Chronic Nitrogen Deposition Induces Phosphorus Limitation of Aquatic, But Not Terrestrial, Decomposition

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ABSTRACT

Elevated deposition of atmospheric nitrogen (N) has shifted nutrient availability in terrestrial and aquatic habitats of ecosystems, but rarely are ecosystem processes in those components examined simultaneously. We used a multi-decadal, whole, paired watershed experiment to determine how chronic N enrichment with $(\text{NH}_4)_2\text{SO}_4$ alters litter decomposition in terrestrial and stream systems. We also used short-term phosphorus (P) enrichment experiments within both watersheds to determine whether chronic N enrichment enhances P limitation of decomposition. Leaves from N-treated and reference watersheds were used in a reciprocal design to parse effects of altered nutrient availability in leaves and in the environment. We found divergent responses of terrestrial and stream decomposition to altered nutrient regimes. Chronic experimental N enrichment increased N and P concentrations in post-abscission leaves which decayed faster than leaves from the reference watershed in the terrestrial environment. Experimental N enrichment also did not induce P limitation of

terrestrial decomposition. In contrast, litter decomposition rate in the two streams was not enhanced by elevated N in stream water or by altered leaf chemistry. Instead, chronic experimental N enrichment shifted decomposition in streams from co-limitation to strong P limitation. Microbial respiration and extracellular enzyme production responded to altered nutrient availability in a manner consistent with resource allocation models. Divergent responses of terrestrial and aquatic decomposition to elevated N deposition likely arise from differences in water bioavailability. Our work highlights the value of simultaneously considering ecosystem processes in terrestrial and aquatic systems to understand the consequences of integrated landscape processes operating on large spatial scales.

Key words: nitrogen; phosphorus; deposition; decomposition; litter; leaf; stream; terrestrial; limitation; Bear Brook Watershed Maine.

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HIGHLIGHTS

- Elevated chronic atmospheric nitrogen deposition shifts the stoichiometric relationship between nitrogen and phosphorus in whole

watersheds.

- Elevated atmospheric nitrogen deposition shifts decomposition from nitrogen to phosphorus limitation in streams, but not terrestrial systems.
- Phosphorus enrichment in addition to nitrogen deposition accelerated decomposition, enhanced microbial respiration and altered the stoichiometry of enzyme activity in streams.
- Water availability likely modulates responses by influencing microbial stress and nutrient availability.

INTRODUCTION

Availability of nitrogen (N) and phosphorus (P) can strongly limit ecosystem processes in terrestrial and aquatic systems. In temperate regions, terrestrial systems have typically been observed to be N limited (Vitousek and Howarth 1991), whereas aquatic systems have been considered to be P limited. Growing recognition of stoichiometric linkages among multiple nutrients and broader analyses of nutrient limitation have eroded these paradigms. For example, meta-analyses have shown N, P and co-limitation can be similarly prevalent in terrestrial and aquatic systems (Elser and others 2007; Vadeboncoeur 2010). These historical paradigms have led to focus on different limiting nutrients in terrestrial and aquatic systems, which are rarely considered simultaneously even though they are intimately connected, and similar processes occur within them. Explicitly considering terrestrial and aquatic habitats of ecosystems together is important, particularly in regard to landscape-scale phenomena where connections between terrestrial and aquatic systems are important (Grimm and others 2003).

The doubling of bioavailable N on the planet has altered many processes across terrestrial and aquatic systems and spurred considerable interest in the role of nutrient limitation in ecosystem processes (Vitousek and others 1997). Increasing N from human activity is a large-scale phenomenon that acts simultaneously on linked terrestrial and aquatic systems by changing the elemental stoichiometry of organic and inorganic pools of nutrients moving between land and water. These changes in nutrient availability have altered the magnitude of critical ecosystem processes such as biological productivity and decomposition (Knorr and others 2005; Thomas and others 2010). In addition, stoichiometric theory suggests that enrichment with N should interact with other coupled nutrients, shifting the balance of nutrient

limitation and cycling rates of other elements (Perring and others 2008). For example, elevated atmospheric N deposition increases P limitation of phytoplankton in lakes (Elser and others 2009) and forest plants (Gress and others 2007). This phenomenon is not universal (Groffman and Fisk 2011; Crowley and others 2012), but few studies have explicitly addressed the issue simultaneously in linked terrestrial and aquatic contexts.

