Environmental DNA
SPECIAL ISSUE Environmental DNA Traveling by Air

WILEY

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

ORIGINAL ARTICLE OPEN ACCESS

Spider Webs as Efficient Passive Samplers for Airborne Fungal eDNA in Forests: A Case Study With *Hymenoscyphus fraxineus*

Polona Kogovšek[1] | Nikica Ogris[2] | Maja Ferle[1] | Janko Šet[3] | Barbara Piškur[2] | Tjaša Lokovšek[3] | Denis Kutnjak[1] | Matjaž Gregorič[3,4]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia | [2]Slovenian Forestry Institute, Ljubljana, Slovenia | [3]Jovan Hadži Institute of Biology, Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia | [4]Postgraduate School ZRC SAZU, Ljubljana, Slovenia

Correspondence: Polona Kogovšek (polona.kogovsek@nib.si)

Received: 10 January 2026 | **Revised:** 8 May 2026 | **Accepted:** 18 May 2026

Keywords: airborne eDNA | ash dieback | forest pathogens | *Hymenoscyphus fraxineus* | passive sampling | qPCR | spider webs

ABSTRACT

Monitoring airborne inoculum of pathogens is important for plant disease surveillance. Here, we evaluate spider webs as passive environmental DNA (eDNA) samplers for detecting the pathogenic fungus *Hymenoscyphus fraxineus*, the causal agent of ash dieback, in a forest environment. In a temperate mixed forest, we compared two types of spider webs (orb and sheet webs) with a conventional passive sampler (filter paper) over matched day (orb) and week (sheet) deployments. Laboratory validation confirmed that webs exposed to airborne spores from apothecia yield positive qPCR signals. Across seven field sampling campaigns, both a species-specific qPCR (Hfrax) and a broad fungal assay (FQ) detected fungal eDNA on spider webs more reliably and at higher relative quantities than on filter paper. In daily and weekly pairs, orb and sheet web vs. filters, the median ΔCq (filter − web) was above 4 for both qPCR assays (Wilcoxon paired, $p < 0.05$), which corresponds to more than a 10-fold difference in the amount of target DNA. This study provides proof of concept for using spider webs as scalable, low-cost tools suitable for targeted (qPCR) surveillance to complement aerobiological networks.

1 | Introduction

Monitoring plant pathogens' airborne inoculum is an important part of plant disease surveillance frameworks (Van der Heyden et al. 2021). Spore trapping from the air, combined with molecular diagnostics, enables early detection of plant pathogens before symptoms appear, thereby supporting disease forecasting and management strategies (Aguayo et al. 2021; Schweigkofler et al. 2004).

Aerobiological networks, originally developed for pollen monitoring and allergy risk assessment, have been adapted for forest pathogen surveillance (Aguayo et al. 2021). Passive and active sampling approaches are used to collect airborne spores, including sticky rods from rotary arm spore collectors, glass fiber filters (Bérubé et al. 2018), filter paper (Schweigkofler et al. 2004), and Hirst-type volumetric air samplers that collect spores and pollen on microscope slides (Aguayo et al. 2021). Subsequent targeted and non-targeted molecular analyses of these samples provide spatial and temporal insights into pathogen presence and prevalence (Aguayo et al. 2021; Larsson et al. 2025; Nicolaisen et al. 2017). Importantly, seasonal patterns of specific pathogens have been shown to correlate with disease incidence reports.

Recent advances in environmental DNA (eDNA) research offer new opportunities for biodiversity studies, invasive species

detection, pathogen surveillance, and agroecological applications (Tulloch et al. 2025). Spider webs have emerged as efficient natural collectors of airborne particles, including pollen from various trees, shrubs, herbs, pteridophytes, as well as fungal spores (Oraon et al. 2022). Their adhesive and electrostatic properties facilitate the collection efficiency of bioaerosols, and viable bacteria and fungi have been recovered from webs in urban environments (Mattei 2009). eDNA studies confirm that webs contain amplifiable DNA from multiple domains of life (Gregorič et al. 2022) and are a promising tool for terrestrial biodiversity monitoring (Berard et al. 2025). Therefore, using spider webs as passive eDNA samplers could complement existing aerobiological networks, especially in habitats where conventional spore trapping is logistically challenging (Newton et al. 2025).

In this study we evaluated spider webs in natural forest environments as a passive, surveillance-oriented substrate for targeted detection of plant pathogenic fungi. As a proof-of-concept we focused on *Hymenoscyphus fraxineus*, a pathogenic fungus causing ash dieback and known to occur in the experimental area (Baral et al. 2014; Ogris et al. 2009). *H. fraxineus* is an ascomycete whose asexual and sexual stages are both associated with ash trees (*Fraxinus* spp.), on which it causes characteristic disease symptoms of leaf loss and crown dieback (Kowalski 2006). This pathogen spreads via ascospores produced in apothecia on infected ash rachises in the leaf litter. Spore release is strongly influenced by humidity, leaf wetness, and temperature, with diurnal peaks linked to radiation and moisture conditions (Burns et al. 2022). Sporulation peaks between June and August in Europe, with the majority of the inoculum deposited within 50 m of infected trees, although wind dispersal can carry spores up to 50–100 km ahead of the disease front (Grosdidier et al. 2018). These dispersal characteristics make *H. fraxineus* an ideal model for testing passive aerial eDNA surveillance, because collectors within ash stands will frequently encounter *H. fraxineus* spores and eDNA. Demonstrating reliable detection of spider web eDNA therefore provides a practical proof of concept for a scalable, low-cost early warning approach that could complement existing aerobiological monitoring and support forest disease surveillance.

