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PRIMARY RESEARCH PAPER

Influence of watershed suburbanization on leaf litter decomposition and microbial activity

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Abstract Urbanization can result in multiple stressors for freshwater ecosystems including altered flow regimes, higher sediment loads and increased inorganic nutrient supply. The effects of urbanization on ecosystem processes including organic matter decomposition are poorly understood. Relationships between decomposition and nutrient levels are inconsistent, possibly due to interactions among stimulatory and inhibitory factors. We incubated leaf litter from two tree species (maple and oak) that differ in foliar chemistry in four streams that varied in the extent of suburbanization (human population density, concentrations of nitrate and chloride) and assessed decomposition rates and microbial activity. We found both stream and leaf species effects but the response of maple to the gradient of suburbanization was much stronger than oak. Although suburban streams were associated with reduced interspecific variability in decomposition rates, fungal biomass only responded to differences among leaf species. We observed high rates of mineralization suggesting that decomposition was not nutrient limited. Results show that multiple stressors may act antagonistically to create a muted signal of suburbanization, conditions where the effects of leaf species emerge to drive microbial activity. Understanding how decomposition responds to multiple factors associated with urbanization is essential for predicting how detrital-based food webs will respond to the Anthropocene.

Keywords Urban stream syndrome · Stream ecosystems · Nutrient enrichment · Nitrate

Introduction

Leaf litter provides an essential source of energy and nutrients to stream ecosystems due to low rates of primary production in many headwater and low-order

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streams (Tank et al., 2010). At the annual scale, approximately 50% of the energy input into small streams derives from leaf litter (Fisher & Likens, 1973). Decomposition of leaf litter drives nutrient cycles (Cheever et al., 2012) and is responsible for transferring energy through aquatic trophic levels (Siders et al., 2018). Many factors control rates of litter decomposition and decomposer communities including leaf chemistry (Zhang et al. 2019), and stream-level differences in physical and chemical conditions including sediment structure (Navel et al., 2010), nutrients (Woodward et al., 2012; Ferreira et al., 2015), hydrology (Paul et al., 2006) and temperature (Griffiths & Tiegs, 2016).

The urbanization of watersheds alters many ecosystem-scale processes. Recognized widely as the Urban Stream Syndrome (Paul and Meyer, 2001; Walsh et al., 2005; Booth et al., 2016), ecosystems under anthropogenic forcing often demonstrate degraded physical, chemical, and biological conditions (Chadwick et al., 2006, 2012). Urbanization often leads to increased inorganic nutrients concentrations and changes in nutrient stoichiometry (McDowell et al., 2019), altered hydrology (Paul & Meyer, 2001), variable redox conditions (Blaszczak et al., 2019), reduced habitat complexity (Booth, 1991), reduced leaf litter inputs (Paul & Meyer, 2001), and changes in the composition of the microbial community (Wang et al., 2011). The effects of the urban stream syndrome, however, on ecosystem and biogeochemical processes remain largely unresolved in part due to complex interactions among factors which may stimulate or inhibit rates of transformation. Since many stream ecosystems are fueled by the decomposition of detritus, a deeper understanding of how the urban stream syndrome interacts with the species composition of the riparian zone is needed to develop a robust predictive framework for how freshwater ecosystems will respond to future perturbations and global change.

One common symptom of urban and human-impacted streams is the increased concentration of solutes that can have variable effects on decomposition. For example, elevated concentrations of inorganic nitrogen (N) may alleviate nutrient limitation during leaf litter decomposition as microbial communities can obtain nutrients from the water column (Cheever et al., 2013; Pastor et al., 2014). Extracting nutrients from coarse organic matter can be metabolically expensive for microbes due to the energy requirements of extracellular enzyme production; thus, ecosystems that receive additional nutrient inputs may be associated with accelerated decomposition rates (Gulis & Suberkropp, 2003; Woodward et al., 2012; Ferreira et al., 2015). In contrast, streams and rivers with extreme nutrient pollution (e.g., $> 10^3$ μg DIN l^{-1}; Woodward et al., 2012) often contain concurrent changes in other water quality parameters such as chloride and low levels of dissolved oxygen which inhibit decomposition and microbial activity (Pascoal et al., 2003; Pascoal & Cássio, 2004; Ferreira et al., 2015). Differential responses of leaf litter decomposition to elevated nutrients are often associated with experimental conditions and whether studies are performed in highly polluted streams where multiple factors have changed (i.e., nutrients and additional forms of pollution; Lecerf et al., 2006; Woodward et al., 2012) or highly controlled studies where a single factor (i.e., nutrients) is manipulated (Rosemond et al., 2015). This variation among studies has led to the detection of multiple non-linear response of leaf litter decomposition to elevated nutrient concentrations including parabolic (Friberg et al., 2011; Woodward et al., 2012) and saturation response curves (Kominoski et al., 2015; Jabiol et al., 2019). Whether switches in the response of decomposition are due to increased nutrients alone or antagonistic effects of other factors remains unknown.

The overarching goal of this study was to examine the effects of the urban stream syndrome in a suburbanized watershed on organic matter decomposition and microbial activity. Much of the urban stream syndrome literature has emphasized macroinvertebrates due to their utility as a metric of biotic integrity. We emphasize in this study the microbial community which is relatively understudied yet critical for the maintenance of ecosystem function in both natural and human-impacted systems (Hilderbrand et al., 2020). We use the term suburbanization to describe the low-intensity impact of development that occurs in smaller towns and cities and which interfaces with rural landscapes (Burcher & Benfield, 2006; Airgood-Obrycki & Rieger, 2019). Within the context of this research and study basin, suburbanization is defined as less than 2300 people/km^2 (Airgood-Obrycki & Rieger, 2019), reflected primarily through increased salinization and electrical conductivity (Daley et al., 2009) and nutrient impairment of the receiving estuary, which is in violation of the Clean

Water Act (Burack et al., 2009), in a landscape that remains partially forested. We investigated how the urban stream syndrome interacted with variation in foliar chemistry, a widely recognized control on decomposition rates (Webster & Benfield, 1986; Gessner & Chauvet, 1994; LeRoy et al., 2007), microbial community composition (Wymore et al., 2016) and the fate of leaf-derived elements (Siders et al., 2018). To achieve this goal, we incubated leaf litter of two tree species with different initial foliar chemistries and decomposition rates (Evans-White et al., 2020) in four streams that provided a gradient of anthropogenic influence characterized by variability in human population density, concentrations of nitrate (NO_3^-) and chloride (Cl^-), and specific conductance. These three water quality parameters are frequently associated with watersheds experiencing anthropogenic stress (Paul & Meyer, 2001). We hypothesized that litter decomposition rates, fungal biomass and microbial respiration would significantly decrease in the more impacted streams due to degraded environmental conditions which can counteract the stimulatory effects of high nutrient concentrations. We also hypothesized that fungal biomass would be greatest on the slowly decomposing leaf species due to longer residence time while respiration would be greatest on the faster decomposing leaf species due to the lability of the organic matter (Siders et al., 2018). Lastly, we expected significant interactions between the factors leaf species and stream with the lower initial lignin leaf species showing the greatest response to stream-level variation. Understanding how a key ecosystem process such as litter decomposition responds to anthropogenic perturbations is critical to managing aquatic ecosystems under increased global change.

