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Spider webs as eDNA samplers: Biodiversity assessment across the tree of life

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Abstract

The concept of environmental DNA (eDNA) utilizes nucleic acids of organisms directly from the environment. Recent breakthrough studies have successfully detected a wide spectrum of prokaryotic and eukaryotic eDNA from a variety of environments, ranging from ancient to modern, and from terrestrial to aquatic. With their diversity and ubiquity in nature, spider webs might act as powerful biofilters and could thus represent a promising new source of eDNA, but their utility under natural field conditions is severely understudied. Here, we bridge this knowledge gap to establish spider webs as a source of eDNA with far reaching implications. First, we conducted a field study to track specific arthropod targets from different spider webs. We then used high-throughput amplicon sequencing of taxonomic barcodes to investigate the utility of spider web eDNA for biodiversity monitoring of animals, fungi and bacteria. Our results show that genetic remains on spider webs allow the detection of even the smallest target organisms. We also demonstrate that eDNA from spider webs is useful in research of community compositions across the different domains of life, with potentially highly detailed temporal and spatial information.

KEYWORDS
biodiversity monitoring, environmental DNA, metabarcoding, microbial communities

1 | INTRODUCTION

The continuous loss of biodiversity is among the most critical challenges facing society (Ceballos et al., 2015; Thomsen & Willerslev, 2015; Waters et al., 2016). Biodiversity data, crucial to countering these trends, still mostly rely on traditional taxonomic species identification, such as morphological and behavioural species-diagnostic traits. These traditional methods have well-known shortcomings; for example, the majority of biodiversity is undescribed (May, 1992; Mora et al., 2011), phenotypic diagnoses are often insufficient (Coddington & Levi, 1991), and crucial taxonomic expertise is declining (Agnarsson & Kuntner, 2007; Hopkins & Freckleton, 2002; Wheeler et al., 2004). As a result, traditional approaches to conducting efficient and standardized biodiversity surveys are often inadequate. In addition, traditional sampling techniques are invasive. They are likely to kill the target organisms and may further harm their ecosystems (Goldberg et al., 2015; Thomsen & Willerslev, 2015).

Novel molecular approaches, such as sampling of environmental DNA (eDNA), that is obtaining intra- or extracellular genetic material directly from environmental samples, has the potential to overcome many limitations of traditional biodiversity monitoring (Baird & Hajibabaei, 2012; Deiner et al., 2017; Gorički et al., 2017; Taberlet et al., 2018; Thomsen & Willerslev, 2015). eDNA is present across a wide diversity of media (e.g., soil, sediment, water, and permafrost), and depending on the medium, the preservation of eDNA varies from a few weeks to hundreds of thousands of years (Dejean et al., 2011; Thomsen & Willerslev, 2015; Willerslev et al., 2014). eDNA can be detected either using targeted tests within a single-species approach, or using generic high-throughput sequencing in a multiple-species approach, such as by DNA metabarcoding (Ji et al., 2013). Accordingly, eDNA is increasingly used to address fundamental questions in basic and applied research fields such as ecology, molecular biology, nature conservation and palaeontology (Deiner et al., 2017; Taberlet et al., 2012; Taberlet et al., 2012; Taberlet, Prud'homme, et al., 2012; Thomsen & Willerslev, 2015). The idea of eDNA arose three decades ago with the aim of obtaining nucleic acids of microbes directly from the environment, while recent studies using eDNA have assessed plant (Taberlet, Prud'homme, et al., 2012; Yoccoz et al., 2012), fungal (Buee et al., 2009; O'Brien et al., 2005), earthworm (Bienert et al., 2012), amphibian (Pilliod et al., 2013), and fish (Thomsen et al., 2012) communities, characterized the functional potential of microbial and viral communities (Dinsdale et al., 2008), monitored invasive species (Dejean et al., 2012; Ficetola et al., 2008), and detected difficult-to-find species such as cave olms (Gorički et al., 2017), brown bears (Skrbinšek et al., 2012), and whale sharks (Sigsgaard et al., 2016).

In this paper, we argue that spider webs represent a powerful tool for obtaining eDNA. Spiders are among the dominant predators of arthropod communities (Coddington & Colwell, 2000). Not all spiders construct capture webs, but those that do show enormous diversity and ecological abundance (Coddington & Colwell, 2000). Ranging from millimetres to metres in size, being diverse in architecture and in the type of silk they consist of, and in the microhabitat they are suspended in, as well as being ubiquitous in natural and anthropogenic ecosystems (Blackledge et al., 2011), spider webs are likely to be powerful biofilters, potentially supplementing human air-sampling materials. That spider webs could represent a promising new source of eDNA has been proposed only recently. One pioneering study in controlled laboratory conditions demonstrated that widow spider webs contain genetic traces of the host and its single prey (Xu et al., 2015). Another preliminary study confirmed these findings by amplifying spider DNA from silk of two spider species (Blake et al., 2016). Recently, a DNA metabarcoding study, aimed at the optimization of diet analysis from several predator sources, including spider webs, demonstrated that spider webs accumulate genetic traces of insects, rotifers, fungi, amoebozoans and other eukaryotes, indicating their potential to represent a useful source of eDNA for biodiversity sampling in general (Corse et al., 2019). However, these early studies have limitations (see Supporting Information 1) and we believe that the utility of eDNA from spider webs far surpasses simple identification of spiders and their prey.

