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 analyzed 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 (1) labile and recalcitrant leaf litter would act as sinks at different points in the breakdown process, (2) plant and microbial biomass would not account for the entire mass of retained nutrients, and (3) 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 to 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.

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: 1) nutrient immobilization through microbial colonization and biomass accrual (Cross *et al.* 2005; Cleveland & Liptzin 2007), and 2) accumulation of inorganic sediments containing aluminum (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

69 decomposers and detrital consumers, nutrients bound to Al and Fe may be largely unavailable
70 (Reynolds & Davies 2001).

71 Here we focus on nutrient uptake associated with terrestrially-derived leaf litter since it is
72 a common and sometimes dominant form of organic matter in aquatic ecosystems. Uptake of
73 nutrients from the surrounding water by litter-inhabiting fungi and bacteria (Suberkropp &
74 Chauvet 1995) may lead to net nutrient sequestration, but the overall ability of leaf litter to serve
75 as a sink for nutrients (accumulating a greater mass of N or P than that initially present in the
76 litter) may also depend on its rate of breakdown (i.e., mass loss). Thus, while labile litter usually
77 supports higher microbial biomass and therefore greater initial microbial uptake of nutrients than
78 recalcitrant litter, labile litter itself is lost more rapidly from the system via decomposition. As a
79 consequence, recalcitrant litter may serve as a larger long-term sink for nutrients, due to lower
80 rates of mass loss. Additionally, relatively recalcitrant pools of N may develop in litter over time,
81 whereby phenols and lignin form complexes with plant proteins and N-containing microbial
82 exoenzymes (Suberkropp, Godshalk & Klug 1976; Schlesinger & Hasey 1981). Chitin in fungal
83 tissue constitutes another N-containing pool that may not decompose rapidly (Gleixner *et al.*
84 2002). An understanding of these dynamic nutrient pools is critical to assessing how forest
85 composition and consequent litter inputs affect nutrient cycling in ecosystems.

86 We examined breakdown, litter structural chemistry, fungal and bacterial biomass, and nutrient
87 and metal immobilization associated with three leaf litter species of differing physicochemical
88 characteristics in an intermittent blackwater stream. Our research asked two questions: (1) how
89 does tree species influence nutrient uptake and retention and the accumulation of inorganic
90 material on leaf litter, and (2) what are the relative contributions from biotic (fungi and bacteria)
91 and abiotic (accumulation of inorganic material) mechanisms to nutrient uptake. The incubation

period spanned more than one 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: (1) labile and recalcitrant leaf litter would act as sinks at different points in the breakdown process, (2) biotic pools would not account for the entire mass of retained nutrients, which would change with litter type and timing, and (3) 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.

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 (31º41'32"N, 83º42'09"W) drains a 2,200 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). 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 southeastern 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,

115 hereafter referred to as "tupelo"). The three litter species also differed in surface roughness;
116 maple leaves are pubescent below (Bicknell 1913) (Fig. S1A), while tupelo's leaves are "velvety
117 hairy" (Duncan & Duncan 1988) (Fig. S1B), and oak leaves are mostly smooth (Brown &
118 Kirkman 2000) (Fig. S1C). Single-species leaf litter bags containing 10 g were incubated in the
119 stream. Leaf litter from each species was collected immediately after abscission, air-dried in the
120 laboratory, and placed into plastic coarse mesh pecan bags (19 × 38 cm, 25 mm^2 mesh; Cady
121 Industries Inc., Georgia) following Benfield (1996). Leaf litter bags were deployed in study
122 reaches and were grouped in arrays affixed to PVC tubing on the bottom of the stream channel.
123 Each array consisted of three bags, each containing leaf litter from a different tree species. Bags
124 were organized into a randomized complete block design, with arrays grouped into five blocks
125 based on longitudinal distance downstream in the stream channel. Five bags of each leaf litter
126 species treatment (one from each block) were removed from the stream on each sampling date
127 (Fig. 1).

128 *In situ* rates of microbial respiration were estimated from dissolved oxygen (DO) uptake
129 by leaf disks at ambient stream water temperatures in darkness, using methods and equipment
130 identical to those described by Suberkropp *et al.* (2010). Leaf disks collected for microbial
131 respiration and fungal and bacterial biomass (described later) were gently rinsed in a beaker of
132 stream water to remove loosely-adhered sediments before any measurements were made.
133 Additional leaf disks were also preserved in HPLC-grade methanol and sterile-filtered 2%
134 phosphate buffered formalin for determination of fungal and bacterial biomass, respectively. All
135 samples were immediately placed on ice and transported to the laboratory where they were
136 stored in the dark at -20ºC (fungal biomass) and 4°C (bacterial biomass) until analyzed.

Remaining litter bag material was placed into clean, re-sealable 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 macroinvertebrates and loosely adhering sediments. Leaves were dried at 60ºC to a constant mass, and a sub-sample combusted at 500ºC to determine ash-free dry mass (AFDM). The mass of leaf disks 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 analyzed 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, New York, USA). To analyze temporal changes in leaf litter phosphorus and metal (aluminum, 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 analyzer (Alpkem 300 Series Autoanalyzer, ortho-PO4 manifold, EPA method 365.1, APHA (1999)). Metal content of extracts was analyzed 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 analyzed 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 minutes at 80ºC 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 analyzed 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 minutes using a Bransonic 150 probe sonicator (Buesing & Gessner 2002), and stained with SYBR Gold (Patel *et al.* 2007). Twenty images were randomly captured from each filter at 1000X magnification using an Olympus BH-2 microscope and an Olympus Qcolor 3 digital camera (Olympus ®, Melville, NY), and analyzed using the MatLab (v 7.9) image processing toolbox. 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).

Statistical analysis – The effect of leaf litter species and incubation length (days) on microbial respiration, fungal biomass and bacterial biomass were analyzed 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 and 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 dataset 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 didn't 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 1). Rather than use 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 (Appendix 2, 3).

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 1). 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 fiber N, ADF-N), as well as N contained in labile (non-fibrous) leaf tissue fractions (non-ADF-N) (Appendix 1).

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, USA) except for the informal exploratory exercise, which was conducted in R software (R Development Core Team, 2008). Sample calculations are available in Appendix 1, and Sample R code is available in Appendices 2 and 3.

