

# Convergence of detrital stoichiometry predicts thresholds of nutrient-stimulated breakdown in streams

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**Abstract.** Nutrient enrichment of detritus-based streams increases detrital resource quality for consumers and stimulates breakdown rates of particulate organic carbon (C). The relative importance of dissolved inorganic nitrogen (N) vs. phosphorus (P) for detrital quality and their effects on microbial- vs. detritivore-mediated detrital breakdown are poorly understood. We tested effects of experimental N and P additions on detrital stoichiometry (C:N, C:P) and total and microbial breakdown (i.e., with and without detritivorous shredders, respectively) of five detritus types (four leaf litter species and wood) with different initial C:nutrient content. We enriched five headwater streams continuously for two years at different relative availabilities of N and P and compared breakdown rates and detrital stoichiometry to pretreatment conditions. Total breakdown rates increased with nutrient enrichment and were predicted by altered detrital stoichiometry. Streamwater N and P, fungal biomass, and their interactions affected stoichiometry of detritus. Streamwater N and P decreased detrital C:N, whereas streamwater P had stronger negative effects on detrital C:P. Nutrient addition and fungal biomass reduced C:N by 70% and C:P by 83% on average after conditioning, compared to only 26% for C:N and 10% for C:P under pretreatment conditions. Detritus with lowest initial nutrient content changed the most and had greatest increases in total breakdown rates. Detrital stoichiometry was reduced and differences among detritus types were homogenized by nutrient enrichment. With enrichment, detrital nutrient content approached detritivore nutritional requirements and stimulated greater detritivore vs. microbial litter breakdown. We used breakpoint regression to estimate values of detrital stoichiometry that can potentially be used to indicate elevated breakdown rates. Breakpoint ratios for total breakdown were 41 (C:N) and 1518 (C:P), coinciding with total breakdown rates that were ~1.9 times higher when C:N or C:P fell below these breakpoints. Microbial and shredder-mediated breakdown rates both increased when C:N and C:P were reduced, suggesting that detrital stoichiometry is useful for predicting litter breakdown dominated by either microbial or shredder activity. Our results show strong effects of nutrient enrichment on detrital stoichiometry and offer a robust link between a potential holistic nutrient loading metric (decreased and homogenized detrital stoichiometry) and increased C loss from stream ecosystems.

**Key words:** breakpoint regression; carbon; detritus; ecological stoichiometry; fungi; nitrogen; phosphorus; shredders; threshold elemental ratios; Coweeta Hydrologic Laboratory; Long Term Ecological Research (LTER); Appalachian Mountains; Macon County; North Carolina; USA.

## INTRODUCTION

Nutrient pollution from nonpoint sources affects aquatic ecosystems worldwide (Carpenter et al. 1998). Among these systems, streams are particularly vulnerable to the effects of nonpoint nutrient pollution due to their

connection to the surrounding landscape via groundwater and/or surface water runoff (Mulholland 1992, Sudduth et al. 2013). Human activities, such as urbanization and agriculture, have dramatically increased nutrient concentrations (i.e., nitrogen [N] and phosphorus [P]) in streams in recent decades (Alexander and Smith 2006), contributing to degradation of water quality (Brown and Froemke 2012). Increased availability of nutrients can have fundamental effects on ecosystem-level processes, such as macroinvertebrate production (Cross et al. 2006) and particulate organic matter breakdown (Ferreira et al. 2015). However, nutrient effects on particulate organic matter pathways are typically outside of the scope of nutrient pollution

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management practices, which tend to rely on metrics related to primary production (e.g., Evans-White et al. 2013).

Particulate organic matter (hereafter detritus) is a crucial resource for stream ecosystems (Wallace et al. 1997, Hall et al. 2000), and nutrient loading from watershed sources can alter its availability (Kominoski and Rosemond 2012). Recent evidence suggests that increased nutrients can rapidly deplete detritus (Rosemond et al. 2015), but development of robust relationships between added nutrients and the mechanisms driving detrital breakdown is lacking. In contrast, relationships between nutrients and production of algal carbon (C) or stream periphyton community structure are more established (e.g., Elser et al. 2007, Taylor et al. 2014). A potential indicator of nutrient pollution effects in stream ecosystems is the rate of detrital breakdown (Gessner and Chauvet 2002). However, stressors that occur in parallel with excessive nutrient loading (e.g., toxic pollutants) may reduce a key trophic guild, detritivores (e.g., shredding macroinvertebrates, hereafter shredders), causing inconsistent effects of nutrient concentrations on detrital breakdown and impeding its use as an indicator of ecosystem health in streams affected by multiple stressors (Woodward et al. 2012). Stronger mechanistic links between consistent nutrient-mediated changes to ecosystem structure and function are needed to adequately assess integrity of detritus-based streams (Palmer and Febria 2012).

Detrital breakdown in streams is driven by interacting abiotic and biotic factors, including abrasion, microbial conditioning, and consumption by shredders (Tank et al. 2010). In terrestrial and aquatic systems, detrital breakdown rates can be partially explained by initial detrital quality (i.e., C:N, or C:P), with slower rates of decomposition for detritus that are nutrient-poor (i.e., high C:N, C:P; Cornwell et al. 2008, Hladysz et al. 2009). Nutrient enrichment can increase the nutrient content of detritus (Rosemond et al. 2010, Scott et al. 2013, Prater et al. 2015), potentially homogenizing formerly diverse detrital resources in terms of elemental stoichiometry (i.e., C:N or C:P). These effects occur through microbial colonization and biomass accrual on detritus and associated immobilization of dissolved inorganic nutrients (Suberkropp 1995, Cheever et al. 2012, Tant et al. 2013, Mehring et al. 2015), such that wide-ranging detrital C:nutrient content becomes increasingly homogenous via microbial conditioning (e.g., Rosemond et al. 2010, Fraier et al. 2015; Fig. 1).

Reduction and homogenization of detrital C:nutrient content driven by microbial decomposers in streams (especially aquatic hyphomycetes; Findlay et al. 2002) can affect the activity of shredders. Specifically, microbial enhancement of detrital nutrient content can cause detrital C:N or C:P to more closely match the shredder threshold elemental ratio (TER), or the point at which the consumer switches from nutrient limitation to C limitation (Sternler and Elser 2002). The implication

