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# Non-perennial rivers and streams in extreme hydrological conditions—comparing the effectiveness of amplicon sequencing and digital microscopy for diatom biodiversity appraisal

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**Introduction:** Non-perennial rivers and streams are increasingly present, in part because of climate change, even in the temperate climate. However, how the loss of connectivity and complete drying affect microphytobenthos in general and diatom communities in particular has gone mostly unstudied.

**Methods:** With this paper, we aim to close this gap, identifying diatom biodiversity through manual digital microscopy and rbcL amplicon sequencing, to observe a) which method is better suited to it and b) how the ecotone flow-pool-dry affects diatom diversity under duress. Three karstic, non-perennial rivers and streams with a gradient from natural to anthropogenically disturbed were sampled under flooding conditions and after a long and intense drought in 2022.

**Results:** Our results show that digital microscopy shows a higher diversity and species richness than amplicon sequencing. We posit that this might be due to a reduced pool of subaerophile taxa having been sequenced and being part of the reference database. Furthermore, the effect of drying only resulted in a reduction in diversity after this drought, although the biofilm was still alive under these conditions.

**Discussion:** To use amplicon sequencing for non-perennial river diatom diversity monitoring, the reference databases will have to be adapted to such systems, as most rivers may be subjected to drying regularly in the future.

## KEYWORDS

IRES, intermittent rivers and ephemeral streams, temporary rivers, benthic diatoms, freshwater diatoms, metabarcoding, molecular method, ASV

# 1 Introduction

Non-perennial rivers and streams make up over 60% of all riparian systems (Messenger et al., 2021). There might even be a prospect of increase, leaving most rivers affected by it due to climate change (Blöschl et al., 2019; Bonaldo et al., 2023). As non-perennial streams have an important impact on ecosystems because of their changing hydrology, it is important to understand how they work (Datry et al., 2017). However, in the case of phyto-benthic communities in general and diatom communities in particular, their study has been underrepresented and very localized (Delgado et al., 2012; Novais et al., 2014; B-Béres et al., 2019).

As the loss of longitudinal connectivity and subsequent loss of surface water creates hydrologically very distinct habitats, ecological communities are shaped by them. These habitats contain new ecological niches to fill: the riparian flow would favor phyto-benthic communities, loss of connectivity creates pools, which further the addition of phytoplankton, and dry riverbeds should facilitate survival of sub-aerophile species (Burfeid Castellanos, 2018). Thus, non-perennial streams have the potential of being hotspots of diversity (Datry et al., 2014) and as such would be important to conserve and understand.

Furthermore, the European Water Framework Directive (European Commission, Directive 2000/60/EC, European Commission, 2000) ensures the ecological quality of flowing rivers. It has, nonetheless, neglected to cover drying and intermittent rivers outside the Mediterranean realm (Stubbington et al., 2018). Due to the previously described problematics and ever-increasing proportion of dry falling rivers at all latitudes, the biodiversity and ecological quality of the rivers are in danger. How these ecological parameters must be analyzed has not yet been assayed.

The traditional way of measuring this diatom biodiversity entails microscopy as a semi-quantitative tool (Szczepocka and Żelazna-Wieczorek, 2018). However, as an alternative to capture even more diversity, molecular tools such as amplicon sequencing can help to get a non-quantitative (presence-absence) overview of the taxa (Vasselon et al., 2017a; Bailet et al., 2019). For diatoms, two primer sets are most used, 18S [V4 or V9, mostly used in Germany because of availability of reference barcodes] and rbcL [mostly used outside of Germany] (Bailet et al., 2019). As rbcL is a more changing molecule, part of the RubisCo located in the chloroplast, it required the creation of five primers (three forward and two reverse) (Vasselon et al., 2017b; Bruce et al., 2021). Because of this, a higher variability and diversity might be caught, which is why we have selected rbcL for this study. Furthermore, as this marker has been most frequently used, a quantification coefficient has been created and could increase similarity to microscopy results (Vasselon et al., 2018; Tapolczai et al., 2019), and has been found to do so (Rimet et al., 2018).

However, similar the results of the methods might be, the combination of amplicon sequencing of benthic diatom communities in non-perennial streams and its functionality is yet unstudied. To fill this gap, we sampled 10 sites within three karstic, naturally drying rivers twice in the year 2022. We aimed to observe

differences between high flow spring and a severely dry summer on the effectivity of both methods to capture biodiversity. Finally, we aimed to monitor the effects that the extreme changes had on the diatom community composition.

Thus, in this study, we have focused on how the diatom biodiversity of three non-perennial streams might be best monitored, using digital microscopy and one amplicon sequencing marker (rbcL) to identify the biodiversity along the changing hydrology. We hypothesize that the diatom community divergence will be determined by the hydrological state (moment of sampling) to a higher degree than because of the method that was used for the identification. Changes in connectivity have been found to significantly alter the biodiversity through the creation of new ecological niches (Datry et al., 2017). Regarding biodiversity, we expect that only connectivity will create the framework for correct and coherent identification using the different methods, as the amplicon sequencing method was developed for monitoring purposes and has been developed for flowing rivers. In non-flow and dry conditions, we expect a divergence of diatom biodiversity according to the method.

