

Leaf litter nutrient uptake in an intermittent blackwater river: influence of tree species and associated biotic and abiotic drivers

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Summary

1. Organic matter may sequester nutrients as it decomposes, increasing in total N and P mass via multiple uptake pathways. During leaf litter decomposition, microbial biomass and accumulated inorganic materials immobilize and retain nutrients, and therefore, both biotic and abiotic drivers may influence detrital nutrient content. We examined the relative importance of these types of nutrient immobilization and compared patterns of nutrient retention in recalcitrant and labile leaf litter.

2. Leaf packs of water oak (*Quercus nigra*), red maple (*Acer rubrum*) and Ogeechee tupelo (*Nyssa ogeche*) were incubated for 431 days in an intermittent blackwater stream and periodically analysed for mass loss, nutrient and metal content, and microbial biomass. These data informed regression models explaining temporal changes in detrital nutrient content. Informal exploratory models compared estimated biologically associated nutrient stocks (fungal, bacterial, leaf tissue) to observed total detrital nutrient stocks. We predicted that (i) labile and recalcitrant leaf litter would act as sinks at different points in the breakdown process, (ii) plant and microbial biomass would not account for the entire mass of retained nutrients, and (iii) total N content would be more closely approximated than total P content solely from nutrients stored in leaf tissue and microbial biomass, due to stronger binding of P to inorganic matter.

3. Labile litter had higher nutrient concentrations throughout the study. However, lower mass loss of recalcitrant litter facilitated greater nutrient retention over longer incubations, suggesting that it may be an important long-term sink. N and P content were significantly related to both microbial biomass and metal content, with slightly stronger correlation with metal content over longer incubations.

4. Exploratory models demonstrated that a substantial portion of detrital nutrients was not accounted for by living or dead plant and microbial biomass, especially in the case of N. This suggests increased importance of both N and P sorption to inorganic matter over time, with possible additional storage of N complexed with lignin. A better understanding of the influence of these mechanisms may improve our understanding of detrital nutrient uptake, basal resource quality and retention and transport of nutrients in aquatic ecosystems.

Key-words: aquatic hyphomycete, biofilm, chitin, coupled biogeochemical cycle, fungi, glucosamine, metal oxide, stoichiometry

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Introduction

Globally, streams and rivers export over 43 Tg of nitrogen (N) and 8 Tg of phosphorus (P) to the ocean each year (Boyer *et al.* 2006; Mayorga *et al.* 2010). However, large quantities of N and P are also temporarily retained within streams and rivers, and understanding the sequestration of these nutrients via biotic and abiotic drivers is critical to estimating fluxes and their corresponding effects within and across ecosystem boundaries. One important mechanism of temporary nutrient retention in streams is through uptake associated with organic materials, such as terrestrially derived wood and leaf litter. This process is influenced simultaneously by biotic and abiotic factors that include (i) nutrient immobilization through microbial colonization and biomass accrual (Cross *et al.* 2005; Cleveland & Liptzin 2007), and (ii) accumulation of inorganic sediments containing aluminium (Al), iron (Fe), manganese (Mn) and calcium (Ca) (Meyer 1980; Cameron & Spencer 1989; Chamier, Sutcliffe & Lishman 1989) and their associated complexation with N (Triska *et al.* 1994; Aufdenkampe *et al.* 2001) and P (Sigg & Stumm 1981; Hesterberg *et al.* 2011). The relative contributions of biotic and abiotic mechanisms to nutrient uptake by organic matter have rarely been quantified simultaneously, although each mechanism may differentially impact the bioavailability of nutrients to consumers in detrital food webs. For example, while microbial nutrients are readily available to decomposers and detrital consumers, nutrients bound to Al and Fe may be largely unavailable (Reynolds & Davies 2001).

Here, we focus on nutrient uptake associated with terrestrially derived leaf litter since it is a common and sometimes dominant form of organic matter in aquatic ecosystems. Uptake of nutrients from the surrounding water by litter-inhabiting fungi and bacteria (Suberkropp & Chauvet 1995) may lead to net nutrient sequestration, but the overall ability of leaf litter to serve as a sink for nutrients (accumulating a greater mass of N or P than that initially present in the litter) may also depend on its rate of breakdown (i.e. mass loss). Thus, while labile litter usually supports higher microbial biomass and therefore greater initial microbial uptake of nutrients than recalcitrant litter, labile litter itself is lost more rapidly from the system via decomposition. As a consequence, recalcitrant litter may serve as a larger long-term sink for nutrients, due to lower rates of mass loss. Additionally, relatively recalcitrant pools of N may develop in litter over time, whereby phenols and lignin form complexes with plant proteins and N-containing microbial coenzymes (Suberkropp, Godshalk & Klug 1976; Schlesinger & Hasey 1981). Chitin in fungal tissue constitutes another N-containing pool that may not decompose rapidly (Gleixner *et al.* 2002). An understanding of these dynamic nutrient pools is critical to assessing how forest composition and consequent litter inputs affect nutrient cycling in ecosystems.

We examined breakdown, litter structural chemistry, fungal and bacterial biomass, and nutrient and metal immobilization associated with three leaf litter species of differing

physicochemical characteristics in an intermittent blackwater stream. Our research asked two questions: (i) how does tree species influence nutrient uptake and retention and the accumulation of inorganic material on leaf litter, and (ii) what are the relative contributions from biotic (fungi and bacteria) and abiotic (accumulation of inorganic material) mechanisms to nutrient uptake. The incubation period spanned more than 1 year and included a natural period where the stream channel dried completely. Nutrient concentrations were incorporated into linear model comparisons as well as informal exploratory models, to estimate relative contributions of biotic and abiotic pools to total detrital nutrient content. Overall, we predicted that (i) labile and recalcitrant leaf litter would act as sinks at different points in the breakdown process, (ii) biotic pools would not account for the entire mass of retained nutrients, which would change with litter type and timing, and (iii) total N content would be more closely approximated than total P content solely from nutrients stored in leaf tissue and microbial biomass, due to stronger binding of P to inorganic matter.

Materials and Methods

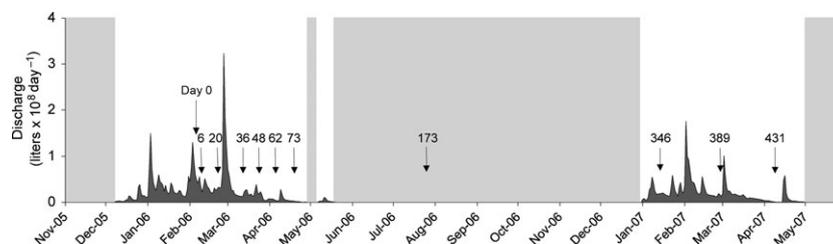
STUDY SITE

This study was conducted in a heavily forested third-order reach of the Little River, a blackwater river in Turner County, Georgia, USA, which drains the Atlantic coastal plain and is part of the Little River Experimental Watershed (LREW). The study reach (31°41'32"N, 83°42'09"W) drains a 2200 ha catchment and meanders through a second-growth forest floodplain with variable discharge and long periods of drought during the summer and fall months when the stream channel completely dries (Fig. 1). Clay-textured soils rich in metals are prevalent throughout the region (Lowrance & Vellidis 1995) (Table S1, Supporting information). Chemical and physical characteristics of the study reach are summarized in Table 1.