Methods

Site description

We selected four 1st-order streams as litter incubation sites. Streams were located within the Great Bay watershed of southeastern New Hampshire either within the Lamprey River or Oyster River basins (Fig. 1). We selected four experimental streams that varied in human population density, and solute concentrations, while controlling for stream order and minimizing differences in basin size (Table 1). Regional town size ranges between 4500 and 15,000 (US Census Bureau). College Brook (CB) represents one of the most developed watersheds and flows through the center of the University of New Hampshire's main campus (Durham, New Hampshire, USA) with a small riparian buffer and high electrical conductivity, sediment and NO_3^- concentrations. Wednesday Hill Brook (WHB) is a primarily forested watershed with high NO_3^- levels due to a high density of septic tanks within the basin. Rum Brook (RB) and Perkins Brook (PEB) represent less impacted forested watersheds (Table 1; for detailed watershed description Wymore et al., 2021). Common tree species found across the experimental watersheds include red maple (*Acer rubrum* L.), sugar maple (*Acer saccharum* Marshall), red oak (*Quercus rubra* L.), American beech (*Fagus grandifolia* Ehrh.), eastern white pine (*Pinus strobus* L.), eastern hemlock [*Tsuga canadensis* (L.) Carrière] and white (*Betula papyrifera* Marshall) and black birch (*Betula lenta* L.)

Water chemistry

We collected stream water samples monthly, beginning in October 2006 and continuing through the end of the study in April 2007 ($n = 7$ per stream). All samples were filtered in the field using a 0.7 µm glass fiber filter (Whatman GF/F), stored in 60 ml High-Density Polyethylene sample bottles, transported to the lab on ice and frozen until chemical analysis. Sample filters and collection bottles were rinsed three times with filtered stream water prior to sample collection. We also measured pH, water temperature, dissolved oxygen, and electrical conductivity with a YSI 556 field probe (YSI, Yellow Springs, OH) to characterize physical and chemical conditions during decomposition.

All water chemistry analyses took place in the Water Quality Analysis Laboratory at the University of New Hampshire. Nitrate (as NO_3^-–N), ammonium (as NH_4^+–N), and phosphate (as PO_4^{3-}–P) were analyzed using a SmartChem discrete colorimetric analyzer (Westco Scientific Instruments, Inc., Brookfield, CT, USA) following US EPA methods 353.2 (Cd–Cu reduction), 350.1 (phenate), and 365.2 (ascorbic acid), respectively. Chloride (Cl^-) was analyzed using a Dionex Ion Chromatograph (Dionex Corp., Sunnyvale, CA, USA). Dissolved organic carbon

