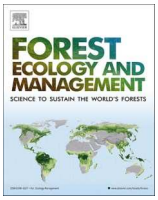
Forest Ecology and Management 585 (2025) 122674

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Harnessing stemflow as a diagnostic tool for canopy disease detection and monitoring

D. Alex R. Gordon [a], David J. Burke [b], Sarah R. Carino-Kyker [b], Claudia Bashian-Victoroff [b], Adam I. Mabrouk [a], John T. Van Stan II [a,*]

[a] Department of Biological, Geological, and Environmental Sciences, Cleveland State University, Cleveland, OH 44115, USA
[b] The Holden Arboretum, Kirtland, OH 44094, USA

ARTICLE INFO

Keywords:
Ecohydrology
Stemflow
Foliar nematode
Disease ecology
Pathogen
Method

ABSTRACT

Monitoring diseases within tree canopies is challenging due to their inaccessibility and the complexity of canopy ecosystems. Here, we explore the potential of stemflow sampling as a novel, ground-based method for detecting and monitoring canopy-associated pathogens. In a case study focused on *Litylenchus crenatae* ssp. *mccannii* (LCM), the nematode associated with Beech Leaf Disease (BLD), we collected stemflow samples from 18 *Fagus grandifolia* Ehrh. (American beech) trees across 12 storm events. eDNA assays detected LCM presence in 7 of those storms, with quantitative PCR-derived gene concentrations ranging from 80 to 158,000 copies mL^{-1}. Higher detections and concentrations coincided with leaf senescence and bud formation periods, and they correlated conditionally with event rainfall amount and pre-storm changes in relative humidity. Although based on a single site and season, these findings demonstrate the potential for stemflow sampling to capture a pathogen's eDNA (*i.e.*, canopy distress signals) at ground level. This method could complement traditional monitoring, offering another affordable, non-invasive tool for pathogen detection. Additional validation, particularly regarding live versus dead organisms and across varied site conditions, will be essential to evaluate the breadth of value stemflow eDNA offers for canopy disease management and ecological research.

1. Introduction

Monitoring disease-afflicted tree canopies is challenging due to their physical inaccessibility (Duarte et al., 2022; Fang et al., 2023). Canopies also host diverse microhabitats, shaped by a unique set of sunlight, humidity, and other microclimatic variables (Ehbrecht et al., 2019; Larrieu et al., 2018; Merrick et al., 2021). These microhabitats can serve as incubators or transport media for diseases, especially when facilitated by water-related vectors such as rain splash, branchflows, or storm-associated windiness (Magyar et al., 2021; Van Stan et al., 2021). Direct study of canopy systems often requires specialized climbing equipment and expertise at significant temporal and economic costs (Anderson et al., 2015; Cannon et al., 2021). Remote sensing technologies can circumvent some needs for physical canopy access, but these systems also demand financial resources and advanced data analysis skills (Duarte et al., 2022; Fang et al., 2023; Zhang et al., 2019). These hurdles impede both disease monitoring and our broader understanding of canopy disease ecology. How can we more effectively study this elevated and intricate world while minimizing the associated costs, labor, and logistical challenges?

Storms offer a promising solution: as precipitation falls upon a forest, tree branches intercept a portion and drain it down their stems to the ground. This "stemflow" process can result in a single tree transporting hundreds of liters of water during a storm (Van Stan and Gordon, 2018). We introduce stemflow sampling as a cost-effective method for monitoring canopy health. Much like how runoff scours watersheds, collecting information as it flows (Hosen et al., 2021; Sprenger et al., 2019; Wilkinson et al., 2022), stemflow serves as a sentinel for the canopies from which it drains (*sensu* Mabrouk et al., 2022). As stemflow scours branches and stems (and some leaves), it can capture disease agents—bacteria, oomycetes, mites, nematodes, etc.—and their presence or abundance in stemflow may provide insights into disease prevalence and transmission dynamics. Moreover, pathogen temporal variability within stemflow can reveal ecological patterns, offering valuable data on environmental conditions that influence disease dynamics. By leveraging this naturally occurring 'remote sensing'

* Corresponding author.
E-mail address: j.vanstan@csuohio.edu (J.T. Van Stan).

https://doi.org/10.1016/j.foreco.2025.122674
Received 17 January 2025; Received in revised form 13 March 2025; Accepted 14 March 2025
Available online 25 March 2025

mechanism, stemflow presents a promising ground-based tool for monitoring canopy health without the high costs or technical and logistical challenges associated with traditional methods.

Although no single stemflow sampler design has yet achieved universal acceptance (Levia and Germer, 2015; Sadeghi et al., 2020), available designs share a common minimalistic approach. Typically, they consist of a simple gutter-like collar, flexible tubing, and a collection bin. While some designs have relied on nails or staples, there is a decades-long tradition of approaches that leave the bark surface intact (Butler and Likens, 1995; Carpenter, 1982; Honda et al., 2015; Likens and Eaton, 1970; Noren et al., 2023; Schroth et al., 2001). This streamlined configuration minimizes potential damage while offering significant flexibility for customization (see photographs in Levia and Germer 2015 and Sadeghi et al. 2020). Components can be adjusted to accommodate various stem sizes, site conditions, or aesthetic requirements. Consequently, although no standard stemflow collection method has emerged, non-invasive systems are recognized as highly adaptable and suitable for diverse settings—from urban landscape to natural forests (see brief review of stemflow reviews in Van Stan and Pinos (2024)—without compromising tree health or appearance.

To illustrate stemflow's feasibility for detecting 'distress signals' from diseased tree canopies, we present a case study focused on *Litylenchus crenatae* ssp. *mccannii* (LCM), a foliar nematode associated with Beech Leaf Disease (BLD). Since 2012, BLD has spread from Lake County, Ohio to Canada and 12 more U.S. states (Kantor et al., 2022; Reed et al., 2020; Vieira et al., 2023b). Nematodes infect beech leaves, diminishing photosynthetic capacity and eventually leading to substantial canopy decline and tree mortality over successive years (Ewing et al., 2019; Reed et al., 2022); no known cure exists (Faubert, 2023). Recent studies further show that LCM's life cycle is tightly coupled to bud and leaf phenology, overwintering in buds and resuming feeding after leaves expand (Vieira et al., 2023a). Evidence also suggests that humidity, wind, and incidental carriers (*e.g.*, insects) can facilitate local dispersal, highlighting the complexity of BLD epidemiology and its threat to North American beech forests (Goraya et al., 2024). Given *Fagus grandifolia*'s ecological importance—ranging from soil stabilization to wildlife habitat (Leak et al., 2014; Stephanson and Coe, 2017)—BLD has emerged as a formidable forest disease threatening both native beech stands and ornamental or urban plantings throughout northeastern North America (Ewing et al., 2019; Reed et al., 2022). Early detection and monitoring are critical to understanding the factors that contribute to BLD's spread and to developing effective management strategies (Reed et al., 2020; Zhao et al., 2023). While the mechanisms of LCM nematode spread are unknown, nematode activity on canopy surfaces (including LCM) is generally facilitated by "free water" (rain, fog, or dew) (Carta et al., 2023). However, stormy canopies are hard to study directly. If stemflow sampling can effectively yield insights to LCM's spatial and temporal patterns, it may offer a ground-based tool for canopy disease monitoring.

This study tests whether (1) LCM is detectable in stemflow collected from trees spanning a range of BLD symptom classes, and (2) LCM gene concentrations in stemflow vary with meteorological conditions. We sampled stemflow from 18 *F. grandifolia* Ehrh. (American beech) trees of varying BLD symptomology (Table 1), quantified LCM presence using species-specific primers, and measured gene copy numbers with quantitative PCR (qPCR) (Burke et al., 2023). Demonstrating LCM detection in stemflow would validate it as a cost-effective, ground-based tool for detecting BLD and potentially other canopy diseases. Linking LCM stemflow variability in response to environmental conditions could provide temporal insights into the factors influencing pathogen presence or abundance within canopy ecosystems. Beyond BLD, we propose stemflow as a scalable, efficient approach for canopy disease research and management.