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 $p < 0.0001$) (Figs. 2A, 2B). 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 to 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 ($F_{1,43} = 105.85$, $R^2_{adj} = 0.70$, $p < 0.0001$) and P ($F_{1,43} = 62.98$, $R^2_{adj} = 0.58$, $p < 0.0001$) were stronger than correlations between ergosterol and N ($F_{1,43} = 59.04$, $R^2_{adj} = 0.57$, $p < 0.0001$) and P ($F_{1,43} = 28.95$, $R^2_{adj} = 0.39$, $p < 0.0001$).

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 litter 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: Modeling results

An informal exploratory modeling 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 if observed increases in nutrient content can be explained solely from N and P in plant tissue 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, 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 ± 20% [95% C.I.]) that oak litter N (compared to other leaf litter species) can be explained by biotic drivers (average across all incubation times). The discrepancy between modeled and

observed detrital N stocks was positively correlated to litter-associated Al ($t_{1,13}$ = 8.39, p < 0.0001, $r^2_{adj.}$ = 0.74), Fe ($t_{1,13}$ = 7.13, p < 0.001, $r^2_{adj.}$ = 0.67), and Mn ($t_{1,13}$ = 5.88, p < 0.0001, $r^2_{adj.}$ = 0.60) contents, bulk inorganic matter ($t_{1,13}$ = 7.86, p < 0.001, $r^2_{adj.}$ = 0.67), glucosamine ($t_{1,13}$ = 5.18, p < 0.001, $r^2_{adj.}$ = 0.65), and % lignin ($t_{1,11}$ = 3.86, p < 0.05, $r^2_{adj.}$ = 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% (range 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 stoichiometry 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 and Statzell-Tallman (1982); and 7-16, Leach and Gulis, personal communication). 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

297 highest for oak litter (57 ± 13% [95% C.I.], averaged across sampling dates). Unlike estimates of
298 detrital N, which were dominated by nutrients contained in leaf tissue, microbial P accounted for
299 the largest estimated relative contribution to observed P in over ¼ of all estimates. The
300 probability of accounting for observed detrital P when allowing for flexible fungal and bacterial
301 C:P ratios rather than Redfield ratios was higher in 14/15 of all estimates, as direct measurements
302 of fungal (40-203, Leach and Gulis 2011, personal communication) and bacterial (8-260) C:P
303 ratios allow for higher P content than the Redfield ratio (106). The discrepancy between
304 estimated and observed detrital P stocks was positively correlated to mg of Al ($t_{1,13} = 4.56$, $p <$
305 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 ($t_{1,13} = 4.27$,
306 $p < 0.001$, $r^2_{adj.} = 0.55$) per litter pack.

307 Median bacterial P contributions to observed detrital P were small (average 1%, range
308 0.25% in oak, day 6 to 5% in tupelo, day 62), although higher than bacterial contributions to
309 observed N. A Redfield C:P ratio (106) resulted in lower potential bacterial contributions (0.13-
310 3%) to detrital P. Estimated fungal P accounted for the largest relative proportion (36 ± 8% [± 1
311 95% C.I.]) of observed P. Median potential contributions by living fungal biomass to observed
312 detrital P ranged from 16-68%, 26-65%, and 21-43% in oak, tupelo, and maple litter, and were
313 highest in oak litter during the dry period (73%).

314 *Microbial respiration*

315 Overall differences in microbial respiration rates among litter species were best explained
316 by fungal and bacterial biomass and ambient temperature, with 1.75× higher weight of evidence
317 for fungal biomass than bacterial biomass (Fig. 6, Table 4). Glucosamine was rejected from the
318 candidate set of respiration models ($\Delta_i > 10$), and was less correlated to total microbial

319 respiration ($F_{1,11} = 5.08$, $R^2_{adj} = 0.25$, $p < 0.05$) when compared to ergosterol ($F_{1,11} = 12.47$, R^2_{adj}
320 $= 0.49$, $p < 0.01$) as single predictors.

321 **Discussion**

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

334 *Nitrogen not accounted for by plant-derived N or microbial cellular N*

335 Deficits between observed and estimated values were greater for N than for P. This may
336 be partially explained by complexation of phenolic compounds in the plant tissue by N-
337 containing microbial exoenzymes (Suberkropp, Godshalk & Klug 1976; Rice 1982). Some
338 proteins are bound to phenolics near the end of the growing season or during senescence in
339 deciduous tree leaves (Davies *et al*. 1964; Feeney 1970), forming a pool of N that is resistant to
340 microbial degradation. We accounted for this initial plant-derived N by assuming that fibrous
341 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 one 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 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 toward the later stages of 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

364 recalcitrant species and occurred earliest in labile litter species. Therefore, recalcitrant leaf litter
365 may slow nutrient export to downstream reaches more effectively than labile litter over time.
366 Leaf litter chemistry influences initial colonization and growth of N- and P-sequestering
367 microorganisms, but physical structures on leaf surfaces may play a role as well. Litter species in
368 the current study differed greatly in the density of hairs (pubescence) on their surfaces (Fig. S1).
369 Pubescence and surface roughness likely facilitate the initial attachment stage of microbial
370 biofilm development (Donlan 2002) and may also increase the accumulation of suspended
371 particles from stream water (Dang, Gessner & Chauvet 2007). The most pubescent litter species
372 (maple and tupelo) had the greatest total amount of inorganic matter (including metals and
373 nutrients) per gram and per unit area of leaf surface throughout the study. The importance of
374 species as a model parameter suggests that differences in initial nutrient content as well as traits
375 more difficult to quantify, such as surface roughness, may contribute to nutrient dynamics.
376 *Microbial stoichiometry and its influence on detrital nutrient content* – Estimates of
377 microbial nutrient content based solely on measured cellular components (i.e. chitin, ATP, or
378 ergosterol) involve a great deal of uncertainty. Ergosterol is an estimate, but not an exact
379 measurement of fungal biomass, since ergosterol:dry mass ratios are known to vary among
380 species and also within a species depending on age, oxygen, and nutrient availability (Gessner &
381 Chauvet 1993; Charcosset & Chauvet 2001). The upper limits of the confidence intervals in
382 figures 5 and 6 illustrate the extreme scenario where the additive effects of all biotic factors are
383 making their maximum possible contributions to nutrient content (e.g., high microbial nutrient
384 content, low ergosterol:dry mass ratios in fungi, low leaching rates of leaf nutrients, and high
385 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, 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 (Figs. 3G, 3H). 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 aluminum content were strongly correlated in this study, and all three metals may have been accumulating in the detrital matrix as co-precipitates in metal oxides, as coatings on larger particles, or as clay particles mobilized from surrounding soils. Analyzing 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 [$Al_2Si_2O_5(OH)_4$], goethite [FeOOH], hematite [Fe_2O_3], 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 favor 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 to 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 microorganisms 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 microorganisms 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 1 and 2, respectively). All other data are archived in the Dryad Digital Repository: http://doi:10.5061/dryad.bt502, (Mehring *et al*. 2014).

