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METHOD

It's raining species: Rainwash eDNA metabarcoding as a minimally invasive method to assess tree canopy invertebrate diversity

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Abstract

Forest canopies are highly diverse ecosystems, but despite several decades of intense research, there remain substantial gaps in our knowledge of their biodiversity and ecological interactions. One fundamental challenge in canopy research is the limited accessibility of the ecosystem. Consequently, previous studies have relied on the application of either highly invasive methods such as chemical knockdown, or on time-consuming and expensive setups such as canopy walkways or cranes. Therefore, time- and cost-efficient, ideally minimally invasive yet comprehensive applications are required to help close this knowledge gap. High-throughput metabarcoding of environmental DNA (eDNA) collected from water, soil, or air provides a minimally invasive method for biodiversity assessment, yet its potential for canopy biodiversity monitoring has not been explored. Herein, we conducted metabarcoding of eDNA washed off the canopy via rainwater to explore its potential for biodiversity monitoring and ecological research. We placed four 1 m^2 rain samplers beneath the canopies of four different trees (beech, oak, larch, and pine) prior to a major rain event, filtered eDNA from the collected rainwater, and performed cytochrome c oxidase subunit I (COI) gene metabarcoding to profile the invertebrate community. Additionally, we collected and identified all specimens present in the rainwater to assess if eDNA only came from specimens physically present in the rainwater. We detected 50 invertebrate species by eDNA metabarcoding, of which 43 were not physically present in the water sample, thus likely representing true canopy biodiversity signals. Furthermore, we observed distinct species occurrence patterns corresponding to the four trees, suggesting that ecological patterns such as host specificity can potentially be assessed using the method. In conclusion, our study provides a proof of principle that rainwash eDNA metabarcoding offers a minimally invasive and comprehensive method for biodiversity monitoring in tree canopies.

Till-Hendrik Macher and Robin Schütz contributed equally to this study.

KEYWORDS
Arthropoda, biomonitoring, environmental DNA, forest ecosystem, insect diversity

1 | INTRODUCTION

The forest canopy is a particularly species-rich zone. However, despite many decades of intense canopy research, there remain substantial gaps in our knowledge of the biodiversity and ecological interactions of canopy communities (Nakamura et al., 2017). This includes both tropical rainforests with their vast array of undescribed species (Basset et al., 2012), and temperate forests (Sallé et al., 2021). The degradation and loss of forests are accelerating in many regions of the world due to global climate change. Assessing this loss in forest cover is relatively straightforward with remote sensing tools, and the consequences on net global carbon balance and other biogeochemical processes can be modeled (e.g., Bondeau et al., 2007). However, estimating the effects on species diversity and interactions remains challenging, especially for the canopy community, which is difficult to access. To address this knowledge gap, reliable data on species occurrence are needed across spatial and temporal scales, as well as across different trophic levels (Seibold, Cadotte, et al., 2018). Yet, this is difficult to achieve with classical canopy habitat assessment methods.

Ozanne (2005) reviewed established techniques and methods to sample and assess canopy arthropods. The simplest technique is to access the canopy directly via rope climbing, canopy walkways, or canopy cranes to collect samples. But these methods require experience in tree climbing or setting up permanent platforms and walkways (Parker et al., 1992). Further commonly applied techniques are based on chemical knockdown using insecticides (Leroy et al., 2022). The chemicals are distributed through fogging (i.e., hot clouds of chemical droplets rising upwards) or mist blowing (i.e., blowing an air current with chemical droplets into the canopy). The stunned, falling insects are caught in collection hoops and can be identified by morphological assessment (Floren et al., 2022; Pedley et al., 2016) or bulk sample DNA metabarcoding (Creedy et al., 2019). Another approach is branch bagging and clipping (i.e., covering the branch in a cloth or bag and cutting the branch), which has the advantage of directly correlating species richness or density with plant or leaf biomass (Krehenwinkel et al., 2022). One of the challenges with bagging and clipping is that only the low canopy can be sampled unless it is done by a tree climber, and that sample sizes are hence often small. Additionally, it has a negative bias toward flying insects which might fly off when being disturbed in the process of sampling. Trapping methods with defined entry areas, such as canopy malaise traps (Skvarla et al., 2021) and flight interception traps (Kowalski et al., 2011), have also been applied to record canopy insects, and vertically stratified artificial substrates have been employed to sample and analyze the distribution of arthropods such as deadwood beetles (Seibold, Hagge, et al., 2018). However, malaise and flight interception traps are prone to miss non-flying or generally less mobile insects.