2. Methodological approach

2.1. Study site and tree selection

The study was conducted in a beech plantation 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 (Fig. 1), 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. The study period spanned July to December 2022 (until all leaves had fallen), aligning both with the time of year without snowfall, and when BLD-associated nematodes are believed to be most active.

All 18 study trees had grown from seed in their source locations and later accessioned as plants at the Holden Arboretum plantation site. Study trees originated from two provenances (Maine and Michigan), were planted at the arboretum in 2006, and were similarly sized but exhibited different levels of BLD symptom severity, as assessed during September 2020 using the Bashian-Victoroff et al. (2023) classification system. Trees displaying visible symptoms of BLD in < 10 %, 10–25 %, 26–50 %; 51–75 %; and > 75 % of their leaves were given a rating of 1 through 5, respectively (see Table 1). The sample included six trees each with severe symptoms (ratings of 3–4), moderate symptoms (rating of 1), and trees treated with TREE-äge R10 pesticide (9.7 % emamectin benzoate, Arborjet Inc., Woburn, MA) which exhibited low-level BLD symptoms. Meteorological data were sourced from the nearest Goldstar

Table 1
Tree ID for each study tree and its symptomatology (based on calculated blight scores from September 2020). Trees in symptom classes 3–4 are considered severely infected, class 1 is considered moderately infected, and class 'P' are the pesticide-treated trees. The 12 sampled rain dates (month/day) are provided, and their magnitudes (mm) are indicated in brackets. The x's indicate from which trees stemflow was sample-able during which events. Bold, upper-case X's indicate samples where *Litylenchus crenatae mccannii* genes were detected in agarose gels.

Tree ID:	57-J	61-P	59-C	60-A	61-B	59-B	56-B	57-O	58-A	61-E	94-L	94-R	57-A	57-F	59-D	60-D	61-T	94-P
Symptom:	4	4	3	3	3	3	1	1	1	1	1	1	P	P	P	P	P	P
07/22 [7.9]	x	x		x	x	x	x	x	x	x	x	x		x	x		x	
08/04 [15.9]		**X**	x	x			x	**X**		x	x	x	x	x	x	x		
08/09 [22.8]		x	x		x		x		x	x	x		x	x	x	x	x	
08/29 [24.8]				x	x	x			**X**	x	**X**	**X**	**X**		x	**X**	x	x
09/11 [18.8]		x	x		x				x			x	x			x	x	x
09/21 [5.9]		x	**X**	x	x		x			**X**		x	**X**	x	**X**		x	**X**
10/13 [43.2]		x	**X**	x	x	x	**X**	**X**	**X**		**X**	x	x		**X**	**X**		x
10/19 [113.8]		**X**	**X**	x	x	x	**X**				**X**		**X**	**X**	**X**	**X**	**X**	x
11/14 [69.5]		x	x		**X**	x	**X**			**X**	**X**	x	x	x	x		x	
11/17 [6.8]	x		x	x	x	x	x				x	x	x			x		
11/28 [11.1]	x		**X**		x	x	x		x	**X**	x		**X**		x		**X**	x
12/01 [4.1]			x	x	x	x	x		x				x	x	x			

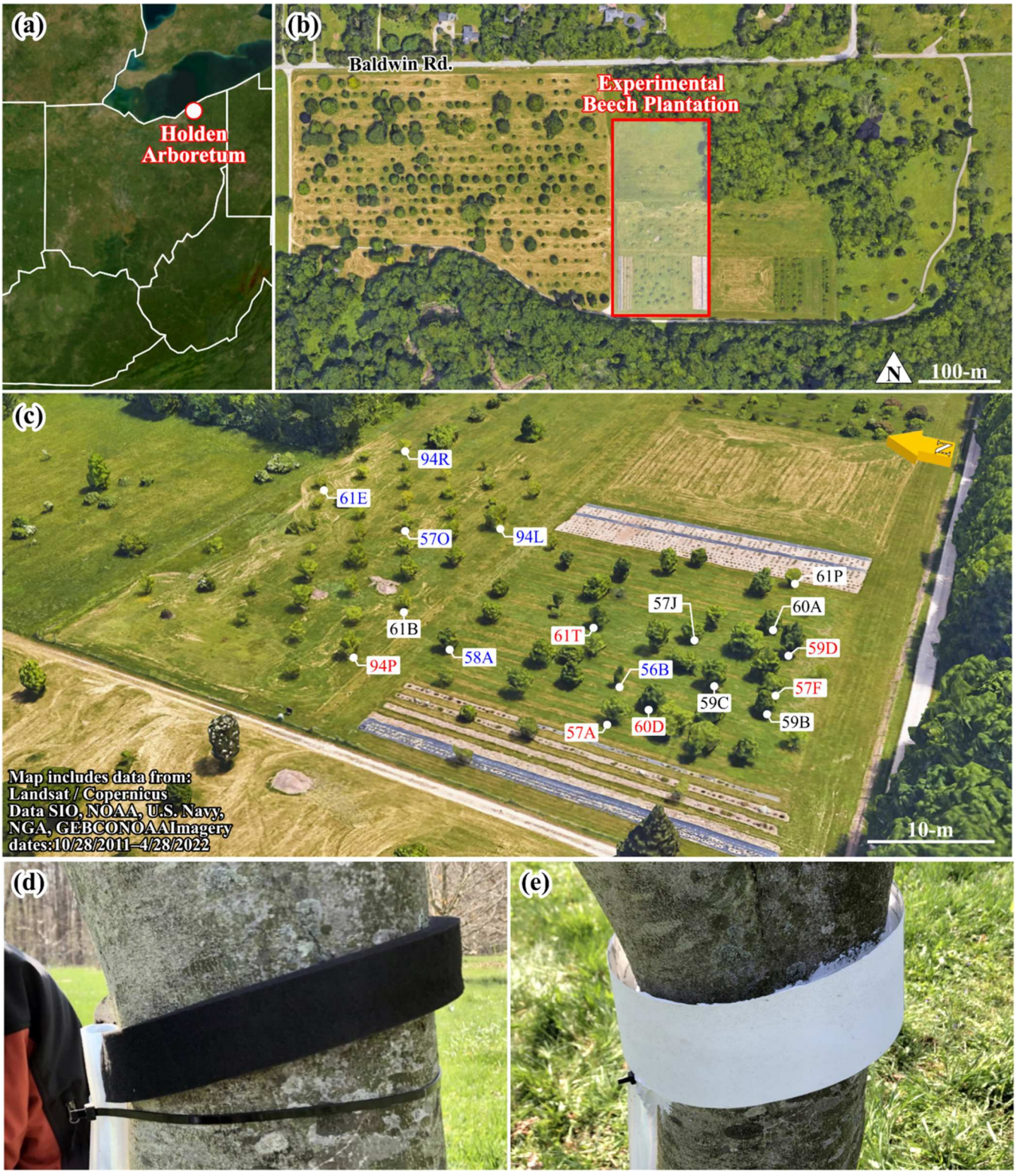


Fig. 1. Site location mapping and stemflow collar details. (a) Aerial location maps showing Holden Arboretum in northeastern Ohio (USA), (b) the experimental beech plantation, (c) the 18 trees for which stemflow collars were installed (red, blue, and black indicate pesticide-treated, moderate symptoms of beech leaf disease (BLD), and severe BLD symptoms, respectively. The stemflow collaring method consisted of (d) weatherproofed foam tape with an adhesive backing as the base of the collar to drain stemflow to a low point, where a silicon tube could convey the waters to a collection bin and (e) flexible plastic sheeting to prevent stemflow from flowing off the edge. The inside of the collar was completely sealed with silicone sealant, including the connection areas between the bark surface, foam tape, and the plastic sheeting.