2 | Methods

2.1 | Study Overview

We combined (i) a laboratory exposure validating binding and detection of *H. fraxineus* DNA on spider webs and (ii) field comparisons of spider web versus filter paper samplers in a temperate mixed forest in Central Europe (Ljubljana Marsh, Slovenia). Two spider web types were used: orb webs, vertical wheel-shaped webs made of dry silk threads as well as glue-coated capture threads, typically rebuilt daily by spiders; and sheet webs, long-lasting webs consisting exclusively of dry silk, a horizontal sheet, interconnected with horizontal threads (Foelix 2025). Sampling was organized during periods without heavy rain (based on weather forecasts) to avoid wash-off of collected material.

2.2 | Laboratory Exposure of Webs to *H. fraxineus* Apothecia and Spores

A laboratory experiment was set up to check if the *H. fraxineus* spores that are released from the apothecium can be collected on spider webs. To obtain the two web types, we housed female hermit spiders *Nephilingis cruentata* in 35 × 35 × 10 cm polymethyl methacrylate frames, and female cellar spiders *Pholcus phalangioides* in plastic boxes with 20 × 13 × 11 cm metal construction for web support (Kralj-Fišer and Gregorič 2019). *H. fraxineus* spores were obtained from infected ash tree (*Fraxinus* spp.) leaf rachises collected in a humid forest (Ljubljana Marsh, Slovenia). Leaf rachises were incubated at room temperature exposed to natural light conditions on wet paper towels in a 15 cm wide glass petri dish for 2 months to obtain fresh apothecia.

The initial laboratory experiment included three groups: (i) in the experimental group, we placed a petri dish containing two fresh *H. fraxineus* apothecia on the bottom of a large plastic container, together with a fresh *N. cruentata* orb web and a fresh *P. phalangioides* sheet web. To avoid apothecium desiccation, we closed the container and kept the relative humidity around 90% by wetting the petri dish once a day. The daily opening of the plastic container created air circulation that we expected was enough to render the *H. fraxineus* spores airborne. We sampled the two webs after 7 days. (ii) In the negative control group, we placed a fresh *N. cruentata* and a fresh *P. phalangioides* web in a clean plastic container of the same size and type as in the experimental group, but without any *H. fraxineus* apothecia. We kept the closed container next to the experimental group container for the entire 7 days, when we sampled the webs. (iii) In the positive control group, we collected a fresh *N. cruentata* and a fresh *P. phalangioides* web. We cut a *H. fraxineus* apothecium in half and added one half to each of the web silk samples. From all collected samples, we extracted DNA and tested it with qPCR assays (see below) to confirm successful collection of fungal DNA on spider webs and proceed with field experiments.

2.3 | Field Sampling Design and Comparative Analysis of Passive Samplers

Prior to the onset of the field experiment, we surveyed several localities for *H. fraxineus* occurrence and habitat suitability. As the study site, we selected the forest Vrbovci (lat. 45.95070, lon. 14.56387) on Ljubljana Marsh (Slovenia) that was humid enough and contained abundant *H. fraxineus* apothecia, where we ran seven sampling campaigns (early summer to late summer across 2 years), starting in June 2023 (sampling dates available in Table 3).

Webs of both types were each collected within a fixed 30 min intense search effort, by one collector, who collected 14–46 orb webs and 21–71 sheet webs during each sampling campaign (Table S2, File S1). Webs were sampled within approximately 25 m of the passive sampler stands and 0.5 m to 1 m above ground. Webs were wound on a clean disposable needle and placed in a microcentrifuge tube that represented one pooled orb or sheet web sample. In an initial field comparison, we evaluated aerial fungal eDNA sampling with simple passive samplers: microscope slides covered with petroleum jelly, petri dishes covered

with petroleum jelly, and cellulose filter paper (File S2) (West and Kimber 2015). Filter paper (Ø 15 cm; MN 617, Macherey-Nagel, Düren, Germany) was housed in Ø 19 cm glass petri dish and set on a 0.5 m tall table. Three visits of the sampling location were needed for each sampling campaign, for (i) installment of two filter papers, (ii) collection of one filter paper after 24 h of exposure to match orb web exposure, and (iii) collection of the second filter paper after 7 days of exposure to match sheet web exposure.

2.4 | Sampling Controls

We tested for possible *H. fraxineus* contaminations in our sampling process, that is, spider webs, and on fresh filter paper. On 13. September 2023, at the field experiment locality, we collected two adult female *Metellina segmentata* and two adult female *Linyphia triangularis* spiders. Both species are abundant at the locality and build orb and sheet webs, respectively. We housed these spiders in disinfected polyacrylate frames in the laboratory to allow web construction (Kralj-Fišer and Gregorič 2019). We then used laboratory webs of these spiders as negative controls. Additionally, we used filter paper directly from the package as negative control.

2.5 | Sample Handling and DNA Extraction

For DNA extraction, we transferred web silk samples into 2 mL Fast Prep tubes (MP Biomedicals) containing 1 g of zirconium oxide beads (Next Advance) and one ceramic sphere bead (MP Biomedicals), with added 700 μL of CSPL buffer and 20 μL of Proteinase K (Mag-Bind Plant DNA DS 96 Kit, Omega Bio-tek). Filter paper samples required a larger volume tube for processing. We cut filter paper into four equal parts and put one of the pieces into a 15 mL tube (Corning) containing 3 g of zirconium oxide beads and three ceramic sphere beads, 2100 μL of CSPL buffer, and 60 μL of Proteinase K. All samples were first thoroughly vortexed and then homogenized using the FastPrep-24 bead beater (MP Biomedicals) for 1 min at 6 m/s. Extraction then followed the manufacturer-provided Mag-Bind Plant DNA DS Kit Protocol, configured for the KingFisher Apex (Thermo Fisher Scientific). We eluted DNA in 100 μL and stored it at −20°C until further analysis. We employed negative extraction control (nuclease-free water only). To monitor for amplification inhibition, we tested different dilutions of the DNA samples (undiluted, 3-, 10- or 100-fold diluted), and 3-fold dilutions were used for comparisons (least inhibition without loss of sensitivity). Dilutions were prepared in nuclease-free water (Sigma-Aldrich) and tested in three technical replicates.