To establish spider web DNA as a standard eDNA methodology, we need to advance our understanding of two critical aspects. The first involves single species detection from spider web DNA. The performance of species-specific detection of eDNA from spider webs must be assessed over a wide dynamic range of target DNA concentrations and include appropriate DNA isolation and amplification controls. The second aspect is whether webs, as aerial filters, can be used to identify multiple taxa. Given that temporal and spatial factors affect spider web biology, comparative metabarcoding should yield sufficient variation in species composition, as well as abundances, for biodiversity estimations. For example, many spider species build webs in specific microhabitats, which could provide fine spatial information. Also, orb web spiders completely renew their webs daily, while webs of other spider families are longer lasting (Foelix, 2011), which can provide useful temporal information. Furthermore, some but not all spider webs contain capture threads that are coated in viscous glue (e.g., orb webs; Blackledge et al., 2011), and could thus function differently in accumulating genetic material compared to webs that only contain "bare" silk threads (e.g., sheet webs).

Here, we use single- and multispecies approaches to estimate the utility of eDNA from spider webs. Within our single-species approach, we conducted field tests and applied rigorous laboratory protocols to test whether model prey can be detected from spider webs. These tests use quantitative PCR (qPCR) to detect prey DNA in the webs of the garden spider *Araneus diadematus* and the common hammock-weaver *Linyphia triangularis*, two species with different web types. Our multispecies approach then establishes metabarcoding protocols. To investigate how eDNA from spider webs may be used for biodiversity monitoring, we sampled webs of the above two spider species across two distinct forest types and in two subsequent years. The diversities of animals, fungi, and bacteria detected from spider web eDNA strongly support our prediction that eDNA accumulated in spider webs contains important biodiversity information.

2 | MATERIALS AND METHODS

2.1 | Studied sites and sampling for single-species eDNA detection

In a forest in Slovenia (45.575873, 13.856277), we selected five webs of both the garden spider *Araneus diadematus* and the common hammock-weaver *Linyphia triangularis*. The garden spider builds a typical two-dimensional orb web, consisting of nonsticky, as well as sticky threads (Blackledge et al., 2011). The viscous glue of these sticky capture threads is among the best bio-adhesives known (Sahni et al., 2010), probably making orb webs efficient in capturing parts of impacting arthropods and airborne particles. The common hammock-weaver builds a three-dimensional web, consisting of a

hammock-like segment, interlaced with a looser mesh of silk above and below it. These webs do not contain sticky capture threads (Blackledge et al., 2011). We introduced two small house crickets *Acheta domestica* (~5 mg body mass) into each web. We then collected the webs several hours later. The collected silk contained no visible prey remains. We collected an additional five webs of each spider species, without introducing the cricket, as control samples. We collected each web onto the tip of a sterile plastic inoculation loop, breaking the loop off into a sterile microcentrifuge tube immediately after collection. We stored all web samples at −80°C.

2.2 | Studied sites and sampling for biodiversity surveys

We sampled two forests in Slovenia from different climates, one submediterranean, the other continental. In both, the above described spider species *A. diadematus* and *L. triangularis* co-occur in close proximity. In each forest, we collected five webs of each species as described above. In the submediterranean forest, we repeated the sampling of *L. triangularis* webs across two subsequent years (25 samples in total, see Supporting Information 2: Table S7).

2.3 | DNA extraction

We isolated DNA from all samples for qPCR analysis and DNA metabarcoding using an adapted protocol of the PowerLyzer PowerSoil DNA Isolation Kit (Qiagen; Supporting Information 2).

2.4 | qPCR analysis for single-species eDNA detection

We used qPCR for sensitive detection of house crickets in a single-species experiment. To test the detection of house crickets in web samples, we designed and tested two TaqMan chemistry-based assays targeting 102- and 127-bp fragments of the mitochondrial gene cytochrome *c* oxidase subunit I (COI; Supporting Information 2: Table S1). We provide amplification protocols and additional information in Supporting Information 2.2 text and Tables S2–S5. Throughout laboratory work, we employed rigorous protocol controls (Table 1): a negative isolation control, a positive amplification control, no template control during qPCR amplification, and an internal control of the DNA isolation process (using the "Eukaryotic 18S rRNA Endogenous Control"; Applied Biosystems), while during qPCR amplification we performed two technical replicates of both undiluted and diluted samples.

To design well-working assays for detection of *A. domestica* from a variety of webs in nature, we first tested the two assays in the laboratory. For this, we used our laboratory populations of two spider species, the African hermit spider (*Nephilingis cruentata*) that builds sticky orb webs, and the Mediterranean black widow (*Latrodectus tredecimguttatus*) that builds nonsticky cobwebs. We performed all assay tests on webs and tissue of both these species. We tested the assays for DNA isolated from webs only (negative control) and from target (house cricket) tissue only (positive control). The total amount of DNA in undiluted web samples was 150–900 ng (75-ml elutions), at a purity of A_{260}/A_{280} = 1.8–2.0. We employed assay specificity controls by testing the amplification of the host (spider) and nontarget prey (mealworms) DNA. To control for potential inhibitors in the amplification matrix (spider silk), we tested the assays on samples of target tissue with added spider silk. To test the dynamic range of the assays, we tested both assays on a range of target DNA concentrations, by performing a dilution series of our test samples' (webs of both species that were fed with house crickets) DNA in nuclease-free water, from 10^{-1} to 10^{-9}.

