2015

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Ecology, 96(8), 2015, pp. 2214–2224

Detrital stoichiometry as a critical nexus for the effects of streamwater nutrients on leaf litter breakdown rates

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Abstract. Nitrogen (N) and phosphorus (P) concentrations are elevated in many freshwater systems, stimulating breakdown rates of terrestrially derived plant litter; however, the relative importance of N and P in driving litter breakdown via microbial and detritivore processing are not fully understood. Here, we determined breakdown rates of two litter species, *Acer rubrum* (maple) and *Rhododendron maximum* (rhododendron), before (PRE) and during two years (YR1, YR2) of experimental N and P additions to five streams, and quantified the relative importance of hypothesized factors contributing to breakdown. Treatment streams received a gradient of P additions (low to high soluble reactive phosphorus [SRP]; ~10–85 μg/L) crossed with a gradient of N additions (high to low dissolved inorganic nitrogen [DIN]; ~472–96 μg/L) to achieve target molar N:P ratios ranging from 128 to 2. Litter breakdown rates increased above pre-treatment levels by an average of 1.1–2.2× for maple, and 2.7–4.9× for rhododendron in YR1 and YR2. We used path analysis to compare fungal biomass, shredder biomass, litter stoichiometry (nutrient content as C:N or C:P), discharge, and streamwater temperature as predictors of breakdown rates and compared models containing streamwater N, P or N + P and litter C:N or C:P using model selection criteria. Litter breakdown rates were predicted equally with either streamwater N or P ($R^2 = 0.57$). In models with N or P, fungal biomass, litter stoichiometry, discharge, and shredder biomass predicted breakdown rates; litter stoichiometry and fungal biomass were most important for model fit. However, N and P effects may have occurred via subtly different pathways. Litter N content increased with fungal biomass (N-driven effects) and litter P content increased with streamwater P availability (P-driven effects), presumably via P storage in fungal biomass. In either case, the effects of N and P through these pathways were associated with higher shredder biomass and breakdown rates. Our results suggest that N and P stimulate litter breakdown rates via mechanisms in which litter stoichiometry is an important nexus for associated microbial and detritivore effects.

Key words: carbon loss; decomposition; detritus; ecological stoichiometry; litter breakdown; nitrogen; path analysis; phosphorus; processing; streams.

Introduction

Understanding biogeochemical cycles and the impacts of human activity on ecosystem dynamics requires the consideration of interactions among multiple elements (Schlesinger et al. 2011). Nitrogen (N) and phosphorus (P) both limit autotrophic production (Elser et al. 2007), but less is known about the relative importance of N and P for heterotrophic processes such as breakdown of detrital organic matter (but see Woodward et al. 2012). Increased anthropogenic mobilization of N and P often occurs in disproportionate amounts, driving the relative availability of N or P in recipient ecosystems (e.g., atmospheric N deposition vs. P-rich livestock waste [Arbuckle and Downing 2001]). Thus, there is a need to understand the specific effects of N and P on fundamental ecosystem processes such as detrital organic matter breakdown.

Processing of detrital carbon (C) in aquatic ecosystems is a function of interacting abiotic and biotic factors, including temperature, physical abrasion, litter stoichiometry, microbial conditioning, and detritivore biomass (Hieber and Gessner 2002, Hladyz et al. 2009). Under low-nutrient conditions, litter species identity can be used to predict litter breakdown rates, as initial nutrient content and other physical and chemical traits can affect microbial colonization and consumption by detritivores (Petersen and Cummins 1974). Nutrient enrichment has been shown to increase nutrient content

Manuscript received 19 August 2014; revised 28 January 2015; accepted 29 January 2015. Corresponding Editor: S. Findlay.

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of decomposing litter, thereby reducing the natural variation in litter stoichiometry (i.e., C : nutrient ratios; C:N, C:P) between litter species (Rosemond et al. 2010). This effect may relax consumer resource constraints on detritivore growth and consumption of litter to affect breakdown rates (Cross et al. 2003, Tant et al. 2013). Aquatic fungi play a generally larger role in breakdown of coarse particulate organic matter such as leaf litter than bacteria (Findlay et al. 2002, Tant et al. 2013). Fungi can affect nutrient content of conditioned litter by incorporating both streamwater- and litter-derived nutrients into their biomass (Suberkropp and Chauvet 1995, Cheever et al. 2013). The relative influence of streamwater N and P on microbial- and detritivore-mediated processes and on the links among fungi, litter stoichiometry, and detritivores that drive breakdown of C remains poorly understood.

Streamwater N and P may similarly affect the links between fungal and shredder pathways of detrital C loss, and therefore either nutrient may limit the rate of litter breakdown (e.g., Ferreira et al. 2014). For example, P may be critically important for the growth and biomass accrual of fungi on litter, given that P-rich RNA is needed for rapid metabolism (i.e., the growth rate hypothesis [Sterner and Elser 2002, Grimmett et al. 2013]). Alternatively, N has been linked to increased fungal biomass (Ferreira et al. 2006), and may be important for fungi to produce N-rich enzymes to acquire C from polymers (Sinsabaugh et al. 2009). Increases in fungal biomass may alter litter stoichiometry (both C:N and C:P) via immobilization of dissolved nutrients to substantially increase litter nutrient content. This altered stoichiometry may affect shredder consumption and litter breakdown rates (Cheever et al. 2013, Scott et al. 2013). Shredder growth and consumption rates have been associated with litter N (Rosemond et al. 2010) or P content of detritus (Danger et al. 2013). Thus, increased litter breakdown rates may occur when N or P are elevated alone or together through similar stimulatory effects on fungal biomass and activity, increased litter nutrient content, and ensuing shredder activity (Ferreira et al. 2014); but the relative contributions of N or P to these processes are unknown.

