Microbial Resilience in Changing Environments: Evaluating Anthropogenic Stress and Monitoring Strategies

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Summary in English

Microbial communities play essential roles in the intricate processes that cycle nutrients in both aquatic and terrestrial ecosystems. These microorganisms establish the very foundation that upholds ecosystem stability, supporting not only their own existence but also the survival of various other organisms, humans included. However, the intricate repercussions of global change and human-induced stressors on these communities and their intricate functions remain unclear. This lack of clarity extends to fundamental knowledge, thereby posing a challenge in determining the most appropriate methodological and experimental approaches. This dissertation aims to delve into the exploration of stressor impacts on these vital microbial communities. Additionally, it seeks to carefully analyze and compare the diverse methodological approaches available, elucidating their respective strengths and limitations in this context.

I revealed the ubiquity of microbial communities and unveiled significant geographical variations among them. In an extensive investigation covering 217 European freshwater lakes, we provided evidence that protists exhibit restricted geographical distribution patterns and that centers of high taxon richness differ from the centers of putative endemic microeukaryotes. Furthermore, the study demonstrates that lowland areas can be examined independently of high-altitude regions and are suitable for generalizable statements.

Another study focused on individual cryptic taxa associated with Ochromonadales and mock communities when exposed to elevated temperature and salt using FISH. I revealed that, while elevated temperature alone does not affect the growth of monocultures of cryptic taxa linked to Ochromonadales, salt exposure influences *Spumella* growth. Yet, within mock communities, increased temperature selectively enhances *Poteriospumella* growth, while combined salt and increased temperature decrease growth rates overall. Thus, morphological similar protists exhibit different tolerances, suggesting that molecular data are rather appropriate to analyze protistan communities.

In another comprehensive German-wide study of 30 freshwater lakes and corresponding soil catchments I uncovered commonalities, differences, and the diffuse exchange of microeukaryotes between habitats. Aside from general variations in community composition, soil harbors greater diversity than freshwater lakes. Analyses of distribution patterns reveal that the majority of microeukaryotes exhibit specificity to particular habitats, and their co-occurrence is largely driven by random drift. This implies that freshwater and soil communities can be viewed as closed systems, supporting the idea that when studying microeukaryotes, it is valid to study these two ecosystems separately.

It is crucial to acknowledge that microbial communities thrive in dynamic environments and are interconnected with these ever-changing surroundings, actively responding to the dynamic shifts around them. The reasons behind these ever-changing environments are diverse, with a significant portion arising from human activities. On one hand, these factors include issues like global warming and deforestation, while on the other hand, they involve various non-point source inputs. Although our understanding of potential stressors on microbial communities and the environment has increased in recent decades, it is far from being comprehensive. This work further aims to identify and monitor the response of microbial diversity and functions to non-point source inputs and environmental stressors using high-throughput methods.

Following, I uncovered that the effects of tire wear proxies primarily stem from leaching substances rather than inert nanoplastics. While diversity indices remain generally unchanged due to tire wear proxies, the structure and function of the community, particularly photosynthesis, are notably impaired by zinc but not by nanoplastics. However, functional redundancy was clearly detectable since photosynthesis is performed by chlorophytes instead of bacillariophytes after exposure to tire wear proxies.

In an extensive mesocosm experiment, I further examined freshwater rivers that received treated wastewater. Utilizing 16S rRNA and strain-resolved metagenomics, I discovered that although a diverse array of bacteria and viruses is initially introduced, their numbers decline over time. This finding underscores the remarkable resilience of the river's microbial community to treated wastewater.

It was not yet investigated whether different high-throughput monitoring methods show similar patterns and exhibit similar sensitivity in detecting environmental changes. In the former large scale mesocosms experiment I additionally analyzed freshwater rivers receiving treated wastewater by means of 16S rRNA and 18S V9 rRNA metabarcoding, and non-target screening. I found that each employed high-throughput method is useful to detect treated wastewater and that the impact of treated wastewater is decreasing. Further, the obtained datasets strongly covary, but their individual strength differs. I observed that 18S V9 rRNA metabarcoding exhibited superior detection sensitivity throughout the study, while non-target screening was also effective throughout the experiments. However, 16S rRNA metabarcoding only showed a reliable detection sensitivity in the first hour.

During these experiments, I recognized that interdisciplinary work and cooperation are crucial for conducting proper research. Because of this, I introduced a measurement for interdisciplinarity based on a bibliometric approach, analyzing metadata from articles in SCOPUS in the final study of this thesis. I demonstrated that, despite being advocated as a

driver of scientific progress, interdisciplinarity is rather absent or primarily conducted by a few individual scientific disciplines.

Summary in German

Mikrobielle Gemeinschaften spielen eine entscheidende Rolle bei den komplexen Prozessen, die Nährstoffe in aquatischen und terrestrischen Ökosystemen zyklisieren. Mikroorganismen bilden das Fundament, das die Stabilität des Ökosystems gewährleistet und nicht nur ihre eigene Existenz, sondern auch das Überleben verschiedener anderer Organismen, einschließlich des Menschen, gewährleistet. Die komplexen Auswirkungen des globalen Wandels und anthropogener Stressoren auf diese Gemeinschaften und ihre komplexen Funktionen bleiben jedoch unklar. Diese Unklarheit erstreckt sich auf grundlegendes Wissen, was eine Herausforderung für die Bestimmung der geeignetsten methodischen und experimentellen Ansätze darstellt. Diese Dissertation zielt darauf ab, die Auswirkungen von anthropogenen Stressoren auf mikrobielle Gemeinschaften zu erforschen. Darüber hinaus wird sorgfältig analysiert und verglichen, welche vielfältigen methodischen Ansätze verfügbar sind und ihre jeweiligen Stärken und Schwächen in diesem Kontext aufzeigen.

Ich habe die Allgegenwärtigkeit mikrobieller Gemeinschaften aufgezeigt und signifikante geografische Variationen zwischen ihnen enthüllt. In einer umfassenden Untersuchung von 217 europäischen Süßwasserseen habe ich nachgewiesen, dass Protisten eingeschränkte geografische Verteilungsmuster aufweisen und dass Zentren hoher Taxonvielfalt von den Zentren vermeintlich endemischer Mikroeukaryoten abweichen. Die Studie zeigt, dass Tieflandgebiete unabhängig von Hochgebieten untersucht werden können und sich für verallgemeinerbare Aussagen eignen.

Eine weitere Studie konzentrierte sich auf einzelne kryptische Taxa, die zu den Ochromonadales gehören, wenn sie erhöhten Temperaturen und Salz ausgesetzt sind, unter Verwendung von FISH. Ich habe aufgedeckt, dass erhöhte Temperaturen allein das Wachstum von Monokulturen kryptischer Taxa, die mit Ochromonadales verbunden sind, nicht beeinflussen, während Salzexposition das Wachstum von *Spumella* beeinflusst. Innerhalb von künstlich erstellten Gemeinschaften fördert erhöhte Temperatur selektiv das Wachstum von *Poteriospumella*, während Salz und erhöhte Temperatur kombiniert insgesamt das Wachstum verringern. Somit zeigen morphologisch ähnliche Protisten unterschiedliche Toleranzen, was darauf hindeutet, dass molekulare Daten eher geeignet sind, mikroeukaryotische Gemeinschaften zu analysieren.

In einer umfassenden deutschlandweiten Studie von 30 Süßwasserseen und den entsprechenden Bodeneinzugsgebieten habe ich Gemeinsamkeiten, Unterschiede und den diffusen Austausch von Mikroeukaryoten zwischen Lebensräumen aufgedeckt. Ich habe festgestellt, dass neben

allgemeinen Variationen in der Gemeinschaftszusammensetzung der Boden eine größere Taxondiversität aufweist als Süßwasserseen. Die Analyse von Verteilungsmustern zeigt, dass die Mehrheit der Mikroeukaryoten eine Spezifität für bestimmte Lebensräume aufweist und ihr gleichzeitiges Auftreten weitgehend durch zufällige Drift getrieben wird. Dies legt nahe, dass Süßwasser- und Bodengemeinschaften als geschlossene Systeme betrachtet werden können, was die Idee unterstützt, diese beiden Ökosysteme getrennt zu untersuchen, sofern Mikroeukaryoten untersucht werden.

Es ist wichtig zu betonen, dass mikrobielle Gemeinschaften in dynamischen Umgebungen gedeihen und mit diesen sich ständig ändernden Umgebungen verbunden sind. Die Gründe für diese ständig wechselnden Umgebungen sind vielfältig und umfassen einen erheblichen Anteil menschlicher Aktivitäten. Auf der einen Seite gehören dazu Probleme wie die globale Erwärmung und Entwaldung, auf der anderen Seite verschiedene diffuse Einflüsse. Obwohl unser Verständnis potenzieller Stressoren für mikrobielle Gemeinschaften und die Umwelt in den letzten Jahrzehnten zugenommen hat, ist es bei weitem nicht vollständig. Diese Arbeit zielt darauf ab, die Reaktion der mikrobiellen Diversität und Funktionen auf diffuse Einflüsse und Umweltstressoren mithilfe Hochdurchsatzmethoden zu identifizieren und zu überwachen.

Im Zuge meiner Untersuchungen konnte ich feststellen, dass die Auswirkungen von Reifenabrieb hauptsächlich auf das Auslaugen von Substanzen zurückzuführen sind, anstatt auf inerte Nanoplastikpartikel. Obwohl Diversitätsindices aufgrund von Reifenabrieb im Allgemeinen unverändert bleibt, sind Struktur und Funktion der mikrobiellen Gemeinschaft, insbesondere die Photosynthese, durch Zink, jedoch nicht durch Nanoplastik, spürbar beeinträchtigt. Allerdings war eine funktionale Redundanz deutlich erkennbar, da die Photosynthese nach Reifenabrieb Exposition von Grünalgen anstelle von Kieselalgen durchgeführt wurde.

In einem umfangreichen Mesokosmos-Experiment habe ich zudem Süßwasserflüsse untersucht, die mit behandeltem Abwasser gespeist werden. Durch die Anwendung von 16S rRNA Metabarcoding und Metagenomik konnte ich feststellen, dass obwohl zunächst Bakterien und Viren eingeführt werden, deren Anzahl im Laufe der Zeit abnimmt. Diese Erkenntnis unterstreicht die bemerkenswerte Widerstandsfähigkeit der mikrobiellen Gemeinschaft des Flusses gegenüber behandeltem Abwasser.

Es wurde noch nicht untersucht, ob verschiedene Hochdurchsatzüberwachungsmethoden ähnliche Muster zeigen und eine vergleichbare Empfindlichkeit bei der Erfassung von Umweltveränderungen aufweisen.

In dem vorherigen groß angelegten Mesokosmos-Experiment habe ich zusätzlich

Süßwasserflüsse, die behandeltes Abwasser erhielten, durch 16S rRNA- und 18S V9 rRNA-Metabarcoding sowie durch non-target screening analysiert. Ich stellte fest, dass jede verwendete Hochdurchsatzmethode dazu geeignet ist, behandeltes Abwasser zu detektieren, und dass der Einfluss von behandeltem Abwasser über die Zeit abnimmt. Die erhaltenen Datensätze korrelieren stark miteinander, jedoch unterscheiden sich die individuellen Detektions-Stärken der Methoden. Ich beobachtete, dass 18S V9 rRNA-Metabarcoding während der gesamten Studie eine überlegene Nachweissensitivität aufwies, während non-target screening ebenfalls effektiv war. Allerdings zeigte 16S rRNA-Metabarcoding nur in der ersten Stunde eine zuverlässige Nachweissensitivität.

Während dieser Experimente erkannte ich, dass interdisziplinäre Zusammenarbeit entscheidend ist, um adäquate Forschung durchzuführen. Aus diesem Grund führte ich eine Messung für Interdisziplinarität auf Basis eines bibliometrischen Ansatzes ein, wobei ich Metadaten aus Artikeln in SCOPUS in der abschließenden Studie meiner Dissertation analysierte. Ich konnte zeigen, dass trotz der Forderung nach Interdisziplinarität, als treibende Kraft des wissenschaftlichen Fortschritts, diese eher fehlt oder hauptsächlich von einigen wenigen wissenschaftlichen Disziplinen durchgeführt wird.

General introduction

Earth's ecosystems are the very bedrock of life on this planet, providing not only the essential resources and environmental conditions that support all living beings but also the foundation upon which industries are built (Calvin et al., 2023; Cavicchioli et al., 2019; Gleick & Cooley, 2021). These ecosystems provide habitats, shelter and sustenance for countless species, including humans, to thrive. However, Earth's ecosystems are undergoing unprecedented and often rapid changes, driven by factors such as climate change, habitat destruction, pollution, and many more (Rogowska et al., 2020; Tolkkinen et al., 2015). These changes are occurring on a global scale and have far-reaching consequences for the tiniest microbes to the grandest trees. Microbial communities, often overlooked but of paramount importance, are the very cornerstone of these ecosystems. These tiny yet powerful organisms play a vital role in maintaining ecosystem stability, nutrient cycling, and the overall health of our planet. Understanding and preserving these microbial communities is not only essential for the survival of countless species but also for safeguarding the balance of our own existence.

Behind the term microbial communities hides a tremendously complex world. While everyone has heard the word ‘microbe’, it is incredibly difficult to put into words what is hidden behind. Microbial communities, microeukaryotic and prokaryotic, can be characterized by the fact that they are of small size and are involved in almost every process on Earth. Microbes are found everywhere, from water to soil to air, and even in extreme environments like deserts and thermal springs (Geisen et al., 2015; Grossmann et al., 2016). They are ubiquitous in these habitats, existing on inanimate matter and occurring on or within other organisms, including other microbes (Taylor et al., 2009; Whitman et al., 1998). Microbes are not only ubiquitous; they also represent the most abundant and diverse life forms on our planet (Brown et al., 2009; Whitman et al., 1998). It might seem, at first glance, that larger organisms like mammals and arthropodes dominate in terms of biomass, but it is the microbes, along with higher plants, that play the predominant role in biomass distribution (Bar-On et al., 2018; Dobrovol’skaya et al., 2015; Pomeroy et al., 2007; Whitman et al., 1998). Due to their considerable abundance and biomass, their photosynthetic representatives (phototrophic/mixotrophic protists and cyanobacteria) contribute to approximately half of the primary production, with a substantial proportion taking place within marine environments (Field et al., 1998; Robinson & Williams, 2005) which lifts them at least to the same importance as higher plants when just referring to primary production, but with microbes comes even more value.

Role of microbes

Prokaryotes play a pivotal role in maintaining aquatic and terrestrial ecosystem function and stability owing to their diverse ecological functions (Coleman et al., 2018). Their significance becomes apparent in their roles as decomposers, nutrient recyclers, and primary producers within the microbial loop (Azam et al., 1983; Fenchel, 2008; Geisen et al., 2020; Pomeroy et al., 2007; Sherr & Sherr, 1988). Dissolved organic matter (DOM) is introduced in multiple ways into the environment, due to sloppy feeding, fecal pellets, algal exudates, dead biomass and its decomposition (Bertilsson & Jones, 2003; Fogg, 1983; Møller et al., 2003). While prokaryotes contribute to the decomposition of organic matter (Kirchman et al., 1982; Kirchman, 2004), microeukaryotic fungi take on the vital role of decomposition, facilitating the vital flow of carbon in ecosystems (Dumack, 2016).

This decomposition process releases essential nutrients like carbon, nitrogen, and phosphorus into the aquatic environment in the form of dissolved organic matter (Williams, 1981), which needs to be reintroduced in a utilizable form into the food web to maintain ecosystem sustainability (Kirchman, 2004). Heterotrophic prokaryotes thrive on dissolved organic matter, effectively converting it into a usable form for other organisms. This subsequently enables prokaryotes to serve as a vital food source for organisms incapable of directly utilizing DOM. In this manner, prokaryotes and fungi are essential intermediaries in nutrient cycling, facilitating the flow of energy and nutrients within ecosystems.

While also some microeukaryotic microbes, protists, are able to make use of dissolved organic matter, most of their heterotrophic representatives, like flagellates, ciliates and amoebas, feed on prokaryotes, channeling bacterial secondary production to higher trophic levels (Azam et al., 1983; Boenigk & Arndt, 2002; Zubkov & Tarran, 2008) and then themselves, combined with their phototrophic representatives, represent a food source for higher trophic levels (Lampert et al., 1986; Verity, 1986).

It is important to note that the distinction between phototrophs and heterotrophs is not always clear-cut, as some organisms exhibit mixotrophic behavior, blurring the boundaries between these nutrition modes (Laval-Peuto et al., 1986; Mitra et al., 2014). Mixotrophs possess the ability to perform both photosynthesis and phagotrophy, providing them with a versatile approach to meet their nutritional needs. In the complex food web, all these microorganisms serve as vital sources of food for various heterotrophic microorganisms, as well as higher trophic levels, such as zooplankton and nematodes. The consumption and transfer of biomass through these trophic levels sustain entire ecosystems and support the growth and survival of organisms at different stages of the food web (Azam et al., 1983; Dumack, 2016; Pomeroy et al., 2007).

Microorganisms form the essential foundation of food webs, making them irreplaceable. Both prokaryotes and microeukaryotes are fundamentally important, however, prokaryotes have garnered more extensive research attention than microeukaryotes (Caron et al., 2009; Geisen et al., 2020). Hence, the responsibility falls upon the scientific community to bridge this knowledge gap by conducting focused research on microeukaryotes, encompassing protists and fungi.

Biogeography and dispersal of microeukaryotes

In ecosystems worldwide and across all habitat types, these group of organisms have similar importance, although the individual taxa may not necessarily be the same due to different tolerances and the presence of dispersal barriers.

The concept of protist dispersal has undergone significant evolution, with notable contributions from various scientists throughout history. Ehrenberg, in the mid-19th century, proposed that protist populations were geographically isolated, and their dispersal was primarily governed by chance. This early perspective emphasized the apparent isolation of protist communities. Fast forward to 1913 and 1934, when Beijerinck and later Baas-Becking formulated a famous statement that encapsulated the idea of dispersal: "Everything is everywhere, but the environment selects" (Baas Becking, 1934; Beijerinck, 1913). This phrase introduced the notion that each protist could potentially be found on a global scale but were subject to selection processes specific to their respective habitats. This concept garnered renewed attention in the 1980s, igniting a contemporary debate regarding the dispersal and endemism of protists, in which the moderate endemicity model and the everything-is-everywhere model were under discussion. This discussion was led by prominent researchers, such as Finlay, Fenchel, and Foissner (Aguilar et al., 2014; Bass & Boenigk, 2011; Chao et al., 2006; Fenchel & Finlay, 2004; Finlay et al., 1996; Foissner, 1999, 2004, 2007; Weinbauer & Rassoulzadegan, 2007).

It is important to acknowledge that the original formulation of the "everything is everywhere" concept, as well as many subsequent interpretations, relied on morphological characteristics to define the operational taxonomic unit (OTU). This often took the form of morphospecies, as discussed by Finlay et al. in 1996 (Finlay et al., 1996). Furthermore, in 1998, Finlay and Esteban proposed that morphospecies possess ecological significance, emphasizing the idea that "form determines function" (Boenigk, 2014; Finlay & Esteban, 1998). This mechanistic view suggested that the similarities in morphology among protists might lead to similar ecological roles and community structures in various environments, reinforcing the concept of global dispersal. Evidently, many protist morphospecies display a cosmopolitan distribution, leading to the assumption that the presence of similar communities in various locations is due to their

shared morphological traits, but distribution is different for molecular defined species (Aguilar et al., 2014; Chao et al., 2006; Katz et al., 2005).

In summary, the study of protist dispersal has progressed from the initial notion of chance-based isolation to the recognition that protists can be found globally, with their dispersal influenced by habitat-specific selection processes. The reliance on morphological traits has played a significant role in these discussions.

In retrospect, the heavy reliance on morphological traits to understand protist dispersal was a product of its time, mainly because it was the only feasible approach. However, in the contemporary era, we have been granted a remarkable array of advanced tools, with Next-Generation Sequencing (NGS) among the most powerful. These molecular techniques have revolutionized our understanding of protist dispersal. By analyzing DNA sequences, we are no longer restricted to morphology as our sole determinant. Instead, we can delve deeper into the genetic complexity of protists, providing us with a more comprehensive view of their global distribution and ecological roles (Chao et al., 2006; Scheckenbach et al., 2006). While the same morphospecies can be well found in different habitats, e.g. marine and freshwater, and their function may also be very similar, they may be ancestrally related, but are phylogenetically distinct from the respective other lineage (Scheckenbach et al., 2006). Morphological similarity, but genetically differences are not a novel concept, as convergent evolution is not a new concept to biology, but the development of morphological similarities can be based on homology, but also analogy.

Dispersal barriers are different for macro- and microorganisms. Contrary to the dispersal capabilities of higher organisms like plants and animals, the knowledge about microeukaryotic dispersal capabilities is limited. On the one hand distances between continents undoubtedly present dispersal challenges for animals and microeukaryotes alike, even smaller spatial gaps, such as adjacent soils and lakes or forests and neighboring meadows still create considerable dispersal obstacles for microeukaryotes. This emphasizes that dispersal barriers for these microorganisms can exist even within seemingly similar environments. On the other hand the distribution by chance is higher due to their small size.

Several recent molecular studies have provided evidence of restricted distribution and endemism in different microeukaryotic groups (Dunthorn et al., 2012; Segawa et al., 2018; Siver & Lott, 2012; Tedersoo et al., 2014).

Microorganisms can be dispersed through anthropogenic factors like ballast water, aquaculture, fishing, and water sports (Hallegraeff & Bolch, 1992; Pagenkopp Lohan et al., 2017). Moreover, the dormant stages of microorganisms exhibit remarkable resilience, enabling them to survive extended journeys and prolonged transport periods (Ekelund & Rønn, 1994; Katz et al., 2005;

Khan et al., 2015). Non-anthropogenic factors, such as chance events, animal interactions, and wind currents, can also act as vectors for microorganism dispersal (Jauss et al., 2021; Solarz et al., 2020). However, it is important to note that being dispersed differs from actively establishing and thriving in a new environment. For a microorganism to successfully colonize, it must possess specific adaptations and tolerances to abiotic and biotic environmental conditions and effectively navigate the interplay of these factors.

These adaptations and tolerances vary among different protists. Some are generalists, capable of thriving in vastly different habitats, such as aquatic and terrestrial habitats. In contrast, specialists are narrowly adapted to specific environments, like certain altitudes (Olefeld et al., 2020). While there is a distinction between specialists and generalists, the boundaries between them can be flexible. As a result, microbial communities exhibit variability according to their habitats. Accordingly, it is evident that different environmental conditions give rise to distinct protist communities. It is important to acknowledge that there can be some degree of overlap, as certain protists possess similar tolerances, allowing them to exist in different habitats. Furthermore, habitats may even act as a "seedbank" for adjacent habitats, helping initiate or reestablish populations in neighboring areas (Crump et al., 2007, 2012). However, at a fundamental level, one can differentiate between aquatic and terrestrial protist communities, which can be further categorized into marine, sedimentary, and freshwater communities (Bates et al., 2013; Geisen et al., 2018; Grossmann et al., 2016; Seppey et al., 2017). To delve deeper, one can continue to refine these distinctions, for instance, one may consider rainforests and agricultural lands or alpine lakes and eutrophic lakes (Bates et al., 2013; Lentendu et al., 2014; Mahé et al., 2017). As we refine these distinctions, the differences between protist communities become more pronounced or subtle. The interconnectedness of habitats underscores a crucial consideration when investigating protists. It is not self-evident that the analyzed protist community is considered as an independent entity, especially when the surrounding terrestrial or aquatic environments can significantly influence the dynamics of these communities (Crump et al., 2007, 2012; Monard et al., 2016). For instance, when examining river's protist community, it is imperative to acknowledge that the adjacent terrestrial habitat may serve as a buffer, potentially reintroducing protist species into the river. In such cases, studies should address both habitats together, as they are interacting in a manner that blurs the boundaries between them. This approach ensures a comprehensive understanding of the intricate relationships between protists and their surrounding ecosystems, offering valuable insights into the broader ecological context of these microorganisms.

Changing environments

Habitats themselves undergo transformations, such as those driven by seasonal and diurnal cycles. These natural changes and varying habitat conditions contribute to habitat heterogeneity and thus to the creation of a huge diversity of habitats each with its own ecological niches, ultimately fostering microbial diversity (Chesson, 1986; Ettema & Wardle, 2002; Tews et al., 2004; Torsvik et al., 2002). Nevertheless, changing environments have only a positive impact on diversity up to a certain degree. Especially when considering human-induced changes, which, instead of nurturing biodiversity, often act as stressors surpassing the range of naturally occurring variations and may result in fatal consequences. As microbes often find themselves at the forefront of man-made stressors, it is crucial to recognize that microbial diversity is equally, if not more, vulnerable compared to the diversity of higher organisms (Rocca et al., 2019; Vibha & Neelam, 2012; Weinbauer & Rassoulzadegan, 2007). While many ecosystem functions can likely be taken over by other microorganisms through functional redundancy (Allison & Martiny, 2008; Banerjee et al., 2016; Caron & Countway, 2009; Huang et al., 2020), a continuous loss of biodiversity ultimately leads to a loss of ecosystem functions.

Human activities, such as urbanization or industrialization, have a profound impact on the environment, introducing a host of distinctive factors and fluctuations that deviate significantly from natural occurrences or variations (Huang et al., 2020; Min et al., 2011). These changes often introduce pollutants into the environment, which have profound and detrimental effects on ecosystems, microbial communities, and both their diversity (Calvin et al., 2023). To highlight some human-induced changes, it is important to acknowledge factors such as climate change, salinization, treated wastewater, and the presence of tire particles along with associated chemicals.

The impact of these stressors on the environment is multifaceted; an illustrative example is the effect of climate warming, which prompts the shift of numerous species accustomed to warmer habitats towards northern territories (Ostfeld, 2009; Short et al., 2017). This shift presents a dual impact: these newcomers may serve as stressors to the already vulnerable native biota if the latter do not undergo a similar northward shift. Concurrently, the migration of these species also introduces a new dimension of concern as it brings along various protistan parasites, including those dominant in rainforests (Mahé et al., 2017; Ostfeld, 2009; Short et al., 2017). These parasites are entering territories where the indigenous biota lack any previous exposure or adaptations (Marcogliese, 2008; Pickles et al., 2013). This serves as merely one instance demonstrating the substantial impact of climate change on the environment. Therefore, this scenario illustrates how a single factor can significantly influence biodiversity by initiating

disruptions in established ecological systems and introducing novel stressors, ultimately impacting the delicate balance of various ecosystems.
The situation is further complicated by the fact that multiple stressors interact, exhibiting additive, synergistic, or antagonistic effects (Crain et al., 2008; Vos et al., 2023).

The intricate interactions between microorganisms and their environment make them sensitive responders to changes, including those induced by human activities (Karpouzas et al., 2022; Pawlowski et al., 2016). By comprehending how stressors influence microorganisms, we can anticipate broader ecological consequences and potentially mitigate harmful effects. However, despite their pivotal importance, the intricate effects of these stressors on especially microeukaryotes remain largely uncharted territory. The gaps in our understanding underscore the critical need for comprehensive research and analyses. Unraveling these intricate relationships can provide insights into safeguarding ecosystem resilience, promoting sustainable practices, and ultimately ensuring the vitality of our environment for generations to come.

Technological progress

But how comes that, despite their central role, microorganisms are still understudied and potential effects on their diversity and dispersal are not known? One must be aware that, in contrast to macroorganisms, microorganisms have only recently become truly investigable and distinguishable. While centuries of knowledge have already been accumulated about macroorganisms, microorganisms remained literally invisible to the human eye for a long time until van Leeuwenhoek described the “Animalcules” (Dobell, 1932; Lane, 2015). Although microscopy allowed the investigation of microorganisms, comprehensive research was still far from being achieved, especially as diversity of microorganisms surpasses that of macroorganisms by far. The vast diversity of microorganisms, combined with their microscopic size and intricate variations in morphology and cryptic species made detecting, differentiating, studying and comprehending them a tremendous challenge.
Microscopy stands as an indispensable tool in unraveling the world of microorganisms. Its evolution from traditional light microscopy to encompass advanced techniques such as phase contrast, differential interference contrast, and dark-field microscopy has empowered researchers to observe live microorganisms (Boenigk, 2014). However, a significant leap occurred with the introduction of electron microscopy. Although in existence for some time, its accessibility to biologists was initially limited until more cost-effective options emerged (Boenigk, 2014; Pawley, 1997; Silveira, 2010). Despite the vast potential for exploration,

differentiating cryptic species remained challenging. These cryptic species, morphologically similar yet genetically and environmentally distinct (Fenchel, 2005), presented an obstacle until the emergence of PCR. The advent of PCR filled this gap, transforming the analysis of DNA sequences into a routine method. Molecular methodologies seamlessly integrate into microscopy, whether through fluorescence microscopy paired with fluorescent dyes and molecular probes for fluorescence in situ hybridization (FISH) or in collaboration with entirely different techniques. This combination of techniques highlights the synergy between traditional and molecular approaches, offering an expanded toolkit to navigate the intricacies of microbial study.

Beyond microscopy and PCR, the emergence of Next-Generation Sequencing (NGS) has fundamentally transformed our capacity to unravel the genetic diversity and functional capabilities within complex microbial communities. NGS technologies, encompassing high-throughput DNA and RNA sequencing, empower researchers to swiftly sequence and analyze extensive genetic material derived from environmental samples. Particularly in metagenomic studies, the extraction and sequencing of DNA from entire microbial communities in intricate ecosystems have fostered an in-depth exploration of microbial presence, interactions, and networks, employing techniques such as 16S rRNA or 18S rRNA gene sequencing. Through the sequencing of specific marker genes or distinct regions within those markers, researchers can effectively identify and categorize microbial taxa, significantly aiding in the comprehension of community structure and dynamics (MacLean et al., 2009; Medinger et al., 2010).

The integration of microscopy and NGS technologies has significantly advanced our understanding of microorganisms, providing unprecedented insights into their morphological traits, interactions, and genomic composition in recent times. However, comprehensive analysis entails not only microscopy and NGS but also other methods like non-target analysis and various applied techniques. These new methods allow for a more comprehensive and successful monitoring of the environment. Monitoring is an exceedingly important task in today's context, as much is changing due to human influences (Friberg et al., 2011; Woodward et al., 2013). In order to take countermeasures for environmental protection, it is necessary to first identify any negative impacts on the environment before addressing it.

While recognizing and monitoring these impacts form fundamental prerequisites, diverse subsequent actions are necessitated, thereby introducing another challenge. Effective solutions require interdisciplinary collaboration among various stakeholders (Ledford, 2015). Although interdisciplinary collaboration is frequently discussed, its practical implementation is often constrained. Consequently, potential issues are frequently addressed using limited measures, underscoring the room for enhancement through enhanced interdisciplinary cooperation.

Objectives and structure

This doctoral thesis is divided into three chapters, encompassing seven articles. **Article I** demonstrates that altitudinal geographic barriers seem to have limited relevance for microeukaryotes, and lowland habitats contain fewer endemic microeukaryotes. This implies that for understanding microbial responses to stressors, focusing on lowland samples yields meaningful and generalizable results. **Article II** sheds light on a crucial finding: despite similar morphology, microeukaryotes exhibit varying levels of tolerance. This implies that molecular data offer an advantage over morphological data. **Article III** shows that freshwater and soil communities can be considered closed systems, reinforcing the notion that studying microeukaryotes separately within these ecosystems is justified.

Hence, when investigating community responses, it becomes evident that employing molecular techniques is a suitable approach, and focusing on aquatic communities is justified for **articles**, **IV**, **V**, and **VI**.

Finally, **article VII** introduces a method for measuring interdisciplinarity. While conducting research, it became clear that stepping beyond subject boundaries was often necessary to complete research effectively or at all. Despite the widespread talk about interdisciplinarity, the study investigated how it is actually practiced. Surprisingly, the findings showed that it is not as commonly done as one might think.

Chapter I: „*Protistan dispersal and barriers*“

Article I: „*Centers of endemism of freshwater protists deviate from pattern of taxon richness on a continental scale*“ aims to provide insights into the distribution patterns of taxonomic diversity and endemism among microeukaryotes across various taxonomic groups, while shedding light on the impacts of geographic barriers and distances. The study implies that studying lowland areas is meaningful for generalizable conclusions. **Article II**: „*Effects of stressors on growth and competition between different cryptic taxa affiliated with Ochromonadales (Chrysophyceae)*“ observes that morphologically similar species have differing stressor tolerances, hence offering an advantage when using molecular datasets. **Article III**: „*Protistan and fungal diversity in soils and freshwater lakes are substantially different*“ demonstrates a strict separation between water and soil microeukaryotic communities, making it evident that focusing on water communities is sufficiently meaningful for subsequent studies.

Chapter II: *„Monitoring anthropogenic stressors and their impact on microbial communities“*
Article IV: „*Microbial community shifts induced by plastic and zinc as substitutes of tire abrasion*“ investigates the impact of tire wear off proxies on microeukaryotic communities. **Article V**:„*Temporal disturbance of a model stream ecosystem by high microbial diversity from treated wastewater*“ focuses on microbial resilience to a substantial introduction of treated wastewater. **Article VI**: „*Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater*“ focuses on a method comparison of whole length 16S nanopore, short fragment 18S V9 Illumina sequencing and non-target screening for monitoring treated wastewater in rivers.

Chapter III: *„The need for interdisciplinarity“*
Article VII: „*The Premise of Interdisciplinarity and Its Actual Absence—A Bibliometric Analysis of Publications on Heavy Rainfall*“ describes a new measure for interdisciplinarity with a case example on heavy rainfall events.

Chapter I:

“Protistan dispersal and barriers”

Article I:

„Centers of endemism of freshwater protists deviate from pattern of taxon richness on a continental scale“

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Analyses & interpretation	20%	Analyses, interpretation, and discussion of the data
Manuscript & tables/figures	20%	Writing and review of the manuscript and creating figures

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Centers of endemism of freshwater protists deviate from pattern of taxon richness on a continental scale

Jana L. Olefeld[1], Christina Bock[1], Manfred Jensen[1], Janina C. Vogt[2], Guido Sieber[1], Dirk Albach[2] & Jens Boenigk[1]✉

Here, we analyzed patterns of taxon richness and endemism of freshwater protists in Europe. Even though the significance of physicochemical parameters but also of geographic constraints for protist distribution is documented, it remains unclear where regional areas of high protist diversity are located and whether areas of high taxon richness harbor a high proportion of endemics. Further, patterns may be universal for protists or deviate between taxonomic groups. Based on amplicon sequencing campaigns targeting the SSU and ITS region of the rDNA we address these patterns at two different levels of phylogenetic resolution. Our analyses demonstrate that protists have restricted geographical distribution areas. For many taxonomic groups the regions of high taxon richness deviate from those having a high proportion of putative endemics. In particular, the diversity of high mountain lakes as azonal habitats deviated from surrounding lowlands, i.e. many taxa were found exclusively in high mountain lakes and several putatively endemic taxa occurred in mountain regions like the Alps, the Pyrenees or the Massif Central. Beyond that, taxonomic groups showed a pronounced accumulation of putative endemics in distinct regions, e.g. Dinophyceae along the Baltic Sea coastline, and Chrysophyceae in Scandinavia. Many other groups did not have pronounced areas of increased endemism but geographically restricted taxa were found across Europe.

Restricted distribution and endemism has been demonstrated for numerous protist taxa by now[1–4]. However, the geographic patterns underlying protist distribution are not yet resolved. Here, we aim at disentangling areas of high and low protist diversity on a European scale and further test dependencies between local taxon richness and local accumulation of endemics.

Many factors influence the diversity and distribution of species, resulting in a heterogeneously distributed taxon richness across the world[5]. For microscopic species the assumption of a ubiquitous distribution changed towards a general acceptance of a "moderate endemicity"[6–8]. Even though restricted dispersal and distribution pattern at least for some taxa are unquestioned[9–12] and large-scale variation between protist communities have been shown[13–15] the resulting pattern as well as areas of high and low protist diversity are largely unresolved. Distinct habitats may differ considerably in taxon richness[14,16–18], which is often related to environmental factors such as pH, altitude and trophy. Examples for regional differences in taxon richness of animals and plants as well as of protists are mountain ranges[18–22] which, however, basically reflect effects of one of the formerly mentioned abiotic factors, i.e. altitude and covarying factors, rather than geographic differentiation. Even though high mountain ranges have been suggested to hinder dispersal and thus to structure protist diversity between remote regions[7,23], taxon richness on both sides of mountain ranges do not necessarily differ despite the above-mentioned altitudinal gradients within the mountain ranges[18]. Aside from such azonal areas and special habitats, it remains unclear where regional areas of high protist diversity are located and whether these correspond to regions of high biodiversity known from macro-organisms. It is further unclear whether such areas are universally valid for all taxonomic groups or specific for distinct taxa. Only for a few taxonomic groups a regionally outstandingly high species richness has so far been suggested and only for few regions such as the Aquitaine region (France)

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for Synurales (Chrysophyceae)[24] or the Antarctic and sub-Antarctic for *Stauroneis* (Bacillariophyta) floras[25]. The importance both of geographic distance and of geographic barriers for structuring pattern of protist taxon richness and in particular the relative importance of these two factors on continental scales remain disputable.

Likewise, it is unclear where regional centers of protist endemism are located and whether these regions correspond to areas of high protist taxon richness. Mechanisms allowing for a high local or regional taxon richness can be different from those which restrict dispersal[26,27]. For instance, local habitat heterogeneity allows for a high regional taxon diversity[28] whereas geographic barriers are considered to limit dispersal[29]. Areas with high numbers of endemic protist taxa, thus, can but do not necessarily merely covary with taxon richness, i.e. regions of high taxon richness may deviate from regions holding high numbers of endemic taxa as it has been inferred for animals and plants[30,31]. For protists such relations between taxon richness and endemism are not known. To date it is unclear (i) where to expect regions of high protist taxon richness and (ii) where to expect regions holding high numbers of endemic protist taxa. Studies so far strongly center around azonal habitats and regions with island character: In particular, high mountain ranges have been suggested to act as islands for endemic taxa both for macro- and microorganisms[32,33]. Further, elevation seems to be a crucial factor structuring species distribution and diversity for animals and plants, but also for protists[18,20,22,34].

Aside from such special and azonal habitats, investigations for centers of protist endemism are again scarce and mostly restricted to local or regional studies on selected taxonomic groups[24,25]. In the marine environment (e.g.[15,16,35]) and to a lesser extent also in fresh waters (e.g.[14,18,27,36]) recent large scale surveys revealed some trends of protist distribution but general patterns of protist endemism in fresh waters are still unclear on continental scales. It is even more vague whether areas of high endemism are congruent for different taxonomic groups. Here, we thus analyze congruencies and discrepancies between patterns of taxon richness and endemism for protists and differences between taxonomic groups.

Signs of restricted distribution may already be apparent for subpopulations below species level, i.e. distribution areas of the subpopulations may be considerably smaller than that of the taxon[13,37,38]. Consequently, beyond the analysis of SSU-based OTU diversity we further applied paired sequencing of SSU and ITS sequences to test whether geographic constraints also apply to populations unresolved by SSU and thus address the issue of population structure and population genetics of free-living protists. In contrast to the relatively conserved SSU rRNA, the ITS-region is highly variable and allows differentiation of very closely related species or of intraspecific subpopulations[39,40]. Unfortunately, large differences in sequence length and the high degree of sequence diversity complicates analyses of these region[7,41]. A dataset with population-like structure can be generated using the SSU-V9- and ITS1-region, resulting in a microdiversity of ITS-sequences with identical V9-sequences differing in their geographic distribution[13].

We here hypothesize that (i) areas of high taxon diversity can be separated from areas of low taxon diversity, (ii) taxa (as well as subpopulations of taxa) tend to have a restricted geographic distribution and are not randomly distributed across Europe, (iii) patterns of protist endemism deviate from patterns of protist taxon richness and that (iv) these patterns vary between taxonomic groups. We further hypothesize that geographic distance and geographic barriers differentially affect distribution pattern.

Results

Filtering resulted in a dataset comprising 13,640 V9-groups with 285,755 ITS-variants occurring in 217 natural freshwater lakes. After clustering of the ITS-variants 46,302 ITS-SWARMS remained for analyses.

Taxa based on V9-sequences (V9-groups) are geographically restrained. 56% (7,643) of V9-groups were only found in a single lake, in particular Dinophyceae had a noticeably high number of V9-groups, which occurred in only one lake. The majority of OTUs, which occurred in more than one lake, showed indications for a geographically restricted distribution (distribution maps of V9-groups, which occurred in at least 5 lakes, are available under https://doi.org/10.5281/zenodo.3674717), i.e. the mean distance between the respective lakes was smaller than would have been expected for random distribution ($p < 0.001$; Fig. 1, Table 1). Hence, a geographical restriction of protist V9-groups is present in many taxa, but the extent of geographical restriction varies between taxonomic groups.

For instance, 52% of the V9-groups affiliated with Chytridiomycetes and 44% of the V9-groups affiliated with Fungi were geographical restricted. In contrast, only 30% of the V9-groups affiliated with Dinophyceae were geographically restricted. For all other taxa the fraction of geographically restricted V9-groups ranged between 39% (Eustigmatophyceae) and 50% (Chloroplastida) (Table 1).

Regions of high taxon richness (V9-richness) differ between taxonomic groups. Diversity patterns vary between protist groups both with respect to taxonomy and with respect to the nutritional strategy. Differential patterns of taxon richness are particularly pronounced for Dinophyceae and Chrysophyceae (Fig. 2). Whereas Dinophyceae do have regions of high taxon richness located around the Massif Central and towards Eastern Europe, Chrysophyceae have a high taxon richness in Scandinavia (Fig. 2). Less pronounced but still a significant unequal distribution of taxon richness was found for phototrophic and mixotrophic taxa, in particular for Viridiplantae and Bacillariophyceae (Figs. 2, 3), Dictyochophyceae and Eustigmatophyceae (Figure S2). Taxon richness was more homogeneously distributed across Europe for heterotrophic and substrate-bound taxa, such as Ciliophora, Apicomplexa, Fungi, Cercozoa and Chytridiomycetes (Figs. 2, 3, S1). An exception were the Oomycetes with a more unevenly distributed pattern of taxon richness. Interestingly, all taxa that do show a more homogenous distribution of taxon richness had a pronounced peak of taxon richness in the grid including Lake Constance.

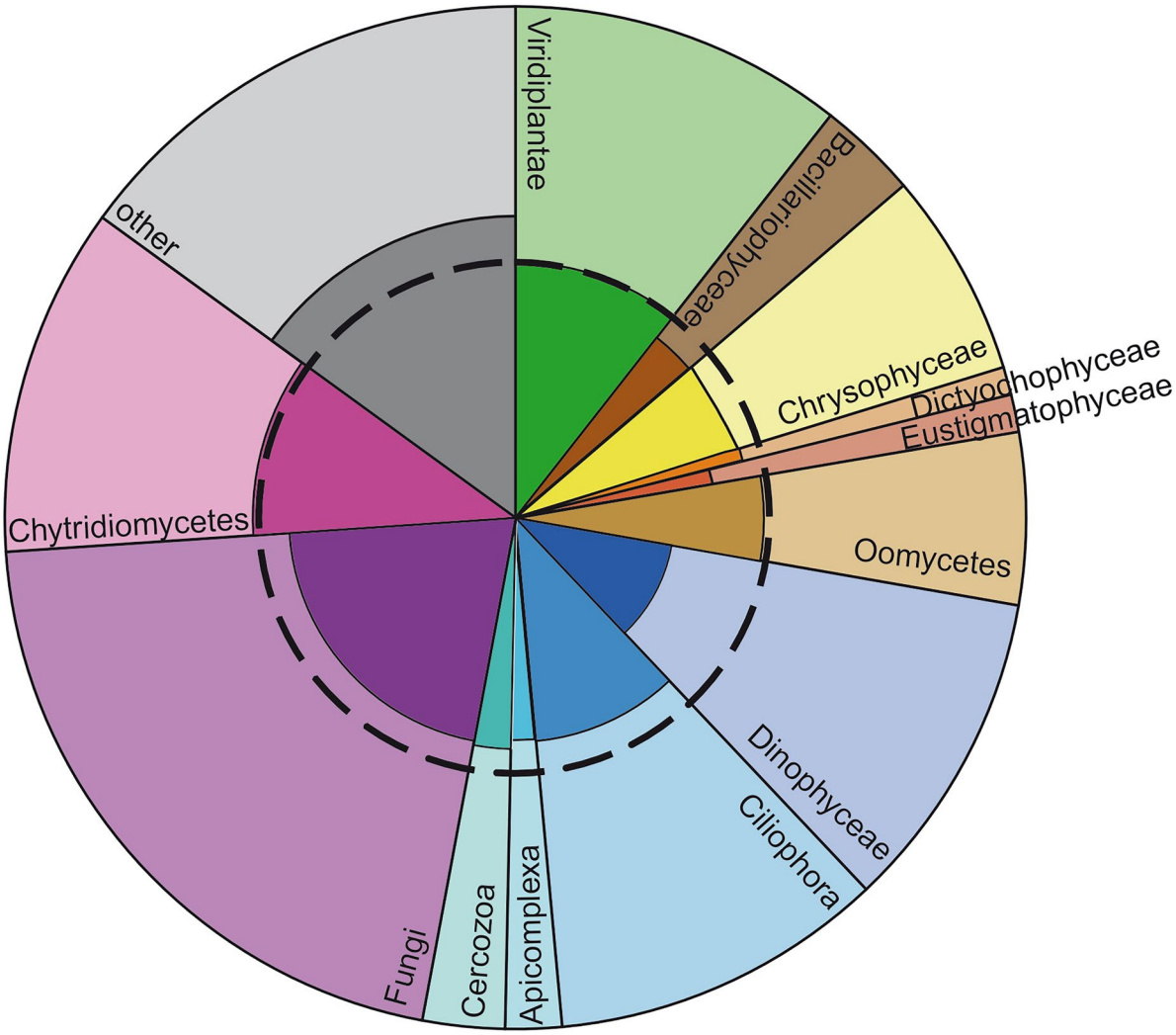

Figure 1. Relative read abundance of different taxonomic groups and fraction of V9-groups showing a geographically restricted occurrence (dark shaded) for each of these groups. The dashed line indicates the 50% line. Graphic was created using R with the package ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html) and manually edited with Adobe Illustrator2020.

Centers of (putative) endemism (based on V9-groups) deviate from regions of high taxon richness. The number of V9-groups with putatively endemic distribution (see methods for definition in the context of this study) varies greatly between the groups studied. Only a very small proportion of the analyzable V9-groups with putative endemic distribution was found within the Eustigmatophyceae (3%), whereas nearly one quarter (17%) of the V9-groups affiliated with Cercozoa met our criteria for putatively endemic distribution (Table 1).

Similar to patterns of taxon richness, regions harboring a particularly high number of groups with putatively endemic distribution differed between taxonomic groups and nutritional strategies but did not necessarily overlap with observed richness patterns (for correlation see Figure S3). In general, many taxa with restricted distribution occurred in mountain regions like the Alps, the Pyrenees or the Massif Central (Figs. 2 and 3 and Figures S1, S2, S8). Beyond such general areas with many putatively endemic taxa, some taxonomic groups were pronouncedly accumulated in distinct regions: For Dinophyceae a region with a high number of putatively endemic taxa is the area near the Baltic Sea coastline (Fig. 2). This clearly deviates from their richness pattern, since taxon richness in this area is only slightly higher compared to other regions. Chrysophyceae exhibited a very different pattern with both, a high number of putatively endemic taxa and a high regional taxon richness in Scandinavia (Fig. 2). The Viridiplantae, Fungi, Oomycetes, Chytridiomycetes and Cercozoa did not show specific areas with taxa of restricted distribution but these were found across the whole sampling area (Fig. 3 and Figures S1 and S2). This was, in principle, similar in diatoms but in this taxon the fraction of taxa with putatively endemic distribution was remarkably low in general. For Ciliates, centers of taxa with putatively endemic distribution are condensed along the Pyrenees and Alps but start to fan out towards Eastern Europe (Fig. 3). For Dictyochophyceae and Eustigmatophyceae only a small number of endemic taxa with putatively restricted distribution were found (Figure S2).

Taxonomy	Biogeographic analyses					
	V9-groups (total)	ITS-SWARMS (total)	Average ITS-SWARMS/ V9-group	Geographical restricted V9-groups	Geographical restricted ITS-SWARMS	Putativly endemic V9-groups
Chloroplastida	635 (1,080)	1666 (4,432)	2.62 (4.10)	315 (50%)	859 (52%)	65 (10%)
Bacillariophyceae	189 (394)	902 (3,199)	4.78 (8.12)	81 (43%)	552 (61%)	21 (11%)
Chrysophyceae	390 (775)	1,260 (4,741)	3.23 (6.12)	172 (44%)	710 (56%)	42 (11%)
Dictyochophyceae	53 (80)	455 (1,245)	8.58 (15.56)	24 (45%)	302 (66%)	6 (11%)
Eustigmatophyceae	71 (170)	241 (844)	3.39 (4.96)	28 (39%)	144 (60%)	2 (3%)
Oomycetes	326 (597)	557 (1522)	1.71 (2.55)	158 (48%)	262 (47%)	30 (9%)
Dinophyceae	612 (1755)	1,415 (4,878)	2.31 (2.78)	186 (30%)	588 (42%)	50 (8%)
Ciliophora	635 (1,219)	1,395 (3,641)	2.20 (2.20)	286 (45%)	630 (45%)	62 (10%)
Apicomplexa	107 (280)	273 (1,071)	2.55 (3.83)	46 (43%)	130 (48%)	11 (10%)
Cercozoa	156 (418)	291 (1,226)	1.87 (2.93)	71 (46%)	142 (49%)	27 (17%)
Fungi	1,260 (3,044)	2026 (7,918)	1.61 (2.60)	556 (44%)	867 (43%)	140 (11%)
Chytridiomycetes	664 (1537)	1,245 (4,109)	1.88 (2.67)	347 (52%)	535 (43%)	88 (13%)
	Environmental analyses					
	V9-groups comprising differently adapted ITS-SWARMS					
Taxonomy	Examined V9-groups	pH	Conductivity	Temperature	Altitude	
Chloroplastida	419	7 (1.67%)	19 (4.53%)	13 (3.10%)	9 (2.15%)	
Bacillariophyceae	155	2 (1.29%)	6 (3.87%)	6 (3.87%)	7 (4.52%)	
Chrysophyceae	299	5 (1.67%)	13 (4.34%)	12 (4.01%)	7 (2.34%)	
Dictyochophyceae	40	3 (7.50%)	3 (7.50%)	5 (12.50%)	3 (7.50%)	
Eustigmatophyceae	41	0 (0 .00%)	0 (0.00%)	1 (2.44%)	1 (2.44%)	
Oomycetes	226	3 (1.33%)	2 (0.88%)	2 (0.88%)	4 (1.77%)	
Dinophyceae	288	6 (2.08%)	9 (3.13%)	9 (3.13%)	8 (2.78%)	
Ciliophora	384	9 (2.34%)	9 (2.34%)	11 (2.86%)	10 (2.60%)	
Apicomplexa	69	1 (1.45%)	2 (3.00%)	2 (3.00%)	1 (1.45%)	
Cercozoa	103	1 (0.97%)	3 (2.91%)	2 (1.94%)	1 (0.97%)	
Fungi	824	4 (0.49%)	5 (0.61%)	4 (0.49%)	10 (1.21%)	
Chytridiomycetes	394	3 (0.76%)	5 (1.27%)	9 (2.28%)	16 (4.06%)	

Table 1. Diversity of V9 groups and ITS-SWARMS as reflected by the richness and the number of taxa with restricted or even putatively endemic distribution. The number of V9 groups comprising ecologically differently adapted subpopulation as reflected by ITS-SWARMS are provided with respect to pH, conductivity, temperature and altitude. As this latter analysis was restricted to V9 groups only which occurred in several lakes and comprised several ITS-SWARMs, the total number of V9 groups subjected to this analysis is also given for comparison.

High mountain ranges are home to many specialist taxa. Taxon richness differed strongly between mountain ranges (altitude > 1,500 m) and lowland (altitude < 1,500 m) regions. Significantly lower richness was found in mountain ranges (V9-groups: $p = 9.7e^{-07}$; ITS-SWARMS: $p < 0.001$; Fig. 4A,B). In contrast, the intraspecific variation in terms of ITS variants within V9-groups is higher in mountain ranges than in lowland areas ($p < 0.001$; Figure S4).

The fraction of OTUs exclusive to the respective altitudinal range is significantly higher in mountain ranges (V9-groups: $p < 0.001$; ITS-variants: $p < 0.001$; Fig. 4C,D) even though the absolute number of exclusive V9-groups and ITS-SWARMS is higher in the lowlands (654 V9-groups and 2,275 ITS-SWARMS are exclusive for mountain ranges, 11 675 V9-groups and 42 248 ITS-SWARMS are exclusive for lowland areas). An intermediate number of OTUs occurred independently of altitude (1 311 V9-groups and 1779 ITS-SWARMS occur in both regions).

Geographic restriction and physicochemical differentiation of subpopulations as reflected by ITS-SWARMS. Approximately 70% of the ITS-SWARMS were only found in one lake (Table 1). Beyond the geographical patterns reflected by SSU sequences (V9-group level) a restricted geographical distribution of subpopulations (ITS-SWARMS associated with distinct V9-groups) could be confirmed ($p < 0.001$; Figure S5). The ratio of diversification within the ITS-SWARMS varied between the different taxonomic groups and nutritional strategies. In osmotrophic and saprophytic taxa, the number of ITS-SWARMS per V9-group was generally low (average number of 1.6–1.9) and the fraction of ITS-SWARMS with a geographical restriction was comparatively low, e.g. only 43% in Fungi and Chytridiomycetes and 47% in Oomycetes. In contrast, the predominantly phototrophic Stramenopiles, i.e. the Ochrophyta (Chrysophyceae, Bacillariophyceae, Dictyochophyceae, Eustigmatophyceae) generally had a higher number of ITS-SWARMS per V9-group (average number of 3.2–8.6) and a higher percentage of ITS-SWARMS with restricted geographical distributions (56–66%). Viridiplantae and

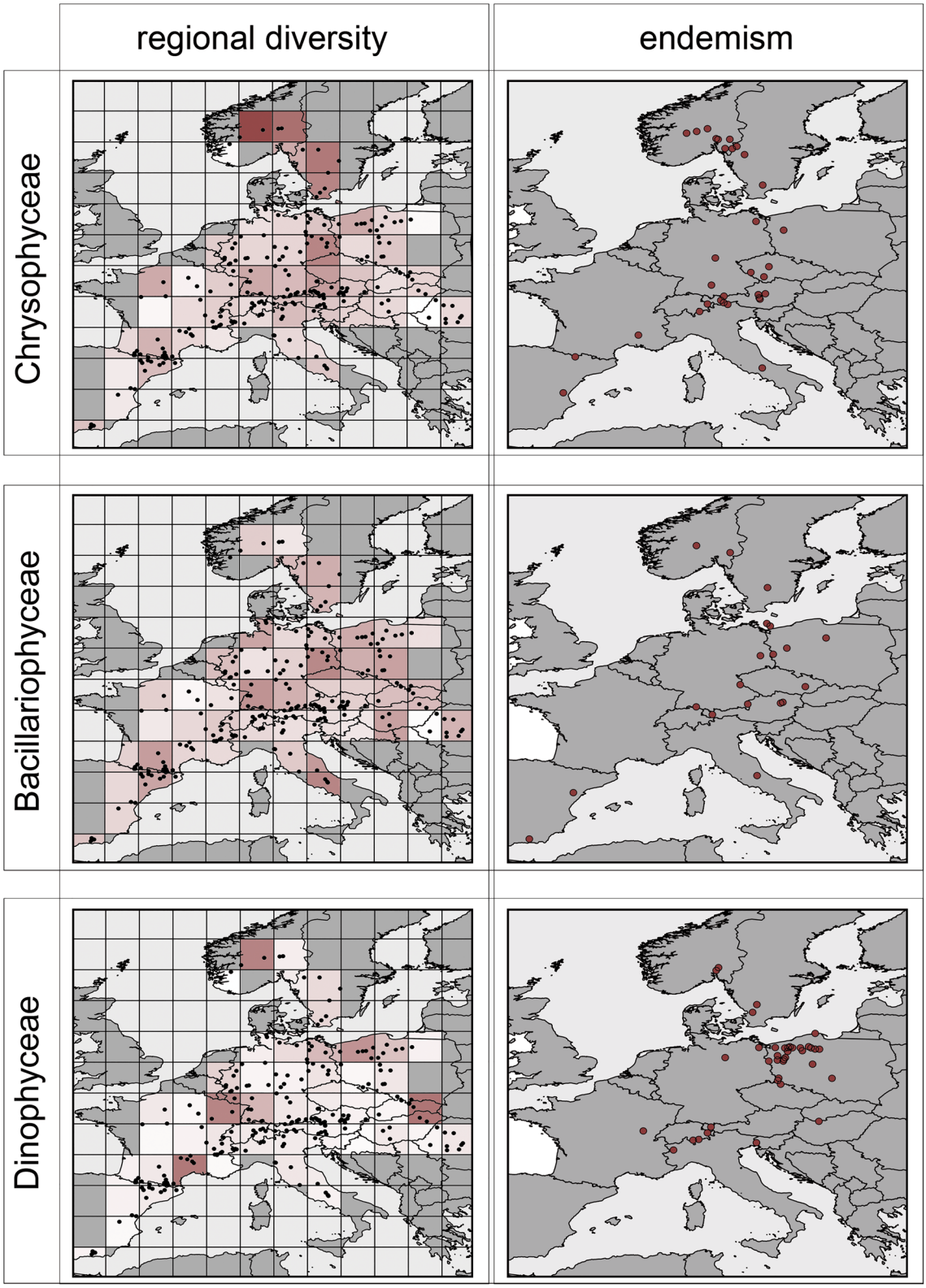

Figure 2. Regional diversity of Chrysophyceae, Bacillariophyceae and Dinophyceae. Left: Regional richness of the respective taxon where color shading indicates low (white) to high (red) regional richness. Maximal shading corresponds to 23 interpolated V9-groups per lake for Chrysophyceae, to 15.4 interpolated V9-groups per lake for Bacillariophyceae and to 17.5 interpolated V9-groups per lake for Dinophyceae. Right: centers of distribution areas for putatively endemic taxa within the respective group. Please note that the centers of the distribution area are geometric centers and do not coincide with a location of a distinct lake. Maps were created using *R* with package *rworldmap* v. 1.3–6 (https://cran.r-project.org/web/packages/rworldmap/index.html) and modified using ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html) and Adobe Illustrator2020.

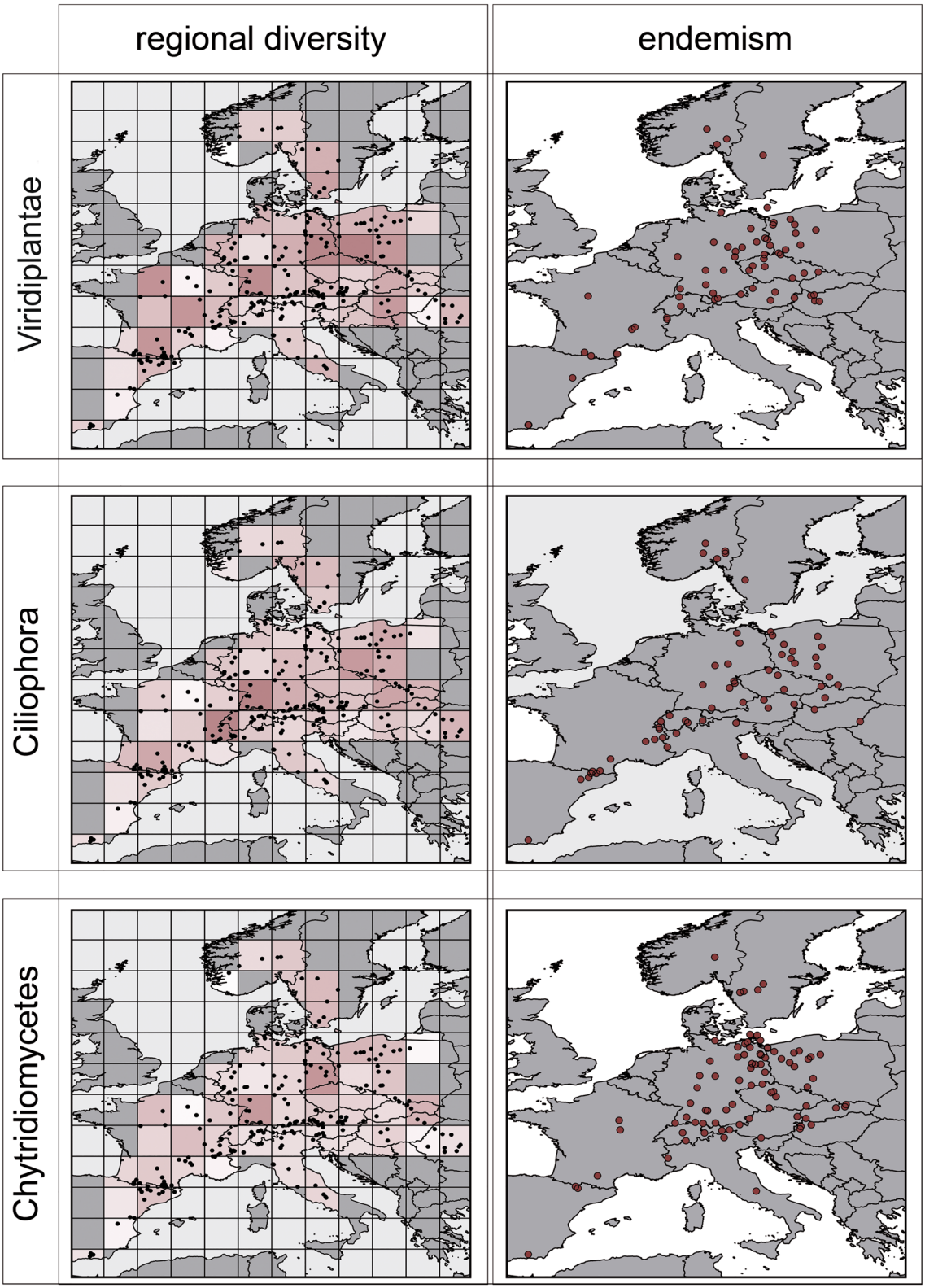

Figure 3. Regional diversity of Viridiplantae (green algae), Ciliophora and Chytridiomycetes. Left: Regional richness of the respective taxon where color shading indicates low (white) to high (red) regional richness. Maximal shading corresponds to 15.4 interpolated V9-groups per lake for Viridiplantae, to 15.1 interpolated V9-groups per lake for Ciliophora and to 13.3 interpolated V9-groups per lake for Chytridiomycota. Right: centers of distribution areas for putatively endemic taxa within the respective group. Please note that the centers of the distribution area are geometric centers and do not coincide with a location of a distinct lake. Maps were created using *R* with package *rworldmap* v. 1.3–6 (https://cran.r-project.org/web/packages/rworldmap/index.html) and modified using ggplot2 (https://cran.r-project.org/web/packages/ggplot2/index.html) and Adobe Illustrator2020.

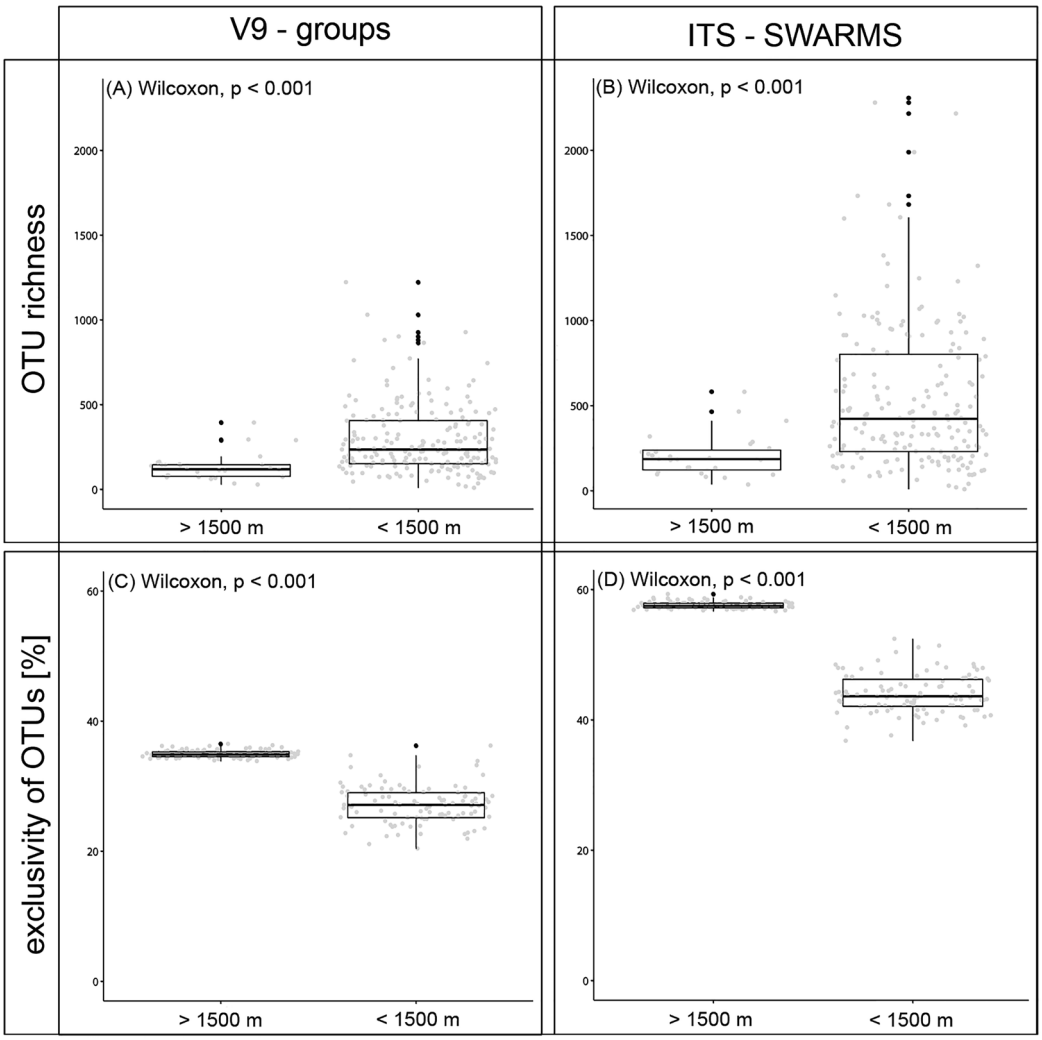

Figure 4. OTU richness (upper graphs) and exclusivity of OTUs (lower graphs) to either high altitude (above 1,500 m) or low altitude (below 1,500 m) lakes. Richness and exclusivity are shown for two different phylogenetic resolutions, i.e. for V9 groups (left) and for ITS-SWARMS (right). Richness is lower but exclusivity is higher in lakes above 1,500 m both for V9-groups and for ITS-SWARMS.

Alveolata had an intermediate number of ITS-SWARMS per V9-group (average number of 2.2–2.6) and a low to intermediate geographical restriction of ITS-SWARMS (42% to 52% respectively). In all taxonomic groups some taxa included certain geographically restricted ITS-SWARMS (Figure S5[42]). Aside from indications for a geographical restriction of some subpopulations, the ITS-SWARMS also indicate an ecophysiological differentiation of clades below the resolution of V9-groups (Table 1). 3,242 V9-groups associated with the main taxon groups of interest were analyzed. The most significant differences of the examined V9-groups and their associated ITS-SWARM were found concerning altitudinal differences (77), followed by temperature and conductivity differences (76, respectively) and pH (44). Overviews on ecophysiological parameters of sampled lake are found in Figure S6.

Discussion

Here, we reveal conspicuous patterns of protist endemism on a European scale, which differ between taxa. However, they do not necessarily correlate with patterns of protist taxon richness.

A surprisingly high number of V9-groups indicated a putatively restricted distribution (putatively endemics) (Fig. 1, Table 1) corresponding to the expectations derived from the moderate endemicity hypothesis[1,43,44]. Our results correspond to this hypothesis insofar as taxa (V9-groups) spanned the whole range from restricted to ubiquitous distribution.

The number of taxa with narrower distribution than expected by chance was surprisingly high and is not reflected by community studies, which report a significant but low effect of geography for structuring protist communities[14,18,45]. In contrast, many studies addressing the distribution of distinct species confirm a geographic restriction for numerous species (e.g.[4,11,43,46–48]) and different degrees of endemism are known for several taxa[12,49]. For instance, our data demonstrate that a large fraction of Chytridiomycetes have a restricted distribution

(Figs. 1,3). This corresponds to a study from the Antarctic[50]. A high proportion of taxa with restricted distribution seems plausible for Chytridomycetes since many species are specific to a particular host or group of hosts (algae), further reducing the ability of wide dispersal[51].

Our analyses show that distribution patterns of taxa with restricted distribution vary considerably both within and between taxonomic groups and do not follow a uniform regional pattern. Despite a high fraction of taxa with restricted distribution, patterns are therefore not or hardly visible at the community level as they are not consistent between taxa. This may explain the somewhat deviating view between taxon-specific investigations and community studies discussed above.

For protists the relations between taxon richness and centers of endemism have not, based on our knowledge, been investigated on a continental scale so far. Studies on animal and plant richness pattern imply that simple correlations are an oversimplification and that centers of endemism may be found both in areas with high and in areas with low or intermediate taxon richness[30,31]. However, for protists the distribution pattern of endemics and relations between the richness of taxa and of endemics may deviate from those known for macro-organisms. In particular, as patterns of taxon richness point to a lower importance of geographic barriers for protists, centers of endemism may also be less dependent on such barriers or even not exist at all (or simply reflect a fixed fraction of taxon richness).

Our data point to a low overlap between areas of increased taxon richness and increased endemism for protists. Indicating that centers of endemism (based on V9-groups) deviate from regions of high taxon richness. Rapid population growth and the combination of asexual and sexual reproduction could lead to a fast adaption to local environmental conditions[52] and may therefore explain the observed missing overlap. For protist taxa with restricted distribution we revealed that distribution areas vary considerably, and boundaries of distribution areas do not necessarily coincide with geographic barriers such as mountain ranges (see additional information on https://doi.org/10.5281/zenodo.3674717). Thus, even though our data substantiate the hypothesis that geographic distance is relevant for dispersal, geographic barriers (which are important for structuring distribution ranges of macro-organisms[53]) seem to be of minor importance. However, it has to be considered that passive dispersal is limited by the dispersal capacity of the transport vector. It is beyond the scope of this study to analyze the differential adaptations between taxa with narrow and such having a wide distribution. Nevertheless, this aspect clearly is a promising objective for future research since our data indicate that dispersal limitations are important for protists even though the geographic barriers that restrict distribution ranges of macro-organisms may be of little relevance for protists.

High mountain ranges as azonal habitats harbor a deviating protistan diversity. Irrespective of their presumably low significance for protist dispersal, high mountain ranges are special habitats deviating in taxon richness and endemism from the surrounding lowlands[54]. Beyond a general decrease of taxon richness with altitude as predicted by species-area-relationships[55], a common pattern for most protist taxa was a certain increase in the number of taxa exclusive to high mountain lakes (Fig. 4) and a certain cumulation of putatively endemic taxa within mountain ranges (Figs. 2, 3). Apart from the apparently low importance as barriers hindering dispersal, high mountain ranges provide azonal environmental conditions and thus can be expected to harbor taxa of restricted distribution and potentially endemic to the mountain range[33]. Further, we also observed an increase of taxa exclusive to high mountain lakes but not necessarily restricted to a distinct orogene. High mountain ecosystems are traditionally considered as extreme for life[56]. In high mountain lakes organisms are confronted with short growing seasons, low food nutrient availability or high incident solar radiation[57]. Harmful wavelengths of UV radiation can reach down to the lake bottom, in particular in clear water with low concentrations of humic substances and suspended particles[58,59]. The harsh environmental conditions of high mountain lakes require special adaptions to survive[14,60] and may thus result in a high degree of taxa specializing on high mountain environment[61,62]. For instance, DNA repair mechanisms are important in adapting to high UV radiation levels and particularly for algae also photoprotection mechanisms[63]. The low nutrient availability and low water temperature further cause low densities of organisms and consequently low encounter rates which are require adaptations of foraging organisms to low food densities[14].

Regions of high taxon richness (V9-richness) and centers of endemism differ between taxonomic groups. Apart from the azonal mountain habitats, we demonstrate that patterns of richness and endemism vary considerably between taxonomic groups. Patterns of two taxa, i.e. the Chrysophyceae and the Dinophyta may deserve special attention.

Taxon richness of Chrysophyceae was exceptionally high in Scandinavia, which corresponds to a pre-dominance of Chrysophyceae in many Scandinavian lakes[64]. Since many Scandinavian lakes are nutrient poor, slightly acidic and rich in humic substances the high taxon richness may reflect abiotic conditions favoring this taxonomic group[65–67].

In contrast, the pattern of endemism was unexpected for Dinophyta. The number of putatively endemic taxa restricted to the area was particularly high near the Baltic Sea coastline while taxon richness in this area was only moderate. We can only speculate that due to the reduced salinity levels of the Baltic Sea[68] the high degree of endemism in this area may result from a migration of taxa along environmental (salinity) gradients and subsequent speciation, while further dispersal might be limited by the niche width of individual taxa[69]. An increased phytoplankton diversity is known for the Baltic Bays and Bodden regions[70] but it remains speculative why such a pronounced richness of putatively endemic taxa in this area was only found for Dinophyta. However, this region is strongly recommended for taxonomic follow-up studies as it likely holds numerous taxa waiting for discovery.

In contrast to these taxa with pronounced centers of endemism, diatoms had a remarkably low fraction of geographically restricted and endemic V9-groups, which may indicate either a generally wider distribution of diatom taxa or a general lack of taxonomic resolution in this group, i.e. that V9-groups reflect clusters of diatom species rather than individual species. This would support the observation that for diatoms the V9 region is well suited to explore genus-level diversity but has limited resolution at the species level[35]. The conspicuously low

phylogenetic resolution within diatoms may be due to the fact that diatoms are a relatively young group, i.e. their diversification in the fossil record starting in the mid-mesozoic and their expansion paralleling the evolution and expansion of grasslands not before the mid-cenozoic[71,72]. Due to this (in geological terms) recent increase both in relative abundance and in diversity may be responsible for low phylogenetic resolution as the differentiation of gene sequences between species may be relatively recent.

Our results show that geographic restriction and physicochemical differentiation are already visible for subpopulations as reflected by ITS-SWARMS. We observed a generally high ITS-diversity. Subpopulations as reflected by ITS-SWARMS associated with given V9-groups provided meaningful information both with respect to geography and to abiotic adaptations. ITS sequence variation within V9 groups was particularly high in high mountain areas. Presumably, the loss of rare genotypes is lower and/or speciation rates are higher in mountain ranges as compared to lowland areas. This is not surprising since mutation rates are likely to be higher due to high UV radiation[73] and population effects, e.g. the bottleneck effect, are presumably less important[74] since generation times are likely to be prolonged due to the generally low temperatures and to nutrient limitations. Further, the pronounced environmental gradients and heterogeneous topographies may limit gene flow and colonization opportunities, thus leading to a stronger genetic differentiation[75,76].

Interestingly, phototrophic and mixotrophic taxa exhibited a more pronounced geographical restriction of ITS-SWARMS than those of heterotrophic taxa. We assume that general differences in the growth and[77] nutritional strategies are responsible for this pattern. Phototrophic taxa strongly depend on the surrounding physicochemical conditions[78]. Free living heterotrophic taxa are presumably less dependent on physicochemical conditions and thus less sensitive to changes. Niche specialization, requiring adaption and therefore genetic differentiation might therefore be infrequent in heterotrophs but frequent in phototrophs and mixotrophs. However, the differential geographic restriction of ITS-SWARMS between nutritional modes co-varied with the average number of ITS-SWARMS per V9 group. Differential dispersal can therefore not clearly be separated from differential diversity and sequence evolution and thus remains an issue for future research.

Contrary to our initial expectations, we could observe abiotic differentiation between subpopulations and it may, thus, be promising to include markers with higher phylogenetic resolution in future surveys of protist diversity. Independent on whether the ITS-SWARMS may represent species, which are not resolved by V9 variation or subspecies/subpopulations, we clearly demonstrate that ecological and geographical differentiation becomes evident on this level of resolution. Beyond the knowledge gain in basic reserach and the promises for protist population genetics our findings of a significant ecophysiological differentiation of subpopulations opens so far unexpected options for biomonitoring and the development of molecular and microbial bioindicators.

Conclusion/summary

In summary, we demonstrated (i) that patterns of taxon richness and of endemism deviate for protists, (ii) that these patterns further deviate between different taxonomic groups, (iii) that high mountain ranges harbor a diverging protist diversity but presumably have only a low effect on protist dispersal as derived from distribution pattern in lowland areas, and (iv) that different taxonomic classes and phyla differ in microdiversity as reflected by ITS variants.

We demonstrate that geographic distance is relevant for protist dispersal but geographic barriers have only a low impact on structuring distribution pattern. These findings challenge the general validity of biogeographic patterns (mostly) derived from studies on macro-organisms. We thus propose that the proportion of the relative importance of geographic distance and of geographic barriers for structuring biodiversity on a continental scale systematically changes with size of the organism and biological traits (e.g., mode of nutrition). We further propose to incorporate this idea as dispersal disparity hypothesis and the respective scaling effects into future studies on differential distribution, dispersal and biogeography of organisms strongly differing in organismic size and in population size.

Materials and methods

Eukaryotic amplicon sequences from 217 freshwater lakes across Europe (including Norway, Sweden, Germany, Poland, Romania, Austria, Italy, France, Spain, and Switzerland) were used in this study from the NCBI Bioproject PRJNA414052[18]. Sampling, DNA-isolation, sequencing and bioinformatic procession were conducted as described in detail in[18]. The sampled lakes reflect the predominant water body type in the different regions.

Briefly, samples were taken near the shore of the first 50 cm water column. Each lake was sampled once in august 2012 at one sampling point. For the DNA extraction and sequencing, the water was filtered onto 0.2 µm nucleopore filters, air dried and frozen in liquid nitrogen (Cryoshippers) and later stored at − 80 °C in the laboratory until further processing.Water temperature, pH and conductivity were determined directly in the field at the same sampling point and time by use of a Waterproof Tester "Combo" (Hanna Instruments, Vöhringen, Germany). Each measurement was performed 3 times and the mean value was calculated. The lakes were unequally distributed throughout Europe. The maximum distance (2,880,648 km) is between lake Nordmesna (Norway) and Embalse de Béznar (Spain); median distance is 781.045 km, mean distance 861.566 km with a standard deviation of 506.958 km.

The V9-ITS1 region of the 18S SSU and ITS region of the rDNA were amplified using a forward primer (5′-GTACACACCGCCCGTC-3′) and a combination of two reverse primers with different wobble positions (5′-GCTGCGCCCTTCATCGKTG-3′ (ITS2_Dino; 10%) and 5′-GCTGCGTTCTTCATCGWTR-3′ (ITS2_broad; 90%)) with an annealing temperature of 52 °C. Samples were equimolar pooled and commercially sequenced using an Illumina HiSeq 2,500 rapid run applying 2 × 300 bp reads with subsequent adapter trimming, quality trimming and demultiplexing (FASTERIS; Geneva, Switzerland). After quality filtering, assembly of reads and chimera removal[18], sequences were filtered using the AmpliconDuo pipeline[79]. Sequencing results were dereplicated based

on 100% identity including length variability (CD-HIT-EST algorithm;[80]). For each ITS-sequence, the most abundant assembled V9-sequence was kept as presumably correct sequence. Pairings of other V9-sequences with this specific ITS-sequence were excluded as likely erroneous sequence parings. Thus, each V9-sequence can be affiliated with multiple ITS-sequence pairings whereas each ITS-sequence after filtering was affiliated with only one distinct V9-sequence. Finally, reads were clustered based on identical V9-sequences (first 150 bp, R-Script "V9_Clust.R";[81]), and taxonomically assigned based on the SSU fragment by searching the NCBI database using BLASTn (version 2.7.1). ITS sequences assigned to the same V9 sequence are denoted ITS variants of the respective V9. Reads assigned to Metazoans or higher plants were excluded from further analyses.

Samples with less than 15,069 reads after filtering, (corresponding to less than 15% of the median number of reads per lake) were excluded from further analysis. ITS-variants with a read abundance of less than 0.001% of total reads per lake (corresponding roughly to 10 reads per lake) were also excluded for the corresponding lake. Remaining ITS-variants belonging to the identical V9-sequences were independently clustered using SWARM (= ITS-SWARM; version 2.2.2;[82]). All subsequent statistical analyses were based on presence-absence data of V9-groups or ITS-SWARMS using the software R (version 3.6.0;[83]).

Restrictive geographical distribution of V9-groups. All V9-groups, which occurred in at least two lakes, were used for identifying areas harboring high numbers of taxa with potentially restricted distribution. For each of these V9-groups the average distances between lakes in which the respective V9-group occurred was calculated and tested using a paired t-test against the average distance between an equal number of randomly selected lakes (based on 1,000 random drawings per V9-group); a spatial distance matrix was calculated based on the GPS data using distm() and the "distHaversine" option (geosphere; version 1.5–10;[84]).

In the context of this study we considered a V9-group (or ITS variant) as being geographically restricted when the mean distance between the lakes harboring this V9-group (or ITS variant) was smaller than would have been expected for random distribution. Further, in the context of this study we considered a V9-group as putatively endemic when two criteria were met: (i) the distance of lakes that contain a given V9-group was smaller than that of randomly chosen lakes in at least 90% of the simulations (based on 1,000 simulations as outlined above) and (ii) all lakes that contained the given V9-group were found within a maximum (rectangular) area of 1,000,000 km.

Regions of high richness and regions harboring a high number of V9-groups with restricted distribution were analyzed based on a geographic grid with a grid size of 2.5° longitude × 2.5° latitude. The number of lakes per grid are displayed in Figure S7. Regional diversity was independently analyzed for all taxonomic groups (classes or phyla) comprising at least 50 V9-groups (Apicomplexa, Bacillariophyceae, Cercozoa, Viridiplantae, Chrysophyceae, Chytridiomycetes, Ciliophora, Dictyochophyceae, Dinophyceae, Eustigmatophyceae, Fungi, Oomycetes). In order to account for different numbers of lakes per grid, we calculated an interpolated number of OTUs per lake for each taxonomic group and each grid ($N_{interpolated}$), i.e. we applied non-linear regression ($N_{interpolated} = a * \log(N + 1) + b$ with N: number of lakes) to rarefaction curves for each grid separately. Prior tests demonstrated that the chosen regression formula reflected best the relation between OTU number and number of lakes (data not shown). Based on this regression we calculated for each grid and each taxonomic group the expected number of OTUs for one lake in the respective grid, for grids containing a single lake only the original number of OTUs was used.

Differences between low-altitude and high mountain lakes were assessed by comparing lakes at high elevations (above 1,500 m altitude) and lakes below 1,500 m altitude (Figure S8 shows the largest mountain ranges in the study area). Total numbers of V9-groups and ITS-SWARMS per lake, as well as the number of ITS- SWARMS per V9-group were compared using the Wilcoxon-test. Since the total number of low-altitude lakes was much higher than that of high mountain lakes (n = 27), the exclusiveness of V9-groups and ITS-SWARMS in low-altitude and mountain lakes was compared using equivalent numbers of low-altitude lakes (n = 27), i.e. subsets of randomly chosen low-altitude lakes (based on 100 randomly permutated drawings; the diversity of both, lowland and high mountain lakes was tested against the remaining lowland lakes (n = 127)).

Geographic restriction and ecophysiological adaptation of subpopulations based on ITS-SWARMS assigned to distinct V9-groups was analyzed by simulations and paired t-tests (as outlined above for V9-groups). For geographic restriction, the average distances between lakes were compared for all ITS-SWARMS, which occurred in at least two different lakes, and tested against average distances between equal numbers of random lake combinations (1,000 random drawing per ITS-SWARM; for these analyses all lakes were considered as reference in which the respective V9-group occurred). Analyses concerning the differential distribution of ITS-SWARMS with respect to physicochemical factors were further restricted to V9-groups with at least two associated ITS-SWARMS. Differences in environmental parameters (pH, conductivity, temperature and altitude) between lakes affiliated with a distinct ITS-SWARMS of one V9-group were tested using Kruskal-Wallice tests[85] followed by Dunns-Test[86].

Data availability

Eukaryotic amplicon sequences from 217 natural freshwater lakes across Europe were used in this study from the NCBI Bioproject PRJNA414052[18]. Additional material (distribution maps of V9 groups) can be found under https://doi.org/10.5281/zenodo.3674717.

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Author contributions
J.L.O. collected and analyzed the data and drafted the first manuscript; C.B. provided critical feedback, and wrote the manuscript; J.C.V. provided critical feedback; G.S. and M.J. helped with the analyses and statistics; D.A. provided critical feedback, helped to shape the analyses, designed the overall study, J.B. designed the overall study and wrote the manuscript; all authors have given their final approval for the manuscript.

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Article II:

„Effects of stressors on growth and competition between different cryptic taxa affiliated with Ochromonadales (Chrysophyceae)“

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Effects of stressors on growth and competition between different cryptic taxa affiliated with Ochromonadales (Chrysophyceae)

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Abstract: Morphologically similar flagellate taxa comprise a high cryptic diversity. This diversity evolved partly through parallel evolution in several phylogenetically distinct taxa. Here we investigate the effects of heat waves and salinization on growth and competition between cryptic taxa. For this purpose, we developed specific FISH probes targeting the phylogenetic clades comprising *Pedospumella encystans*, *Spumella rivalis*, and *Poteriospumella lacustris*, respectively. Exposure to salt resulted in a decreasing growth rate for all three taxa. In contrast, a sudden increase in temperature to 27 °C stimulated particularly the growth of *P. lacustris*. This species showed a high competitive strength and the taxon–specific responses to stressors lead to a shift of community composition. This turn–over of differently adapted cryptic species with presumably similar feeding preferences and predator–prey interactions may stabilize microbial food webs facing environmental change.

Key words: anthropogenic stressors, Chrysophyceae, cryptic species, fluorescence in situ hybridization, heat wave, heterotrophic flagellate, microbial food web, multiple stressors, salinization

Introduction

In the face of rapid environmental change the stability of food webs and ecosystem functions becomes an increasingly pressing issue (Saint–Beat et al. 2015; van Meerbeek et al. 2021). In particular, microbial components are of special interest due to their high abundances and their link with geochemical cycles. On the one hand, microbial components may respond quickly to environmental changes due to short generation times, on the other hand, a high functional redundancy between taxa may dampen effects of environmental change. As cryptic diversity is high in many microbial groups the effects of stressors on taxon–turnover and the significance of stressor–induced shifts in the competitive strength of distinct (cryptic) taxa is largely unresolved. Here we address the competitive strength of such cryptic taxa using temperature and salinity as environmental stressors.

Significance of heat waves and salinization

Freshwater ecosystems are under severe pressure by anthropogenic impacts and climate change, factors of particular concern in inland waters are heatwaves and salinization (Sun & Arnott 2022). Salt is introduced to freshwater ecosystems by various routes including the mobilization of natural salts (Herczeg et al. 2001; Cañedo–Argüelles et al. 2013) and anthropogenic salinization including e.g., human–accelerated weathering, mining, vegetation removal and application of fertilizers, irrigation practices, and road de–icing salts (Cañedo–Argüelles et al. 2013; Dugan et al. 2017). Eukaryotic microbial community shifts caused by salinity are well documented (von Alvensleben et al. 2016; Castillo et al. 2018; Li et al. 2018; Nuy et al. 2018). Similarly, effects on the growth rates of individual taxa are well documented. The range of tolerated salt concentrations is highly variable (e.g., Boenigk 2008) but generally increasing salt concentrations are negatively correlated with growth rates (Jones et al. 2017; Lam et al. 2019; Park et al. 2020; Arnott et al. 2022). Salt stress can reduce the cell size, cease motility, and trigger morphological changes (Shetty et al. 2019; Kamakura et al. 2022).

Long–term temperature increases are expected due to the warming climate (Ipcc 2013; Rasconi et al. 2015). But heat waves, i.e., rapid short–term increases of temperature, may be more problematic and can impact all trophic levels including microorganisms (Szymczak et al. 2020; Polazzo et al. 2021). Again, severe eukaryotic

microbial community shifts following heat stress have been demonstrated (Hao et al. 2018; Nuy et al. 2018; Thomson et al. 2019). With respect to individual taxa increasing temperatures are expected to increase growth rate but above a critical threshold further temperature increases damage proteins and eventually cause cell death. Both, the extent of growth rate acceleration and the critical maximum temperatures are species– and strain–specific and may therefore affect the competitive strength of individual taxa even within the range of well–tolerated temperatures (Boenigk et al. 2007).

Even though salinization and heat waves are important stressors in freshwater ecosystems, effects of these stressors on the competitive strength and taxon–turn–over in cryptic species and with that the potential preservation of functions despite a considerable taxon turn–over stay secret.

Significance of cryptic diversity

Eukaryotic microbial communities are composed of numerous taxa which are in ecological studies often roughly categorized into functional groups such as heterotrophic nanoflagellates, microalgae, etc. (e.g., Fernandez–Leborans & Fernandez–Fernandez 2002; Latorre et al. 2021). Small protists, largely heterotrophic nanoflagellates (HNF) are the major link connecting dissolved organic material, bacteria, and higher trophic levels (Jürgens & Matz 2002; Sherr & Sherr 2002). They are consumers of suspended as well as of attached bacteria (for reviews see Fenchel 1986; Sandersa et al. 1992; Laybourn–Parry & Parry 2000). Despite their importance in food web architecture the functional role of heterotrophic nanoflagellates is often limited to black box approaches while the ecological importance of taxon diversity in this functional group still stays largely secret. During the last decades, molecular surveys proved the diversity behind these black boxes. On the one hand, many taxa comprise a high molecular diversity (e.g., Lowe et al. 2005), on the other hand, taxa from different lineages have similar morphological forms which evolved independently through parallel evolution (e.g., Graupner et al. 2018). The functional role of the high molecular (and taxon) diversity behind this similar morphology is not well understood. It is likely that different lineages are adapted to different environmental conditions. As current environmental change is speeding up deciphering the ecological significance of cryptic diversity may be a key for stabilizing ecosystem functions and services under changing conditions.

Target group

The chrysophytes (cf. Kristiansen & Škaloud 2017) are a particularly well–suited model flagellate group for addressing these issues: Chrysophytes are widespread and abundant, particularly in oligotrophic freshwaters and can reach abundances of several thousand cells.ml^{-1} (Sandgren 1988; Carrick & Fahnenstiel 1989; Kristiansen & Preisig 2001; Boenigk & Arndt 2002). Further, they are among the most important grazers of bacteria–sized microorganisms (Finlay & Esteban 1998). Within the chrysophytes a high cryptic diversity of colourless non–scaled taxa evolved through parallel evolution in several chrysophyte lineages (Graupner et al. 2018). Colourless non–scaled forms comprise the genera *Spumella* Cienkowsky 1870, *Poteriospumella* Boenigk et Findenig 2010, *Pedospumella* Boenigk et Findenig 2010, *Atacamaspumella* Pietsch, Nitsche et Arndt 2022, *Vivaspumella* Pietsch, Nitsche et Arndt 2022, and many others (Grossmann et al. 2016; Pietsch et al. 2022). Specifically, the main target organisms of this study – members of the clades C1, C2, and C3 within Ochromonadales (in particular *Spumella* spp., *Pedospumella* spp., and *Poteriospumella* spp.), are abundant in many habitats (Boenigk et al. 2005; Pfandl et al. 2009; Nolte et al. 2010; Grossmann et al. 2016) and tolerate a wide range of physico–chemical conditions (Boenigk 2008).

Identifying and separating distinct cryptic nanoflagellate lineages is of particular significance in colourless non–scaled chrysophytes as a similar and in many cases microscopically indistinguishable morphological form evolved many times independently in several lineages (Grossmann et al. 2016; Graupner et al. 2018; Pietsch et al. 2022). Examples comprise the genera within Ochromonadales (e.g., *Poteriospumella*, *Atacamaspumella*, *Chlorospumella* Pietsch, Nitsche et Arndt 2022, *Spumella*, *Pedospumella*, and many so far not taxonomically assigned lineages), within the Apoikiida (e.g., *Apoikiospumella* Boenigk et Grossmann 2016, *Pseudapoikia* Pietsch, Nitsche et Arndt 2022), within the Chromulinales (e.g., *Chromulinospumella* Boenigk et Grossmann 2016), and in several other lineages (e.g., *Vivaspumella* Pietsch, Nitsche et Arndt 2022) (Grossmann et al. 2016; Pietsch et al. 2022). Here we address the competitive strength and ecophysiological differentiation between morphologically indistinguishable cryptic nanoflagellate taxa facing temperature and salt stress. We investigated the growth response at food concentrations in the range of field conditions, i.e., 2×10^6 bacteria.ml^{-1}, in single culture and mixed culture of cryptic taxa affilitated with *Spumella*, *Pedospumella*, and *Poteriospumella* using lineage–specific fluorescently labelled probes (FISH–probes) for differentiation.

Fluorescence in situ hybridization (FISH) is a robust and widely applied method for detection, identification, and quantification of microbes. The identification of single–celled organisms by hybridizing the ribosomal RNA with fluorescently labelled probes was first shown by Delong et al. (1989). Standard FISH probes are 15–25 nt long oligonucleotides that are labelled with a fluorescent dye at the 5'end and can be visualized by epifluorescence or confocal laser scanning microscopy (Amann et al. 1990; Wagner et al. 1994). Because the probes are designed to target specific phylogenetic groups,

FISH allows a more detailed and reliable identification than morphological characteristics.

We hypothesize that sudden shifts in environmental conditions as applied in our experiments decrease the growth rate. However, as the temperature stressor is within the optimal temperature range for the flagellates (Boenigk et al. 2007; Boenigk 2008) we expect that a potential negative effect is overcompensated by a positive relation of temperature and thus growth rates will increase. Based on experimental (Boenigk et al. 2007) and environmental seasonal abundance data (Nolte et al. 2010) we expect that the stimulating effect of temperature increase is strongest in *Poteriospumella*. With respect to salt we expect strong differences between the investigated strains as high variability in salt tolerance has been demonstrated for different strains of colourless chrysophytes (Pfandl et al. 2009). The study by Pfandl et al. (2009) showed that variability is similar in all three clades. We therefore cannot make predictions on the tolerance of the individual taxa but suspect that *Pedospumella* might be the least sensitive as this strain originated from soil while the other taxa originated from freshwater (Grossmann et al. 2016).