References

APHA (1999) *Standard methods for the examination of water and wastewater,* American Public Health Association Publication, APHA, AWWA, WEF.

Aufdenkampe, A.K., Hedges, J.I., Richey, J.E., Krusche, A.V. & Llerena, C.A. (2001) Sorptive fractionation of dissolved organic nitrogen and amino acids onto fine sediments within the Amazon Basin. *Limnology and Oceanography,* **46**, 1921-1935.

Bärlocher, F. & Porter, C.W. (1986) Digestive enzymes and feeding strategies of three stream invertebrates. *Journal of the North American Benthological Society*, **5**, 58-66.

Battin, T.J., Kaplan, L.A., Newbold, J.D. & Hansen, C.M.E. (2003) Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature,* **426**, 439-442.

Benfield, E.F. (1996) Leaf breakdown in stream ecosystems. *Methods in Stream Ecology* (eds F.R. Hauer & G.A. Lamberti), pp. 579-589. Academic Press, New York, NY.

Bicknell, E.P. (1913) The Ferns and Flowering Plants of Nantucket-XI. *Bulletin of the Torrey Botanical Club,* **40**, 605-624.

Bligh, M.W. & Waite, T.D. (2011) Formation, reactivity, and aging of ferric oxide particles formed from Fe(II) and Fe(III) sources: Implications for iron bioavailability in the marine environment. *Geochimica et Cosmochimica Acta,* **75**, 7741–7758.

498 Boyer, E.W., Howarth, R.W., Galloway, J.N., Dentener, F.J., Green, P.A. & Vörösmarty, C.J.
499 (2006) Riverine nitrogen export from the continents to the coasts. *Global Biogeochemical*
500 *Cycles*, **20**, GB1S91.
501 Brown, C.L. & Kirkman, L.K. (2000) *Trees of Georgia and Adjacent States*, Timber Press,
502 Portland, OR.
503 Buesing, N. & Gessner, M.O. (2002) Comparison of detachment procedures for direct counts of
504 bacteria associated with sediment particles, plant litter and epiphytic biofilms. *Aquatic*
505 *Microbial Ecology*, **27**, 29-36.
506 Burgin, A.J., Yang, W.H., Hamilton, S.K. & Silver, W.L. (2011) Beyond carbon and nitrogen:
507 how the microbial energy economy couples elemental cycles in diverse ecosystems.
508 *Frontiers in Ecology and the Environment*, **9**, 44-52.
509 Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical*
510 *Information-Theoretic Approach*, Springer, New York.
511 Cameron, G.N. & Spencer, S.R. (1989) Rapid leaf decay and nutrient release in a chinese tallow
512 forest. *Oecologia*, **80**, 222-228.
513 Chamier, A.-C., Sutcliffe, D.W. & Lishman, J.P. (1989) Changes in Na, K, Ca, Mg and Al
514 content of submersed leaf litter, related to ingestion by the amphipod *Gammarus pulex*
515 (L.). *Freshwater Biology*, **21**, 181-189.
516 Charcosset, J.-Y. & Chauvet, E. (2001) Effect of culture conditions on ergosterol as an indicator
517 of biomass in the aquatic hyphomycetes. *Applied and Environmental Microbiology*, **67**,
518 2051-2055.
519 Cleveland, C.C. & Liptzin, D. (2007) C:N:P stoichiometry in soil: is there a "Redfield ratio" for
520 the microbial biomass? *Biogeochemistry*, **85**, 235-252.

521 Crews, T. E., Farrington, H. & Vitousek, P. M. (2000) Changes in asymbiotic, heterotrophic
522 nitrogen fixation on leaf litter of *Metrosideros polymorpha* with long-term ecosystem
523 development in Hawaii. *Ecosystems*, **3**, 386-395.
524 Cross, W.F., Benstead, J.P., Frost, P.C. & Thomas, S.A. (2005) Ecological stoichiometry in
525 freshwater benthic systems: recent progress and perspectives. *Freshwater Biology*, **50**,
526 1895-1912.
527 Dang, C.K., Gessner, M.O. & Chauvet, E. (2007) Influence of conidial traits and leaf structure
528 on attachment success of aquatic hyphomycetes on leaf litter. *Mycologia*, **99**, 24-32.
529 Das, A. , Prasad, R., Srivastava, A.,Giang, P.H., Bhatnagar, K. & Varma, A. (2007) Fungal
530 siderophores: Structure, functions and regulation. *Soil Biology*. (eds A. Varma & S.B.
531 Chincholkar), pp. 1-42. Springer, New York, New York.
532 Davies, R.I., Coulson, C.B. & Lewis, D.A. (1964) Polyphenols in plant, humus, and soil III.
533 Stabilization of gelatin by polyphenol tanning. *Journal of Soil Science*, **15**, 299-309.
534 Donlan, R.M. (2002) Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*, **8**, 881-
535 890.
536 Duncan, W.H. & Duncan, M.B. (1988) *Trees of the southeastern United States*, University of
537 Georgia Press, Athens, Georgia.
538 Feeny, P. (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding
539 by winter moth caterpillars. *Ecology*, **51**, 565-581.
540 Ferris, F.G., Konhauser, K.O., Lyven, B. & Pedersen, K. (1999) Accumulation of metals by
541 bacteriogenic iron oxides in a subterranean environment. *Geomicrobiology Journal*, **16**,
542 181-192.

