

Community composition of microbial eukaryotes transported by stemflow from *Fagus grandifolia* Ehrh. (American beech) trees in northeastern Ohio (USA)

D. Alex R. Gordon

Cleveland State University

David J. Burke

The Holden Arboretum

Sarah R. Carino-Kyker

The Holden Arboretum

Claudia Bashian-Victoroff

The Holden Arboretum

Adam I. Mabrouk

Cleveland State University

John T. Van Stan II

j.vanstan@csuohio.edu

Cleveland State University

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Abstract

Stemflow, the concentrated fraction of rainfall that drains down tree trunks, can translocate canopy biota to the forest floor, but its eukaryotic composition remains uncharacterized via eDNA methods. We collected stemflow from 16 *Fagus grandifolia* (American beech) trees during ten storms in northeastern Ohio (USA) and analyzed 18S rRNA eDNA to resolve transported microbial-eukaryote communities. Over 12 million reads (83 samples) revealed 920 zero-radius OTUs spanning fungi, algae, protists and metazoans. Community composition differed significantly among storm events (PERMANOVA $F = 3.6$, $r^2 = 0.31$, $p < 0.001$) and among NOAA HYSPLIT modeled air-mass back-trajectories ($F = 8.9$, $r^2 = 0.36$, $p < 0.001$). Summer storms were dominated by fungal taxa (Entomophthoromycota, Basidiomycota and Ascomycota comprised up to 90% of reads); whereas late-autumn and winter storms carried mainly algal stramenopiles (Ochrophyta). Large storms ($> 60 \text{ mm event}^{-1}$) mobilized conspicuously higher relative abundances of larger metazoans (tardigrades, arthropods). We infer from stemflow eDNA that (i) seasonal resource shifts in tree canopies favor parasitic fungi in summer and saprotrophic fungi in autumn; (ii) northerly winter storms entrain Great Lakes aerosol algae that deposit onto canopies; and (iii) rainfall intensity and duration jointly control the detachment of well-attached canopy eukaryotes. Together, our results establish stemflow eDNA as a non-invasive window into storm-mediated linkages between aboveground and surface biodiversity, offering new scope for monitoring canopy microbiomes under intensifying hydro-climatic regimes.

1. Introduction

When rainfall contacts a forest canopy, a portion is captured by canopy surfaces and drained down the stem to the soil surface. This “stemflow” delivers not only water and solutes (Parker, 1983; Ponette-González et al., 2020) but also a diverse array of microbial and meiofaunal passengers from the canopy to the soil (Van Stan et al., 2021a). Aboveground leaf and bark surfaces represent one of the largest terrestrial habitats, surpassing the global land surface area (Van Stan et al., 2021; Vorholt, 2012). These surfaces host an abundance of microorganisms, including bacteria, fungi, and other eukaryotic organisms, that fulfill essential ecophysiological roles, such as nutrient cycling and pathogen defense (Kembel and Mueller, 2014; Koskella, 2020; Redford et al., 2010). Stemflow may be especially significant in mobilizing these organisms from the phyllosphere to the soil, as it provides a concentrated, directed flow of water (Allen and Van Stan, 2021; Carlyle-Moses et al., 2020; Van Stan and Allen, 2020). However, we still lack a community-level understanding of which small metazoans (i.e., those with microbial eukaryotic lineages) are transported from the phyllosphere to the ground by stemflow, and how this community composition varies across storms. Exploring this transport pathway is merited, as these organisms could influence soil microbial dynamics and overall forest health upon reaching the ground (Aslani et al., 2022; Schmidt et al., 2016).

Several hydrodynamic features suggest that stemflow is well suited to mobilize small particles. As branchflow accelerates along inclined twigs and trunks, velocities of $0.1\text{--}0.6 \text{ m s}^{-1}$ can scour bark (Zhang et al., 2022), while instabilities, like waves and rivulet meandering (Puri et al., 2024), further

increase stemflow capability to entrain and transport particles. Empirical work already shows that stemflow harbors far higher abundances of microbes than open rain or throughfall: up to 10^{16} bacterial cells $\text{ha}^{-1} \text{y}^{-1}$ (Bittar et al., 2018), $>10^9$ fungal spores $\text{ha}^{-1} \text{y}^{-1}$ across a diversity of tree species (Magyar et al., 2021), and tens to hundreds of metazoans m^{-2} of canopy y^{-1} at the single plant scale (Lima et al., 2023). Guidone et al. (2021), although focusing on an understory plant rather than trees, reported 10^5 – 10^7 flagellated microorganisms L^{-1} of stemflow. Ptatscheck et al. (2018) provide compelling evidence of how stemflow transports significant numbers of small metazoans, specifically, estimating that an average *Fagus sylvatica* L. (European beech) tree can transfer about 1.6 million metazoans to the forest floor each year through stemflow. This estimate includes approximately 1.2 million rotifers, 216,000 nematodes, 160,000 tardigrades, 73,000 mites, and 25,000 collembolans (Ptatscheck et al., 2018). At the hectare scale, beech stemflow at that site could annually wash 154 million small metazoans to the soil—numbers approaching standing soil inventories of these organisms (Devetter, 2007; Schaefer, 1990; Sohlenius, 1979; Yeates, 1972). What remains unknown is the taxonomic breadth and compositional dynamics of these eukaryotic passengers in stemflow.

Fagus species are ideal for probing this knowledge gap because their smooth, thin bark stores little water and initiates stemflow under modest rain conditions (Sadeghi et al., 2020). This relatively low bark water storage capacity is complemented with steeply-inclined branch angles (Levia and Germer, 2015; Pypker et al., 2011), yielding some of the highest tree-level stemflow volumes reported in the literature (André et al., 2008; Chang and Matzner, 2000; Levia et al., 2010; Van Stan et al., 2016). This further enhances the potential for mobilizing particles and organisms from the canopy via stemflow. Stemflow fluxes, however, have never been paired with molecular surveys of the eukaryotic biota they convey; to date, no study has resolved the community composition of microbial eukaryotes in stemflow from any tree species.