Materials and Methods

Strains and cultivation. The strains *Pedospumella encystans* JBM/S11 Boenigk et Findenig 2010, *Spumella rivalis* AR4A6 Boenigk et Findenig 2010, and *Poteriospumella lacustris* JBM10 Boenigk et Findenig 2010 are affiliated with the 18S rRNA subclades C1, C2, and C3, respectively, and were obtained from the culture collection of the working group and the Central Collection of Algal Cultures (CCAC) at the University of Duisburg–Essen. The strains have previously been isolated from soil or freshwater samples from different geographical origins (Table 1). The xenic strains JBM/S11and AR4A6 were routinely grown in inorganic basal medium (IB Hahn et al. 2003) in cell culture flasks (25 cm^3 with filter screw cap, TTP Techno Plastic Products AG) at 15 °C with 14h:10h light–dark cycle in a climate chamber (SANYO Electric Co. Ltd., Osaka, Japan). The axenic strain JBM10 was routinely grown in NSY medium (Nutrient broth, Peptone from soybean (Bacto Soytone), Yeast extract; Hahn et al. 2003) in 100 ml Erlenmeyer flasks at 15 °C with 14h:10h light–dark cycle. The bacterial strain *Linmohabitans* spp. IID5 was grown in 100 ml Erlenmeyer flasks at room temperature (RT) with constant shaking (96 rpm, orbital shaker, LAUDA–Brinkmann, LP, New Jersey, USA).

FISH–probe design and optimization. Specific probes for fluorescence in situ hybridization (FISH) targeting the 18S rRNA were designed for the C1 clade, the C2 clade, the C3 clade, and the entire group of *Ochromonadales* using the DECIPHER's design web tool (Wright et al. 2014). To optimize the hybridization conditions a formamid series from 0% to 70% was carried out for all four probes in 10% steps. Pictures with a fixed exposure time of 1 s for the TRITC channel were taken for comparison of signal intensities in samples hybridized with different formamid concentrations. Signal intensities were measured with the program "Daime" (Daims et al. 2006) by measuring the mean signal intensity of all FISH–stained cells on each picture. Cells were identified by dark–light contrast with an object size threshold of 28 and including internal dark regions into the object area. In the range of optimal formamide concentrations, a second test in steps of 5% was applied in order to determine the optimal formamide concentration for each probe. In addition, formamide curves were generated using mathFISH (Yilmaz & Noguera 2007) for comparing calculated and experimentally determined concentrations for each newly designed probe. Sequences, specificity and hybridization conditions determined for each probe are

Table 1. Origin and affiliation of strains used in this study.

Strain	Species designation	18S rRNA clade	Geographical origin	Habitat origin	Media	Nutrition mode
JBMS11	*Pedospumella encystans*	C1	Austria, Mondsee, near Rauchhaus	soil	IB + WK	heterotrophic
AR4A6	*Spumella rivalis*	C2	Austria, River Fuschler Ache	freshwater	IB + WK	heterotrophic
JBM10	*Poteriospumella lacustris*	C3	Austria, Mondsee	freshwater	NSY (3g/L)	heterotrophic

Table 2. Target region and hybridization conditions for FISH–probes.

Probe	Sequence (5' to 3')	Specificity	Formamide (%) for hybridization at 46 °C	18S rRNA position
O1C531	CCGAGGATGGATTCAGACAACTGGT	C1 clade	15	18S, 531–555
O2C613	GCCTGCTTTGAACACCCTATTT	C2 clade	50	18S, 613–634
O3C723	ACCCCCAACTGTCCC	C3 clade	50	18S, 723–737
Och1268	CTGTTATTGCCCCCAACTTC	Ochromonadales	45	18S, 1268–1287

shown in Table 2. Coverage of the probes was evaluated with the Silva rRNA database (August 2022) using TestProbe 3.0 (http://www.arb–silva.de/search/testprobe; Quast et al. 2013).

Stressor and competition experiments. Food bacteria (*Linmohabitans* spp. strain IID5) were grown in NSY medium, collected by centrifugation for 10 min at 15000 g at RT and the pellet was resuspended in IB medium. Prior to the experiments, flagellate cultures were also collected by centrifugation for 10 min at 2820 g at RT to remove the initial medium. The resulting pellet was then resuspended in 50 ml IB medium and flagellate cultures were fed with one ml of washed bacteria every three to four days and incubated for 17 days at 23 °C with 14h:10h light–dark cycle.

All experiments were run in five replicates in 75 ml IB medium in 100 ml Erlenmeyer flasks following a three–day acclimatization period. At the beginning of the acclimatization phase, flagellates abundance was adjusted to 2400 cells.ml^{-1}. For the competition experiments density of each strain was adjusted to 800 cells.ml^{-1}, i.e., total flagellate abundance was the same for the single strain experiments and the competition experiments. Bacterial food concentration was adjusted to 2×10^6 bacteria.ml^{-1}. Then, all cultures were incubated at 23 °C (14h:10h hours light–dark cycle) for three days to acclimatize to lower food concentrations. On day 3, cultures were exposed to the respective stressors, i.e., the temperature treatments, as well as the combined treatments, were incubated at 27 °C, and sodium chloride was added to the salt and the combined treatment (final concentration corresponding to 2 g chlorid.l^{-1}). Subsamples were taken each day (on day 3 samples were taken before and after onset of stressors). Single–strain samples were fixed with Lugol's solution and stored at 4 °C until further use. Mixed–culture samples were fixed with 2% paraformaldehyde (PFA) for 1h at RT or overnight at 4 °C and 4–5 ml of each fixed sample were filtered onto white polycarbonate filters (diameter 25 mm, pore size 0.2 µm, Millipore GTTP 02500, Eschborn, Germany). The filters were air–dried and stored at –20 °C until further use.

FISH protocol and cell enumeration. Total flagellate abundance was counted in Sedgewick–Rafter chambers using 1 ml of the Lugol–fixed subsamples. In the competition experiment the fractions of C1, C2, and C3 clade were counted on the filters after FISH–staining as outlined in the following: sections of the filters were cut out with a razor blade and samples were dehydrated through an ethanol series of 50%, 80%, and 100%. For each step, the filters were incubated for 3 min at RT. The cells were then hybridized with fluorescently mono–labelled oligonucleotide probes (O1C531, O2C613, O3C723, or Och1268, 50 ng DNA. µl^{-1}, Eurofins Genomics Germany GmbH, Ebersberg, Germany) as described previously (Glöckner et al. 1996). To optimize signal detection rates the concentration of SDS was increased to 0.02% in hybridization buffer and hybridization time was extended to 3 h at 46 °C. To determine the total cell count in the mixed cultures, the filter sections were counterstained by incubating them in a 4,6–diamidino–2–phenylindole (DAPI) solution (0,1 mg.ml^{-1}) for 10 min at RT. To remove unspecific staining the filters were washed in sterile distilled water for 1 min at RT, then dehydrated with 100% ethanol for several seconds at RT and air–dried. The filters were mounted in the non–hardening and anti–bleaching mounting medium CitiFluor™ AF2 (Citifluor, Ltd., London, United Kingdom), and the cells were counted using a Nikon Eclipse 80i microscope (Nikon Corp., Tokyo, Japan) with a 20× objective and appropriate sets of fluorescence filters for detecting DAPI– and Cy3–signals. Images were acquired using the NIS–Elements BR ("Basic Research") software (Nikon Corp., Tokyo, Japan). Growth rates between day 3 and day 6 were calculated for single strains and mixed cultures. Statistical data processing was carried out with R version 4.2.1 (R core team 2022). Growth rates were statistically compared using ANOVA, post–hoc Tukey's test, and Welch's two–sample T–tests using R–packages „broom", „tidyverse", „dplyr" and base R (R core team 2022; Robinson et al. 2022; Wickham et al. 2022).

Results

Specificity of probes

We developed probes specific for the C1, the C2, and the C3 clade within Ochromonadales (Fig. 1). Tests of the probes using strains affiliated with other clades within Ochromonadales (Fig. 1) and outside Ochromonadales (data not shown) proved the specificity of the probes. Formamide concentration in the hybridization buffer affected the signal intensity and specificity (Fig. S1). Optimal hybridization conditions were similar for the probes O2C613 and O3C723, while for probe O1C531 the hybridization conditions differed. Additionally, the experimentally determined hybridization conditions notably differed from the calculated optimal formamide concentration (Fig. S1).

Effect of temperature and salinization on growth rates

Growth rates were significantly different both for the application of stressors and between the taxa. This was consistent in the single strain experiments (ANOVA, stressors: $p < 0.001$; taxa: $p < 0.001$) as well as in the competition experiment (ANOVA, stressors: $p < 0.001$; taxa: $p = 0.009$). Further, the interaction between stressor application and taxa was also significant (single strains: $p = 0.002$; competition: $p < 0.001$).

In the single strain experiments, increased temperature alone did not have a significant effect on the growth rate for any of the strains (*Pedospumella*: $p = 1.0$; *Spumella*: $p = 0.92$; *Poteriospumella*: $p = 1.0$). The application of salt significantly reduced growth rate for *Spumella* ($p < 0.001$) but not for the two other taxa (*Pedospumella*: $p = 0.99$; *Poteriospumella*: $p = 0.99$). Similarly, the effects of combined stressors significantly decreased growth rate for *Spumella* ($p < 0.001$) but not for *Pedospumella* ($p = 0.99$) or *Poteriospumella* ($p = 1.0$).

In mixed cultures, higher temperature resulted in a significantly increased growth rate for *Poteriospumella* ($p < 0.001$) but not for the two other species (*Pedospumella*: $p = 0.99$; *Spumella*: $p = 0.97$). Increased salinity resulted in significantly lower growth rate for all taxa (*Pedospumella*: $p < 0.001$; *Spumella*: $p < 0.001$; *Poteriospumella*: $p < 0.001$). Application of both stressors reduced growth of *Pedospumella* ($p = 0.001$) and *Spumella* ($p = 0.03$). Growth rates for *Poteriospumella* were also lower but not significant ($p = 0.15$).

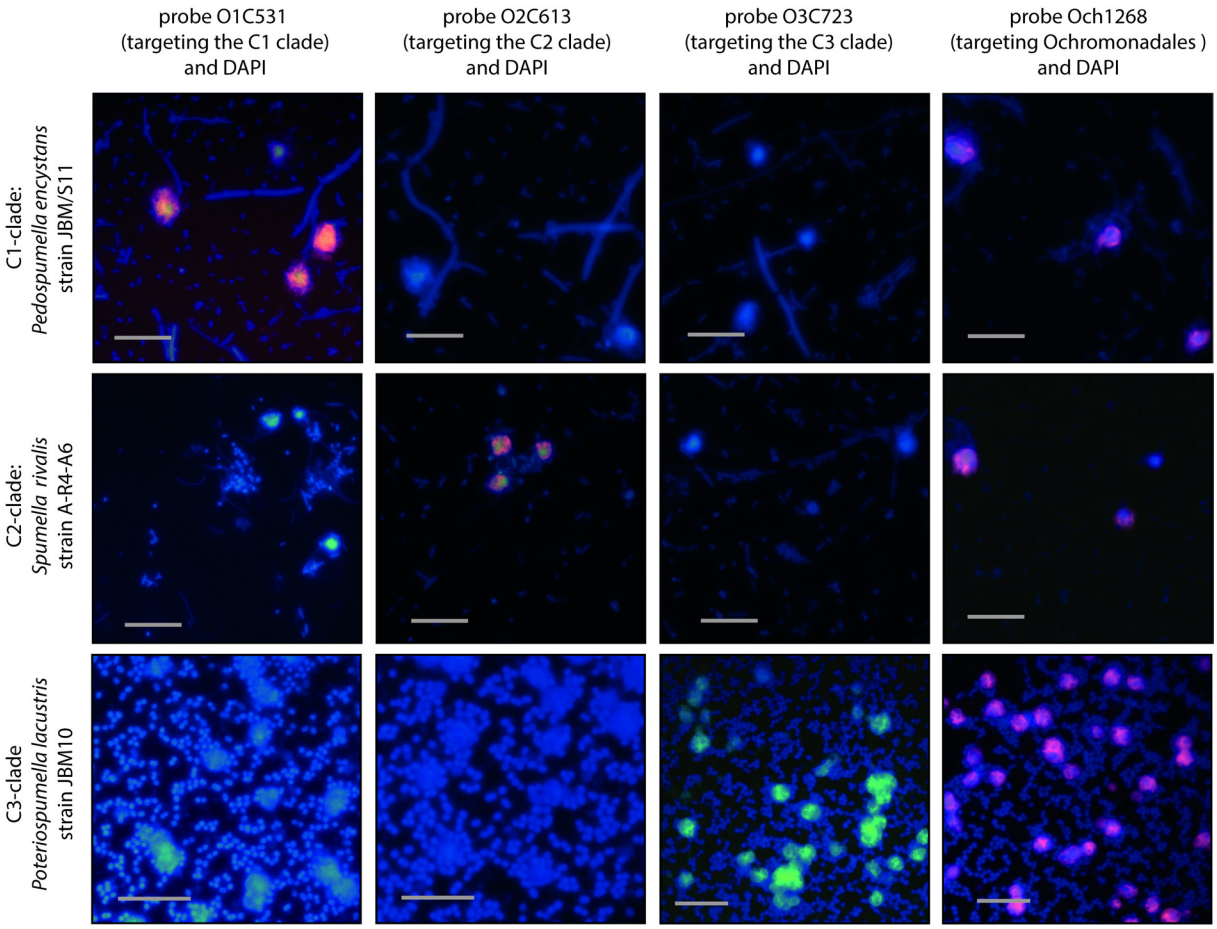

Fig. 1. Fluorescence overlay images (DAPI and Cy3 for probes O1C531, O2C613, and Och1268; DAPI and FITC for probe O3C723) using probes specific to the C1, C2, and C3 clade applied to *Pedospumella encystans* JBM/S11, *Spumella rivalis* A–R4–A6, *Poteriospumella lacustris* JBM10. Contrast was enhanced using the autocontrast function combined with a reduction of the blue channel range to 150 using Adobe Photoshop. All scale bars are 10 µm.

Effect of temperature and salinization on competition in the mixed treatment

Regarding competition between the three strains, the relative share of *Poteriospumella* increased in all treatments (Fig. 2) but the extent differed as well as the effect on the other species. The share of *Poteriospumella* slightly but significantly increased in the control ($p = 0.005$) but the associated decrease of both other taxa was not significant and could therefore not be attributed to any of the two species (*Pedospumella*: $p = 0.955$; *Spumella*: $p = 0.917$). In the temperature treatment, the share of *Poteriospumella* increased stronger ($p = 0.002$). This was largely compensated by a decrease of the share of *Spumella* ($p < 0.001$), while the decrease of the share of *Pedospumella* was not significant ($p = 0.103$). The salt treatment resulted in a significant increase of the share of *Poteriospumella* ($p = 0.001$), while it had no significant effect on both *Pedospumella* ($p = 0.969$) and *Spumella* ($p = 0.220$). The combination of both stressors (salt and temperature) also resulted in a significant increase of the relative share of *Poteriospumella* ($p = 0.002$). In contrast to the temperature treatment, the increased share of *Poteriospumella* was largely compensated by a decrease of the share of *Pedospumella* ($p = 0.004$) while the decrease of the share of *Spumella* was not significant ($p = 0.205$).

For all three species competition had a significant effect on growth (ANOVA; *Pedospumella*: $p = 0.001$; *Spumella*: $p = 0.008$; *Poteriospumella*: $p < 0.001$). Despite the significant overall effect of competition, results differed when comparing the effect of the treatments in single–strain experiments and in mixed cultures. For *Pedospumella* differences between single–strain and competition experiment were not significant (control: $p = 0.191$; temperature: $p = 0.564$; salt: $p = 0.080$; treatment combination: $p = 0.341$). For *Spumella* effects of competition were significant in the control ($p = 0.014$) and the combined treatment ($p = 0.005$) but not for temperature ($p = 0.093$) and salt ($p = 0.070$). For *Poteriospumella* the effect of competition was significant for the temperature treatment ($p < 0.001$) but not for the other treatments (control: $p = 0.06$; salt: $p = 0.47$; combination treatment: $p = 0.67$).

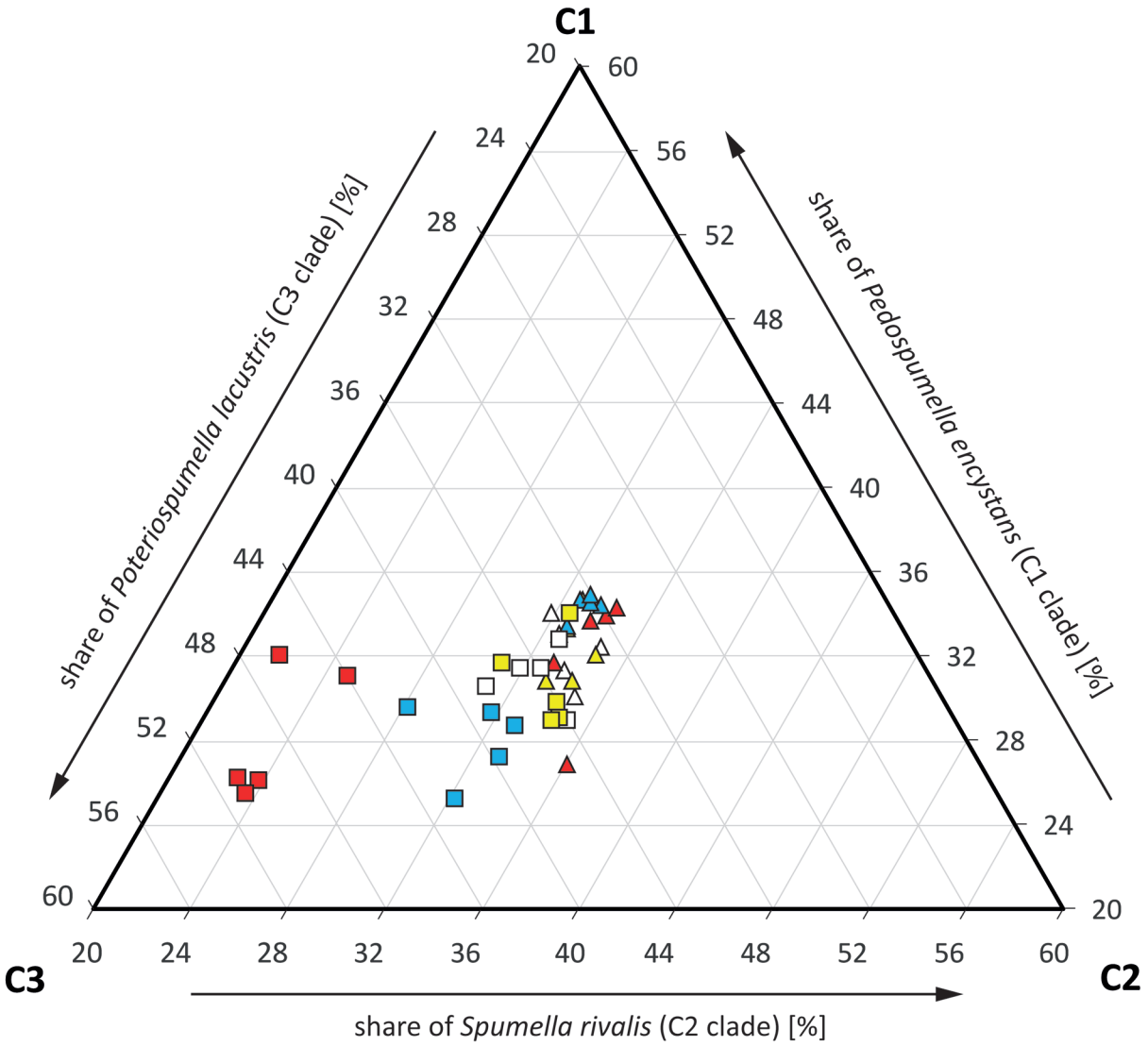

Fig. 2. Ternary plot showing the relative share of *Pedospumella encystans* (C1), *Spumella rivalis* (C2), and *Poteriospumella lacustris* (C3) in the competition experiments. Triangles refer to the community immediately before the application of stressors (day 3) and squares to day 6. White: control; red: temperature; yellow: salt; blue: combination of salt and temperature. Symbols of the same shape and colour represent replicates of the same treatment.

Discussion

Colourless non–scaled chrysophytes are abundant in nearly any habitat, i.e., in soils, freshwater, and the marine habitats, and often make up a share of several percent of the total eukaryotic abundance (Foissner 1987; Sandgren 1988; Boenigk & Arndt 2002). As outlined above, this morphological form combines different distinct molecular lineages which are separated by taxa deviating in form and function such as e.g. the mixotrophic genus *Dinobryon*. Inferring the ecological role of this high molecular diversity and of the parallel evolution of this form requires a separation of these lineages based on molecular and morphological data. Quantifying different morphologically similar protist taxa both in the field and in competition experiments remains a tricky task. While morphologically such taxa can usually not be separated, molecular studies based on amplicon diversity do not provide absolute cell counts and the relative differences in read abundance are subjects to numerous biases including primer bias, different selectivity for sequences during processing and sequencing etc. (Medinger et al. 2010). The differentiation of cells by FISH offers a way for combining the advantage of molecular differentiating different cryptic taxa with absolute cell counts. We here focused on the order Ochromonadales as representatives of this order are often dominating in natural environments (Bock et al. 2022).

Specificity of FISH–probes and suitability for competition experiments

We developed FISH–probes specific to clade C1 (comprising the genus *Pedospumella*), clade C2 (comprising

Table 3. Growth rates [day^{-1}] after stressor application (mean ± standard deviation). Negative growth rates correspond to mortality rates.

		***Pedospumella* (C1)**	***Spumella* (C2)**	***Poteriospumella* (C3)**
Single–strain experiment	Control	0.019 ± 0.052	0.164 ± 0.063	–0.261 ± 0.099
	Temperature	0.061 ± 0.180	0.005 ± 0.068	–0.254 ± 0.063
	Salt	–0.041 ± 0.182	–0.301 ± 0.034	–0.344 ± 0.063
	Combination	–0.075 ± 0.203	–0.350 ± 0.033	–0.256 ± 0.295
Competition experiment	Control	–0.038 ± 0.039	–0.053 ± 0.037	–0.056 ± 0.034
	Temperature	–0.014 ± 0.110	–0.104 ± 0.076	0.139 ± 0.093
	Salt	–0.366 ± 0.065	–0.361 ± 0.034	–0.379 ± 0.046
	Combination	–0.220 ± 0.038	–0.194 ± 0.064	–0172 ± 0.048

the genus *Spumella* sensu stricto), and clade C3 (comprising the genus *Poteriospumella*). Tests of the probes with taxa affiliated to other clades prove the specificity of the probes (Fig. 1) and thus enable the separation of these clades in morphological community analyses. We successfully used the probes, following the standard FISH protocol (Glöckner et al. 1996), to specifically detect and quantify the differed clades in mixed cultures. The application of the probes on environmental samples, however, remains to be tested. Surprisingly, the predicted optimal formamid concentration differed notably from the optimal hybridization conditions that were determined experimentally (Fig. S1). The hybridization conditions resulting in highest fluorescence intensity, and consequently most efficient probe binding and clade–specific binding did not coincide with the predicted formamid concentration for all three clade–specific probes (Fig. S1). The general Ochromonadales probe did show high signal intensities when hybridized with the predicted formanid concentration in some but not all tested strains (data not shown). Signal intensities for Och1268, however, were stable when hybridized with a formamid concentration higher than predicted. Consequently, experimental testing enabled us to find the best hybridization conditions for the newly designed probes.

Effects of stressors and food concentration on the growth of individual strains

Data so far indicates that different members of the C1, C2, and C3 clades are ecophysiologically differently adapted (Boenigk et al. 2007; Boenigk 2008; Nolte et al. 2010). However, in our single–strain experiments, we found only low differences and significant effects only for *Spumella* exposed to salt. Before considering the effects of stressors and competition, a word of caution is needed with respect to the food concentrations applied in our experiments: all experiments were run at low food and potentially food–limiting conditions in order to mimic natural conditions and to focus on food–limiting conditions where competition should be expected to be more pronounced. Therefore, even slight changes in bacteria abundances affect growth rates and these differences need to be considered when comparing results from strains in the single strain experiments. We did not find any significant effects of the treatments on bacterial growth. Bacterial abundance was significantly higher ($p < 0.001$) in *Spumella* cultures that were exposed to increased salinity compared to the control, however, it is likely that this resulted from the reduced growth rate of *Spumella* during salt treatment. *Poteriospumella* generally proved to be the fastest–growing and potentially best–adapted strain to the experimental conditions. However, due to the stronger growth of this strain the food concentration was more strongly reduced, i.e., was lowest in the single–strain experiments with *Poteriospumella* ($2.8 \pm 3.5 \times 10^6$ bacteria.ml^{-1} on day 3), followed by the mixed community experiment ($5.79 \pm 0.6 \times 10^6$ bacteria.ml^{-1} on day 3) and the single–strain experiments using *Spumella* ($6.47 \pm 0.4 \times 10^6$ bacteria.ml^{-1} on day 3) and *Pedospumella* ($6.0 \pm 0.3 \times 10^6$ bacteria.ml^{-1} on day 3). In the direct comparison, this is reflected by lower growth rates of *Poteriospumella* in the single–strain experiments while this species proved to be best–adapted to the experimental conditions as reflected by the stronger growth during acclimatization (data not shown) and the higher growth rates in the competition experiment.

Corresponding to the expectations, *Poteriospumella* performed best under the experimental conditions and particularly profited from the temperature increase in the competition experiment, while the effect of temperature treatment on growth rates for the other taxa was less pronounced. This is in accordance with previous laboratory growth experiments that showed that *Poteriospumella* tolerated higher temperatures than members of the two other clades (Boenigk et al. 2007) and thus suggested a competitive advantage of the C3 clade at high temperatures. Except for strains originating from Antarctica (Boenigk et al. 2007) differences between members of the two other clades were not very pronounced but may

hint to a higher competitive strength of the C1 clade at low temperatures as compared to the C2 clade. In a seasonal study on Lake Fuschlsee the relative abundance of members of the C3 clade (*Poteriospumella* and allies) was highest in summer while the relative abundance of members of the C2 clade (*Spumella* and allies) was highest in the colder seasons (Nolte et al. 2010). The C1 clade (*Pedospumella* and allies) showed an intermediate pattern in this study. Regarding the C1 and C2 clades the results from environmental data and lab experiments thus slightly deviate. Although, a study focusing on the maximal temperature tolerated (Boenigk 2008) indicated a similar pattern as the environmental data, i.e., lowest temperature tolerance for the C2 clade (with some strains showing exceptions), followed by the C1 clade, and highest temperature tolerance for the C3 clade. However, this latter study contributes only data on the maximum temperatures survived but no data on growth rates of the distinct strains. In our experiments, the temperature treatment had no significant effect on growth rates for *Spumella* (C2) and *Pedospumella* (C1) in single or mixed cultures. As outlined above, taxa affiliated with the C2 and C1 clades seem to be adapted to lower or moderate temperatures, respectively.

Consequently, *Spumella* and *Pedospumella* did not profit from the temperature treatment but were able to tolerate it. Additionally, food concentrations in our experiments may have been too low to permit significantly increased growth rates for either of the two taxa (Boenigk et al. 2007). Competition experiments have so far not been conducted, the potential temperature dependence of the competitive strength can only be inferred from seasonal distribution data, which may, however, be affected by other factors. In our competition experiment increased share of *Poteriospumella* was largely compensated by a decreased share of *Spumella* which further implies a competitive advantage of the C3 clade and a competitive disadvantage of the C2 clade at high temperature.

In our study, salinity had a similar effect for all three species. In contrast, literature suggests strongly deviating salinity tolerances between protist taxa: while some taxa have been reported to tolerate freshwater as well as marine conditions, other taxa seem to have quite restricted tolerances (Finley 1930; Ekelund 2005). Brackish and at least some marine species tolerate a wide range of salinities while freshwater species seem more restricted to low salinities: some freshwater protist taxa show decreased growth rates or become non–viable already at concentrations around salinities of 1 $g.l^{-1}$ (e.g., Finley 1930; Lam et al. 2019), other taxa are only negatively affected at salinities above 5 $g.l^{-1}$ (Finley 1930; Zuo et al. 2014; Park et al. 2020). However, for some freshwater species exceptional tolerance ranges have been reported: for some freshwater bodonid taxa and some colourless non–scaled freshwater chrysophytes tolerance of marine conditions has been reported (Finley 1930). Our experiments showed less variability in salt tolerance between the investigated taxa as expected: in single strain cultures salinity of 2 $g.l^{-1}$ negatively affected the growth of *Spumella* (C2) but had no significant effect on growth rates for *Poteriospumella* (C3) or *Pedospumella* (C1) and in mixed cultures the effect on growth rates was similar for all investigated taxa. For distinct strains of colourless non–scaled chrysophytes salt tolerance has been tested but data so far are largely restricted to the maximum (short–term) tolerance of strains (Boenigk 2008). Salt tolerance of members of the C3 clade (including *Poteriospumella*) seem to be rather similar with a maximum around 5 $g.l^{-1}$ while the tolerance varied considerably (i.e., between 1 and 7 $g.l^{-1}$) for different members of the C2 and C1 clade. Bock et al. (2022) inferred from the distribution pattern in a pan–European data set that both *Pedospumella* and *Poteriospumella* were among the taxa with a wide tolerance to conductivity but the sampling site in this study covered only the lower of salinities. Ekelund (2005) suggested that soil protists may be more salt–tolerant than freshwater forms. But in contrast to our expectation pattern of soil–borne *Pedospumella* did not deviate from the water–borne taxa (*Poteriospumella lacustris* and *Spumella rivalis*). Comparative data on growth rate are not available for these strains and a potential niche separation with respect to salt concentration is therefore not clear. However, growth rates seem to be severely affected already at salt concentrations far below the maximum concentration survived (based on cultivation experience; unpublished data). Irrespective of a potential survival at high salinities we therefore expected a negative effect on growth rate already at moderate salt concentration. While older literature suggests a high salinity tolerance for many species (e.g., Finley 1930) more recent studies reported an increasing number of species with limited salinity tolerances (cf. Mylnikov 1983; Ekelund 2005). Several non–exclusive aspects may contribute to these findings: firstly, during progress in protistology the number of cultivated taxa and strains strongly increased, early isolates were often affiliated with 'ubiquitous' species and early cultivation protocols may have selected for taxa with broader tolerances. Secondly, evidence from environmental samples as well as evidence–based on mixed cultures (such as the study by Finley 1930) may contain several cryptic strains with different ecophysiological requirements as species identities in such mixed samples were hard to prove in early studies which rely on morphological inspection only. As most of these common species are now known to be highly diverse it is indeed likely that a high salinity tolerance inferred from (morphological) field studies erroneously concluded on a high salinity tolerance of the respective species while in fact, the distinct strains (and possibly species) had a much narrower (but different) salinity tolerance. Thirdly, strains from culture collections may show low salinity tolerances but may be able to

gradually acclimatize to higher salinities (Mylnikov 1983). In sum, literature findings over time may reflect to a certain extent the improving methodologies in isolation and cultivation as well as in the differentiation of taxa and strains. But even considering such potential uncertainties regarding some of the early studies the variation in salinity tolerance between taxa seems to be remarkably high. Many protist taxa originating from soil and freshwater survive salinities of 5 $g.l^{-1}$ or even of 10 $g.l^{-1}$ (Mylnikov 1983; Ekelund 2005). However, salinity tolerances of strains affiliated with the same species may considerably deviate and thus salinity may be an efficient reproductive barrier possibly questioning current species delimitations (Ekelund 2005).

Taken together the increasing growth rate in response to a sudden increase in temperature corresponded largely to our expectations while the similar effect of salinization for all three taxa was unexpected. Presumably, the applied salt concentration was in a range which effectively prevented growth but was not lethal. When both stressors were applied together, growth rates were higher as in the control and in the salt treatment but lower as in the temperature treatment. Temperature therefore is likely to stimulate growth but this effect is impaired by salinization.

Effects of stressors on competitive strength

Competition experiments using different lineages of colourless non–scaled chrysophytes have so far not been performed or were restricted to analyzing the relative shifts in amplicon abundances (e.g., Nolte et al. 2010). In lab experiments, the difficulties in separating cryptic taxa can leave unclear whether the presence of other taxa affects the observed pattern or not. Besides, the challenges of performing experiments at near–natural food concentrations further hamper such analyses. Both seems plausible: on the one hand, the investigated taxa are morphologically similar and most likely their ecological niches overlap to a considerable extent. Therefore, adverse effects due to competition might be expected. On the other hand, since the formulation of the 'paradox of the plankton' (Hutchinson 1961) numerous mechanisms for maintaining plankton diversity have been proposed (Roy & Chattopadhy 2007).

Our experiments provided evidence that the different cryptic taxa responded differently to the stressors and responded differently in competition as compared to single–strain experiments. It is likely that *Poteriospumella* can deal best with low food concentrations and thus outcompetes the two other taxa in particular at higher temperatures. At lower temperatures, i.e., in the control and the salt treatment, this relative advantage was less pronounced but still significant. Our results therefore imply that competition is a relevant issue but that its strength depends on the abiotic conditions. The different taxa responded differently to stressors thus shifting the relative abundance of these taxa in response to environmental change. Our results therefore suggest that the seeming persistence of common morphological flagellate types in natural communities masks a turn–over of cryptic taxa differently adapted to abiotic factors. Such a cryptic species turn–over affects food web stability: many aspects of predator–prey interactions such as food size preferences and feeding mechanisms largely correlate with morphology (Boenigk & Arndt 2000a, b) while adaptations to many abiotic environmental factors do not. Cryptic species diversity and turn–over thus stabilize trophic interactions and food web architecture despite a potentially high taxon turn–over due to environmental change or the (sudden) occurrence of stressors.

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Supplementary material

The following supplementary material is available for this article:

Fig. S1. Signal intensity as a function of formamide concentrations. The grey dashed line indicates the calculated optimal formamide concentration (mathFISH) and the black dashed line the selected formamide concentration in our protocols based on signal intensity and probe specificity.

This material is available as part of the online article (http://fottea.czechphycology.cz/contents)

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Article III:

„*Protistan and fungal diversity in soils and freshwater lakes are substantially different*“

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Protistan and fungal diversity in soils and freshwater lakes are substantially different

G. Sieber✉, D. Beisser, C. Bock & J. Boenigk

Freshwater and soil habitats hold rich microbial communities. Here we address commonalities and differences between both habitat types. While freshwater and soil habitats differ considerably in habitat characteristics organismic exchange may be high and microbial communities may even be inoculated by organisms from the respective other habitat. We analyze diversity pattern and the overlap of taxa of eukaryotic microbial communities in freshwater and soil based on Illumina HiSeq high-throughput sequencing of the amplicon V9 diversity. We analyzed corresponding freshwater and soil samples from 30 locations, i.e. samples from different lakes across Germany and soil samples from the respective catchment areas. Aside from principle differences in the community composition of soils and freshwater, in particular with respect to the relative contribution of fungi and algae, soil habitats have a higher richness. Nevertheless, community similarity between different soil sites is considerably lower as compared to the similarity between different freshwater sites. We show that the overlap of organisms co-occurring in freshwater and soil habitats is surprisingly low. Even though closely related taxa occur in both habitats distinct OTUs were mostly habitat–specific and most OTUs occur exclusively in either soil or freshwater. The distribution pattern of the few co-occurring lineages indicates that even most of these are presumably rather habitat-specific. Their presence in both habitat types seems to be based on a stochastic drift of particularly abundant but habitat-specific taxa rather than on established populations in both types of habitats.

Despite the central importance of protists and fungi at the basis of soil and aquatic food webs comparative analyses of protists and fungi community composition in soils and freshwaters are rare. In both habitats protists are a very diverse and ubiquitously distributed group of organisms. They fulfill important ecosystem functions[1–3] channel bacterial secondary production from the microbial food web to higher trophic levels[4,5] thereby interacting indirectly and directly with other taxa such as fungi[6,7]. Particularly in aquatic habitats they are also the dominant primary producers[8]. But constraints structuring their diversity differ fundamentally between both habitats: for instance, freshwater habitats are more homogeneous than soil habitats due to mixing of the water body[9,10], the availability of water in soils is constrained by e.g. evaporation and soil irrigation[11–13], and soils are more heterogeneous than aquatic habitats consisting of various microhabitats[14–17]. As diversity is largely sustained and maintained by habitat heterogeneity[18–22] soils are therefore expected to harbor a higher protists richness even in small volumes[23,24] as compared to aquatic habitats.

Beyond habitat heterogeneity the distinct habitat properties of soil and water put different demands upon organisms inhabiting these habitats. Microbial organisms are differentially challenged by environmental factors of soil and freshwater habitats[25–28]. Their adaptations may therefore systematically differ eventually leading to exclusive communities (at least in part) of soils and freshwaters. In fact, protist communities in aquatic habitats comprise a high share of phototrophs such as diatoms and green algae while these groups are less important in soil communities[29]. But also the heterotrophic protist communities differ between both habitat types. In soils gliding and substrate attached taxa such as amoeba and cercozoans have a high share[29]. In contrast, free swimming taxa such as katablepharids and (heterotrophic) dinoflagellates as well as the filter-feeding choanoflagellates are more abundant in the pelagial of aquatic habitats[30,31]. However, several studies suggest that both habitats may not be that different for microorganisms. First, even soil pores have been suggested to be basically aqueous environments for microbial organisms and soil protists therefore to be basically aquatic organisms[32–34]: in both habitats protists move by gliding or swimming and they feed by similar mechanisms such as interception feeding, filter feeding and grazing. Secondly, as there is no clear boundary between soil and freshwater habitats organisms may further easily be exchanged between both habitats. Dispersal via passive mechanisms like surface run-offs, interstitial and groundwater flow and flooding is well known[35–38]. Further, active dispersal enhances

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exchange of organism between different habitats. Microorganisms are dispersed by anthropogenic factors like ballast water, aquaculture, fishing and watersport[39,40]. Resting stages do even survive long distance transport and long transport times[41–43]. Also non-anthropogenic factors such as animals act as vector[44,45]. Thus, due to the similar microhabitat properties with respect to movement and feeding and potentially easy exchange between both habitats they may be inhabited by basically the same protist species[37]. However, easy dispersal does not necessarily result in establishment. Dead organisms and resting stages may wrongly indicate the presence of newly introduced microorganisms. But even microorganisms which survive in the new habitat may be out-competed by adapted/acclimatized taxa. In particular the potential dessication of soil pores may pose environmental constraints selecting against freshwater organisms while soil organisms may miss adaptations for buoyancy required for staying in the euphotic zone.

Thus, although the dominant microbial eukaryotes differ considerably between different habitats[33,44–46], organisms considered as typical for aquatic environments may occur also in soil (e.g. Choanoflagellata[47]) and vice versa fungi (e.g. soil fungi occur also on submerged material[48]).

Based on the high diversity of microhabitats in soils and the capacity of soils and sediments as seed bank the (active) freshwater communities may represent merely subfractions of the more diverse soil communities[49,50]. These findings suggest an inoculation of the (freshwater) habitats with individual taxa or the existence of habitat–generalistic taxa which occur in both, soil and fresh water. Studies on bacterial and archaeal taxa showed that upslope soil environments contain the core community, which inoculate downslope surface waters (58%, 43% respectively), but only 18% of the upslope eukaryotic microbes were found downslope in the arctic tundra[49–51]. Accordingly, protist freshwater communities may be expected as subsets of protist soil communities possibly further modified by environmental constraints.

Here we address the community overlap between soil and freshwater protists using a geographically consistent set of 30 sampling sites comprising samples from lakes and ponds and adjoined soil samples from the respective catchment areas. We studied the molecular diversity of communities based on Illumina amplicon sequencing of the hypervariable 18S SSU V9 rRNA gene region[3,29,52,53].

We expect that OTU richness in soils is much higher as in freshwaters[21,54,55], and we hypothesize that the freshwater communities are to a large extent composed of taxa present also in soils, i.e. a subset of the soil communities, even though a certain fraction of taxa may be habitat-specific[56,57]. Following this idea, we further hypothesize that the community is composed mostly of habitat-generalists, occurring in both habitat types and comprising only few rather habitat-specific organisms[29,58–60].

However, we expect the relative abundance of taxa to be considerably different between both habitat types.

Methods

Sampling and sample processing. We sampled freshwater lakes and corresponding soils from a geographic consistent set in Germany. Site selection focused on natural lakes (and reservoirs) and corresponding soil sites which were typical for the respective area (Fig. S1).

All samples were taken during mid-summer, soil samples were taken 2016 and freshwater samples were taken 2012 (see Table S1 and Fig. S1 for details on sampling sites). Samples were taken years apart, as the idea of the study was to compare freshwater and soil habitats on a similar geographic scale, i.e. use soil samples from the (direct) catchment area of the respective freshwaters, but to reduce effects of short-term cross-contaminations between both habitat types due to flooding, intense rainfall, recently performed watersports, etc. which may have blurred the results. Freshwater sampling and sample processing is described in detail in Boenigk et al. (Genbank, PRJNA414052)[46]. Briefly, the freshwater samples were collected two meters from the waterside and between 0.2 and 0.8 m below the water surface. Freshwater samples were filtered on Isopore 0.2 µm polycarbonate filters (Merck Chemicals GmbH, Darmstadt, Germany) until the filters were clogged (biomass normalized). The filters were air dried and subsequently frozen in liquid nitrogen (Cryoshippers). The filters were stored at − 80 °C in the laboratory until DNA extraction[46].

Soil samples were taken as top soil composite samples from the upper 5 cm of the surface soil (A horizon) with a distance of around 50 m from the corresponding freshwater lake to avoid collecting samples from the direct floodplain. For each soil sample three subsamples within one square meter were taken and roots, as well as other larger particles like stones and fir needles were manually removed. The three subsamples were combined, mixed thoroughly and filled in 1.5 ml tubes. Samples were immediately preserved in a cryoshipper and stored at − 80 °C until DNA extraction.

Soil DNA was extracted by using the Power Soil DNA Isolation Kit (MoBio, Germany) according to the instructions of the supplier with the following modification: vortexing at maximum speed subsequent centrifugation and transfer of the supernatant to a new tube was repeated until no new pellet was formed. Subseqently, two washing steps with C5 solution (MoBio, Germany) were performed and a final dry centrifugation was conducted two times. For PCR we used the forward primer Euk1391F (5′-GTACACACCGCCCGTC-3′[61,62]) and the reverse primerbased on Bock et al.[63], i.e. a combination of the primers ITS2_Dino (5′-GCTGCGCCCTTC ATCGKTG-3′) and ITS2_broad (5′-GCTGCGTTCTTCATCGWTR-3′) in a ratio of 10%:90%. Primers used for freshwater and soil samples were identical.

The mixture for the PCR of the soil samples consisted of: 0.5 µl DNA template (depending on the concentration dilutions of 1:1, 1:10, 1:50 or 1:100 were used) in 25 µl reactions with 0.25 units Phusion Taq (Thermo Fisher Scientific), 0.75 µM primers, 0.5 µl of 0.4 mM dNTPs and 5× Phusion HF buffer. The PCR-cycling conditions included an initial denaturation step at 98 °C for 3 min followed by 35 cycles each including a denaturation step at 98 °C for 30 s, annealing step at 61 °C for 75 s, and an elongation step at 72 °C for 60 s. The PCR was completed by a final extension step at 72 °C for 10 min.

The quality and quantity of the DNA was checked using a Thermo Scientific NanoDrop ND-2000 UV–Vis spectrophotometer (Thermo Fisher Scientifics), electrophoresis in 1% agarose gel stained with ethidium bromide (0.2 µg mL^{-1}) and ImageJ (v. 1.51d)[64]. Equimolar subsamples were pooled and commercially sequenced using paired-end HiSeq 2500 sequencing, applying 2 × 300 bp reads using the "rapid run" mode on the Illumina platform of a sequencing provider (Fasteris, Geneva, CH)[46].

The sequencing reads are available through the project PRJNA675443.

Bioinformatical processing

Sequence filtering. Adapter-, quality trimming and demultiplexing using MID sequences were performed by the sequencing company (Fasteris). The base quality of the sequence reads was checked using FastQC[65]. A split-sample filtering protocol for Illumina amplicon sequencing was used by two technical replicates per DNA sample[62]. The raw sequences were quality filtered (PRINSEQ-lite v.0.20.4)[66] to remove reads with an average Phred quality score below 25. The paired-end reads were assembled and quality filtered with PANDASeq (v2.10)[67]. All reads with uncalled bases, an assembly quality score below 0.9, a read overlap below 20, or a base with a recalculated Phread-score below 1 were removed. After dereplicating chimeras were identified and filtered using UCHIME (v7.0.1090)[68] with default settings. Sequences that were not present in both sample branches were discarded[62]. The bioinformatical pipeline is available on github (https://github.com/MW55/Natrix).

Statistical analyses. Data processing was carried out with R[69] version 3.6.1. Remaining reads after the filtering were clustered using SWARM (v 2.1.9)[70], then clustered by identical V9 sequences (first 150 bp, identity = 100%, to remove the ITS1 region from the sequences to obtain OTUs which are based on the V9 region) ("V9_Clust.R" by Jensen 2017 available on https://github.com/manfred-uni-essen/V9-cluster)[71] and aggregated to OTUs. Taxonomic assignment was done by searching against the NCBI nt database using BLASTn (Dec 05th 2017)[72] using an 85% identity value for the best hit and adjusting the taxonomy according to Adl et al.[73]. All sequences assigned to Metazoa and Embryophyta were discarded, as protists and fungi are the targets of this study. OTUs that represent less than 0.0005% of a respective site were discarded (total number of reads/OTUs). For habitat comparisons we restricted the analysis to OTUs that occurred in at least two sites. Rarefaction curves were created by the R-package vegan (v2.5.6.)[74] and samples that did not reach saturation were discarded from further analyses.

True diversities are based on the Shannon index and were computed from the raw OTU table using R-package RAM as well as Pielou's evenness[75]. True diversity were chosen as a measurement for diversity as it is not a non-linear diversity index (e.g. Shannon index, Simpson index) but is suitable for comparisons. Diversities (true diversities, evenness and richness) were statistically compared using a Mann–Whitney-U-test. For distance decay relationships, we replaced zeroes in our raw dataset based on a Bayesian-multiplicative replacement (cmultRepl, R zCompositions package[76]) and calculated the Aitchison distance, as we are dealing with compositional data[77]. Aitchison distance is used as an community dissimilarity proxy. Linear regression slopes of distance decay relationships were tested against zero with an ANOVA.

Figures were prepared with R (R Core Team) version 3.6.1, CorelDRAW × 8 and ArcGIS Pro 2.6.

Results

Differential pattern of diversity between soil and freshwater. Total number of assembled reads after filtering was 35,445,831 which grouped in 33,745 OTUs. Of these 18,745 OTUs (corresponding to 13,957,146 reads) were found exclusively in soil sites and 14,337 (corresponding to 13,429,169 reads) exclusively in freshwater sites. OTU richness was 1212 ± 420 OTUs per sample in soil and 852 ± 427 OTUs per samples in freshwater. For habitat comparisons we further restricted the analyses to OTUs occurring in at least two samples resulting in 10,515 OTUs (34,139,127 reads) with an OTU richness of 918 ± 334 per sample in soil and of 588 ± 290 per sample in freshwater.

Community composition and richness strongly differed between soil and freshwater (Fig. 1). In soil Ascomycota, Basidiomycota, remaining Opisthokonta and Ciliophora dominated while Ciliophora and algae dominated freshwater samples, in particular by Chlorophyta and Dinophyta. While in soil 4530 of the 6744 OTUs were affiliated with fungi, in freshwaters only 938 of the 4434 OTUs were affiliated with fungi.

Estimates of the effective number of eukaryotic microbial OTUs (true diversity) revealed that the soil community is more diverse than the freshwater community ($p < 0.05$, Fig. S2). This was largely due to the higher OTU richness in soil samples while evenness was rather similar between freshwater and soil sites (Fig. S5). When restricting the analysis to either protists or fungi we found different pattern. We found a higher diversity in soil when the analysis was restricted to protists excluding fungi ($p < 0.05$), even though soil protist richness was lower than freshwater protist richness (Fig. S2). The higher diversity in soils is thus to a large extend related to a higher evenness (Fig. S5) while protist communities in freshwater are rather dominated by individual OTUs. In contrast, the diversity of fungi was similar in freshwater and in soil ($p > 0.05$) (Fig. S2) but the architecture of fungal community composition differed between soils and freshwater. While richness of fungi was higher in soils, evenness was higher in freshwater resulting in similar diversity indices (Figs. S2, S5).

Corresponding to the effective OTU number we also found that richness was significant higher in soil sites compared to fresh water ($p < 0.05$). Richness revealed a deviating pattern for protists and for fungi: richness of fungi was significantly higher in soil than in freshwater ($p < 0.05$) while richness of protists was significantly higher in freshwater ($p < 0.05$).

Soil did not only hold a higher richness but also a higher dissimilarity among samples as compared to freshwater: The community dissimilarity analyses clearly revealed a generally higher dissimilarity among soil samples (Fig. S3). However, neither soil nor freshwater community dissimilarity changed significantly with

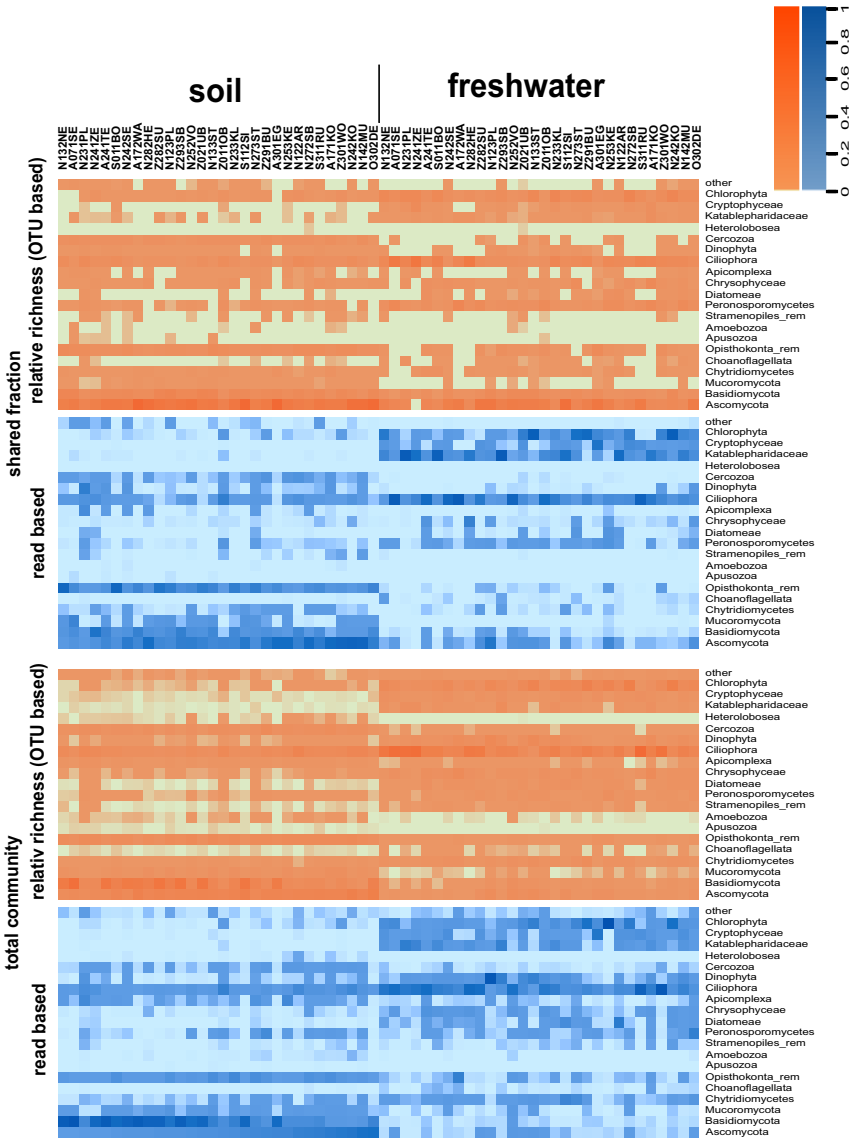

Figure 1. Relative total community composition (bottom) and relative total composition of the overlapping OTUs (top). Taxonomic composition based on relative abundance of OTUs that are present in at least two sites (blue) and taxonomic composition based on presence absence data of OTUs that are present in at least two sites (orange). The sites are sorted by the abundance of Ascomycota in soil sites. Remaining OTUs which could be assigned to a rough taxonomic level were marked as _rem. Created with the R-package gplots[78].

increasing distance up to 800 km, i.e. both slopes of the linear regressions slopes are not significantly different from 0 (ANOVA, $p < 0.001$).

Community overlap between aquatic and terrestrial habitats. The vast majority of the OTUs were exclusive to either soil or freshwater. Only 6.3% of the OTUs (663) were shared between both habitat types (Fig. 2B) while 35.9% (3771) occured exclusively in freshwater and 57.8% (6081) exclusively in soil (Fig. 2A). Even though the fraction of shared OTUs was already low, the distribution of most of these shared OTUs showed strong preferences to either soil or freshwater indicating that the fraction of habitat generalists is considerably smaller (Fig. 2C; please refer also to Tables S2 and S3 for an overview on the presumably generalistic taxa, i.e. taxa with a relative read abundance between 25 and 75% in soil and in freshwater, respectively. For an overview of all shared OTUs see Table S4). It is noteworthy that the analysis of corresponding sites revealed an average of 20 ± 18 shared OTUs (min. 3, max. 87).

Even though only 6.3% of the OTUs were shared between soil and freshwater, their relative share of the OTU richness in individual samples was considerably higher reflecting a comparatively wide distribution of these OTUs: shared OTUs on average account for 14.6% of the OTUs in soil sites (min. 6.9%, max. 25.42%) and for 13.32% in freshwater sites (min. 5.25%, max. 32.74%). With respect to average relative read abundance, the

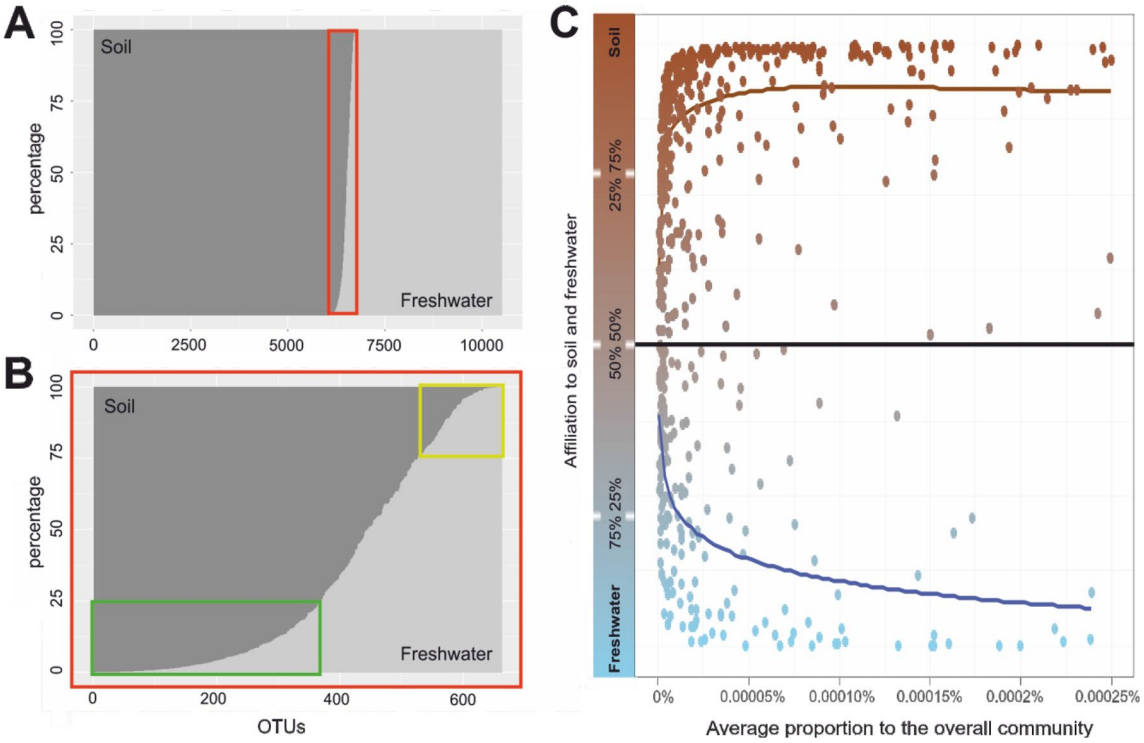

Figure 2. (**A**) Distribution of OTUs across soil (dark) and fresh water (light). The red frame marks overlapping OTUs. (**B**) Is an enlarged view of (**A**), namely OTUs that occur in soil and freshwater (red frame). The green frame indicates OTUs that have their origin rather in soil and the yellow frame indicates OTUs that have their origin rather in freshwater. (**C**) Abundance distribution pattern of 663 shared OTUs across soil (brown) and fresh water (blue) sites. X-Axis represents the average proportion of the shared OTUs in the complete soil and freshwater community and Y-axis represents the affiliation to soil and freshwater. A cutoff at 0.00025% is chosen as shared OTUs that represent more are spurious. Curve fitting was done with a generalized additive model ($y \sim s(\log(x))$).

importance of the shared OTUs is even higher representing 24.28% of the reads in soil sites (min. 3.52%, max. 56%) and 31.26% in freshwater sites (min. 1.13%, max. 68.06%).

The importance of highly abundant OTUs among those shared between both habitat types together with the slope of the curve in Fig. 2B indicate that in particular soil OTUs may be randomly dispersed to freshwaters (Fig. 2B green frame): most of the shared OTUs strongly dominate in soils (with low fractions of reads in freshwaters) indicating potential soil origin of these OTUs while considerably less OTUs showed the opposed pattern of high read fractions in freshwater and low fractions in soils (Fig. 2B yellow frame). Interestingly this tendency to specialize (with respect to habitat type) differed between OTUs with low and high read abundances: while many OTUs with low to moderate read abundances occurred in similar fractions in both habitat types, those with high read abundances strongly dominated in just one of the habitat types (Fig. 2C), i.e. may be considered potential habitat specialists (randomly dispersed to the other habitat type).

Irrespective of the presence of the shared OTUs in both habitat types, the richness of shared taxa within distinct samples is systematically affiliated with different taxonomic groups in soil and in freshwater (Fig. 1): In soil samples the majority of OTUs detected in both habitat types were affiliated with opisthokonts (especially fungi), followed by Ciliophora, Cercozoa and Stramenopiles. In contrast, in freshwater samples the majority of shared OTUs was affiliated with Chlorophyta, Cryptophyceae, Katablepharidaceae and Ciliophora.

Beyond this general pattern we also observed a systematic difference between rare and abundant OTUs: Shared OTUs with high abundances were predominantly affiliated with taxonomic groups considered to be dominant in the respective habitat, e.g. fungi and gliding taxa in soil, while shared OTUs with low abundances were affiliated to a wider (and more stochastic) selection of taxa (Fig. S4). For instance, in soils the relative contribution of opisthokonts (in particular fungi) to the fraction of shared OTUs was high within the abundant OTUs while the relative contribution of Chlorophyta and Chrysophyceae but also of Apicomplexa and Peronosporomycetes was higher within the rare OTUs (i.e. OTUs with low abundances). The contribution of Cercozoa and Ciliophora was similar for abundant and rare OTUs (Fig. S4).

In contrast, in freshwater the share of opisthokonts was high in the fraction of OTUs with low abundances but low within the abundant OTU. In contrast, Ciliophora, Oomycota and Chrysophyceae are more important in the fraction of OTUs with high read abundances. It is noteworthy that Apicomplexa and Cercozoa seem to play a minor role of the taxonomic overlap in freshwater (Fig. S4).

Discussion

Differential pattern of diversity between soil and freshwater. It is well known that morphological and molecular community analyses systematically deviate regarding the relative importance of distinct taxa. For instance, a higher relative abundance has been demonstrated for ciliates in molecular surveys which is due to different factors including copy number variation of the ribosomal genes[79]. Nevertheless, morphological as well as molecular surveys reveal corresponding trends in richness as long as the methodology within one study is consistent. For instance, community composition of soil clearly differed from that of freshwater in our study (Fig. 1). This is consistent with molecular[28,29,38,46,80] and morphological surveys[24,81,82].

Irrespective of a generally high community dissimilarities both for soil and for freshwater (which was more pronounced in soils), we found a higher richness in soils which is consistent with the literature (e.g.[14–17,24]). Interestingly, evenness of protists was generally higher in soil as compared to freshwater while it was the other way round for fungi. In other words the dominance of few protist taxa is more pronounced in freshwater lakes while dominance of few fungi is more pronounced in soils. This finding is noteworthy as it supports a differential role for community and ecosystem stability in soils and freshwater for protists and fungi with respect to the local extinction of distinct species (cf.[83–85]).

Community overlap between aquatic and terrestrial habitats. It is well documented that soils and lakes host different protist communities as their environmental characteristics are fundamentally different[24,29,47,86]. However, as boundaries between habitats are diffuse, exchange of taxa and shared taxa between both compartments are proven[38,49,50]. For instance, Crump et al.[50] showed that an arctic freshwater lake harbors 18% of the microbial eukaryotic upslope community and Graupner et al.[38] demonstrated the non-permanent exchange of taxa between the compartments as a result of flooding. Here we show for a set comprising 30 sites that the number of shared microbial eukaryotic OTUs between soil and freshwater lakes is, however, very low (6.31%).

The small fraction of taxa occurring in both habitats indicate that communities presumably largely consists of taxa which are typical for either soil or freshwater and for which probably only few cells were dispersed to the other habitat type by chance. This view is supported by a strongly uneven share of most of these taxa to soil and freshwater communities (Fig. 2C). Among those OTUs which were found in both habitats in particular the most abundant ones were strongly unevenly distributed indicating that they are characteristic for one habitat type and just few cells may have been dispersed by chance. Only a few taxa (and interestingly predominantly taxa with low overall relative abundances) seem to be of similar importance in both habitat types and can thus presumably be considered as habitat generalists (Tables S2 and S3). For instance, within the taxa shared between both habitats we found sequences affiliated with taxa known to occur in soil and freshwater such as the ciliate *Microdiaphanosoma arcuatum*[87], the ascomycete *Tetracladium maxilliforme*[88,89] and the diatom *Fistulifera pelliculosa*[90,91]. In contrast, some other OTUs found within the shared fraction were previously known from only one habitat type, e.g. the OTUs related to the ascomycete *Podosphaera fusca*[92] and the ciliate *Phialina salinarum*[93]. This is not necessarily contradictory to our results as sequence similarities of our OTUs to these latter species were often rather low and may not sufficiently resolve closely related species varying in their environmental demands[94]. Further, the resolution of the V9 region may be not appropriate for separating individual fungal taxa[95] and therefore inferring information from the assigned taxa may be misleading.

Our data indicate that in particular taxa affiliated with Opisthokonta, Cercozoa and Apicomplexa may rather be specific for soils and their presence in freshwater samples is presumably largely due to random dispersal[96–98]. This is consistent with the study of Graupner et al.[38] which demonstrated that despite an exchange of these taxa between soil and water, most of the exchanged taxa fail to establish in the new environment. As Opisthokonta (especially fungi), Apicomplexa and Cercozoa are highly abundant in terrestrial habitats[29,99–101] the chance of random dispersal to freshwaters is high for these taxa.

In contrast, taxa assigned to Chlorophyta, Peronosporomycetes and Chrysophytceae may rather disperse from freshwater to soil habitats. This seems also conclusive as in particular Chlorophyta and Chrysophyceae are more abundant in freshwater than in soil[29,99]. This is possibly also true for Chytridiomycetes as their abundance and diversity in freshwater is slightly higher—again dispersal from freshwater to soil has been demonstrated[38]. Our results also indicate a predominant exchange of Peronosporomycetes from water to soil. For this taxon, however, published data indicate an exchange from soil to water[38,102]. Possibly, this hints to differential routes of dispersal for different taxa[94] but data so far are inconclusive. Peronosporomycetes may nevertheless be an interesting taxon for further studies on habitat specificity and dispersal.

For Ciliophora the dominant direction of dispersal between the two habitat types is even less clear. Numerous ciliate OTUs occurred in both habitat types and these taxa made up for a similar share in freshwater and in soil communities[38,46,47,99,103,104].

Overall, our data indicate that the direction of dispersal is predominantly from soil to freshwater (Fig. 2B): A majority of the shared OTUs occurred predominantly in soils with only low read numbers in freshwater. At first sight, this may seem to confirm the idea of soil protists as aqueous organisms[33], with an aquatic origin which may therefore be able to cope with aquatic environments while freshwater protists lack an adaption to terrestrial habitats[105]. However, our data demonstrate that the vast majority of OTUs is habitat specific with only a very minor fraction capable of maintaining in both habitat types. Even for the fraction of shared OTUs our data indicate that the majority of taxa presumably is not established in both habitat types and that the presence of taxa in both habitats may largely be due to random dispersal rather than a broad niche adaptation[96,106].

Nevertheless, despite the small number of shared OTUs they account for an integral part (up to ~ 68%) of the read abundances. This does not necessarily indicate a high abundance of generalistic taxa but may be rather due to a higher chance of random dispersal and subsequent random detectability of these taxa in both habitats.

This view is supported by the high fraction of shared taxa with a strongly biased distribution towards either soil or freshwater and by the fact that particularly OTUs with high read abundances show such strongly biased distributions (Fig. 2C). Further, these latter taxa mostly belong to taxonomic groups considered either typical for soils (such as fungi) or freshwater (such as distinct algae).

Only some taxa with low to moderate read abundances show a rather uniform pattern across soil and fresh water, indicating that these may be true generalists without a distinct habitat preference (Table S3). For some of these taxa the presence in aquatic and terrestrial habiats was already shown as e.g. for *Cladosporium cladosporioides*[107], *Gomphonema parvulum*[108] and *Pythium capillosum*[109,110], while other presumably generalistic taxa so far were known only from one habitat type (e.g. *Boeremia exigua* (terrestrial) and *Articulospora proliferata* (aquatic)).

As we cannot exclude the possibility that some of the OTUs in our data set represent inactive cells (e.g. resting stages, dead organisms) the number of shared OTUs may in fact be even smaller. Our results provide evidence that either the exchange of organisms is very low, the survival of these organisms in the other habitat type is low or both. However, we have to admit that the sampling depth of our study (as any such study) is restricted to the sampling volume. According to the sampling volume of several hundred milliliters of water and several grams of soil in our study we most likely have missed taxa which are very rare in a compartment (few individuals per liter of water / per gram of soil). That may explain why the overlap between corresponding soil and freshwater sites is very low and commends the general investigation of all freshwater sites and all soil sites. Thus, several of the taxa found to be habitat-specific may occur in the other habitat types at low abundance which may reflect, however, most likely random drift of some cells rather than true occurrence as an active member of the respective community. We also cannot exclude the possibility that some taxa were missing in either habitat due to spatial or seasonal variability in particular as soil communities differ over scales ranging from hectares to square millimeters, even when topography and texture are relatively uniform[20,84,111–113] and samples were taken a few years apart. We are aware that soil and freshwater campaigns did not take place in the same year. Still, Gilbert et al. and Bock et al. showed that microbial communities show repeatable seasonal patterns and Shade et al. showed that microbial communities can stay relatively stable over time and as we sampled during mid summer we expect that the abundances may changed, but the mere presence of OTUs should be stable[9,114,115]. The intention of our study was not to link active interacting communities but rather to reflect differences and commonalities between soil and aquatic communities on a comparable spatial (geographic) scale. We therefore consider the temporal difference to be neutral (if not advantageous) as the temporal and logistic separation decreases the chance of (natural and artificial) cross contamination between aquatic and terrestrial samples while taxon coverage may be rather stable due to resting stages in the seedbank of soils[9,41,42].

However, in future studies the inclusion of freshwater sediments seems reasonable as conditions between soil and aquatic sediments may be more similar and soil organisms may deposit to and dwell in freshwater sediments even if they cannot compete in the pelagial. Further, sampling over a long time period could provide valuable information about the long-time establishment of exchanged species.

Conclusions

Our study showed that the community in soil and freshwater is fundamentally different and that co-occurring OTUs are rare. In addition, abundant shared OTUs are rather affiliated with one habitat type and most likely dispersed to the other habitat by chance. Only few rare shared OTUs may represent true habitat generalists. This gives evidence , that soil and freshwater communities are rather closed communities and that an establishment of taxa from the respective other habitat type is unlikely even though inoculation occurs and, in particular freshwater habitats seem regularly to be inoculated by individual OTUs originating from soil.

Further, soil habitats show a significant higher OTU richness and higher diversity compared to freshwater habitats, which is also reflected by a higher community dissimilarity compared to freshwater habitats. However, true diversity was similar for fungi as richness was higher in soils but evenness was higher in freshwater.

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G.S. processed the soil samples, analyzed the data and wrote the manuscript; C.B. provided critical feedback and helped to interpret the data; D.B. helped with the analyses and statistics and provided critical feedback, J.B. designed the overall study and wrote the manuscript; all authors gave their final approval for the manuscript.

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Chapter II:

„Monitoring anthropogenic stressors and their impact on microbial communities”

Article IV:

„*Microbial community shifts induced by plastic and zinc as substitutes of tire abrasion*“

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Microbial community shifts induced by plastic and zinc as substitutes of tire abrasion

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Aquatic environments serve as a sink for anthropogenic discharges. A significant part of the discharge is tire wear, which is increasingly being released into the environment, causing environmental disasters due to their longevity and the large number of pollutants they contain. Main components of tires are plastic and zinc, which therefore can be used as substitutes for tire abrasion to study the effect on microbial life. We investigate environmentally realistic concentrations of plastic and zinc on a freshwater microeukaryotic community using high-throughput sequencing of the 18S V9 region over a 14-day exposure period. Apart from a generally unchanged diversity upon exposure to zinc and nanoplastics, a change in community structure due to zinc is evident, but not due to nanoplastics. Evidently, nanoplastic particles hardly affect the community, but zinc exposure results in drastic functional abundance shifts concerning the trophic mode. Phototrophic microorganisms were almost completely diminished initially, but photosynthesis recovered. However, the dominant taxa performing photosynthesis changed from bacillariophytes to chlorophytes. While phototrophic organisms are decreasing in the presence of zinc, the mixotrophic fraction initially benefitted and the heterotrophic fraction were benefitting throughout the exposure period. In contrast to lasting changes in taxon composition, the functional community composition is initially strongly imbalanced after application of zinc but returns to the original state.

Pollution is a globally growing problem, triggering unforeseen consequences and incalculable harm to biodiversity[1–6]. One important pollution source is tire abrasion[7,8]. Tires contain numerous substances like carbon black, additives, fabric, curing agents, sulfur, metals, but also styrene and butadiene, which are precursors of polystyrene[9–12]. Wearing off the tire leaves particles on the surface, which, due to abrasion and UV radiation, degrade to micro- and nanoplastic[4,13–16]. During the next heavy rainfall event, these particles are then carried into the water bodies (Fig. 1).

The particles can remain in the environment for centuries, if not millennia, due to their long half-life, attributable to their low biodegradability[18]. One of the most important toxic compounds in tires in terms of quantity is zinc, i.e., tires contain around 1% zinc[19]. Some adverse effects are already described[20–24], but the overall impact on microeukaryotic communities are insufficiently investigated yet[5,25–29] as the effects of nano- and microparticles and associated toxic substances are mostly unknown[30].

Further, for tire abrasion it is unclear to what extent potential effects are caused by the nanoplastics or by toxic compounds adsorbed to or embedded in the nanoplastic particles[4,7,8,13,28,31,32].

Strong effects on microeukaryotic communities are likely as both direct and indirect harmful effects via physical contact, phagocytosis, feeding and accumulation via the food web are likely[37–40]. A shift in bacterial communities has been shown by Fu et al. suggesting indirect effects on microeukaryotes i.e. through feeding interactions[41,42]. Effects of micro- and nanoplastics are known to increase with decreasing particle size[43–47]. Nanoplastics can permeate into lipid membranes[48] leading to lipid peroxidation[49] and to cell membrane disruption by direct contact, physical piercing, plasmolysis and physiological stress[20,50–54]. Nano- and microplastics have further been shown to cause a decrease in chlorophyll content and photosynthetic activity in microalgal cultures, independent of growth inhibition and shading effects[43,52,55,56]. The underlying effects are diverse, ranging from

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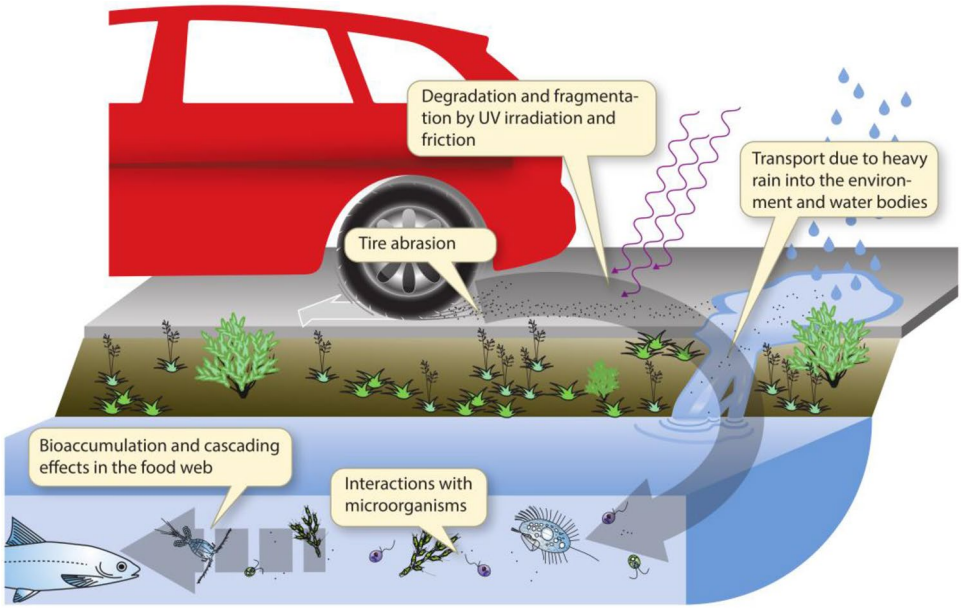

Figure 1. Pictorial representation showing how pollutants from tire abrasion enter aquatic ecosystems. While driving, the tire wears off and leaves particles on the road surface, which are further degraded to micro- and nanoparticles by friction and UV radiation. These particles are discharged with the next heavy rainfall into aquatic and terrestrial ecosystems. There they interact and affect microbial organisms and are further accumulated along the food chain[17].

reduced CO_2 uptake to increased reactive oxygen species (ROS) production, distorted thylakoids and negatively affected photosynthesis genes[43,52,55,56].

Zinc, on the other hand, is one of the most common and abundant metals in the environment[57]. Even though essential for cells at low concentration, it is toxic at higher concentrations due to several effects on the physiology of cells[58,59]. For instance, excess zinc can lead to lipid peroxidation, decreasing cellular chlorophyll content, photosynthesis reduction and reduced diversity in microorganisms[59–61]. Zinc excess can further negatively affect the activities of carbon-, nitrogen-, and phosphorus-acquiring enzymes[62]. In order to cope with zinc individual microeukaryotes activate defense mechanisms such as the production of antioxidants and metal chelators[63].

In this study, we examined the effects of 100 nm polystyrene nanoparticles and zinc as a substitute for tire abrasion, and its effects on a natural freshwater microbial community using mesocosms in conjunction with next generation amplicon sequencing.

The objectives of this study were to determine effects on the community composition both for the polystyrene nanoparticles and for zinc. Regarding nanoplastic we particularly hypothesize a decrease of microeukaryotes lacking cell walls or possessing (thin) cellulose but hardly an effect on taxa with robust cell walls such as diatoms and fungi and thus an increase of the relative abundance of the latter. Nanoplastics may invade the cells of the former taxa more easily, damage the cellulose wall and thylakoid membranes, while the latter taxa should be better protected from such effects[52]. We conjecture effects on phototrophs and heterotrophs, however we assume that heterotrophic microeukaryotes are affected more severely due to weaker defenses, mistaking plastic for food and accumulation compared to phototrophic microeukaryotes and fungi which should be reflected by a relative decrease of phagotrophs in the microbial community. Nevertheless, negative effects on photosynthesis have also been shown for cultivated microorganisms[52,56]. Such effects may decrease the abundance of phototrophic microorganisms and counterbalance the expected community shifts.

With respect to zinc, we expect to see a greater impact onphototrophic microorganisms than on heterotrophic or mixotrophic ones as it impacts nutrient acquiring enzymes and negatively affects photosynthesis. The impact of zinc on cells, however, may be modulated by interactions with outer structures such as cell walls. Biomolecules present in cell walls such as mannans, glucans, phosphomannans, chitin, chitosan and melanin are known to act as bioabsorbants of metals[64]. We therefore expect that organisms possessing thick and robust cell walls, in particular fungi, are more tolerant to zinc and that consequently their relative abundance will increase[65]. Thinking along these lines, we hypothesize strong negative effects for phagotrophic taxa as most of these taxa lack cell walls and further accumulate zinc along the food chain. In consequence, we further hypothesize a decrease of heterotrophs, an increase of fungi and a presumably intermediate response of phototrophic taxa. However, as metal binding to cell walls should eventually reduce the free zinc concentration, the mid- to long-term effects may be the other way around and organisms lacking zinc-binding molecules on the surface may benefit.

Methods

Lake water was taken from lake Baldeney (Germany, Essen 51.402484 N, 7.007840 E) on the 22 nd of August 2019 5 m from the shoreline. Water was filtered through a stainless steel filter (2 mm) and subsequently through a 100 µm mesh to exclude larger metazoa and debris. The water was stored indoors in an open 2400 L stainless steel tank. To compensate for missing substrate we added an amount of 0.025% of NSY medium (Nutrient broth, peptone from soybean and yeast extract media) and WC medium (freshwater medium) (modified from Guillard & Lorenzen[66]) (Table S1) every day. The water was exposed to daylight, gently stirred for 30 s once a day and ventilated by four aquarium aerators. After two days of initial acclimatization, we transferred the water into 25 glass aquariums (45 L each) with one aquarium aerator each and allowed for a secondary acclimatization for one day. The experiment was run under natural light conditions in an air-conditioned greenhouse at 19 °C air temperature. Basic chemical parameters, such as pH, conductivity and water temperature, are shown in the supplement (Table S2).

In the experiment we tested the effects of polystyrene nanoparticles (Micromod, Germany, 100 nm polystyrene, micromer®-F green) and zinc (applied as zinc chloride) in a replicated factorial design[57,67]. In addition, we ran acontrol containing silicate nanoparticles (Micromod, Germany, 100 nm silica, sicastar®-F). We applied nanoparticles in a concentration of 3×10^8 particles ml^{-1} and zinc chloride in a concentration corresponding to 1 mg Zn l^{-1} (15.3 µmol/l). The experimental setup therefore consisted of five different treatments with five replicates each. Namely, control (without any exposure), zinc exposure (Zn), nanoplastic exposure (NP), zinc & nanoplastic (ZnNP), and the additional silica nanoparticle exposure (Si). The silica nanoparticle treatment was applied to differentiate between effects due to particle concentration from effects due to particle composition (polystyrene and silica). The nanoplastic concentration (0.158 µg/l) applied corresponds to concentrations found in the environment[68]. Baselines of metals and actual zinc concentrations are shown in the supplement (Tables S3, S4). Dissolved metals were measured by measuring filtered samples (using Whatmann™ 0.45 µm cellulose nitrate filters). The metals concentrations were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The analyses were carried out with a quadrupole ICP-MS system (Perkin Elmer Sciex Elan DRC-e) operating at 1000 W plasma power, 14 l/min plasma gas flow and 0.95 l/min nebuliser gas flow and an auto sampler system (Perkin Elmer AS-90) connected with a peristaltic pump with a sample flow of 1 ml/min. To avoid contamination and memory effects the wash time between measurements was set to 10 s (with 1% HNO_3, suprapure). Before analyses, the samples were diluted 1:10 using a solution of 1% HNO_3 (suprapure) with a concentration of 10 ng/l of yttrium (Y) as internal standard. In order to control the accuracy and stability during measurements a standard solution of all analytes in concentration of 10 µg/l was analyzed after every 10 samples. The calibration was carried out with a series of 1 1 dilutions of a multielement standard solution (ICP Multielementstandard solution, Merck, Darmstadt, Germany) using additionally Molybdenum standard (AAS-standard, Bernd Kraft, Duisburg, Germany). Element concentrations were calculated as mg l^{-1} using corresponding regression lines (correlation factor ≥ 0.999)[69,70]. A lower dissolved zinc concentration than calculated is present under natural conditions, as zinc is bound to organisms and particles and is therefore not freely available (Fig. S1). Pre-experiments confirmed that no zinc adsorption to the aquariums was detectable. Formation of nanoplastic aggregates, however, occurs naturally and cannot be overcome without addition of surfactants to ensure initial dispersion, which is not feasible in the experiment; thus particles were sonicated in 0.1 M Na_2CO_3 for 15 min[71,72] (Manufacturer) prior to the experiment. Corresponding amounts of Na_2CO_3 were added to the treatments without nanoparticles to exclude effects caused by Na_2CO_3.

Sample processing. Samples were taken after 1 h, 12 h, 24 h, 48 h/2 days, 96 h/4 days, 168 h/7 day and 336 h/14 days after stirring and before addition of nutrient media. This sampling scheme was chosen to capture immediate effects at the beginning with a dense sampling and medium-term effects, which become rather visible only after a few generations, with a more broader sampling towards the end. Briefly, we withdrew 400 ml (sampling points 1, 12 and 24 h) and 200 ml (sampling points 48, 96, 168, 336 h) with sterile 1 L glass bottles and filtered the sample on a polycarbonate filter (0.2 µm). Sampling volume was less for the last four samplings as filters clogged earlier.Saturation curves were used to manually rule out that samples were under-sampled. This sampling scheme is considered biomass normalized because filters that are close to clogging were sampled, so that sampled biomass is comparable.

From each sample, 4 ml subsamples were taken and adapted to darkness (30 min) for measuring the photosynthetic activity using the AquaPen-C AP100 QJIP protocol (Photon System Instruments, Brno). Each subsample was measurement twice (2 times 2 ml) and the average was taken for analysis (Table S5).The Fv/Fm quotient describes the maximum photochemical quantum yield of the photosystem II (PSII) in a dark adapted state, with Fv being the variable fluorescence or difference between maximum and minimum yields ($Fm-F_0$), of chlorophyll a fluorescence. The measured `Fv/Fm`- ratio is related to the photochemical conversion efficiency of the PSII and describes the proportion of functional PSII indicating stress and vitality. The `Fix Area` value measures the area under the induction curve of fluorescence during a saturation light flash (1 s), which serves as an indirect proxy for the chlorophyll a content[73–75]. Photosynthesis proxies were statistically compared against the control using Wilcoxon tests.

Filters were air dried and immediately stored in 1.5 ml eppendorf tubes with 400 µl RNA/DNA Shield (Zymo Research) and frozen at -80 C° until DNA extraction. DNA was extracted using the Zymo Quick DNA/RNA microprep plus kit with a modified protocol. Briefly, each step was performed at room temperature and samples were centrifuged until the fluid passed the columns (30–70 s) at 10.000 rpm.Two filters with the surrounding DNA/RNA shield solution were transferred in a bashing bead tube (Zymo BashingBeads Lysis Tubes (0.1 & 0.5 mm)) and homogenized using the FastPrep instrument (MP Biomedical). Homogenization was run five times for 30 s at 5.5 m/s. Between homogenization steps the samples rested 1 min on ice. The bashing bead tubes were

centrifuged for 30 s, the supernatant was transferred in a 1.5 ml tube and mixed with 400 µl lysis buffer. After 30 s the sample was transferred onto a Zymo-Spin IC-XM column in a 1.5 ml RNAse free tube and was centrifuged.

400 µl preperation buffer was added to the DNA carrying column and the column was centrifuged. Followed by two washing steps with 700 and 400 µl wash buffer and a final dry centrifugation for 2 min. The dry columns were transferred to a 1.5 ml tube and 30 µl RNAse/DNAse free water was added. After 5 min an elution centrifugation was performed. DNA was frozen at − 20 °C.

PCR amplification targeted the 18S V9 region. Forward primers are based on 1391f. (5`-GTACACACCGCC CGTC-3`)[76]. Reverse primers are based on EukR (5`-TGATCCTTCYGCAGGTTCACCTAC-3`)[77]. The final concentrations in all of the PCR reactions were as follows: 0.5 µl of DNA template in 25 µl PCR reactions with 0.02 units/µl of Q5 DNA polymerase (NEB), 5 µl Q5 buffer, primers at a final 0.5 µm concentration, dNTPs at 0.2 µm final concentration and 16.25 µl water. The PCRs conditions included an initial denaturation at 98 °C for 30 s and 25 cycles of: 98 °C for 30 s, annealing for 10 s at 60 °C, extension for 15 s at 72 °C, followed by a final extension step at 72 °C for 2 min.

DNA-Sequencing: Equimolar subsamples were pooled and commercially sequenced using a NovaSeq6000, yielding 150 bp-long paired-end reads (Fasteris, Geneva, CH).

Bioinformatics. Adapter removal, quality trimming and demultiplexing using molecular identifier (MID) sequences were performed by the sequencing company (Fasteris). The base quality of the sequence reads was checked using FastQC[78]. A split-sample filtering protocol for Illumina amplicon sequencing was used for two technical replicates per DNA sample[79]. The raw sequences were quality filtered[80] (PRINSEQ-lite v.0.20.4) to remove reads with an average Phred quality score below 25. The paired-end reads were assembled and quality filtered with PANDASeq[81] (v2.10). All reads with uncalled bases, an assembly quality score below 0.9, a read overlap below 20, or a base with a recalculated Phread-score below 1 were removed. After dereplicating chimeras were identified and filtered using UCHIME[82] (v7.0.1090) with default settings. Sequences that were not present in both sample branches were discarded[79]. Operational taxonomic units (OTUs) were created swarm with default settings[83] and amplicon sequencing variants (ASVs) were created with DADA2[84] (1.18.0). Sequences can barely be tracked down to represent distinct species or even strains, thus, the use of taxonomic units (OTUs or ASVs) is mandatory to perform analyses. The tools were used within the modular bioinformatic pipeline Natrix[85]. Sequences wereBLAST (Basic Local Alignment Search Tool) aligned against the National Center for Biotechnology Information (NCBI) nucleotide database (nt) (downloaded Feb. 2020). OTUs with less than 100 reads in total, occurring in less than 10 samples, having a BLAST e-value larger than 1×10^{-5} or a percentage of identity less than 90% were discarded. Sequencing depth waschecked with rarefaction curves and samples that did not reach saturation were manually curated. Namely, the samples NP1_336h, NP5_168h, ZnNP5_168h, Zn5_336h, C2_336h were removed before analysis.

True diversities and functional group abundances were computed from rarefied reads (function rrarefy from the vegan R Package[86], version 2.5.7[87]). Reads were rarefied to the mean read amount (419,158) from all samples. True diversities are based on the Simpson index using the R package RAM[88] as well as Pielou`s evenness. True diversity, also known as Hill numbers or effective number of species, were chosen as a measurement for diversity as it is not a non-linear diversity index (e.g. Shannon index, Simpson index) but is suitable for linear comparisons between samples. True diversity and OTU richness was statistically compared against the control using Wilcoxon tests.

We assigned the nutritional mode to OTUs by using their BLAST based percentage identity values to species/taxa where the nutritional mode is known. We accepted individual thresholds for correctly resolving the assigned taxonomy to the phylum, clade, class, order, genus, species and strain level, while referring to Table S6. For the phylum/clade/class/order level we accepted a percentage identity of 90%, for the family level 92%, for the genus level 94.5%, for the species level 98.7% and for the strain level 99%[89]. These thresholds were derived from research on bacteria and archaea and were used here for eukaryotes, since similar, universally usable, thresholds are not available for the V9 18S region in eukaryotic microorganisms. Differential abundant OTUs were calculated by their $\log_2$fold changes using the R Package DESeq2[90] on raw count data with an accepted adjusted p value below 0.01. The design formula accounts for effects caused by individual treatments and not by temporal effects (design = ~ time + treatment).

For the non-metric multidimensional scaling (NMDS) we replaced zeroes in our dataset based on a Bayesian-multiplicative replacement (cmultRepl, R zCompositions package[91]) and calculated the Aitchison distance, as we are dealing with compositional data[92]. Aitchison distance is used as a community dissimilarity indicator. This distance matrix was used to compare the treatments among each other using a Benjamini–Hochberg p-adjusted pairwise adonis (adonis padj). The same test wasapplied to functional community compositions of individual treatments. Figures were created with R and Adobe Illustrator[17,87].

Data availability. The datasets generated and analyzed during the current study are available in the Genbank repository (PRJNA844210) available after the manuscript was accepted.

Results

Sequencing results. The total number of assembled reads after filtering was 70,807,761 which formed 1410 OTUs. Of these 419,158 ± 182,342 assembled reads and 571 ± 189 OTUs were found in individual samples. Results shown here are based on OTUs as patterns were inconclusive for ASVs (Fig. S2).

Effects of treatments on the community structure. Microeukaryotic communities in the different treatments differed and the separation increased over time. Besides a general temporal development we

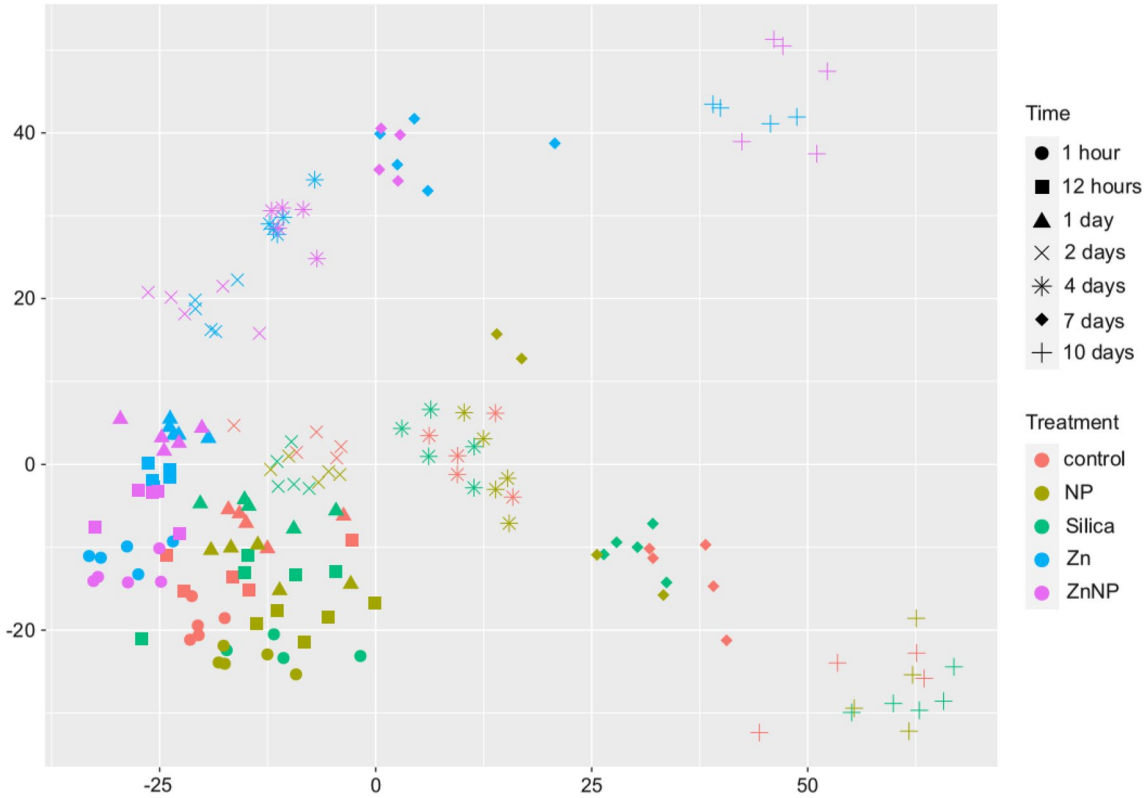

Figure 2. Non-metric multidimensional scaling (NMDS) ordination plot of individual treatments over time showing their community development and differentiation during the time-course of the experiment. A stress value of 0.105 denotes a decent ordination representation. Treatments are color-coded and sampling points are shape-coded. It can be observed that the x-axis represents time and the y-axis individual treatments Samples close to each other are more similar, thus their community structure is more similar than the community structure of more distant samples. The distance between individual samples is rank-based and not linear. The differentiation of the Zn and ZnNP treatment compared to the control becomes obvious over time, while there is no differentiation of Si and NP treatment, when compared to the control.

observed a pronounced effect of zinc, i.e. the shift of the community composition was stronger in treatments exposed to zinc while exposure to silicate and polystyrene nanoparticles did not or hardly affect the development of the community compared to the control treatment (Fig. 2). The community turnover and the impact of zinc became visible both on the level of individual OTUs (Fig. 2) as well as on the level of higher-level groups (S3).

While community composition developed similarly within treatments without additional Zn (control, silicate and NP) as well as within zinc-treatments (Zn and ZnNP) the development was different between these two groups (pairwise adonis padj > 0.01; Fig. 2). The communities in the zinc treatments started diverging from the zinc-free treatments already within the first hour after exposure (Figs. 2, 3) and continued to diverge until the end of the experiment. In particular, the relative abundances of Chrysophyta and Euglenozoa were initially higher in the zinc-treatments but later replaced by a higher abundance of Choanoflagellata, Cercozoa, Amoeba and Chlorophyta. While there are generally less Bacillariophyta, Chytridiomycota and Dinophyta (Fig. S3) compared to zinc-free treatments.

The exposure of silica and polystyrene nanoparticles did not significantly affect the development of the community. Likewise, we did not find indications for an interactive effect of nanoplastic and zinc on the community structure.

Diversity. While taxon composition was changing during the course of the experiment and developed differently between zinc and non-zinc treatments (Fig. 2) the diversity was hardly affected: OTU richness did not significantly change in the course of the experiment (padj > 0.01) (Fig. S4, Table S8). Similarly, the true diversity, as well as evenness, was not significantly affected throughout the experiment (wilcoxon $p > 0.01$) (Figs. S5, S6). Further, diversity indices were similar between zinc and non-zinc treatments (wilcoxon $p > 0.01$). Diversity is thus decoupled from the taxonomic community profile. Although the relative abundances of individual taxa/OTUs changed, the evenness did not.

Functional attributes. Roughly 90% of the OTUs (1273 OTUs, i.e. 702 heterotrophic, 26 mixotrophic, 41 parasitic, 504 phototrophic) were appointable to a trophic mode, i.e. phototrophic, mixotrophic, heterotrophic or parasitic, while 137 OTUs could not be assigned to a trophic mode.