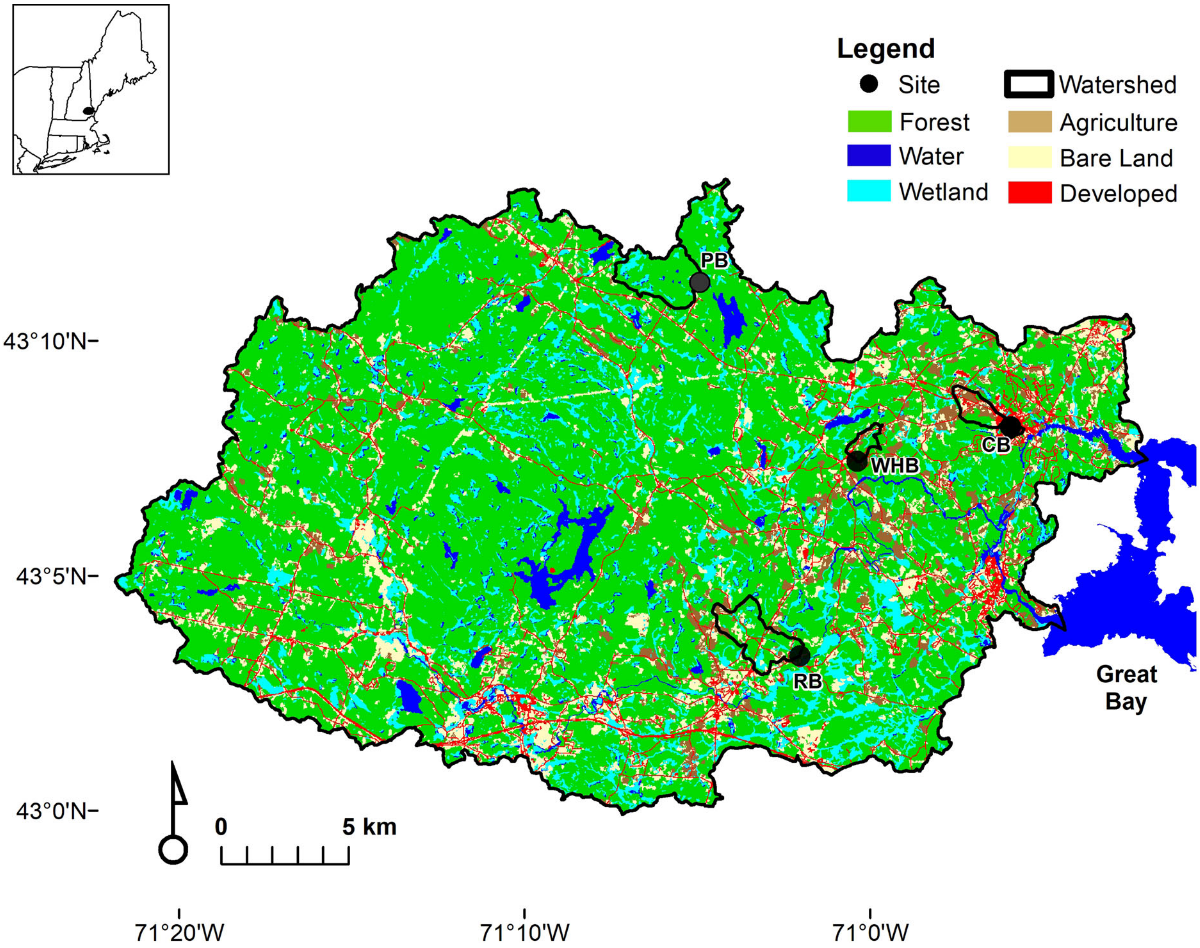

Fig. 1 Map of the study region in southeastern New Hampshire (USA) and the four study watersheds: *PEB* Perkins Brook; *RB* Rum Brook; *WHB*:Wednesday Hill Brook; *CB* College Brook. Streams are ordered by increasing suburbanization

(measured as non-purgeable organic carbon) and total dissolved N (TDN) were measured using a Shimadzu TOC-5000 (Shimadzu Corp, Kyoto, Japan) coupled with Antek 720C Chemiluminescent N detector (Antek Instruments, Inc., Houston, TX, USA) using protocols described in Merriam et al. (1996). Dissolved organic N (DON) concentrations were calculated as the difference between TDN and dissolved inorganic N (DIN) with DIN as the sum of NO_3^- and NH_4^+.

Experimental set up and decomposition

Litter of red maple (*A. rubrum*) and northern red oak (*Q. rubra*) were collected soon after abscission in the first week of October 2006 in College Woods, Durham, NH. These tree species were selected as they represent common riparian species throughout northern New England (Alden et al., 1998) while also contrasting in decomposition rate (Evans-White et al., 2020). Collected litter was brought back to the laboratory, air dried to constant mass and stored until use.

We constructed litter bags (21.6 × 30.5 cm^2) from fine mesh (1 mm) fiberglass screen material to minimize top-down pressure from the macroinvertebrate community and litter mass loss due to physical abrasion. Portions of air-dried litter material (7 g) were pre-weighed into each litter bag and the bags were sewn shut using nylon thread. On 19 October 2006, litter bags were transported to the streams. To minimize breakage due to transport and handling, litter packs were lightly misted with deionized water prior to transport. Bags were randomly tied in groups

Table 1 Watershed characterization and physical and chemical measurements (mean ± 1SE at four streams across a gradient of suburbanization

Characteristics	PEB	RB	WHB	CB
Basin size (km^2)	5.94	4.76	1.47	1.86
Population density (people km^2)	23	51	151	289
Forested %	87.3	67.1	59.2	26.2
Developed %	0.2	6.7	16.9	24.4
Agriculture %	0.0	7.2	9.8	29.8
Wetland %	5.3	3.5	0.0	0.0
Temperature (°C)	5.28^{a} ± 2.45	5.37^{a} ± 2.30	5.66^{a} ± 1.68	5.51^{a} ± 1.94
pH	4.87^{c} ± 0.35	6.55^{b} ± 0.31	7.14^{a} ± 0.17	7.14^{a} ± 0.25
Electrical Conductivity (µS/cm@25 °C)	24.9^{d} ± 1.11	139^{c} ± 8.34	303^{b} ± 17.3	777^{a} ± 42.2
Dissolved Oxygen (mg/l)	12.21^{a} ± 1.23	13.11^{a} ± 1.03	13.20^{a} ± 0.67	14.64^{a} ± 1.10
Cl (mg/l)	3.61^{b} ± 0.32	23.1^{b} ± 1.81	47.0^{b} ± 3.07	159^{a} ± 27.0
TDN (µg/l)	210^{b} ± 20	430^{b} ± 50	1,120^{a} ± 140	1,.170^{a} ± 120
NO_3^-–N (µg/l)	20^{b} ± 10	190^{b} ± 50	940^{a} ± 130	870^{a} ± 100
NH_4^+–N (µg/l)	21.5^{a} ± 6.4	42.5^{a} ± 13.7	51.0^{a} ± 17.1	45.6^{a} ± 13.8
PO_4^{3+}–P (µg/l)	1.85^{a} ± 0.88	4.56^{a} ± 1.51	2.81^{a} ± 0.76	11.7^{a} ± 7.9

Streams are ordered by human population density in the watershed: *PEB* Perkins Brook; *RB* Rum Brook; *WHB* Wednesday Hill Brook; *CB* College Brook. Superscripted letters reflect significant differences among streams as assessed by Analysis of Variance and Tukey's HSD posthoc comparisons

of eight to ten bags to nylon rope attached to either rebar driven into the stream banks or to nearby trees and secured to the stream bottom using nails. A total of 72 bags were installed at each stream for a total of 288 incubation litter bags subdivided into 192 decomposition litter bags [2 species × 4 streams × 3 replicates × 8 harvest dates] and 96 fungal litter bags [2 species × 4 streams × 3 replicates × 4 harvest dates] which we used for assessments of both biomass and activity. Three extra litter bags for each species were returned to the laboratory without stream incubation to correct for handling loss and were used to determine initial ash-free dry mass (AFDM) as described below and initial leaf chemistry. In total 294 litter bags were used.

To measure decomposition rates, we sampled litter bags after 2, 11, 27, 62, 98, 126, 162 and 189 days of in-stream incubation. These harvest dates were selected to capture multiple short- and long-term dynamics including those associated with early-mass loss due to leaching (Webster & Benfield, 1986). On each sampling day, three replicate bags for each species were collected at each stream, rinsed with stream water and transported back to the laboratory in coolers on ice packs. In the laboratory, bags were further rinsed with deionized water to remove attached sediments (e.g., Benfield et al., 2017). The enclosed leaf material was removed, placed into paper bags, dried to constant weight at 65 °C, weighed and ground in a Wiley mill. Sub-samples (~ 2 g) were combusted at 550 °C for 5 h and weighed again to calculate ash free dry mass (AFDM) for each litter bag. Mass remaining was expressed as percentage of initial mass.