Here we address that knowledge gap by analyzing environmental DNA (eDNA) in stemflow from 16 *Fagus grandifolia* Ehrh. (American beech) trees. Using high-throughput Illumina sequencing, we ask:

1. Which eukaryotic microbial lineages are transported by stemflow?
2. Do these stemflow community profiles vary across storms events?
3. Can we explain inter-storm variability in this community's composition using storm conditions or back-trajectory information?

By coupling canopy hydrology with modern metabarcoding, this study shifts the focus from flux magnitude to community dynamics, offering the first taxonomically resolved portrait of eukaryotic microbes riding stemflow from canopy to soil. Such knowledge will refine our understanding of how precipitation links above- and below-ground biodiversity and may open a new, non-invasive avenue for tree canopy ecosystem surveillance.

2. Methods

2.1. Study site and tree selection

The study was conducted in a beech orchard established in 2006 and located at the Holden Arboretum in Kirtland, Ohio, USA (Koch et al., 2015). Located roughly 15 km south of the Lake Erie shoreline in the western reach of the Allegheny Plateau, the site experiences a hot summer continental (Köppen *Dfa*), with a mean annual temperature of 10.8°C and mean annual precipitation of 990 mm year⁻¹. Rainfall events occur relatively frequently throughout the year (156 rain days year⁻¹) and are evenly spread across the non-winter months. Significant winter snowfall (not monitored in this study) occurs at the site each year, predominantly during the months of January through April. All 18 study trees had grown from seed in their source locations and were later accessioned as plants at the Holden Arboretum orchard site. Study trees originated from two provenances (Maine and Michigan), which were planted at the arboretum in 2006 and were similarly sized at the time of sampling. Further Meteorological data were sourced from the nearest Goldstar Weather Underground station (KOHMENT0112, Ambient Weather WS-2902 [Ambient, LLC, Chandler, AZ USA], elev. 224 m, 41.66 °N, 81.33 °W), providing 5-minute resolution data for each rainfall event. Further details on the site and the individual trees (including a detailed study site map) may be found in Gordon et al. (2025).

2.2. Stemflow method, sampling, and processing

Each study tree was equipped with a non-invasive stemflow collector to collect stemflow. For this, we wrapped a ring of 3.8 × 3.8 cm platinum expandable weather-seal foam around each trunk, positioning it on a slight downslope so that water naturally converged at the lowest point. The foam strip was cut a few centimeters shy of the tree's full circumference, leaving a narrow gap into which a 2.54 cm-diameter silicone tube could be slipped. Once the tube was seated, we sheathed the entire collar in an 8 mm flexible plastic band to keep water from spilling over the sides, then sealed every junction – bark to foam, and foam to plastic – with silicone. The drain tube was gently zip-tied to the bark, guiding flow into a 113 L high-density polyethylene tote equipped with a snap-locking lid. All components could be installed without harming the tree, and weekly checks allowed us to reseal or adjust the system whenever minor leaks appeared. More details and an image of the stemflow collector set up can be found in Gordon et al. (2025).

Stemflow was sampled within 48 h of each qualifying storm—any rainfall event exceeding 3 mm after at least 72 h with no precipitation, a threshold known to trigger stemflow in *F. grandifolia* of comparable size (Van Stan and Levia, 2010). At each tree, we gently shook the collection tote to resuspend settled particles, then, wearing nitrile gloves, drew one 50 mL aliquot per tote into sterile vials. Surplus water was discarded and the tote rinsed with deionized water before the next storm. Samples travelled on ice to the Holden laboratory, where they were filtered immediately through a vacuum manifold equipped with a 300 mL glass funnel and 500 mL filter flask. Each aliquot passed through a 47 mm, 0.45 µm mixedcelluloseester membrane (gridded, sterile; Membrane Solutions). Filters, with all retained eukaryotic cells, were sealed in sterile bags and frozen at – 80°C until DNA extraction. The filtration assembly was triplerinsed with deionized water between samples to eliminate carryover.

2.3. Assessment of microbial eukaryotic lineages in eDNA

DNA was extracted from half of each sample's filter using a protocol where the filter was transferred into a 1.5-ml bead beating tube containing glass beads (300 mg of 400 μ M sterile glass beads; VWR, West Chester, PA, USA and 200 mg of 1 mm sterile glass beads; Chemglass, Vineland, NJ, USA) and CTAB (cetyltrimethylammonium bromide) buffer. Cells were lysed by bead beating (Precellys homogenizer; Bertin Technologies, France) and then DNA was extracted using a phenol-chloroform procedure detailed in Burke et al. (2020). Extracted DNA from each sample was suspended in 100 μ l Tris EDTA buffer and stored at -80°C in 1.5-mL low retention centrifuge tubes (Fisher Scientific, Pittsburgh, PA). Extraction controls using chemicals only were run alongside the samples to ensure there was no contaminating DNA.

To broadly amplify eukaryotes from filtered stemflow with a focus on microbial eukaryotic lineages, we targeted the 18S SSU rRNA gene using primers originally described by Amaral-Zettler et al. (2009): Euk1391f (5'-GTACACACCGCCCGTC-3') and EukBr (5'-TGATCCTTCTGCAGGTTACCTAC-3'). These primers were designed for Illumina sequencing and aligned with the Earth Microbiome Project's protocols for sequencing the 18S rRNA gene (EMP 18S). This study did not include a mammalian blocking primer (Vestheim and Jarman, 2008), since the eDNA was not derived from a host. Each primer contained an Illumina overhang adapter (as in Burke et al. (2019)).

PCR was performed using Fast Start Taq DNA polymerase (Sigma Aldrich, Saint Louis, MO, USA) at a final concentration of 0.5 unit, 0.8mM dNTPs, 0.2 μ M of each primer, and 0.5 μ g/ μ l bovine serum albumin in a total reaction volume of 25 μ l. Thermocycling included an initial denaturation step of 95°C for 5 min, 25 cycles of 95°C for 30 sec, 54°C for 60 sec for primer annealing, and 72°C for 90 sec for fragment elongations, and a final extension of 72°C for 5 min on an Applied Biosystems Veriti 60 Well Thermocycler (ThermoFisher, Waltham, MA, USA). PCR products were quantified and sequenced as 2 x 250 bp reads on an Illumina Mi-Seq V3 sequencer (Illumina Inc., San Diego, CA, USA) through the Case Western Reserve University Genomics Core facility.