FIELD PROCEDURES

We examined the breakdown, nutrient and metal content, and microbial dynamics associated with decaying leaf litter of three common south-eastern coastal plain tree species that differ in their initial litter chemistry (Table 2). The three species selected, in order from most recalcitrant to most labile, were water oak (*Quercus nigra* L., hereafter referred to as 'oak'), trident red maple (*Acer rubrum* var. *trilobum* Torr. & Gray ex K. Koch, hereafter referred to as 'maple') and Ogeechee tupelo (*Nyssa ogeche* Bartram ex Marsh, hereafter referred to as 'tupelo'). The three litter species also differed in surface roughness; maple leaves are pubescent below (Bicknell 1913) (Fig. S1a, Supporting information), while tupelo's leaves are 'velvety hairy' (Duncan & Duncan 1988) (Fig. S1b), and oak leaves are mostly smooth (Brown & Kirkman 2000) (Fig. S1c). Single-species leaf litter bags containing 10 g were incubated in the stream. Leaf litter from each species was collected immediately after abscission, air-dried in the laboratory and placed into plastic coarse mesh pecan bags (19 × 38 cm, 25 mm² mesh; Cady Bag Company, LLC, Pearson, GA, USA) following Benfield (1996). Leaf litter bags were deployed in study reaches and were grouped in arrays affixed to PVC tubing on the bottom of the stream channel. Each array consisted of three bags, each containing leaf litter from a different tree species. Bags were

Fig. 1. Discharge and sampling dates in the study reach of the LREW. Arrows indicate sampling dates, with incubation time in days listed above each arrow. Shaded areas indicate dry periods when flow ceased and the stream channel dried.



organized into a randomized complete block design, with arrays grouped into five blocks based on longitudinal distance downstream in the stream channel. Five bags of each leaf litter species treatment (one from each block) were removed from the stream on each sampling date (Fig. 1).

In situ rates of microbial respiration were estimated from dissolved oxygen (DO) uptake by leaf discs at ambient stream water temperatures in darkness, using methods and equipment identical to those described by Suberkropp *et al.* (2010). Leaf discs collected for microbial respiration and fungal and bacterial biomass (described later) were gently rinsed in a beaker of stream water to remove loosely adhered sediments before any measurements were made. Additional leaf discs were also preserved in HPLC-grade methanol and sterile-filtered 2% phosphate-buffered formalin for the determination of fungal and bacterial biomass, respectively. All samples were immediately placed on ice and transported to the laboratory where they were stored in the dark at -20°C (fungal biomass) and 4°C (bacterial biomass) until analysed. Remaining litter bag material was placed into clean, resealable plastic bags filled with stream water, placed on ice and immediately transported to the laboratory for further processing.

LABORATORY PROCEDURES

Upon returning to the laboratory, remaining leaf material within litter bags was gently rinsed over a 1-mm mesh size sieve to remove

Table 1. Physical and chemical stream water data (mean \pm 1 SE) within the Little River Experimental Watershed averaged across sampling dates. For DOC (mg L^{-1}), N ($\mu\text{g L}^{-1}$) and P ($\mu\text{g L}^{-1}$), $n = 31$; for DO (mg L^{-1}), pH and temperature ($^{\circ}\text{C}$), $n = 10$; and for Fe and Mn (both in $\mu\text{g L}^{-1}$), $n = 7$

DO	7.51 \pm 0.72
DOC	10.76 \pm 0.50
pH	6.82 \pm 0.28
Temperature	13.91 \pm 1.15
Total P	28.92 \pm 6.77
PO ₄ ³⁻	7.21 \pm 2.21
Total N	562.38 \pm 139.90
NO ₃ ⁻	9.29 \pm 2.64
NH ₄ ⁺	26.88 \pm 3.69
Fe	65.71 \pm 15.54
Mn	31.49 \pm 5.57

macroinvertebrates and loosely adhering sediments. Leaves were dried at 60°C to a constant mass, and a subsample combusted at 500°C to determine ash-free dry mass (AFDM). The mass of leaf discs removed for microbial biomass and respiration measurements was added to total mass. Breakdown rate (k) was determined from the slope of the natural log of mass remaining versus time in days (Webster & Benfield 1986). Remaining litter was ground to a powder and C and N concentrations analysed using a Carlo Erba 1500N CHN Analyzer (Carlo Erba, Milan, Italy). Cellulose, hemicellulose and lignin concentrations were determined using an Ankom A200 Fiber Analyzer (Ankom, Macedon, NY, USA). To analyse temporal changes in leaf litter phosphorus and metal (aluminium, iron and manganese) content, 10 mg of ground dried litter was weighed, combusted at 500°C , extracted with 0.25 mL of aqua regia and diluted with 10 mL of deionized water. Phosphorus was measured from diluted extracts using a colorimetric analyser (Alpkem 300 Series Autoanalyzer, ortho-PO₄ manifold, EPA method 365-1, APHA (1999)). Metal content of extracts was analysed by atomic absorption spectroscopy (AAS, Perkin Elmer AAnalyst 200) and inductively coupled plasma mass spectroscopy (ICP-MS, Perkin Elmer Elan 6000). On days 36, 173 and 431, one replicate extract from each litter species was also analysed for Ca, Mg and potassium (K) content using ICP-MS.

Fungal biomass was estimated from ergosterol concentrations in preserved leaf discs, and glucosamine concentrations (an indicator of living + dead fungal mass) in ground litter. Ergosterol was extracted in alcoholic KOH (0.8% KOH in methanol, total extraction volume 10 mL) for 30 min at 80°C in tightly capped tubes with constant stirring. The resultant crude extract was partially cleaned by solid phase extraction, and ergosterol quantified by high-pressure liquid chromatography (HPLC) (Gessner 2005). Glucosamine concentrations from ground litter were analysed using procedures described by Kuehn *et al.* (2011).

Bacterial biomass was estimated using epifluorescence direct count microscopy and analysis of captured microscope images. Bacteria attached to preserved leaf litter samples were removed by ultrasonication for 1.5 min using a Branson 150 probe sonicator (Buesing & Gessner 2002) and stained with SYBR Gold (Patel *et al.* 2007). Twenty images were randomly captured from each filter at $1000\times$ magnification using an Olympus BH-2 microscope and an Olympus Qcolor 3 digital camera (Olympus[®], Melville, NY, USA) and analysed using MATLAB (v 7.9) and the Image Processing Toolbox (The MathWorks, Inc., Natick, MA, USA). Biovolume estimates (μm^3) were calculated from bacterial cell length (l) and width (w) measurements and converted to biomass following published protocols (First & Hollibaugh 2008).