Weather Underground station (KOHMENTO112, 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, including the preceding 24 hours, to assess the influence of pre-event meteorological conditions on LCM presence or DNA abundance in stemflow samples.

2.2. Stemflow method, sampling, and processing

Each study tree was equipped with a non-invasive stemflow collector (Fig. 1) consisting of three components: (1) an adhesive collar that captures and redirects the stemflow water draining down the outside of the stem bark toward (2) a flexible tube to direct captured stemflow into (3) a collection bin from which stemflow may be sampled. The base of each stemflow collar was constructed from adhesive-backed expandable waterproofed foam insulation tape: 3.8 cm× 3.8 cm Platinum Expandable Foam Weatherseal (M-D Building Products, Inc., Oklahoma City, OK, USA). Prior to installation, the foam tape was unpackaged and allowed to expand to its full width and height. Sections of the foam tape were cut to lengths of a few centimeters smaller than each study tree's circumference. This permits the collar base to be installed at a moderate slope around the stem to facilitate water collection, but leaves a small opening at the lowest point, where a 2.54-cm (internal diameter, 3.18-cm outer diameter) flexible silicone tube may be inserted. To guide the collar placement, the silicone tubing was zip-tied to the tree stem at the location where it was easiest to place a collection bin. To prevent stemflow water from flowing off the side edges of the foam tape, it was wrapped with flexible 8-mm thick plastic sheets (Arrow Home Products Co., Elk Grove Village, IL, USA) (Fig. 1e). Silicon sealant was applied to all connection points (bark-to-foam tape and foam tape-to-plastic sheeting) and applied to the top of the foam tape. The tubing connected to a 113.5 L HDPE storage tote with snap-locking lid (HDX, Model #2130–4415707). The entire process was noninvasive and maintained through weekly inspections and repairs as needed.

Stemflow samples were collected within 48 h after the end of a discrete rain event, defined as (1) any cumulative rainfall over 3 mm (2) following a 72 h antecedent dry period (Fig. 2). The minimum cumulative rainfall threshold aligns with previous findings indicating that 3 mm of rain initiates stemflow in similarly sized *F. grandifolia* trees (Van Stan and Levia, 2010). Despite efforts to collect stemflow samples for every storm, some were lost due to field logistics, including displaced bins and disconnected tubing during storms (Table 1). These gaps reflect common challenges in ground-based storm sampling, but the dataset remains robust for assessing stemflow across diverse conditions and symptom classes. Collection involved shaking bins to suspend particulates (which potentially contained BLD nematodes), filling 50 mL vials with water using gloves to prevent contamination, discarding excess water, and cleaning bins with deionized water. Vials were placed in a cooler and the samples were filtered immediately upon arrival at the Holden lab using a vacuum filtration apparatus (Fristaden Lab, Reno NV, USA), consisting of a 500 mL filtering glass flask and joined 300 mL graduated glass funnel, connected to a vacuum pump. A 47 mm diameter, 0.45 μm pore size, sterile mixed cellulose esters membrane gridded filter (Membrane Solutions, LLC, Auburn WA, USA) was placed in the funnel. The filter and its retained materials were stored in sterile bags at –80 °C until analysis. Between each sample, the entire apparatus was triple rinsed with deionized water.

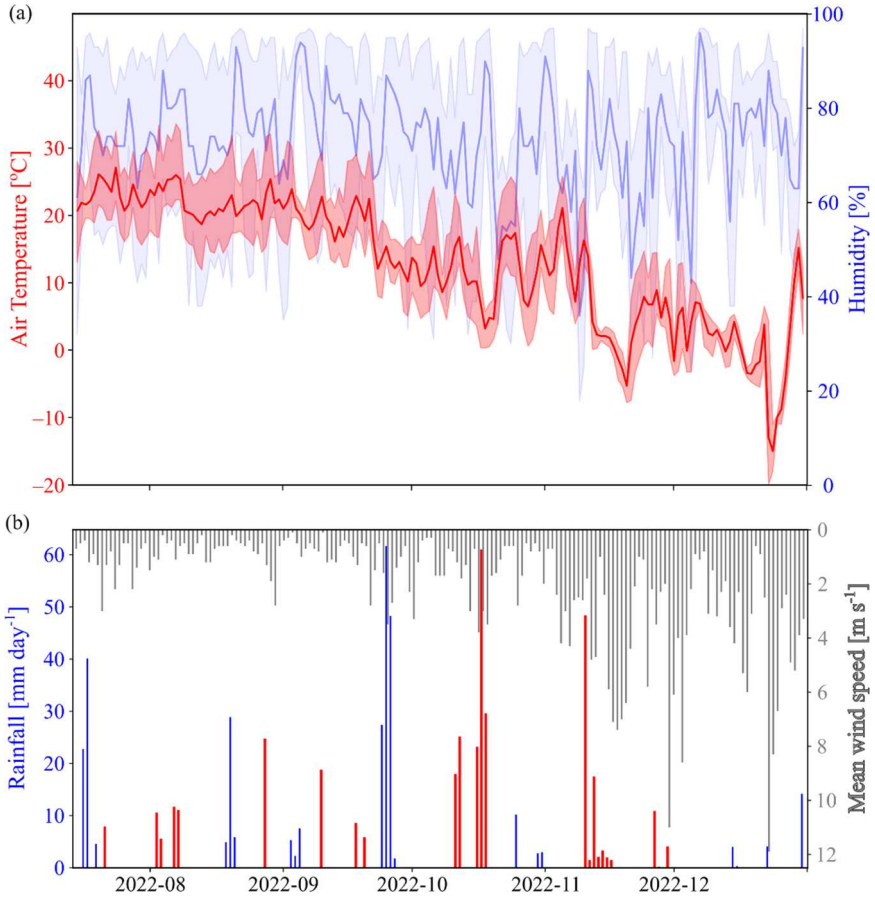


Fig. 2. Seasonal trends in weather variables over the study period. (a) Daily mean air temperature (°C, red line) and relative humidity (% RH, blue line) with shaded regions indicating daily min-max ranges. (b) Daily rainfall (mm day^{-1}, blue bars) and mean wind speed (m s^{-1}, black line, right axis). Red bars in (b) highlight rainfall events.

2.3. Quantification of BLD nematode genes (qPCR)

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 and CTAB (cetyltrimethylammonium bromide) buffer, and then phenol-chloroform was used to purify DNA (see Burke et al., 2020). Extracted DNA from each sample was suspended in 100 µl Tris EDTA buffer and stored at –80 °C in 1.5-mL low retention centrifuge tubes (Fisher Scientific, Pittsburgh PA, USA). This study applied a quantitative PCR (qPCR) method developed by Burke et al. (2023) to DNA extracted from stemflow water samples for the identification and quantification of LCM. This method employed two newly developed ITS primers: 33 F and 234 R, designed to amplify the ITS1 region of the rRNA gene of LCM specifically (Burke et al., 2023). These primers showed high specificity for LCM and no amplification of non-target nematode species (Burke et al., 2023). In serial dilution tests, the assay consistently detected LCM DNA from solutions containing as little as 1.5 nematodes, indicative of robust sensitivity and reproducibility (Burke et al., 2023). Thus, the method can detect very small amounts of LCM DNA, making it well suited for diagnostic or monitoring efforts.

Standard PCR was used for addressing question 1 (presence or absence of LCM in stemflow). Each 25 µl PCR mixture comprised 0.25 units of FastStart Taq DNA polymerase (Roche Diagnostics Corporation, Indianapolis, IN, USA), 10X buffer with 2 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 µg/µl BSA, 0.2 µM of each primer, and 1 µl of the extracted DNA derived from stemflow filtered water samples. The PCR thermocycling protocol included an initial denaturation step of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 sec for DNA denaturation, 56 °C for 45 sec for primer annealing, and 72 °C for 1 min for product extension, and concluded with a final extension phase of 72 °C for 5 min (Burke et al., 2023). The presence of PCR product was visualized using 1 % agarose gels stained with ethidium bromide and were compared to the Invitrogen™ Low DNA Mass ladder (ThermoFisher Scientific, Waltham MA, USA) alongside a positive control for LCM. Samples that contained PCR product of the correct size (approximately 200 base pairs) without non-specific amplification were scored as positive for the presence of LCM.