2.6 | qPCR Assays and Controls

To test for the presence and relative amount of fungal DNA in samples, we used specific qPCR assay for detection of *H. fraxineus* (Hfrax) (Ioos et al. 2009) and a general FungiQuant (FQ) assay (Liu et al. 2012). FQ assay was used to control for successful extraction of DNA and control for the presence of fungi in general and possible plant DNA (cross-reaction with FQ assay is not ruled out). The qPCR reaction mixture for Hfrax consisted of TaqMan Environmental master mix 2.0 (Applied Biosystems), 300 nM of each primer and 150 nM TaqMan probe. The qPCR reaction mixture for FQ consisted of PerfeCTa qPCR ToughMix Low ROX master mix (QuantaBio, 95114) and 900 nM of each primer and 250 nM TaqMan probe. Two μl of DNA were tested in a final reaction mixture volume of 10 μL. The reactions were incubated for 2 min at 50°C, followed by 10 min at 95°C, and 45 cycles at 95°C for 15 s and at 65°C for 1 min, on 7900HT Fast or Viia7 Real-time PCR System (Applied Biosystems). In each qPCR run, we included a negative control of amplification (nuclease-free water instead of sample). For the positive control of amplification, we used DNA extracted from 100 *H. fraxineus* spores (Brittain 2017). The generated data were analyzed using SDS software (version 2.4.1., Applied Biosystems) or QuantStudio Real-Time PCR Software (version 1.6.1, Applied Biosystems). Same threshold was set for all qPCR runs on the same device, positive and negative controls and the appearance of sigmoid amplification curves were checked for each analysis. We evaluated performance of both qPCR assays to meet MIQE guidelines: dilution series of positive control was tested in up to 10 sequential dilutions (each in 5 technical replicates) to determine linearity of the assays (Bustin et al. 2025). Number of target copies in the positive control was determined by droplet digital PCR using the Hfrax assay (Ioos et al. 2009) and reaction conditions as previously described (Dobnik et al. 2019). Three consecutive 10-fold dilutions of DNA were tested in duplicate, and average number of copies was determined from them. Acceptance criteria were $>10{,}000$ positive droplets, NTC <3 positives, coefficient of variation between replicates $<25\%$. We report eDNA sampling, controls, metadata, and sample processing following the eDNA reporting guidance (Gagné et al. 2021). All qPCR raw data and data on qPCR assay performance are available on are openly available in Zenodo repository at https://doi.org/10.5281/zenodo.20285349 and (Kogovšek et al. 2026).

2.7 | Data Analysis

The obtained Cq values were further analyzed in MS Excel (Microsoft, WA, USA): we calculated the average Cq value and standard deviation of the three technical replicates. A standard curve was prepared from the Cq values obtained by testing dilution series of positive control, plotted against the logarithmic value of the number of copies of the target sequence per reaction. Slope, intercept, and efficiency of amplification (E) were calculated from the standard curve. The limit of detection (LOD) of the assays was determined at the dilution when at least two of the replicates were positive (out of five tested), and the limit of quantification (LOQ) was determined at the lowest dilution within the linear range of the standard curve (regression line $R^2 \geq 0.99$, SD (Cq) ≤ 0.8). All data on qPCR assay performance are openly available in Zenodo repository at https://doi.og/10.5281/zenodo.20285349 (Kogovšek et al. 2026). We used the Wilcoxon signed-rank test (Statistics Kingdom 2017) across seven sampling events to evaluate the statistical significance of the difference in Cq between web and filter paper samples and between orb and sheet webs. For field comparisons, we computed $\Delta Cq = Cq(filter) - Cq(web)$, separately for daily (orb) and weekly (sheet) pairs and for both assays. For easier interpretation, Cq values that were out of the linear range of the assays were included in the calculations. Median ΔCq was

calculated from all seven paired sampling dates and converted to fold-difference using the standard-curve slope.

3 | Results

3.1 | qPCR Assay Evaluation

Before testing the samples, the performance of both qPCR assays was evaluated by testing dilution series of positive control with approximately 250.000 copies of target DNA per reaction (https://doi.org/10.5281/zenodo.20285349; Kogovšek et al. 2026). The Hfrax assay showed broad linearity over six orders of magnitude (Table 1). The limit of quantification for the Hfrax assay was determined to be 2.5 copies/reaction (Cq 34.5), and the limit of detection was 0.25 copies/reaction (Cq 35.1). The FQ assay showed narrower linear response (four orders of magnitude), with a limit of quantification at 250 copies/reaction (Cq 36.9) and a limit of detection at 25 copies/reaction (Cq 39.4). Later signals observed with FQ assay in samples with lower copy numbers were considered stochastic or artifacts and were ignored, as the assay is known to react with DNA originating from the environment and laboratory procedures, and can give positive signals in negative controls. Even though the assays allowed for quantification, the results of the testing of samples from webs and filters were not quantified, since the Cq values from the samples were or on the limit of quantification or detection (filter paper samples tested with Hfrax assay) and gave stochastic Cq values, or were out of the range of the standard curve (FQ assay).

TABLE 1 | Performance of Hfrax assay (Ioos et al. 2009) and FQ assay (Liu et al. 2012) determined by testing a 10-fold serial dilution prepared from DNA extracted from 100 *Hymenoscyphus fraxineus* spores.

Assay	Range of detection (copies/reaction)	Dynamic range (copies/reaction)	*k*	*E* (%)	R^2
Hfrax	0.25–250,000	2.5–250,000	−3.44	95	0.998
FQ	25–250,000	250–250,000	−3.37	98	0.999

Note: Range of detection (between lowest and highest detected copy number) and dynamic range (linear part of detection range, with lowest to highest quantifiable copy number) are given as range of number of copies of target gene in qPCR reaction that can be detected with each of the assays. *k*—slope of the linear regression line in the plot of Cq against log [copy number]; *E* (%) efficiency of amplification; R^2—mean square regression coefficient.