Table 2. Mean breakdown rates and initial per cent concentrations of leaf litter structural compounds, carbon and nutrients. Standard errors (\pm 1 SE) are provided in parentheses next to mean values. For breakdown rates (k , day^{-1}) and initial (pre-incubation) per cent concentrations of leaf litter structural compounds, $n = 5$. For nutrient concentrations, $n = 3$

Leaf litter species	k	Lignin	Hemicellulose	Cellulose	C	N	P	Lignin: N
Ogeechee tupelo	0.0053 (0.0006)	8.18 (0.48)	13.04 (0.40)	19.07 (0.49)	48.67 (0.02)	1.04 (0.08)	0.038 (0.006)	8.48 (0.95)
Trident red maple	0.0024 (0.0002)	13.29 (0.23)	10.52 (0.52)	20.08 (0.82)	50.04 (0.19)	0.97 (0.06)	0.038 (0.002)	13.86 (0.52)
Water oak	0.0013 (0.0002)	13.56 (0.51)	12.72 (0.54)	22.76 (1.06)	50.57 (0.11)	0.83 (0.11)	0.021 (0.005)	16.71 (1.46)

STATISTICAL ANALYSIS

The effect of leaf litter species and incubation length (days) on microbial respiration, fungal biomass and bacterial biomass was analysed with multivariate analysis of covariance (MANCOVA). Time (days) was used as a covariate, leaf litter species as a treatment effect and longitudinal location in the stream channel as a blocking factor. Planned pairwise comparisons (Bonferroni method, $\alpha = 0.05$, Milliken & Johnson 1992) among leaf litter species were conducted when main effects were significant. Data were transformed whenever necessary to meet the assumptions of normality and homoscedasticity.

To determine the factors explaining nutrient immobilization and microbial respiration (O_2 uptake) in leaf litter, we compared candidate multiple regression models using Akaike's information criterion (AIC) and an information theoretic approach (Burnham & Anderson 2002). Akaike weights (w_i) were calculated for all candidate models with Δ_i (difference between a candidate model's AIC_c and that of the top model) not greater than ten. For regression models dealing with respiration, samples of microbial biomass and measurements of microbial respiration were treated as subsamples and averaged per litter species on each sampling date. For each nutrient (N or P), the analysis was conducted for the full data set and also separately for the first wet period (days 6, 36 and 62), to compare the importance of abiotic and biotic drivers of nutrient immobilization during short-term and long-term incubations. Leaf litter species (maple, tupelo and oak) was coded as two binary variables (dummy variables 'oak' and 'tupelo' = 0 or 1), with a value of one for either variable signifying species identity and zeroes for both variables indicating that the species was maple. To correct for multicollinearity in nutrient immobilization models, Al, Fe and Mn were combined into a single summed parameter (Al+Fe+Mn), and bacterial biomass (positively correlated with both metal content and fungal biomass) was excluded from models.

We used an informal exploratory exercise similar to methods used by Wenger *et al.* (2013), to estimate how nutrients within leaf litter are partitioned into fungal and bacterial biomass and leaf tissue and to determine whether these nutrient pools can account for total leaf litter N and P. We reasoned that if the nutrients in leaf litter were derived solely from plant tissue and microbial cells, the total leaf litter nutrient content would be the sum of all those pools. While we did not have direct measures of nutrients from each of these pools, we did have measures of total detrital (including associated microbial cells) N and P, the mass of total leaf litter and structural compounds (lignin, cellulose, hemicellulose), and fungal (ergosterol, glucosamine) and bacterial biomass on each sampling date. We used literature values of leaf litter nutrient leaching rates, microbial stoichiometric C: N and C: P ratios, fungal ergosterol: C ratios and fungal dry mass: glucosamine ratios to convert these to masses of nutrients (Appendix S1, Supporting information). Rather than using a single value for these conversions, we identified a range of values from multiple literature sources and used a Monte Carlo approach to sample across these different possible literature values, while simultaneously randomly sampling from our empirical data on biomass (Appendices S2 and S3, Supporting information).

When converting microbial biomass to N and P, literature values were compared with Redfield C: N (6.625) and C: P (106) molar ratios, to assess whether flexible or fixed stoichiometric molar ratios could better account for accumulated nutrient content in leaf litter. For detrital P, estimated biotic nutrient pools were leaf, fungal and bacterial biomass. For detrital N, an additional pool of excess glucosamine (not contained in living fungal tissue) was estimated as the difference between total measured glucosamine, and the fraction potentially in living fungal biomass estimated with ergosterol, according to a range of literature values (calculations available in Appendix S1). All other leaf litter N pools were the same, but the leaf tissue nutrient pool included both N initially complexed with lignin and cellulose (hereafter

referred to as acid detergent fibre N, ADF-N), as well as N contained in labile (non-fibrous) leaf tissue fractions (non-ADF-N) (Appendix S1).

The probability that estimated nutrient content was less than actual nutrient content was calculated by comparing differences in 10 000 randomly paired estimated and observed values. All analyses were conducted in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) except for the informal exploratory exercise, which was conducted in R software (R Development Core Team 2008). Sample calculations are available in Appendix S1, and Sample R code is available in Appendices S2 and S3.

Results

NUTRIENT AND METAL CONTENT

Leaf litter N and P content differed among tree species (Wilks' $\lambda = 0.17$, $F_{2,57} = 50.33$ and 62.41 , respectively, all $P < 0.0001$) and increased over time (Wilks' $\lambda = 0.11$, $F_{1,57} = 76.75$ and 177.98 , respectively, all $P < 0.0001$) (Fig. 2a and b). All three leaf litter species differed significantly in N content ($P \leq 0.0007$, Bonferroni) with tupelo litter containing the most and oak the least. Maple and tupelo litter had significantly higher P content than oak litter ($P < 0.0001$), but were not significantly different from one another ($P = 0.35$).

Leaf litter N and P content over the entire study period were best related to fungal biomass (ergosterol) and metal content (Al+Fe+Mn), with some limited weight of evidence (0.01–0.20) for models excluding ergosterol but none that excluded metal content (Table 3, 'N' and 'P' candidate models). However, during the first wet season (Table 3, 'N year 1' and 'P year 1' candidate models), metal content was not significantly related to N content, and roughly equivalent weight of evidence was found for ergosterol and metals as parameters explaining P content. Bacterial biomass was excluded from regression models due to multicollinearity with total inorganic matter, metal (Al+Fe+Mn) content and glucosamine content, but it was also strongly correlated with both N and P ($R = 0.86$ and 0.82 , respectively). Glucosamine was also too highly correlated with ergosterol, bacterial biomass, metal (Al+Fe+Mn) and total inorganic matter content to be included in models containing those parameters, but correlations between glucosamine and N ($F_{1,43} = 105.85$, $R^2_{\text{adj}} = 0.70$, $P < 0.0001$) and P ($F_{1,43} = 62.98$, $R^2_{\text{adj}} = 0.58$, $P < 0.0001$) were stronger than correlations between ergosterol and N ($F_{1,43} = 59.04$, $R^2_{\text{adj}} = 0.57$, $P < 0.0001$) and P ($F_{1,43} = 28.95$, $R^2_{\text{adj}} = 0.39$, $P < 0.0001$).

