Streamwater nutrients stimulate respiration and breakdown of standardized detrital substrates across a landscape gradient: Effects of nitrogen, phosphorus, and carbon quality

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Abstract: Elevated streamwater nitrogen (N) and phosphorus (P) concentrations can stimulate microbial activity on detrital C and accelerate its breakdown in stream ecosystems. Our study evaluated whether nutrient–detrital relationships are robust across a moderately altered land-use gradient and can be used to identify functional impairment of stream ecosystems. We tested the relative importance of N vs P as likely drivers of these responses, whether responses differed for labile or recalcitrant standardized substrates, and whether responses were detectable across streams with other stressors, which can potentially mask nutrient effects. Two studies were conducted in 23 sites in southeastern US streams. These streams differed in land use and exhibited low-to-moderate gradients in N and P. In study 1, we used 9 sites to compare the relationships between nutrient (N and P) concentrations and microbial respiration and breakdown of 2 standardized C substrates: recalcitrant oak wood veneer and labile cellulose sponge. Both of these substrates are low in nutrient content but differ structurally. In the best supported models, respiration and breakdown rates were positively related to streamwater P, but not N, after 4 wk of stream incubation. Microbial respiration increased 4.2 and 1.2× and breakdown increased 1.8 and 2.3× on cellulose and wood, respectively, across the P gradient. Temperature (+) and specific conductivity (−) were also in top models for wood respiration. Respiration and breakdown were highly correlated for both substrates, indicating the importance of microbial processing in driving breakdown rates. In study 2, we used 23 sites to test for association between landscape nutrient (N and P) gradients and wood veneer breakdown and whether detrital stoichiometry was a better predictor of breakdown than streamwater nutrient concentrations. Wood breakdown was related to P, but not N, and increased 4.1× in 12 wk across the P gradient. Wood nutrient content (increased %N and %P, reduced C:N and C:P) was also related to streamwater P and better predicted breakdown (C:P $r^2 = 0.75$, C:N $r^2 = 0.87$) than streamwater nutrient concentrations. Streamwater P concentrations appeared to stimulate breakdown to a degree that indicates impaired stream function. Our study showed that standardized detrital substrates responses to nutrients 1) were greater to streamwater P than N concentration gradients, 2) occurred on both labile and recalcitrant substrates, and 3) were detectable across landscape gradients with other stressors (e.g., temperature, specific conductivity). These responses likely reflect effects of excess nutrients on diverse C resources in these streams. Wood veneers integrated streamwater nutrient effects, were resistant to physical abrasion, and exhibited significant mass loss even when detritivores were excluded, indicating their value in stream functional assessments under a wide range of stream conditions.

Key words: streams, detrital carbon loss, detritus stoichiometry, nutrient pollution, wood veneer, cellulose sponge, Upper Oconee River Basin, Georgia USA

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Detrital C from terrestrial vegetation is a key energy source for aquatic organisms (Tank et al. 2010, Walther and Whiles 2011, Venarsky et al. 2017, Marks 2019). Detritus facilitates nutrient uptake and sequestration in stream ecosystems (Aldridge et al. 2009) but is altered in its quantity and quality by a variety of global change stressors. Stressors can reduce the diversity and quantity of detrital inputs and alter the retention and processing of detritus within aquatic ecosystems (Kominoski and Rosemond 2012). These stressors include the altered hydrology, increased temperature, and increased nutrient concentrations that are associated with land use and climate change. Stressor–detritus relationships that focus on nutrients are necessary to develop for streams, given the importance of detritus in supporting stream organisms and the role of detritus-based functions in protecting downstream ecosystems from nutrient enrichment (Wurtsbaugh et al. 2019).

To develop the use of detrital responses to assess effects of streamwater nutrient concentrations, other factors contributing to the integrative functions of detrital respiration and breakdown need to be considered. The main questions to address are whether all biological factors have been included and whether additional physical or chemical factors suppress or increase responses, potentially masking nutrient effects. These factors include the role of detritivores, which increase breakdown rates, and contaminants, which potentially suppress breakdown rates. Breakdown rates also typically increase with high flows and high temperatures (Paul et al. 2006, Manning et al. 2018). Biological, physical, and chemical factors affecting breakdown rates can also interact. For example, nutrient effects on detrital breakdown rates can change if toxins or other factors affect detritivore or microorganism activity (Woodward et al. 2012, Magali et al. 2016). Accordingly, tools to assess nutrient–detritus relationships that focus on microbial responses (e.g., respiration, microbially-driven breakdown) will be robust to factors that affect potentially more sensitive metazoan detritivores. In addition, quantifying and accounting for other factors that contribute to detrital processing will help in determining the effects of nutrients on detritus in field studies.

Characteristics of detrital substrates also affect nutrient–detritus relationships, including nutrient content and carbon lability. Standardized detrital substrates of low-nutrient content facilitate detection of nutrient effects (Chauvet et al. 2016). Microorganisms obtain nutrients from both the substrate and the water column, so microorganisms are more dependent on water column nutrients when substrate nutrient concentrations are low (Ferreira et al. 2006, Greenwood et al. 2007). Therefore, low-nutrient substrates facilitate detection of nutrient effects because they typically respond more to exogenous nutrients than high-nutrient substrates (Ferreira et al. 2015). Microbial sequestration of nutrients elevates N and P content and changes the carbon-to-nutrient stoichiometry of detrital materials (Manning et al. 2015). These changes, which will be greatest on low-nutrient substrates, can also be indicators of greater breakdown rates (Manning et al 2016).

The effects of nutrients may also differ based on other aspects of detrital quality. Because stream C resources vary widely in their composition, it is important to determine nutrient effects on a range of detrital materials. Typically, aspects of detrital quality, including lignin concentration, high concentrations of polyphenolics, and thick plant cuticle, can affect responses to nutrient enrichment and can be confounded with detrital nutrient content (Ferreira et al. 2015). Thus, to test the effects of C quality in this study, we used 2 substrates that differed in C lability (they were high vs low in lignin) but were similarly low in nutrient content.

The use of standardized substrates to determine stressor–detritus responses should also ideally reflect natural stream processes. Substrates such as cotton strips or agar-based pellets reduce the variability in functional responses and can identify factors that contribute to heterotrophic processes in streams, but they are limited in how well they can be extrapolated to natural system functions (Chauvet et al. 2016). Thus, results from low-nutrient standardized substrates such as wood may be best to use in functional assessments because these substrates are colonized and processed by stream organisms, may be comparable in responses to natural detrital materials, and are sensitive to water column nutrient availability (Chauvet et al. 2016).

We tested whether respiration, breakdown rates, and associated stoichiometric changes in detrital C have a consistent functional response to landscape-level enrichment. We did this with 2 complementary studies that examined detrital breakdown and respiration across a gradient of nutrient concentrations in the Upper Oconee River watershed in the southeastern US (Fig. S1). In the 1st study, we tested if respiration and breakdown rates of 2 low-nutrient C sources that differed in C quality (recalcitrant wood veneers and labile cellulose sponges) varied across a gradient in streamwater nutrient concentrations. This test determined whether nutrient effects were similar in magnitude on standardized substrates that differed in C quality. We hypothesized that the more labile substrate would respond more strongly than recalcitrant substrates, as they are more easily mineralized by microorganisms. Detritivores were excluded in this study to avoid their potentially-confounding effects. Our 2nd study quantified how respiration and breakdown rates of wood substrates varied across a broader range of N and P concentrations, water temperatures, and flow environments under natural field conditions, which also allowed colonization of detritivores. We used wood in this field test of detrital functional response to nutrients because wood is less influenced by hydrology and more likely to be adopted as an assessment tool than are cellulose sponges. We also tested whether changes in substrate nutrient content (e.g., C:N and C:P) were better predictors of breakdown than streamwater nutrients. We hypothesized that because C:N and C:P ratios integrate the colonization of microorganisms and uptake of streamwater nutrients over time rather than measurements of streamwater nutrients at limited time points, these responses would be more predictive of breakdown than streamwater nutrients alone. Such responses can give managers alternative tools (changes in detrital stoichiometry as well as breakdown rates) for functional assessments of nutrient effects in streams.