This study used crossed streamwater N and P concentration gradients in five headwater streams to test the effects of N and P on litter breakdown rates and identify the mechanisms by which they occurred. We used path analysis to test hypothesized causal links among streamwater N and P concentrations, conditioned litter stoichiometry (C:N, C:P), fungal biomass, shredder biomass, discharge, temperature, and litter breakdown rates; breakdown of litter is hypothesized to occur through microbial processing (e.g., litter mass loss due to respiration, biomass production, and spore production in the case of fungi) and shredder feeding (Fig. 1). Our experimental design precluded testing for the isolated effects of N and P, but allowed us to examine the relative strength of their effects on these pathways. We predicted that the effects of dissolved N and P on stream detrital food webs would propagate through microbial pathways, whereby increases in fungal biomass increase litter nutrient content and enhance shredder biomass (Fig. 1). We tested path models with N, P, and N + P to assess the relative strength of their singular or combined effects on litter breakdown. We also tested path models using stoichiometry of conditioned litter, as either C:N or C:P, to evaluate the importance of litter N or P content for explaining litter breakdown rates.

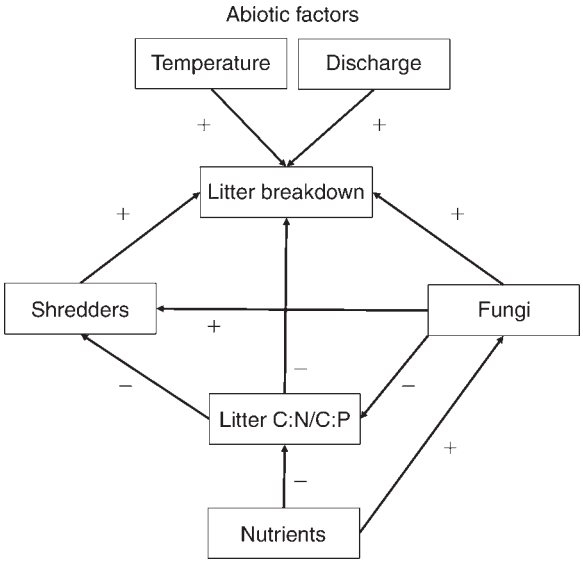

FIG. 1. Hypothesized path model describing how nutrients affect litter breakdown rates. Arrows indicate hypothesized causal links between variables, with the direction of the effect denoted by a (+) or (−) symbol. The structured set of linear equations that correspond to each response variable can be described based on the links associated with each variable (e.g., shredders ~ fungal biomass + litter C:N/C:P, litter C:N/C:P ~ fungal biomass, etc.). We hypothesized that aquatic fungi play a central role in mediating the effects of nutrients on leaf breakdown due to their direct positive effects on shredders, and positive indirect effects on shredders due to increased microbially mediated litter nutrient content.

METHODS

Study site and experimental nutrient additions

This study was conducted at the Coweeta Hydrologic Laboratory (CWT), a United States Forest Service research station located in Macon County, North Carolina, USA. Coweeta is a heavily forested 2185-ha basin with mixed hardwoods (maple, poplar, and oak) that are common in the Blue Ridge physiographic region of the southern Appalachian Mountains (Swank and Crossley 1988). The basin contains a network of low-order streams that are heavily shaded year-round by *Rhododendron maximum*. Reaches (70 m in length) in five first-order streams within the Dryman Fork catchment were identified for the nutrient manipulations used in this study (35°02′ N, 83°45′ W). These five

streams were physically and chemically similar in terms of elevation (~1200 m above sea level), aspect (four out of five on east-facing slopes, one northeast), gradient, pH, and temperature and were in close proximity (<0.5 km apart). Experimental additions of aqueous 21% ammonium nitrate and 85% phosphoric acid occurred continuously for two years (July 2011 through July 2013, following a year of pretreatment data collection, hereafter: PRE, YR1, and YR2). Solar-powered metering pumps (LMI Milton Roy, Ivyland, Pennsylvania, USA) delivered concentrated nutrient solutions into gravity-fed irrigation lines according to a program based on continuously measured discharge using a CR800 data-logger (Campbell Scientific, Logan, Utah, USA) and a Nanolevel pressure transducer (Keller America, Newport News, Virginia, USA). Each irrigation line had drip spouts placed approximately every 5 m throughout the experimental reach to ensure sufficient mixing. Each stream was assigned a unique target concentration of N (as dissolved inorganic nitrogen, DIN [nitrate + ammonium], including both mean background and added N; 81, 244, 366, 488, and 650 μg/L) that corresponded to a unique decreasing concentration of P (as soluble reactive phosphorus, SRP; 90, 68, 51, 34, and 11 μg/L), which resulted in five molar ratios of dissolved N:P very close to target values (2, 8, 16, 32, and 128, respectively). Therefore, N and P concentrations were elevated above background concentrations in each stream (target N concentrations were between ~2× and ~12× background, and target P concentrations were between ~5× and ~31× background) and reflected low-to-moderate enrichment consistent with observed concentrations in streams experiencing land-use change in the region (Scott et al. 2002).

Water samples for nitrate (as NO_3^--N), ammonium (NH_4^+-N), and SRP were collected biweekly within each experimental reach ($n = 2$) and upstream of the nutrient-dosing system ($n = 2$), filtered in the field using 0.45-μm nitrocellulose membrane filters (Millipore, Billerica, Massachusetts, USA) and frozen until analysis. Nitrate-N, NH_4^+-N, and SRP concentrations were measured with an Alpkem Rapid Flow Analyzer 300 (DIN; Alpkem, College Station, Texas, USA) at the University of Georgia Analytical Chemistry Laboratory (Athens, Georgia, USA) or spectrophotometrically (SRP) using the ascorbic acid method (APHA 1998; Shimadzu UV-1700, Tokyo, Japan).