BREAKDOWN RATE (K) AND NUTRIENT RETENTION

Breakdown rates differed among tree species ($F_{2,8} = 40.48$, $P < 0.0001$, Table 2), with tupelo losing mass significantly faster than maple and oak (Fig. 3, $P < 0.001$). Leaf litter N and P stocks (mg pack^{-1} , Fig. 4 'observed total N', Fig. 5 'observed total P') differed significantly among species and over time (Wilks' $\lambda = 0.11$, $F_{24,82} = 6.94$, $P < 0.0001$). All three leaf litter species showed a net loss of N by the end of the first wet season, although tupelo lit-

ter briefly immobilized N after 36 days of incubation (Fig. 4 'observed total N'). During the dry period (173 days incubation), maple and oak litter both immobilized N, retaining significantly greater N stocks when compared to tupelo litter (all $P < 0.01$, Bonferroni) and retaining more N than the mass present prior to submergence in the stream. During the second wet season, oak was the only litter still retaining a greater stock of N than it contained prior to incubation (Fig. 4 'observed total N'). Patterns of P immobilization were similar to those observed for N among litter species; after longer periods of decomposition, oak was the only litter to retain a greater stock of P than initially present in 10 g of litter prior to incubation (Fig. 5 'observed total P').

POTENTIAL BIOTIC CONTRIBUTIONS TO DETRITAL N AND P: MODELLING RESULTS

An informal exploratory modelling exercise was used to compare estimated nutrient stocks (sum of fungal, bacterial and leaf tissue N or P) to observed total detrital nutrient stocks, to determine whether observed increases in nutrient content can be explained solely from N and P in plant tis-

sue and microbial biomass. For both N and P, estimated biotic pools could not fully account for the entire mass of nutrients measured directly (Figs 5 and 6), but the discrepancy between estimated and observed nutrient content was greater for N than for P, especially after long incubations (Fig. 4). This result differs among leaf litter species, with a greater probability [$34 \pm 20\%$ (95% CI)] that oak litter N (compared to other leaf litter species) can be explained by biotic drivers (average across all incubation times). The discrepancy between modelled and observed detrital N stocks was positively correlated with litter-associated Al ($t_{1,13} = 8.39$, $P < 0.0001$, $r^2_{\text{adj.}} = 0.74$), Fe ($t_{1,13} = 7.13$, $P < 0.001$, $r^2_{\text{adj.}} = 0.67$) and Mn ($t_{1,13} = 5.88$, $P < 0.0001$, $r^2_{\text{adj.}} = 0.60$) contents, bulk inorganic matter ($t_{1,13} = 7.86$, $P < 0.001$, $r^2_{\text{adj.}} = 0.67$), glucosamine ($t_{1,13} = 5.18$, $P < 0.001$, $r^2_{\text{adj.}} = 0.65$) and % lignin ($t_{1,11} = 3.86$, $P < 0.05$, $r^2_{\text{adj.}} = 0.41$).

Leaf tissue (ADF + non-ADF fractions) held the majority of observed N and remained the dominant pool even after long incubations, whereas median bacterial contributions were low, averaging 0.4% (ranging from 0.05% in oak litter day 6 to 1.84% in tupelo litter day 62) across incubation times and litter species. Microbial biomass was converted to nutrient content using both flexible stoichiom-

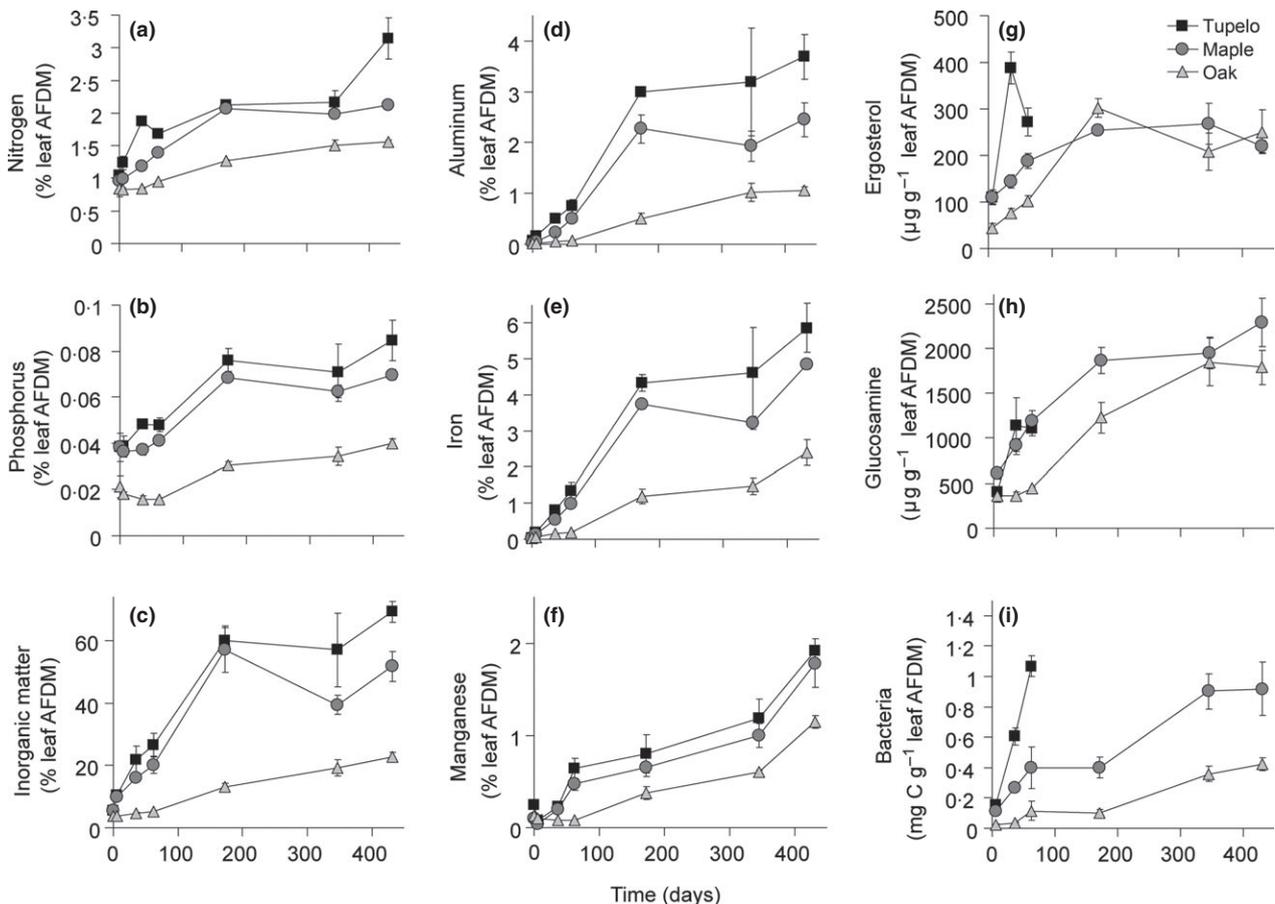


Fig. 2. Changes in concentrations of leaf litter nutrients, metal oxides, inorganic matter, fungal lipids and structural compounds, and bacterial biomass over time. Mean leaf litter (a) nitrogen, (b) phosphorus, (c) inorganic matter ($n = 5$), (d) aluminum, (e) iron, (f) manganese, (g) ergosterol ($n = 5$), (h) glucosamine, and (i) bacterial biomass are expressed per leaf litter AFDM. Vertical bars around means (sometimes obscured by symbols) signify ± 1 standard error. All $n = 3$ unless otherwise noted.