## 2 Materials and methods

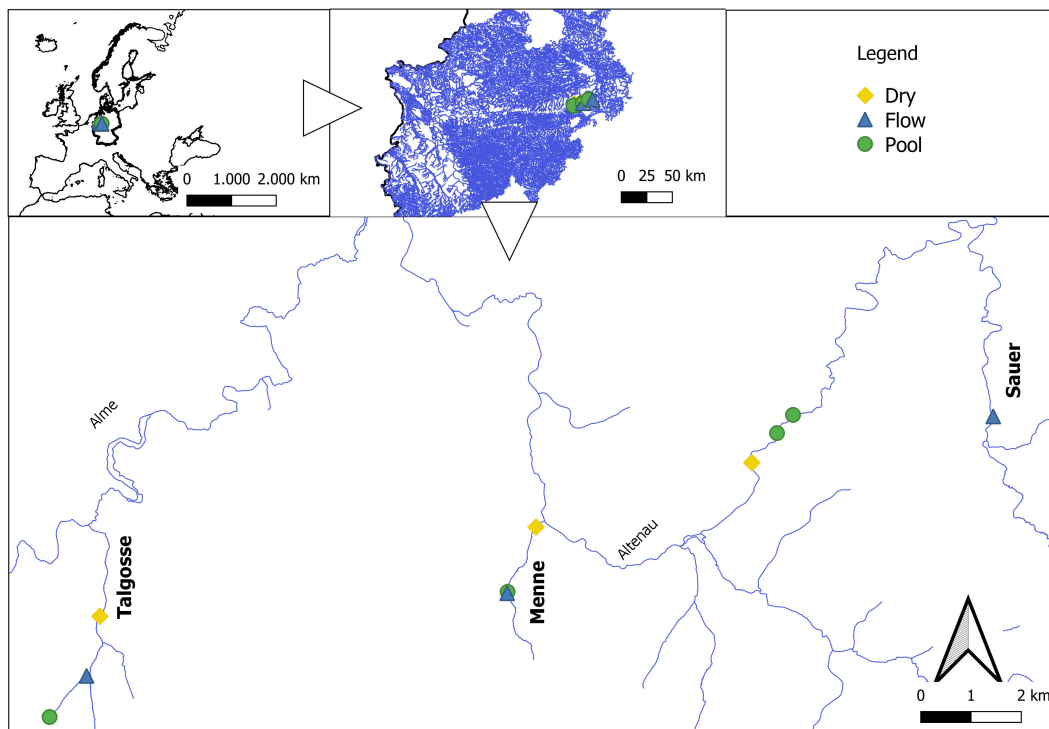
### 2.1 Sampling sites

Diatoms were sampled in three streams of the Paderborn High Plateau (North-Rhine Westphalia, Germany) twice during the year 2022 (Figure 1). The streams (Menne, Talgosse, and Sauer) are karstic and characterized by a longitudinal and temporal drying pattern (Meyer and Meyer, 2000; Meyer et al., 2003). In the smaller and natural streams (Menne and Talgosse), three sampling sites were selected, namely, dry (located at the lower reach), pool, and flowing sites, based on the characterization of Meyer et al. In the Sauer river, a bigger catchment of approximately 110,000 km<sup>2</sup> located under anthropogenic and agricultural influence, four sites were selected: a dry site at the lower reach; two pools, one of which was under direct influx of farmyard effluent; and a flowing site at the upper reach. The samples were taken in April, under flowing conditions with high flow (relative flooding), and in September, after an extreme drought affected Germany.

To characterize the streams, the physicochemical conditions were measured in the field with a Multiméter sonde (PCE-PHD1, PCE Instruments, Meschede, Germany). Fresh water samples were taken at each site with flowing or standing surface water and analyzed spectrophotometrically in the laboratory after being filtered. The results are listed in Supplementary Table 1.

### 2.2 Diatom sampling

Diatoms were sampled from the periphyton following the CEN/TR 17244:288 (CEN, 2018). The stones to be sampled were first measured with a Benthotorch (BBE+ Moldaenke GmbH, Schwentinental, Germany) to confirm fluorescence and thus



**FIGURE 1**  
Sampling sites along the Alme catchment. The sites are described at the majoritarian aquatic state, not necessarily the state that the sites were found in.

functional chlorophyll activity within the biofilm (Supplementary Table 2). A total of 100 cm<sup>2</sup> of biofilm extracted from five stones was scraped with a new toothbrush for each site, pooled in a Falcon tube and directly fixated using molecular grade ethanol to a final concentration of 75%. In the dry sites, stones were wetted with deionized water to scrape the sample. The fixated sample was then kept cool during the sampling and put into the -20°C freezer upon arrival.