We considered a sample positive if it produced an exponential amplification curve that was distinguishable from the negative controls. In such cases, we determined the quantification cycles (Cq). For fluorescence acquisition and determination of Cq, we used the SDS 2.4 software (Applied Biosystems). For this, we set the baseline between the 3rd and the 8th amplification cycle, and we set the fluorescence threshold manually at 0.05, that is at a level that was

TABLE 1 Controls used for testing the performance of the qPCR assays used for the detection of *Acheta domestica*

		Adom1 (Cq)		Adom2 (Cq)	
Sample type	**Control type**	**Undil.**	**10× dil.**	**Undil.**	**10× dil.**
Cobweb, no target	Negative, primer specificity	neg.	neg.	neg.	neg.
Orb web, no target	Negative, primer specificity	neg.	neg.	neg.	neg.
Web host tissue (widow spider)	Primer specificity	neg.	neg.	neg.	neg.
Web host tissue (hermit spider)	Primer specificity	neg.	neg.	neg.	neg.
Laboratory prey (mealworm)	Primer specificity	neg.	neg.	neg.	neg.
Target (house cricket)	Primer specificity	11.96	18.06	12.99	19.56
Fresh cobweb + target tissue	Amplification inhibition	12.31	18.87	13.30	20.39
Fresh orb web + target tissue	Amplification inhibition	15.09	22.01	16.33	23.49
Cobweb from spider fed with target	Positive	23.88	29.73	25.88	31.50
Orb web from spider fed with target	Positive	17.35	23.93	18.73	25.69

above the baseline and sufficiently low to be within the exponential increase region of the amplification curve.

2.5 | DNA metabarcoding and sequencing (biodiversity surveys)

For extracted DNA from the samples collected to test the biodiversity survey approach, we amplified specific regions using primers commonly used in metabarcoding studies of arthropods, fungi and bacteria using a KAPA HiFi HotStart ReadyMix (2×) PCR kit (Kapa Biosystems; conditions in Supporting Information 2: Table S9). For amplification of markers specific for animal DNA, we used the primer set mlCOIintF and jgHCO2198 that target a 313-bp fragment of the mitochondrial COI (Leray et al., 2013). For amplification of fungal DNA, we used the primer set ITS3_KYO2 and ITS4 that target a 300–400-bp fragment containing the Internal transcribed spacer 2 region (ITS; Toju et al., 2012). For amplification of bacterial DNA, we used the primer pair S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 that targets a 464-bp fragment of 16S rRNA (16S; Klindworth et al., 2013). We provide primer and adapter details, and the amplification protocol, in Supporting Information 2 (Tables S8 and S9).

Before sample submission for sequencing, we measured the amount of amplified DNA for all samples, using microfluidic capillary electrophoresis on the Labchip GX (PerkinElmer), where we used the DNA High Sensitivity Assay. Based on these DNA amplicon amounts, we sequenced bacterial amplicons separately, as the amount of amplified bacterial DNA was several-fold larger compared to that of fungi and animals. For sequencing, we pooled amplified DNA of fungi and animals in a ratio of 3:1, as we expected more fungal amplicon sequence variants (ASVs) than animal ASVs. We sent amplicons for library preparation and high-throughput sequencing through the commercial provider Eurofins Genomics Europe Applied Genomics (HRB 207710). There, the amplicons were cleaned up, and indexes and Illumina adapters were added in a second PCR using in-house company methods in a way that each sample was assigned a unique index. Final amplicon libraries were cleaned up again, quantified and pooled in equimolar ratios. The resulting final library pool was quantified and sequenced in an Illumina MiSeq run using v3 chemistry (2 × 300-bp paired-end reads) using the company's in-house sample multiplexing strategy.

In all parts of sample preparation and sequencing, we included four negative control samples, obtained from individual DNA isolation procedures, and a microbial mock community sample ("ZymoBIOMICS Microbial Community Standard," Zymo Research) that we used as a positive control in the metabarcoding experiment.

2.6 | DNA metabarcoding data analysis (biodiversity surveys)

We analysed sequence data using the QIIME2 2019.4 software package (Quantitative Insights Into Microbial Ecology; Bolyen et al., 2019). We trimmed primers, adapters and regions with quality score <20. Assembly of the paired-ends failed for a large number of reads, presumably due to little overlap between the forward and reverse reads. As ITS length varies among taxa, this failure was probably nonrandom but predominantly occurred in taxa with longer amplicons. To avoid this bias, albeit with a somewhat reduced identification power, we used only single-end forward reads for analysis. We denoised the reads using DADA2, aligned representative sequences using MAFFT and constructed the midpoint-rooted tree using FASTTREE. We calculated alpha and beta diversity indices. We used a trained feature classifier to assign the sequences to taxonomic categories using three databases: Silva release 132 (bacteria; Quast et al., 2012), dynamically clustered UNITE ITS database version 8.0 (fungi; Abarenkov et al., 2010) and MIDORI-UNIQUE CO1 release 244 downloaded on August 5, 2021 (animals; Machida et al., 2017). Due to the large number of nonanimal DNAs in the COI sequences, we manually identified the representative sequences by blasting them against all barcode records on the BOLD database. We then manually removed all nonanimal representative sequences from the QIIME2 representative sequences file, which was finally used to filter the feature table with the option "qiime feature-table filter-features."

For analyses of alpha and beta diversity, we normalized our samples by rarefaction to an equal number of sequences. Any samples with a total number of reads lower than the chosen rarefaction value were removed from these analyses. We retained 15,900 reads for bacterial, 1360 reads for fungal and 490 reads for animal samples, maximizing the number of samples rather than the number of reads (Supporting Information 2: Table S10). To investigate alpha diversity, we calculated the Chao1 (Chao, 1984) and Shannon (Shannon, 1948) indices. We assessed differences in alpha diversity compositions using the Kruskal–Wallis *H* test. We determined the distance and dissimilarity matrices to visualize the ordination and clustering of the bacterial, fungal and animal community compositions for beta diversity analyses, through weighted and unweighted Unifrac distance metrics (Lozupone et al., 2007), and with the Jaccard similarity coefficient and Bray–Curtis dissimilarity. We evaluated the ordination patterns based on phylogenetic distance metrics using principal coordinate analysis (PCoA). We assessed differences in community compositions between sample types by nonparametric permutational analysis of variance (PERMANOVA), using the unweighted UniFrac distance metric and other settings at default within the QIIME2 pipeline.