Table 3. Comparison of candidate multiple regression models explaining variation in nitrogen (N) and phosphorus (P) content of leaf litter for full data sets (N, P) and during the first wet season only (N year 1, P year 1). The ‘oak’ term is a binary variable (0, 1) that specifies whether the leaf litter is from oak or from another species (maple/tupelo). *Parameters* indicate the number of terms in the multiple regression model (including *y*-intercept and error), C_p provides a measurement of model error (Mallows’ C_p), R^2_{adj} is adjusted for sample size and number of parameters, AIC_c is Akaike’s second-order information criterion (corrected for small sample size), Δ_i is the difference between the candidate model and the best model’s AIC_c , L is the likelihood value of each model, and w_i is the relative strength of evidence for each candidate model (between 0 and 1)

N	<i>Parameters</i>	C_p	R^2_{adj}	AIC_c	Δ_i	L	w_i
Candidate model							
Ergosterol, Al+Fe+Mn, oak	5	2.67	0.92	-189.64	0	1	0.99
Al+Fe+Mn, oak	4	13.49	0.90	-179.68	9.96	0.01	0.01
N year 1	<i>Parameters</i>	C_p	R^2_{adj}	AIC_c	Δ_i	L	w_i
Candidate model							
Ergosterol, oak	4	13.32	0.87	16.56		1	0.98
Ergosterol	3	28.23	0.82	24.23	7.66	0.02	0.02
P	<i>Parameters</i>	C_p	R^2_{adj}	AIC_c	Δ_i	L	w_i
Candidate model							
Ergosterol, Al+Fe+Mn, oak	5	4.00	0.95	-785.93		1	0.80
Al+Fe+Mn, oak	4	7.17	0.93	-783.19	2.75	0.25	0.20
P year 1	<i>Parameters</i>	C_p	R^2_{adj}	AIC_c	Δ_i	L	w_i
Candidate model							
Ergosterol, Al+Fe+Mn, oak	5	4.00	0.89	-539.37		1	0.54
Al+Fe+Mn, oak	4	6.26	0.88	-537.82	1.55	0.46	0.25
Ergosterol, oak	4	6.58	0.86	-537.51	1.86	0.39	0.21

etry and also fixed Redfield C: N: P ratios. Assuming a Redfield C: N ratio (6.625), bacterial contributions (0.04–1.13%) to detrital N were much lower than when using flexible C: N ratios from published literature values. Potential contributions by living fungal biomass to observed detrital N were highest in oak litter during the dry period (19%). Fixed ergosterol: fungal dry mass (0.0055) and Redfield C: N (6.625) ratios provided higher estimates of fungal contributions to detrital N (5–27%) than when assuming flexible nutrient stoichiometry, primarily because the Redfield C: N ratio is at the low end of values measured directly (6–14, Newell & Stutzell-Tallman (1982); and 7–16, Leach and Gulis, pers. comm.). Glucosamine not contained in living fungal biomass made contributions to total N roughly equivalent to those of bacterial N earlier in the decomposition process. However, from the dry period (day 173) until the end of the incubation period, N contributions from glucosamine not contained in living fungal biomass were roughly 2 × greater than bacterial N in oak litter.

Estimated biotic nutrient pools had a higher probability of accounting for total detrital P (Fig. 5) than detrital N,

although as was the case for N, the probability decreased after longer incubations, and the probability that biotic contributions could account for all accumulated P was highest for oak litter [$57 \pm 13\%$ (95% CI), averaged across sampling dates]. Unlike estimates of detrital N, which were dominated by nutrients contained in leaf tissue, microbial P accounted for the largest estimated relative contribution to observed P in over ¼ of all estimates. The probability of accounting for observed detrital P when allowing for flexible fungal and bacterial C: P ratios rather than Redfield ratios was higher in 14/15 of all estimates, as direct measurements of fungal (40–203, Leach and Gulis 2011, pers. comm.) and bacterial (8–260) C: P ratios allow for higher P content than the Redfield ratio (106). The discrepancy between estimated and observed detrital P stocks was positively correlated with mg of Al ($t_{1,13} = 4.56$, $P < 0.001$, $r^2_{adj} = 0.59$) Fe ($t_{1,13} = 3.81$, $P < 0.005$, $r^2_{adj} = 0.49$) and bulk inorganic matter ($t_{1,13} = 4.27$, $P < 0.001$, $r^2_{adj} = 0.55$) per litter pack.

Median bacterial P contributions to observed detrital P were small (average 1%, range 0.25% in oak, day 6 to 5%

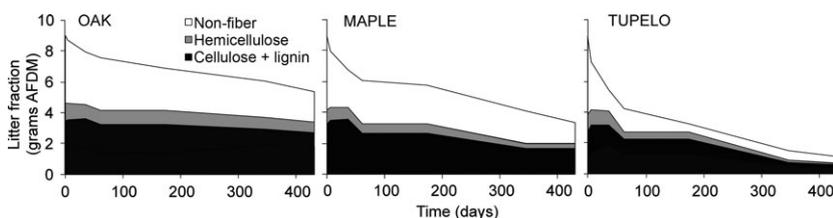


Fig. 3. Mean mass of leaf litter structural compounds (non-fibrous, cellulose, hemicellulose, lignin) remaining over time per pack of leaf litter species. On day 173, fibrous fractions were not measured directly and were assumed equal to those on day 62.

in tupelo, day 62), although higher than bacterial contributions to observed N. A Redfield C: P ratio (106) resulted in lower potential bacterial contributions (0.13–3%) to detrital P. Estimated fungal P accounted for the largest relative proportion [$36 \pm 8\%$ (± 1 95% CI)] of observed P. Median potential contributions by living fungal biomass to observed detrital P ranged from 16 to 68%, 26 to 65% and 21 to 43% in oak, tupelo and maple litter and were highest in oak litter during the dry period (73%).

MICROBIAL RESPIRATION

Overall, differences in microbial respiration rates among litter species were best explained by fungal and bacterial biomass and ambient temperature, with $1.75 \times$ higher weight of evidence for fungal biomass than bacterial biomass (Fig. 6, Table 4). Glucosamine was rejected from the candidate set of respiration models ($\Delta_i > 10$) and was less correlated with total microbial respiration ($F_{1,11} = 5.08$, $R^2_{\text{adj}} = 0.25$, $P < 0.05$) when compared to ergosterol ($F_{1,11} = 12.47$, $R^2_{\text{adj}} = 0.49$, $P < 0.01$) as single predictors.