To test question 2 (relative abundance of gene copies per sample), qPCR was performed on a Bio-Rad CFX Connect Real-Time System (Bio Rad Laboratories, Inc. Hercules, CA) on stemflow samples where LCM was detected with standard PCR. qPCR reactions used iTaq Universal SYBR Green Supermix (Bio-Rad) and the 33 F and 234 R primers as previously described (Burke et al., 2023). Briefly, the thermal cycling conditions for the qPCR were as follows: an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds (denaturation), 59 °C for 60 seconds (annealing), and 72 °C for 60 seconds (extension). Plate reads were conducted after every extension phase and melt curves were generated at the end of the run by increasing the temperature from 65 °C to 95 °C and conducting a plate read at every 0.5 °C interval. The Bio-Rad CFX Maestro Software (version 4.1.2433.1219) was used first to examine melt curves and assure specificity of the qPCR reactions. In addition, this software was used to quantify the starting quantity of ITS1 genes in each stemflow sample. For quantification, the threshold cycle (Cq) of each sample was compared to the Cq values of qPCR reactions that made up a standard curve. The multi-point standard curve was generated using a transformed plasmid containing the ITS1 region of LCM and included 100, 1000, 10,000, 100,000, and 1000,000 copies (Burke et al., 2023). Cq values were determined manually with the CFX Maestro Software (Bio-Rad) to ensure optimized reaction efficiency and the r^2 of the standard curve. The qPCR runs had efficiencies that ranged from 88.8 % to 93.4 % and standard curve r^2 values that ranged from 0.993 to 0.997.

2.4. Data analysis

Data analysis began with the computation of descriptive statistics for each BLD symptom class, accompanied by visualizations (boxplots and scatterplots) to illustrate the distribution of nematode gene concentrations. To evaluate significant differences in LCM gene concentrations among the symptom classes, ANOVAs were employed. Additionally, possible correlations between LCM gene presence/absence or qPCR concentrations with weather variables across the sampled storm events were assessed.

To identify which storm and pre-storm variables best predict the presence or absence of nematodes in stemflow samples, we coded nematode presence (1) or absence (0) and considered ten predictors (see variable columns in Table 2). We first used Recursive Feature Elimination (RFE) with a logistic regression model (*LogisticRegression* and *RFE* functions from *scikit-learn* in Python) to systematically rank predictors, removing the least informative ones and checking for cross-correlation among the top-ranked variables (using *pearsonr* from *scipy.stats*) to avoid multicollinearity. This procedure retained two predictors—rain amount (R) and pre-storm change in relative humidity (ΔRH)—which showed low and insignificant correlation with each other. These predictors were then fit via a ridge (L2-regularized) logistic regression (using *LogisticRegression* with *penalty='l2'* from *scikit-learn*) to mitigate overfitting (Pedregosa et al., 2011), especially given our modest dataset of 12 storms. Finally, we used bootstrapping (*numpy* for resampling, *accuracy_score* from *sklearn.metrics*) to assess the robustness of the model, drawing 1000 random samples (with replacement) from the original dataset. In each iteration, we recorded the model coefficients, accuracy, and their 95 % confidence intervals (using *numpy.percentile*). This approach—a simple RFE, ridge logistic regression, and bootstrapping—allowed us to identify which storm variables most reliably predict nematode presence in stemflow samples for our data.

3. Results and discussion

3.1. Detection of Litylenchus crenatae mccannii (LCM) in stemflow samples

Litylenchus crenatae mccannii (LCM) eDNA was detected in stemflow from across multiple rainfall events (Tables 1–2) and symptom classes (Fig. 3), thereby rejecting the null hypothesis that LCM would not be detectable in stemflow. This suggests that stemflow sampling for eDNA may be useful as a ground-based and inexpensive tool for detecting BLD nematodes in individual trees. Daily weather data of the study period are presented in Fig. 2, where the 12 sampled rainfall events are highlighted in red. Seven of the 12 events resulted in positive detections in stemflow samples from at least one tree (Table 2). The majority of detections occurred from late September to mid-November 2022 (Fig. 4). Storms with positive detections varied widely in magnitude, with rainfall amounts ranging from 4 mm to 114 mm and durations spanning from 1 to 82 h (Table 2). This range encompasses a broad spectrum of typical storm conditions for northeastern Ohio. Rainfall intensity ranged from 0.1 to 4.4 mm h^{-1}, while pre-storm atmospheric conditions included relative humidity (RH) changes from 11 % to 51 %, and pressure changes (ΔP) from 2.5 to 19.3 hPa (Table 2). LCM presence was observed under both moderate and severe storm conditions, indicating that nematode eDNA mobilization via stemflow is feasible across varied meteorological scenarios.

Stemflow samples from storms with rainfall amounts as low as 5.9 mm successfully flushed nematode eDNA from six different beech trees, encompassing all BLD symptom classes (Tables 1–2). This rainfall threshold aligns closely with the minimum rainfall needed to exceed the bark water storage capacity of *Fagus* species, which triggers the onset of stemflow. Bark water storage capacity for *Fagus* species ranges from approximately 1.5 mm to 6 mm of precipitation, depending on tree characteristics and bark roughness (Berger et al., 2009; Dezhban et al., 2020; Staelens et al., 2007; Van Stan et al., 2014). Conversely, stemflow samples from a 4.1 mm storm, which were collected at the minimum sample volume (~100 mL), did not yield detectable levels of LCM eDNA

Table 2
Summary of storm and 24-h pre-storm conditions. Storms in bold are those for which the eDNA from *Litylenchus crenatae mccannii* was detected in stemflow samples collected from *Fagus grandifolia* trees. Storm conditions include rainfall amount (R), duration (D), intensity (I), mean wind speed and max sustained gust (Wavg and Wgst, respectively). Pre-storm conditions include the following values estimated from the 24-h period preceding the first recorded rainfall: mean relative humidity (RH), change from min-to-max RH (ΔRH), change from min-to-max atmospheric pressure (ΔP), and the mean and standard deviation of solar radiation (S_{avg} and S_{std}, respectively).

	Storm conditions					24-h pre-storm conditions				
Date	R [mm]	D [h]	I [mm h^{-1}]	Wavg [m s^{-1}]	Wgst [m s^{-1}]	RH [%]	ΔRH [%]	ΔP [hPa]	Savg [W m^{-2}]	Sstd [W m^{-2}]
07–22	7.9	1.0	7.9	0.4	1.7	69.3	36.0	8.2	254.7	311.8
08–04	**15.9**	**16.0**	**1.0**	**0.1**	**1.4**	**69.8**	**38.0**	**4.9**	**216.5**	**276.2**
08–09	22.8	23.6	1.0	0.3	2.4	80.1	33.0	4.8	207.6	291.9
08–29	**24.8**	**11.5**	**2.2**	**0.4**	**2.8**	**68.1**	**38.0**	**4.1**	**161.8**	**244.6**
09–11	18.8	16.5	1.1	0.0	0.6	69.6	34.0	2.5	144.8	213.6
09–21	**5.9**	**7.1**	**0.8**	**0.2**	**1.2**	**73.5**	**44.0**	**5.8**	**113.3**	**194.6**
10–13	**43.2**	**9.8**	**4.4**	**0.4**	**1.9**	**68.8**	**20.0**	**15.2**	**52.1**	**76.5**
10–19	**113.8**	**50.3**	**2.3**	**1.0**	**3.3**	**73.7**	**43.0**	**7.6**	**72.9**	**125.7**
11–14	**69.5**	**82.0**	**0.8**	**0.9**	**3.9**	**50.6**	**41.0**	**11.2**	**42.6**	**78.5**
11–17	6.8	51.3	0.1	1.4	4.7	66.8	11.0	6.3	20.2	32.0
11–28	**11.1**	**26.9**	**0.4**	**1.2**	**3.6**	**52.2**	**51.0**	**17.8**	**28.4**	**52.9**
12–01	4.1	2.4	1.7	2.9	6.7	74.1	29.0	19.3	33.2	57.5