3.2 | *H. fraxineus* DNA Can Be Detected on Laboratory Exposed Spider Webs

With the preliminary laboratory experiment we tested whether airborne *H. fraxinues* spores can be detected on two different types of spider webs. A low amount of *H. fraxineus* DNA (high Cq values) was detected in the experimental group (Table 2), where webs were not in contact with the apothecium but exposed to airborne spores. No *H. fraxineus* DNA was detected in the negative control. The high amount of *H. fraxineus* DNA (low Cq values) detected in positive control samples confirmed that *H. fraxineus* DNA can be detected in the spider web sample using the qPCR method. Comparing the collection efficiency between the web types, orb webs captured higher quantities of both *H. fraxineus* and general fungal DNA than sheet webs (Table 2). The general FQ assay gave higher values in the experimental group in comparison to the positive control, likely a consequence of the daily opening of the container and high humidity increasing the exposure of webs to environmental fungi.

3.3 | Initial Comparison and Selection of Filter Paper for Passive Sampler of eDNA

Established passive samplers were evaluated in the initial field comparison. Petri dishes with petroleum jelly gave the strongest signal with FQ assay, but filter paper recovered comparable *H. fraxineus* DNA and offered simpler processing; we therefore selected filter paper as the conventional sampler for subsequent web comparisons (detailed values summarized in File S2).

TABLE 2 | qPCR results of the initial laboratory experiment of *H. fraxineus* detection from spider webs (orb and sheet), when exposed to airborne spores (experimental group) and the apothecium (positive control) for 7 days.

	Web type	FQ (Cq)	Hfrax (Cq)
Experimental group	Orb	18.06 ± 0.14	30.97 ± 0.17
	Sheet	28.95 ± 0.04	36.61 ± 0.20[a]
Negative control	Orb	28.32 ± 0.13	Neg
	Sheet	38.06[b]	Neg
Positive control	Orb	26.44 ± 0.29	16.95 ± 0.08
	Sheet	31.95 ± 0.21	20.43 ± 0.03

Note: Average Cq values (calculated from three technical replicates, with corresponding standard deviations (SD) obtained with FQ and Hfrax assays) are reported. SD is not given when less than two technical replicates yielded a Cq. Lower Cq values correspond to higher amounts of target DNA.
Abbreviation: Neg, negative.
[a]Two out of three replicates positive.
[b]One out of three replicates positive.

TABLE 3 | qPCR results of the FQ assay and Hfrax assay for presence of *H. fraxineus* DNA on filter papers and spider webs collected in forest during 7 sampling campaigns (1–7).

Sampling campaign		1	2	3	4	5	6	7	
qPCR assay	**Sample type**	**Daily sample date**							***p*-value/median ΔCq**
		15.6.2023	**8.9.2023**	**29.9.2023**	**5.7.2024**	**1.8.2024**	**14.8.2024**	**23.8.2024**	
FQ	Filter paper	27.69 ± 0.13	23.10 ± 0.19	24.78 ± 0.36	26.95 ± 0.06	26.07 ± 0.02	24.94 ± 0.07	25.98 ± 0.05	0.0156
	Orb web	22.46 ± 0.06	19.67 ± 0.21	17.30 ± 0.34	16.47 ± 0.07	21.13 ± 0.10	16.86 ± 0.15	15.02 ± 0.06	
ΔCq		5.22	3.43	7.48	10.48	4.94	8.08	10.97	7.48
Hfrax	Filter paper	33.48 ± 0.50	Neg	Neg	34.62 ± 0.40	37.24[b]	Neg	36.32 ± 0.61[a]	0.0313
	Orb web	33.72 ± 1.13	35.76 ± 0.42[a]	33.45[b]	30.14 ± 0.20	33.06 ± 0.20	35.13 ± 0.43	32.24 ± 0.26	
ΔCq		−0.23	4.24[c]	6.55[c]	4.49	4.18	4.87[c]	4.08	4.24
qPCR assay	**Sample type**	**Weekly sample date**							***p*-value/median ΔCq**
		22.6.2023	**13.9.2023**	**4.10.2023**	**11.7.2024**	**7.8.2024**	**22.8.2024**	**28.8.2024**	
FQ	Filter paper	23.15 ± 0.17	20.84 ± 0.12	19.56 ± 0.07	22.82 ± 0.05	18.99 ± 0.04	16.27 ± 0.04	22.98 ± 0.04	0.0223
	Sheet web	21.17 ± 0.06	16.28 ± 0.09	14.15 ± 0.34	14.67 ± 0.06	13.58 ± 0.08	13.66 ± 0.04	16.21 ± 0.05	
ΔCq		1.98	4.56	5.41	8.16	5.40	2.61	6.77	5.40
Hfrax	Filter paper	30.04 ± 0.36	35.34 ± 0.09[a]	35.15[b]	31.90 ± 0.16	34.82 ± 0.50	33.60 ± 0.05	35.35 ± 0.31	0.0313
	Sheet web	30.81 ± 0.33	29.65 ± 0.23	32.08 ± 0.17	28.08 ± 0.05	27.87 ± 0.01	29.25 ± 0.03	31.33 ± 0.21	
ΔCq		−0.76	5.69	3.07	3.82	6.95	4.35	4.02	4.02

Note: Orb webs were collected after 1 day of exposure to forest, together with daily filter paper. Sheet webs were collected after 1 week of exposure to the forest, together with weekly filter paper. Average Cq values with corresponding standard deviation (SD) are presented, calculated from three technical replicates. SD is not given when less than two technical replicates yielded a Cq. Lower Cq values correspond to higher amounts of target DNA. Median was determined for ΔCq, which was calculated as the difference between filter paper Cq and web Cq values. *p*-value (Wilcoxon signed-rank test) shows statistical significance of difference in Cq values between sample pairs.
Abbreviation: Neg, negative result.
[a]Two positive signals out of three.
[b]One positive signal out of three.
[c]To calculate ΔCq, Cq 40 was used for negative results.