We assessed changes in litter carbon (C) and N concentration over time for each species using subsamples from decomposing litter bags. C and N concentrations were determined at the WQAL lab using a CHN analyzer (PerkinElmer, Inc., Waltham, MA, USA) and C and N concentrations were adjusted for pack AFDM. We present leaf N data as the fraction of initial N remaining to assess whether N mineralization or immobilization was the predominant N-cycle process throughout decomposition and to provide insight into the nutrient limitation of decomposition (Cheever et al., 2012). Initial lignin concentration for each species of leaves was measured at the University of Vermont Agricultural Lab following a wet-chemistry method developed by Ankom Technology.

Fungal biomass

We estimated fungal biomass throughout decomposition by measuring ergosterol concentrations (Newell et al., 1988; Sinsabaugh, personal comm.). Three replicate litter bags of each leaf litter type were removed at each stream after 11, 27, 98 and 161 days of incubation and transported to the laboratory on ice packs. Within 48 h of collection, five leaf disks (13 mm diameter) were taken from each of the replicate bags, placed in 5 ml methanol in centrifuge vials and stored at 4 °C until analysis. Leaf disks were refluxed in 5 ml methanol at 65 °C for 2 h with each sample shaken at 30-min intervals. At the end of 2 h, samples were cooled, 1 ml of 4% KOH in ethanol solution was added to each tube and the samples were refluxed for an additional 30 min at 65 °C. Following refluxing, samples were cooled, transferred to 10 ml centrifuge tubes, centrifuged at 5000 rpm for 10 min to remove any solids, and extracts was transferred to clean vials. Water (1 ml) and pentane (2 ml) were added to each vial and vortexed. The pentane fraction, plus 2 successive 1 ml aliquots of pentane were removed, consolidated and evaporated to dryness in a fume-hood overnight. Residues were re-dissolved in 1.5 ml of methanol, sonicated for 5 min and filtered (pore size 0.20 µm, Millipore). Prepared samples were injected into reverse-phase HPLC (Shimadzu), equipped with a C18 column, using methanol as mobile phase (flow rate 1.4 ml min^{-1}), and a UV absorbance detector set at 282 nm. Ergosterol concentration was estimated by comparing absorbance with standard concentrations of ergosterol (elution, 3.6 min). Ergosterol concentrations were converted to fungal biomass by assuming a factor of 5.5 mg ergosterol g^{-1} fungal dry mass (Gessner & Chauvet, 1994) and expressed as mg C per gram AFDM.

Microbial respiration

Respiration of the metabolically active microbial biomass was assessed as CO_2 production per gram leaf AFDM. Within 24 h of collection a litter subsample, equivalent to approximately 1.0 g dry weight, was taken from each of the same litter bag used for fungal biomass analysis, rinsed with de-ionized water to remove sediment and placed into a 500-ml mason jar. Autoclaved water (30 ml) from the stream of origin, cooled at 4 °C, was added to each jar and incubated at 4 °C overnight. The following day, jars were allowed to warm to room temperature and sealed with mason jar caps fitted with septa and incubated at 25 °C for approximately 2.5 h. CO_2 production was measured using Li-COR infrared gas analyzer (IRGA) and presented as µg C/g leaf AFDM.

Statistical analysis

We tested for differences in physical and chemical properties among streams using a 1-way ANOVA on background temperature, pH, dissolved oxygen, and electrical conductivity, and concentrations of TDN, NO_3^-, NH_4^+, PO_4^{3+}, and Cl^- followed by Tukey's HSD. Post-hoc results were confirmed with Bonferroni correction (which produced identical results). We estimated decomposition rate (k) assuming an exponential decay by linear regression of ln transformed fraction of AFDM remaining against time (Benfield, 1996). Due to the spatial and temporal non-independence of the data, we used a 2-way Analysis of Variance (ANOVA: stream [$n = 4$], leaf species [$n = 2$]) to test for difference in response variables. The advantages of ANOVA in this application are two-fold. First, it allows us to test for differences among streams while not assigning any causality (spatial non-independence). Second, and to remove the effect of repeated measures (temporal non-independence), we calculated new response variables that quantify the magnitude of change from the first harvest (day 11) to the last harvest (day 161) rather than analyze changes in the measured parameters through time. Thus, to examine the effects of watershed suburbanization on decomposition and microbial community activity we use the decomposition coefficient (k; $n = 24$), the fraction of initial foliar N remaining after 161 days ($n = 24$), the change in fungal biomass C as the difference between days 161 and 11 ($n = 21$) and peak fungal biomass ($n = 23$), the change in litter C:N ratios between days 161 and 11 ($n = 24$), and the change in microbial respiration between days 161 and 11 ($n = 24$) and peak microbial respiration ($n = 24$). When appropriate, we followed these ANOVAs with Tukey's HSD post hoc comparisons. (See Table S1 for full suite of ANOVA results). Microbial respiration data were ln transformed prior to statistical analyses. All analyses were performed in R (version 4.0.1) using the base functions with plots produced in ggplot.

Results

Water chemistry

The four streams provided a gradient in suburbanization evidenced by significant differences in electrical conductivity ($P < 0.001$), Cl^- ($P < 0.001$), and inorganic N, primarily in the form of NO_3^- ($P < 0.001$) (Table 1). These differences are likely the result of an approximately 12-fold difference in population density and variation in land use and land cover among streams (Fig. 1, Table 1). Mean electrical conductivity values ranged over an order of magnitude from 24.9 to 777 (µS cm^{-1}) at PEB and CB, respectively. Concentrations of Cl^- ranged over three orders of magnitude with CB recording the highest values at 159 mg l^{-1}. Mean NO_3^- levels among the four streams ranged from 0.02 to 0.94 mg NO_3–N l^{-1} with concentrations 4.5 to 47 × greater in CB and WHB than RB and PEB. NH_4^+ ranged from 21.5–51.2 µg NH_4–N l^{-1} with no significant differences among streams ($P = 0.44$). Phosphate concentrations were relatively low across streams and ranged from < 5.0 to 11.7 µg PO_4-P l^{-1} and did not significantly differ among streams ($P = 0.27$). Molar DIN: PO_4-P ratios varied from 58 at PEB to 1408 at WHB, with variation in the ratio driven largely by changes in DIN. However, variation among sites was not significantly different ($P = 0.11$). pH was significantly different among streams ($P < 0.001$) Stream water temperature (mean = 5.5 °C) and dissolved oxygen (mean = 13.29 mg l^{-1}) did not vary ($P = 0.99$ and $P = 0.44$, respectively) by streams.