METHODS

Study sites

We studied streams and rivers with a variety of land uses, potential for nutrient inputs, and land-use-associated stressors in the Upper Oconee River watershed (Georgia, USA; Fig. S1). We studied 9 sites in study 1 and 23 sites in study 2 in unique locations in 15 streams (Table 1). The sites ranged from 1 to 45% impervious surface cover, 0 to 38% agricultural cover, 11 to 100% urban cover, and 0 to 76% forest cover. Watershed size ranged from 0.14 to 1185 km^2. For both studies, we calculated % land use/land cover from the National Land Cover Database's 2011 statistics (NLCD 2011). Urban land cover was calculated as the sum of all urban categories, forest as the sum of all forested categories, and agriculture/pasture as the sum of all agriculture, livestock, and pasture categories from the NLCD dataset (Table 1).

Study 1: N and P effects on microbial respiration and breakdown from substrates that differed in C quality

We quantified relationships between metabolic responses (respiration and breakdown rates) and gradients in dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) on 2 types of C-rich, nutrient-poor substrates across 9 sites. We measured these metabolic responses on standardized (2.5 × 2.5 cm) white oak wood veneers (Constantine's Wood Center, Fort Lauderdale, Florida) and reinforced cellulose sponge (sponge cloth; The Coburn Company, Whitewater, Wisconsin). The wood veneers had a starting C:N of 167, a starting C:P of 10,215, and were 45% C. The cellulose sponges had a starting C:N of 466, a starting C:P of 38,750, and were 30% C (see Martínez et al. 2017). In comparison, leaf litter generally has a C:N of ~40 to 120 and a C:P of ~1500 to 8000 (Manning et al. 2016). Thus, both substrates were lower in nutrient content than most leaf litter. We created 1 array of 16 wood veneer pieces and 1 array of 16 cellulose sponge pieces by attaching the substrate pieces to glass slides and suspending them inside a PVC channel ~45 cm long and 15 cm wide. We covered each array with a 250-μm nylon mesh cover to reduce scour and exclude consumers (Fig. S2). One array for each substrate was placed ~15 cm below the water surface at each site, and we removed accumulated debris weekly. Arrays were deployed between 16 June and 16 July 2015. Although mass loss of substrates was primarily microbially driven and could be referred to as decomposition, we used the term breakdown to express mass loss over time.

We sampled each site once every wk for 4 wk. Each wk we randomly selected 4 wood and 4 cellulose pieces from each array. Substrates were removed from the stream, placed in centrifuge tubes with stream water, and transported on ice to the lab where we measured respiration rates within 3 h of collection (see methods below). We measured stream temperature, pH, dissolved oxygen (DO), and specific conductivity (SPC) weekly (n = 5, wk 0–4). At wk 0, 2, and 4, we collected 40-mL water samples and filtered them through 0.45-μm nitrocellulose membrane filters (Millipore, Billerica, Massachusetts) into acid-washed polypropylene bottles in the field. We placed the water samples on ice and froze them until processing.

We used the water samples collected on wk 0, 2, and 4 to measure DIN (NH_4-N + NO_3-N) and SRP (PO_4-P) concentrations with continuous flow colorimetry with an Alpkem Rapid Flow Analyzer 300 (College Station, Texas) at the University of Georgia Center for Applied Isotope Studies in Athens, Georgia. Unpublished data on total nitrogen–DIN and total phosphorous–SRP relationships (from samples taken in the same year for a different study in the Upper Oconee Watershed) indicate dissolved and total nutrients are relatively similar within a given stream (more so for N than for P) (total nitrogen = 1.075 [DIN] + 249.66; r^2 = 0.93, n = 36; total phosphorous = 0.744 [SRP] + 17.93, r^2 = 0.28, n = 36) (SJW, PMB, and ADR, unpublished data). Thus, relationships we report based on dissolved nutrient concentrations might be generally applicable to total nutrient concentrations.

We measured substrate-specific respiration rates as the change in mg DO g^{-1} ash-free dry mass (AFDM) h^{-1} in a walk-in incubator maintained at 20°C (the ~mean stream temperature during our study) without light. We placed each substrate in a 30-mL glass respiration chamber filled with streamwater from the respective study site. We recorded DO readings every ~5 min over a 25 to 35 min period with a YSI™ 5100 Dissolved Oxygen Meter (Yellow Springs, Ohio). We also measured respiration in chambers with streamwater but no substrate to account for ambient streamwater respiration (see Statistical analyses). Both wood and sponge substrates were then dried for 24 h at 60°C, weighed, ashed at 500°C for 4.5 h, and weighed again to determine AFDM.

Study 2: Response of wood nutrient content and breakdown to changes in streamwater nutrient concentrations

We conducted our 2nd study the year following our 1st study at a greater number of sites (23) including the 9 sites from study 1. This 2nd study further tested 1) whether there were discernable relationships between detrital breakdown and streamwater nutrient concentration and 2) whether breakdown was associated with changes in wood nutrient content. Here, we used coarse mesh to hold the wood veneers to

Table 1. Mean (±SE) site characteristics for the 9 sites used in study 1 (A) and the 23 sites used in study 2 (B) located in the Upper Oconee River watershed, Georgia, USA. Physical and chemical characteristics measured include temperature (Temp; °C), specific conductivity (SPC; μs/cm), soluble reactive phosphorus (SRP; μg/L), and dissolved inorganic nitrogen (DIN; μg/L). Study 1 Temp and SPC are averages from wk 0 to 4 ($n = 5$), whereas SRP and DIN are averages from wk 0, 2, and 4 ($n = 3$), and all data were collected between 23 June and 16 July 2015. In study 2, Temp, SPC, SRP, and DIN represent the averages from wk 0, 8, and 12 ($n = 3$), and all data were collected between 24 January and 8 April 2016. For both studies we calculated % land use/land cover from NLCD (2011) data (ISC = % impervious surface cover). Urban is the sum of all urban categories, forest is the sum of all forested categories, and agriculture/pasture (Ag) is the sum of all agriculture, livestock, and pasture categories from the NLCD dataset. Watershed area (Area) is shown for each sample site (km^2).