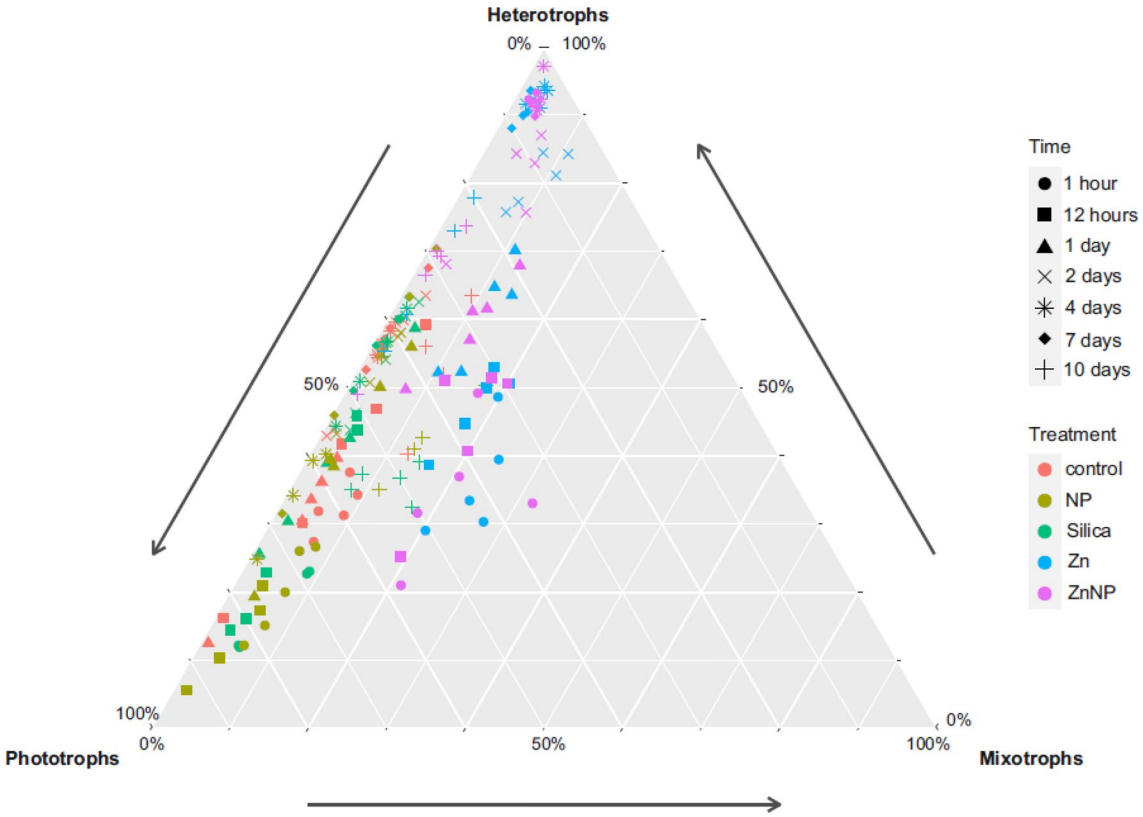

Figure 3. Ternary plot showing the functional community composition and its shift. Treatments are color-coded and sampling points are shape coded. Consumers include parasitic and fungal OTUs. Ternary plots are three dimensional composition plots. Relative abundances of samples always sum up to 100%. The closer a sample is located towards one corner (nutrition type), the more organisms affiliated with this nutritional type are represented in the community in terms of relative abundance. Samples close to each other are similar in their functional community composition. Distances are based on the relative abundance of the three functional groups, thus distances between samples behave linear.

The relative importance of trophic modes changed over time and the strength of this change depended on the treatment, i.e. the shift was stronger in the zinc treatments as in the non-zinc treatments. Within groups, i.e. between control, Si and NP on the one hand and between Zn and ZnNP on the other, no significant differences were observed (pairwise adonis, $p > 0.01$), while the difference between these groups is significant (pairwise adonis, $p < 0.01$).

Initially phototrophs dominated in all treatments. During the course of the experiment, the community shifted towards a rather heterotrophic dominated community and subsequently back to a more balanced community with a slight dominance of heterotrophs. In the non-zinc treatment the shift of nutritional modes was moderate while in the zinc-treatments the initial shift was sharp and phototrophs decreased drastically. In consequence, the relative importance of mixotrophs increased initially in the zinc treatment and later on the community was nearly exclusively composed of heterotrophs. Towards the end of the experiment, phototrophs became more frequent again and functional diversity became similar to the control in the zinc treatment as well. Thus, the ratio of consumers, mixotrophs and phototrophs is largely balanced towards the end and became similar to the starting conditions in all experiment. However, taxon composition changed strongly (Tables S8, S9, S10, S11).

In the zinc treatments (Zn and ZnNP) the dominant phototrophs of the initial community, i.e., Bacillariophyta, were nearly entirely replaced by Archaeplastida as the experiment progressed (Fig. S10). It is noteworthy that the Chlorophyta species *Halochlorella rubescens* made up a major part of the phototrophic fraction. Considering the last sampling day this OTU made up 16.8 ± 9.9% of the total reads in the Zn and 13.3 ± 9.1% in the ZnNP treatment, representing 63.5 ± 31.8% and 40% ± 14.2 of the Chlorophyta fraction, respectively.

The mixotrophic subcommunity is diminished in the zinc treatments (Zn and ZnNP) and consisted mostly of Chrysophyta with a small share of Dinophyta. In contrast, Dinophyta dominated this fraction almost exclusively in the non-zinc treatments (control, Si and NP) (Table S10).

In all treatments heterotrophs were initially dominated by Chrysophyta and Ciliophora while they were Cercozoa and Ciliophora dominated towards the end of the experiment. However, their relative importance differed, as the zinc treatments contained a higher fraction of Amoebozoa and Cercozoa but a lower fraction of Ciliophora and Chytridiomycota. Interestingly, the NP treatment contained more Cercozoa than any other treatment (Table S11) which is one of the few deviations of the NP treatment from the control.

The relative abundance of parasites decreased in the zinc treatments and that of fungi increased in the non-zinc treatments towards the end of the experiment.

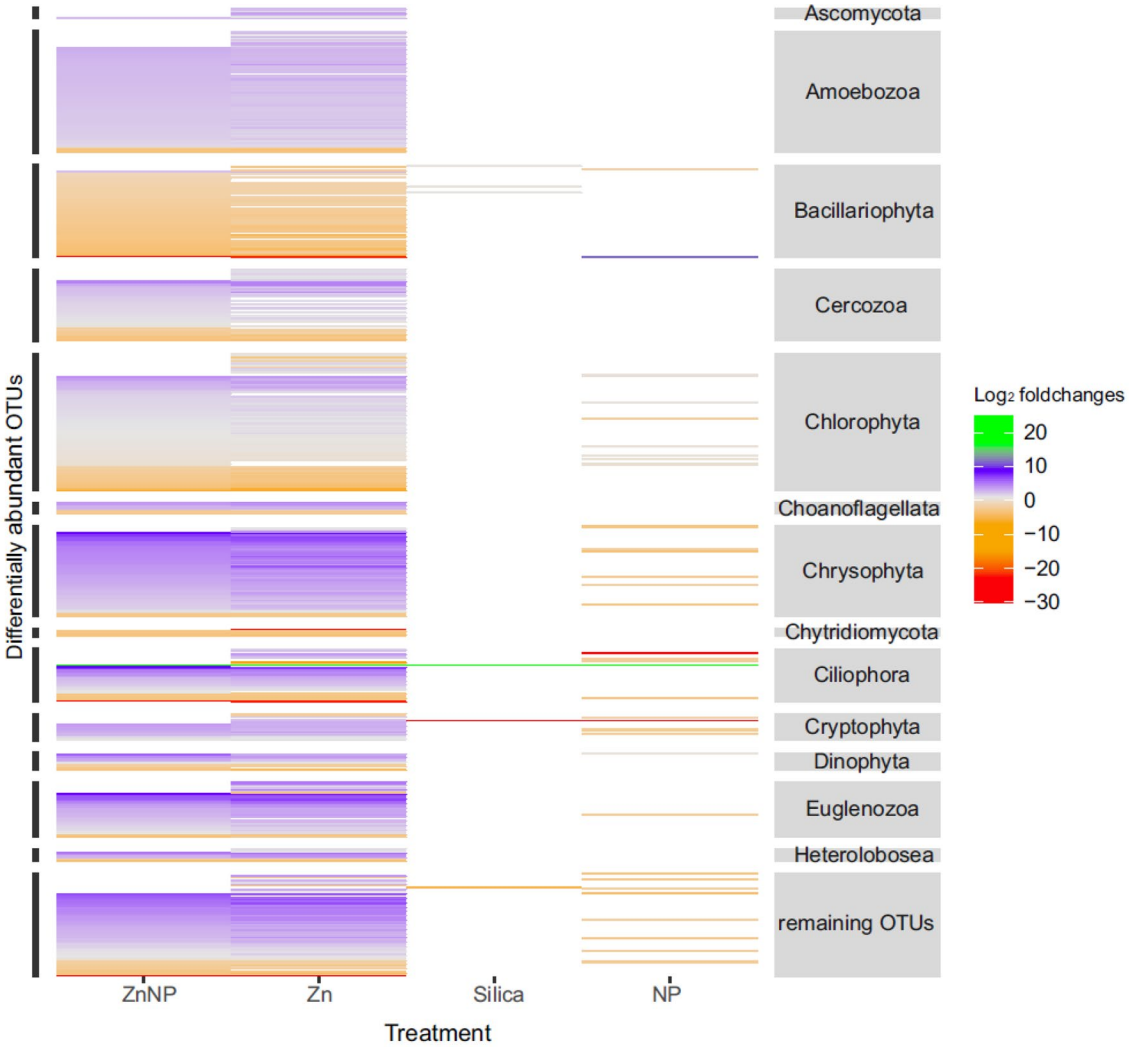

Figure 4. Log_2-fold changes of differentially abundant OTUs and their affiliation to subgroups compared to the control. Only differences with an adjusted *p* value of 0.01 are shown. Each line represents one OTU. OTUs are sorted in descending order based on their log_2-fold changes in the ZnNP treatment. Log_2fold changes are color-coded, the descending order is: green, blue, grey, orange, red. Differences visible here are solely based on the factor treatment.

Fv/Fm photosynthesis proxy. The shift of trophic modes is supported by photosynthesis proxies (Fig. S7). While photosynthesis proxies do not significantly vary in the non-zinc treatments (control, Si, and NP) (wilcoxon $p > 0.01$), they develop parabolically in the zinc treatments, i.e., an initial strong decrease is followed by a plateau phase and a full recovery towards the end. Compared to the control, photosynthesis proxies were lower in zinc treatments throughout the experiment (wilcoxon $p < 0.01$) with the exceptions of the last sampling (336 h) for the ZnNP treatment and the last two samplings for the Zn treatment.

Differentially abundant OTUs. Community composition shifted differently in treatments and so did individual OTUs. Compared to the control, 519 OTUs (36.81%) were differentially abundant in other treatments (Fig. 4, Table S12) (padj < 0.01). This was most pronounced in the Zn treatment with 351 more abundant and 116 less abundant OTUs and in the ZnNP treatment with 315 more abundant and 118 less abundant OTUs. Patterns between these two treatments were similar as they shared 397 of the differentially abundant OTUs (78.93%). In contrast, in the non-zinc treatments only a minor fraction of OTUs deviated from the control, i.e. 11 OTUs were more abundant and 26 were less abundant and in the NP treatment 4 OTUs were more abundant and 2 were less in the Si treatment.

The differentially developing OTUs again demonstrate the strong effect of zinc and a minor (but present) effect of nanoparticles. Thus, while nanoplastic particles have no effect on the diversity or community composition (Fig. 2) they have an effect on few individual OTUs (accounting for 2.62% of the reads).

In general, the majority of differentially abundant OTUs which were affiliated with Ascomycota, Amoebozoa, Chrysophyta, Cryptomycota and Euglenozoa had a higher abundance as compared to the control with only few exceptions. In contrast, most of the differentially abundant OTUs which were affiliated with Bacillariophyta and

Chytridiomycota had lower abundances in the treatments while OTUs affiliated with Cercozoa, Chlorophyta, Choanoflagellata and Dinophyta showed a mixed pattern with a balanced occurrence of some more and some less abundant OTUs.

Some strongly differential abundant OTUs occur with a $\log_2$fold change of − 30 to 28 (Fig. 4), each with a percentage identity value of less than 98.7%. Therefore reliable affiliations to species are questionable, namely, an unclassified fungi (98.33%), an uncultured Chytriomycota (93.02%) *Holosticha diademata* (93.86%), *Stephanodiscus hantzschii* (98.43%), *Apouronema harbinensis* (98.2%) and *Hemiselmis virescens* (92.59%) (Table S12).

Interestingly, the OTUs most strongly deviating from the control were mostly affiliated with common taxa (Tablr 1). Noteworthy is that *Cryptomonas* sp. seem to react differently to zinc (Zn and ZnNP) as compared to NP.

Discussion

The environment is subject to many types of pollution, with heavy metals and plastics being of particular importance[93,94]. These pollutants are being released in particular from tires[7,22,34] and affect microbial communities, especially microeukaryotes[95–97]. Zinc is bound initially to suspended natural particles[98–100], which explains the deviation from the calculated value (Table S2). Effects on the community structure by zinc are conclusive, as excessive zinc concentrations are toxic[57,101–103] and lead to lipid peroxidation, decreased chlorophyll content, and reduced activities of carbo-nitrogen and phosphorus acquiring enzymes[59,62].

The striking impact of zinc after just one hours is especially noteworthy. Zinc entails major cell damages leading to disturbed osmosis, causing the cells to disintegrate in a matter of minutes[104]. Thus the differences between the communities of zinc and non-zinc communities after one hour presumably reflect immediate death and lysis of some individuals following the addition of zinc. This suggests that even short pulses of zinc pollution cause devastating effects, thus, threatening microbial life, ecosystem balance and their function. Further, following this short-term effect is a mid-term effect, as biosorption of metal by microeukaryotes increases with time of exposure leading to enhanced toxicity[60,105–107].

In general, the community composition changed over time as was to be expected for mesocosm systems[108] (Fig. 2). However, we observed pronounced differences between treatments indicating significant effects of the tire abrasion substitutes. In particular, community structure was strongly affected by zinc, but not significantly by nanoparticles. In contrast, diversity in terms of OTU richness and effective number of species was barely affected, demonstrating the stability of diversity upon exposure to nanoplastic, zinc and their combination (Figs. S5, S6). In the plastic and silicate nanoparticle treatments we also did not observe strong deviations in the development of the community composition as compared to the control indicating a generally weak effect of nanoparticles. This is in contrast to our initial hypothesis but not necessarily surprising as microeukaryotes are surrounded by a high concentration of particles, including nanoparticles, in their natural environment and microeukaryotic communities should be adapted to varying nanoparticle concentrations[109,110]. In contrast, in the zinc treatments community composition changed over time, i.e. taxon composition changed (Fig. 2) but community structure with respect to species richness and evenness stayed stable. This finding is congruent with discoveries on effects of arsenic, microplastic and nanoplastics in soil[25].

In all treatments the functional diversity shifted initially towards a stronger contribution of heterotrophic taxa and later back to a more balanced composition, which agrees with the literature regarding functional redundancy[111,112]. As soon as ecosystem functions are disturbed by unfavorable conditions, which lead to the decline of the organisms performing them, the ecosystem strives to compensate by having the functions taken over by other organisms which can cope with the present conditions. Still, this is valid in an open ecosystem with high diversity[111,113]and is more limited in closed mesocosm experiments with limited diversity and dispersion especially regarding the inability of new species to colonize the mesocosms.However, this functional diversity shift was more pronounced in the zinc treatments.

A major finding therefore is that while diversity indices (e.g. effective number of species and evenness) barely reflect an effect of zinc, taxon composition and functional composition do. The functional community composition with respect to trophic modes was strongly imbalanced by the applied tire wear substitutes (Fig. 3). We observed a shift in the functional composition over time in all treatments with a general trend from phototrophic to heterotrophic organisms. The impact of zinc accelerated and amplified this functional shift considerably while there was no corresponding effect for nanoparticles. This was unexpected but conclusive as it is possible that the inert nanoplastic particles formed aggregates and were therefore no longer bioavailable for microeukaryotes[13,114–116]. Alternatively, microorganisms may be used to cope with particles of all size[117–121]. Further the idea that plastics are just a vector for harmful substances could provide an explanation for nanoplastics alone not having a strong effect[2,30,122]. The relative abundance of phototrophs sharply decreased initially in the zinc-treatments causing a short-term increase of mixotrophs and heterotrophs. The relative increase of mixotrophs presumably reflects the abrupt loss of many phototrophic individuals, which were directly affected by the applied substitutes (e.g. cell disintegration)[104,113]as a strong growth of mixotrophs and heterotrophs is unlikely due to the average generation time of around 26 hours[123]. In contrast, despite certain losses of individuals as well, the heterotrophs as a functional group seem to profit early from the zinc addition—most likely due to increased carbon and food availability due to the increased number of dying and dead individuals[46,113]. Within the first days of the experiment, community composition develops towards a community strongly dominated by heterotrophs (≥ 90%). After a couple of days, the detectable increase of heterotrophs is likely based on the decrease of phototrophs and at the same time the actual increase of heterotrophs. Towards the end of the experiment the community composition drifts back towards a more balanced community composition. Thus, the strong

initial effect on the phototrophic community may not be explicitly linked to photosynthesis pathways but may be rather taxon-specific and linked to properties, which are not directly related to photosynthesis. A general link to impaired photosynthesis would not allow another organisms group to peform photosysnthesis (i.e. Chlorophyta), but would have a negative effect on all photosynthesis performing organisms.

Based on the initial response, it is not possible to decide whether the decrease in photosynthetic taxa and in community photosynthesis reflects a general effect of zinc on photosynthesis pathways or rather a sharp decrease of the dominating phototrophic taxa (i.e. diatoms), potentially caused by zinc acting on other structures or pathways. Regarding a general effect on phototrophs, photosynthesis and photosynthesis pathways can additionally be directly affect by zinc[60,124]. Further, phototrophic taxa may be more susceptible to zinc for other reasons, i.e. pathways and structures not directly linked to photosynthesis may be affected. For instance, one could argue that while phototrophs have detoxifying mechanisms like osmotic adjustment, excretion of complexing compounds, metal binding compounds, chelation and an antioxidant protection system[60,63,125,126], heterotrophs may further use particle excretion as an additional way to deal with toxic substances therefore may be slightly better at detoxifying, hence, tolerating zinc[57,127–130]. However, the recovery of phototrophic fraction towards the end of the experiment indicates that there are some photosynthetic taxa capable of dealing with high zinc concentrations. In particular, the dominant algae at the onset of the experiment, i.e. Bacillariophyta, were largely replaced by green algae. This suggests that the observed effect may be limited to or more pronounced in taxa like the Bacillariophyta while other taxa like the (or at least some) green algae rather thrive under high zinc conditions (Fig. S3, S10). This may be linked, for instance, to cell wall properties and cell wall synthesis rather than photosynthesis itself, i.e. silicate cell walls and synthesis pathways may be more strongly impacted by zinc as compared to cell walls made of sugars (cellulose or chitin) in many other taxa. In fact, it is documented that Bacillariophyta are rather sensitive to heavy metals and build deformed frustules, while Archaeplastida, especially Chlorophyta, can better cope with metal pollution[60,63,105,124,130–133]. For instance, the chlorophyte species *Halochlorella rubescens* thrived well in the zinc treatments. The high abundance is coherent with the literature, since this species was used in the wastewater sector[134] and was investigated especially for zinc removal from wastewater[135].

The relative abundance of heterotrophic and mixotrophic fractions increased initially. One could assume that the mixotrophs took over the photosynthetic ecosystem function during this period until the obligate phototrophs eventually recovered. The photosynthesis proxies contraindicate this, i.e. the Fv/Fm values are collapsing (Fig. S7). Thus, mixotrophs probably rely largely on their ability to feed heterotrophically during this period.

This observed switch to a predominantly heterotrophic community is reasonable assuming that the phototrophs in the original community are vulnerable to zinc and that heterotrophs profit from increased food availability in form of dead algal biomass, i.e. it is likely that especially photosynthetic organisms serve as food source[43,52,56,59] (Fig. S7). The assumed selective advantage of heterotrophs during the initial phase of the experiment is consistent with a decreasing importance of mixotrophs and a further increasing importance of obligate heterotrophs.

The addition of zinc also resulted in a lower relative abundance of parasites (Fig. S9). As most parasites are host-specific, the relative abundance of parasite and host is likely coupled[136–138]. As zinc is causing a decline in phototrophs, it will directly (e.g. disturbed osmosis) or indirectly (e.g. killing the host) affect parasite relative abundance[139]. The decrease of parasites thus suggests that many taxa were parasites of diatoms[138,140,141].

We did not observe a strong effect on fungi (Fig. S9) and fungal diversity is not affected by zinc, suggesting that a direct impact is unlikely[142]. This is not surprising as the fungal fraction is low in pelagic freshwaters[143,144] and the nature of compositional data may obscure shifts especially in rare taxa. In summary, it is evident that zinc is strongly unsettling the balance between heterotrophs and phototrophs.

In contrast to our expectations, nanoparticles did not strongly affect microeukaryotic communities or functional composition. We observed no combined effect of zinc and nanoplastic. Even though we have to reject our hypothesis of direct effects on the community and functional level, this finding is plausible: Microeukaryotes aresurrounded by a high concentration of particles with different surface properties (e.g. clay, silt, bacteria). Thus, they are likely well adapted to cope with them[117–121]. Further, nanoparticles cluster easily to hetero- and homoaggregates[13,114–116] and are therefore less likely to have direct interactions with microeukaryotes. Still, plastic leachates and surfactants are known to negatively affect microorganisms in various ways[145,146] but rather due to the respective surfactants or leachates than the nanoparticles themselves[29,147,148]. Our results on tire wear substitutes back this idea as zinc (being a leachate from tire wear) has a strong impact while nanoplastic (as potential vector) is rather neutral (Fig. 2, 3, S7). It should also to be noted that nanoplastic remains in the environment for centuries[18], thus a short- to medium-term exposure likely does not reveal all effects. However, with an average generation time of 26 h the investigated runtime allows to observe effects over multiple generations[123]. Furthermore, it should be noted that nanoplastics normally occur in combination with microplastics, through this combination the effects in natural environment can deviate[113]. With the given half-life of tires, which are made to persist, it becomes obvious that the pollution caused by tires is increasing. With increasing pollution from tires, the chances are rising that at some point microorganisms develop ways to utilize and break down their components, thus playing an important role in biodegradation and bioremediation[149–151]. However, this should not be relied upon, because even if this is the case the pollution level will be so advanced that damage caused to biodiversity is already irreversible.

Differential abundant OTUs. On the level of individual OTUs zinc caused large scale community modulations (pairwise adonis $p < 0.01$, DESeq2 Wald test $p < 0.01$) (Fig. 2, 3, 4), while effects of nanoparticles were less prominent. In particular, a difference between polystyrene and silica nanoparticles became apparent, which was

only detectable on this level. Corresponding to the community and functional group level effects most phototrophic taxa decreased after exposure to zinc. This comprises species affiliated with e.g. the chrysophyte genera *Mallomonas* and *Synura*[152,153], the green algal genus *Micractinium*[154] as well as many diatom genera (e.g. *Sellaphora*, *Fistulifera* and *Nitzschia)*[131,132,155]. While the energy providing photosynthesis as well as cellular integrity is impaired, the degree of impairment is not uniform among taxa[43,52,56,59,104]. As an exception, the diatom *Conticribra weissflogiopsis,* increased in relative abundance and may be an interesting candidate for restoration and treatment of zinc polluted waters. Again in concert with the community and functional group level findings OTUs affiliated with preferably heterotrophic taxa such as Amoebozoa, (heterotrophic) Chrysophyta and Euglenozoa were generally increasing after zinc exposure[96], which is conclusive as they benefit from impaired organisms (e.g. *Spumella*, *Poteriospumella*, *Pedospumella*, *Chlamydomonas*)[156–158] and several of these species are rather metal insensitive (e.g. *Vannella simplex*, *Bodo saltans*, *Rhynchomonas nasuta)*[159–161]. While zinc affected a broad spectrum of OTUs, nanoparticles affected only few. Silica nanoparticles lead to only a few differentially abundant OTU (e.g. *Cyclotella meneghiniana*), while polystyrene nanoparticles lead to several differentially abundant OTUs. The weak effect in particular of silica nanoparticles is plausible as silica particles (e.g. clay minerals) occur naturally in the environment and protists presumably are adapted to dealing with such particles.

Nanoplastic may not cause large scale community modulations, but individual species can be affected as already shown for bacterial phytoplankton[131,162]. Interestingly, a number of OTUs (e.g. affiliated with *Cryptomonas* sp., *Acrispumella msimbaziensis*, *Neobodo designis*, *Spumella* sp., *Chlorella* sp.) (Tables 1, S12) which increased in relative abundance in the zinc-containing treatments were decreasing when exposed just to NP. It is likely that drawbacks of nanoplastic become rather visible on higher trophic levels as nanoplastic accumulated due to feeding on microeukaryotes[30,116,163,164].

However, the individual groups show generally a clear pattern with increasing (e.g. Amoebozoa, Chrysophyta) or decreasing (e.g. Bacillariophyta) abundances with individual exception due zinc exposure. While different patterns for the remaining, Chlorophyta, Dinophyta, Cercozoa, Choanoflagellata and Heterolobosea, are present. The relative amount of increasing and decreasing OTUs isbalanced implying unclear patterns for these groups. Which is conclusive as microdiversity is contrasting among certain phyla or groups and is therefore not a trait specific for certain phyla or groups, therefore, tolerances and deficiencies are different even for closely related taxa. Individual OTUs show different reactions while being within the same genus (e.g. *Acanthamoeba*, *Cercomonas*)[96,165,166]. After all, when working with compositional data one has to note that increasing abundances can have three reasons: (i) the abundance is actually increasing, (ii) the abundance of other groups are decreasing, thus, an ostensible increase is visible or (iii), a mixture of both. This peculiarity may lead to a distorted perception and masking of effects.

The effects of tire abrasion as derived from tire abrasion substitutes, however, may behave different in the environment, as tire wear abrasion is a non-point source of pollution that tends to occur in pulses, in particular after long dry period followed by heavy rainfall[22,167–172]. It is therefore likely that immediate effects (e.g. disintegration of cells) are more important than mid- to long-term effects for pelagial organisms[104] and more pronounced than indicated by our study. Finally, the used tire abrasion substitutes presumably provide a simplified sight, as tires consist of far more potential hazardous substances[170,173–175]. Due to the quantitative importance of tire abrasion and the here and elsewhere already documented[176,177] significance of different tire abrasion substitutes the impact of tire abrasion on the surrounding environments is drastic with unpredictable long term consequences requiring further examination.

Conclusion

Ongoing pollution by tire abrasion contaminants, like nanoplastic and zinc, will accumulate, reach high concentrations in the environment, and trigger effects in biological communities[30,116]—it is just a matter of time. Here we demonstrated the significance of tire abrasion substitues, in particular zinc, for eukaryotic microbial communities with a pronounced short-term functional imbalance and lasting effects on species composition. Tire abrasion strongly affects microeukaryotic communities, but predominantly due to the release of leachates, while the tire wear particles have a minor effect. Zinc has an immediate effect on susceptible cells (death, photosynthesis) and a medium-term effect on the community structure and functional diversity. However, the functional diversity is restored over time and general community diversity is not affected at any point. Still, long-term effects may occur causing unpredictable harm to protists especially if nanoparticles contain surfactants or release leachates.

It would be interesting to investigate how the microeukaryotic community behaves after stressor release, in particular after zinc exposure, and if the potential to shift back to the initial community profile is still present or if it is irreversible lost due to stressor exposures. In future studies methods such as RNA based analyses (metatranscriptomics), mass spectrometry (proteomics, metabolomics) and ecophysiological methods may provide more insights into the functional basis of the community response.

	Zn	ZnNP	NP	Si
Ascomycota	*Phaeosphaeria graminis*	*Leptosphaeria* sp. *Fuscohilum siciliana* *Phaeosphaeria graminis*		
Amoebozoa	*Vanella simplex* *Vexillifera bacillipedes* *Ripella* sp. GP2 *Acanthamoeba culbertsoni*	*Acanthamoeba* sp. *Ripella* sp. GP2 *Ripella* sp. DP13 *Acanthamoeba culbertsoni*		
Bacillariophyta	*Conticribra weissflogiopsis* *Sellaphora* sp. 108 *Sellaphora* cf. *seminulum* *Fistulifera saprophila*	*Conticribra weissflogiopsis* *Sellaphora* sp. 108 *Fistulifera saprophila* *Nitzschia dissipata*	*Cymbella tumida*	*Cyclotella meneghiniana* *Cyclotella meneghiniana*
Cerczoa	*Cercomonas* sp. *Rhogostoma* sp. CCAP 1966/4 *Rhogostoma* sp. CCAP 1966/4 *Cercomonas rapida*	*Cercomonas* sp. *Rhogostoma* sp. CCAP 1966/4 *Rhogostoma* sp. *Cercomonas rapida* *Paracercomonas sp. Panama101*		
Chlorophyta	*Chlamydomonas bilatus* *Chlamydomonas raudensis* *Chlamydomonas pumilio* var. *pumilio* *Micractinium singularis* *Micractinium* sp. SAG 48.93 *Micractinium* sp. CCAP 248/14	*Chlamydomonas bilatus* *Haematococcus lacustris* *Chlamydomonas raudensis* *Micractinium singularis* *Micractinium* sp. SAG 48.93 *Micractinium* sp. CCAP 248/14	*Mychonastes* sp. 6A3 *Mychonastes homosphaera* *Desmodesmus* sp. HH 10203 *Chlorella* sp. BUM11008	
Choanoflagellata	*Codosiga botrytis* *Salpingoeca fusiformis*	*Codosiga botrytis* *Salpingoeca fusiformis*		
Chrysophyta	*Poteriospumella* sp. *Pedospumella encystans* *Spumella* sp. TGS6 *Mallomonas* sp. *Synura* sp.	*Poteriospumella* sp. *Spumella* sp. TGS6 *Poterioochromonas malhamensis* *Mallomonas* sp. *Synura* sp.	*Paraphysomonas* sp. HD *Acrispumella msimbaziensis* *Spumella* sp. TGS6	
Chytridiomycota	*Rhizophlyctis rosea* *Zygophlyctis planktonica* *Rhizophydium patellarium*	*Zygophlyctis planktonica* *Rhizophlyctis rosea* *Rhizophydium patellarium*		
Ciliophora	*Paramecium putrinum* *Cyclidium vorax* *Cyclidium varibonneti* *Vorticella* sp. 4 PS-2013	*Paramecium tetraurelia* *Paramecium putrinum* *Cyclidium vorax* *Vorticella* sp. 4 PS-2013	*Vorticella campanula*	
Cryptophyta	*Cryptomonas pyrenoidifera* *Cryptomonas* sp. NIES-3916 *Cryptomonas* sp. Yeonra43011B *Teleaulax amphioxeia*	*Cryptomonas pyrenoidifera* *Cryptomonas* sp. NIES-3916 *Cryptomonas* sp. Dumo2 100310C	*Cryptomonas* sp. Dumo2 100310C *Cryptomonas* sp. Yeonra43011B *Cryptomonas sp. NIES-3916*	
Dinophyta			*Unruhdinium penardii*	
Euglenozoa	*Bodo saltans* *Neobodo designis* *Rhynchomonas nasuta* *Lepocinclis oxyuris*	*Bodo saltans* *Rhynchomonas nasuta* *Neobodo designis*	*Neobodo designis*	
Heterolobosea	*Naegleria clarki* *Naegleria* sp. KDN1 *Naegleria* sp. J14Z1	*Naegleria clarki* *Naegleria* sp. KDN1 *Naegleria* sp. J14Z1		

OTUs are only considered when the species/genera level is resolved properly with a percentage identity above 98.7%. Thus, sometimes less than 3 OTUs are present. OTUs with an identical species association can occur more than once as the NCBI database have multiple entries for individual species.

Table 1. Showing the top 3 increased (black) and top 3 decreased (red) differential abundant OTUs for individual groups and treatments.

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Author contributions
G.S. planned the experiment, performed the experiment, processed the samples, did the analyses and wrote the manuskript.D.B. has done bioinformatic work, analyses and reviewed the manuskript.J.L.R.. performed the experiment.M.S. contributed bioinformatic knowlegde.M.S. processed and measured the samples for metal measurements.B.S. planned the experiment and reviewed the manuskript.J.B. planned the experiment, wrote the manuskript and reviewed the manuskript.

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Article V:

„Temporal disturbance of a model stream ecosystem by high microbial diversity from treated wastewater“

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ORIGINAL ARTICLE

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Temporal disturbance of a model stream ecosystem by high microbial diversity from treated wastewater

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Abstract

Microbial communities in freshwater streams play an essential role in ecosystem functioning via biogeochemical cycling. Yet, the impacts of treated wastewater influx into stream ecosystems on microbial strain diversity remain mostly unexplored. Here, we coupled full-length 16S ribosomal RNA gene Nanopore sequencing and strain-resolved metagenomics to investigate the impact of treated wastewater on a mesocosm system (AquaFlow) run with restored river water. Over 10 days, community Bray–Curtis dissimilarities between treated and control mesocosm decreased (0.57 ± 0.058 to 0.26 ± 0.046) based on *ribosomal protein S3* gene clustering, finally converging to nearly identical communities. Similarly, strain-resolved metagenomics revealed a high diversity of bacteria and viruses after the introduction of treated wastewater; these microbes also decreased over time resulting in the same strain clusters in control and treatment at the end of the experiment. Specifically, 39.2% of viral strains detected in all samples were present after the introduction of treated wastewater only. Although bacteria present at low abundance in the treated wastewater introduced additional antibiotic resistance genes, signals of naturally occurring ARG-encoding organisms resembled the resistome at the endpoint. Our results suggest that the previously stressed freshwater stream and its microbial community are resilient to a substantial introduction of treated wastewater.

KEYWORDS
ARG, freshwater, mesocosm, metagenomics, microbiome, stressor

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1 | INTRODUCTION

Freshwater stream ecosystems are characterized by high biodiversity. Micro- and macroorganisms in these environments are responsible for essential ecosystem services with a major impact on biogeochemical cycling (Cardinale et al., 2012; Maavara et al., 2020; Ripl, 2003). These ecosystems have been exposed to environmental fluctuations on a daily, seasonal, and interannual timescale for centuries (Bucci et al., 2014; Burns et al., 1998; Hamid et al., 2020; Portillo et al., 2012). Over time, organisms have adapted to these natural disturbances (Crump et al., 2009; Lytle & Poff, 2004). Especially starting from the 20th century, additional anthropogenic stressors have been introduced to stream ecosystems such as water pollution, flow modification, habitat degradation, and climate change-derived effects (Beattie et al., 2020; Dudgeon et al., 2006; Grill et al., 2019; Reid et al., 2019). One major anthropogenic stressor in stream ecosystems, particularly in urban areas, is the inflow of treated wastewater from wastewater treatment plants. The percentage of treated wastewater in the water body of an urban stream can reach from up to 30% during normal conditions to over 50% during low-flow conditions, for example, during dry seasons (Drewes et al., 2018).

Although microbial activity has been considered important for ecosystem integrity (Cotner & Biddanda, 2002; Fasching et al., 2020), most studies have not considered microbial communities when studying the biome of streams (Gautam et al., 2022; Zeglin, 2015). Typically, experiments on stressor effects in streams have focused solely on higher organisms such as fish or invertebrates (Kim et al., 2020; Wright & Ryan, 2016), thus neglecting a major part of biodiversity, that is, prokaryotes and viruses. The latter are even considered to be a "blank spot on the map" in stream research (Peduzzi, 2016). Despite exceeding other microbes in diversity and particle number, information on viral interaction with organisms for example is missing so far (Bar-On et al., 2018; Whitman et al., 1998). Thus, how microbial and viral communities are influenced by treated wastewater are a major gap of knowledge, which is nevertheless of great importance as river water serves as a major source of potable water in densely populated regions (Strathmann et al., 2016).

The AquaFlow mesocosm setup has previously been used to study the effects of flooding events on microeukaryotic communities (Graupner et al., 2017). In this study, the AquaFlow system was used to simulate an inflow of treated wastewater to understand its effect on microbial communities in river water. We studied prokaryotic and viral communities in the water phase for 10 consecutive days and in 6 mesocosms in parallel (three control systems and three treatment systems). The reference water used in our study was sourced from the near-natural stream Boye, which had been used as an open sewer from the beginning of the last century until 2017. After a series of renaturation procedures, it has been fully restored since 2021 (Prati et al., 2022; Winking et al., 2014, 2016). In this study, we used a combination of full-length 16S ribosomal RNA (rRNA) genes sequenced via Oxford Nanopore Technology (ONT) and genome-resolved metagenomics on an Illumina NovaSeq. 6000 platform to conduct an in-depth analysis of the prokaryotic community, ranging from community changes over time to strain-resolved bacterial and viral analyses. Our results show that treated wastewater introduces a new microbial community to the existing stream ecosystem. This community differed not only at the bacterial phyla level but also strain level of the near-identical bacterial and viral metagenome-assembled genomes (MAGs) of cooccurring phyla. During an incubation period of 10 days in the AquaFlow systems, both treated and control experiments developed highly similar microbial communities after 10 days, suggesting the resilience of the river water community used herein regarding a pulse disturbance by treated wastewater.

2 | MATERIALS AND METHODS

2.1 | Study design and mesocosm setup

The AquaFlow mesocosm system was used to investigate the effects of treated wastewater on a natural stream ecosystem. The individual flow mesocosm systems are described in detail by Graupner et al. (2017). In short, one mesocosm system included three water tanks (~40, ~40, and ~270 L) connected by two steel channels (10 cm width and 4 and 2 m long) and a pump (10 L/min) to maintain a circular flow (shown as a scheme in Figure A1). The channels were filled with 60 L sediment taken from the river Boye (Germany; 51°33′19.7″ N and 6°56′38.3″ E, used as an open sewer until 2017 and fully restored in 2021) and homogenized in a concrete mixer before filling in. Per system, 350 L stream water from the same location as the sediment was prefiltered (200 µm pore size) and filled into the system. Throughout the experiment, the water temperature was kept at 19°C and natural sunlight was used as a light source. All six systems, three controls, and three treatments were run in parallel in February and March 2021.

Before the start of the experiment, an acclimatization phase of 14 days was performed, that is, running the systems as described and interconnecting the mesocosms each day until approximately 700 L were exchanged. Afterward, treated wastewater from the municipal wastewater treatment plant Schwerte (North Rhine-Westphalia, Germany) was filled into three systems to reach a percentage of 33% treated wastewater (~120 L) per mesocosm after removing the same volume of control water beforehand in treatment systems. The wastewater treatment plant (WWTP) operated as explained in Rothe et al. (2021) with the following measurements at the effluent on the day of sampling: turbidity 3.4 NTU; chemical oxygen demand 16 mg/L; total phosphorus 0.23 mg/L; temperature 9.1°C; pH 6.81; and conductivity 928 µS/cm.

Thus, three systems served as controls ("C") and three systems as treatments ("T"). Water samples were taken after 1 h, 12 h, 24 h, 2 days, 4 days, 7 days, and 10 days (samplings S1–S7). For each sample, 0.4 L water was filtered onside on 0.2 µm polycarbonate filters (Nucleopore; Cytiva) in duplicates, air-dried, submerged in 400 µl DNA/RNA shield (Zymo Research), frozen in liquid nitrogen, and stored at −80°C until DNA extraction. DNA extraction was done at room

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temperature using the Zymo Quick DNA/RNA microprep plus kit (Zymo Research). Two filters with DNA/RNA shield solution were transferred in a Zymo BashingBeads Lysis Tube and homogenized using a FastPrep (MP Biomedical) with five steps at 5.5 m/s for 30 s and a resting step on the ice for 1 min after each step. Bashing bead tubes were centrifuged for 30 s, the supernatant was transferred to a 1.5 mL tube, and mixed with 400 µL lysis buffer. After transferring the samples to a Zymo-Spin IC-XM column, steps were performed according to the protocol until the addition of 30 µL RNAse/DNAse free water, incubation for 5 min, and centrifugation to elute the DNA. The latter was stored at −20°C until further processing.

2.2 | DNA amplicon sequencing and processing

Full-length 16S rRNA sequencing was performed for all 42 samples (systems C1–C3 and T1–T3; samplings S1–S7 each) using the 16S Barcoding kit (SQK-RAB204; version RAB_9053_v1_rev-M_14Aug2019; Oxford Nanopore). The protocol was followed at each step if not mentioned otherwise using the LongAmp Taq 2× Master Mix (NEB) for PCR setup and Agentcourt AMPure XP beads (Beckman Coulter) for clean-up. Instead of 10 ng genomic DNA, 15 ng was used as an input for the PCR. Sequencing was done on a MinION Mk1B using an FLO-MIN106D flow cell, controlled by MinKNOW (v21.02.1). Per sample, at least 100,000 reads were sequenced. Raw sequencing signals were base-called and demultiplexed using guppy (v5.0.7; dna_r9.4.1_450bps_hac.cfg).

Basecalled and demultiplexed 16S rRNA gene sequences were processed using the NanoCLUST pipeline (v1.0dev; UMAP settings --cluster_sel_epsilon 0.5 –umap_set_size 100000) (Rodríguez-Pérez et al., 2021) based on the NCBI 16S rRNA database (v28.04.2021). Statistical analysis of operational taxonomic unit counts was performed using the R script MC_Stats (v1.2) as described in Weinmaier et al. (2015). Rarefaction and calculation of the Bray–Curtis dissimilarities were iterated 100 times and the average distance was calculated. Based on these dissimilarities principal coordinate analysis, nonmetric multidimensional scaling (NMDS), hierarchical clustering, rarefaction curve, diversity index, and multiple response permutation procedure (MRPP) were calculated using the R vegan package (Oksanen et al., 2013).

2.3 | Metagenomic DNA sequencing, assembly, and annotation

Samples from the first and last sampling points (S1 and S7) were sent for metagenomic sequencing to the West German Genome Center (Cologne, Germany). Sequencing was done according to the Illumina PCR-Free Protocol for Thermal Cycler, Low Input with a sequencing depth of 20 Gbp (Gigabase pair) (150 bp paired-end reads) on a NovaSeq. 6000 with an S4 FlowCell.

Metagenomic sequences were quality checked using BBduk (Bushnell; https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/) and Sickle (Joshi & Fass, 2011) (quality score ≥ 20 and minimum read length ≥ 20 bp). The microbial community coverage by short-read metagenomic sequences was estimated using Nonpareil3 (v3.4.1) (Rodriguez-R et al., 2018).

Metagenomic paired-end reads were individually assembled using a combined approach of metaviralSPAdes (Antipov et al., 2020) and metaSPAdes (Nurk et al., 2017) (SPAdes version 3.14.0). In short, reads were first assembled using metaviralSPAdes, generating scaffolds of viral origin. All metagenomic reads mapping (using Bowtie2; Langmead & Salzberg, 2012) to these viral scaffolds were excluded from the following assembly with metaSPAdes. Finally, both metaviralSPAdes and metaSPAdes assemblies were combined. Prediction of open reading frames was done for scaffolds equal or larger than 1 kbp using Prodigal (Hyatt et al., 2010) in meta mode and annotated using DIAMOND blast (Buchfink et al., 2015) (DIAMOND version 0.9.9; blastp -e 0.00001 -k 1) against an in-house database called FunTaxDB based on UniRef100 (state February 2021; Bornemann et al., 2023; Suzek et al., 2007). The taxonomy of each scaffold was assigned as described in Bornemann et al. (2022). In brief, the scaffold taxonomy was assigned based on all proteins detected on the scaffold and the lowest taxonomic rank when more than 50% of the protein taxonomies agree. To calculate the average scaffold coverage, quality-checked reads were mapped to scaffolds using Bowtie2 (Langmead & Salzberg, 2012). For all scaffolds, length, and GC content were calculated.

2.4 | Comparison of taxonomic classification based on amplicon sequencing and metagenomics

Three approaches were compared to retrieve abundances of prokaryotes in our samples, that is, 16S rRNA gene data from ONT sequencing (described above), blasting of metagenomic reads to 16S rRNA genes from ONT, and *rpS3* (ribosomal protein S3) gene analysis from metagenomic data. The *rpS3* gene is a single-copy gene containing both conserved and discriminatory regions and has been used for prokaryotic community analysis in metagenomic studies (Finstad et al., 2017; Smith & Wrighton, 2019; Zhong et al., 2022).

For blasting the quality-checked short metagenomic reads to the results from 16S rRNA gene sequencing, the consensus sequence per cluster from NanoCLUST was taken as a basis. Using USEARCH, the short reads were blasted against the reference sequences (*e* value: 0.00001 and -top_hits_only), and all hits with more than three mismatches were discarded. Then, the average number of hits per cluster was calculated and the NCBI TaxID was added based on the NanoCLUST output.

RpS3 genes were identified using a phylosift HMM (Hidden Markov Model) set (DNGNGWU00028; date: January 20, 2022) (Darling et al., 2014) in combination with hmmsearch (v3.2) at an *e* value cutoff of 1E−28. Additionally, annotation results using UniRef100 (described above) were searched for *rpS3* genes, and the respective taxonomy was attached. For statistical analyses of *rpS3* genes, representative genes were determined by clustering using USEARCH (-cluster_fast -id 0.99). If the centroid of the cluster could be extended by 1 kb in both directions the sequence was

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chosen as a representative for the cluster. Otherwise, a noncentroid longest sequence that could be extended or just the longest sequence was selected. Then, quality-passed reads were mapped to those representative *rpS3* gene sequences. To compare all three approaches, the TaxID and TaxString of the same NCBI database (date: January 2020) was used for taxonomic classification.

2.5 | Screening for antibiotic resistance genes (ARGs)

Detection of ARGs was done as described by Rahman et al. (2018). In brief, ARGs were annotated to predicted genes using the Resfams HMM database (Gibson et al., 2015) using hmmsearch (v3.3) with the *--cut_ga* flag. The latter was used to apply manually optimized profile-specific gathering thresholds for reporting and inclusion of results (Gibson et al., 2015). Based on the Resfam output and scaffold coverage, a sample resistance gene summary was normalized to the number of sequenced reads. The Resfam metadata was used to translate the Resfam IDs to resistance mechanisms. Taxonomic annotation of scaffolds encoding for ARGs based on FunTaxDB (described above) was extracted to infer the underlying community harboring the resistome. For analysis of ARGs in MAGs, scaffolds encoding for resistances were matched to binned scaffolds, and hits of respective resistance mechanisms per MAGs were calculated.

2.6 | Virus detection and analysis

Viral scaffolds were identified using VirSorter2 (v2.2.3; --high-confidence-only; Guo et al., 2021), deepvirfinder (v1.0.0; -l 1000) (Ren et al., 2020), and VIBRANT (v1.2.1) (Kieft et al., 2020). All hits were collected and checked using checkV (v0.7.0) (Nayfach et al., 2021). Only those viral scaffolds with a completeness higher than 25% were used for further analyses. All steps from raw reads until the detection of viral sequences were run in a Snakemake pipeline (Mölder et al., 2021).

2.7 | Binning of assembled metagenomes and analysis of MAGs

Scaffolds equal or larger than 1 kb were used to generate MAGs in all 12 metagenomic samples. In short, ABAWACA (Brown et al., 2016), MaxBin2 (Wu et al., 2016), and MetaBAT2 (Kang et al., 2019) were run on all samples and an optimized set of bins was produced using DASTool (Sieber et al., 2018). For MaxBin2, both marker sets were used and a cross-mapping over all samples using Bowtie2 was performed to use the differential coverage information as an input. The listed tools were wrapped in a Snakemake pipeline and run under Snakemake version 6.13.1 (Mölder et al., 2021).

All initial bins were manually curated using uBin (v0.9.14) (Bornemann et al., 2023) based on GC, coverage, taxonomy, and single-copy core genes. After curation, all remaining non-prophage viral sequences identified by checkV were removed from the MAGs as they likely represented contaminations. The resulting viral clean MAGs from all samples were dereplicated using dRep (v3.0.0) (Olm et al., 2017) using default settings and quality checked. In short, quality metrics were calculated using checkM (Parks et al., 2015) and Quast (Gurevich et al., 2013), transfer RNA (tRNA) genes were detected using tRNAscan-SE (v2.0.7) (Chan et al., 2021), and rRNA genes were identified using RNAmmer (v1.2) (Lagesen et al., 2007). All results were combined and the quality of the MAG was assessed according to MIMAG standards by Bowers et al. (2017).

A standardized taxonomy assignment was done using GTDB-Tk (v2.0.0, database release R207) (Chaumeil et al., 2020). To retrieve the taxonomy, the "classify_wf" pipeline was run, and an unrooted tree was created using the "infer" workflow.

2.8 | Strain analysis of MAGs and viral genomes

Quality-checked reads of all samples were mapped to the set of dereplicated MAGs using Bowtie2 and genes of MAGs were predicted using prodigal in normal mode. The inStrain (v1.5.5) (Olm et al., 2021) "profile" was run for each sample using the same set of dereplicated genomes followed by "inStrain compare" in database mode.

Viral genomes equal or larger than 3 kb and with minimal completeness of 25% were clustered using VIRIDIC (Moraru et al., 2020) with default parameters. From the resulting clusters, the centroid sequence was determined using USEARCH (v10.0.240) at an identity of 0.99. If more than one sequence remained per cluster, the longest sequence was selected for downstream analysis. These representative virus genomes were prepared as described before to run inStrain "profile" and "compare" over all samples.

2.9 | Data visualization and significance testing

Final data polishing and visualization were done in R (R Core Team, 2022) using the packages ggplot2 (Wickham, 2016), tidyverse (Wickham et al., 2019), data.table (Dowle & Srinivasan, 2021), ggtree (Yu, 2020), ape (Paradis & Schliep, 2019), ggtreeExtra (Xu et al., 2021), nonpareil (Rodriguez-R et al., 2018), and UpSetR (Gehlenborg, 2019). Paired *t* tests were performed to test for significant differences between 16S rRNA and *rpS3* gene analyses and AMR hits using the R package stats (R Core Team, 2022).

3 | RESULTS

3.1 | Wastewater-induced shift in microbial community as revealed by full-length 16S rRNA and rpS3 gene analysis

To determine the effect of wastewater on the microbial communities, we investigated 42 samples, that is, three controls and three treated

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samples each across seven time points, via 16S rRNA Nanopore sequencing. NMDS (Figure 1) revealed a change in the bacterial community with time along NMDS axis 1 and a separation of control and treatment along NDMS axis 2, indicating that time has a greater effect on the community than the addition of wastewater. This was confirmed via MRPP comparing the two sets of samples with a chance corrected within-group agreement of 0.0675 for the addition of wastewater and 0.3775 for time, respectively ($p = 0.001$ for both tests).

Based on these results the first and last sampling time points (12 samples in total, three for each time point with two time points for treatment and control, respectively) were selected for an in-depth comparison via metagenomics. When comparing the 16S rRNA gene analysis with the *rpS3* gene analysis of these nine samples, we observed a similar pattern in ordination analyses (Figure A2).

Although beta-diversity analyses revealed a similar pattern when using 16S rRNA and *rpS3* gene, 16S rRNA Nanopore sequencing results were further compared to the diversity detected via metagenomics using two independent approaches. First, we used USEARCH to assign short reads to the 16S rRNA genes from Nanopore sequencing to determine their relative abundance in the metagenomes. This approach enabled us to elucidate the amplification bias during 16S rRNA gene PCR. Yet, the bias in 16S rRNA gene analysis resulting from the possibility of multiple copies of the gene per genome is not reduced. Therefore, we directly used the relative abundances of *rpS3* genes, present as single copies in prokaryotic genomes, in assembled metagenomes and compared them to 16S rRNA gene data at the taxonomic level to determine the effect of primer bias combined with amplification bias in the analyses (Figure 2). Proteobacteria was the dominant phylum in relative abundance ranging from 59.04% based on *rpS3* genes analysis, to an average of 86.9% in abundance-corrected 16S rRNA gene analysis up to 98.20% when using 16S rRNA genes from Nanopore only. Correction of relative abundance of Proteobacteria in 16S rRNA gene analysis using short reads revealed a lower abundance compared to Nanopore sequencing ($p < 0.0001$; paired t test), indicating an amplification bias favoring Proteobacteria. For all samples except C1_S1 and C3_S1, the detected relative abundance of Proteobacteria was lowest for *rpS3* gene analysis, highlighting a combinatorial effect of primer bias and PCR bias ($p < 0.01$ compared to 16S rRNA gene and $p < 0.05$ compared to abundance-corrected 16S rRNA gene; both paired t test). Particularly the primer bias in 16S rRNA gene analysis was supported by the fact that *rpS3*-based diversity analysis retrieved 44.4% more phyla (relative abundance ≥ 0.1%) on average over all 12 samples compared to 16S rRNA gene diversity, especially in the case of samples taken at the start of the experiment (56.3%).

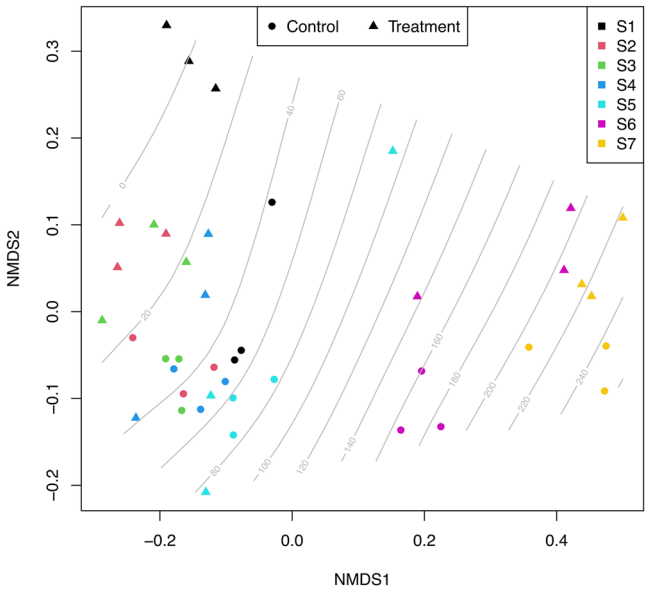

FIGURE 1 Nonmetric multidimensional scaling (NMDS) of full-length 16S ribosomal RNA (rRNA) gene sequencing with curve fitting on incubation time in hours revealing a separation dependent on time and type. Sampling points refer to the time after the introduction of wastewater (S1–S7: 1 h, 12 h, 24 h, 2 days, 4 days, 7 days, and 10 days).

3.2 | Control and treatment develop the same strain clusters over time

Genome-resolved metagenomics was used to investigate the community structure on a deeper taxonomic level. Overall samples, 376 manually curated MAGs were recovered and were dereplicated resulting in 100 reference MAGs (completeness ≥ 75%, contamination ≤ 25%) (Supporting Information: 2). Sixty-six MAGs fulfilled the MIMAG criteria (Bowers et al., 2017) to be at least medium quality MAGs (e.g., selecting MAGs with contamination ≤ 10%) and were present in at least two samples according to inStrain and thus comparable at the strain level.

Similar to marker gene analyses (see above), Proteobacteria represented the highest occurring phylum also among MAGs (Figure 3). Of the 66 MAGs in total, 28 were only detected in the initial sampling and 11 only in endpoint sampling (labeled with empty and filled triangles, respectively). Seventeen MAGs were exclusively detected in treatment samples and seven only in control samples. The number of strain clusters per MAG reduced from 1.8 ± 0.90 ($n = 40$ MAG clusters) to 1.29 ± 0.63 ($n = 34$) for control and from 2.0 ± 0.91 ($n = 49$) to 1.27 ± 0.52 ($n = 33$) for treatment from S1 to S7. MAGs belonging to lower abundant classes (Figure 3) were mostly found in samples treated with wastewater, for example, Acidimicrobiia, Nitrospiria, or Bacteroidia.

This strain analysis of bacteria was also performed on quasi-species of viruses detected in the metagenomes. Overall, 2336 representative viral scaffolds were identified, of which 1713 were present in at least two samples and used for further analyses. 128 viral scaffolds were detected in control samples only, whereas 1164 viral scaffolds were exclusively detected in samples treated with wastewater. InStrain detected 2434 subclusters of viral scaffolds, that is, viral strains, across different samples (Figure 4).

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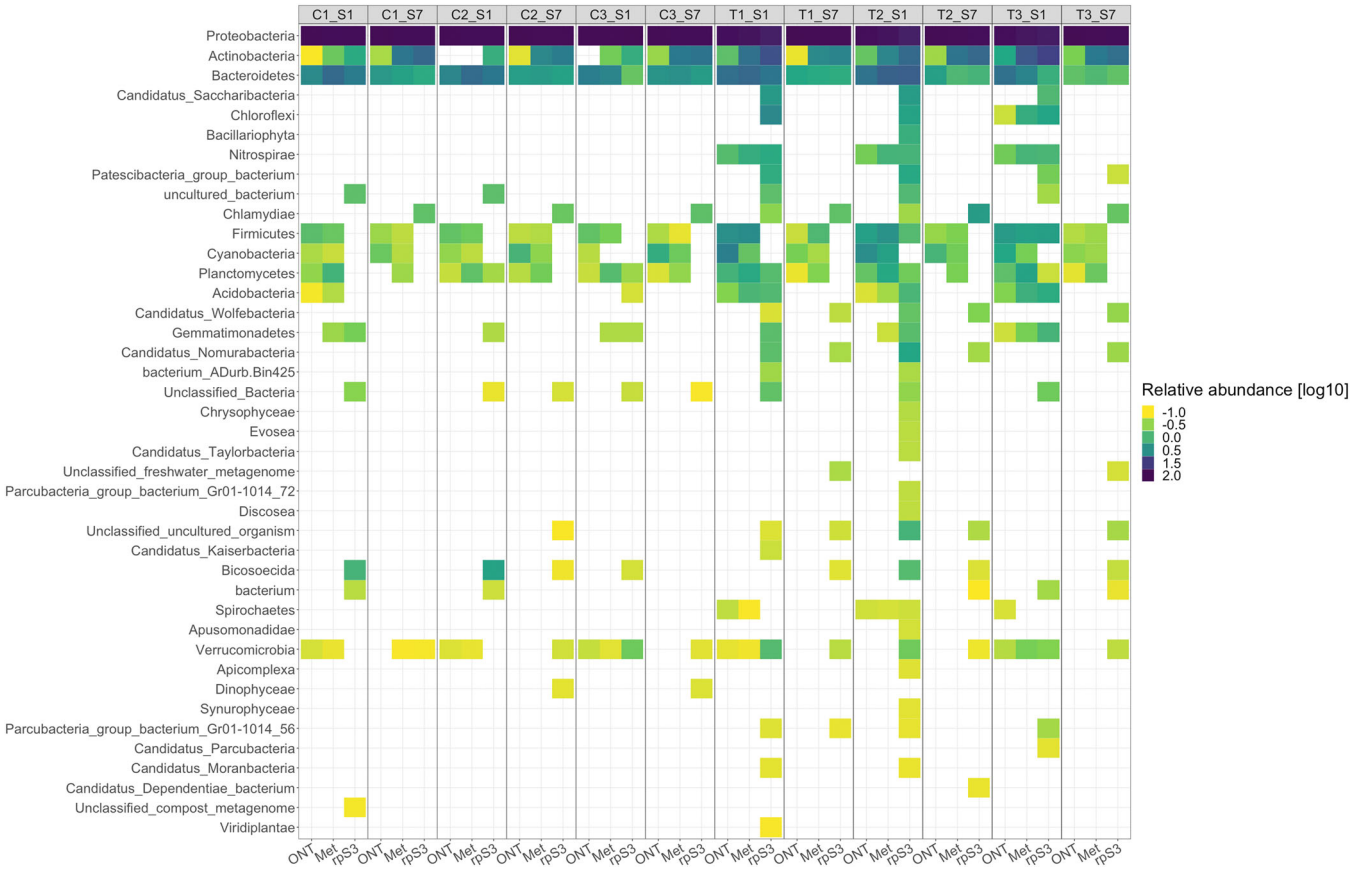

FIGURE 2 Phylum level relative abundance (≥0.1% abundance) of start and end point samples ("S1" and "S7") of control ("C") and treatment ("T") systems based on three methods, that is, whole-length 16S rRNA gene sequencing ("ONT"), blasting of metagenomic reads against reference sequences obtained by whole-length 16S rRNA gene sequencing ("Met"), and rpS3 gene analysis based on metagenomics ("rpS3").

In treatment samples, 1767 (72.6%) viral strains were present either at the start or end point or detected in both with the highest percentage at the start (955; 39.2%). In contrast to treatment samples, viral strains present in control samples only made up 9.7%, highlighting a high diversity of viral strains introduced via treated wastewater.

3.3 | Stress response increase in control and treatment samples

Assembled metagenomes were scanned for antimicrobial resistance genes using the Resfams HMM database (Gibson et al., 2015), resulting in ATP-binding cassette transporter (ABC transporter), acetyltransferase, major facilitator superfamily transporter (MFS Transporter), and β-lactamases with the highest counts per million reads (Supporting Information: 1). Sums of counts were significantly higher for endpoint samples compared to the start of the experiment ($p = 2.13 \times 10^{-14}$; Figure 5a). The taxonomic profiles of scaffolds carrying these resistance genes were determined using the uBin package (Bornemann et al., 2023), and their distribution is shown in Figure 5b. In wastewater-treated samples, 57.98%–61.74% of resistance gene hits were assigned to Proteobacteria at the initial sampling, in contrast to 90.20%–94.92% in control samples. The share of less abundant phyla encoding for resistance genes is reduced from S1 to S7 in treatment samples, resulting in the same distribution compared to the control samples. This is also supported by the ARG analysis in MAGs (Figure 3). Genomes encoding for resistances were introduced by treated wastewater contributing to a more diverse resistome at the beginning, for example, MAG AF21_PE0_T1_S1_Chloroflexi_54_10. At sampling point S7 however, mainly MAGs belonging to the Proteobacteria remain in both control and treatment, forming the resistome.

4 | DISCUSSION

Stream ecosystems harbor a rich biodiversity, shape and connect ecosystems, and serve humankind in many ways (Dudgeon et al., 2006; Honey-Rosés et al., 2013). Such ecosystem services are dependent on healthy microbial communities, which can be stressed by natural and anthropogenic influences (Lin et al., 2019). Effluents of WWTPs represent a ubiquitous stressor for freshwater streams, a general source of drinking water production. While the microbial community of WWTP effluents has already been investigated directly

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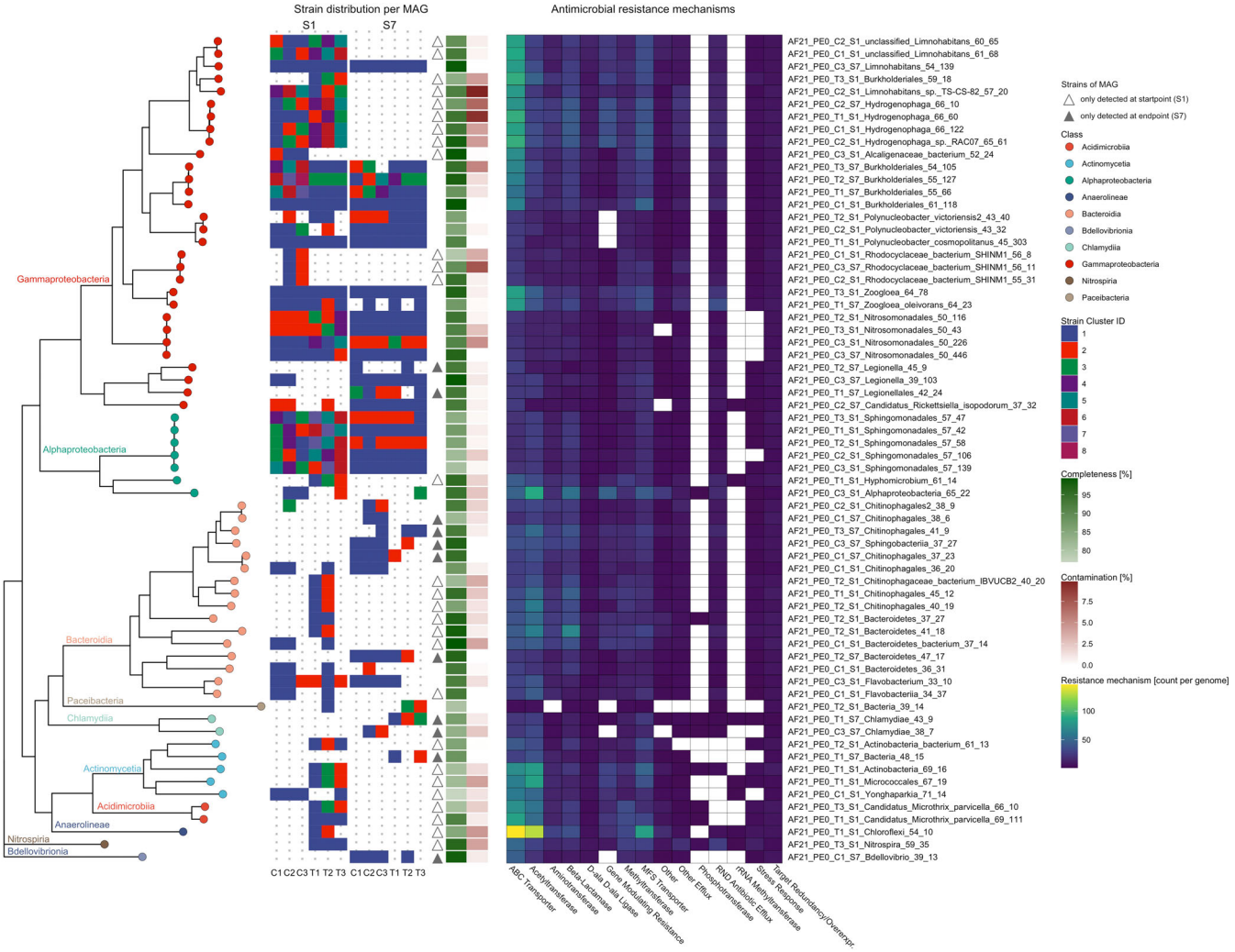

FIGURE 3 Distribution of metagenome-assembled genome (MAG) strain clusters in metagenomic samples faceted by start and endpoint sampling. Strain cluster IDs are referring to the respective MAG only. For example, if the same strain of a certain MAG is present in all samples, they are shown as strain cluster one and if a sample contains another strain of the same MAG, this sample is color coded as strain cluster two, and so on. A genome is considered present if at least 50% of the genome is covered (breadth = 0.5). Only MAGs fulfilling MIMAG requirements for medium-quality MAGs present in at least two samples are considered (Bowers et al., 2017). MAGs that were only detected at the start and endpoint sampling were labeled with empty and filled triangles, respectively. Antimicrobial resistance mechanisms were assigned to the MAGs based on Resfams HMMs.

at the outlet (Newton et al., 2022) as well as in an upstream/downstream approach (Chaudhary et al., 2018), the temporal development of the microbial community for a longer period after addition of wastewater has not been investigated so far. Surveying such a disturbance in real stream systems is challenging due to the heterogeneous nature of fluvial ecosystems, for example, different flow conditions, influence from the shore (e.g., leaf litter) or changing weather conditions (Bastias et al., 2020; Beisel et al., 2000). Mesocosm systems like the AquaFlow system, however, enable such investigations with the trade-off of studying a closed system (Graupner et al., 2017; Röhl et al., 2018). In this study, we replaced one-third of the water in three AquaFlow systems with treated wastewater from a municipal WWTP and investigated the microbiome for 10 days using 16S rRNA gene and shotgun sequencing. The control system was only filled with water from the stream Boye, a previous open sewer that has been wastewater free since 2017 and restored since 2021.

As expected, the introduction of treated wastewater resulted in a significant disturbance of the microbial community as revealed by both marker genes used in this study, with commonly known phyla introduced by treated wastewater (Newton et al., 2022). Yet, only *rpS3* gene analysis based on metagenomic sequencing was able to detect also lower abundant phyla, which we attribute to primer biases in amplicon analyses. Treated wastewater introduced not only new phyla but also new strains of MAGs and viruses, highlighting its impact on the microbial community composition. As presented, counts of both bacterial and viral clusters introduced by treated wastewater, but also those which were already present in the

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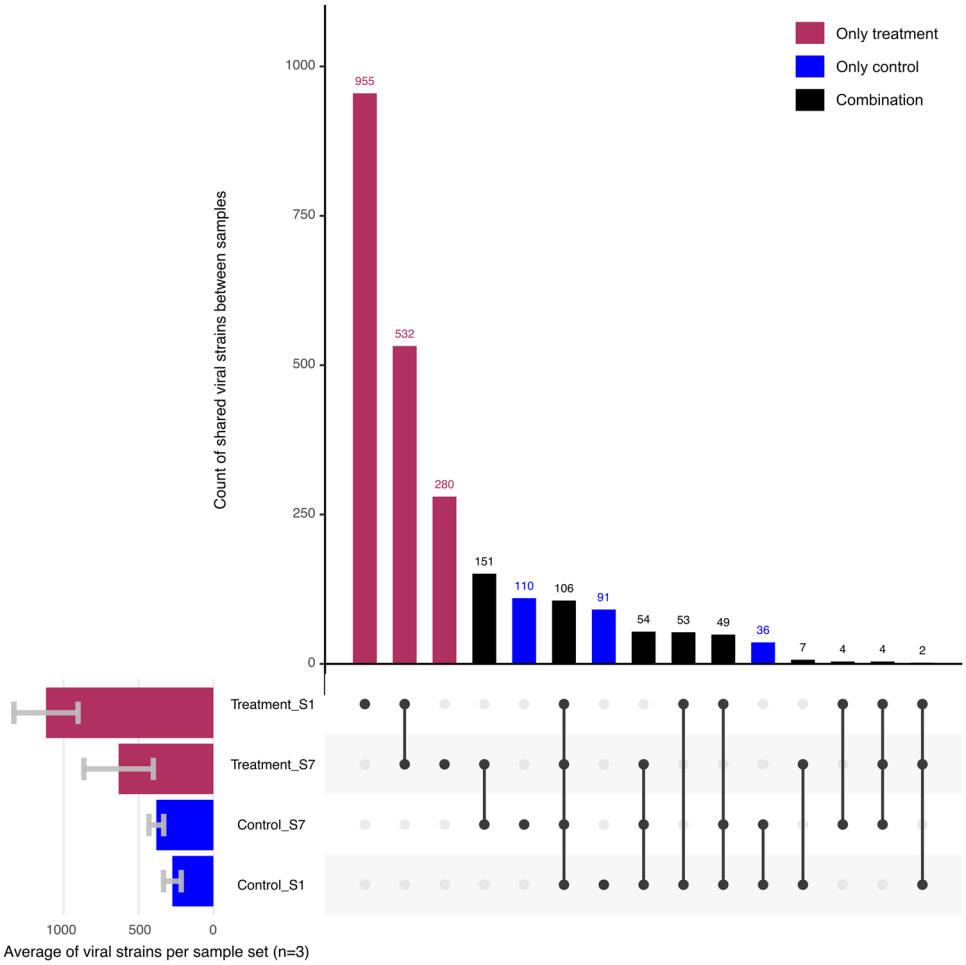

FIGURE 4 Counts of viral subclusters, that is, viral strains, present in treatment and control at start and endpoint sampling as detected by inStrain (module compare). Connected samples indicate the count of shared viral strains of respective samples.

near-natural stream, were reduced over the incubation period of ten days in the mesocosm system. This is also reflected by the decrease of Nonpareil diversity (Figure A3) from start to endpoint for all systems and the increase of estimated sequencing coverage at comparable sequencing depth (22.4 ± 3.5 Gbp), suggesting that the setup in combination with the factor time itself serves as a driver for the microbial community.

A major public concern about WWTP effluents released into streams is the introduction of antibiotic-resistant bacteria and ARGs along with their propagation in natural ecosystems (Araújo et al., 2010; Ferreira da Silva et al., 2007; Sekizuka et al., 2022). Our results showed that the added treated wastewater introduced new phyla encoding for stress response systems including antimicrobial resistances. The total count of stress response genes did not increase compared to control systems, suggesting that the natural resistome was only changed to a more diverse resistome originating from the treated wastewater. After 10 days of incubation, the near-natural stream microbiome was solely responsible for the composition of the resistome resulting in an increase of stress response genes. One explanation could be that the control stream water originated from a previously stressed ecosystem that was under influence of wastewater for centuries and only freed from wastewater 4 years before sampling and restored in the year of sampling. Thus, the restored stream microbiome should still be adapted to the environmental change that the mesocosm setup represents. However, the increase of stress response systems over time along with the factor time as the major driver of the microbial community in 16S rRNA gene analyses, suggests that the community in the AquaFlow system is under constant development. Nevertheless, the effect of stressors like wastewater introduction could be elucidated and the introduction of strains of bacteria and viruses and stress response systems could be detected.

Our results suggest a limited temporal disturbance of microbial communities by wastewater bacteria, viruses, and ARGs in restored ecosystems. Yet, the persistence of biological entities was short, arguably because fecal organisms, for example, have a limited survival time in open water columns (Brooks et al., 2015). It has also been shown previously that highly polluted urban rivers might have a higher resilience to such a pulse disturbance than more natural rivers (García-Armisen et al., 2014). Our results suggest that despite comprehensive measures the river Boye is likely still in a stressed state and the microbial community is resilient to pulse disturbances

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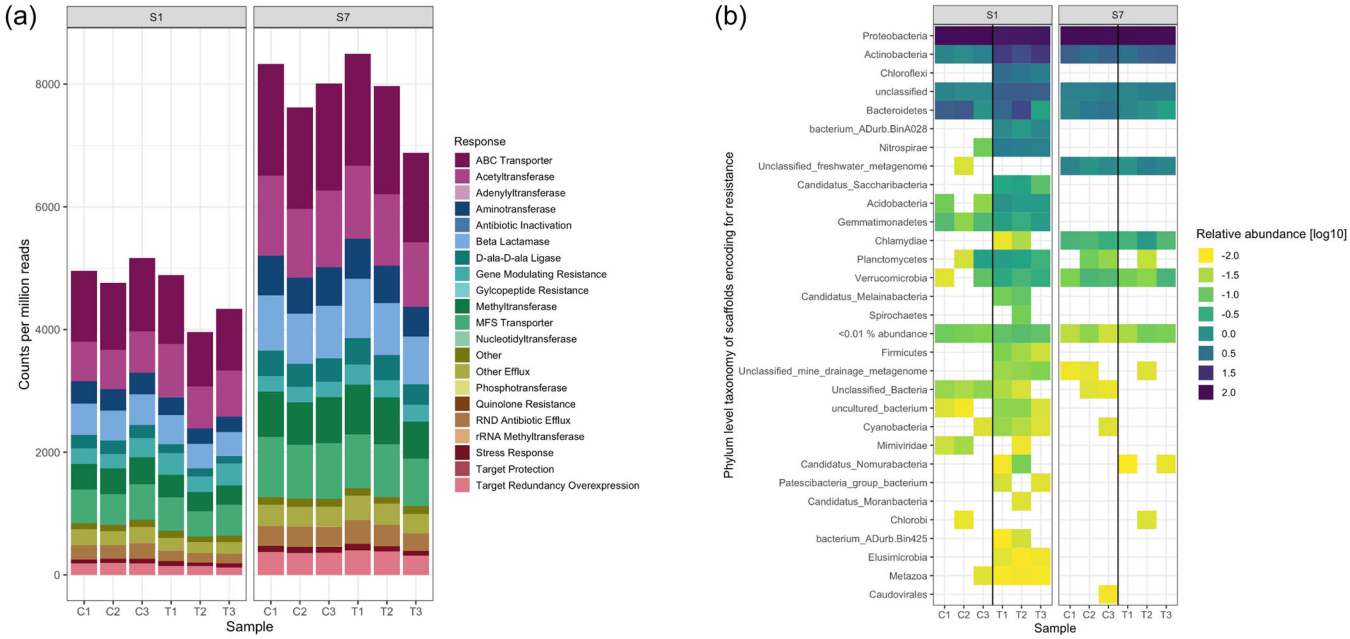

FIGURE 5 Stress response encoded on metagenomic scaffolds. Total counts (a) were normalized using sequencing depth and scaffold coverage. Scaffolds encoding for stress response were extracted and a phylum-level taxonomy was annotated (b). A relative abundance of 0.5 would reflect that 50% of counts were detected on scaffolds assigned to that phylum.

of treated wastewater. Future studies including a pristine freshwater stream should be able to verify this hypothesis. From a public health perspective, the return to the equilibrium state of the control counters concerns about the impacts of WWTP effluents, however, it is unclear if the same holds true for rivers not previously stressed. In addition, the duration of the temporary disturbance might impact the microbiome resilience.

5 | CONCLUSIONS

In conclusion, investigation of long-term effects caused by anthropogenic disturbances in stream systems is currently only possible using mesocosm systems like the presented AquaFlow setup. Here, we show that the introduction of treated wastewater resulted in a drastic temporal disturbance of a restored freshwater stream microbiome by introducing bacterial phyla, viruses, and a more diverse resistome. Yet, the introduction showed no lasting effect on the microbial community, and both control and treatment developed to a similar endpoint community. This suggests that the microbiome of a previously stressed river might be resilient to a drastic, but temporary disturbance.

AUTHOR CONTRIBUTIONS

Tom L. Stach: Data curation (lead); formal analysis (lead); investigation (equal); visualization (lead); writing—original draft (lead); writing—review and editing (equal). **Guido Sieber**: Conceptualization (equal); methodology (equal); writing—review and editing (supporting). **Manan Shah**: Conceptualization (equal); methodology (equal); writing—review and editing (supporting). **Sophie A. Simon**: Methodology (equal); writing—review and editing (supporting). **André Soares**: Methodology (supporting); writing—review and editing (supporting). **Till L. V. Bornemann**: Methodology (supporting); writing—review and editing (supporting). **Julia Plewka**: Methodology (equal); writing—review and editing (supporting). **Julian Künkel**: Investigation (supporting); writing—review and editing (supporting). **Christian Becker**: Resources (supporting). **Folker Meyer**: Writing—review and editing (supporting). **Jens Boenigk**: Conceptualization (equal); funding acquisition (lead); resources (equal); supervision (equal); writing—review and editing (supporting). **Alexander J. Probst**: Conceptualization (lead); funding acquisition (lead); resources (equal); supervision (equal); writing— original draft (lead); writing—review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

None declared.

DATA AVAILABILITY STATEMENT

Raw sequencing data and MAGs have been deposited at SRA and GenBank, respectively, and are accessible under the BioProject PRJNA874517: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA874517.

ETHICS STATEMENT

None required.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX

Scheme of an AquaFlow mesocosm system

Figure A1

Statistical analyses of start and end point samples based on rpS3 gene clustering

Figure A2

Coverage of shotgun metagenomic sequencing diversity

The diversity of start- and endpoint samples estimated by Nonpareil3 (Rodriguez-R et al., 2018) ranged for initial treatment samples from Nonpareil diversity (Nd) 21.39 to 21.56 ($n = 3$) and for initial control samples from Nd 20.10 to 20.88 ($n = 3$). All endpoint samples ranged from Nd 17.89 to 18.82 ($n = 6$). The sequencing coverage was significantly higher for endpoint samples based on the redundancy estimation from Nonpareil ($n = 12$, t test $p = 3.008 \times 10^{-8}$) with an average of 0.69 ± 0.019 for start-point and 0.86 ± 0.017 for end-point samples. Corresponding Nonpareil curves are shown in Figure A3.

Rarefaction curve and diversity of 16S rRNA gene analysis

Figure A4

Relative abundances of antimicrobial resistance mechanism

Figure A5

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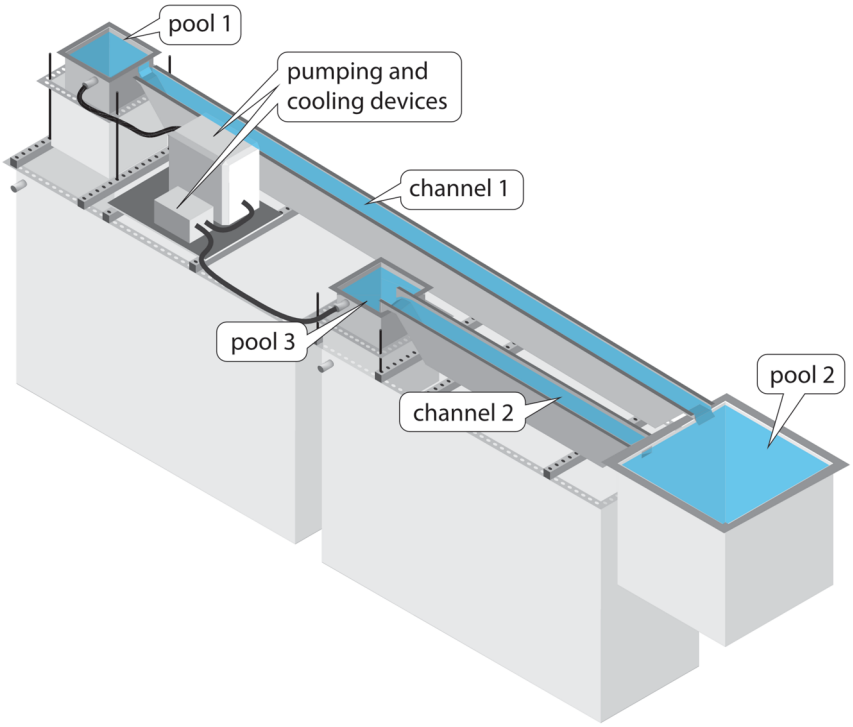

FIGURE A1 Schematic representation of the AquaFlow mesocosm setup as used in the presented study. One mesocosm system included three water tanks (~40, ~40, and ~270 L) connected by two steel channels (10 cm width and 4 and 2 m long) and a pump (10 L/min) to maintain a circular flow.

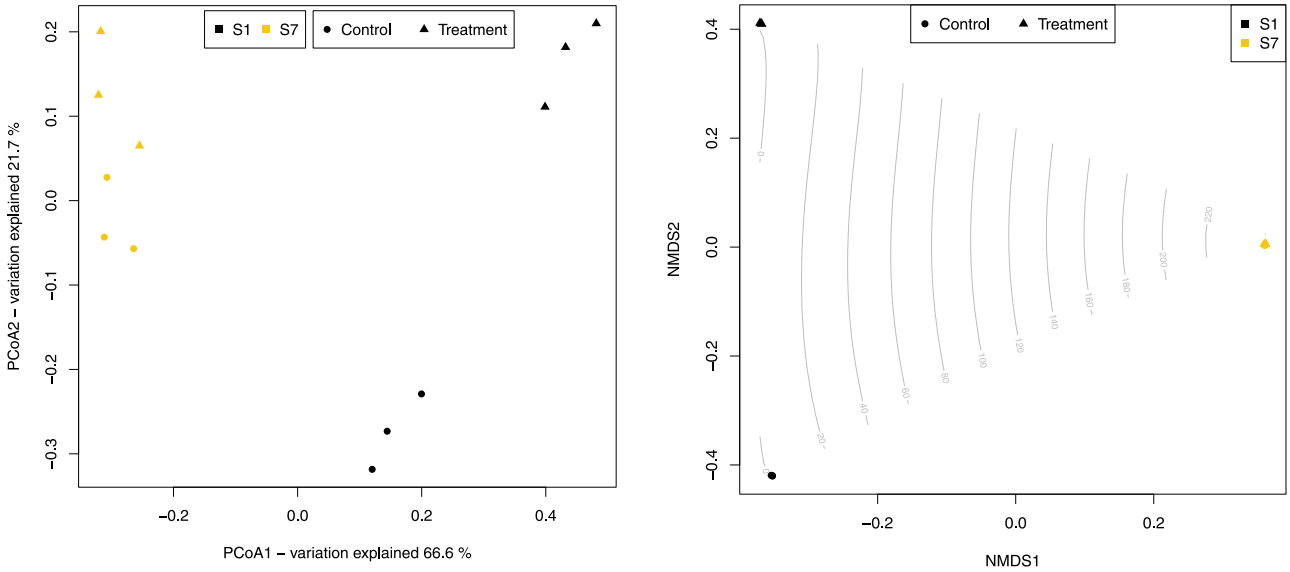

FIGURE A2 Principal coordinate analysis (PCoA) (left) and nonmetric multidimensional scaling (NMDS) (right) based on clustering of *rpS3* genes detected on metagenomic scaffolds. Samples are separated by treatment and time.

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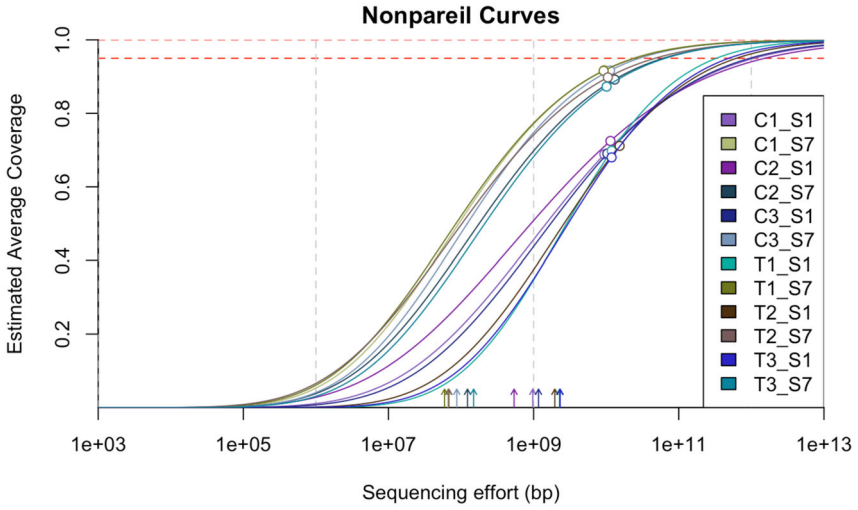

FIGURE A3 Nonpareil curves for metagenomic samples. Empty circles indicate the actual sequencing effort and arrows indicate the community diversity. Horizontal red dashed lines represent 95% and 99% coverage. All samples investigated had more than 60% coverage in diversity.

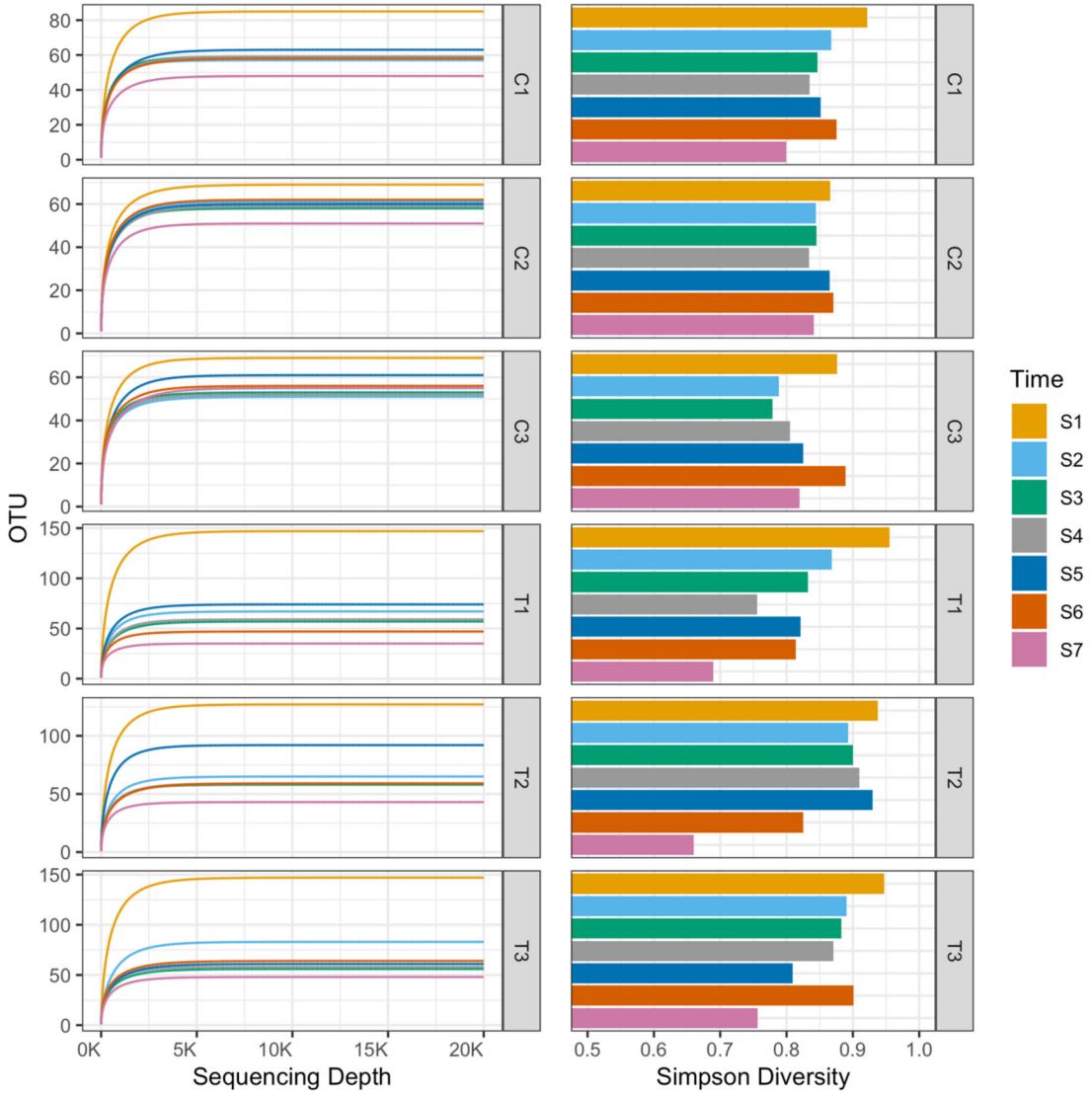

FIGURE A4 Rarefaction curve (left) and Simpson diversity (right) based on 16S ribosomal RNA (rRNA) gene analysis. Especially for treatment samples, diversity was highest at the start and reduced towards the end of the experiment. OTU, operational taxonomic unit.

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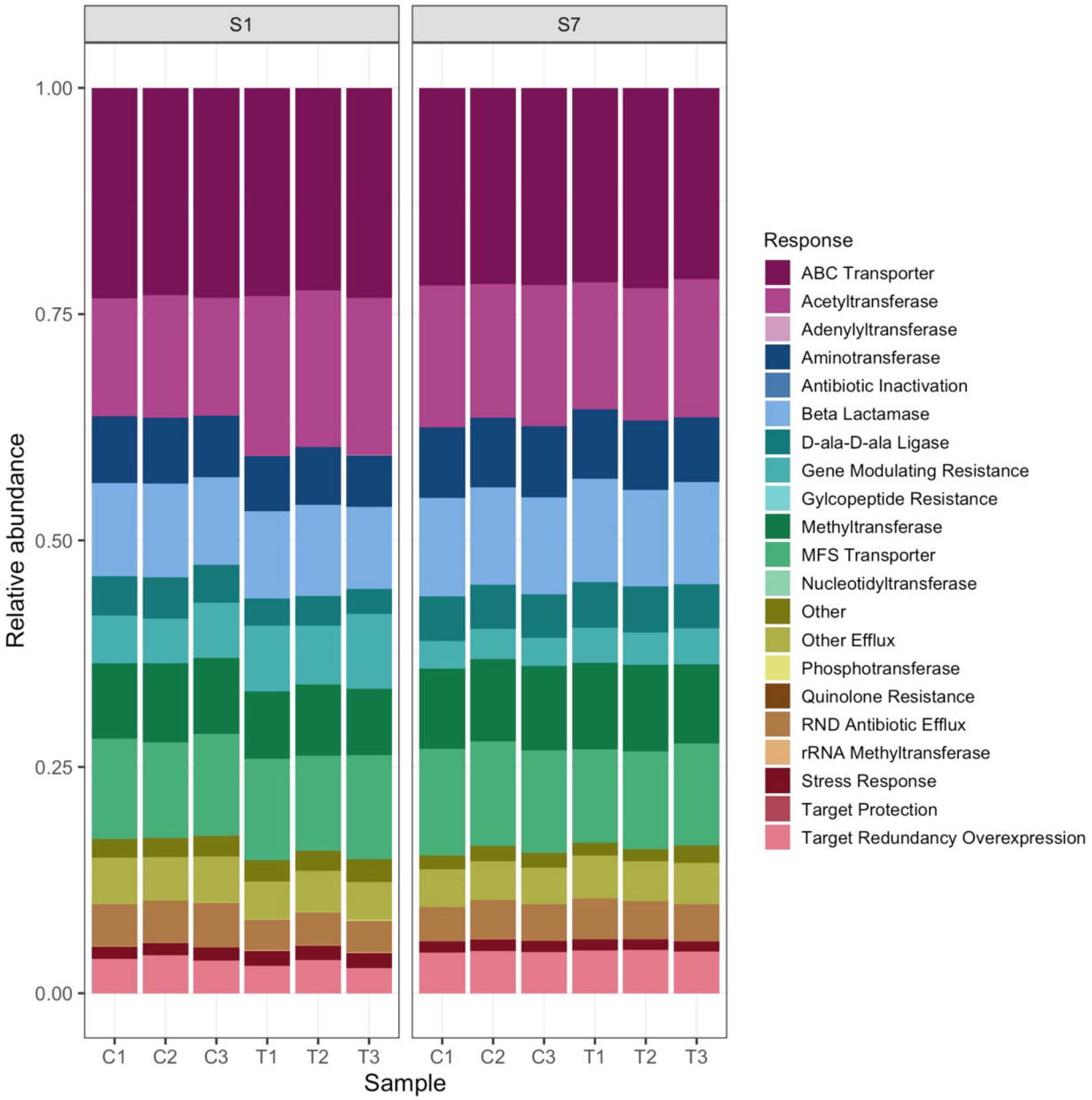

FIGURE A5 Stress response encoded on metagenomic scaffolds with relative abundances per mechanism and sample. ABC, ATP-binding cassette; MFS, major facilitator superfamily; rRNA, ribosomal RNA; RND, resistance nodulation division.

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Article VI:

„Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater“

Guido Sieber, Felix Drees, Manan Shah, Tom L. Stach, Lotta Hohrenk-Danzouma, Christina Bock, Maryam Vosough, Mark Schumann, Bernd Sures, Alexander Probst, Torsten C. Schmidt, Daniela Beisser & Jens Boenigk

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Contribution to this publication:

Conception & planning	70%	Conception and planning of study
Experiments & methods	40%	Wet and dry lab work
Analyses & interpretation	70%	Analyses and interpretation of data
Manuscript & tables/figures	75%	Writing and review of the manuscript and creation of tables and figures

Signature doctoral candidate

Signature of scientific supervisor

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Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater

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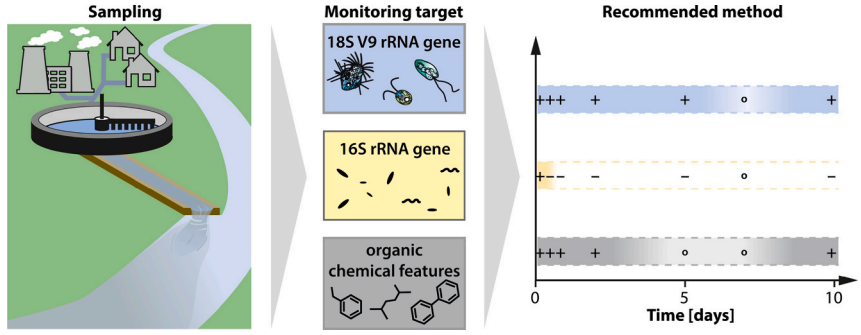
HIGHLIGHTS

- Treated wastewater causes chemical and biological disturbances, which decrease over time.
- Non-Target screening and metabarcoding data covary
- Non-Target screening and metabarcoding are complementary for environmental monitoring.
- Non-Target screening and metabarcoding exhibit distinct detection sensitivities and capabilities for treated wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater treatment processes can eliminate many pollutants, yet remainder pollutants contain organic compounds and microorganisms released into ecosystems. These remainder pollutants have the potential to adversely impact downstream ecosystem processes, but their presence is currently not being monitored. This study was set out with the aim of investigating the effectiveness and sensitivity of non-target screening of chemical compounds, 18S V9 rRNA gene, and full-length 16S rRNA gene metabarcoding techniques for detecting treated wastewater in receiving waters. We aimed at assessing the impact of introducing 33 % treated wastewater into a triplicated large-scale mesocosm setup during a 10-day exposure period. Discharge of treated wastewater significantly altered the chemical signature as well as the microeukaryotic and prokaryotic diversity of the mesocosms. Non-target screening, 18S V9 rRNA gene, and full-length 16S rRNA gene metabarcoding detected these changes with significant covariation of the detected pattern between methods.

