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RESOURCE ARTICLE

Plant-derived environmental DNA complements diversity estimates from traditional arthropod monitoring methods but outperforms them detecting plant–arthropod interactions

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Abstract

Our limited knowledge about the ecological drivers of global arthropod decline highlights the urgent need for more effective biodiversity monitoring approaches. Monitoring of arthropods is commonly performed using passive trapping devices, which reliably recover diverse communities, but provide little ecological information on the sampled taxa. Especially the manifold interactions of arthropods with plants are barely understood. A promising strategy to overcome this shortfall is environmental DNA (eDNA) metabarcoding from plant material on which arthropods leave DNA traces through direct or indirect interactions. However, the accuracy of this approach has not been sufficiently tested. In four experiments, we exhaustively test the comparative performance of plant-derived eDNA from surface washes of plants and homogenized plant material against traditional monitoring approaches. We show that the recovered communities of plant-derived eDNA and traditional approaches only partly overlap, with eDNA recovering various additional taxa. This suggests eDNA as a useful complementary tool to traditional monitoring. Despite the differences in recovered taxa, estimates of community α- and β-diversity between both approaches are well correlated, highlighting the utility of eDNA as a broad scale tool for community monitoring. Last, eDNA outperforms traditional approaches in the recovery of plant-specific arthropod communities. Unlike traditional monitoring, eDNA revealed fine-scale community differentiation between individual plants and even within plant compartments. Especially specialized herbivores are better recovered with eDNA. Our results highlight the value of plant-derived eDNA analysis for large-scale biodiversity assessments that include information about community-level interactions.

KEYWORDS
biodiversity, eDNA, high-throughput sequencing, monitoring, plant–arthropod interaction

Sven Weber and Manuel Stothut shared first authorship.

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1 | INTRODUCTION

In response to worrisome reports of a global insect decline, the monitoring of arthropod communities has become a key priority of ecological research in recent years (van der Sluijs, 2020; Wagner et al., 2021). Passive sampling methods such as Malaise or pitfall traps are commonly employed for arthropod monitoring (Montgomery et al., 2021), resulting in significant information on temporal and spatial changes of biodiversity, thus indicating the current state of an arthropod population (Crossley et al., 2020; Hallmann et al., 2017; Sánchez- Bayo & Wyckhuys, 2019; Seibold et al., 2019). However, these trapping methods provide limited information on the ecology of sampled taxa and are primarily used to provide an overview of local taxonomic diversity. To fully understand the consequences of biodiversity loss, the ecological role of individual species and their manifold interactions with other taxa in the ecosystem must be understood (Hines & Pereira, 2021). Studying plant–arthropod interactions is of particularly high relevance, as arthropods are among the most important pollinators and herbivores of plants (Crawley, 1989; Haddad et al., 2009; Knops et al., 1999; Schaffers et al., 2008; Siemann et al., 1998). For example, a single invasive herbivore or the loss of an important pollinator can have devastating effects on local plant and arthropod communities (Myers & Sarfraz, 2017; Valiente-Banuet et al., 2015).

Active assessment methods typically involve visual observations of interactions to identify plant–arthropod interactions. However, these methods come with high sampling effort (Hurlbert et al., 2018; Novotny et al., 2002; Volf et al., 2019). Another approach involves collecting arthropod community samples from individual plants through techniques such as vegetation beating or branch clipping, and assuming interactions based on the presence of a species on a plant (Graham et al., 2022; Harris et al., 1972; Moir et al., 2005). However, the presence of an arthropod does not necessarily indicate an interaction, as the specimen collected by traditional methods might simply be a vagrant species resting on the plant. Moreover, visual censuses or sampling from plants may overlook arthropod taxa living within the plant tissue, as many taxa spend their entire lives inside their host plants, only emerging briefly as adults, mate and die. Given the vast array of plant–arthropod interactions and the complex life cycles of many arthropods, this research area remains significantly understudied (Gardarin et al., 2018).

A possible solution to solve this problem and to reliably recover community-level plant–arthropod interactions is environmental DNA (eDNA) analysis. When an arthropod interacts with a plant, it leaves behind traces of its DNA, for example, through chewing, puncturing or defecation (Beng & Corlett, 2020; Kudoh et al., 2020; Thomsen & Willerslev, 2015). Consequently, DNA will primarily be deposited by taxa which closely interact with the plant and much less by vagrant taxa. Hence, eDNA holds the potential to outperform traditional monitoring approaches to detect plant–arthropod interactions.

eDNA is typically recovered from plant surfaces by washing it off with water and filtering the wash-off (Allen et al., 2022; Macher et al., 2023; Valentin et al., 2020). However, washes are probably limited to taxa from the surface of the plant. Therefore, the diverse arthropod community that resides inside leaf tissue may be omitted by surface washes. A possible alternative to surface washes is the homogenization of whole plant compartments and DNA isolation from the ground plant material (Krehenwinkel, Weber, Broekmann, et al., 2022; Krehenwinkel, Weber, Künzel, & Kennedy, 2022), which possibly recovers more endophytic species.

Considering this background, monitoring arthropod communities using plant-derived eDNA holds the promise to elevate arthropod monitoring of spatiotemporal taxonomic composition with community-level interaction networks, thereby addressing a critical shortfall in biodiversity research (Hortal et al., 2015). However, a detailed comparative analysis of the performance of plant-derived eDNA in relation to traditional arthropod monitoring approaches for the recovery of arthropod diversity patterns and plant–arthropod interactions is still lacking. To close this gap, we test the following hypotheses:

1. Plant-derived eDNA complements traditional monitoring approaches by recovering additional taxa. Particularly, eDNA analysis from ground plant material will provide an important complement over surface washes to recover arthropods living inside plant tissue.
2. Plant-derived eDNA outperforms traditional approaches for monitoring plant–arthropod interactions. Plant-specific or plant tissue-specific arthropod taxa will be recovered more reliably with eDNA than with traditional monitoring. eDNA should thus provide a more fine-scaled image of community differentiation than traditional monitoring.
3. Plant-derived eDNA will recover similar patterns of community diversity as traditional arthropod trapping approaches. While the different methods will not detect identical taxa lists, patterns of diversity within a site (α-diversity) as well as community differences between sites (β-diversity) should be comparable between eDNA and traditional monitoring approaches.

To test these hypotheses, we designed a series of four experiments in which we directly compared plant-derived eDNA and traditional trapping methods. We (1) tested whether the recovered arthropod community composition between traditional trapping methods and plant-derived eDNA differs. We sampled eDNA from nine individual plant species in parallel to arthropod bulk samples from Malaise traps, pitfall traps and sweep netting of a grassland site. (2) We tested whether vegetation beating and plant-derived eDNA display the same community composition and recover similar ecological groups, by comparing the community composition recovered by individual shrubs and trees. (3) To explore the level of fine-scale community differentiation recovered by eDNA, we tested whether arthropod communities of the stem including leaves, flowers and roots of a single plant species differ. We assumed that eDNA will recover a unique community from each compartment of an individual plant. (4) We then tested the comparative performance

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of three different sampling approaches to recover plant-associated arthropod communities: eDNA from surface washes, eDNA from ground plant material and traditional sweep netting. The three different sampling strategies were analysed across 10 grassland sites to explore the recovery of α- and β-diversity patterns. We particularly explored the added benefit of ground plant material to recover arthropod taxa from inside the plant.

2 | MATERIALS AND METHODS

2.1 | Study sites and sampling

The first three experiments were conducted at an approx. 2.5 ha big semi-dry grassland site 'Kimmlingen', located at 310 m above sea level on a south-east exposed hill (Hang am Hohen Göbel, Rhineland-Palatinate, Germany; 49°49′58.4″ N 6°36′05.8″ E; Figure S9 and Table S1). The grassland area was interspersed by hedgerows with common shrub and tree species. The site was chosen, because the area was rich in typical plant species, a diverse arthropod community was expected, and it was rather small and therefore ideal for comprehensive assessments.