Initial leaf litter chemistry

The two litter species varied in their initial chemistry expressed as % AFDM (Table 2). Oak had the highest concentration of lignin. The two litter species were similar in carbon concentration ($P = 0.09$). Nitrogen concentration varied between species with maple having the highest concentrations ($P < 0.01$). Variation in N concentration led to a nearly threefold and significant difference in initial litter C:N ratios between species ($P < 0.01$).

Table 2 Initial lignin, carbon (C), and nitrogen (N) concentrations and carbon to nitrogen (C: N) ratios in leaf litter from two tree species

	Maple	Oak
Lignin	12.9	20.3
C (% AFDM)	0.53 ± 0.001^a	0.52 ± 0.002^a
N (% AFDM)	0.010 ± 0.001^a	0.012 ± 0.001^b
C: N (Molar)	64.0 ± 4.1^a	50.5 ± 5.1^b

Data are means ± 1SE. Superscript letters represent significant differences between leaf species. Replicate data is not available for lignin

Litter decomposition rates

The percent AFDM remaining at the end of the study varied among species across the four streams (Maple: 8.5–48.9%, Oak: 33.6–64.5%; Fig. S1) We detected both significant effects of species ($P < 0.0001$; Fig. 2A) and stream ($P < 0.0001$) on decomposition rates. We also detected a significant species × stream interaction ($P < 0.01$). This interaction term was driven by maple litter which was the only species that had significantly different decomposition rates across the four streams (Table S1). Specifically, maple had a unimodal distribution with respect to decomposition rates increasing between PEB and RB and the lowest mean decomposition rates found in the two impacted streams WHB and CB. We also found that the magnitude of interspecific variation in decomposition rates decreased in the two urban streams with rates for both leaf species converging (Fig. 2A) with decomposition ratios (k_{maple}:k_{oak}) lowest in CB (PB: 1.88; RB: 2.40; WHB: 2.23; CB: 1.41).

Changes in leaf litter chemistry

The fraction of initial N remaining did not vary by species ($P = 0.15$) but did significantly vary by stream ($P = 0.003$) (Fig. 2B). We detected a significant interaction term ($P = 0.04$). Leaves decomposing in CB had a significantly higher proportion of initial N remaining relative to the two less impacted streams (PEB and RB) with patterns driven primarily by changes in maple across the suburbanization gradient.

In contrast to changes in foliar N, we detected significant changes in C:N ratios only between species (Fig. 3; $P < 0.0001$). No significant stream level ($P = 0.10$) or interaction terms were detected

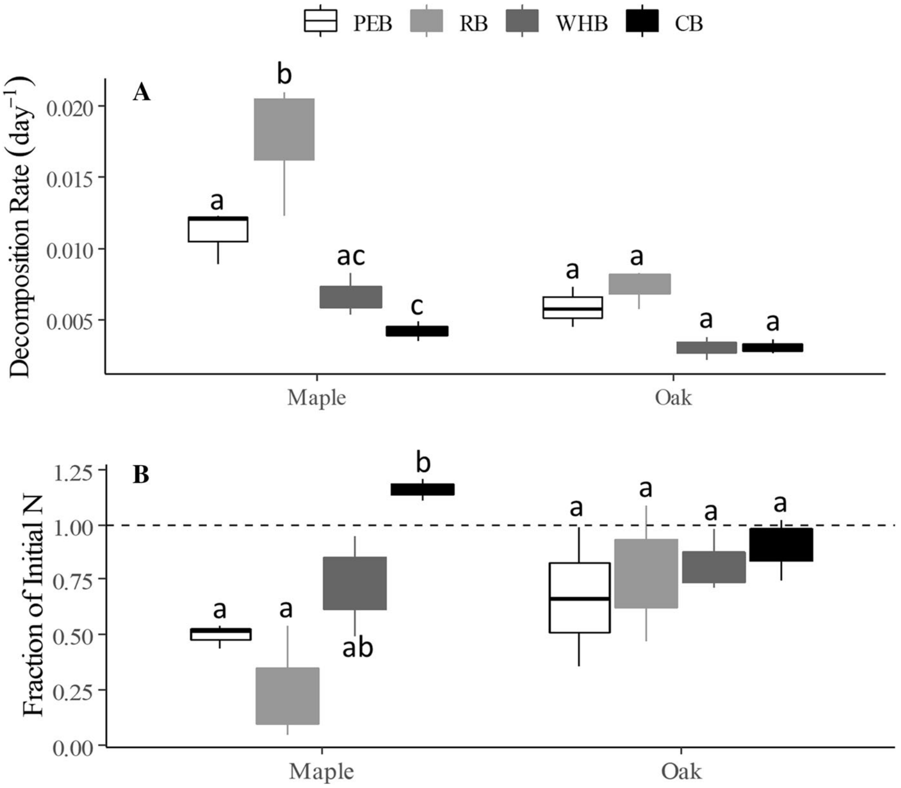

Fig. 2 **A** Decomposition rate of maple and oak litter incubated in four streams across a gradient of suburbanization. **B** Fraction of initial nitrogen mass remaining for maple and oak litter incubated in four streams across a gradient of suburbanization. Streams are ordered by human population density: *PEB* Perkins Brook; *RB* Rum Brook; *WHB* Wednesday Hill Brook; *CB* College Brook. Boxes represent the 25th, 50th, and 75th quantile, and lines represent 1.5 times the interquartile range. Lowercase letters represent significant differences for each leaf species among streams

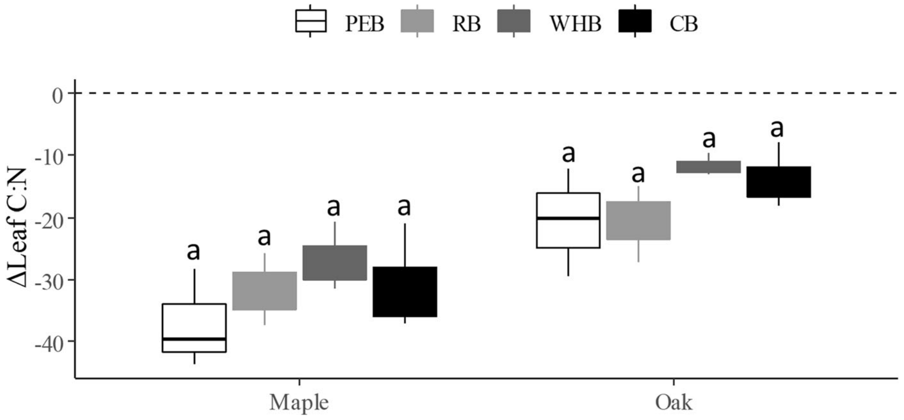

Fig. 3 Change in C:N ratios for maple and oak litter incubated in four streams across a gradient of suburbanization. Streams are ordered by human population density: *PEB* Perkins Brook; *RB* Rum Brook; *WHB* Wednesday Hill Brook; *CB* College Brook. Boxes represent the 25th, 50th, and 75th quantile, and lines represent 1.5 times the interquartile range. Lowercase letters represent significant differences for each leaf species among streams

(P = 0.86). Throughout decomposition C:N ratios generally declined for both maple and oak. Across steams, the ordering of the magnitude of change in foliar C:N ratios was consistent, with maple litter experiencing greater change in C:N ratios compared to oak. On average, maple litter decreased by 65% to a mean C:N ratio of 23.4 while oak decreased by 50% to a mean C:N ratio of 25.7.