Site	Temp	SPC	SRP	DIN	ISC	Urban	Forest	Ag	Area
A									
BRC	20.8 (0.3)	47.5 (0.5)	5.7 (1.0)	188.1 (39.7)	1	11	76	2	2.10
BIG 2	22.5 (0.4)	140.8 (2.9)	8.6 (1.3)	570.1 (98.6)	2	11	35	38	22.95
TAL	21.9 (0.4)	32.4 (0.3)	2.4 (1.1)	254.9 (12.0)	3	24	57	12	1.99
MCN1	23.2 (0.3)	53.4 (2.9)	5.3 (0.7)	319.6 (68.2)	8	32	32	23	149.22
HUN	22.7 (0.3)	88.8 (2.9)	16.0 (3.6)	408.8 (42.5)	19	70	27	0	6.62
BRY	22.6 (0.3)	140.8 (2.9)	16.2 (1.7)	925.0 (231.3)	21	81	13	3	3.60
CAR	22.9 (0.3)	393.0 (86.6)	3.8 (0.2)	2485.2 (495.3)	23	67	13	0	4.04
TAN 1	22.9 (0.3)	199.0 (1.9)	31.4 (2.1)	2061.7 (429.6)	41	99	1	0	2.12
LIL	23.6 (0.3)	184.8 (3.4)	16.9 (3.7)	1600.9 (145.1)	42	98	2	0	1.53
B									
BRC	13.1 (3.0)	36.9 (5.2)	4.5 (0.6)	198.4 (43.1)	1	11	76	2	2.10
BIG 2	10.7 (2.1)	65.8 (8.4)	5.0 (0.4)	798.8 (186.1)	2	11	35	38	22.95
TAL	12.3 (2.1)	27.9 (0.6)	3.8 (0.5)	396.5 (195.4)	3	24	57	12	1.99
NOR 1	11.9 (2.6)	68.9 (14.5)	8.0 (2.0)	1108.3 (209.8)	4	17	44	24	756.70
NOR 2	12.0 (2.6)	69.9 (6.5)	7.8 (3.5)	1339.5 (232.1)	4	18	44	24	767.13
SHC	10.2 (1.6)	42.5 (7.3)	4.3 (0.2)	458.6 (121.5)	4	27	27	35	2.39
MCN 3	12.4 (2.2)	51.0 (6.0)	4.0 (0.8)	356.9 (4.8)	6	30	39	20	15.55
MID 3	13.1 (3.5)	48.5 (19.5)	5.7 (0.6)	1084.6 (81.2)	6	26	40	22	914.69
MID 1	12.3 (2.7)	75.0 (3.8)	5.9 (0.4)	1286.3 (64.4)	7	27	40	21	1028.91
MID 2	13.3 (3.5)	68.3 (18.8)	11.7 (0.9)	702.4 (235.6)	7	28	39	21	1185.02
MCN 1	10.1 (2.4)	40.4 (9.4)	6.5 (1.5)	638.4 (137.0)	8	32	32	23	149.22
TUR	12.1 (2.4)	53.4 (4.0)	5.7 (0.3)	325.3 (54.2)	8	42	44	6	10.04
MCN 2	10.6 (2.5)	48.2 (8.2)	4.9 (1.3)	772.9 (186.6)	10	42	32	16	27.87
LHR	11.5 (2.7)	52.4 (2.3)	3.5 (0.6)	778.8 (362.2)	13	53	43	0	0.37
HUN	12.1 (2.3)	79.8 (2.6)	17.2 (3.4)	571.2 (9.4)	19	70	27	0	6.62
BRY	12.2 (1.7)	123.9 (5.0)	8.2 (1.2)	537.1 (72.3)	21	81	13	3	3.60
BRK2	15.9 (1.9)	160.9 (5.3)	4.5 (0.8)	1097.8 (224.6)	22	97	3	0	0.93
CAR	10.8 (2.4)	441.6 (6.1)	6.7 (3.1)	2355.4 (1077.0)	23	67	13	0	4.04
TAN 2	13.3 (1.4)	120.5 (5.9)	3.9 (0.5)	1705.0 (312.9)	26	97	3	0	0.44
BRK1	12.5 (1.4)	87.5 (0.9)	6.7 (0.8)	682.8 (61.6)	32	100	0	0	0.14
TAN 1	13.0 (2.0)	196.9 (12.3)	3.6 (1.9)	1741.3 (290.6)	41	99	1	0	2.12
LIL	13.1 (2.0)	219.7 (15.6)	1.4 (1.4)	1727.6 (656.2)	42	98	2	0	1.53
TAN 3	13.2 (1.5)	182.9 (15.4)	7.7 (0.3)	2078.2 (1118.6)	45	100	0	0	0.83

allow for a more realistic assessment of other stream conditions such as invertebrate colonization and flow. In each stream, we enclosed 4 standardized white oak wood veneers (2.5 × 10 cm) in commercially-available coarse plastic mesh (used for rain gutters; 1-cm^2 mesh, 300-cm^2 section) secured to the stream bed with rebar. These substrates remained submerged for the duration of the study (Fig. S3). We deployed wood veneers between 24 and 29 January 2016 and retrieved them between 28 March and 8 April 2016. We collected data on physical and chemical variables (temperature, pH, DO, and SPC) and water samples for nutrient analyses at wk 0, 8, and 12. We processed and analyzed water samples as described above in study 1.

At the end of the ~12-wk incubation period, we removed wood veneers from the mesh and lightly rinsed them with tap water in the lab to remove any inorganic sediment deposition. Samples were dried at 60°C for 48 h, weighed, and then ground in a ball pestle impact grinder for 60 s. We measured C and N concentrations of each sample with a Carlo Erba 1500 CHN Analyzer (Milan, Italy). We measured P content with the plant dry ash/acid extraction method followed by spectrophotometric analysis of the extracted solution with the ascorbic acid method (Allen et al. 1974, APHA 1998). Nutrient content analysis was done at the University of Georgia Center for Applied Isotope Studies.

Statistical analyses

In study 1, weekly site respiration was based on the average of 4 individual wood or cellulose sponge samples. We calculated microbial respiration (mg O_2 g^{-1}AFDM h^{-1}) for each sample from the slope of the DO readings collected over the incubation period, adjusted for meter drift and chamber volume, and divided by AFDM. For each substrate, we used mixed-effects regression analysis to determine the relationship between d 28 respiration and average concentrations of DIN and SRP. We then determined the best predictor of wk 4 (d 28) respiration by comparing mixed-effects models that included temperature, DIN, SRP, and SPC. We standardized these predictors by subtracting the mean and dividing by the standard deviation. We included SPC as a proxy of urban pollution that might suppress microbial activity. We compared models of all additive permutations of DIN, SRP, SPC, and temperature except models that included both SPC and DIN because these variables were collinear ($r = 0.96$; correlations above 0.7 were excluded). All models included site as a random effect. We ranked the models based on Akaike Information Criterion adjusted for small sample size (AIC_c). We also calculated the marginal and conditional R^2 as a metric of goodness-of-fit of each model. The marginal R^2 describes the variance explained by the fixed effects and the conditional R^2 includes the fixed plus random effects (Nakagawa and Schielzeth 2013). We calculated variance inflation factors for all models with more than 1 predictor variable to assess multicollinearity.

We examined breakdown of cellulose and wood substrates from study 1 based on 4 samples of % AFDM remaining of each substrate on sampling wk 0 through 4. We used hierarchical Bayesian models to calculate breakdown rates (as k/d) while simultaneously testing the ability of predictor variables to explain variation in breakdown rates among sites. Models had the form:

$$\ln(R_{i,t}) = \ln(100) + k_i \times day_{i,t} + \varepsilon_1 \qquad \text{(Eq. 1)}$$

$$k_i = b0 + b1 \times x + \varepsilon_2, \qquad \text{(Eq. 2)}$$

where ln(R) is the natural log of the % mass remaining in site (i) and time (t), k is the decay rate for i, day is the number of days after the start of the experiment the sample was taken, x is the predictor variable to be tested, $b0$ (intercept) and $b1$ are parameters to be estimated, and ε_1 and ε_2 are normally-distributed error terms. We tested the same 4 standardized predictor variables used in the prior analysis (SRP, DIN, SPC, and temperature), but here we tested each predictor variable individually rather than in combination to limit model complexity. We limited model complexity for this analysis because there was only 1 estimate of breakdown rate/site, so the sample size was low ($n = 9$). Models were fit in JAGS software (version 3.4.0; Plummer 2003) and the R package *runjags* (Denwood 2013) with 5 chains and a burn-in period of 10,000 iterations, each followed by 200,000 iterations to estimate posterior distributions. Parameter estimates were considered significant if the 95% credible interval around the mean did not include 0. We also tested the relationship between respiration and breakdown rates for both substrates with mixed-effects linear regression (see Fig. S4).