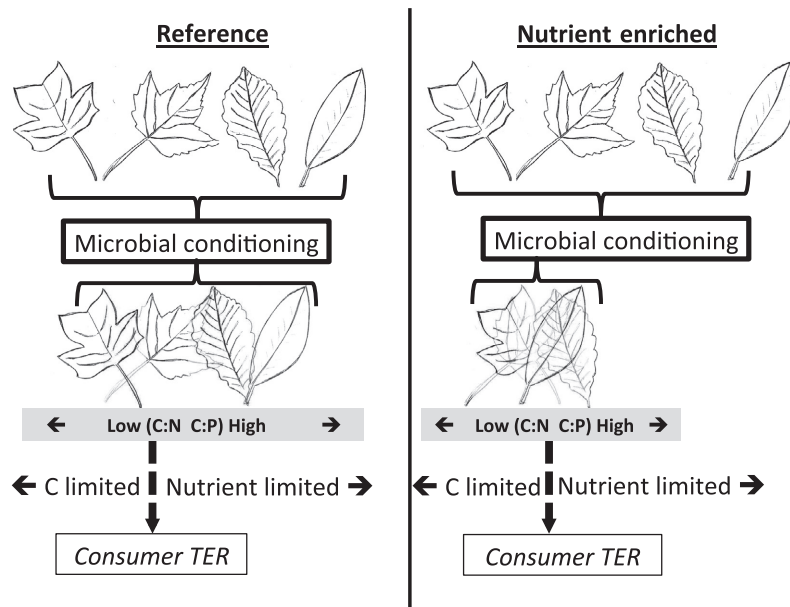


FIG. 1. A conceptual representation of how nutrients could affect microbially mediated conditioning and detrital stoichiometry, and the quality of the resource for shredders, inspired by and modified from Wickings et al. (2012). We show reference and nutrient-enriched conditions, where the leaf litter species used in this study (poplar, maple, oak, and rhododendron) converge to a similar C:nutrient stoichiometry and approach shredder threshold elemental ratios (TERs) under nutrient enrichment. We expected that detrital breakdown rates would be increased under nutrient enrichment and could be predicted based on litter C:N and C:P approaching optimal C:nutrient content after nutrient-enhanced microbial conditioning.

of such reduced imbalances between detritus and detritivore TERs is increased consumption (Frainer et al. 2015), and/or growth by individuals (Frainer et al. 2015, Fuller et al. 2015, Halvorson et al. 2015), and potentially increased reproduction and survival at the population level (e.g., Danger et al. 2013). Combined, these effects of increased nutrient content on shredder growth, consumption, and survival could affect the rate at which detritus is processed in streams. However, the association between nutrient-stimulated changes to detrital C:N and C:P and breakdown rates remains poorly characterized, particularly with respect to differential effects of streamwater N vs. P on detrital C:nutrient content and subsequent microbial vs. shredder-mediated effects on breakdown rates.

Our objective was to assess if detrital C:nutrient stoichiometry affected by nutrient pollution could be linked to increased microbial and shredder-mediated detrital breakdown rates. We explored these relationships using multiyear, whole-ecosystem enrichments of five headwater streams with a crossed gradient of N and P concentrations. We used a set of five substrates (four leaf litter species and wood veneers) that spanned a wide range of initial C:N and C:P ratios. We predicted that nutrient enrichment would enhance microbial conditioning and stoichiometric homogenization of diverse detritus types (Fig. 1) and that litter nutrient content would depend on streamwater nutrient concentrations (i.e., that P would have stronger negative effects on C:P and that N would have stronger negative effects on C:N; Kominoski et al. 2015). We also predicted that microbial effects on litter nutrient concentrations would produce C:nutrient content that approached estimated shredder TERs and thereby facilitate shredder-mediated breakdown (Frost et al. 2006, Tant et al. 2013). If shredder activity is stimulated by such convergence of detrital stoichiometry toward shredder TERs, we would also expect breakdown rates to exhibit threshold responses at or around detrital C:N and/or C:P ratios that matched detritivore TERs.

## METHODS

### *Site description and experimental design*

Our study took place at Coweeta Hydrologic Laboratory (CWT), a USDA Forest Service research station and Long Term Ecological Research (LTER) site located in the southern Appalachian Mountains in Macon County, North Carolina, USA (see Swank and Crossley 1988). We selected five 70-m reaches in first-order streams within the Dryman Fork catchment to receive continuous dosing of N and P for two years. The streams were similar physically and chemically and contained similar abundance and biomass of shredders prior to nutrient additions based on analysis of similarity (ANOSIM) of shredder community data from

litterbags (see *Methods: Detrital breakdown rates*; ANOSIM  $R = -0.005$  and  $0.004$ , for shredder abundance and biomass, respectively;  $P > 0.05$  in both cases). Following a year of pretreatment data collection, we began dosing the entire length of each 70-m reach on 11 July 2011 with solutions of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) and phosphoric acid ( $\text{H}_3\text{PO}_4$ ) using solar-powered metering pumps (LMI Milton Roy, Ivyland, Pennsylvania, USA) connected to gravity-fed irrigation lines supplied with streamwater. The dosing system in each stream was programmed to be proportional to discharge measured continuously using pressure transducers (Keller America, Newport News, Virginia, USA) and CR800 dataloggers (Campbell Scientific, Logan, Utah, USA). Dripper spouts were placed ~5 m apart along the irrigation line to ensure adequate mixing along each 70-m reach.

Each stream reach received a unique concentrated solution of N and P to target five increasing concentrations of N (added + background = 81, 244, 365, 488, 650  $\mu\text{g/L}$  as dissolved inorganic nitrogen [DIN]) and corresponding decreasing concentrations of P (added + background = 90, 68, 51, 33, and 11  $\mu\text{g/L}$  as soluble reactive phosphorus [SRP]), resulting in a unique target N:P ratio for each stream (2, 8, 16, 32, and 128, respectively). Multiple streamwater samples ( $n = 4$ ) were taken every ~15 m along each of the 70-m reaches on days 1, 4, 7, 14, 23, 29, and 34 of enrichment to confirm adequate mixing of added nutrients. After day 34 of enrichment, streamwater was collected above ( $n = 1$ ) and below ( $n = 3$ , at 10, 17, and 70 m) the nutrient dosing system biweekly, filtered in the field (0.45- $\mu\text{m}$  nitrocellulose membrane filters; Millipore Billerica, Massachusetts, USA), frozen within 24 h after retrieval, and analyzed for DIN ( $\text{NH}_4\text{-N} + \text{NO}_3\text{-N}$ ) and SRP concentrations within 28 d (Alpkem Rapid Flow Analyzer 300 for DIN, spectrophotometric method with UV-1700 spectrophotometer, Shimadzu, Kyoto, Japan, for SRP). Two-year average measured DIN concentrations during enrichment (83, 198, 330, 363, and 309  $\mu\text{g/L}$ ; all values respective to treatment targets previously discussed) were close to but lower than target concentrations. Two-year average measured SRP concentrations (49, 55, 36, 22, and 7  $\mu\text{g/L}$ ) were also close to but lower than target concentrations. Measured concentrations reflected the effects of uptake, so we also calculated the actual amounts of DIN and SRP that were added to the streams; the latter values were used for the analyses presented here. These enriched concentrations were determined using the quantity of nutrients added to each stream based on detailed nutrient solution refill records, total daily discharge, and measured background concentrations from samples collected above the nutrient dosing system. Further details about the experimental design, infrastructure, and stream physicochemical characteristics can be found in Manning et al. (2015) and Rosemond et al. (2015).