In order to monitor canopy invertebrate diversity and trace biodiversity change reliably, all the above-mentioned methods have drawbacks. Therefore, novel approaches are required that are ideally fast, taxonomically comprehensive, non-invasive, and simultaneously cost-efficient. We here propose and test one such potential canopy invertebrate monitoring method that involves the collection of rainwash environmental DNA (eDNA). eDNA can be extracted from environmental samples such as soil (Drummond et al., 2015; Pansu et al., 2015), water (Harper et al., 2019; Nakagawa et al., 2018), or air (Lynggaard et al., 2022; Roger et al., 2022), without first isolating any target organisms (Taberlet et al., 2012). Today, eDNA metabarcoding is an established method in marine, freshwater, and terrestrial biodiversity research (Deiner et al., 2017). However, the potential of eDNA metabarcoding of rainwater to assess canopy insect diversity has not been explored. Valentin et al. (2021) investigated the effect of rain on the fate of arthropod eDNA and found that rainfall or mist removes most terrestrial eDNA present on vegetation surfaces. Building on this idea, we performed a simple proof-of-principle analysis and hypothesized that using a rainwash sampler, canopy invertebrates can be detected reliably by eDNA metabarcoding of the collected water shortly after a rain event.

2 | MATERIAL AND METHODS

2.1 | Rain sampler

Four rain samplers were built using 1 m^2 of 0.5 mm PVC pond liner (Sika), eight 1 m PVC tubes with a 50 mm diameter, four PVC three-way tube connectors with a 50 mm diameter (HT CONNECT), 20 reusable zip ties, and 22 copper eyelet rings (Vastar). First, the pond liner was cut to 1 × 1 m, five holes were punched on all four sides, and copper eyelet rings were used to support the holes (Figure S1). Two additional holes as overflow outlets were implemented and supported with eyelet rings 25 cm from the center of the liner. This allowed for ~4 L of water to be collected while protecting the liner from tearing due to weight. The liner was sterilized by applying 1% bleach, which was then washed off using 80% ethanol, followed by deionized water. The liner was then sterilized using UV radiation for 30 min, folded with sterile gloves, and placed in a plastic bag. In the field, four of the 1 m PVC tubes were connected to a square using the three-way tube connectors. The liner was then placed in the frame using sterile gloves and fastened using 20 reusable zip ties while leaving enough room for the liner to expand when water is collected. The other four PVC tubes were then inserted into the three-way connector as legs.

2.2 | Sampling sites

The sampling sites were located within a >1000 ha forest area in the lower Rhine region of Germany (N 51.707104, E 6.549781), which includes the "Diersfordter Wald" nature protection area and the "Großes Veen" EU Special Area of Conservation (92/43/EEC). Multiple different forest types with different tree species occur in this area. Four rain samplers were set up the evening before a major rain event on June 19, 2021 (Figure 1). One rain sampler each was placed under the canopy of a beech tree (*Fagus sylvatica*, site S1), an oak tree (*Quercus robur*, S4), a larch tree (*Larix* sp., S2), and a pine tree (*Pinus sylvestris*, S3). Site S1 was an old-growth beech forest, and S2 was a planted larch monoculture (see Figure S2). The sites were between 200 m (S3 and S4) and 3 km (S1 and S3) apart (Figure 2).

2.3 | Verification specimens

To confirm whether the collected eDNA originated from the canopy (washed from leaves and branches) or solely from organisms falling or flying into the rain sampler, all organisms present in the rain samplers were collected. For this, invertebrate specimens were picked using forceps and stored in 80% ethanol. Specimen collection was conducted after eDNA sampling to prevent contamination of the rainwater. All collected specimens were morphologically identified to at least order level using a ZEISS Stemi SV 11 stereo microscope (Oberkochen, Germany).