For study 2, we calculated breakdown as % mass loss of wood veneers as ((initial dry mass − final dry mass)/initial dry mass) × 100 of each veneer. We used mixed-effects regression analysis to determine the relationship between either streamwater nutrients or wood veneer stoichiometry (%P, %N, C:N, C:P) and % mass loss. We also used mixed-effects regression and AIC_c to ascertain the best predictors of % mass loss of wood veneers and the best predictors of wood C:N, C:P, and N:P at the end of the study period. For wood stoichiometry, we considered a suite of 6 models that included SRP, DIN, SPC, and additive combinations of each (excluding variable groupings that were highly correlated [$>r = 0.70$]; Table S3). We included site as a random effect and analyzed each suite of models separately for C:N, C:P, and N:P. We determined the relationship between % mass loss and streamwater nutrients with mixed-effects linear regression with individual substrate samples as well as basic linear regression with site-level means. To determine if streamwater nutrients or substrate stoichiometry was a better predictor of % mass loss, we again used AIC_c model selection and considered a suite of 26 (Table S4) competing models that included DIN, SRP, C:N, C:P, and SPC. To simplify interpretations, we included only 1 wood stoichiometry and 1 dissolved nutrient predictor in a given model and did not include N:P in any models. We also included temperature and SPC to account for differences among sites. DIN and SPC were not included in the same models because they were correlated ($r = 0.72$). Wood stoichiometry variables were natural-log transformed to meet assumptions of linearity. All predictor variables were standardized by subtracting the mean and dividing by the standard deviation. We calculated variance inflation factors for all models with more than 1 predictor variable to assess multicollinearity. All analyses were done in R (version 3.5.1; R Project for Statistical Computing, Vienna, Austria) with the *lme4* package (Bates et al. 2015).

RESULTS

Study 1: Substrate respiration and breakdown responses to streamwater nutrients

The 9 study sites had average DIN concentrations ranging from 188 to 2485 μg/L and average SRP concentrations ranging from 2 to 31 μg/L. Average SPC ranged from 32 to 393 μs/cm, streamwater temperatures ranged from 20.8 to 23.6°C, and pH was roughly circumneutral across sites (Table 1A).

Respiration and breakdown rates of wood and cellulose sponge tended to be positively correlated with streamwater SRP across the nutrient gradient but weakly correlated with DIN (Table 2, Fig. 1A–D). For both cellulose sponge and wood, the best supported models to predict respiration rates included a positive effect of SRP (Tables 3, S1). The top model for wood respiration included a negative effect of DIN and a positive effect of temperature in addition to a positive effect of SRP (Table 3). Alternative models were also plausible, especially for wood respiration. The best supported model for wood respiration had an AIC weight of 0.24, and alternative models that omitted SRP or omitted temperature had weights of 0.10 (Table S1). For cellulose respiration, however, SRP appeared in all models with a weight greater than 0.01 (Table S1). For breakdown rates of both cellulose sponge and wood, SRP was the only predictor with credible intervals that did not include 0 (Tables 4, S2). Variance inflation factors for all parameters for all models were <2, indicating limited effect of multicollinearity on inferences.

P appeared to stimulate respiration and breakdown rates of both substrates, but to differing degrees. P concentrations were more strongly related to cellulose sponge respiration than wood respiration (4.2 vs 1.2×) but were more strongly related to wood breakdown rates than cellulose sponge breakdown rates (2.3 vs 1.8×; Fig. 1A, C). Overall, cellulose had much higher rates of breakdown and respiration than wood, as predicted based on its higher lability (Fig. 1A, C).

We examined the relationship between respiration and breakdown for both substrates by converting breakdown rates to g C/d (C mass loss) and respiration rates to g C/d (C respired). The relationship between C mass loss and C respired was significantly positive for both substrates (overall model: marginal $R^2 = 0.97$; Fig. S4). Additionally, for both substrates the C respired was often greater than could be accounted for based on C mass loss. This difference occurred at all sites for wood but at only 5 sites for cellulose (as indicated by points below the 1:1 line in Fig. S4). The amount of C respired that was greater than the amount of C mass loss could have resulted from overestimates of respiration because of the aggregation of respiration data, which was extrapolated over 28 d. Alternatively, this discrepancy could be from microorganisms associated with the substrates respiring other forms of C (e.g., water-derived dissolved organic carbon). Wood had a significantly-steeper slope than cellulose sponge (cellulose sponge slope = 0.172, t-value = 7.5; wood slope = 0.522, t-value = 3.3; Fig. S4), indicating that respired C and C mass loss were more tightly coupled for wood than cellulose. Four of the cellulose values indicated greater C mass loss than respired C (as indicated by points above the 1:1 line in Fig. S4). It is likely that physical abrasion caused greater mass loss in these instances because we observed very few invertebrates colonizing these substrates and cellulose sponges were physically fragile. The average mean mass loss in the 28-d period was 55% for cellulose sponge and 8.5% for wood.

Study 2: Wood nutrient content and breakdown responses associated with streamwater nutrients

Over the sampling period, the 23 study sites in study 2 had DIN concentrations ranging from 198 to 2078 μg/L and SRP concentrations from <2 to 17 μg/L. Average SPC ranged from 28 to 442 μs/cm, and streamwater temperatures ranged from 10.1 to 15.9°C (Table 1B).

The best supported models indicated that streamwater SRP, but not DIN, was related to changes in the nutrient content of wood veneers (Table 2, Fig. 2A–H). Variance inflation factors for all parameters for all models were <2, indicating limited effect of multicollinearity on inferences. SRP concentration tended to be positively related to %N and %P and negatively related to wood veneer C:N (Table 2, Fig. 2B, D, F, H). Wood C:P and N:P were best predicted by the model that included only streamwater SRP, although SRP explained little variance and was only marginally better than the intercept-only model for C:P (C:P marginal $R^2 = 0.10$, N:P marginal $R^2 = 0.17$; Tables 5, S3). Wood C:N was best predicted by the model that included SRP and SPC, which explained 34% of the variance in wood C:N (marginal $R^2 = 0.34$; Tables 5, S3). The ratio of C:N decreased as SRP increased and SPC decreased. The model that included just SRP carried similar AIC weight but explained only half of the variation. Streamwater N concentrations alone were not strongly correlated with any measure of wood nutrient content (Tables 2, S3, Fig. 2A, C, E, G). Wood C:N was reduced to a greater extent (47%) than C:P (30%) relative to initial stoichiometry, although C:P exhibited greater absolute change. Across all sites, wood veneer C:N and C:P reached values of 88 and 7166, respectively, compared with initial values (mean ± SE) of C:N = 167 ± 10 and C:P of 10,215 ± 1188.