### 2.3 Diatom microscopy preparation and manual digital microscopy

Diatoms were pre-washed after sampling using deionized water and centrifuged for 4 min at 1,300 rpm. This process was repeated three times. After this, the diatom samples were digested using the hot hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)– hydrochloric acid (HCl) method (Taylor et al., 2007). After the diatom samples were cleaned of organic and carbonic debris, the washing step was repeated equivalently to the pre-wash seven times. After a quantification step, the diluted samples were then dripped onto coverslips with an additional ammonium chloride (NH<sub>4</sub>Cl) and dried overnight in the lateral flow chamber. The coverslip was later attached to the slide using the artificial mounting resin, Naphrax<sup>®</sup> (Thorns Biologie Bedarf) and let to harden for 2 weeks.

The prepared slides were then scanned into the Olympus Slideview VS200 (EVIDENT, Tokyo, Japan) using the pipeline

described in the paper of Burfeid-Castellanos et al. (2022) on a surface of 16–25 mm<sup>2</sup> depending on density. The stitched and stacked virtual slides were then uploaded onto the browser-based annotation platform BIIGLE 2.0 (Langenkämper et al., 2017). An average of 420 diatom valves (half-shells) were identified manually in each slide for the counting using general bibliography (Bey and Ector, 2013; Cantonati et al., 2017; Peeters and Ector, 2017). As the dry locations presented sub-aerial diatom genera such as *Luticola*, *Humidophila*, and *Gomphonema*, we used genus-specific bibliography for their identification (Levkov et al., 2013; Kopalová et al., 2015; Levkov et al., 2016). The identified samples are available on here: <https://biigle.de/project-invitations/b087efa0-37fc-445e-bfcb-2ccc4ea33d7d>.

### 2.4 Diatom molecular preparation: amplicon sequencing

Diatom DNA was extracted with a NucleoSPIN soil Mini Kit (Macherey & Nagle). The primers used were rbcL primers (Vasselon et al., 2017a). The first PCR was performed using the protocol from Vautier et al. (2020). After the quality and quantity of DNA were confirmed electrophoretically, a second PCR was made to attach the MiSeq indices (Illumina) following a modified protocol for 16S Sequencing Library preparation (Illumina) for the amplicon attachment and cleaning of rbcL DNA. The PCR was adapted to the rbcL primer (see Supplementary Table). The cleaned and labeled

DNA was then sent to CeGAT (<https://cegat.com/>) for sequencing. The data are available on Zenodo: <https://doi.org/10.5281/zenodo.10351139>.

## 2.5 Bioinformatic pipeline

The taxonomic assignment and statistics were made using RStudio (v. 2023.06.1 + 524) with R version 4.2.2. We followed the bioinformatic pipeline from Canino et al., modified from Keck (2020). Based on DADA2, it utilizes the diat.barcode database for taxonomic classification of the amplicon sequence variants (ASVs). Harmonization of the taxonomic names was made with the taxonomy tool and DNA barcodes reference library named Phytool (Canino et al., 2021). The unassigned diatom sequences were blasted on NCBI (<https://www.ncbi.nlm.nih.gov/>) and the assignments that surpassed 95% of similarity with over 98% if length were taken as species. The ASVs were clustered into taxa, and further unassigned to species or genus were taken out of the dataset.

## 2.6 Statistical analysis

The microscopy data were standardized with a logarithm for comparability; the amplicon reads were first calculated to proportion before standardization and analysis. Biodiversity measures were calculated following the Rimet and collaborator study setup (Rimet et al., 2018), in which alpha and gamma diversity equals the Shannon index using natural logarithms (Shannon, 1948). The diatom biodiversity measures (species richness and Shannon entropy), Jaccard dissimilarities, Bray–Curtis distances, Mantel test, and PCA were calculated using the vegan package (Oksanen et al., 2015). The bar- and boxplots were created using the ggplot2 package (Wickham, 2009). The heatmap distance matrices were calculated with FactoMineR (Husson et al., 2015).

# 3 Results

## 3.1 General methodology differences

Using microscopy, a total of 187 diatom taxa were identified, 99.99% to species (Supplementary Table 3). The division of taxa proportions according to sampling can be seen in Table 1. Through microscopy identification, the main diatom species were *Achnanthes minutissimum* (Kützing) Czarnecki, *Amphora pediculus* (Kützing) Grunow, and *Gomphonema micropus*

TABLE 1 Number of species found using each method at each point in time.

	Microscopy	rbcl marker
April	155	109
September	99	91

Kützing, which coincided with the predominant results of the rbcl dataset (Figure 2A). Microscopy had a predominance of subaerial taxa pertaining to *Luticola* and *Humidophila*, which were not found when amplicon sequencing.