### Detrital breakdown rates

We determined breakdown rates of red maple (*Acer rubrum* L.), tulip poplar (*Liriodendron tulipifera* L.), chestnut oak (*Quercus prinus* L.), rhododendron (*Rhododendron maximum* L.), and white oak wood veneers (*Quercus alba* L.) prior to nutrient enrichment (PRE) and during two consecutive years of nutrient addition (YR1, YR2). We collected freshly abscised leaf litter of each type during peak leaf fall (October 2010, 2011, and 2012). Collected litter was air-dried for several weeks in the laboratory. Air-dried litter was then weighed into  $10 \pm 0.1$ -g litter packs and placed into 5-mm plastic mesh bags to allow macroinvertebrate access ( $22 \times 40$  cm; Cady Bag, Pearson, Georgia, USA). To prevent macroinvertebrate access to litter, we placed one leaf (typically 0.5–1 g) into 0.5-mm mesh bags (20-cm right triangles, Industrial Netting, Minneapolis, Minnesota, USA) within corresponding coarse-mesh litterbags. Initial mass of the litter in coarse- or fine-mesh litterbags was determined to the nearest 0.01 g. Wood veneers were cut into  $\sim 2.5 \times 15$ -cm pieces and weighed to the nearest 0.01 g. Three veneers were fastened to  $12 \times 17$ -cm nylon gutter mesh rafts with cable ties. Seven sets of litterbags of each species and two wood veneer rafts were anchored into four 17.5-m sub-reaches within the 70-m experimental reach in each of the five streams ( $n = 7$  sampling dates  $\times 4$  sub-reaches  $\times 5$  streams = 140 per year for leaf litter types;  $n = 3$  sampling dates  $\times 4$  sub-reaches  $\times 2$  rafts  $\times 5$  streams = 120 per year for wood veneers). Thus, the number of substrates sampled was 140 litterbags  $\times 4$  litter species + 120 wood veneers per year for a total of 2040 substrates used during the three years of the study.

After incubation in the stream began (day 0 = 1 December 2010, 27 November 2011, and 29 November 2012 for PRE, YR1, and YR2, respectively), litterbags were collected on seven dates (PRE = day 7, 14, 21, 70, 109, 160, 187; YR1, YR2 = day 7, 14, 21, 34, 55, 63, 77 [maple, poplar] or day 7, 14, 21, 63, 110, 126, 143 [oak, rhododendron]), placed into individual plastic bags, and transported to the laboratory on ice. Wood veneers were placed in the streams at the same time as litterbags and collected on day 21, 109, and 160 (PRE) and day 21, 109, and 143 (YR1, YR2). For both leaf litter and wood veneers, sampling dates differed in PRE vs. YR1 and YR2 because of accelerated breakdown during nutrient enrichment. Within 24 h, the litter was removed from the coarse-mesh bags, rinsed over nested 1-mm and 0.25-mm sieves, subsampled for microbiological analyses (see *Methods: Fungal biomass*) and then dried for 24 h at 55°C. Litter in the fine-mesh bags was rinsed over the nested sieves and dried while still inside the mesh bag to minimize loss of litter material. Once dry, the litter from coarse-mesh bags was weighed to determine dry mass remaining and then ground with a ball mill (Spex Certiprep 8000D, Metuchen, New Jersey, USA). A 1–2-g subsample was weighed and combusted for 4.5 h at 500°C to determine ash-free dry mass (AFDM). The entire sample from each fine-mesh bag was weighed to determine

dry mass remaining and combusted in a similar manner. After subsampling for fungal biomass, wood veneers were also dried for 24 h at 55°C, ground using a ball mill, and  $\sim 0.5$ -g subsamples combusted to determine AFDM.

### Detrital stoichiometry

We measured C:N and C:P at early, middle, and late stages of leaf litter decay to target litter with different levels of microbial conditioning. We compared litter C:N and C:P relative to initial values (i.e.,  $\Delta$  initial) and for the same stage of decay (i.e.,  $\Delta$  conditioned) among all three years to develop potential benchmarks for nutrient-stimulated changes in litter C:N and C:P relative to known initial ratios or relative to the mean detrital C:N or C:P observed at the same stage of decay before enrichment. Sampling schedules for middle and late stages of decay differed depending on litter species and year due to faster processing of detritus under nutrient enrichment (PRE = day 14, 70, 160; YR1 and YR2 = day 14, 34, 77 [maple, poplar]; day 14, 63, 126 [oak, rhododendron]). Wood veneers were sampled for nutrient analyses at middle stages of decay (day 109) for all years. The majority of the analyses presented here uses only detrital stoichiometry data from middle stages of decay to assess the effect of nutrient concentrations on stoichiometric homogenization of detrital C:N and C:P after substantial microbial conditioning. We chose to focus on middle stages of decay to coincide with the observed timing of substantial changes to litter nutrient content (e.g.,  $\sim$ day 60; Rosemond et al. 2010) and peak fungal biomass on litter types we used in this study (e.g., day 30–60 for maple; Gulis and Suberkropp 2003). A 2–4-mg subsample of the dried and ground litter or wood was used to determine conditioned litter %C and %N with a Carlo Erba 1500 CHN analyzer (Milan, Italy). Phosphorus content of conditioned leaf litter and wood was determined using the plant dry ash/acid extraction method (Allen 1974) followed by spectrophotometric analysis (Shimadzu UV-1700) of the extracted solution using the ascorbic acid method (APHA 1998).

### Fungal biomass

We analyzed ergosterol concentrations to estimate fungal biomass associated with leaf litter and wood veneers during middle stages of decay for leaf litter (day 34 [YR1, YR2 for maple, poplar], 63 [YR1, YR2 for oak, rhododendron], or 70 [all leaf litter during PRE]), and day 109 [wood veneers]) that corresponded with the samples used for detrital stoichiometry (as described previously). We subsampled and froze  $\sim 2 \times 2$ -cm pieces from rinsed leaf litter or wood until analysis. Lipids were extracted using methanolic KOH solution from freeze-dried, weighed leaf litter and wood pieces using liquid-to-liquid extraction (Gulis and Suberkropp 2006). 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 (Gulis and Suberkropp 2006). We used external ergosterol standards (Acros Organics, Geel, Belgium), and ergosterol concentrations were converted to fungal biomass using a standard conversion factor of 5.5  $\mu\text{g}$  of ergosterol/mg of fungal dry mass (Gessner and Chauvet 1993).

#### Data analysis

Total leaf litter and wood breakdown rates (i.e., microbial + shredder =  $k_{\text{total}}$ ) were estimated based on the percent litter or wood mass remaining over time according to the negative-exponential model (Petersen and Cummins 1974)

$$m_t = m_0 e^{-kt},$$

where  $m_t$  is the detrital mass remaining at time  $t$ ,  $m_0$  is the initial litter or wood mass, and  $k$  is the breakdown rate. We determined a unique decay rate  $k$  for each leaf litter type and wood veneers from each sub-reach within the 70-m experimental reach ( $n = 4$  sub-reaches  $\times$  5 streams  $\times$  5 detritus types  $\times$  3 yr = 300). Breakdown rates of litter in fine-mesh bags were determined in the same way, and we considered these estimates to reflect microbial breakdown rates ( $k_{\text{microbe}}$ ). We determined shredder contributions to breakdown rates by subtracting  $k_{\text{microbe}}$  from total breakdown rates (i.e.,  $k_{\text{total}} - k_{\text{microbe}} = k_{\text{shredder}}$ ; Woodward et al. 2012).