2.4 | eDNA sampling

A 2 L volume of water was collected from each rain sampler in two sterile 1 L bottles (Nalgene) 45 h after setting up the samplers. Within this timeframe, the closest weather station (20 km distance) reported a total precipitation of 35 mm, while the temperatures were between 16°C at night and 26°C during the day (data from wetteronline.de for Duisburg-Baerl, June 2021). Two individual bottles per site were filled with water that was discharged through the overflow outlet in the pond liner. The water was immediately filtered on site next to the rain sampler by pumping the water from the bottle using a Vampire Sampler peristaltic pump (Buerkle) and collecting eDNA using Whatman Polydisc AS disk filters (PES, 50 mm diameter, 0.45 µm pore size, sterile, Maidstone, UK). After filtration, TNES buffer (50 mM Tris, 0.4 M NaCl, 100 mM EDTA, 0.5% SDS) was added to the disk filters using 1 ml syringes (Braun), and filters were closed using sterile rubber plugs. Additionally, one field blank was taken by filtering 1 L of deionized water from a sterile 1 L bottle on site. The field blank was otherwise handled similarly to the samples. The filters and the field blank were stored on ice in the field and placed at −20°C in a freezer until extraction.

2.5 | eDNA extraction

All wet lab steps were conducted under sterile conditions in a dedicated sterile laboratory (UV lights, sterile benches, overalls, gloves, and face masks). Extraction and PCR were conducted under different sterile benches to prevent cross-contamination. First, 100 µl TNES buffer and 10 µl Proteinase K (300 U/ml, 7BioScience,) were added to the inlet of each filter, and all filters were incubated at 55°C with shaking at 1000 rpm for 3 h on an Eppendorf ThermoMixer C instrument (Eppendorf AG). After lysis, liquid was extracted from the filter using a sterile 5 ml syringe (Braun) and transferred to a new 2 ml Eppendorf tube. Subsequently, DNA was extracted using an adapted NucleoMag tissue kit (Macherey Nagel, Appendix S1). In total, a volume of 400 µl per sample was extracted.

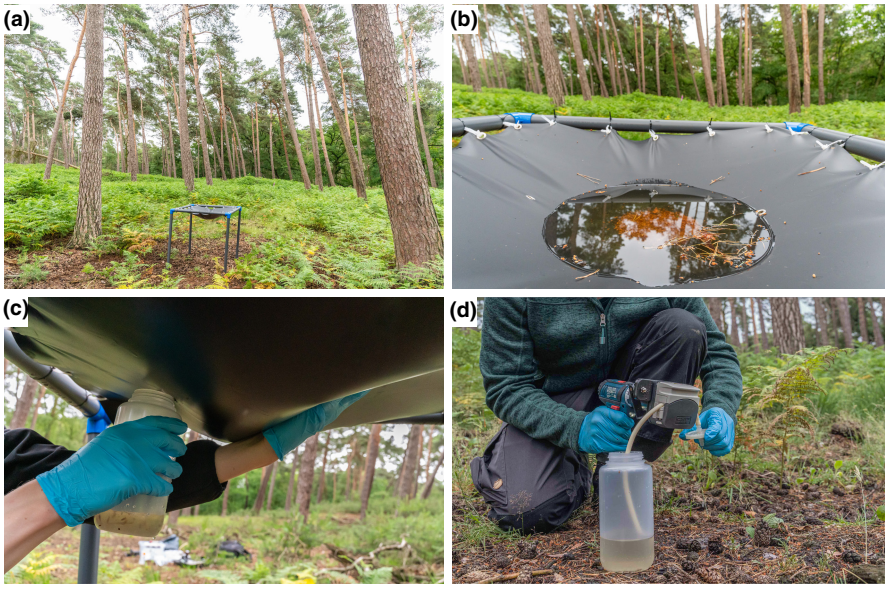

FIGURE 1 Setup of the rain sampler prototype at sampling site S3 in a pine forest (a). Approximately 4 L of rainwater was collected in the liner (b) and was transferred into sterile bottles by pushing the water toward the overflow hole (c). A 2 L volume of rainwater was then filtered using a hand-held pump and encapsulated filters (d).

FIGURE 2 Map of the sampling sites located near Wesel, Germany. Rain samplers were placed below four different tree taxa: beech (*Fagus sylvatica*), larch (*Larix* sp.), pine (*Pinus sylvestris*), and oak (*Quercus robur*). Orthophotos Geobasis NRW, TIM-online 2.