In total, our sequencing effort yielded over 12 million reads from 83 samples that were processed following the UNOISE pipeline (R. C. Edgar, 2016). USEARCH, version 11.0.667 (Edgar, 2010) was used to first merge forward and reverse reads with the *fastq_mergepairs* command and then remove control PhiX reads with the *filter_phiX* command. Reads were trimmed of PCR primers using Cut Adapt (v4.4; Martin 2011) where up to 15% mismatches were allowed during primer removal. Reads less than 100bp in length or with one or more sequence errors were removed with the *fastq_filter* command. The *unoise3* command was used to create error-corrected and chimera-filtered sequence variants (i.e, zero radius OTUs or zOTUs) where exact sequence matches (i.e., unique sequences) with fewer than 8 reads were removed (per the default settings) prior to mapping. The merged reads from each leaf sample with control PhiX and primers removed were then mapped to the zOTUs with the *otutab* command. Taxonomic assignments for the zOTUs were made with the SINTAX algorithm (R. Edgar, 2016) by comparing against the Silva 18S eukaryotic database (version 123, Glöckner et al. 2017; Quast et al.

2013; Yilmaz et al. 2014). Two extraction controls (see above) were also sent for sequencing and any zOTU that had more than 500 reads that matched to these controls were removed prior to statistical analysis, as these are likely contaminants of the extraction procedure.

2.4. Data Analysis.

All statistical analyses were conducted in R (version 4.2.1) with significance set at $\alpha = 0.05$. To account for varying sequencing depths among samples, raw sequence read counts were normalized using the *estimateSizeFactors* function in DESeq2 (version 1.36.0; Love et al. 2014), as recommended by McMurdie and Holmes (2014). This normalization step ensures that differences in eukaryotic community composition are not artifacts of sequencing depth.

Community analyses, including Permutational Multivariate Analysis of Variance (PERMANOVA) and Principal Coordinates Analysis (PCoA), were performed on Bray-Curtis dissimilarity matrices calculated with the *vegdist* function in the *vegan* package (version 2.6-4; Oksanen et al., 2022). PERMANOVA was conducted using the *adonis2* function with 4999 permutations to assess the effects of storm events and other environmental factors on community composition. PCoA was performed with the *capscale* function in an unconstrained mode (*dist_matrix* ~ 1) to explore patterns in eukaryotic community structure without environmental constraints, leveraging *capscale*'s flexibility for handling both constrained and unconstrained ordinations.

Ordination scores were extracted with the *scores* function (*display* = "sites") to visualize sample positions along PCoA axes, with the first two axes explaining the primary variance in community composition. For visualization, *ggplot2* was used to plot sample scores along PCoA1 and PCoA2, supplemented with covariance ellipses generated by the *veganCovEllipse* function to represent sample grouping by storm event. These ellipses are based on covariance, not 95% confidence intervals, providing a visual representation of variability within groups. The plot was color-coded by storm back-trajectory to explore potential influences of atmospheric origins on eukaryotic communities. This approach integrates *vegdist*, *capscale*, and *ggplot2*, offering a comprehensive framework for analyzing and visualizing microbial diversity in canopy-derived stemflow samples.

Storm dates were used to develop back-trajectory categories. Back-trajectories were determined using the NOAA HYSPLIT (Hybrid Single-Particle Lagrangian Integrated Trajectory) model to calculate backward air mass trajectories for storm events as part of our analysis (Stein et al. 2015). Using the parameters available on the HYSPLIT platform (<https://www.ready.noaa.gov/hypub-bin/trajtype.pl>), air mass movements up to 72 hours prior to each storm were generated, determining potential atmospheric origins and transport paths of eukaryotic microbes found in stemflow. The model was configured with backward trajectories, modeling vertical motion based on vertical velocity to trace air movements and possible sources of microbial input. Key inputs included the study site latitude (41.631819°), longitude (-81.304278°), and an initial height of 500 meters above ground level (AGL) for back-trajectory elevation, with a temporal resolution of 6-h intervals over a 1° grid and using meteorological data from the GDAS1 (Global Data Assimilation System) dataset. The dominant back-trajectory direction (N, NE, E, SE, S, SW,

W, NW) was identified and added to the storm condition dataset (Table 1). This approach helps contextualize the origins of eukaryotic communities, correlating microbial presence with atmospheric transport patterns, which can provide insights into regional and long-range transport processes influencing microbial deposition in forest canopies.

Table 1
Storm events included in the DNA analysis of eukaryotic microbial communities. Rainfall characteristics such as amount, duration, and intensity were recorded, and storm back-trajectories were determined using the NOAA HYSPLIT model.

Event	Date	Amount	Duration	Intensity	Back
[#]	[DD-MM-YYYY]	[mm]	[h]	[mm h ⁻¹]	trajectory
1	22-07-2022	7.9	1.0	7.9	SW
2	04-08-2022	15.9	16.0	1.0	SW
3	11-09-2022	18.8	16.5	1.1	SW
4	19-10-2022	113.8	50.3	2.3	N
5	14-11-2022	69.5	82.0	0.8	NW
6	17-11-2022	6.8	51.3	0.1	SW
7	28-11-2022	11.1	26.9	0.4	NW
8	01-12-2022	4.1	2.4	1.7	S
9	12-06-2023	63.0	14.1	4.5	W
10	14-06-2023	27.4	5.2	5.3	SW

The relative abundance of each Eukaryotic phylum detected in stemflow was calculated with the phyloseq package (version 1.40.0; McMurdie and Holmes, 2013) with the function transform_sample_counts on the raw sequence reads (*i.e.*, no normalization). The function glom was used to agglomerate the relative abundance at the level of phylum. For further visualization and analysis, the 15 most abundant phyla were retained individually, while the relative abundane of other less abundant phyla were summed together at higher taxonomic ranks. To test how back trajectories influenced the phyla, Kruskal-Wallis non-parametric tests were performed with the function kruskal.test in R. In total, 26 Kruskal-Wallis tests were conducted, one for each phylum or larger taxonomic group. To correct for multiple comparisons, a Bonferroni correction was used where tests with p-values below 0.0019 were considered significant. This value was calculated by dividing 0.05 by 26 (the number of comparisons). Dunn post-hoc tests were used to determine significant differences between the five back-trajectories of the storm events (N, NW, S, SW, and W) with the function dunnTest in the FSA package (version 0.9.5; Ogle et al., 2023).