Litter breakdown rates and stoichiometry

We measured breakdown rates of maple (*Acer rubrum*) and rhododendron (*Rhododendron maximum*) litter from December to June (PRE) and from December to April (YR1, YR2). Maple and rhododendron represent dominant riparian tree species at CWT, have distinct initial nutrient content (maple C:N/C:P was ~78/2645; rhododendron C:N/C:P was ~145/7552 [D. W. P. Manning and A. D. Rosemond, *unpublished data*]) and have been used extensively for studying litter breakdown rates in southern Appalachian streams (Webster et al. 1999, Kominoski et al. 2007). Litter bags were constructed using 5-mm plastic mesh pecan bags (22 × 40 cm; Cady Bag, Incorporated, Pearson, Georgia, USA) to allow access by shredders and to maintain known quantities of litter. Freshly abscised litter was collected during peak leaf-fall in October 2010, 2011, and 2012, air-dried in the laboratory for several weeks, and weighed into 10 ± 0.1 g packs. The litterbags were anchored in the five experimental reaches on 1 December 2010, 27 November 2011, and 29 November 2012 for PRE, YR1, and YR2, respectively. Each of those dates was designated as day 0 for their respective years. Within each experimental reach, we delineated four 17.5-m sub-reaches where seven arrays (one for each sampling date) of the single-species litterbags were deployed for a total of 280 bags for each year (5 streams × 4 sub-reaches × 7 sampling dates × 2 species). Five additional litterbags of each species were taken to the sites, submerged in the stream, and immediately collected to account for handling losses (Benfield 2006).

Incubated leaf litter was removed over time and processed for mass remaining and litter stoichiometry. In the PRE year, we collected leaf packs on days 7, 14, 21, 70, 109, 160, and 187 from each sub-reach. During YR1 and YR2, we expected higher breakdown rates, in particular for maple, so we used a shorter sampling schedule (maple sampled on days 7, 14, 21, 34, 55, 63, 77; rhododendron sampled on days 7, 14, 21, 63, 110, 126, 143). On each sampling date, litterbags were removed from the streams, placed into individual plastic bags, and transported to the laboratory on ice. In the laboratory, litter was rinsed over nested 1-mm and 250-μm sieves to remove sediments and macroinvertebrates, placed into paper bags, and dried for a minimum of 24 h at 55°C. The entire sample was weighed to determine dry mass and ground using an 8000-D ball mill (Spex SamplePrep, Metuchen, New Jersey, USA). A subsample was combusted at 500°C for 4.5 h to determine ash-free dry mass (AFDM). We estimated conditioned litter C:N or C:P content for litter material collected on day 70 (PRE) or day 63 (YR1, YR2). Conditioned litter C and N content were determined using a Carlo Erba NA 1500 CHN Analyzer (Carlo Erba, Milan, Italy). Phosphorus content of the conditioned litter was determined using the plant dry ash/acid extraction method followed by spectrophotometric analysis using the ascorbic acid method (Allen 1974, APHA 1998).

Fungal biomass

Fungal biomass was estimated by measuring ergosterol concentration associated with five ~2 × 2 cm litter pieces subsampled from each litterbag early in the breakdown experiments (day 14). We measured ergosterol concentrations early in the breakdown process because early fungal colonization of litter is indicative of subsequent fungal community development and effects on litter breakdown rates (e.g., Duarte et al. 2008,

Sridhar et al. 2009). Briefly, lipids were extracted from the freeze-dried, weighed litter pieces using liquid-to-liquid extraction (Gulis and Suberkropp 2006), and ergosterol concentrations were determined by HPLC (LC-10VP, Shimadzu, Columbia, Maryland, USA) equipped with a Kinetex C18 column (Phenomenex, Torrance, California, USA) and a UV detector set at 282 nm. External ergosterol standards (Acros Organics, Geel, Belgium) were used. Ergosterol concentrations were converted to fungal biomass using a conversion factor of 5.5 μg/mg of mycelial dry mass (Gessner and Chauvet 1993).

Macroinvertebrate biomass

We focused our macroinvertebrate sampling efforts for both maple and rhododendron litter collected on day 70 (PRE) and day 63 (YR1 and YR2), such that we captured the time to ~50% mass loss for maple and ~15% mass loss for rhododendron under pretreatment conditions. After rinsing the litter, the two size classes of macroinvertebrates were removed from the nested sieves and preserved separately in 70% ethanol. The macroinvertebrates in each sample were sorted, identified to the lowest taxonomic unit (typically genus [Merritt et al. 2008]), and measured to the nearest millimeter. Biomass was then determined using previously established length–mass regressions for CWT stream taxa (Benke et al. 1999; J. B. Wallace, *unpublished data*). We estimated shredder biomass per gram of litter AFDM remaining in each corresponding litterbag based on the classification of specific taxa as shredders (Merritt et al. 2008).

Data analyses

Breakdown rate, k, was estimated using a linear regression of the ln-transformed fraction of AFDM remaining vs. time (negative exponential model; sensu Benfield 2006). Specifically, the model is $M_t = M_0 \times e^{-kt}$, where M_0 is the initial litter mass, M_t is the litter mass on a given sampling day, and t is time (number of days incubated in the stream). We estimated a specific k value in four sub-reaches within each experimental stream, such that our total number of litter breakdown rate estimates was 120 (4 sub-reaches × 5 streams × 2 species × 3 years). The primary predictor variables used in this study were either ambient (PRE) or enriched (YR1, YR2) DIN or SRP concentrations. For enriched values, we used calculated concentrations based on experimental additions of N and P. Evidence of concentration-dependent nutrient uptake in the treatment reaches indicated that concentration estimates based on the amounts of nutrients actually added were better than measured streamwater concentrations to characterize the experimental treatments (A. D. Rosemond, *unpublished data*). Enriched concentrations were determined based on the quantity of N or P added to each stream, using records of concentrated nutrient solution refills, measured ambient water nutrient concentrations, and total daily discharge.