Across the study sites, % mass loss of wood veneers ranged from slight gains (~5% gain) to larger losses (45% loss). Breakdown of wood veneers was related to both wood nutrient content and streamwater nutrients. The natural log of wood C:P was negatively correlated with breakdown

Table 2. Study 1 mixed-effects regression parameter estimates (not standardized) between soluble reactive phosphorus (SRP) or dissolved inorganic nitrogen (DIN) concentration and wk 4 microbial respiration rates (mg O_2 g^{-1} AFDM h^{-1}) of wood and cellulose sponge substrates. Study 1 took place at 9 sites over 4 wk; SRP and DIN are averages from wk 0, 2, and 4 ($n = 3$). Parameter estimates of factors predicting breakdown rates from study 1 were analyzed differently and are shown in Table 4. Study 2 mixed-effects regression parameter estimates (not standardized) between SRP or DIN concentration and wood veneer %P, %N, C:P, and C:N, and % mass loss ([initial mass − final dry mass] / initial mass) × 100 are also shown. Study 2 took place at 23 sites over 12 wk; SRP and DIN are averages from wk 0, 8, and 12 ($n = 3$). We also report the intercept and slope estimates (and SE), t-values, and marginal (R^2 marg) and conditional R^2 (R^2 cond). All models included a random effect of site.

Study/Response	Parameter	Estimate	SE	t	R^2 marg	R^2 cond	Parameter	Estimate	SE	t	R^2 marg	R^2 cond
Study 1 wk 4			**SRP**						**DIN**			
Wood respiration	intercept	0.067	0.025	2.7			intercept	0.110	0.003	3.7		
	slope	0.004	0.001	2.2	0.30	0.72	slope	0.000003	0.00002	0.13	0.001	0.71
Cellulose respiration	intercept	0.086	0.044				intercept	0.021	0.007	2.8		
	slope	0.014	0.003	4.5	0.52	0.65	slope	0.00004	0.00005	0.7	0.04	0.64
Study 2, wk 12												
Wood %P	intercept	−0.0001	0.006	0.02			intercept	0.026	0.008	3.3		
	slope	0.005	0.0009	4.99	0.5	0.95	slope	0.00001	.000007	0.2	0.002	0.95
Wood %N	intercept	0.406	0.088	4.6			intercept	0.719	0.0968	7.4		
	slope	0.047	0.013	3.66	0.34	0.89	slope	−0.0003	0.00008	0.30	0.004	0.88
Wood C:P	intercept	10920	2429	4.5			intercept	7287	2262	3.2		
	slope	−605	351	1.72	0.1	0.87	slope	−0.122	1.97	0.06	0.001	0.87
Wood C:N	intercept	115	13	8.4			intercept	83	13	6.3		
	slope	−4.46	1.98	2.2	0.17	0.9	slope	0.005	0.01	0.4	0.008	0.9
Wood % mass loss	intercept	3.93	4.31	0.9			intercept	18.5	4.64	3.9		
	slope	2.14	0.62	3.4	0.32	0.94	slope	−0.0013	0.004	0.32	0.004	0.94

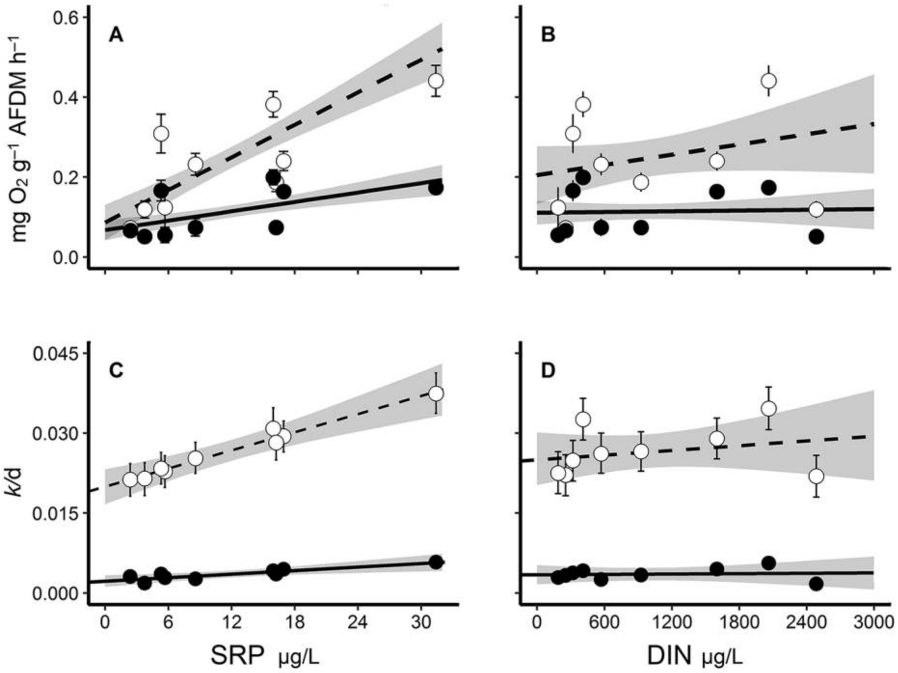

Figure 1. Study 1 relationships between mean d 28 microbial respiration (mg O_2 g^{-1} AFDM h^{-1}; ±SE) and stream water soluble reactive phosphorus (SRP) (A) and dissolved inorganic nitrogen (DIN) (B) and between mean breakdown rate (k/d; ±SE) and SRP (C) and DIN (D). Results for wood substrates are represented by a solid line. Cellulose sponge substrates are represented by a dashed line. SRP and DIN represent an average of 3 samples taken over the study period at wk 0, 2, and 4 (n = 3). Shading indicates the 95% confidence interval. See Table 2 for linear regression results. See Table 4 for breakdown rate parameter estimates.

(parameter estimate = −10.3, t = −7.6; Fig. 3A). The natural log of C:N ratio was also negatively correlated with breakdown (parameter estimate = −20.3, t = −9.7; Fig. 3B) and explained 6% more of breakdown than did C:P (marginal R^2 = 0.62 vs 0.56, respectively). The N:P of wood veneers was a poor predictor of breakdown within a site, as evidenced by the relatively-low parameter estimate (−3.0) and the low marginal R^2 of 0.013 based on the mixed-effect model that used individual substrates. However, when analyzed with site level means, breakdown was negatively related to all

Table 3. Study 1 standardized mixed-effects regression parameter estimates for models with Akaike Information Criterion (AIC) less than from the top model for predicting microbial respiration rates (mg O_2 g^{-1} AFDM h^{-1}) on wood veneer and cellulose sponge substrates across 9 sites after 4 wk. Predictor variables included in these models were: soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), stream water temperature (Temp), and specific conductivity (SPC). Averaged values of variables as described in Table 1 were used in the models. All permutations of these variables were tested except combinations with both DIN and SPC because they were collinear (r = 0.96). Thus, we evaluated 12 total models. We report the marginal R^2 (R^2 marg), conditional R^2 (R^2 cond), intercept and slope estimates with SE, and t-values. The 2 models listed are the top models for wood and cellulose respectively. See Table S1 for AIC results of all candidate models.

Model	Intercept	DIN	SRP	Temp	SPC	R^2 marg	R^2 cond
Wk 4 wood respiration							
SRP + DIN + Temp	0.114 (0.010)	−0.031 (0.012)	0.034 (0.011)	0.038 (0.012)		0.57	0.72
SPC + SRP + Temp	0.114 (0.010)		0.026 (0.011)	0.036 (0.012)	−0.027 (0.012)	0.56	0.72
SPC + Temp	0.114 (0.015)			0.046 (0.015)	−0.027 (0.015)	0.43	0.72
SRP	0.114 (0.015)		0.035 (0.015)			0.30	0.72
Temp	0.114 (0.015)			0.034 (0.015)		0.28	0.72
SRP + Temp	0.114 (0.013)		0.026 (.014)	0.024 (0.014)		0.42	0.72
Wk 4 cellulose respiration							
SRP	0.247 (.026)		0.121 (0.027)			0.52	0.65

Table 4. Study 1 mean, standard deviation (SD), and 95% credible intervals for parameter estimates of candidate variables to predict wood and cellulose breakdown rates. Each variable was tested independently (i.e., each row represents a distinct model). Variables included soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), specific conductivity (SPC), and water temperature (Temp). Averaged values of variables as described in Table 1 were used in the models. All values are $\times10^{-3}$. See Table S2 for estimated k of each model and site.