543 Ferris, F.G., Schultze, S., Witten, T.C., Fyfe, W.S. & Beveridge, T.J. (1989) Metal interactions
544 with microbial biofilms in acidic and neutral pH environments. *Applied and*
545 *Environmental Microbiology*, **55**, 1249-1257.
546 First, M.R. & Hollibaugh, J.T. (2008) Protistan bacterivory and benthic microbial biomass in an
547 intertidal creek mudflat. *Marine Ecology Progress Series*, **361**, 59-68.
548 Gensemer, R.W. & Playle, R.C. (1999) The bioavailability and toxicity of aluminum in aquatic
549 environments. *Critical Reviews in Environmental Science and Technology*, **29**, 315-450.
550 Gessner, M.O. (2005) Ergosterol as a measure of fungal biomass. *Methods to Study Litter*
551 *Decomposition: A Practical Guide.* (eds M.A.S. Graça, F. Bärlocher & M.O. Gessner),
552 pp. 189-196. Springer, Dordrecht, The Netherlands.
553 Gessner, M.O. & Chauvet, E. (1993) Ergosterol-to-biomass conversion factors for aquatic
554 hyphomycetes. *Applied and Environmental Microbiology*, **59**, 502-507.
555 Gleixner, G., Poirier, N., Bol, R. & Balesdent, J. (2002) Molecular dynamics of organic matter in
556 a cultivated soil. *Organic Geochemistry*, **33**, 357-366.
557 Gubernatorova, T.N. & Dolgonosov, B.M. (2010) Modeling the biodegradation of
558 multicomponent organic matter in an aquatic environment: 3. Analysis of lignin
559 degradation mechanisms. *Water Resources*, **37**, 332-346.
560 Gulis, V., Kuehn, K. & Suberkropp, K. (2006) The role of fungi in carbon and nitrogen cycles in
561 freshwater ecosystems. *Fungi in Biogeochemical Cycles* (ed G.M. Gadd), pp. 404-435.
562 Cambridge University Press, Cambridge, UK.
563 Hall, E.K., Maixner, F., Franklin, O., Daims, H., Richter, A. & Battin, T. (2011) Linking
564 microbial and ecosystem ecology using ecological stoichiometry: A synthesis of
565 conceptual and empirical approaches. *Ecosystems*, **14**, 261-273.

566 Henderson, R., Kabengi, N., Mantripragada, N., Cabrera, M., Hassan, S. & Thompson, A. (2012)
567 Anoxia-induced release of colloid- and nanoparticle-bound phosphorus in grassland soils.
568 *Environmental Science and Technology*, **46**, 11727–11734.
569 Hesterberg, D., Duff, M.C., Dixon, J.B. & Vepraskas, M.J. (2011) X-ray microspectroscopy and
570 chemical reactions in soil microsites. *Journal of Environmental Quality*, **40**, 667-678.
571 Jørgensen, B.B. & N.P. Revsbech. (1985) Diffusive boundary layers and the oxygen uptake of
572 sediments and detritus. *Limnology and Oceanography*, **30**, 111-122.
573 Konhauser, K. & Urrutia, M.M. (1999) Bacterial clay authigenesis: a common biogeochemical
574 process. *Chemical Geology*, **161,** 399-413.
575 Kuehn, K.A., Ohsowski, B.M., Francoeur, S.N. & Neely, R.K. (2011) Contributions of fungi to
576 carbon flow and nutrient cycling from standing dead *Typha angustifolia* leaf litter in a
577 temperate freshwater marsh. *Limnology and Oceanography*, **56**, 529-539.
578 Lalonde, S., Amskold, L., Warren, L. & Konhauser, K. (2007) Surface chemical reactivity and
579 metal adsorptive properties of natural cyanobacterial mats from an alkaline hydrothermal
580 spring, Yellowstone National Park. *Chemical Geology,* **243**, 36-52.
581 Lowrance, R. & Vellidis, G. (1995) A conceptual-model for assessing ecological risk to water-
582 quality function of bottomland hardwood forests. *Environmental Management*, **19**, 239-
583 258.
584 Mayorga, E., Seitzinger, S.P., Harrison, J.A., Dumont, E., Beusen, A.H.W., Bouwmand, A.F.,
585 Fekete, B.M., Kroeze, C. & Drecht, G.V. (2010) Global Nutrient Export from
586 WaterSheds 2 (NEWS 2): Model development and implementation. *Environmental*
587 *Modelling & Software*, **25**, 837–853.

588 Mehring, A.S., Kuehn, K.A., Thompson, A., Pringle, C.M., Rosemond, A.D., First, M.R.,
589 Lowrance, R.R. & Vellidis, G. (2014) Data from: Leaf litter nutrient uptake in an
590 intermittent blackwater river: Influence of tree species and associated biotic and abiotic
591 drivers. Dryad Digital Repository. http://doi:10.5061/dryad.bt502
592 Meyer, J.L. (1980) Dynamics of phosphorus and organic matter during leaf decomposition in a
593 forest stream. *Oikos,* **34**, 44-53.
594 Milliken, G.A. & Johnson, D.E. (1992) *Analysis of Messy Data*, Van Nostrand Reinhold, New
595 york, New York.
596 Newell, S.Y. & Statzell-Tallman, A. (1982) Factors for conversion of fungal biovolume values to
597 biomass, carbon and nitrogen: Variation with mycelial ages, growth conditions, and
598 strains of fungi from a salt marsh. *Oikos,* **39**, 261-268.
599 Patel, A., Noble, R.T., Steele, J.A., Schwalbach, M.S., Hewson, I. & Fuhrman, J.A. (2007) Virus
600 and prokaryote enumeration from planktonic aquatic environments by epifluorescence
601 microscopy with SYBR Green I. *Nature Protocols*, **2**, 269-276.
602 Reynolds, C.S. & Davies, P.S. (2001) Sources and bioavailability of phosphorus fractions in
603 freshwaters: a British perspective. *Biological Reviews of the Cambridge Philosophical*
604 *Society,* **76**, 27-64.
605 Rice, D. (1982) The detritus nitrogen problem: New observations and perspectives from organic
606 geochemistry. *Marine Ecology Progress Series*, **9**, 153-162.
607 Schlesinger, W.H. & Hasey, M.M. (1981) Decomposition of chaparral shrub foliage: Losses of
608 organic and inorganic constituents from deciduous and evergreen leaves. *Ecology,* **62**,
609 762-774.