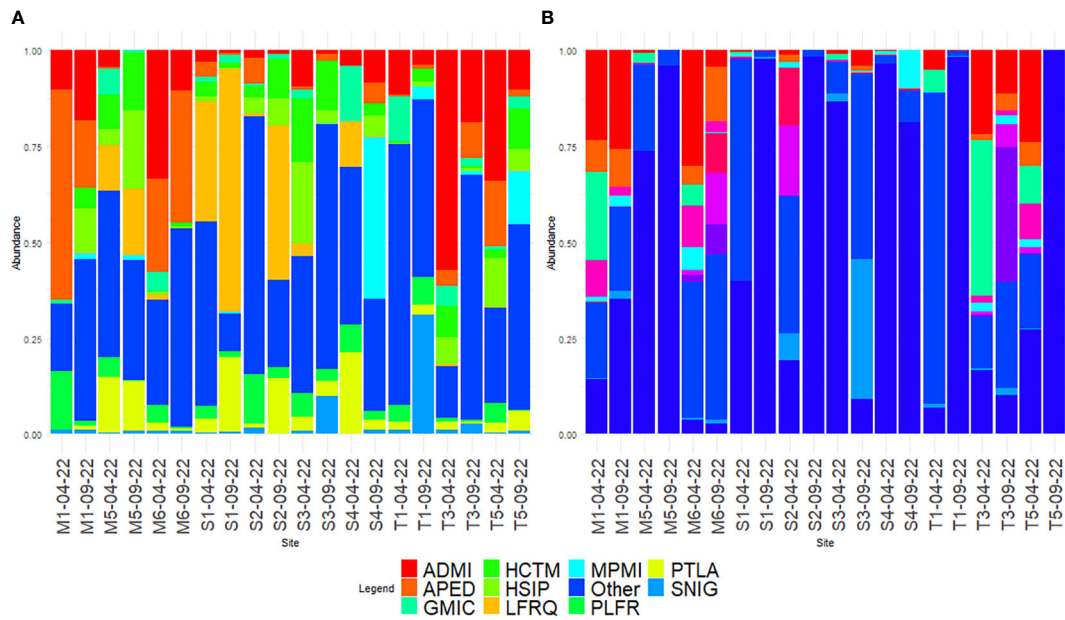
Based on amplicon sequencing, a total of 124 taxa were identified [43.36% species, 172 after blasting from a total of 286 molecular species (amplicon sequence variants, ASVs)] using a distance threshold of 95% for assignment through the diat.barcode reference library (v. 10.1). Further blasting only added 10 reads of low proportions to the before-mentioned subaerial taxa *Luticola* and five counts of *Humidophila*. A high proportion of the diatoms, including 100% of the T5 September sample, were not assigned to a taxon at dry locations (Figure 2B). However, the taxa assigned had some overlap with the microscopy results, as the three predominant taxa were the same. Some big taxa (*Navicula tripunctata* (O.F. Müller) Bory and *Melosira varians* C. Agardh) were found to be part of the predominant taxa with the amplicon assignment, although they were present in low numbers when using microscopy. A comparison of the proportions of the predominant diatoms according to the method of identification is listed in Table 2.

To compare the distances between the diatom communities identified with the different methods, a mantel test was made on the Bray–Curtis distances from each site as calculated from DNA or microscopy method, which showed significant differences ( $r = 0.39$ ,  $p$ -value = 0.00007). To confirm the similarities between methods, we compared the species and richness and Shannon diversity found using each method. The result showed that rbcl was usually not significantly different to the microscopy result (paired Student's  $t$ -test:  $p$ -value > 0.0001). With further analysis, the differentiation of the spring sample and the summer samples showed that only summer had significant differences between the communities ascertained through microscopy or barcoding (Wilcoxon = 53,  $p$ -value = 0.002).

We calculated two principal component analyses (PCA) of the diatom composition as calculated from microscopy and amplicon sequencing (Supplementary Figure 1). The first two axes of the microscopy PCA represented 53.37% of the cumulative proportion, while the amplicon sequencing represented 73.64%. However, the correlation of both first axes of each method was not significant ( $\rho = 0.19$ ,  $p$ -value > 0.05). An NMDS calculation (Figure 3) resulted in a clear separation of the methods used and, to some extent, the state of the sampled river site (Stress = 0.15). To better follow the site distribution, see Supplementary Figure 3.

## 3.2 Biodiversity capture in extreme hydrological conditions

When comparing the local alpha-biodiversity measured in September 2022 with the values measured in April, there is a clear tendency of reduction (Figure 4). This had also been seen through the reduction in functional biomass measured *in situ* (Supplementary Table 2) and the increase in broken frustule and valve fragments (not shown). Both alpha and gamma diversities were significantly reduced using the amplicon sequencing method of diatom identification.



**FIGURE 2** Comparison of the 10 predominant diatoms as identified with (A) microscopy and (B) rbcL amplicon sequencing, the rest is assigned to the “Other” category. Species codes based on OMNIDIA coding: ADMI, *Achnanthydium minutissimum*; APED, *Amphora pediculus*; GMIC, *Gomphonema micropus*; GPUM, *Gomphonema pumilum*; HCTM, *Humidophila contemnata*; HSIP, *Humidophila simplex*; LFRQ, *Luticola frequentissima*; MPMI, *Mayamaea permitis*; MVAR, *Melosira varians*; NTPT, *Navicula tripunctata*; PLFR, *Planothidium frequentissimum*; PTLA, *Planothidium lanceolatum*; SNIG, *Sellaphora nigri*; XXXX, not assigned to taxon.