2.1.1 | Experiment 1—Traditional monitoring versus plant-derived eDNA

For the first experiment, plant-derived eDNA was sampled between 10th and 13th June 2021. Plant species identification was done with an excursion flora (Jäger, 2016) and the help of expert botanists. For the first experiment, we took the following grassland species: *Arrhenatherum elatius* (bulbous oat grass), *Brachypodium pinnatum* (heath false brome), *Campanula rapunculus* (rampion bellflower), *Genista tinctoria* (dyer's greenweed), *Helianthemum nummularium* (common rock-rose), *Lotus corniculatus* (bird's-foot trefoil), *Plantago media* (hoary plantain), *Poa angustifolia* (narrow-leaved meadow-gras) and *Vicia tenuifolia* (fine-leaved vetch). Six replicates of 25 g plant material of each plant species were cut right above the soil, including the stem, leaf and flower compartments. The replicates were sampled from six different locations where the traditional sampling was also carried out. For the comparison of plant-derived eDNA with traditional monitoring approaches, bulk samples from three Malaise traps (Townes, 1972) were taken at the grassland site from 2nd to 16th of June 2021. The samples were only taken from this 2-week period, but the Malaise trap was already set up 2 weeks earlier. The collection bottles of the Malaise traps were filled with 750 mL of 99.9% propylene glycol (Isolatech, Langenfeld, Germany) and approximately 2 mL of household soap. Simultaneously, five pitfall traps were set up around each of the three Malaise traps, filled with 100 mL propylene glycol and one drop of household soap. When the pitfall traps were collected, the five pitfall traps around each Malaise trap were pooled together ($n=3$). On 26th June 2021, two transects left and right of each Malaise trap were sampled with a sweep net ($n=6$). The transects were walked three times back and forth with the run forth sweeping in the upper vegetation layer and on the run back in the lower vegetation layer. During each run, approx. 30 sweeps were conducted.

2.1.2 | Experiment 2—Vegetation beating versus eDNA to detect plant–arthropod interactions

For the second experiment, we took leaves from the following shrub and tree species of the hedgerows: *Acer campestre* (field maple), *Corylus avellana* (common hazel), *Crataegus monogyna* (common hawthorn), *Quercus robur* (pedunculate oak) and *Viburnum lantana* (wayfarer). Here, we took approx. 25 g leaves from three protruding branches of each plant individual, then homogenized and mixed equal amounts of these leaves from three spatially associated plant individuals into one 25 g sample. We took a total of six sample replicates. For the traditional assessment of plant-associated arthropods, we used vegetation beating. Beating was carried out between the 29th of June and 9th of July, between 9:00 am and 2:00 pm to collect samples while the arthropods were most active. The same plant individuals and plant species as previously sampled for plant-derived eDNA were used, and all the recovered arthropods of the three plant individuals were mixed into one sample corresponding to the eDNA sample. We only used plants that could be sampled individually, without contact to the neighbouring plants, to prevent sampling of host-specific or vagrant arthropods from other plant individuals. Per individual, three branches were hit four times and arthropods were caught on a white beat sheet (Ø 90 cm, Bioform, Nuremberg, Germany). After each branch, arthropods were collected with aspirators. After each plant individual, the arthropods were transferred to a 50-mL tube and stored in 99.8% ethanol (VWR, Pennsylvania, USA).

2.1.3 | Experiment 3—Community differentiation between plant compartments

For the third experiment in Kimmlingen, we collected four replicates of 25 g of the flower, stem including leaves and roots compartments of *Campanula rapunculus* separately.

2.1.4 | Experiment 4—Comparative performance of sweep netting, plant-derived eDNA from surface washes or ground plant material to recover arthropod diversity

For the fourth experiment, we chose different grassland societies at 10 sites close to Trier, hereinafter called 'Trier' (Figure S9 & Table S1). The sites were chosen, because they represent different grassland sites of the landscape: We sampled at a vineyard fallow, a wetland area, four fertilized and two rough pastures, a

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nardus grassland and an extensively used apple orchard. We compared sweep netting with plant-derived eDNA using two eDNA sampling methods. The sites were sampled between 12th and 16th of June 2022. First, a 30-m long transect was measured out, along which four 400 cm^2 square plots were placed at 10 m distance. The wooden plot frames were alternately thrown to 3–5 m distance from the middle of the plot without the targeted vegetation in the field of view to prevent selective choices. Next to each plot, a second 400 cm^2 square frame was placed on similar vegetation composition. For the first eDNA method, which involved grounding of plants to archive a homogenate, the plant material of four plots was cut close to the ground and transferred to one paper bag. For the second eDNA method, which involved surface washes of plants, all plant materials from the second plots were collected in a plastic bag, 1 L of deionized water was added, and the bag shaken for 1 min. The water was then transferred to a sterile container. Sweep netting was performed like in Kimmlingen, but the two transects were run only two times back and forth in close proximity to the middle of the main transect and pooled together at each site.

2.1.5 | Sampling considerations

In all experiments, eDNA samples were collected at least three days after the last rain and during dry weather to provide enough time for DNA accumulation and prevent the DNA from being washed off the plant surfaces. To avoid any cross contamination, eDNA and bulk community samples were collected separately. Sampling was performed with single-use gloves and the plant samples were placed in paper bags. Beating was performed when the leaves were fully dried and under dry conditions. All samples were immediately stored on dry ice in the field and transferred to a laboratory freezer at −28°C until further processing.

2.2 | Sample processing and DNA isolation

All plant materials were dried in a freeze-dryer and then stored on silica gel. Depending on the sample size of the experiments, the plant material was ground with two blenders (300 mL, AEG, MiniMixer SB 2700, Nürnberg Germany; 2 L, Arendo Multi Mixer, Hannover, Germany) for 1 min, transferred to a 15-mL tube and stored at −28°C. The blender bottles and the belonging blades were cleaned according to Buchner et al. (2021). To check for cross contamination during the process, the bottles were filled with approx. 20 mL homemade lysis buffer (10 mM Tris pH 8, 100 mM NaCl, 10 mM EDTA pH 8, 0.5% SDS). DNA of 150 ± 5 mg homogenate was extracted by adding 1500 μL CTAB-buffer. The following extraction steps followed the OPS Diagnostics CTAB protocol (New Jersey, USA), but no RNase A solution was added.

The bulk samples of Malaise traps, pitfall traps and beating were sieved over an aluminium bowl using a 1-mm stainless steel sieve, then cleaned with 96% denatured ethanol (Carl Roth, Karlsruhe, Germany) to remove remaining propylene glycol and allowed to dry. Small arthropods, which passed through the sieve, were transferred back into the sample as well. The arthropods from the nets were sorted out of the bags. In all bulk samples, arthropods with a body size <20 mm were directly stored into 15 mL tubes. Individuals with a body size >20 mm were sorted out and we removed a leg which was then added back to the sample. Additionally, since it was found that the Malaise traps were filled with many individuals of *Melanargia galathea* (marbled white), they were removed likewise, counted and 10% of all complete individuals were put back into the sample. We used a nondestructive DNA isolation protocol (Kennedy et al., 2022) for the bulk community samples. After sorting, the samples were fully covered with home-made lysis buffer and stored at −28°C. Depending on their sample size, bulk samples contained 5–250 mL of lysis buffer. For each millilitre lysate, 6 μL of proteinase K was added. Afterwards, the sample remained in an incubator at 55°C for 16 ± 0.5 h. The reaction was subsequently stopped by transferring 600 μL of the solution into a 1.5-mL tube placed on ice. The isolation was executed as recommended by the manufacturer's protocol of the Gentra Puregene Cell Kit (Qiagen, Hilden, Germany).

Using a vacuum pump, the water samples derived from surface washes were filtered through a 0.45 μL cellulose nitrate filter (Thermo Fisher Scientific Inc., Waltham, USA) and then stored at −28°C. DNA from the filters was extracted using the Qiagen PureGene Blood & Tissue Kit (Qiagen, Hilden, Germany). First, the filters were cut into small pieces and bead-beated for 45 s at 1000 rpm (SPEX 1600 MiniG, Metuchen, New Jersey, USA). The extraction was started by adding 540 μL of ATL buffer to the samples. The other steps were conducted according to the manufacturer's protocol.