610 Sigg, L. & Stumm, W. (1981) The interaction of anions and weak acids with the hydrous
611 goethite (α-FeOOH) surface. *Colloids and Surfaces*, **2**, 101-117.
612 Stief, P. & Eller, G. (2006) The gut microenvironment of sediment-dwelling *Chironomus*
613 *plumosus* larvae as characterised with O_2, pH, and redox microsensors. *Journal of*
614 *Comparative Physiology B*, **176**, 673-683.
615 Stief, P., Poulsen, M., Nielsen, L.P., Brix, H. & Schramm, A. (2009) Nitrous oxide emission by
616 aquatic macrofauna. *Proceedings of the National Academy of Sciences of the United*
617 *States of America*, **106**, 4296-4300.
618 Suberkropp, K. & Chauvet, E. (1995) Regulation of leaf breakdown by fungi in streams:
619 Influences of water chemistry. *Ecology*, **76**, 1433-1445.
620 Suberkropp, K., Godshalk, G.L. & Klug, M.J. (1976) Changes in the chemical composition of
621 leaves during processing in a woodland stream. *Ecology*, **57**, 720-727.
622 Suberkropp, K., Gulis, V., Rosemond, A.D. & Benstead, J.P. (2010) Ecosystem and
623 physiological scales of microbial responses to nutrients in a detritus-based stream:
624 Results of a 5-year continuous enrichment. *Limnology and Oceanography*, **55**, 149-160.
625 Tate, C.M., Broshears, R.E. & Mcknight, D.M. (1995) Phosphate dynamics in an acidic
626 mountain stream: Interactions involving algal uptake, sorption by iron oxide, and
627 photoreduction. *Limnology and Oceanography*, **40**, 938-946.
628 Triska, F.J., Jackman, A.P., Duff, J.H. & Avanzino, R.J. (1994) Ammonium sorption to channel
629 and riparian sediments: a transient storage pool for dissolved inorganic nitrogen.
630 *Biogeochemistry*, **26**, 67-83.
631 Vu, A.T., Nguyen, N.C. & Leadbetter, J.R. (2004) Iron reduction in the metal-rich guts of wood-
632 feeding termites. *Geobiology*, **2,** 239-247.

633 Webster, J.R. & Benfield, E.F. (1986) Vascular plant breakdown in freshwater ecosystems.
634 *Annual Review of Ecology and Systematics*, **17,** 567-594.
635 Wenger, S.J., Som, N.A., Dauwalter, D.C., Isaak, D.J., Neville, H.M., Luce, C.H., Dunham, J.B.,
636 Young, M.K., Fausch, K.D. & Rieman, B.E. (2013) Probabilistic accounting of
637 uncertainty in forecasts of species distributions under climate change. *Global Change*
638 *Biology*, **19**, 3343–3354.
639 Woitchik, A.F.; Ohowa, B.; Kazungu, J.M.; Rao, R.G.; Goeyens, L. & Dehairs, F. (1997)
640 Nitrogen enrichment during decomposition of mangrove leaf litter in an east African
641 coastal lagoon (Kenya): Relative importance of biological nitrogen fixation.
642 *Biogeochemistry*, **39**,15–35.
643
644

Figure 1.

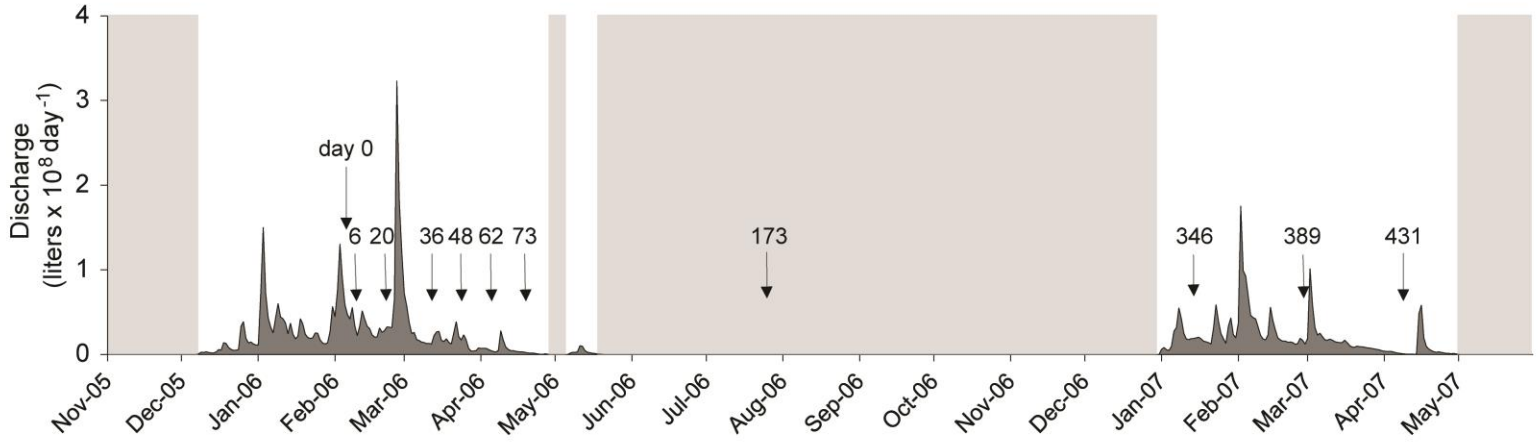

Discharge
(liters x 10^8 day^{-1})
4
3
2
1
0
day 0
6
20
36
48
62
73
173
346
389
431
Nov-05
Dec-05
Jan-06
Feb-06
Mar-06
Apr-06
May-06
Jun-06
Jul-06
Aug-06
Sep-06
Oct-06
Nov-06
Dec-06
Jan-07
Feb-07
Mar-07
Apr-07
May-07

Figure 2.