**TABLE 2** Genus distribution according to method used for the biodiversity identification and median proportion.

	Microscopy	rbcl
<i>Achnanthydium</i>	r–vf	vr
<i>Amphora</i>	r–f	vr
<i>Caloneis</i>	vr	–
<i>Fistulifera</i>	vr	vr
<i>Gomphonema</i>	vr–f	vr
<i>Humidophila</i>	r–vf	–
<i>Luticola</i>	vr–vf	–
<i>Mayamaea</i>	vr	vr
<i>Melosira</i>	–	vr
<i>Meridion</i>	vr–r	vr
<i>Navicula</i>	vr	r
<i>Nitzschia</i>	vr–r	vr
<i>Pinnularia</i>	–	vr
<i>Planothidium</i>	r–f	r
<i>Psammothidium</i>	vr	–
<i>Sellaphora</i>	vr–f	r
<i>Surirella</i>	vr–r	vr
<i>Not assigned</i>	–	vr–vf

0 < vr < 5; 5 < r < 20; 20 < f < 50; 50 < vf.

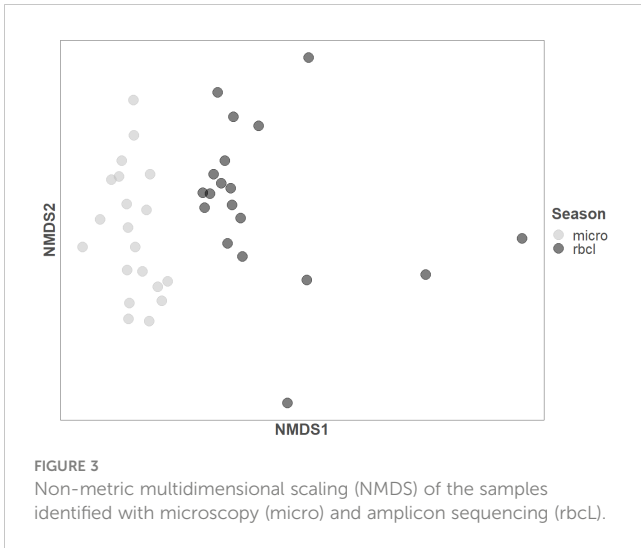
Looking at Jaccard similarities of the molecular and microscopic workflow (Figure 5), differences become clear. A clearer subdivision of diatom communities according to time sampled is found in the microscopy-identified samples, while the rbcL-amplicon-identified samples have more random dissimilarities, rarely following the drying pattern. Furthermore, when comparing the whole dataset (molecular and microscopic), the sites were clustered by methodology, with only the rbcL T1-09-22 sample clustering with the microscopy (Supplementary Figure 2).

The biodiversity was highest in the pool locations, followed by the flowing river state. The dry river had the lowest biodiversity in general (Figure 6). Further comparing the methods of identification, microscopy usually showed a higher value of diversity and a narrower range, which was more spread for amplicon sequencing. In the dry state, the proportion of alpha diversity was even significantly higher. Table 3 shows the alpha, beta, and gamma diversity according to the method and time of sampling.

## 4 Discussion

This study aimed to confirm and to compare the effectiveness of both (digital) microscopy and amplicon sequencing in determining the biodiversity in diatom communities of intermittent rivers. We have found that river intermittency has a big impact on the taxon assignment accuracy of the molecular method, confirming the stated hypotheses. This will mainly be due to the reference library being mostly based on fully humidophilous taxa and lacking the subaerial and terrestrial diatoms that are characteristic of these