2.6 | eDNA amplification and sequencing

A tagged two-step PCR approach (Bohmann et al., 2022) was applied for amplifying the extracted DNA. In the first PCR step, tagged versions of primers fwhF2 and fwhR2n (Vamos et al., 2017) were used, which are optimized for invertebrates, target a 205 bp cytochrome c oxidase subunit I (COI) gene fragment, and are known to reliably amplify DNA from terrestrial insects (Elbrecht et al., 2019). In total, 20 first-step PCR amplifications were conducted, including two replicates per sample, two negative PCR controls, and one field blank to control for potential cross-contaminations. The reaction volume was 25 µl, consisting of 4 µl PCR-grade water, 2.5 µl CoralLoad dye, 12.5 µl Multiplex Mastermix (Qiagen Multiplex PCR Plus Kit, Qiagen), 0.5 µl fwhF2 forward primer (10 µm), 0.5 µL fwhR2n reverse primer (10 µM), and 5 µL of DNA template. The first PCR step was carried out at 95°C for 5 min, followed by 10 touchdown cycles at 94°C for 30 s, 68–59°C for 90 s (with a decrease of 1°C per cycle), and 72°C for 30 s, followed by 25 cycles at 94°C for 30 s, 58°C for 90 s, and 72°C for 30 s. The final elongation was carried out at 68°C for 10 min. In the second PCR step, Illumina sequence adapters with a dual twin-indexing system were added (Bohmann et al., 2022; Buchner et al., 2021). For each sample, the second-step PCR mix contained 4 µl PCR-grade water, 2.5 µl CoralLoad dye, 12.5 µl Multiplex Mix, 1 µl combined primer (10 µM), and 5 µl first-step product. PCR conditions were 95°C for 5 min, followed by 15 cycles at 94°C for 30 s and 72°C for 120 s. The final elongation was carried out at 68°C for 10 min. Following second-step PCR, products were visualized on a 1% agarose gel to evaluate amplification success. Negative controls did not produce bands on the gel. Thus, PCR products were subsequently normalized to 25 ng per sample using a SequalPrep Normalization Plate (Applied Biosystems) following the manufacturer's protocol. Subsequently, normalized PCR products were pooled into one library with samples from a different project. The pooled library was concentrated using a NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel) following the manufacturer's protocol. The final elution volume of the library was 40 µl. The library was then analyzed using a Fragment Analyzer (High Sensitivity NGS Fragment Analysis Kit; Advanced Analytical) to check for potential primer dimers and co-amplification, and to quantify the DNA concentration of the library. Primer dimers were removed by cutting the library from a 2% agarose gel and extracting the DNA from the gel using a NucleoSpin Gel and PCR Clean-up kit (Macherey Nagel). Subsequently, samples were normalized and pooled, and the final library was concentrated using a NucleoSpin Clean-up kit. The resulting library (together with samples from a different project) was sequenced on a HiSeq X platform using the 150 bp PE kit at Macrogen (Seoul, Rep. of Korea).

2.7 | Bioinformatics

Raw reads were received as demultiplexed fastq files. All samples were processed with the APSCALE-GUI pipeline v1.2.0 (https://github.com/TillMacher/apscale_gui), which is based on VSEARCH (Rognes et al., 2016) and cutadapt (Martin, 2011). All settings were kept as default, and OTUs were clustered with a 97% percentage similarity threshold. Subsequently, taxonomy was assigned using BOLDigger (Buchner & Leese, 2020), which automatically performs an identification search against the BOLDsystems COI database (www.boldsystems.org). The resulting taxonomy table was filtered using the "JAMP filtering" option (98%: species level, 95%: genus level, 90%: family level, 85%: order level, <85%: class level). Both the taxonomy and read table were then converted to a TaXon table (Table S1) for downstream analyses in TaxonTableTools v1.4.1 (Macher et al., 2021).

Initially, PCR replicates were merged, and only OTUs present in both PCR replicates were kept. To account for potential contamination, a strict read filter was applied where the sum of reads per OTU that were present in negative controls were subtracted from the reads per OTU of each sample. The dataset was then filtered by taxonomic groups, and only OTUs with similarity ≥85% to the nearest reference sequence and that were assigned to the phyla Arthropoda, Nematoda, and Tardigrada were kept. This TaXon table was used for all downstream analyses (Table S2). Additionally, a taxon list was created in which OTUs with the same taxonomy were merged to a single entry (Table S3).

Despite read abundances being distorted by factors such as differences in primer binding efficiencies or DNA shedding rates, they

hold some quantitative information (Krehenwinkel et al., 2017). Thus, relative read abundances per invertebrate species were analyzed for distribution patterns across the four tree taxa. Therefore, species with a relative read abundance of ≥70% assigned to one of the four trees were categorized as mainly detected on "beech," "oak," "larch," "pine," or "unspecified" if a species did not show ≥70% relative read abundance to one of the trees. The distribution of fungi OTUs (Ascomycota and Basidiomycota) was investigated accordingly.

