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Fake Webs, Real Results: Artificial Spiderwebs for eDNA Collection

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ABSTRACT

Environmental DNA (eDNA)-based detection is a valuable biomonitoring tool that is well-developed for water, soil, and scat substrates. Emergent research is focusing on air as a new substrate, including opportunistically collected natural spiderwebs which may have negative impacts on local spider diversity. Here, we design novel artificial spiderwebs and compare their effectiveness with natural spiderwebs and aquatic eDNA approaches for biomonitoring of terrestrial taxa. A total of 33 eDNA samples (18 water, 6 natural spiderwebs, 9 artificial spiderwebs) were collected from a rural property in Palmerston North (Aotearoa New Zealand). Three amplicons (COI, 16S, and ITS) were sequenced for each sample to evaluate the performance of each collection method for detecting invertebrates, vertebrates, and plant/algal taxa. The 16S amplicon performed best in terms of sequencing output and consistency, as well as species accumulation curves, with the COI dataset performing worst for all eDNA collection methods. Alpha diversity varied by amplicon and collection method in both value and consistency among samples, with 16S and ITS retrieving higher diversity for water samples and both artificial and natural webs outperforming water in fungal COI diversity recovery. Ordination plots showed clear differences in sample similarity across biomes, with all three amplicons showing differentiation between water and either web type. However, specialist species were recovered by each of the two web types, with artificial webs consistently recovering more unique diversity than natural webs. Our results suggest that artificial spiderwebs could be a promising new method in the eDNA biomonitoring toolbox, providing biodiversity data that complements water-based collections and, depending on the research question, may serve as a sufficient proxy for natural spiderweb studies.

1 | Introduction

Environmental DNA (eDNA)-based detection has emerged as a valuable biomonitoring tool across various ecosystems, with a recent rapid surge in the uptake of eDNA methods occurring globally, owing to their relative ease of use, increased throughput, and versatility across a range of applications (Takahashi et al. 2023). For example, eDNA-based detection can be used to capture changes in biodiversity across space and time, to detect invasive or endangered species, trophic interactions, and dietary preferences, and for overall monitoring of ecosystem health (Beng and Corlett 2020)—all without requiring direct observation, physical capture, or invasive sampling.

While water-based and some terrestrial (e.g., soil, scat) eDNA methods are well-developed (Ruppert et al. 2019), new environmental substrates are currently being investigated to target terrestrial species, such as swabbed plant material (e.g., Thomsen and Sigsgaard 2019; Lynggaard et al. 2023), and invertebrate-derived DNA (e.g., Drinkwater et al. 2021; Fernandes et al. 2023). Airborne DNA (airDNA) has also been shown to successfully detect a range of vertebrates in

zoological parks (Clare et al. 2022; Lynggaard et al. 2022; Newton et al. 2024), in a mixed forest (Lynggaard et al. 2024), and in bat roosts (Garrett et al. 2023). These methods hold great promise for biomonitoring in terrestrial systems, though many are still at a proof-of-concept stage of research, with outstanding questions relating to the ecology of eDNA in these different substrates, as well as their spatial and temporal representation of biodiversity (e.g., Barnes and Turner 2016; Tulloch et al. 2025).

Spiderwebs are found in diverse environments, from natural microhabitats to anthropogenic ecosystems, and represent an inexpensive passive air-based biofilter that has recently been shown to harbor extractable eDNA corresponding to the host spider and its prey (Blake et al. 2016; Xu et al. 2015), as well as to a range of vertebrates, invertebrates, fungi, and bacteria (Corse et al. 2019; Gregorič et al. 2022; Newton et al. 2024). Spiderwebs are known to collect endogenous eDNA, that is bound to airborne cells or tissues of organisms that may come in contact with the web, such as pollen, fungal spores, hair, feathers, skin cells or prey insects (Newton et al. 2024). Due to their naturally sticky quality, spiderwebs may also attract free-floating molecules of DNA, although studies quantifying this are scarce. But eDNA collection from spiderwebs is dependent on their presence in the sampling vicinity at the specific sampling time point (Newton et al. 2024), and there is no control over their placement, size, or deployment duration (e.g., webs can vary from two-dimensional orbs to three-dimensional sheets, and can be renewed daily or less frequently, depending on the spider host; Vollrath 2005; Gregorič et al. 2022). Moreover, rampant sampling of spiderwebs may have detrimental impacts on their resident spiders.

To address the challenges associated with natural spiderwebs, artificial spiderwebs present a promising alternative. In this proof-of-concept study, we explored the potential of using artificial spiderwebs as an eDNA substrate for detecting terrestrial species. We explored three different metabarcoding amplicons to target a broad range of terrestrial taxa, and collected eDNA from natural and artificial spiderwebs, and from water, to assess the potential of artificial spiderwebs as a new eDNA substrate for terrestrial species detection.