2.3 | PCR amplification, DNA sequencing and data analysis

PCR amplification, Index PCR and amplicon checks on all samples were conducted as described in Krehenwinkel, Weber, Broekmann, et al. (2022) using the primer combination fNoPlantF_270 (forward primer, RGCHTTYCCHCGWATAAAYAAYATAAG) and mlCOIintR_W (reverse primer, GRGGRTAWACWGTTCAWCCWGTNCC) in one PCR replicate, with a fragment length of 116 bp of the COI gene (Krehenwinkel, Weber, Künzel, & Kennedy, 2022). Illumina Truseq libraries were prepared using dual index PCRs as described in Lange et al. (2014). The libraries were quantified by the intensity of the gel images and pooled in approximate proportions. All products were finally cleaned with magnetic beads (1× sample to beads ratio, AMPure XP, Beckman Coulter, California, USA). The Kimmlingen and Trier libraries were then sequenced on an Illumina MiSeq using V3 chemistry and 300 cycles (Illumina Inc, San Diego, California, USA). Negative controls of all DNA isolations and PCR amplifications were run along all experiments and sequenced as well. Additionally, we repeated the isolation and PCR of eight samples once and obtained a high replicability of the methods as we received high correlations

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of zOTU-richness (LM; $R^2_{Isolation} = .93$, $p < .001$ and $R^2_{PCR} = .83$, $p < .001$; Figure S8).

The samples were demultiplexed using CASAVA v1.8.2 (Illumina Inc., San Diego, California, USA) with no mismatches allowed. Demultiplexed fastq files were merged using PEAR (Zhang et al., 2014) with a minimum overlap of 50 and a minimum phred quality score of 20. The merged reads were additionally filtered for a minimum quality of Q33 over >90% of the sequence and then transformed to fasta files using the FASTX-Toolkit (Gordon & Hannon, 2010). PCR primer sequences were then trimmed off from the merged reads using grep and sed in UNIX scripts, allowing degenerate bases to vary in the search patterns. The processed reads were dereplicated and clustered into zOTUs using USEARCH (Edgar, 2010). The minimum size cluster was determined with eight occurrences. A de novo chimera removal was included in the clustering pipeline. All resulting zOTUs were searched against the NCBI database using BLASTn with a maximum of 10 target sequences (Altschul et al., 1990). Taxonomy was then assigned to the resulting BLAST output using a custom python script (de Kerdrel et al., 2020), with a minimum of 90% similarity to a reference being used to classify a sequence. Order level was filtered to >93%, family level was filtered to >95% and species level was filtered to >98% reference hit to the database. The zOTU table was then constructed for all samples using USEARCH. Non-arthropod zOTUs were removed, which mostly consisted of fungi amplified by the primers. Then, using the R package GUniFrac (Chen & Chen, 2018), the zOTU tables were rarefied according to the minimum read number of the Kimmlingen and Trier samples, as well as eDNA or bulk samples separately (Figures S1 and S2).

2.4 | Statistical analysis

The statistical analysis was performed in R 4.2.2 (R Core Team, 2021) and RStudio, 2022.7.2.576 (RStudio, 2022), which were extended with the R package vegan 2.5–7 (Oksanen et al., 2020) and tidyverse v1.3.2 (Wickham et al., 2019). The feeding type, herbivore specialization and habitat preferences of arthropods living inside or outside plants and preference for specific plant parts were assigned to the finest taxonomic level using the database of Ellis (2020) and a variety of different open databases and papers, while making sure that different life cycles of the season were considered. The habitat preference was primarily based on larval life history for most species, with all miner and gall midges characterized as living inside the plant and groups such as Formicidae, Araneae and Opiliones characterized as living on the plant.

For the calculations of α- and β-diversity, we used the zOTU-richness and binary Jaccard dissimilarity to avoid using abundance-based variables in the analysis. The zOTU level dissimilarity or clustering of arthropod communities into certain groups was tested by PERMANOVA, pairwise PERMANOVA and ANOSIM and visualized by three- and two-dimensional nonmetric multidimensional scaling (NMDS). Differences in the mean zOTU-richness were tested with Kruskal–Wallis test or pairwise-Wilcoxon's test. Fisher's exact test and pairwise Fisher tests were used to check differences between order compositions or the ecological groups of feeding type, herbivore specialization and habitat preferences. The heat trees were calculated and plotted with MetacodeR (Foster et al., 2017).

3 | RESULTS

3.1 | Comparison of arthropod communities sampled by traditional methods or plant-derived eDNA

In the first experiment, we compared traditional trapping methods with plant-derived eDNA. The recovered arthropod communities were significantly differentiated by all sampling methods (PERMANOVA, $p < .001$). Even though we detected similar arthropod orders, the order composition differed significantly between all approaches (Fisher's exact test, $p < .001$; Figure 1a). The traditional trapping methods provided methodologically expected arthropod communities, as Malaise traps mainly captured flying insects like Diptera (67%), Hymenoptera (10%) and Lepidoptera (9%), whereas pitfall traps also effectively detected ground and plant-dwelling Orthoptera (16%) and Coleoptera (14%) and sweep netting of the meadow was most efficient in capturing Orthoptera (27%). Otherwise, Acari was only widely detected by eDNA (13%), while Orthoptera were underrepresented with eDNA (Figure 1a). Among all methods, 71% of the zOTUs were unique to Malaise traps, 62% to sweep netting, 43% to pitfall traps and 65% to eDNA. The mean zOTU-richness of Malaise traps, sweep netting, pitfall traps and eDNA was $440 \pm 41 > 255 \pm 13 > 155 \pm 7 > 26 \pm 1$, respectively, and differed significantly (Kruskal–Wallis test, $p < .001$; Figure 1b & Figure S4), indicating that more zOTUs were found in bulk than by eDNA samples. In contrast to the mean recovered zOTU richness, the total zOTU-richness of all samples was 835, 668, 315 and 536 respectively. The large difference between mean and total zOTU-richness in eDNA resulted from the little overlap among plant species, as each species hosted unique arthropod communities (PERMANOVA, $p < .001$) that differed greatly from each other (ANOSIM, $R = .916$, $p < .001$; Figure 1c). We detected rare and host-specific arthropod species associated with certain plant species. For example, the pollen beetle *Meligethes solidus* was found on *Helianthemum nummularium*, the sawfly *Rhogogaster picta* was found on *Genista tinctoria* and the two beetles *Genistogethes corniculatus* and *Tychius squamulatus* were present on *Lotus corniculatus*.

3.2 | Comparison of the plant-associated arthropods recovered by vegetation beating or plant-derived eDNA

In the second experiment, in order to directly compare the effectiveness of two methods for plant-associated arthropod assessments, we collected bulk samples from hedgerows by vegetation

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FIGURE 1 Comparison of traditional trapping methods and plant-derived eDNA for the assessment of arthropod communities at a common grassland site (Kimmlingen). (a) The 100% bar charts display the taxonomic composition captured by traditional trapping methods, including sweep netting (Netting), Malaise traps (Malaise) and pitfall traps (Pitfall), as well as those detected through plant-derived eDNA (eDNA). Orders with less than 3% occurrence are summarized under Other. All orders of Acari and Collembola were summarized under their corresponding taxon. (b) Venn diagram on zOTU-level, showcasing the overlap between traditional sampling methods and plant-derived eDNA. (c) The three-dimensional nonmetric multidimensional scaling (NMDS) plot is based on Jaccard dissimilarity and shows how arthropod communities are differentiated by plant species sampled with eDNA. Notably, all plant species were clustered into distinguishable groups.

beating and collected plant-derived eDNA of the same plant species and individuals. Beating detected a higher number of taxa compared to eDNA (1095/421 zOTUs, consisting of 18/13 orders, 143/98 families and 296/167 species, respectively) and the mean richness differed significantly by 145 ± 7 and 32 ± 1.7 zOTUs respectively (pairwise Wilcoxon tests, $p < .001$). Regarding the arthropod orders, both methods recovered similar compositions, but eDNA was more successful at sampling Acari (9%) and Lepidoptera (17%), while beating detected Araneae (5%) and Orthoptera (8%) more efficiently (Fisher's exact test, $p < .01$; Figure 2c). The sampling methods were significantly different (PERMANOVA, $p < .001$; Figure S3), showing that both methods recover unique arthropod communities. Moreover, the plant species within both methods were significantly differentiated as well (PERMANOVA, $p < .001$). However, eDNA showed very strong differentiation between plant species (ANOSIM, $R = .894$, $p < .001$; Figure 2b), while the groups were only moderately differentiated by beating (ANOSIM, $R = .284$, $p < .001$; Figure 2a), showing that eDNA captured a more specific plant-associated arthropod community than beating. This methodologically determined trend is underpinned by the ecological interaction types of the recovered arthropod communities. Beating resulted in the detection of approx. 39% herbivorous and 61% non-direct interacting arthropods. In contrast, eDNA detected approx. 51% herbivorous and 49% non-direct interacting arthropods (Fisher's exact test, $p < .01$; Figure 2d). When examining the composition of feeding types, herbivores captured with eDNA were significantly closer associated with the host plant (Fisher's exact test, $p < .05$; Figure 2e), which was due to the presence of approx. 45% monophagous species (29% oligophagous and 25% polyphagous), while in beating 29% of the species were monophagous (34% oligophagous and 37% polyphagous). eDNA also recovered similar diversity trends between individual plant species in comparison to beating. However, this trend was only significant for β-diversity (LM, $R^2 = .34$, $p < .01$; Figure S7).