Based on existing ecological information (Appendix S2), species were categorized according to their host-tree association (phytophagous on oak, larch, pine, or broad-leaved trees), which was compared to their respective read abundance-based patterns. To assess the larval ecology association based on the eDNA metabarcoding results, the detected species were categorized by their respective traits and occurrence patterns (relative read abundance).

To investigate whether the species detected by eDNA metabarcoding were true rainwash eDNA signals or mostly signals derived from specimens that fell into the rain sampler during sample collection (verification specimens), both species lists were compared and visualized in a Venn diagram.

3 | RESULTS

3.1 | eDNA metabarcoding

Sequencing yielded a total of 30,005,824 raw reads. In total, 10,631,775 quality-filtered reads were clustered into 982 OTUs. The average number of reads per sample was 664,485 reads (±197,104). An average of 647 reads (±315) were assigned to field blanks and negative controls (8 OTUs), of which 635 reads were assigned to *Baetis rhodani* (Table S1). After PCR replicate merging and subtraction of the sum of reads per OTU that were present in the field blanks and negative controls, 389 OTUs with similarity ≥85% to reference sequences remained. For downstream analyses, we kept only OTUs of the phyla Arthropoda (103 OTUs assigned to 48 species), Tardigrada (6 OTUs assigned to 2 species), and Nematoda (2 OTUs not assigned on species level). Most species belonged to the orders Lepidoptera (17) and Coleoptera (13), while the remaining 17 orders were represented by fewer than three species each (Figure S3). Additionally, we found 299 OTUs that were assigned to Ascomycota, Basidiomycota, or Zygomycota in the pre-filtered data.

In total, we detected 21 species under both broadleaf trees (beech and oak) and conifers (larch and pine), while 20 and nine species were exclusively detected under broadleaf trees and conifers, respectively (Table S2). When using relative read abundances as a proxy, more distinct occurrence patterns were observed; 20 species were detected mostly under the oak tree, while eight species were mainly found under the beech tree (Figure 3). For conifers, nine species were detected predominantly under the larch tree, and seven species mainly beneath the pine tree. The remaining six species showed no distinct occurrence patterns toward any specific host tree. However, four of the taxa were predominantly found under the two conifer trees. Accordingly, host-tree specific occurrence patterns were found for fungi OTUs.

In total, 25 phytophagous species were detected in the eDNA data. Of these, five species are known to be monophagous on oak, compared with two on pine and two on larch. A further 16 detected species are described as being associated to broad-leaved trees, while the habitat of one phytophagous species is not specified. In 21 cases, the association was congruent with the species occurrence