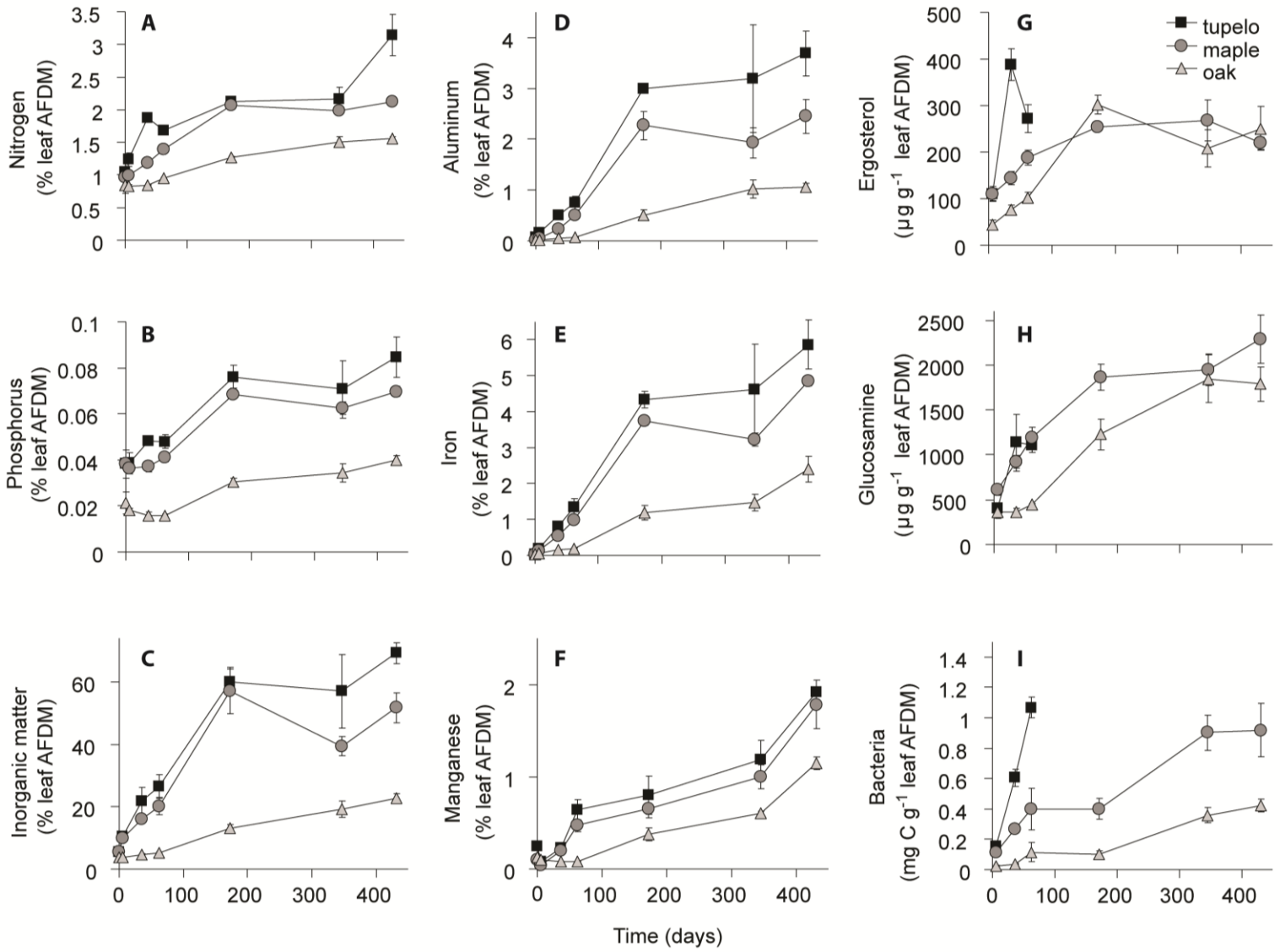

A
Nitrogen (% leaf AFDM)
B
Phosphorus (% leaf AFDM)
C
Inorganic matter (% leaf AFDM)
D
Aluminum (% leaf AFDM)
E
Iron (% leaf AFDM)
F
Manganese (% leaf AFDM)
G
Ergosterol (µg g-1 leaf AFDM)
H
Glucosamine (µg g-1 leaf AFDM)
I
Bacteria (mg C g-1 leaf AFDM)
tupelo
maple
oak
Time (days)

Figure 3.

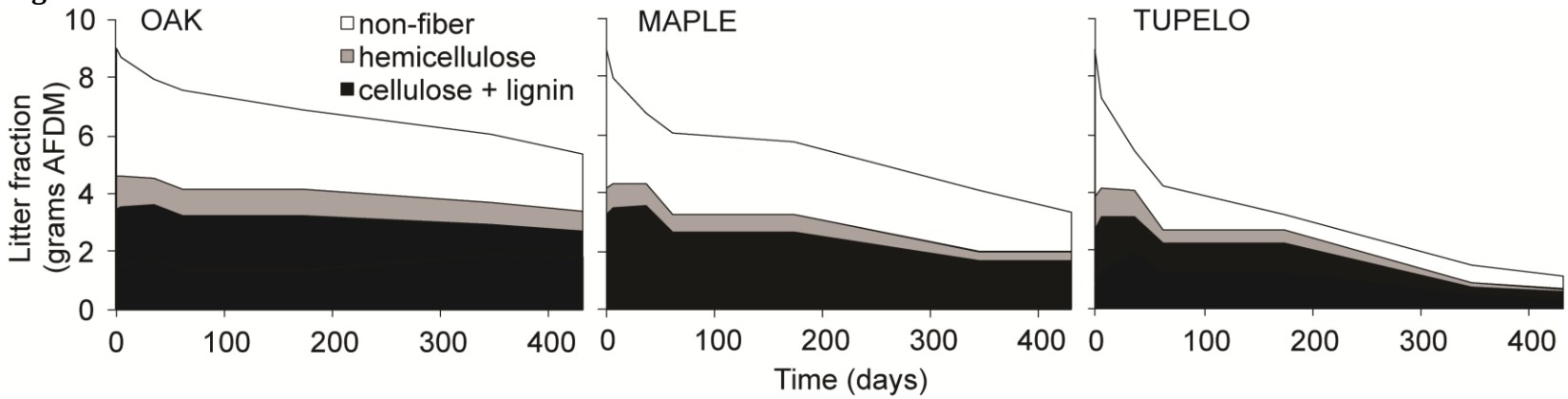

OAK
MAPLE
TUPELO
non-fiber
hemicellulose
cellulose + lignin
Litter fraction (grams AFDM)
0
2
4
6
8
10
0
100
200
300
400
Time (days)

Figure 4.