3.3 | Associations of arthropods with plant compartments

In the third experiment, we could detect 27, 103 and 117 zOTUs on the flower, stem including leaves and roots compartments of *Campanula rapunculus* respectively. Even though the most zOTUs were unique in all compartments, the flowers were singled out due to the small overlap found with the other compartments (Figure 3a,b). The flower was the only compartment where

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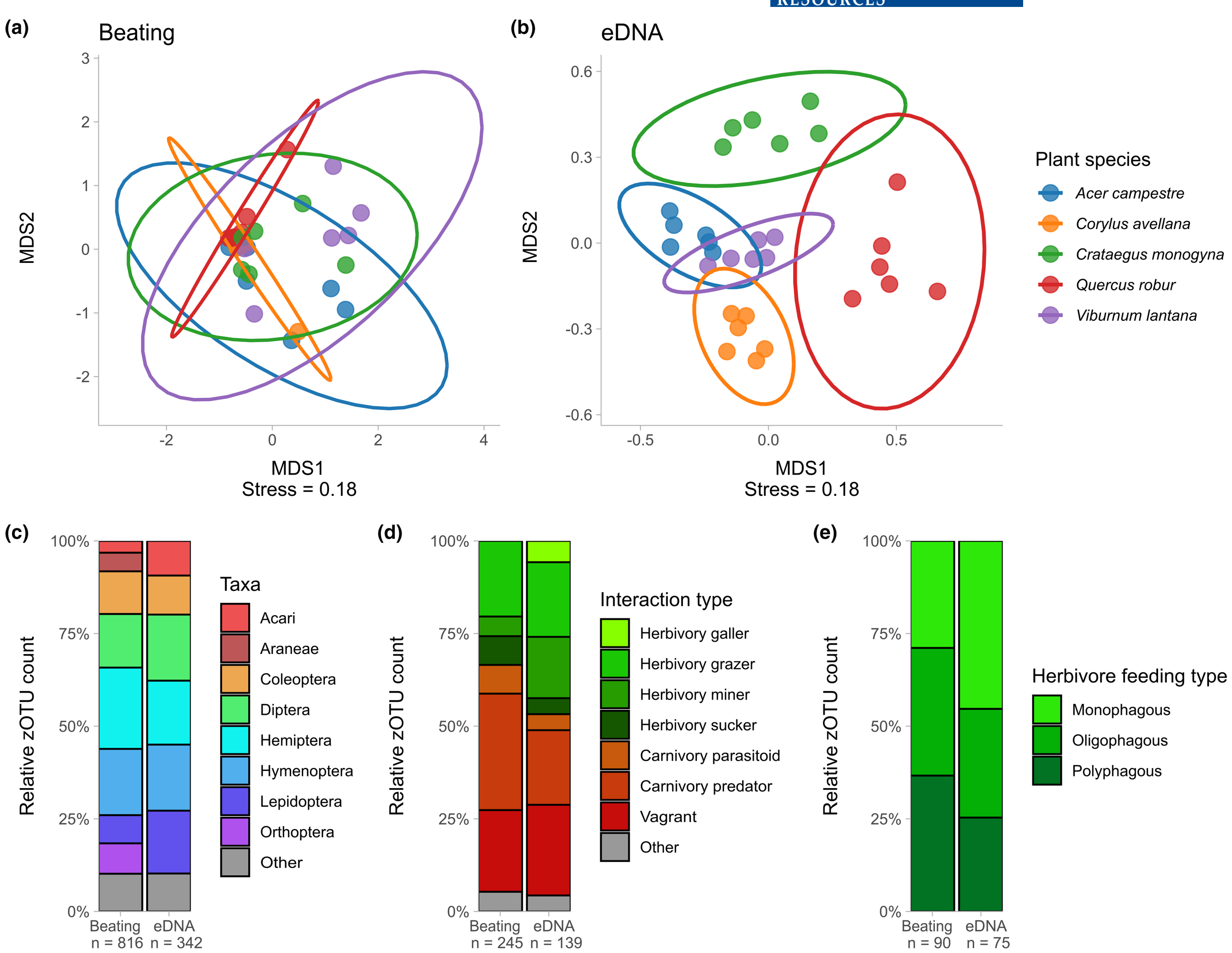

FIGURE 2 The utility of vegetation beating (Beating) and plant-derived eDNA (eDNA) to capture plant–arthropod associations when the same plant species and individuals were assessed. The nonmetric multidimensional scaling (NMDS) plot of (a) beating and (b) plant-derived eDNA is based on Jaccard dissimilarity. The rings around the plant species indicate the 95% coefficient interval of the groups. (c) The 100% bar charts show a similar taxonomic composition of vegetation beating and plant-derived eDNA samples by the relative number of zOTUs that are annotated to the corresponding taxon. (d) The 100% bar charts show the interaction type of the arthropod species found on the plants, insofar that all annotated herbivores directly interact with the plants and carnivores interact with other arthropods, while vagrant species could not be annotated to be directly interact with the plants or other arthropods. (e) The 100% bar charts provide a view at the herbivore feeding types and shows the tightness of their associations to the host plant. In all bar charts (c–e), we summarized orders or interaction types under 3% of the total occurrence to other. All orders of Acari and Collembola were summarized under their corresponding taxon.

no Collembola and only one zOTU of Acari were found, but the highest proportions of Diptera (41%), Hymenoptera (22%) and Coleoptera (19%) occurred. In contrast, higher proportions of 30% and 27% Acari and of 13% and 8% Collembola occurred in the root and stem including leaves respectively (Figure S5). Therefore, the arthropod community of all three compartments was significantly differentiated (PERMANOVA, $p<.001$; ANOSIM, $R=.692$, $p<.01$). Overall, we could annotate 36 arthropod species to specific plant compartments, which were each mostly detected on expected plant compartments (Fisher's exact test, $p<.001$; Figure 3c). As an example, *Meligethes subrugosus*, a monophagous pollen beetle on Campanulaceae, was mostly detected on the flowers and on some of the stem including leaf samples and is known to inhabit the flower and flower buds. Also, *Phytomyza cichorii*, a leaf miner fly that burrows deeply into the tissues of stems and leaves, but is also able to descend to the root and pupate there, was mostly found in the root samples.

3.4 | Similarity of diversity measurements recovered by sweep netting and eDNA derived from surface washes or ground plant material over different sites

In the fourth experiment, we compared two eDNA sampling approaches (surface washes of plants and ground plant material