The path model

We constructed a path model with hypothesized causal links based on previous studies of how nutrients, other abiotic drivers, and biological factors are predicted to affect litter breakdown rates (e.g., Hieber and Gessner 2002, Hladyz et al. 2009; Fig. 1). We used six predictor variables for litter breakdown: temperature, discharge, streamwater nutrient concentrations, fungal biomass, shredder biomass, and conditioned litter stoichiometry (C:N or C:P); a link between fungal biomass and litter breakdown is included to imply fungal contribution to C losses via respiration. We assessed model fit based on comparisons of the implied model covariance structure and observed covariance structure using χ^2 tests (Grace 2006). A path model was deemed to be consistent with the data when modeled covariance structure and observed covariance structure were not statistically different (i.e., nonsignificant χ^2 test). If assessment of the χ^2 test suggested that a model was inconsistent with the data, we reevaluated model structure using one degree of freedom χ^2 criteria and inspection of residual covariance matrices to test the improvement in model fit gained by adding a specific link to the model (Grace et al. 2012). We removed links from the model to improve model parsimony in cases where maintaining a specific link had negligible impact on overall model fit based on nonsignificant parameter estimates.

Once we arrived at an acceptable model to predict litter breakdown rates, we compared models with this underlying structure using N alone, P alone, or N and P combined as predictors. We tested for the importance of stoichiometry of decomposing litter (C:N or C:P) in the same manner, such that we had two sets of three models (i.e., N, P, and N + P for litter C:N and C:P, respectively). These six models allowed us to test for the importance of N, P, and C:N vs. C:P for predicting litter breakdown rates. We evaluated the support for each model based on Akaike's Information Criterion (AIC; Burnham and Anderson 2002). In addition to an overall model that included results from PRE, YR1, and YR2, we analyzed models using a separate group (hereafter: single-year) approach, to compare PRE, YR1, and YR2, separately. To contrast the path coefficients before and after experimental nutrient enrichment, we compared model fit when all path coefficients were allowed to differ to model fit when coefficients were held constant among years using χ^2-difference tests. For single-year modeling, we focused on addressing differences in path coefficients using the underlying structure of the best-supported overall models.

Parameters of each model are reported as standardized path coefficients to allow for direct comparison of variables measured at different scales and are indicative of the weight of each predictor variable for explaining

TABLE 1. Mean (±SE) ambient (PRE) and enriched (YR1, YR2) nutrient concentrations (μg/L) during each litter breakdown experiment for the five treatment reaches used in this study ($n = 9$, 11, 23, respectively).

Target N:P	Year	Nutrients (μg/L)			Discharge (L/s)			Temperature (°C)
		N:P	DIN (±SE)	SRP (±SE)	Mean	Max	Min	Mean (±SE)
2	PRE	12.5	17.0 (2.0)	3.0 (0.0)	6.3	16.4	2.2	7.09 (0.29)
	YR1	3.0	120.5 (15.5)	90.1 (6.5)	5.2	16.5	1.7	7.79 (0.19)
	YR2	2.6	80.4 (7.9)	69.4 (6.5)	8.3	34.6	1.6	6.48 (0.17)
8	PRE	127.6	173.0 (10.0)	3.0 (0.3)	21.9	43.2	13.2	7.66 (0.27)
	YR1	14.3	302.8 (26.2)	46.9 (4.1)	18.1	75.7	6.1	8.28 (0.15)
	YR2	8.6	149.1 (10.7)	38.6 (2.7)	17.9	74.5	8.3	7.35 (0.13)
16	PRE	27.1	49.0 (8.0)	4.0 (1.0)	9.6	25.2	3.1	6.69 (0.26)
	YR1	18.0	429.5 (51.2)	52.8 (7.3)	5.7	26.0	3.0	7.22 (0.18)
	YR2	16.0	409.1 (85.3)	56.7 (11.9)	5.7	8.9	2.1	6.32 (0.16)
32	PRE	125.1	238.0 (22.0)	4.0 (0.4)	12.0	23.0	6.3	7.06 (0.27)
	YR1	42.7	362.8 (26.5)	18.8 (1.9)	6.1	16.0	3.8	8.00 (0.17)
	YR2	30.6	388.1 (12.0)	28.1 (1.2)	7.5	17.8	3.6	6.98 (0.16)
128	PRE	57.5	78.0 (9.0)	3.0 (0.3)	18.7	118.4	6.7	6.38 (0.29)
	YR1	103.3	366.9 (43.1)	7.9 (1.0)	9.8	45.2	2.1	6.95 (0.20)
	YR2	105.6	494.1 (32.6)	10.4 (0.5)	10.7	44.2	0.1	5.72 (0.17)

Notes: Nutrient concentrations for PRE were measured ambient concentrations; YR1 and YR2 concentrations are based on the amounts of dissolved inorganic nitrogen (DIN) or soluble reactive phosphorus (SRP) added to each stream estimated using records of total daily discharge, concentrated nutrient solution refills, and background nutrient concentrations. Also reported are the mean, maximum, and minimum daily discharge (L/s) observed for each treatment reach, in addition to the mean (±SE) daily temperature (°C) recorded during each litter breakdown experiment (PRE, YR1, YR2).

variation in the response variables. (Unstandardized coefficients are reported in Appendix A: Table A4.) Standardized coefficients were obtained through *z*-transformations such that means and variances of the variables are adjusted to zero and one, respectively. Because path analysis is a structured set of linear regressions, basic assumptions of linear regression apply; thus, we ln-transformed our predictor and response variables to meet assumptions of normality and linearity. All analyses were conducted using the statistical software R, version 3.0.1 (R Development Core Team 2013) and the package 'lavaan' (version 0.5-16; Rosseel 2012).

RESULTS

Whole-stream nutrient additions

Experimental enrichment of the five study reaches resulted in elevation of DIN and SRP, which generally reflected target concentrations (Table 1). Enriched DIN and SRP levels were on average between 0.85–8× and 3–28× background (PRE) concentrations, respectively, during the enrichment period. Mean temperature during each breakdown experiment differed, at most, by 2.6°C across streams and years (mean temperature for all streams and years = 7.1°C); within each stream, temperature changed <15% compared to pretreatment (Table 1). Mean discharge ranged from 4.1–20.0 L/s and changes in discharge ranged from 3% to 78% of pretreatment depending on stream and year (Table 1).