3.4 | Spider Webs Outperform Conventional Filter Paper in Collection Efficiency of *H. fraxineus* DNA in Forest Environment

We got a positive signal with the general FQ assay across all 28 field collected samples, confirming successful collection and amplification of fungal and/or plant DNA (Table 3, Table S1). Control samples (laboratory webs, unused filter paper) yielded no *H. fraxineus* detection, demonstrating the absence of contamination and cross-reactivity with the Hfrax assay.

The relative amount of collected *H. fraxineus* DNA is expressed as average Cq values obtained with Hfrax assay (Table 3, Figure 1). A considerably higher amount of *H. fraxineus* DNA was detected in all spider web samples than on filter paper, except in June 2023, when the amount was similar between sample types. This pattern was observed for both web types (orb, sheet), exposure durations (daily, weekly), and with both assays (general FQ, specific Hfrax). The difference in Cq values between sample pairs (spider webs vs. filter paper) was statistically significant (Wilcoxon test, $p < 0.05$) for both types of samples

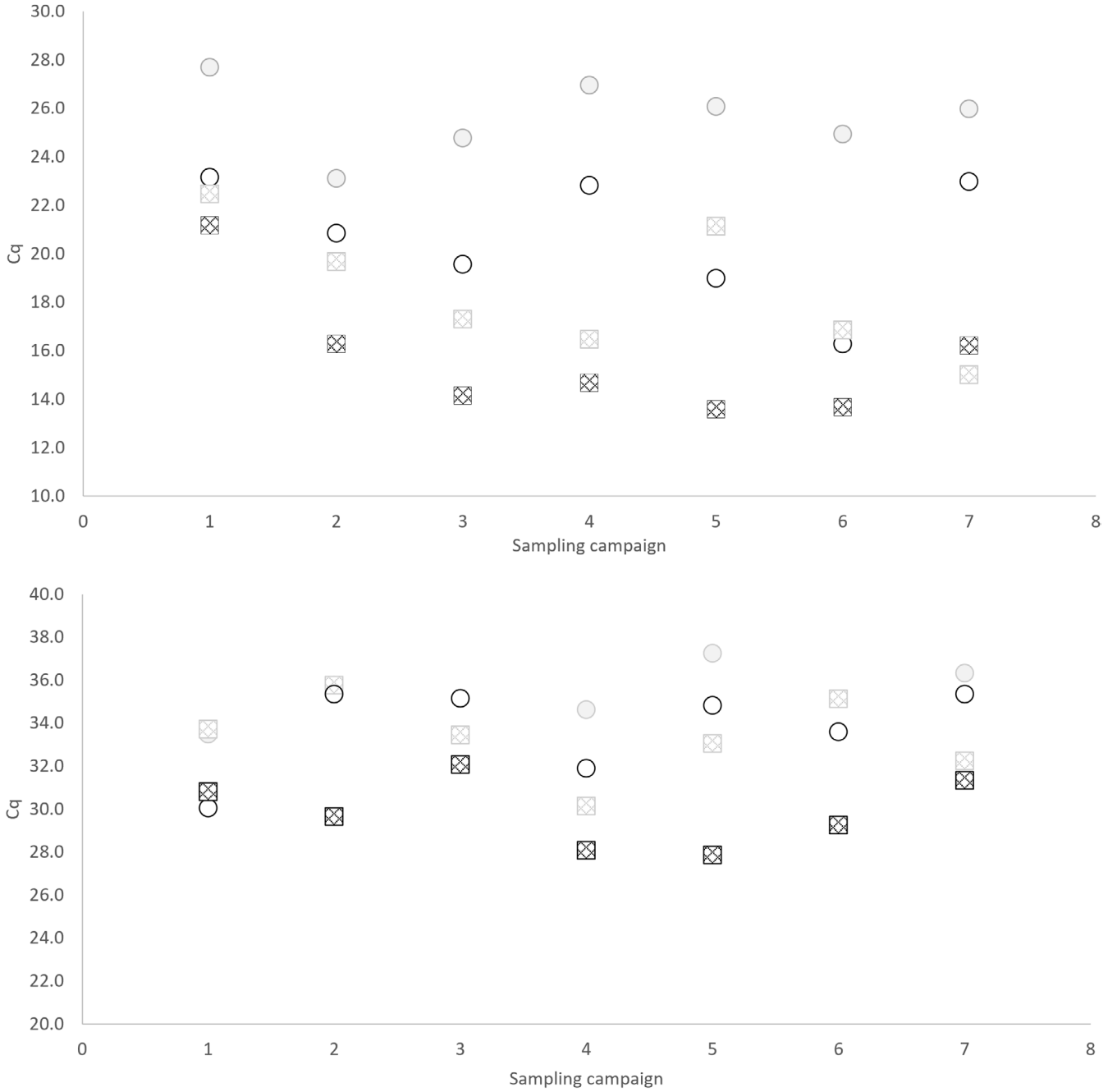

FIGURE 1 | Schematic presentation of results of forest-collected filter paper (circle) and spider webs (square with pattern) tested with general FQ assay (upper panel) and for presence of *H. fraxineus* (Hfrax assay, lower panel) DNA using qPCR. For each sampling campaign (1–7), orb webs and daily filter paper (gray) or sheet webs and weekly filter paper (black) were collected. Average Cq values are presented, calculated from three technical replicates.

tested with both assays (Table 3). The median difference in Cq

between all seven sample pairs (ΔCq) is above 4 (Table 3), in favor of spider webs. Taking into account the standard curve slopes, ΔCq > 4 corresponds to more than a 10-fold difference in the amount of *H. fraxineus* and total fungal and/or plant DNA in spider webs compared to filter paper. As expected, significantly higher total and *H. fraxineus* DNA amounts were detected in samples exposed for longer durations, that is, sheet webs and weekly exposed filter paper, compared to daily samples (Wilcoxon test, $p = 0.0223$ for FQ assay, $p = 0.0313$ for Hfrax assay). In three daily exposed filter paper samples, *H. fraxineus* DNA was not detected and in one sample detection was positive in only one out of three replicates (Table 3, Table S1), indicating collection of a low amount of target DNA, which leads to unreliable detection.