3. Results

Eukaryotic microbial community composition is plotted for each tree and storm in Fig. 1. Notably, dominant phyla of fungi (i.e., Basidiomycota and Ascomycota), algae (i.e., Ochrophyta and other stramenopiles), and ciliated protists (i.e., Ciliophora and Alveolata) show varying relative abundances across storms, suggesting differential responses of these groups to changing environmental conditions associated with each storm (Fig. 1). No significant differences were statistically detected in eukaryotic community composition across trees (PERMANOVA: $F = 1.13$, $p = 0.08$). However, the observed variation in community composition across storm events (Fig. 2a) supports the significant differences identified by PERMANOVA ($F = 3.58$, $R^2 = 0.31$, $p < 0.001$), indicating that storm events contribute substantially to the observed variability in eukaryotic community structure.

Summer storms (events 1, 2 in 2022; events 9, 10 in 2023) were principally characterized by a high relative abundance of fungi, often comprising 60–90% of the total eukaryotic community composition (Fig. 1). These storms also exhibited elevated levels of Ciliophora, particularly evident during event 2 on August 5, 2022. Notably, the intense summer storm (event #9; 63 mm with a rainfall rate of $\sim 5 \text{ mm h}^{-1}$; Table 1) was associated with an increased relative abundance of Arthropoda, reaching 15–25% in several samples. The back-trajectories for these four summer storms were primarily from the southwest or west, which may have influenced the community composition observed (Table 1). Indeed, the relative abundance of both Ciliophora and Arthropoda were significantly affected by storm back-trajectory when tested with Kruskal-Wallis tests (Table 2). Stemflow eDNA samples collected in September 2022 (event #3), which was a transitional period between the summer and fall storms (i.e., those storms occurring during leaf budding and senescence), displayed eukaryotic taxa common to both seasons. For instance, some samples (e.g., from trees 61T, 94P, 94L, 59C) were dominated by fungi, aligning with trends seen in summer storms, while other samples (e.g., from trees 57F, 60D) showed increased relative abundances of Ochrophyta and Euglenozoa, taxa typically more prevalent in fall storms from October through December (Fig. 1).

Table 2

Average percent abundance ($\pm$ standard error) of each eukaryotic phylum found in stemflow after storms that originated from different cardinal directions.

Phylum	N	NW	S	SW	W
Ochrophyta*	38.47 $\pm$ 10.81^{a,b}	47.46 $\pm$ 8.17^a	69.09 $\pm$ 9.21^a	14.88 $\pm$ 5.51^{b,c}	40.20 $\pm$ 3.73^c
Peronosporomycetes	0.11 $\pm$ 0.059	0.073 $\pm$ 0.061	0.021 $\pm$ 0.011	0.34 $\pm$ 0.31	0.062 $\pm$ 0.024
Other Stramenopiles*	0.071 $\pm$ 0.035^{a,c}	0.0075 $\pm$ 0.0038^{a,b}	0 ^b	0.12 $\pm$ 0.057^c	1.67 $\pm$ 1.66^{a,b,c}
Cercozoa	1.3 $\pm$ 0.39	0.89 $\pm$ 0.25	0.60 $\pm$ 0.35	2.8 $\pm$ 1.3	1.1 $\pm$ 0.60
Euglenozoa*	0.56 $\pm$ 0.0023^a	0.25 $\pm$ 0.0024^{b,c}	3.81e⁻⁰⁴ $\pm$ 2.48e⁻⁰⁴ b	0.17 $\pm$ 0.11^{b,c}	0.063 $\pm$ 0.023^{a,c}
Other Discoba	0.0096 $\pm$ 0.0055	0.015 $\pm$ 0.010	0.011 $\pm$ 0.0093	0.011 $\pm$ 0.0026	0.0056 $\pm$ 0.0027
Discosea	0.046 $\pm$ 0.015	0.027 $\pm$ 0.0099	0.017 $\pm$ 0.0017	0.044 $\pm$ 0.014	0.012 $\pm$ 0.0061
Schizoplasmodiida	0.027 $\pm$ 0.019	0.027 $\pm$ 0.018	0.093 $\pm$ 0.074	0.020 $\pm$ 0.016	0.0015 $\pm$ 8.6e ⁻⁰⁴
Other Amoebozoa*	0.023 $\pm$ 0.0057^{a,b}	0.024 $\pm$ 0.0080^a	0.0048 $\pm$ 0.0026^a	0.058 $\pm$ 0.011^b	0.042 $\pm$ 0.013^{a,b}
Ciliophora*	1.94 $\pm$ 0.61^{a,b}	1.16 $\pm$ 0.39^a	0.49 $\pm$ 0.12^a	8.27 $\pm$ 3.55^b	3.87 $\pm$ 2.18^{a,b}
Apicomplexa*	0.13 $\pm$ 0.035^a	0.027 $\pm$ 0.013^{b,c}	0.056 $\pm$ 0.050^{a,b}	0.11 $\pm$ 0.024^a	0.32 $\pm$ 0.079^a
Protaveolata	0.0081 $\pm$ 0.0064	0.043 $\pm$ 0.038	0.0011 $\pm$ 0.0011	0.014 $\pm$ 0.0066	5.95e ⁻⁰⁴ $\pm$ 4.03e ⁻⁰⁴
Other Alveolata	0.052 $\pm$ 0.013	0.028 $\pm$ 0.010	0.011 $\pm$ 0.0054	0.13 $\pm$ 0.038	0.029 $\pm$ 0.0071
Arthropoda	2.62 $\pm$ 1.75^a	0.26 $\pm$ 0.16^b	0.071 $\pm$ 0.035^{a,b}	4.50 $\pm$ 1.97^a	1.12 $\pm$ 0.88^{a,b}

*Bold face indicates taxa that had significantly different relative abundance between storm directions with Kruskal-Wallis tests. A Bonferroni correction for multiple comparisons was used and differences were considered significant if the p-value of the Kruskal-Wallis test was below 0.0019 (determined by dividing 0.05 by 26, which was the number of comparisons). Different superscript letters indicate significant differences between the storm directions as determined with Dunn tests where adjusted p-values (Bonferroni method) below 0.05 indicated significance.