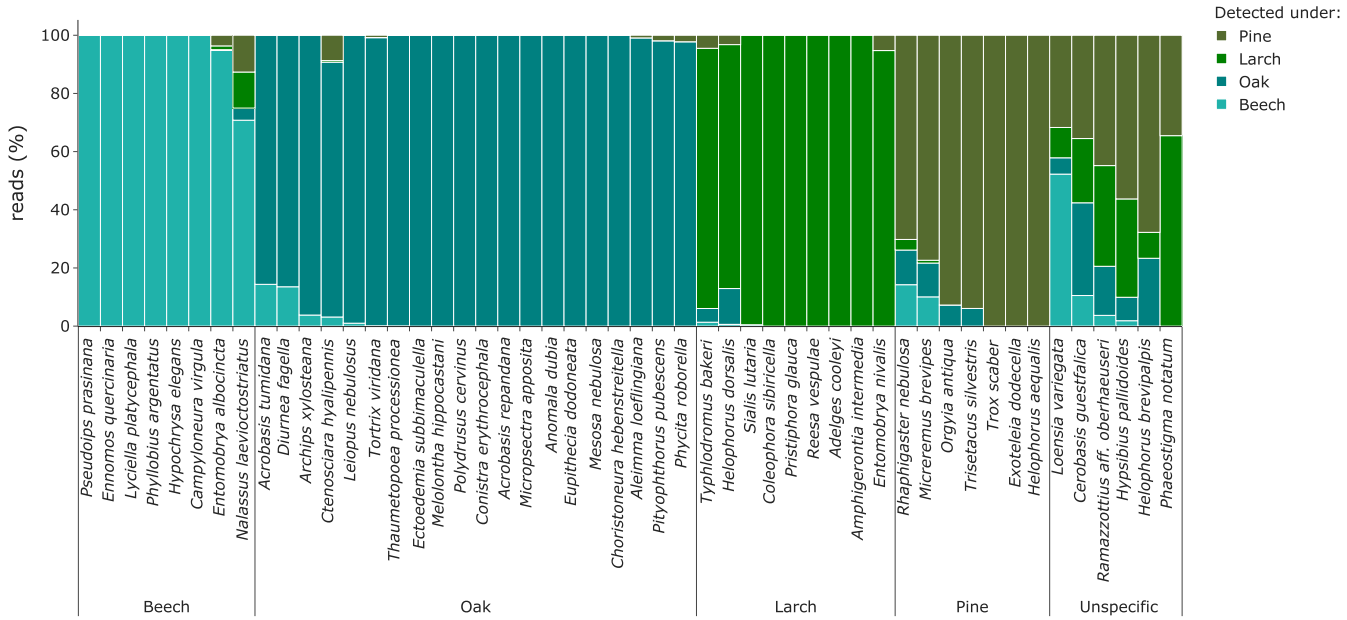

FIGURE 3 Relative read abundances per host tree for all detected invertebrate species. Species are grouped by tree host according to their relative read abundance (threshold ≥70% relative read abundance).

patterns observed in the eDNA results (Figure 4). For three species, detection was less congruent with their ecology.

3.2 | Verification specimens

We sampled 220 invertebrate specimens from the rain samplers after eDNA filtration and identified 18 invertebrate taxa (13 of which could be assigned to species level) based on morphological identification (Table S3). The specimens belonged to the orders Coleoptera (4 species), Hymenoptera (4), Diptera (1), Hemiptera (1), Isopoda (1), Julida (1), and Lepidoptera (1). When comparing the identified species that were present in the water to the species detected by eDNA analysis, 43 species were detected only by eDNA, seven species were observed with both methods, and six species were only detected through morphological identification (Figure 5).

4 | DISCUSSION

Our results support our hypothesis that eDNA metabarcoding of rainwater collected below the tree canopy can detect many canopy invertebrate species. Despite the small number of samples collected in our pilot study, we detected a remarkable number of invertebrate species from rainwater eDNA. We found significantly more species through eDNA metabarcoding than through morphological identification, highlighting that most of the detected signal derived from the canopy, rather than from specimens that fell into the rain-sampler. However, six species were exclusively identified through morphological identification. All these species were present in low abundances, which generally reduces their detectability with eDNA. The detected aquatic beetles of the genus *Helophorus*, found with both methods, likely colonized the small water bodies created in the rain samplers as they usually inhabit puddles. Overall, these results suggest that most species detected within the rainwash water were eDNA signals washed into the sampler from the tree canopy. This aligns with results reported by Valentin et al. (2021) showing that invertebrate eDNA resides on vegetation surfaces and can be washed off and detected. However, it is highly likely that only a very small proportion of the eDNA released by the invertebrate community in the canopy was detected in rainwash eDNA, since the samplers only covered an area of 1 m^2. Additionally, the observed species richness will most likely vary considerably with different rain intensities. The minimal amount of precipitation needed to recover most of the canopy community via rainwater metabarcoding needs to be explored in future studies. Nevertheless, our results revealed a large number of canopy invertebrate species in the rainwater. By comparison, Leroy et al. (2022) reported 1213 putative species (i.e., barcode index numbers in the BOLD database) in 24 trees and 757 of them were assigned to a described species in the BOLD database. Further, morphology-based studies reported, for example, 143 ichneumon wasp species in canopies of 31 oak trees (Horstmann & Floren, 2001), 33 xylobiont coleopteran species in 10 pine trees (Schmidl et al., 2004) or 297 lepidopteran species over a course of two summer sampling campaigns (Erlacher et al., 2009). However,