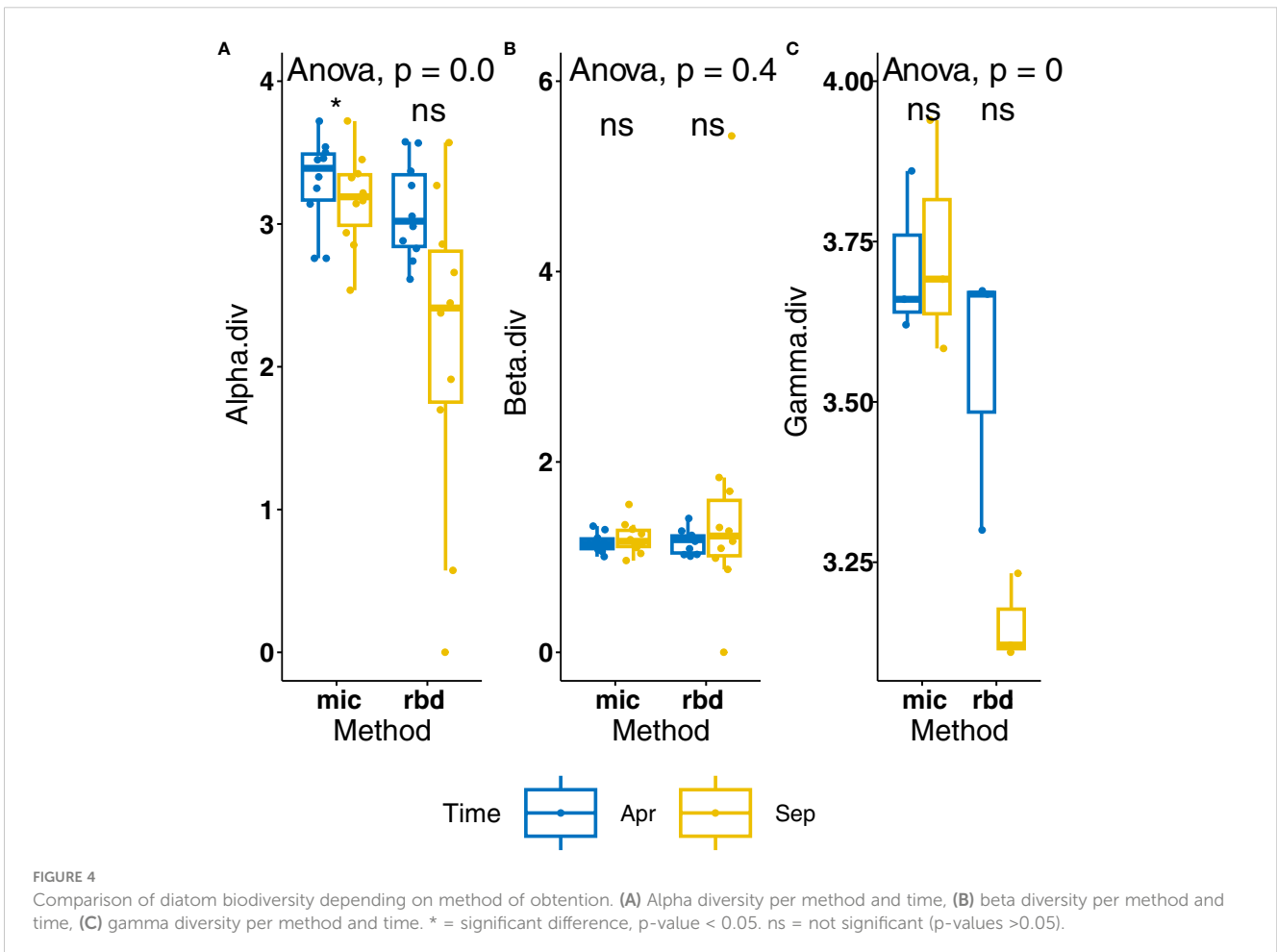


### 4.1 General methodology differences

Even though amplicon sequencing resulted in a higher quantity of amplicon sequence variants (ASVs), the total of assigned taxa was smaller than the number of diatom taxa identified using digital microscopy. Furthermore, even after blasting, the number of assigned diatom taxa was still smaller. In addition, some differences were found between the datasets; for instance, a higher proportion of centric diatoms was found in the amplicon sequences (Supplementary Material). This can be due to the differential quantity of chloroplasts between centric (high quantity and small) and pennate diatoms (low quantity and big, Nonoyama et al., 2019). Although the DADA2 pipeline contains the quantification coefficient (Vasselon et al., 2018) to avoid quantitative errors, the quantity of chloroplasts might still be affecting the read count.

Nevertheless, the chloroplast and biovolume difference might also be the reason why the species composition was partially differential. The higher proportion of big taxa, *Navicula tripunctata* and *Melosira varians*, although present, were found in a lower proportion in the microscopy dataset. However, the predominant taxa, *Achnantheidium minutissimum*, *Amphora pediculus*, and *Gomphonema micropus* were found in all sites, although the variability of percentage found according to the method of identification soared (Supplementary

types of streams. Until now, only a handful of studies have investigated intermittent streams using metabarcoding (Pissaridou et al., 2021). Although the assignation rate was not ascertained, the level of assignation was mostly at taxonomic class level.



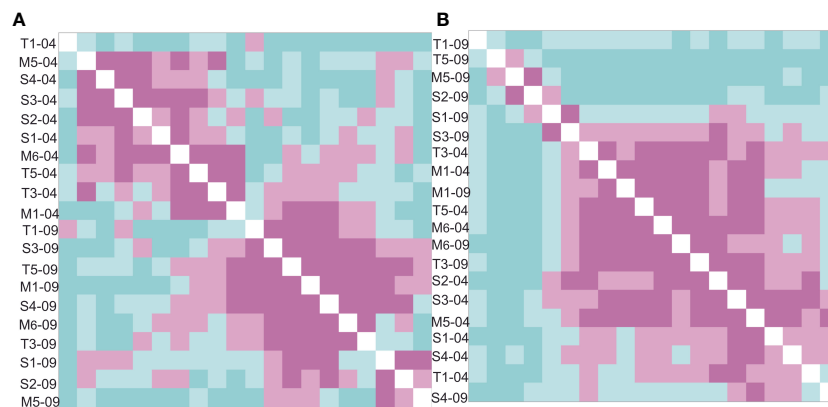


FIGURE 5

Ordered Jaccard dissimilarity matrix of the (A) microscopic and (B) rbcL-amplicon-detected diatom communities. The microscopy shows a clearer time separation (April = black, September, color) than the rbcL-amplicon-identified community. Microscopy also shows a better similarity of similar flow state. Menne = hexagons and circles, Sauer = triangles and reptiles, Talgosse = fish scales (black and color).