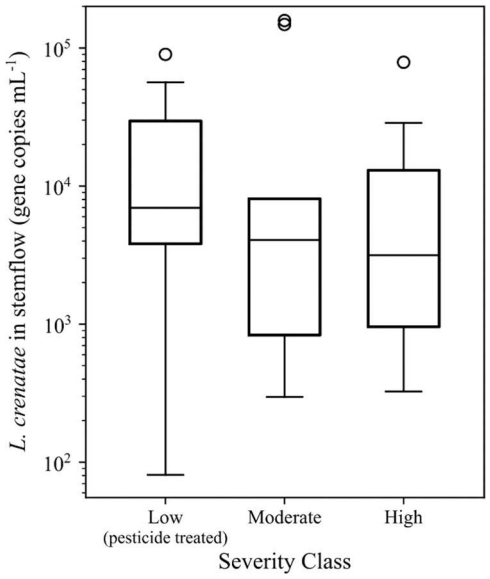


Fig. 3. Boxplots presenting gene copies per mL^{-1} of stemflow for the BLD-associated nematode, *Litylenchus crenatae mccannii*. Samples from *Fagus grandifolia* trees with differing symptom severities ($n = 6$ trees $class^{-1}$). Symbols show median (line), interquartile range (box), non-outlier range (whiskers) and outliers (open dots).

(Tables 1–2). This may be due to insufficient rainfall to generate enough stemflow to mobilize nematode genes from the canopy or bark surfaces, or unfavorable pre-storm or seasonal conditions. These findings suggest that stemflow sampling may detect LCM eDNA in storms that exceed the canopy's minimum water storage capacity (*i.e.*, >6 mm). Under conditions where pathogens are available to be mobilized by rain, stemflow sampling may serve as an effective, ground-based, and cost-efficient method for monitoring canopy-dwelling pathogens. By capturing eDNA during natural precipitation events, this method complements, and in some cases may circumvent, the logistical and financial barriers associated with direct canopy access and traditional remote sensing technologies. However, the variability in detection underscores that further research is needed to clarify the environmental conditions that optimize LCM detection.

Of note, eDNA assays, like the method employed here, amplify target genetic materials as long as it remains sufficiently intact, regardless of whether it originates from live or nonviable individuals (Barnes and Turner, 2016). Consequently, a positive detection confirms the presence of nematode DNA in stemflow but does not necessarily indicate actively infecting, viable nematodes. Such limitations are common to eDNA-based methods, given that DNA can be released from excreted, sloughed, or decomposing tissue, and can persist or be transported in the environment long after the nematode, in this case, dies (Bass et al., 2023; Rees et al., 2014). A potential explanation for elevated gene concentrations in pesticide-treated trees (Fig. 3) could be that the treatments induce nematode lysis, thereby releasing additional DNA. However, our field observations suggest that the infestation itself was not suppressed by pesticide application as symptoms continued to spread, indicating active or persistent infection. This pesticide (Emamectin benzoate) works by disrupting the insects nervous system, and can kill insects in 2–4 days following exposure. Trees were treated in 2018–2019, ~1.5 years prior to stemflow sampling. Although the pesticide could have had effects on tree health, it is unlikely to have resulted in insect death and lysis that could have impacted our eDNA work. While stemflow eDNA detections are encouraging for cost-effective and ground-based disease monitoring, further research is needed to clarify how well eDNA concentrations correlate with active nematode infections and canopy-level disease severity.

3.2. Influence of meteorological conditions on LCM presence in stemflow

Recursive Feature Elimination (RFE) analysis using logistic regression identified the top five predictors for LCM detection as R, pre-storm ΔRH, storm duration, pre-storm mean RH, and mean windspeed. Both R and pre-storm ΔRH were ranked highest (rank = 1) and correlation tests identified them as the most significant and uncorrelated predictors of LCM presence in stemflow. A Ridge Logistic Regression model using R and ΔRH as predictors estimated coefficients of 0.448 and 0.691, respectively, indicating that increases in both storm rain amount and pre-storm humidity changes are associated with a higher likelihood of nematode presence in stemflow samples (Table 3). The model achieved an overall classification accuracy of 91.67 % in predicting nematode presence based on the 12 sampled storms (Table 3); yet, cross-validation revealed large variability in accuracy scores (0.50–1.00), suggesting potential overfitting. Thus, bootstrapping was performed with 1000 iterations, yielding mean coefficients of 0.389 (95 % CI: [0.0011, 0.7169]) for R, and 0.756 (95 % CI: [0.3239, 1.1761]) for ΔRH (Table 3), confirming that these variables may increase the likelihood of nematode presence in stemflow. The mean classification accuracy after bootstrapping was also high, with a mean of 95.39 % and a 95 % confidence interval ranging from 75 % to 100 % (Table 3). These results confirmed robust coefficients and high accuracy, suggesting that larger

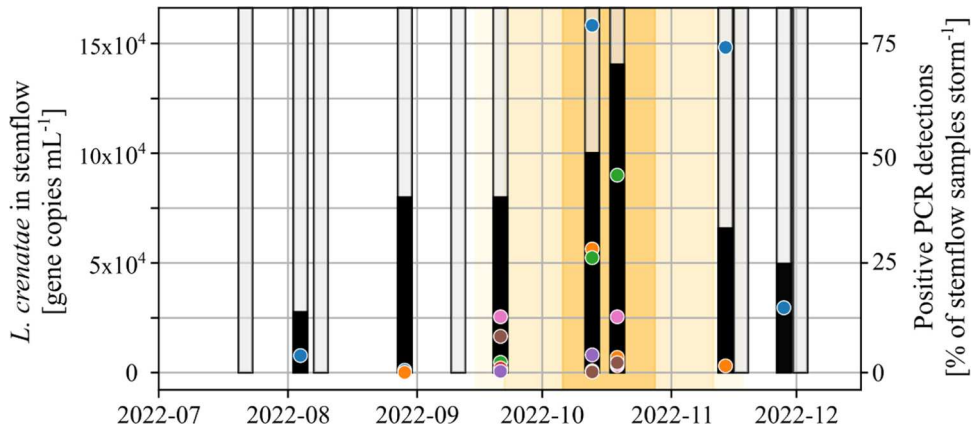


Fig. 4. Presence (per PCR detection) and qPCR concentrations of *Litylenchus crenatae mccannii* gene copies in stemflow from beech trees over the study period (July through December 2022). (Left axis) Each circular point represents nematode gene concentrations (qPCR) from individual storm-tree samples (colored by tree). (Right axis) Each bar represents how many stemflow samples (out of all collected samples from each storm) yielded positive PCR detections. Note that some storms have fewer collected samples (see Table 1). The majority of gene copies and PCR-based detections occurred during or near leaf senescence and bud production, approximately October 6–28, 2022 (dark orange shading). Lighter orange and lightest orange shading represent the two-week, then three-week periods, respectively, before and after that window (based on *Fagus grandifolia* phenological observations reported by the USA National Phenology Network at the nearest site, Talawanda Natural Areas [site #34167] in Ohio).

Table 3
Ridge Logistic Regression and Bootstrapping Results. The coefficients (β) for storm rain amount and pre-storm changes in relative humidity (ΔRH) are presented for the overall (ridge) model and their descriptive statistics are presented for a bootstrapped model performed with 1000 iterations.

	Ridge	Bootstrapped	
	overall	mean	95 % CI
Rain β	0.448	0.389	0.001–0.717
ΔRH β	0.691	0.756	0.324–1.176
Accuracy:	0.917	0.954	0.750–1.000

rainfall and greater pre-storm ΔRH enhance nematode mobilization by stemflow.