2 | Material and Methods

2.1 | Study Site

Water and spiderweb (artificial and natural) eDNA samples were collected between 30 July and 2 August 2024 from sites at a rural property in Aokautere, Palmerston North, Aotearoa New Zealand. The property consisted of ~16 acres of native forests and cultivated gardens, with known wildlife including domestic animals (e.g., *Capra hircus* goat, *Ovis aries* sheep, *Sus scrofa* pig) and native and introduced birds (e.g., *Prosthemadera novaeseelandiae* tūī, *Hemiphaga novaeseelandiae* kererū, *Passer montanus* Eurasian tree sparrows). A stream flowed through the native forest, nestled in a small valley.

2.2 | Spiderweb Construction and/or Collection

Artificial spiderweb samplers ($n = 9$) were constructed by carefully stretching 20 g of polyester (Halloween spiderweb decorations) over sterile plastic coat hangers (surface area ~562 cm^2)—both of which were purchased from a local home supplier—that were first decontaminated using 10% bleach (Figure 1). The polyester was secured by wrapping it around the hanger's strap notches, then manually teased to achieve a thin, web-like consistency. Sterile gloves (sprayed with 10% bleach) were used throughout the assembly, deployment, and collection.

The artificial spiderwebs were secured and evenly distributed along a rope fastened across a gazebo at two proximate sites ~10 m apart, for a deployment duration of 5 days (Figure 1). Collection—including that of trapped arthropods—involved peeling the web off the coat hanger using sterile gloves and a BD spinal needle (Becton Dickinson, Auckland, New Zealand) and storing the material in 50 mL falcon tubes.

Natural spiderwebs—including trapped arthropods and plant material—were opportunistically collected on 2 August 2024 (distance of up to 25 m from artificial webs) by twirling a sterile barrier pipette tip around the web and storing all contents in a 50 mL falcon tube (ThermoFisher Scientific New Zealand Ltd.). A total of 10 natural spiderwebs were collected, nine of

FIGURE 1 | Images showing the artificial (left-hand side) and natural (right-hand side) spiderwebs sampled for eDNA in the current study. Photos: S Bird.

which were situated in trees, and one of which was suspended over a plastic water tank (Figure 1). Due to the opportunistic nature of collection, web size and type varied; no spiders were observed on natural webs; hence no spider species were identifiable.

All spiderweb samples (artificial and natural) were stored at −20°C immediately after collection and later transported on ice to Bioeconomy Science Institute (Lincoln, Aotearoa New Zealand), where they were stored at −15°C until DNA extraction.

2.3 | Water Collection

Water was collected on three separate occasions (30 July, 31 July, 1 August 2024; i.e., $n = 18$ total) from the same site at a stream located ~50 m away from the farthest artificial spiderwebs. Six 1 L replicates were filtered through a 5 μm active syringe sampler (wetland eDNA kits; Wilderlab New Zealand Ltd), with a DNA/RNA Shield preservative agent added to the filter before storage at room temperature until DNA extraction.

2.4 | DNA Extraction and Sequencing

Artificial spiderwebs were submerged (up to the 45 mL mark) in 0.1 M phosphate-buffered saline (PBS) solution (ThermoFisher Scientific New Zealand Ltd.) to prevent cell rupture/shriveling in their respective 50 mL falcon tube (ThermoFisher Scientific New Zealand Ltd.), sealed with parafilm, and secured in a shaking incubator at 16°C, 140 RPM for 1 h and 10 min. After mixing, the PBS solution was pushed through a 0.22 μm filter (13 mm, PES membrane; JET BIOFIL) using a 30 mL syringe, followed by passing 30 cc of air through the filter to remove excess liquid. Filters were stored at −20°C (for approximately 16 h, until the protocol could be resumed) before the outer plastic lip encapsulating the filter was trimmed using sterilized secateurs, and the filter was removed with sterilized forceps and placed into bead tubes for DNA extraction. Natural spiderwebs were scraped from the barrier pipette tip into bead tubes using sterilized forceps. Only nine natural spiderwebs were recovered, as one was too fine. The secateurs and forceps were sanitized using 0.5% bleach, milli-Q water, and 70% ethanol between each spiderweb sample. To extract the water sample lysates from the encapsulated filter, the assembly was held vertically with the filter at the top, and the 3 cc syringe was drawn back to create a suction for 5 s to allow the lysate to pool in the 3 cc syringe barrel. Sample lysates were then transferred into bead tubes. All samples were stored at −15°C until DNA extraction. DNA was extracted from all samples using a Qiagen Blood and Tissue kit (ThermoFisher Scientific New Zealand Ltd.), including negative controls, following the manufacturer's protocols. Negative controls ($n = 3$) were performed identically to the sample extractions, but without the addition of any sample material. A further three sampling controls were included in the extractions (one for each substrate); in each case, the extraction protocol proceeded identically to the sample extractions, but without the substrate having been deployed in the field. For water and natural spiderwebs, sampling blanks consisted of open bead tubes (part of the Qiagen Blood and Tissue kit) that were briefly exposed in a fume hood to simulate handling. For the artificial spiderweb, a 0.22 μm filter from the normal extraction procedure was used as a sampling blank.