3 | RESULTS

To test the performance of the two assays designed for detection of *Acheta domestica*, we employed negative controls, primer specificity controls and amplification controls. Both assays were positive for the house cricket (Table 1). In the dilution series, both assays showed consistent detection on a broad linear range (5–6 logs down from undiluted samples, see Figure 1). The limit of detection of the assays was determined at the last dilution

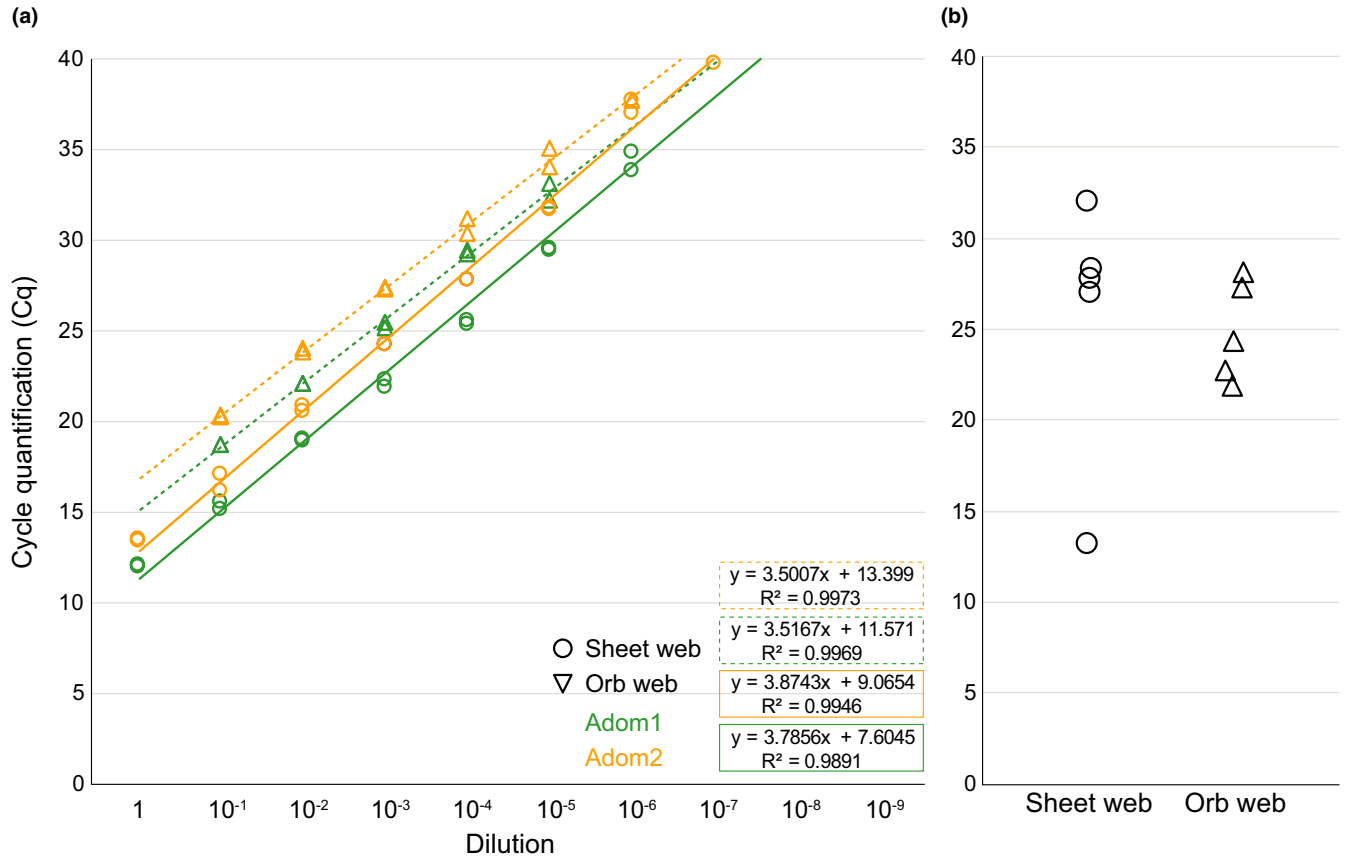

FIGURE 1 Targeted detection of house crickets from webs in nature. (a) The dilution series of DNA isolated from two web types (designated by architecture) containing traces of prey sample (*Acheta domestica*) performed using the Adom1 and Adom2 qPCR assays (designated by colour). (b) Detection success of the target, showing the Cq values for Adom1 for samples of two web types containing traces of *A. domestica* DNA