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The 18S V9 rRNA gene metabarcoding exhibited superior sensitivity immediately following the introduction of treated wastewater and remained one of the top-performing methods throughout the study. Full-length 16S rRNA gene metabarcoding demonstrated sensitivity only in the initial hour, but became insignificant thereafter. The non-target screening approach was effective throughout the experiment and in contrast to the metabarcoding methods the signal to noise ratio remained similar during the experiment resulting in an increasing relative strength of this method. Based on our findings, we conclude that all methods employed for monitoring environmental disturbances from various sources are suitable. The distinguishing factor of these methods is their ability to detect unknown pollutants and organisms, which sets them apart from previously utilized approaches and allows for a more comprehensive perspective. Given their diverse strengths, particularly in terms of temporal resolution, these methods are best suited as complementary approaches.

1. Introduction

Freshwater and associated ecosystems are a finite resource (Gleick and Cooley, 2021; Gleick and Palaniappan, 2010), essential for ecological and economic sustainability, and therefore, closely linked to human concerns. Freshwater ecosystems and water resources encompass various elements such as rivers, lakes, groundwater, and even wastewater, which represents a wide spectrum of water utilization, including runoff from industrial, household, road, hospital, and agricultural sources. The natural generation of freshwater hardly meets human demands (Gleick and Cooley, 2021; Jiang, 2009). Thus, to prevent water shortages, it is essential to reuse wastewater. However, reuse is accompanied by obstacles, as treated and untreated wastewater can contain thousands of potentially harmful compounds such as pharmaceuticals, plant protection agents, and chemicals as well as pathogens (Adegoke et al., 2018; Bonetta et al., 2022; Freudenthal et al., 2022; Pohl et al., 2021; Rogowska et al., 2020; Schaider et al., 2017; Schollée et al., 2015; Verkh et al., 2018). On the one hand, these compounds are challenging to neutralize, and on the other hand, they can lead to human infections with pathogens and, e.g., increased microbial resistance to antibiotics (Adegoke et al., 2018; Gatica and Cytryn, 2013; Petterson et al., 2002; Sorinolu et al., 2021).

It is crucial to emphasize the significance of monitoring treated wastewater (TWW) since it can contribute to a considerable proportion of up to 77 % in rivers (Guillet et al., 2019). Thus, the dilution of released compounds in receiving rivers is often low. Admittedly, it is unfeasible to monitor all microorganisms and chemicals before releasing treated wastewater. Many organisms and substances and in particular potential associated risks and biologically harmful concentrations are unknown (Garnaga, 2012; Rogowska et al., 2020). In particular, monitoring of microeukaryotes, prokaryotes, viruses, and chemicals is not obligatory, unless a special order is issued depending on the sources of wastewater input. With such a special order, mostly coliform prokaryotes are monitored (e.g., *Escherichia coli*), as they indicate risks to human health (Bonetta et al., 2022; Edberg et al., 2000; Glassmeyer et al., 2005; Osuolale and Okoh, 2017). Other potentially harmful microorganisms and micro-pollutants are usually not monitored (Adegoke et al., 2018). In many countries only the monitoring of bulk parameters such as total organic carbon, nitrogen-bound, chemical and biochemical oxygen demand, metals like lead and copper, and of individual chemicals such as cyanide and chlorate, is mandatory before releasing water into receiving rivers (AbwV, 2004; BGBl 186, 1996).

Treated wastewater has inevitable different characteristics than receiving waters because it contains deviating microorganisms (prokaryotes, fungi, microeukaryotes), chemical substances, and their transformation products, and abiotic factors (e.g., pH, conductivity) differ (Glassmeyer et al., 2005) (Fig. 1). Currently, several approaches are in use for the detection of chemical pollutants (target screening, suspect screening, and non-targeted screening) and microorganisms (cultivation, microscopy, metabarcoding, denaturing gradient gel electrophoresis) (Geisen et al., 2015; Gran-Stadniczeňko et al., 2019; Hird et al., 2014; Muyzer, 1999; Pourchet et al., 2020; Smit et al., 2001; West and Whitman, 2022). Most established techniques require at least a rough previous knowledge of the compounds (Franco-Duarte et al., 2019; Krauss et al., 2010; Prakash et al., 2007; Stewart, 2012), and even fewer are suitable for high-resolution high-throughput analysis (Alain and Querellou, 2009; Krauss et al., 2010; Muyzer et al., 1993). Therefore, emerging techniques such as metabarcoding and non-target screening have a high potential for analyzing the unknown compositions of microorganisms and chemicals in treated wastewater (Fig. 1).

On the one hand, metabarcoding identifies organisms based on the amplification of DNA sequences in the sample without prior knowledge of the sample's composition. The targeted DNA region can be specific to any of the three domains of life: Eukarya, Bacteria, or Archaea (Bock et al., 2014, 2022; Gołębiewski and Tretyn, 2020). Beyond proving the presence of a distinct sequence (often referred to as operational taxonomic unit, OTU), metabarcoding can determine the relative abundance of these OTUs based on sequence counts (Gloor et al., 2017). However, assigning OTUs to distinct species can be challenging, as most microorganisms have not yet been formally described to serve as a reference and sequencing short fragments may result in limited phylogenetic resolution. Nonetheless, OTU diversity reflects community composition and can thus be used for identifying environmental changes (Bock et al., 2020; Sieber et al., 2020). On the other hand, non-target screening (NTS) is a technique for screening unknown chemical compounds in a sample. Unlike metabarcoding, NTS uses high-resolution mass spectrometry (HRMS) coupled with liquid chromatography (LC) to detect organic chemicals (referred to as chemical features). Chemical features are described based on their specific retention time and peak intensity (Hohrenk-Danzouma et al., 2022; Hohrenk et al., 2019; Itzel et al., 2020; Schymanski et al., 2015). Like metabarcoding, NTS does not require prior knowledge of the sample composition. In contrast to conventional target screening approaches that concentrate on a limited number of predetermined pollutants, non-targeted screening has the ability to detect compounds that have not been reported previously. Similarly, metabarcoding has the capacity to assess OTUs that have not yet been described. Therefore, both methods can be used to describe and monitor environmental samples but with a different perspective (e.g. biologically and chemically). While metabarcoding characterizes the sample based on the OTUs present, NTS characterizes it based on the chemical features present. As treated wastewater can introduce both (eukaryotic and prokaryotic) OTUs and micro-pollutants into receiving waters, both techniques are theoretically suitable for detecting environmental disturbances caused by treated wastewater.

Here we compare the efficacy of both techniques, and particularly the sensitivity of both approaches for detecting disturbances caused by treated wastewater over short- and long-term periods. In the context of this study, we refer to sensitivity as the signal-to-noise ratios (SNR). Beyond environmental monitoring, SNR are straightforward for analyzing sensitivity in many scientific disciplines such as engineering, medical diagnostics, communication, and signal processing (Price and Goble, 1993; Widrow and Kollár, 2008; Zeng and Liao, 2023).

For an investigation period of ten days, we analyzed and compared non-target screening data with microeukaryotic 18S V9 rRNA and prokaryotic 16S rRNA metabarcoding data from six large-scale circular freshwater mesocosms. Three of these received treated wastewater

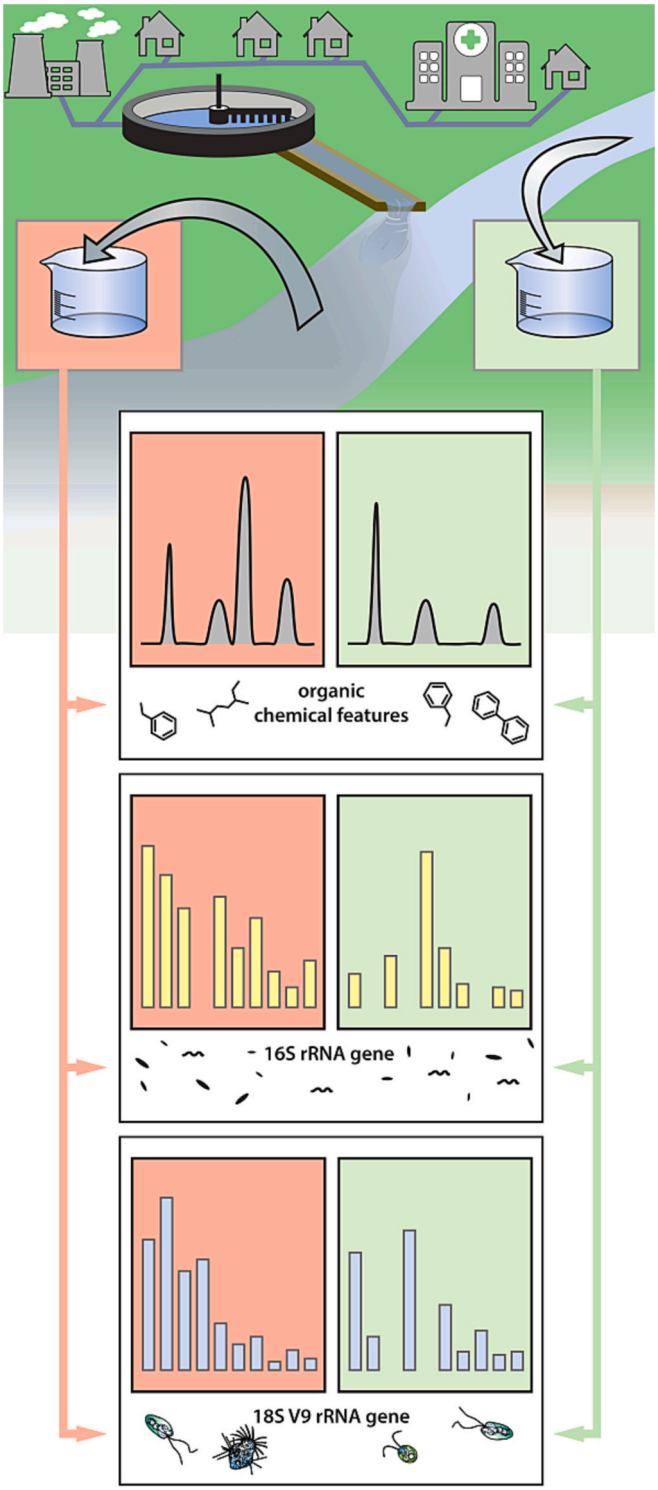

Fig. 1. Schematic illustration of a wastewater discharge pathway and the conception of study. Effects of treated wastewater versus an unaffected control are investigated using non-target screening and 16S and 18S V9 metabarcoding.

while another three systems served as control. Our objectives are: first to test whether the addition of treated wastewater (TWW) results in altered chemical and microbial fingerprints. Therefore, we hypothesize that each method is useful for environmental monitoring. Second, we aim at revealing a shared pattern of environmental disturbances detected by the three methods. Specifically, we anticipate a strong correlation between microbial diversity and the chemical signature since chemicals and microorganisms interact and affect each other in various ways (Duan et al., 2009; Hu and Hartmann, 2021; Staley et al., 2014). Since NTS also detects chemical features released from microorganisms, we lastly expect the highest response of environmental changes using NTS compared to metabarcoding. Due to the short generation times of prokaryotes we expect an initially more pronounced effect on prokaryotes as compared to microeukaryotes. However, the prokaryotic community disturbance may also level off more rapidly, resulting in mid- to long-term effects being more visible within microeukaryotic data.

2. Material and methods

2.1. Experimental setup

The experiments were conducted in six identical large-scale circular flow mesocosms, namely, the AquaFlow systems located in the greenhouses of the University of Duisburg-Essen (Graupner et al., 2017; Röhl et al., 2018; Stach et al., 2023). Each circular flow system comprises three water tanks (40 L, 40 L, 270 L) interconnected by two sediment-filled channels (10 cm width, 4 m and 2 m long, respectively). For each system, 365 L of river water and 60 L (~150 kg) of natural sediment from the river Boye (Germany, 51°33′19.7″N 6°56′38.3″E) were added. The sediment was homogenized in a concrete mixer to ensure similar starting conditions, and the water was prefiltered (200 μm) to exclude larger metazoa. A pump (Eheim compactON 1000, 10 L/min) maintained the circular flow, and the temperature was kept constant at 19 °C, while sunlight served as the light source. All systems were operated under identical abiotic conditions from 15th February to 11th March 2021. The mesocosms are not a full representation of natural conditions as they are circulating systems, however, this provides an important advantage because it allows treated wastewater to be tracked for longer periods of time.

Three systems (T_1, T_2, T_3) simulated a river receiving 33 % of treated wastewater (TWW) from a municipal wastewater treatment plant (WWTP) (Schwerte, North Rhine-Westphalia, Germany), while the remaining three systems served as controls (C_1, C_2, C_3). The basic parameters of the TWW, as well as a process flow diagram, were provided by the WWTP (see Table S1 and Fig. S1 in the Supplementary material).

Both the river water and sediment were acclimatized for 14 days prior to the experiment. During this period, all systems were interconnected daily with pumps (Wingard SWP 5225) to create a circular flow between all six systems. The water in each system was completely replaced twice during the daily acclimatization process. Thus, 700 L were replaced in each system. This homogenization method was found suitable based on preliminary experiments conducted with the same setup. The last interconnection between the systems was performed directly before the first sampling, thus the initial sampling of the controls may also be considered the initial status. After acclimatization, 33 % of the river water was replaced by TWW in three systems, and the experiment itself was run for ten days.

2.2. Sampling

Samples were collected after 1 h, 12 h, 1 day, 2 days, 4 days, 7 days, and 10 days. In detail, 400 mL of water was filtered using a 0.2 μm polycarbonate filter (Cytiva, Nucleopore, Freiburg), air-dried, submerged in 400 μL of DNA/RNA Shield (Zymo Research), frozen in liquid nitrogen, and stored at −80 °C until DNA extraction. For the non-target analyses, 1 mL of 0.2 μm filtered subsamples of the water samples undergoing metabarcoding were utilized. These subsamples were stored in an opaque vial kept at a low temperature on ice and promptly measured.

2.3. DNA extraction

DNA was extracted using the Zymo Quick DNA/RNA microprep plus kit (Sieber et al., 2022). Each step was carried out at room temperature, and the samples were centrifuged until the fluid passed through the columns (30–70 s) at 10,000 rpm. Briefly, two filters with DNA/RNA shield solution were transferred to a bashing bead tube (Zymo BashingBeads Lysis Tubes, 0.1 & 0.5 mm) and homogenized (FastPrep instrument, MP Biomedical) five times for 30 s at 5.5 m/s. The samples were rested for 1 min on ice between homogenization steps. The bashing bead tubes were centrifuged for 30 s, and the supernatant was transferred to a 1.5 mL tube and mixed with 400 μL of lysis buffer. After 30 s, the samples were transferred onto a Zymo-Spin IC-XM column in a 1.5 mL tube and centrifuged. Then, 400 μL preparation buffer was added to the DNA-carrying column before the next centrifugation, followed by two washing steps with 700 μL and 400 μL of wash buffer and a final dry centrifugation for 2 min. The dry columns were transferred to a 1.5 mL tube, and 30 μL of RNAse/DNAse-free water was added. After 5 min, an elution centrifugation was performed, and the DNA was stored at −20 °C. The same DNA was used for 16S rRNA full length and 18S V9 rRNA sequencing.

2.4. Processing of microeukaryotes

Eukaryotic PCR amplification was conducted to target the 18S V9 rRNA region, which is typically around 150 base pairs in length. The amplification used forward primers based on 1391f (5′-GTACACACCGCCCGTC-3′) (Amaral-Zettler et al., 2009) and reverse primers based on EukR (5′-TGATCCTTCYGCAGGTTCACCTAC-3′) (Medlin et al., 1988). The PCR were carried out using the QIAGEN Multiplex PCR Kit (Qiagen, Germany) with 12.5 μL 2× Qiagen Multiplex PCR Master Mix, 2.5 μL of each primer at a final concentration of 0.2 μM, 5 μL 5× Q-Solution, 1 μL template DNA, and 4 μL RNase-free water. A corresponding negative control was performed for each PCR. The PCR conditions consisted of an initial denaturation/heat activation step at 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, annealing for 90 s at 60 °C, extension for 60 s at 72 °C, and a final extension step at 72 °C for 2 min. Equimolar subsamples were pooled and commercially sequenced using a NovaSeq6000, which yielded 150 bp-long paired-end reads (Fasteris, Geneva, CH).

The DNA sequencing company (Fasteris, Geneva, CH) performed adapter removal, quality trimming, and demultiplexing using MID sequences. The base quality of the sequence reads was checked using FastQC (Andrews et al., 2012). Two technical replicates per DNA sample were used, and a split-sample filtering protocol for Illumina amplicon sequencing was applied (Lange et al., 2015). The raw sequences were quality filtered using PRINSEQ-lite v.0.20.4 (Schmieder and Edwards, 2011) to remove reads with an average Phred quality score below 25. The paired-end reads were assembled and subjected to quality filtering using PANDASeq v2.10 (Masella et al., 2012). Reads with uncalled bases, an assembly quality score below 0.9, a read overlap below 20, or a base with a recalculated Phread-score below 1 were removed. After dereplicating, chimeras were identified and filtered using UCHIME v7.0.1090 (Edgar et al., 2011) with default settings. Sequences that were not present in both sample branches were discarded (Lange et al., 2015). Operational taxonomic units (OTUs) were created using swarm with default settings v2.1.9 (d = 1) (Mahé et al., 2014). All sequences were blasted against the NCBI nucleotide database (downloaded on May 17th, 2022), and OTUs assigned to Metazoa, prokaryotes, Archaea, and Embryophyta were discarded. The fastq files are available at: PRJNA1019091. The above tools were used within the snakemake-based amplicon pipeline Natrix (Welzel et al., 2020).

2.5. Processing of prokaryotes

Full-length 16S rRNA sequencing was performed as described in Stach et al. (Stach et al., 2023) using the 16S rRNA barcoding kit (SQK-RAB204; Version RAB_9053_v1_revM_14Aug2019) on a MinION Mk1B with a FLO-MIN106D flow cell from Oxford Nanopore. Amplification was based on specific 16S rRNA gene primers (27F: 5′-AGACTTTGATCMTGGCTCAG-3′ and 1492R: 5′-TACGGYTACCTTGTTACGACTT-3′) (Lane, 1991) with LongAmp Taq 2× Master Mix (NEB) for PCR setup and Agentcourt AMPure XP beads (Beckman Coulter) for clean-up. Deviating from the protocol, 15 ng DNA instead of 10 ng was used as input for the PCR. A corresponding negative control was performed for each PCR. There was one PCR per replicated system per time point. A minimum of 100,000 reads were sequenced for each sample, followed by basecalling and demultiplexing via guppy (v5.0.7, dna_r9.4.1_450bps_hac.cfg). The 16S rRNA gene sequences were then processed using the NanoCLUST pipeline (v1.0dev; UMAP settings –cluster_sel_epsilon 0.5 –umap_set_size 100000) (Rodríguez-Pérez et al., 2021), utilizing the NCBI 16S rRNA database (v28.04.2021) as a reference. The 16S rRNA raw data is available in the Genbank repository (PRJNA 874517). To validate that the nanopore sequencing accurately represents the environmental pattern we ran the nanopore dataset with a second pipeline (Natrix[2], https://github.com/dbeisser/Natrix2) and compared the diversity pattern with a coinertia analysis (RV: 0.87, $p < 0.05$). Additionally, we compared our results with the pattern based on available rpS3 gene counts (RV: 0.94, $p < 0.05$) (Stach et al., 2023). This highlights the robustness of the nanopore sequencing approach in capturing diversity patterns within the environmental samples.

2.6. Filtering of metabarcoding data

To ensure data quality, we applied filtering steps to both metabarcoding datasets. We removed OTUs that were present in only one sample and those with a joint abundance of <100 reads (Sieber et al., 2022). In addition, we rarefied the reads per sample to the mean read amount per sequencing strategy, which was 97,129 for whole 16S rRNA and 1,850,045 for 18S V9 rRNA, to compensate for uneven sequencing depth. Rarefaction curves were used to verify sufficient sequencing depth, as shown in Fig. S2A and B (Oksanen et al., 2022).

2.7. Metal measurements

The measurements of present metal concentrations were carried out by measuring filtered samples (using Whatmann™ 0.45 μm cellulose nitrate filters). The dissolved metal concentrations were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The analyses were carried out with a quadrupole ICP-MS system (Perkin Elmer Sciex Elan DRC-e) operating at 1000 W plasma power, 0.95 L/min nebuliser gas flow, 14 L/min plasma gas flow and an auto sampler system (Perkin Elmer AS-90) connected with a peristaltic pump with a sample flow (1 mL/min). To avoid memory effects and contamination a wash time between measurements of 10 s (with 1 % HNO_3, suprapure) was set. Before analyses, the samples were diluted 1:10 with a solution of 1 % HNO_3 (suprapure) with a concentration of 10 ng/L of yttrium (Y) as internal standard. After 10 samples a standard solution of all analytes in a concentration of 10 μg/L was analyzed to control the accuracy and stability. The calibration was carried out with a series of dilutions of a multielement standard solution (ICP Multielementstandard solution, Merck, Darmstadt, Germany) using additionally Molybdenum standard (AAS-standard, Bernd Kraft, Duisburg, Germany). Dissolved metal concentrations were calculated as mg/L using corresponding regression lines (correlation factor ≥ 0.999) (Erasmus et al., 2020; Schertzinger et al., 2018). Heavy metal concentrations were not further analyzed, but are presented as environmental information (Table S2).

2.8. Non-target screening, instrumental conditions and analysis

Chromatographic separation was performed using a Dionex UltiMate 3000 HPLC system (Thermo Scientific, Bremen, Germany), which was equipped with a modified C_{18} column (XSelect® 2.5 μm HSS T3 2.1 × 100 mm + 2.1 × 5 mm guard column, Waters, Dublin, Ireland). Ultra-pure water and methanol, both LC-MS grade and spiked with 0.1 % formic acid, were used as eluents and run in gradient mode (Fig. S3). Samples were analyzed in triplicates, whereby the injection volume was 100 μL. The HPLC system was coupled to a high resolution orbitrap mass spectrometer (QExactive Thermo Scientific, Bremen, Germany). Compounds were ionized by an atmospheric pressure electrospray ionization (IonMax with H-ESI II probe, Thermo Scientific, Bremen, Germany) in positive mode. Subsequently, HRMS analysis was performed in full scan spectra acquisition mode (*m/z* 80–1000) with a resolution of 70,000. Detailed information on ESI source parameters and full scan MS^1 settings are listed in table S3. Mass calibration of the HRMS was performed with a respective standard for positive ionization mode (Pierce LTQ Velos Positive Ions Calibration Solution, Thermo Scientific, Bremen, Germany). Software-wise, the HPLC was operated using Chromeleon 7.2 (Thermo Scientific, Germany), while the HRMS was run with Xcalibur™ 4.3 (Thermo Scientific, Germany). Xcalibur software (Thermo Fischer Vendor) was used to acquire raw chromatographic data in profile mode, which were then converted via msConvert software (ProteoWizard) (Kessner et al., 2008) into mzXML file format (Table S4).

Subsequent data compression steps were then performed in R (R Core Team, 2023), following a non-target screening workflow developed by the Bundesanstalt für Gewässerkunde (Köppe et al., 2020).

In order to set data-processing parameters in terms of peak picking and alignment (Table S5), the false positive rate (Supplemental Eq. (1)) of the 300 least intense extracted features from one treatment surface water sample was determined based on their chromatographic profiles. All samples were spiked with four isotope labeled internal standards (Table S6). In addition, eight quality control samples were spiked with 11 compounds for the optimization of parameter settings during data-processing and to monitor measurement stability. Lastly, this also allowed to determine a true positive rate (Supplemental Eq. (2)). Final NTS parameters (Table S6) were evaluated with a false positive rate of 0.05 and a true positive rate of 1.00. Subsequently, three respective samples of ultra-pure water, were used as system blanks. Thereby, features were considered authentic in case they showed at least a tenfold intensity compared to the blank. In addition, only features that were present in all three replicate measurements were kept in the data set. The total number of achieved features was 2548. We excluded features that appeared in only one sample, resulting in a final set of 1790 chemical features. The NTS control replicates from day 4 were discarded due to strong deviation across the replicates (Fig. S4), except for chemical feature richness analysis, as this is a presence-absence based analysis and relatively robust to strong peak intensity variation. For comparative analysis across the different datasets these samples were also discarded in the corresponding other dataset (both metabarcoding datasets).

2.9. Statistics

The metabarcoding datasets were transformed using the Hellinger transformation with the R-package vegan 2.6-4 (Oksanen et al., 2022), and chemical features were log transformed (Dixon et al., 2006; Hohrenk-Danzouma et al., 2022). To ensure comparability between datasets, dissimilarity analyses were conducted using Bray-Curtis dissimilarity (Kew et al., 2022; Oksanen et al., 2022; Riedel et al., 2016). In order to validate the effectiveness of the methods in detecting environmental perturbations, a pairwise Adonis analysis was performed with Benjamini-Hochberg corrected *p*-values for each dissimilarity matrix, comparing the groups of control and treatment. The SNR was calculated by dividing the mean dissimilarity between each treatment and control pairing by the mean dissimilarity between the three control replicates pairings (referred to as measured noise or natural variation) (Price and Goble, 1993). Calculating the Signal-to-Noise Ratio (SNR) provides a comparable value that quantifies the change as a multiple of the measured noise. SNRs were compared using a *t*-test ($p < 0.01$) (R Core Team, 2023). The assumptions for the *t*-tests were checked using the Bartletts test and Shapiro-Wilk test ($p < 0.01$) (R Core Team, 2023). Cross comparisons between the individual datasets and their SNR over time were calculated using a Wilcoxon test, as the t-test assumptions were violated ($p < 0.01$) (R Core Team, 2023). A three-dimensional principal coordinate analysis (PCoA) was performed to visualize the Bray-Curtis dissimilarity between all samples, with distances reflecting the level of dissimilarity (Paradis and Schliep, 2019). The further away the samples were, the more dissimilar they were. The explained variance per axis reflected the importance of each axis, with higher explained variance indicating greater importance in a linear manner. Coinertia analyses were run based on principal component analysis using the R-package ade4 1.7–22 (Dray and Dufour, 2007). A Coinertia analysis is a multivariate method to detect co-relationships in different datasets by finding covariances, which are based on the same samples. The resulting RV value (global similarity) varies between 0 (no similarity) and 1 (full similarity).

3. Results

3.1. Achieved metabarcoding and NTS datasets

After filtering, the total number of assembled reads for eukaryotes was 77,701,891, which formed 3088 operational taxonomic units (OTUs). On average, 1224 ± 330 OTUs were found per sample. For prokaryotes, the total number of assembled reads after filtering was 4,079,415, which formed 231 OTUs. On average, 62 ± 20 OTUs were found per sample. The non-target analysis resulted in a total of 1790 features, with a mean peak area of 306,180. On average, there were 464 ± 171 features per sample.

3.2. Community composition

The microeukaryotic community composition reveals both shared characteristics and distinctions between control and treatment replicates at a higher taxonomic level (Fig. S5A). Both encompass a substantial representation of Stramenopiles and Fungi; however, the taxonomic identification of these groups relies on uncharacterized OTUs (e.g., labeled as "environmental sample"). Ciliophora and Choanoflagellata exhibit greater prevalence in control samples, while Chrysophyta show higher abundance in treatment samples. The presence of phototrophic organisms is relatively modest, yet Bacillariophyta are initially more abundant in treatment samples.

The prokaryotic community composition exhibits similarity between control and treatment samples at a higher taxonomic level, particularly in the later sampling periods (Fig. S5B). Betaproteobacteria emerge as the dominant presence within the community. Notably, *Limnohabitans* and Burkholderiales (Betaproteobacteria) show higher abundances in treatment samples, whereas *Polynucleobacter* demonstrates greater prevalence in control samples. However, initial comparisons reveal elevated levels of Cyanobacteria, Deltaproteobacteria, and Firmicutes in treatment samples compared to controls.

3.3. OTU and feature richness

Treated wastewater (TWW) introduced microeukaryotic and prokaryotic OTUs as well as chemical features, which were unique in TWW (Table S7, Fig. S6). However, the unique OTUs from TWW, both microeukaryotic and prokaryotic, decreased over time (respectively

from 774.4 ± 198.5 to 262.9 ± 23.8 and from 73.6 ± 5.0 to 7.4 ± 2.5), but features unique from TWW oscillated over time and did not show any decrease (from 270.1 ± 25.5 to 291.3 ± 32.4) (Table S7, Fig. S6). A trend of generally increasing chemical features was observed both in the controls and the TWW treatments, regardless of the relatively stable features that are unique to TWW, as the richness of chemical features in the control replicates also increased, indicating parallel offset development of the control (Fig. S7). However, the increase in chemical features was more pronounced in the control replicates compared to treatment replicates (Fig. S6).

The increase in richness was significant for the non-target screening for all time points ($p < 0.01$ except 168 h). In contrast, for the 16S rRNA data richness was only significantly affected initially ($p < 0.01$ for time points 1 h and 12 h) while for 18S V9 rRNA data richness was not significantly affected (even though showing a similar trend) (Fig. S7).

3.4. Prokaryotic, eukaryotic and feature sample composition

The addition of treated wastewater (TWW) had an impact on the composition of the eukaryotic and prokaryotic communities, as well as the chemical feature composition, as shown in Fig. 2. Treatment (Fig. 2: blue) and control (Fig. 2: red) were clearly separated for prokaryotes, eukaryotes, and chemical features (PCoA, $p < 0.01$ for each method). Treatment and control develop in parallel manner over time. For each time point replicates clustered closely together both for treatment and for control. Over time, the differences introduced by the TWW decreased (Fig. S7). This was a steady process for eukaryotes and chemical features, while for prokaryotes the decrease of the TWW effect was more pronounced in the beginning (Figs. 2, S7).

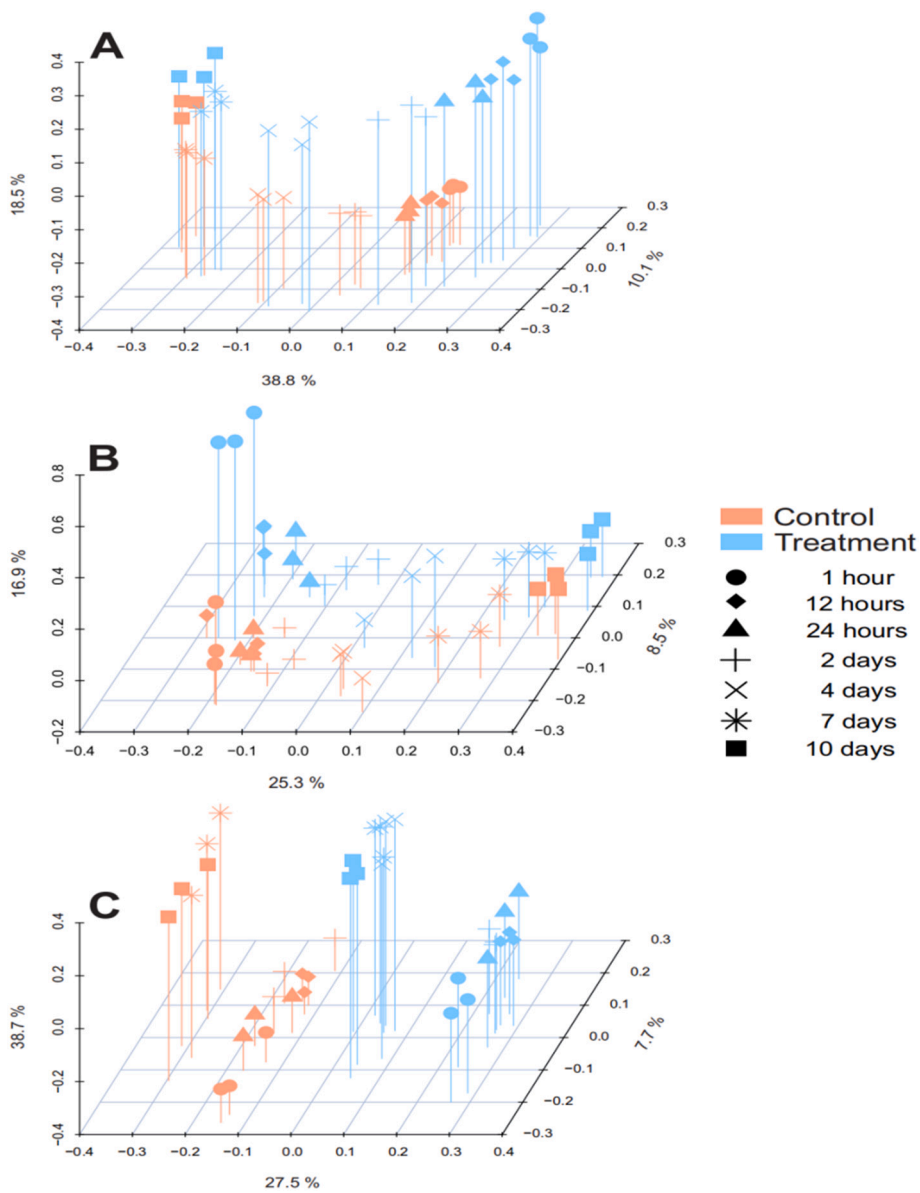

Fig. 2. Three-dimensional PCoA of A) 18S V9 rRNA, B) 16S rRNA, C) non-target screening. Blue represents the treatment and red the control replicates. The sampling points were differentiated using different shapes. The first three axes with the highest explained variance were arranged in the order that would best illustrate their effects, rather than according to their explained variance. While one axis is referring to the time (A: x-axis, B: x-axis, C: y-axis) another axis is referring to the treatment (A: y-axis, B: z-axis, C: x-axis).

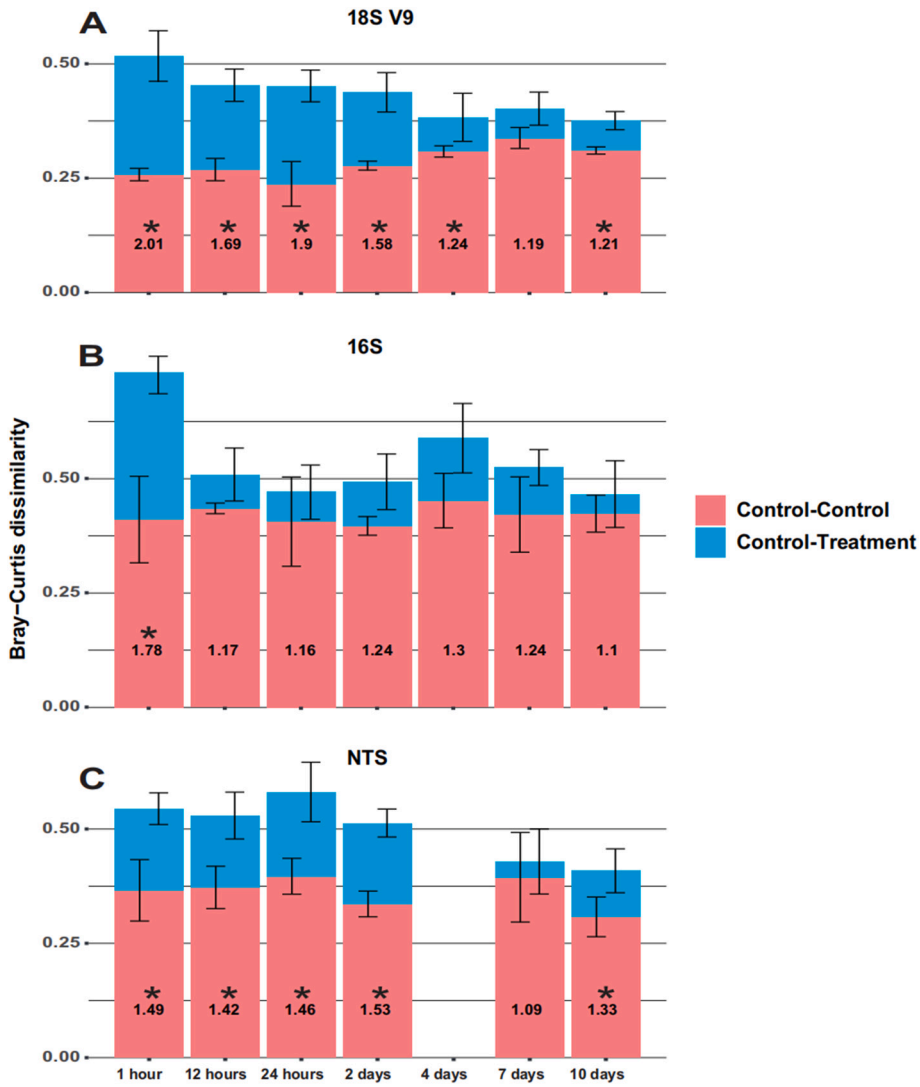

Fig. 3. Signal to noise ratios (SNR) based on Bray-Curtis dissimilarity for A: 18S V9 rRNA, B: full length 16S rRNA, and C: NTS. Red bars denote the measured noise. Blue bars denote the added signal introduced by TWW, namely the mean dissimilarity of the treatment replicates to the control replicates. Asterisks represent a significant difference between noise and signal deflection ($p < 0.01$). The numbers within the bars denote the SNR. The missing values after 96 h for non-target screening in C is due to two removed control replicates.

3.5. Overarching pattern across methods

The pattern of effects caused by TWW was highly similar between the methods (Coinertia analysis $p < 0.001$: Fig. S9, A-C) with highest resemblances between 18S V9 rRNA and 16S rRNA (RV: 0.849), followed by 18S V9 rRNA and NTS (RV: 0.816) and 16S rRNA and NTS (RV: 0.676).

3.6. TWW detection and signal to noise ratio

The measured noise levels differed significantly between the methods. Noise of 18S V9 rRNA data was significantly lower than measured noise of 16S rRNA data after 12, 48, 96, and 240 h ($p < 0.01$). Measured noise of 18S V9 rRNA was also lower than that of NTS dataset but this was only significant after 24 h ($p < 0.01$). There was no significant difference in measured noise between the NTS data and the 16S rRNA data.

All methods detected an effect of TWW (SNR > 1), but significance varied over time and across methods (Fig. 3A-C). 16S rRNA data performed well for detecting short-term disturbances of up to 12 h ($p < 0.01$) (Fig. 3B), while 18S V9 rRNA and NTS data performed well throughout the experiment ($p < 0.01$) with one exception after 168 h (Fig. 3A, C). Immediately after TWW introduction, both 18S V9 rRNA and 16S rRNA performed best in terms of SNR compared to NTS ($p < 0.01$, Table 1). After 12 h and 24 h 18S V9 rRNA data had the highest SNRs compared to 16S rRNA and NTS ($p < 0.01$) while after 48 h to 240 h, NTS and 18S V9 rRNA performed similar but better than 16S rRNA ($p < 0.01$).

However, the disturbance caused by TWW was diminishing in each method, which is evident by the general trend of decreasing SNRs (Fig. 3A-C).

4. Discussion

4.1. Potential of metabarcoding and non-target screening for environmental monitoring

Running water systems are often subjected to various types of disturbances, with treated wastewater being a major concern due to its continuous generation and discharge into freshwater systems (Karakurt et al., 2019; Li et al., 2016). The monitoring of treated wastewater released from wastewater treatment plants is necessary, as it can

Table 1
Comparison of the SNRs between different methods to identify the best-performing methods to detect treated wastewater over time. Statistical testing ($p < 0.01$) was conducted only for comparisons where both methods exhibited a significant difference between noise and the detected signal (filled circle). Otherwise, the method that demonstrated a significant difference between noise and signal was selected as the superior one and the one where no significant difference between signal and noise (empty circle) was selected as inferior one (Fig. 3).

Time	18S V 9	16S	NTS	Best-performing method
1 h	●	●	●	18S V9 $>^{n.s.}$ 16S $>$ * NTS
12 h	●	○	●	18S V9 $>$ * NTS
24 h	●	○	●	18S V9 $>$ * NTS
48 h	●	○	●	18S V9 $>^{n.s.}$ NTS
96 h	●	○	–	18S V9
168 h	○	○	○	–
240 h	●	○	●	18S V9 $>^{n.s.}$ NTS

potentially affect microbial communities and the chemical composition of receiving waters, thereby having implications for the ecosystem and its processes. Although wastewater treatment processes remove many pollutants and harmful organisms, many other compounds and organisms may persist in the treated wastewater (Baresel et al., 2019; Freudenthal et al., 2022; Li et al., 2008; Lira et al., 2020; Martínez-alcalá et al., 2017; Rüdel et al., 2020). In fact, the introduction of TWW to receiving waters leads to significant changes in microbial and chemical diversity (Figs. 2, S7) as treated wastewater contains microorganisms and chemicals that are not commonly found in rivers (Fig. S7, Table S7).

We demonstrate that chemical compounds can be detected using non-target screening methods, while metabarcoding can be used to detect microorganisms originating from treated wastewater (Fig. S6, Table S7). Each method separately was used for environmental monitoring (Hollender et al., 2019; Stoeck et al., 2018; Tian et al., 2020). However, the comparative strengths and sensitivities of the three methods have so far not been addressed. Here we show that both 18S V9 and full-length 16S metabarcoding, as well as non-target screening can be used effectively for monitoring treated wastewater. We demonstrate that all three methods are appropriate for freshwater monitoring and in particular for detecting treated wastewater (Figs. 2A-C, S7, S8).

It is important to note that chemical features as well as microeukaryotic and prokaryotic sequences have been processed differently which explains for the apparent difference in richness estimates (Fig. S7): for prokaryotes we applied full-length 16S rRNA sequencing. Even though partial sequencing of 16S rRNA conserves sample relationships, full-length 16S rRNA provides superior resolution (Gehrig et al., 2022; Jeong et al., 2021; Matsuo et al., 2021). For microeukaryotes we focused on the 18S rRNA V9 region, which is considered a suitable marker (Choi and Park, 2020; Pawlowski et al., 2011; Stoeck et al., 2010). Established bioinformatic protocols were applied, but these varied due to the different target groups. A direct comparison of prokaryotic and eukaryotic richness is therefore not meaningful (as also for comparing to richness of chemical features). However, despite some limitations and uncertainties associated with each method, patterns are comparable between methods. The strong correlation between the results of the three methods (Fig. S9) confirm this.

4.2. Changes in chemical and microbial diversity over time in treated and non-treated water samples

Differences between TWW exposed and non-exposed samples may decrease over time, as micro-pollutants are degraded through biotic and abiotic processes and microbial communities adapt to changing conditions (Li et al., 2008; Martínez-alcalá et al., 2017; Petterson et al., 2002). In fact, altered microbial and chemical diversity leveled off in our study (Figs. 2, S7, S8, Table S7).

Chemicals can degrade completely thereby decreasing chemical richness. Alternatively, chemicals can degrade into transformation products, ultimately increasing chemical richness (Li et al., 2008; Montagner et al., 2022). Chemical richness in the TWW treatment was generally higher than in the control. However, changes in chemical richness developed similar over time both in the TWW treatment and in the control. This indicates that degradable chemicals in the TWW are already largely degraded during the wastewater treatment before release to receiving waters (West and Whitman, 2022). Shifts in chemical richness were presumably due to degradation and transformation of chemicals present in the receiving water (Figs. S6, S7).

We observed temporal trends for each method (Fig. 2). While the temporal trend was more uniform in both metabarcoding datasets, it was more abrupt in the non-target screening dataset. However, the time interval between samplings, during which the abrupt compositional shift was observed, was relatively large (between samplings 4 and 5, equivalent to 2 days). Therefore, transitional stages may not have been resolved in our study but may be better detectable with more frequent sampling. For studies aiming at process understanding we therefore suggest a higher sampling frequency. Interestingly, the chemical composition underwent alterations in both control and treatment samples, leading to the hypothesis that the degradation or transformation of commonly occurring chemicals drove this change. Microbial diversity decreased over time in our experiment both in the TWW treatment and in the control (Fig. S7). The overall trend of decreasing microbial diversity in mesocosms corresponds to findings in previous studies (Graupner et al., 2017; West and Whitman, 2022). This decrease can be attributed to accidental extinction of microorganisms, competition for resources, and the prevention of new species from recolonization. Interestingly, the prokaryotic diversity in the TWW treatments converged rapidly towards a community similar to the control conditions. This may indicate that the majority of prokaryotes in TWW basins are specialized to their environment (Liu et al., 2021; Stach et al., 2023) and may struggle to compete in receiving waters (Fig. S6). Prokaryotes may adapt more quickly than microeukaryotes due to their shorter generation times, resulting in faster adaptation (Figs. 2, 3, S8). Correspondingly, the convergence to control was less pronounced for microeukaryotes. Possibly microeukaryotes are more generalistic (Liu et al., 2021) and occur in both TWWs and rivers, or their richness is limited in TWWs, so in principle less diversity (e.g., richness) could be introduced to receiving rivers compared to prokaryotes (Fig. S8).

Notably, community differences among microeukaryotes are more pronounced at higher taxonomic levels, while such distinctions are less prominent among prokaryotes (Fig. S5A, B). Stach et al. (2023) demonstrated that prokaryotic community differences resulting from treated wastewater are based on the strain level, providing an explanation for the observed patterns at higher taxonomic levels. Moreover, our findings reveal that differences induced by treated wastewater are already evident at a relatively high taxonomic level for microeukaryotes. Consequently, high-resolution sequencing for microeukaryotes may be less imperative in theory.

4.3. Covariation of patterns detected with non-target screening and metabarcoding

Our findings demonstrate that there is significant covariation between metabarcoding and non-target screening data (Fig. S9). The covariation between prokaryotic and microeukaryotic communities is complex. For example, microeukaryotes can exert direct or indirect impacts on prokaryotic populations through predation, competition for nutrients, or modification of the environment (Bock et al., 2020; Heck et al., 2023; Tsao et al., 2019).

Another way in which prokaryotic and microeukaryotic communities interact is through the production and consumption of extracellular organic matter. Both, prokaryotes and microeukaryotes produce various extracellular organic compounds, which can serve as a food source for the respective other (Bennett et al., 1988; Duan et al., 2009; Sauvage et al., 2022; Šimek et al., 1997; Wyatt and Turetsky, 2015). Thus, the conclusive joint pattern between prokaryotes and microeukaryotes is attributed to a range of interactions.

The high degree of global similarity between the microeukaroytic data and non-target screening data can be attributed to several factors. First, NTS can detect a wide range of chemicals present in environmental samples, including those that may be harmful to microeukaryotes, such as herbicides. As a result, the presence of certain chemicals in an environmental sample can impact the abundance and diversity of microeukaryotes within that sample (Amacker et al., 2018; Ibtissem et al., 2012; Melero-Jiménez et al., 2021). Second, microeukaryotes can serve as bioindicators of environmental contamination (Karpouzas et al., 2022; Payne, 2013; Schreiber and Brink, 1989). Changes in the abundance and diversity of microeukaryotes in a given environment can indicate the existence of contaminants or pollutants in that environment. Therefore, the similarity between the NTS dataset and the microeukaryote dataset may be due to the presence of contaminants in the environment. Third, certain microeukaryotes have the ability to biotransform or biodegrade certain chemicals, which can also impact the similarity between the NTS dataset and the microeukaryote dataset. For example, some algae can absorb and metabolize pollutants such as polycyclic aromatic hydrocarbons, leading to their removal or transformation (García de Llasera et al., 2016; Srivastava and Kumar, 2019). Finally, microeukaryotes actively produce chemicals, with some of them producing toxic compounds as a defense mechanism against predators or competitors (Pierce and Kirkpatrick, 2001; Strom et al., 2003; Tillmann et al., 2008).

The high degree of covariation observed between the non-target screening dataset and the prokaryotic dataset can be attributed to the critical role that prokaryotes play in the transformation and degradation of environmental chemicals. Certain prokaryotes are highly adaptable and can thrive in a wide range of environments, including those that are contaminated with pollutants. These prokaryotes have developed various mechanisms to break down and detoxify chemicals such as pesticides and hydrocarbons (Galitskaya et al., 2021; Haritash and Kaushik, 2009; Joy et al., 2017). Therefore, the presence of specific chemicals in the environment can lead to the proliferation or suppression of particular prokaryotic species, which can be reflected in the prokaryotic dataset (Galitskaya et al., 2021; Patel et al., 2014; Wu et al., 2019). As a result, the global similarity between NTS and prokaryotic datasets can indicate the presence of environmental contaminants and the impact they have on prokaryotic populations. Therefore, the observed similarity serves as a useful indicator for monitoring environmental pollution and assessing the associated risks to human health. By detecting changes in either the composition of prokaryotes or the presence of chemicals, we can infer that corresponding changes have likely occurred in the other composition as well. These changes can have additional impacts on humans and ecosystems.

Indeed, it should be noted that the overall covariation between datasets can also result from indirect effects (White et al., 2006). For instance, prokaryotes may biodegrade chemicals into transformation products (Zhang and Bennett, 2005) that may have an impact on microeukaryotes (la Farré et al., 2008). Even if the original chemical does not directly interact with microeukaryotes, the transformation product produced by prokaryotes can still affect them. Therefore, the presence of specific transformation products in the environment can also impact the abundance and diversity of microeukaryotes, leading to covariation between the NTS and microeukaryote datasets.

4.4. Recommendation for environmental treated wastewater signal detection

The presence of treated wastewater in receiving water can be detected through metabarcoding of both prokaryotes and microeukaryotes, as well as non-target screening. These methods not only provide a means to identify the presence of treated wastewater but also offer a more holistic perspective on its detection. By employing these approaches, a comprehensive understanding of the composition and potential impacts of treated wastewater on the receiving water and associated ecosystems can be obtained. While non-target screening was expected to be the most sensitive method due to its ability to detect chemicals originating from treated wastewater and those produced by microorganisms, the results showed that this expectation only holds true to some extent (Fig. 3, Table 1).

Interestingly, signal-to-noise ratio indicated that all three methods detected TWW even though the significance of noise, i.e., variation between replicates, differed between the methods (Fig. 3). Microeukaryotes performed best and had the lowest noise, suggesting that their communities developed similar in the replicates. Noise was highest for non-target data. Thus, despite high signal levels the non-target data were not necessarily sensitive as noise partly masked the signal. Due to the method-specific different noise levels, the relative strength of the methods is therefore best characterized by the signal-to-noise-ratio (Fig. 3, Table 1).

Based on SNR, metabarcoding of prokaryotes and microeukaryotes were found to be initially more sensitive, possibly due to high abundance of specialized microorganisms in treated wastewater. Surprisingly, the sensitivity of prokaryotic metabarcoding data already decreased after 12 h while the sensitivity of microeukaryotic metabarcoding data remained high throughout the experiment. This difference may be related on the one hand to the degree of specialization of the organisms, on the other hand to differences in growth rate and generation time. Some prokaryotes are presumably highly specialized to the treated wastewater environment (Gratia et al., 2009; Guo et al., 2010; Muller et al., 2014) and may go extinct in the mesocosms quickly. In contrast, microeukaryotes appear to be more generalist and introduced taxa therefore may be detectable in the system for longer. However, many introduced microeukaryotes eventually also went extinct in the systems. The decline in specialized microorganisms is supported by the decrease in microbial richness, suggesting that the microbial community is adapting to the new environment (Fig. S7). Thus, the initial advantage of biotic screening over non-target screening fades over time.

Overall, the choice of method depends on the specific research question, the circumstances surrounding the contamination event and particularly on the time frame under consideration. The impact of treated wastewater on downstream ecosystems is a complex issue that depends on various factors, including the quality of the effluent, chemicals and their transformation products, the characteristics of the receiving water body, and the sensitivity of the downstream biota. Given the unpredictability of wastewater composition, all three approaches have their justification and complement each other. As stand-alone method biotic approaches seem to be more sensitive in particular on short time scales, while chemical features seem to be more persistent presumably allowing for signal detection even after longer periods of time.

5. Conclusion

The results of our study have highlighted the significant impact of treated wastewater on the chemical and microbial diversity and composition of receiving waters. We have also shown that non-target screening, microeukaryotic and prokaryotic metabarcoding are effective approaches for detecting these impacts, and all have potential as monitoring methods. Although these methods significantly co-vary in their detection capabilities, they differ in their temporal resolution. Microeukaryotic metabarcoding consistently showed exceptional detection capabilities and we recommend this method particularly for short- and mid-term effects. For initial detection, we can also recommend the use of prokaryotic metabarcoding, but the sensitivity of this methods decreases strongly within the first hours after TWW introduction. Non-target screening performed well throughout the experiment event though this method is outperformed by the metabarcoding methods during the initial phase. Consequently, the relative strength of non-target analysis is better for mid- to long-term detection. However, it would be of great interest and importance to generalize these findings by analyzing treated wastewater from different source in a follow-up study.

CRediT authorship contribution statement

Guido Sieber: Writing – original draft, Methodology, Validation, Conceptualization, Visualization, Formal analysis. **Felix Drees:** Writing – review & editing, Methodology, Validation, Visualization, Formal analysis. **Manan Shah:** Methodology, Conceptualization, Writing – review & editing. **Tom L. Stach:** Writing – review & editing, Methodology, Validation, Formal analysis. **Lotta Hohrenk-Danzouma:** Methodology, Validation, Formal analysis. **Christina Bock:** Writing – review & editing. **Maryam Vosough:** Writing – review & editing, Validation. **Mark Schumann:** Methodology, Formal analysis. **Bernd Sures:** Writing – review & editing. **Alexander J. Probst:** Supervision, Writing – review & editing, Funding acquisition, Methodology. **Torsten C. Schmidt:** Supervision, Writing – review & editing, Validation, Funding acquisition, Methodology. **Daniela Beisser:** Supervision, Writing – review & editing, Validation, Formal analysis, Methodology. **Jens Boenigk:** Supervision, Writing – original draft, Writing – review & editing, Validation, Conceptualization, Funding acquisition, Visualization, Methodology.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Statement

During the preparation of this work the authors used ChatGPT in order to enhance readability and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167457.

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Chapter III:

“The need for interdisciplinarity”

Article VII:

„The Premise of Interdisciplinarity and Its Actual Absence—A Bibliometric Analysis of Publications on Heavy Rainfall“

Guido Sieber, Benjamin Freudenberg, Leon Netzel, Torsten C. Schmidt & Frederik Brandenstein

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Experiments & methods	30%	Development of an applicable methodology based on the SCOPUS database
Analyses & interpretation	30%	Analyses and interpretation of data
Manuscript & tables/figures	30%	Writing and review of the manuscript and creation of tables and figures

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Article

The Premise of Interdisciplinarity and Its Actual Absence—A Bibliometric Analysis of Publications on Heavy Rainfall

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Abstract: Working together across disciplinary boundaries is considered to be the gold standard for conducting meaningful research tackling complex problems. As this is the nature of many issues concerning water, one would assume interdisciplinarity as being a widespread trait of water research. To review this assumption, we chose to conduct an analysis of research output considering issues of stormwater management and heavy precipitation, as reflected in the meta-information for more than 300,000 documents supplied by Elsevier's Scopus literature database. For this purpose, we applied a bibliometric measure based on Jaccard similarity determining the level of interdisciplinary cooperation between different fields of research on the topic above. Contrary to interdisciplinarity being depicted as highly desirable, it turns out to be a relatively marginal phenomenon, only growing slowly over the last 50 years.

Keywords: bibliometrics; interdisciplinarity; stormwater; flood; heavy rainfall; co-citation

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1. Introduction

Following the Fifth Intergovernmental Panel on Climate Change (IPCC) synthesis report, heavy precipitation events are expected to increase in many parts around the globe [1]. This is of particular concern in urban areas with many impervious surfaces resulting in large volumes of stormwater during precipitation events. Furthermore, ongoing soil compression and sealing enhances pluvial flood risk as infiltration capacity decreases while surface water runoff increases [2,3]. The potential damage, both economic and humanitarian, is enormous. Tragic evidence of this is provided by recent floods, e.g., in Western and Central Europe in 2021 and in Pakistan just this year [4,5]. Furthermore, due to economic reasons, the retention volume of sewer systems is restrained, which has consequences for the environment when the planned for rainfall intensity is exceeded [6]. In order to minimize flooding events, any volume that exceeds the designed limit is released into the environment, often into surface water bodies, consequently polluting aquatic ecosystems [7,8]. The spike in discharge not only causes problems in terms of water quality, but also the sudden increase in shear forces causes erosion, thus destroying aquatic habitats and promoting particle loads [8]. Policy objectives as laid out in the European Union's Water Framework Directive require consequences from heavy precipitation to be minimized [9]. Thus, nature-based solutions for stormwater management are going to be more important

in addition to water retention measures in urban areas, such as multifunctional water plazas. This adaptation challenges the common practices of water management, and its implementation relies on its acceptance in politics and society [10]. Thus, the challenges of the water sector are diverse and complex. Environmental protection and maintaining or improving living conditions require an intensive interdisciplinary collaboration.

Nevertheless, contemporary research is institutionalized, mainly in a disciplinary manner [11]. Literature on interdisciplinary research acknowledges that disciplinary approaches are and will always be an important part of any problem-solving strategy [12]. However, to tackle environmental challenges, disciplinary approaches, knowledge and skills need to be integrated to address problems in their full complexity [12–14]. Experts from many disciplines must assist society in a collaborative way, developing solutions for pressing sustainability challenges [11,15]. Tackling complex and multifaceted problems is therefore one key argument for interdisciplinary research [16].

Whether and to what extent research is shifting away from its institutional disciplinary orientation are the subjects of this paper. Here, publications as the subject of scientific output will serve as a measure of interdisciplinary research. We apply a statistical similarity metric to evaluate whether research disciplines are approaching the increasing challenges of water management in an interdisciplinary manner. Using three different resolutions that delineate research fields with increasing levels of detail, we discuss the importance, challenge and opportunities of interdisciplinary research.

2. Understanding Interdisciplinary Research

Although the term interdisciplinarity is used ubiquitously, there is no universal definition [11,17,18]. According to Clark and Wallace, interdisciplinarity is one way to address knowledge specialization in order to solve complex problems [12]. While generally organized in disciplines, interdisciplinarity aims to integrate academic knowledge derived from usually distinct disciplines. Being therefore more demanding than the additive approach of multidisciplinarity, it is less sophisticated than transdisciplinarity. The latter focuses on transcending disciplines to a point where boundaries between them are blurred [12,19], which would achieve the highest degree of interdisciplinarity

Assessing interdisciplinarity faces the challenge of accounting for the degree to which the research output was conducted in an integrative manner [17]. Bibliometric approaches provide a diverse set of quantitative measures to describe research outputs in this regard and have been established as a reliable method (e.g., term counts, publication counts, citation analysis) [11,18,20–22]. The most common bibliometric approach is citation analysis [11,18]. It infers the relation between disciplines from the frequency of accompanying citations. It uses publications as the major research output, although other types of research outputs (e.g., publication counts or keyword counts) may be used [11,23,24].

A requirement for citation analysis is the subdivision of research into subject areas that correspond to disciplinary structures. Here, many bibliometric analyses inherit the categorization provided by scientific literature databases, e.g., Web of Science or Scopus [11,21,25–27]. While this is sufficient for investigating the distribution of subject areas, this does not account for any cooperation of researchers of different disciplinary fields. The subject areas of publications treating a certain issue could mirror the interest of a vast diversity of disciplines; however, all the underlying research may well be conducted in strict disciplines. Therefore, accounting for interdisciplinarity needs a more elaborate measurement indicating the cooperation between disciplines in publishing research outputs.

The core research objectives of this manuscript therefore are:

1. Introduce a measure of interdisciplinarity from citation analysis.
2. Generate a representation of interdisciplinary collaboration ("links") between individual research disciplines.
3. Gain insights into the interdisciplinary nature of research about heavy precipitation.
4. Account for the temporal development of interdisciplinary research on heavy precipitation.

3. Method

The extent of integration of knowledge from different research fields is an indicator for assessing interdisciplinarity, which is a real synthesis of approaches, whereas in multidisciplinarity, different units from different research fields conduct separate research on the same topic [11,23,24]. It can be measured by the differentiation of research fields of cited references. One approach is the integration score I (Equation (1). It implies that greater variety in cited research fields results in greater interdisciplinarity [28].

$$I = 1 - \sum(s_{ij} \cdot p_i \cdot p_j) \; 0 \leq I \leq 1 \tag{1}$$

s_{ij}: cosine similarity of research field ij
p_i: proportion of referenced research field i
p_j: proportion of referenced research field j

The integration score is based on the application of cosine similarity, expressed by the factor s_{ij}. It determines the similarity of two research fields based on the geometrically mappable distribution of referenced research fields [29]. The integration score is thus an expression of interdisciplinary research in one research field in relation to the entirety of all research fields.

In order to map the collaboration between two individual research disciplines ("exclusively"), integration score and cosine similarity are not appropriate. By aggregating I as an association to all research disciplines s_{ij}, the information of functional composition is lost.

To avoid impairment of these relationships, they can instead be mapped using the Jaccard similarity matrix (Equation (2)).

$$\mathrm{J}(\mathrm{D_i}, \mathrm{D_j}) = \frac{|D_i \cap D_j|}{|D_i \cup D_j|} \tag{2}$$

It expresses the proportion of the intersection of two research fields $|D_i \cap D_j|$ to the entirety of their representations (union) $|D_i \cup D_j|$ in a data corpus. The multiplication of the Jaccard matrix J with the proportional parts p_i and p_j analogous to Equation (1) is omitted. The proportionality is already considered in the denominator (Equation (2)).

Both similarity metrics are based on frequency vectors or matrices that numerically capture the relationship between the research fields. Commonly, similarity metrics are calculated by strictly separating papers and references expressed by co-citation profiles [28,30]. A major drawback of this method is that integration is not properly mapped as the relation between research fields of papers and those of references is missing (Figure 1). Neither the interaction among the research fields of the paper (multidisciplinary), nor the interaction between the research fields of the different literature sources are taken into account (no integration, see Figure 2). Instead, a primary–secondary relationship between paper and the reference research field is induced. In the frequency matrix, only the relationship between paper and referenced research field is mapped as (unique) tuples. Each tuple ij leads to an increment in cells ij and ji of the frequency matrix (Figure 2).

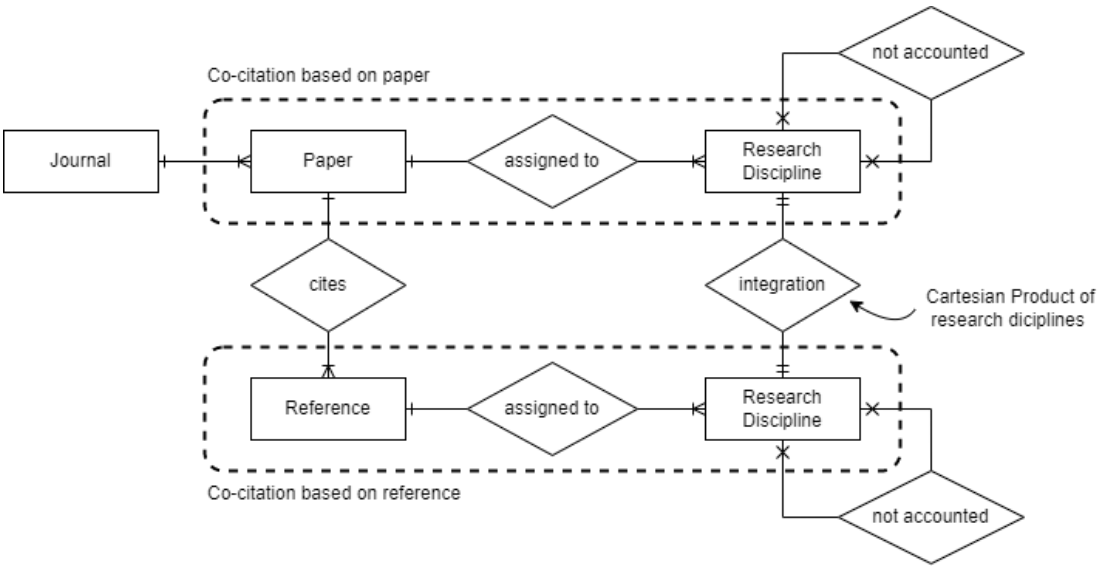

Figure 1. Primary–secondary relation between paper and its references. In conventional co-citation based on paper research disciplines the Cartesian relation between all research disciplines of a paper would be accounted for (upper dashed rectangle). Likewise, in conventional co-citation based on references the Cartesian relation between all referenced research disciplines would be considered (lower dashed rectangle). In this work, solely the Cartesian product between paper research disciplines and referenced research disciplines is considered.

Table 1. Corresponding frequency matrix for Figure 2.

	1 (Diamond)	**2 (Circle)**	**3 (Star)**	**4 (Triangle)**
1 (diamond)	2	1	1	1
2 (circle)	1	2	1	1
3 (star)	1	1	1	0
4 (triangle)	1	1	0	1

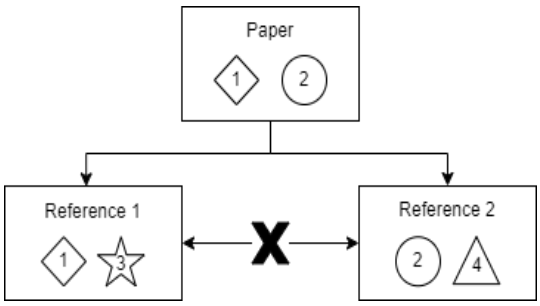

Figure 2. An arbitrary paper assigned to research disciplines 1 (Square) and 2 (Circle). It is assumed that integration of knowledge takes place between the research disciplines of the paper and those of the references, 1 (Square), 3 (Star) and 2 (Circle), 4 (Triangle), respectively. In common co-citation profiles the relation of references 1 (Square) and 2 (Circle) as common references of paper 1 are accounted for (line marked with X). However, there is no similarity between reference 1 (Square) and 2 (Circle), if it was not for the citing paper. Their relationship fails to satisfy the prerequisite of integration. Thus there is no increment for the relation of 3 (star) to 4 (triangle) in the corresponding frequency matrix (Table 1). On the main diagonal, the absolute occurrence of a research discipline is denoted.

The data basis for the frequency matrices $|D_i \cap D_j|$ and $|D_i \cup D_j|$ according to Jaccard is an excerpt from the Scopus database. The excerpt is based on the search string (Figure 3). On the one hand, the search string must be specific enough to represent the key topics of the chain of effects of a heavy rain event up to the urban flash flood. On the other hand, it has been chosen to be abstract enough to include all disciplines and as many subdisciplines as possible. Therefore, commonly used terms were used as hints for the database query. The coverage of the disciplines in the search hits was checked manually. Random 5% of the search hits (881 of 17,622) were manually checked for false positives, and 1.25% (11) of the subset was flagged accordingly. Assuming equal distribution of false-positive hits, the proportion was found to be negligible.

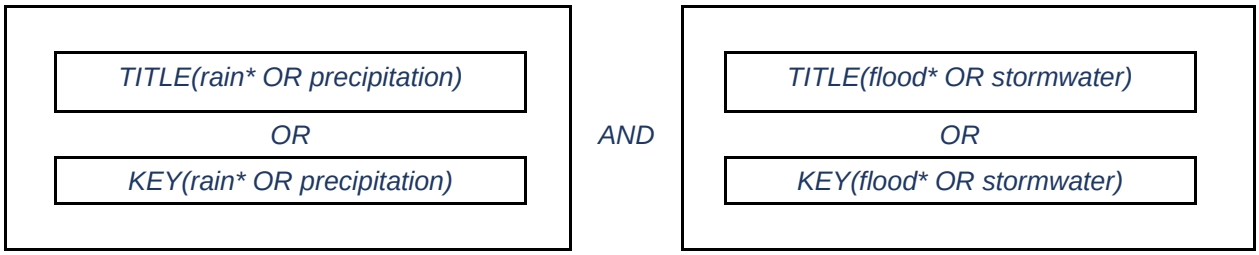

Figure 3. Search string to delimit the research field.

The disciplines are determined based on the Scopus categorization and are presented in three resolutions. Four clusters are divided into 27 areas and the latter are aggregated from 318 classifications (Table S1).

The bibliometric data were retrieved from Scopus via its API using Python 3.7 and the library pybliometrics [31]. Jaccard matrix was computed using Python. Statistical analyses were performed with R (v. 4.1.0). Non-metric multidimensional scaling (NMDS) was performed based on the Jaccard matrix with 1000 permutations using the R package Vegan [32]. The mean of the subject clusters was statistically compared using a pairwise Wilcox test [33,34]. Results were visualized with the R package ggplot2 [35] and CorelDraw21.

4. Results

A total of 17,622 articles from the period 1972 to 2020 with a total of 300,918 references were recorded using the search string. In total, 4,936,622 subject–area relationships were determined from the paper–reference ratios. The frequency distribution among the 27 subject areas can be seen in Table S2.

4.1. Resolution of Interdisciplinarity

The method results in a symmetric Jaccard matrix (Tables S3 and S4). Each cell of the matrix describes pairwise similarity between two disciplines. The hierarchical relationship between a paper and its references (Figure 1) induces integration; thus, each cell is a unitless measure of interdisciplinarity. The larger the entry, the higher the interdisciplinarity between these two subject areas. The column or row sum is an expression of the overall interdisciplinarity of a discipline. A completely transdisciplinary discipline would have the value 1 in each field, D_{ij} for $i \neq j$, in the column- or row-wise consideration of the Jaccard matrix (Equation (3)) as transdisciplinarity is defined as a fully integrative manner of knowledge without boundaries between individual research fields [11]. The maximum interdisciplinarity is then represented with $I = n - 1$ (with n = number of research fields).

$$\sum_{i=1,j=const.}^{i=n,j=const.} D_{ij} \text{ or } \sum_{i=const.,j=1}^{i=const.,j=n} D_{ij} \text{ for } \; i \neq j \tag{3}$$

For the $n = 27$ subject areas of the studied dataset, I can be represented with values between 0 and 26. Here, no subject area pairing has a transdisciplinary relation since all $D_{ij} < 1$.

Expression I behaves linearly, and larger k-fold expressions signal k-fold interdisciplinarity. The actual summed interdisciplinarity of the subject areas ranged from $I_{min} = 1.121 \times 10^{-4}$ to $I_{max} = 6.839 \times 10^{-1}$ (Figure 4A). Thus, compared to the potential maximum value, the results are low. The mean value is $I_m = 2.030 \times 10^{-1}$. Based on the average aggregated interdisciplinarity of subject areas, a hierarchy can be derived for the disciplines. The frontrunner physical sciences is 3.724 times more interdisciplinary than health science, 2.492 times more than social sciences and humanities, and 1.625 times more than life sciences.

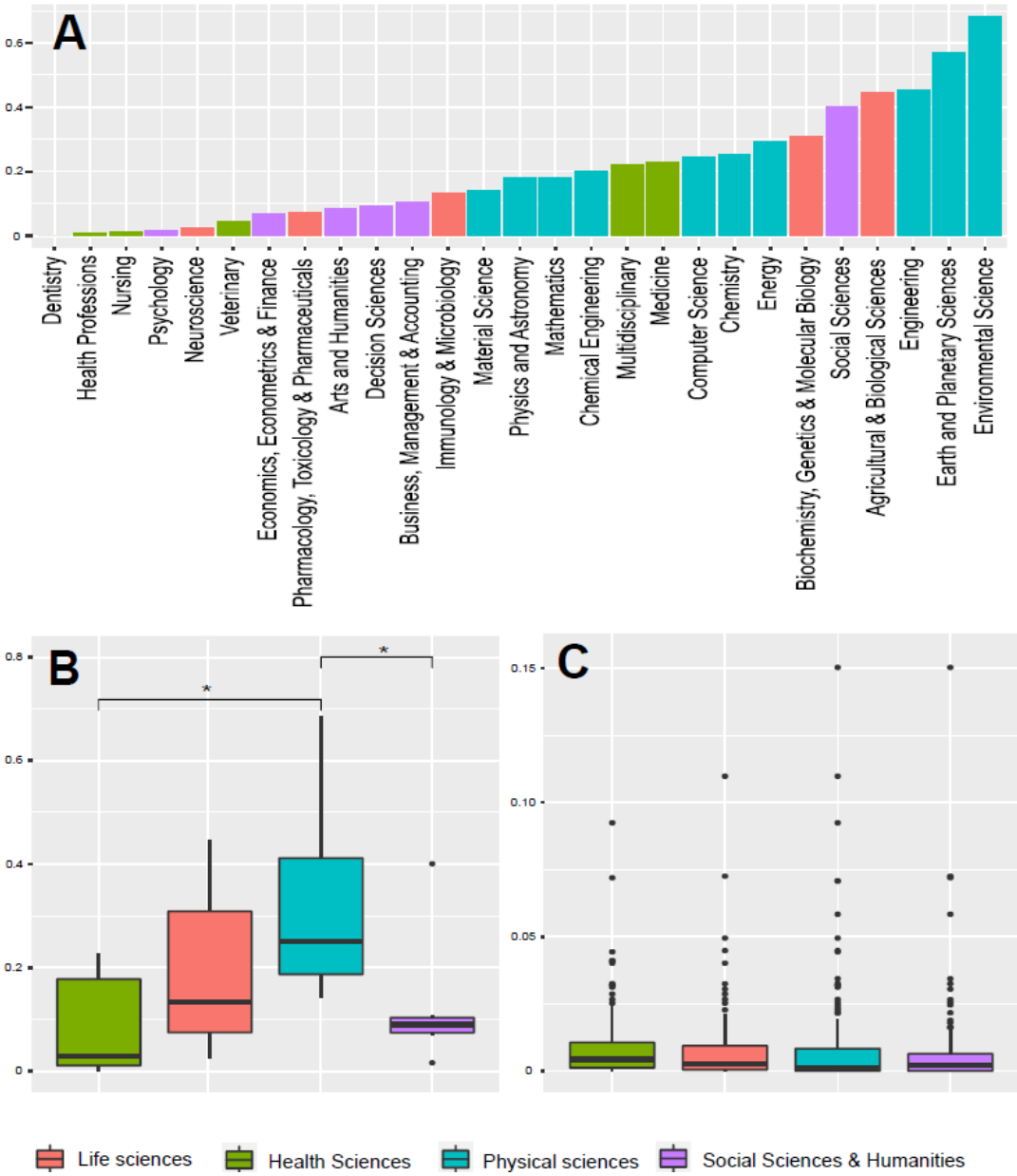

Figure 4. (**A**) Aggregated Jaccard similarities per "Subject Area" for the whole dataset ranging from 1972–2020. Showing the total interdisciplinarity per "Subject Area". Colors denote the affiliation to scientific disciplines. (**B**) Boxplots of the aggregated Jaccard similarity per discipline (Table S1) for the complete time span. (**C**) Boxplots of the Jaccard similarity per "Classifications" per discipline. Significant differences are marked with an asterisk and denote a p-value of less than 0.05.

Statistical relevance is tested by comparing the aggregated Jaccard values per subject area among the scientific disciplines (Figure 4B). Pairwise comparison of the subject area means shows that physical sciences are significantly more interdisciplinary than health sciences and social sciences and humanities ($p < 0.05$), while there is no significant difference between physical sciences and life sciences ($p = 0.093$).

However, the upper whiskers or upper outliers and their difference to the median indicate that this observation is based on pioneers of interdisciplinarity (Figure 4B) (e.g., the "Subject Area": "Social Sciences"). This observation is confirmed in the finest resolution ("Classifications") as shown by the median close to the lower quartiles (Figure 4C). Here, there are clear outliers upwards but not downwards, as the median and 75% of the values are close to zero. This gives evidence that despite all indications of interdisciplinarity, the default mode of research is disciplinary.

4.2. Temporal Development of Interdisciplinarity

Hierarchy and statistical relevance (Figure 4) of the clusters is constant over time with the data split into ten-year time intervals from 1983 on (Figure 5). This analysis indicates a trend to higher interdisciplinarity over time. A steep rise took place from 1983 to 1992, after which the development plateaued over the next three decades. The most recent decade has shown another growth in interdisciplinarity.

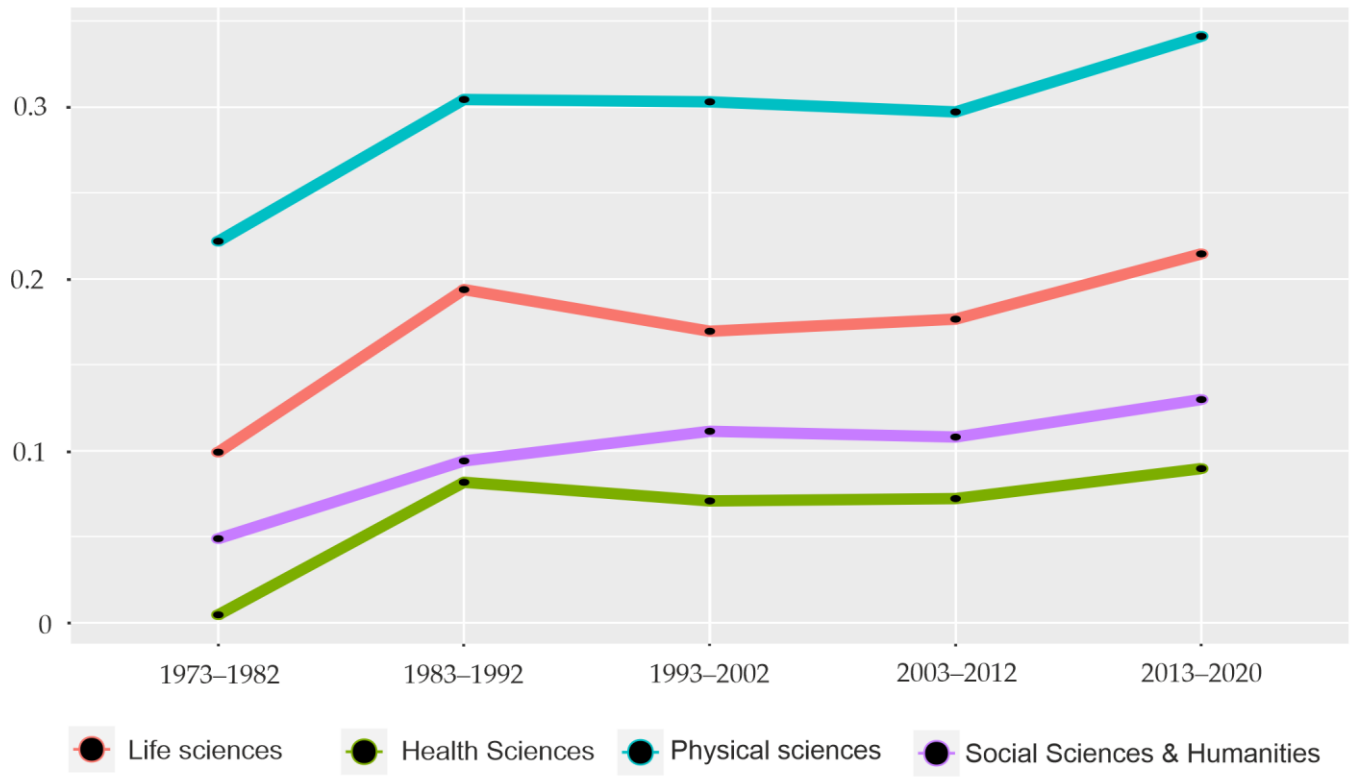

Figure 5. Aggregated Jaccard similarities per "Subject Area" over time. Dots denote the aggregated Jaccard similarity per time span and colors denote the affiliation to distinct "Clusters".

4.3. Interdisciplinary Placing

Figure 6 shows the interdisciplinarity of individual subjects and their affiliation to individual scientific disciplines. The stress value of 0.137 denotes a decent ordination. The ordination is determined by the number of edges and their rank-based strength. Nodes close to each other are more similar, thus more interdisciplinary. Generally, nodes in the center are more interdisciplinary as their ordination in the center is based on a relatively high similarity, thus interdisciplinarity, with all other nodes. A clear pattern is observable as distinct scientific disciplines form clusters. Namely, "Physical Sciences" are rather

located in the middle and the other clusters are rather close to nodes from their own cluster. This ordination shows that subject areas are rather more collaborative within their cluster than with other clusters. Physical sciences form the center of the ordination, thus distinguishing it as the most interdisciplinary cluster. Taking the strength of edges into account points out that the strongest connections are between the physical sciences, social sciences and Humanities and life sciences, indicating that these disciplines exhibit a higher interdisciplinarity. The results stay relatively stable when using a higher resolution, namely, scientific classification instead of scientific areas (Figure S1).

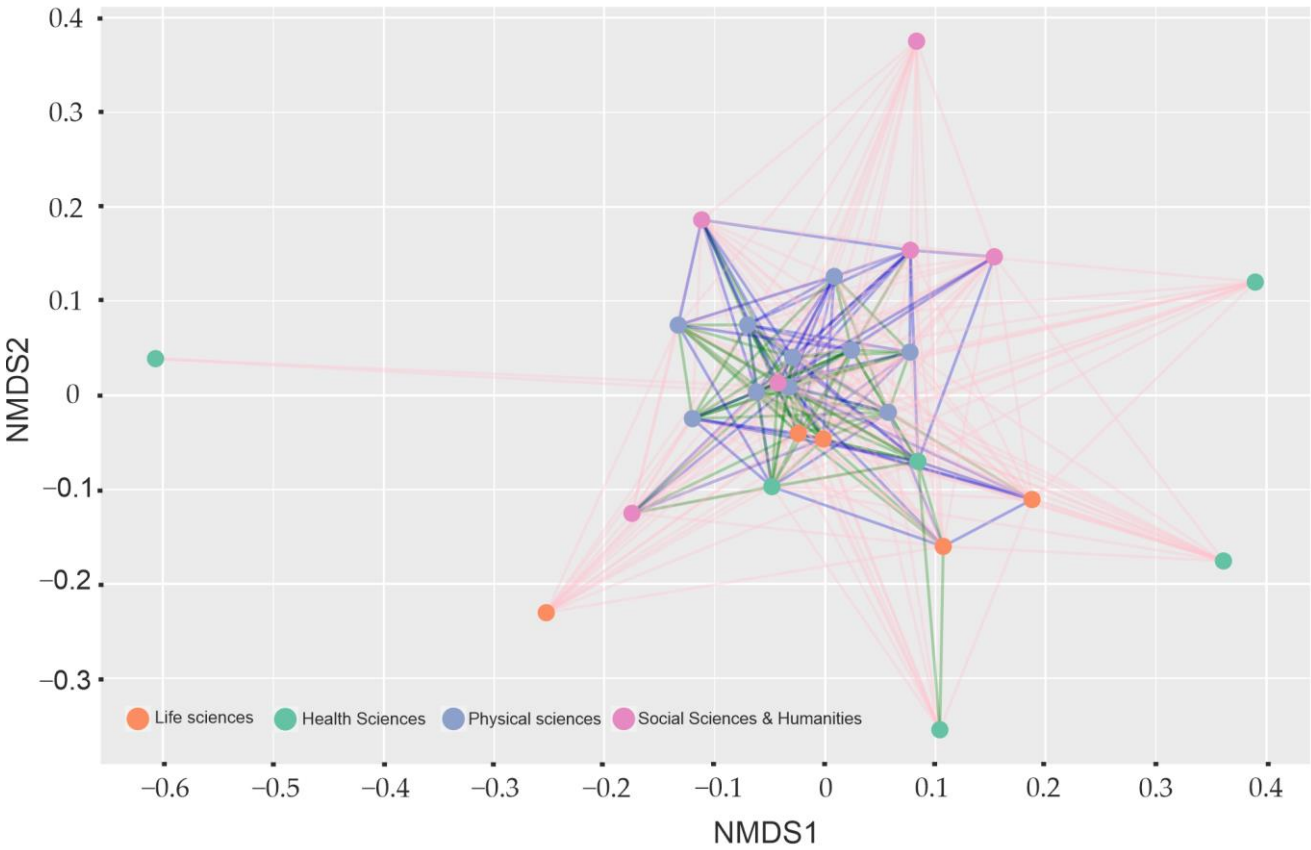

Figure 6. NMDS of the scientific disciplines based on Jaccard dissimilarities. Stress value: 0.137. Point colors denote the affiliation of individual "Subject Areas" to scientific disciplines. Orange: life sciences, pink: social sciences and humanities, blue: physical sciences, green: health sciences. Lines denote the pairwise Jaccard similarity between nodes. Red: below the median of the dataset (0.02967), blue: below the mean (0.06486), green: higher than the mean.

5. Discussion

A bibliometric method assessing interdisciplinarity based on papers and their cited references was introduced, based on Scopus entries between 1972 and 2020. Interdisciplinary collaboration was mapped by a co-citation matrix based on integration employing a bottom-up approach [9]. For the first time, a picture of interdisciplinary research in the field of heavy precipitation is drawn.

The literature agrees on interdisciplinarity as a highly desirable paradigm for multisectoral research. In recent years the demand and application of interdisciplinary collaboration has gained popularity [36–39]. However, the analysis presented here highlights this being only partly implemented in research on heavy precipitation (Figure 4A). Although interdisciplinarity has increased, its actual numerical increase is negligible (Figure 5). A small number of "Classifications" account for a large share of the research area's interdisciplinarity (Figure 4C), indicating that relatively few areas of research are characterized by interdisciplinary work. At the same time, this implies that the majority of research areas

show a lack of interdisciplinarity. Since many universities, research institutes and funding bodies are still structured in a disciplinary manner, it is not surprising that the results reveal the described pattern [40,41]. Furthermore, it proves that even in the digital age with extensive information availability, disciplinary glasses are worn and interdisciplinarity is not the modus operandi (Figure 6).

As with other challenging research topics, the collaborative research output related to heavy precipitation events has experienced an increase over the last 50 years [42]. However, an appreciable temporal development of interdisciplinarity is not detectable (Figure 5). The level of interdisciplinarity has remained relatively constant over the last 40 years with an initial increase 50 years ago, while the number of publications has generally increased over time. Hence, based on these results, it is obvious that despite the simplicity in exchange and availability of information in the age of the Internet, complex issues in socio-economic contexts are often not addressed in an interdisciplinary manner.

This result becomes even more intriguing considering the ever-growing number of branches of research. With newly emerging research fields, an increase in interdisciplinarity is mandatory for the subject disciplines as any collaborative effort leads to more interdisciplinarity. Thus, measured by the initial and final values, which behave linearly, the actual increase over the last 40 years is trivial. Thus, the assumption of a growth in interdisciplinarity over time can be refuted. However, increased interdisciplinarity could still be particularly true for individual disciplines (Figure 4C).

The analysis detected some collaborations and placing between and within clusters. "Physical Sciences" were found to be the most interdisciplinary cluster (Figure 6 and Figure S1, but also see Figure 4A,B and Figure 5). Thus, it is pivotal for research on heavy rainfall. For a long time, the issue of heavy precipitation events have been an important topic in many subject areas in physical sciences, particularly in "Environmental Science", "Earth and Planetary Science" and "Engineering", for which environmental-related scenarios are at the core of their research. The hierarchy of the four subject areas with respect to heavy rainfall events is coherent, namely, in descending order: physical sciences, life sciences, social sciences and humanities and, finally, health sciences. Heavy rain events have the very first point of contact with physical sciences, as they deal with the immediate problem; be it solutions for the drainage of large water volumes or the interim storage of water. However, after the failure or the overuse of physical strategies, which can happen for various reasons (e.g., climate change, growing population), other areas are affected to a greater scale. Certainly, this is generalized, as for the physical solutions, but the encouragement of the other areas is needed.

Life sciences, second in the order, are affected after the management of heavy rainfall fails, even for short time events or even single events. Stormwater runoff is known for carrying particular loads and pollutants, thus having eventual toxic and anaerobic effects for aquatic organisms. The impairment of biological services and functions (e.g., ecosystem services, unusable recreational areas) call for the third subject area, social sciences and humanities, to come into play, which ensures that public understanding is sensitized and that the acceptance of making the scarce resource of money available is increased. Health sciences are last in line; however, that does not imply that it is of less importance. Heavy rainfall events cause threats to human life and not just by physical factors. Pathogens are known to be discharged into the environment due to sewage overflow, thus posing risk to human health. At this point one has to acknowledge that the used search string may be selective for individual scientific fields or even subject areas, which does not make the results less trustworthy, but makes them highly specific for the heavy rainfall events. Analyzing another dataset created with a different search string will certainly change the hierarchy of subject areas regarding their interdisciplinarity. However, it was not the objective of this study to make a general statement about interdisciplinarity or the interdisciplinarity of another challenging research field, but to investigate the interdisciplinarity regarding research affiliated with heavy rainfall events by using a new approach.

Certainly, bibliometric analyses are affected by limitations [43]. Drawing general conclusion of interdisciplinarity is not possible when applying pre-selective search strings. For doing that, one has to use either a neutral search string, which is barely possible due to the unique and biased language in different research fields, or one has to work with randomly chosen publications, which are large enough in number to be representative for the used database. Using the shown method here, with a representative and randomly chosen publication dataset, would be a promising approach to make a statement about the general interdisciplinarity. Bibliometric databases such as Scopus or Web of Science vary concerning their coverage of research areas and types of publications [23,26]. Authors from the "Social Sciences", "Humanities" and some applied fields often publish in books, reports, proceedings and journals, which are not recorded by bibliometric databases [11]. Furthermore, "Social Sciences" and "Humanities" results are often published in the language in which they were collected. Therefore, Scopus and Web of Science are unbalanced between countries and languages [26]. Furthermore, scientific databases such as Scopus, Web of Science or Google Scholar use different assignments of scientific fields. Therefore, interdisciplinarity varies based on the underlying codification [11]. In addition, this study is limited due to the defined search term. The pre-selection, performed by the use of search terms, enabled it to perform a bibliometric analysis on the research areas dealing with heavy rainfall events. However, at the same time a search term always either defines the topic too narrowly or is too general to precisely cover the desired research field.

6. Conclusions

The method introduced here makes interdisciplinarity more tangible and measurable. Furthermore, this approach is replicable and based on already accessible data structures. It shows how the current state of research is still struggling to accomplish multisectoral outcomes. While interdisciplinarity is required and encouraged, we show here that the actual interdisciplinarity of research fields dealing with heavy rainfall events is mostly considered to be low. This implies that the vast majority of research fields are not satisfactorily interdisciplinary and that despite the recent popularity of interdisciplinarity, it is a rather rare phenomenon. In addition, we show that when different research fields collaborate, most of them work under a common overarching research cluster (e.g., life sciences and health sciences).

However, crossing disciplinary boundaries in a more ambitious way is feasible. This is proven by a few fields of research excelling over others in terms of their interdisciplinary collaboration.

If interdisciplinarity is the gold standard for complex challenges such as research regarding heavy rainfall events, one would have expected a more dynamic growth. Over the last five decades, interdisciplinarity has increased, but, especially under the premise of extensive information availability, this development can be neglected. Accordingly, the underlying potential to address a wide variety of issues (e.g., heavy rainfall events) is enormous, creating tremendous opportunities as soon as a truly interdisciplinary approach is employed. Starting from the results of this analysis, obstacles must be identified and ways of overcoming them have to be sketched out.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14193001/s1. Table S1: Dictionary of the existing Scopus hierarchy of individual subject areas, associated classification abbreviation and codes, Table S2: Frequency distribution of the 27 subject areas within the data corpus, Table S3: Symmetric Jaccard similarity matrix of the 27 subject areas, Table S4: Symmetric Jaccard dissimilarity matrix of the 318 Classifications, Figure S1: NMDS of the "Classifications" based on their affiliation to their superordinate "Subject Areas" and their Jaccard dissimilarities, after removing outliers to enhance the resolution, as individual single "Classifications" had a dissimilarity of 1 to every other "Classification". Stressvalue: 0.0586. Point color denote the affiliation of individual "Classifications" to "Clusters". Orange: life sciences, pink: social sciences & humanities, blue: physical sciences, green: health sciences.

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General discussion

The Anthropocene poses one of the most pressing questions: how to detect and comprehend the human impact on ecosystems. This comprehension is essential for implementing effective countermeasures, as addressing a problem requires both recognition and understanding (Geisen et al., 2019; He & Silliman, 2019; Wu et al., 2022). Neglecting the health of our ecosystems carries a cost, ultimately borne by living organisms, including ourselves. The fascinating world of microorganisms, though frequently overlooked, harbors promising potential as indicators for environmental disturbances and for a profound understanding of ecosystem functioning (Curds & Cockburn, 1970; Foissner, 1999, 2016; Pawlowski et al., 2016; Uhlmann, 1998). Yet, there is a lack of clarity regarding which specific techniques, organisms, and habitats should be prioritized when utilizing microbes to assess the impacts of stressors and monitor environmental changes. One of the most crucial aspects for adequate research is delineating the scope, ensuring focused and meaningful investigations. Once a research question is posed, scientists must strive to define the appropriate methods that ultimately enable them to answer the question. Here, I present a systematic approach to using microorganisms as indicators for environmental changes and assessing the impacts of stressors on them.

The initial step was to determine the target group of organisms as the research basis. While various organismic groups could have been chosen (Zaghloul et al., 2020), it is pivotal to commence with the bedrock of our ecosystems, focusing on organisms that form the cornerstone of higher trophic levels (Azam et al., 1983; Calbet & Landry, 2004; Caron et al., 2009; Pomeroy et al., 2007). Hence, microorganisms, both prokaryotic and eukaryotic, stand out as ideal candidates for analyzing the impact of human-induced stressors (Foissner, 1999, 2016; Pawlowski et al., 2016; Sumampouw & Risjani, 2014). These tiny yet essential life forms wield immense influence on the entire ecosystem, making them key organisms for assessing the repercussions of human activities.

„*Centers of endemism of freshwater protists deviate from pattern of taxon richness on a continental scale*“

However, the choice of microbial communities necessitates a more nuanced approach due to their ubiquitous presence and remarkable diversity. Therefore, I began by pinpointing a specific group of microbial communities to establish a solid foundation for the origin of the community, later enabling generalizable conclusions. It is crucial to think about the habitat of origin, whether it should be aquatic or terrestrial. Furthermore, variations in microbial communities may be influenced by altitude, introducing an additional layer of complexity to the delineation

process. As demonstrated in article I, I provided evidence that communities indeed exhibit discernible differences based on the altitude at which they are situated. Highland habitats encompass various factors, including elevated UV radiation, lower temperatures, limited nutrients, and shorter growing seasons when contrasted with lowland habitats. These factors play a pivotal role in shaping the communities (Sommaruga, 2001). While human influences are affecting highland areas and lowlands at the same time, lowland areas have a higher population density and more human activities like agriculture, urban development, and industrial zones (Kummu et al., 2016; Underwood et al., 2009). This increased human presence leads to a greater introduction of pollutants and stressors. Additionally, lowlands often collect and channel water and pollutants from higher areas due to natural drainage patterns (Koga et al., 2022). This runoff carries pollutants, pesticides, and other contaminants from various sources to lowland ecosystems. While these points may on the one hand indicate that lowlands areas are already heavily impacted and therefore it would be beneficial to rather analyze pristine highland communities, one could, on the other hand, argue that highland areas rather inhabit different microbial communities, which are less likely to be impacted compared to lowland areas. This was shown by my research. Highland microbial diversity differs from that of lowlands, with highlands being more inhabited by putative endemic microeukaryotes. Therefore, in order to get a more generalizable picture, it is preferable to investigate lowland areas, which have fewer endemic microeukaryotes.