Litter breakdown rates

Across all five streams, maple and rhododendron breakdown rates were higher compared to PRE in YR1 and YR2 (Table 2). Rhododendron breakdown rates were affected by nutrients to a greater extent than maple, and were 3.1–6.4× higher in YR1 and 2.4–4.7× higher in YR2 than PRE. Maple breakdown rates were 1.1–1.8× higher in YR1, and 1.1–2.7× higher in YR2 than PRE. Two-year averages for increases in rhododendron breakdown rates tended to be highest in the two lowest N:P treatments (N:P = 2 and 8; rates were 4.5× and 4.9×, respectively), with decreasing response to nutrients in higher N:P treatments (Table 2). Two-year averages for increases in maple breakdown rates were highest when treatment N:P was 128 (2.2×), but breakdown rates also increased when treatment N:P was <16 (Table 2).

Litter stoichiometry

Maple and rhododendron C:N and C:P were reduced during YR1 and YR2 compared to PRE (Table 3) for all treatments, with relatively greater differences in C:P for both species. Rhododendron C:N and C:P decreased ~1.2–1.8× and 1.8–4.8× compared to PRE, which were relatively greater changes than those of maple. Maple C:N and C:P decreased ~1.2–1.4× and 1.1–2.5×, respectively (Table 3).

Path model: nutrient effects in an overall model

We arrived at a general model structure that indicated that the primary influences of nutrients on litter breakdown rate were propagated through effects on fungal biomass, conditioned litter stoichiometry, and shredders (Fig. 2a, b). The final model structure was similar to our original hypothesized model (Fig. 1), except for an added link between discharge and

TABLE 2. Mean breakdown rates (±SE) reported as decay coefficients (k, d^{-1}) of the negative exponential model.

		Maple k			Rhododendron k		
Target N:P	Year	Mean	±SE	YRx/PRE	Mean	±SE	YRx/PRE
2	PRE	0.0106	0.004		0.0019	0.000	
	YR1	0.0115	0.004	1.09	0.0099	0.003	5.27
	YR2	0.0215	0.005	2.04	0.0069	0.003	3.70
			mean:	1.56		mean:	4.48
8	PRE	0.0133	0.004		0.0047	0.001	
	YR1	0.0207	0.002	1.56	0.0300	0.001	6.36
	YR2	0.0252	0.000	1.90	0.0159	0.001	3.37
			mean:	1.73		mean:	4.86
16	PRE	0.0096	0.001		0.0020	0.000	
	YR1	0.0124	0.002	1.30	0.0083	0.002	4.06
	YR2	0.0191	0.003	2.00	0.0095	0.003	4.70
			mean:	1.65		mean:	4.38
32	PRE	0.0152	0.003		0.0039	0.001	
	YR1	0.0177	0.001	1.16	0.0210	0.006	5.35
	YR2	0.0166	0.002	1.09	0.0093	0.001	2.37
			mean:	1.12		mean:	3.86
128	PRE	0.0074	0.001		0.0035	0.001	
	YR1	0.0135	0.002	1.83	0.0109	0.002	3.08
	YR2	0.0195	0.003	2.65	0.0080	0.001	2.26
			mean:	2.24		mean:	2.67

Notes: Also reported are the YR1/PRE and YR2/PRE ratios (and their means) that indicate the multiplicative increase in breakdown rate between PRE and enrichment years (i.e., YRx/PRE = 2 indicates an increase in k by 2×).

TABLE 3. Mean litter C:P and C:N ratios on day 70 and standard error for *Acer rubrum* (maple) and *Rhododendron maximum* (rhododendron) leaves during PRE, YR1, and YR2.

		Litter C:P			Litter C:N		
Target N:P	Year	Mean	±SE	YRx/PRE	Mean	±SE	YRx/PRE
Maple							
2	PRE	2746	172		55	3	
	YR1	1102	33	0.40	40	2	0.72
	YR2	1448	255	0.53	42	3	0.75
8	PRE	2254	244		47	3	
	YR1	986	87	0.44	38	5	0.81
	YR2	1025	119	0.45	36	2	0.75
16	PRE	2124	201		51	3	
	YR1	1324	175	0.62	44	6	0.85
	YR2	1170	106	0.55	37	2	0.73
32	PRE	2107	211		51	3	
	YR1	1175	163	0.56	34	4	0.67
	YR2	1825	510	0.87	35	1	0.69
128	PRE	2331	123		52	5	
	YR1	1234	180	0.53	44	2	0.84
	YR2	2062	273	0.88	40	2	0.76
Rhododendron							
2	PRE	6223	683		112	1	
	YR1	1312	87	0.21	66	4	0.58
	YR2	1873	151	0.30	64	4	0.57
8	PRE	5827	614		73	23	
	YR1	1294	109	0.22	59	2	0.81
	YR2	2066	253	0.35	59	3	0.81
16	PRE	5023	217		103	3	
	YR1	1886	141	0.38	64	3	0.63
	YR2	2442	198	0.49	63	2	0.62
32	PRE	4430	384		101	6	
	YR1	1626	119	0.37	60	3	0.59
	YR2	1425	114	0.32	63	4	0.63
128	PRE	5026	72		113	n.a.	
	YR1	2875	90	0.57	69	1	0.61
	YR2	2293	645	0.46	64	2	0.57

Notes: Also reported are the YR1/PRE and YR2/PRE ratios, indicating the magnitude of change in C:P or C:N compared to PRE. n.a. means "not available."