Phylum	N	NW	S	SW	W
Nematoda	0.35 ± 0.17^a	0.0039 ± 0.0016^b	0^b	0.20 ± 0.096^{a,b}	0.011 ± 0.0069^{a,b}
Tardigrada	0.23 ± 0.22	0.29 ± 0.22	4.71e ⁻⁰⁴ ± 4.71e ⁻⁰⁴	5.66e ⁻⁰⁵ ± 4.23e ⁻⁰⁵	7.10e ⁻⁰⁴ ± 6.24e ⁻⁰⁴
Rotifera	0.11 ± 0.064	0.090 ± 0.046	0.087 ± 0.025	0.035 ± 0.012	0.0086 ± 0.0057
Other Metazoa	0.058 ± 0.026	0.043 ± 0.015	0.0088 ± 0.0019	0.047 ± 0.013	0.012 ± 0.0075
Phragmoplastophyta	0.25 ± 0.080^a	0.38 ± 0.20^a	0.065 ± 0.011^a	0.78 ± 0.42^a	2.92 ± 0.83^b
Klebsormidiophyceae	0.017 ± 0.014	0.054 ± 0.023	0.062 ± 0.037	0.023 ± 0.013	0.019 ± 0.0090
Other Chloroplastida	5.43 ± 1.77^{a,c}	1.81 ± 0.55^b	2.76 ± 0.52^{a,b}	14.42 ± 3.12^a	17.60 ± 2.48^{b,c}
Ascomycota	26.33 ± 5.52	23.06 ± 4.33	11.96 ± 2.93	28.42 ± 3.57	50.49 ± 5.45
Basidiomycota	17.94 ± 6.38	19.74 ± 3.60	11.06 ± 4.50	17.78 ± 3.09	14.67 ± 2.87
Chytridiomycota	0.18 ± 0.053	0.27 ± 0.14	0.074 ± 0.051	0.051 ± 0.012	0.054 ± 0.021
Entomophthoromycota	0.011 ± 0.0044	0.15 ± 0.15	0.0041 ± 0.0019	0.011 ± 0.0078	0.0031 ± 0.0016
Other Fungi	0.53 ± 0.12	0.64 ± 0.11	0.19 ± 0.033	0.82 ± 0.14	0.43 ± 0.078
*Bold face indicates taxa that had significantly different relative abundance between storm directions with Kruskal-Wallis tests. A Bonferroni correction for multiple comparisons was used and differences were considered significant if the p-value of the Kruskal-Wallis test was below 0.0019 (determined by dividing 0.05 by 26, which was the number of comparisons). Different superscript letters indicate significant differences between the storm directions as determined with Dunn tests where adjusted p-values (Bonferroni method) below 0.05 indicated significance.					

The largest storm event (~ 114 mm) with a distinct northerly back-trajectory on October 10, 2022 (event #4: Table 1), showed considerable variability in dominant taxa across samples, similar to event #3. However, this storm also resulted in stemflow with a greater relative abundance of Chloroplastida, and some samples contained over 10% Arthropoda, as seen in samples from tree 56B (Fig. 1). Additionally, tardigrades appeared in noticeably higher relative abundance during this event. The next largest storm (~ 70 mm on November 14, 2022, event #5) exhibited a similar eukaryotic community composition to the largest storm, with notable increases in the relative abundance of tardigrades from some trees (Fig. 1).

In general, larger storm events (> 60 mm) consistently produced stemflow with higher relative abundances of Arthropoda and other relatively larger (in body size) taxa.

Finally, stemflow eDNA from late November and early December storms predominantly consisted of Ochrophyta, which accounted for 50–90% of the relative abundance across sampled trees (Fig. 1). Elevated levels of other taxa, such as Cercozoa and Arthropoda, were observed in specific samples—tree 56B and 57A, respectively—during event #6. These observations suggest that storm size and back-trajectory direction play a significant role in shaping the eukaryotic community composition in stemflow samples.

The Principal Coordinates Analysis (PCoA) plot illustrates the differences and similarities among stemflow samples and storms (Fig. 2, top). Distinct clustering patterns are observed, indicating variation in eukaryotic communities associated with specific storm events. For example, just as the taxa relative abundance presentation (in Fig. 1) shows similarities among summer storms (from June, July, and August), these storms also cluster together in the PCoA suggesting similar community compositions within these storms (see yellow, blue, red and white markers in top panel of Fig. 2). Similarly, storms from fall plot similarly in the PCoA (Fig. 2, top) and the relative abundance plot (Fig. 1). These cross-event groupings suggest some event characteristic's influence on eukaryote community composition.

Another PCoA plot was developed based on the HYSPLIT back-trajectories (Fig. 2, bottom). A PERMANOVA found a significant effect ($F = 8.9$, $R^2 = 0.36$, $p < 0.00001$) on community composition (accounting for about 5% more of the variation than with just storm events), suggesting that air masses from different regions and trajectories may carry distinct sets of microorganisms. Overlapping groups of storms with differing back-trajectories tend to share a directional element (i.e., the overlap between S and SW, or between N and NW). Despite this overlap, ten phyla detected in our sequencing showed significant relative abundances differences between the storm back-trajectories (Table 2). This supports the idea that atmospheric transport pathways contribute to the diversity and structure of eukaryotic communities in tree canopies, likely due to differing source regions and environmental conditions encountered along each trajectory.