Table 3). The change in proportion and even inversion of proportion of two taxa are not uncommon in taxa not pertaining to the most abundant ones (Vidakovic et al., in prep.). This might already point to the unsuitability of the amplicon-sequencing-based identification for biodiversity studies in non-perennial rivers as long as these biomass divergences persist.

Because of these differences between the identification method results, it is not surprising that the similarities between the site and method were quite small (Supplementary Figure 2). Only the site T1 sampled in September, the original pool site found in a dry state, was too different to anything else and clustered only with the microscopy data. Meanwhile, T5, original flowing state sampled dry in September, did not even feature because of the amplicon sequences only amplifying other algae sequences (mostly of the order Vaucheriales). As these sites were dry at the time of sampling, after a heavy drought, the quality of the DNA sampled could have deteriorated, showing the death of the diatoms within the biofilm (Smith et al., 2013; Smith et al., 2019). The dissimilarity method used did not change the pattern of dissimilarity, as no variability was observed when re-calculating the distances of the sites with other formulas (not shown).

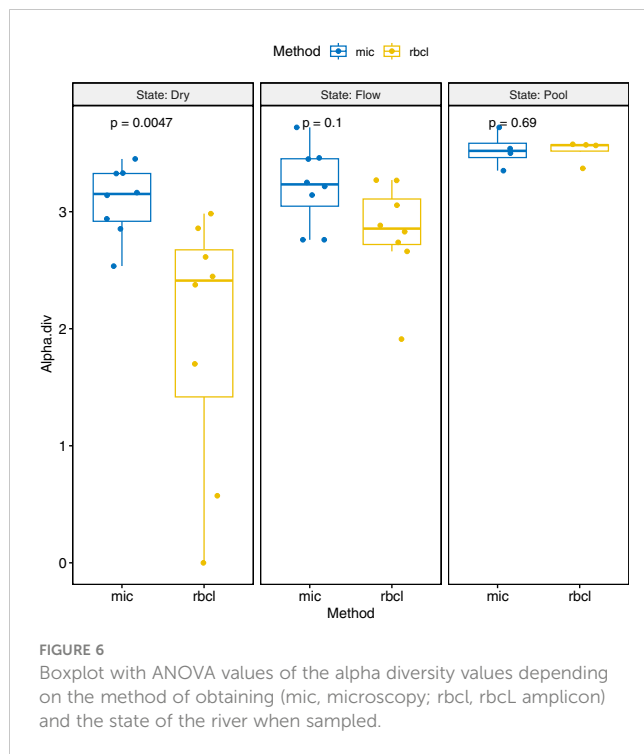
This leads to the question, how many of the diatoms were still alive at the time of sampling? Both methods have some degree of bias to identify the living diatoms from the dead ones. As diatom microscopy looks at diatoms only after the oxidation of the cells, the identification of diatom state can, to some extent, be ascertained through the brokenness of the frustules (shells). To circumvent this, before preparation, we observed an aliquot of each sample under the microscope to confirm the proportion of living cells and used the Benthotorch for biomass estimation in the field (Supplementary Table 2). Through this, we can confirm that although the biomass and general quantity of diatoms were low at these sites, a significant proportion of diatoms was still alive at the time of sampling and should have been caught by amplicon sequencing. However, as explained above, the subaerial taxa are mostly missing from the reference databases and such can be the explanation of the differences (Kulaš et al., 2022).

Due to the low proportion of assigned taxa, alpha and gamma diversity were significantly reduced when using amplicon sequencing (Figure 3). This means that at the moment of this analysis, the use of amplicon sequencing to ascertain biodiversity would have dramatically undershot. This may be a reason for the increase in use of taxonomy-free amplicon sequencing (Apothéloz-Perret-Gentil et al., 2017; Tapolczai et al., 2019; Gregersen et al., 2023). Taxonomy assignments work best where they have been created, and a geographical bias is already visible in permanent rivers. In the case of the rbcL marker, the proportion of assigned taxa names gets gradually smaller the higher the distance is to France, where the marker was initially created (Mugnai et al., 2023). This phenomenon is enhanced when the riparian lotic ecosystem changes to lentic, confirming our first and second hypotheses. Therefore, at this stage, amplicon sequencing would not be encouraged to sole identification of diatom biodiversity.

In order to be able to use amplicon sequencing meaningfully in diatom biodiversity monitoring in non-perennial rivers, the reference databases will need to be updated with the subaerial and aerial taxa. Furthermore, to equate it to “traditional” monitoring based on proportional species distribution, the biomass correction factors should be expanded and updated accordingly.