„*Protistan and fungal diversity in soils and freshwater lakes are substantially different*“

The differentiation between aquatic and terrestrial habitats represents a fundamental classification. These habitats may be interconnected, potentially sharing organisms, exchanging nutrients or acting as protective buffers for each other (Graupner et al., 2017; Lennon & Pfaff, 2005). Therefore, a comprehensive examination of both habitats simultaneously would be crucial to ensure the validity of statements. While this aspect was partially explored for fungi and prokaryotes (Crump et al., 2007, 2012; Monard et al., 2016), there has been a lack of information until now regarding the interconnection of microeukaryotic communities across habitat boundaries. My research has shown that freshwater lakes and adjacent terrestrial areas of the direct catchment area exhibit separate protist communities, as the overlap of organisms is very low. This implies that for analyzing human-induced stressors, it is acceptable to selectively study either freshwater lakes or soils, as their communities are clearly separated entities. Thus, studying both habitats simultaneously is not mandatory for obtaining valid and generalizable results.

However, it is essential to note that, even though there is no prevalent link based on their communities, they do affect each other in other aspects. Nearby lakes influence the soil humidity in the catchment area, and events like flooding or heavy rainfall introduce nutrients into water bodies (Baldwin & Mitchell, 2000; Beltman et al., 2007). While it has been demonstrated that flooding does not have a long-term impact on the respective communities (Graupner et al., 2017), there are other indirect effects (Junk et al., 1989; Vannote et al., 1980). Abiotic factors may lead to the introduction of pollutants into lakes (Koga et al., 2022; Lennon & Pfaff, 2005). Agricultural runoff, containing herbicides and pesticides, is a potential source of these pollutants. Nonetheless, it should be acknowledged that some pollutants, such as those found in treated wastewater, can also transition from freshwater lakes to soil (Durán–Álvarez & Jiménez–Cisneros, 2014; Likens & Bormann, 1974). However, the predominant direction of pollutant introduction is from soil to freshwater, as many pollutants originate from terrestrial sources (e.g., pesticides, herbicides, tire abrasion) (Schulz et al., 2015; Schwarzenbach et al., 2006). Hence, selecting exclusively freshwater lakes for the analysis of anthropogenic influences is a justified choice. One must emphasize the valid point that the impact extends beyond freshwater ecosystems; marine habitats are undoubtedly affected, and due to their vast expanse, they represent the largest ecosystems on Earth (Aryal et al., 2015; Pedrós-Alió, 2006; Pettipas et al., 2016; Sarkar et al., 2006; Sogin et al., 2006; Turner, 2010; Yanko et al., 1999). It is crucial to recognize the fundamental differences between marine and freshwater habitats, as community structuring and shaping factors such as physio-chemical properties are fundamentally different (Aryal et al., 2015; Blomqvist et al., 2004). Consequently, both marine and freshwater habitats require separate in-depth study. In my research, I specifically focused on freshwater ecosystems. This emphasis arises from recognizing freshwater as not only one of the most essential but also constrained resources on Earth (Calvin et al., 2023; Gleick & Cooley, 2021). The significance is underscored by the fact that most inland waters are freshwater bodies.

„*Effects of stressors on growth and competition between different cryptic taxa affiliated with Ochromonadales (Chrysophyceae)*“

Studying microorganisms involves various techniques such as microscopy and Next-Generation Sequencing (NGS) (Eiler et al., 2013), but determining the most suitable method is not immediately clear-cut. Each technique has its advantages and disadvantages, requiring careful consideration in selection (Foissner, 1999; Varma & Sharma, 2017; Zamyadi et al., 2019). The extensive diversity of microeukaryotes presents both an opportunity and a challenge. Microscopy offers information on absolute abundance and morphology but demands specialized taxonomic and morphological knowledge, posing challenges, especially given time

constraints (Foissner, 1999, 2007; Weber & Pawlowski, 2013). My findings, as outlined in article III, revealed that morphologically indistinguishable species, referred to as cryptic species, exhibit distinct responses to stressors. This poses a significant challenge, as different species may be concealed within a single morphospecies, potentially leading to misleading results. This is where NGS stands out, as it can differentiate morphospecies into molecular defined OTUs (Bickford et al., 2007). Additionally, morphological specialist knowledge is not mandatory, and processing hundreds of samples and thousands of OTUs is possible. Given my discovery that similar morphospecies react differently to stressors, I suggest that the use of high-throughput sequencing is a suitable approach to study anthropogenic stressors on microbial communities. Nonetheless, it is essential to bear in mind that morphological characteristics remain elusive to NGS. The decision to incorporate microscopic data hinges directly on the specific research question at hand, given that morphological traits are otherwise impervious to detection. Relying solely on NGS poses the risk of overlooking information concerning structural adaptations and variations that can significantly impact a microorganism's resilience to stress. Nevertheless, while NGS stands as a robust standalone method for analyzing community patterns, relying solely on microscopy introduces complexities. Microorganisms may elude correct identification using microscopy, resulting in the ascription of morphological traits not to individual taxa but to morphologically indistinguishable species complexes (Bickford et al., 2007; Weber & Pawlowski, 2013). The challenges of routine light microscopy, methodological intricacies, operator fatigue, varying operator expertise, and the potential for taxonomic bias contribute to uncertainties. Consequently, taxonomic records derived from microscopy often lack comprehensiveness (Stern et al., 2018), while molecular data prove more adept at detecting a broader range of taxa (Charvet et al., 2012; de Vargas et al., 2015). Additionally, NGS provides the option to expand, reanalyze and to store datasets indefinitely. This allows data to be structured in a modular way and, at the same time, theoretically comparable to other studies. This advantage is notably absent in microscopy due to the limited durability of samples, numerous species lacking morphological descriptions, a scarcity of user-friendly identification literature, and the introduction of bias by taxonomic experts (Foissner, 1999).

Equipped with the appropriate molecular tools, I delved into the effects of anthropogenic non-point source inputs on microbial communities. Additionally, I conducted a comparative analysis of different methods to assess their potential for monitoring environmental pollution.

„*Microbial community shifts induced by plastic and zinc as substitutes of tire abrasion*“

Plastics, being ubiquitous and persistent pollutants, are a prevalent concern (Azevedo-Santos et al., 2021; Dusaucy et al., 2021; Smith et al., 2018; Thushari & Senevirathna, 2020). Tire abrasion represents a significant pathway for plastic entering the environment (Kole et al., 2017; Sommer et al., 2018). In article IV, I scrutinized the impact of tire abrasion proxies on microbial communities. My findings revealed that while inert nanoplastic particles had no discernible effect on the microbial community, the accompanying substances, such as zinc in my study, exerted a substantial impact. This suggests that microeukaryotes possess mechanisms to deal with nanoparticles of various materials (e.g., silicate, polystyrene), and it is the leaching substances that trigger impairments (Amelia et al., 2021; Bradney et al., 2019; Buffle, 2006; Nowack & Bucheli, 2007; Zhu et al., 2021).

Although diversity indices remained unaffected, exposure to zinc resulted in a pivotal community shift. Therefore, my study highlighted that diversity indices, like Hill number and species richness, may not consistently indicate community stress. While a change in diversity signals stress, unchanged diversity does not necessarily imply an unstressed community. I demonstrated that zinc induced shifts in functional abundance, with organisms capable of phagotropy benefiting, while the phototrophic community diminished. However, my research provided evidence of functional redundancy as photosynthesis rebounded, now driven by green algae instead of diatoms. Moreover, the functional community composition, particularly nutrition modes, began to recover towards the end of the 14-day exposure experiment.

In essence, my study indicates that while anthropogenic factors do impact communities, these communities exhibit a certain resilience. However, it is crucial to acknowledge that tires contain numerous leaching substances (Councell et al., 2004; Klöckner et al., 2021; Wik & Dave, 2006, 2009), and simultaneous exposure to other stressors like climate change could result in significant community impairments (Vos et al., 2023).

„*Temporal disturbance of a model stream ecosystem by high microbial diversity from treated wastewater*“

Human-induced non-point source pollutants are diverse, with one in particular being highly prevalent and directly correlated with population densities and urban areas (Borck & Schrauth, 2021; Chen et al., 2016; Kocasoy, 1995). This pollutant predominantly affects freshwater and coastal ecosystems (Creel, 2003; Osorio et al., 2016). Treated wastewater, often reintroduced into receiving water at levels reaching 77%, is a major contributor to the water level and pollution (Drewes et al., 2018; Guillet et al., 2019). Surprisingly, most studies on treated

wastewater overlook its impact on microbial communities (Adegoke et al., 2018). My research addressed this gap by examining the effects on microbes.

I found that treated wastewater introduces microbes, viruses, and antibiotic resistance genes into receiving waters, causing a significant and temporary disturbance in the freshwater stream microbiome. Notably, human pathogens like *Legionella* were among the introduced microbes. However, my 10-day exposure experiment revealed that the microbial and viral community structure recovered, trending towards an unstressed state. These results suggest that freshwater streams and their microbial and viral communities exhibit a considerable resilience, even in the face of a substantial influx of treated wastewater. It is important to highlight that my study, conducted in circular large-scale mesocosms, might not fully capture the resilience of freshwater rivers under entirely natural conditions, such as a continuous stream. In a natural setting, continuous dilution would likely mitigate the effects, making the impact on a natural river potentially even less pronounced.

„*Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater*“

I demonstrated, through findings presented in article V, that treated wastewater introduces prokaryotes. Building on this, article VI showcased that microeukaryotes and a range of organic chemicals are also introduced with treated wastewater, as assessed through NGS and non-target screening. I utilized these datasets with the overarching goal of evaluating the effectiveness of non-target screening, 18S V9 rRNA gene, and full-length 16S rRNA gene metabarcoding techniques. This comprehensive assessment occurred within the framework of environmental monitoring, aiming to deepen our understanding of how these methods perform in detecting disturbances in the environment. Notably, I provided evidence that all methods are capable of detecting environmental disturbances, although their individual strengths vary.

In showcasing the initial sensitivity of full-length 16S rRNA gene metabarcoding as a monitoring method, I further unveiled that the non-target screening approach remained effective throughout the experiment. In contrast to the metabarcoding methods, the signal-to-noise ratio of non-target screening remained stable over the experiment's duration, resulting in an permanent and relative strength of this method. Additionally, I determined that 18S V9 rRNA gene metabarcoding displayed superior sensitivity throughout the study. The distinctive factor among these methods lies in their capacity to detect unknown pollutants and organisms, setting them apart from previously employed approaches in environmental monitoring and providing a more comprehensive perspective. Given their diverse strengths, particularly in terms of

temporal resolution, these methods prove most beneficial when employed in tandem as complementary approaches.

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Article VI demonstrates that employing multiple diverse methods is advantageous, as they complement each other and contribute to a more comprehensive understanding. Essentially, interdisciplinarity emerges as a pivotal catalyst for driving research, fostering innovation, and facilitating progress (Clark & Wallace, 2015; Röhlig, 2018). Despite the acknowledged significance of interdisciplinarity, the challenge lies in the absence of a standardized method for its description and quantification (Abramo et al., 2018; Pellegrino & Musy, 2017; Wagner et al., 2011). In response to this gap, I introduced a methodology in article VII to render interdisciplinarity more tangible and measurable. This approach relies on meta-information and Jaccard similarity, offering a means to assess and quantify the degree of interdisciplinarity. To assess the practical manifestation of interdisciplinarity, I applied this measure to an extensive dataset comprising over 300,000 documents sourced from Elsevier's Scopus database detailed with topics related to heavy rainfall events. Surprisingly, even in a field inherently conducive to interdisciplinary collaboration, my findings show that the actual implementation of interdisciplinarity is constrained and predominantly steered by a select few disciplines. Despite the advocacy of interdisciplinarity as a gold standard for scientific inquiry, my research suggests that its realization is somewhat limited. This revelation underscores the existence of ample room for improvement, especially when addressing complex problems. Therefore, enhancing the genuine integration of diverse disciplines remains a crucial endeavor for advancing the effectiveness of scientific efforts tackling intricate challenges.

Conclusion

In the era of the Anthropocene, grappling with the consequences of human activities on ecosystems has become an essential pursuit for the well-being of our planet and its inhabitants. This study embarked on a systematic exploration, shedding light on the often-overlooked yet profoundly influential world of microorganisms—a promising frontier for unraveling the intricate web of environmental disturbances caused by human actions.

Our systematic journey began with the selection of microorganisms as indicators to assess human-induced stressors. By leveraging advanced techniques such as Next-Generation Sequencing, we obtained detailed insights into the impacts across diverse habitats — from highlands to lowlands, freshwater lakes to soils, and treated wastewater. Focusing on microscopic organisms provided a profound understanding of ecosystem health.
The examination of morphologically similar species revealed disparate reactions to stressors, emphasizing the efficacy of Next-Generation Sequencing for large-scale analyses. The prevalence of potentially endemic taxa in mountains underscored the validity of studying lowlands for generalizable insights. Additionally, since aquatic and terrestrial habitats exhibited taxonomic independence in their microeukaryotic communities, focusing on aquatic habitats is reasonable. In these habitats, inert nanoplastic particles had minimal effects, while zinc substantially impacted diversity. Ecosystem resilience to treated wastewater contrasted with lasting changes from tire wear-off proxies. Detecting ecosystem disturbances became crucial, and our study demonstrated the effectiveness of 18S V9 rRNA, full-length 16S rRNA, and non-target screening methods. These methods exhibited strong covariance while deviating in individual strengths. Finally, my research highlighted a gap in interdisciplinary collaboration to address complex challenges posed by human-induced environmental changes.

In essence, this body of work highlights the pressing need for interdisciplinary collaboration. In the Anthropocene, where environmental challenges frequently surpass disciplinary boundaries, the imperative for collaboration across diverse fields becomes evident. Many of the challenges we encounter inherently require a multidisciplinary approach, underscoring the necessity for collaborative efforts that integrate expertise from various scientific domains to formulate comprehensive solutions. This study not only exposes the shortcomings in the present execution of interdisciplinary approaches but also concurrently illustrates the advantages of interdisciplinary collaboration.

This work provides a thoughtful and well-founded approach for investigating anthropogenic stressors, which is extendable to environmental disturbances, primarily based on microeukaryotes. In this context, informed decisions are made regarding the selection of suitable habitats and methods to yield statements that are both generalizable and precise. While this study indeed brings forth crucial new insights, particularly concerning tire abrasion and treated wastewater and their impacts on the environment, subsequent experiments should follow to investigate more anthropogenic stressors, their interactions, and additional habitats. After all, anthropogenic stressors are diverse and far from being adequately researched. However, armed with the insights gleaned from this thesis, we are better equipped to monitor the environment, make informed decisions, and implement effective measures, navigating the Anthropocene to safeguard our ecosystems and secure our collective future.

Appendix

List of abbreviations

°C	Degree Celsius
ANOVA	Analysis of variance
API	Application Programming Interface
ARGs	Antibiotic resistance genes
ASVs	Amplicon sequencing variants
ATP	Adenosine triphosphate
bp	Base pair
CCAC	Central Collection of Algal Cultures
cf.	Confer
cm	Centimeter
CO_2	Carbon dioxide
cond	Conductivity
DAPI	4,6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DOM	Dissolved organic matter
F_0	Ground Fluorescence
FA	Formic acid
FISH	Fluorescence *in situ* hybridization
Fm	Maximum Fluorescence
Fv	Variable Fluorescence
g	Gram
Gbp	Giga base pairs
GC	Guanine-cytosine
h	Hour
HMM	Hidden Markov Model
HNF	Heterotrophic nanoflagellates
HNO_3	Nitric acid
HPLC	High pressure liquid chromatography
HRMS	High-resolution mass spectrometry
IB	Inorganic basal medium
ICP-MS	Inductively Coupled Plasma- Mass Spectrometry
ID	Identifier
IPCC	Intergovernmental Panel on Climate Change
ITS	Internal transcribed spacer
kbp	Kilo base pairs
kg	Kilogram
km	Kilometer
kV	Kilovolt
l	Liter
log	Logarithm
LSA	Liquid chromatography
m	Meter
M	Molar
MAGs	Metagenome-assembled-genomes
MFS	Major facilitator superfamily

mg	Milligram
MID	Molecular identifier
MIMAG	Minimum information metagenome-assembled genome
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
MRPP	Multiple response permutation procedure
Na_2CO_3	Sodium Carbonate
NCBI	National Center for Biotechnology Information
ng	Nanogram
NGS	Next-generation sequencing
nm	Nanometer
NMDS	Non-metric multidimensional scaling
NP	Nanoplastic
NSY	Nutrient broth, Peptone from soybean, yeast extract medium
nt	Nucleotide
NTS	Non-Target Screening
NTU	Nephelometric turbidity unit
ONT	Oxford Nanopore Technology
OTU	Operational taxonomic unit
PC	Principal component
PCoA	Principal coordinates analysis
PCR	Polymerase chain reaction
ppb	parts per billion
ppm	Parts per million
PSII	Photosystem II
rDNA	Ribosomal DNA
RNA	Ribonucleic acid
RND	Resistance nodulation division
ROS	Reactive oxygen species
rpm	Rounds per minute
rpS3	Ribosomal protein S3
rRNA	Ribosomal ribonucleic acid
RT	Room temperature
RV	RV coefficient
s	Second
S	Svedberg unit
sd	Standard deviation
sds	Sodium dodecyl sulfate
Si	Silica
SNR	Signal-to-noise ratio
sp.	Species
SSU	Small subunit ribosomal ribonucleic acid
TaxID	Taxonomic identifier
td	True diversity
Temp	Temperature
TWW	Treated wastewater
UV	Ultraviolet
WC	Wright's Chu medium
WK	Grain of wheat
WW	Wastewater

WWTP	Wastewater treatment plant
Zn	Zinc
ZnNP	Zinc and Nanoplastic
µg	Microgram
µl	Microliter
µm	Micrometer
µmol	Micromolar
µS	Microsiemens

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Article I supplement: „*Centers of endemism of freshwater protists deviate from pattern of taxon richness on a continental scale*“

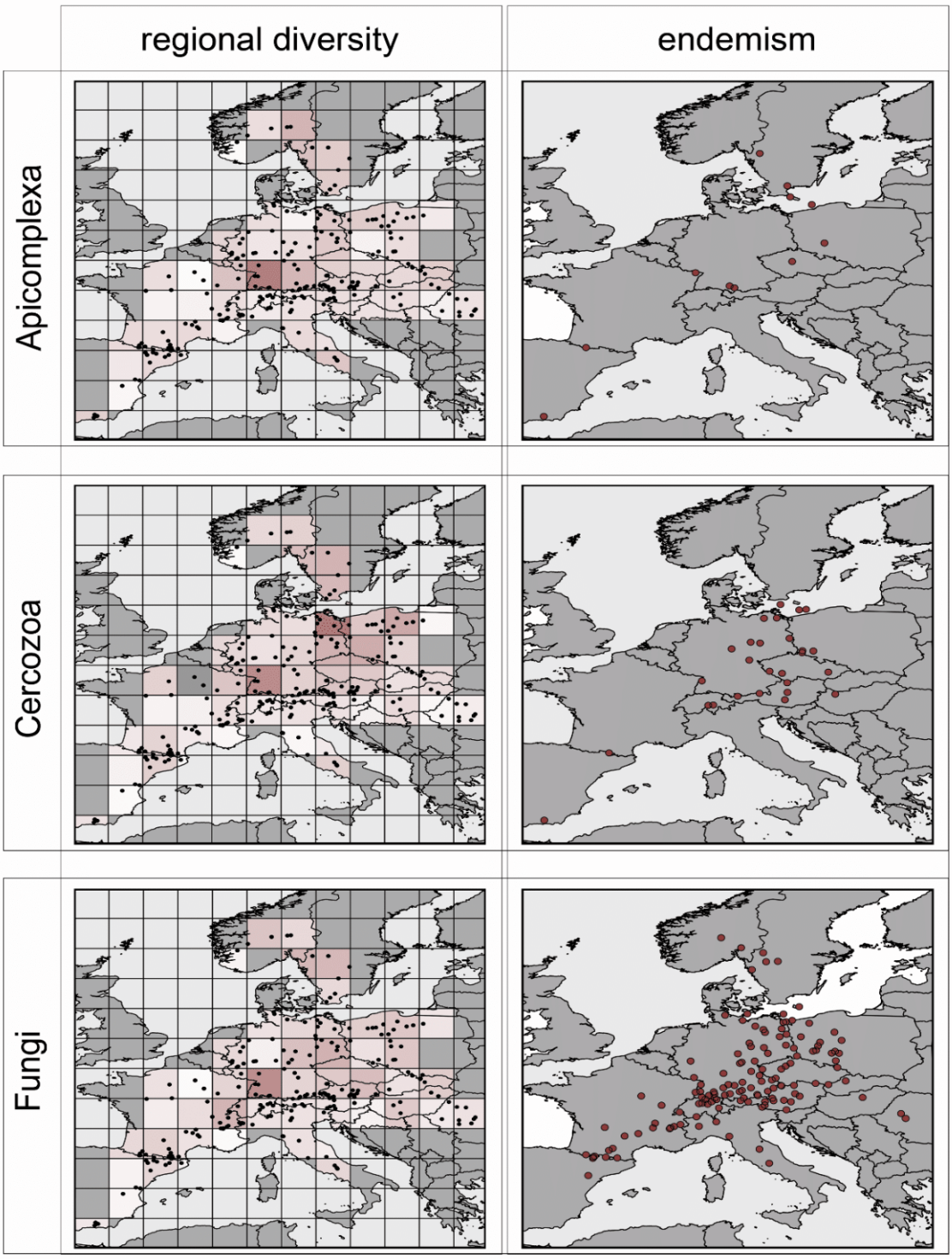

Figure S1: Regional diversity of Apicomplexa, Cercozoa and Fungi. Left: Regional richness of the respective taxon where color shading indicates low (white) to high (red) regional richness. Maximal shading corresponds to 16.2 interpolated V9-groups per lake for Apicomplexa, to 17.3 interpolated V9-groups per lake for Cercozoa and to 14.2 interpolated V9-groups per lake for Fungi. Right: centers of distribution areas for putatively endemic taxa within the respective group. Please note that the centers of the distribution area are geometric centers and do not coincide with a location of a distinct lake.

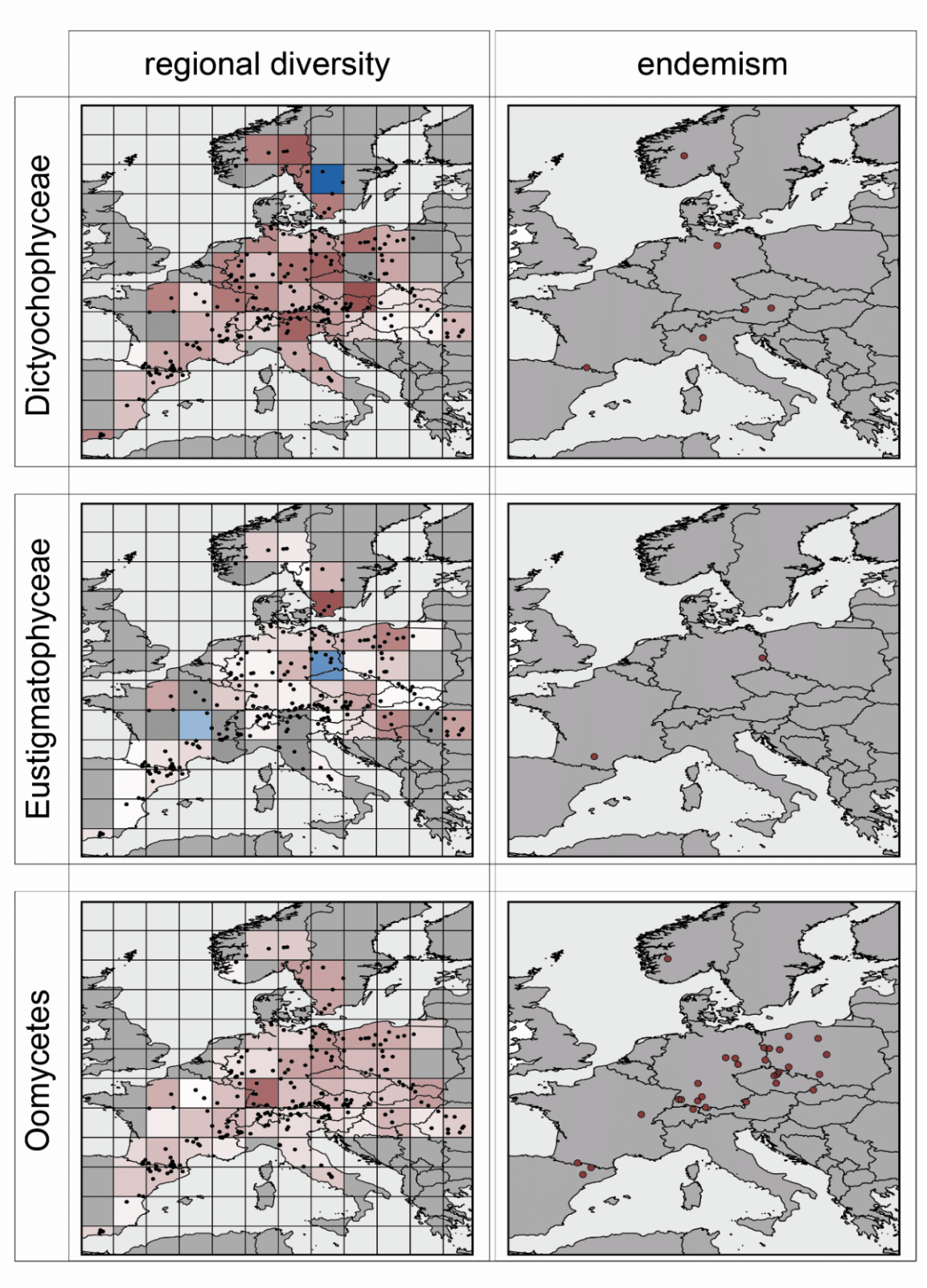

Figure S2: Regional diversity of Dictyochophytes, Eustigmatophytes and Oomycetes. Left: Regional richness of the respective taxon where color shading indicates low (white) to high (red) regional richness (blue shading indicates an exceptionally high richness). Maximal shading corresponds to 41.8 (blue) for Dictyochophyceae, to 34.5 (blue) for Eustigmatophyceae and 18.5 for Oomycetes. Right: centers of distribution areas for putatively endemic taxa within the respective group. Please note that the centers of the distribution area are geometric centers and do not coincide with a location of a distinct lake.

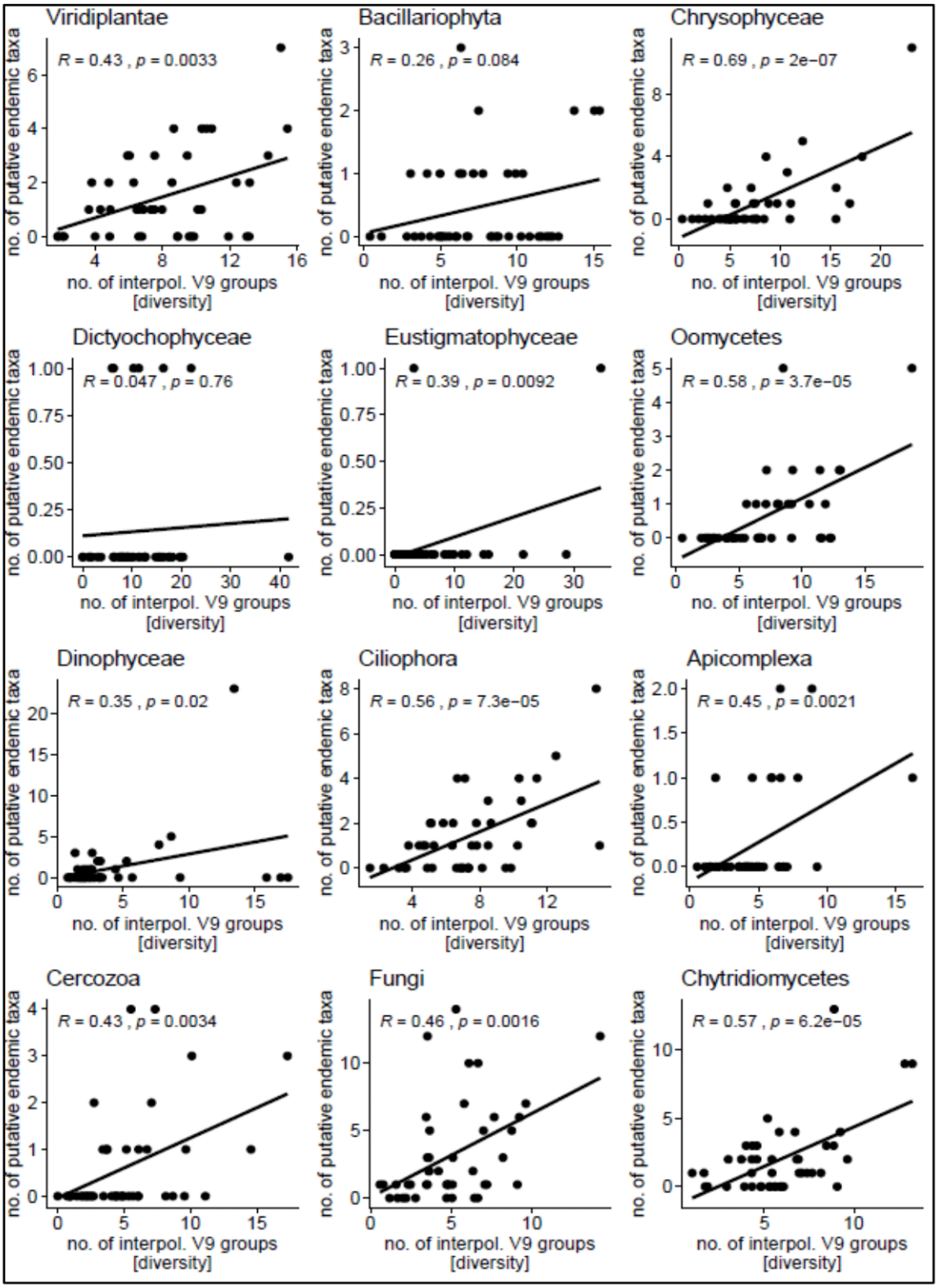

Figure S3

Correlation between diversity of the interpolated V9 groups per grid and putatively endemic centers within different taxonomic groups (visually displayed in Figures 2, 3, S1, S2). Pearson's correlation coefficient (R) was calculated using ggpubr' (vs 0.2.3) and tested for significance (*p*).

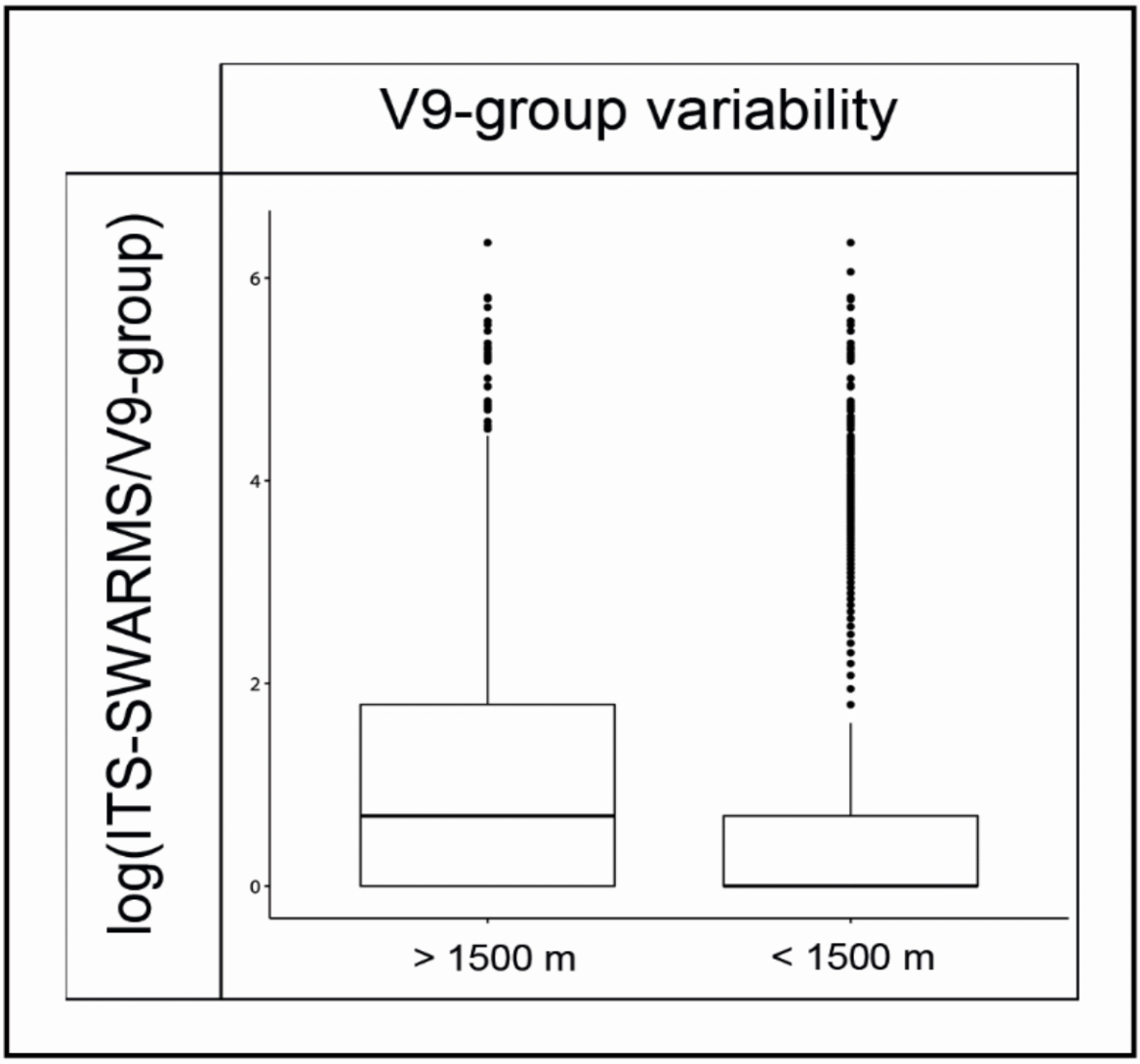

Figure S4: Microdiversity of V9-groups, i.e. the number of ITS-SWARMS affiliated with a distinct V9 group, in lakes above and below 1500m.

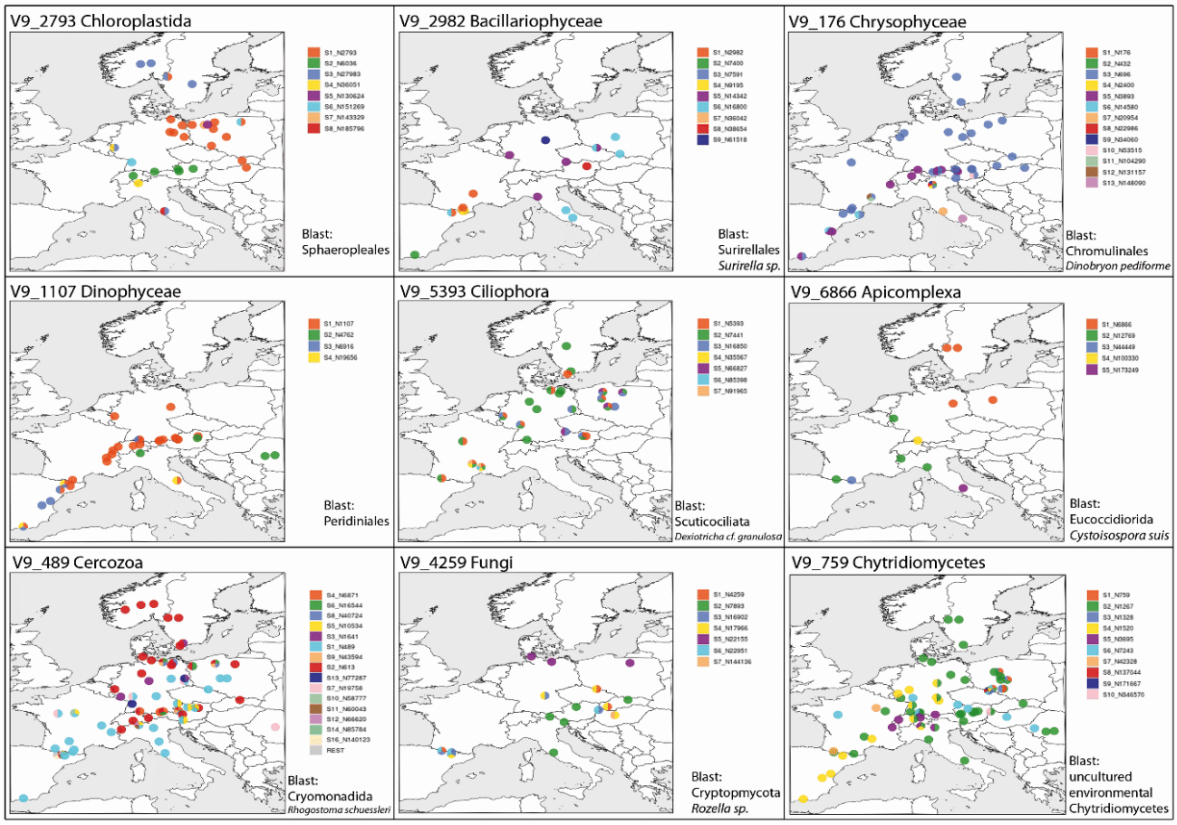

Figure S5: Examples for V9 groups affiliated with different phyla which show a geographically differential distribution of associated ITS_SWARMS.

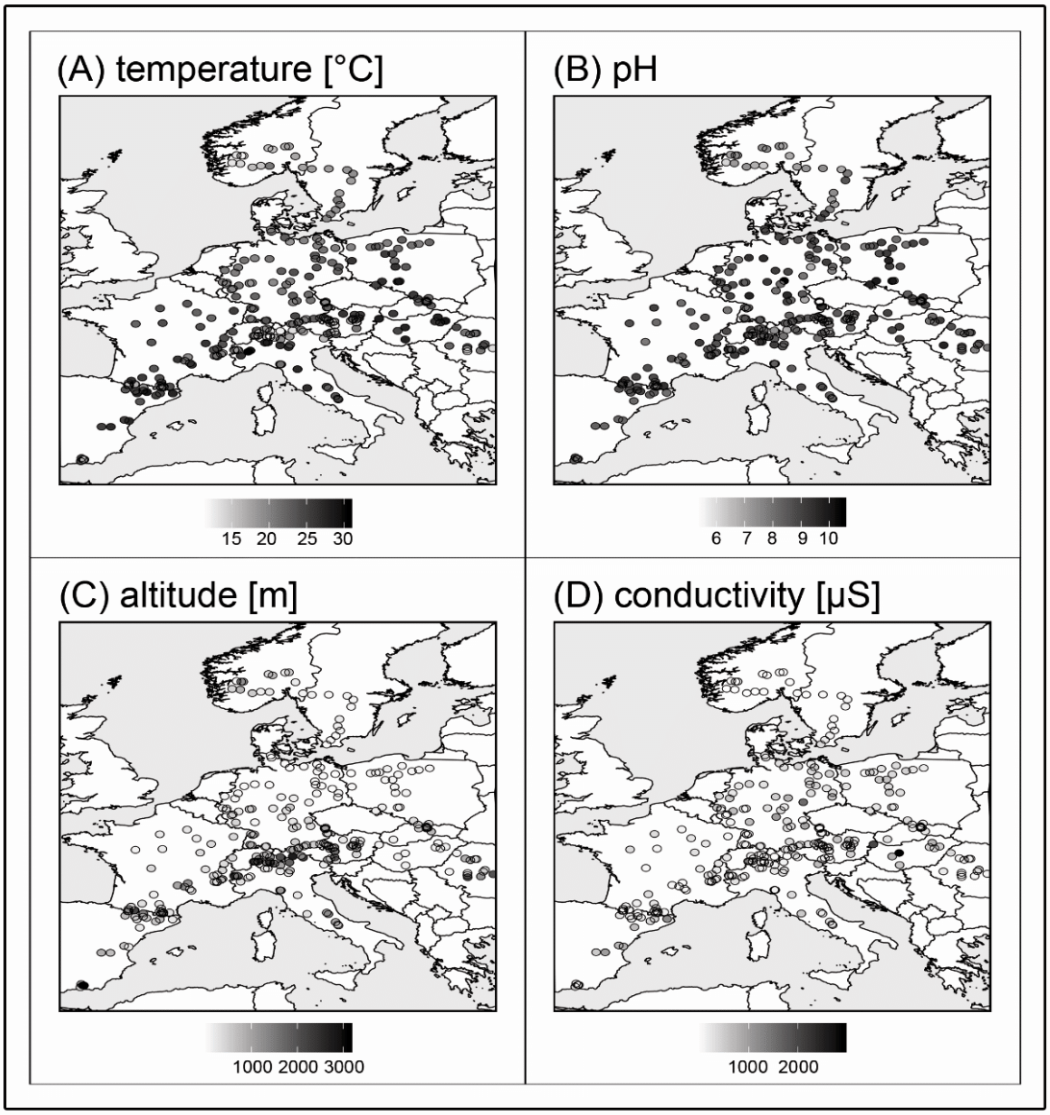

Figure S6: Temperature, pH, altitude and conductivity for the sampling sites.

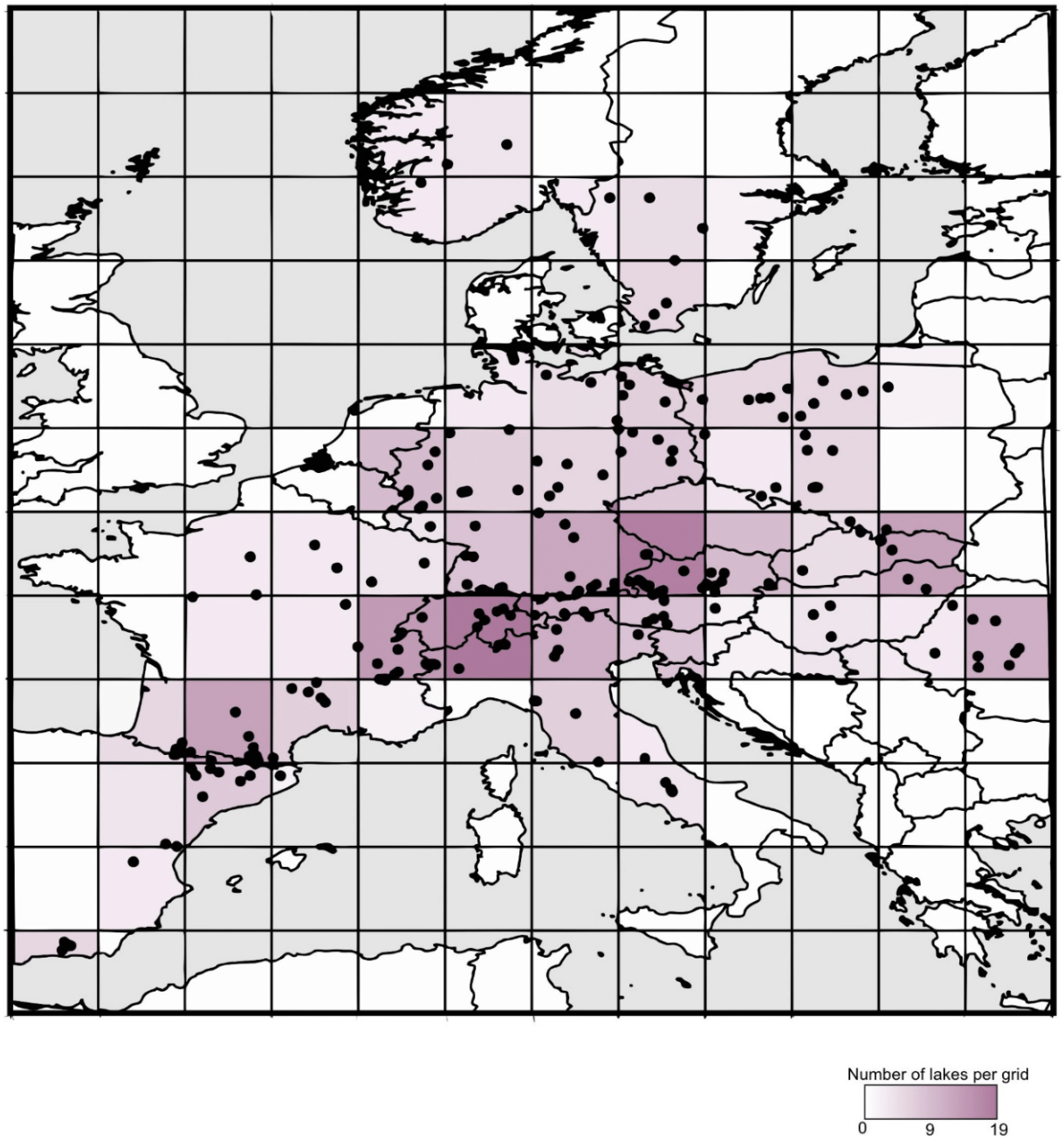

Figure S7:

Regional distribution of the sampled lakes. Shading corresponds to number of lakes per grid.

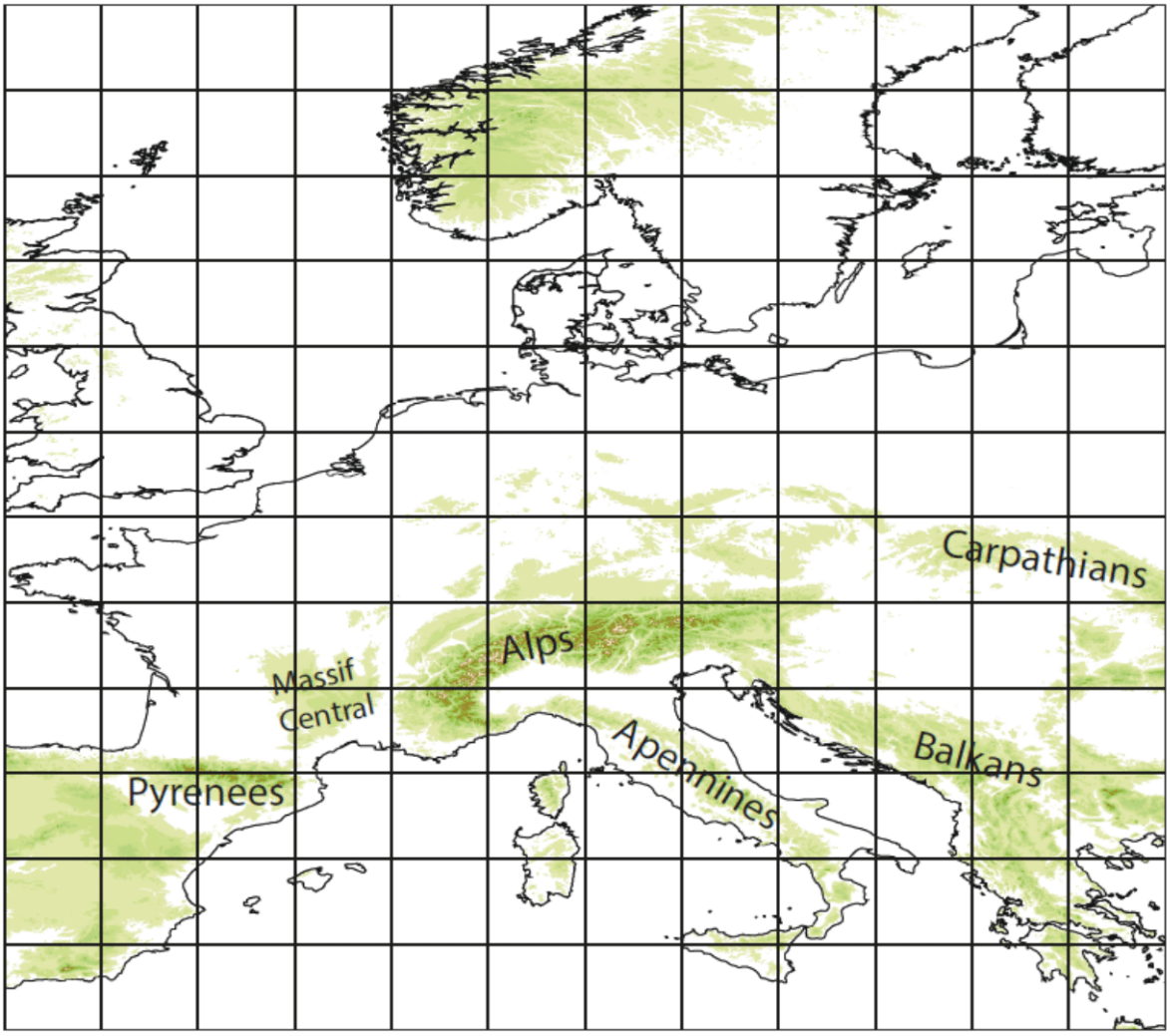

Figure S8

Geographical elevations within the sampling area.

Article II supplement: „*Effects of stressors on growth and competition between different cryptic taxa affiliated with Ochromonadales (Chrysophyceae)*“

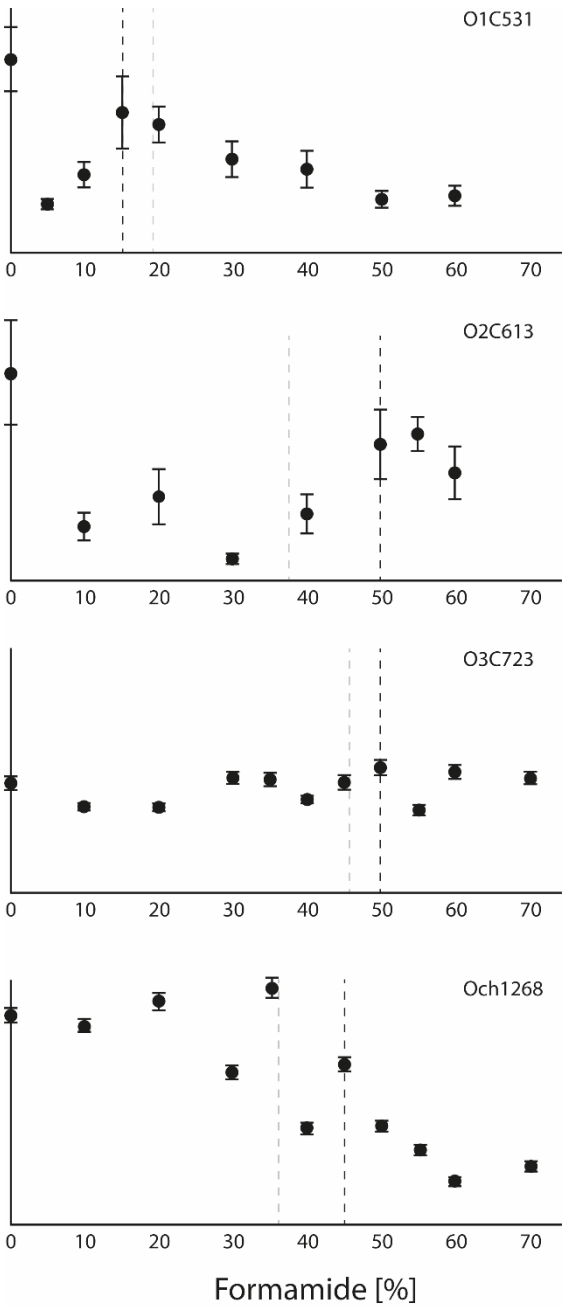

Figure S1: Signal intensity as a function of formamide concentrations. The grey dashed line indicates the calculated optimal formamide concentration (mathFISH) and the black dashed line the selected formamide concentration in our protocols based on signal intensity and probe specificity.

Article III supplement: „*Protistan and fungal diversity in soils and freshwater lakes are substantially different*“

Name of the lake	Water Sample ID	Water Sampling	Soil Sample ID	Soil Sampling	Location	Country	CoordinatesN	CoordinatesE	Altitude	Soil Carbonate Content	Soil pH	Soil Conductivity	Water pH	Water Conductivity	Water Temp. C°
Rurtalsperre	S311RU.y	August 2012	S311RU.x	August 2018	Eifel	Germany	6.440619	50.638739	320	0	4.21	276	8.5	101	20.3
Bostalsee	S011BO.y	August 2012	S011BO.x	August 2018	Saarland	Germany	7.074703	49.562925	450	0	3.49	156	9	112	22.6
See bei Worms (Altrheinsee)	Z301WO.y	August 2012	Z301WO.x	August 2018	Frankenthal/Rheinland-Pfalz	Germany	8.369489	49.57846	84	7	6.79	588	9.7	694	22.4
See bei Bühl (Pond)	Z291BU.y	August 2012	Z291BU.x	August 2018	Bühl/Schwarzwald BW	Germany	8.093199	48.695527	129	3	6.7	450	8.4	369	23.8
Schwarzenbachtalsperre	Z293SB.y	August 2012	Z293SB.x	August 2018	Bühl/Schwarzwald BW	Germany	8.313016	48.661996	659	0	2.63	486	8	47	21.5
Schluchsee	Z282SU.y	August 2012	Z282SU.x	August 2018	BW	Germany	8.13464	47.832144	940	0	2.89	588	8.7	150	20.8
Untersee-Bodensee	Z021UB.y	August 2012	Z021UB.x	August 2018	Allensbach/BW	Germany	9.073561	47.712049	399	9	6.83	690	8.5	256	23.6
Obersee-Bodensee	Z011OB.y	August 2012	Z011OB.x	August 2018	Dingelsdorf/BW	Germany	9.178352	47.722003	400	5	6.89	228	8.6	283	23.4
Dechsendorferweiher	O302DE.y	August 2012	O302DE.x	August 2018	Erlangen/Bayern	Germany	10.958067	49.6303	284	0	2.79	225	9.6	353	22.7
Talsperre Kelbra	N253KE.y	August 2012	N253KE.x	August 2018	Kelbra/Sachsen-Anhalt	Germany	11.01694444	51.427778	165	7	6.96	438	8.9	791	23.9
Seeburger See	N272SB.y	August 2012	N272SB.x	August 2018	Seeburg/Niedersachsen	Germany	10.1664734	51.5647996	150	8	6.79	651	9.2	481	22.9
Silbersee II	S112SI.y	August 2012	S112SI.x	August 2018	Haltern/Münsterland	Germany	7.21525	51.796667	88	0	2.75	273	8.6	241	20.8
Heiliges Meer	N282HE.y	August 2012	N282HE.x	August 2018	Recke/NRW	Germany	7.632777778	52.348889	40	0	2.5	318	8	248	21.3
Steinhuder Meer	N273ST.y	August 2012	N273ST.x	August 2018	Steinhude/Niedersachsen	Germany	9.349722222	52.452222	38	0	4.75	1050	9.5	281	21.1
Arenholzer See	N122AR.y	August 2012	N122AR.x	August 2018	Neuberend/Schleswig	Germany	9.486944444	54.535833	27	0	4.1	123	8.9	316	21.1
Großer Plöner See	N123PL.y	August 2012	N123PL.x	August 2018	Plön/Schleswig	Germany	10.42027778	54.085833	34	0	3.19	525	8.9	332	23
Neukloster See	N132NE.y	August 2012	N132NE.x	August 2018	Neukloster	Germany	11.70388889	53.864167	36	0	3.23	285	8.5	464	20.2
Stassower See	N133ST.y	August 2012	N133ST.x	August 2018	Thelkow/Meckl-Vorpomm	Germany	12.59055556	54.034444	33	1	5.35	375	8.5	205	21.3
Müritz	N142MU.y	August 2012	N142MU.x	August 2018	Klink/Meckl-Vorpomm	Germany	12.62416667	53.478889	70	3	6.69	168	8.7	397	20.2
Kleßener See	N233KL.y	August 2012	N233KL.x	August 2018	Klessen/Brandenburg	Germany	12.46083333	52.731944	12	0	3.96	525	8.8	421	23.5
Großer Plessower See	N231PL.y	August 2012	N231PL.x	August 2018	Werder/Brandenburg	Germany	12.90861111	52.373056	28	0	6.23	1765	8.6	458	22.3
Zemminsee	N241ZE.y	August 2012	N241ZE.x	August 2018	Groß Köris/Brandenburg	Germany	13.64388889	52.156944	20	2	6.45	921	9.1	243	21.9
Kossateich	N242KO.y	August 2012	N242KO.x	August 2018	Raddusch/Brandenburg	Germany	14.06527778	51.83	53	0	4.82	333	7.7	689	22.4
Senftenberger See	N242SE.y	August 2012	N242SE.x	August 2018	Senftenberg/Brandenburg	Germany	14.01583333	51.5125	91	0	4.51	144	8.3	406	23.2
Vollertsee	N252VO.y	August 2012	N252VO.x	August 2018	Trebnitz/Sachsen-Anhalt	Germany	12.05277778	51.104444	181	2	6.79	180	8	1599	24.1
Eginger See	A301EG.y	August 2012	A301EG.x	August 2018	Eging a.See/Bayern	Germany	13.27135	48.719483	378	0	2.09	285	10	143	21.7
Seehamer See	A071SE.y	August 2012	A071SE.x	August 2018	Seeham. Rosenheim	Germany	11.858833	47.841817	649	0	3.16	422	8.4	359	18.4
Tegernsee	A241TE.y	August 2012	A241TE.x	August 2018	Gmund/Bayern	Germany	11.717767	47.736	742	0	5.44	375	8.4	302	20.4
Kochelsee	A171KO.y	August 2012	A171KO.x	August 2018	Kochel a.See	Germany	11.356917	47.6424	587	0	6.44	336	8.4	313	19.1
Walchensee	A172WA.y	August 2012	A172WA.x	August 2018	Einsiedl/Bayern	Germany	11.304667	47.567933	799	0	3.43	831	8.5	275	21.6

Table S1: Additonal sampling information. The soil pH-value was measured according to DIN ISO 10390 with downscaled quantity (1g soil : 2.5ml double distilled water). Soil Conductivity was measured according to DIN ISO 11265 with downscaled quantity (2.5g soil : 12.5ml double distilled water). Soil Carbonate content was measured semi-qualitative with 10% HCl and estimated as accurately as possible (Benzler et al. 1982). Benzler J.H.. Finnkern. H.. Müller. W.. Roeschmann. G.. Will. K.H.. Wittmann. O. : Bodenkundliche Kartieranleitung 3. Verbesserte und erweiterte Auflage Hannover 1982

Identity %	Low abundant taxa with similar importance																
100	Eukaryota	Rhizaria	Cercozoa	unclassified Cer	Gymnophrys	Gymnophrys sp. ATCC 50923											
99.007	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae	Hannaella	Hannaella coprosmae						
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Bulleribasidiaceae	Bulleribasidium	Bulleribasidium oberjochense						
99.702	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Saccharomycotina	Saccharomycetes	Saccharomycetales	Lipomycetaceae	Lipomyces	Lipomyces aromalus					
97.452	Eukaryota	Rhizaria	Cercozoa	Cercomonadida	Cercomonadidae	Cercomonas	Cercomonas celer										
99.679	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricomycetes incertae se	Corticiales	Corticiaceae	Hyphodontia	Hyphodontia pallidula					
100	Eukaryota	Viridiplantae	Chlorophyta	Trebouxiophycea	Chlorellales	Chlorellaceae	Chloroidium	Chloroidium ellipsoideum									
98.726	Eukaryota	Rhizaria	Cercozoa	Cercomonadida	Cercomonadidae	Cercomonas	Cercomonas rotunda										
100	Eukaryota	Alveolata	Apicomplexa	Conoidasida	Gregarinasina	Eugregarinorida	Monocystidae	Monocystis	unclassified Monocys	Monocystis sp. R1_02							
99.31	Eukaryota	Alveolata	Ciliophora	Intramacronucle	Colpodea	Bryometopida	Kreyellidae	Microdiaphanosoma	Microdiaphanosoma arcuatum								
97.674	Eukaryota	Stramenopiles	Diatomeae	Bacillariophycea	Bacillariophycidae	Naviculales	Stauroneidaceae	Craticula	Craticula accomoda								
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	Eurotiomycetes	Eurotiomycetidae	Eurotiales	Aspergillaceae	Thysanophora	Thysanophora penicillioides			
98.575	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	Pleosporineae	Pleosporaceae	Alternaria	Alternaria alte	Alternaria alternata
97.872	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	Pleosporineae	Didymellaceae	Epicoccum	Epicoccum sp. 43-13-1	
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Pucciniomycotina	Pucciniomycetes	Pucciniales	Pucciniaceae	Puccinia	Puccinia coronata	Puccinia coronata v	Puccinia coronata var. avenae f. sp. avenae				
98.802	Eukaryota	Alveolata	Ciliophora	Intramacronucle	Litostomatea	Haptoria	Haptorida	Dileptida	Dileptidae	Dileptus	Dileptus costaricanus						
98.958	Eukaryota	Viridiplantae	Chlorophyta	Chlorophyceae	Chlamydomonadales	Chlamydomonadales incerta	Tetracystis	Tetracystis viratzeri									
98.413	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Dothideomycetidae	Capnodiales	Teratosphaeriaceae	Austroafricana	Austroafricana parva		
99.67	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricomycetidae	Agaricales	Psathyrellaceae	Coprinopsis	Coprinopsis cinerea					
98.925	Eukaryota	Stramenopiles	Peronosporomyc	Peronosporales	Phytophthora	Phytophthora plurivora											
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	Trimorphomycetacea	Saitozyma	Saitozyma flava						
99.595	Eukaryota	Alveolata	Dinophyceae	Peridiniales	Peridiniopsidaceae	Peridiniopsis	Peridiniopsis polonicum										
99.385	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	unclassified Ascomycota	Ascomycota sp. LM221										
97.97	Eukaryota	Viridiplantae	Chlorophyta	Trebouxiophycea	Microthamniales	Symbiochloris	Symbiochloris symbiontica										
100	Eukaryota	Stramenopiles	Diatomeae	Bacillariophycea	Bacillariophycidae	Naviculales	Naviculaceae	Fistulifera	Fistulifera pelliculosa								
98.78	Eukaryota	Viridiplantae	Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	Desmodesmus	Desmodesmus insignis									
97.638	Eukaryota	Alveolata	Ciliophora	Intramacronucle	Litostomatea	Haptoria	Haptorida	Lacrymariidae	Lacrymaria	Lacrymaria marina							
97.708	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	Ascomycota incertae sedis	Tetracladium	Tetracladium maxilliforme									
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Pucciniomycotina	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	Rhodotorula	Rhodotorula babjevae						
100	Eukaryota	Stramenopiles	Chrysophyceae	unclassified Chry	Spumella-like flagellate JB	Spumella-like flagellate JBAF33											
99.392	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	Pleosporineae	Didymellaceae	Epicoccum	Epicoccum sp. 43-13-1	
98.88	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	sordariomyceta	Leotiomycetes	Erysiphales	Erysiphaceae	Podosphaera	Podosphaera fusca			
99.624	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	unclassified Ascomycota	Ascomycota sp. D7										
99.723	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Dothideomycetes incert	Botryosphaeriales	Botryosphaeriaceae	Diplodia	Diplodia seriata		
97.638	Eukaryota	Alveolata	Ciliophora	Intramacronucle	Litostomatea	Haptoria	Haptorida	Lacrymariidae	Phialina	Phialina salinarum							
98.795	Eukaryota	Stramenopiles	Chrysophyceae	Chromulinales	Dinobryaceae	Poteriospumella	Poteriospumella sp.										
98.466	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Dothideomycetidae	Capnodiales	Mycosphaerellaceae	Xenoramularia	Xenoramularia neerlandica		
99.225	Eukaryota	Alveolata	Ciliophora	Intramacronucle	Litostomatea	Haptoria	Haptorida	Acropisthiidae	Fuscheria	Fuscheria sp. VBOEF-2011							
97.554	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	Pleosporineae	Pleosporaceae	unclassified Pleos	Pleosporaceae sp. LM282	
99.528	Eukaryota	Viridiplantae	Chlorophyta	Chlorophyceae	Sphaeropleales	Scenedesmaceae	Coelastrum	Coelastrum microporum									
99.048	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	unclassified Ascomycota	Ascomycota sp. 20302										
99.731	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	Pleosporineae	Pleosporineae incerta	Camarosporium	Camarosporium brabeji	
98.394	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	unclassified Pleospo	Pleosporales sp.			
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	Eurotiomycetes	Eurotiomycetidae	Eurotiales	Aspergillaceae	Aspergillus	Aspergillus amstelodami			

Table S2 Low abundant and equally important shared OTUs in soil and freshwater lakes. Defined as distributed in a 25% to 75% ratio and represents less than 0.00005% according to Figure 2C. Only OTUs with a sequence similarity greater than 97% (NCBI BLASTn) are shown. Uncultured and environmental OTUs are discarded.

Identity %	High abundant taxa with similar importance																
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Dothideomycetidae	Capnodiales	Cladosporiaceae	Cladosporium	Cladosporium cladosporioides		
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	Pleosporineae	Didymellaceae	Boeremia	Boeremia exig	Boeremia exigua var. Exigua
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	dothideomyceta	Dothideomycetes	Pleosporomycetidae	Pleosporales	Pleosporineae	Pleosporaceae	Bipolaris	Bipolaris multiformis	
97.765	Eukaryota	Opisthokonta	Fungi	Dikarya	Ascomycota	saccharomyceta	Pezizomycotina	leotiomyceta	sordariomyceta	Leotiomycetes	Helotiales	Helotiaceae	Articulospora	Articulospora proliferata			
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricomycetes incertae se	Russulales	Bondarzewiaceae	Heterobasidion	Heterobasidion ann	Heterobasidion annosum				
100	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Agaricomycotina	Tremellomycetes	Tremellales	unclassified Tremella	Tremellales sp. DU26							
99.685	Eukaryota	Opisthokonta	Fungi	Dikarya	Basidiomycota	Ustilaginomycotina	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	Ustilago	Ustilago striiformis						
100	Eukaryota	Stramenopiles	Diatomeae	Bacillariophycea	Bacillariophycidae	Cymbellales	Gomphonemataceae	Gomphonema	Gomphonema parvulum								
97.493	Eukaryota	Stramenopiles	Peronosporomyc	Pythiales	Pythiaceae	Pythium	Pythium capillosum										
100	Eukaryota	Stramenopiles	Synurophyceae	Ochromonadales	Ochromonadaceae	Poterioochromonas	Poterioochromonas malhamensis										

Table S3: High abundant and equally important shared OTUs in soil and freshwater lakes. Defined as distributed in a 25% to 75% ratio and represents more than 0.00005% according to Figure 2C. Only OTUs with a sequence similarity greater than 97% (NCBI BLASTn) are shown. Uncultured and environmental OTUs are discarded.

Table S4: Shared OTUs

Identity %	Shared OTUs between soil and freshwater habitats
99.333	Eukaryota Katablepharidaceae Katablepharidaceae environmental samples uncultured Katablepharidaceae
99.736	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Eurotiomycetes Chaetothyriomycetidae Chaetothyriales Herpotrichiellaceae environmental samples uncultured Herpotrichiellaceae
98.209	Eukaryota Opisthokonta Fungi Dikarya Ascomycota Taphrinomycotina Taphrinomycetes Taphrinales environmental samples uncultured Taphrinaceae
100	Eukaryota Stramenopiles Peronosporomycetes Peronosporales Phytophthora Phytophthora hydropathica
90.379	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Microascales Microascaceae Petriella Petriella sp. P3
87.73	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.363	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.351	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Scenedesmus Scenedesmus sp. HH 10205
98.367	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
96.599	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Scuticociliatia Philasterida Philasteridae Miamiensis Miamiensis avidus
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Eurotiomycetes Eurotiomycetidae Eurotiales Aspergillaceae Penicillium Penicillium bialowiezense
97.647	Eukaryota environmental samples uncultured eukaryote
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Didymellaceae Phoma Phoma herbarum
99.611	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
96.341	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae environmental samples uncultured Trebouxiophyceae
95.299	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Colpodida Colpodidae Colpoda Colpoda cucullus
86.624	Eukaryota Alveolata Dinophyceae Peridiniales Heterocapsaceae environmental samples uncultured Heterocapsa
95.652	Eukaryota Rhizaria Cercozoa Cercomonadida environmental samples Cercomonadida environmental sample
99.194	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Helotiales incertae sedis Chalara Chalara hyalina
85.994	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Helotiaceae Claussenomyces Claussenomyces sp. HB9300b
93.956	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlorophyceae incertae sedis Pleurastrum Pleurastrum sp. CCCryo 205-05
98.837	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Desmodesmus unclassified Desmodesmus Desmodesmus sp. GM4i
85.276	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida Plasmodiophoridae Ligniera Ligniera sp. F69
93.976	Eukaryota Stramenopiles Chrysophyceae Chromulinales Chromulinaceae Pedospumella Pedospumella encystans
92.647	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
92.089	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae environmental samples uncultured Oxytrichidae
98.476	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Cordycipitaceae Isaria Isaria fumosorosea
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Polyporales unclassified Polyporales Polyporales sp. 1 SR-2012
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae Bipolaris Bipolaris multiformis
96.97	Eukaryota Heterolobosea Schizopyrenida Vahlkampfiidae Tetramitus Tetramitus dokdoensis
99.553	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
95.425	Eukaryota Stramenopiles Hyphochytriomycetes Hyphochytriaceae Hyphochytrium Hyphochytrium catenoides
100	Eukaryota Rhizaria Cercozoa unclassified Cercozoa Gymnophrys Gymnophrys sp. ATCC 50923
100	Eukaryota Alveolata Ciliophora environmental samples uncultured ciliate
86.943	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.171	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Magnaporthales Magnaporthaceae Gaeumannomyces Gaeumannomyces tritici
100	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
95.238	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.554	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
90.476	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
95.541	Eukaryota Stramenopiles Chrysophyceae Chromulinales Dinobryaceae Poteriospumella Poteriospumella sp.
97.319	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium monospermum
100	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Cymbellales Gomphonemataceae Gomphonema Gomphonema parvulum
99.66	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Xylariomycetidae Xylariales Microdochiaceae Microdochium Microdochium bolleyi
95.238	Eukaryota Viridiplantae Chlorophyta prasinophytes Mamiellophyceae Mamiellales Bathycoccaceae Ostreococcus Ostreococcus sp. BCC7000
98.714	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Cystofilobasidiales Cystofilobasidiaceae Cystofilobasidium Cystofilobasidium infirmominiatum
97.05	Eukaryota Opisthokonta Fungi Mucoromycota Mortierellomycotina Mortierellales Mortierellaceae Mortierella Mortierella hyalina
89.439	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales unclassified Tremellales Tremellales sp. LM490
90.446	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
95.765	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Stichotrichida environmental samples uncultured stichotrichid
100	Eukaryota Opisthokonta Fungi Fungi incertae sedis Olpidiaceae Olpidium Olpidium brassicae
88.701	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae environmental samples uncultured Dothideomycetidae
91.954	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota

89.655	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales unclassified Hypocreales Hypocreales sp. GMG_PPb3
96.845	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas Chlamydomonas bacca
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Russulales Russulaceae environmental samples uncultured Russula
95.616	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Diaporthales Diaporthaceae Diaporthe Diaporthe miriciae
100	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Urostylida Urostylidae Urostyla Urostyla grandis
100	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Brevilegnia Brevilegnia minutandra
91.541	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Capnodiales Mycosphaerellaceae Staninwardia Staninwardia suttonii
99.51	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Sordariales Lasiosphaeriaceae environmental samples uncultured Lasiosphaeriaceae
97.143	Eukaryota environmental samples uncultured eukaryote
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Capnodiales Cladosporiaceae Cladosporium Cladosporium cladosporioides
94.318	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Thelephorales Thelephoraceae environmental samples uncultured Tomentella
91.843	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
100	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Pythiopsis Pythiopsis intermedia
97.129	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.697	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae Hemiurosomoida Hemiurosomoida longa
99.007	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Bulleribasidiaceae Hannaella Hannaella coprosmae
96.403	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Vorticellidae Vorticella Vorticella sp. 4 PS-2013
98.204	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
97.403	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Massarineae Trematosphaeriaceae Trematosphaeria Trematosphaeria sp. HR-2-1
93.75	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
96.354	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Sellaphoraceae Sellaphora Sellaphora laevissima
86.687	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Phallomycetidae Phallales Phallaceae Phallus Phallus hadriani
99.085	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Glomerellales Plectosphaerellaceae Plectosphaerella Plectosphaerella sp. sedF4
87.975	Eukaryota Cryptophyceae Pyrenomonadales Chroomonadaceae Chroomonas Chroomonas sp. M1312
95.055	Eukaryota Stramenopiles Peronosporomycetes Peronosporales Phytopythium Phytopythium mirpurense
85.89	Eukaryota Opisthokonta Fungi Mucoromycota Mortierellomycotina Mortierellales Mortierellaceae Mortierella Mortierella sp. 1 ZHNL-2013
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Bulleribasidiaceae Bulleribasidium Bulleribasidium oberjochense
90.576	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Paracercomonas unclassified Paracercomonas Paracercomonas sp. DB-1602-30
99.359	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Rhizophydiales Rhizophydiaceae Rhizophydium Rhizophydium sp. JEL317
94.388	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chloromonas Chloromonas fonticola
98.013	Eukaryota Opisthokonta Choanoflagellata Craspedida Salpingoecidae Salpingoeca Salpingoeca helianthica
92.222	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Vorticellidae Vorticella Vorticella convallaria
98.551	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetes incertae sedis Venturiales Venturiaceae environmental samples uncultured Venturia
93.464	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae Chlorellales Chlorellaceae Chloroidium Chloroidium angustoellipsoideum
94.313	Eukaryota environmental samples uncultured eukaryote
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes environmental samples uncultured Cryptococcus
92	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Scuticociliatia Philasterida Pseudocohnilembidae Pseudocohnilembus Pseudocohnilembus persalinus
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Didymellaceae Boeremia Boeremia exigua Boeremia exigua var. exigua
92.384	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
95.57	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Cercomonas Cercomonas radiata
99.574	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Sordariales Lasiosphaeriaceae Cercophora Cercophora sp. TMS-2011
99.702	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Saccharomycotina Saccharomycetes Saccharomycetales Lipomycetaceae Lipomyces Lipomyces anomalus
97.452	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Cercomonas Cercomonas celer
97.72	Eukaryota Stramenopiles Diatomeae environmental samples uncultured freshwater diatom
89.796	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Sarcocystidae Sarcocystis Sarcocystis bovini
99.679	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Corticiales Corticiaceae Hyphodontia Hyphodontia pallidula
87.898	Eukaryota Alveolata Dinophyceae Peridiniales Heterocapsaceae environmental samples uncultured Heterocapsa
98.966	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Lecanoromycetes environmental samples uncultured Lecanoromycetidae
96.689	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae Microthamniales Trebouxia environmental samples uncultured Trebouxia photobiont
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporales incertae sedis Monodictys Monodictys arctica
100	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae Chlorellales Chlorellaceae Chloroidium Chloroidium ellipsoideum
99.693	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
91.791	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas unclassified Chlamydomonas Chlamydomonas sp. HH 10204
89.068	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida Plasmodiophoridae Woronina Woronina pythii
94.663	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium kashmirense
93.711	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiogetonaceae Pythiogeton Pythiogeton zizaniae
100	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.048	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida Plasmodiophoridae Polymyxa Polymyxa graminis Polymyxa graminis f. sp. temperata

95.376	Eukaryota Stramenopiles Chrysophyceae Chromulinales Chromulinaceae Ochromonas Ochromonas villosa
93.029	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetes incertae sedis Phyllachorales Phyllachoraceae unclassified Phyllachoraceae Phyllachoraceae sp. LM508
93.789	Eukaryota Stramenopiles Chrysophyceae Chromulinales Paraphysomonadaceae Paraphysomonas Paraphysomonas sp.
88.889	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.593	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Sordariales Lasiosphaeriaceae environmental samples uncultured Podospora
96.418	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Fusarium Fusarium oxysporum species complex Fusarium oxysporum
99.676	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Russulales Russulaceae Russula Russula vesca
98.726	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Cercomonas Cercomonas rotunda
87.931	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
97.727	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Rhytismatales Rhytismataceae Rhytisma Rhytisma acerinum
90.351	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
99.692	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Filobasidiales Filobasidiaceae Goffeauzyma Goffeauzyma gastrica
89.103	Eukaryota Rhizaria Cercozoa Imbricatea Silicofilosea Euglyphida Trinematidae Trinema Trinema enchelys
90.196	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae Oxytrichinae Oxytricha Oxytricha longigranulosa
91.304	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
90.045	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.708	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Desmodesmus Desmodesmus insignis
96.319	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.455	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
99.563	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Leptolegnia Leptolegnia chapmanii
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Xylariomycetidae Xylariales Apiosporaceae Arthrinium Arthrinium arundinis
97.512	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
98.84	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Hydrodictyaceae Pseudopediastrum Pseudopediastrum boryanum
92.429	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae environmental samples uncultured Oxytrichidae
92.339	Eukaryota Alveolata Dinophyceae Prorocentrales Prorocentraceae Prorocentrum Prorocentrum triestinum
97.765	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Helotiaceae Articulospora Articulospora proliferata
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Russulales Bondarzewiaceae Heterobasidion Heterobasidion annosum species complex Heterobasidion annosum
87.675	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium anandrum
98.964	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Choreotrichia Tintinnida Tintinnidae Tintinnidium Tintinnidium fluviatile
100	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina Eugregarinorida Monocystidae Monocystis unclassified Monocystis Monocystis sp. R1_02
99.306	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Grossglockneriida Grossglockneriidae Pseudoplatyophrya Pseudoplatyophrya nana
89.726	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
96.694	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Glomerellales Glomerellaceae Colletotrichum Colletotrichum dematium
95.722	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Naviculaceae Navicula unclassified Navicula Navicula sp. ETS 07
100	Eukaryota Opisthokonta Fungi Blastocladiomycota Blastocladiomycetes Blastocladiales Catenariaceae Catenophlyctis Catenophlyctis variabilis
99.31	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Bryometopida Kreyellidae Microdiaphanosoma Microdiaphanosoma arcuatum
86.667	Eukaryota Katablepharidaceae Katablepharidaceae Katablepharis environmental samples uncultured Katablepharis
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetidae Agaricales Cortinariaceae Alnicola Alnicola sp. MG 2011c
95.172	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
91.857	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae Oxytrichinae Oxytricha Oxytricha longigranulosa
87.821	Eukaryota Rhizaria Cercozoa Imbricatea Silicofilosea Euglyphida Trinematidae Trinema Trinema enchelys
97.674	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Stauroneidaceae Craticula Craticula accomoda
86.747	Eukaryota Katablepharidaceae Katablepharidaceae Katablepharis environmental samples uncultured Katablepharis
99.425	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Bacillariales Bacillariaceae Cylindrotheca Cylindrotheca sp. RCC4823
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Massarineae Dictyosporiaceae Jalapriya Jalapriya pulchra
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Dactylonectria Dactylonectria torresensis
94.937	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Polyporales Polyporaceae Daedaleopsis Daedaleopsis confragosa
89.326	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
96.316	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
96.842	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
87.43	Eukaryota Stramenopiles Peronosporomycetes Peronosporomycetes incertae sedis Haptoglossa Haptoglossa zoospora
85.87	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
88.312	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Sarcocystidae Besnoitia Besnoitia besnoiti
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Eurotiomycetes Eurotiomycetidae Eurotiales Aspergillaceae Thysanophora Thysanophora penicillioides
89.655	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Scuticociliatia Philasterida Loxocephalidae Dexiotricha Dexiotricha cf. granulosa
95.205	Eukaryota Amoebozoa Tubulinea Euamoebida Echinamoebidae Echinamoeba Echinamoeba silvestris
89.818	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
92.288	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Thelephorales Thelephoraceae environmental samples uncultured Tomentella

94.561	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Urostylida Urostylidae Uroleptus Uroleptus pisces
88.816	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Hypotrichia unclassified hypotrichs Hypotrichida sp. Florida
94.828	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
98.575	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae Alternaria Alternaria alternata group Alternaria alternata
91.413	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Saprolegnia unclassified Saprolegnia Saprolegnia sp. SAP4
93.506	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Stichotrichida environmental samples uncultured stichotrichid
86.096	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium salpingophorum
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Fusarium Gibberella sp.
95.833	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota unclassified Basidiomycota Basidiomycota sp. 7-LS-4-C-44(M)-A
85.35	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Cymbellales Gomphonemataceae Gomphonema Gomphonema parvulum
99.676	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Russulales Russulaceae Russula Russula vesca
94.595	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Paracercomonas unclassified Paracercomonas Paracercomonas sp. DB-1602-28
95.139	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
97.688	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Sellaphoraceae Sellaphora Sellaphora laevissima
95.225	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chaetophorales Chaetophorales incertae sedis Stigeoclonium Stigeoclonium aestivale
91.205	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida Plasmodiophoridae Woronina Woronina pythii
95.954	Eukaryota environmental samples uncultured eukaryote
99.668	Eukaryota Cryptophyceae environmental samples uncultured Cryptophyceae
99.668	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Bulleribasidiaceae Dioszegia Dioszegia hungarica
97.872	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Didymellaceae Epicoccum Epicoccum sp. 43-13-1
93.711	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiogetonaceae Pythiogeton Pythiogeton zizaniae
98.925	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Sellaphoraceae Sellaphora Sellaphora laevissima
85.034	Eukaryota Alveolata Dinophyceae Peridiniales Heterocapsaceae environmental samples Heterocapsaceae environmental sample
94.813	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales environmental samples uncultured Helotiales
91.761	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Leotiomycetes incertae sedis Leohumicola Leohumicola minima
96.605	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
87.054	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Pucciniomycetes Pucciniales Pucciniaceae Puccinia Puccinia coronata Puccinia coronata var. avenae Puccinia coronata var. avenae f. sp. avenae
94.857	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes environmental samples uncultured Leotiomycetes
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Cylindrocarpon Cylindrocarpon sp. AC-2011a
87.528	Eukaryota Stramenopiles Chrysophyceae Chromulinales Chromulinaceae Pedospumella Pedospumella encystans
100	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Stichotrichida environmental samples uncultured stichotrichid
97.241	Eukaryota Alveolata Dinophyceae Peridiniales Heterocapsaceae environmental samples Heterocapsaceae environmental sample
97.468	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Sellaphoraceae Sellaphora Sellaphora laevissima
95.172	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
99.537	Eukaryota environmental samples uncultured eukaryote
90.909	Eukaryota Katablepharidaceae Katablepharidaceae environmental samples uncultured Katablepharidaceae
85.818	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Trichostomatia Vestibuliferida Balantidiidae Balantioides Balantioides coli
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Filobasidiales Piskurozymaceae Solicoccozyma Solicoccozyma terricola
96.985	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
98.802	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Dileptida Dileptidae Dileptus Dileptus costaricanus
100	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Coelastrum Coelastrum microporum
99.578	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Phaeosphaeriaceae environmental samples uncultured Phaeosphaeria
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Thelephorales Thelephoraceae environmental samples uncultured Thelephoraceae
100	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.958	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadales incertae sedis Tetracystis Tetracystis vinatzeri
98.466	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
98.413	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Capnodiales Teratosphaeriaceae Austroafricana Austroafricana parva
94.175	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiales incertae sedis Paralagenidium Paralagenidium sp. C09-TL95
94.332	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
98.83	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
99.162	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Saprolegnia unclassified Saprolegnia Saprolegnia sp. SAP4
95.541	Eukaryota Stramenopiles Chrysophyceae Chromulinales Dinobryaceae Poteriospumella Poteriospumella sp.
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Polyporales environmental samples uncultured Polyporales
98.611	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Colpodida Bryophidae Bryophrya Bryophrya gemmea
92.994	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
99.618	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes environmental samples uncultured Myrmecridium
100	Eukaryota Opisthokonta Fungi Mucoromycota Mucoromycotina Mucorales Mucorineae Mucoraceae Mucor Mucor hiemalis

88.298	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Thyridariaceae Parathyridaria Parathyridaria ramulicola
87.898	Eukaryota Alveolata Dinophyceae Peridiniales Heterocapsaceae environmental samples uncultured Heterocapsa
95.367	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Microascales Halosphaeriaceae environmental samples uncultured Periconia
88.321	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes environmental samples uncultured Tremellomycetes
99.39	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporomycetidae incertae sedis Gloniaceae Cenococcum environmental samples uncultured Cenococcum
91.139	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Phaeosphaeriaceae Phaeosphaeriopsis Phaeosphaeriopsis glaucopunctata
98	Eukaryota Stramenopiles Chrysophyceae Chromulinales Dinobryaceae Epipyxis Epipyxis pulchra
91.083	Eukaryota Alveolata Dinophyceae Suessiales Symbiodiniaceae Symbiodinium Symbiodinium psygmophilum
100	Eukaryota Alveolata Ciliophora environmental samples uncultured ciliate
91.411	Eukaryota Stramenopiles Labyrinthulomycetes Amphitremida Diplophrys Diplophrys sp. ATCC_50360
99.625	Eukaryota Opisthokonta Fungi Dikarya Ascomycota unclassified Ascomycota Ascomycota sp. UNEX FECRGA 2012E425
97.126	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Leotiomycetes incertae sedis Myxotrichaceae environmental samples uncultured Geomyces
86.909	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
100	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Eocercomonas Eocercomonas sp. 8VMO
91.139	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.429	Eukaryota Opisthokonta Fungi Dikarya Ascomycota unclassified Ascomycota ascomycete sp. RH 10-1
100	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas Chlamydomonas applanata
99.67	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetidae Agaricales Psathyrellaceae Coprinopsis Coprinopsis cinerea
97.959	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales unclassified Tremellales Tremellales sp. LM334
98.919	Eukaryota Opisthokonta Fungi Blastocladiomycota environmental samples uncultured Blastocladiomycota
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
96.894	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
87.417	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Stichotrichida Cladotrichidae Engelmanniella Engelmanniella mobilis
89.294	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas Chlamydomonas dorsoventralis
99.379	Eukaryota Stramenopiles Synurophyceae Ochromonadales Ochromonadaceae Poterioochromonas Poterioochromonas malhamensis
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Hymenochaetales Hymenochaetales incertae sedis Resinicium Resinicium bicolor
89.568	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
91.575	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
100	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Coelastrella Coelastrella rubescens
94.82	Eukaryota Stramenopiles Chrysophyceae Chromulinales Chromulinaceae Pedospumella Pedospumella sp. JBM19
99.306	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Colpodida Colpodidae Colpoda Colpoda sp. PRA-118
95.979	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium hydnosporum
95.568	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium dissimile
97.382	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
89.203	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chloromonas Chloromonas subdivisa
89.744	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas unclassified Chlamydomonas Chlamydomonas sp. HH 10204
85.117	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiaceae Lagenidium Lagenidium sp. PWL-2010e
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae Alternaria Alternaria alternata group Alternaria alternata
99.306	Eukaryota Alveolata Ciliophora Intramacronucleata Nassophorea Nassulida Nassulidae Obertrumia Obertrumia georgiana
89.855	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
86.735	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiaceae Lagenidium Lagenidium giganteum Lagenidium giganteum f. caninum
96.96	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Microascales Microascaceae unclassified Microascaceae Microascaceae sp. LM278
97.245	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Naviculaceae Navicula unclassified Navicula Navicula sp. ETS 07
92.222	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Vorticellidae Vorticella Vorticella chlorostigma
100	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida environmental samples uncultured Plasmodiophoridae
100	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Vorticellidae Vorticella Vorticella fusca
94.048	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Cystobasidiomycetes Erythrobasidiales Erythrobasidiaceae unclassified Erythrobasidiaceae Erythrobasidium clade sp. LM485
96.1	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Dothideales Saccotheciaceae Pseudosydowia Pseudosydowia eucalypti
99.206	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
96.124	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Polyporales Polyporaceae Ganoderma Ganoderma multiplicatum
94.595	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiaceae Lagenidium Lagenidium giganteum Lagenidium giganteum f. caninum
99.673	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae Oxytrichinae Oxytricha Oxytricha granulifera
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Polyporales Polyporaceae Ganoderma Ganoderma sp. MU-2009-1
99.772	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.686	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
97.143	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Epistylidae Rhabdostyla Rhabdostyla sp. 5 PPS-2010

92.701	Eukaryota Alveolata Ciliophora environmental samples uncultured ciliate
98.925	Eukaryota Stramenopiles Peronosporomycetes Peronosporales Phytophthora Phytophthora plurivora
90.217	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae Trebouxiophyceae incertae sedis Hindakia Hindakia tetrachotoma
88.286	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Mariannaea Mariannaea elegans Mariannaea elegans var. punicea
88.599	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Rhynchogastremataceae Rhynchogastrema Rhynchogastrema noutii
99.631	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Leotiomycetes incertae sedis Pseudeurotiaceae Geomyces Geomyces auratus
95.313	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Cystofilobasidiales Mrakiaceae Tausonia Tausonia pullulans
93.016	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae environmental samples uncultured Oxytrichidae
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
99.306	Eukaryota Alveolata Ciliophora Intramacronucleata Prostomatea Prorodontida Prorodontidae Prorodon Prorodon teres
95.765	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Halteriidae Halteria Halteria grandinella
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Trimorphomycetaceae Saitozyma Saitozyma flava
99.595	Eukaryota Alveolata Dinophyceae Peridiniales Peridiniopsidaceae Peridiniopsis Peridiniopsis polonicum
97.403	Eukaryota Stramenopiles Chrysophyceae Chromulinales Chromulinaceae Ochromonas Ochromonas danica
99.385	Eukaryota Opisthokonta Fungi Dikarya Ascomycota unclassified Ascomycota Ascomycota sp. LM221
94.606	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae environmental samples uncultured Sordariomycetidae
95.957	Eukaryota Stramenopiles Peronosporomycetes Lagenaceae Lagena Lagena radicicola
97.101	Eukaryota Rhizaria Cercozoa Cercomonadida environmental samples Cercomonadida environmental sample
98.457	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Cystobasidiomycetes Cystobasidiomycetes incertae sedis Buckleyzyma Buckleyzyma aurantiaca
97.97	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae Microthamniales Symbiochloris Symbiochloris symbiontica
99.14	Eukaryota Opisthokonta Fungi Dikarya Ascomycota Ascomycota incertae sedis Tetracladium Tetracladium maxilliforme
97.093	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Dermateaceae Phlyctema Phlyctema vagabunda
87.919	Eukaryota Alveolata Dinophyceae Gymnodiniales Gymnodiniaceae Gymnodinium unclassified Gymnodinium Gymnodinium sp. Dino1
89.041	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
95.745	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
97.683	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Rhizophydiales Rhizophydiaceae environmental samples uncultured Rhizophydium
86.131	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
90.741	Eukaryota Opisthokonta Fungi Mucoromycota Glomeromycotina Glomeromycetes Diversisporales Diversisporaceae Corymbiglomus Corymbiglomus tortuosum
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Microbotryomycetes Leucosporidiales Leucosporidiaceae Leucosporidium Leucosporidium intermedium
94.043	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae environmental samples uncultured Oxytrichidae
92.348	Eukaryota Viridiplantae Chlorophyta Ulvophyceae Ulotrichales Ulotrichales incertae sedis Planophila Planophila bipyrenoidosa
99.659	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Cantharellales Ceratobasidiaceae Rhizoctonia Rhizoctonia solani
85.87	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Trichostomatia Vestibuliferida Balantidiidae Balantioides Balantioides coli
100	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Naviculaceae Fistulifera Fistulifera pelliculosa
95.238	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peniculida Parameciidae Paramecium Paramecium duboscqui
92.994	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Fusarium Fusarium sp. 08025
98.78	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Desmodesmus Desmodesmus insignis
87.456	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Choreotrichia Tintinnida Tintinnidae Amphorellopsis Amphorellopsis quinquealata
90.446	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae Oxytrichinae Oxytricha unclassified Oxytricha Oxytricha sp. Steamboat Hot Springs
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Glomerellales Plectosphaerellaceae Plectosphaerella Plectosphaerella sp. sedF4
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae Pyrenophora Pyrenophora tritici-repentis
93.363	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Cymbellales Gomphonemataceae Gomphonema Gomphonema parvulum
94.888	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Cucurbitariaceae Pyrenochaeta Pyrenochaeta sp. 14009
97.872	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Cystobasidiomycetes Cystobasidiomycetes incertae sedis Sakaguchia Sakaguchia lamellibrachiae
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Sordariales Chaetomiaceae Humicola Humicola grisea
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Rhynchogastremataceae Papiliotrema Papiliotrema flavescens
98.734	Eukaryota Rhizaria Cercozoa Thecofilosea Cryomonadida Rhogostomidae Rhogostoma Rhogostoma schuessleri
99.063	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes environmental samples uncultured Dothideomycetes
97.638	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Lacrymariidae Lacrymaria Lacrymaria marina
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetes incertae sedis Arxiella Arxiella dolichandrae
97.403	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Cercomonas Cercomonas jendrali
99.306	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Colpodida Colpodidae Bresslaua Bresslaua vorax
88.636	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Hypotrichia unclassified hypotrichs Hypotrichida sp. Florida
95.897	Eukaryota Opisthokonta Choanoflagellata environmental samples uncultured choanoflagellate

99.617	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
93.878	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina Eugregarinorida Monocystidae Monocystis Monocystis agilis
93.82	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Leotiomycetes incertae sedis Myxotrichaceae environmental samples uncultured Oidiodendron
97.5	Eukaryota Opisthokonta Fungi Mucoromycota Mortierellomycotina Mortierellales Mortierellaceae Mortierella Mortierella globalpina
94.483	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Urostylida Urostylidae Tunicothrix Tunicothrix brachysticha
99.582	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Torulaceae Torula Torula herbarum
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Dothideales Saccotheciaceae Aureobasidium Aureobasidium pullulans
100	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Scuticociliatia Philasterida Orchitophryidae environmental samples Orchitophryidae environmental sample
93.114	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Glomerellales Plectosphaerellaceae Plectosphaerella Plectosphaerella sp. sedF4
98.089	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Cercomonas Cercomonas sp. C-83
97.493	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium capillosum
99.174	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
96.311	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.684	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Cymbellales Gomphonemataceae Gomphonema Gomphonema parvulum
95.745	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Bacillariales Bacillariaceae Nitzschia Nitzschia microcephala
96.257	Eukaryota Opisthokonta Opisthokonta incertae sedis Ichthyosporea Ichthyophonida Anurofeca Anurofeca sp. LAH-2003
97.093	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiaceae Lagenidium Lagenidium deciduum
99.449	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes environmental samples uncultured Agaricomycetes
98.697	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae Oxytrichinae Oxytricha unclassified Oxytricha Oxytricha sp. Steamboat Hot Springs
96.296	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Paracercomonas unclassified Paracercomonas Paracercomonas sp. DB-0802-29
97.708	Eukaryota Opisthokonta Fungi Dikarya Ascomycota Ascomycota incertae sedis Tetracladium Tetracladium maxilliforme
92.784	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Dermateaceae Pezicula Pezicula rubi
100	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae Trebouxiophyceae incertae sedis Neocystis Neocystis brevis
100	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas Chlamydomonas dorsoventralis
100	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.62	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Polyporales Dacryobolaceae Oligoporus Oligoporus balsameus
96.682	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Xylariomycetidae Xylariales unclassified Xylariales Xylariales sp. MU-2009-1
89.568	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
93.314	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.077	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
86.577	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae environmental samples uncultured Trebouxiophyceae
88.064	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.598	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
98.592	Eukaryota Amoebozoa Archamoebae Entamoebidae Entamoeba Entamoeba nuttalli
99.608	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Eurotiomycetes Eurotiomycetidae Eurotiales Aspergillaceae environmental samples uncultured Penicillium
97.931	Eukaryota Rhizaria Cercozoa unclassified Cercozoa Gymnophrys Gymnophrys sp. ATCC 50923
97.561	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
95.014	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
86.076	Eukaryota Viridiplantae Chlorophyta prasinophytes Mamiellophyceae Mamiellales Mamiellaceae Micromonas Micromonas pusilla
96.599	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina environmental samples uncultured gregarine
94.118	Eukaryota Stramenopiles Hyphochytriomycetes Hyphochytriaceae Hyphochytrium Hyphochytrium catenoides
93.704	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Epistylidae Epistylis Epistylis plicatilis
100	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
87.037	Eukaryota Katablepharidaceae Katablepharidaceae environmental samples uncultured Katablepharidaceae
93.464	Eukaryota Apusozoa Apusomonadidae Amastigomonas Amastigomonas mutabilis
89.888	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Rosculus Rosculus cf. ithacus CCAP 1571/3
100	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
85.766	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Trichostomatia Vestibuliferida Balantidiidae Balantioides Balantioides coli
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Microbotryomycetes Sporidiobolales Sporidiobolaceae Rhodotorula Rhodotorula babjevae
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Erysiphales Erysiphaceae Erysiphe Erysiphe sp. Wisley-2007
89.333	Eukaryota Cryptophyceae Cryptomonadales Goniomonadaceae Goniomonas Goniomonas sp. ATCC 50108
89.106	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Sordariales Chaetomiaceae Thermothelomyces Thermothelomyces thermophila Thermothelomyces thermophila
88.372	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota

100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Auriculariales Auriculariaceae environmental samples uncultured Auriculariaceae
97.283	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Grossglockneriida Grossglockneriidae Mykophagophrys Mykophagophrys terricola
91.613	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales unclassified Tremellales Tremellales sp. DU26
90.146	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
96.923	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadales incertae sedis Tetracystis Tetracystis sp. 14601-7.1
89.091	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
97.783	Eukaryota Stramenopiles Eustigmatophyceae Eustigmatales Eustigmataceae Eustigmatos Eustigmatos vischeri
98.225	Eukaryota environmental samples uncultured eukaryote
90.683	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Hypocreales incertae sedis Cephalosporium Cephalosporium sp. BRO-2013
85	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Cercomonas Cercomonas parincurva
93.671	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.654	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Rhizophydiales Rhizophydiales incertae sedis Operculomyces Operculomyces laminatus
99.563	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.065	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadales incertae sedis Ettlia Ettlia pseudoalveolaris
92.727	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Stachybotryaceae Albifimbria Albifimbria verrucaria
87.898	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
96.154	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
93.671	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae environmental samples uncultured Oxytrichidae
99.213	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.497	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.696	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
96.689	Eukaryota Stramenopiles Chrysophyceae Chromulinales Chromulinaceae Chromulina Chromulina chionophila
100	Eukaryota Stramenopiles Chrysophyceae unclassified Chrysophyceae Spumella-like flagellate JB Spumella-like flagellate JBAF33
93.952	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
93.836	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina Eugregarinorida Lecudinidae Psychodiella Psychodiella sp. JJV-2009b
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Fusarium Fusarium oxysporum species complex Fusarium oxysporum Fusarium oxysp
96.765	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales unclassified Hypocreales Hypocreales sp. LM421
99.66	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Sordariales Sordariaceae environmental samples uncultured Sordaria
100	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida environmental samples uncultured Plasmodiophoridae
96.526	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
86.861	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia environmental samples uncultured peritrich ciliate
85.902	Eukaryota Breviatea Breviata Breviata anathema
98.061	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Achlya Achlya bisexualis
90.057	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
95.58	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Cordycipitaceae Parengyodontium Parengyodontium album
89.937	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.694	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae environmental samples uncultured Pleosporaceae
99.701	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.69	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
91.946	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Paracercomonas unclassified Paracercomonas Paracercomonas sp. Panama83
99.32	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina environmental samples uncultured gregarine
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales environmental samples uncultured Chalara
93.711	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Chytridiales Chytridiaceae Phlyctochytrium Phlyctochytrium planicorne
96.023	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.621	Eukaryota Alveolata Dinophyceae Peridiniales Heterocapsaceae environmental samples Heterocapsaceae environmental sample
95.105	Eukaryota Stramenopiles Labyrinthulomycetes Thraustochytriaceae Aplanochytrium Aplanochytrium kerguelense
95.522	Eukaryota Stramenopiles Labyrinthulomycetes Amphitremida Diplophrys Diplophrys mutabilis
98.575	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium anandrum
100	Eukaryota Rhizaria Cercozoa Cercomonadida Cercomonadidae Cercomonas Cercomonas celer
93.038	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.643	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetidae Agaricales Bolbitiaceae Agrocybe Agrocybe erebia
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Mariannaea Mariannaea elegans Mariannaea elegans var. punicea
93.75	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Saprolegnia unclassified Saprolegnia Saprolegnia sp. SAP4
98.444	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Scenedesmus Scenedesmus sp. HH 10206
96.377	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Scuticociliatia Philasterida Orchitophryidae environmental samples Orchitophryidae environmental sample

99.754	Eukaryota Stramenopiles Peronosporomycetes Peronosporales Peronosporaceae Hyaloperonospora Hyaloperonospora parasitica species group Hyaloperonospora parasitica
96.97	Eukaryota Alveolata Dinophyceae Gonyaulacales Gonyaulacaceae Gonyaulax Gonyaulax spinifera
100	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae environmental samples uncultured Apatococcus
100	Eukaryota Stramenopiles Chrysophyceae Chromulinales Dinobryaceae Poteriospumella Poteriospumella sp.
94.118	Eukaryota unclassified eukaryotes Telonemida Telonema Telonema subtile
100	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas Chlamydomonas globosa
94.444	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
99.185	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Dothideales Dothioraceae Dothiora Dothiora maculans
100	Eukaryota Stramenopiles Synurophyceae Ochromonadales Ochromonadaceae Poterioochromonas Poterioochromonas malhamensis
98.256	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Naviculales Sellaphoraceae Sellaphora Sellaphora laevissima
99.392	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Didymellaceae Epicoccum Epicoccum sp. 43-13-1
88.462	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiaceae Lagenidium Lagenidium giganteum Lagenidium giganteum f. caninum
98.276	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
97.436	Eukaryota Stramenopiles Labyrinthulomycetes Amphitremida Diplophrys Diplophrys sp. ATCC_50360
96.333	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales unclassified Tremellales Tremellales sp. LM630
100	Eukaryota Opisthokonta Fungi Mucoromycota Mucoromycotina Mucorales Mucorineae Mucoraceae Mucor Mucor racemosus
96.057	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Epistylidae Rhabdostyla Rhabdostyla sp. 5 PPS-2010
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae environmental samples uncultured Sordariomycetidae
99.27	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Russulales Russulaceae environmental samples uncultured Russula
95.062	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
96.522	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Desmodesmus Desmodesmus insignis
97.436	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Rhizophlyctidales Rhizophlyctidaceae Rhizophlyctis Rhizophlyctis harderi
95.833	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
93.151	Eukaryota Alveolata Ciliophora Intramacronucleata Colpodea Cyrtolophosidida Cyrtolophosididae Pseudocyrtolophosis Pseudocyrtolophosis alpestris
94.41	Eukaryota Opisthokonta Choanoflagellata environmental samples uncultured choanoflagellate
95.804	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae environmental samples uncultured Chlamydomonas
95.789	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Bionectriaceae Clonostachys Clonostachys rosea
98.993	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peniculida Parameciidae Paramecium Paramecium tetraurelia
98.94	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.31	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiaceae Lagenidium Lagenidium sp. SLG-2014b
93.811	Eukaryota Alveolata Dinophyceae Prorocentrales Prorocentraceae Prorocentrum Prorocentrum micans
94.005	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Coniochaetales Coniochaetaceae Coniochaeta Coniochaeta ligniaria
99.676	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Russulales Russulaceae Russula Russula vesca
99.701	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Microbotryomycetes Microbotryomycetes incertae sedis Curvibasidium Curvibasidium cygneicollum
99.659	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Phallomycetidae Phallales Phallaceae Mutinus Mutinus caninus
98.88	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Erysiphales Erysiphaceae Podosphaera Podosphaera fusca
98.611	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Oligotrichia Strombidiidae Strombidium Strombidium sp. HCB-2005
97.794	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Ophrydiidae Ophrydium Ophrydium versatile
88.211	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina Eugregarinorida Monocystidae Monocystis unclassified Monocystis Monocystis sp. R1_20
100	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida environmental samples uncultured Plasmodiophoridae
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota unclassified Ascomycota Ascomycota sp. LM221
99.606	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Bionectriaceae environmental samples uncultured Bionectria
88.043	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
93.557	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Helotiaceae Articulospora Articulospora proliferata
89.701	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
90.685	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Xylariomycetidae Xylariales Xylariaceae Lopadostoma Lopadostoma americanum
99.231	Eukaryota Rhizaria Cercozoa Imbricatea Plasmodiophorida environmental samples uncultured Plasmodiophoridae
96.691	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Sordariales Lasiosphaeriaceae environmental samples uncultured Lasiosphaeriaceae
93.831	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Oxytrichidae environmental samples uncultured Oxytrichidae
100	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Stichotrichida environmental samples uncultured stichotrichid
97.929	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Microbotryomycetes Sporidiobolales unclassified Sporidiobolales Sporidiobolales sp. LM531
99.624	Eukaryota Opisthokonta Fungi Dikarya Ascomycota unclassified Ascomycota Ascomycota sp. D7
97.126	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Thelebolales Thelebolaceae environmental samples uncultured Thelebolaceae
94.154	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Cystobasidiomycetes Cystobasidiales Cystobasidiaceae Cystobasidium Cystobasidium psychroaquaticum
99.329	Eukaryota Stramenopiles Synurophyceae Synurales Mallomonadaceae Mallomonas Mallomonas annulata
91.216	Eukaryota Rhizaria Cercozoa Cercomonadida Metopion Metopion-like sp. (SA)
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae environmental samples uncultured Pleosporaceae

95.012	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.723	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetes incertae sedis Botryosphaeriales Botryosphaeriaceae Diplodia Diplodia seriata
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
99.346	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
97.368	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chlamydomonas Chlamydomonas splendida
93.238	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Choreotrichia Tintinnida Tintinnidae Tintinnidium Tintinnidium fluviatile
98.956	Eukaryota Viridiplantae Chlorophyta Ulvophyceae Ulotrichales Ulotrichales incertae sedis Planophila Planophila laetevirens
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Didymellaceae Epicoccum Epicoccum sp. 43-13-1
100	Eukaryota Opisthokonta Choanoflagellata environmental samples uncultured choanoflagellate
87.879	Eukaryota Stramenopiles Peronosporomycetes Lagenidiales Lagenidiaceae Lagenidium Lagenidium sp. SLG-2014a
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Phaeosphaeriaceae Paraphoma Paraphoma sp.
90.909	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chloromonas Chloromonas sp. CCCryo273-06
97.638	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Lacrymariidae Phialina Phialina salinarum
88.208	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadales incertae sedis Tetracystis Tetracystis aplanospora
96.386	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Urostylida Holostichidae Holosticha Holosticha diademata
86.982	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomyctina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Capnodiales Mycosphaerellaceae Pallidocercospora Pallidocercospora crystallina
95.251	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Phaeosphaeriaceae Setophoma Setophoma vernoniae
99.425	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae Chloromonas Chloromonas fonticola
95.641	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadales incertae sedis Tetracystis Tetracystis sp. 14601-7.1
90.859	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Aphanomyces unclassified Aphanomyces Aphanomyces sp. APH2
99.338	Eukaryota Stramenopiles Synurophyceae Synurales Mallomonadaceae Mallomonas Mallomonas papillosa
92.533	Eukaryota Stramenopiles Peronosporomycetes Lagenaceae Lagena Lagena radicicola
98.795	Eukaryota Stramenopiles Chrysophyceae Chromulinales Dinobryaceae Poteriospumella Poteriospumella sp.
95.522	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina Neogregarinorida Syncystidae Syncystis Syncystis mirabilis
98.675	Eukaryota Stramenopiles Chrysophyceae Chromulinales Chromulinaceae Chromulina Chromulina chionophila
92.638	Eukaryota Stramenopiles Chrysophyceae Chromulinales Dinobryaceae Dinobryon Dinobryon sp.
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Sclerotiniaceae Botrytis Botrytis cinerea Botrytis cinerea B05.10
100	Eukaryota Opisthokonta Fungi unclassified Fungi fungal sp.
97.846	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
95.309	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlorococcaceae Chlorococcum unclassified Chlorococcum Chlorococcum sp. CCAP 11/52
97.853	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
91.824	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.023	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Stichotrichida environmental samples uncultured stichotrichid
92.025	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Microascales Microascaceae Lomentospora Lomentospora prolificans
98.466	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Dothideomycetidae Capnodiales Mycosphaerellaceae Xenoramularia Xenoramularia neerlandica
85.027	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Lecanoromycetes OSLEUM clade Umbilicariomycetidae Umbilicariales Umbilicariaceae Umbilicaria Umbilicaria nylanderiana
99.038	Eukaryota Rhizaria Cercozoa Kraken Kraken carinae
99.324	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
96.032	Eukaryota Alveolata Dinophyceae Syndiniales environmental samples uncultured Amoebophrya
96.914	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
92.8	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Rhizophydiales Alphamycetaceae Betamyces Betamyces americaemeridionalis
92.157	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Diaporthales Gnomoniaceae Apiognomonia Apiognomonia cf. errabunda MU-2009
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Massarineae Morosphaeriaceae Rhizopycnis Rhizopycnis sp. 12227
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Xylariomycetidae Xylariales Hypoxylaceae Daldinia Daldinia childiae
92.308	Eukaryota environmental samples uncultured eukaryote
92.308	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
89.619	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Phaeotremellaceae Phaeotremella Phaeotremella neofoliacea
99.655	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.225	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Acropisthiidae Fuscheria Fuscheria sp. VBOEF-2011
98.556	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Vorticellidae Vorticella Vorticella sp. 4 PS-2013
89.493	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
98.905	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
93.048	Eukaryota Stramenopiles Peronosporomycetes Myzocytiopsidales Myzocytiopsidaceae Myzocytiopsis Myzocytiopsis humicola
94.643	Eukaryota Alveolata Ciliophora Intramacronucleata Oligohymenophorea Peritrichia Sessilida Epistylidae Rhabdostyla Rhabdostyla sp. 5 PPS-2010
99.262	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Leotiomycetes incertae sedis Pseudeurotiaceae Geomyces Geomyces auratus
99.548	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae environmental samples uncultured Apatococcus
97.554	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae unclassified Pleosporaceae Pleosporaceae sp. LM282
92.647	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae

86.744	Eukaryota Opisthokonta Fungi Dikarya Ascomycota unclassified Ascomycota Ascomycota sp. LM165
95.833	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
87.662	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Saccharomycotina Saccharomycetes Saccharomycetales Metschnikowiaceae Metschnikowia Metschnikowia caudata
96.286	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Phaeosphaeriaceae Sclerostagonospora Sclerostagonospora sp. CBS 118152
98.093	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
99.528	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Coelastrum Coelastrum microporum
93.558	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Filobasidiales Filobasidiaceae Filobasidium Filobasidium magnum
88.398	Eukaryota Stramenopiles Peronosporomycetes Saprolegniales Saprolegniaceae Aphanomyces unclassified Aphanomyces Aphanomyces sp. APH2
93.418	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Sphaeropleales Scenedesmaceae Coelastrella Coelastrella rubescens
98.551	Eukaryota Rhizaria Cercozoa Cercomonadida environmental samples Cercomonadida environmental sample
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Cordycipitaceae Isaria Isaria farinosa
98.046	Eukaryota Alveolata Ciliophora Intramacronucleata Spirotrichea Stichotrichia Sporadotrichida Halteriidae Halteria Halteria grandinella
99.725	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Helotiaceae Meliniomyces Meliniomyces variabilis
99.706	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
99.048	Eukaryota Opisthokonta Fungi Dikarya Ascomycota unclassified Ascomycota Ascomycota sp. 20302
87.679	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Rhizophydiales Rhizophydiaceae Rhizophydium Rhizophydium patellarium
89.212	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
95.897	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
91.319	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlamydomonadaceae environmental samples uncultured Chlamydomonas
95.484	Eukaryota environmental samples uncultured eukaryote
90.842	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
98.338	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium pyrilobum
99.707	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
95.361	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Thelephorales Thelephoraceae environmental samples uncultured Tomentella
97.122	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Tremellales Phaeotremellaceae Phaeotremella Phaeotremella skinneri
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Massarineae Periconiaceae Periconia Periconia macrospinosa
98.27	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Cantharellales Ceratobasidiaceae environmental samples uncultured Ceratobasidium
98.339	Eukaryota Cryptophyceae environmental samples uncultured Cryptophyceae
90.123	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
98.429	Eukaryota Stramenopiles Diatomeae Bacillariophyceae Bacillariophycidae Mastogloiales Achnanthaceae Achnanthes unclassified Achnanthes Achnanthes sp. CCAC 2681 B
90.184	Eukaryota Stramenopiles Chrysophyceae Chromulinales Paraphysomonadaceae Paraphysomonas Paraphysomonas sp.
99.731	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporineae incertae sedis Camarosporium Camarosporium brabeji
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Neonectria environmental samples uncultured Neonectria
94.444	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
96.703	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiaceae Pythium Pythium graminicola
94.615	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Diaporthales Valsaceae Cytospora Cytospora pruinosa
100	Eukaryota Alveolata Ciliophora environmental samples uncultured ciliate
95.954	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
94.104	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
94.444	Eukaryota Alveolata Apicomplexa Conoidasida Coccidia Eucoccidiorida Eimeriorina Eimeriidae environmental samples uncultured Eimeriidae
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales unclassified Pleosporales Pleosporales sp. MU-2009-4
94.752	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
93.939	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Spiculogloeomycetes Spiculogloeales Spiculogloeaceae Phyllozyma Phyllozyma linderae
91.195	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
99.405	Eukaryota Opisthokonta Fungi Chytridiomycetes Chytridiomycetes Spizellomycetales Spizellomycetaceae Spizellomyces Spizellomyces lactosolyticus
98.394	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales unclassified Pleosporales Pleosporales sp.
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99.005	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
98.452	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
93.611	Eukaryota Stramenopiles Peronosporomycetes Peronosporales Phytopythium Phytopythium helicoides
99.355	Eukaryota Rhizaria Cercozoa environmental samples uncultured Cercozoa
96.914	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Pucciniomycotina Cystobasidiomycetes Cystobasidiales Cystobasidiaceae Cystobasidium Cystobasidium minutum
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95.562	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Hypocreomycetidae Hypocreales Nectriaceae Fusarium Fusarium lateritium species complex Fusarium lateritium
87.5	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Saccharomycotina Saccharomycetes Saccharomycetales Metschnikowiaceae Metschnikowia Metschnikowia caudata
97.006	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Leotiomycetes Helotiales Sclerotiniaceae Sclerotinia Sclerotinia sp. BF105
99.583	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Pleosporaceae environmental samples uncultured Dendryphion
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota

100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta Eurotiomycetes Eurotiomycetidae Eurotiales Aspergillaceae Aspergillus Aspergillus amstelodami
97.17	Eukaryota Viridiplantae Chlorophyta Trebouxiophyceae Chlorellales Chlorellaceae Chlorella Chlorella vulgaris
99.013	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Russulales Russulaceae Russula Russula vesca
99.383	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
99.685	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Ustilaginomycotina Ustilaginomycetes Ustilaginales Ustilaginaceae Ustilago Ustilago striiformis
100	Eukaryota Stramenopiles Synurophyceae Synurales Mallomonadaceae Synura Synura glabra
99.468	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetidae Agaricales Lycoperdaceae environmental samples uncultured Lycoperdon
99.449	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
96.732	Eukaryota Stramenopiles Hyphochytriomycetes Hyphochytriaceae Hyphochytrium Hyphochytrium catenoides
90	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
92.013	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta dothideomyceta Dothideomycetes Pleosporomycetidae Pleosporales Pleosporineae Cucurbitariaceae Pyrenochaeta Pyrenochaeta sp. 14009
98.272	Eukaryota Viridiplantae Chlorophyta Chlorophyceae Chlamydomonadales Chlorococcaceae Chlorococcum unclassified Chlorococcum Chlorococcum sp. CCAP 11/52
96.552	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
90.698	Eukaryota Opisthokonta Fungi Chytridiomycetes environmental samples uncultured Chytridiomycetes
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94.375	Eukaryota Opisthokonta Fungi Fungi incertae sedis environmental samples uncultured zygomycete
94.97	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
87.599	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus
98.769	Eukaryota Opisthokonta Fungi Dikarya Ascomycota saccharomyceta Pezizomycotina leotiomyceta sordariomyceta Sordariomycetes Sordariomycetidae Diaporthales Valsaceae Phomopsis Phomopsis sp. OOW7
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota environmental samples uncultured Basidiomycota
100	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Agaricomycetes Agaricomycetes incertae sedis Auriculariales Auriculariaceae environmental samples uncultured Auriculariaceae
99.711	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Agaricomycotina Tremellomycetes Filobasidiales Piskurozymaceae Solicoccozyma Solicoccozyma terreus
93.175	Eukaryota Alveolata Dinophyceae Peridiniales Peridiniaceae Peridinium Peridinium inconspicuum
100	Eukaryota Opisthokonta Fungi Dikarya Ascomycota environmental samples uncultured Ascomycota
86.38	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
95.918	Eukaryota Alveolata Apicomplexa Conoidasida Gregarinasina environmental samples uncultured gregarine
93.711	Eukaryota Stramenopiles Peronosporomycetes Pythiales Pythiogetonaceae Pythiogeton Pythiogeton zizaniae
87.956	Eukaryota Alveolata Ciliophora Intramacronucleata Litostomatea Haptoria Haptorida Spathidiidae Epispathidium Epispathidium amphoriforme
98.649	Eukaryota Opisthokonta Fungi Dikarya Basidiomycota Ustilaginomycotina Ustilaginomycetes Urocystidales Urocystidaceae Urocystis Urocystis agropyri
99.632	Eukaryota Opisthokonta Fungi environmental samples uncultured fungus

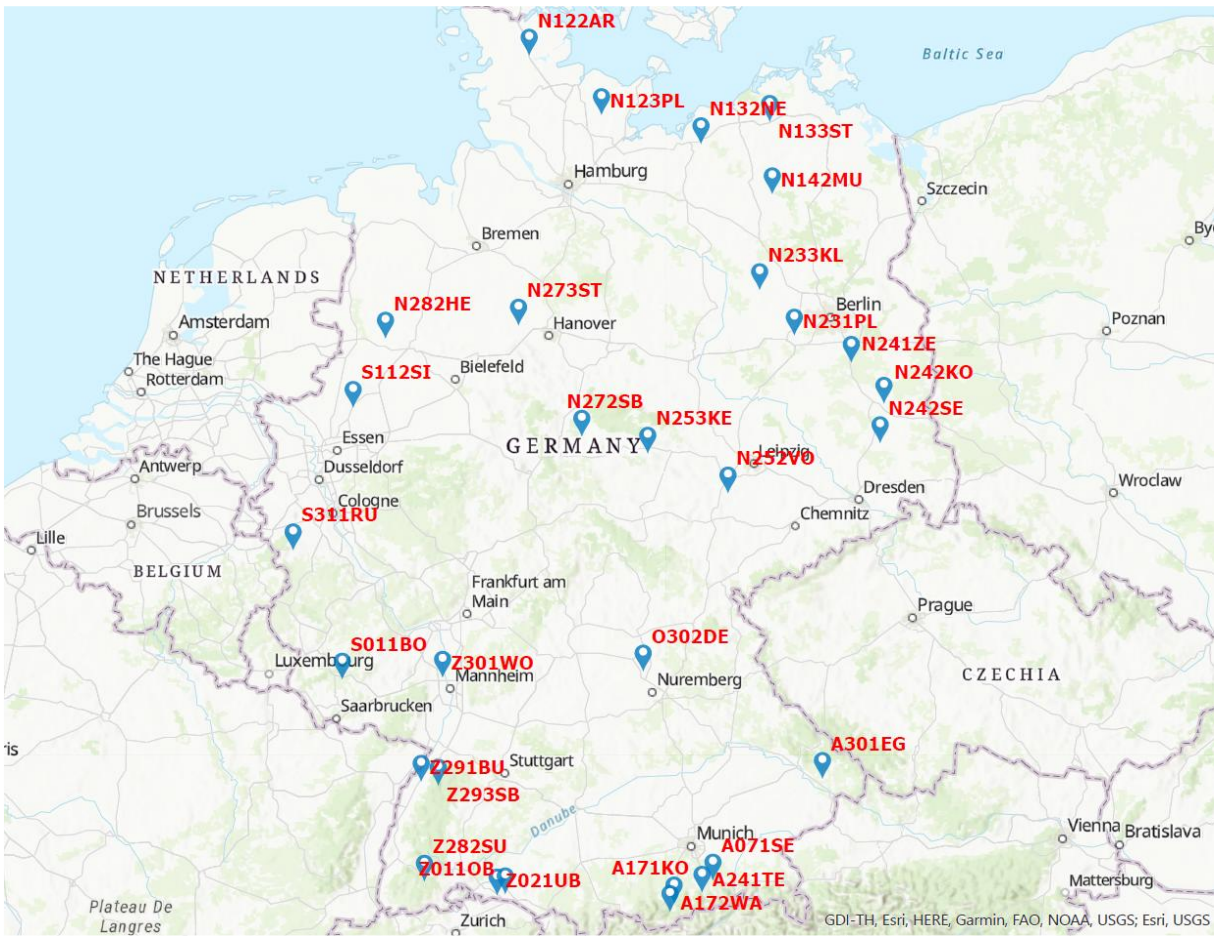

Figure S1: Figure of the study area with each sampling site based on the coordinates from table S1. Map was created with ESRI ArcGIS Pro 2.6 and its topographic base maps (GDI-TH, Esri, HERE, Garmin, FAO, NOAA, USGS; ESRI, USGS) https://www.arcgis.com/home/item.html?id=eaee8760ed754019a964a6d785613a50.

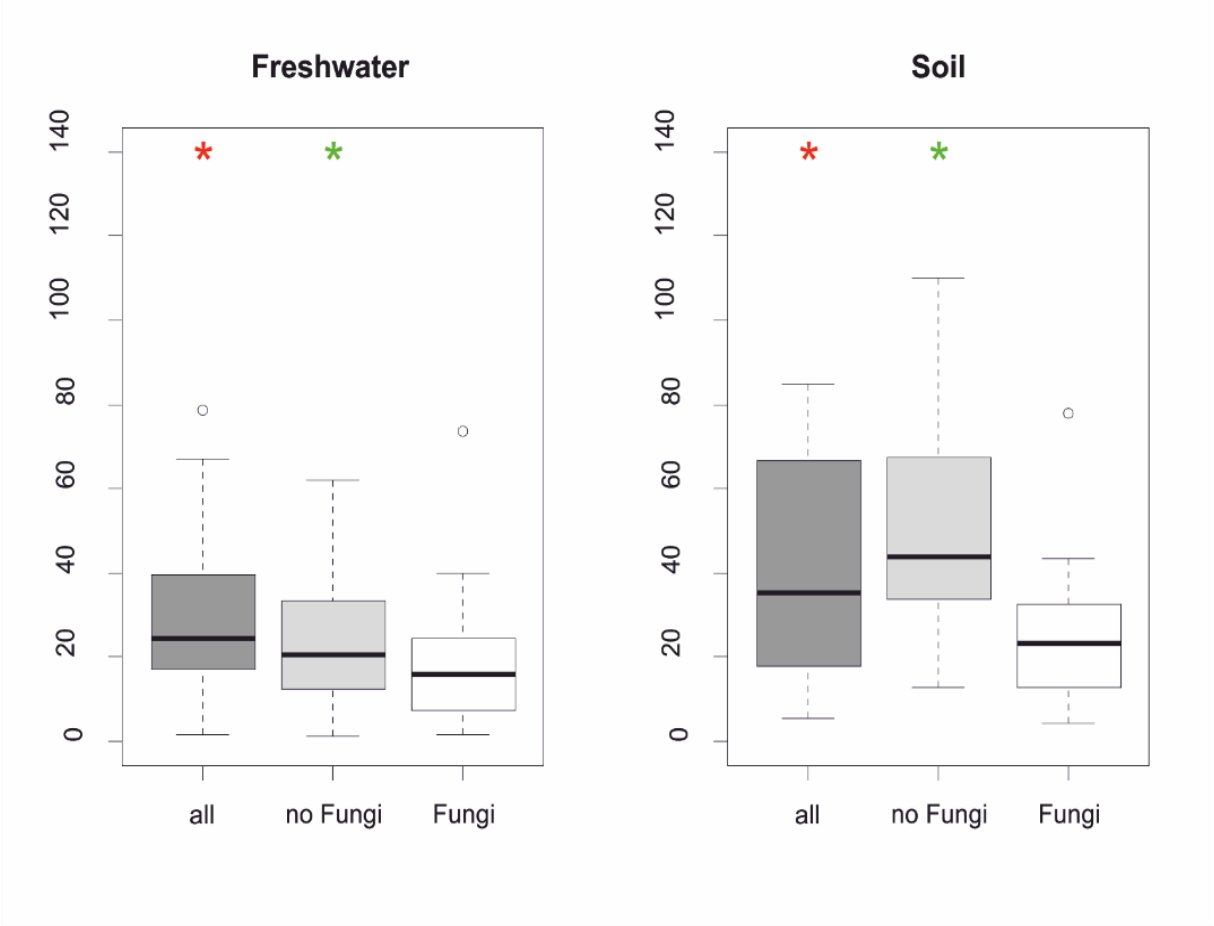

Figure S2: True diversities based on the Shannon-Index. Left fresh water. right soil. The gray boxplot represents the complete dataset. the greyish boxplot represents a subset without Fungi and the white boxplot represents a subset including only Fungi. Identical colored asterisks mark significant differences ($p<0.05$).

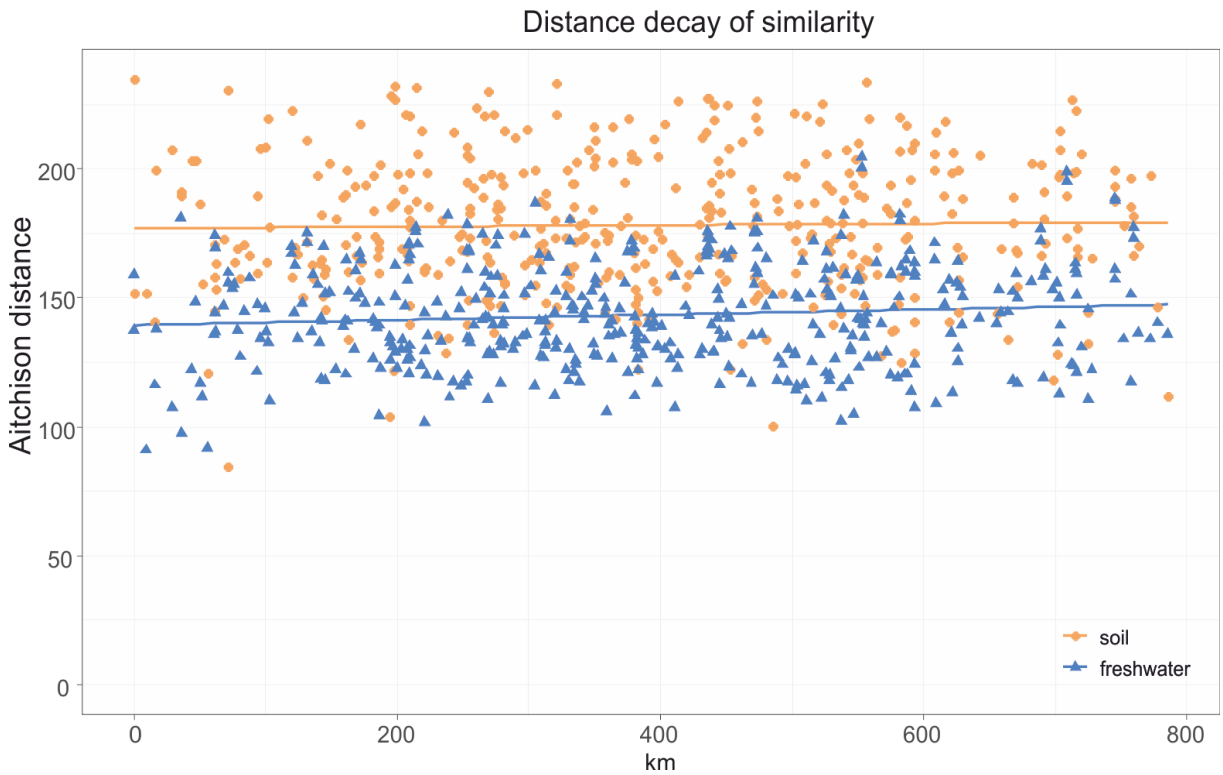

Figure S3: Aitchison-Distance-dissimilarity-decay-relationship of soil and freshwater sites. Each dot represents the Aitchison-dissimilarity between two sites of the same habitat-type (blueish fresh water. brownish soil). Best fit lines are given in respective colors.

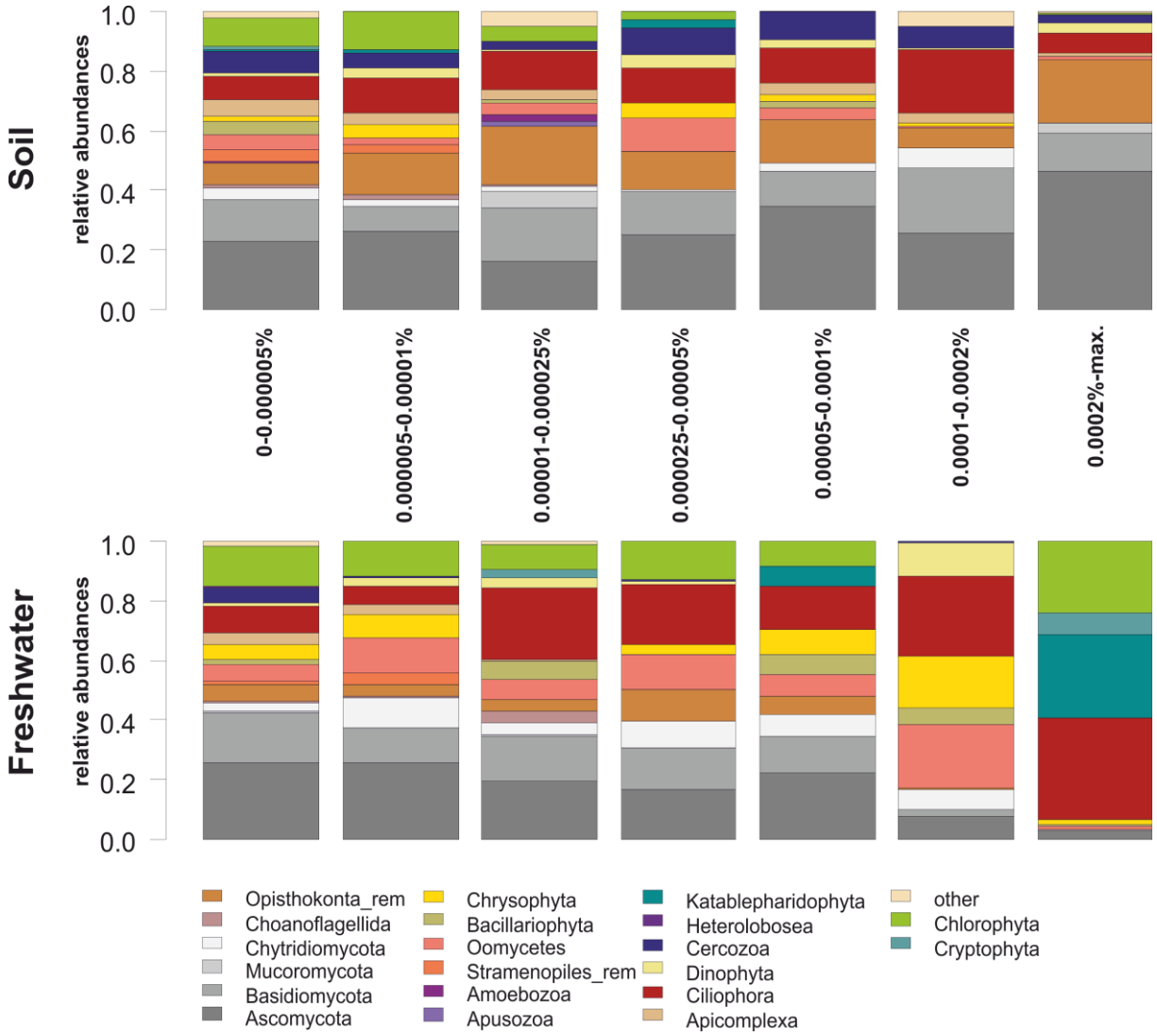

Figure S4: Relative total taxonomic composition of the overlap sorted according to the average share that the shared OTUs make up in the overall soil or freshwater community.

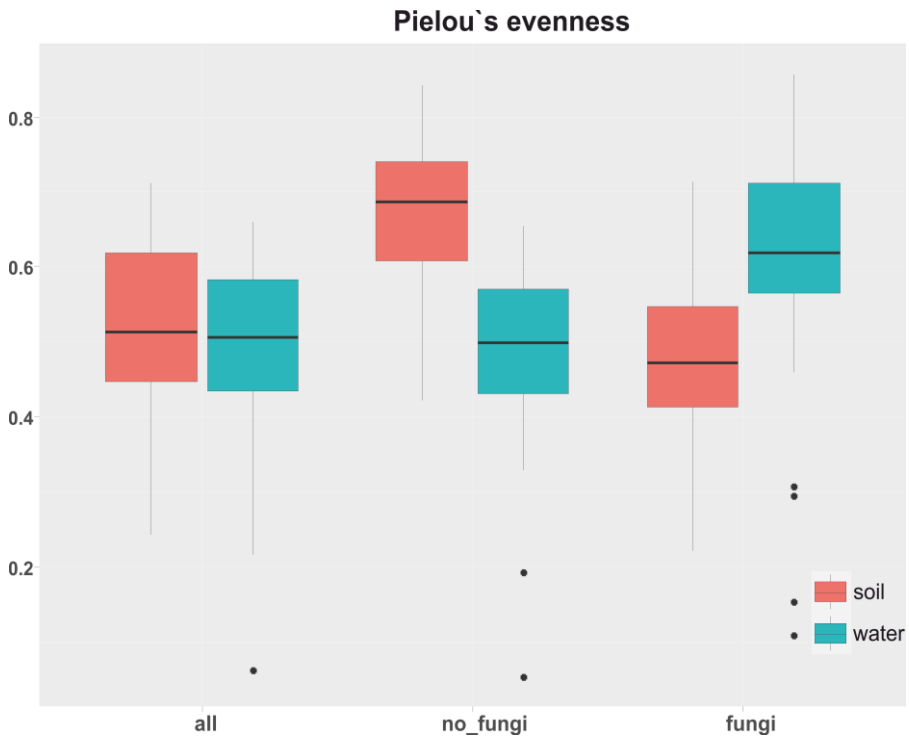

Figure S5: Pairwise Pielou`s evenness index of soil (red) fresh water (blue) based on the raw OTU table. Complete dataset (right). dataset without Fungi (middle). dataset including only Fungi (left).

Article IV supplement: „*Microbial community shifts induced by plastic and zinc as substitutes of tire abrasion*“

Table S1: Basic chemical parameters. Temperature, conductivity and pH are measured three times per sample and per timepoint.

Treatment	Temp 1	Temp 2	Temp 3	cond1	cond2	cond3	pH1	pH2	pH3
C1_1H	19,3	19,9	20,2	453	450	450	7,15	7,29	7,35
C2_1H	18	17,8	17,7	435	438	440	7,76	7,86	7,88
C3_1H	17,7	17,9	17,8	441	440	439	7,61	7,8	7,8
C4_1H	18,6	18	18	441	442	442	7,5	7,55	7,62
C5_1H	18,7	18,5	18,7	437	439	438	7,64	7,7	7,65
C1_24H	19,7	18	17,8	458	467	440	7,9	7,97	7,86
C2_24H	17,3	17,4	17,5	444	443	443	7,7	7,85	7,87
C3_24H	17,3	17,4	17,5	444	442	443	7,7	7,85	7,87
C4_24H	17,8	17,7	17,7	441	442	441	7,99	8,03	9,03
C5_24H	18,2	18,2	18,2	439	441	445	7,77	7,83	7,84
C1_48H	17,1	17,3	17,3	438	438	439	8,39	8,44	8,45
C2_48H	16,7	16,8	16,8	441	441	441	8,49	8,54	8,56
C3_48H	17	17	17	441	441	441	8,52	8,54	8,56
C4_48H	17,3	17,4	17,4	439	439	438	8,63	8,65	8,63
C5_48H	17,8	17,8	17,8	437	436	437	8,63	8,65	8,67
C1_96H	17,4	17,3	17,3	431	434	442	8,4	8,19	8,4
C2_96H	17	16,9	16,9	443	445	446	8,43	8,5	8,51
C3_96H	17	17,1	17,1	445	447	441	8,57	8,59	8,61
C4_96H	17,5	17,4	17,4	445	447	8,61	8,51	8,55	8,57
C5_96H	17,8	17,7	17,7	439	440	442	8,48	8,66	8,7
C1_168H	17,3	17,4	17,4	466	467	464	9,22	9,35	9,3
C2_168H	16,8	16,8	16,8	464	463	464	9,22	9,25	9,29
C3_168H	17,3	17,1	17,2	464	464	466	8,97	9,07	9,07
C4_168H	17,5	17,6	17,6	455	455	457	9,04	9,07	9,08
C5_168H	17,6	17,5	17,4	438	440	448	9,11	9,12	9,12
C1_336H	17,2	17,3	17,4	493	493	494	9,21	9,25	9,28
C2_336H	16,9	16,9	17	492	492	492	9,37	9,38	9,39
C3_336H	17	17	17	499	498	498	9,39	9,42	9,43
C4_336H	17,5	17,5	17,6	485	483	484	9,41	9,43	9,44
C5_336H	17,4	17,4	17,5	459	457	456	9,44	9,44	9,45
Si1_1H	19,5	19,4	19,3	444	444	440	7,4	7,52	7,54
Si2_1H	18	18	18	439	440	440	7,88	7,91	7,9
Si3_1H	18,3	18,3	17,9	440	438	438	7,8	7,8	7,8
Si4_1H	17,8	17,7	17,9	440	438	444	7,79	7,79	7,79
Si5_1H	18,2	18,2	18,2	438	440	439	7,74	7,74	7,76
Si1_24H	17,7	17,7	17,7	440	442	443	7,7	7,72	7,77
Si2_24H	18,1	17,7	17,7	440	443	442	7,7	7,76	7,77
Si3_24H	18	17,9	17,8	435	439	440	7,63	7,67	7,7
Si4_24H	17,6	17,8	17,4	441	441	440	7,7	7,68	7,72
Si5_24H	18	18	18	433	434	438	7,72	7,78	7,8
Si1_48H	17	17	17	441	446	441	8,52	8,51	8,5
Si2_48H	17	16,9	16,9	440	440	440	8,54	8,55	8,57

Si3_48H	16,9	16,9	16,9	439	439	438	8,62	8,63	8,64
Si4_48H	16,8	16,9	16,9	441	441	442	8,63	8,62	8,64
Si5_48H	17,1	17	16,9	437	438	437	8,62	8,63	8,63
Si1_96H	17	17	17	438	443	448	8,2	8,29	8,32
Si2_96H	16,9	16,8	16,9	442	446	447	8,46	8,46	8,48
Si3_96H	17,2	17	17	438	442	440	8,47	8,51	8,48
Si4_96H	16,8	16,8	16,9	449	449	559	8,94	8,97	8,98
Si5_96H	17,2	17,2	17,2	442	442	442	9	9,03	9,03
Si1_168H	17,2	16,9	16,9	449	458	460	9,01	9,06	9,07
Si2_168H	17	16,9	16,8	462	464	464	9,08	9,1	9,09
Si3_168H	17,2	17	17	458	460	455	9,07	9,12	9,13
Si4_168H	16,9	16,8	16,9	464	467	466	9,15	9,17	9,16
Si5_168H	17,3	17,3	17,2	444	455	455	9,06	9,12	9,13
Si1_336H	17,1	17	17,1	484	484	484	9,34	9,34	9,36
Si2_336H	17	17	17	488	488	488	9,37	9,37	9,38
Si3_336H	17,3	17,2	17,2	483	484	484	9,37	9,38	9,39
Si4_336H	17	16,9	16,9	502	504	503	9,36	9,37	9,38
Si5_336H	17,5	17,5	17,6	466	466	466	9,37	9,38	9,38
Zn1_1H	18,9	18,8	18,9	443	445	448	7,49	7,51	7,56
Zn2_1H	17,9	18	18,1	446	440	445	7,63	7,66	7,67
Zn3_1H	18,2	18	18,1	438	440	445	7,69	7,7	7,7
Zn4_1H	17,7	17,7	17,9	439	442	444	7,67	7,67	7,69
Zn5_1H	18,7	18,8	18,8	441	445	443	7,61	7,61	7,64
Zn1_24H	17,6	17,7	17,7	444	445	446	7,72	7,74	7,81
Zn2_24H	17,4	17,3	17,4	444	444	445	7,79	7,8	7,81
Zn3_24H	17,5	17,6	17,6	440	446	446	7,65	7,69	7,81
Zn4_24H	17,5	17,5	17,6	445	444	445	7,8	7,84	7,86
Zn5_24H	18,4	18,5	18,5	444	447	445	7,91	7,9	7,96
Zn1_48H	16,8	17,2	17,3	447	445	440	8,49	8,5	8,44
Zn2_48H	17,4	17,3	17,4	444	444	445	7,79	7,8	7,81
Zn3_48H	17,5	17,6	17,6	440	446	446	7,65	7,69	7,81
Zn4_48H	17,5	17,5	17,6	445	444	445	7,8	7,84	7,86
Zn5_48H	18,4	18,5	18,5	444	447	445	7,91	7,9	7,96
Zn1_96H	16,8	17,2	17,3	447	445	440	8,49	8,5	8,4
Zn2_96H	17	16,8	16,6	446	443	445	8,01	8,17	8,2
Zn3_96H	17	17	17	453	451	453	8,85	8,82	8,88
Zn4_96H	17	17	17	449	448	448	8,84	8,89	8,89
Zn5_96H	17,9	17,9	17,9	447	447	445	8,94	8,91	8,9
Zn1_168H	16,8	16,9	16,8	465	465	464	8,87	8,93	8,96
Zn2_168H	16,7	16,7	16,9	462	466	466	8,99	9	9,03
Zn3_168H	17,2	17,2	17,3	472	472	471	9,11	9,12	9,14
Zn4_168H	17,1	17,1	17,1	461	461	460	9,15	9,16	9,17
Zn5_168H	17,9	18	18,1	457	458	457	9,21	9,22	9,2
Zn1_336H	17	17	17,1	502	505	505	9,29	9,31	9,32
Zn2_336H	16,9	17,1	17,2	500	505	598	9,37	9,36	9,37
Zn3_336H	17,4	17,4	17,4	516	514	514	9,34	9,36	9,36
Zn4_336H	17,2	17,2	17,2	488	489	488	9,36	9,37	9,35
Zn5_336H	17,7	17,8	17,8	481	482	481	9,44	9,47	9,48

NP1_1H	19,1	19,3	19,7	443	442	441	7,47	7,5	7,55
NP2_1H	18,4	18,4	18,5	439	444	441	7,85	8	8,02
NP3_1H	17,7	17,7	17,7	436	440	440	7,72	7,74	7,73
NP4_1H	17,6	17,7	17,7	443	437	440	7,56	7,63	7,66
NP5_1H	18,8	18,3	18,3	439	440	441	7,79	7,72	7,78
NP1_24H	18	18	18	433	435	437	7,86	7,88	7,9
NP2_24H	17,8	17,6	17,6	433	437	439	7,86	7,88	7,92
NP3_24H	17,6	17,6	17,7	440	443	442	7,94	7,96	7,93
NP4_24H	17,7	17,7	17,7	434	437	438	7,78	7,89	7,88
NP5_24H	18,3	18,3	18,3	429	431	429	7,78	7,81	7,81
NP1_48H	17,3	17,3	17,4	435	435	437	8,24	8,26	8,31
NP2_48H	17,1	17	16,9	440	442	442	8,19	8,27	8,3
NP3_48H	17,1	17	16,6	440	443	445	8,34	8,36	8,39
NP4_48H	16,9	16,9	17	439	440	439	8,4	8,44	8,45
NP5_48H	17,7	17,7	17,7	438	438	438	8,37	8,34	8,38
NP1_96H	17,3	17,3	17,3	443	443	442	8,96	8,96	8,99
NP2_96H	16,9	16,9	16,9	449	450	448	8,96	8,97	8,99
NP3_96H	16,8	16,8	16,8	448	448	447	8,99	9,01	9,01
NP4_96H	17	17,1	17,2	442	441	442	8,99	9,02	9,02
NP5_96H	17,6	17,7	17,7	441	440	442	9	8,99	8,98
NP1_168H	17,4	17,5	17,5	457	458	458	9,19	9,2	9,21
NP2_168H	17,2	17,2	17,2	465	466	466	9,19	9,2	9,21
NP3_168H	17	17	17	464	464	464	9,2	9,2	9,24
NP4_168H	17	17,1	17,1	453	453	452	9,22	9,23	9,22
NP5_168H	17,3	17,3	17,3	449	448	450	9,25	9,25	9,26
NP1_336H	17,3	17,3	17,4	484	484	485	9,4	9,41	9,41
NP2_336H	17,2	17,2	17,2	510	509	508	9,44	9,43	9,43
NP3_336H	17	17	17,1	499	495	497	9,44	9,46	9,45
NP4_336H	17,1	17,1	17,1	479	479	478	9,44	9,44	9,45
NP5_336H	17,5	17,5	17,5	468	470	469	9,45	9,45	9,45
ZN_NP1_1H	19	19,1	19,1	443	445	443	7,62	7,63	7,65
ZN_NP2_1H	17,8	17,7	17,6	441	443	440	7,85	7,87	7,83
ZN_NP3_1H	17,9	17,7	17,7	443	445	444	7,72	7,6	7,77
ZN_NP4_1H	18,1	18,1	18,2	446	443	441	7,73	7,75	7,74
ZN_NP5_1H	18,7	187	18,7	444	440	442	7,9	7,91	7,94
ZN_NP1_24H	17,6	17,6	17,6	447	444	444	7,97	8	8
ZN_NP2_24H	17,6	17,8	17,5	436	436	439	7,94	7,97	8,01
ZN_NP3_24H	17,3	17,3	17,2	440	440	444	7,88	7,91	7,93
ZN_NP4_24H	17,8	17,9	18	440	446	445	8,01	8,02	8,04
ZN_NP5_24H	18,4	18,3	18,4	443	441	441	7,97	8,02	8,09
ZN_NP1_48H	17,2	17,1	17	441	442	445	8,2	8,23	8,27
ZN_NP2_48H	17,3	16,9	16,9	443	447	450	8,23	8,29	8,28
ZN_NP3_48H	17	16,7	16,6	442	446	446	8,25	8,29	8,29
ZN_NP4_48H	17	16,9	17	445	446	448	8,27	8,31	8,28
ZN_NP5_48H	17,8	17,8	17,8	441	443	442	8,33	8,37	8,34
ZN_NP1_96H	16,9	16,9	16,9	446	445	445	8,9	8,91	8,92
ZN_NP2_96H	16,6	16,6	16,6	449	449	449	8,89	8,91	8,92
ZN_NP3_96H	16,6	16,6	16,6	449	449	449	8,9	8,91	8,91

ZN_NP4_96H	16,8	16,8	16,8	449	451	451	8,89	8,9	8,9
ZN_NP5_96H	17,7	17,7	17,7	447	446	446	8,92	8,94	8,95
ZN_NP1_168H	16,8	16,8	16,8	460	462	462	9,17	9,17	9,17
ZN_NP2_168H	16,7	16,6	16,7	464	464	463	9,16	9,16	9,17
ZN_NP3_168H	16,9	16,9	17	460	460	461	9,16	9,16	9,15
ZN_NP4_168H	17,1	17,1	17,1	467	468	468	9,15	9,16	9,15
ZN_NP5_168H	17,4	17,4	17,4	457	457	456	9,2	9,19	9,19
ZN_NP1_336H	17,2	17,3	17,3	505	506	507	9,39	9,39	9,39
ZN_NP2_336H	17	17	17,1	508	508	507	9,37	9,38	9,38
ZN_NP3_336H	17	17,1	17,2	509	510	510	9,39	9,36	9,35
ZN_NP4_336H	17,4	17,4	17,5	522	522	523	9,35	9,38	9,4
ZN_NP5_336H	17,5	17,5	17,5	486	488	487	9,4	9,4	9,39

Table S2: Recipes for media

WC-Medium (modified)

Compound	Stock Solution	For 80ml Stock	Quantity für 1l
TES-Buffer			0,115g
$NaNO_3$	42,5 g/L dH_2O	3,4g	1 mL
$MgSO_4 \cdot 7H_2O$	36,97 g/L dH_2O	2,96g	1 mL
$CaCl_2 \cdot 2H_2O$	36,76 g/L dH_2O	2,94g	1 mL
K_2HPO_4	4,35 g/L dH_2O	0,35g	1 mL
$NaHCO_3$	31,5 g/L dH_2O	2,52g	1 mL
$Na_2SiO_3 \cdot 5H_2O$	21,2 g/L dH_2O	1,70g	1 mL
trace metal solution	(see recipe, right)		1 mL
vitamin solution f/2**	(see recipe, right)		1 mL**

WC Trace metal solution

	Compound	Stock Solution	Quantity/100ml Aqua dest	Quantity/100ml Aqua dest
1	Na_2EDTA (Titriplex III)	---	4,36 g	0,436g
2	$FeCl_3 \cdot 6H_2O$	---	3,15 g	0,315g
3	$CuSO_4 \cdot 5H_2O$	10 g/L dH_2O	1 mL	0,1ml
4	$ZnSO_4 \cdot 7H_2O$	22 g/L dH_2O	1 mL	0,1ml
5	$CoCl_2 \cdot 6H_2O$	10 g/L dH_2O	1 mL	0,1ml
6	$MnCl_2 \cdot 4H_2O$	180 g/L dH_2O	1 mL	0,1ml
7	$Na_2MoO_4 \cdot 2H_2O$	6 g/L dH_2O	1 mL	0,1ml
8	H_3BO_3	---	1,3 g	0,13g

WC Vitamin solution

Compound	Stock Solution	Quantity für 1l	Concentration in final Medium
Thiamine · HCl (Vit. B1)	-	100 mg	$2,96 \times 10^{-7}$ M
Biotin (Vit. H)	0.2 g/L dH_2O	2,5 mL	$2,05 \times 10^{-9}$ M (0,20mg/l)
Vitamin B_{12} (Cyanocobalamin)	0.5 g/L dH_2O	1 mL	$3,69 \times 10^{-10}$ M (0,50mg/l)

NSY Media

Compound	Stock Solution	For 80ml Stock	Quantity used for 1l Medium
$MgSO_4 \cdot 7H_2O$	75 g/L dH_2O	6g	1 mL
$Ca(NO_3)_2 \cdot 4H_2O$	43 g/L dH_2O	3,44g	1 mL
$NaHCO_3$	16 g/L dH_2O	1,28g	1 mL
KCL	5 g/L dH_2O	0,4g	1 mL
K_2HPO_4	2,8 g/L dH_2O	0,224g	1 mL
trace metal solution	(see right)		1 mL
Yeast Extract			1g/L
Soyotone			1g/L
Nutrient Broth			1g/L

NSY Trace metal solution

Compound	Stock solution	Quantity used/1000ml aqua dest
$Na_2EDTA \cdot 2H_2O$ (Titriplex III)	---	4,4 g
$FeCl_3 \cdot 6H_2O$	---	3,2 g
H_3BO_3	---	1 g
$MnCl_2 \cdot 4H_2O$	---	200 mg
$ZnSO_4 \cdot 7H_2O$	---	20 mg
$CuSO_4 \cdot 6H_2O$	10 g/L dH_2O	1 mL
$CoCl_2 \cdot 6H_2O$	10 g/L dH_2O	1 mL
$Na_2MoO_4 \cdot 2H_2O$	6 g/L dH_2O	1 mL

Table S3: Zinc concentrations during the experiment. NpZn: Nanoplastic particles and zinc treatment, Zn: Zinc treatment. First number represents the replicate and second number represent the sample point in hours

Zn in ppb	Treatment
713,39	NpZn_1_1h
698,10	NpZn_2_1h
745,91	NpZn_3_1h
776,50	NpZn_4_1h
770,51	NpZn_5_1h
750,33	NpZn_1_12h
711,67	NpZn_2_12h
783,03	NpZn_3_12h
744,32	NpZn_4_12h
694,39	NpZn_5_12h
1366,50	NpZn_1_24h
808,48	NpZn_2_24h
798,31	NpZn_3_24h
807,25	NpZn_4_24h
796,29	NpZn_5_24h
811,59	NpZn_1_48h
824,72	NpZn_2_48h
843,34	NpZn_3_48h
913,21	NpZn_4_48h
926,18	NpZn_5_48h
790,17	NpZn_1_96h
723,88	NpZn_2_96h
780,01	NpZn_3_96h
802,84	NpZn_4_96h
754,45	NpZn_5_96h
711,32	NpZn_1_168h
669,09	NpZn_2_168h
745,05	NpZn_3_168h
738,92	NpZn_4_168h
677,74	NpZn_5_168h
686,85	NpZn_1_336h
671,06	NpZn_2_336h
684,42	NpZn_3_336h
744,34	NpZn_4_336h
672,82	NpZn_5_336h
730,02	Zn_1h_1h
731,36	Zn_2_1h
812,39	Zn_3_1h

736,61	**Zn_4_1h**
712,68	**Zn_5_1h**
735,90	**Zn_1h_12h**
752,66	**Zn_2_12h**
834,52	**Zn_3_12h**
730,56	**Zn_4_12h**
716,17	**Zn_5_12h**
774,31	**Zn_1_24h**
793,48	**Zn_2_24h**
864,65	**Zn_3_24h**
777,60	**Zn_4_24h**
812,43	**Zn_5_24h**
835,43	**Zn_1_48h**
839,65	**Zn_2_48h**
896,10	**Zn_3_48h**
847,28	**Zn_4_48h**
800,93	**Zn_5_48h**
782,53	**Zn_1_96h**
821,20	**Zn_2_96h**
850,51	**Zn_3_96h**
783,77	**Zn_4_96h**
716,91	**Zn_5_96h**
713,65	**Zn_1h_168h**
732,89	**Zn_2_168h**
737,45	**Zn_3_168h**
725,07	**Zn_4_168h**
672,46	**Zn_5_168h**
707,70	**Zn_1_336h**
707,84	**Zn_2_336h**
678,21	**Zn_3_336h**
779,63	**Zn_4_336h**
667,00	**Zn_5_336h**

Table S4: Heavy metal concentrations from the lake Baldeneysee. Acting as baseline conditions for the experiment

	µg/l (ppb)
Al27	10691,48
V51	4461,282
Mn55	30910,79
Fe56	1372026
Fe57	9642,006
Co59	345,223
Ni60	5427,566
Cu63	2647,458
Cu65	1365,844
Zn66	14544,24
Zn67	2359,875
As75	277,165
Se82	-6,418
Y89	174577
Mo95	827,198
Mo98	1464,665
Ag107	18,827
Ag109	12,346
Cd111	14,352
Cd114	35,398
Sn118	705,737
Sn120	1032,904
Sb121	544,152
Sb123	421,054
Tm169	79127,5
Au197	12,5
Pb206	3759,113
Pb207	3435,22
Pb208	8195,678
U238	468,068

Table S5: Photosynthesis proxies. Measured twice per replicate.

fv/fm	fixarea	sampling	Sample
0.717	2157340	1h	c1.1_1h
0.71	2411918	1h	c1.2_1h
0.705	1554187	1h	c2.1_1h
0.701	1737208	1h	c2.2_1h
0.702	1805021	1h	c3.1_1h
0.703	2001417	1h	c3.2_1h
0.685	1467028	1h	c4.1_1h
0.682	1486899	1h	c4.2_1h
0.686	2173751	1h	c5.1_1h
0.69	2146116	1h	c5.2_1h
0.711	1238744	1h	si1.1_1h
0.72	1189665	1h	si1.1_1h
0.727	1509758	1h	si2.1_1h
0.729	1499279	1h	si2.2_1h
0.725	1654596	1h	si3.1_1h
0.73	1960984	1h	si3.2_1h
0.734	1646229	1h	si4.1_1h
0.72	1515182	1h	si4.2_1h
0.722	1939380	1h	si5.1_1h
0.732	1935542	1h	si5.2_1h
0.59	644228	1h	zn1.1_1h
0.583	496812	1h	zn1.2_1h
0.49	452529	1h	zn2.1_1h
0.535	590255	1h	zn2.2_1h
0.505	490434	1h	zn3.1_1h
0.518	512345	1h	zn3.2_1h
0.597	892632	1h	zn4.1_1h
0.593	713126	1h	zn4.2_1h
0.586	880020	1h	zn5.1_1h
0.57	1012353	1h	zn5.2_1h
0.589	803331	1h	znnp1.1_1h
0.562	763736	1h	znnp1.2_1h
0.616	724857	1h	znnp2.1_1h
0.585	738992	1h	znnp2.2_1h
0.465	385453	1h	znnp3.1_1h
0.449	428648	1h	znnp3.2_1h
0.518	383256	1h	znnp4.1_1h
0.501	363369	1h	znnp4.2_1h
0.536	551624	1h	znnp5.1_1h
0.519	455660	1h	znnp5.2_1h
0.725	1117322	1h	np1.1_1h
0.728	1077994	1h	np1.2_1h

0.734	1389764	1h	np2.1_1h
0.735	1352929	1h	np2.2_1h
0.66	28067	1h	np3.1_1h
0.636	1476186	1h	np3.2_1h
0.648	1529368	1h	np4.1_1h
0.641	1380048	1h	np4.2_1h
0.655	1593581	1h	np5.1_1h
0.669	1671304	1h	np5.2_1h
0.668	1743744	12h	c1.1_12h
0.675	1650478	12h	c1.2_12h
0.686	1802542	12h	c2.1_12h
0.65	1404633	12h	c2.2_12h
0.672	1855451	12h	c3.1_12h
0.658	1984073	12h	c3.2_12h
0.661	1892768	12h	c4.1_12h
0.66	1818507	12h	c4.2_12h
0.655	1772336	12h	c5.1_12h
0.65	1941403	12h	c5.2_12h
0.678	2250210	12h	si1.1_12h
0.674	2043507	12h	si1.1_12h
0.649	1594933	12h	si2.1_12h
0.662	1633886	12h	si2.2_12h
0.668	2415296	12h	si3.1_12h
0.654	2136630	12h	si3.2_12h
0.665	1992678	12h	si4.1_12h
0.666	1852553	12h	si4.2_12h
0.655	1175534	12h	si5.1_12h
0.637	1204037	12h	si5.2_12h
0.538	532413	12h	zn1.1_12h
0.493	494275	12h	zn1.2_12h
0.472	415089	12h	zn2.1_12h
0.426	353199	12h	zn2.2_12h
0.477	449817	12h	zn3.1_12h
0.47	447285	12h	zn3.2_12h
0.492	408051	12h	zn4.1_12h
0.451	429503	12h	zn4.2_12h
0.474	445432	12h	zn5.1_12h
0.476	433232	12h	zn5.2_12h
0.46	254281	12h	znnp1.1_12h
0.429	277056	12h	znnp1.2_12h
0.518	659097	12h	znnp2.1_12h
0.498	621783	12h	znnp2.2_12h
0.466	619984	12h	znnp3.1_12h
0.455	604164	12h	znnp3.2_12h
0.413	405294	12h	znnp4.1_12h

0.451	412262	12h	znnp4.2_12h
0.455	445517	12h	znnp5.1_12h
0.464	402585	12h	znnp5.2_12h
0.655	1100201	12h	np1.1_12h
0.656	1386534	12h	np1.2_12h
0.651	1341595	12h	np2.1_12h
0.646	1423127	12h	np2.2_12h
0.664	2241747	12h	np3.1_12h
0.667	2247704	12h	np3.2_12h
0.628	838981	12h	np4.1_12h
0.644	769268	12h	np4.2_12h
0.644	733028	12h	np5.1_12h
0.645	832576	12h	np5.2_12h
0.679	1896168	24h	c1.1_24h
0.664	1880270	24h	c1.2_24h
0.688	1645036	24h	c2.1_24h
0.679	1681033	24h	c2.2_24h
0.665	1624354	24h	c3.1_24h
0.673	1622888	24h	c3.2_24h
0.662	2514851	24h	c4.1_24h
0.656	2389182	24h	c4.2_24h
0.664	1636600	24h	c5.1_24h
0.659	1596976	24h	c5.2_24h
0.652	1216081	24h	si1.1_24h
0.655	1336798	24h	si1.1_24h
0.67	1440772	24h	si2.1_24h
0.678	1576466	24h	si2.2_24h
0.656	1312850	24h	si3.1_24h
0.661	1459021	24h	si3.2_24h
0.684	2716790	24h	si4.1_24h
0.671	2025016	24h	si4.2_24h
0.663	1446958	24h	si5.1_24h
0.659	1465235	24h	si5.2_24h
0.457	412168	24h	zn1.1_24h
0.46	425770	24h	zn1.2_24h
0.43	266935	24h	zn2.1_24h
0.359	237459	24h	zn2.2_24h
0.449	211874	24h	zn3.1_24h
0.377	225406	24h	zn3.2_24h
0.395	385800	24h	zn4.1_24h
0.416	495500	24h	zn4.2_24h
0.43	269496	24h	zn5.1_24h
0.384	264775	24h	zn5.2_24h
0.379	321289	24h	znnp1.1_24h
0.417	345144	24h	znnp1.2_24h

0.469	538387	24h	znnp2.1_24h
0.467	575285	24h	znnp2.2_24h
0.397	342422	24h	znnp3.1_24h
0.425	375703	24h	znnp3.2_24h
0.423	287737	24h	znnp4.1_24h
0.42	276578	24h	znnp4.2_24h
0.392	314824	24h	znnp5.1_24h
0.399	320018	24h	znnp5.2_24h
0.673	762246	24h	np1.1_24h
0.692	825177	24h	np1.2_24h
0.671	944404	24h	np2.1_24h
0.66	876465	24h	np2.2_24h
0.65	927841	24h	np3.1_24h
0.659	996183	24h	np3.2_24h
0.656	1150030	24h	np4.1_24h
0.656	1862495	24h	np4.2_24h
0.673	2210721	24h	np5.1_24h
0.646	736013	48h	c1.1_48h
0.663	1427188	48h	c1.2_48h
0.68	1718437	48h	c2.1_48h
0.678	2519467	48h	c2.2_48h
0.675	2598272	48h	c3.1_48h
0.68	2546663	48h	c3.2_48h
0.64	972418	48h	c4.1_48h
0.659	1900744	48h	c4.2_48h
0.653	1104872	48h	c5.1_48h
0.652	1184236	48h	c5.2_48h
0.671	1884409	48h	si1.1_48h
0.628	614112	48h	si1.1_48h
0.665	1488560	48h	si2.1_48h
0.671	1433007	48h	si2.2_48h
0.678	2430849	48h	si3.1_48h
0.663	2200793	48h	si3.2_48h
0.622	2622018	48h	si4.1_48h
0.633	2667998	48h	si4.2_48h
0.662	2594094	48h	si5.1_48h
0.66	2842139	48h	si5.2_48h
0.445	408520	48h	zn1.1_48h
0.417	431803	48h	zn1.2_48h
0.43	428892	48h	zn2.1_48h
0.393	370646	48h	zn2.2_48h
0.362	296203	48h	zn3.1_48h
0.377	300093	48h	zn3.2_48h
0.426	272570	48h	zn4.1_48h
0.433	279874	48h	zn4.2_48h

0.42	423668	48h	zn5.1_48h
0.451	350942	48h	zn5.2_48h
0.355	340530	48h	znnp1.1_48h
0.339	302587	48h	znnp1.2_48h
0.382	367635	48h	znnp2.1_48h
0.401	362203	48h	znnp2.2_48h
0.444	216010	48h	znnp3.1_48h
0.29	180050	48h	znnp3.2_48h
0.402	224152	48h	znnp4.1_48h
0.456	226750	48h	znnp4.2_48h
0.387	294256	48h	znnp5.1_48h
0.371	295221	48h	znnp5.2_48h
0.707	2179028	48h	np1.1_48h
0.701	3189041	48h	np1.2_48h
0.666	961118	48h	np2.1_48h
0.642	944635	48h	np2.2_48h
0.656	1067460	48h	np3.1_48h
0.664	1338260	48h	np3.2_48h
0.626	2800425	48h	np4.1_48h
0.655	1503210	48h	np4.2_48h
0.645	906904	48h	np5.1_48h
0.613	751625	48h	np5.2_48h
0.67	2801250	96h	c1.1_96h
0.667	2774871	96h	c1.2_96h
0.644	2141966	96h	c2.1_96h
0.644	2774059	96h	c2.2_96h
0.635	2479937	96h	c3.1_96h
0.647	2147588	96h	c3.2_96h
0.624	2332263	96h	c4.1_96h
0.619	1695741	96h	c4.2_96h
0.635	991056	96h	c5.1_96h
0.641	902741	96h	c5.2_96h
0.652	1167394	96h	si1.1_96h
0.659	1065244	96h	si1.1_96h
0.657	2469409	96h	si2.1_96h
0.659	2347843	96h	si2.2_96h
0.588	2006396	96h	si3.1_96h
0.593	1939471	96h	si3.2_96h
0.636	2667352	96h	si4.1_96h
0.662	2481985	96h	si4.2_96h
0.678	3576576	96h	si5.1_96h
0.67	3621695	96h	si5.2_96h
0.301	195098	96h	zn1.1_96h
0.416	186288	96h	zn1.2_96h
0.445	339481	96h	zn2.1_96h

0.393	305481	96h	zn2.2_96h
0.417	364215	96h	zn3.1_96h
0.411	378664	96h	zn3.2_96h
0.5	170623	96h	zn4.1_96h
0.313	169839	96h	zn4.2_96h
0.232	157999	96h	zn5.1_96h
0.36	177105	96h	zn5.2_96h
0.399	215179	96h	znnp1.1_96h
0.341	187839	96h	znnp1.2_96h
0.403	405531	96h	znnp2.1_96h
0.412	357425	96h	znnp2.2_96h
0.412	326226	96h	znnp3.1_96h
0.408	316025	96h	znnp3.2_96h
0.384	457064	96h	znnp4.1_96h
0.414	413907	96h	znnp4.2_96h
0.37	450654	96h	znnp5.1_96h
0.415	403762	96h	znnp5.2_96h
0.666	1264658	96h	np1.1_96h
0.66	1248201	96h	np1.2_96h
0.638	3745746	96h	np2.1_96h
0.661	1448942	96h	np2.2_96h
0.645	2445903	96h	np3.1_96h
0.637	2036468	96h	np3.2_96h
0.634	3571711	96h	np4.1_96h
0.626	3560749	96h	np4.2_96h
0.667	3561540	96h	np5.1_96h
0.664	3020534	96h	np5.2_96h
0.678	1920201	168h	c1.1_168h
0.671	1995227	168h	c1.2_168h
0.632	1861381	168h	c2.1_168h
0.635	1914224	168h	c2.2_168h
0.651	2449187	168h	c3.1_168h
0.637	2639369	168h	c3.2_168h
0.662	2096682	168h	c4.1_168h
0.673	2295457	168h	c4.2_168h
0.673	2222995	168h	c5.1_168h
0.678	2311753	168h	c5.2_168h
0.654	2625230	168h	si1.1_168h
0.673	2452083	168h	si1.1_168h
0.61	1950606	168h	si2.1_168h
0.609	1726306	168h	si2.2_168h
0.621	2027014	168h	si3.1_168h
0.64	1905987	168h	si3.2_168h
0.624	1419183	168h	si4.1_168h
0.617	1613810	168h	si4.2_168h

0.632	2314481	168h	si5.1_168h
0.639	2243820	168h	si5.2_168h
0.468	522953	168h	zn1.1_168h
0.453	572277	168h	zn1.2_168h
0.485	451355	168h	zn2.1_168h
0.464	451829	168h	zn2.2_168h
0.45	417328	168h	zn3.1_168h
0.471	398891	168h	zn3.2_168h
0.466	436542	168h	zn4.1_168h
0.514	552399	168h	zn4.2_168h
0.481	584573	168h	zn5.1_168h
0.466	502930	168h	zn5.2_168h
0.445	486514	168h	znnp1.1_168h
0.415	365907	168h	znnp1.2_168h
0.463	555465	168h	znnp2.1_168h
0.49	586091	168h	znnp2.2_168h
0.448	417663	168h	znnp3.1_168h
0.457	471755	168h	znnp3.2_168h
0.449	519430	168h	znnp4.1_168h
0.489	547827	168h	znnp4.2_168h
0.462	486666	168h	znnp5.1_168h
0.473	496810	168h	znnp5.2_168h
0.652	3760440	168h	np1.1_168h
0.659	1772344	168h	np1.2_168h
0.643	1645862	168h	np2.1_168h
0.641	1715346	168h	np2.2_168h
0.644	1554235	168h	np3.1_168h
0.621	1453345	168h	np3.2_168h
0.62	1150738	168h	np4.1_168h
0.633	1427880	168h	np4.2_168h
0.676	3540690	168h	np5.1_168h
0.681	3377774	168h	np5.2_168h
0.577	880159	336h	c1.1_336h
0.523	610635	336h	c1.2_336h
0.666	2022791	336h	c2.1_336h
0.679	1810943	336h	c2.2_336h
0.636	1453275	336h	c3.1_336h
0.656	1368853	336h	c3.2_336h
0.593	969124	336h	c4.1_336h
0.621	1343401	336h	c4.2_336h
0.687	2172031	336h	c5.1_336h
0.68	1977665	336h	c5.2_336h
0.68	3461968	336h	si1.1_336h
0.662	2087197	336h	si1.1_336h
0.695	4755612	336h	si2.1_336h

0.69	4405679	336h	si2.2_336h
0.677	3203802	336h	si3.1_336h
0.678	3762233	336h	si3.2_336h
0.624	1547589	336h	si4.1_336h
0.64	1501480	336h	si4.2_336h
0.678	2894385	336h	si5.1_336h
0.673	2983878	336h	si5.2_336h
0.579	1035196	336h	zn1.1_336h
0.606	983172	336h	zn1.2_336h
0.648	1746121	336h	zn2.1_336h
0.63	1352581	336h	zn2.2_336h
0.664	1229293	336h	zn3.1_336h
0.671	1332492	336h	zn3.2_336h
0.635	2061229	336h	zn4.1_336h
0.64	2119574	336h	zn4.2_336h
0.746	4091332	336h	zn5.1_336h
0.743	4046810	336h	zn5.2_336h
0.655	1644281	336h	znnp1.1_336h
0.635	1522039	336h	znnp1.2_336h
0.672	1405826	336h	znnp2.1_336h
0.663	1507527	336h	znnp2.2_336h
0.662	1300717	336h	znnp3.1_336h
0.656	1295072	336h	znnp3.2_336h
0.634	2613989	336h	znnp4.1_336h
0.644	1519229	336h	znnp4.2_336h
0.653	1604916	336h	znnp5.1_336h
0.652	1310281	336h	znnp5.2_336h
0.673	1704769	336h	np1.1_336h
0.664	1487483	336h	np1.2_336h
0.656	2390549	336h	np2.1_336h
0.662	2368424	336h	np2.2_336h
0.667	1889372	336h	np3.1_336h
0.658	2011509	336h	np3.2_336h
0.674	2103831	336h	np4.1_336h
0.662	2072732	336h	np4.2_336h
0.606	873282	336h	np5.1_336h
0.595	868646	336h	np5.2_336h
0.642	1380341	336h	c1.1_336h
0.628	877312	336h	c1.2_336h
0.691	1645184	336h	c2.1_336h
0.701	1618208	336h	c2.2_336h
0.582	659700	336h	c3.2_336h
0.705	2780750	336h	c4.1_336h
0.701	4991251	336h	c4.2_336h
0.706	4149142	336h	c5.1_336h

0.709	3911331	336h	c5.2_336h
0.689	3107914	336h	si1.1_336h
0.68	2265256	336h	si1.1_336h
0.709	4683622	336h	si2.1_336h
0.712	4201706	336h	si2.2_336h
0.69	3403617	336h	si3.1_336h
0.696	3933073	336h	si3.2_336h
0.69	3541813	336h	si4.1_336h
0.706	5237803	336h	si4.2_336h
0.672	2090529	336h	si5.1_336h
0.666	1962474	336h	si5.2_336h
0.703	2035381	336h	zn1.1_336h
0.704	1957417	336h	zn1.2_336h
0.713	2915783	336h	zn2.1_336h
0.712	2202475	336h	zn2.2_336h
0.715	2896078	336h	zn3.1_336h
0.717	2982913	336h	zn3.2_336h
0.724	2268389	336h	zn4.1_336h
0.716	2201167	336h	zn4.2_336h
0.76	10693000	336h	zn5.1_336h
0.762	9903777	336h	zn5.2_336h
0.706	3399766	336h	znnp1.1_336h
0.703	3214343	336h	znnp1.2_336h
0.723	3921645	336h	znnp2.1_336h
0.714	3707218	336h	znnp2.2_336h
0.739	3206390	336h	znnp3.1_336h
0.739	3568778	336h	znnp3.2_336h
0.69	2595513	336h	znnp4.1_336h
0.706	2464347	336h	znnp4.2_336h
0.731	3382180	336h	znnp5.1_336h
0.728	3108269	336h	znnp5.2_336h
0.681	2378325	336h	np1.1_336h
0.704	3336328	336h	np1.2_336h
0.704	3183737	336h	np2.1_336h
0.684	1942706	336h	np2.2_336h
0.638	1136803	336h	np3.1_336h
0.682	2085908	336h	np3.2_336h
0.578	819567	336h	np4.1_336h
0.594	816629	336h	np4.2_336h
0.689	3573431	336h	np5.1_336h
0.68	2214437	336h	np5.2_336h

Table S6: List of individual phyla, classes, orders, families, genera and species and their affiliation to individual nutrition modes.

Phylum	class	order	family	genus	species		Mode
Amoebozoa							consumer
Apicomplexa							parasitic
Bacillariophyta							phototrophic
Bigyra	**Opalinata**						parasitic
	rem. Bigyra						consumer
Cercozoa	**Endomyxa**						Parasitic
Ciliophora	**Litostomatea**	**Cyclotrichida**	Cyclotrichida	Mesodiniidae	Mesodinium rubrum		mixotrophic
					Mesodinium major		mixotrophic
					Mesodinium rubra		mixotrophic
	Spirotrichea	**Oligotrichia**	Strombidiidae	Strombidium			mixotrophic
			Strombidiidae	Laboea	Laboea strobila		mixotrophic
			Tontoniidae	Tontonia			mixotrophic
	rem. Ciliophora						consumer
Chlorophyta	**Chlorophyceae**	**Sphaeropleales**	Neochloridaceae	Botryosphaerella	Botryosphaerella sudetica		mixotrophic
	Mamiellophyceae	**Mamiellales**	Mamiellaceae	Micromonas			mixotrophic
	Trebouxiophyceae	**Chlorellales**	Chlorellaceae	Dictyosphaerium	Dictyosphaerium chlorelloides		mixotrophic
	Pyramimonadophyceae	**Pyramimonadales**	Pyramimonadaceae	Pyramimonas			mixotrophic
	rem. Chlorophyta						phototrophic
Choanoflagellata							consumer
Choanozoa	**Ichthyosporea**						parasitic
Chytridiomycota	**Chytridiomycetes**	**Chytridiomycetes**	Zygophlyctidales	Zygophlyctis	Zygophlyctis planktonica		parasitic
					Zygophlyctis melosirae		parasitic
			Zygorhizidiales	Zygorhizidium			parasitic
CruMs	**Rigifilida**						consumer
	Collodictyonidae						consumer
Cryptophyta	**Kathablepharidaceae**	**Kathablepharidales**	Kathablepharidae				consumer
	Cryptophyta rest						phototrophic
Discoba	**Heterolobosea**	**Schizopyrenida**	Vahlkampfiidae	Naegleri	Naegleria fowleri		parasitic
	Heterolobosea rest						consumer
Dinoflagellata	**Dinophyceae**	**Coccidiniales**	Chytriodiniaceae	Chytriodinium			parasitic
		Dinophysiales	Dinophysiaceae	Dinophysis	Dinophysis acuminata		mixotrophic
					Dinophysis norvegica		mixotrophic
		Gonyaulacales	Pyrocystaceae	Alexandrium	Alexandrium tamarense		mixotrophic
			Amphidomataceae	Azadinium			mixotrophic
			Ceratiaceae	Ceratium	Ceratium fusus		mixotrophic
					Ceratium macroceros		mixotrophic
					Ceratium arcticum		mixotrophic
					Ceratium longipes		mixotrophic
				Tripos	Tripos fusus		mixotrophic
					Tripos arcticus		mixotrophic
			Amphidomataceae				mixotrophic
			Lingulodiniaceae	Lingulodinium	Lingulodinium polyedrum		mixotrophic
		Gymnodiniales	Gymnodiniaceae	Akashiwo	Akashiwo sanguinea		mixotrophic
				Gyrodinium	Gyrodinium galatheanum		mixotrophic
				Gymnodinium	Gymnodinium catenatum		mixotrophic
					Gymnodinium impudicum		mixotrophic
				Gymnoxanthella			parasitic
			Kareniaceae	Karlodinium	Karlodinium veneficium		mixotrophic
					Karlodinium micrum		mixotrophic
		Haplozoonales					parasitic
		Lophodiniales	Lophodiniaceae	Woloszynskia	Woloszynskia leopoliensis		mixotrophic
		Peridiniales	Amphidiniopsidaceae	Archaeperidinium			consumer
			Glenodiniaceae	Glenodinium			mixotrophic
			Heterocapsaceae	Heterocapsa	Heterocapsa triquetra		mixotrophic
					Heterocapsa rotundata		mixotrophic
			Oodiniaceae				parasitic
			Peridiniaceae	Scrippsiella	Scrippsiella trochoidea		mixotrophic
					Scrippsiella masanensis		phototrophic
			Peridiniaceae	Pentapharsodinium			mixotrophic
			Peridiniopsidaceae	Peridiniopsis	Peridinopsis kevei		mixotrophic
				Peridiniopsis rest			phototrophic
			Pfiesteriaceae	Aduncodinium			consumer
			Kryptoperidiniaceae	Unruhdinium			mixotrophic
		Prorocentrales	Prorocentraceae	Prorocentrum	Prorocentrum minimum		mixotrophic
					Prorocentrum donhaiense		mixotrophic
					Prorocentrum triestinum		mixotrophic
					Prorocentrum micans		mixotrophic
					Prorocentrum minimum		mixotrophic
					Prorocentrum triestinum		mixotrophic
					Prorocentrum dentatum		mixotrophic
					Prorocentrum cordatum		mixotrophic
		Suessiales	Suessiaceae	Asulcocephalium			phototrophic
				Leiocephalium			phototrophic
			Symbiodiniaceae	Zooxanthella			parasitic
				Symbiodinium			parasitic
		Syndiniales					parasitic
		Thoracosphaerales	Thoracosphaeraceae	Amyloodinium			parasitic
Euglenozoa	**Euglenoidea**	**Petalomonadida**	Scytomonadidae	Petalomonas			consumer
				Scytomonas			consumer
		Heteronematina					consumer
		Peranemea	Peranemida	Peranema			
	Euglenophyceae	**Euglenales**					consumer
		Euglenales rest					phototrophic
	Kinetoplastea	Eubodonida					consumer
		Neobodonida					consumer
		Parabodonida	Bodonidae	Cryptobia			Parasitic
				Bodo			consumer
		Trypanosomatida					Parasitic
Fungi							consumer
Haptophyta	**Prymnesiophyceae**	**Prymnesiales**	Chrysochromulinaceae	Chrysochromulina			mixotrophic
	Centroplasthelida						consumer
	Haptophyta rest						phototrophic
Metamonada							Parasitic
Ochrophyta	**Bolidophyceae**	**Parmales**					phototrophic
	Chrysophyceae	**Apoikiales**	Apoikiaceae	Apoikia			consumer
		Chromulinales	Chromulinaceae	Ochromonas			mixotrophic
				Paraphysomonas			consumer
				Pedospumella			consumer
				Chrysonephele	Chrysonephele palustris		mixotrophic
				Spumella			consumer
				Uroglena			mixotrophic
				Uroglenopsis			mixotrophic
				Chromulina	Chromulina chionophila		mixotrophic
				Cyclonexis	Cyclonexis annularis		mixotrophic
			Chrysoamoebidaceae	Chrysamoeba	Chrysamoeba pyrenoidifera		mixotrophic
				Chrysamoeba	Chrysamoeba mikrokonta		mixotrophic
			Chrysocapsaceae	Chrysocapsa	Chrysocapsa vernali		mixotrophic
					Chrysochaete britannica		mixotrophic
					Chrysocapsa vernalis		mixotrophic
					Chrysocapsa paludosa		mixotrophic
				Kremastochrysopsis	Kremastochrysopsis americana		mixotrophic
			Chrysolepidomonadac	Chrysolepidomonas	Chrysolepidomonas dendrolepidota		unknown
		Chromulinales	Dinobryaceae	Epipyxis	Epipyxis pulchra		mixotrophic
					Epipyxis aurea		mixotrophic
				Poteriospumella			consumer
				Dinobryon			mixotrophic
			Oikomonadaceae	Oikomonas			parasitic
		Chrysosaccales					mixotrophic
		Hydrurales	Hydruraceae	Chrysonebula	Chrysonebula flava		mixotrophic
				Hydrurus	Hydrurus foetidus		mixotrophic
		Hibberdiales	Hibberdiaceae	Chromophyton			mixotrophic
				Hibberdia	Hibberdia magna		mixotrophic
		Segregatospumellale	Segregatospumellacea	Segregatospumella			consumer
		Spumella-like-flagellate					consumer
	Eustigmatophyceae						phototrophic
	Raphidophyceae						phototrophic
	Synurophyceae	**Ochromonadales**	Ochromonadaceae	Acrispumella			consumer
				Cornospumella			consumer
				Poterioochromonas	Poterioochromonas malhamensis		mixotrophic
					Poterioochromonas stipitata		mixotrophic
				Urostipulosphaera			mixotrophic
		Synurales	Synuraceae	Chrysosphaerella			mixotrophic
		Synurales rest					phototrophic
Oomycota							parasitic
Perkinsozoa							parasitic
Rhizaria							consumer
Rotosphaerida							consumer
Stramenopile	unclassified				Stramenopile sp. MAST-12 KKTS_D3		consumer
Sulcozoa	**Ancyromonadida**						consumer
Viridiplantae	**Viridiplantae rest**						phototrophic
Incertae sedis		**Pirsoniales**	Pirsonia				parasitic
			Rhynchobodo				consumer

Table S7: Table showing individual OTU richness and standard deviation per treatment.