All analyses were conducted using the statistical software R v. 3.0.1 (R Core Team 2013). Response variables were ln-transformed when appropriate to meet assumptions of normality or linearity. We used an ANOVA model to test for overall nutrient enrichment effects on detrital C:N and C:P across years and detritus types. We then applied a linear model to assess the effects of N and P concentrations (as continuous variables) on detrital C:N and C:P, respectively. We expected that N and P effects would be additive, but fungal biomass effects would interact with nutrient concentrations (e.g., Kominoski et al. 2015, Manning et al. 2015). This model also included a categorical predictor for detritus type, such that we tested for differences in mean C:N or C:P among the five detritus types (i.e., differences in the intercept for the detritus). Therefore, our model predicting detrital C:N and C:P included N and P concentrations, their interaction with fungal biomass, and detritus type. We standardized predictor variables using  $z$  scores to compare predictor variables measured at different scales and to aid the interpretation of interactions between continuous predictors (i.e., N and P concentrations and fungal biomass; Gelman and Hill 2007). Slope coefficients for this model can be interpreted as the expected change in detrital C:N or C:P for an increase in N, P, or fungal biomass concentration by one standard deviation. Because we centered our data, main effects in the models can be interpreted as the predicted effect of a given parameter on detrital C:N or C:P for detritus with mean fungal biomass or in streamwater with mean N or P concentration.

We analyzed the contributions of microbial and shredder-mediated breakdown rates (i.e.,  $k_{\text{microbe}}$  vs.  $k_{\text{total}} - k_{\text{microbe}} = k_{\text{shredder}}$ ; wood  $k$  was excluded for this analysis) with respect to nutrient enrichment (i.e., by year) and detritus type. The relative contribution of microorganisms vs. shredders can be determined based on the ratio of  $k_{\text{shredder}}/k_{\text{microbe}}$ , where a ratio  $< 1$  indicates greater microbial contributions (Hladyz et al. 2010). We tested for differences in contributions of microbial vs. shredder-mediated breakdown using ANOVA, with year as the main predictor of ln-transformed  $k_{\text{shredder}}/k_{\text{microbe}}$ . Finally, we used breakpoint regression to pinpoint possible thresholds in the relationship between leaf litter breakdown and detrital C:N or C:P (Dodds et al. 2010). Breakpoint regression allows for the estimation of piecewise linear relationships and can be used to pinpoint the value where the linear relationship between two variables changes (Muggeo 2003). We specifically used these breakpoint models to identify the point at which the relationship between C:N or C:P and leaf litter breakdown changed significantly. We then compared the breakpoint C:N and C:P values identified by the models to previously reported shredder TERs (e.g., Tant et al. 2013). Specifically, we compared breakpoints ad hoc to the values reported in Tant et al. (2013), which was specific to a dominant shredder in our system (larvae of a caddisfly in the genus *Pycnopsyche*, TER C:N = 27, C:P = 1992) and is also comparable to detritivore TER C:P values from Frost et al. (2006; mean  $\pm$  SD TER C:P = 1,495  $\pm$  1,154) and Halvorson et al. (2015; *Pycnopsyche lepida* TER C:P = 1620). We used the R package segmented (Muggeo 2003) to analyze our data for possible breakpoints that could correspond to shredder TERs.

## RESULTS

### Nutrient enrichment effects on detrital stoichiometry

Decreases in detrital C:N and C:P relative to initial C:N and C:P (i.e.,  $\Delta$  initial) were enhanced by nutrient enrichment (Appendix S1: Table S1; Fig. 2). In the pretreatment year, there were relatively small changes from initial stoichiometry through time. Specifically, in the pretreatment year, leaf litter C:N and C:P were reduced 30% and 24% compared to initial, respectively, and wood C:N and C:P were reduced 11% and increased 47%, respectively (Appendix S1: Table S1). Under nutrient-enrichment (YR1 and YR2), leaf litter C:N was reduced by ~50% and leaf litter C:P was reduced by ~60% compared to initial C:N and C:P. Wood exhibited the sharpest reductions in C:N and C:P compared to initial ratios under nutrient-enriched conditions and was reduced by >70% for both C:N and C:P in YR1 and YR2 (Appendix S1: Table S1).

Nutrient-stimulated reductions in C:N and C:P also resulted in pronounced differences in detrital nutrient content between the pretreatment and nutrient-enriched years for the same stage of decay (i.e.,  $\Delta$  conditioned; Appendix S1: Table S1). Nutrient enrichment led to