DNA extracts were PCR-amplified in a single reaction per sample/amplicon using Illumina-tailed primers for the detection of invertebrates, vertebrates, and plants: cytochrome c oxidase subunit I (COI, mlCOIintF/HCO2198; Leray et al. 2013; Folmer et al. 1994), 16S mitochondrial DNA (16S, V16S-U-FV16S-U-R; Wang et al. 2023), and internal transcribed spacer (ITS, ITS-S2F/ITS4R; Chen et al. 2010; White et al. 1989), respectively (see Table S1 for master mix and PCR cycling conditions). Negative PCR controls were included for each PCR assay, and one was selected at random for sequencing. All PCR products were visualized using a 1% agarose gel, and a subset of samples was quantified using a Qubit fluorometer (Thermo Fisher Scientific New Zealand Ltd.). Samples for which PCR products had low concentrations and/or faint gel bands (8, 29, and 12 samples for COI, 16S, and ITS, respectively) were re-amplified, with their PCR products then pooled. These, and all other samples, Bioeconomy Science Institute were then pooled by amplicon prior to clean-up (PCR master mix volumes were 15 or 25 μL; see Table S1).

All PCR products were cleaned and size-selected with solid-phase reversible immobilization (SPRI) beads following the standard bead clean-up protocol, with two 80% ethanol washes in a 1:2 ratio of PCR product:beads used to select for product sizes of ~300–400 bp. Cleaned PCR products (8 μL elution volume) for each amplicon were finally pooled in equal volumes into a single tube per sample, with library preparation and sequencing carried out by SeQuench Ltd. (Nelson, New Zealand) on an Illumina MiSeq v2 (500-cycle kit; 2 × 251 bp) using Nextera XT v2 Index Kit Set C dual indices (C-N701-N712 and C-N714-N715 for i7 and C-S513, C-S515-S518, C-S520-S522 for i5). The final library concentration for all pooled samples was 6 pM, with a 15% PhiX spike-in, and the sequencing run generated a total of 5.83 Gbp of data, with an effective yield of 5.16 Gbp (across a total of 96 samples), though only 41 were from this study, including $n = 8$ controls (three extraction blanks, three sampling blanks, one PCR negative, one SeQuench sequencing blank).

2.5 | Data Analysis

Pooled amplicons were first demultiplexed into individual samples per amplicon using the clsplitseq function and default settings in Claident v.0.2.2019.05.10 (Tanabe and Toju 2013).

Demultiplexed DNA sequence reads were analyzed using DADA2 v1.34.0 (Callahan et al. 2016) in R v4.2.1 (R Core Team 2024). Reads were first truncated to remove relevant primer sequences and then further trimmed according to the quality plot profile (trimming up to 30 bp from either end of the sequence based on the sequencing quality, Phred score). Further filtering removed all reads with 1 or more ambiguous bases and > 2 expected errors (i.e., maxN = 0 and maxEE = c(2,2), respectively). Next, error rates were learned for each amplicon separately, and reads were de-replicated and denoised. Denoised reads were then merged and chimeras *de novo* removed to produce a table of amplicon sequence variants (ASVs).

Taxonomy was assigned to the ASVs using bespoke DNA databases, which were first created on 17/03/25 (COI) and 19/03/25 (16S) using CRABS v.1.7.6 (Jeunen et al. 2023), following the tutorial steps provided at: https://github.com/gjeunen/reference_database_creator. The COI database was created using the National Center for Biotechnology Information (NCBI) and the query "COI[All Fields] OR COXI[All Fields] OR CO1[All Fields] OR COX1[All Fields] OR cytochrome oxidase subunit i[All Fields] OR cytochrome oxidase subunit 1[All Fields] OR cytochrome oxidase subunit I[All Fields] AND (mitochondrion[filter] AND ('200'[SLEN]: '50000'[SLEN]))," and the 16S database was similarly obtained from NCBI using the query "16S[All Fields] AND (mitochondrion[filter] AND ('100'[SLEN]: '50000'[SLEN]))". Both COI and 16S databases were then imported to CRABS format using the --import command, while the ITS database was generated in CRABS format by importing the sh_general_release_dynamic_s_all_04.04.2024 fasta file from unite (available at: https://unite.ut.ee/repository.php; Abarenkov et al. 2023). Next, amplicon regions were extracted from each database, pairwise global alignment was performed to retrieve amplicons without primer regions, and the database was de-replicated and filtered using default settings before being exported to DADA2 format.