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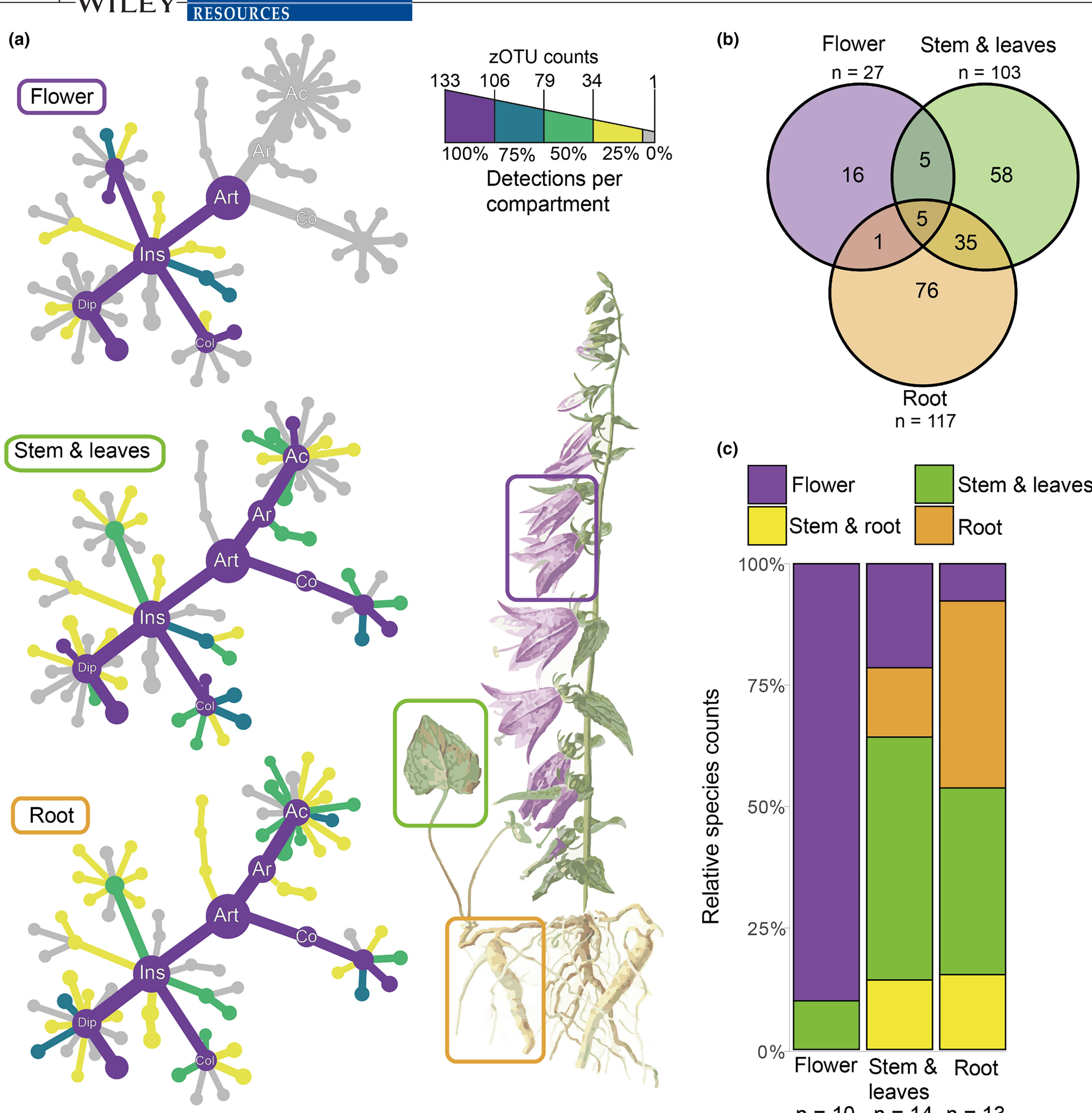

FIGURE 3 Plant-derived eDNA captured different arthropod communities at multiple taxonomic levels in the plant compartments flower, stem including leaves and root of *Campanula rapunculus*. (a) The heat trees show the taxonomic composition of arthropods to the family level of the finest branches. Ac = Acari, Ar = Aranea, Art = Arthropoda, Co = Coleoptera, Col = Collembola, Dip = Diptera, Ins = Insecta. (b) The Venn diagram shows the overlap between plant compartments at zOTU-level. (c) The 100% bar charts show that arthropods annotated to the different compartments were particularly found on them.

for plant homogenates) with each other and to traditional sweep netting to estimate biodiversity trends over different sites. All three methods recovered similar patterns of α- and β-diversity. The α-diversity was strongly related between homogenates with sweep netting and surface washes (LM, $R^2 = .70$, $p < .01$ and $R^2 = .55$, $p < .05$ respectively) and also showed a trend between sweep netting and surface washes which, however, was not significant (LM, $p > .05$; Figure 4a). Regarding the β-diversity (Figure 4b), we observed moderate correlations when comparing homogenate with sweep netting and surface washes (LM, $R^2 = .11$, $p < .05$ and $R^2 = .20$, $p < .01$ respectively) and a strong correlation between sweep netting and surface washes (LM, $R^2 = .63$, $p < .001$). Sweep netting achieved higher zOTU richness than the eDNA methods and within eDNA methods, the homogenate yielded more zOTUs than surface washes (232 ± 22, 62 ± 5 and 48 ± 4 mean zOTUs respectively; Kruskal–Wallis test,

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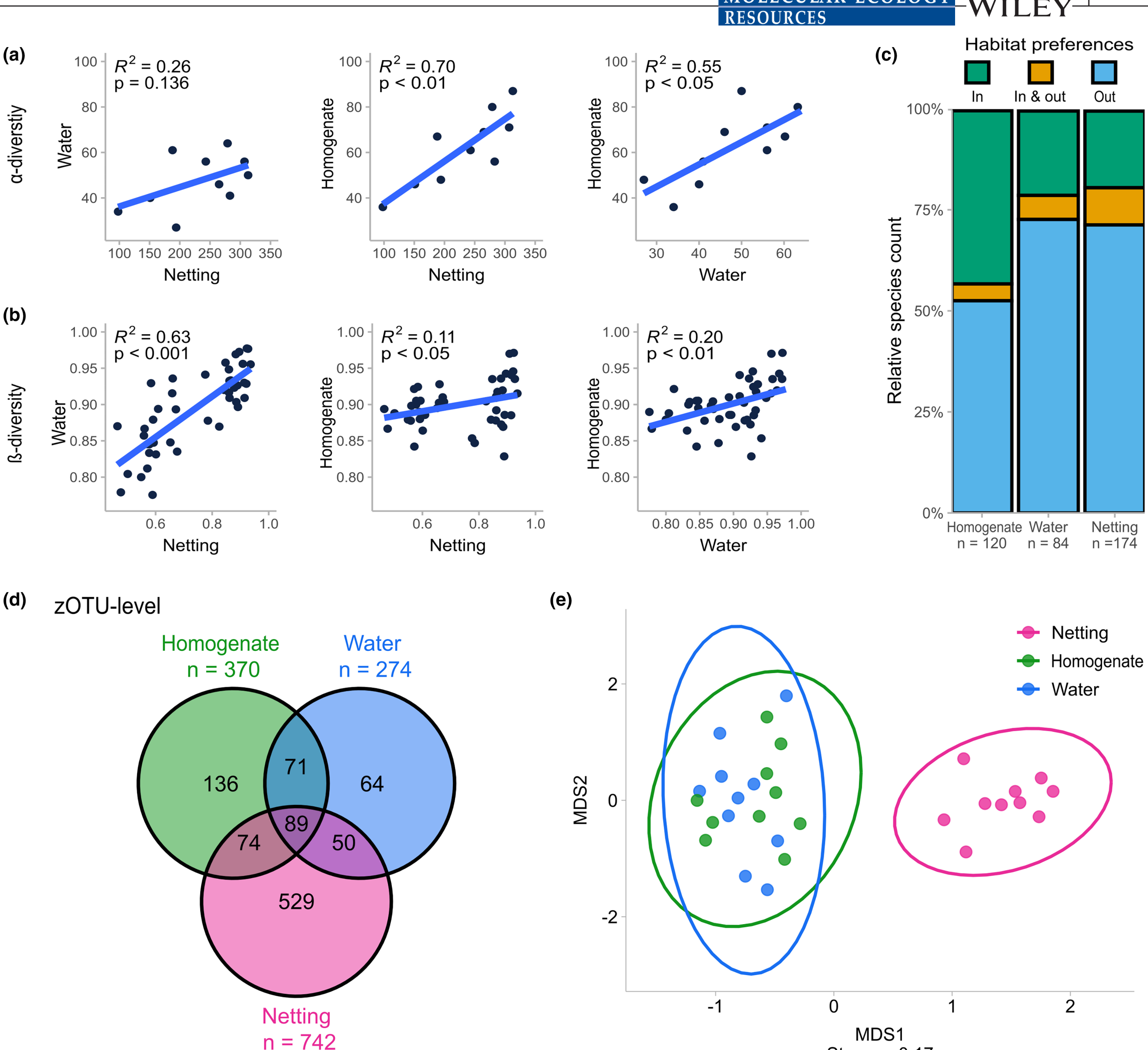

FIGURE 4 Comparison of two plant-derived eDNA methods and traditional sweep netting between grassland sites for the evaluation of arthropod diversity measurements and the recovery of arthropods from inside or outside of the sampled plants. Linear models show the relation of (a) α-diversity (zOTU-richness) and (b) β-diversity (Jaccard dissimilarity) between sweep netting (Netting), eDNA derived from ground plant material (Homogenate) and eDNA derived from plant surfaces washes by water (Water). (c) The 100% bar charts show the annotation of arthropod species that typically occur inside of plants (In), in and outside of plants (In & Out) or outside plants (Out). (d) The Venn diagram shows the overlap between the methods and the number (*n*) of zOTU recovered by each method. (e) The nonmetric multidimensional scaling (NMDS) plot is based on Jaccard dissimilarity and shows the differentiation between arthropod communities recovered by the two eDNA methods and sweep netting.