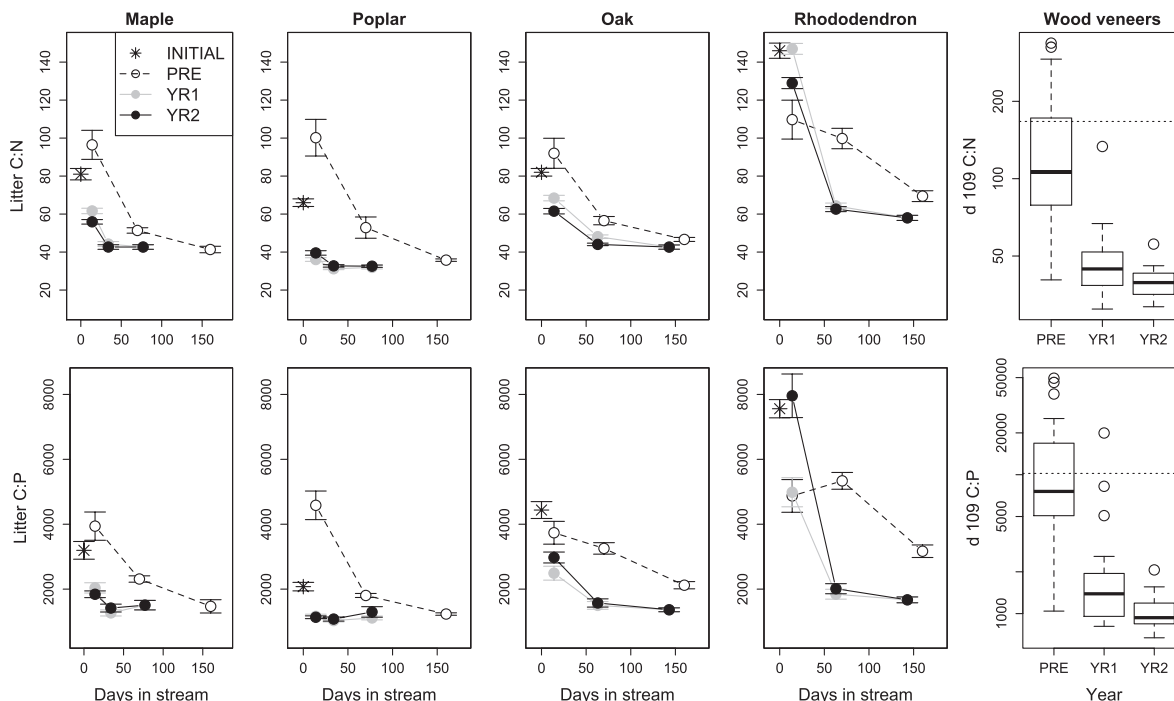


FIG. 2. Mean C:N (top row) and C:P (bottom row) for leaf litter collected during pretreatment (PRE) (open circles) or nutrient-enriched conditions (YR1 and YR2; filled gray and black circles, respectively). Early (d 14), middle (d 70 [PRE], 34, or 62) and late (d 160 [PRE], 77, or 143) C:N and C:P are shown depending on the given leaf litter type and year. Asterisks indicate mean initial C:N or C:P across all three years ( $n = 15$ ). All error bars indicate  $\pm 1$  standard error. Lines connect points from the same year (dashed = PRE; solid gray = YR1; solid black = YR2). Conditioned C:N or C:P were only measured on wood veneers on d 109 in all years, and are compared among years using boxplots (rightmost graph on top and bottom rows of the figure; note different y-axis scales). Solid horizontal lines, open boxes, and whiskers in the boxplots indicate median, interquartile range, and 1.5 times the 1st and 3rd quartile, respectively. Outliers  $> 1.5$  times the 3rd quartile are shown with open circles outside the whiskers. Dotted lines denote mean ( $n = 5$ ) initial C:N or C:P ratios for wood veneers. All ratios are molar. Note: Increased C:N or C:P on day 14 compared to initial for most litter types occurred PRE but not during enrichment years, likely reflecting initial leaching of N and P relative to C under those conditions.

27% and 29% reductions in mean detrital C:N for YR1 and YR2 compared to pretreatment values at middle stages of decay across leaf litter species (Appendix S1: Table S1). For wood, nutrient enrichment led to 66% and 73% reductions in mean detrital C:N for YR1 and YR2 at middle stages of decay compared to pretreatment. Significantly greater reductions in mean detrital C:N at middle stages of decay were observed for poplar, rhododendron, and wood veneers compared to red maple leaf litter in YR1 and YR2 (Appendix S1: Table S1).

Detrital C:P was reduced to a greater degree than C:N during YR1 and YR2 (Appendix S1: Table S1; Fig. 2). Conditioning under nutrient enrichment led to 52% and 48% reductions in mean C:P for YR1 and YR2 compared to pretreatment values across leaf litter species (Appendix S1: Table S1). Nutrient enrichment led to 80% and 93% reductions in wood C:P compared to pretreatment. Significantly greater reductions in mean detrital C:P were observed for wood veneers and rhododendron leaf litter compared to red maple leaf litter, but not other leaf litter species in YR1 and YR2 (Appendix S1: Table S1).

#### *Microbial and streamwater nutrient effects on stoichiometric homogenization of detritus*

The effects of N and P concentrations, fungal biomass, their interactions, and detritus type explained 54% and 58% of the variation in conditioned detrital C:N and C:P, respectively, based on adjusted  $R^2$  values from each model. The effects of fungal biomass on detrital C:N and C:P were dependent on N and P availability (Table 1); specifically, there was a positive association between added N and P concentrations, fungal biomass, and detrital stoichiometry corresponding to stronger negative effects of fungal biomass on detrital C:N and C:P with increased N and P concentrations. The combined effects of N and P on detrital C:N and C:P were comparable to the effects of fungal biomass alone based on the scaled coefficients in the models. That is, the N and P effects on detritus with mean fungal biomass were comparable to fungal biomass effects given mean N and P concentrations (Table 1).

The C:N ratio of leaf litter and wood was driven by fungal biomass, streamwater N, streamwater P, and the interaction between fungal biomass and streamwater N.

TABLE 1. Parameter estimates based on linear models for drivers of leaf litter and wood stoichiometry. Intercepts correspond to mean ln-transformed maple (M), poplar (P), oak (O), rhododendron (R), and wood (W) C:N or C:P, and standardized slopes correspond to effects of added N (N conc.) or P (P conc.) concentrations on detrital stoichiometry, and their interactions with fungal biomass (i.e.,  $N \times \text{fungi}$  and  $P \times \text{fungi}$ ).

C:N model	Estimate	SE	C:P model	Estimate	SE
<b>Intercepts</b>					
M	<b>3.789</b>	0.040	M	<b>7.276</b>	0.068
P	<b>-0.202</b>	0.054	P	<b>-0.199</b>	0.091
O	0.071	0.056	O	0.147	0.094
R	<b>0.408</b>	0.057	R	<b>0.400</b>	0.098
W	<b>0.243</b>	0.054	W	<b>0.385</b>	0.092
<b>Slopes</b>					
Fungi	<b>-0.123</b>	0.021	Fungi	<b>-0.263</b>	0.035
N conc.	<b>-0.049</b>	0.020	N conc.	-0.043	0.034
P conc.	<b>-0.081</b>	0.020	P conc.	<b>-0.238</b>	0.034
$N \times \text{fungi}$	<b>0.078</b>	0.021	$N \times \text{fungi}$	<b>0.159</b>	0.035
$P \times \text{fungi}$	<b>0.070</b>	0.021	$P \times \text{fungi}$	<b>0.166</b>	0.036

*Notes:* These predictors explained 54% and 58% of the variation in detrital C:N and C:P, respectively. All slopes are based on relationships between scaled predictors (using  $z$  scores) and ln-transformed response variables (i.e., middle-stage C:N, C:P). Standardized slopes are useful for direct comparisons of parameters measured at different scales and for interpreting interactions between continuous variables (Gelman and Hill 2007). Bold text corresponds to significant differences ( $P < 0.05$ ) between intercepts for different detritus types in comparison to maple or significant slope estimates (i.e., slope estimates are for fungi, N conc., P conc., and their interactions).

Based on comparisons among the scaled coefficients, the strength of the effect of streamwater N on detrital C:N was weaker than the effect of streamwater P on detrital C:N for detritus with mean fungal biomass (Table 1). In general, the most N-poor substrates (i.e., rhododendron, wood) tended to gain the most N and showed significant decreases in C:N compared to relatively nutrient-rich detritus (e.g., red maple; Appendix S1: Table S2), although poplar also showed decreased C:N compared to maple. As a result, differences between rhododendron and wood C:N vs. maple, poplar, and oak C:N were reduced under nutrient-enriched conditions (Appendix S1: Table S3). For example, mean wood C:N was ~2.9 times greater than maple C:N under pretreatment conditions, whereas mean wood C:N was essentially equivalent to maple C:N (~1.0 times) under nutrient-enriched conditions (Appendix S1: Table S3). Likewise, the range of C:N values was reduced by 65% under nutrient-enriched conditions; C:N values of all five detritus types spanned a range between 27 and 133 compared to 37 and 337 under pretreatment conditions. Across our N:P treatments, we found that the greatest reductions in detrital C:N occurred when dissolved N:P  $\geq 32$ , but the differences between reduced C:N values among streams were small (mean C:N = 48 vs. C:N = 43 for treatment N:P = 2 and 128, respectively).