Predictors of k/d	5%	Mean	95%	SD
Wood				
SRP	0.353	1.024	1.72	0.341
DIN	−1.107	0.102	1.317	0.607
SPC	−1.396	−0.171	1.045	0.606
Temp	−0.723	0.435	1.580	0.574
Cellulose				
SRP	3.163	5.235	7.314	1.041
DIN	−2.175	1.178	4.500	1.700
SPC	−3.321	0.038	3.385	1.703
Temp	−1.047	2.270	5.497	1.662

3 substrate nutrient content measurements (Fig. 3A–C). Again, C:N explained the most variation ($R^2 = 0.87$), followed by C:P ($R^2 = 0.75$) and N:P ($R^2 = 0.53$). Breakdown was correlated with streamwater SRP ($t = 3.4$; however, with 2 strong leverage points, Fig. 4B) but not with DIN concentrations ($t = -0.32$; Table 2, Fig. 4A). Breakdown of wood veneers in the highest P site (40.7%) was 4.1× higher than breakdown in the lowest P site (9.9%).

When we evaluated models of breakdown to determine the relative influence of wood nutrient content vs streamwater nutrients, wood nutrient content was the better predictor variable. Breakdown was best predicted by the model that included wood C:N and streamwater SRP concentration (Table 6). Substrate C:N was included in the top 8 models (Table S4). SPC and temperature were included with C:N in the 2nd and 5th best models, but only the 2nd model had a AIC_c <2 from the top model (Table S4). Temperature had a small effect relative to SRP and C:N in the 2nd best model. Wood C:P alone explained 56% of the variation in breakdown but was not included in a model that received any weight (Table S4).

DISCUSSION

Streamwater P was associated with increased microbial processing and subsequent breakdown of standardized detritus substrates in our studies, indicating that nutrients may stimulate processing of diverse C resources in streams. These associations were of relatively-high magnitude and were detectable across a landscape with moderate variation in land use. The observation of elevated detrital breakdown with higher P concentrations in our study is consistent with impaired stream function in the higher-nutrient streams (e.g., >2× change; Gessner and Chauvet 2002). Streamwater nutrients were more strongly associated with C breakdown of recalcitrant substrates than of labile substrates,

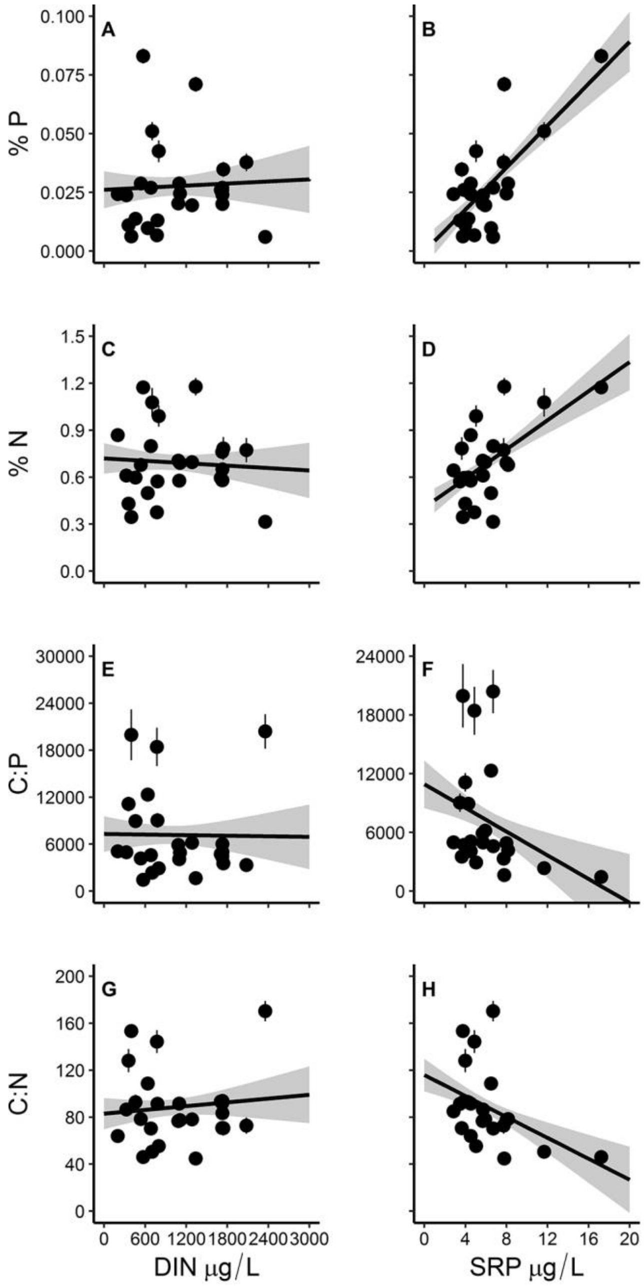

Figure 2. Relationships between wk 12 average (±SE) %P (A and B), average (±SE) %N (C and D), average (±SE) C:P (E and F), and average (±SE) C:N (G and H) ratio of 4 wood veneers with average stream water dissolved inorganic nitrogen (DIN; left columns) and soluble reactive phosphorus (SRP; right columns). SRP and DIN represent an average of 3 samples taken over the study period at wk 0, 8, and 12 ($n = 3$). Shading indicates the 95% confidence interval from the mixed effects model. See Table 2 for linear regression results.

Table 5. Study 2 standardized mixed-effects regression parameter estimates for the top models predicting C:N, C:P, and N:P of wood veneer substrates across 23 sites after 12 wk. Shown are models with an Akaike Information Criteria (AIC) less than 2 from the top model. Predictor variables included soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), and specific conductivity (SPC). Averaged values of variables as described in Table 1 were used in the models. DIN and SPC were not included in the same model because they were correlated ($r = 0.72$), so we evaluated 6 models for each response variable. Each model included site as a random effect. The marginal R^2 (R^2 marg), conditional R^2 (R^2 cond), parameter estimates, standard error (SE), and *t*-values are included in this table. See Table S3 for AIC results of all models.

Model	Intercept	DIN	SRP	SPC	R^2 marg	R^2 cond
C:P Wood						
SRP	7166 (1082)		−1877 (1088)		0.1	0.87
Intercept	7166 (1150)				0	0.87
SRP + SPC	7166 (1063)		−1849 (1069)	963 (1069)	0.13	0.87
C:N Wood						
SRP + SPC	88.21 (5.79)		−13.55 (5.82)	9.85 (5.82)	0.34	0.9
SRP			−13.83 (6.17)		0.17	0.9
N:P Wood						
SRP	72.482 (5.51)		−12.31 (5.53)		0.15	0.79
DIN + SRP	72.482 (5.35)	−6.27 (5.41)	−12.92 (5.41)		0.19	0.79

but in both substrates we detected a relationship with nutrients despite variation in other potential stressors (e.g., SPC, temperature). Temperatures were typically positively related with both respiration and breakdown, indicating that they likely stimulated microbial activity. In contrast, increased SPC was negatively related to both wood respiration and increased wood C:N, possibly indicating suppression of microbial activity. Our 1st study showed that respiration and breakdown of both substrates increased as streamwater P increased. This finding is consistent with the underlying mechanism of nutrient-stimulated fungal and bacterial C mineralization of detrital substrates in streams (Gulis and Suberkropp 2003). Further corroborating this mechanism, we found that relatively-large differences in detrital breakdown were highly correlated with microbial respiration rates (study 1) as well as changes in microbially-mediated substrate stoichiometry (study 2). These findings indicate that microbial activity on a variety of detrital substrates is likely stimulated in systems with