Decomposition of organic matter is a critical ecosystem process in terrestrial and aquatic systems, and there are many similarities in processes and constraints in both habitats, including sensitivity to nutrient availability (Wagner and others 1998). Decomposition in terrestrial environments can be accelerated by fertilization with N or P (Craine and others 2007). However, atmospheric N deposition can enhance, suppress or have no effect on terrestrial decomposition rates across systems (reviewed by Knorr and others 2005). Variable response to N enrichment has been linked to differences in N deposition rate, litter chemistry and suppression of taxa that produce lignin-degrading enzymes (Carreiro and others 2000). In streams, microcosm and reach-scale experiments have shown enrichment with N (Ferreira and others 2006) and P (Elwood and others 1981) alone, and in combination (Greenwood and others 2007; Rosemond and others 2015), can accelerate decomposition rates. Stream decomposition response to atmospheric N deposition has received little direct attention, with few exceptions (for example, Chadwick and Huryn 2003).

The process of decomposition relies on a microbial consortium deploying a suite of enzymes that catalyze decomposition of organic matter. These enzymes acquire carbon (C), N and P, and they are responsive to shifts in organic matter chemistry and environmental chemistry, particularly N and P (Allison and Vitousek 2005). Because production of these enzymes is resource intensive, there are trade-offs in resource allocation toward enzymes that acquire C, N and P (Sinsabaugh and Moorhead 1994); the balance of enzyme production reflects the underlying microbial and environmental stoichiometry (Sinsabaugh and others 2009). Much like decomposition rate, the response of terrestrial enzymes to simulated N deposition ranges from enhancement to suppression (Carreiro and others 2000). However, resource trade-offs following allocation models are apparent. For example, simulated N enrichment can stimulate activity of enzymes that acquire C (Carreiro and others 2000) and P (Marklein and Houlton 2012), suggesting increased decomposition capacity and P demand,

respectively. Consequently, variation in enzyme activity can explain the underlying stoichiometry of microbial metabolism, and enzymes are increasingly used in models designed to explain patterns of decomposition (for example, Moorhead and others 2012).

We used a long-term, whole-watershed experiment to determine whether terrestrial and aquatic systems follow the same trajectory in response to shifting nutrient availability. We hypothesized that N availability constrains decomposition rate and N enrichment would lead to increasing P limitation of decomposition. Because atmospheric N deposition shifts nutrient availability from organic matter decomposition to additional sources of available inorganic nutrient inputs to the system, we segregated these effects and compared them. We tested whether changes in decomposition rates were underlain by shifts in microbial metabolism and enzyme activity consistent with expectations of shifting resource availability to define a microbial mechanism behind altered decomposition. We hypothesized that P amendment would have stronger effects on aquatic decomposition, but that chronic N enrichment would lead to increased P limitation in both terrestrial and aquatic environments.

MATERIALS AND METHODS

Site Description

The Bear Brook Watershed in Maine (BBWM) is a long-term, paired watershed experiment composed of two gauged watersheds, one of which serves as a reference site (East Bear Brook, REF) and a second, contiguous watershed that has been treated bi-monthly with N and acidifying chemicals (as $(\text{NH}_4)_2\text{SO}_4$) from 1989 to 2016 (West Bear Brook, TRT) (Norton and others 2010). The bedrock is quartzite and meta-pelite, intruded by dikes of granite. The soils, developed from till, are podzols (coarse, loamy, mixed, frigid typic Haplorthods). Vegetation includes American beech (*Fagus grandifolia*), maple (*Acer* spp.) and birch (*Betula* spp.) at lower elevations and spruce-fir-hemlock (*Picea* spp., *Abies* spp., *Tsuga* spp.) forest in upper elevations. The streams draining each watershed are first order and consist of steep channels of alternating riffles, pools and debris dams (see Chadwick and Huryn 2003 for detailed description). During 2008–2009, the duration of our experiments reported here, most vegetation in the treatment watershed had become enriched with N (Elvir and others 2010). Soils responded to treatments by accumu-

lating N and S, mobilizing Al and being depleted in exchangeable base cations, primarily Ca (Fernandez and others 2003; Norton and others 2010; Patel and others 2019). Stream water in the treatment catchment had seasonally high concentration of NO_3^- ($\sim 250 \mu\text{g N/l}$) compared to low, constant values ($< 5 \mu\text{g N/l}$) in the reference stream (Navrátil and others 2010). The stream water in both streams was acidic but more so in the TRT watershed ($\text{pH} \sim 5.2$) than in the REF watershed ($\text{pH} 5.5$). Dissolved P concentrations were typically $3 \mu\text{g P/l}$ in both streams (Reinhardt and others 2004). Total P commonly exceeded $100 \mu\text{g P/l}$ at high flow in the treated watershed; most of this total P was adsorbed to amorphous $\text{Al}(\text{OH})_3$ leached from the soil and precipitated in the stream.