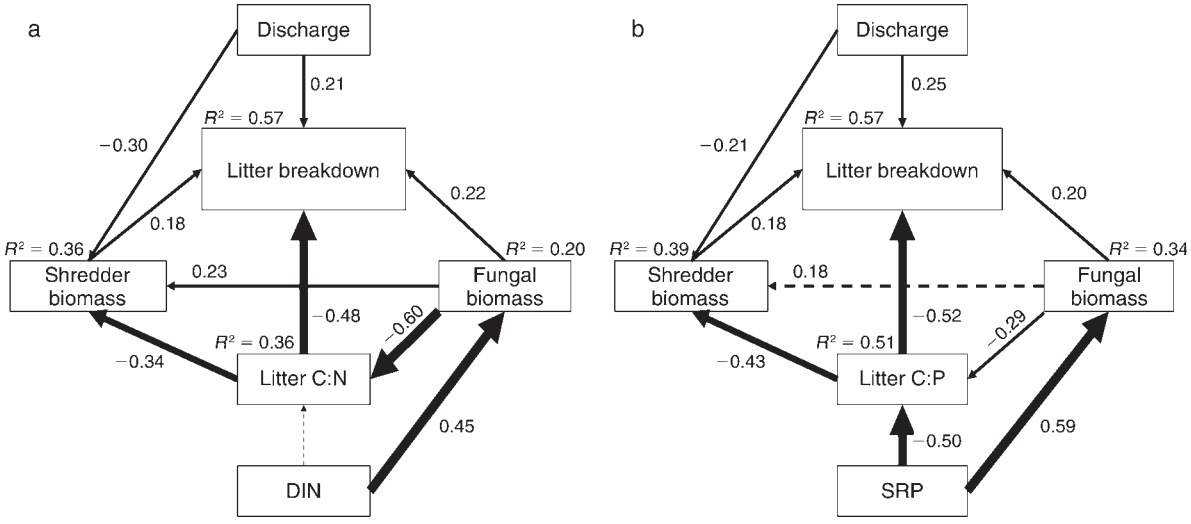

FIG. 2. The best-supported models for PRE, YR1, and YR2 relating (a) N or (b) P concentrations to drivers of litter breakdown rates. Standardized path coefficients are reported, and the sign of the coefficient indicates the direction of the correlation between variables. The models explained 57% of the variation in litter breakdown rates. Weights of the arrows correspond to path coefficients adjusted based on standard deviations, with strength of the correlations indicated by arrow width. Small, medium, and large arrows denote adjusted coefficients <0.30, <0.45, and >0.45, respectively. Dashed arrows indicate nonsignificant path coefficients.

shredders, and a pruned link between temperature and litter breakdown (Fig. 2a, b). Thus, candidate models maintained this general model structure, and excluded temperature as a predictor variable. We tested six candidate models containing N, P, N + P and C:N and C:P that had 13 to 15 path coefficient estimates (Appendix A: Table A1). Of these six candidate models, five were found to have good agreement between modeled and observed covariance matrices based on χ^2 tests (Appendix A: Table A1). These five models included the three models with litter C:N (and streamwater N, P, and N + P) and two models with litter C:P (and streamwater P and N + P) (Appendix A: Table A1). The model with the best support based on AIC included N and litter C:N ($\chi^2 = 9.3$, df = 5, $P = 0.10$; Appendix A: Table A1). Although the model with the most support based on AIC contained N and C:N, we also found support for a path model containing P and C:P based on χ^2 tests ($\chi^2 = 0.7$, df = 5, $P = 0.95$; Appendix A: Table A1).

We tested the importance of specific parameters (stoichiometry, discharge, fungal and shredder biomass) for the fit of the overall path model by fixing path coefficients to zero, and then ranked the importance of each parameter based on ΔAIC when the full and reduced models were compared. For both C:P/P and C:N/N models, removing any of the four parameters resulted in significantly reduced model fit (χ^2 difference test $P < 0.05$ in all cases; Appendix A: Table A2). For the C:P/P model, conditioned litter stoichiometry (C:P) was the most important parameter for model fit (ΔAIC = −47; Appendix A: Table A2), while for C:N/N, the most important parameter was fungal biomass (ΔAIC = −271; Appendix A: Table A2), followed by litter stoichiometry (C:N; ΔAIC = −34; Appendix A: Table A2).

Nitrogen effects on litter breakdown

Nitrogen concentrations affected litter breakdown rates through positive effects on fungal biomass, which were linked to decreases in litter C:N and positive indirect effects on shredder biomass. Overall, the C:N/N model explained 57% of the variation in litter breakdown rates, and 20%, 36%, and 36% of the variation in fungal biomass, shredders, and litter C:N, respectively (Fig. 2a). Streamwater N positively affected fungal biomass, which was linked to reduced litter C:N, that then positively affected litter breakdown rates through increased shredder biomass (Fig. 2a). There was a strong link between litter C:N and litter breakdown (Fig. 2a) and significant positive effects of fungi through C:N on litter breakdown (compound path = −0.60 × −0.48 = 0.3; $P < 0.05$). Fungal biomass, discharge, and shredder biomass had comparable influence on litter breakdown rates, but litter C:N was 2.2–2.7× more important compared to these three variables (Fig. 2a).

Phosphorus effects on litter breakdown

Similar to N effects on litter breakdown, P concentrations affected litter breakdown rates through fungal biomass, litter stoichiometry, and shredder pathways. Overall, the C:P/P model explained 57% of the variation in litter breakdown, and 34%, 39%, and 51% of the variation in fungal biomass, shredders, and litter C:P, respectively. Streamwater P positively affected fungal biomass, which was linked to reduced litter C:P, that

then positively affected litter breakdown rates through increased shredder biomass (Fig. 2b), although the strength of this path was lower compared to that of the C:N/N model. In contrast to the C:N/N model, the C:P/P model included a direct link between SRP and litter C:P, and there was a strong link between litter C:P and litter breakdown (Fig. 2b), and significant positive effects of fungi on breakdown rates via C:P (compound path $= -0.29 \times -0.52 = 0.15$; $P < 0.05$). As with the C:N/N model, fungal biomass, discharge, and shredder biomass had similar influence on litter breakdown rates, but litter C:P was 2.1–2.9× more important compared to these three variables (Fig. 2b).