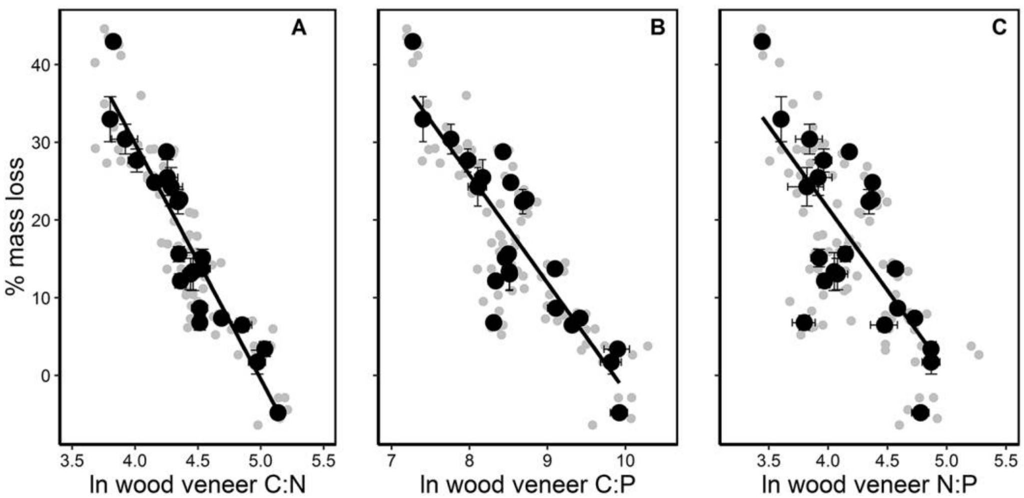

Figure 3. Relationship between % mass loss of wood veneers and the ln(C:N) (A), ln(C:P) (B) and ln(N:P) (C) of wood veneers at the end of study 2. The lines show the regressions based on site-level means. Each graph shows 23 sites (black points) with 4 samples/site (gray points) for a total of 92 samples. Line equations for site regressions of site-level means: A.—$F = 150_{1,21}$, $p = <0.005$, $R^2 = 0.87$. B.—$F = 68_{1,21}$, $p = <0.005$, $R^2 = 0.75$. C.—$F = 25.3_{1,21}$, $p = <0.005$, $R^2 = 0.53$.

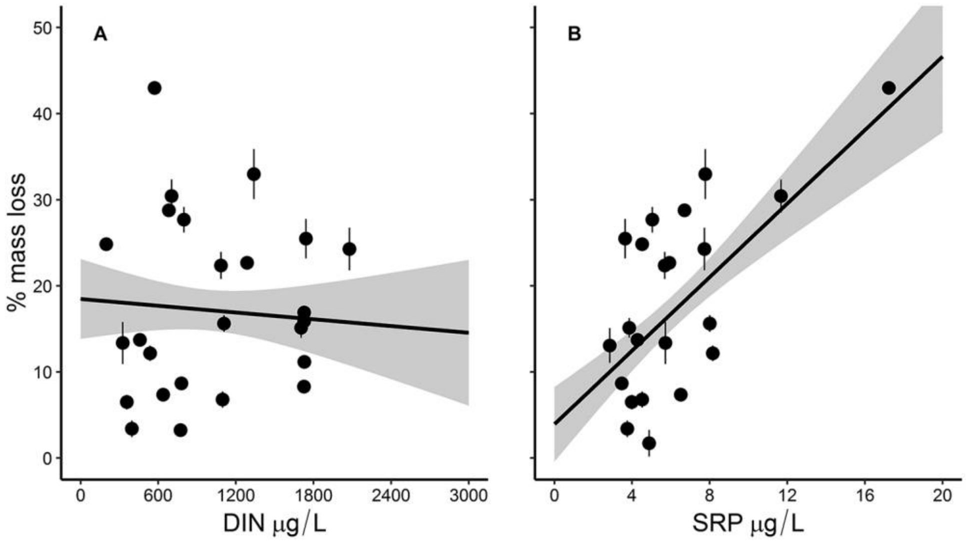

Figure 4. Wk 12 average (±SE) % mass loss of wood veneers ([initial mass − final dry mass/initial mass] × 100) related to average stream water dissolved inorganic nitrogen (DIN; A) or soluble reactive phosphorus (SRP; B). Shading indicates the 95% confidence interval from the mixed-effects model. See Table 2 for linear regression results.

elevated nutrients, which results in accelerated breakdown of organic matter and hence a reduction in important ecosystem services in streams.

Response of labile vs recalcitrant C

Detrital recalcitrance influenced the strength of relationships between nutrients and both respiration (greater for cellulose sponge) and breakdown (greater for wood). Wood veneers consist of more structurally-complex C that takes a longer time to mineralize than the less structurally-complex C in cellulose sponges. However, microorganisms apparently drove breakdown on both substrates, as evidenced by the significant relationships between C mass and respiratory losses for both wood and cellulose sponge. The substrate-specific relationships between streamwater nutrients and breakdown were slightly different than our prediction that labile substrates would increase most in both respiration and breakdown across the nutrient gradient. The hypothesized pattern occurred for respiration, which increased by 4.2× across the P gradient for cellulose sponge but by 1.2× for wood. However, the effect of nutrients on breakdown was smaller for the labile substrate than for wood. These results suggest that detrital C resources that are labile, low in structural compounds (e.g., crop residues such as maize), or both, may be susceptible to nutrient enrichment (Taylor et al. 2017). On labile substrates, nutrients appeared to stimulate microbial respiration (and associated fates of carbon as CO_2 flux) more than they did breakdown, suggesting that labile carbon may be primarily lost as respiration in high nutrient environments.

The close alignment of microbial respiration and breakdown in wood veneers is consistent with breakdown being largely microbially driven on these substrates, but wood substrates were less susceptible to site-specific physical fragmentation than cellulose sponge. The close allignment of

Table 6. Study 2 standardized parameter estimates for the top model predicting % mass loss on wood veneer substrates ([initial mass − final dry mass] / initial mass) × 100 across 23 sites after 12 wk. Shown are models within 2 Akaike Information Criterion (AIC) of the top model. Predictor variables included soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), stream water temperature (Temp), and specific conductivity (SPC). Averaged values of these variables as described in Table 1 were used in the models. Values of C:P and C:N were from individual wood veneers. DIN and SPC were not included in the same models because they were correlated ($r = 0.72$). C:N and C:P were not included in models together for simplicity. Each nutrient content covariate was only paired with 1 dissolved nutrient covariate at a time for a total of 26 models. Each model included site as a random effect. We report the parameter estimates, standard error (SE), *t*-values, and the marginal R^2 (R^2 marg; fixed effect) and conditional R^2 (R^2 cond; fixed and random effect). See Table S4 for AIC results for all models.

Model	Intercept	SRP	Temp	C:N	R^2 marg	R^2 cond
C:N + SRP	0.17 (.010)	0.031 (0.011)		−0.069 (0.008)	0.74	0.93
C:N + SRP + Temp	0.17 (.010)	0.031 (0.010)	−0.007 (0.010)	−0.07 (0.007)	0.75	0.93

responses of respiration and breakdown to nutrients for wood compared with cellulose sponge suggests that the fates of C to CO_2 vs other fates (particles, consumers) was more balanced for recalcitrant substrates. Recalcitrant substrates such as wood can be incubated for longer periods of time than is possible with labile substrates, and we observed measurable relationships between breakdown rates and substrate stoichiometry. These characteristics indicate that wood is useful as a standardized substrate to assess nutrient effects in streams and that changes in wood nutrient content may alone be used as an indicator of detrital responses to nutrients without the measurement of breakdown, as previously suggested in Manning et al. (2016).