Following taxon assignment in DADA2, ASVs were further annotated to species level using BLAST searches in the nucleotide database of NCBI. Only ASVs with e-values $> 1e^{-50}$ were used for this purpose.

Per-sample ASV counts, final taxonomic assignments, and associated metadata tables for each amplicon were read into R v.4.4.2 for analysis and visualization of ASV data using phyloseq v1.50.0 (McMurdie and Holmes 2013). The number of raw reads ranged from 514–28,849, 1156–74,413, and 10–67,560 per sample for COI, 16S, and ITS, respectively (Table S2) and the number of initial ASVs was 4955 (COI), 421 (16S), and 2574 (ITS). First filtering steps involved the removal of any samples with total read counts < 5, as well as taxa for which phylum-level information was uncharacterized, and for which family corresponded to Hominidae. This resulted in a final number of taxa of 2663 (COI), 356 (16S), and 2268 (ITS). Reads for the COI dataset were additionally subset by phyla into four groups for separate analysis: fungi (Ascomycota, Basidiomycota, Mucoromycota): 2162 taxa; invertebrates (Annelida, Arthropoda, Cnidaria, Gastrotricha, Mollusca, Nematoda, Platyhelminthes, Porifera, Rotifera, Tardigrada): 566 taxa; vertebrates (Chordata): 25 taxa; and plants/algae (Bacillariophyta, Chlorophyta, Haptophyta, Rhodophyta, Streptophyta): 91 taxa. However, as the number of retained taxa for vertebrates and plants/algae was low for the COI dataset, we focused mainly on the fungal and invertebrate subsets for the COI analyses.

In addition to the filtering above, we examined the eight control samples to identify any clear patterns of contamination that could potentially be removed. We first examined the number of reads for each control type and amplicon (Table S3). As sample read numbers varied by amplicon and were sometimes present in high abundance (up to 55,097; Table S3), we could not reliably filter them out. However, we next produced MDS plots (see below) on filtered data to examine where controls clustered with respect to samples (Figure S1). For 16S, only two control types made it past filters (an extraction control and the control for water samples—both clustered with the main water sample cluster in the MDS plot). For COI, five control types made it past filters—an extraction and PCR control fell outside the main clusters in the MDS plot, while another extraction and the water control fell into the main water sample cluster, and the web natural control fell within the web natural cluster. For ITS, an extraction control fell within the web cluster (both natural and artificial), while the water control sat within the water cluster.

Initial data exploration following filtering involved plotting relative read abundance (top 20 taxa) by taxonomic group. Subsequently, patterns of Shannon's alpha diversity were examined by calculating taxonomic richness for all ASVs for each collection type (water, natural spiderweb, artificial spiderweb). Following rarefaction to sample sizes of 100–3500, depending on the dataset, multivariate analyses (PCoA/MDS, PERMANOVA) based on the abundance of ASVs (Bray–Curtis) were conducted using the ordinate function in phyloseq and the vegan v.2.6-8 package (Oksanen et al. 2018). Rarefaction resulted in too few taxa/samples for the subset COI dataset, so the geometric means function from DESeq2 v1.46.0 (Love et al. 2014) was used to normalize prior to ordination analysis for the fungal and invertebrate COI data (2162 and 566 taxa; and all samples retained for fungal and invertebrate datasets, respectively). Statistical significance was tested using the adonis2 function, with Bray's distance, 999 permutations, and a confidence level of 0.05. Beta dispersion was also calculated from the Bray's distance matrix, using the betadisper function from vegan. Rarefaction and species accumulation curves were generated on non-rarefied data using the rarecurve (100 steps) and accu_plot (10 steps) functions in vegan and MiscMetabar v0.14.2 (Taudière 2023) R packages. Finally, to assess differences in the taxa recovered by natural and artificial spiderwebs, the clamtest function was applied to rarefied data using the clamtest function in vegan. All plots were generated using base R or ggplot2 v.3.5.1 (Wickham 2016).

3 | Results

3.1 | General Dataset Characteristics

Of the 37 total samples, $n = 4$ natural spiderwebs did not pass filtering thresholds, resulting in a final total of 33 samples (18 water, 9 artificial spiderwebs, 6 natural spiderwebs). After filtering, the COI dataset retained 2663 taxa (622 taxa when rarefied to a sample size of 100); the 16S dataset retained 356 taxa (280 taxa when rarefied to a sample size of 1000); and the ITS dataset retained 2268 taxa (1083 taxa and 31 samples when rarefied to a sample size of 3500).