Discussion

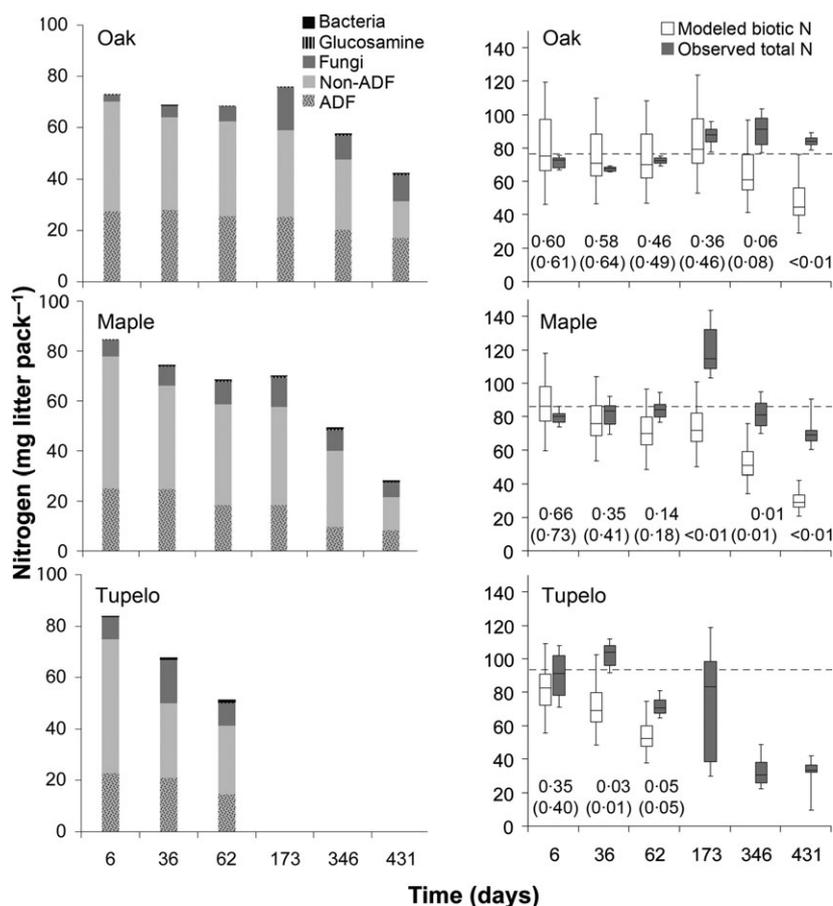
Current knowledge suggests that the degree to which leaf litter acts as a sink for nutrients over time is determined

by the tree species from which it was derived, with litter species traits modifying a complex set of primarily biotic processes occurring in the detrital matrix during decomposition. The potential effects of inorganic material on nutrient uptake in detritus have been incorporated into a few earlier studies (Meyer 1980), but are generally ignored. Here, we provide evidence suggesting that inorganic matter may be an important component of nutrient accumulation in detritus. Nutrient uptake and accumulation in leaf litter are facilitated by microbial growth and activity, but it may also be influenced by the degree to which litter intercepts inorganic matter from the surrounding water column. Our exploratory models reveal that a large portion of detrital nutrients cannot be accounted for by N and P stored in microbial biomass or by plant-derived nutrients, even when propagating substantial variability in the factors that regulate biotic processes.

NITROGEN NOT ACCOUNTED FOR BY PLANT-DERIVED N OR MICROBIAL CELLULAR N

Deficits between observed and estimated values were greater for N than for P. This may be partially explained by complexation of phenolic compounds in the plant tissue by N-containing microbial exoenzymes (Suberkropp, Godshalk & Klug 1976; Rice 1982). Some proteins are bound to phenolics near the end of the growing season or during

Fig. 4. Median observed and modeled detrital N in oak, maple, and tupelo leaf litter packs over time are provided in panels on the left. For each incubation time (in days), columns show N predicted with flexible ergosterol:fungal dry mass ($2.3\text{--}11.5 \mu\text{g g}^{-1}$), fungal C:N ($6.083\text{--}16$), bacterial C:N ($2.62\text{--}17.1$), glucosamine:fungal dry mass ($2.3\text{--}53.5 \mu\text{g g}^{-1}$) ratios, and N concentration in acid-detergent fiber (ADF) and non-ADF tissues in leaf litter. Glucosamine shown here is that which is not contained in living fungal biomass (see Methods and Appendix S1 for calculations). In right-hand panels, box plots of observed and modeled detrital N over time are presented for each leaf litter species. The probability that N modeled with flexible stoichiometric ratios (or the Redfield ratio, in parentheses) is equal to or greater than observed N is provided below each set of box plots. The horizontal dashed line indicates the average total N (mg) per litter pack at day 0. Error bars represent 95% quantile ranges. At $t = 173$ days, the stream channel was completely dry. Tupelo litter had too little material remaining after the dry period for measurement of all biotic and abiotic model parameters.