Experimental Design

To address effects of altered leaf chemistry and altered environmental chemistry caused by the long-term N treatment at BBWM, we used a reciprocal design in which leaves sourced from each watershed were incubated in terrestrial plots and the streams of both watersheds. To test for P limitation, and how it interacts with altered leaf and environmental chemistry, leaves were incubated under ambient conditions and experimentally elevated P availability in terrestrial plots and in reaches of the streams in each watershed. We use ‘treatment’ to refer to the experimental watershed N enrichment and ‘amendment’ to refer to experimentally elevated P we created in each watershed.

Leaves from American beech and sugar maple (*Acer saccharum*) were collected from the forest floor shortly after abscission of the REF and TRT catchments, yielding two leaf types (REF leaf, TRT leaf) for each species. We chose leaves of these species because they are common on the watersheds, and contrast in chemistry and decomposition rate, with beech typically having higher lignin and a slower decomposition rate than maple (Melillo and others 1982). Leaves of both species were N-enriched in the TRT catchment (Table 1). Leaves were returned to the laboratory and air-dried for two weeks. Packs of each leaf type were constructed by placing about 4 g of dried leaves into plastic net bags (1-cm mesh). Sets of bags were created by joining one bag of each of the four leaf types with a plastic tie. The sets were transported to the field for deployment on September 23, 2008; four extra sets were returned to the laboratory and processed to determine handling loss, a conversion factor for initial air-dry mass to ash-free dry mass (AFDM) and initial leaf chemistry.

Table 1. Initial Carbon, Nitrogen and Phosphorus Concentration of Maple and Beech Leaves Collected from the Reference (REF) and Treatment (TRT) Watersheds

Leaf type	C (%)	N (%)	P (mg/kg)	C:N	C:P	N:P
REF maple	47.4 (0.3)	0.52 (0.00)	160 (9)	106 (1)	7670 (360)	72 (4)
TRT maple	47.1 (0.4)	0.70 (0.01)	192 (9)	78 (2)	6357 (347)	81 (3)
REF beech	48.2 (0.2)	0.75 (0.04)	212 (26)	75 (4)	6050 (724)	80 (6)
TRT beech	48.4 (0.2)	0.86 (0.04)	234 (19)	66 (3)	5406 (456)	82 (4)

Values are means ($n = 3$) with standard errors in parentheses. Concentrations are per unit ash-free dry mass. Ratios are molar.

In the REF and TRT streams, two contiguous 40-m reaches were established. The upstream reach was maintained under ambient conditions, while the downstream reach was amended with P for 63 days. Phosphorus was added at the top (0 m) of the amended reaches as KH_2PO_4 to a target of 150 $\mu\text{g P/l}$ above ambient using a battery-powered fluid metering pump (FMI QBG, Fluid Metering Inc.) to saturate P availability to microbes. Pump rates were held constant; thus, the magnitude of P enrichment varied over time with changing stream discharge. In each reach, multiple leaf bag sets were anchored to the stream bed at three stations located 10, 20 and 30 m from the top of the reach. One set was collected from each station at 16, 35, 47 and 63 days after deployment and returned to the laboratory on ice for processing.

For terrestrial decomposition, leaves were deployed into three stations in the hardwood zone of each catchment. Each station was comprised of a 10 m $\times$ 10 m area in which ten leaf pack sets were anchored to the forest floor at 2-m grid spacing. Sets of packs in each station were randomly assigned to ambient or P amendment conditions. Ambient sets were sprayed with distilled water, whereas P-amended sets were sprayed with an equal volume of the mixture of KH_2PO_4 in distilled water. The water or P mixture was applied to a 0.25 m $\times$ 0.25 m area encompassing each set using a hand-held sprayer twice each month except between late December and mid-April when snow-pack covered the leaves. Phosphorus was applied at rate of 100 kg P $\text{ha}^{-1} \text{y}^{-1}$, a value in the range of enrichments used by others in decomposition experiments (67–150 kg P $\text{ha}^{-1} \text{y}^{-1}$; (McGroddy and others 2004; Cleveland and others 2006; Hobbie 2008)). One control and one P-amended set were collected from each station after 35, 233, 268, 328 and 399 days and returned to the laboratory on ice for processing. The duration of the sampling was targeted to yield a similar proportional mass loss as occurred in the stream experiment based on prior decomposition studies in the watersheds.