$p<.001$; Figure S6). The arthropod communities of both eDNA methods were similar to each other (pairwise PERMANOVA, $p>.05$), while sweep netting differed significantly from both (pairwise PERMANOVA, $p<.01$), showing that both eDNA methods resulted in a similar arthropod community and sweep netting shares a smaller overlap with eDNA than both methods within eDNA (Figure 4d,e). Furthermore, homogenates detected significantly more arthropods that were annotated to the interior of the plant than sweep netting or surface washes (Fisher exact test, $p<.01$; Figure 4c).

4 | DISCUSSION

Using a series of four experiments, we conducted tests to assess the effectiveness of plant-derived eDNA in monitoring arthropod biodiversity and retrieving plant–arthropod interactions. We employed commonly utilized methods of large-scale arthropod monitoring programmes, such as Malaise traps, pitfall traps and sweep netting (Arribas et al., 2022). By doing so, we provided a very comprehensive baseline of arthropod community diversity at our study sites, which we could then compare to the diversity recovered by eDNA.

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Our data clearly support our first hypothesis: Plant-derived eDNA does not recover identical taxon lists compared to traditional trapping approaches, but instead complements them by capturing additional taxa, of which many were galling or mining species and Acari. Consequently, eDNA offers the ability to uncover cryptic arthropod habitats that were previously overlooked by traditional methods (Saccaggi et al., 2016), such as leaf galls and mines. It is well known that a combination of traditional trapping methods significantly enhances the observed diversity in arthropod monitoring (Missa et al., 2009). Consistent with this understanding, plant-derived eDNA can likewise function as a complementary method to saturate the taxonomic diversity of a site (Kestel et al., 2023; van der Heyde et al., 2022).

In accordance with our initial hypothesis, eDNA extracted from ground plant material recovers more arthropod taxa compared to eDNA derived from plant surface washes, as the tissue homogenization enabled a simultaneous detection of the inner and outer arthropod communities of the plants (Krehenwinkel, Weber, Künzel, & Kennedy, 2022). Particularly, the data show that endophytic taxa were more effectively detected through the utilization of ground plant material. Nevertheless, it is important to note that the process of drying and grinding the plant material introduces additional effort into the processing, contrasting with the relative simplicity of filtering the surface washes. The method of choice should thus be selected based on the specific requirements of the study. While surface washes will suffice, for example, for monitoring of pollinators, monitoring of whole plant community pests, for example, gall inducing or mining arthropods, requires disruption of the plant material.

Our data provide clear evidence that each plant species harbours a unique arthropod community, even when different plant species in close proximity were sampled. In this study, we focused on a few plant species and were able to recover several hundred arthropod OTUs, while a typical European meadow can contain close to a hundred plant species (Petermann & Buzhdygan, 2021). Thus, expanding the range of sampled plant taxa would undoubtedly result in a substantial increase in the diversity of arthropods detected. Alternatively, bulk samples of multiple plant species can be collected from a site and surface washed as a single sample of a composite plant community, as we have shown for different grassland sites. The recovered diversity can be significantly increased this way, while reducing the required number of samples and the sampling effort.

The recovery of plant-specific arthropod communities from eDNA is well in line with our second hypothesis. In contrast to traditional methods, eDNA analysis of individual plant species additionally provides detailed insights into plant–arthropod interactions. Moreover, eDNA outperformed vegetation beating in recovering plant–arthropod interactions, an approach frequently used to identify plant-specific arthropod communities (Graham et al., 2022). As expected, both beating and plant-derived eDNA yielded a broad spectrum of ecological groups, including predators, parasitoids and herbivores (Johnson et al., 2023; Krehenwinkel, Weber, Broekmann, 2022; Thomsen & Sigsgaard, 2019). However, in our experiment, only eDNA was able to consistently differentiate specific arthropod communities between individual plant species. Pronounced differences in community composition between beating and eDNA were observed, particularly for oligophagous and monophagous herbivores. Given their longer and more intensive interactions with plants, specialist herbivores are likely to deposit a considerably higher amount of eDNA on the plant surface compared to vagrant species and predators (Kucherenko et al., 2018; Kudoh et al., 2020). In contrast, the dominance of vagrant species in a beating sample makes the recovery of plant-specific communities much more challenging. These results underline that eDNA analysis is a preferable option over traditional approaches to assess the interaction ecology of herbivorous arthropods and highlight the significance of individual plants as microhabitats for arthropod communities within an ecosystem (Schuldt et al., 2019).

Even more, our data did not only suggest that eDNA is a superior approach to detect differentiation between arthropod communities of plant species. The analysis of different compartments of *Campanula rapunculus* revealed that eDNA analysis can precisely target specific communities in terrestrial environments, even beyond the level of plant individuals. Interestingly, the majority of arthropods were not located on the flowers. Hence, solely relying on flower-derived eDNA will not provide a complete analysis of the arthropod community. This approach, however, can assess plant–pollinator interactions, one of the most important ecosystem services in agricultural systems (Brown et al., 2016; Kremen et al., 2007).

Our experiments also provided us to compare the recovery of α-diversity patterns within sites and β-diversity patterns between sites using eDNA and traditional sampling approaches. By comparing eDNA derived from plant homogenates, eDNA derived from plant surface washes and traditional sweep netting, we observed well-correlated α- and β-diversity trends, thus supporting our third hypothesis. Although eDNA does not capture an identical community composition as traditional monitoring methods, it effectively captures the overall diversity trends, which hold significant importance for the assessment of the ecological status (Hortal et al., 2015). This suggests that plant-derived eDNA can not only be used as a complement to traditional monitoring but may also serve very well as a stand-alone approach for monitoring patterns of community diversity. Plant-derived eDNA, therefore, may be preferable over traditional trapping methods, especially for fast and reproducible study designs.

In summary, plant-derived eDNA offers three advantages over traditional monitoring using passive trapping methods. First, eDNA is less invasive as it does not require large-scale killing of organisms to access the specimens being monitored, while still yielding comparable diversity patterns and diverse taxa lists. However, plant-derived eDNA recovers narrowly associated taxa and therefore yields a lower taxonomic richness compared to traditional methods. Since arthropod eDNA is naturally more degraded than

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DNA from fresh organisms, we used a short primer (Krehenwinkel, Weber, Künzel, & Kennedy, 2022). Hence, the primer could have amplified degraded DNA recovered by traditional methods, for example, from primary and secondary predation (Cuff et al., 2021) or contamination with arthropod eDNA during traditional sampling. Second, eDNA sampling is a rapid, cost-effective, and easily standardized procedure (Bálint et al., 2018; Smart et al., 2016). Collecting and processing, for example, surface washes from plants is significantly easier than analysing a Malaise trap sample containing thousands of specimens. And last, plant-derived eDNA has the potential to address a critical shortfall in our understanding of biological communities by providing detailed information on the interaction diversity of arthropods within an ecosystem, particularly by providing detailed information on their associations with plants. To fully understand and predict the responses of a community to global environmental change, understanding these complex interactions is paramount. Our data impressively show that arthropod communities are tightly associated with only one single plant, and such specialized arthropods will likely never interact directly with each other, even despite living on directly adjacent plants. We showed that these plant–arthropod associations can be more reliably assessed by eDNA metabarcoding than with traditional methods. Incorporating plant-derived eDNA metabarcoding into large-scale arthropod monitoring programmes could therefore quickly generate comprehensive whole community interaction networks. This integration of interaction networks is crucial for developing next-generation biodiversity assessment designs (Grimm et al., 2017; Thompson et al., 2012), which are, for example, implemented into conservation management (Decker et al., 2017), protection of agricultural systems (Punt et al., 2016) and the exploration of ecosystem network stability (McDonald-Madden et al., 2016).