The C:P ratio of leaf litter and wood was also driven by fungal biomass, streamwater P, and the interaction between fungal biomass and streamwater N and P (Table 1). The strongest driver of conditioned detrital C:P ratios was fungal biomass, followed by nearly equal control by streamwater P (Table 1). The most P-poor substrates (i.e., rhododendron, wood) gained the most P, leading to sharp decreases in C:P compared to more nutrient-rich substrates (e.g., maple; Appendix S1: Table S2). As a result, differences between rhododendron and

wood C:P vs. maple, poplar, and oak C:P were reduced under nutrient-enriched conditions. For instance, mean wood C:P was ~6.5 times greater than mean maple C:P under pretreatment conditions, whereas wood C:P was only ~1.5 times higher than maple C:P under nutrient-enriched conditions (Appendix S1: Table S3). Likewise, the range of C:P values was reduced by 60% under nutrient-enriched conditions; C:P values of all five detritus types spanned a range between 669 and 19,956 compared to 1042 and 49,496 under pretreatment conditions. Across our N:P treatments, we found that the differences in litter stoichiometry values among streams were larger for C:P compared to C:N (mean C:P = 1,205 vs. C:P = 2,705 for treatment N:P = 2 and 128, respectively).

#### *Effects of nutrient enrichment and initial stoichiometry on detrital breakdown rates*

Reduced and homogenized detrital stoichiometry corresponded to increased detrital breakdown rates, especially for  $k_{\text{total}}$ . Across litter types and years, total breakdown rates were ~2.5 times pretreatment rates, whereas microbial breakdown rates in YR1 and YR2 were only ~1.4 times pretreatment rates (Appendix S1: Table S4). Similar to reductions in detrital C:nutrient ratios, increases in total breakdown rates were more pronounced for recalcitrant leaf litter species: average  $k_{\text{total}}$  for oak and rhododendron was 2.4 times and 3.9 times higher during nutrient enrichment, compared to 1.8 times and 2.3 times higher breakdown rates for maple and poplar, respectively (Appendix S1: Table S4). We found a positive, linear relationship between the magnitude of the increase in breakdown rate under nutrient enrichment (mean YR1 and YR2  $k_{\text{total}}/\text{PRE } k_{\text{total}}$ ; Fig. 3A, B; Appendix S1: Table S4) and the initial C:N or C:P of the detritus, such

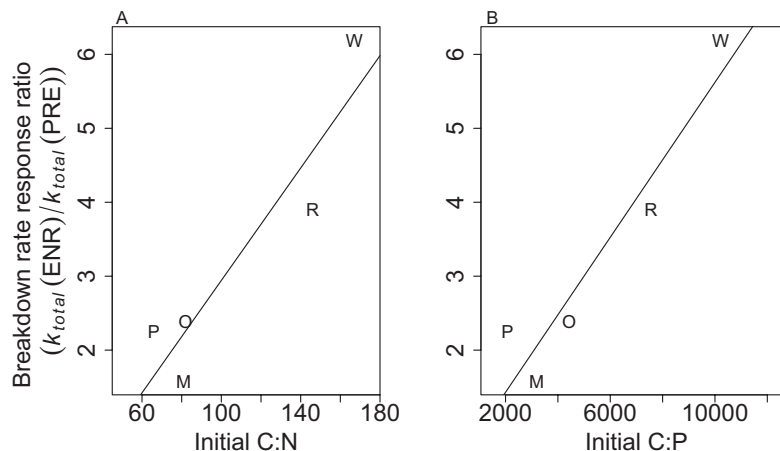


FIG. 3. Breakdown rates of leaf litter and wood increased as a function of initial (A) C:N and (B) C:P; solid lines indicate linear relationships between breakdown rate response ratios and initial C:N or C:P ( $P < 0.05$  in both cases,  $R^2 = 0.86, 0.90$ , respectively). The magnitude of the increase in breakdown rates was calculated as the ratio of the average YR1 and YR2 (ENR)  $k_{total}/PRE k_{total}$  (i.e., the response ratio). Letters correspond to each detritus type: maple (M), poplar (P), oak (O), rhododendron (R), and wood (W).

that the most pronounced increases in breakdown rates relative to PRE were for the most nutrient-poor substrates (i.e., highest initial C:N, C:P; Fig. 3A, B).

#### *Effects of shredders on detrital breakdown rates*

We found that shredder contributions increased more than microbial contributions to breakdown in YR1 and YR2 based on the ln-transformed ratio,  $k_{shredder}/k_{microbe}$  (Fig. 4A–D). We tested for differences in shredder contributions between years for each detritus type because we found no evidence for interactions between year and detritus type, or differences among streams in terms of the contribution of shredders vs. microorganisms to breakdown rates. We found significantly increased shredder contributions to breakdown for all leaf litter types in all years, with the exception of rhododendron in YR2 (Tukey's HSD, all  $P < 0.05$ ; Fig. 4A–D). Maple, poplar, and oak switched from greater relative microbial vs. shredder contribution to breakdown under pretreatment conditions to greater relative shredder contribution in YR1 and YR2 (i.e.,  $\ln k_{shredder}/k_{microbe} > 0$ ; Fig. 4A–C). For rhododendron, shredder contributions were greater than for other litter types under pretreatment conditions; shredder contributions increased in YR1, but not in YR2, compared to pretreatment conditions (Fig. 4D).

#### *Identifying stoichiometrically explicit breakpoints for detrital breakdown rates*

Breakpoints were found in the relationship between total breakdown rates and C:N ratios (Fig. 5A;  $P < 0.05$ , adjusted  $R^2 = 0.25$ ) of decaying leaf litter. The identified breakpoint for litter C:N and total litter breakdown was  $41 (\pm 2)$ , which represents a 56% decrease from initial C:N averaged across leaf litter species. Total breakdown

rates differed above and below breakpoints for C:N. Total breakdown rates were 1.8 times higher when C:N was below the breakpoint (Fig. 5A). There were also significant breakpoints in the relationship between total litter breakdown rates and litter C:P ratios (Fig. 5B;  $P < 0.05$ , adjusted  $R^2 = 0.31$ ). The breakpoint for C:P and total litter breakdown was  $1,518 (\pm 167)$ , which represents a 65% decrease from initial C:P averaged across leaf litter types. As with C:N, total breakdown rates differed above and below breakpoints for C:P. Total breakdown rates were 1.9 times higher when C:P was below the breakpoint (Fig. 5B). These breakpoint C:N and C:P ratios for total breakdown rates differed considerably from mean leaf litter C:N (63) and C:P (3,167) values for pretreatment conditions, while mean C:N (46) and C:P (1,453) during enriched conditions were more similar to breakpoint C:N and C:P for total breakdown rates. Mean C:N and C:P of leaf litter as well as the breakpoint ratios were comparable to TERs for C:N (27) and C:P (1,992) of an important shredder taxon in our study system (caddisfly larvae in the genus *Pycnopsyche*; Tant et al. 2013) and to the mean TER C:P reported for detritivores (mean  $\pm$  SD TER C:P =  $1,495 \pm 1,154$ ; Frost et al. 2006).