Leaf and Water Chemistry

Initial leaf C, N and P concentrations were determined from packs used for handling loss and on leaves from the final collection date. Total C and N concentration of leaves was measured by combustion on a LECO CN-2000 analyzer (LECO Corporation, St-Joseph, MI). Leaf P concentration was determined by ICP-AES (IRIS-1000 Thermo Fisher Scientific, Waltham, MA) after ashing leaves and dissolving the ash in HCl. Elemental concentration was expressed as % dry mass. Stream water samples were collected at the top and bottom of each reach approximately twice each week during the experiment. Water samples were filtered (Whatman GF/F, 0.8 μm) in the field, frozen and later analyzed for NH_4^+ , NO_3^- and soluble reactive phosphorus (SRP) concentrations using a Lachat Quikchem 8500 analyzer (Hach company, Loveland, CO USA) following standard methods (Rice and Bridgewater 2012).

Leaf Mass Loss

In the laboratory, packs were opened and pieces of multiple leaves were cut, avoiding large veins and removed for measurement of microbial respiration and enzyme activity. The remainder of the pack was frozen until processing for mass loss. Packs were later thawed, rinsed over a 1-mm sieve and sorted by hand to separate leaves from debris. The leaves were dried at 60 $^{\circ}\text{C}$ for 48 h, weighed, combusted at 500 $^{\circ}\text{C}$, wetted with distilled water, dried at 60 $^{\circ}\text{C}$ and weighed. Leaf pieces used in microbial assays were similarly processed after use in assays. Ash-free dry mass (AFDM) of leaves was calculated as the difference between dry mass and ash remaining after combustion. The proportion of leaf mass remaining in each pack, including that used for microbial assays, was determined as the difference between initial AFDM and AFDM remaining on each collection date with correction for handling loss. Decomposition rates of terrestrial leaves were calculated from regression of the nat-

ural log (ln) of proportion AFDM remaining over time (R^2 on regressions averaged 0.86; range = 0.68–0.98). We calculated decomposition rates of stream leaf decomposition as the change from initial to final mass on the last date because of the shorter time frame and relatively small mass loss.

Microbial Respiration

We measured rates of respiration as an indicator of microbial metabolic activity contributing to decomposition. We interpolated respiration data from each collection date to calculate and estimate the ‘total’ microbial respiration that reflected changes over time. Rates of microbial respiration on leaves from the streams were determined by oxygen consumption in sealed vials on each collection date. Approximately 3 g wet mass of leaves from each pack was sealed in 26-ml serum vials containing water at 100% oxygen saturation from their representative stream reach and incubated at 10 °C for 6 to 10 h with gentle stirring. Respiration rates on the leaf pieces were determined as differences between dissolved oxygen concentrations in the vials containing leaves and that in control vials that contained only stream water over the incubation period. Dissolved oxygen concentration was measured with an electronic meter (HQ40D with LDO probe, Hach Company). Preliminary trials were used to select incubation times that yielded linear rates of oxygen consumption.

For terrestrial leaves, rates of microbial respiration on each collection date were measured using a Gilson differential respirometer. For each leaf pack, approximately 0.25 g wet mass of leaves was placed in 25-ml Warburg vials. All leaves were moistened with 1.0 ml of water from the REF stream to standardize leaf moisture. The stream water may have added some N and P, but dissolved concentrations in the REF stream are very low (Table 1), so this was likely negligible. Vials were incubated for approximately 2 h, and oxygen consumption was measured by volume change in the vials. Volumetric oxygen consumption was converted to mass using the ideal gas law.

Microbial Enzymes

We assayed potential activity of four extracellular enzymes associated with acquisition of C (β -1,4-glucosidase, BG), P (acid phosphatase, AP) and N (leucine aminopeptidase, LAP and n-acetylglucosaminidase, NAG) on the final collection date. We note that LAP and NAG can also be involved in