4. Discussion

4.1. Temporal variability of stemflow eukaryotic community composition.

Stemflow eDNA exhibited pronounced seasonal shifts in eukaryotic community composition, a pattern that likely captures in-canopy (phyllosphere) dynamics of our beech trees, varying atmospheric inputs, and meteorological conditions affecting canopy rainfall capture and drainage. Seasonal insights reported here are limited to summer, fall, and winter. Fungal reads (principally Entomophthoromycota, Basidiomycota, and Ascomycota) accounted for up to 90% of the community during summer storms (Fig. 1). Summer storms align well with the life cycle of canopy-dwelling fungi, possibly explaining their

dominance in beech-tree stemflow during that time. Entomophthoromycota, known to be insect, arthropod, and nematode pathogens (Humber, 2016), thrive when their insect hosts multiply in warm, humid months. High temperatures and moisture spur spore production and dispersal (Benny et al., 2014), while storm-heightened humidity and rainfall ease aerial release and canopy wash-off (Magyar et al., 2016) then likely flushes both spores and the occasional infected insect to the forest floor. Their resilient resting spores further ensure survival between hosts and splash events (Eilenberg et al., 2013). Intense, short-lived summer downpours therefore coincide with peak fungal activity and act as efficient conveyors, redistributing Entomophthoromycota across the stand (Skrzecz et al., 2024).

During fall, the relative abundance of Ascomycota and Basidiomycota rises, plausibly because senescing leaves, pollen, leaf exudates, and insect frass enrich leaf surfaces with substrates that favor saprotrophic fungi (*sensu* Kembel and Mueller 2014). Decomposition is further encouraged by canopy-retained litter, often substantial in forest crowns (Nadkarni and Matelson, 1991; Van Stan et al., 2021), even though litter mass was not quantified in the present beech stands. These observations support a seasonal transition from summer-dominated parasitic fungal reads in stemflow to a fall emphasis on decomposition, likely driven by shifts in canopy resource availability and microclimate. By late fall and winter, stemflow communities shift again, with fungi giving way to Ochrophyta. Back-trajectory analysis shows that many cold-season storms approached from the north and northwest, crossing the Great Lakes (Table 1); such paths can entrain aerosolized algal cells that subsequently deposit onto forest canopies, a phenomenon documented for stemflow measured from other lake-adjacent vegetation (Guidone et al., 2021). The Great Lakes can be a relevant winter sources of airborne algae, which northerly winds can disperse over land before precipitation washes them into stemflow. Leaf drop during this period also increases light penetration to bark surfaces, potentially stimulating *in situ* algal growth. Atmospheric transport is a recognized route for delivering algal propagules to terrestrial phyllospheres (Warren, 2022) and, in conjunction with enhance winter bark insolation, may together explain the observed late-season increase in algal reads in stemflow.

Most phyllosphere algae studies, including work on Ochrophyta, have been conducted in tropical settings, where warm, humid air fosters diverse algal assemblages on bark and leaves (Liu et al., 2023; Manikandan et al., 2024; Zhu et al., 2018). In our winter study system, however, *F. grandifolia* is leafless; the relevant phyllosphere is therefore the bark surface alone; called the cortisphere (Pfanz et al., 2002) or dermosphere (Lambais et al., 2014). The contribution of Ochrophyta to winter phyllospheres in temperate forests remains largely unresolved. Nevertheless, overcast, moisture-rich winters near large lakes may offer microclimates conducive to their establishment. Subtropical work shows that winter soils can harbor elevated algal abundance, Ochrophyta included (Wei et al., 2023). In temperate canopies, retained leaf litter in branch forks, bark pores that hold water, consistently high humidity, and greater bark radiation receipt could provide similarly suitable microsites, while northerly air masses crossing the Great Lakes can supply aerosolised algal cells to tree crowns. It is therefore plausible that Ochrophyta occupy this winter habitat on beech cortispheres via this atmospheric pathway. Targeted winter sampling of bark biofilms, coupled with stemflow eDNA, will be required to test this hypothesis and to track seasonal algal dynamics in arboreal habitats that are otherwise difficult to monitor directly.

4.2. Stemflow as a lens on storm-driven shifts in canopy eukaryotes under a warming climate?

These results demonstrate the potential stemflow offers to study how discrete storm conditions (through their magnitude, intensity, and air mass origins) influence community composition on bark surfaces (including incoming atmospheric recruits) (Mabrouk et al., 2022), thereby tracing storm-driven shifts in canopy eukaryotes under a warming climate. Importantly, stemflow eDNA integrates at least three source pools: (1) resident cortisphere (and some leaf) taxa mobilized by rivulet scouring; (2) dry-deposited atmospheric cells that settle on bark before the storm; and (3) in-drop passengers already suspended in rain before canopy contact. Our sequencing cannot assign individual reads to one pool or another. Instead, storm traits and back-trajectory directions here serve as proxy indicators of relative source strength. For example, storms delivering $> 60 \text{ mm event}^{-1}$ (Table 1) carried markedly higher relative abundances of large metazoans—tardigrades, arthropods, and related taxa—than smaller events (Fig. 1). Intense rainfall likely generates greater scouring velocities along bark and leaf surfaces, mobilizing organisms that smaller flows leave undisturbed. Laboratory and field work show that rainfall intensity governs the detachment of particulate matter and microbes from canopy substrates (Cayuela et al., 2019; Guidone et al., 2021; Levia et al., 2013; Xu et al., 2017), and the additional kinetic energy of heavy downpours can overcome even strong attachment forces (Van Stan et al., 2021). The large storms in this study also lasted longer, keeping the canopy saturated and perhaps triggering behavioral responses in motile micro-metazoans that make them easier to flush away, as discussed in Van Stan et al. (2023). Together, storm magnitude, duration, and organism behavior emerge as key, testable controls on which eukaryotes stemflow exports from tree crowns.