Nematodes requiring free water for movement (Yeates, 2010) are likely sensitive to rapid changes in relative humidity, which often signal approaching storms and may increase the opportunity for nematode dispersal through rain-related water films. Similarly, greater rainfall amounts can enhance the availability of free water sources, such as branchflows and stemflow (Van Stan et al., 2021, 2020), facilitating nematode transport across canopy surfaces. This hypothesis is consistent with prior observations that the BLD-associated nematodes likely move via rain films to spread and locate new host buds (Reed et al., 2020; Walker, 2020). Nevertheless, these correlations are based on a dataset limited to one case study. Further research is needed to confirm whether changes in humidity specifically trigger LCM nematode movement and whether rainfall consistently ensures their transport. Despite these limitations, our findings illustrate the potential of stemflow monitoring to serve as a complementary tool in detecting plant disease within canopy environments.

3.3. Gene concentrations of LCM in stemflow

LCM gene copy concentrations in stemflow samples spanned four orders of magnitude (80–158,000 copies mL^{-1}: Fig. 3) but showed no strong link to BLD symptom severity ($F = 1.6$, $p = 0.2$), nor any significant correlation with specific weather variables ($p > 0.1$ for all variables in Table 2). This suggests that while LCM presence in stemflow may be influenced by pre-storm changes in relative humidity and storm size, concentration may not be strongly influenced by meteorological conditions included here. Using USA-NPN phenological data (site #34167), leaf senescence (falling leaves, color change) and bud production by *F. grandifolia* in southwestern Ohio spanned ~6–28 October 2022. At Holden Arboretum, bud formation typically occurs in August, while leaf senescence in 2022 extended from late October to early December (unpublished data). Most LCM gene copies and PCR-positive amplifications in stemflow coincided with these phenophases (Fig. 4). To account for climatic variation, we shifted the USA-NPN window in Fig. 4 by two- and three-week intervals. Regardless of BLD symptom severity, LCM peaked during this period, indicating a strong temporal association with canopy phenology, though further research is needed for statistical confirmation. The peak in LCM gene concentrations in our case study aligns with what little is known about the biological lifecycle of this nematode, which overwinters in buds and detached leaves (Reed et al., 2020). This temporal association indicates that stemflow may capture LCM's migratory behavior as it exits senescing leaves to colonize new buds for overwintering. Although the temporal pattern (Fig. 4) and bootstrapped logistic regression model results (Table 3) support the idea that live nematode emergence might drive the observed eDNA signals in stemflow, these findings are not conclusive. Alternative explanations for these temporal and statistically significant meteorological associations include (a) leaf decomposition releasing dead nematode DNA into stemflow, (b) other organisms consuming nematodes or nematode-carrying leaf materials and excreting their DNA, or (c) possible vectors delivering nematodes to the canopy. Hence, while our data highlight a strong temporal and meteorological association with nematode eDNA, further research is necessary to confirm if it fully represents live nematode activity.

4. Conclusions and future directions: harnessing 'distress signals' in stemflow

In this study eDNA of the nematode associate with beech leaf disease, *Litylenchus crenatae* ssp. *mccannii* (LCM), was detected in stemflow samples. It was more frequently detected in samples from storms exceeding ~6 mm (an amount consistent with the estimated canopy water storage capacity of similarly-sized *Fagus grandifolia* trees); however, this threshold can differ among individual trees due to variations in bark and canopy structure. While canopy water storage thresholds offer a practical guideline for scheduling stemflow sampling, we stress that 6 mm is not a universal cutoff. For those using canopy water storage capacity to guide sampling efforts, local site-specific values should be measured or otherwise assessed. Stemflow monitoring also yielded possible insights into the target nematode's ecology, where a

bootstrapped logistic regression suggested that pre-storm relative humidity changes and higher rainfall amounts correlate with an increased likelihood of LCM presence during key phenological periods (i.e., leaf senescence and bud formation). These factors may largely reflect the mobilization and transport of nematodes within the canopy rather than confirming that LCM is consistently present or active. Further data, including direct viability assays, will be necessary to confirm the true predictive value of these weather variables in estimating actual nematode activity. Future research should focus on refining the conditions under which stemflow carries biological signals. Importantly, the potential to detect other pathogens in stemflow eDNA opens exciting avenues for diagnosing and monitoring canopy diseases. Traditional methods for monitoring canopy pests like the hemlock woolly adelgid (*Adelges tsugae*)—including sticky traps, remote sensing with machine learning, and velcro-covered racquetballs (Fidgen et al., 2021; Kantola et al., 2016)—remain valuable but can be constrained by precision and spatial resolution. Stemflow sampling can complement existing techniques, leveraging natural canopy hydrological processes to provide organism-specific eDNA signals drained directly from individual trees. Additionally, stemflow eDNA can offer broader spatial coverage than some standard eDNA protocols (Natural Resources Canada, 2022), making it a versatile addition to the diagnosing and monitoring toolkit for canopy diseases.

CRediT authorship contribution statement

Bashian-Victoroff Claudia: Writing – review & editing, Visualization, Resources, Data curation. **Carino-Kyker Sarah R.:** Writing – original draft, Visualization, Software, Methodology, Formal analysis. **Burke David J.:** Writing – original draft, Supervision, Resources, Project administration, Formal analysis, Conceptualization. **Gordon D. Alex R.:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Van Stan John:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Mabrouk Adam I.:** Writing – review & editing, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge the support of US NSF DEB-2213623, the staff at Holden Arboretum, and the service of DARG's thesis committee members (Robert Krebs and Kevin E. Mueller at Cleveland State University).

Data availability

Data will be made available on request.

References

Anderson, D.L., Koomjian, W., French, B., Altenhoff, S.R., Luce, J., 2015. Review of rope-based access methods for the forest canopy: safe and unsafe practices in published information sources and a summary of current methods. Methods Ecol. Evol. 6, 865–872.

Barnes, M.A., Turner, C.R., 2016. The ecology of environmental DNA and implications for conservation genetics. Conserv. Genet. https://doi.org/10.1007/s10592-015-0775-4.

Bashian-Victoroff, C., Brown, A., Loyd, A.L., Carrino-Kyker, S.R., Burke, D.J., 2023. Beech leaf disease severity affects ectomycorrhizal colonization and fungal taxa composition. J. Fungi 9. https://doi.org/10.3390/jof9040497.

Bass, D., Christison, K.W., Stentiford, G.D., Cook, L.S.J., Hartikainen, H., 2023. Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. Trends Parasitol. https://doi.org/10.1016/j.pt.2022.12.010.

Berger, T.W., Untersteiner, H., Toplitzer, M., Neubauer, C., 2009. Nutrient fluxes in pure and mixed stands of spruce (Picea abies) and beech (Fagus sylvatica). Plant Soil 322, 317–342. https://doi.org/10.1007/s11104-009-9918-z.

Burke, D., Hoke, A., Reed, S., Martin, D., Kyker, S., Pitts, M., Battinger, S., 2023. Development of primers specific for detection of Litylenchus crenatae, the causal agent of beech leaf disease, in plant tissue. Plant Dis. https://doi.org/10.1094/PDIS-12-22-2911-SR.

Burke, D.J., Hoke, A.J., Koch, J., 2020. The emergence of beech leaf disease in Ohio: probing the plant microbiome in search of the cause. Pathol 50. https://doi.org/10.1111/efp.12579.

Butler, T.J., Likens, G.E., 1995. A direct comparison of throughfall plus stemflow to estimates of dry and total deposition for sulfur and nitrogen. Atmos. Environ. 29, 1253–1265.