C acquisition (Mori 2020). Enzyme activity was assayed using methylumbelliferone-labeled substrates (Enzyme/Substrate: BG /4-MUF- β -D glucoside, phosphatase/4-MUF-phosphate, NAG/4-MUF-N-acetyl- β -glucosaminide) or methylcoumarin-labeled substrate (LAP/L-Leucine 7-amido-4-methylcoumarin). Approximately 2 g wet mass of leaves was homogenized in 30 ml of 50 mM acetate buffer that was adjusted to pH 5.0. Two hundred microliters of the leaf slurry was added to each of 96-well microplates. Fifty microliters of a 200 μ M substrate solution was added to each well, and fluorescence was measured at 25 °C using a microplate fluorometer (Fluoroskan Ascent FL, Thermo Fisher Scientific, Inc.). Reference methylumbelliferone or methylcoumarin standards and controls for particle quench and sample and substrate fluorescence were run on each plate. A series of test runs was used to establish incubation times that yielded linear increases in fluorescence over time and substrate concentrations that saturated enzyme kinetics. Enzymatic rates were expressed per unit dry mass of leaves.

Data Analysis

Initial chemistry of the leaves sourced from the reference and treatment watersheds was compared using t tests. We used linear models to examine the response of leaf decomposition rates, microbial respiration and enzyme activities to P amendment, incubation watershed, leaf watershed source, leaf species and habitat (terrestrial or stream) using the `lm()` function in R (R Core Team 2021). We first fitted a model specified as $response \sim phosphorus * watershed * source * species + habitat:phosphorus$ to determine whether the terrestrial and stream habitats differed in response to phosphorus amendment (that is, significant `habitat:phosphorus` interaction term). When `habitat:phosphorus` interactions were apparent, we split the data and used separate models, specified as $response \sim phosphorus * watershed * source * species$, for terrestrial and stream habitats. Our design is inherently pseudo-replicated as we had only one reference watershed and one treated watershed. However, our design permits consideration of 26 years of combined terrestrial and stream treatment that is neither easily replicated nor reproducible with smaller scale experiments. Model residuals were checked and data were log10 transformed, as needed, to meet assumptions of the linear models. All statistical analyses were performed in R v4.1.0 (R Core Team 2020).

RESULTS

Leaf and Stream Chemistry

Maple leaves from the treated watershed had 34% higher initial N concentration ($P < 0.001$) and a 26% lower C:N ratio ($P < 0.001$) than maple leaves from the reference watershed (Table 1). Initial phosphorus concentration ($P = 0.063$) and C:P ($P = 0.059$) were similar in maple leaves from the two watersheds. Beech leaves from the two watersheds did not differ in their initial C, N and P concentration or elemental ratios (Table 1, $P > 0.103$). By the end of the experiment, leaves incubated in the TRT stream had 13–60% higher N than leaves in the REF stream (Table S1). Phosphorus amendment increased leaf P concentration by about 160% in the REF stream and about 820% in the TRT stream. There were no consistent differences in N of terrestrial leaves between the watersheds after incubation (Table S2). P amendment increased leaf P concentration by 120–280% on leaves in terrestrial plots with no consistent difference between watersheds.

Nitrate concentration in the stream water was, on average, two orders of magnitude higher in the TRT stream than the REF stream during the experiment (Table 2). Ammonium concentration was low and similar among the four stream reaches. Experimental addition of P elevated SRP more than 50-fold in the downstream reaches of the two streams compared to the low, similar concentrations in the upstream reference reaches ($\sim 3 \mu\text{g P/l}$). The mean DIN:SRP ratios among the four stream reaches spanned four orders of magnitude from 0.2 to 202.

Decomposition Rate

Total mass loss ranged from 30 to 65% for terrestrial leaves and 3 to 52% for stream leaves. Decomposition rates responded differently to P amendment in terrestrial and stream habitats (P:habitat $P < 0.001$; Table S3). In terrestrial

plots, there was no difference between watersheds ($P = 0.252$) and no effect of P amendment ($P = 0.460$) on decomposition rate (Figure 1A; Table S4). There was a significant leaf source effect ($P = 0.001$), but this differed by species (source $\times$ species $P = 0.003$). Beech leaves sourced from the TRT watershed decayed about 20% faster than leaves from the REF watershed, but there was no clear difference for maple (Figure 1A). In streams, the effect of P amendment differed between watersheds (P $\times$ watershed $P = 0.005$; Table S5). In particular, P amendment accelerated decomposition rate by 17–180% in the N-rich TRT stream but had little effect in the N-poor REF stream (Figure 1B). In contrast to the terrestrial habitat, there was no leaf source effect ($P = 0.251$) on leaf decomposition in the streams.