Library size (i.e., sequence output per sample) varied by collection method and amplicon (Figure S2). In particular, library size was highest for the 16S data, followed closely by the ITS data, while the COI data returned higher library sizes for fungal and invertebrate taxa and very low library sizes (< 200) for chordate and plant/algal taxa. Library size patterns across collection methods were largely similar for ITS and 16S, but highly variable for COI, with less variability across samples for water collection across all three amplicons (Figure S2). For the COI dataset, neither chordate nor plant/

algal subsets returned high sequencing output and were excluded from certain subsequent analyses.

Rarefaction curves showed that the 16S dataset plateaued quickly for all ASVs, indicating saturation. ITS data did not show saturation for some ASVs, suggesting greater sampling effort would have returned a greater number of taxa, while COI data showed a more extreme version of this pattern. These patterns appeared consistent regardless of the sample collection method (Figure S3).

Similarly, species accumulation curves reached saturation for 16S, approached saturation for ITS, and suggested that large portions of species diversity were not captured for COI fungal (especially for water) and invertebrate (especially for water and artificial webs) samples (Figure S4).

3.2 | Biodiversity Assessment

Relative abundance plots for the top 20 taxa showed that biome recovery differed across amplicons (Figure S5). For the COI data subset, eight phyla were detected overall, with the majority represented in the water samples and fungi dominating both web types. For the 16S data, the phylum Chordata dominated all sample types. Finally, for the ITS dataset, the phylum Streptophyta dominated all sample types (Figure S5).

Shannon's alpha diversity plots further showed patterns of diversity that varied by amplicon and collection method (Figure 2). For 16S data, alpha diversity was higher for water compared to either web type and was similarly variable among samples. ITS data showed a tighter distribution of higher alpha diversity for water samples, while diversity was lower and more variable for artificial and natural webs. For the COI data, the chordate subset is shown in the plot for comparison, but diversity was too low for consideration. Meanwhile, alpha diversity was higher, and more variable for COI invertebrate and plant/algal data subsets for water samples compared to either web type. In contrast, the fungal COI dataset returned higher alpha diversity for artificial webs, with a low range of values (all around 4) per sample. Fungal alpha diversity of natural spiderwebs was also higher and more variable than that recovered from water samples (Figure 2).

3.3 | Biome Comparisons

Ordination plots showed clear differences in sample similarity across biomes. The separation between water and either web type was clearest for the ITS dataset (PERMANOVA: $F_{2,28} = 4.8285$, $p = 0.001$; BETADISPER: $F_{2,28} = 3.9694$, $p = 0.025$), where both web types partially overlapped but water-based samples formed a discrete cluster (Figure 3; Figure S6). Pairwise differences in the dispersion analysis indicated that water was statistically different from both natural ($p = 0.048$) and artificial ($p = 0.036$) webs, while the two web types were not significantly different ($p = 0.815$).

The variation on axis 1 for the 16S dataset was substantial (43.6%) and reflected the discrimination between water and