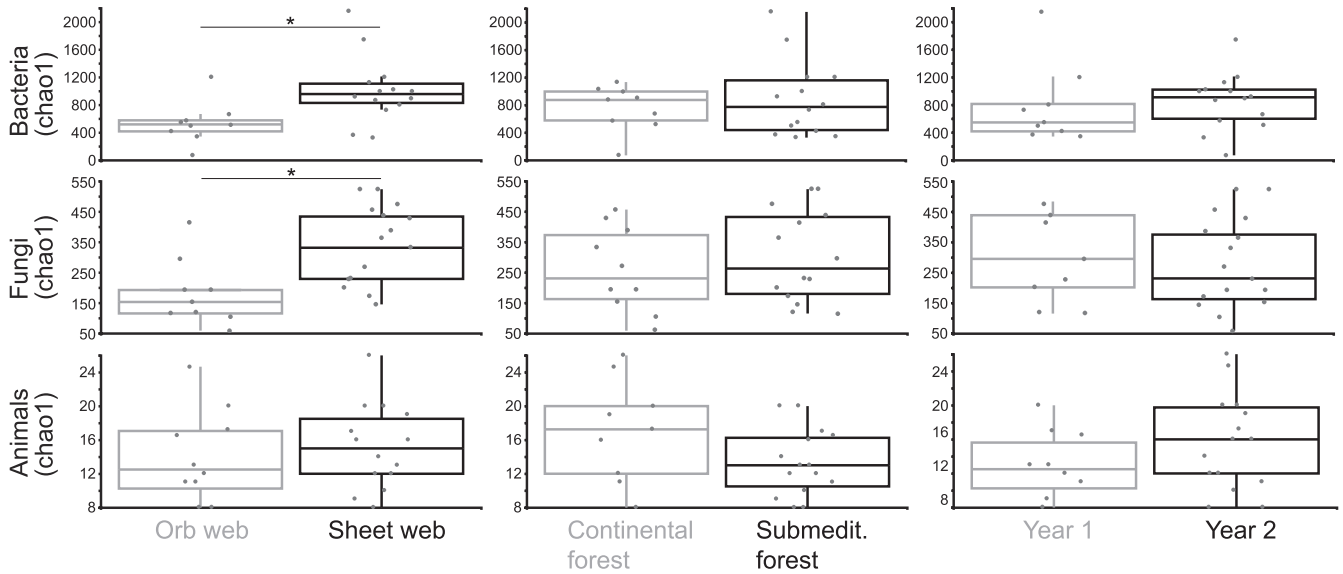

FIGURE 2 Alpha diversities of bacteria, fungi and animals, inferred from spider web eDNA using a DNA metabarcoding approach, and compared between web types (a), forests (b) and years sampled (c). Asterisks mark statistically significant differences

point, which was positive in all replicates, and was at Cq =33 for Adom1 and Cq =35 for Adom2. The details on the performance characteristics of the used assays according to MIQE guidelines are summarized in Table S6. As the two assays were similar in performance, we used only Adom1 in field tests. We successfully detected house crickets in all 10 webs, with Cq values ranging from

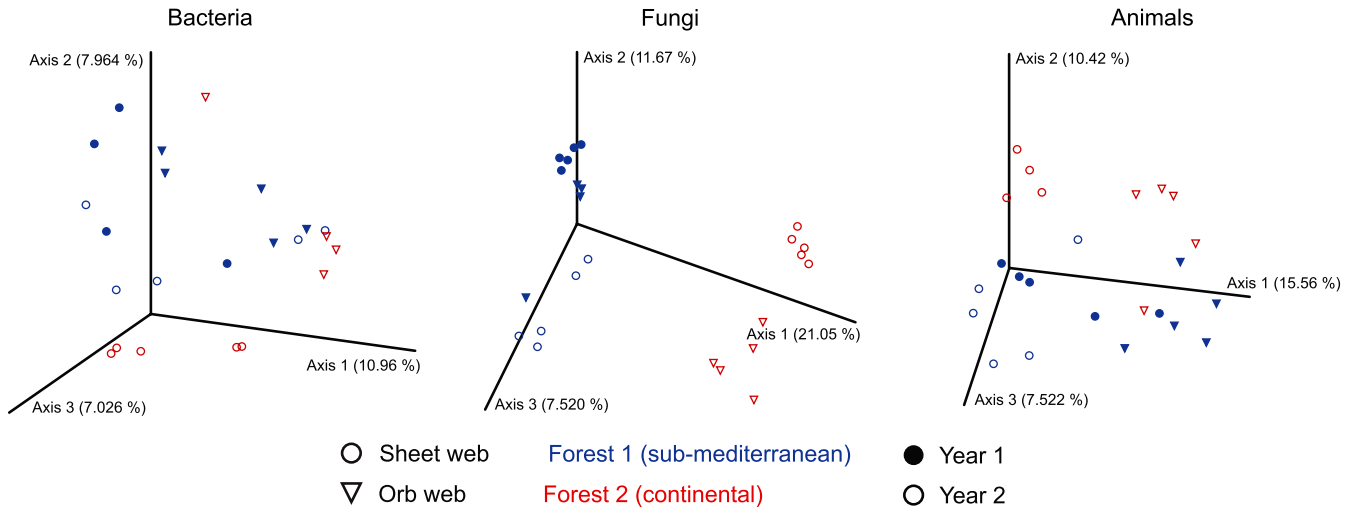

FIGURE 3 Beta diversities (community composition) of bacteria, fungi and animals, inferred from spider web eDNA using a DNA metabarcoding approach. The PCoA plots visualize beta diversities estimated using the unweighted Unifrac distance metric

14 to 32 (Figure 1b). We also successfully traced house crickets in all 10 samples when diluted 10-fold. All negative controls were negative, while the internal isolation control (18S assay) was positive in all samples.