#### DISCUSSION

Linking resource structure and ecosystem function is a crucial step toward developing robust metrics of ecosystem integrity (Gessner and Chauvet 2002, Palmer and Febria 2012), particularly with regard to detecting and predicting responses to widespread ecosystem stressors, such as nutrient pollution. Increases in streamwater nutrient concentrations are known to increase litter and wood nutrient content (Stelzer et al. 2003, Gulis et al. 2004); here, we explicitly linked these stoichiometric changes to causative experimental gradients of N and P



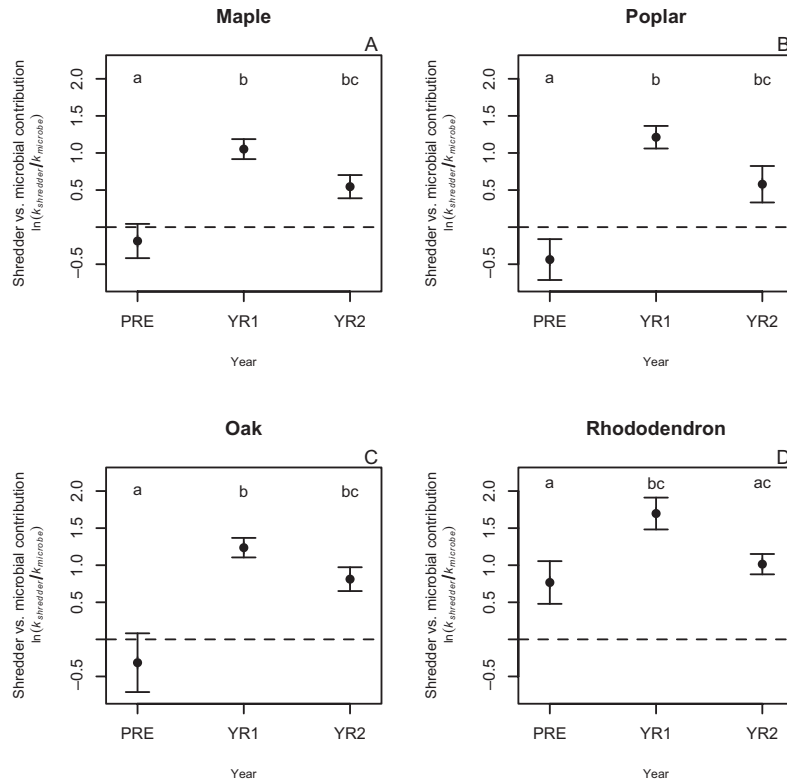


FIG. 4. The contribution of shredder-mediated breakdown increased with nutrient enrichment for all leaf litter types, with the exception of rhododendron in YR2. We tested for differences in shredder contributions between years for each detritus type because the interaction between year and detritus type was not significant. Shown are mean (error bars indicate  $\pm$  SE)  $\ln$ -transformed  $k_{\text{shredder}}/k_{\text{microbe}}$  for each leaf litter type: (A) maple, (B) poplar, (C) oak, and (D) rhododendron, and year. The dashed horizontal line at  $k_{\text{shredder}}/k_{\text{microbe}} = 1$  corresponds to the point at which contributions of shredders and microorganisms are equivalent (i.e.,  $\ln(1) = 0$ ). Differing letters between years denote significant differences in  $\ln(k_{\text{shredder}}/k_{\text{microbe}})$  between years based on ANOVA and Tukey's HSD post hoc tests at  $P < 0.05$ .

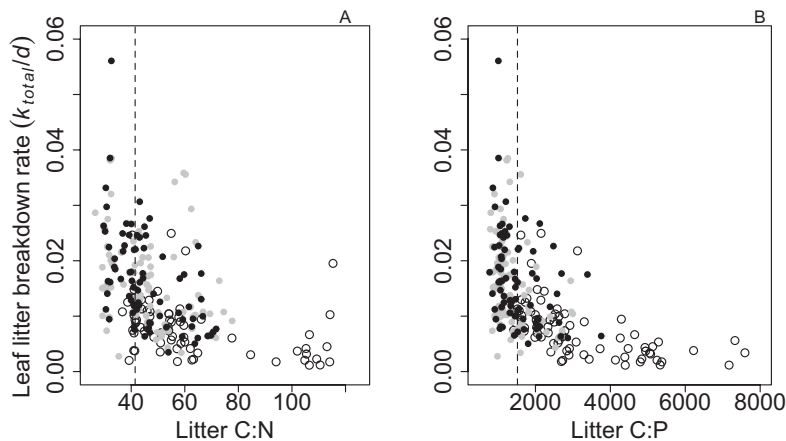


FIG. 5. Total breakdown rates as a function of middle-stage (A) C:N or (B) C:P ratios of all leaf litter species used in this study (wood veneers not included). Vertical dashed lines depict breakpoints for these relationships. Identified breakpoints were 41 and 1518 for C:N and C:P, respectively. Open, filled gray, and filled black circles represent PRE, YR1, and YR2, respectively.

and resulting ecosystem function (i.e., increased rates of detrital breakdown).

Our study targeted low-to-moderate nutrient enrichment common in human-modified landscapes

(Scott et al. 2002, Alexander and Smith 2006), but these concentrations were sufficient to induce decreased detrital stoichiometry and increased breakdown rates. Thus, we suggest standardized, nutrient-poor detrital

substrates (e.g., wood veneers) could be used to indicate nutrient pollution and its effects on an ecosystem function. For instance, we found that after ~100 d of conditioning, wood veneers had C:N and C:P that was 73% and 80% lower than initial under nutrient-enriched conditions, compared to only 10% lower than initial for C:N and no difference for C:P under pretreatment conditions. Thus our data suggest that increased rates of ecosystem-scale C loss may be expected if detrital C:N and C:P are found to be substantially reduced (e.g., >70%) compared to corresponding initial C:N and C:P. These findings offer a potentially significant and useful metric of nutrient-mediated changes to a critical basal resource that can be used to predict where and when accelerated C loss might occur in stream ecosystems in response to nutrient pollution. More studies at the level of stream mesocosms and/or whole streams are needed to further develop the predicted linkage we make here between changes in nutrient content of detritus and detrital loss rates.

#### *Streamwater nutrient and microbial effects on detrital stoichiometry*

Nutrient loading from distinct land use can result in skewed N and P availability in streams, thus, the concentrations and ratios we used mimicked such patterns of N and P loading (e.g., high N:P from N-rich fertilizers vs. low N:P from sewage effluent; Arbuckle and Downing 2001, Peñuelas et al. 2012). Detrital C:N and C:P were reduced across our N:P treatments, but there were differences in this response when comparing detrital C:N and C:P. For example, our findings suggest that C:N can be affected by increases in either N or P concentrations, as evidenced by similar C:N values across our treatment N:P ratios. In contrast, P effects were stronger than N effects on C:P, indicating that changes in detrital C:P are more dependent on the availability of P when N is also available. These different responses of C:N and C:P to streamwater N compared to P could be important in the context of detritivore nutrient limitation when N and P loading are skewed. Several studies have observed both N- and P-limitation of decomposers (Rosemond et al. 2002, Ferreira et al. 2006) and detritivores (Danger et al. 2013, Fraimer et al. 2015). Thus, our findings suggest that N-limitation via detrital resources could be alleviated across elevated N or P concentrations, while P-limitation could be alleviated more when N:P loading is skewed toward lower N:P ratios.