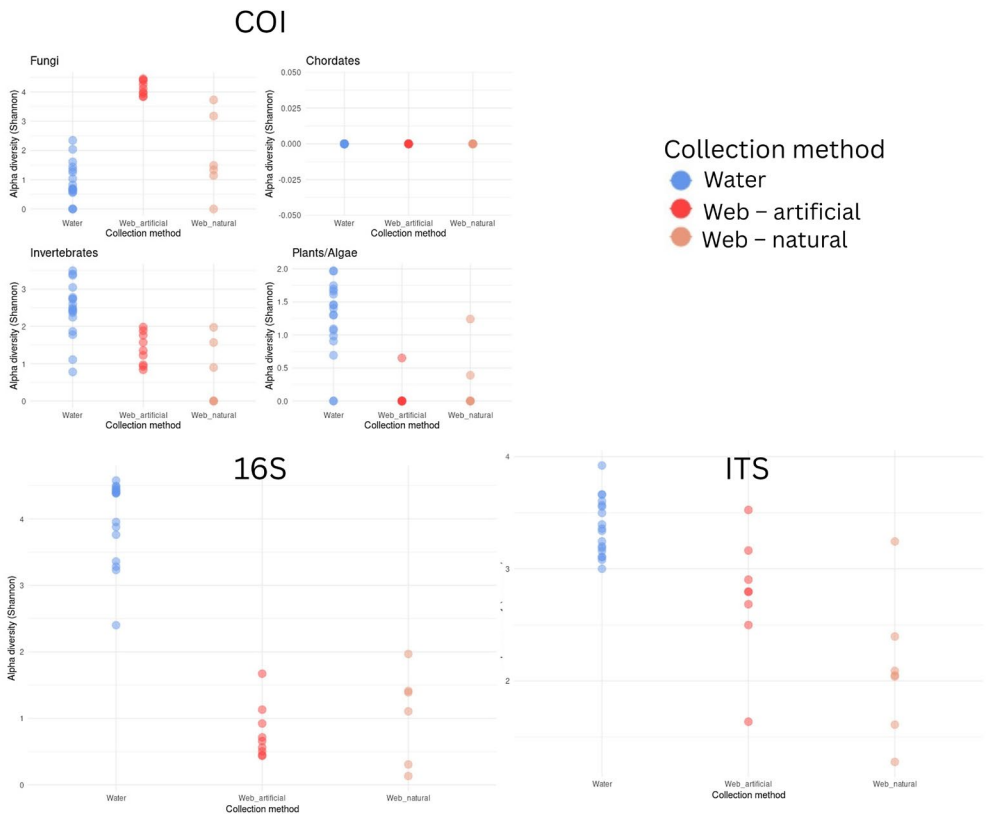

FIGURE 2 | Plots of Shannon's alpha diversity for each eDNA collection method and amplicon, as indicated. The number of taxa for each group/amplicon = 2663 (COI), 356 (16S), and 2268 (ITS).

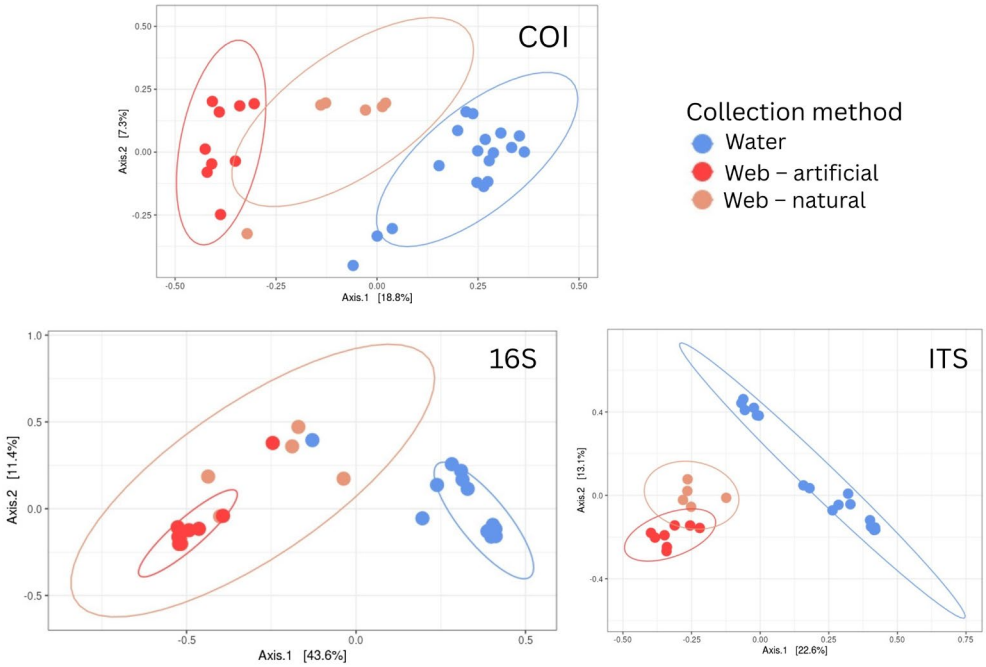

FIGURE 3 | Ordination plots for each eDNA sampling method and amplicon, as indicated.

web samples; however one outlier water sample overlapped with the two web 95% confidence clusters in the plot, and the artificial web samples mostly clustered tightly as a subset within the natural web cluster (PERMANOVA: $F_{2,30}=11.0960$, $p=0.001$; BETADISPER: $F_{2,30}=4.0484$, $p=0.026$). Indeed, pairwise distances in the dispersion analysis did not reach significance for water versus natural ($p=0.098$) and artificial ($p=0.061$) webs, while the two web types were significantly different ($p=0.046$) (Figure 3, Figure S6).