Fungal biomass

We assessed differences in fungal biomass, expressed as fungal C, using two metrics: change in fungal biomass (day 161–day 11) and maximum biomass. We found strong species effects on the change in fungal biomass (Fig. 4A; $P < 0.01$). And while oak litter was consistently associated with the largest change in fungal biomass, there was no significant stream effect on fungal biomass (P = 0.40). No effect of leaf species (P = 0.09) or stream (P = 0.91) was detected for maximum biomass (Fig. 4B). Maximum biomass for oak and maple litter was typically found after 98 days of decomposition.

Microbial respiration

We found significant species effects ($P < 0.001$) and stream-level effects (P = 0.03) on microbial respiration assessed as the change in respiration between days 11 and 161 (Fig. 5A). No interactive effects were detected. Stream level significance was driven by differences between the two impacted streams, WHB and CB (P = 0.023). Peak respiration (Fig. 5B) for both species was primarily detected at day 11. Maximum microbial respiration was generally highest on low lignin and high C:N maple leaf litter (Fig. 5b). Microbial respiration correlated negatively with fungal biomass for both maple (Fig. S2; r^2 = 0.12, P = 0.01) and oak litter (Fig. S2; r^2 = 0.30, $P < 0.001$).

Discussion

Suburbanization, decomposition, and species effects

Evidence from this study indicates that leaf species respond uniquely to the suburbanization of watersheds. While stream effects were common, they were heavily influenced by maple. The non-linear (unimodal) response of maple decomposition rates may reflect the stimulation of decomposition between PEB and RB where nutrients are elevated. Differences in decomposition rates between PEB and RB may also be associated with variation in pH. Acidification can inhibit decomposition (Ferreira & Guérold, 2017) and differences between PEB and RB may reflect the interaction between nutrient concentrations and variability in acidification. The inhibition of decomposition occurring in WHB and CB is likely due to concomitant changes in other stream chemistry metrics associated with increased suburbanization. This unimodal distribution, and variation among leaf species, has been previously observed (Pereira et al., 2016) reiterating the importance of the leaf species x stream interactions. The observed unimodal distribution of decomposition rates, which is most pronounced in maple litter, could be the result of two different mechanisms. The first possibility is other factors associated with the urban stream syndrome acted antagonistically against the effects of increased nutrient concentrations. Although the moderately elevated levels of NO_3^- may have acted to accelerate decomposition, higher concentrations of Cl^-, sodium and sedimentation (see additional discussion below) may have resulted in a diminution of decomposition rates at the more impacted streams (Júnior et al., 2020). We note that within the Lamprey River basin that salinization and specific conductance have increased over recent decades (Daley et al. 2009). Second, phosphate limitation may have constrained the stimulatory effects of elevated NO_3^- concentrations in the impacted streams reflected by their high DIN:P ratios. We recognize that these proposed mechanisms are speculative, nevertheless they offer explanations that account for the distribution of decomposition rates across our suburbanized watersheds.

We also observed a decrease in variability in rates of decomposition rates between the two leaf species in both suburbanized streams. This is particularly evident in CB, which drains a large university campus, where decomposition rates of maple and oak converge evidenced by CB's lowest decomposition ratios (k_{maple}:k_{oak}). This pattern is primarily driven by changes in decomposition rates for maple leaf litter which showed strong interactions with the factor stream. Although suspended sediment concentrations