Cannon, C.H., Borchetta, C., Anderson, D.L., Arellano, G., Barker, M., Charron, G., LaMontagne, J.M., Richards, J.H., Abercrombie, E., Banin, L.F., Tagle Casapia, X., Chen, X., Degtjarenko, P., Dell, J.E., Durden, D., Guevara Andino, J.E., Hernández-Gutiérrez, R., Hirons, A.D., Kua, C.-S., La Vigne, H., Leponce, M., Lim, J.Y., Lowman, M., Marshall, A.J., Michaletz, S.T., Normark, B.B., Penneys, D.S., Schneider, G.F., Strijk, J.S., Tiamiyu, B.B., Trammell, T.L.E., Vargas-Rodriguez, Y.L., Weintraub-Leff, S.R., Lussier Desbiens, A., Spenko, M., 2021. Extending Our scientific reach in arboreal ecosystems for research and management. Front. For. Glob. Change 4. https://doi.org/10.3389/ffgc.2021.712165.

Carpenter, S.R., 1982. Stemflow chemistry: effects on population dynamics of detritivorous mosquitoes in tree-hole ecosystems. Oecologia 53. https://doi.org/10.1007/BF00377128.

Carta, L.K., Li, S., Mowery, J., 2023. Beech leaf disease (BLD), Litylenchus crenatae and its potential microbial virulence factors. in: Forest Microbiology. Elsevier, pp. 183–192. https://doi.org/10.1016/B978-0-443-18694-3.00018-3.

Dezhban, A., Attarod, P., Zahedi Amiri, G., Pypker, T.G., Nanko, K., 2020. The variability of stemflow generation in a natural beech stand (Fagus orientalis Lipsky) in relation to rainfall and tree traits. Ecohydrology 13. https://doi.org/10.1002/eco.2198.

Duarte, A., Borralho, N., Cabral, P., Caetano, M., 2022. Recent advances in forest insect pests and diseases monitoring using UAV-based data: a systematic review. Forests 13, 911. https://doi.org/10.3390/f13060911.

Ehbrecht, M., Schall, P., Ammer, C., Fischer, M., Seidel, D., 2019. Effects of structural heterogeneity on the diurnal temperature range in temperate forest ecosystems. Ecol. Manag. 432, 860–867. https://doi.org/10.1016/j.foreco.2018.10.008.

Ewing, C.J., Hausman, C.E., Pogacnik, J., Slot, J., Bonello, P., 2019. Beech leaf disease: an emerging forest epidemic. Pathol 49. https://doi.org/10.1111/efp.12488.

Fang, S., Cui, R., Wang, Y., Zhao, Y., Yu, K., Jiang, A., 2023. Application of multiple spectral systems for the tree disease detection: a review. Appl. Spectrosc. Rev. 58, 83–109. https://doi.org/10.1080/05704928.2021.1930552.

Faubert, H., 2023. Beech leaf disease in Rhode Island 2023 update. Kingston, RI (USA).

Fidgen, J.G., MacQuarrie, C.J.K., Turgeon, J.J., 2021. Technical Note 118: Sampling techniques to detect canopy infestations of the hemlock woolly adelgid. Ontario.

Goraya, M., Kantor, C., Vieira, P., Martin, D., Kantor, M., 2024. Deciphering the vectors: unveiling the local dispersal of Litylenchus crenatae ssp. mccannii in the American beech (Fagus grandifolia) forest ecosystem. e0311830 PLoS One 19. https://doi.org/10.1371/journal.pone.0311830.

Honda, E.A., Mendonça, A.H., Durigan, G., 2015. Factors affecting the stemflow of trees in the Brazilian Cerrado. Ecohydrology 8. https://doi.org/10.1002/eco.1587.

Hosen, J.D., Allen, G.H., Amatulli, G., Breitmeyer, S., Cohen, M.J., Crump, B.C., Lu, Y., Payet, J.P., Poulin, B.A., Stubbins, A., Yoon, B., Raymond, P.A., 2021. River network travel time is correlated with dissolved organic matter composition in rivers of the contiguous United States. Hydrol. Process 35. https://doi.org/10.1002/hyp.14124.

Kantola, T., Lyytikäinen-Saarenmaa, P., Coulson, R.N., Holopainen, M., Tchakerian, M. D., Streett, D.A., 2016. Development of monitoring methods for Hemlock Woolly Adelgid induced tree mortality within a Southern Appalachian landscape with inhibited access. IForest 9. https://doi.org/10.3832/ifor1712-008.

Kantor, M., Handoo, Z., Carta, L., Li, S., 2022. First report of beech leaf disease, caused by *Litylenchus crenatae mccannii*, on American Beech (*Fagus grandifolia*) in Virginia. Plant Dis. 106, 1764. https://doi.org/10.1094/PDIS-08-21-1713-PDN.

Koch, J., Allmaras, M., Barnes, S., Berrang, P., Hall, T., Iskra, A., Kochenderfer, J., Macdonald, W., Rogers, S., Rose, J., 2015. Beech seed orchard development: Identification and propagation of beech bark resistant American beech trees. In: Potter, K.M., Conkling, B.L. (Eds.), Forest Health Monitoring: National Status, Trends and Analysis; General Technical Report SRS-209. U.S. Department of Agriculture's Forest Service, Southern Research Station, Asheville, NC, USA, pp. 103–108.

Larrieu, L., Paillet, Y., Winter, S., Bütler, R., Kraus, D., Krumm, F., Lachat, T., Michel, A. K., Regnery, B., Vandekerkhove, K., 2018. Tree related microhabitats in temperate and mediterranean European forests: a hierarchical typology for inventory standardization. Ecol. Indic. 84, 194–207. https://doi.org/10.1016/j.ecolind.2017.08.051.

Leak, W.B., Yamasaki, M., Holleran, R., 2014. Silvicultural guide for northern hardwoods in the northeast. USDA For. Serv. Gen. Tech. Rep. NRS–132.

Levia, D.F., Germer, S., 2015. A review of stemflow generation dynamics and stemflow-environment interactions in forests and shrublands. Rev. Geophys. 53, 673–714.

Likens, G.E., Eaton, J.E., 1970. A polyurethane stemflow collector for trees and shrubs. Ecology 51. https://doi.org/10.2307/1933996.

Mabrouk, A.I., Gordon, D.A., Gotsch, S.G., Van Stan, J.T., 2022. Conceptual analysis: What signals might plant canopies send via stemflow? Front. Water 4. https://doi.org/10.3389/frwa.2022.1075732.