AUTHOR CONTRIBUTIONS

S.W., H.K & M.S. conceived the ideas and designed the survey. S.W., L.M., A.K., L.H., A.J., N.L. & M.S. collected & generated the data. S.K. generated the sequences. S.W., H.K., M.S. & L.M. analysed the data. M.S. led the writing of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The fastq files as well as tables with sheets including raw OTU tables, rarefied OTU tables, taxonomic annotation and corresponding meta data of all experiments conducted in this study are available at DataDryad (DOI: 10.5061/dryad.79cnp5j2c).

BENEFIT SHARING STATEMENT

This study was done under applicable law and permission by the Struktur- und Genehmigungsdirektion Nord, Rhineland-Palatinate, Germany (425-104-235-005/2021).

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REFERENCES

Allen, M. C., Lockwood, J., Kwait, R., Vastano, A., Peterson, D., Tkacenko, L., Angle, J., & Jaffe, B. (2022). Using surface environmental DNA to assess arthropod biodiversity within a forested ecosystem. *Environmental DNA*, 1–15. https://doi.org/10.1002/edn3.487

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410.

Arribas, P., Andújar, C., Bohmann, K., DeWaard, J. R., Economo, E. P., Elbrecht, V., Geisen, S., Goberna, M., Krehenwinkel, H., Novotny, V., & Zinger, L. (2022). Toward global integration of biodiversity big data: A harmonized metabarcode data generation module for terrestrial arthropods. *GigaScience*, *11*, 1–12.

Bálint, M., Nowak, C., Márton, O., Pauls, S. U., Wittwer, C., Aramayo, J. L., Schulze, A., Chambert, T., Cocchiararo, B., & Jansen, M. (2018). Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources*, *18*(6), 1415–1426.

Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodiversity and Conservation*, *29*(7), 2089–2121.

Brown, M. J., Dicks, L. V., Paxton, R. J., Baldock, K. C., Barron, A. B., Chauzat, M.-P., Freitas, B. M., Goulson, D., Jepsen, S., & Kremen, C. (2016). A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ*, *4*, e2249.

Buchner, D., Haase, P., & Leese, F. (2021). Wet grinding of invertebrate bulk samples–a scalable and cost-efficient protocol for metabarcoding and metagenomics. *Metabarcoding and Metagenomics*, *5*, e67533.

Chen, J., & Chen, M. J. (2018). *Package 'GUniFrac'*. The Comprehensive R Archive Network (CRAN). Available from https://cran.r-project.org/package=GUniFrac

Crawley, M. J. (1989). Insect herbivores and plant population dynamics. *Annual Review of Entomology*, *34*(1), 531–562.

Crossley, M. S., Meier, A. R., Baldwin, E. M., Berry, L. L., Crenshaw, L. C., Hartman, G. L., Lagos-Kutz, D., Nichols, D. H., Patel, K., Varriano, S., Snyder, W. E., & Moran, M. D. (2020). No net insect abundance and diversity declines across us long term ecological research sites. *Nature Ecology & Evolution*, *4*, 1368–1376.

Cuff, J. P., Windsow, F. M., Tercel, M. P. T. G., Kitson, J. J. N., & Evans, D. M. (2021). Overcoming the pitfalls of merging dietary metabarcoding

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into ecological networks. *Methods in Ecology and Evolution*, *13*(3), 545–559.

de Kerdrel, G. A., Andersen, J. C., Kennedy, S. R., Gillespie, R., & Krehenwinkel, H. (2020). Rapid and cost-effective generation of single specimen multilocus barcoding data from whole arthropod communities by multiple levels of multiplexing. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-019-54927-z

Decker, E., Linke, S., Hermoso, V., & Geist, J. (2017). Incorporating ecological functions in conservation decision making. *Ecology and Evolution*, *7*(20), 8273–8281.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Ellis, W. N. (2020). *Plant parasites of Europe: Leafminers, galls and fungi*. Available from https://bladmineerders.nl

Foster, Z. S., Sharpton, T. J., & Grünwald, N. J. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Computational Biology*, *13*(2), e1005404.

Gardarin, A., Plantegenest, M., Bischoff, A., & Valantin-Morison, M. (2018). Understanding plant–arthropod interactions in multitrophic communities to improve conservation biological control: Useful traits and metrics. *Journal of Pest Science*, *91*, 943–955.

Gordon, A., & Hannon, G. (2010). *Fastx-toolkit. FASTQ/A short-reads preprocessing tools*. (unpublished) http://hannonlab.cshl.edu/fastx_toolkit

Graham, N. R., Krehenwinkel, H., Lim, J. Y., Staniczenko, P., Callaghan, J., Andersen, J. C., Gruner, D. S., & Gillespie, R. G. (2022). Ecological network structure in response to community assembly processes over evolutionary time. *Molecular Ecology*, 1–18. https://doi.org/10.1111/mec.16873

Grimm, V., Ayllón, D., & Railsback, S. F. (2017). Next-generation individual-based models integrate biodiversity and ecosystems: Yes we can, and yes we must. *Ecosystems*, *20*, 229–236.

Haddad, N. M., Crutsinger, G. M., Gross, K., Haarstad, J., Knops, J. M., & Tilman, D. (2009). Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecology Letters*, *12*(10), 1029–1039.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D., & de Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One*, *12*(10), e0185809.

Harris, J., Collis, D., & Magar, K. (1972). Evaluation of the tree-beating method for sampling defoliating forest insects. *The Canadian Entomologist*, *104*(5), 723–729.

Hines, J., & Pereira, H. M. (2021). Biodiversity: Monitoring trends and implications for ecosystem functioning. *Current Biology*, *31*(20), R1390–R1392.

Hortal, J., de Bello, F., Diniz-Filho, J. A. F., Lewinsohn, T. M., Lobo, J. M., & Ladle, R. J. (2015). Seven shortfalls that beset large-scale knowledge of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *46*(1), 523–549. https://doi.org/10.1146/annurev-ecolsys-112414-054400

Hurlbert, A. H., Hayes, T. E., McKinnon, T. N., & Goforth, C. L. (2018). *Caterpillars count! A citizen science project for monitoring foliage arthropod abundance and phenology*. bioRxiv, 257675.

Jäger, E. J. (2016). *Rothmaler-Exkursionsflora von Deutschland*. Grundband. Springer-Verlag.

Johnson, M. D., Katz, A. D., Davis, M. A., Tetzlaff, S., Edlund, D., Tomczyk, S., Molano-Flores, B., Wilder, T, Sperry, J. H. (2023). Environmental DNA metabarcoding from flowers reveals arthropod pollinators, plant pests, parasites, and potential predator–prey interactions while revealing more arthropod diversity than camera traps. *Environmental DNA*.*5*(3), pp.551-569.

Kennedy, S., Calaor, J., Zurápiti, Y., Hans, J., Yoshimura, M., Choo, J., Andersen, J. C., Callaghan, J., Roderick, G. K., Krehenwinkel, H., Rogers, H., & Krehenwinkel, H. (2022). Richness and resilience in the Pacific: DNA metabarcoding enables parallelized evaluation of biogeographic patterns. *Molecular Ecology*, 1–14. https://doi.org/10.1111/mec.16575

Kestel, J., Bateman, P., Field, D., White, N., Lines, R., & Nevill, P. (2023). EDNA metabarcoding of avocado flowers:'Hass' it got potential to survey arthropods in food production systems? *Molecular Ecology Resources*., *23*, 1540–1555.

Knops, J. M., Tilman, D., Haddad, N. M., Naeem, S., Mitchell, C. E., Haarstad, J., Ritchie, M. E., Howe, K. M., Reich, P. B., Siemann, E., & Groth, J. (1999). Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters*, *2*(5), 286–293.

Krehenwinkel, H., Weber, S., Broekmann, R., Melcher, A., Hans, J., Wolf, R., Hochkirch, A., Kennedy, S. R., Koschorreck, J., Künzel, S., & Müller, C. (2022). Environmental DNA from archived leaves reveals widespread temporal turnover and biotic homogenization in forest arthropod communities. *eLife*, *11*, e78521.