Increased microbial biomass has been implicated as a driver of increased detrital nutrient content (Gulis and Suberkropp 2003, Tant et al. 2013). Our study found that streamwater nutrient availability and fungal biomass together control detrital C:nutrient stoichiometry. Specifically, the effects of streamwater N and P on detrital C:N and C:P were dependent on fungal biomass, but streamwater P concentration effects were stronger than the interaction of streamwater P with fungal biomass.

These results suggest that while DIN effects on detrital C:N are particularly dependent on fungal biomass accrual, streamwater P appears to independently control detrital C:P to a greater degree, as fungi can likely store P without considerable increases in biomass when streamwater P availability is elevated (V. Gulis, *unpublished data*). Aquatic fungi typically contribute >95% of microbial biomass on coarse detritus, such as leaf litter and wood (Gessner et al. 2007), and thus were likely the major drivers of stoichiometric changes of detritus in this study. Detrital stoichiometry could be affected in a similar way in other systems where fungal decomposers are predominant (e.g., soils/terrestrial detritus; Barantal et al. 2014). However, we cannot rule out the potential effects of bacteria (but see Tant et al. 2013), abiotic sorption (e.g., Mehring et al. 2015), or differential allocation of resources to fungal sporulation in response to N vs. P availability in affecting detrital stoichiometry.

#### *Shredder-driven effects on leaf litter breakdown rates*

We found that relatively recalcitrant detritus showed greater responses to streamwater nutrients in terms of reduced C:nutrient content and that shredders contributed more to the breakdown of all four leaf litter species under nutrient enrichment. These findings combined with our breakpoint ratios for increased total breakdown rates suggest that reduced C:N and C:P of detritus is an important driver of increased breakdown rates, particularly when nutrient-poor resources approach shredder nutrient requirements. Thus, the effects of nutrient enrichment on detrital breakdown could be amplified in systems characterized by intact shredder communities and nutrient-poor detritus. Although the presence of shredders may increase nutrient-enrichment effects on detrital breakdown, our analysis demonstrated that microbial breakdown rates also increased. These findings imply that, across diverse systems with and without detritivores, nutrient enrichment will predictably increase detrital C loss rates.

#### *Breakpoints for detrital breakdown*

Shredder growth, reproduction, and survival are related to the nutrient content of basal resources consumed, particularly if the food resource meets shredder nutrient demands (i.e., approaches the TER; Danger et al. 2013, Halvorson et al. 2015). Our findings support the existence of a causative link between (1) nutrient-mediated microbial processing that drives detrital C:N or C:P toward shredder TERs and (2) subsequent stimulation of shredder activity leading to increased litter breakdown rates. As a result of effects of streamwater N and P and fungal biomass, greater than 78% of conditioned detrital C:P was found to be below the estimated C:P TER for a dominant shredder in our study system (larvae of the caddisfly *Pycnopsyche*; C:P TER = 1992; Tant et al. 2013) under enriched conditions compared to

17% falling below this threshold during pretreatment. Other detritivores can exhibit a range of C:P TERs depending on diet or taxon-specific physiology (e.g., larvae of the crane fly *Tipula* C:P TER = 1,000–2,500; Fuller et al. 2015) and other reported values of shredder TERs tend to fall in the range of reduced detrital stoichiometry found in this study (Frost et al. 2006). However, it is known that some shredders selectively consume and assimilate patches of detritus that contain greater amounts of fungal biomass and nutrients (Arsuffi and Suberkropp 1985, Dodds et al. 2014), complicating the inferences made here based on bulk detrital resources. Nevertheless, we show that nutrient enrichment did increase the prevalence of low C:nutrient detritus that could be consumed by shredders and that these patterns likely correspond to the breakpoints we have identified in the relationship between total litter breakdown rates and detrital C:N and C:P ratios.

Breakpoints in litter breakdown rates were also observed when detritivores were excluded from litter using fine-mesh litterbags (data not shown). In this case, large changes in detrital stoichiometry indicate increased colonization of detritus by microorganisms. Consequently, changes in detrital stoichiometry may indicate increased detrital loss from either decomposers and/or detritivores and thus be applicable to a range of stream types and conditions. Thus, we expect that in areas where shredder abundance is low due to biogeographic factors (Boyero et al. 2011) or stressors (Griffiths et al. 2009, Woodward et al. 2012), microbially driven breakdown will be more important and detrital loss rates would be increased with nutrient enrichment unless decomposers are suppressed by associated contaminants.

#### *Comparing nutrient-induced changes to detrital vs. biofilm stoichiometry*

We found that changes in detrital stoichiometry were induced at low to moderate nutrient concentrations. The changes in detrital stoichiometry we observed were similar to changes in biofilm stoichiometry across gradients of N and P concentrations (Taylor et al. 2014). Specifically, Taylor et al. (2014) found that biofilm C:N decreased by 26% and biofilm C:P decreased by 38% when total P increased from 10 to 20  $\mu\text{g/L}$ . When comparing our data across this range in P concentrations, we found similar responses; detrital C:N decreased by 10% and detrital C:P decreased by 38%. These findings indicate that changes in basal resource stoichiometry can occur due to effects on either autotrophic (e.g., biofilm) or heterotrophic microbial communities and that they may respond to nutrient gradients similarly. Further, as a result of our experimental nutrient additions, detrital stoichiometry that formerly spanned large ranges also became more similar and approached TERs for certain detritivores. A similar pattern occurred in the Taylor et al. (2014) study, where biofilms were more variable in

terms of nutrient content at low nutrient concentrations and more homogeneous at high nutrient concentrations. This similar pattern suggests that the diversity in nutrient content of detritus or biofilms can be reduced by nutrient enrichment, which may affect detritivore and biofilm consumer communities (LeRoy and Marks 2006, Taylor et al. 2014). Given that diverse detritus or biofilm nutrient content typically supports diverse consumer assemblages, nutrient-induced reduction and homogenization of basal resource stoichiometry could potentially lead to low diversity of food quality and associated reduced diversity of consumers (e.g., Evans-White et al. 2009). Thus, nutrient enrichment may diminish stream consumer biodiversity related to either heterotrophic or autotrophic food web pathways in some cases.

We identified relevant breakpoints for a key ecosystem function in response to microbial homogenization of a key detrital resource trait under nutrient enrichment, a pattern similar to observations made for stream biofilms. Thus, the response of detrital or autotrophic stoichiometry could be an important tool for predicting ecosystem-scale consequences of nutrient enrichment. Such assessments of changes in basal resource structure (i.e., reduced and homogenized detrital stoichiometry associated with stream carbon loss) could be useful if incorporated into management strategies for mitigating the effects of nutrient pollution on the functioning of stream ecosystems.

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