For the overall COI dataset, there was some minor overlap between artificial and natural spiderwebs and a clear separation of water and both web types (PERMANOVA: $F_{2,30}=4.1753$, $p=0.001$; BETADISPER: $F_{2,30}=24.6530$, $p=0.001$), and all pairwise differences were highly significant ($p=8.067e^{-06}$, $p=6.337e^{-02}$, $p=3.914e^{-05}$, for water versus artificial spiderwebs, water versus natural spiderwebs, and artificial versus natural spiderwebs, respectively) (Figure 3, Figure S6). For the subset COI data, despite overlaps in the ordination space, fungal diversity differed by collection method (PERMANOVA: $F_{2,30}=3.2388$, $p=0.001$; BETADISPER: $F_{2,30}=23.9830$, $p=0.001$), and all pairwise differences were highly significant ($p=1.365e^{-06}$, $p=2.6011e^{-01}$, $p=4.3167e^{-03}$, for water versus artificial webs, water versus natural webs, and artificial versus natural webs, respectively), while the invertebrate dataset showed differences by collection method (PERMANOVA: $F_{2,30}=3.1050$, $p=0.001$; BETADISPER: $F_{2,30}=6.5360$, $p=0.008$), with all pairwise differences highly significant ($p=0.015$, $p=0.011$, $p=0.556$, for water versus artificial webs, water versus natural webs, and artificial versus natural webs, respectively) (Figure S7).

3.4 | Artificial Versus Natural Spiderwebs

Clamtest analysis was used to investigate the taxonomic patterns driving any differences between artificial and natural spiderwebs, categorizing specialist species (present in either one web type or the other) for all three amplicons (Table 1, Figure S8; Table S4). For the COI fungal dataset, both web types recovered Ascomycota, but the artificial webs also recovered Basidiomycota ($n=13$). Patterns of taxa recovery were similar for the COI invertebrate data, with $n=6$ and 5 taxa unique to each natural and artificial web, respectively and arthropods and rotifera recovered for natural spiderwebs, while the artificial spiderweb recovered arthropods, and a poriferan and annelid (possible misidentifications or contamination). The ITS dataset recovered similar groups of unique taxa for both spiderweb types, with the main difference driven by a higher diversity of Streptophyta recovered with the artificial webs ($n=61$ versus $n=41$ for the natural spiderwebs). Finally, the 16S dataset revealed higher numbers of birds ($n=10$ versus $n=4$) and similar numbers of mammalian ($n=3$, $n=4$) taxa that were unique to natural and artificial webs, respectively in terms of ASVs, but matched the same species for cattle (*Bos taurus*) and gray parrot (*Psittacus erithacus*). Interestingly, natural webs recovered native birds (*H. novaeseelandiae* and *P. novaeseelandiae*), as well as the common brushtail possum (*Trichosurus vulpecula*), while artificial webs recovered domestic animals (*C. hircus*, *O. aries*, *S. scrofa*, *Gallus gallus domesticus* chicken). All recovered 16S taxa were confirmed as being present in the near vicinity of the web collection sites (Table 1, Figure S8; Table S4).

4 | Discussion

Our research demonstrates for the first time that artificial spiderwebs may be a viable substrate for the collection of eDNA, recovering unique biodiversity compared to both water-based and natural spiderweb eDNA substrates.

Sequencing performance in our study was amplicon- and collection method-specific, with differences likely driven at least

TABLE 1 | Clamtest results, showing the unique phyla detected from artificial and natural spiderwebs for each amplicon.

	Natural spiderwebs		Artificial spiderwebs	
Amplicon	**No. unique taxa**	**Phyla counts**	**No. unique taxa**	**Phyla counts**
COI—Fungi	23	Ascomycota: 23	51	Ascomycota: 38 Basidiomycota: 13
COI—Invertebrates	6	Arthropods: 5 Rotifers: 1	5	Annelids: 1 Arthropods: 3 Rotifers: 1
16S	13	Aves: 10 Mammalia: 3	8	Aves: 4 Mammalia: 4
ITS	51	Ascomycota: 8 Chlorophyta: 2 Streptophyta: 41	69	Ascomycota: 5 Basidiomycota: 1 Chlorophyta: 2 Streptophyta: 61

in part by our sampling methodology. For example, natural spiderwebs represented six independent sampling points, while water was collected three times from the same site and artificial spiderwebs were placed at two proximate locations. Despite the higher independent replication for natural webs, both web types returned high diversity for the COI fungal dataset. Other research has similarly shown that fungal diversity is well detected from natural spiderwebs, with >2 million fungal reads (4005 ASVs) detected from 25 webs collected from two forests in Slovenia (Gregorič et al. 2022).