Krehenwinkel, H., Weber, S., Künzel, S., & Kennedy, S. R. (2022). The bug in a teacup—Monitoring arthropod–plant associations with environmental DNA from dried plant material. *Biology Letters*, *18*(6), 20220091.

Kremen, C., Williams, N. M., Aizen, M. A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S. G., Roulston, T. A., Steffan-Dewenter, I., & Vázquez, D. P. (2007). Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change. *Ecology Letters*, *10*(4), 299–314.

Kucherenko, A., Herman, J. E., Everham, E. M., III, & Urakawa, H. (2018). Terrestrial snake environmental DNA accumulation and degradation dynamics and its environmental application. *Herpetologica*, *74*(1), 38–49.

Kudoh, A., Minamoto, T., & Yamamoto, S. (2020). Detection of herbivory: EDNA detection from feeding marks on leaves. *Environmental DNA*, *2*(4), 627–634.

Lange, V., Böhme, I., Hofmann, J., Lang, K., Sauter, J., Schöne, B., Paul, P., Albrecht, V., Andreas, J. M., Baier, D. M., Nething, J., Ehninger, U., Schwarzelt, C., Pingel, J., Ehninger, G., & Schmidt, A. H. (2014). Cost-efficient high-throughput HLA typing by MiSeq amplicon sequencing. *BMC Genomics*, *15*(63), 1–11.

Macher, T., Schütz, R., Hörren, T., Beermann, A. J., & Leese, F. (2023). It's raining species: Rainwash eDNA metabarcoding as a minimally invasive method to assess tree canopy invertebrate diversity. *Environmental DNA*, *5*(1), 3–11.

McDonald-Madden, E., Sabbadin, R., Game, E. T., Baxter, P. W., Chades, I., & Possingham, H. P. (2016). Using food-web theory to conserve ecosystems. *Nature Communications*, *7*(1), 10245.

Missa, O., Basset, Y., Alonso, A., Miller, S. E., Curletti, G., De Meyer, M., Eardley, C., Mansell, M. W., & Wagner, T. (2009). Monitoring arthropods in a tropical landscape: Relative effects of sampling methods and habitat types on trap catches. *Journal of Insect Conservation*, *13*, 103–118.

Moir, M., Brennan, K., Koch, J., Majer, J., & Fletcher, M. (2005). Restoration of a forest ecosystem: The effects of vegetation and dispersal capabilities on the reassembly of plant-dwelling arthropods. *Forest Ecology and Management*, *217*(2–3), 294–306.

Montgomery, G. A., Belitz, M. W., Guralnick, R. P., & Tingley, M. W. (2021). Standards and best practices for monitoring and benchmarking insects. *Frontiers in Ecology and Evolution*, *8*, 513.

Myers, J. H., & Sarfraz, R. M. (2017). Impacts of insect herbivores on plant populations. *Annual Review of Entomology*, *62*, 207–230.

Novotny, V., Basset, Y., Miller, S. E., Weiblen, G. D., Bremer, B., Cizek, L., & Drozd, P. (2002). Low host specificity of herbivorous insects in a tropical forest. *Nature*, *416*(6883), 841–844.

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Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Sólymos, P., Stevens, M. H. H., & Wagner, H. (2020). *Vegan: Community ecology package*. Available from https://CRAN.R-project.org/package=vegan

Petermann, J. S., & Buzhdygan, O. Y. (2021). Grassland biodiversity. *Current Biology*, *31*(19), R1195–R1201.

Punt, A. E., MacCall, A. D., Essington, T. E., Francis, T. B., Hurtado-Ferro, F., Johnson, K. F., Kaplan, I. C., Koehn, L. E., Levin, P. S., & Sydeman, W. J. (2016). Exploring the implications of the harvest control rule for Pacific sardine, accounting for predator dynamics: A MICE model. *Ecological Modelling*, *337*, 79–95.

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. URL: https://www.R-project.org/

RStudio. (2022). *RStudio: Integrated development for R*. RStudio.

Saccaggi, D. L., Karsten, M., Robertson, M. P., Kumschick, S., Somers, M. J., Wilson, J. R., & Terblanche, J. S. (2016). Methods and approaches for the management of arthropod border incursions. *Biological Invasions*, *18*, 1057–1075.

Sánchez- Bayo, F., & Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: A review of its drivers. *Biological Conservation*, *232*, 8–27.

Schaffers, A. P., Raemakers, I. P., Sýkora, K. V., & Ter Braak, C. J. (2008). Arthropod assemblages are best predicted by plant species composition. *Ecology*, *89*(3), 782–794.

Schuldt, A., Ebeling, A., Kunz, M., Staab, M., Guimarães-Steinicke, C., Bachmann, D., Buchmann, N., Durka, W., Fichtner, A., Fornoff, F., & Härdtle, W. (2019). Multiple plant diversity components drive consumer communities across ecosystems. *Nature Communications*, *10*(1), 1460.

Seibold, S., Gossner, M. M., Simons, N. K., Blüthgen, N., Müller, J., Ambarlı, D., Ammer, C., Bauhus, J., Fischer, M., Habel, J. C., Linsenmair, K. E., Nauss, T., Penone, C., Prati, D., Schall, P., Schulze, E.-D., Vogt, J., Wöllauer, S., & Weisser, W. W. (2019). Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature*, *574*, 671–674.

Siemann, E., Tilman, D., Haarstad, J., & Ritchie, M. (1998). Experimental tests of the dependence of arthropod diversity on plant diversity. *The American Naturalist*, *152*(5), 738–750.

Smart, A. S., Weeks, A. R., van Rooyen, A. R., Moore, A., McCarthy, M. A., & Tingley, R. (2016). Assessing the cost-efficiency of environmental DNA sampling. *Methods in Ecology and Evolution*, *7*(11), 1291–1298.

Thompson, R. M., Brose, U., Dunne, J. A., Hall, R. O., Jr., Hladyz, S., Kitching, R. L., Martinez, N. D., Rantala, H., Romanuk, T. N., Stouffer, D. B., Tylianakis, J. M., & Stouffer, D. B. (2012). Food webs: Reconciling the structure and function of biodiversity. *Trends in Ecology & Evolution*, *27*(12), 689–697.

Thomsen, P. F., & Sigsgaard, E. E. (2019). Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. *Ecology and Evolution*, *9*(4), 1665–1679.

Thomsen, P. F., & Willerslev, E. (2015). Environmental DNA–an emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation*, *183*, 4–18.

Townes, H. (1972). A light-weight malaise trap. *Entomological News*, *83*(9), 239–247.

Valentin, R. E., Fonseca, D. M., Gable, S., Kyle, K. E., Hamilton, G. C., Nielsen, A. L., & Lockwood, J. L. (2020). Moving eDNA surveys onto land: Strategies for active eDNA aggregation to detect invasive forest insects. *Molecular Ecology Resources*, *20*(3), 746–755.

Valiente-Banuet, A., Aizen, M. A., Alcántara, J. M., Arroyo, J., Cocucci, A., Galetti, M., García, M. B., García, D., Gómez, J. M., Jordano, P., & Medel, R. (2015). Beyond species loss: The extinction of ecological interactions in a changing world. *Functional Ecology*, *29*(3), 299–307.

van der Heyde, M., Bunce, M., & Nevill, P. (2022). Key factors to consider in the use of environmental DNA metabarcoding to monitor terrestrial ecological restoration. *Science of the Total Environment*, *848*, 157617.

van der Sluijs, J. P. (2020). Insect decline, an emerging global environmental risk. *Current Opinion in Environmental Sustainability*, *46*, 39–42.

Volf, M., Klimeš, P., Lamarre, G. P., Redmond, C. M., Seifert, C. L., Abe, T., Auga, J., Anderson-Teixeira, K., Basset, Y., Beckett, S., & Butterill, P. T. (2019). Quantitative assessment of plant-arthropod interactions in forest canopies: A plot-based approach. *PLoS One*, *14*(10), e0222119.

Wagner, D. L., Fox, R., Salcido, D. M., & Dyer, L. A. (2021). A window to the world of global insect declines: Moth biodiversity trends are complex and heterogeneous. *Proceedings of the National Academy of Sciences*, *118*(2), e2002549117.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., & Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina paired-end reAd mergeR. *Bioinformatics*, *30*(5), 614–620. https://doi.org/10.1093/bioinformatics/btt593

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