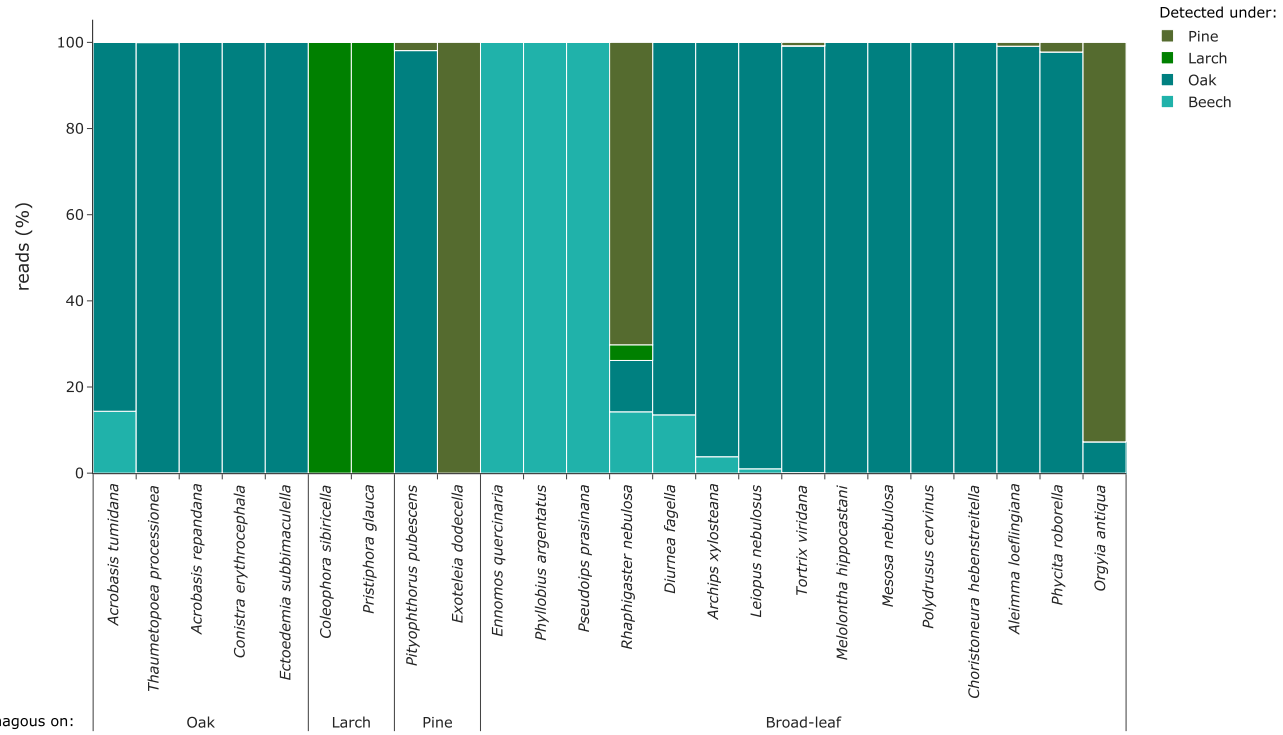

FIGURE 4 Relative read abundances per host tree for all detected phytophagous invertebrate species. Species are grouped by their host–plant associations traits: phytophagous on "oak," "larch," "pine," and "broad-leaf trees."

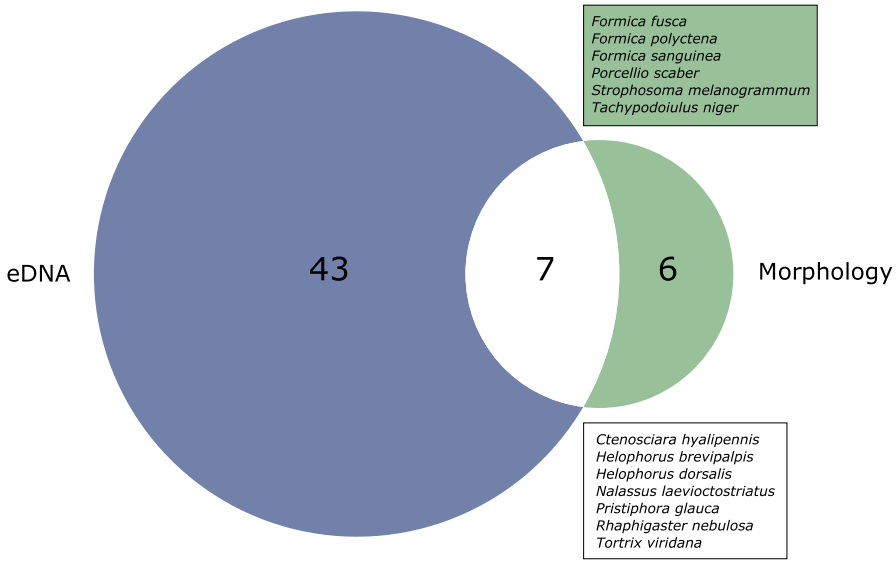

FIGURE 5 Number of species that were detected by eDNA metabarcoding alone (blue), by both methods (white box), or exclusively by morphological identification of specimens collected from the rain sampler (green box).