Previous work has shown that spiderwebs do a good job of recovering terrestrial species, with 85 vertebrate taxa detected from two sampling locations in a wildlife sanctuary in Perth, Australia—including mammals, birds, one reptile, and one amphibian (Newton et al. 2024). Our results confirmed this pattern, differentiating water and web samples regardless of the amplicon. This suggests that passive airDNA methods could be employed to complement water-based eDNA sampling if the study objectives include the recovery of terrestrial species, a more holistic overview of the entire ecosystem, and/or rarer species that are otherwise swamped by more abundant sequence reads from common species in eDNA datasets. Indeed, though water and soil are the most common eDNA substrates in current use (Cowgill et al. 2025), new methods are regularly being introduced and/or have been around but less popular for a long time (e.g., scat), and these are collectively showing greater terrestrial biodiversity representation (e.g., insect-derived 'iDNA' has the potential to recover DNA not just from the captured insects, but also local vertebrates and plants that they have fed on; Kocher et al. 2017; Ji et al. 2022; Carvalho et al. 2022), as well as being applied to novel questions related to population genetics and animal behavior (Zhao and Andermann 2024; Newton et al. 2025).

A key objective of our study was to evaluate the potential of artificial spiderwebs to serve as a proxy for natural spiderwebs that might enable biodiversity recovery without the adverse effects on spiders. Our results confirmed that artificial spiderwebs are capable of collecting eDNA—a particularly encouraging finding was the presence of various spider taxa on the artificial webs when they were being collected. Moreover, artificial webs consistently recovered unique subsets of biodiversity compared to natural webs for all three amplicons. This is a promising result given that other advantages apply to the use of artificial over natural spiderwebs, including the ability to standardize and reproduce various methodological components such as web size, shape, and deployment location and duration. For example, artificial webs can be deployed under weather-proof shelters at any required height. They can also be replicated with ease in aid of a well-designed biomonitoring study, and are simple and cheap to construct and deploy, with opportunities for educational and citizen science programmes.

Despite the promise of this proof-of-concept study, there are some caveats to consider. First, there are likely to be different biases associated with the tested amplicons in terms of DNA extraction and PCR amplification/replication and we did not explore these here (though we included controls and normalized reads as appropriate during data analysis). Quite possibly, shorter amplicons could increase the amplification efficiency and further refinement of the sampling technique to optimize spiderweb capture of more degraded fragments may be necessary (e.g., Anmarkrud et al. 2025). Second, we included extraction, sample, PCR, and sequencing controls, and found that these contained sometimes high numbers of sequence reads, with results differing by control type and amplicon. As is typical for most eDNA surveys, we recommend that these and other considerations, such as web material and hanger material controls, be included in future studies. Third, we used a relatively small number of samples and a single study location; future studies should expand the scope of our preliminary analyses to better investigate the impacts of sample size (e.g., to 30 per factor, per environment assessed), environmental differences, and temporal and spatial signals. Fourth, we focused our analysis on key taxonomic groups (e.g., excluding bacteria). Along with these outstanding aspects, we look forward to further refinement of artificial spiderwebs (e.g., collection methods, as well as methods of web creation) as an eDNA substrate for terrestrial applications. This includes the impact of the protective roof we used here versus natural canopy, which on the one hand may have enabled more airDNA to fall on the webs but on the other

may have been severely impacted by DNA losses to rainfall events (e.g., Condachou et al. 2025).

Finally, we propose that artificial spiderwebs may offer a useful, tractable method, more conducive to managed study design than natural spiderwebs may offer. Natural spiderwebs, while effective at eDNA capture, also introduce increased variability arising from spider species-specific differences in web location, building and enzymatic material, web suspension time, and rebuilding frequency, with additional stochasticity associated with finding spiderwebs in the studied environment. Conversely, artificial spiderwebs can be installed in predetermined locations across a GPS-marked survey grid to optimize against the biomonitoring questions and ensure web recovery. As discussed above, further development of artificial spiderweb methods, including testing of other artificial materials, degradation rates, impacts of canopy, and impact of wind speed and direction on the effectiveness of eDNA recovery would be beneficial for refinement of this methodology. Paired with their ease of the readily available, inexpensive materials, artificial spiderwebs offer a standardizable and common tool for trapping eDNA from terrestrial organisms for biomonitoring surveys led by agencies, scientists, communities, and citizen scientists.

Author Contributions

A.M. and M.K.D. conceived the research project. A.M., M.K.D., and S.B. designed the initial experiment and later adjustments. S.B. collected eDNA samples. A.M. led the main manuscript writing, with S.B. writing some sections and M.K.D. providing feedback. All authors approved the final version of the manuscript. M.K.D. de-mutiplexed the data and co-ran some pilot DADA2 analyses with A.M. A.M. ran the subsequent analyses and generated all final outputs.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequence reads are available on the SRA (Bioproject number: PRJNA1251198). All scripts and input files required to reproduce the analyses in this paper are available at https://github.com/invasomics/spiderwebs.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** edn370194-sup-0001-Supinfo.docx.