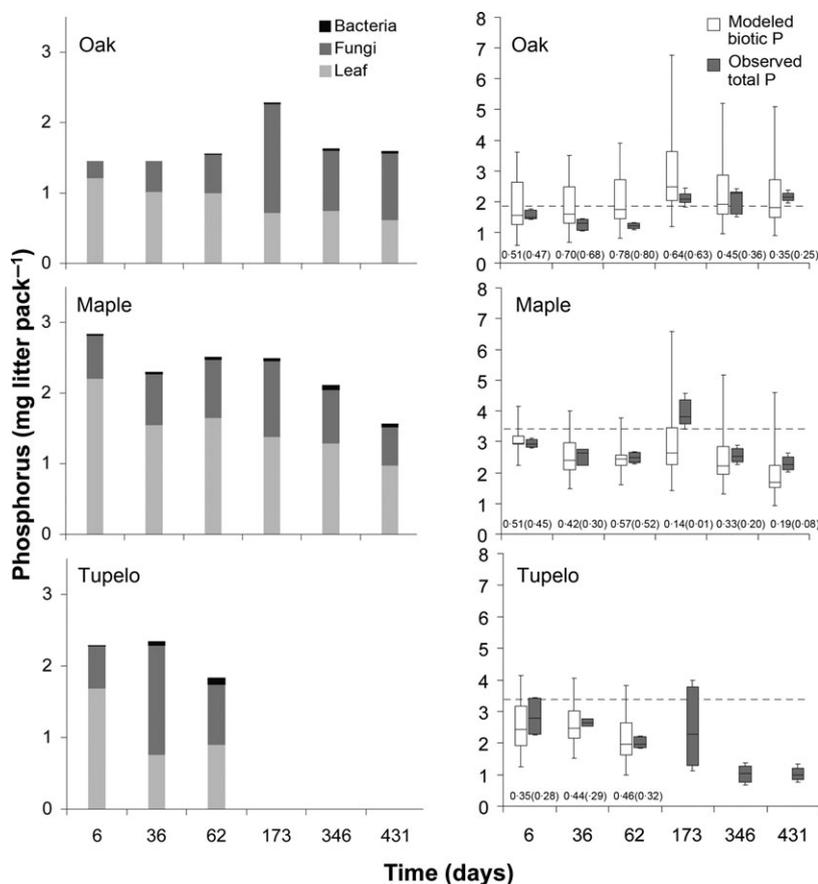


Fig. 5. Median observed and modeled detrital P in oak, maple, and tupelo leaf litter packs over time are provided in panels on the left. For each incubation time (in days), columns show P predicted with flexible ergosterol:dry mass (2.3–11.5 $\mu\text{g g}^{-1}$), fungal C:P (40–203), and bacterial C:P (8–260) ratios. In right-hand panels, box plots of observed and modeled detrital P over time are presented for each tree species. The probability that P modeled with flexible stoichiometric ratios (or the Redfield ratio, in parentheses) is equal to or greater than observed P is provided below each set of box plots. The horizontal dashed line indicates the average total P (mg) per litter pack at day 0. Error bars represent 95% quantile ranges. At $t = 173$ days, the stream channel was completely dry.

senescence in deciduous tree leaves (Davies, Coulson & Lewis 1964; Feeny 1970), forming a pool of N that is resistant to microbial degradation. We accounted for this initial plant-derived N by assuming that fibrous material contained a small concentration of N (ADF-N). However, our model did not account for the fact that the concentration of N in the ADF fraction of litter may increase substantially over time when N-containing microbial exoenzymes complex the breakdown products of lignin. This can drive the accumulation of a recalcitrant biotic pool of N neither derived from plant tissue nor from microbial cellular N. Previous work suggests enzyme–lignin complexes can account for 13–35% of the total N in detritus (Suberkropp, Godshalk & Klug 1976; Woitchik *et al.* 1997).

Allowing the N concentration bound to lignin in our model to increase over time could explain a substantial proportion of the unexplained N in our models. However, if the concentration of ADF-N reached 35% of total observed N, the maximum recorded by Suberkropp, Godshalk & Klug (1976), it would still not be sufficient to account for the total observed N in the current study. The study by Suberkropp, Godshalk & Klug (1976) involved submerging litter for 28 weeks, while our study lasted for more than 1 year and spanned an extended period of complete drying. Drying has been shown in other studies to greatly enhance N immobilization in leaf litter (Woitchik *et al.* 1997). Additionally, the availability of other nutrients has also been shown to enhance N fixation in leaf

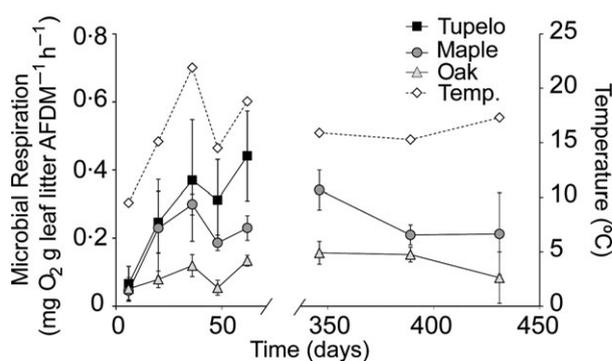


Fig. 6. Mean microbial oxygen uptake rates over time per leaf litter species. Error bars signify ± 1 standard error. For all dates and leaf litter species, $n = 5$.

litter (Crews, Farrington & Vitousek 2000), and as litter continued to accumulate nutrients such as Fe and P over time in the current study, N fixation may have been further enhanced.

INFLUENCE OF LEAF CHEMISTRY AND STRUCTURE ON NUTRIENT AND METAL DYNAMICS IN DETRITUS

Litter recalcitrance has the potential to have long-lasting ecosystem effects on nutrient retention. Although oak had lower concentrations of nutrients than other litter species, it decayed slowly enough that towards the later stages of

Table 4. Comparison of candidate multiple regression models explaining variation in oxygen uptake generated by leaf litter. *Parameters* indicates the number of parameters in the multiple regression model (including y -intercept and error), C_p provides a measurement of model error (Mallows' C_p), AIC_c is Akaike's second-order information criterion (corrected for small sample size), Δ_i is the difference between the candidate model and the best model's AIC_c , L is the likelihood value of each model, and w_i is the relative strength of evidence for each candidate model (between 0 and 1). Parameter importance weights are calculated as the sum of the values of w_i for all models containing the parameter of interest

Candidate model	<i>Parameters</i>	C_p	AIC_c	Δ_i	L	w_i
Ergosterol, temp	4	4.00	-31.35		1	0.46
Ergosterol, bacteria, temp	5	4.59	-30.83	0.52	0.77	0.36
Bacteria, temp	4	7.12	-28.80	2.55	0.28	0.13
Ergosterol	3	11.25	-25.66	5.69	0.06	0.03
Ergosterol, bacteria	4	12.76	-25.20	6.14	0.05	0.02
Bacteria	3	17.70	-22.43	8.92	0.01	0.01
Parameter	Temp		Ergosterol		Bacteria	
Importance weight	0.95		0.84		0.48	

breakdown, it became an important net sink for N and P. At different points in time, all three species became sinks for N (% initial remaining greater than 100%), and maple and oak also became sinks for P, but this was delayed for more recalcitrant species and occurred earliest in labile litter species. Therefore, recalcitrant leaf litter may slow nutrient export to downstream reaches more effectively than labile litter over time.

Leaf litter chemistry influences initial colonization and growth of N- and P-sequestering micro-organisms, but physical structures on leaf surfaces may play a role as well. Litter species in the current study differed greatly in the density of hairs (pubescence) on their surfaces (Fig. S1). Pubescence and surface roughness likely facilitate the initial attachment stage of microbial biofilm development (Donlan 2002) and may also increase the accumulation of suspended particles from stream water (Dang, Gessner & Chauvet 2007). The most pubescent litter species (maple and tupelo) had the greatest total amount of inorganic matter (including metals and nutrients) per gram and per unit area of leaf surface throughout the study. The importance of species as a model parameter suggests that differences in initial nutrient content as well as traits more difficult to quantify, such as surface roughness, may contribute to nutrient dynamics.

MICROBIAL STOICHIOMETRY AND ITS INFLUENCE ON DETRITAL NUTRIENT CONTENT

Estimates of microbial nutrient content based solely on measured cellular components (i.e. chitin, ATP or ergosterol) involve a great deal of uncertainty. Ergosterol is an estimate, but not an exact measurement of fungal biomass, since ergosterol: dry mass ratios are known to vary among species and also within a species depending on age, oxygen and nutrient availability (Gessner & Chauvet 1993; Charcosset & Chauvet 2001). The upper limits of the confidence intervals in Figs 5 and 6 illustrate the extreme scenario where the additive effects of all biotic factors are making their maximum possible contributions to nutrient content

(e.g. high microbial nutrient content, low ergosterol: dry mass ratios in fungi, low leaching rates of leaf nutrients and high concentrations of N contained in recalcitrant leaf tissue). Therefore, while it is theoretically possible to account for the entire mass of nutrients contained in leaf litter with the living and dead microbial and plant biomass included here, it is not highly probable.