Relative importance of streamwater N, P, and temperature

In this study, P appeared to be more important than N in driving both breakdown and respiration rates. However, our experimental design was likely better suited to detecting effects of P than N. Low P likely limited N responses, but N may have been at sufficiently-high concentrations when P was highest (e.g., >400 μg/L DIN; see Kominoski et al. 2015 and references therein). Our studies support a dual control hypothesis in which background N concentrations (188–2485 μg/L DIN) enabled a P response over small P gradients (<~30 μg/L SRP in study 1, <17 μg/L in study 2) and are consistent with previous work showing dual control of N and P on detrital loss rates (Woodward et al. 2012, Ferreira et al. 2015, Manning et al. 2015, Rosemond et al. 2015). Detrital responses to nutrients have been observed in systems with high and low baseline concentrations of N and P. Tensile strength of cotton was associated with land-use change across large gradients of DIN (up to 1.8 mg/L) and dissolved P (up to 260 μg/L) (Young and Collier 2009), but a meta-analysis of litter breakdown responses to N and P also found relatively-strong effects of nutrients in correlative field studies where background reference concentrations of nutrients were sometimes low (e.g., ~2 to ~10 μg/L; Ferriera et al. 2015). These findings are significant to water quality management because they attest to the need to manage both N and P in streams. In our studies, data from study 2 show that land cover variables were related to DIN concentrations (DIN vs impervious surface cover, $R^2 = 0.41$), but not to P concentrations. Therefore, sources of P might be more difficult to predict from land cover than sources of N.

The weight of evidence from our models indicates that SRP effects were stronger than other factors, including temperature. Respiration rates of cellulose sponge in study 1 were positively associated with temperature, as they were for SRP. For this response, temperature occurred in a similar number of models as for SRP and had equivalent standardized parameter estimates as SRP (Table 3). However, SRP was the only variable in models predicting respiration rates from wood. Further, SRP was the only variable for which there were credible parameter estimates predicting breakdown of both cellulose sponge and wood compared with DIN, SPC, and temperature (Table 4). Respiration and breakdown were closely aligned for both of these substrates, which argues that SRP was the consistent driver of these processes. In study 2, SRP was much more strongly related to breakdown rates than temperature.

Detrital stoichiometry as a predictor of breakdown

Changes in detrital C:N and C:P were more predictive of breakdown than streamwater nutrient concentrations. We attribute this difference to the temporally-integrative nature of detrital stoichiometry that combines the effects of microbial biomass and microbial sequestration of nutrients in contrast to streamwater nutrients that are snapshot measurements and can be temporally variable. An assessment of nutrient effects on detritus could include the magnitude change in detrital stoichiometry, but it is unknown what stoichiometric changes signal impaired function. The changes in stoichiometry from initial values in our studies (change C:N = 47%; change C:P = 30%) were of relatively-large magnitude in our 12-wk study, but were smaller than observed in an experimental nutrient enrichment study that observed 73 and 93% changes in C:N and C:P, respectively, of wood veneers over 109 days (Manning et al. 2016; Appendix S1, Table S1). In our studies, changes in C:P were observed despite very small concentration gradients in P. This result suggests that tight control of watershed P inputs is necessary because nutrients, particularly P (Gulis et al. 2017), can be sequestered by microorganisms. Higher N and P content of detritus not only reflects greater microbial colonization but can potentially further stimulate losses of detritus caused by higher rates of detritivory (Evans-White and Halvorson 2017).

The effects of nutrients on detritus when detritivores are scarce vs abundant

The relative abundance of detritivores changes across systems depending on a variety of extrinsic factors, and these differences can contribute to how strongly nutrients affect breakdown rates of detritus. Across the P gradient, the range in breakdown rates in wood in our study that excluded consumers (2.3×; Study 1) was nearly ½ of the range of breakdown rates of wood in our study that did not exclude consumers (4.1×; Study 2). However, study 2 also ran for a longer period of time, and substrates likely experienced higher flow effects than in study 1. In cases where nutrient–detritus relationships are tested with abundant detritivores, nutrient effects can be amplified. Greater detrital nutrient content (lower C:N, C:P) is associated with increased detritivore feeding (Hladyz et al. 2009, Halvorson et al. 2017). Alternatively, detrital stoichiometric changes may affect consumer growth due to higher resource quality rather than affecting detritivore feeding (Frainer et al. 2015). Although nutrient enrichment may thereby stimulate growth of and energy flow to consumers in some cases (Cross et al. 2006), longer-term studies show nutrient-stimulated energy flow to consumers can fundamentally change stream trophic relationships (Davis et al. 2010). Thus, detection of nutrient effects on detrital mass loss may indicate larger-scale phenomena.

Opportunities for assessment and management

The pioneering concept and rationale for the use of leaf litter breakdown as an integrative functional response in streams (Webster and Benfield 1986, Gessner and Chauvet 2002) was tested extensively by the European Union's *RivFunction* consortium (see Chauvet et al. 2016). Chauvet et al. (2016) elegantly and comprehensively summarized insights from these studies and cited the intrinsic role of substrate quality in determining detrital breakdown rates. We found that wood veneers, a recalcitrant substrate, were useful in assessing breakdown rates that varied 2.3 to 4.1× across relatively-small gradients in P in our 2 studies, suggesting that similar effects of accelerated detrital loss occur across moderately nutrient-enriched landscapes. These results align with recommendations by Chauvet et al. (2016) for the use of low-nutrient standardized substrates (that can also include the potential impacts of invertebrate consumers, albeit in this case xylophagous consumers) for detection of these effects across landscapes. Chauvet et al. (2016) also highlighted the need to quantify variation in extrinsic factors to improve the predictive use of litter breakdown in management. We were able to detect nutrient–detritus relationships in this study because our sites were within a relatively-small area (~300 km^2, presumably reducing other sources of variation) and because we tested for other easily-measured extrinsic drivers (e.g., temperature, SPC) in our models. Other metrics for assessing functional impairment have also been proposed including absolute values of breakdown, predictability of breakdown rates, and changes in detrital stoichiometry (Gessner and Chauvet 2002, Chauvet et al. 2016, Manning et al. 2016). Of these metrics, our studies corroborated the use of stoichiometry as a predictor of wood breakdown.

Effective management of nutrients should include consideration of detrital C, which is critical for the conservation of ecosystem services provided by streams and rivers within urban and agricultural landscapes. Understanding the effects of nutrients on detrital C dynamics (e.g., respiration rate and breakdown) informs management strategies to maintain detrital resources for aquatic ecosystem services (production of fishes, habitat, and substrates for nutrient uptake). With current trends towards increased nutrient enrichment of aquatic systems, there is an urgent need for proactive management to sustain freshwater ecosystems that considers whole ecosystem perspectives, including detrital endpoints. Our studies suggest that deployable arrays made of low-cost materials and standardized substrates can be effective tools to detect nutrient pollution effects across a diversity of landscapes.

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Author contributions: ADR, JW, RLU, and PMB designed the experiments. RLU and JW conducted the experiments. RLU, PMB, and SJW analyzed the data. RLU and ADR wrote the first draft of the manuscript, and all authors contributed to revisions.

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