Microbial Respiration

Microbial respiration rates also responded differently to P amendment in terrestrial and stream habitats (P:habitat $P < 0.001$; Table S6). In the terrestrial habitat there was little evidence of an effect of P amendment on respiration ($P = 0.046$; Table S7; Figure 2A) nor were there differences related to incubation watershed ($P = 0.540$) or leaf source ($P = 0.692$). In contrast, phosphorus amendment influenced microbial respiration in streams, but the effect depended on the watershed (P $\times$ watershed $P < 0.001$; Table S8). As was the case for decomposition rate, P amendment increased respiration rate in the TRT stream by 33–73% but had little effect in the REF stream (Figure 2B). Microbial respiration rates were higher (roughly double or more) in the TRT watershed than in the REF watershed ($P < 0.001$). Leaves sourced from the TRT watershed also tended to have higher respiration rates than leaves from the REF watershed ($P = 0.011$), but the difference was small compared to the effect of P and leaf source (Figure 2B).

Table 2. Dissolved Inorganic Nitrogen and Phosphorus Concentrations and Ratio in the Stream Water of the Upstream Ambient (amb) and Downstream P Enriched (+ P) Reaches of the Streams Draining the Reference (REF) and Treatment (TRT) Watersheds

Reach	NH_4^+ ($\mu\text{g N/l}$)	NO_3^- ($\mu\text{g N/l}$)	SRP ($\mu\text{g P/l}$)	DIN:SRP (molar)
REF amb	7 (< 2–14)	1 (< 1–2)	3 (< 1–6)	8.4 (3–28)
REF + P	6 (< 2–13)	1 (< 1–2)	221 (9–597)	0.2 (0.01–3)
TRT amb	7 (< 2–13)	126 (3–395)	3 (< 1–7)	202.0 (12–1057)
TRT + P	7 (< 2–15)	126 (3–389)	138 (4–405)	13.7 (0.11–198)

Values are means of 17 dates with ranges in parentheses. DIN = dissolved inorganic nitrogen ($\text{NH}_4^+ + \text{NO}_3^-$). SRP = soluble reactive phosphorus.

Figure 1. Leaf decomposition rates (k) in the terrestrial (**A**) and stream (**B**) environment in the reference (REF) and treated (TRT) watersheds under ambient and elevated P. Decomposition rates of leaves originating from each watershed (REF leaf, TRT leaf) are shown. Small symbols are raw data and large symbols are means.

Enzymes

The activity of all enzymes responded differently to P amendment in terrestrial and stream habitats (P:habitat $P < 0.001$; Tables S9, S12 and S15). Activity of BG on terrestrial leaves was unresponsive to P amendment ($P = 0.099$) and similar in both watersheds ($P = 0.588$) and on leaves sourced from the different watersheds ($P = 0.559$; Table S10; Figure 3A). In streams, BG activity was influenced by P amendment ($P = 0.008$) and incubation watershed ($P < 0.001$), but unresponsive to change in leaf source ($P = 0.139$; Table S11). Amendment with P tended to increase BG activity, but the effect was small (Figure 3B). Activity of BG was consistently higher ($\sim$ twofold) in the TRT stream than in the REF stream (Fig-

ure 3B) for both species under ambient or elevated P availability (Figure 3B).

Amendment with P reduced AP activity on terrestrial leaves by up to 80% ($P < 0.001$; Table S13) and the effect was the same in both watersheds and for both species regardless of the watershed source (Figure 4A). There was little evidence that incubation watershed ($P = 0.044$) or leaf source ($P = 0.181$) had much effect on AP activity on terrestrial leaves. In streams, the effect of P depended on the watershed ($P \times$ watershed $P < 0.001$; Table S14). In particular, AP activity was higher in the TRT watershed streams under ambient conditions and it declined to levels similar to that in the REF stream when amended with P (Figure 4B). Amendment with P reduced AP activity in the REF stream, but the change was

Figure 2. Microbial respiration rates in the terrestrial (**A**) and stream (**B**) environment in the reference (REF) and treated (TRT) watersheds under ambient and elevated P. Rates on leaves originating from each watershed (REF leaf, TRT leaf) are shown. Small symbols are raw data and large symbols are means.

much smaller than in the N-rich TRT stream (Figure 4B). We also found differences by leaf source ($P < 0.001$) and an interaction between leaf source and P amendment ($P = 0.035$; Table S9). Activity of AP tended to be higher on the N-enriched leaves sourced from the TRT watershed, and the effect of P amendment was stronger on TRT leaves (Figure 4B).