Our metabarcoding investigation from the 25 web and control samples retrieved a total of 2,663,604 bacterial, 2,093,028 fungal and 2,093,028 animal reads, of which 2,284,297 bacterial reads, 778,249 fungal reads and 174,006 animal reads could be taxonomically assigned. Totalling 11,285 ASVs, bacteria represented the largest diversity, followed by fungi with 4005 ASVs and animals with 314 ASVs (Supporting Information 3: Tables S11, S12 and S14). Among bacteria, we identified 30 phyla (Supporting Information 3: Table S11). Among fungi, we identified nine phyla, containing 31 classes, 108 orders, and 307 families. Ascomycota and Basidiomycota were by far the most diverse, encompassing 297 of these 307 families (Supporting Information 3: Table S12). Among animals, we identified three phyla, containing five classes, 16 orders, and 50 families. Insects were the most diverse animal group, representing 33 of the 50 families (Supporting Information 3: Table S15). The microbial mock community sample that we used as a control contained eight bacterial and two fungal species, of which we detected all but one bacterial species. As predicted, we detected no animal sequences in the mock community. All negative controls appear negative, as we detected no animal and fungal sequences, and only single bacterial sequences (Supporting Information 2: Table S10).

Statistical analysis of the chao1 index of alpha diversity showed that in the two forests, webs accumulate a similar diversity of bacterial ($p = .950$, $H = 0.004$), fungal ($p = .412$, $H = 0.672$) and animal ($p = .143$, $H = 2.148$) eDNA (Figure 2). The sampling did not significantly affect eDNA diversity across the three taxonomic groups (bacteria: $p = .314$, $H = 1.016$; fungi: $p = .456$, $H = 0.556$; animals: $p = .207$, $H = 1.595$). Compared to orb webs, sheet webs accumulated a higher diversity of bacterial ($p = .014$, $H = 6.036$) and fungal ($p = .022$, $H = 5.270$), but not animal ($p = .518$, $H = 0.418$) eDNA. Statistical analysis of the Shannon index of alpha diversity showed similar results (Supporting Information 3). The described diversity pattern was reflected in the average ASV number per web (Supporting Information 3: Figure S1). The different biology of the two web types was reflected in the taxonomic representation of taxa on sheet versus orb webs. For example, no orb webs contained nematodes or rotifers, while 20% of sheet webs contained nematodes and 65% contained rotifers. Similarly, of the nine insect orders, all were found on sheet webs, but only four on orb webs. Detailed results of ASV and taxonomic representations are given in Supporting Information 3 (Tables S11–S15) and Supporting Information 4.

Sampling locality, web type and sampling time all affected the inferred beta diversities (community composition) of bacteria (web type: $p = .006$, $F = 1.48165$; locality: $p = .003$, $F = 1.52083$; year: $p = .011$, $F = 1.37463$), fungi (web type: $p = .001$, $F = 5.73776$; locality: $p = .013$, $F = 2.0729$; year: $p = .001$, $F = 3.86025$) and animals (web type: $p = .008$, $F = 1.83303$; locality: $p = .001$, $F = 3.27745$; year: $p = .007$, $F = 1.7966$). The PCoA plots revealed differences in community compositions, and both indices using presence/absence information only (unweighted Unifrac, Jaccard index), as well as those incorporating abundance data (weighted Unifrac, Bray–Curtis coefficient), show similar results (Figure 3; Supporting Information 3: Figure S2).

4 | DISCUSSION

Our study shows that spider webs are suitable for detecting genetic traces of organisms in both a targeted search of a specific species and within a metabarcoding approach. We successfully detected prey introduced into spider webs and show that the targeted search for eDNA from individuals of a single species is a valid concept. In addition, we show that spider webs act as passive filters of the air column by accumulating genetic traces of diverse

organisms. These results suggest spider webs are an effective biofilter and a promising tool for general biodiversity monitoring through DNA metabarcoding.

The idea of using organisms and their extended phenotypes, such as spiders and their webs, to sample genetic material of other local organisms parallels recent studies using bloodsucking insects to identify the local fauna. For example, mosquito blood had traces of their avian, mammalian and amphibian hosts (Townzen et al., 2008), tick blood was used to identify their rodent and stoat hosts (Gariepy et al., 2012), blood from leeches identified a range of mammalian species (Rodgers et al., 2017; Schnell et al., 2012), and carrion flies successfully identified diverse mammals (Calvignac-Spencer et al., 2013). Similarly, the contents of spider intestines can assess local arthropod biodiversity (Kennedy et al., 2020). By accumulating DNA of the hosts' prey, spider webs resemble the intestine contents of bloodsucking and predatory invertebrates. However, spiders are generalist predators, and furthermore their webs are passive traps (Blackledge et al., 2011). We therefore expected that spider webs would accumulate a more general sample of organisms compared to intestine contents. Indeed, our results show that spider webs efficiently capture diverse "aerial plankton" from all domains of life, thus supporting our expectation. In this respect, spider webs better resemble aquatic filter-feeding organisms. Recently, for example, sponge tissue was used to obtain DNA of thousands of marine fish (Mariani et al., 2019), microorganism biofilms were used to identify macroinvertebrates in rivers (Rivera et al., 2021), while other organisms of interest include bivalves, bryozoans, and other active and passive suspension feeders.

4.1 | Single-species eDNA detection

Our tests used two spider species that construct different webs. The capture threads of the hammock web are dry, while the garden spider's classical orb contains glue-coated spirals. Although this would intuitively suggest easier prey detection in the gluey web, this was not the case. The successful detection of prey in all our samples (Figure 1b) indicates that even strikingly different web types accumulate enough genetic traces of arthropods to allow for successful amplification. Furthermore, our approach allows the tracing of DNA of even the smallest-bodied arthropods. Having fed spiders 5 mg crickets, we used a size class below which potential prey is often ignored due to low nutritional value (Blackledge, 2011; Riechert & Luczak, 1982; Uetz & Hartsock, 1987; Watanabe, 2000). Both these arthropod "leftovers," and larger prey are thus expected to be easily detected.