Little previous work has examined eukaryotic responses to storm scouring, but bacterial studies hint at differential sensitivities. On *Typha latifolia* (cattail) leaves, rain scarcely altered bacterial composition, (Stone and Jackson, 2021), whereas on subtropical oaks some taxa were readily removed (Teachey et al., 2018), while others, potentially supported by biofilms (Flemming and Wuertz, 2019; Morris and Monier, 2003) or sheltered by micro-depressions in the leaf cuticle (Vorholt, 2012), remained. Our data suggest a comparable gradient for eukaryotes: firmly attached or endophytic microbes may require high-intensity events to enter stemflow, whereas loosely deposited aerosols depart even in moderate rain. Testing these mechanistic insights gains urgency in the context of climate change. Models and observations converge on a future with more powerful storms separated by longer dry spells (Creed et al., 2015; Gloor et al., 2013; Huntington, 2006; Lian et al., 2022; Madakumbura et al., 2019). Prolonged drying can increase bark and leaf hydrophobicity, and subsequent intense rainfall enhances scouring efficiency (Van Stan and Pinos, 2024). Shifts in storm regime could therefore reorder canopy microbiomes, altering functions linked to nutrient cycling, plant health, and forest resilience. Because stemflow integrates canopy wash-off at event scale, eDNA profiles from contrasting storms can reveal which organisms move under which hydrometeorological conditions, information essential for forecasting biotic change in increasingly volatile climates.

5. Conclusions

This study provides the first taxonomically resolved portrait of eukaryotic organisms transported by stemflow from any tree species and demonstrates how storm characteristics modulate that flux. Three principal insights emerge. First, there may be a seasonal re-ordering of canopy guilds. Fungal reads dominated summer stemflow, reflecting peak activity of entomopathogenic and saprotrophic taxa that prosper when insect hosts and leaf exudates are plentiful. Autumn senescence elevated Ascomycota and Basidiomycota, consistent with a shift toward decomposition of litter retained in beech crowns. By winter, fungal prevalence waned and algal stramenopiles surged, a pattern best explained by northerly air masses crossing the Great Lakes and depositing aerosolized Ochrophyta. These findings suggest that temperate winter canopies may harbor overlooked algal niches. Second, storm mechanics can influence the eukaryotic community in stemflow. Events delivering > 60 mm rainfall, and therefore higher kinetic energy and longer saturation, exported more tardigrades, collembolans and other metazoans than smaller storms. The combination of greater scouring velocities and possible behavioral escape responses appears pivotal for dislodging well-anchored organisms. Such mechanistic links imply that projected increases in storm intensity, coupled with longer inter-storm drying that raises bark hydrophobicity, could restructure canopy microbiomes in the coming decades. Finally, stemflow may be a practical surveillance tool. Because stemflow integrates wash-off over entire crowns yet is easy to collect at the trunk base, it offers a scalable, low-impact method for tracking canopy biodiversity in real time. Pairing eDNA with routine meteorological and back-trajectory data allowed us to attribute community shifts to specific storm properties—an approach readily transferable across sites, species and climates. Future directions should include (i) simultaneous sampling of canopy surfaces and soil recipients to quantify actual dispersal success; (ii) incorporation of quantitative PCR or microscopy to convert relative read abundance into organismal fluxes; and (iii) coupling stemflow microbiomics with functional assays that link community turnover to nutrient cycling, pathogen pressure and forest health metrics. Ultimately, integrating hydrology, aerobiology and molecular ecology will refine our predictions of how changing storm regimes propagate biological change from the treetops to the rhizosphere.

Declarations

Author Contribution

D.A.R.G. contributed to the original draft writing, visualization, methodology, investigation, formal analysis, and data curation. D.J.B. contributed to review and editing, supervision, resources, conceptualization, and project administration. S.R.C.-K. contributed to review and editing, methodology, software, and formal analysis. C.B.-V. contributed to review and editing, visualization, resources, and data curation. A.I.M. contributed to field work, review and editing, data curation, and conceptualization. J.T.V.S. contributed to original draft writing, review and editing, supervision, methodology, investigation, resources, project administration, funding acquisition, and formal analysis. All authors reviewed the manuscript text and visuals.

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Data Availability

Sequence data will be made publicly available through the sequence read archive upon publication.