Response of N-acquiring enzymes in the terrestrial habitat was largely unresponsive to P amendment ($P = 0.529$), incubation watershed ($P = 0.326$) or change in leaf source ($P = 0.307$; Table S16, Figure 5A). In streams, LAP + NAG was consistently higher ($P < 0.001$, Table S17) by 40–100% in the TRT stream than in the REF stream. Amendment with P influenced activity, but it interacted with leaf source ($P < 0.001$). Amendment increased LAP + NAG activity on

leaves from the TRT watershed but not on leaves from the REF watershed (Figure 5B).

DISCUSSION

Anthropogenic supply of N can drive terrestrial and aquatic systems toward P limitation, a phenomenon typically examined for primary producers (Elser and others 2009; Vitousek and others 2010). Because decomposition is sensitive to nutrient availability and follows general stoichiometric theory we expected shifting nutrient limitation in response to long-term N fertilization (Mooshammer and others 2012). In our study, terrestrial and aquatic decomposition displayed divergent responses to chronic N and acute P experimental enrichment. The effect of the long-term N enrichment on terrestrial decomposition

Figure 3. BG activity on leaves in the terrestrial (**A**) and stream (**B**) environments in the reference (REF) and treated (TRT) watersheds under ambient and elevated P. Activity on leaves originating from each watershed (REF leaf, TRT leaf) is shown. Small symbols are raw data and large symbols are means.

was small and manifested largely through alteration in leaf chemistry and decomposition did not shift to P limitation in the N-enriched watershed. In stark contrast, in streams leaf chemistry played a minor role and watershed N enrichment induced P limitation.

The response pattern of stream decomposition among the four experimental stream reaches was indicative of co-limitation by N and P under ambient conditions (that is, N and P together increased decomposition). This result mirrors patterns observed in other studies that have manipulated N and P at smaller spatial and shorter temporal scales. For example, N and P enrichment of stream reaches for several years (Rosemond and others 2015) and in stream mesocosms for a few months (Kominoski and others 2015) has shown co-limitation of N and P with several litter types in

forested watersheds. The enrichment of stream water and leaves with N (TRT watershed) did not alter decomposition rates at BBWM, a result also observed by (Chadwick and Huryn 2003) in a prior study of the same streams. It did, apparently, saturate N demand and switch decomposition to P limitation as evident in the large increase in decomposition rates of beech and maple in the treatment streams augmented with P. The concentration of dissolved inorganic N in the TRT stream ($\sim 190 \mu\text{g N/l}$) was within the range ($\sim 50\text{--}520 \mu\text{g N/l}$) found to saturate N limitation in other studies of stream litter decomposition (Kominoski and others 2015).

The effect of chronic experimental watershed N enrichment on streams was manifest largely through altered water chemistry as the relatively N-rich maple leaves from the treated watershed

Figure 4. AP activity on leaves in the terrestrial (**A**) and stream (**B**) environments in the reference (REF) and treated (TRT) watersheds under ambient and elevated P. Activity on leaves originating from each watershed (REF leaf, TRT leaf) is shown. Small symbols are raw data and large symbols are means.

decayed at the same rate as leaves from the reference watershed. Leaf chemistry can influence decomposition rate in streams (Webster and Benfield 1986), but the change in chemistry of recently abscised leaves at BBWM was apparently insufficient to influence decomposition rate. Leaf chemistry also had little influence on the response of decomposition to changes in stream water chemistry. While stream water nutrient enrichment can have stronger effects on leaves with lower nutrient content (Kominoski and others 2015), maple and beech leaves, which differed in decomposition rate and initial chemistry, responded similarly to stream N and P enrichment in our experiment.

The patterns in decomposition we observed in streams were underlain by consistent microbial responses. Microbial respiration rates were higher in the N-enriched TRT stream than in the REF

stream and acute amendment with P further increased respiration. While microbial activity was spurred by stream water N enrichment, it was insufficient to alter decomposition rate. Decomposition is driven by enzymes and the patterns we observed were consistent with predictions from resource allocation models (Sinsabaugh and Moorhead 1994). This was evident as higher allocation toward C-acquiring enzymes as a response to P amendment, but only under high N availability.