4 | Discussion

This study demonstrates that spider webs are effective passive collectors of fungal spores that can be used for molecular surveillance of plant pathogens. In a forest with high incidence of *Hymenoscyphus fraxineus*, we evaluated the collection efficiency of *H. fraxineus* in terms of detectable DNA using a species-specific qPCR assay (Ioos et al. 2009). In parallel, a broad control assay targeting diverse fungi (Liu et al. 2012) was used to prove successful extraction of fungal and/or plant DNA from all samples. The strong qPCR signals obtained from spider web samples (low Cq values) indicate that high amounts of DNA accumulate on webs and that eDNA from spider webs is useful for detecting various fungal taxa.

Spider webs occur in high abundances across most terrestrial environments. The molecular structure of their glue droplets assures maintained adhesiveness across wide ranges of relative humidity, including those common in forests and agroecosystems (Jain et al. 2018). Additionally, spider webs display electrostatic behavior, actively attracting charged particles and even deforming towards them, which increases interception efficiency (Ortega-Jimenez and Dudley 2013; Vollrath and Edmonds 2013). These characteristics, combined with long exposure times of certain web types, likely contribute to the collection efficiency of spider webs for pollen (Oraon et al. 2022), bacteria, and fungi (Gregorič et al. 2022; Mattei 2009). In our field comparison, spider webs, both orb and sheet types, outperformed filter paper in collection efficiency of *H. fraxineus* and total fungal and/or plant DNA for more than 10 times. In a study investigating diverse eDNA substrates to target vertebrate genetic remains, collecting only two to three spider webs proved more efficient in obtaining vertebrate eDNA than swabbing leaves with a combined surface area of 100 cm^2 (one to three leaves), while both decisively outperformed the sampling of topsoil, highlighting the utility of passive samples of airborne eDNA (Berard et al. 2025). A key advantage of spider webs is their positioning and orientation within the environment, allowing them to collect airborne material from all directions as it is carried by wind currents, while passive sampling with filter paper relies solely on gravitational deposition of particles.

As spider webs accumulate genetic material over days or weeks (and in some species, months; Foelix 2025) and act as natural collectors of transient inoculum events, they can support monitoring of episodic or weather-driven sporulation and dispersal. In our experimental setup near ash (*Fraxinus*) trees, a significantly higher amount of *H. fraxineus* and total fungal and/or plant eDNA was collected on long-lasting sheet webs that likely reflects their longer exposure time. Those webs are permanent installations and spiders maintain and repair them (Foelix 2025). On the contrary, orb webs are rebuilt by spiders daily in the evening or in the morning, meaning that the exposure time of those webs is much lower, which is reflected in less reliable detection of *H. fraxineus* DNA. Nevertheless, orb webs can be collected daily and used to fine-scale the temporal resolution of spore presence and amount. In terms of sampling effort needed to collect passive samplers, a considerably lower sampling effort was needed to collect daily orb and weekly sheet webs (30 min for each) in contrast to 3 visits to the site for filter setup and collection on two sampling events (day, week).

Several filter-paper samples yielded Hfrax Cq values close to the assay's LOQ or LOD, and some daily filter-paper samples were negative, consistent with increased stochasticity and higher replicate-to-replicate variability at low target copy numbers. Under such near-limit conditions, converting Cq values into absolute copy numbers using the standard curve would be associated with substantial uncertainty (and, for non-detects, would not be possible), thereby reducing the reliability of absolute quantification and potentially obscuring differences between sampling substrates. On the other side, a presence/absence approach would discard the consistent shifts in signal strength across paired samples and would underestimate the differences between substrates. We therefore used Cq values as a consistent, assay-internal measure of signal strength and compared paired web and filter samples using ΔCq. Interpreting ΔCq as a fold-difference assumes comparable amplification efficiency and log-linear behavior across samples. Even though near the LOQ these assumptions weaken, making ΔCq-based fold-change approximate, we observed that spider webs consistently produced earlier signals than filter paper across all seven paired campaigns (median ΔCq > 4 for both assays). Our observations thus support a robust qualitative conclusion that webs outperform filter paper for passive interception of airborne fungal eDNA under the tested conditions.

In conclusion, spider webs represent a simple, low-cost, and logistically flexible tool for passive collection of airborne biological material. Because they are ubiquitous in most habitats, sampling can be opportunistic, without prior installation of equipment and detailed site planning. Previous work has demonstrated that webs capture eDNA from bacteria, plants, fungi, and invertebrate and vertebrate animals (Gregorič et al. 2022; Newton et al. 2024; Oraon et al. 2022) and can even yield higher detection probabilities compared to other passive substrates (Berard et al. 2025). Our results extend these findings to plant pathogenic fungi, showing that spider webs can outperform conventional passive samplers such as filter paper. While air sampling as a method for plant pathogen detection is a vivid field with numerous recent innovations (Van der Heyden et al. 2021), the web eDNA approach could potentially complement it or make it more accessible under certain conditions. Because this approach

relies on the same mechanism by which spores disperse, that is, via air currents that carry particles from a few metres to hundreds or even thousands of kilometers, depending on turbulence, wind speed, and spore characteristics (Golan and Pringle 2017). Detecting fungal and other organismal DNA from spider webs thus offers a simple yet powerful approach for biodiversity monitoring, documentation of pathogen presence, and supporting early-warning systems for prediction of emerging plant diseases.