Path models: single-year models

The models above include data from PRE, YR1, and YR2 together. These models reflect how similar variation in N and P concentrations, due to time or space, would affect litter breakdown. Insights into the effects of N vs. P were also obtained by contrasting model structure between PRE (no added nutrients) to YR1 or YR2. We analyzed the two best-supported models for C:N/N and C:P/P (Appendix A: Table A1) with each year treated as a subset of the data. For both C:N/N and C:P/P models, the model structure was consistent among years (C:P/P, $\chi^2 = 3.1$, 11.6, and 2.1 for PRE, YR1, YR2, df = 12; C:N/N, $\chi^2 = 5.4$, 4.7, and 8.0 for PRE, YR1, and YR2, df = 15; all $P > 0.05$). For the C:P/P model, we found significant reduction in model fit when path coefficients were held constant (χ^2 difference test; $P \ll 0.05$). We found marginal evidence for differences in model fit when path coefficients were held constant for the C:N/N model (χ^2 difference test; $P = 0.07$). Prior to enrichment for both the C:N/N and C:P/P models, conditioned litter C:N or C:P was the central predictor of litter breakdown rates and shredder biomass, which in this case was solely determined by litter species identity, not streamwater nutrient concentrations (Appendix A: Table A3). During the enrichment years for both C:P/P and C:N/N, fungal biomass, shredders, and discharge became stronger predictors of litter breakdown rates (Appendix A: Table A3). Conditioned litter C:P, and to some extent C:N, were weaker predictors of litter breakdown rates in YR1 and YR2 compared to PRE, but remained a nexus of the paths linking fungal biomass, shredders, and breakdown rates (Appendix A: Table A3). The amount of variation in litter breakdown rates explained by the single-year models differed from year to year, although in each case the models explained >30% of the variation in litter breakdown.

Discussion

Our study showed that streamwater N and P affected litter breakdown through stimulation of fungal biomass and changes in litter stoichiometry, which were associated with higher shredder biomass and litter breakdown rates. These effects occurred via multiple pathways and included a collection of interactions with litter stoichiometry at their center. Path analysis indicated that the strength of N and P as predictors of these C loss pathways was similar. Our study adds to evidence that N and P loading accelerates detrital C loss from ecosystems, thereby reducing standing stocks of an important energy source (Benstead et al. 2009, Suberkropp et al. 2010, Woodward et al. 2012), and reveals some of the fundamental mechanisms by which these effects occur.

Nitrogen and phosphorus effects on litter breakdown pathways

Previous studies have revealed that a key effect of nutrient enrichment of detritus-based systems is increased detrital quality for consumers, and our results emphasize the central importance of this effect for predicting litter breakdown rates (Cross et al. 2003, Rosemond et al. 2010, Scott et al. 2013). Based on the overall models, litter breakdown in streams could largely be predicted using conditioned litter stoichiometry (using either C:N or C:P) across gradients of low-to-moderate nutrient enrichment, given that large ranges in N and P availability will be reflected in corresponding gradients of litter C:N and C:P. By examining single-year path models, we were able to ascertain that the strongest driver of litter breakdown before nutrient enrichment was conditioned litter stoichiometry (C:N and/or C:P), owing to the large range in litter C:N and C:P content driven by species differences and associated microbial activity. During YR1 and YR2, we observed substantial decreases in conditioned maple and rhododendron litter C:N and C:P, by as much as 1.8× for rhododendron C:N and 4.8× for rhododendron C:P. As a result, litter species differences in terms of C:N and C:P were weaker predictors of litter breakdown rates during YR1 and YR2.

The streamwater nutrient-mediated convergence of C:P content of different litter species facilitated by microbial pathways is likely an important determinant of shredder biomass and activity, because of reduced consumer resource stoichiometric imbalances (Cross et al. 2003). Consumer resource imbalances are typically determined using threshold elemental ratios (TERs; Sterner and Elser 2002), in this case, the C:P or C:N threshold at which growth limitation by either element is minimized (e.g., Frost et al. 2006, Danger et al. 2013). The results of this study support TER predictions, as we observed the highest shredder biomass in litterbags containing litter with C:N and C:P content that approached or matched reported stream shredder TERs for C:N and C:P (Frost et al. 2006, Tant et al. 2013) (Appendix B: Fig. B1). The effects we observed based on fungal biomass measured at early stages of decay support the idea that the initial (approximately two-week) fungal colonization of litter is an important predictor of litter stoichiometry at later stages of decay, shredder colonization, and breakdown rates (Duarte et al. 2008, Sridhar et al. 2009).

Path analysis showed that N and P had similar effects on litter breakdown via both fungal biomass and litter stoichiometry, but the similar consequences of N and P on breakdown rates appear to be driven by subtly different mechanisms. The key difference between the overall C:N/N and C:P/P models was the inclusion of an apparent link between streamwater P and litter C:P in the overall C:P/P model. In contrast, litter C:N was not predicted by streamwater N, but was strongly predicted by fungal biomass. The differences in the models imply two alternative mechanisms driving the effects of N and P on litter breakdown. First, the absence of direct effects of streamwater N on litter C:N suggests that reductions in litter C:N are driven indirectly by positive N effects on fungal biomass. This result is consistent with previous studies that showed increased fungal biomass and increased litter N content due to elevated streamwater N (e.g., Ferreira et al. 2006, Rosemond et al. 2010). Microcosm studies complementary to this study also demonstrated that fungal growth rates were more strongly related to N concentrations compared to P, indicating that N may be more important for fungal biomass accrual on litter (V. Gulis, *unpublished data*). Second, the apparent direct effect of streamwater P on litter C:P in the overall model suggests that litter C:P and fungal biomass may be decoupled, presumably because fungi may exhibit flexible cellular C:P via P storage (e.g., as polyphosphate granules [Beever and Burns 1980; V. Gulis, *unpublished data*]). However, we cannot rule out increased litter P due to abiotic sorption, microbial community shifts (e.g., Gulis and Suberkropp 2004), or the effects of bacteria (but see Gulis and Suberkropp 2003, Tant et al. 2013).