Since spiders tend to cut fallen debris out of their webs, but usually ignore the presence of small, dust-like particles such as plant pollen, fungal spores, and bacteria (Blackledge et al., 2011), these organisms should also be detectable as single-species eDNA in spider webs. While each spider web in our experiments represented a single sample, future studies could pool several webs in a habitat of interest, thereby even increasing the detection power of such an approach.

4.2 | DNA metabarcoding

While alpha diversity provides information about the number of taxa in samples, beta diversity analyses compare compositions of taxa across samples. A good eDNA tool for assessing local biodiversity should result in beta diversity reflecting differences in sample types and sample collection. Although the alpha diversity differed only across web types, but was similar across forests and sampling years (Figure 2), our results show that forest type (submediterranean vs. continental), time of collection (same season in two consecutive years), and web type (sticky and rebuilt daily vs. nonsticky and long-lasting) all affect the recovered beta diversity of animal, fungal, and bacterial communities. Thus, the information obtained from a few individual webs appears to be sufficient to show differences in community compositions among and even within habitats (i.e., among web types). The differentiation of samples based on web type, locality, and year is particularly pronounced for fungal communities (Figure 3). This result is in accordance with research on fungal communities of soil samples, where metabarcoding of fungal taxa has high discrimination power (Young et al., 2014). Similarly, the airborne microbiomes fluctuate across time and space, and our data thus probably reflect actual differences rather than stochastic variability (Šantl-Temkiv et al., 2018; Vestergaard et al., 2018). Thus, in particular, our results indicate that eDNA from spider webs could be useful in tracking biodiversity across time and space. For example, using a general sampling of spider webs, general biodiversity monitoring could be carried out throughout a season or over several years. On the other hand, by sampling spider webs in specific microhabitats (i.e., vegetation layers), useful spatial information could be obtained in research of community compositions.

As a relatively new approach, DNA metabarcoding is simple in concept, but poses substantial methodological challenges that need to be considered for accurate biological interpretation (Zinger et al., 2019). Because spider webs are almost unknown as eDNA samplers, we need to carefully consider both the field/experimental steps, as well as appropriate laboratory standards and controls. For example, the variable sources, transport and degradation of eDNA could all influence metabarcoding results, but are at the same time environment-specific (Harrison et al., 2019). Regarding spider webs, these variables could be shaded vs. sun-exposed habitats, day vs. night active spiders, and web of various types. In the laboratory, unequal PCR amplification of different taxa can skew abundances, while primer biases, PCR inhibitors, reagent contaminants and tag-jumps sometimes lead to false negatives and positives (reviewed by Zinger et al., 2019). Thus, general considerations for laboratory work connected with eDNA research should also be followed when using spider webs as eDNA samplers (van der Loos & Nijland, 2021; Van den Bulcke et al., 2021; Zinger et al., 2019). Additionally, specific controls could also be used to, for example, estimate the "background" eDNA associated with spiders, the producers of webs; these could be controls containing spider webs of corresponding species built in controlled laboratory conditions. The inclusion of such controls

can be considered and carried out in metabarcoding experiments when testing specific hypotheses using spider webs for biodiversity assessments to help with final interpretations of the results. For all these reasons, before screening of eDNA from spider webs can be implemented as an established biodiversity monitoring approach, future studies should include careful considerations of what genetic markers can best answer specific research questions, what are the specifics of the eDNA matrix (web types and their environments) and how to include rigorous laboratory controls.

Regarding our study design, we acknowledge the reservations of using COI in metabarcoding of animal communities (Collins et al., 2019; Deagle et al., 2014); however, potentially better performing genetic markers like 12S rRNA lack large reference data sets, and some studies suggest that the combination of several COI primer sets outperforms single sets that amplify potentially better suited genetic markers (Corse et al., 2019). Thus, the choice of genetic markers and primers might depend largely on the focal organismal group. Because our study tested a methodological concept rather than addressing specific organismal hypotheses, the COI marker was chosen to provide good taxonomic resolution, given the availability of a reliable reference database and longer amplicons. However, analyses of the sequenced data showed that amplicon length was indeed a shortcoming, as it prevented efficient assembly of paired-end reads. To avoid taxonomic bias by excluding taxa with longer (and therefore unassembled) COI amplicons, we present the results based on single-end reads only. Future studies may therefore benefit from using a combination of PCR primers that produce a shorter amplicon of either COI or another taxonomic marker. The use of shorter amplicons could also lead to more efficient amplification due to template degradation, which is often a problem with eDNA. Alternatively, the problem of suboptimal paired-end read assembly can be circumvented by using sequencing technologies that generate longer read lengths.

4.3 | Utility of eDNA from spider webs

When using eDNA to draw conclusions about the proximity of organisms relative to the traces of their DNA, it is crucial to also consider the spatial and temporal distribution and persistence of eDNA (Barnes & Turner, 2016). For example, traces of genetic material remain in water for up to several weeks, but can persist for decades or even hundreds of thousands of years in soil or in permafrost (Dejean et al., 2011; Thomsen & Willerslev, 2015; Willerslev et al., 2014). The fast degradation of DNA in aquatic ecosystems makes eDNA useful in addressing topics such as nature conservation, where positive detection reflects the contemporary presence of species and populations. However, in aquatic eDNA samples, the release of older genetic material from bottom sediments and the transport of eDNA in flowing and marine waters are potential sources of contamination (Goldberg et al., 2015; Thomsen & Willerslev, 2015). Similar to aquatic ecosystems, eDNA collected from the air column via spider webs represents a contemporary presence of organisms in the environment, with potentially more accurate spatial and temporal information. Also, we consider temporal contamination to be unlikely. For example, some web types (e.g., sheet webs) are suspended for several weeks or even months, while most orb webs are rebuilt daily (Blackledge et al., 2011; Foelix, 2011). In addition, many spider species choose web-specific microhabitats (Blackledge et al., 2011). While further studies are needed to elucidate the degeneration rate of genetic traces on longer lasting spider webs, it is clear that spider webs are not only a new tool for "filtering out" genetic material from the air column, but are also unique in the precise spatial and temporal information they provide.