Author Contributions

The conception and design of the study: P.K., N.O., B.P., D.K., M.G.; the acquisition, analysis, and interpretation of the data: P.K., M.F., J.Š., T.L.; and writing of the manuscript: P.K., N.O., B.P., D.K., M.G.

Acknowledgments

Authors would like to thank Ian W. Brittain for providing *H. fraxineus* isolate used for positive control in qPCR reactions. The study was funded by Slovenian Research and Innovation Agency programs P4-0463, P4-0107, P1-0236 and project J1-1703; research infrastructure grant IO-0004; and by the Slovenian Ministry of Agriculture, Forestry and Food (Public Forestry Service).

Funding

The study was supported by Slovenian Research And Innovation Agency programs P4-0463, P4-0107, P1-0236 and project J1-1703; research infrastructure grant IO-0004; and by the Slovenian Ministry of Agriculture, Forestry and Food (Public Forestry Service).

Ethics Statement

No vertebrates were handled. Invertebrate husbandry followed best-practice guidance for spider welfare.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Additional supporting information on field experiment setup, web types and passive samplers comparison are provided as part of the online supporting material (Files S1 and S2). Additional information on qPCR results of filter paper and spider webs is provided in Table S1. All qPCR results (including assay evaluation) and sampling metadata (web type, exposure duration, date, weather conditions, controls) that support the findings of this study are openly available in the Raw data file at https://zenodo.org/ repository under DOI: https://doi.org/10.5281/zenodo.20285349.

References

Aguayo, J., C. Husson, E. Chancerel, et al. 2021. "Combining Permanent Aerobiological Networks and Molecular Analyses for Large-Scale Surveillance of Forest Fungal Pathogens: A Proof-Of-Concept." *Plant Pathology* 70, no. 1: 1. https://doi.org/10.1111/ppa.13265.

Baral, H.-O., V. Queloz, and T. Hosoya. 2014. "Hymenoscyphus Fraxineus, the Correct Scientific Name for the Fungus Causing Ash Dieback in Europe." *IMA Fungus* 5, no. 1: 79–80. https://doi.org/10.5598/imafungus.2014.05.01.09.

Berard, A., J. Pradel, N. Charbonnel, and M. Galan. 2025. "Spider Webs, Soil or Leaf Swabs to Detect Environmental DNA From Terrestrial Vertebrates: What Is the Best Substrate?" *Molecular Ecology Resources* 25: e70037. https://doi.org/10.1111/1755-0998.70037.

Bérubé, J. A., P. N. Gagné, J. P. Ponchart, É. D. Tremblay, and G. J. Bilodeau. 2018. "Detection of Diplodia Corticola Spores in Ontario and Québec Based on High Throughput Sequencing (HTS) Methods." *Canadian Journal of Plant Pathology* 40, no. 3: 378–386. https://doi.org/10.1080/07060661.2018.1498394.

Brittain, I. W. 2017. "Towards Early Automated Detection of Pre-Symptomatic Pathogen Risk: Mitigating the Impact of Airborne Fungal Plant Pathogens." University of Nottingham. https://eprints.nottingham.ac.uk/50491/2/Ian%20Brittain%20PhD%202013-2017_redacted.pdf.

Burns, P., V. Timmermann, and J. M. Yearsley. 2022. "Meteorological Factors Associated With the Timing and Abundance of Hymenoscyphus Fraxineus Spore Release." *International Journal of Biometeorology* 66, no. 3: 493–506. https://doi.org/10.1007/s00484-021-02211-z.

Bustin, S. A., J. M. Ruijter, M. J. B. van den Hoff, et al. 2025. "MIQE 2.0: Revision of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines." *Clinical Chemistry* 71, no. 6: 634–651. https://doi.org/10.1093/clinchem/hvaf043.

Dobnik, D., P. Kogovšek, T. Jakomin, et al. 2019. "Accurate Quantification and Characterization of Adeno-Associated Viral Vectors." *Frontiers in Microbiology* 10: 1570. https://doi.org/10.3389/fmicb.2019.01570.

Foelix, R. 2025. *Spider Biology*. 1st ed. Springer Cham. https://doi.org/10.1007/978-3-031-96011-6.

Gagné, N., L. Bernatchez, D. Bright, et al. 2021. "Environmental DNA (eDNA) Reporting Requirements and Terminology [CSA W214:21]." Canadian Standards Association. https://www.csagroup.org/store/product/CSA%20W214%3A21/.

Golan, J. J., and A. Pringle. 2017. "Long-Distance Dispersal of Fungi." *Microbiology Spectrum* 5, no. 4: 1–24. https://doi.org/10.1128/microbiolspec.funk-0047-2016.

Gregorič, M., D. Kutnjak, K. Bačnik, et al. 2022. "Spider Webs as eDNA Samplers: Biodiversity Assessment Across the Tree of Life." *Molecular Ecology Resources* 22, no. 7: 2534–2545. https://doi.org/10.1111/1755-0998.13629.

Grosdidier, M., R. Ioos, C. Husson, O. Cael, T. Scordia, and B. Marçais. 2018. "Tracking the Invasion: Dispersal of Hymenoscyphus Fraxineus Airborne Inoculum at Different Scales." *FEMS Microbiology Ecology* 94, no. 5: fiy049. https://doi.org/10.1093/femsec/fiy049.

Ioos, R., T. Kowalski, C. Husson, and O. Holdenrieder. 2009. "Rapid In Planta Detection of *Chalara fraxinea* by a Real-Time PCR Assay Using a Dual-Labelled Probe." *European Journal of Plant Pathology* 125, no. 2: 2. https://doi.org/10.1007/s10658-009-9471-x.

Jain, D., G. Amarpuri, J. Fitch, T. A. Blackledge, and A. Dhinojwala. 2018. "Role of Hygroscopic Low Molecular Mass Compounds in Humidity Responsive Adhesion of Spider's Capture Silk." *Biomacromolecules* 19, no. 7: 3048–3057. https://doi.org/10.1021/acs.biomac.8b00602.