OTU Richness	C	Si	NP	ZN	ZnNP
Day 1	542±99	435±110	514±77	588±55	630±71
Day 7	750±67	699±145	430±181	595±99	591±56
Day 14	479±77	584±46	548±33	416±13	453±87

Table S8: Functional modes of each treatment. Values represent the average of all replicates from and individual timepoint

	consumer	mixotrophic	Parasitic	phototrophic	unknown
control_1h	0,276169899	0,07191455	0,039922639	0,58703554	0,02495737
control_12h	0,358937927	0,037577475	0,01869597	0,56181251	0,02297612
control_24h	0,286503979	0,031764406	0,013553017	0,65355721	0,01462139
control_48h	0,51219347	0,017874662	0,00912358	0,43992789	0,0208804
control_96h	0,524690996	0,013568279	0,035492248	0,41343855	0,01280993
control_168h	0,533110121	0,01235921	0,039776692	0,39558869	0,01916529
control_336h	0,527260088	0,098895026	0,0012198	0,36998994	0,00263515
silica_1h	0,139583646	0,067930227	0,032516079	0,74514644	0,01482361
silica_12h	0,262459489	0,034815933	0,015900088	0,66582342	0,02100107
silica_24h	0,366752279	0,028325595	0,018716956	0,5692887	0,01691647
silica_48h	0,497315486	0,027188069	0,022101395	0,42436405	0,029031
silica_96h	0,499689267	0,015149846	0,033134684	0,43940229	0,01262392
silica_168h	0,546862979	0,011451097	0,022228741	0,40456966	0,01488752
silica_336h	0,358912649	0,121482075	0,001130849	0,51437552	0,00409891
NP_1h	0,159960509	0,065116102	0,036619759	0,72047094	0,01783269
NP_12h	0,117331178	0,035040577	0,010175255	0,82461015	0,01284284
NP_24h	0,365139711	0,039065082	0,032058684	0,54134276	0,02239377
NP_48h	0,502033477	0,023051003	0,013825355	0,43782549	0,02326468
NP_96h	0,364082312	0,014303259	0,020313309	0,59675578	0,00454534
NP_168h	0,522270593	0,009022705	0,004503006	0,45944791	0,00475579
NP_336h	0,409446833	0,137159547	0,011870694	0,43809365	0,00342927
zinc_1h	0,341325588	0,224716036	0,010085112	0,39547279	0,02840048
zinc_12h	0,446287539	0,17081971	0,010352204	0,33654655	0,035994
zinc_24h	0,57221002	0,116485068	0,010030262	0,26289208	0,03838257
zinc_48h	0,772982203	0,087070596	0,005728172	0,10026018	0,03395885
zinc_96h	0,901021866	0,029814156	0,007788597	0,04138163	0,01999375
zinc_168h	0,899157944	0,021619675	0,003345806	0,06945301	0,00642357
zinc_336h	0,645211659	0,020198841	0,004080309	0,3040164	0,02649279
ZnNP_1h	0,320564423	0,211210714	0,012815652	0,4268461	0,02856312
ZnNP_12h	0,40160446	0,169150862	0,014265103	0,36735991	0,04761966
ZnNP_24h	0,559753989	0,10589582	0,012490372	0,28591161	0,03594821
ZnNP_48h	0,770074714	0,060535472	0,007557276	0,13820156	0,02363097
ZnNP_96h	0,910916197	0,028873611	0,005442002	0,04301137	0,01175682
ZnNP_168h	0,905486954	0,026278763	0,008757402	0,05494049	0,00453639
ZnNP_336h	0,642341253	0,020064341	0,004240564	0,31735836	0,01599548

Table S9: Relative abundance of the phototrophic fraction consisting of individual subgroups per timepoint

	Bacillariophyta	Chlorophyta	Chrysophyta	Cryptophyta	Dinophyta	Euglenozoa	rest
control_1h	0,780499199	0,157177666	0,000818972	0,04549683	0,00693283	0,00294359	0,00613092
control_12h	0,84289349	0,092430176	0,002024743	0,05314081	0,00635778	0,00247554	0,00067746
control_24h	0,935014201	0,040603262	0,000770643	0,01896249	0,00388897	0,00052398	0,00023645
control_48h	0,947034672	0,040952886	0,001009349	0,00754464	0,00272448	0,00051389	0,00022008
control_96h	0,886743544	0,104668923	0,0006218	0,00448411	0,00123206	0,00190347	0,00034609
control_168h	0,865407222	0,107643599	0,000149503	0,01196147	0,00054617	0,01367955	0,00061248
control_336h	0,547624611	0,426145638	4,51E-05	0,02274599	0,00052853	0,00073962	0,0021705
silica_1h	0,830108685	0,14248247	0,000149618	0,01426809	0,00560066	0,00197543	0,00541504
silica_12h	0,879898453	0,081183265	0,001524353	0,03129007	0,00431805	0,00116977	0,00061604
silica_24h	0,937918167	0,040733008	0,001053115	0,01621143	0,00332607	0,00051525	0,00024296
silica_48h	0,899673502	0,075899275	0,001908412	0,01603156	0,00513855	0,00103738	0,00031133
silica_96h	0,89239695	0,100347887	0,000681664	0,00432444	0,00115601	0,00078153	0,00031152
silica_168h	0,861222551	0,127359731	0,000273507	0,00673981	0,00061893	0,00330801	0,00047746
silica_336h	0,564189202	0,411734052	1,58E-05	0,02126906	0,00029764	0,00096433	0,00152995
NP_1h	0,803002028	0,1642026	0,000267447	0,01932636	0,00567531	0,00191913	0,00560712
NP_12h	0,882361447	0,096656705	0,000257964	0,0129491	0,00586781	0,00138179	0,00052518
NP_24h	0,819434912	0,118794653	0,000999111	0,04606836	0,01189506	0,00222377	0,00058414
NP_48h	0,903076248	0,079363246	0,001348629	0,00958529	0,00476051	0,00154798	0,00031809
NP_96h	0,923892515	0,074309997	3,60E-05	0,00042999	0,00061861	0,00056266	0,00015026
NP_168h	0,919755321	0,076279159	2,34E-05	0,0031597	0,00015961	0,00035295	0,0002699
NP_336h	0,418168952	0,554155071	1,36E-06	0,02423842	0,0002885	0,00094444	0,00220325
zinc_1h	0,901882605	0,034132928	0,002701497	0,05620802	0,0028016	0,00010975	0,00216361
zinc_12h	0,845044514	0,088598428	0,001009039	0,05620431	0,00505086	0,00132507	0,00276777
zinc_24h	0,827071775	0,107339703	0,000355592	0,05481917	0,00621923	0,00140785	0,00278668
zinc_48h	0,753780725	0,204932187	0,000104657	0,02731541	0,00617475	0,00307786	0,00461441
zinc_96h	0,353802888	0,555962795	0,000737642	0,06578841	0,01108768	0,00685776	0,00576283
zinc_168h	0,132819206	0,691372692	0,00129104	0,15199253	0,00678483	0,00693591	0,0088038
zinc_336h	0,022408782	0,927186656	9,81E-06	0,03289249	0,00073539	0,00288076	0,01388611
ZnNP_1h	0,84396988	0,058109457	0,002536457	0,08895591	0,00325046	0,00084809	0,00232974
ZnNP_12h	0,819294831	0,091714521	0,001280142	0,07588153	0,0067941	0,00166315	0,00337173
ZnNP_24h	0,798918356	0,122592408	0,000286926	0,0651255	0,00751846	0,00196177	0,00359658
ZnNP_48h	0,776512367	0,186923016	3,80E-05	0,02339169	0,00921104	0,00130797	0,00261595
ZnNP_96h	0,403570637	0,530616545	0,00062098	0,04422267	0,00862719	0,00433577	0,00800621
ZnNP_168h	0,1600599	0,623636778	0,001942422	0,19618461	0,00672794	0,00415613	0,00729222
ZnNP_336h	0,01876641	0,922758198	1,35E-05	0,03869604	0,00079953	0,00534122	0,01362507

Table S10: Relative abundance of the mixotrophic fraction consisting of individual subgroups per timepoint

	Chlorophyta	Chrysophyta	Dinophyta	rest
control_1h	1,33E-05	0,788645709	0,21134103	0
control_12h	0	0,687714978	0,31228502	0
control_24h	0	0,66622622	0,33377378	0
control_48h	2,67E-05	0,632361182	0,36761214	0
control_96h	3,52E-05	0,699978909	0,29998594	0
control_168h	0	0,697487747	0,30193339	0,00057886
control_336h	0,000343624	0,017898589	0,98147445	0,00028334
silica_1h	0	0,80414425	0,19585575	0
silica_12h	0	0,748455416	0,25154458	0
silica_24h	0	0,799424136	0,20057586	0
silica_48h	0	0,55799593	0,4419339	7,02E-05
silica_96h	3,15E-05	0,740051631	0,25991689	0
silica_168h	8,33E-05	0,68032821	0,31942188	0,0001666
silica_336h	0,000294457	0,023167888	0,97640024	0,00013741
NP_1h	0	0,804454829	0,19554517	0
NP_12h	0	0,661389993	0,33861001	0
NP_24h	0	0,467816766	0,53218323	0
NP_48h	0	0,500351748	0,49944134	0,00020691
NP_96h	0,000166728	0,813431592	0,18613492	0,00026676
NP_168h	0,000132153	0,788489494	0,21137835	0
NP_336h	0,000326	0,012005512	0,98749897	0,00016952
zinc_1h	0	0,818014144	0,18198586	0
zinc_12h	0	0,929303365	0,07069664	0
zinc_24h	1,23E-05	0,939302867	0,06068485	0
zinc_48h	0	0,988003747	0,01199625	0
zinc_96h	1,60E-05	0,968820989	0,03116301	0
zinc_168h	0,000926559	0,875223367	0,12385007	0
zinc_336h	0,016292798	0,922461629	0,06115702	8,85E-05
ZnNP_1h	0	0,839353621	0,16064638	0
ZnNP_12h	0	0,899368675	0,10063132	0
ZnNP_24h	0	0,927373698	0,0726263	0
ZnNP_48h	0	0,957517215	0,04248278	0
ZnNP_96h	1,65E-05	0,971720242	0,02826324	0
ZnNP_168h	0,000136122	0,923249694	0,07661418	0
ZnNP_336h	0,015070838	0,883569459	0,1013597	0

Table S11: Relative abundance of the heterotrophic fraction consisting of individual subgroups per timepoint

	Amoebozoa	Apicomplexa	Ascomycota	Cercozoa	Chlorophyta	Choanoflagellata	Chrysophyta	Chytridiomycota	Ciliophora	Cryptophyta	Dinophyta	Euglenozoa	Heterobolosea	Oomycetes	rest
control_1h	0,043945034	0,004172092	0,004822426	0,06637973	0,001041137	0,011375553	0,320406134	0,009037069	0,25275637	8,80E-05	0,011721948	0,046021127	0,012018394	0,002020441	0,127374811
control_12h	0,050701721	0,002528518	0,002253185	0,077233323	0,007091899	0,007721959	0,512619858	0,024406139	0,176097704	3,79E-05	0,02027774	0,056775477	0,015787455	0,007527275	0,056525454
control_24h	0,069084239	0,001486212	0,001524361	0,16270697	0,000356055	0,006881084	0,471339193	0,033273673	0,106774184	5,56E-05	0,014061	0,039686692	0,019493986	9,15E-05	0,019219997
control_48h	0,106075633	0,000257085	0,000397979	0,380261331	0,000709511	0,008261667	0,327616462	0,011739908	0,06939224	4,76E-05	0,004228864	0,039686692	0,019439386	9,15E-05	0,019219997
control_96h	0,095997398	0,000788413	0,000749991	0,386923579	0,000721151	0,008965433	0,192447751	0,053787491	0,211664226	6,05E-05	0,002797761	0,011705305	0,005339324	0,000116641	0,027940414
control_168h	0,020898946	0,005409834	0,000449743	0,245639581	0,001174711	0,006437186	0,068337238	0,063141367	0,525231925	5,74E-05	0,004077777	0,008024484	0,006030344	3,16E-05	0,037887115
control_336h	0,00498741	0,001090989	0,000197421	0,178361115	0,00529313	0,006094921	0,005268331	0,024990976	0,77096833	0	0,000559677	0,001291917	0,001993924	0	0,009142277
silica_1h	0,049354446	0,002851036	0,007981515	0,102516048	0,002241337	0,010912228	0,154298898	0,015213774	0,208388889	3,46E-06	0,019673119	0,036786833	0,012072773	0,002020231	0,287837379
silica_12h	0,054029649	0,001961688	0,001387788	0,082815306	0,001199402	0,007931476	0,470796266	0,029030677	0,16693712	4,11E-05	0,024837791	0,070478786	0,012869559	0,000800017	0,074912296
silica_24h	0,069187425	0,001075472	0,001401888	0,124192646	0,000413266	0,007989389	0,489010092	0,037686403	0,076740051	3,34E-05	0,008334861	0,129524277	0,014343061	0,000322794	0,039744407
silica_48h	0,108895469	0,006132385	0,000478404	0,257539223	0,00047014	0,006864774	0,337676004	0,034264185	0,141132882	8,17E-05	0,009175071	0,074778984	0,010725198	0,000165238	0,025138711
silica_96h	0,14199773	0,000575573	0,000656416	0,339782328	0,001041044	0,014534332	0,165663815	0,054496808	0,211086621	4,21E-05	0,002422224	0,034480803	0,008768765	0,000165918	0,025237348
silica_168h	0,022645197	0,001844637	0,000402283	0,383261998	0,001882885	0,007605669	0,080917608	0,035904622	0,433729228	5,03E-05	0,002045578	0,004557513	0,008160044	4,44E-05	0,021219777
silica_336h	0,014549197	0,000830588	0,000476893	0,122756709	0,015204925	0,002188407	0,000837211	0,077234076	0,728883893	1,32E-05	0,000993259	0,005337722	0,006055221	1,32E-05	0,025188655
NP_1h	0,045440981	0,006051033	0,008072232	0,074955551	0,001940989	0,013630596	0,154165484	0,145013272	0,250867799	3,88E-05	0,023828807	0,048367702	0,012046226	0,00303328	0,213358837
NP_12h	0,036812216	0,002369323	0,003411425	0,112805186	0,004897701	0,007010736	0,360159649	0,047374662	0,192835226	0	0,035594226	0,050484432	0,008993924	0,001645586	0,134272477
NP_24h	0,055780102	0,002277891	0,001323266	0,107596411	0,001105924	0,005539225	0,430970991	0,058730433	0,179887832	3,48E-05	0,030723331	0,066597798	0,011974724	0,000596679	0,03973352
NP_48h	0,083803624	0,000446657	0,000676789	0,384441991	0,000450268	0,006064288	0,298744057	0,020917585	0,121055553	2,87E-05	0,008289874	0,049142091	0,008835524	6,19E-05	0,019041462
NP_96h	0,111395939	0,000240711	0,000392086	0,568575173	0,001181222	0,010907692	0,039368819	0,049493452	0,18668852	6,20E-06	0,001609929	0,012299985	0,006767121	3,35E-05	0,016261665
NP_168h	0,030190025	0,009395971	0,000237672	0,421905452	0,001436614	0,017662598	0,090297317	0,007358783	0,411945586	2,15E-05	0,000550024	0,001379563	0,00133321	0	0,015388984
NP_336h	0,009189371	0,007146683	0,000195523	0,274441548	0,010864797	0,001324492	0,000691962	0,05712155	0,623337504	0	0,000682026	0,001774481	0,00394234	0	0,015680223
zinc_1h	0,008550639	0,001127581	0,000762771	0,010328263	0,000104508	0,003451472	0,764926634	0,010985536	0,082707076	0,00145215	0,003964451	0,053019339	0,001336869	0,000158838	0,059429666
zinc_12h	0,062031552	0,001090435	0,001393329	0,049856071	0,003781011	0,009650977	0,550684967	0,012161902	0,094971261	0,00017965	0,006092344	0,151217444	0,006470754	0,000333111	0,053570231
zinc_24h	0,079393412	0,000628299	0,000972793	0,063943486	0,000246568	0,006866231	0,514702762	0,006845331	0,068485831	0,00013434	0,007393977	0,198361334	0,005580961	0,000182677	0,028111125
zinc_48h	0,175548193	0,000225395	0,000682512	0,183942176	0,000159247	0,004798838	0,123513238	0,004608345	0,446083854	0,00079641	0,005905853	0,305780359	0,007964117	3,00E-05	0,011813041
zinc_96h	0,233596167	0,000188377	0,000689826	0,37535044	0,000186831	0,013644985	0,123887167	0,000107061	0,159825503	6,35E-05	0,007213295	0,039306448	0,005651112	1,26E-05	0,010335287
zinc_168h	0,127750247	0,000448146	0,000773158	0,334339373	0,000865113	0,005206044	0,08582214	4,70E-05	0,349021455	0,000109923	0,001107681	0,086087754	0,00480833	3,17E-06	0,032991081
zinc_336h	0,043349711	0,003522362	0,000424214	0,206805237	0,008585891	0,003093483	0,024485962	0,005426663	0,585121447	0,000322292	0,000223313	0,011997361	0,000847351	0	0,03340458
ZnNP_1h	0,006430772	0,000257291	0,000618041	0,010345503	0,000247502	0,003340568	0,659886738	0,020092048	0,149088031	0,000313312	0,007350683	0,042626622	0,00191421	0,000251179	0,060967115
ZnNP_12h	0,040884928	0,000829319	0,001521902	0,040938831	0,000529856	0,003349246	0,570370498	0,017533417	0,12240943	0,000215612	0,011083339	0,120029452	0,005593243	0,000402255	0,05768232
ZnNP_24h	0,094401646	0,000964328	0,000949326	0,078904883	0,000325888	0,007152031	0,512732549	0,008394739	0,08217461	0,000165861	0,010807641	0,162823111	0,006595595	0,000253004	0,032993886
ZnNP_48h	0,217595279	0,000174188	0,000355735	0,245802631	0,000207921	0,006444941	0,231478154	0,014545835	0,06839133	6,87E-05	0,007544465	0,197909841	0,012973707	9,20E-06	0,009591975
ZnNP_96h	0,208128718	0,000166035	0,000186854	0,508826115	0,000231616	0,008506277	0,089963655	5,62E-05	0,14311813	4,16E-05	0,005320265	0,02488167	0,009469833	4,68E-06	0,005736781
ZnNP_168h	0,143819564	0,000209327	0,000183895	0,275442065	0,000676238	0,053458866	0,068153303	6,06E-05	0,411685842	0,000166288	0,008873911	0,005338035	0,008016011	3,26E-06	0,028921077
ZnNP_336h	0,039188404	0,000884387	0,000692652	0,221753406	0,012958097	0,057162605	0,013126457	0,000297272	0,523094967	0,001110899	0,003648403	0,010861614	0,008010246	2,21E-06	0,014337775

Table S12: Individual differential abundant OTUs and their log2fold changes compared to the control with a p-value of <0.01. Calculated with DESeq2. NA values represent no significant difference calculated for this OTU.

high resolution taxonomy	group	percentage identity	ZnNP Treamtent	Zinc Treatment	Silica nanoparticles	nanoplastic particles
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured Cercozoa	Cercozoa	100	1,689266432	NA	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchomonadidae; Rhynchomonas; Rhynchomonas nasuta	Euglenozoa	95.652	2,789421035	3,256731901	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas fottii	Chlorophyta	100	1,763714093	1,994500528	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Pleuronematida; Cyclidiidae; Cyclidium; Cyclidium marinum	Ciliophora	100	3,538273932	3,43420998	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured freshwater cercozoan	Cercozoa	96.094	2,867423146	2,401616597	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	92.913	2,177976953	1,90026903	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchomonadidae; Rhynchomonas; Rhynchomonas nasuta	Euglenozoa	98.261	3,914859632	4,419028281	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	100	2,609234249	2,487771412	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Thalassiosiraceae; Conticribra; Conticribra weissflogiopsis	Bacillariophyta	99.213	2,627197207	3,149607996	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Euglenida; Spirocuta; Euglenophyceae; Euglenales; Phacaceae; Lepocinclis; Lepocinclis oxyuris	Euglenozoa	100	NA	-2,638872922	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia acicularis	Bacillariophyta	100	NA	-1,633712998	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Dinobryaceae; Poteriospumella; unclassified Poteriospumella; Poteriospumella sp.	Chrysophyta	97.6	NA	4,457832808	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia dissipata	Bacillariophyta	100	-3,126041805	-2,521683988	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	95.161	2,628349889	2,46825833	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Scenedesmaceae; Desmodesmus; Desmodesmus communis	Chlorophyta	100	NA	NA	NA	0,603292914
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas parallestriata	Chlorophyta	100	1,738559158	1,562740751	NA	NA
cellular organisms; Eukaryota; Haptista; Centroplasthelida; Pterocystida; Pterista; Pterocystidae; Pterocystis; unclassified Pterocystis; Pterocystis sp. JJP-2003	rem.OTUs	92.308	-1,455947572	-1,384779333	NA	-1,8776939
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	92.857	3,780358752	4,28154651	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Coscinodiscophycidae; Melosirales; Melosiraceae; Melosira; Melosira varians	Bacillariophyta	100	-0,807743829	NA	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Hemiselmidaceae; Hemiselmis; Hemiselmis virescens	Cryptophyta	92.593	NA	3,40993107	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; environmental samples; uncultured Glenodinium	Dinophyta	95.868	1,376496032	1,052942881	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Cyclotella; Cyclotella cryptica	Bacillariophyta	99.206	-1,762163765	-1,376562099	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; Sessilida; Vorticellidae; Vorticella; Vorticella campanula	Ciliophora	100	NA	NA	NA	-1,591916005
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Selenastraceae; Monoraphidium; Monoraphidium subclavatum	Chlorophyta	100	0,428576471	NA	NA	0,543761991
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Cercomonas; unclassified Cercomonas; Cercomonas sp.	Cercozoa	97.71	NA	1,334570986	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	90.323	1,272799051	1,212094435	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; environmental samples; uncultured Trebouxiophyceae	Chlorophyta	100	-2,132299665	-2,742708278	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Raphidophyceae; Chattonellales; Chattonellaceae; Chattonella; Chattonella subsalsa	rem.OTUs	99.187	4,25047861	3,944683481	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Cyclotella; Cyclotella meneghiniana	Bacillariophyta	100	-1,073263536	-1,058969261	0,864326746	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Fungi incertae sedis; Chytridiomycota; environmental samples; uncultured Chytridiomycota	Chytridiomycota	93.023	NA	-23,78126009	NA	NA

cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Chlorella; unclassified Chlorella; Chlorella sp. BUM11008	Chlorophyta	100	1,538055758	1,72210841	NA	-1,486416975
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Astomatida; Haptophryidae; Haptophrya; Haptophrya planariarum	Ciliophora	92.562	2,397434517	2,647277197	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	99.206	2,745981144	2,654187432	NA	NA
cellular organisms; Eukaryota; environmental samples; uncultured eukaryote	rem.OTUs	100	-1,952429548	-1,156386948	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Cyclotella; Cyclotella meneghiniana	Bacillariophyta	100	NA	NA	1,118864594	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Lobomonas; Lobomonas monstruosa	Chlorophyta	100	2,949080669	2,749255497	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	90.625	3,000136795	4,454965294	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	98.333	-30	-28,27832235	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; unclassified Cercozoa; Gymnophrys; unclassified Gymnophrys; Gymnophrys sp. ATCC 50923	Cercozoa	100	NA	1,999297905	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; Eurotiomycetes; Eurotiomycetidae; Eurotiales; Aspergillaceae; Penicillium; Penicillium roqueforti	Ascomycota	100	NA	1,764707397	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	99.115	6,536409399	6,578766666	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured Cercozoa	Cercozoa	99.219	-1,582353585	-1,197537535	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; Sessilida; Vorticellidae; Carchesium; Carchesium polypinum	Ciliophora	100	5,127758407	NA	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Tubulinea; environmental samples; uncultured Lobosea	Amoebozoa	98.462	-2,952884642	-2,91672879	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Naviculales; Sellaphoraceae; Sellaphora; unclassified Sellaphora; Sellaphora sp. 108	Bacillariophyta	99.2	-3,832259022	-3,41931572	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; environmental samples; uncultured Chlorophyceae	Chlorophyta	97.581	1,912063922	1,586985825	NA	1,068073922
cellular organisms; Eukaryota; Amoebozoa; Discosea; Longamoebia; Centramoebida; Acanthamoebidae; Acanthamoeba; Acanthamoeba culbertsoni	Amoebozoa	100	-2,531691235	-3,271227308	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Hymenostomatida; Tetrahymenina; Tetrahymenidae; Tetrahymena; Tetrahymena glochidiophila	Ciliophora	100	2,876340987	3,223096264	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas bilatus	Chlorophyta	100	4,494897674	4,252857723	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	93.701	3,544298611	3,945113207	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp.	Cercozoa	100	1,869636974	NA	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JBNZ43; JBNZ43	Chrysophyta	98.425	2,906746363	2,99705247	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; environmental samples; uncultured Trebouxiophyceae	Chlorophyta	97.674	-2,615889265	-2,680707756	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	92.742	2,881803346	NA	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Suessiales; Suessiaceae; Asulcocephalium; Asulcocephalium miricentonis	Dinophyta	96.825	1,512487257	1,43928001	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Rotosphaerida; Nucleariidae; Nuclearia; Nuclearia thermophila	rem.OTUs	96.154	3,928231078	3,984580823	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peniculida; Parameciidae; Paramecium; Paramecium putrinum	Ciliophora	100	7,857931408	7,550801603	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Pleodorina; Pleodorina illinoisensis	Chlorophyta	99.2	0,755319265	0,604432402	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	95.161	2,812996954	2,753734094	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chloromonadinia; Chloromonas; Chloromonas fonticola	Chlorophyta	98.425	NA	1,291382216	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Spirotrichea; Choreotrichia; Tintinnida; Codonellidae; Tintinnopsis; unclassified Tintinnopsis; Tintinnopsis sp. HCB-2005	Ciliophora	94.215	NA	1,881443181	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas pitschmannii	Chlorophyta	99.213	1,358122338	1,542756219	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peniculida; Parameciidae; Paramecium; Paramecium tetraurelia	Ciliophora	99.167	8,886371501	NA	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	92.913	2,205077882	2,142203841	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia palea	Bacillariophyta	100	-1,243574081	-1,104536358	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas oviformis	Chlorophyta	100	NA	0,776698676	NA	NA

cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Syndiniales; environmental samples; uncultured Amoebophrya	Dinophyta	94.167	3,61841363	3,415729046	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Spumella; unclassified Spumella; Spumella sp. TGS6	Chrysophyta	100	3,390236024	3,726711793	NA	-0,988358709
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Thalassiosiraceae; Thalassiosira; Thalassiosira Tenera	Bacillariophyta	100	-2,586881854	-2,682201121	NA	NA
cellular organisms; Eukaryota; Haptista; Centroplasthelida; Pterocystida; Pterista; Pterocystidae; Chlamydaster; Chlamydaster sterni	rem.OTUs	93.182	NA	NA	NA	-2,550162669
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Cyclotella; Cyclotella striata	Bacillariophyta	100	-0,675185094	-0,610383563	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JBAS36	Chrysophyta	100	4,163836108	4,726464482	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; Vannella simplex	Amoebozoa	96.97	2,163647512	2,584851559	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	94.915	4,733794898	5,24076402	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Spirotrichea; Stichotrichia; Urostylida; Holostichidae; Holosticha; Holosticha diademata	Ciliophora	93.86	-22,864339662	-21,788406766	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Paracercomonas; Paracercomonas crassicauda	Cercozoa	96.85	-2,013618927	-1,269202318	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; unclassified Vannella; Vannella sp. ELH3	Amoebozoa	95.556	NA	2,21085002	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Cyclotella; Cyclotella striata	Bacillariophyta	98.425	-1,262295997	-1,18239761	1,161726007	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Eubodonida; Bodonidae; Allobodo; Allobodo chlorophagus	Euglenozoa	92.035	-2,60732752	-3,166135642	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; unclassified Paraphysomonas; Paraphysomonas sp. SW02	Chrysophyta	100	3,739859263	4,100681789	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Choanoflagellata; environmental samples; uncultured Choanoflagellida	Choanoflagellata	100	-1,912218084	-1,6021001	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	100	3,101871481	3,903248526	NA	-1,830921302
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; Skeletonema subsalsum	Bacillariophyta	98.425	-2,825671572	-2,74008374	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Ochromonas; unclassified Ochromonas; Ochromonas sp.	Chrysophyta	93.548	3,580232488	4,321838472	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Cercomonas; unclassified Cercomonas; Cercomonas sp. Tempisque	Cercozoa	97.521	2,757470156	3,170318294	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Choanoflagellata; Craspedida; Salpingoecidae; Codosiga; Codosiga botrytis	Choanoflagellata	100	4,053404651	4,507740559	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Hymenostomatida; Tetrahymenina; Tetrahymenidae; Tetrahymena; Tetrahymena glochidiophila	Ciliophora	100	2,667648706	2,927889601	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; Vannella planctonica	Amoebozoa	99.237	2,441065621	2,531508852	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; unclassified Cryptomonas; Cryptomonas sp. Dumo2 100310C	Cryptophyta	97.436	3,857937603	3,460422017	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas pumilio; Chlamydomonas pumilio var. pumilio	Chlorophyta	100	2,98980755	3,567599717	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; Skeletonema subsalsum	Bacillariophyta	100	-1,38838773	-1,576456212	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Rotosphaerida; Nucleariidae; Nuclearia; Nuclearia pattersoni	rem.OTUs	100	3,198263648	3,372432623	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Scenedesmaceae; Desmodesmus; unclassified Desmodesmus; Desmodesmus sp. HSJ717	Chlorophyta	100	0,566847838	NA	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Fragilariophyceae; Fragilariophycidae; Licmophorales; Ulnariaceae; environmental samples; uncultured Ulnaria	Bacillariophyta	100	-0,812317811	NA	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	100	NA	1,747387917	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchomonadidae; Rhynchomonas; Rhynchomonas nasuta	Euglenozoa	100	1,218556336	1,745300023	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Cercomonas; unclassified Cercomonas; Cercomonas sp.	Cercozoa	98.462	-2,675507674	-2,848722509	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Fungi incertae sedis; Chytridiomycota; Chytridiomycota incertae sedis; Chytridiomycetes; Rhizophydiales; Rhizophydiaceae; Rhizophydium; Rhizophydium patellarium	Chytridiomycota	100	-2,561988619	-2,500657821	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Synurophyceae; Ochromonadales; Ochromonadaceae; Poterioochromonas; Poterioochromonas malhamensis	Chrysophyta	100	4,698886994	5,252990269	NA	NA

cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; environmental samples; uncultured Bacillariophyceae	Bacillariophyta	100	-1,306220825	NA	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Eutetramitia; Psalteriomonadidae; Monopylocystis; Monopylocystis visvesvarai	Heterolobosea	95.652	NA	1,46793016	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Euglenida; Spirocuta; Euglenophyceae; Euglenales; Euglenaceae; Euglena; Euglena agilis	Euglenozoa	92.969	NA	2,744721666	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. NIES-4296	Chlorophyta	100	1,422441736	1,788508194	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Eutetramitia; Vahlkampfiidae; Naegleria; unclassified Naegleria; Naegleria sp. KDN1	Heterolobosea	100	4,442165251	4,665258814	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; Vannella miroides	Amoebozoa	100	NA	2,142645613	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain SUM1S/I	Amoebozoa	100	2,382909262	2,271984519	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	100	2,564085319	2,542065551	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; environmental samples; uncultured diatom	Bacillariophyta	100	-2,041403946	-2,32631579	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	100	0,892626918	0,852792475	NA	NA
cellular organisms; Eukaryota; environmental samples; uncultured eukaryote	rem.OTUs	95.146	-2,827532919	-2,569688854	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Micractinium; unclassified Micractinium; Micractinium sp. SAG 48.93	Chlorophyta	98.438	-5,730370341	-5,610834048	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Cymbellales; Gomphonemataceae; Gomphonema; unclassified Gomphonema; Gomphonema sp. 12	Bacillariophyta	100	-1,622401368	-1,096438908	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; Paraphysomonas foraminifera	Chrysophyta	97.638	7,518759807	6,075903822	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Himatismenida; Cochliopodiidae; Cochliopodium; Cochliopodium minus	Amoebozoa	100	2,547865326	2,598607187	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	99.213	-2,310695533	-2,321388948	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Oocystaceae; Oocystoideae; Oocystis; unclassified Oocystis; Oocystis sp. NIES-4297	Chlorophyta	100	-1,661830899	-1,935784788	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Pirsoniales; Pirsonia	rem.OTUs	100	NA	NA	NA	-3,715633894
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Stephanodiscus; Stephanodiscus hantzschii	Bacillariophyta	98.425	-2,507948007	-2,431342959	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Neochloridaceae; Neochloris; unclassified Neochloris; Neochloris sp. GM4e	Chlorophyta	99.206	1,215076069	1,57112438	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; unclassified Cryptomonas; Cryptomonas sp. NIES-3916	Cryptophyta	100	3,730199066	3,184266128	NA	-1,728383433
cellular organisms; Eukaryota; Discoba; Euglenozoa; Euglenida; Spirocuta; Euglenophyceae; Euglenales; Colacium; unclassified Colacium; Colacium sp. Jjockji033107A	Euglenozoa	99.145	1,875621637	2,026873765	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Micractinium; unclassified Micractinium; Micractinium sp. CCAP 248/14	Chlorophyta	100	-2,286358467	-2,800298293	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	92.562	2,7720479	3,372944951	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Thalassiosiraceae; Conticribra; Conticribra guillardii	Bacillariophyta	100	-0,971020766	-1,117148254	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Glissomonadida; Viridiraptoridae; Bodomorpha; Bodomorpha minima	Cercozoa	96.85	3,647396926	4,985559943	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Tubulinea; Echinamoebida; Vermamoeba; Vermamoeba vermiformis	Amoebozoa	100	1,777055599	1,792217735	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Fungi incertae sedis; Chytridiomycota; Chytridiomycota incertae sedis; Chytridiomycetes; Zygophlyctidales; Zygophlyctidaceae; Zygophlyctis; Zygophlyctis planktonica	Chytridiomycota	100	-2,86480599	-3,030136949	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	95.276	5,080286249	4,552668963	NA	-1,471453375
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas fasciata	Chlorophyta	100	1,427059676	1,355183704	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Fragilariophyceae; Fragilariophycidae; Licmophorales; Ulnariaceae; environmental samples; uncultured Ulnaria	Bacillariophyta	100	-1,226126701	-1,093450717	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Ochromonas; unclassified Ochromonas; Ochromonas sp.	Chrysophyta	92.742	NA	1,996424938	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	98.413	1,349043123	0,849931961	NA	NA

cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	96.85	3,140099024	3,692783701	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; environmental samples; uncultured Trebouxiophyceae	Chlorophyta	99.219	-4,281719512	-5,654586833	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Oocystaceae; unclassified Oocystaceae; Oocystaceae sp. NIES 3919	Chlorophyta	99.107	-1,900049187	-2,181006132	NA	NA
cellular organisms; Eukaryota; environmental samples; uncultured eukaryote	rem.OTUs	97.087	NA	1,300382789	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; Cryptomonas marssonii	Cryptophyta	100	1,354163935	1,270338964	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JBNZ43	Chrysophyta	100	4,916941246	5,193940342	NA	-2,946188185
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; unclassified Peritrichia; Peritrichia sp. aOmb2	Ciliophora	100	1,802327384	1,756904506	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Ochromonas; Ochromonas villosa	Chrysophyta	95.276	2,359058993	2,685484098	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	100	2,91023883	NA	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	95.122	2,881900661	2,75501353	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Euglenida; Spirocuta; Euglenophyceae; Euglenales; Euglenaceae; Euglena; Euglena agilis	Euglenozoa	90.625	2,664430641	NA	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas isabeliensis	Chlorophyta	100	1,957359837	1,990647944	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	97.619	1,763528081	1,622852257	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	92.857	NA	3,512317033	NA	NA
cellular organisms; Eukaryota; Haptista; Centroplasthelida; Pterocystida; Pterista; Pterocystidae; Pterocystis; unclassified Pterocystis; Pterocystis sp. JJP-2003	rem.OTUs	90.769	NA	NA	NA	-1,303146684
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	91.27	3,343607361	3,125035138	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	98.23	5,000077984	5,918467374	NA	NA
cellular organisms; Eukaryota; Haptista; Centroplasthelida; Pterocystida; Pterista; Pterocystidae; Chlamydaster; Chlamydaster sterni	rem.OTUs	100	1,535650941	1,510531542	NA	-1,388864558
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Dactylopodida; Vexilliferidae; Vexillifera; Vexillifera bacillipedes	Amoebozoa	100	NA	3,322654273	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Meyerella; Meyerella planktonica	Chlorophyta	97.674	-2,801765614	-3,307789619	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. DP13	Amoebozoa	100	3,104640859	2,83724679	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	91.803	NA	NA	-4,602451719	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Tetracystaceae; Tetracystis; Tetracystis pampae	Chlorophyta	99.213	1,628246943	1,226594229	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	93.023	3,34892206	2,866891678	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Naviculales; Stauroneidaceae; Craticula; Craticula pseudocitrus	Bacillariophyta	100	-2,42044898	-2,501792098	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Dinobryaceae; Poteriospumella; unclassified Poteriospumella; Poteriospumella sp.	Chrysophyta	97.6	2,644699903	3,325649262	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Cymbellales; Cymbellaceae; Cymbella; Cymbella tumida	Bacillariophyta	100	NA	NA	NA	-0,97187677
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Mychonastaceae; Mychonastes; unclassified Mychonastes; Mychonastes sp. 6A3	Chlorophyta	100	NA	NA	NA	0,884129327
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Lobomonas; Lobomonas francei	Chlorophyta	100	NA	0,685297757	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. YACCYB80	Chlorophyta	100	NA	1,235346103	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas proteus	Chlorophyta	96.032	1,089435016	1,212696005	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp.	Cercozoa	100	1,132771523	NA	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; Vannella simplex	Amoebozoa	99.242	NA	3,647034391	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	96.825	2,208291327	NA	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Evosea; Archamoebae; Rhizomastix; Rhizomastix elongata	Amoebozoa	97.727	2,985942613	4,22899791	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae; Phaeosphaeriaceae; Phaeosphaeria; Phaeosphaeria graminis	Ascomycota	100	2,840108609	3,029490396	NA	NA

cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; unclassified Vannella; Vannella sp. GERL34	Amoebozoa	96.032	2,736307484	2,862175805	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	100	4,060429907	4,955186801	NA	-1,505029064
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Eustigmatophyceae; Eustigmatales; Eustigmataceae; Vischeria; Vischeria magna	rem.OTUs	100	1,412116478	1,567784799	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; environmental samples; uncultured diatom	Bacillariophyta	96.063	-1,983387897	-1,994676623	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	90.551	NA	-2,944197918	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Peridiniales; Pfiesteriaceae; Aduncodinium; Aduncodinium glandula	Dinophyta	96.825	-1,640698567	-1,648801373	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	93.651	NA	3,226022075	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; environmental samples; uncultured diatom	Bacillariophyta	100	-3,140204163	-4,060347846	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas oviformis	Chlorophyta	98.425	NA	1,956420797	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; environmental samples; uncultured Bracteacoccus	Chlorophyta	100	NA	2,163285448	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas simplex	Chlorophyta	96.85	1,874856861	1,964118459	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Discostella; Discostella nipponica	Bacillariophyta	100	-2,026021667	-2,014702426	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; Rhogostoma schuessleri	Cercozoa	93.75	-2,792902031	NA	NA	NA
cellular organisms; Eukaryota; environmental samples; uncultured eukaryote	rem.OTUs	98.039	-2,806859763	-4,026549802	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Pyrenomonadales; Pyrenomonadaceae; Rhodomonas; unclassified Rhodomonas; Rhodomonas sp. 08C1	Cryptophyta	99.206	1,230175101	1,665587614	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; Skeletonema subsalsum	Bacillariophyta	96.85	-1,544696272	-1,436294219	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Litostomatea; Haptoria; Haptorida; Didiniidae; Didinium; Didinium nasutum	Ciliophora	98.077	NA	-1,821637615	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; Vannella simplex	Amoebozoa	100	NA	2,004843724	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; Skeletonema subsalsum	Bacillariophyta	100	-1,702363089	-1,597490695	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	98.387	2,469433476	2,376002323	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; unclassified Vannella; Vannella sp. GERL34	Amoebozoa	96.61	NA	2,336974674	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; Skeletonema potamos	Bacillariophyta	100	-0,773245297	NA	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	97.619	1,92668607	2,328902554	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; environmental samples; uncultured Peritrichia	Ciliophora	100	2,721892362	2,892646031	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; environmental samples; uncultured diatom	Bacillariophyta	98.425	-2,481249412	-2,38444528	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Pedospumella; Pedospumella encystans	Chrysophyta	100	4,27054485	6,557909705	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	100	2,127494064	2,797881065	NA	NA
cellular organisms; Eukaryota; Haptista; Centroplasthelida; environmental samples; uncultured Centroheliozoa	rem.OTUs	92.5	1,252465152	1,470263878	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia amphibia	Bacillariophyta	99.213	-1,55990409	-1,515370692	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; environmental samples; uncultured Trebouxiophyceae	Chlorophyta	100	-1,892898146	-3,018567589	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Litostomatea; Haptoria; Pleurostomatida; Litonotidae; Loxophyllum; unclassified Loxophyllum; Loxophyllum sp. SZ08110101-FW	Ciliophora	96.154	NA	-6,384645212	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Dinobryaceae; Poteriospumella; unclassified Poteriospumella; Poteriospumella sp.	Chrysophyta	100	6,565415839	6,936201577	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Eutetramitia; Vahlkampfiidae; Vahlkampfia; Vahlkampfia avara	Heterolobosea	100	NA	1,645718534	NA	NA

cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchomonadidae; Rhynchomonas; Rhynchomonas nasuta	Euglenozoa	98.261	1,633820555	2,348300749	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	94.915	6,036551527	5,97852628	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; Cryptomonas curvata	Cryptophyta	100	2,96932212	2,636807904	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Longamoebia; Thecamoebida; Stenamoeba; unclassified Stenamoeba; Stenamoeba sp. HP839	Amoebozoa	98.551	NA	1,469316249	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured Cercozoa	Cercozoa	100	1,496510618	2,058890726	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Schroederiaceae; Schroederia; Schroederia setigera	Chlorophyta	100	0,591123714	0,675538006	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Micractinium; unclassified Micractinium; Micractinium singularis	Chlorophyta	100	-2,777305172	-3,446454665	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Diplonemea; Hemistasiidae; Hemistasia; Hemistasia phaeocysticola	Euglenozoa	96	-3,138419005	-2,266933322	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	96.774	NA	3,429958378	NA	NA
cellular organisms; Eukaryota; environmental samples; uncultured eukaryote	rem.OTUs	97.015	NA	1,719393239	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	92.969	2,234496443	2,132071593	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	96.46	6,504326507	7,049576273	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	99.213	6,786852993	6,563750296	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	100	1,651790648	1,324451565	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; environmental samples; uncultured fungus	rem.OTUs	95.868	NA	3,213500067	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Hemiselmidaceae; Hemiselmis; Hemiselmis virescens	Cryptophyta	92.593	NA	NA	-29,84364656	-26,43227787
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Leishmaniinae; Endotrypanum; unclassified Endotrypanum; Endotrypanum sp. 889	Euglenozoa	93.805	2,341797253	3,168302963	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Cryptochilidae; Biggaria; Biggaria bermudensis	Ciliophora	96.46	NA	NA	NA	-1,872050103
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Scenedesmaceae; Desmodesmus; unclassified Desmodesmus; Desmodesmus sp. HH 10203	Chlorophyta	100	0,957065545	0,764973741	NA	0,768973917
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Asteromonadaceae; Halochlorella; Halochlorella rubescens	Chlorophyta	100	0,800147866	0,890387003	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	100	2,65683418	2,517135307	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; unclassified Paraphysomonas; Paraphysomonas sp. 25 JMS-2012	Chrysophyta	98	5,107578843	4,857380197	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia dissipata	Bacillariophyta	98.425	-2,995676433	-2,627403633	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chrysocapsaceae; Kremastochrysopsis; Kremastochrysopsis austriaca	Chrysophyta	93.023	2,287537129	3,136636758	NA	-2,349714933
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Eubodonida; Bodonidae; Bodo; Bodo saltans	Euglenozoa	100	8,53550231	8,428114341	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Thalassiosiraceae; Thalassiosira; Thalassiosira pseudonana	Bacillariophyta	100	-0,407957458	-0,376950872	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	95.161	2,891664032	2,724647039	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; environmental samples; uncultured Chlorophyta	Chlorophyta	96.85	NA	-1,281052241	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; unclassified Paraphysomonas; Paraphysomonas sp. GflagA	Chrysophyta	98.425	5,555425432	5,712099702	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	96.063	2,291709653	2,182553154	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Glissomonadida; Allapsidae; Allantion; Allantion parvum	Cercozoa	95.385	4,978545773	4,935629496	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Nannochloris; unclassified Nannochloris; Nannochloris sp. MBIC10091	Chlorophyta	100	0,470587748	NA	NA	0,548959379
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; unclassified Peritrichia; Peritrichia sp. aOmb2	Ciliophora	100	1,355549537	1,240752142	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; environmental samples; uncultured ciliate	Ciliophora	100	-2,505276497	-2,570640237	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Dactylopodida; Vexilliferidae; Vexillifera; Vexillifera bacillipedes	Amoebozoa	100	2,045861559	2,196310284	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Tubulinea; Elardia; Euamoebida; environmental samples; uncultured Euamoebida	Amoebozoa	100	NA	1,267424592	NA	NA

cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Auxenochlorella; Auxenochlorella protothecoides	Chlorophyta	100	0,987898062	0,954157336	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas raudensis	Chlorophyta	100	3,520636218	3,776227999	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	92.126	4,799145031	5,051620521	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas dorsoventralis	Chlorophyta	100	1,994665657	1,901078025	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Synurophyceae; Synurales; Mallomonadaceae; Synura; Synura sphagnicola	Chrysophyta	100	4,401730237	3,873061112	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	99.174	NA	2,157166203	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. YACCYB80	Chlorophyta	98.214	1,140337216	1,250779853	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JB; Spumella-like flagellate JBC29	Chrysophyta	100	1,510623239	1,149347606	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured Cercozoa	Cercozoa	98.4	-1,753416222	-1,629287752	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Philasterida; Uronematidae; Apouronema; Apouronema harbinensis	Ciliophora	100	3,060545189	3,521606528	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	100	NA	1,43413576	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	97.541	2,880389833	2,755327061	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchobodo; unclassified Rhynchobodo; Rhynchobodo sp. HFCC304	Euglenozoa	93.805	3,232729653	3,611048379	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Fungi incertae sedis; Chytridiomycota; Chytridiomycota incertae sedis; Chytridiomycetes; Rhizophlyctidales; Rhizophlyctidaceae; Rhizophlyctis; Rhizophlyctis rosea	Chytridiomycota	100	-2,837587256	-3,083586361	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	94.737	4,19166875	4,831603258	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. CCAP 11/159	Chlorophyta	98.425	0,94665374	1,057780158	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Scenedesmaceae; Desmodesmus; Desmodesmus costato-granulatus	Chlorophyta	95.276	0,655326787	0,77834722	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas fasciata	Chlorophyta	97.6	1,265338283	1,473318115	NA	NA
cellular organisms; Eukaryota; Kathablepharidacea; Katablepharidaceae; environmental samples; uncultured Katablepharidaceae	rem.OTUs	99.2	-1,791588858	-2,524735876	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	91.736	4,812849206	5,085059814	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Neovahlkampfiidae; Neovahlkampfia; Neovahlkampfia nana	Heterolobosea	94.545	-3,174893727	-3,143381912	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Colpodea; Grossglockneriida; Grossglockneriidae; Pseudoplatyophrya; Pseudoplatyophrya nana	Ciliophora	100	NA	2,516921832	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Spumella; unclassified Spumella; Spumella sp. TGS6	Chrysophyta	100	4,716508911	6,211477937	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	97.345	NA	5,244696814	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Colpodea; Cyrtolophosidida; Cyrtolophosididae; Pseudocyrtolophosis; Pseudocyrtolophosis alpestris	Ciliophora	99.167	NA	3,302891121	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; unclassified Vannella; Vannella sp. GERL34	Amoebozoa	96.721	2,284452051	2,231978217	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Glissomonadida; Allapsidae; Allantion; Allantion parvum	Cercozoa	93.846	3,065083019	3,53915534	NA	NA
cellular organisms; Eukaryota; CRuMs; Collodictyonidae; Diphylleia; Diphylleia rotans	rem.OTUs	100	5,442304158	6,823208991	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; environmental samples; uncultured diatom	Bacillariophyta	99.213	-1,955179296	-1,703563159	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain SUM1S/I	Amoebozoa	92.742	2,210433418	2,030604726	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; Eurotiomycetes; environmental samples; uncultured Eurotiomycetes	Ascomycota	100	NA	1,457577888	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; environmental samples; uncultured Cryptomonadaceae	Cryptophyta	100	3,763131506	3,422377661	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	100	6,568814697	6,955557162	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	98.413	2,248967771	2,032082912	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Euglenida; Spirocuta; Euglenophyceae; Euglenales; Colacium; unclassified Colacium; Colacium sp. Jjockji033107A	Euglenozoa	96.581	NA	2,605359684	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Choanoflagellata; Craspedida; Salpingoecidae; Sphaeroeca; Sphaeroeca leprechaunica	Choanoflagellata	96.939	3,234461032	3,239204468	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Hemiselmidaceae; Hemiselmis; Hemiselmis virescens	Cryptophyta	92.593	NA	2,702852534	NA	NA

cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; unclassified Paraphysomonas; Paraphysomonas sp. GflagA	Chrysophyta	99.213	2,987138793	3,404649475	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	98.333	2,408961054	2,307448232	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. GP2	Amoebozoa	98.438	2,860686646	3,327408508	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Synurophyceae; Synurales; Mallomonadaceae; Mallomonas; unclassified Mallomonas; Mallomonas sp.	Chrysophyta	100	-2,649352115	-2,352319817	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. CCAP 11/132	Chlorophyta	100	1,851438435	1,784662871	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Fragilariophyceae; Fragilariophycidae; Fragilariales; unclassified Fragilariales; Fragilariales sp. endosymbiont of Amphistegina lobifera	Bacillariophyta	99.213	-1,758522789	NA	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Mychonastaceae; Mychonastes; Mychonastes homosphaera	Chlorophyta	100	0,747518466	0,668028435	NA	0,819128771
cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Venturiales; Sympoventuriaceae; Fuscohilum; Fuscohilum siciliana	Ascomycota	100	NA	3,417211193	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Stephanodiscus; Stephanodiscus hantzschii	Bacillariophyta	100	-1,935424902	-2,163569427	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JBNZ43; JBNZ43	Chrysophyta	97.638	4,037779601	4,431602305	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Scenedesmaceae; Desmodesmus; Desmodesmus armatus	Chlorophyta	100	0,622690224	0,564036107	NA	0,666493409
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	95.968	3,067239778	2,981578379	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Spirotrichea; Hypotrichia; Euplotida; Euplotidae; Euplotes; Euplotes woodruffi	Ciliophora	100	1,169751184	NA	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	93.651	4,629230871	5,073265273	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured Cercozoa	Cercozoa	91.339	1,927366168	NA	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Peridiniales; Kryptoperidiniaceae; Unruhdinium; Unruhdinium penardii	Dinophyta	99.153	NA	NA	NA	0,952137624
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Synurophyceae; Ochromonadales; Acrispumella; Acrispumella msimbaziensis	Chrysophyta	99.213	3,697647959	3,549340649	NA	-1,91336189
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. CCAP 11/160	Chlorophyta	96.85	NA	1,018299354	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Cyclotella; Cyclotella cryptica	Bacillariophyta	96.85	NA	-2,425926375	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; unclassified Cryptomonas; Cryptomonas sp. Yeonra43011B	Cryptophyta	100	3,119609094	3,083206661	NA	-1,781414386
cellular organisms; Eukaryota; Sar; Stramenopiles; Bigyra; Opalozoa; Bicosoecida; environmental samples; uncultured Bicosoecida	rem.OTUs	93.846	0,575567045	0,763105367	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	96.032	3,063906591	3,080610851	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; unclassified Vannella; Vannella sp. strain CAZ6/I	Amoebozoa	98.387	2,246053305	2,157817398	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Tubulinea; unclassified Tubulinea; Tubulinea group 04 sp. P18	Amoebozoa	100	1,952955138	2,226029672	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	93.75	1,972538782	1,835435069	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Cyclotella; Cyclotella meneghiniana	Bacillariophyta	100	-1,198037642	-1,123974309	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Uroglena; unclassified Uroglena; Uroglena sp. CCMP2768	Chrysophyta	96.063	3,276893461	3,497812259	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Eutetramitia; Vahlkampfiidae; Naegleria; unclassified Naegleria; Naegleria sp. KDN1	Heterolobosea	98.165	3,210122408	4,392711149	NA	NA
cellular organisms; Eukaryota; Haptista; Centroplasthelida; Pterocystida; Pterista; Pterocystidae; Chlamydaster; Chlamydaster sterni	rem.OTUs	93.893	NA	NA	NA	-2,12928064
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Naviculales; Sellaphoraceae; Sellaphora; Sellaphora cf. seminulum	Bacillariophyta	100	-2,606194134	-2,998784134	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	95.082	4,178682474	4,881540476	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain SUM1S/I	Amoebozoa	93.75	2,856104331	2,872470446	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JB; Spumella-like flagellate JBM08	Chrysophyta	96	2,147642158	2,729414794	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. TgW1001D1P2	Chlorophyta	99.2	2,600229296	2,629162315	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; unclassified Paraphysomonas; Paraphysomonas sp. SW02	Chrysophyta	100	2,879555549	4,007698554	NA	NA

cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	96.063	1,562479507	1,90177532	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	96.063	1,836292564	2,311031598	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	93.651	3,035815865	3,356898205	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Rotosphaerida; Nucleariidae; Nuclearia; Nuclearia simplex	rem.OTUs	96.923	5,830733436	6,740196929	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Colemanosphaera; Colemanosphaera charkowiensis	Chlorophyta	98.4	1,171142912	0,987971046	NA	NA
cellular organisms; Eukaryota; Kathablepharidacea; Katablepharidaceae; environmental samples; uncultured Katablepharidaceae	rem.OTUs	100	-2,563628153	-2,840062064	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia aequorea	Bacillariophyta	99.213	-2,158119706	-1,709919843	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Uroglena; unclassified Uroglena; Uroglena sp. CCMP2768	Chrysophyta	95.902	2,407720105	3,465084103	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	100	3,373223911	3,792014911	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas proteus	Chlorophyta	100	0,786763433	0,789339641	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	91.603	2,635197543	2,428552383	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; environmental samples; uncultured fungus	rem.OTUs	100	NA	-2,378449292	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Philasterida; Uronematidae; Apouronema; Apouronema harbinensis	Ciliophora	98.198	NA	4,073402976	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Ochromonas; unclassified Ochromonas; Ochromonas sp.	Chrysophyta	93.548	5,08704864	5,130083547	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	93.651	2,17210169	2,066544782	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Pleodorina; Pleodorina starrii	Chlorophyta	100	1,489122036	1,215711133	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchomonadidae; Rhynchomonas; Rhynchomonas nasuta	Euglenozoa	100	6,718978784	6,724516444	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	94.444	1,914497489	1,865193665	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Hydrodictyaceae; unclassified Hydrodictyaceae; Hydrodictyaceae sp. HND10-6	Chlorophyta	93.75	1,212320977	1,895330473	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Cylindrotheca; unclassified Cylindrotheca; Cylindrotheca sp. SMS41	Bacillariophyta	98.425	-3,309435131	-1,70303988	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	100	5,555481429	5,976640071	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	92.623	2,103334398	2,347974729	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	100	1,410954635	2,205730873	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; environmental samples; uncultured ciliate	Ciliophora	100	-2,933580772	-2,729633922	NA	-2,589185322
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Trypanosomatida; Trypanosomatidae; Leishmaniinae; Zelonia; Zelonia cf. costaricensis	Euglenozoa	100	NA	1,770695195	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Choanoflagellata; Craspedida; Salpingoecidae; Salpingoeca; Salpingoeca fusiformis	Choanoflagellata	100	-2,245900004	-2,227953243	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; Cryptomonas lucens	Cryptophyta	100	NA	NA	NA	-0,900510693
cellular organisms; Eukaryota; Sar; Stramenopiles; Pirsoniales; Pirsonia; Pirsonia formosa	rem.OTUs	95.349	5,686623063	6,963650979	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; environmental samples; uncultured Trebouxiophyceae	Chlorophyta	100	-2,301902502	-2,595077543	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	97.541	2,846729752	2,647355478	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Peridiniales; Amphidiniopsidaceae; Archaeperidinium; Archaeperidinium saanichi	Dinophyta	95.238	-2,536257766	-4,104820106	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Vannella; unclassified Vannella; Vannella sp. strain CH88/I	Amoebozoa	98.485	2,411215559	2,480441508	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Naviculales; Naviculaceae; Navicula; unclassified Navicula; Navicula sp. NIES-3965	Bacillariophyta	100	-1,301251679	-0,895377135	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Carteria; unclassified Carteria; Carteria sp. KMMCC FC-98	Chlorophyta	98.361	NA	2,134383898	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	95.312	3,140440179	3,02315275	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Ochromonas; environmental samples; uncultured Ochromonas	Chrysophyta	95.276	2,208301657	2,959960408	NA	NA

cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae; Leptosphaeriaceae; Leptosphaeria; unclassified Leptosphaeria; Leptosphaeria sp.	Ascomycota	100	NA	3,86174531	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; environmental samples; uncultured Trebouxiophyceae	Chlorophyta	100	NA	1,751707781	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	95.041	3,236393294	4,144391458	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Fungi incertae sedis; Chytridiomycota; Chytridiomycota incertae sedis; Chytridiomycetes; Rhizophydiales; Terramycetaceae; Boothiomyces; Boothiomyces macroporosum	Chytridiomycota	90.226	-2,678796936	-2,696633114	NA	NA
cellular organisms; Eukaryota; environmental samples; uncultured eukaryote	rem.OTUs	92.437	-2,451413355	NA	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. HyT1601D5	Chlorophyta	96.8	2,07626298	2,386505451	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Spirotrichea; Hypotrichia; Euplotida; Euplotidae; Euplotes; Euplotes estuarinus	Ciliophora	91.489	2,152144297	2,399305568	NA	NA
cellular organisms; Eukaryota; Ancyromonadida; Nutomonas; Nutomonas howeae; Nutomonas howeae lacustris	rem.OTUs	100	-1,956721069	-1,814485887	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Longamoebia; Centramoebida; Acanthamoebidae; Acanthamoeba; unclassified Acanthamoeba; Acanthamoeba sp.	Amoebozoa	100	3,180787872	2,851359429	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. HyT1201C6	Chlorophyta	98.4	0,985263712	0,991282372	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Pedospumella; unclassified Pedospumella	Chrysophyta	98.425	5,74133273	6,395584465	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Bigyra; Opalozoa; Bicosoecida; Bicosoecidae; Bicosoeca; Bicosoeca petiolata	rem.OTUs	91.473	1,978720072	1,76476606	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	96.063	2,563797646	2,645463304	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	96.85	4,923008998	4,850565715	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	97.561	2,814002767	2,685950851	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Eutetramitia; Vahlkampfiidae; Naegleria; Naegleria clarki	Heterolobosea	99.083	5,304265478	5,806032075	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; unclassified Cryptomonas; Cryptomonas sp. Dumo2 100310C	Cryptophyta	100	3,537720234	3,06948904	NA	-2,366528728
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	92.623	3,317839402	3,473731942	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Pandorina; Pandorina morum	Chlorophyta	97.6	1,183108353	1,498668021	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	98.438	1,959774027	1,919184666	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	100	1,446804477	1,490938152	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Pandorina; Pandorina morum	Chlorophyta	100	0,994271932	1,042181415	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	95.238	3,355065816	3,928134483	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Naviculales; Naviculaceae; Navicula; Navicula cryptotenella	Bacillariophyta	100	-2,261420493	-1,626891557	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Haematococcaceae; Haematococcus; Haematococcus lacustris	Chlorophyta	100	3,931954444	3,296085164	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Tubulinea; Elardia; Leptomyxida; Gephyramoeba; unclassified Gephyramoeba; Gephyramoeba sp. ATCC50654	Amoebozoa	91.87	NA	2,783192214	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Dictyosphaerium; unclassified Dictyosphaerium; Dictyosphaerium sp. YN12-4	Chlorophyta	100	-1,201807423	-1,440363424	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Syndiniales; environmental samples; uncultured Amoebophrya	Dinophyta	99.167	3,553487128	2,585320818	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Uroglena; unclassified Uroglena; Uroglena sp. CCMP2768	Chrysophyta	95.935	8,61910107	8,883686322	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Tubulinea; Elardia; Euamoebida; Hartmannellidae; Saccamoeba; Saccamoeba limax	Amoebozoa	93.22	-2,823938486	-2,894863968	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JBNZ43: JBNZ43	Chrysophyta	98.425	4,807867699	5,296467028	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp.	Cercozoa	100	2,003473633	NA	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; unclassified Chlorellaceae; Chlorellaceae sp. CCAP 283/3	Chlorophyta	94.531	NA	-1,293088596	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Actinochloridaceae; Actinochloris; Actinochloris sphaerica	Chlorophyta	96.063	3,246195081	3,907333787	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Spumella; unclassified Spumella	Chrysophyta	96.85	4,558585106	4,934982267	NA	NA

cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	92.742	2,448771373	2,393235705	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Dinobryaceae; Poteriospumella; unclassified Poteriospumella; Poteriospumella sp.	Chrysophyta	100	NA	1,360887266	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	99.115	6,061682529	7,438228453	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. CCAP 11/119	Chlorophyta	100	2,123243031	NA	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; Rhogostoma schuessleri	Cercozoa	92.969	-2,103600557	NA	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchomonadidae; Rhynchomonas; Rhynchomonas nasuta	Euglenozoa	100	1,42039949	1,790571628	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Evosea; Variosea; Flamellidae; Flamella; Flamella fluviatilis	Amoebozoa	100	2,396882719	2,583051721	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Choanoflagellata; Craspedida; Salpingoecidae; Sphaeroeca; Sphaeroeca leprechaunica	Choanoflagellata	96.939	3,250957055	3,840683228	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Pandorina; Pandorina morum	Chlorophyta	100	1,819212072	2,121502341	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Scenedesmaceae; Scenedesmus; Scenedesmus acutus	Chlorophyta	100	1,038754887	1,029836729	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Thalassiophysales; Catenulaceae; Amphora; unclassified Amphora; Amphora sp. TH-2015	Bacillariophyta	100	-2,08161782	-1,945226216	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	96.063	3,283265208	4,051002907	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Choanoflagellata; Craspedida; Salpingoecidae; Sphaeroeca; Sphaeroeca volvox	Choanoflagellata	90.977	3,053045458	2,946758623	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	98.413	2,188037803	NA	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	96.85	4,611565292	4,64667602	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	95.041	2,602536156	3,256137853	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Cryptomonadaceae; Cryptomonas; Cryptomonas pyrenoidifera	Cryptophyta	100	3,990320729	3,386340825	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	100	2,74150765	2,663274809	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchomonadidae; Rhynchomonas; unclassified Rhynchomonas; Rhynchomonas sp.	Euglenozoa	96.522	2,265756552	2,301641704	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; Skeletonema menzelii	Bacillariophyta	95.276	-4,01901656	-4,959807173	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Neovahlkampfiidae; Neovahlkampfia; Neovahlkampfia nana	Heterolobosea	93.636	-1,55564192	-1,802663677	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Tetracystaceae; Tetracystis; Tetracystis pampae	Chlorophyta	96.85	3,096082549	3,481101082	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	92.308	2,671126893	2,498867444	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp.	Cercozoa	100	1,527718788	NA	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	92.913	2,492155447	2,295727406	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	95.968	2,913353441	2,673537668	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	100	2,366409001	2,49353175	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Dinobryaceae; Poteriospumella; unclassified Poteriospumella; Poteriospumella sp.	Chrysophyta	93.6	6,877818659	7,298136396	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	94.915	3,716381412	3,226227912	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain SUM1S/I	Amoebozoa	92.308	3,305395819	3,11377691	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Pedospumella; unclassified Pedospumella; Pedospumella sp. JBM19	Chrysophyta	100	3,655662128	3,797932142	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Dangeardinia; Dangeardinia pseudopertusa	Chlorophyta	100	1,892097957	1,548268448	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Carteria; Carteria lunzensis	Chlorophyta	96.85	NA	1,203277376	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	93.701	2,998560622	3,047392327	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	100	4,508561324	5,196049008	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Micractinium; unclassified Micractinium; Micractinium sp. SAG 48.93	Chlorophyta	100	-2,720052398	-3,327163059	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; unclassified Chrysophyceae; Spumella-like flagellate JBNZ43	Chrysophyta	100	2,796087271	3,47164883	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	100	4,553333859	5,450173485	NA	NA

cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. GP2	Amoebozoa	100	3,161662654	3,082859298	NA	NA
cellular organisms; Eukaryota; Haptista; Centroplasthelida; Pterocystida; Pterista; Pterocystidae; Pterocystis; unclassified Pterocystis; Pterocystis sp. JJP-2003	rem.OTUs	91.603	2,775389406	2,924897916	NA	-2,126921189
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	100	2,893776682	2,721497596	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured Cercozoa	Cercozoa	97.561	NA	2,047890713	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Radiococcaceae; Radiococcus; Radiococcus polycoccus	Chlorophyta	93.798	1,996712084	NA	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	96.46	NA	3,336251665	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Fragilariophyceae; Fragilariophycidae; Licmophorales; Ulnariaceae; Tabularia; Tabularia fasciculata	Bacillariophyta	100	-2,17550927	-1,682473553	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; dothideomyceta; Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae; Didymellaceae; Didymella; Didymella bellidis	Ascomycota	100	NA	2,616632547	NA	NA
cellular organisms; Eukaryota; Discoba; Heterolobosea; Tetramitia; Eutetramitia; Vahlkampfiidae; Naegleria; unclassified Naegleria; Naegleria sp. J14Z1	Heterolobosea	100	3,066344944	3,420963171	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	100	2,631625047	2,249815894	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Pleuronematida; Cyclidiidae; Cyclidium; Cyclidium varibonneti	Ciliophora	100	4,481948667	4,767135743	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; Skeletonema subsalsum	Bacillariophyta	100	-1,319998075	-1,144328567	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Syndiniales; environmental samples; uncultured Amoebophrya	Dinophyta	97.5	6,135351184	3,489438611	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Phyllopharyngea; Phyllopharyngia; Dysteriida; Dysteriidae; Dysteria; Dysteria semilunaris	Ciliophora	91.818	-3,167536591	-2,92822647	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Oocystaceae; Oocystoideae; Oocystidium; Oocystidium polymammilatum	Chlorophyta	100	NA	-2,578148269	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Pyrenomonadales; Pyrenomonadaceae; Pyrenomonas; Pyrenomonas salina	Cryptophyta	91.603	3,382480036	3,875676132	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Cercomonas; unclassified Cercomonas; Cercomonas sp.	Cercozoa	100	4,872422326	5,009714536	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	95.082	2,744564771	2,540701827	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; unclassified Chlamydomonas; Chlamydomonas sp. NIES-3906	Chlorophyta	100	2,284703638	1,679801856	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Spumella; Spumella lacusvadosi	Chrysophyta	100	4,22219832	5,224820546	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Lobochlamys; unclassified Lobochlamys; Lobochlamys sp. Ru-6-5	Chlorophyta	96.094	1,401900576	1,599096092	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; Sessilida; Vorticellidae; Carchesium; Carchesium polypinum	Ciliophora	100	4,520340916	4,664253934	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Peridiniales; Peridiniaceae; Pentapharsodinium; unclassified Pentapharsodinium; Pentapharsodinium sp. CCMP771	Dinophyta	96.032	-2,368019548	NA	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Euglenida; Spirocuta; Euglenophyceae; Euglenales; Colacium; unclassified Colacium; Colacium sp. Songjanggol033107B	Euglenozoa	100	2,631368429	3,003371195	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Cercomonas; Cercomonas rapida	Cercozoa	100	-3,157896537	-3,189739417	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; unclassified Bacillariophyta; Peridiniopsis cf. kevei diatom endosymbiont	Bacillariophyta	98.361	-2,317810403	-2,390064496	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Stephanodiscus; Stephanodiscus hantzschii	Bacillariophyta	100	-2,330905886	-2,846558523	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; unclassified Paraphysomonas; Paraphysomonas sp. HD	Chrysophyta	100	NA	NA	NA	-2,542054992
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Coscinodiscophycidae; Aulacoseiraceae; Aulacoseira; Aulacoseira nyassensis	Bacillariophyta	100	-1,062175299	-0,996856005	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; Syndiniales; environmental samples; uncultured Amoebophrya	Dinophyta	97.5	4,514079064	4,146624071	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	95.161	2,381914628	2,448432187	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Stephanodiscus; Stephanodiscus hantzschii	Bacillariophyta	98.425	-22,60800672	-21,4647745	NA	11,69735073
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Eubodonida; Bodonidae; unclassified Bodonidae; Bodonidae sp. river Thames	Euglenozoa	100	3,049694595	3,581084889	NA	NA

cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	98.413	1,026192964	NA	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Paracercomonas; unclassified Paracercomonas; Paracercomonas sp.	Cercozoa	100	NA	1,661929864	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Litostomatea; Haptoria; Pleurostomatida; Amphileptidae; Hemiophrys; Hemiophrys macrostoma	Ciliophora	93.269	1,633051799	1,672209465	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia microcephala	Bacillariophyta	100	-2,149151096	NA	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	96.825	2,696657132	2,510167465	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Bacillariales; Bacillariaceae; Nitzschia; Nitzschia fonticola	Bacillariophyta	100	-2,823394476	-2,14467789	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Dunaliellaceae; Hafniomonas; Hafniomonas laevis	Chlorophyta	96.581	3,157007071	2,939358343	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Micractinium; unclassified Micractinium; Micractinium sp. MM0001	Chlorophyta	98.438	-2,236063605	-2,431795474	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Naviculales; Naviculaceae; Fistulifera; Fistulifera saprophila	Bacillariophyta	100	-3,184516964	-2,901144671	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Tubulinea; environmental samples; uncultured Lobosea	Amoebozoa	100	-1,823814881	-1,571755548	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Dinophyceae; environmental samples	Dinophyta	100	-1,443477036	-1,731955326	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Rhynchobodo; unclassified Rhynchobodo; Rhynchobodo sp. Hamilton Lake	Euglenozoa	97.321	1,937349583	2,873674592	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	96.825	2,098636394	1,682345454	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	96.063	4,075818885	4,118805123	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Pleuronematida; Cyclidiidae; Cyclidium; Cyclidium vorax	Ciliophora	100	5,202059145	5,147426173	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	92.857	2,500491438	2,321099101	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Glissomonadida; Viridiraptoridae; Bodomorpha; unclassified Bodomorpha; Bodomorpha sp. Panama105	Cercozoa	98.387	2,643187992	4,741211077	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Chlamydomonadaceae; Chlamydomonas; Chlamydomonas cribrum	Chlorophyta	99.2	1,730868892	1,712482696	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Meyerella; Meyerella planktonica	Chlorophyta	100	-1,420610321	-2,403369981	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Astomatida; Haptophryidae; Haptophrya; Haptophrya planariarum	Ciliophora	93.333	-2,312620515	-2,479148629	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Litostomatea; Haptoria; Pleurostomatida; Amphileptidae; Amphileptus; unclassified Amphileptus; Amphileptus sp. PHB08122401	Ciliophora	98.077	NA	NA	NA	-22,08140006
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Spumella; unclassified Spumella; Spumella sp. TGS6	Chrysophyta	96.063	4,033843265	4,520868942	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Stephanodiscus; Stephanodiscus hantzschii	Bacillariophyta	98.425	-2,569836041	-4,070991743	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Philasterida; Uronematidae; Apouronema; Apouronema harbinensis	Ciliophora	100	NA	2,698875832	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Cercomonadida; Cercomonadidae; Paracercomonas; unclassified Paracercomonas; Paracercomonas sp. Panama101	Cercozoa	100	-1,562112617	NA	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; environmental samples; uncultured Cryptophyta	Cryptophyta	100	1,800522728	1,394564688	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Eudorina; Eudorina elegans	Chlorophyta	100	1,706867824	1,728727289	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Platyamoeba; unclassified Platyamoeba; Platyamoeba sp. strain CAZ7/I	Amoebozoa	96.721	2,875555567	2,85529898	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Volvocaceae; Pandorina; Pandorina morum	Chlorophyta	100	0,884841624	0,927591241	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	98.23	3,387303734	3,662637131	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; Thecofilosea; Cryomonadida; Rhogostomidae; Rhogostoma; unclassified Rhogostoma; Rhogostoma sp. CCAP 1966/4	Cercozoa	98.413	1,890228476	1,673148589	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Rotosphaerida; Nucleariidae; Nuclearia; Nuclearia simplex	rem.OTUs	96.154	4,823056201	5,484103279	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	94.69	NA	4,125398198	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Stephanodiscaceae; Discostella; unclassified Discostella; Discostella sp. HYK0210-A2	Bacillariophyta	100	-3,00855296	-2,514767245	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella tribonemae	Amoebozoa	91.538	2,269135908	2,807716832	NA	NA

cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Bacillariophyceae; Bacillariophycidae; Cymbellales; Gomphonemataceae; Gomphoneis; Gomphoneis okunoi	Bacillariophyta	95.2	-2,671688654	NA	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	96.85	-1,739577119	-1,838640335	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	94.444	2,451927917	2,301692575	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	97.345	NA	5,358643352	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Paraphysomonadaceae; Paraphysomonas; unclassified Paraphysomonas; Paraphysomonas sp. GflagA	Chrysophyta	96.85	3,554676503	3,788072379	NA	NA
cellular organisms; Eukaryota; CRuMs; Rigifilida; Micronuclearia; environmental samples; uncultured Micronuclearia	rem.OTUs	97.638	-1,321125255	-1,211159403	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Coscinodiscophyceae; Thalassiosirophycidae; Thalassiosirales; Skeletonemataceae; Skeletonema; environmental samples; uncultured Skeletonema	Bacillariophyta	96.85	-2,554783803	NA	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Chlamydomonadales; Haematococcaceae; Chlorogonium; Chlorogonium euchlorum	Chlorophyta	100	2,214980428	NA	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Bacillariophyta; Mediophyceae; Biddulphiophycidae; Biddulphiales; Biddulphiaceae; Biddulphia; Biddulphia tridens	Bacillariophyta	96.85	-0,557513017	NA	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo curvifilus	Euglenozoa	100	3,910712735	4,473687984	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. 805	Amoebozoa	92.188	2,440116939	2,364970942	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Peritrichia; Sessilida; Vorticellidae; Vorticella; unclassified Vorticella; Vorticella sp. 4 PS-2013	Ciliophora	100	-2,47954779	-2,381247665	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Synurophyceae; Synurales; Mallomonadaceae; Synura; unclassified Synura; Synura sp.	Chrysophyta	100	-2,016668806	-2,026214052	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Eubodonida; Bodonidae; Bodo; Bodo saltans	Euglenozoa	95.575	3,42430921	3,467025724	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Raphidophyceae; Chlorinimonas; Chlorinimonas sublosa	rem.OTUs	97.727	5,29907021	4,876299966	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. PS2	Amoebozoa	96.774	2,755437743	2,534781465	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; unclassified Ripella; Ripella sp. GP2	Amoebozoa	98.438	2,510845624	2,720654128	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	92.063	3,348870121	3,224174596	NA	NA
cellular organisms; Eukaryota; Discoba; Euglenozoa; Kinetoplastea; Metakinetoplastina; Neobodonida; Neobodo; Neobodo designis	Euglenozoa	98.23	1,927046637	2,354036851	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; environmental samples; uncultured stramenopile	rem.OTUs	92.913	5,700796297	5,896369833	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; Ciliophora; Intramacronucleata; Oligohymenophorea; Scuticociliatia; Philasterida; Uronematidae; Apouronema; Apouronema harbinensis	Ciliophora	98.198	24,15972105	24,78677596	18,71976083	19,80160396
cellular organisms; Eukaryota; Cryptophyceae; Cryptomonadales; Hemiselmidaceae; Hemiselmis; Hemiselmis virescens	Cryptophyta	92.593	NA	2,527241658	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Trebouxiophyceae; Chlorellales; Chlorellaceae; Chlorella clade; Micractinium; unclassified Micractinium; Micractinium sp. MM0001	Chlorophyta	100	-1,66428754	-2,036363647	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; environmental samples; uncultured Chrysophyceae	Chrysophyta	98.425	2,640091991	2,9593978	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; unclassified Fungi; fungal sp.	rem.OTUs	100	2,087293933	2,166136024	NA	NA
cellular organisms; Eukaryota; Cryptophyceae; Pyrenomonadales; Geminigeraceae; Teleaulax; Teleaulax amphioxeia	Cryptophyta	99.194	NA	-1,437328754	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Choanoflagellata; environmental samples; uncultured Choanoflagellida	Choanoflagellata	98.45	-2,046818447	-1,838319203	NA	NA
cellular organisms; Eukaryota; Viridiplantae; Chlorophyta; core chlorophytes; Chlorophyceae; Sphaeropleales; Scenedesmaceae; Desmodesmus; unclassified Desmodesmus; Desmodesmus sp. HH 10203	Chlorophyta	96.875	1,363683141	1,227233703	NA	NA
cellular organisms; Eukaryota; Sar; Alveolata; environmental samples; uncultured alveolate	rem.OTUs	90.4	6,313084627	NA	NA	NA
cellular organisms; Eukaryota; Amoebozoa; Discosea; Flabellinia; Vannellidae; Ripella; Ripella decalvata	Amoebozoa	95.2	3,012357708	3,088082615	NA	NA
cellular organisms; Eukaryota; Sar; Rhizaria; Cercozoa; environmental samples; uncultured freshwater cercozoan	Cercozoa	98.4	-2,788432389	-2,291518404	NA	NA
cellular organisms; Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Pezizomycotina; leotiomyceta; Eurotiomycetes; Chaetothyriomycetidae; Chaetothyriales; Herpotrichiellaceae; Exophiala; Exophiala lecanii-corni	Ascomycota	100	NA	2,909326296	NA	NA
cellular organisms; Eukaryota; Sar; Stramenopiles; Ochrophyta; Chrysophyceae; Chromulinales; Chromulinaceae; Spumella; unclassified Spumella; Spumella sp. TGS6	Chrysophyta	96.85	4,776042937	5,235298887	NA	NA

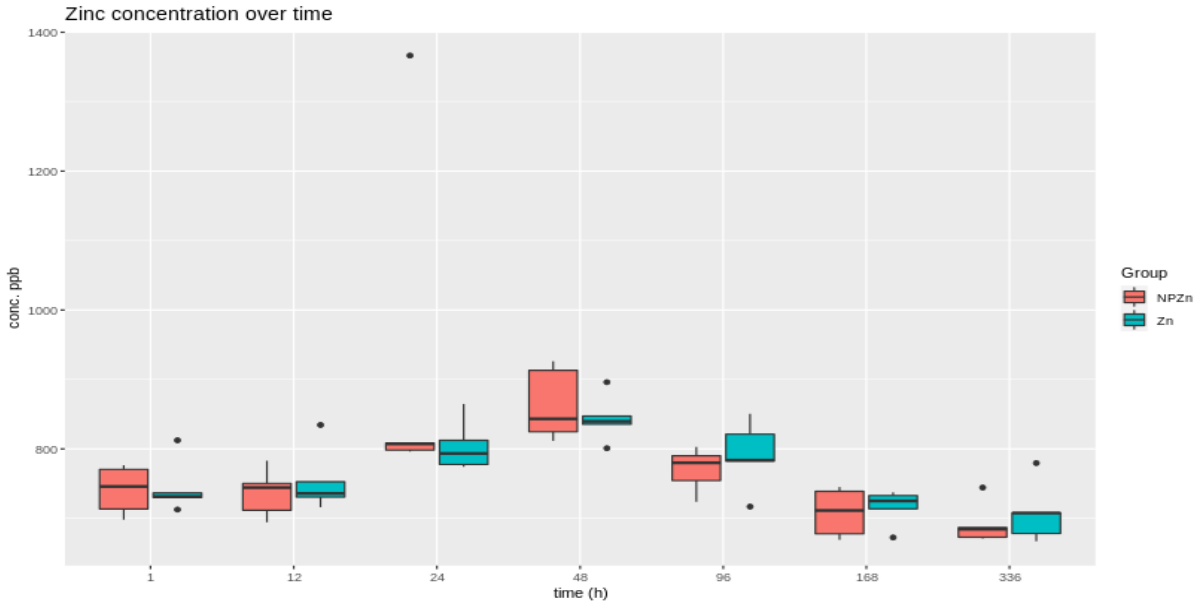

Figure S1: Zinc concentration in ppb (parts per billion) over time for the ZnNP and Zn treatment

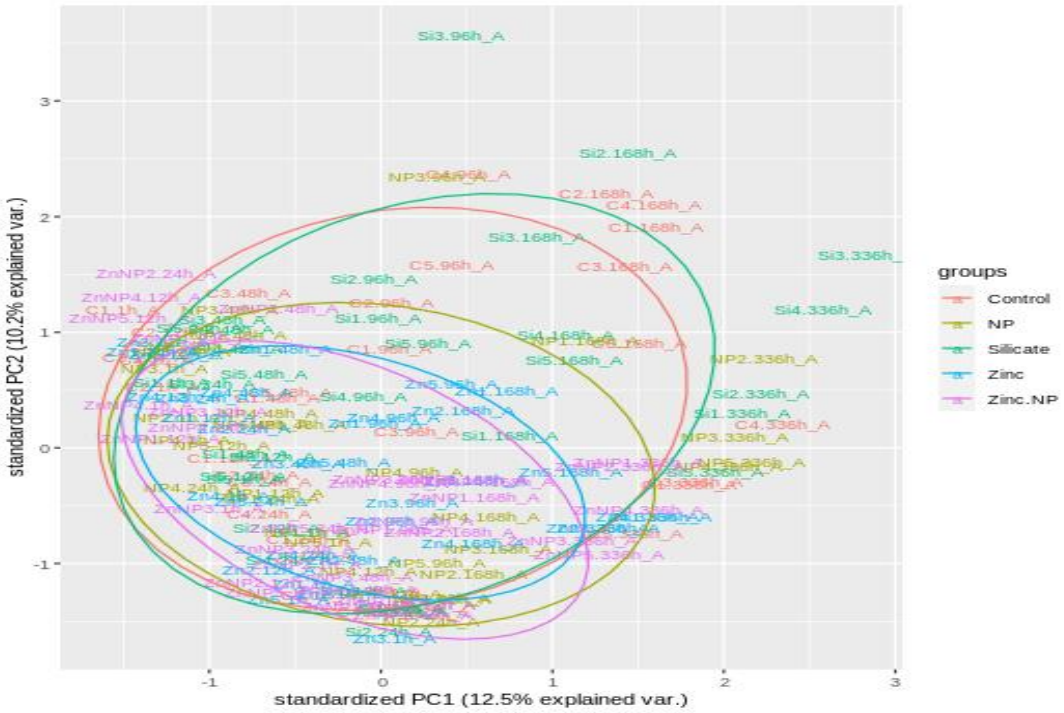

Figure S2: PCoA calculated with ASVs obtained from dada2

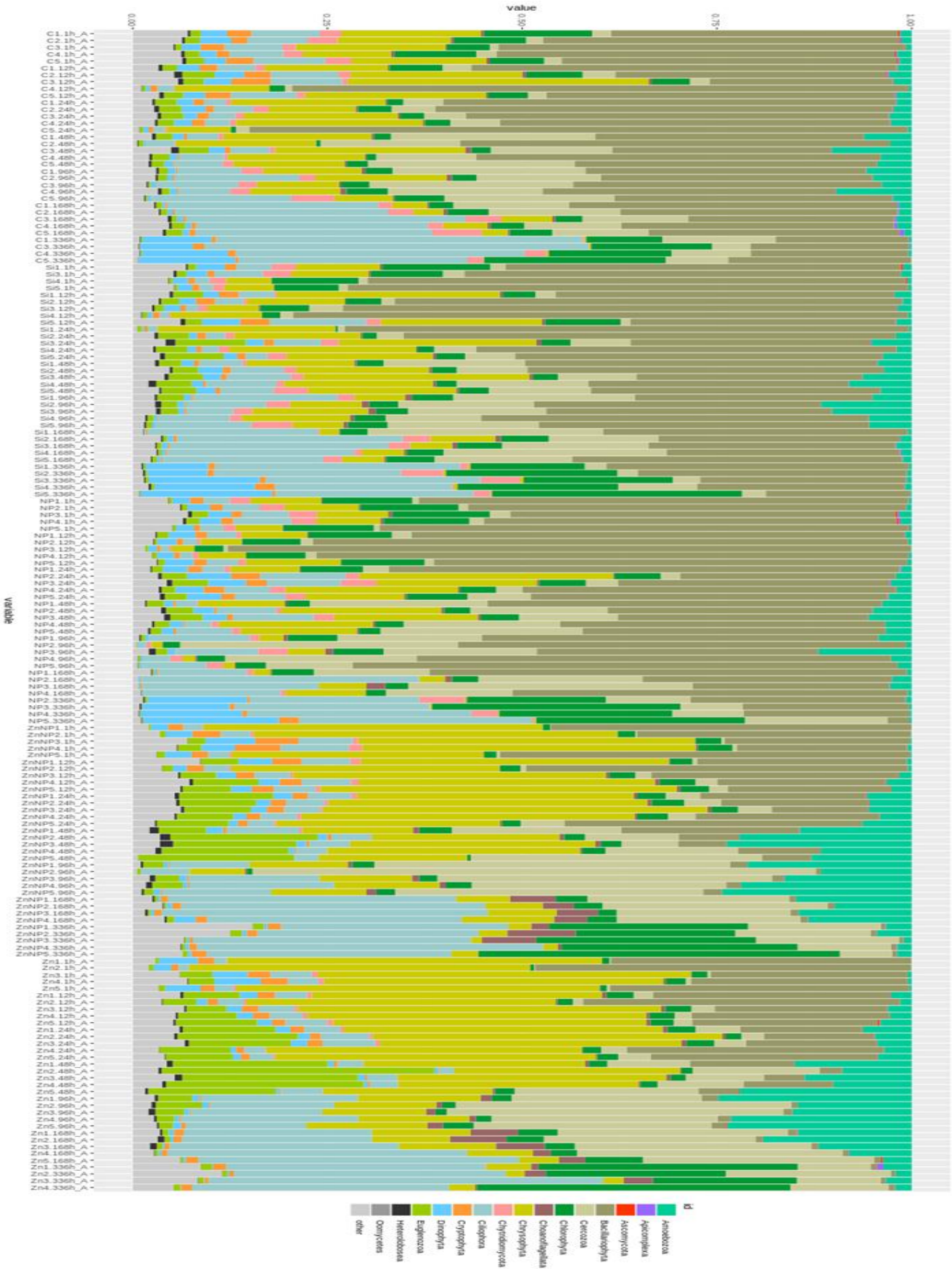

Figure S3: Showing relative communtiy compositions of treatments over time based of higher level groups

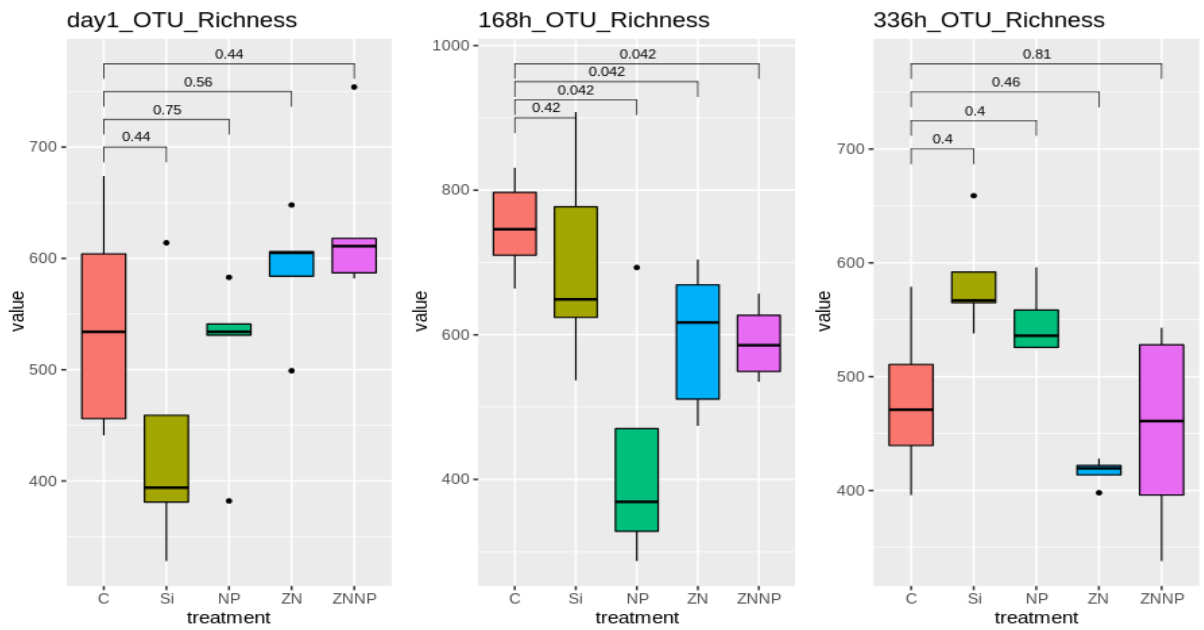

Figure S4: Boxplot depicting OTU richness. Braces show adjusted p-value. Treatments are color-coded. The first sampling day is merged (1h, 12h, 24h), thus portraying the first day.

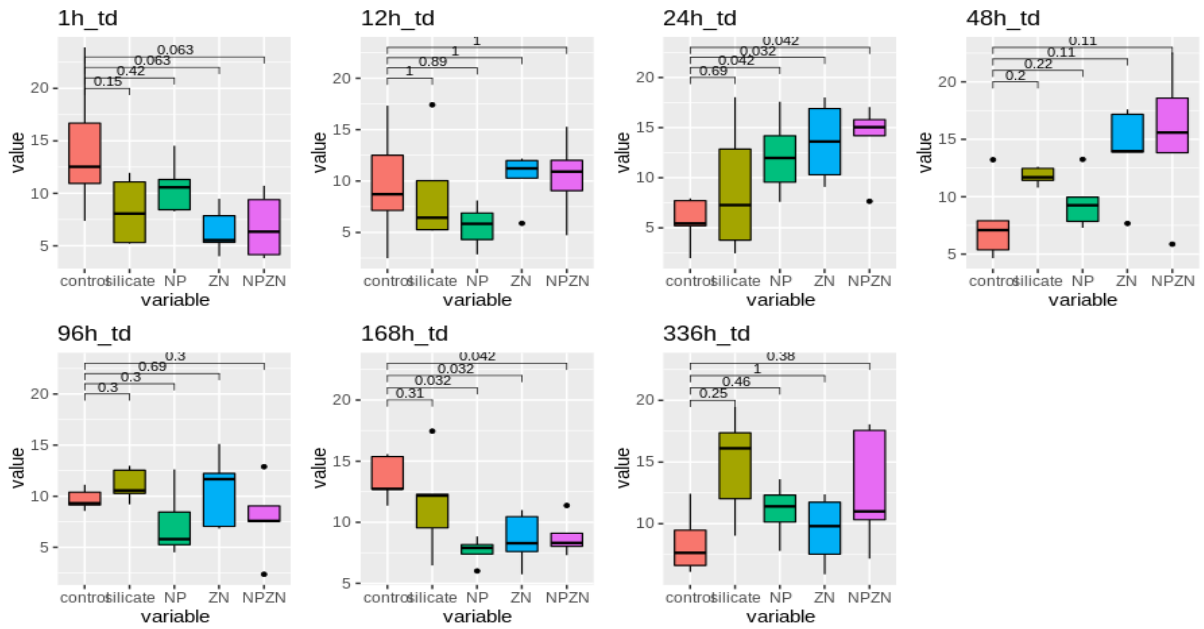

Figure S5: Boxplot of true diversities of the treatments. Braces show p values of the true diversity compared with the control. Treatments are color coded.

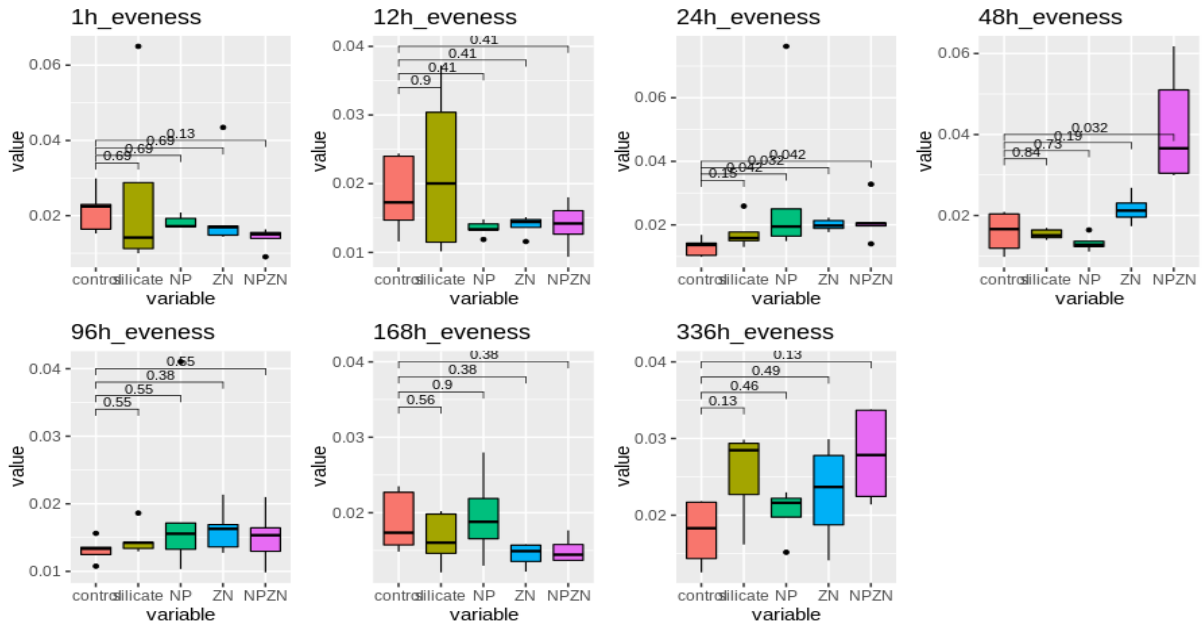

Figure S6: Evenness boxplots of the treatments. Braces show p-values of comparisons with the control. Treatments are color-coded.

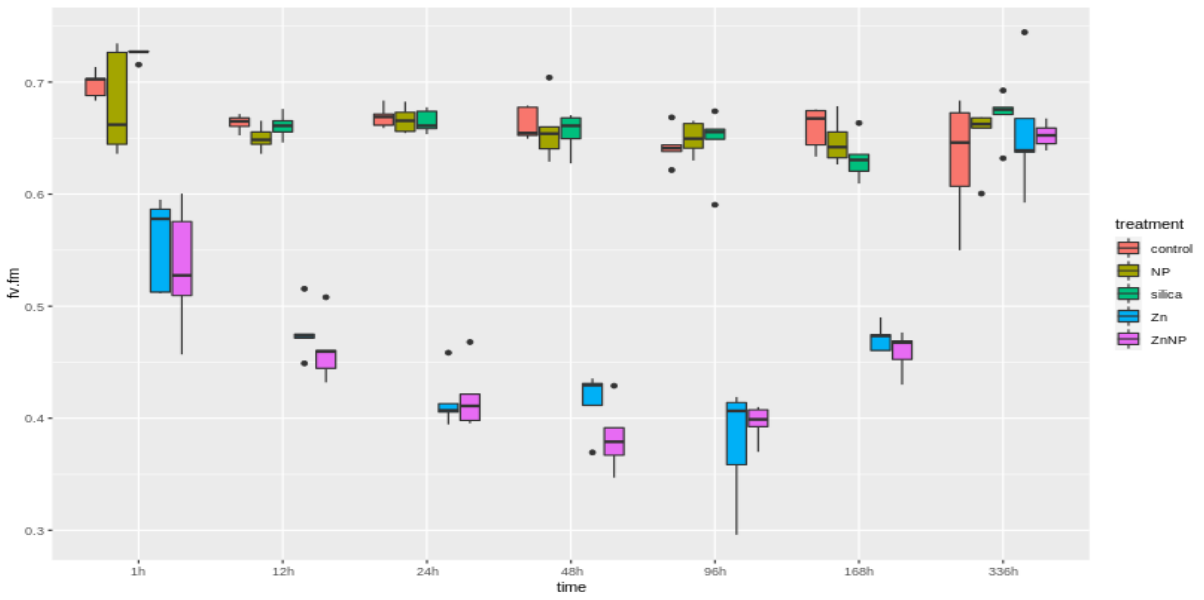

Figure S7: Fv/Fm values of the treatments over time.

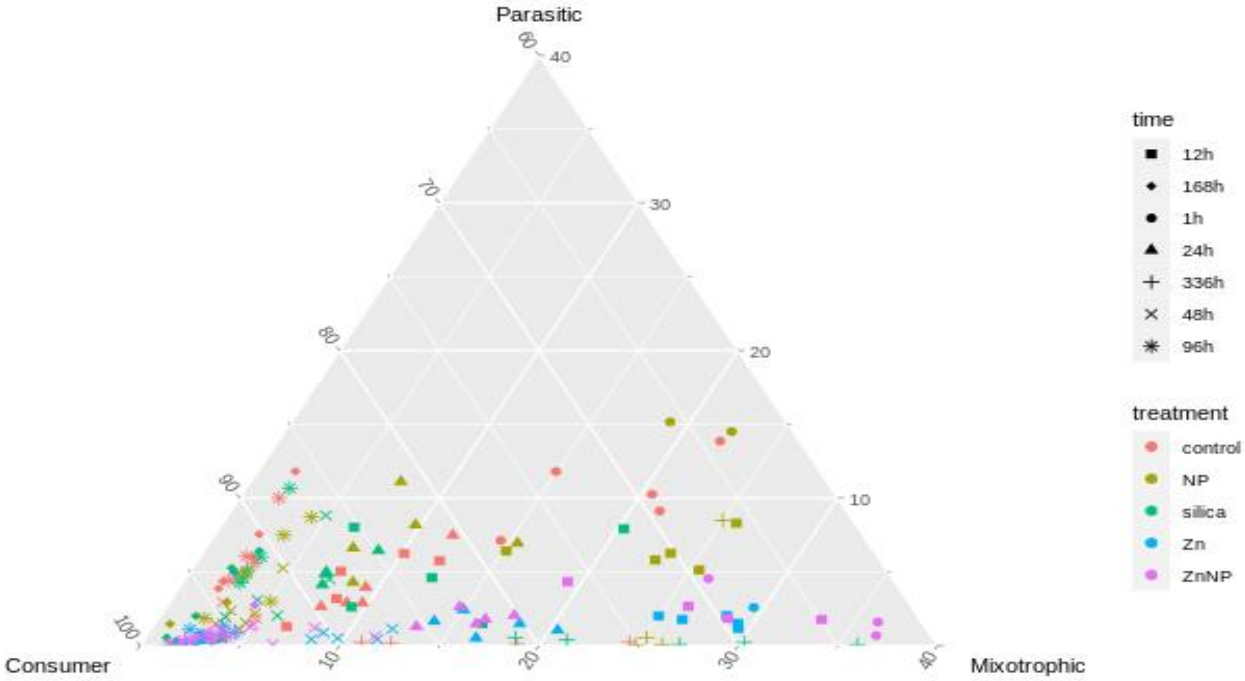

Figure S8: Ternary plot : Showing differences in consumer, parasitic and mixotrophic relative abundance. Based on rarefied data. For better visualization the three groups were considered as 100%

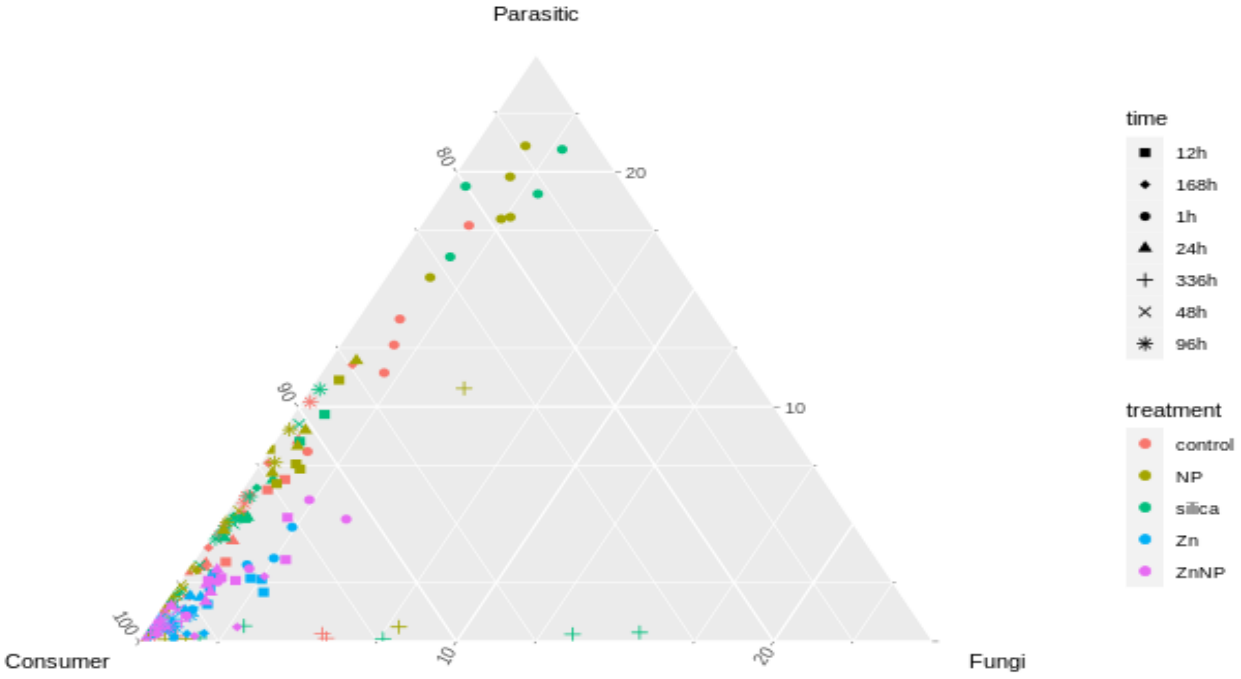

Figure S9: Showing differences in consumer, parasitic and fungal relative abundance. Based on rarefied data. For better visualization the three groups were considered as 100%.