In a straight forward application, eDNA from spider webs could provide a noninvasive and simple method for identifying juvenile spider specimens that cannot be determined morphologically, and could lead to new ways of studying interactions between spiders and their prey without the labour-intensive and biased sampling of potential target prey (Xu et al., 2015). While the prey composition of spiders can be efficiently detected from spider gut contents (Kennedy et al., 2020; Krehenwinkel et al., 2017), eDNA from webs could potentially offer a similar, yet noninvasive approach to infer spider prey. Additionally, it might offer insights into how different webs and details in their structure interact with potential prey, something not possible with conventional observation (Eberhard, 2020). However, our results show a much broader utility of eDNA from spider webs. For example, webs could be used to address questions related to entire communities, such as the distribution and composition of arthropods, plants, fungi, and bacteria over seasons and years, habitats, etc. Immediate applications in nature conservation are also foreseeable, examples being the assessment of declining global insect biodiversity (Hallmann et al., 2017; Lister & Garcia, 2018; Sánchez-Bayo & Wyckhuys, 2019). More precisely, the development of a general pollinator eDNA sampling approach could be used as an information platform to counter the concerning, global pollinator declines exacerbated by the lack of taxonomic expertise (European Commission, 2018). Indeed, among the high diversity of taxa encountered in our web samples, we found several pollinator species such as bees, flies, wasps, and beetles, including the endangered longhorn beetles (Supporting Information 3). In addition to tracking pollinators and endangered species, eDNA from spider webs could also be used to track invasive or pathogen-carrying mosquitoes (Schneider et al., 2016), perhaps as an alternative to direct sampling of water puddles. Furthermore, our results indicate that eDNA from spider webs could be used to investigate species associations. For example, we found co-occurring taxa that are in known relationships, both parasitic and mutualistic (Supporting Information 3).

In our samples, we found several animal, fungal, and bacterial taxa that are of agricultural and medical importance to humans (Supporting Information 3), which highlights a range of possible uses of eDNA from spider webs. Notable examples among plant pathogens and disease-causing agents are genera that include grain rust and wheat curl mites (Eriophyidae; Hartford et al., 1982; Skoracka, 2009), gall and fungus gnats (Cecidomyiidae, Sciaridae; Gagné, 2004; Scheepmaker et al., 1997), aphids (e.g., *Pineus* and *Anoecia*;

Day et al., 2003; McGaving, 1993; van Emden & Harrington, 2017), several fungal representatives that damage wheat crops (e.g., *Fusarium* and *Ustilago*; Andrade et al., 1994; Kämper et al., 2006; Nelson et al., 1994), eudicot plants (*Verticillium*; Barbara & Clewes, 2003), and rice (*Magnaporthe grisea*; Talbot, 2003), as well as several bacterial genera that include plant pathogenic representatives, such as *Pseudomonas*, *Erwinia*, *Dickea* and *Pectobacterium* (Mansfield et al., 2012). Therefore, spider web eDNA could be used for early detection of agricultural pests, even in the absence of disease symptoms, an approach already demonstrated for eDNA from orchards (Guarnaccia et al., 2017; Nicolaisen et al., 2017; Valentin et al., 2018). Moreover, we found several medically important fungal and bacterial representatives, many of which cause respiratory problems and allergies (e.g., *Aspergillus*, *Stachybotrys* and *Botrytis*; Andersen et al., 2003; San-Blas & Calderone, 2008; Williamson et al., 2007), cause food spoilage and gastrointestinal infections (e.g., *Absidia*; Hoffmann et al., 2007), or are associated with livestock (*Salmonella* and *Clostridium*; Wales et al., 2010) and invertebrate vectors (e.g., *Haemophilus*; Ciancio, 2016; Tortora et al., 2020). Perhaps, future use of eDNA from spider webs can extend our understanding of the presence and role of human pathogens in both urban and nonurban environments.

5 | CONCLUDING REMARKS

Spider webs are passive air filters. Utilizing them as eDNA samplers represents a novel technique for obtaining biotic particles from the air masses, an approach equivalent to sampling more established media such as water, ice, and sediments. Our results show that spider webs can become a new-generation biofilter for the targeted tracking and general monitoring of any kind of organisms, from arthropods, rotifers and fungi, to bacteria, plants, and perhaps viruses. As such, the use of eDNA from spider webs offers numerous potential applications from biodiversity monitoring, tracking invasive and pest species, animal diet assessment, obtaining climate change data, to studies on the distribution and niches of arthropods, plants, fungi, and bacteria, whether in a single species or in a metabarcoding context.

AUTHOR CONTRIBUTIONS

M.G., M.K., D.K. and M.R. conceived the study. M.G., K.B., A.P. and C.G. performed experiments and analyses. All authors jointly wrote the paper.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All experimental data are available in the paper and will be available in Supporting Information 4 upon publication.

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