Kogovšek, P., N. Ogris, M. Ferle, et al. 2026. "Data for: Spider Webs as Efficient Passive Samplers for Airborne Fungal DNA in Forests: A Case Study With *Hymenoscyphus fraxineus*." Zenodo [Excel]. https://doi.org/10.5281/zenodo.20285349.

Kowalski, T. 2006. "*Chalara fraxinea* sp. Nov. Associated With Dieback of Ash (*Fraxinus excelsior*) in Poland." *Forest Pathology* 36, no. 4: 264–270. https://doi.org/10.1111/j.1439-0329.2006.00453.x.

Kralj-Fišer, S., and M. Gregorič. 2019. "Spider Welfare." In *The Welfare of Invertebrate Animals*, 1st ed., 248. Springer Cham. https://doi.org/10.1007/978-3-030-13947-6.

Larsson, R., A. Menkis, and Å. Olson. 2025. "Temporal Dynamics of Airborne Fungi in Swedish Forest Nurseries." *Applied and Environmental Microbiology* 91, no. 2: e01306–e01324. https://doi.org/10.1128/aem.01306-24.

Liu, C. M., S. Kachur, M. G. Dwan, et al. 2012. "FungiQuant: A Broad-Coverage Fungal Quantitative Real-Time PCR Assay." *BMC Microbiology* 12: 255. https://doi.org/10.1186/1471-2180-12-255.

Mattei, D. 2009. "The Use of Spider Webs as Passive Bioaerosol Collectors." Air Force Institute of Technology. https://scholar.afit.edu/etd/2457.

Newton, J. P., M. E. Allentoft, P. W. Bateman, M. A. Campbell, M. Mousavi-Derazmahalleh, and P. Nevill. 2025. "Evaluation of Extraction Methods for the Recovery of Vertebrate DNA From Spider Webs." *Environmental DNA* 7, no. 4: e70122. https://doi.org/10.1002/edn3.70122.

Newton, J. P., P. Nevill, P. W. Bateman, M. A. Campbell, and M. E. Allentoft. 2024. "Spider Webs Capture Environmental DNA From Terrestrial Vertebrates." *iScience* 27, no. 2: 108904. https://doi.org/10.1016/j.isci.2024.108904.

Nicolaisen, M., J. S. West, R. Sapkota, G. G. M. Canning, C. Schoen, and A. F. Justesen. 2017. "Fungal Communities Including Plant Pathogens in Near Surface Air Are Similar Across Northwestern Europe." *Frontiers in Microbiology* 8: 1729. https://doi.org/10.3389/fmicb.2017.01729.

Ogris, N., T. Hauptman, and D. Jurc. 2009. "Chalara Fraxinea Causing Common Ash Dieback Newly Reported in Slovenia." *Plant Pathology* 58, no. 6: 1173. https://doi.org/10.1111/j.1365-3059.2009.02105.x.

Oraon, S., S. Pal, P. Bhandari, and S. Mondal. 2022. "Spider Web: A Natural Sampler for Analysis of Airborne Pollen–Spores From Santiniketan, West Bengal." *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 92, no. 4: 919–927. https://doi.org/10.1007/s40011-022-01383-x.

Ortega-Jimenez, V. M., and R. Dudley. 2013. "Spiderweb Deformation Induced by Electrostatically Charged Insects." *Scientific Reports* 3, no. 1: 2108. https://doi.org/10.1038/srep02108.

Schweigkofler, W., K. O'Donnell, and M. Garbelotto. 2004. "Detection and Quantification of Airborne Conidia of *Fusarium circinatum*, the Causal Agent of Pine Pitch Canker, From Two California Sites by Using a Real-Time PCR Approach Combined With a Simple Spore Trapping Method." *Applied and Environmental Microbiology* 70, no. 6: 3512–3520. https://doi.org/10.1128/AEM.70.6.3512-3520.2004.

Statistics Kingdom. 2017. "Wilcoxon Signed Rank Test Calculator." https://www.statskingdom.com/175wilcoxon_signed_ranks.html.

Tulloch, R. L., C. I. M. Adams, M. A. Barnes, et al. 2025. "Winds of Change: Charting a Pathway to Ecosystem Monitoring Using Airborne Environmental DNA." *Environmental DNA* 7, no. 4: e70134. https://doi.org/10.1002/edn3.70134.

Van der Heyden, H., P. Dutilleul, J.-B. Charron, G. J. Bilodeau, and O. Carisse. 2021. "Monitoring Airborne Inoculum for Improved Plant Disease Management. A Review." *Agronomy for Sustainable Development* 41, no. 3: 40. https://doi.org/10.1007/s13593-021-00694-z.

Vollrath, F., and D. Edmonds. 2013. "Consequences of Electrical Conductivity in an Orb Spider's Capture Web." *Naturwissenschaften* 100, no. 12: 1163–1169. https://doi.org/10.1007/s00114-013-1120-8.

West, J., and R. Kimber. 2015. "Innovations in Air Sampling to Detect Plant Pathogens." *Annals of Applied Biology* 166, no. 1: 4–17. https://doi.org/10.1111/aab.12191.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **File S1:** Photographs of field experiment set-up and types of spider webs collected in the forest. **Figure S1:** Field sampling. Cellulose filter paper (Ø 15 cm) housed in Ø 19 cm glass petri dish, set on 0.5 m tall table, deployed in the forest. Petri dish covered with petroleum jelly was set on side during comparative analysis of passive samplers. **Figure S2:** Representative photo of sheet spider web collected in the forest. **Figure S3:** Representative photo of orb spider web collected in the forest. **File S2:** Initial passive sampler comparison. **Table S1:** Results of filter paper and spider webs tested with general fungal (FQ) assay and for presence of *H. fraxineus* (Hfrax) DNA using qPCR. Mean Cq value and its standard deviation (SD) and number of positive replicates (No P.), out of three tested, are shown. Neg, no aplification, negative result.