most morphology-based studies report results on a higher taxonomic level than species (Floren et al., 2022) rendering a comparison to better-resolved DNA metabarcoding results difficult. Overall, the comparison to previous canopy arthropod monitoring results suggests that even a small subsample of the whole rainwater that passes through the canopy can offer substantial and novel insights into the invertebrate community. To further maximize detection, more samplers covering a greater area would be needed. Also, the implementation of optimized or additional eDNA metabarcoding protocols (Alberdi et al., 2018), including additional or improved primers, filtration of larger volumes of water, or more PCR replicates might help to further increase the taxonomic completeness and detect taxonomic groups that remained underrepresented in this study (e.g., Formicidae, Aranea, and Opiliones). Further, at this point false-negative detections due to DNA degradation cannot be excluded, as the study design did not include a direct comparison and secondly there is a lack of information regarding eDNA degradation in the respective terrestrial ecosystems. Studies including several methods such as bagging and clipping next to rainwash analyses are needed to address potential false-negative observations.

Furthermore, our results suggest that eDNA can reveal differences in taxonomic composition between different tree hosts. Despite the limited number of rain samplers used in this study, and hence the limited number of individual trees per species and the limited overall tree canopy coverage, the rainwash eDNA results revealed distinct species occurrence patterns under the four different trees. In total, 88% of detected species (from 50 species under 4 trees) were assigned to a certain tree host based on their relative read abundances. In fact, many of those species have a known host specificity toward distinct tree taxa, such as *Acrobasis repandana* to oak, *Exoteleia dodecella* to pine, and *Pristiphora glauca* to larch (Appendix S2). Other species, such as *Trox scaber*, occupy bird nests and have only a secondary ecological associations with trees (Appendix S2). Additionally, our unfiltered data included a remarkable number of fungal OTUs displaying tree-specific occurrence patterns (Figure S4). This provides evidence that fungal eDNA was washed from the canopy alongside invertebrate eDNA, and that both can be extracted from rainwater, which opens the possibility for multimarker analyses with additional fungi-specific DNA metabarcoding markers. This facilitates the analysis of even more complex multitrophic ecological interactions, such as invertebrate and fungi co-occurrence patterns.

Our results demonstrate the potential of rainwash eDNA metabarcoding as a rapid and minimally invasive method for measuring canopy invertebrate diversity. While our occurrence data have limited statistical power, due to the small sample size, they suggest that local canopy communities can be distinguished using rainwash eDNA. In particular, phytophagous insects that specialize on single host tree species were detected locally in our results, which demonstrates the potential for applications in forestry or forest sciences.

To generate statistically robust results, future studies with more comprehensive designs are required. For example, several rain samplers should be set up per forest type, and several forests of the same type could be investigated. For this, stationary and passive hard-shell rain samplers could be implemented in forest survey areas, with regular emptying. To record forest-specific communities or target communities of specific shrub or tree species, rain samplers could be installed at different heights in the canopy. In urban setups, rain samplers could potentially be installed in water catchment trays that are often used to enhance the supply of water to urban trees. Specific collection of metadata (e.g., tree height, diameter at breast height, crown density) could also generate multivariate data in addition to the samples and could be used for a more standardized setup. Since our rainwash eDNA metabarcoding approach relies on natural rain events, its most promising field of application lies in the canopy biomonitoring of rainforests or areas with regular precipitation. However, in drier regions, actively rinsing eDNA off bushes or tree canopies with a water hose could be an alternative approach, as already conducted by Valentin et al. (2020) for species-specific assessments.

In conclusion, rainwash eDNA metabarcoding has the potential to substantially advance forest canopy biodiversity monitoring. Our results highlight the possibility of a minimally invasive, cheap, and comprehensive approach, which could even be expanded to complex multitrophic ecological analyses. With further improvements, our method could significantly contribute to closing the gaps in our knowledge of biodiversity and ecological interactions of canopy communities.

AUTHOR CONTRIBUTIONS

RS, TM, TH, AB, and FL conceived the study design. RS and TM conducted eDNA sampling. TH collected and identified verification specimens and provided expertise on traits. TM conducted the laboratory workflow and bioinformatic analyses. TM and RS drafted the first version of the manuscript. All authors contributed to the article and approved the version to be published.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The raw data were deposited at the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home) under the accession number PRJEB51872.

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