Terrestrial decomposition rate is commonly positively associated with litter N availability, but experimental N enrichment leads to highly variable outcomes that encompass suppression, enhancement and no effect on decomposition (Knorr and others 2005). Normally, the effect of N fertilization is linked to substrate chemistry with N-suppression of lignin degradation leading to reduced decomposi-

Figure 5. LAP + NAG activity on leaves in the terrestrial (A) and stream (B) environments in the reference (REF) and treated (TRT) watersheds under ambient and elevated P. Activity on leaves originating from each watershed (REF leaf, TRT leaf) is shown. Small symbols are raw data and large symbols are means.

tion, particularly for high-lignin substrates and in late stages of decomposition (Hobbie 2008). Our study focused on early stages of decomposition when we expected N fertilization to be most likely to stimulate decomposition (Hobbie 2008). However, neither beech nor maple decomposition rates under ambient conditions differed between the two watersheds despite 19 years of differences in N availability.

Phosphorus limitation of terrestrial decomposition has been shown in tropical sites (Vitousek and others 1993) and in the laboratory (Craine and others 2007), but generally has not been considered in temperate systems. In one of the few studies in temperate systems, DeForest (2019) found that P addition suppressed decomposition although the mechanism for this is unknown. Phosphorus clearly did not limit decomposition in our watersheds, even after nearly two decades of N enrichment suggesting that changes in stoichiometric

balance between watershed N and P inputs to streams does not influence nutrient limitation patterns of terrestrial decomposition, in striking contrast to what occurs in streams.

We note that the TRT watershed N enrichment was accompanied by acidification that has altered an array of watershed characteristics (Norton and others 2010). Base cations had been depleted and soil pH had declined, but this does not appear to have influenced decomposition rate as we found similar rates in both watersheds. This may be because we examined early decomposition in the uppermost litter layer rather than in deeper soils where chemical change is greater. Acidification does not appear to have directly influenced decomposition in the streams as it typically suppresses decomposition rate (Simon and others 2009), but decomposition rate was similar between watersheds in the absence of P enrichment (our

data and Chadwick and Huryn 2003). We suspect watershed acidification enhances P limitation in the TRT catchment streams by mobilizing Al to the stream where it precipitates and sequesters P, also mobilized from the soil (Navrátil and others 2010). Mobilization of P in the rooting zone and increased uptake may explain the somewhat higher P concentration in beech and maple leaves (Table 1). In prior work we have found that P uptake is higher in the TRT stream and that P addition stimulates uptake of N in both streams (Simon and others 2010). In essence, acidification draws down P supply in the streams through adsorption of PO_4 by $\text{Al}(\text{OH})_3$ in both streams, while N enrichment releases N limitation, amplifying the effect of atmospheric deposition on streams.

Why are terrestrial and stream responses to atmospheric N deposition so different? We suspect the answer at least partly stems from the most obvious difference between these ecosystem compartments, the availability of water. Water scarcity can reduce decomposition in terrestrial (Allison and others 2013) and stream (Chadwick and Huryn 2003) systems. Water availability may set the stage for differential responsiveness of terrestrial and stream systems to shifting nutrient availability by modulating microbial stress and the flux of exogenous nutrients to microbes.

Drying stress alters microbial function (Fierer and Schimel 2002) and moisture influences enzyme activity (Sardans and Peñuelas 2005), often with differing effects among enzymes. Consequently, water stress may limit capacity for enzyme allocation trade-offs that underlie decomposition response to shifting nutrient availability seen in streams. Notably, the loose litter layer which we simulated in our terrestrial plots is particularly prone to a high temporal variability in wetting and drying. Drying may have decoupled nutrient limitation and enzyme resource allocation (that is, adding P stimulated AP, but constrained capacity to increase BG and LAP/NAG that would drive increased decomposition). For example, Sardans and Peñuelas (2005) found drying led to reduced BG and peptidase, but not AP, activity in the terrestrial environment. Our results only hold for initial phases of decomposition in the loose litter layer, but mass loss during the 399 days of our experiment was consequential (that is, ~ 30–65% mass loss in our terrestrial litter bags).

Water may also modulate how interactions between endogenous and exogenous nutrient availability influence decomposition. For example, microbial N mining (Moorhead and others 2012) may explain why increasing exogenous N supply

inhibits decomposition in terrestrial systems. Essentially, increased N supply inhibits lignin degradation that typically is used to support acquisition of N from organic matter. It is possible that this phenomenon does not occur in streams where flowing water creates a continual flux of exogenous nutrients to microbes. To our knowledge nobody has examined mining in a stream context, highlighting the disconnect between how terrestrial and aquatic systems are typically studied. Our work suggests that a more holistic approach that expressly considers linked terrestrial and aquatic processes simultaneously will yield deeper insight into the impact of large-scale phenomena like air pollution.

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