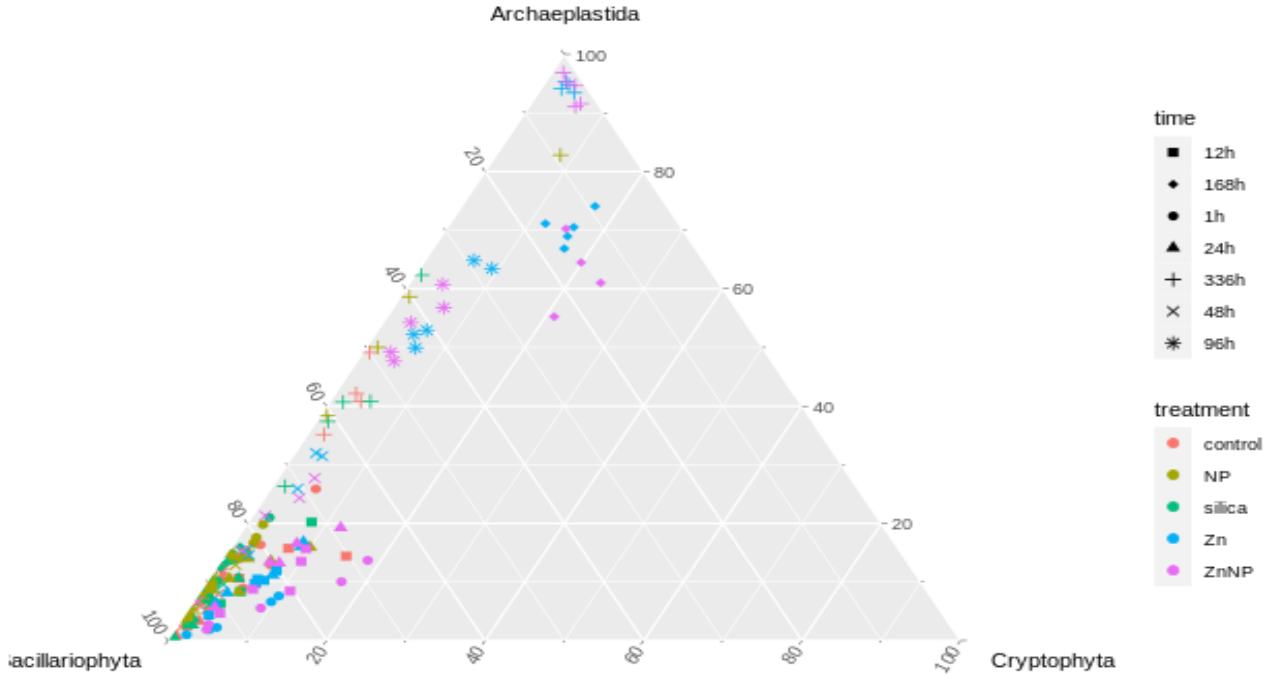

Figure S10: Showing differences in Archaeplastida, Bacillariophyta and Cryptophyta relative abundance. Based on rarefied data. For better visualization the three groups were considered as 100%

Article V supplement: „*Temporal disturbance of a model stream ecosystem by high microbial diversity from treated wastewater*“

The supplement of article V is part of the actual manuscript and therefore not repeated here.

Article VI supplement: „*Exploring the efficacy of metabarcoding and non-target screening for detecting treated wastewater*“

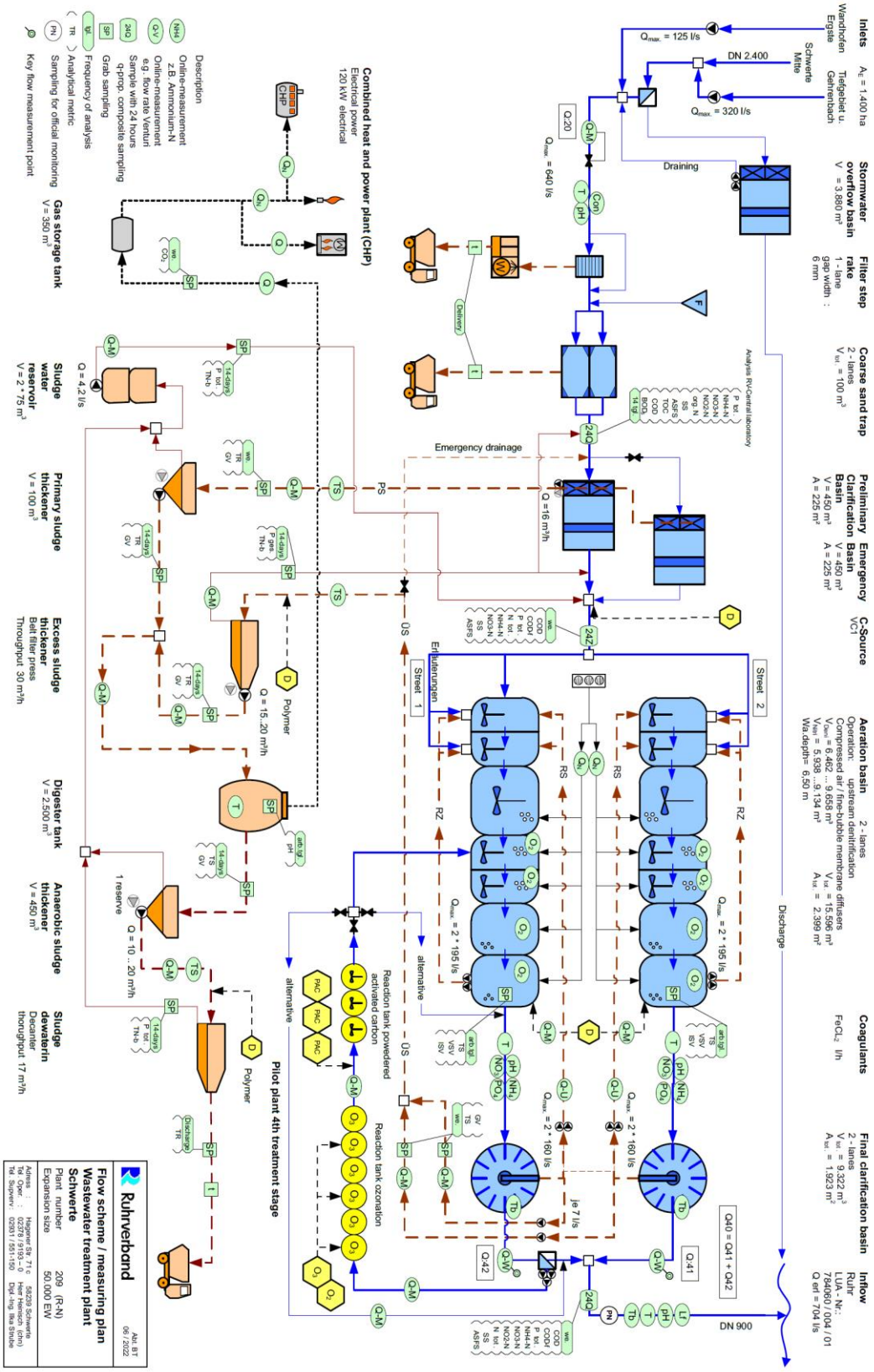

Figure S1: The flowchart was kindly provided by the Ruhrverband. It shows the entire process of the wastewater treatment plant and individual measuring stations can be identified.

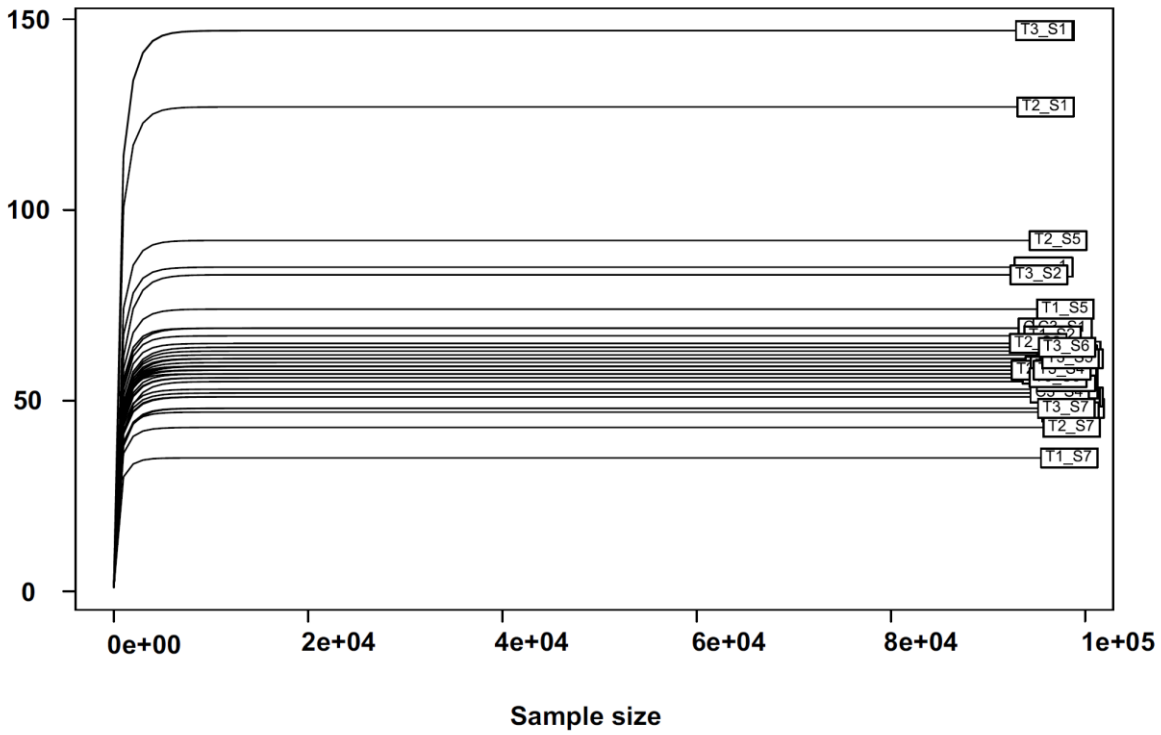

Figure S2 A: Rarefaction curves of full length 16S rRNA. Demonstrating that all samples have been adequately sequenced can be achieved by assessing the saturation of new operational taxonomic units (OTUs). Saturation occurs when additional sequencing efforts do not result in the detection of new OTUs. Therefore, if saturation is reached, it is an indication that the sequencing depth is sufficient to capture the diversity of the sample.

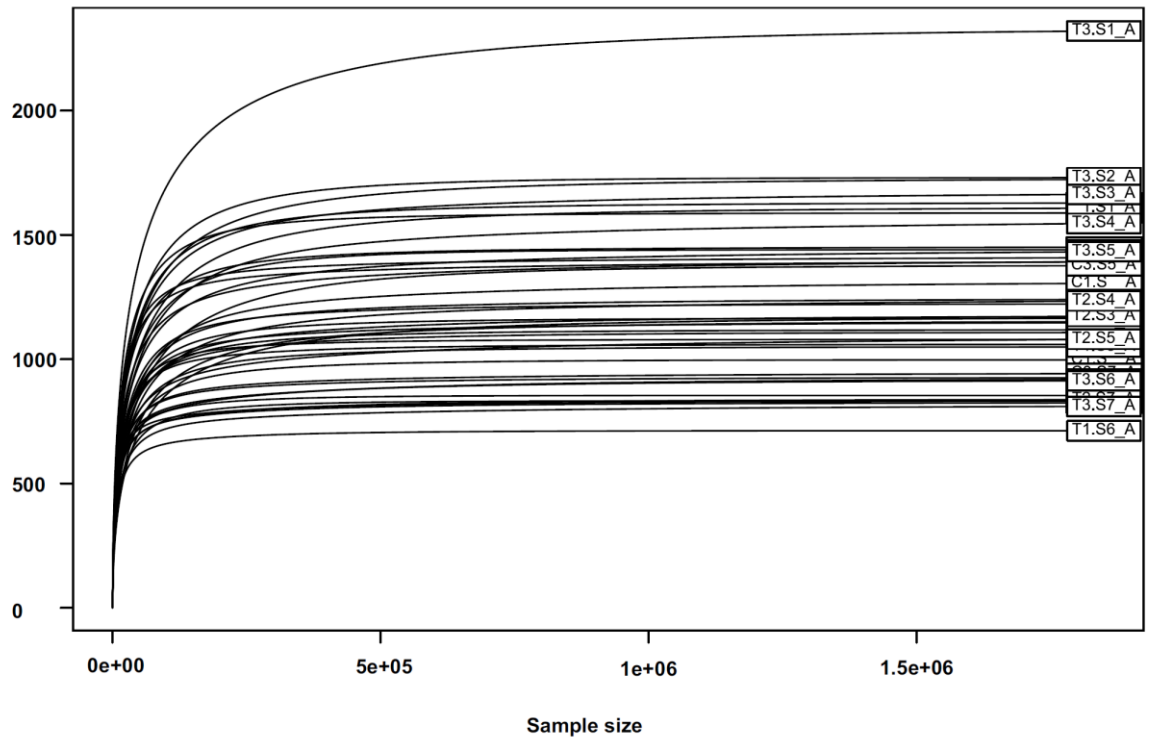

Figure S2 B: Rarefaction curves of 18S V9 rRNA. Demonstrating that all samples have been adequately sequenced can be achieved by assessing the saturation of new operational taxonomic units (OTUs). Saturation occurs when additional sequencing efforts do not result in the detection of new OTUs. Therefore, if saturation is reached, it is an indication that the sequencing depth is sufficient to capture the diversity of the sample.

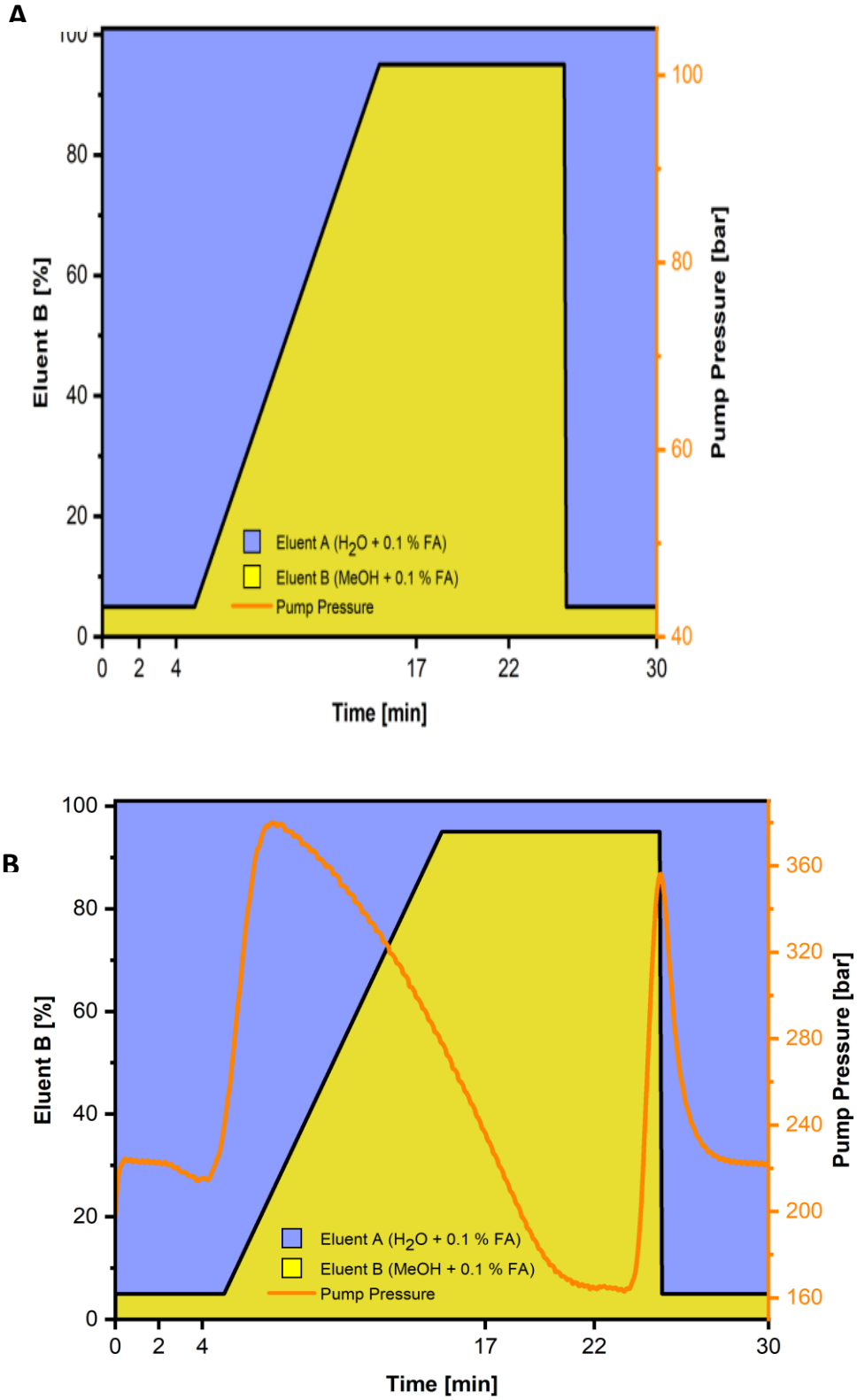

Figure S3: Water and methanol gradient, both spiked with 0.1 % formic acid (FA) over a run time of 30 min. In addition, information on the corresponding pump pressure is given. The flow rate was 0.3 mL/min.

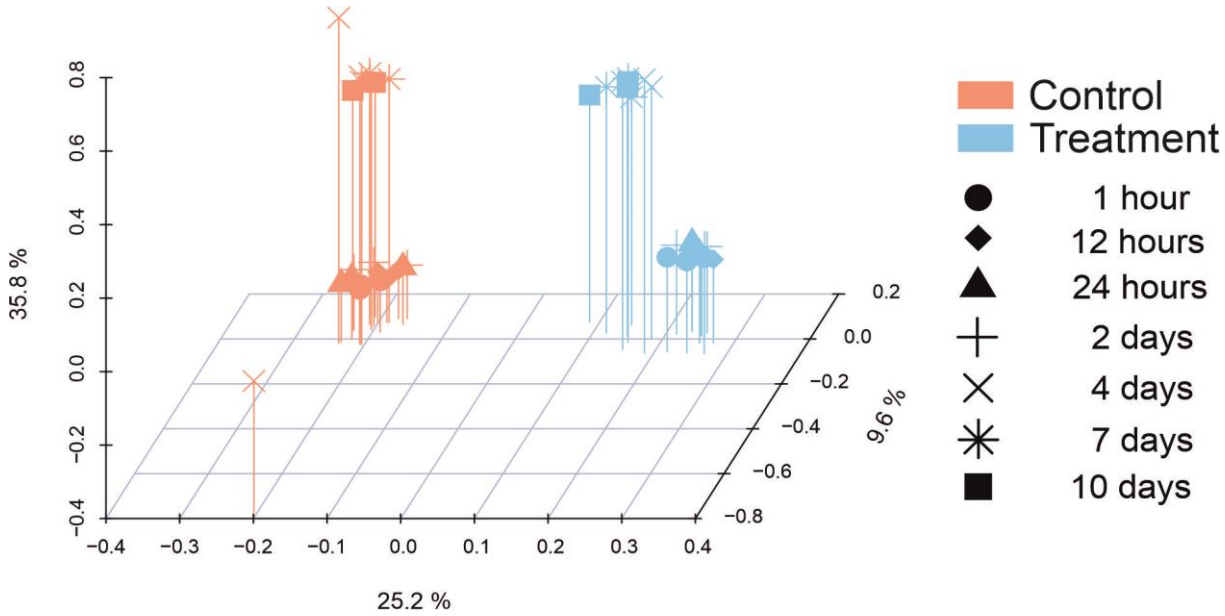

Figure S4: Showing that the control replicates from day 4 (red X) are very dissimilar. Therefore they are leading to a heavily distorted ordination. They were therefore removed to enhance data quality.

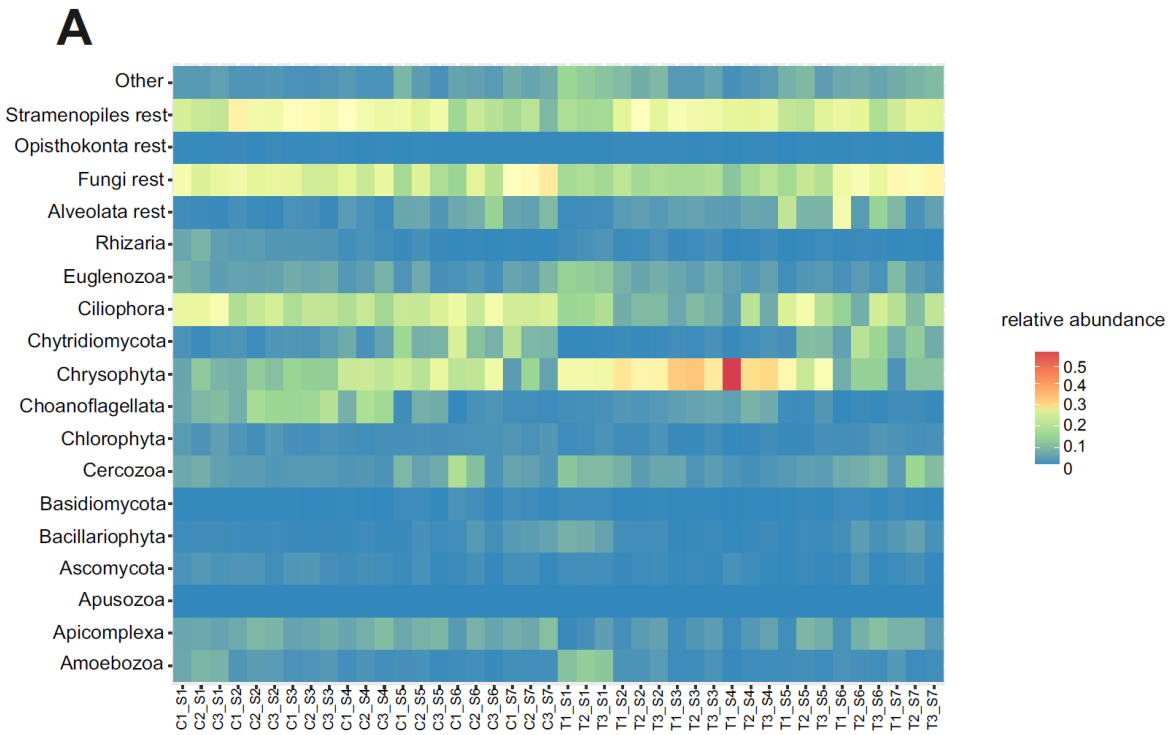

Figure S5: Relative taxonomic composition of all samples. A) Microeukaryotic relative taxonomic composition based on 18SV9 rRNA. B) Bacteria relative taxonomic composition based on full length 16S rRNA.

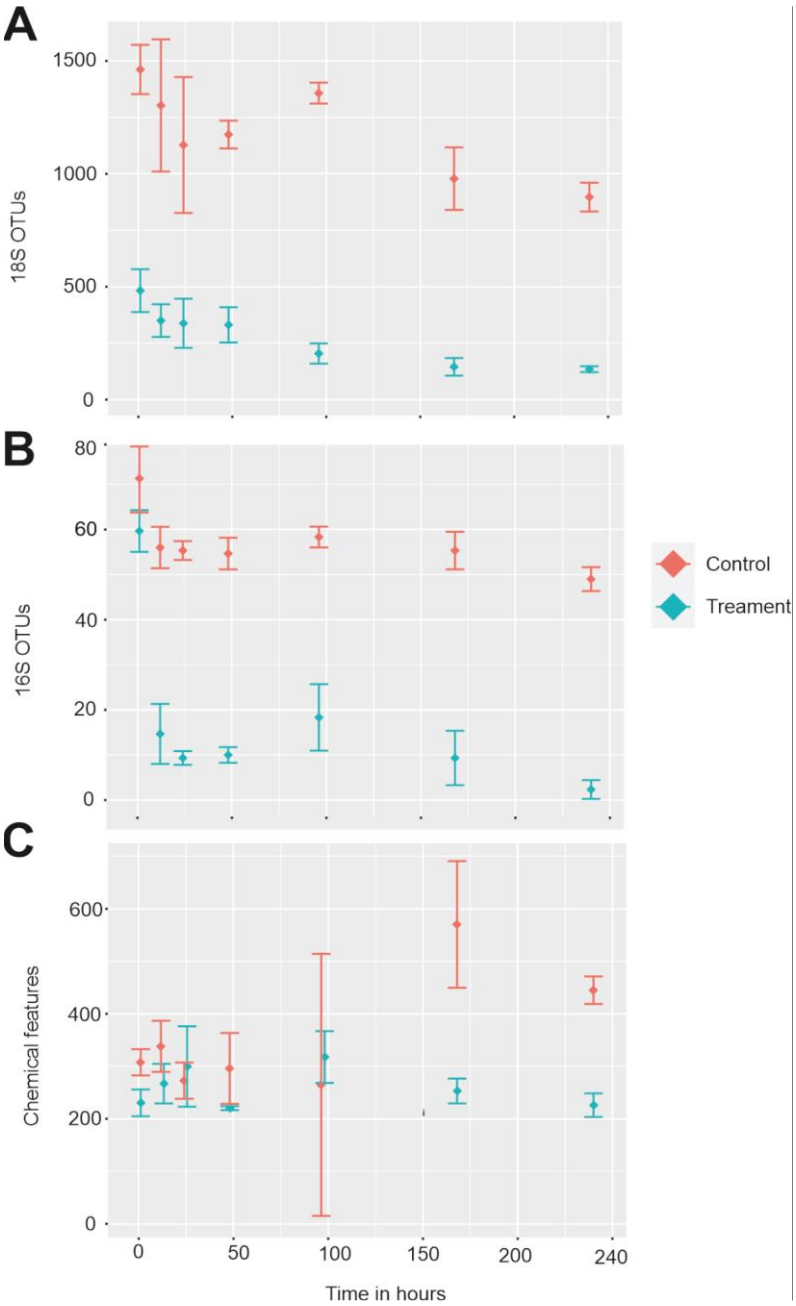

Figure S6: Depicting the fluctuations of the richness of operational taxonomic units (OTUs) or chemical features in the treatment and control samples over time. Blue indicate OTUs or features exclusively present in the treatment samples, which are introduced by treated wastewater, while red represent all remaining OTUs or features, which are either present only in the controls or shared between the control and treatment groups. A) shows a decrease over time in the richness of 18S V9 OTUs introduced by treated wastewater, as well as the remaining OTUs. B) displays a similar trend for 16S OTUs. C) demonstrates a decreasing trend over time in the richness of chemical features introduced by treated wastewater, whereas the chemical features originating from river water or common between the treatment and control groups show an increasing trend.

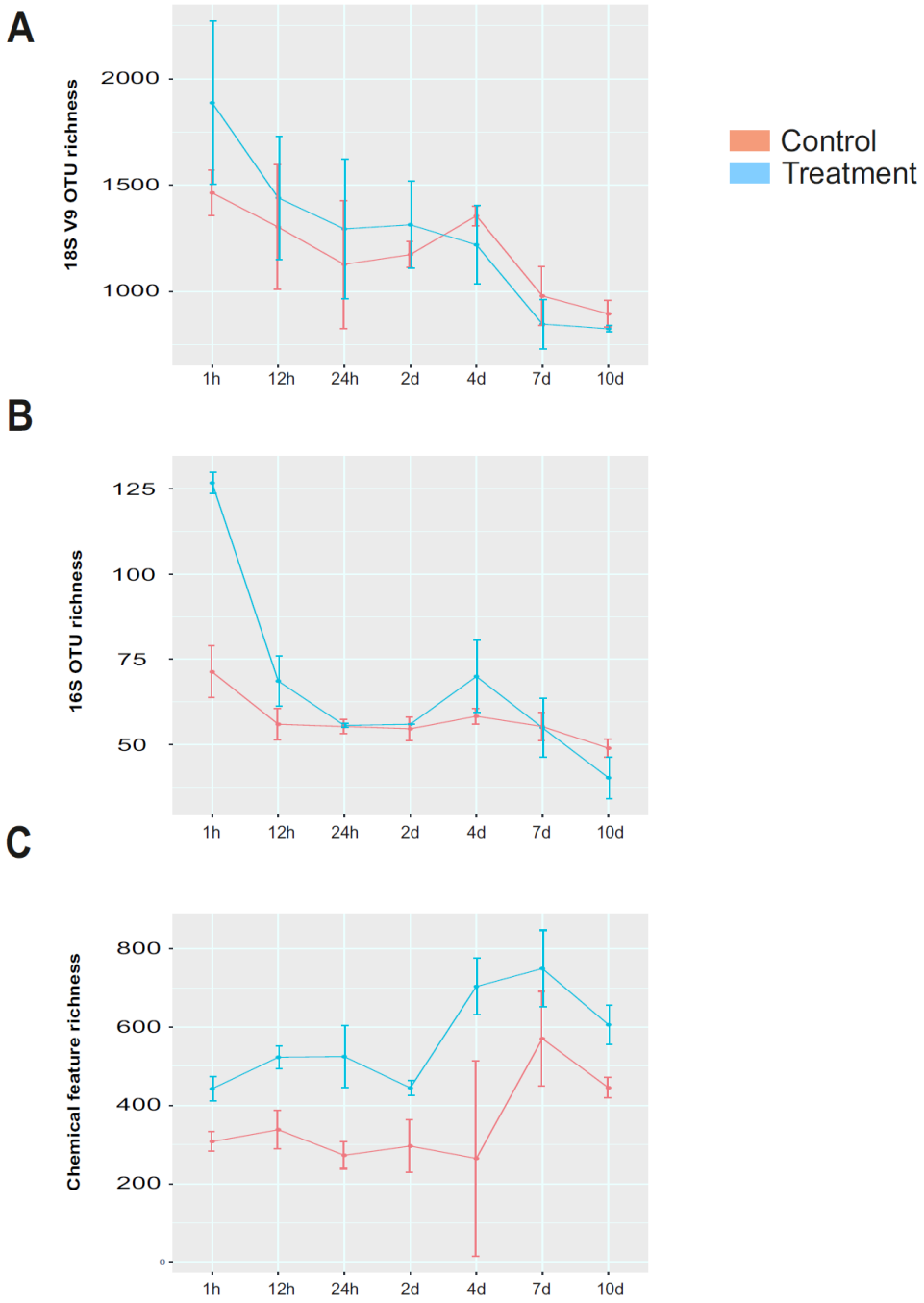

Figure S7: Richness of OTUs and chemical features (y-axis) and sampling points (x-axis). Blue represents treatment replicates and red represents control replicates. Sampling points: S1=1 h, S2 = 12 h, S3 = 24 h, S4 = 48 h, S5 = 4 days, S6 = 7 days, S7 = 10 days. A) 18S V9 OTU richness over time. Introduced OTUs due to treated wastewater were not significant, but a trend. B) 16S OTU richness over time. Introduced OTUs due to treated wastewater were significantly higher after 1 h (S1), and 12 h (S2). C) Chemical feature richness. Introduced features due to treated wastewater were significantly higher after 1 h (S1), 12 h (S2), 24 h (S3), 48 h (S4), 4 days (S5), and 10 days (S7).

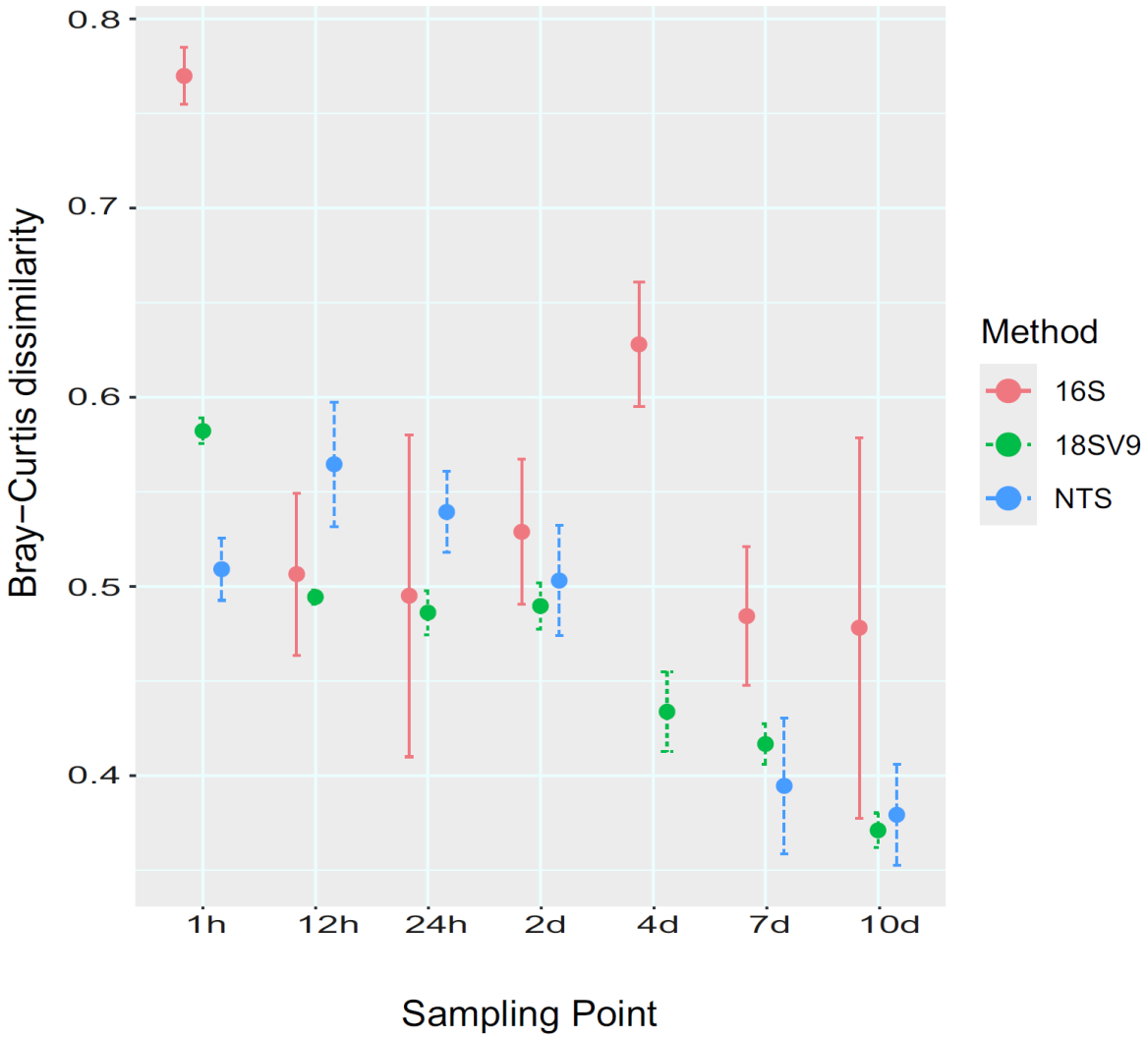

Figure S8: Showing the decreasing Bray-Curtis dissimilarity (thus increasing similarity) between control and treatment samples over time. Each treatment sample was compared to each control sample per method per day. This allows to gain insight into how fast and if the samples with TWW become more similar to the controls again.

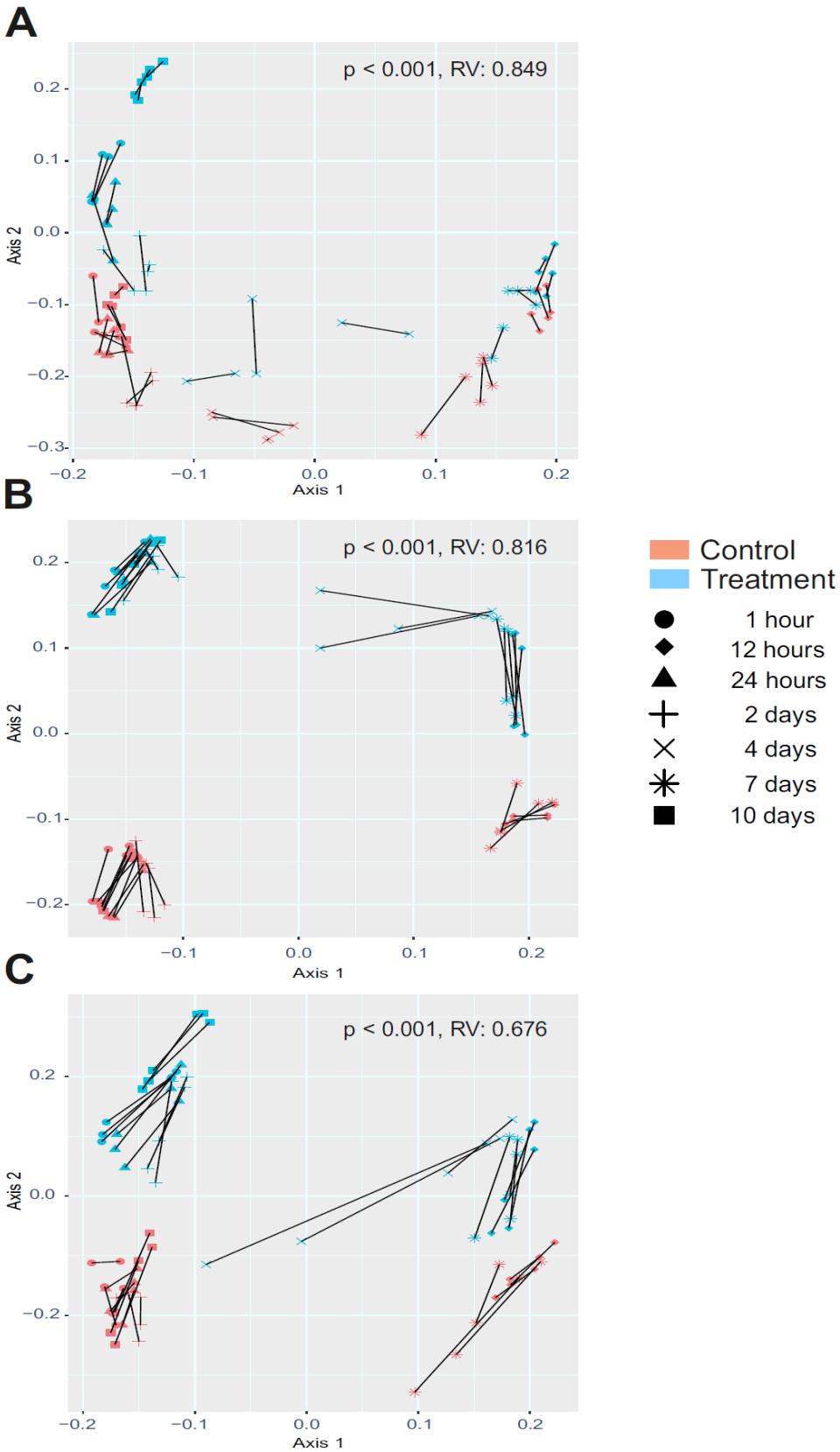

Figure S9: Coinertia analysis to reveal covariation (global similarity) of of each method pairing (18S V9 rRNA, 16S rRNA, NTS). Each arrow is connecting the same sample originating from the both datasets. The length of the arrows indicates thedifference between the two datasets. The colors indicates if the samples belong to control or treatments replicates, while the shape corresponds to the sampling point. A: Coinertia analyses of 18S V9 rRNA data and 16S rRNA

data, showing that the global similarity is significant ($p < 0.001$) and strongly correlated (RV: 0.849). B: Coinertia analyses of 18S V9 rRNA data and non-target screening data, showing that the global similarity is significant ($p < 0.001$) and strongly correlated (RV: 0.816) C: Coinertia analyses of non-target screening data and 16S rRNA data, showing that the global similarity is significant ($p < 0.001$) and strongly correlated (RV: 0.676).

Table S1: Basic parameters of the treated wastewater measured by the wastewater treatment plant. Individual measurements are missing as they are not measured daily or weekly.

	Date	Wastewater volumne	Turbidity	chemical oygen demand	NH4-N	NO3-N	NO2-N	N anorgic	total bound nitroger	P total	Temperature	pH	Conductivity
Units		m³	TE/F	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	°C	pH	µS/cm
Monday	2.1.2021	14.828	3.2	20						0.35	9.5	6.85	845
Tuesday	2.2.2021	21.746	3.9								9.9	6.78	893
Wednesda	2.3.2021	41.136	3.5	12	0.07	4.46	0.12	4.65	7.7	0.26	9.3	6.67	554
Thursday	2.4.2021	35.244	2.5								8.8	6.7	456
Friday	2.5.2021	18.183	2.7	18						0.21	9.5	6.77	700
Saturday	2.6.2021	16.720	3.8								10	6.8	814
Sunday	2.7.2021	18.866	3.4								9.3	6.82	879
Monday	2.8.2021	14.857	3.7								8.8	6.84	1020
Tuesday	2.9.2021	13.861	3.5	13	0.13	5.3	0.03	5.46	6.3	0.39	8.7	6.86	1075
Wednesda	2.10.2021	12.808	3.8								8.7	6.82	1175
Thursday	2.11.2021	13.014	3.2	23						0.4	8.9	6.8	1176
Friday	2.12.2021	11.901	3.3								8.8	6.79	1352
Saturday	2.13.2021	11.498	3.6								8.6	6.78	1251
Sunday	2.14.2021	11.540	3.4								8.6	6.79	1152
Monday	2.15.2021	20.989	3	20	0.2	6.39	0.04	6.63	8.6	0.29	8.6	6.79	1197
Tuesday	2.16.2021	35.527	3.7								7.2	6.68	1502
Wednesda	2.17.2021	31.449	3.2	12						0.14	7.1	6.75	897
Thursday	2.18.2021	21.654	2.6								8.4	6.82	948
Friday	2.19.2021	18.746	3.4	16						0.23	9.1	6.81	928
Saturday	2.20.2021	15.654	3.4								9.7	6.79	964
Sunday	2.21.2021	14.185	3.3								10	6.8	947
Monday	2.22.2021	13.527	3.1								10.1	6.79	942
Tuesday	2.23.2021	12.309	2.7	17						0.22	10.5	6.75	963
Wednesda	2.24.2021	11.580	2.6								10.8	6.77	970
Thursday	2.25.2021	11.758	2.6	20						0.31	10.9	6.74	976
Friday	2.26.2021	15.492	3.7								11	6.73	924
Saturday	2.27.2021	11.554	2.7	20	0.2	5.29	0.06	5.55	6.4	0.32	10.8	6.75	890
Sunday	2.28.2021	11.509	2.8								10.9	6.76	906

Table S2: Heavy metal concentration within distinct samples. Samples were measured as described in the methods part of the manuscript.

ID	V µg/l	Mn µg/l	Fe µg/l	Co µg/l	Ni µg/l	Cu µg/l	Zn µg/l	Mo µg/l	Ag µg/l	Cd µg/l	Sn µg/l	Sb µg/l	Pb µg/l
HNO3a	0,52	1,83	23,5	0,48	5,12	3,76	73,3	1,35	0,41	0,24	0,43	1,52	1,03
C1_S1	0,21	0,87	32,8	0,08	2,08	0,47	21,6	0,07	0,01	0,01	0,01	0,04	0,30
C1_S2	0,24	1,10	41,2	0,10	2,83	0,64	27,4	0,09	0,00	0,02	0,08	0,05	0,34
C1_S3	0,27	1,18	58,8	0,12	2,83	1,18	32,3	0,15	0,00	0,01	0,03	0,08	0,34
C1_S4	0,34	1,01	79,5	0,12	3,42	1,80	33,0	0,22	0,00	0,02	0,02	0,13	0,31
C1_S5	0,26	0,95	57,0	0,10	2,49	1,16	30,8	0,14	0,00	0,01	0,12	0,09	0,41
C1_S6	0,30	0,81	66,7	0,11	2,57	1,59	26,1	0,20	0,00	0,02	0,03	0,11	0,25
C1_S7	0,30	0,65	60,3	0,09	2,04	1,34	17,5	0,19	0,00	0,02	0,02	0,11	0,19
C2_S1	0,21	0,80	36,3	0,08	1,55	0,71	17,9	0,07	0,00	0,01	0,01	0,04	0,27
C2_S2	0,20	0,83	32,5	0,10	1,97	0,50	32,2	0,06	0,00	0,01	0,02	0,03	0,40
C2_S3	0,20	0,69	30,3	0,08	1,53	0,40	19,4	0,06	0,00	0,01	0,02	0,03	0,27
C2_S4	0,20	0,50	31,8	0,07	1,30	0,63	14,7	0,10	0,01	0,01	0,02	0,09	0,20
C2_S5	0,19	0,54	30,3	0,07	1,19	0,52	18,5	0,09	0,00	0,01	0,02	0,07	0,24
C2_S6	0,21	0,62	37,1	0,08	1,62	0,69	26,2	0,10	0,00	0,01	0,05	0,07	0,29
C2_S7	0,22	0,71	47,9	0,08	1,69	1,07	21,2	0,14	0,00	0,01	0,02	0,09	0,20
C3_S1	0,18	0,61	34,9	0,07	1,33	0,88	17,9	0,11	0,00	0,01	0,02	0,04	0,26
C3_S2	0,18	0,91	30,1	0,08	1,60	0,41	19,7	0,06	0,00	0,01	0,01	0,04	0,29
C3_S3	0,17	0,61	28,2	0,07	1,28	0,33	27,5	0,06	0,00	0,01	0,01	0,03	0,38
C3_S4	0,17	0,63	31,9	0,08	1,57	0,56	33,5	0,06	0,00	0,01	0,04	0,04	0,31
C3_S5	0,20	0,68	38,6	0,10	2,20	0,77	32,3	0,09	0,00	0,01	0,02	0,06	0,40
C3_S6	0,17	0,56	31,1	0,06	1,23	0,45	22,0	0,07	0,00	0,01	0,01	0,03	0,29
C3_S7	0,18	0,58	35,2	0,08	1,55	0,59	26,7	0,12	0,01	0,01	0,02	0,10	0,31
T1_S1	0,17	0,99	36,1	0,07	1,32	0,57	23,2	0,10	0,01	0,01	0,02	0,06	0,29
T1_S2	0,18	0,74	36,8	0,07	1,35	0,54	20,8	0,11	0,00	0,01	0,02	0,05	0,28
T1_S3	0,16	0,72	31,0	0,09	1,80	0,39	28,4	0,07	0,00	0,01	0,02	0,04	0,41
T1_S4	0,16	0,68	31,5	0,08	1,60	0,38	25,5	0,07	0,00	0,01	0,02	0,04	0,36
T1_S5	0,17	0,60	33,5	0,08	1,70	0,50	32,5	0,08	0,00	0,01	0,02	0,04	0,40
T1_S6	0,18	0,66	33,8	0,08	1,74	0,54	28,6	0,10	0,00	0,01	0,01	0,04	0,37
T1_S7	0,16	0,63	28,6	0,07	1,26	0,38	20,5	0,07	0,00	0,01	0,01	0,03	0,32
T2_S1	0,19	0,99	35,4	0,12	2,67	0,72	47,8	0,09	0,00	0,02	0,02	0,04	0,56
T2_S2	0,16	1,02	35,8	0,10	2,45	0,58	46,8	0,09	0,00	0,01	0,02	0,04	0,49
T2_S3	0,17	0,77	38,1	0,09	1,93	0,68	33,1	0,15	0,01	0,01	0,03	0,11	0,41
T2_S4	0,19	0,65	51,9	0,10	2,32	1,00	33,8	0,21	0,01	0,01	0,02	0,11	0,33
T2_S5	0,15	0,86	33,8	0,12	1,75	0,56	26,0	0,12	0,01	0,01	0,02	0,06	0,32
T2_S6	0,18	0,67	46,9	0,10	1,91	0,91	27,0	0,16	0,00	0,01	0,02	0,08	0,35
T2_S7	0,16	0,59	38,3	0,10	1,87	0,64	27,3	0,12	0,00	0,01	0,26	0,06	0,31
T3_S1	0,17	0,80	39,5	0,09	1,67	0,74	24,8	0,07	0,01	0,01	0,02	0,05	0,39
T3_S2	0,15	0,75	38,7	0,09	1,84	0,68	24,8	0,12	0,00	0,01	0,02	0,05	0,37
T3_S3	0,14	0,70	29,4	0,10	1,24	0,38	25,8	0,08	0,00	0,01	0,20	0,04	0,27
T3_S4	0,15	0,67	33,7	0,08	1,52	0,50	25,0	0,08	0,00	0,01	0,02	0,04	0,31
T3_S5	0,14	0,81	31,6	0,11	1,39	0,51	21,5	0,08	0,00	0,01	0,02	0,04	0,40
T3_S6	0,16	0,68	33,4	0,08	1,32	0,52	20,1	0,13	0,01	0,01	0,02	0,11	0,28
T3_S7	0,16	0,49	36,6	0,07	1,18	0,59	22,3	0,12	0,01	0,01	0,02	0,08	0,23

Table S3: Atmospheric pressure electrospray Ionization parameters in positive polarity mode.

Atmospheric Pressure Electrospray Ionization Parameters	
Polarity	Positiv
Transfer capillary temperature [°C]	320
Spray voltage [kV]	3,5
Sheath gas flow rate [a. u.]	37
Aux gas flow rate [a. u.]	15
Aux gas heater temperature [°C]	50
Sweep gas flow rate [a. u.]	1
S-lens RF level	50

Table S4: msConvert software settings applied to convert raw chromatographic data from profile mode into mzXML file format.

	msConvert Options
Binary encoding precision	64-bit
Write index	√
Use zlib compression	√
TPP compatibility	√
	Centroidization
Filter	Peak Picking
Algorithm	Vendor
MS Levels	1 - 2
Min SNR	0,1
Min peak spacing	0,1

Table S5: Feature extraction and alignment parameters applied following BfG`s NTS workflow as well as information on system blank correction.

Peak Finding				
Min. Intensity	**S/N**	**Noise (scans)**	**Max peaks per peak**	**Peak width [s]**
100	3	30	10	_x005F_x001E_14
Alignment				
m/z Tolerance [ppm]	**RT Tolerance[s]**	**Replicate-Filter**	**Blank Faktor**	
10	30	3/3	10	

Table S6: List of (isotope labeled) standards including information on molecular formular and exact mass. Each compound was added in a concentration of 1 µg/L.

Compound	Molecular Formular	Exact Mass
Isotope labeled compounds		
Carbamazepine-D8	$C_{15}D_8H_4N_2O$	244,1445
Sulfamethoxazole-D4	$C_{10}D_4H_7N_3O_3S$	257,0765
Metoprolol-D7	$C_{30}D_{14}H_{36}N_2O_6$	274,2272
Diclofenac-$^{13}C_6$	$^{13}C_6C_8H_{10}Cl_2NNaO_2$	323,0180
Spiked compounds		
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	295,0166
Carbamazepine	$C_{15}H_{12}N_2O$	236,0949
1H-Benzotriazole	$C_6H_5N_3$	119,0483
Diethyltoluamide	$C_{12}H_{17}NO$	191,131
Metformin	$C_4H_{11}N_5$	129,1014
Gabapentin	$C_9H_{17}NO_2$	171,1259
Metoprolol	$C_{15}H_{25}NO_3$	267,1834
Acesulfame	$C_4H_4KNO_4S$	200,9498
Hydrochlorothiazide	$C_7H_8ClN_3O_4S_2$	296,9644
Iopromide	$C_{18}H_{24}I_3N_3O_8$	790,8697
Valsartan	$C_{24}H_{29}N_5O_3$	435,227

Table S7: Number of unique OTUs and chemical features introduced by treated wastewater, per sampling point. Control replicates were combined, and each treatment was compared to the combined controls. Features and OTUs present in the treatment but absent in the combined controls were attributed to treated wastewater origin.

	18S V9 OTUs	16S OTUs	Chemical features
Sampling 1	483.7±96.5	59.7±4.6	230.3±25.5
Sampling 2	350.7±71.5	14.7±6.7	267±37.7
Sampling 3	337.3±111	9.3±1.5	299.7±76.6
Sampling 4	332±81.7	10±1.7	220.3±3.8
Sampling 5	203.3±42.3	18.3±7.4	317.7±49.3
Sampling 6	144.7±38.4	9.3±6	253±23.6
Sampling 7	135.3±13	2.3±2.1	226±22.5

Supplemental equation 1

$$\frac{\sum true\ positive\ features}{15} = true\ positive\ rate$$

Supplemental equation 2

$$\frac{\sum true\ positive\ features}{15} = true\ positive\ rate$$

Article VII supplement: „*The Premise of Interdisciplinarity and Its Actual Absence — A Bibliometric Analysis of Publications on Heavy Rainfall*“

Table S1

Dictionary of the existing Scopus hierarchy of individual subject areas, associated classifications abbreviations and codes.

subject cluster,subject area,subject code,subject classification,abbreviation

Health Sciences,Multidisciplinary,1000,Multidisciplinary,MULT

Life Sciences,Agricultural and Biological Sciences,1100,Agricultural and Biological Sciences (all),AGRI

Life Sciences,Agricultural and Biological Sciences,1101,Agricultural and Biological Sciences (miscellaneous),AGRI

Life Sciences,Agricultural and Biological Sciences,1102,Agronomy and Crop Science,AGRI

Life Sciences,Agricultural and Biological Sciences,1103,Animal Science and Zoology,AGRI

Life Sciences,Agricultural and Biological Sciences,1104,Aquatic Science,AGRI

Life Sciences,Agricultural and Biological Sciences,1105,"Ecology, Evolution, Behavior and Systematics",AGRI

Life Sciences,Agricultural and Biological Sciences,1106,Food Science,AGRI

Life Sciences,Agricultural and Biological Sciences,1107,Forestry,AGRI

Life Sciences,Agricultural and Biological Sciences,1108,Horticulture,AGRI

Life Sciences,Agricultural and Biological Sciences,1109,Insect Science,AGRI

Life Sciences,Agricultural and Biological Sciences,1110,Plant Science,AGRI

Life Sciences,Agricultural and Biological Sciences,1111,Soil Science,AGRI

Social Sciences & Humanities,Arts and Humanities,1200,Arts and Humanities (all),ARTS

Social Sciences & Humanities,Arts and Humanities,1201,Arts and Humanities (miscellaneous),ARTS

Social Sciences & Humanities,Arts and Humanities,1202,History,ARTS

Social Sciences & Humanities,Arts and Humanities,1203,Language and Linguistics,ARTS

Social Sciences & Humanities,Arts and Humanities,1204,Archeology (arts and humanities),ARTS

Social Sciences & Humanities,Arts and Humanities,1205,Classics,ARTS

Social Sciences & Humanities,Arts and Humanities,1206,Conservation,ARTS

Social Sciences & Humanities,Arts and Humanities,1207,History and Philosophy of Science,ARTS

Social Sciences & Humanities,Arts and Humanities,1208,Literature and Literary Theory,ARTS

Social Sciences & Humanities,Arts and Humanities,1209,Museology,ARTS

Social Sciences & Humanities,Arts and Humanities,1210,Music,ARTS

Social Sciences & Humanities,Arts and Humanities,1211,Philosophy,ARTS

Social Sciences & Humanities,Arts and Humanities,1212,Religious Studies,ARTS

Social Sciences & Humanities,Arts and Humanities,1213,Visual Arts and Performing Arts,ARTS

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1300,"Biochemistry, Genetics and Molecular Biology (all)",BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1301,"Biochemistry, Genetics and Molecular Biology (miscellaneous)",BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1302,Aging,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1303,Biochemistry,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1304,Biophysics,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1305,Biotechnology,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1306,Cancer Research,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1307,Cell Biology,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1308,Clinical Biochemistry,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1309,Developmental Biology,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1310,Endocrinology,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1311,Genetics,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1312,Molecular Biology,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1313,Molecular Medicine,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1314,Physiology,BIOC

Life Sciences,"Biochemistry, Genetics and Molecular Biology",1315,Structural Biology,BIOC

Social Sciences & Humanities,"Business, Management and Accounting",1400,"Business, Management and Accounting (all)",BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1401,"Business, Management and Accounting (miscellaneous)",BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1402,Accounting,BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1403,Business and International Management,BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1404,Management Information Systems,BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1405,Management of Technology and Innovation,BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1406,Marketing,BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1407,Organizational Behavior and Human Resource Management,BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1408,Strategy and Management,BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1409,"Tourism, Leisure and Hospitality Management",BUSI

Social Sciences & Humanities,"Business, Management and Accounting",1410,Industrial Relations,BUSI

Physical Sciences,Chemical Engineering,1500,Chemical Engineering (all),CENG

Physical Sciences,Chemical Engineering,1501,Chemical Engineering (miscellaneous),CENG

Physical Sciences,Chemical Engineering,1502,Bioengineering,CENG

Physical Sciences,Chemical Engineering,1503,Catalysis,CENG

Physical Sciences,Chemical Engineering,1504,Chemical Health and Safety,CENG

Physical Sciences,Chemical Engineering,1505,Colloid and Surface Chemistry,CENG

Physical Sciences,Chemical Engineering,1506,Filtration and Separation,CENG

Physical Sciences,Chemical Engineering,1507,Fluid Flow and Transfer Processes,CENG

Physical Sciences,Chemical Engineering,1508,Process Chemistry and Technology,CENG

Physical Sciences,Chemistry,1600,Chemistry (all),CHEM

Physical Sciences,Chemistry,1601,Chemistry (miscellaneous),CHEM

Physical Sciences,Chemistry,1602,Analytical Chemistry,CHEM

Physical Sciences,Chemistry,1603,Electrochemistry,CHEM

Physical Sciences,Chemistry,1604,Inorganic Chemistry,CHEM

Physical Sciences,Chemistry,1605,Organic Chemistry,CHEM

Physical Sciences,Chemistry,1606,Physical and Theoretical Chemistry,CHEM

Physical Sciences,Chemistry,1607,Spectroscopy,CHEM

Physical Sciences,Computer Science,1700,Computer Science (all),COMP

Physical Sciences,Computer Science,1701,Computer Science (miscellaneous),COMP

Physical Sciences,Computer Science,1702,Artificial Intelligence,COMP

Physical Sciences,Computer Science,1703,Computational Theory and Mathematics,COMP

Physical Sciences,Computer Science,1704,Computer Graphics and Computer-Aided Design,COMP

Physical Sciences,Computer Science,1705,Computer Networks and Communications,COMP

Physical Sciences,Computer Science,1706,Computer Science Applications,COMP

Physical Sciences,Computer Science,1707,Computer Vision and Pattern Recognition,COMP

Physical Sciences,Computer Science,1708,Hardware and Architecture,COMP

Physical Sciences,Computer Science,1709,Human-Computer Interaction,COMP

Physical Sciences,Computer Science,1710,Information Systems,COMP

Physical Sciences,Computer Science,1711,Signal Processing,COMP

Physical Sciences,Computer Science,1712,Software,COMP

Social Sciences & Humanities,Decision Sciences,1800,Decision Sciences (all),DECI

Social Sciences & Humanities,Decision Sciences,1801,Decision Sciences (miscellaneous),DECI

Social Sciences & Humanities,Decision Sciences,1802,Information Systems and Management,DECI

Social Sciences & Humanities,Decision Sciences,1803,Management Science and Operations Research,DECI

Social Sciences & Humanities,Decision Sciences,1804,"Statistics, Probability and Uncertainty",DECI

Physical Sciences,Earth and Planetary Sciences,1900,Earth and Planetary Sciences (all),EART

Physical Sciences,Earth and Planetary Sciences,1901,Earth and Planetary Sciences (miscellaneous),EART

Physical Sciences,Earth and Planetary Sciences,1902,Atmospheric Science,EART

Physical Sciences,Earth and Planetary Sciences,1903,Computers in Earth Sciences,EART

Physical Sciences,Earth and Planetary Sciences,1904,Earth-Surface Processes,EART

Physical Sciences,Earth and Planetary Sciences,1905,Economic Geology,EART
Physical Sciences,Earth and Planetary Sciences,1906,Geochemistry and Petrology,EART
Physical Sciences,Earth and Planetary Sciences,1907,Geology,EART
Physical Sciences,Earth and Planetary Sciences,1908,Geophysics,EART
Physical Sciences,Earth and Planetary Sciences,1909,Geotechnical Engineering and Engineering Geology,EART
Physical Sciences,Earth and Planetary Sciences,1910,Oceanography,EART
Physical Sciences,Earth and Planetary Sciences,1911,Paleontology,EART
Physical Sciences,Earth and Planetary Sciences,1912,Space and Planetary Science,EART
Physical Sciences,Earth and Planetary Sciences,1913,Stratigraphy,EART
Social Sciences & Humanities,"Economics, Econometrics and Finance",2000,"Economics, Econometrics and Finance (all)",ECON
Social Sciences & Humanities,"Economics, Econometrics and Finance",2001,"Economics, Econometrics and Finance (miscellaneous)",ECON
Social Sciences & Humanities,"Economics, Econometrics and Finance",2002,Economics and Econometrics,ECON
Social Sciences & Humanities,"Economics, Econometrics and Finance",2003,Finance,ECON
Physical Sciences,Energy,2100,Energy (all),ENER
Physical Sciences,Energy,2101,Energy (miscellaneous),ENER
Physical Sciences,Energy,2102,Energy Engineering and Power Technology,ENER
Physical Sciences,Energy,2103,Fuel Technology,ENER
Physical Sciences,Energy,2104,Nuclear Energy and Engineering,ENER
Physical Sciences,Energy,2105,"Renewable Energy, Sustainability and the Environment",ENER
Physical Sciences,Engineering,2200,Engineering (all),ENGI
Physical Sciences,Engineering,2201,Engineering (miscellaneous),ENGI
Physical Sciences,Engineering,2202,Aerospace Engineering,ENGI
Physical Sciences,Engineering,2203,Automotive Engineering,ENGI
Physical Sciences,Engineering,2204,Biomedical Engineering,ENGI
Physical Sciences,Engineering,2205,Civil and Structural Engineering,ENGI
Physical Sciences,Engineering,2206,Computational Mechanics,ENGI
Physical Sciences,Engineering,2207,Control and Systems Engineering,ENGI
Physical Sciences,Engineering,2208,Electrical and Electronic Engineering,ENGI
Physical Sciences,Engineering,2209,Industrial and Manufacturing Engineering,ENGI
Physical Sciences,Engineering,2210,Mechanical Engineering,ENGI
Physical Sciences,Engineering,2211,Mechanics of Materials,ENGI
Physical Sciences,Engineering,2212,Ocean Engineering,ENGI
Physical Sciences,Engineering,2213,"Safety, Risk, Reliability and Quality",ENGI
Physical Sciences,Engineering,2214,Media Technology,ENGI
Physical Sciences,Engineering,2215,Building and Construction,ENGI
Physical Sciences,Engineering,2216,Architecture,ENGI
Physical Sciences,Environmental Science,2300,Environmental Science (all),ENVI
Physical Sciences,Environmental Science,2301,Environmental Science (miscellaneous),ENVI
Physical Sciences,Environmental Science,2302,Ecological Modeling,ENVI
Physical Sciences,Environmental Science,2303,Ecology,ENVI
Physical Sciences,Environmental Science,2304,Environmental Chemistry,ENVI
Physical Sciences,Environmental Science,2305,Environmental Engineering,ENVI
Physical Sciences,Environmental Science,2306,Global and Planetary Change,ENVI
Physical Sciences,Environmental Science,2307,"Health, Toxicology and Mutagenesis",ENVI
Physical Sciences,Environmental Science,2308,"Management, Monitoring, Policy and Law",ENVI
Physical Sciences,Environmental Science,2309,Nature and Landscape Conservation,ENVI
Physical Sciences,Environmental Science,2310,Pollution,ENVI
Physical Sciences,Environmental Science,2311,Waste Management and Disposal,ENVI
Physical Sciences,Environmental Science,2312,Water Science and Technology,ENVI
Life Sciences,Immunology and Microbiology,2400,Immunology and Microbiology (all),IMMU

Life Sciences,Immunology and Microbiology,2401,Immunology and Microbiology (miscellaneous),IMMU
Life Sciences,Immunology and Microbiology,2402,Applied Microbiology and Biotechnology,IMMU
Life Sciences,Immunology and Microbiology,2403,Immunology,IMMU
Life Sciences,Immunology and Microbiology,2404,Microbiology,IMMU
Life Sciences,Immunology and Microbiology,2405,Parasitology,IMMU
Life Sciences,Immunology and Microbiology,2406,Virology,IMMU
Physical Sciences,Materials Science,2500,Materials Science (all),MATE
Physical Sciences,Materials Science,2501,Materials Science (miscellaneous),MATE
Physical Sciences,Materials Science,2502,Biomaterials,MATE
Physical Sciences,Materials Science,2503,Ceramics and Composites,MATE
Physical Sciences,Materials Science,2504,"Electronic, Optical and Magnetic Materials",MATE
Physical Sciences,Materials Science,2505,Materials Chemistry,MATE
Physical Sciences,Materials Science,2506,Metals and Alloys,MATE
Physical Sciences,Materials Science,2507,Polymers and Plastics,MATE
Physical Sciences,Materials Science,2508,"Surfaces, Coatings and Films",MATE
Physical Sciences,Mathematics,2600,Mathematics (all),MATH
Physical Sciences,Mathematics,2601,Mathematics (miscellaneous),MATH
Physical Sciences,Mathematics,2602,Algebra and Number Theory,MATH
Physical Sciences,Mathematics,2603,Analysis,MATH
Physical Sciences,Mathematics,2604,Applied Mathematics,MATH
Physical Sciences,Mathematics,2605,Computational Mathematics,MATH
Physical Sciences,Mathematics,2606,Control and Optimization,MATH
Physical Sciences,Mathematics,2607,Discrete Mathematics and Combinatorics,MATH
Physical Sciences,Mathematics,2608,Geometry and Topology,MATH
Physical Sciences,Mathematics,2609,Logic,MATH
Physical Sciences,Mathematics,2610,Mathematical Physics,MATH
Physical Sciences,Mathematics,2611,Modeling and Simulation,MATH
Physical Sciences,Mathematics,2612,Numerical Analysis,MATH
Physical Sciences,Mathematics,2613,Statistics and Probability,MATH
Physical Sciences,Mathematics,2614,Theoretical Computer Science,MATH
Health Sciences,Medicine,2700,Medicine (all),MEDI
Health Sciences,Medicine,2701,Medicine (miscellaneous),MEDI
Health Sciences,Medicine,2702,Anatomy,MEDI
Health Sciences,Medicine,2703,Anesthesiology and Pain Medicine,MEDI
Health Sciences,Medicine,2704,Biochemistry (medical),MEDI
Health Sciences,Medicine,2705,Cardiology and Cardiovascular Medicine,MEDI
Health Sciences,Medicine,2706,Critical Care and Intensive Care Medicine,MEDI
Health Sciences,Medicine,2707,Complementary and Alternative Medicine,MEDI
Health Sciences,Medicine,2708,Dermatology,MEDI
Health Sciences,Medicine,2709,Drug Guides,MEDI
Health Sciences,Medicine,2710,Embryology,MEDI
Health Sciences,Medicine,2711,Emergency Medicine,MEDI
Health Sciences,Medicine,2712,"Endocrinology, Diabetes and Metabolism",MEDI
Health Sciences,Medicine,2713,Epidemiology,MEDI
Health Sciences,Medicine,2714,Family Practice,MEDI
Health Sciences,Medicine,2715,Gastroenterology,MEDI
Health Sciences,Medicine,2716,Genetics (clinical),MEDI
Health Sciences,Medicine,2717,Geriatrics and Gerontology,MEDI
Health Sciences,Medicine,2718,Health Informatics,MEDI
Health Sciences,Medicine,2719,Health Policy,MEDI

Health Sciences,Medicine,2720,Hematology,MEDI
Health Sciences,Medicine,2721,Hepatology,MEDI
Health Sciences,Medicine,2722,Histology,MEDI
Health Sciences,Medicine,2723,Immunology and Allergy,MEDI
Health Sciences,Medicine,2724,Internal Medicine,MEDI
Health Sciences,Medicine,2725,Infectious Diseases,MEDI
Health Sciences,Medicine,2726,Microbiology (medical),MEDI
Health Sciences,Medicine,2727,Nephrology,MEDI
Health Sciences,Medicine,2728,Neurology (clinical),MEDI
Health Sciences,Medicine,2729,Obstetrics and Gynecology,MEDI
Health Sciences,Medicine,2730,Oncology,MEDI
Health Sciences,Medicine,2731,Ophthalmology,MEDI
Health Sciences,Medicine,2732,Orthopedics and Sports Medicine,MEDI
Health Sciences,Medicine,2733,Otorhinolaryngology,MEDI
Health Sciences,Medicine,2734,Pathology and Forensic Medicine,MEDI
Health Sciences,Medicine,2735,"Pediatrics, Perinatology and Child Health",MEDI
Health Sciences,Medicine,2736,Pharmacology (medical),MEDI
Health Sciences,Medicine,2737,Physiology (medical),MEDI
Health Sciences,Medicine,2738,Psychiatry and Mental Health,MEDI
Health Sciences,Medicine,2739,"Public Health, Environmental and Occupational Health",MEDI
Health Sciences,Medicine,2740,Pulmonary and Respiratory Medicine,MEDI
Health Sciences,Medicine,2741,"Radiology, Nuclear Medicine and Imaging",MEDI
Health Sciences,Medicine,2742,Rehabilitation,MEDI
Health Sciences,Medicine,2743,Reproductive Medicine,MEDI
Health Sciences,Medicine,2744,Reviews and References (medical),MEDI
Health Sciences,Medicine,2745,Rheumatology,MEDI
Health Sciences,Medicine,2746,Surgery,MEDI
Health Sciences,Medicine,2747,Transplantation,MEDI
Health Sciences,Medicine,2748,Urology,MEDI
Life Sciences,Neuroscience,2800,Neuroscience (all),NEUR
Life Sciences,Neuroscience,2801,Neuroscience (miscellaneous),NEUR
Life Sciences,Neuroscience,2802,Behavioral Neuroscience,NEUR
Life Sciences,Neuroscience,2803,Biological Psychiatry,NEUR
Life Sciences,Neuroscience,2804,Cellular and Molecular Neuroscience,NEUR
Life Sciences,Neuroscience,2805,Cognitive Neuroscience,NEUR
Life Sciences,Neuroscience,2806,Developmental Neuroscience,NEUR
Life Sciences,Neuroscience,2807,Endocrine and Autonomic Systems,NEUR
Life Sciences,Neuroscience,2808,Neurology,NEUR
Life Sciences,Neuroscience,2809,Sensory Systems,NEUR
Health Sciences,Nursing,2900,Nursing (all),NURS
Health Sciences,Nursing,2901,Nursing (miscellaneous),NURS
Health Sciences,Nursing,2902,Advanced and Specialized Nursing,NURS
Health Sciences,Nursing,2903,Assessment and Diagnosis,NURS
Health Sciences,Nursing,2904,Care Planning,NURS
Health Sciences,Nursing,2905,Community and Home Care,NURS
Health Sciences,Nursing,2906,Critical Care Nursing,NURS
Health Sciences,Nursing,2907,Emergency Nursing,NURS
Health Sciences,Nursing,2908,Fundamentals and Skills,NURS
Health Sciences,Nursing,2909,Gerontology,NURS
Health Sciences,Nursing,2910,"Issues, Ethics and Legal Aspects",NURS

Health Sciences,Nursing,2911,Leadership and Management,NURS
Health Sciences,Nursing,2912,LPN and LVN,NURS
Health Sciences,Nursing,2913,Maternity and Midwifery,NURS
Health Sciences,Nursing,2914,Medical and Surgical Nursing,NURS
Health Sciences,Nursing,2915,Nurse Assisting,NURS
Health Sciences,Nursing,2916,Nutrition and Dietetics,NURS
Health Sciences,Nursing,2917,Oncology (nursing),NURS
Health Sciences,Nursing,2918,Pathophysiology,NURS
Health Sciences,Nursing,2919,Pediatrics,NURS
Health Sciences,Nursing,2920,Pharmacology (nursing),NURS
Health Sciences,Nursing,2921,Psychiatric Mental Health,NURS
Health Sciences,Nursing,2922,Research and Theory,NURS
Health Sciences,Nursing,2923,Review and Exam Preparation,NURS
Life Sciences,"Pharmacology, Toxicology and Pharmaceutics",3000,"Pharmacology, Toxicology and Pharmaceutics (all)",PHAR
Life Sciences,"Pharmacology, Toxicology and Pharmaceutics",3001,"Pharmacology, Toxicology and Pharmaceutics (miscellaneous)",PHAR
Life Sciences,"Pharmacology, Toxicology and Pharmaceutics",3002,Drug Discovery,PHAR
Life Sciences,"Pharmacology, Toxicology and Pharmaceutics",3003,Pharmaceutical Science,PHAR
Life Sciences,"Pharmacology, Toxicology and Pharmaceutics",3004,Pharmacology,PHAR
Life Sciences,"Pharmacology, Toxicology and Pharmaceutics",3005,Toxicology,PHAR
Physical Sciences,Physics and Astronomy,3100,Physics and Astronomy (all),PHYS
Physical Sciences,Physics and Astronomy,3101,Physics and Astronomy (miscellaneous),PHYS
Physical Sciences,Physics and Astronomy,3102,Acoustics and Ultrasonics,PHYS
Physical Sciences,Physics and Astronomy,3103,Astronomy and Astrophysics,PHYS
Physical Sciences,Physics and Astronomy,3104,Condensed Matter Physics,PHYS
Physical Sciences,Physics and Astronomy,3105,Instrumentation,PHYS
Physical Sciences,Physics and Astronomy,3106,Nuclear and High Energy Physics,PHYS
Physical Sciences,Physics and Astronomy,3107,"Atomic and Molecular Physics, and Optics",PHYS
Physical Sciences,Physics and Astronomy,3108,Radiation,PHYS
Physical Sciences,Physics and Astronomy,3109,Statistical and Nonlinear Physics,PHYS
Physical Sciences,Physics and Astronomy,3110,Surfaces and Interfaces,PHYS
Social Sciences & Humanities,Psychology,3200,Psychology (all),PSYC
Social Sciences & Humanities,Psychology,3201,Psychology (miscellaneous),PSYC
Social Sciences & Humanities,Psychology,3202,Applied Psychology,PSYC
Social Sciences & Humanities,Psychology,3203,Clinical Psychology,PSYC
Social Sciences & Humanities,Psychology,3204,Developmental and Educational Psychology,PSYC
Social Sciences & Humanities,Psychology,3205,Experimental and Cognitive Psychology,PSYC
Social Sciences & Humanities,Psychology,3206,Neuropsychology and Physiological Psychology,PSYC
Social Sciences & Humanities,Psychology,3207,Social Psychology,PSYC
Social Sciences & Humanities,Social Sciences,3300,Social Sciences (all),SOCI
Social Sciences & Humanities,Social Sciences,3301,Social Sciences (miscellaneous),SOCI
Social Sciences & Humanities,Social Sciences,3302,Archeology,SOCI
Social Sciences & Humanities,Social Sciences,3303,Development,SOCI
Social Sciences & Humanities,Social Sciences,3304,Education,SOCI
Social Sciences & Humanities,Social Sciences,3305,"Geography, Planning and Development",SOCI
Social Sciences & Humanities,Social Sciences,3306,Health (social science),SOCI
Social Sciences & Humanities,Social Sciences,3307,Human Factors and Ergonomics,SOCI
Social Sciences & Humanities,Social Sciences,3308,Law,SOCI
Social Sciences & Humanities,Social Sciences,3309,Library and Information Sciences,SOCI
Social Sciences & Humanities,Social Sciences,3310,Linguistics and Language,SOCI
Social Sciences & Humanities,Social Sciences,3311,Safety Research,SOCI

Social Sciences & Humanities,Social Sciences,3312,Sociology and Political Science,SOCI
Social Sciences & Humanities,Social Sciences,3313,Transportation,SOCI
Social Sciences & Humanities,Social Sciences,3314,Anthropology,SOCI
Social Sciences & Humanities,Social Sciences,3315,Communication,SOCI
Social Sciences & Humanities,Social Sciences,3316,Cultural Studies,SOCI
Social Sciences & Humanities,Social Sciences,3317,Demography,SOCI
Social Sciences & Humanities,Social Sciences,3318,Gender Studies,SOCI
Social Sciences & Humanities,Social Sciences,3319,Life-span and Life-course Studies,SOCI
Social Sciences & Humanities,Social Sciences,3320,Political Science and International Relations,SOCI
Social Sciences & Humanities,Social Sciences,3321,Public Administration,SOCI
Social Sciences & Humanities,Social Sciences,3322,Urban Studies,SOCI
Health Sciences,Veterinary,3400,Veterinary (all),VETE
Health Sciences,Veterinary,3401,Veterinary (miscellaneous),VETE
Health Sciences,Veterinary,3402,Equine,VETE
Health Sciences,Veterinary,3403,Food Animals,VETE
Health Sciences,Veterinary,3404,Small Animals,VETE
Health Sciences,Dentistry,3500,Dentistry (all),DENT
Health Sciences,Dentistry,3501,Dentistry (miscellaneous),DENT
Health Sciences,Dentistry,3502,Dental Assisting,DENT
Health Sciences,Dentistry,3503,Dental Hygiene,DENT
Health Sciences,Dentistry,3504,Oral Surgery,DENT
Health Sciences,Dentistry,3505,Orthodontics,DENT
Health Sciences,Dentistry,3506,Periodontics,DENT
Health Sciences,Health Professions,3600,Health Professions (all),HEAL
Health Sciences,Health Professions,3601,Health Professions (miscellaneous),HEAL
Health Sciences,Health Professions,3602,Chiropractics,HEAL
Health Sciences,Health Professions,3603,Complementary and Manual Therapy,HEAL
Health Sciences,Health Professions,3604,Emergency Medical Services,HEAL
Health Sciences,Health Professions,3605,Health Information Management,HEAL
Health Sciences,Health Professions,3606,Medical Assisting and Transcription,HEAL
Health Sciences,Health Professions,3607,Medical Laboratory Technology,HEAL
Health Sciences,Health Professions,3608,Medical Terminology,HEAL
Health Sciences,Health Professions,3609,Occupational Therapy,HEAL
Health Sciences,Health Professions,3610,Optometry,HEAL
Health Sciences,Health Professions,3611,Pharmacy,HEAL
Health Sciences,Health Professions,3612,"Physical Therapy, Sports Therapy and Rehabilitation",HEAL
Health Sciences,Health Professions,3613,Podiatry,HEAL
Health Sciences,Health Professions,3614,Radiological and Ultrasound Technology,HEAL
Health Sciences,Health Professions,3615,Respiratory Care,HEAL
Health Sciences,Health Professions,3616,Speech and Hearing,HEAL

Table S2
Frequency distribution of the 27 subject areas within the data corpus.

	Subject Area	Frequency
1	Agricultural and Biological Sciences	72211
2	Arts and Humanities	3651
3	Biochemistry, Genetics and Molecular Biology	62691
4	Business, Management and Accounting	2917
5	Chemical Engineering	10628
6	Chemistry	24060
7	Computer Science	10771
8	Decision Sciences	1946
9	Dentistry	91
10	Earth and Planetary Sciences	93293
11	Economics, Econometrics and Finance	2669
12	Energy	6889
13	Engineering	25794
14	Environmental Science	107911
15	Health Professions	468
16	Immunology and Microbiology	15064
17	Materials Science	12774
18	Mathematics	8503
19	Medicine	30597
20	Multidisciplinary	8412
21	Neuroscience	4611
22	Nursing	801
23	Pharmacology, Toxicology and Pharmaceutics	6149
24	Physics and Astronomy	12924
25	Psychology	3002
26	Social Sciences	15022
27	Veterinary	889

Table S3

Symmetric Jaccard similarity matrix of the 27 subject areas.

	Agricultural.;	Arts and Hur	Biochemistry	Business, Me	Chemical Eng	Chemistry	Computer Sc	Decision Scie	Dentistry	Earth and Pla	Economics, E	Energy	Engineering	Environment	Health Profe	Immunology	Materials Sci	Mathematics	Medicine	Multidisciplir	Neuroscienc	Nursing	Pharmacolog	Physics and /	Psychology	Social Scienc	Veterinary
Agricultural &	0	0,01039487	0,04098837	0,00425022	0,00564994	0,01411925	0,01741404	0,00592066	0	0,07192978	0,00354458	0,01284247	0,04013666	0,0923735	0,00019083	0,01	0,00425595	0,01054483	0,01428897	0,02520182	0,00163893	0,00031811	0,00476627	0,00895942	0,00250643	0,04434389	0,00214914
Arts and Hur	0	0,00500214	0,00250214	0	0,00026782	0,00292588	0,00118437	0	0	0,00973084	0,00157749	0,00141618	0,00019906	0,00641732	0	0,00216732	0,00075593	0,00158658	0,00242266	0,01343171	0,00349406	0	0	0	0	0,01053114	0
Biochemistry	0,04098837	0,00500214	0	0,00404858	0,00084256	0,01454079	0,014858	0,00689267	0	0,02166096	0,00396616	0,01490916	0,02110178	0,03192519	0,00067935	0,01255841	0,00651782	0,01005474	0,01921286	0,01972205	0,0028141	0,00067946	0,00516953	0,00932401	0,00350251	0,03183854	0,00168806
Business, Me	0,00425022	0,00259067	0,00404858	0	0,00614754	0,00378072	0,00712038	0,00451375	0	0,00543514	0,00967222	0,01365126	0,00636196	0,0073018	0,00169492	0,00180818	0,00512821	0,00301065	0,00647249	0,00114003	0	0	0,00105319	0,00019943	0,00079239	0,03835569	0
Chemical Eng	0,00564994	0,00056677	0,00084256	0,00614754	0	0,03244736	0,00449662	0,00137137	0	0,01128395	0,00027642	0,04949437	0,01226122	0,0120101	0	0,00410196	0,01763429	0,00362713	0,0034443	0,00278897	0,00039216	0,00041964	0,00026025	0,01614639	0	0,03588324	0
Chemistry	0,01411925	0,00267782	0,01454079	0,00378072	0,03244736	0	0,0046582	0,00200682	0	0,01401368	0,00158479	0,03428936	0,01349488	0,02547211	0,00053691	0,00718057	0,01616121	0,00364491	0,01229256	0,00890897	0,00077121	0,00055706	0,0153003	0,01626153	0	0,00384993	0,00256048
Computer Sc	0,01741404	0,00292588	0,014858	0,00712038	0,00449662	0,0046582	0	0,01043171	0	0,02655158	0,00209974	0,00943295	0,02862918	0,03251082	0,00079586	0,00149441	0,00803783	0,02391087	0,00714742	0,00584731	0,00231481	0,00019889	0,00087047	0,01153688	0,00176401	0,02175481	0
Decision Scie	0,00592066	0,00118437	0,00689267	0,00451375	0,00137137	0,00200682	0,01043171	0	0	0,00737415	0,00383693	0,00312229	0,00856732	0,01068946	0	0,00041034	0,00326916	0,00932712	0,00141143	0,00274323	0	0	0,00047304	0,00018797	0,00208528	0,00862651	0
Dentistry	0	0	0	0	0	0	0	0	0	6,80E-05	0	0	0	4,41E-05	0	0	0	0	0	0	0	0	0	0	0	0	0
Earth and Pla	0,07192978	0,00973084	0,02166096	0,00543514	0,01128395	0,01401368	0,02655158	0,00737415	6,80E-05	0	0,00320357	0,02457181	0,07251679	0,15026919	0,00023758	0,00284594	0,00655948	0,01886557	0,01217794	0,03046122	0,0010809	0,00309549	0,00212441	0,01785714	0,00074501	0,05883334	0,00016904
Economics, E	0,00354458	0,00157749	0,00396616	0,00967222	0,00027642	0,00158479	0,00209974	0,00383693	0	0,00320357	0	0,00423729	0,00448889	0,00625982	0	0,00160772	0,00228519	0,00277427	0,00463089	0,00163066	0	0,00121212	0,00125634	0,00062291	0,00110254	0,00621069	0
Energy	0,01284247	0,00141618	0,01490916	0,01365126	0,04949437	0,03428936	0,04943295	0,00312229	0	0,02457181	0,00423729	0	0,02268594	0,01902035	0	0,00180213	0,01641327	0,00762355	0,01165501	0,01045214	0,00085488	0	0,00014723	0,01678807	0,00021753	0,01508249	0,00021603
Engineering	0,04013666	0,00019906	0,02110178	0,00636196	0,01226122	0,01349488	0,02862918	0,00856732	0	0,07251679	0,00448889	0,02268594	0	0,10976696	0,00029453	0,00200145	0,00924143	0,00242885	0,00823171	0,00987441	0,00099241	0,0001178	0,00215151	0,01062104	0,00070381	0,04481975	0,00017553
Environment	0,0923735	0,00641732	0,03192519	0,00073018	0,0120101	0,02547211	0,03251082	0,01068946	4,41E-05	0,15026919	0,00623982	0,01902035	0,10976696	0	0,00046238	0,00749989	0,00848955	0,02344441	0,01802125	0,02661638	0,00153731	0,00046239	0,00775975	0,01269767	0,0013419	0,07076246	0,00072499
Health Profe	0,00019083	0	0,00067935	0,00169492	0	0,00053691	0,00079586	0	0	0,00023758	0	0,00029453	0,00046238	0	0	0	0	0	0,00301811	0,00023719	0	0	0,00117925	0	0	0,00094603	0
Immunology	0,01	0,00216732	0,01255841	0,00180818	0,00410196	0,00718057	0,00149441	0,00041034	0	0,00285394	0,00160772	0,00180213	0,00200145	0,00749989	0	0	0,00236967	0,00115848	0,03119407	0,00708541	0,00074794	0,00342759	0,00478215	0,00014188	0,00159872	0,00021348	0,02146264
Materials Sci	0,00425595	0,00075593	0,00651782	0,00512821	0,01763429	0,01616121	0,00803783	0,00326916	0	0,00655948	0,00228519	0,01641327	0,00924143	0,00848955	0	0,00236967	0	0,00693692	0,00323358	0,00359518	0	0	0,00362483	0,01215324	0,00126984	0,00454986	0
Mathematics	0,01054483	0,00158658	0,00105474	0,00301065	0,00362713	0,00364491	0,00392712	0	0	0,01886557	0,00277427	0,00762355	0,02042885	0,02344441	0	0,00115848	0,00693692	0	0,00441682	0,00504944	0,00087159	0	0,00075094	0,01062106	0,00029771	0,01254813	0
Medicine	0,01428897	0,00242266	0,01921286	0,00647249	0,0034443	0,01229256	0,00714742	0,00141143	0	0,01217794	0,00463089	0,01165501	0,00823171	0,01802125	0,00301811	0,03119407	0,00322358	0,00441682	0	0,01462156	0,00158629	0,0033557	0,00897471	0,00604458	0,00195695	0,01478467	0,01305483
Multidisciplir	0,02520182	0,01343171	0,01972205	0,00114003	0,00278897	0,00890897	0,00584731	0,00274323	0	0,03046122	0,00163066	0,01045214	0,00987441	0,02661636	0,00023719	0,00708541	0,00359518	0,00504944	0,01462156	0	0,00205761	0,00047461	0,00264967	0,0081551	0	0,01779203	0,00161925
Neuroscienc	0,00163893	0,00349406	0,0028141	0	0,00039216	0,00077121	0,00231481	0	0	0,0010809	0	0,00085488	0,00098241	0,00153731	0	0,00074794	0	0,00087159	0,00158629	0,00205761	0	0	0	0,00114155	0	0,00163292	0
Nursing	0,00031811	0	0,00067946	0	0,00041964	0,00055706	0,00019889	0	0	0,00030549	0,00121212	0	0,00011178	0,00046239	0	0,00342759	0	0	0,0033557	0,00047461	0	0	0,00118064	0	0	8,26E-05	0
Pharmacolog	0,00476627	0	0,00516953	0,00105319	0,00026025	0,0153003	0,00087047	0,00047304	0	0,00212441	0,00125634	0,00114723	0,00215151	0,00775975	0,00117925	0,00478215	0,00362483	0,00075094	0,00897471	0,00264967	0	0,00118064	0	0,00220334	0	0,00250215	0,00208333
Physics and /	0,00895942	0,00449807	0,00932401	0,00019943	0,01614639	0,01626153	0,01153688	0,0016797	0	0,01785714	0,00062291	0,01678807	0,01062104	0,01269767	0	0,00114188	0,01215324	0,01062106	0,00604458	0,0081551	0,00114155	0	0,00220334	0	0	0,00958042	0
Psychology	0,00350251	0	0,00050251	0,00079239	0	0,00053691	0,00017401	0,00020528	0	0,00074501	0,00110254	0,00021753	0,00070381	0,0013419	0	0,00158872	0,00126984	0,00029771	0,00195695	0	0	0	0	0	0	0,0010682	0
Social Scienc	0,04434389	0,01053114	0,03183854	0,00835569	0,00388324	0,00834993	0,02175481	0,00662651	0	0,05833334	0,00621069	0,01508249	0,04481975	0,07076246	0,00049603	0,00213348	0,00454986	0,01254813	0,01479467	0,01779203	0,00163292	8,26E-05	0,00250215	0,00958042	0,0010682	0	0,00040095
Veterinary	0,00214914	0	0,00016806	0	0	0,00026048	0	0	0	0,00016904	0	0,00021603	0,00017553	0,00072499	0	0,02146264	0	0,01305483	0,00161925	0	0	0	0,00208333	0	0	0,00040095	0

Table S4
Symmetric Jaccard dissimilarity matrix of the 318 Classifications.

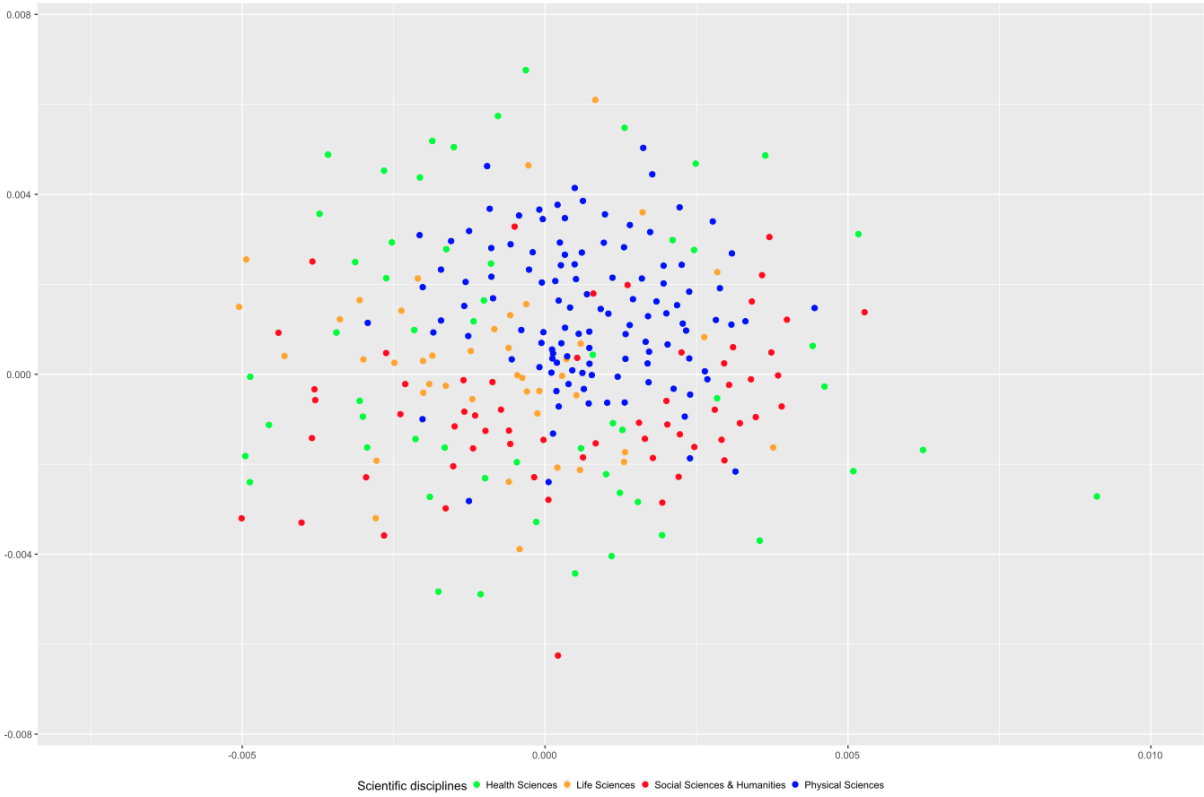

Figure S1
NMDS of the „Classifications“ based on their affiliation to their superordinate „Subject Areas“ and their Jaccard dissimilarities, after removing outliers to enhance the resolution, as individual single Classifications had a dissimilarity of 1 to every other „Classification“. Stressvalue: 0.0586. Point color denote the affiliation of individual „Classifications“ to „Clusters“. Orange: life sciences, pink: social sciences & humanities, blue: physical sciences, green: health sciences.

Acknowledgements

A doctoral thesis bestows a title upon only one person, although the actual effort behind it was carried by a whole armada, and it is this very armada that I want to sincerely thank from the bottom of my heart. It is clear that assistance has come in various ways, some direct, some indirect, some professional, some completely detached from the actual theme of the doctoral thesis. Many people probably do not even realize how indispensable they were during this time.

It is clear that the work became possible only through all the help and encouragement received. It is also clear that it is impossible to thank everyone by name and with anecdotes, as it would make the work twice as large. Let's start in the environment where the doctoral thesis was written. First and foremost, I want to deeply thank my doctoral advisor, Jens. All the professional help and guidance over the years have ultimately brought me to where I am now, but I want to thank you not only for that but also for making the path to a doctorate appealing to me in the first place—without our conversations, I would never have wanted to pursue a doctorate or even believed in my ability to do so. When I began the doctorate, you gave me so much freedom, which is by no means taken for granted and has significantly contributed to maintaining and even increasing my enjoyment of science. Furthermore, you bravely accepted my direct Sauerland style, not just tolerated it. Daniela fits perfectly here too, enduring me with brilliance, always having an open ear, and providing me with all her professional knowledge. Although you knew that as soon as you entered our office, I would ask you at least one question, you bravely stepped in, and your door was always open for me—from the bottom of my heart, I thank you for that. I have skillfully received all the supervision and knowledge from both of you, the main actors in my dissertation. Thank you!

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Now, let's talk about the people who have known and tolerated me for much longer, and fortunately, I can call them my family. My dearest ones. How can I thank you? A few lines of words cannot express how much I thank and love you. You stood by me, never lost faith, and built me up again and again. Beloved father, beloved mother, beloved brother, beloved Charlie, beloved uncle, beloved aunt but as family is not only defined by blood, I include you, Arno, Maike, Robin, Nelly, Tina and Bolle: thank you for always being there for me and accompanying me on this journey.

I thank all of you. With love,

Guido

Curriculum Vitae

The curriculum vitae is not provided due to data protection reasons

The curriculum vitae is not provided due to data protection reasons

Declarations

Declaration: In accordance with § 6 (para. 2, clause g) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I represent the field to which the topic “Microbial Resilience in Changing Environments: Evaluating Anthropogenic Stress and Monitoring Strategies” is assigned in research and teaching and that I support the application of Guido Sieber.

_____ _____

Name of the scientific supervisor/ member of the of the University of Duisburg-Essen	Signature of the supervisor/ member of the University of Duisburg-Essen

Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I have written the herewith submitted dissertation independently using only the materials listed, and have cited all sources taken over verbatim or in content as such.

Essen, date _____ _____

Signature of the doctoral candidate

Declaration: In accordance with § 7 (para. 2, clause e and g) of the Regulations Governing the Doctoral Proceedings of the Faculty of Biology for awarding the doctoral degree Dr. rer. nat., I hereby declare that I have undertaken no previous attempts to attain a doctoral degree, that the current work has not been rejected by any other faculty, and that I am submitting the dissertation only in this procedure.

Essen, date _____ _____

Signature of the doctoral candidate