Nutrient enrichment resulted in increases in litter breakdown rates via pathways that were driven by both microorganisms and shredders. Our findings illustrate that losses due to shredder feeding were stimulated by initial fungal colonization and subsequent changes in litter stoichiometry; thus it is difficult to adequately partition contributions by either microorganisms or shredders. Litter mass loss driven by microorganisms includes multiple mechanisms: production of microbial biomass, respiration, production of exoenzymes, and in the case of fungi, production of spores. We were not able to measure all of these microbial-driven C loss pathways, which together may result in high C loss, particularly in the early stages of litter breakdown, such that less litter C is subsequently available to shredders (Tant et al. 2015). However, comparing a primary measure of microbial-driven C loss, respiration, to shredder-driven C loss illustrates that losses directly attributed to microorganisms alone can be smaller than the effects of microorganisms and shredders combined. Specifically, we found estimated mass (milligrams of C per day) of maple and rhododendron litter respired by microorganisms or consumed by shredders increased by 1.7× and 9.4× under nutrient-enriched conditions, respectively. Our path analyses are consistent with this contrast illustrating the important interactions between microorganisms and shredders in driving litter breakdown rates, which resulted in greater C losses compared to the effect of one microbially driven pathway alone.

Overall effects of gradients of N and P on litter breakdown

Increased litter breakdown rates across the experimental gradient of N:P were likely because of similar effects of N and P on fungi, litter stoichiometry, and eventually shredders, demonstrating that rapid C loss from detritus-based aquatic ecosystems could occur in situations where either N or P is elevated relative to the other nutrient. Our experimental design included treatments with relatively low levels of added P relative to N and vice versa (e.g., ~430 μg N/L and 9 μg P/L vs. 100 μg N/L and 80 μg P/L), suggesting that large changes in breakdown can occur with elevated concentrations of one nutrient and minor alleviation of nutrient limitation by the other. For this reason, the ratio of nutrients was found to be a poor predictor of litter breakdown, as shown by stronger support for models containing N and P separately compared to N and P combined, and poor agreement between observed and modeled covariance matrices when N:P was used as a predictor (D. W. P. Manning and A. D. Rosemond, *unpublished data*).

Species-specific differences in initial litter nutrient ratios may have been important in the context of differential responses to N vs. P enrichment. Breakdown rates for both litter species were elevated across all nutrient treatments, but generally the highest breakdown rates for rhododendron occurred when streamwater P concentrations were greatest and the highest breakdown rates for maple occurred when streamwater N concentrations were greatest. Deficiencies in litter nutrient content may help explain these patterns. Rhododendron litter is much lower in P content than maple, and thus colonizing microorganisms require P from the water column, and respond most when it is available. Rhododendron litter gained much more P in low vs. high N:P treatments (~4× vs. ~2× increase in P content compared to PRE in N:P = 2, 128, respectively). Maple litter may have had adequate P availability for a stronger response to streamwater N in the high N:P treatment. Specifically, maple litter gained similar P content in both low and high N:P treatments (~2× vs. ~1.5× increase in P content compared to PRE in N:P = 2, 128, respectively). Thus, because rhododendron litter was initially more P deficient, differential changes to litter P content created a more defined gradient in litter P content compared to maple and potentially limited the increases in rhododendron breakdown rate where streamwater N:P treatments were high (128) and litter P gains were low.

Our results show that low-to-moderate enrichment of aquatic ecosystems with gradients of N and P concentrations caused substantial acceleration of C loss, and that streamwater N and P and associated effects on litter

C:N and C:P had similar magnitude effects on breakdown rates via microbial and detritivore pathways. We propose that dissolved N and P modulate litter breakdown rates through effects on fungal biomass and litter C:N (N-driven effects), as well as effects on litter C:P owing to abiotic or biotic P immobilization on detritus (P-driven effects). The N and P effects on litter stoichiometry appear to be important for shredder pathways, because litter C:N and C:P can be reduced to levels that approach shredder nutrient demand (Frost et al. 2006). The N and P concentrations we used for this study are comparable or lower than those observed in streams experiencing moderate land-use change across the southern Appalachians (Scott et al. 2002), and are in the lower range of continental nutrient gradients in the United States and Europe (Alexander and Smith 2006, Woodward et al. 2012). Mechanisms for accelerated litter breakdown described in this study likely occur in many systems with similarly elevated nutrient concentrations. Our results imply that elevated N and P throughout river networks could lead to increased litter breakdown rates, reduced C retention, and altered delivery of C to downstream ecosystems (Cole et al. 2007, Benstead et al. 2009, Woodward et al. 2012).

Acknowledgments

This work was supported by NSF (DEB-0918894 to A. D. Rosemond and J. C. Maerz, DEB-0918904 to J. P. Benstead, and DEB-0919054 to V. Gulis). This research leveraged logistical support from the CWT LTER Program at the University of Georgia, which is supported by NSF award DEB-0823293 from the Long Term Ecological Research Program (J. C. Maerz co-PI). Rob Case, Daniel Hutcheson, and Kevin Simpson of YSI Integrated Systems and Services implemented the infrastructure for the nutrient-dosing system. Aqueous ammonium nitrate was provided by The Andersons, Inc. through David Plank. We are grateful for data collection and maintenance of the experimental dosing system by Jason Coombs and Katie Norris. Christian Barrett, Phillip Bumpers, Jason Coombs, John Davis, Hannah Dolan, Kait Farrell, Tom Maddox, Chelsea Norman, Katie Norris, and James Wood helped with fieldwork or in the laboratory. The manuscript was improved by helpful comments from two anonymous reviewers, the Rosemond lab group, and Chao Song.

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Supplemental Material

Ecological Archives

Appendices A and B are available online: http://dx.doi.org/10.1890/14-1582.1.sm