## 4.2 Biodiversity capture in extreme hydrological conditions

When looking at the effects of the hydrological conditions within the catchments, clear differences between them appear. The point in time in sampling had very different effects on the sampled rivers, as in April, the Menne was mainly flooded, homogenizing the flow type of the whole catchment. In the meantime, in September, the drought mostly affected the Sauer and the Talgosse, leaving all visited sites with either extremely reduced flow (T3), hyporheic (S3 and S2), or completely dry (S1, T1, and T5). However, although reduced, the amount of biomass



measured was still high, including a predominance of diatoms (Supplementary Table 2).

Both methods show a significant clustering of the dry sites. The sampling date also creates a clustering of the spring samples for both methods, while the summer samples were clustering better in the microscopy samples. As the summer samples were taken during an intense drought, the taxa found in excess were mostly subaerial. As already mentioned, they were thus underrepresented in the diat.barcode datasets due to it being adapted to flowing river biomonitoring (Keck et al., 2019; Rimet et al., 2019).

General patterns emerged, as the combination of drought and sampling a dry location reduced alpha diversity of the molecular results significantly. In contrast, in wet environments (both pools and flowing river), the alpha diversity was more similar between the used methods. As this includes both sampling efforts, the time since drying did not seem to have a deleterious effect on the diversity. Furthermore, we had expected the flooding to affect the maturity of the biofilm, as increased flow and turbulence could move the substrate, which would be repeatedly colonized as a result, which we did not observe much, other than the (small) increase in colonizing taxa in those sites (Figure 2). We believe that this confirms our hypothesis that drying would create a divergence between the methods.

**TABLE 3** Comparison of the calculated alpha, beta, and gamma diversity according to sample and identification method.

	Site		Aquatic State	Alpha diversity		Beta diversity		Gamma diversity	
				Mic	rbcl	Mic	rbcl	Mic	rbcl
Menne	M1	April	Flow	2.76	2.83	1.34	1.17	Apr 3.66	Apr 3.30
		September	Flow	3.22	2.66	1.15	1.17		
	M5	April	Flow	3.46	2.74	1.05	1.2	Sep 3.69	Sep 3.11
		September	Dry	2.85	0.57	1.29	5.42		
	M6	April	Flow	3.25	3.27	1.13	1.01		
		September	Pool	3.35	3.57	1.10	0.87		
Talgosse	T1	April	Pool	3.54	3.57	1.06	1.03	Apr 3.61	Apr 3.67
		September	Dry	3.14	1.91	1.14	1.69		
	T3	April	Flow	2.76	3.06	1.27	1.20	Sep 3.94	Sep 3.12
		September	Flow	3.72	3.27	0.96	0.98		
	T5	April	Dry	3.14	2.98	1.14	1.23		
		September	Dry	3.45	0	1.04	0		
Sauer	S1	April	Dry	3.33	2.61	1.20	1.41	Apr 3.56	Apr 3.67
		September	Dry	2.54	2.45	1.55	1.28		
	S2	April	Pool	3.50	3.37	1.14	1.09	Sep 3.58	Sep 3.23
		September	Dry	2.94	1.70	1.34	1.84		
	S3	April	Pool	3.72	3.58	1.07	1.03		
		September	Dry	3.33	2.86	1.18	1.09		
	S4	April	Flow	3.45	2.88	1.16	1.27		
		September	Dry	3.16	2.38	1.25	1.31		



Comparing hydrology, we found that through extreme drought (e.g., the dry locations found in the second sampling in September), the diversity suffered. The alpha diversity was found to be significantly lower in the dry locations, and the gamma diversity was somewhat lower, confirming our third hypothesis.

The similarity of amplicon sequencing samples was increased in dry locations and mostly in the September sampling. We posit that we are observing the effect of taxon homogenization due to drying. On the other hand, the recurring impact of missing subaerial taxa, which varied according to the microscopic identification, may also be responsible for this (artificial) similarity. Up to this time few comparative studies of microscopic and amplicon sequencing identifications have been made in intermittent rivers and streams, but those that exist have found similar effects (Kulaš et al., 2022).

In conclusion, drying does affect diatom communities and reduce their diversity after prolonged drought. Using different methods, we have found that digital microscopy showed a higher resolution and diversity in intermittent streams due to the incomplete reference databases for dry(ing) habitats. In the future, the use of both combined methods to calculate bioindex values might be of interest, as regrettably, the trend of intermittency is reaching ever further in the European continent, and as it currently stands, a lot of rivers might fall out of the regulation of the Water Framework Directive because of it. However, for the time being, the unique use of amplicon sequencing for non-perennial rivers would not be advisable, as information on subaerial taxa and proportion of the taxa within the community would be missing.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary Material](#).

## Author contributions

AB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. PM: Data curation, Formal analysis, Writing – review & editing. MD: Data curation, Formal analysis, Writing – review & editing. BB: Funding acquisition, Resources, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2024.1355018/full#supplementary-material>

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