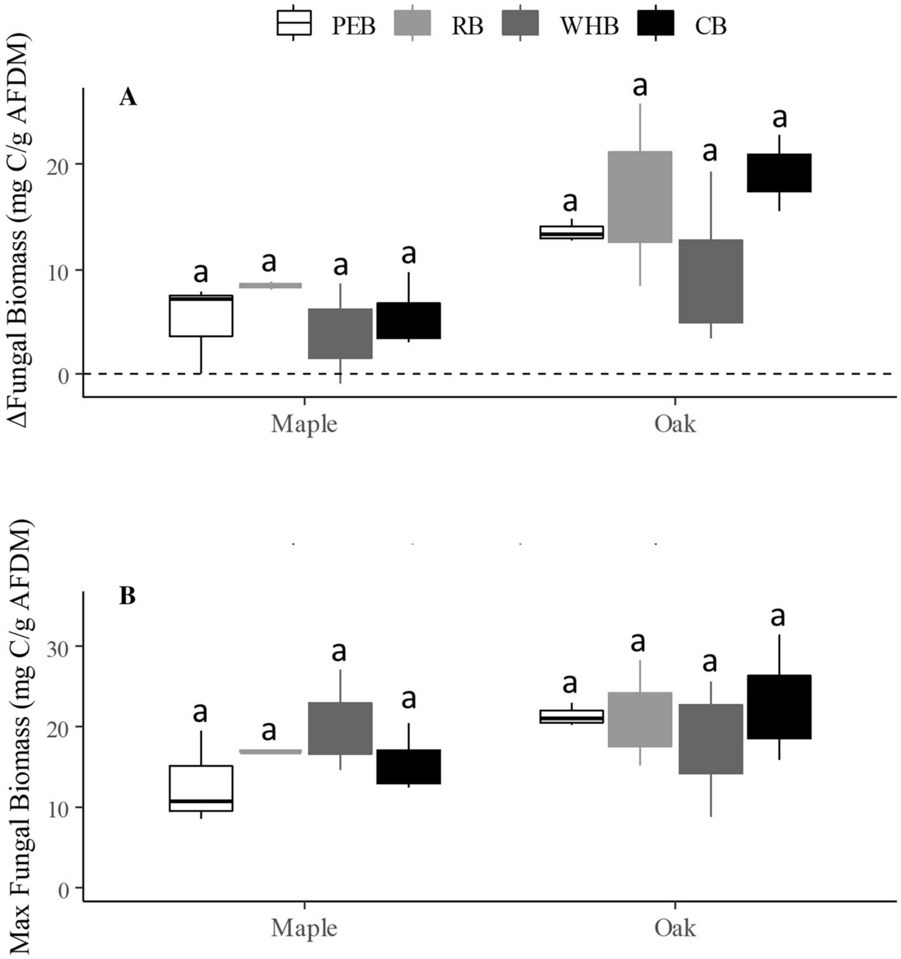

Fig. 4 **A** Change in fungal biomass for maple and oak litter incubated in four streams across a gradient of suburbanization. **B** Maximum fungal biomass. Values represent harvest day when peak fungal biomass was measured. Streams are ordered by human population density: *PEB* Perkins Brook; *RB* Rum Brook; *WHB* Wednesday Hill Brook; *CB* College Brook. Boxes represent the 25th, 50th, and 75th quantile, and lines represent 1.5 times the interquartile range. Lowercase letters represent significant differences for each leaf species among streams

were not quantified in this study, visual examination at the two impacted streams (WHB and CB) revealed that litter packs were either partially or entirely covered with fine sediments (Bourakovsky, personal observation). This observation points to an increased particle load in these two streams, which have the highest percentage of developed land. For example, due the surrounding development, CB has reduced riparian buffer and forest cover with some reaches subterrain. Leaf packs with high sediment loads likely have reduced surface area for microbial colonization and experience anoxic conditions, both of which retard rates of litter decomposition (Herbst, 1980; Sponseller & Benfield, 2001). The effects of sedimentation should be more pronounced later in decomposition as demonstrated in CB, where the rate of mass loss across the two species plateaus after approximately 100 days (Supplemental Fig. 1).

Many studies have detected significant nutrient effects on decomposition rates (Meyer and Johnson, 1983; Cheever et al., 2013; Ferreira et al., 2015; Rosemond et al., 2015) Significant nutrient effects often result from manipulative studies (i.e., nutrient amendment; Ferreira et al., 2015; Manning et al., 2016) where only nutrient levels (N or P) are altered. Often these studies occur in relatively undisturbed watersheds (e.g., Gulis & Suberkropp, 2003; Rosemond et al., 2015). The present study attempted to

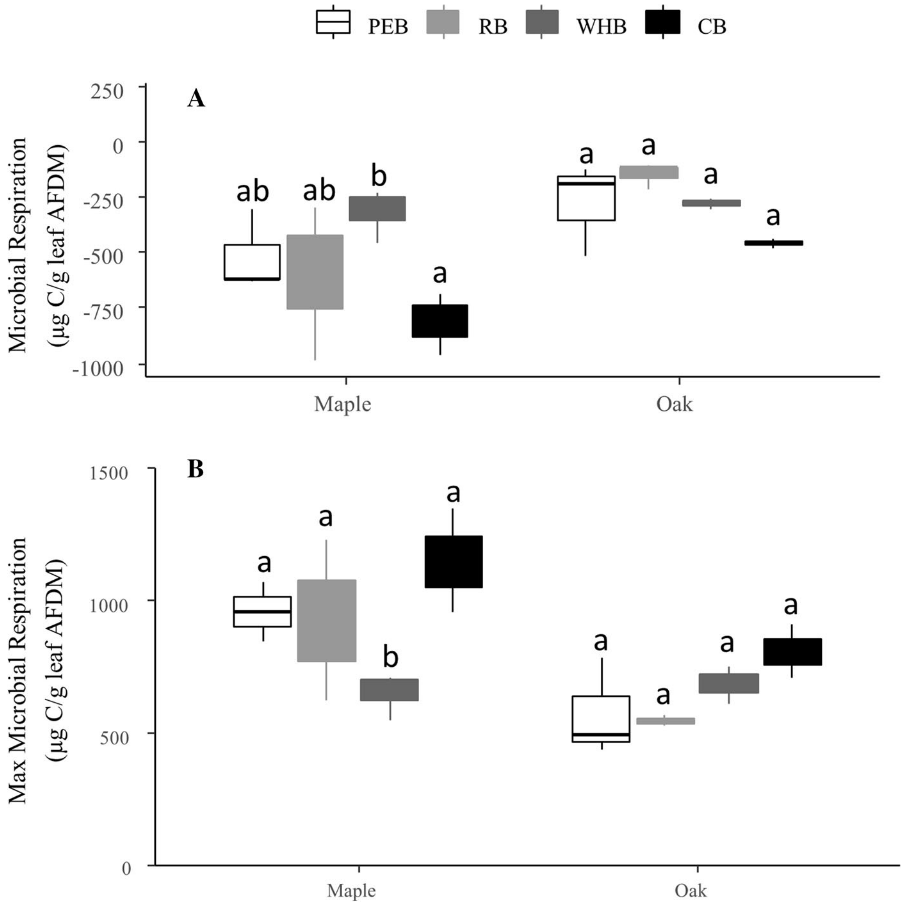

Fig. 5 **A** Change in microbial respiration for maple and oak litter incubated in four streams across a gradient of suburbanization. **B** Maximum microbial respiration. Values represent harvest day when peak fungal biomass was measured. Streams are ordered by human population density: *PEB* Perkins Brook; *RB* Rum Brook; *WHB* Wednesday Hill Brook; *CB* College Brook. Boxes represent the 25th, 50th, and 75th quantile, and lines represent 1.5 times the interquartile range. Lowercase letters represent significant differences for each leaf species among streams

account for the effect of the urban stream syndrome in a multivariate background where factors may positively or negatively affect organic matter decomposition rates and microbial activity. It is likely that interactions among variables influenced our results and help to explain why we observed unimodal distributions in these suburbanized watersheds similar to patterns previously observed in other nutrient impacted systems (Woodward et al., 2012; Pereira et al., 2016). We also note that this study's experimental design did not account for the effect of macroinvertebrates, which play a central role in the leaf litter decomposition processes (Wallace et al., 1997). Macroinvertebrate communities are known to respond to both differences in litter traits (Compson et al., 2013) and degraded environmental conditions (Walters et al., 2009; Martins et al. 2015), including elevated nutrient concentrations (Greig et al., 2012). And while some leaf packs from RB were associated with macroinvertebrates (primarily shredders of order Plecoptera) that were attempting to feed from outside of the packs, decomposition rates were not consistently higher across leaf species in RB suggesting the macroinvertebrate contribution to decomposition in this study was minimal. As a result, this study accounts for the response and contribution of the microbial community in the absence of strong top-down pressure. An experimental design that accounts for the influence of the macroinvertebrate community would help to develop a more complete picture of how detrital-based food webs and the entire decomposer community responds to suburbanization.