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Figures

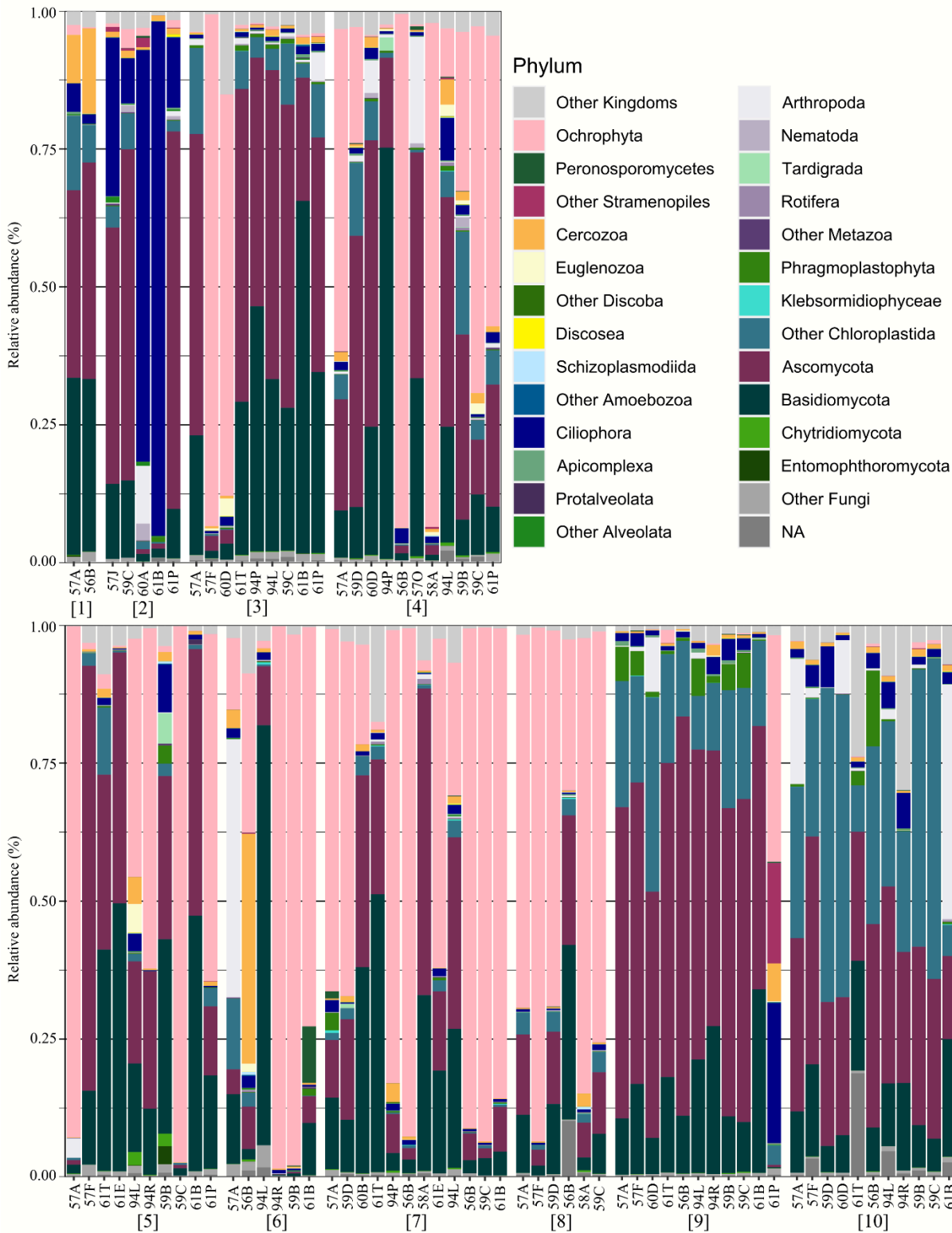


Figure 1

Relative abundance of eukaryotic microbial phyla across ten storm events (refer to Table 1 for event details). Each bar represents the community composition of eukaryotic taxa (color coded by phylum) for individual stemflow samples after a storm.

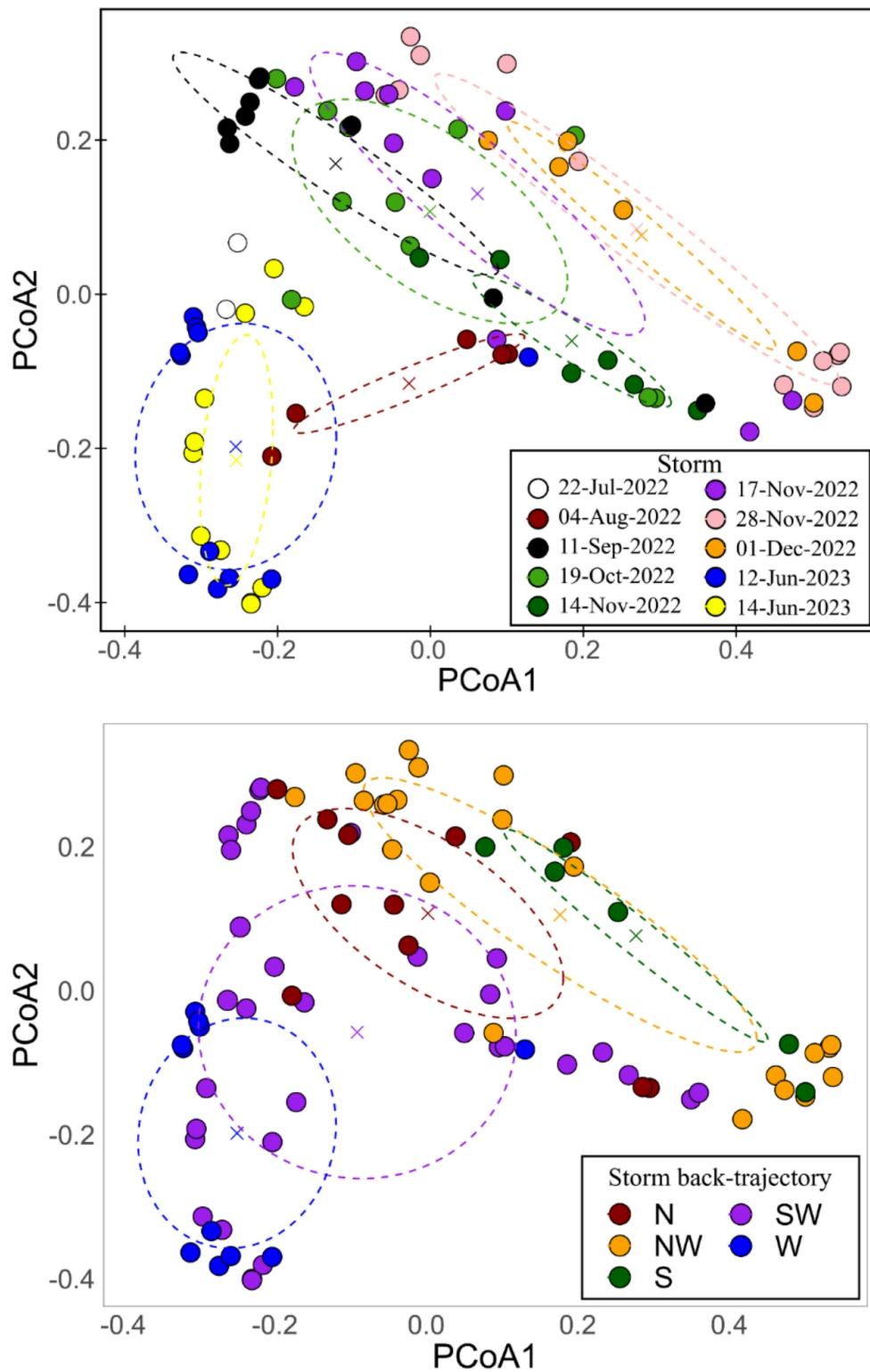


Figure 2

Principal Coordinates Analysis (PCoA) of stemflow eukaryotic community composition categorized by (top) event and (bottom) NOAA HYSPLIT back-trajectories. Each point represents a sample, color-coded by (top) storm dates and (bottom) the back-trajectory direction of the associated storm: North (N), Northwest (NW), Southwest (SW), West (W), and South (S).