Magyar, D., Van Stan, J.T., Sridhar, K.R., 2021. Hypothesis and theory: fungal spores in stemflow and potential bark sources. Front. For. Glob. Change 4, 19.
Merrick, T., Pau, S., Detto, M., Broadbent, E.N., Bohlman, S.A., Still, C.J., Almeyda Zambrano, A.M., 2021. Unveiling spatial and temporal heterogeneity of a tropical forest canopy using high-resolution NIRv, FCVI, and NIRvrad from UAS observations. Biogeosciences 18, 6077–6091. https://doi.org/10.5194/bg-18-6077-2021.
Natural Resources Canada, 2022. Hemlock Woolly Adelgid Monitoring Network: 2022 sampling protocol and instruction manual. Ontario.
Noren, B.J., Lewis, N.R., Tonello, K.C., Ilek, A., Van Stan, J.T., 2023. Hypothesis and theory: do trees "release the tension" in rainwater? Surface tension reduction in throughfall and stemflow from urban trees. Front. For. Glob. Change 6. https://doi.org/10.3389/ffgc.2023.1315936.
Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., Duchesnay, É ., 2011. Ridge coefficients as a function of the L2 Regularization from "Scikit-learn: Machine learning in Python. J. Mach. Learn. Res. 12.
Reed, S.E., Greifenhagen, S., Yu, Q., Hoke, A., Burke, D.J., Carta, L.K., Handoo, Z.A., Kantor, M.R., Koch, J., 2020. Foliar nematode, *Litylenchus crenatae* ssp. *mccannii*, population dynamics in leaves and buds of beech leaf disease-affected trees in Canada and the US. Pathol 50. https://doi.org/10.1111/efp.12599.
Reed, S.E., Volk, D., Martin, D.K.H., Hausman, C.E., Macy, T., Tomon, T., Cousins, S., 2022. The distribution of beech leaf disease and the causal agents of beech bark disease (Cryptoccocus fagisuga, Neonectria faginata, N. ditissima) in forests surrounding Lake Erie and future implications. Ecol. Manag. 503. https://doi.org/10.1016/j.foreco.2021.119753.
Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M., Gough, K.C., 2014. The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.12306.
Sadeghi, S.M.M., Gordon, A.G., Van Stan, J.T., 2020. A Global Synthesis of Throughfall and Stemflow Hydrometeorology. Precip. Partit. Veg.: A Glob. Synth. https://doi.org/10.1007/978-3-030-29702-2_4.
Schroth, G., Elias, M.E.A., Uguen, K., Seixas, R., Zech, W., 2001. Nutrient fluxes in rainfall, throughfall and stemflow in tree-based land use systems and spontaneous tree vegetation of central Amazonia. Agric. Ecosyst. Environ. 87, 37–49.
Sprenger, M., Stumpp, C., Weiler, M., Aeschbach, W., Allen, S.T., Benettin, P., Dubbert, M., Hartmann, A., Hrachowitz, M., Kirchner, J.W., McDonnell, J.J., Orlowski, N., Penna, D., Pfahl, S., Rinderer, M., Rodriguez, N., Schmidt, M., Werner, C., 2019. The demographics of water: a review of water ages in the critical zone. Rev. Geophys. 57, 800–834. https://doi.org/10.1029/2018RG000633.
Staelens, J., Schrijver, A.De, Verheyen, K., 2007. Seasonal variation in throughfall and stemflow chemistry beneath a European beech (Fagus sylvatica) tree in relation to canopy phenology. Can. J. For. Res. 37, 1359–1372. https://doi.org/10.1139/x07-003.
Stephanson, C.A., Coe, N.R., 2017. Impacts of beech bark disease and climate change on American beech. Forests. https://doi.org/10.3390/f8050155.
Van Stan, J.T., Gordon, D.A., 2018. Mini-review: stemflow as a resource limitation to near-stem soils. Front Plant Sci. 9. https://doi.org/10.3389/fpls.2018.00248.
Van Stan, J.T., Levia, D.F., 2010. Inter-and intraspecific variation of stemflow production from Fagus grandifolia Ehrh.(American beech) and Liriodendron tulipifera L.(yellow poplar) in relation to bark microrelief in the eastern United States. Ecohydrology 3, 11–19.
Van Stan, J.T., Pinos, J., 2024. Three Fundamental Challenges to the Advancement of Stemflow Research and Its Integration into Natural Science. Water (Basel) 16, 117. https://doi.org/10.3390/w16010117.
Van Stan, J.T., Van Stan, J.H., Levia, D.F., 2014. Meteorological influences on stemflow generation across diameter size classes of two morphologically distinct deciduous species. Int J. Biometeorol. 58, 2059–2069. https://doi.org/10.1007/s00484-014-0807-7.
Van Stan, J.T., Morris, C.E., Aung, K., Kuzyakov, Y., Magyar, D., Rebollar, E.A., Remus-emsermann, M.N., Uroz, S., Vandenkoornhuyse, P., 2020. Precipitation Partitioning - Hydrologic Highways between Microbial Communities of the Plant Microbiome? Precip. Partit. Veg.: A Glob. Synth.
Van Stan, J.T., Ponette-González, A.G., Swanson, T., Weathers, K.C., 2021. Throughfall and stemflow are major hydrologic highways for particulate traffic through tree canopies. Front Ecol. Environ.
Vieira, P., Kantor, M.R., Jansen, A., Handoo, Z.A., Eisenback, J.D., 2023a. Cellular insights of beech leaf disease reveal abnormal ectopic cell division of symptomatic interveinal leaf areas. PLoS One 18. https://doi.org/10.1371/journal.pone.0292588.
Vieira, P., Kantor, M.R., Medina-Mora, C.M., Sakalidis, M.L., Handoo, Z.A., 2023b. First report of the beech leaf disease nematode *Litylenchus crenatae mccannii* (Nematoda: Anguinidae) in Michigan. Plant Dis. 107, 2266. https://doi.org/10.1094/PDIS-10-22-2468-PDN.
Walker, J.K.M., 2020. Site condition effects on beech leaf disease symptom severity in southwestern Ontario hardwood forests. Lakehead University, Thunder Bay, Ontario.
Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., Adell, A.D., Mondon, J., Metian, M., Marchant, R.A., Bouzas-Monroy, A., Cuni-Sanchez, A., Coors, A., Carriquiriborde, P., Rojo, M., Gordon, C., Cara, M., Moermond, M., Luarte, T., Petrosyan, V., Perikhanyan, Y., Mahon, C.S., McGurk, C.J., Hofmann, T., Kormoker, T., Iniguez, V., Guzman-Otazo, J., Tavares, J. L., Gildasio De Figueiredo, F., Razzolini, M.T.P., Dougnon, V., Gbaguidi, G., Traoré, O., Blais, J.M., Kimpe, L.E., Wong, M., Wong, D., Ntchantcho, R., Pizarro, J., Ying, G.-G., Chen, C.-E., Páez, M., Martínez-Lara, J., Otamonga, J.-P., Poté, J., Ifo, S. A., Wilson, P., Echeverría-Sáenz, S., Udikovic-Kolic, N., Milakovic, M., Fatta-Kassinos, D., Ioannou-Ttofa, L., Belušová, V., Vymazal, J., Cárdenas-Bustamante, M., Kassa, B.A., Garric, J., Chaumot, A., Gibba, P., Kunchulia, I., Seidensticker, S., Lyberatos, G., Halldórsson, H.P., Melling, M., Shashidhar, T., Lamba, M., Nastiti, A., Supriatin, A., Pourang, N., Abedini, A., Abdullah, O., Gharbia, S.S., Pilla, F., Chefetz, B., Topaz, T., Yao, K.M., Aubakirova, B., Beisenova, R., Olaka, L., Mulu, J. K., Chatanga, P., Ntuli, V., Blama, N.T., Sherif, S., Aris, A.Z., Looi, L.J., Niang, M., Traore, S.T., Oldenkamp, R., Ogunbanwo, O., Ashfaq, M., Iqbal, M., Abdeen, Z., O'Dea, A., Morales-Saldaña, J.M., Custodio, M., de la Cruz, H., Navarrete, I., Carvalho, F., Gogra, A.B., Koroma, B.M., Cerkvenik-Flajs, V., Gombač, M., Thwala, M., Choi, K., Kang, H., Ladu, J.L.C., Rico, A., Amerasinghe, P., Sobek, A., Horlitz, G., Zenker, A.K., King, A.C., Jiang, J.-J., Kariuki, R., Tumbo, M., Tezel, U., Onay, T.T., Lejju, J.B., Vystavna, Y., Vergeles, Y., Heinzen, H., Pérez-Parada, A., Sims, D.B., Figy, M., Good, D., Teta, C., 2022. Pharmaceutical pollution of the world's rivers. Proc. Natl. Acad. Sci. 119. https://doi.org/10.1073/pnas.2113947119.
Yeates, G.W., 2010. Nematodes in Ecological Webs, in: Encyclopedia of Life Sciences. https://doi.org/10.1002/9780470015902.a0021913.
Zhang, J., Huang, Y., Pu, R., Gonzalez-Moreno, P., Yuan, L., Wu, K., Huang, W., 2019. Monitoring plant diseases and pests through remote sensing technology: a review. Comput. Electron Agric. 165, 104943. https://doi.org/10.1016/j.compag.2019.104943.
Zhao, Y., Bonello, P., Liu, D., 2023. Mapping the environmental risk of beech leaf disease in the Northeastern United States. Plant Dis. https://doi.org/10.1094/PDIS-12-22-2908-RE.