The role of leaf chemistry

Although the urban stream syndrome homogenized variability in decomposition rates, we still found strong effects of leaf litter species across many of our response variables consistent with expectations regarding the effect of foliar chemistry. As expected, leaf decomposition rates were lower for the species with higher lignin concentration (Melillo et al., 1982; Gessner & Chauvet, 1994; LeRoy et al., 2007; Ramos et al., 2021). This pattern held in all four streams. We also detected a relationship with foliar C:N ratios, where higher C:N maple litter decomposed faster than oak litter. The relationship between decomposition rates and C:N ratios, which was observed across streams, is inconsistent with other studies that show negative (Cornwell et al., 2008; Hladyz et al., 2009) or no relationships (LeRoy et al., 2007; Wymore et al., 2013). These differences are unlikely due to interspecific variability in foliar phosphorus content as oak and maple have similar initial phosphorus concentration (Scott et al., 2013; Evans-White et al., 2020). Coarse measurements of foliar chemistry may not adequately describe how microbes respond to the complexity and bioavailability of the organic compounds found in leaf litter, missing important details such as how N is bound within the leaf tissue. Patterns reported here may reflect how variability in lignin interacts with variability in dissolved nutrient availability to determine decomposition rates (Jabiol et al., 2019) and why species level differences were heavily influenced by maple which has high C:N ratios and low lignin.

Decomposition and N mineralization

Although previous studies observe both uptake of N from the water column and mineralization of leaf litter N (Webster et al., 2009; Cheever et al., 2012; Pastor et al., 2014), evidence suggest that leaf packs generally supplied enough nutrients to the decomposer community from both species and that leaf litter decomposition was not nutrient limited across these study watersheds. In the majority of leaf packs mineralization appears to be the dominant process. The one exception to this was maple litter in the highly suburbanized CB. The effects of leaf pack N mineralization on water column solute levels and other biogeochemical cycles remains an open question (Webster et al., 2009; Duan et al., 2014). For example, is this newly mineralized N (in the form of ammonium) rapidly nitrified into nitrate, or is it rapidly taken up by heterotrophs due to the availability of dissolved organic carbon from leaf litter leaching (e.g., Strauss and Lamberti 2000)? Alternatively, is the potential nitrification of this mineralized N associated with the production of nitrous oxide? Throughout the study basin, peaks of ammonium and nitrous oxide occur during the late fall and early winter months (Herreid et al., 2020) potentially linking leaf litter decomposition, nitrification, and greenhouse gas production.

Microbial dynamics

Differences in fungal biomass and microbial respiration were also consistently associated with species rather than stream, with no clear pattern across the suburban gradient. And while species effects were consistent across measurements of fungal and microbial activity, interspecific differences in litter chemistry were not consistently associated with observed variation. For example, lignin concentrations are approximately 50% higher in oak compared to maple, yet some streams (e.g., suburbanized WHB) had very similar values of fungal biomass and rates of microbial respiration between the two species. And while aquatic fungal communities appear responsive to differences in leaf species (e.g., Gessner & Chauvet, 1994; Wymore et al., 2013), the decoupling of fungal biomass and leaf litter chemistry has been previously observed (LeRoy et al., 2007) potentially the result of variation in leaf area (Bärlocher & Schweizer, 1983), leaf roughness (Kearns & Bärlocher, 2008) and cuticle toughness (Canhoto & Graça, 1999). It is possible that variation in the responses of fungal biomass and microbial respiration to leaf chemistry may be driven by the contribution of the bacterial community that is included in the respiratory metrics and which can respond uniquely to litter chemistry compared to fungi (Wymore et al., 2013). Although we did not measure the proportion of leaf C entering respiratory pathways versus biomass pathways, these results are consistent with recent work demonstrating that more of the C contained in fast decomposition and low lignin leaf species enters the microbial loop and respiratory pathways (Meyer, 1994) rather than food-web based pathways (Siders et al., 2018). Paradigms regarding litter quality have often purported that decomposition rate correlates positively with “litter quality” (Melillo et al., 1982; Gessner & Chauvet, 1994; Hobbie, 2000). Recent studies, however, demonstrate that slowly decomposing leaf species provide relatively more C and nutrients to aquatic food webs (Marks, 2019).

Conclusions

Our findings suggest that understanding and predicting the effects of the urban stream syndrome (Walsh et al., 2005; Booth et al., 2016) requires appreciating the interactive effects of multiple stressors and their interaction with other drivers of ecosystem and microbial activity. Foliar chemistry is recognized as a first order control on decomposition rates (Gessner & Chauvet et al., 1994; Zhang et al., 2019). We show that moderate urban effects may have greater influence on fast decomposing species such as maple and that interspecific differences in a major ecosystem-scale process can become homogenized. The homogenization and convergence of decomposition rates to those of slow decomposition species such as oak may have meaningful effects for energy exchanges and food web dynamics. The diminution of decomposition rates of fast decomposing leaf species such as maple, for example, may shunt more C and N into the macroscopic food web (Siders et al., 2018; Marks, 2019). However, those organisms which are adapted to take advantage of fast decomposing leaf species may be disproportionately affected by these changes (Compson et al., 2018). Although these study watersheds have population densities on the lower end of the definition of “outer suburbs” (e.g., < 2300 people/km^2; Airgood-Obrycki & Rieger, 2019), we are still able to detect some initial signals of altered ecosystem processes. Because we found consistently strong species effects on many metrics of decomposition and microbial growth and respiration, more attention should be devoted to the complex interactions among the multiple stressors due to urbanization and other drivers of organic matter decomposition including variation among leaf litter traits.

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Author contributions AB and WHM designed the project, AB performed the field work and analyzed samples, ASW, AB, HF, and JCM analyzed the data, ASW wrote the first draft of the manuscript, all authors contributed to revising the manuscript.

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Data availability Data is available upon reasonable request to the corresponding author.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interests to declare.

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