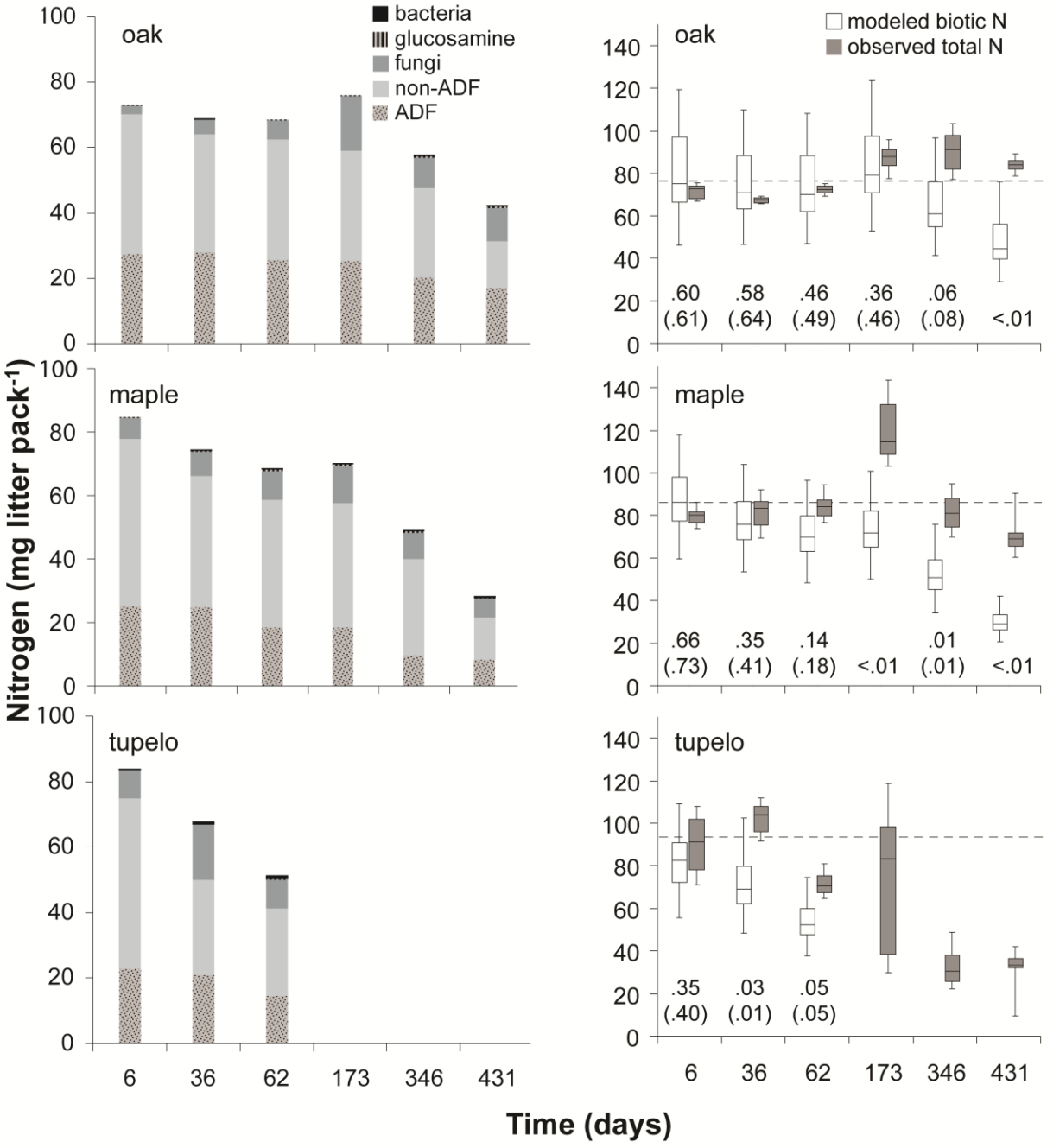

oak
maple
tupelo
bacteria
glucosamine
fungi
non-ADF
ADF
Nitrogen (mg litter pack-1)
modeled biotic N
observed total N
.60 (.61)
.58 (.64)
.46 (.49)
.36 (.46)
.06 (.08)
<.01
.66 (.73)
.35 (.41)
.14 (.18)
<.01
.01 (.01)
<.01
.35 (.40)
.03 (.01)
.05 (.05)
6
36
62
173
346
431
Time (days)

Figure 5.