Although fungal contributions to detrital N and P nutrients have exceeded 50% in other plant decay systems (Kuehn *et al.* 2011), the large fungal contribution to oak litter nutrient content during the dry period was surprising (Figs 5 and 6). Oak leaves were the most recalcitrant in our study, had presumably lower moisture during the dry period and had lower fungal biomass than other litter species during other times of the year (Fig. 3g and h). High fungal biomass (highest for oak litter) during the dry period may be due to the exploitation of high concentrations of lignin and lignin-bound N in oak leaf tissue, the breakdown of which requires oxygen (Gubernatorova & Dolgonosov 2010) that might otherwise be limiting within the leaf interior when submerged (Jørgensen & Revsbech 1985).

ACCUMULATED INORGANIC MATTER AS A NUTRIENT STORAGE POOL

Our findings are consistent with research highlighting a strong influence of microbial growth on detrital nutrient content (Gulis, Kuehn & Suberkropp 2006; Kuehn *et al.* 2011), but suggest that in addition to microbial community structure and nutrient stoichiometry, detrital accumulation of inorganic matter may influence nutrient dynamics (Hall *et al.* 2011). Strong correlation between bacterial biomass, glucosamine, Al, Fe and Mn content suggests that litter-attached biofilms may have been important for the process of suspended particle interception and inorganic matter accumulation. As microbial biofilms develop on submerged litter surfaces, they may enhance adsorption of metals (Ferris *et al.* 1989) and other particles (Battin *et al.* 2003). Microbial activity in leaf litter biofilms can influence

the rate of metal-oxide accumulation (Ferris *et al.* 1999) and thereby indirectly enhance nutrient immobilization. Thus, the potential for inorganic matter accumulation as an additional driver of nutrient uptake should be viewed as a coupled biotic–abiotic process.

Iron, manganese and aluminium content were strongly correlated in this study, and all three metals may have been accumulating in the detrital matrix as coprecipitates in metal oxides, as coatings on larger particles or as clay particles mobilized from surrounding soils. Analysing samples for a broader range of elements across the incubation period, we found Al, Fe, Mn and Si content increased over time, while Ca, Mg and K content decreased (Table S1). This is consistent with the accumulation of the main clay-sized soil minerals of the region, including kaolinite [Al₂Si₂O₅(OH)₄], goethite [FeOOH], haematite [Fe₂O₃] and gibbsite [Al(OH)₃] (Henderson *et al.* 2012). However, the measured leaf Al and Si content was lower than typical for regional soils, while the Fe content was comparatively higher (Table S1). This suggests leaf litter-associated inorganic matter was not simply a passive accumulation of suspended sediment (which would include a strong kaolinite signature with Si–Al ratios close to 1), but rather involved *in situ* precipitation of Fe, Al and Mn oxides. Such *in situ* precipitation is likely to favour the formation of high surface area metal oxides that have a high affinity for carbon and nutrients (Tate, Broshears & McKnight 1995; Bligh & Waite 2011).

In aquatic environments, microbial biofilms have been shown to accumulate cations such as Al, Ca, Fe, Mg and Mn up to 21 000× above stream water concentrations (Lalonde *et al.* 2007) and to precipitate inorganic components comprising Fe and Al-bearing silicates (Konhauser & Urrutia 1999). The correlation in our study of N and P with Al and Fe in leaf litter is consistent with the work of the aforementioned authors as ammonium and dissolved organic nitrogen strongly associate with metal oxides and silicate minerals (Triska *et al.* 1994; Tate, Broshears & McKnight 1995; Aufdenkampe *et al.* 2001). Consistent with this conceptual framework, respiration rates were strongly affected by temperature and were also significantly correlated with fungal (ergosterol) and bacterial biomass, suggesting an active microbial community. Oak litter, which had the least metabolically active microbial community throughout the study, also immobilized significantly less nutrients and metals per gram of litter.

IMPLICATIONS OF METAL-NUTRIENT ADSORPTION

Nutrients adsorbed to inorganic matter may be less bioavailable to micro-organisms and consumers at higher trophic levels, depending upon which metals are most prevalent within the inorganic fraction. Production of Al- and Fe-solubilizing acids has been documented in fungi and bacteria (Gensemer & Playle 1999; Das *et al.* 2007), and iron reduction by bacteria in leaf litter biofilms

may gradually liberate Fe-bound phosphorus as well (Burgin *et al.* 2011). It is possible that metal-adsorbed N and P could also be assimilated in the gut of consumers, depending on the metal to which nutrients are bound. Al only becomes soluble at pH levels lower than those observed in the guts of most aquatic macroinvertebrates (Bärlocher & Porter 1986; Stief & Eller 2006), and it is relatively unaffected by changes in redox conditions. However, iron reduction has been demonstrated in the guts of terrestrial insects (Vu, Nguyen & Leadbetter 2004). The extremely low redox potential in the anoxic guts of many aquatic macroinvertebrates (Stief *et al.* 2009) makes liberation of phosphorus during digestion via an Fe-reduction mechanism possible. This may represent an additional pathway for the flow of leaf litter nutrients into higher trophic levels of aquatic food webs, without directly obtaining nutrients from ingested micro-organisms or plant tissue, but the degree to which this occurs is unknown.

Detrital nutrient content is commonly expressed relative to the dry weight of the organic fraction of litter, although many of the nutrients could be contained in (and partially a function of) the inorganic fraction or a result of complexation of phenolic compounds in plant tissue by N-containing microbial exoenzymes. The dynamics of these potentially substantial components of detritus are rarely examined in aquatic studies, but may be essential to detrital nutrient dynamics. Furthermore, because accumulation rates of inorganic matter and retention of nutrients differ significantly among litter species, our findings suggest that forest composition may be able to influence nutrient and metal cycling across regional scales in streams and rivers.

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Data accessibility

Inorganic constituents of leaf litter and Tifton soils of the Georgia coastal plain, calculations and literature values used in the development of exploratory models and R scripts are available as online supporting information (Table S1, and Appendices S1 and S2, respectively). All other data are archived in the Dryad Digital Repository: <http://doi:10.5061/dryad.bt502>, (Mehring *et al.* 2014).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Leaf surfaces of freshly-shed (A) trident red maple, (B) Ogeechee tupelo, and (C) water oak leaves at 500× magnification.

Table S1. Inorganic constituents of leaf litter (top), and Tifton soils of the Georgia coastal plain (bottom).

Appendix S1. Calculations and literature sources used in the development of predicted detrital nutrient models.

Appendix S2. Sample R code for modeled detrital N, maple litter day 36.

Appendix S3. Sample R code for modeled detrital P, maple litter day 36.