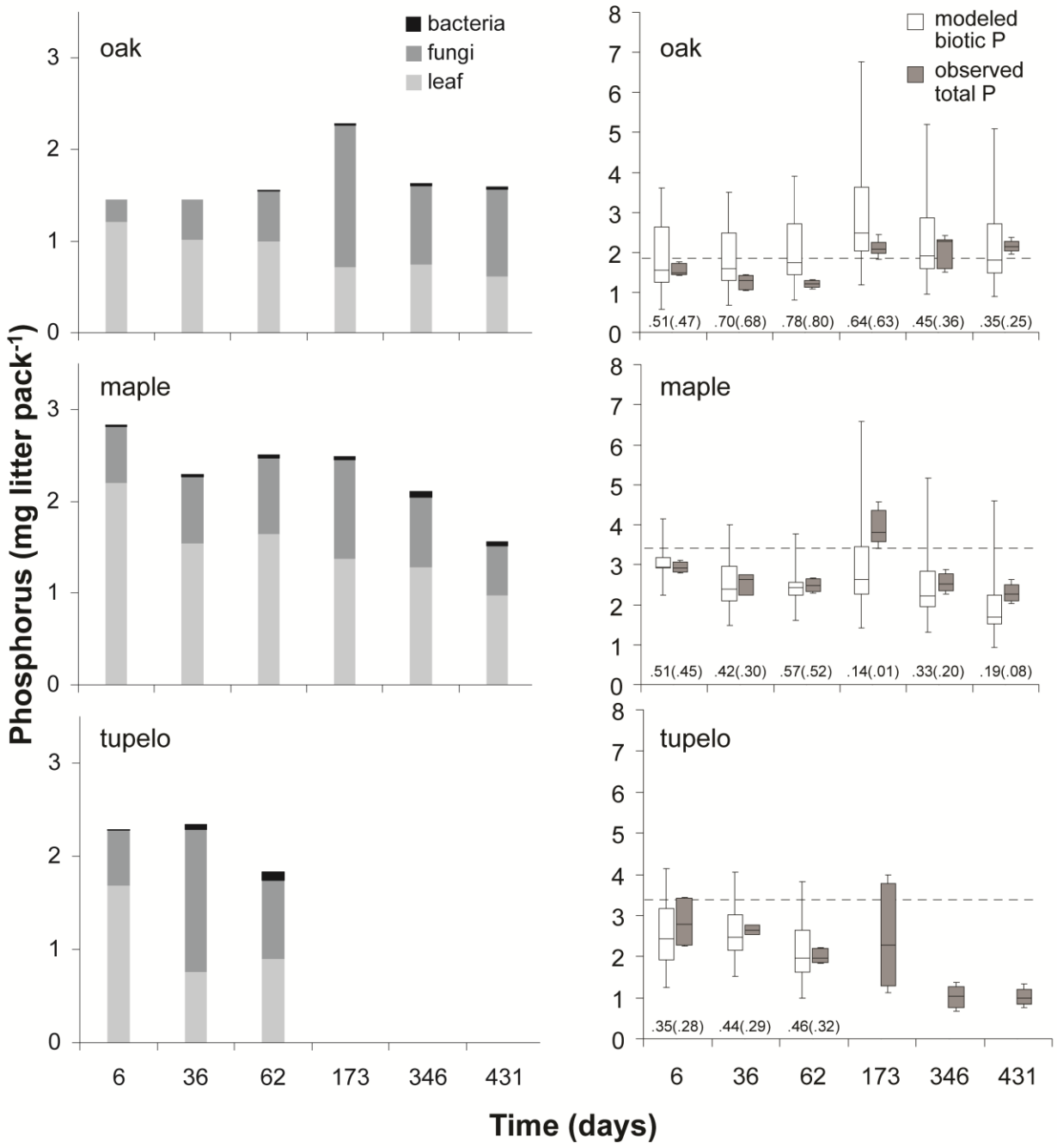
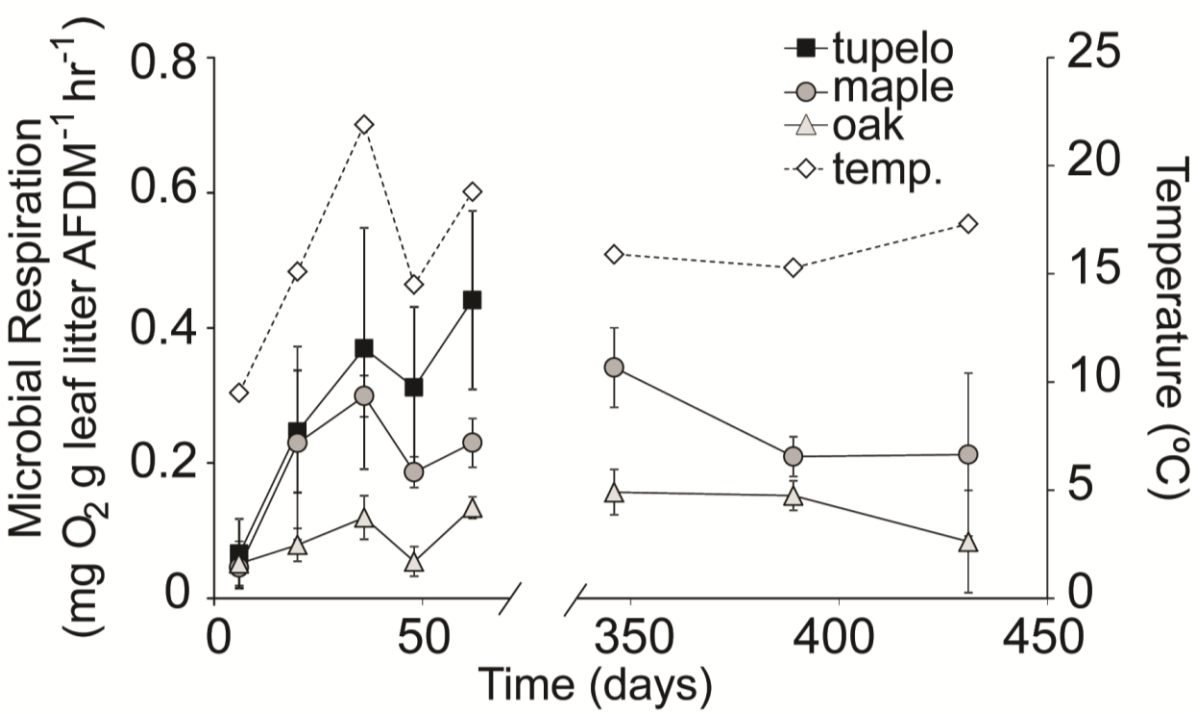

bacteria
fungi
leaf
oak
maple
tupelo
modeled biotic P
observed total P
.51(.47)
.70(.68)
.78(.80)
.64(.63)
.45(.36)
.35(.25)
.51(.45)
.42(.30)
.57(.52)
.14(.01)
.33(.20)
.19(.08)
.35(.28)
.44(.29)
.46(.32)
Phosphorus (mg litter pack-1)
Time (days)
6
36
62
173
346
431

Figure 6.


Microbial Respiration
(mg O_2 g leaf litter AFDM^{-1} hr^{-1})
0.8
0.6
0.4
0.2
0
tupelo
maple
oak
temp.
25
20
15
10
5
0
Temperature (°C)
0
50
350
400
450
Time (days)