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Exogenous nutrient manipulations alter endogenous extractability of carbohydrates in decomposing foliar litters under a typical mixed forest of subtropics

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ABSTRACT

Litter decomposition is a major driver of nutrient availability and carbon (C) cycling in forest ecosystems, and may be susceptible to perturbation by exogenous nutrient inputs from anthropogenic activity. The role of resource limitation in this process is unclear, although some models suggest the macronutrients nitrogen (N) and phosphorus (P) stimulate early-stage decomposition while availability of labile C restricts later-stage decomposition. We studied this interplay in a subtropical mixed forest in southern China through *in situ* incubations of litter samples over a 540-day period. Litter samples were amended with labile C (+C, as sucrose), N (+N, as NH_4NO_3), P (+P, as NaH_2PO_4), all three inputs (+CNP), or no inputs (CK). Litter mass and nutrient content were measured at 90-day intervals, while extractable carbohydrate profiles of the litter samples were assayed at 90, 270, and 450 days of incubation. The +P and +CNP treatments showed greater reductions in litter mass, as well as a larger P pool throughout the incubation. The +N treatment had a larger N pool, but otherwise did not differ from the no input control CK. The concentration of accessible carbohydrate fractions remained constant or increased from 90 to 450 days of incubation, while less accessible fractions thought to be lignocellulose and hemi-cellulose decreased during that period. Total extractable carbohydrates decreased in the +P treatment, but was not significantly different among other treatments. Our results suggest that mixed forest systems in southern China are likely to be minimally perturbed in the short term by exogenous N addition, and that decomposition activity is not regulated by labile C availability over the time period studied. Moreover, increased C inputs due to climate change-induced changes in litter deposition and root exudation will likely have a smaller impact on subtropical forest management than anthropogenic disturbances such as P fertilization.

1. Introduction

Forests are a key element of global biogeochemical cycles, functioning as major terrestrial carbon (C) reservoirs (Pan et al., 2011) and playing a critical role in many nutrient cycles (Attiwill and Adams, 1993). Litter decomposition is one of the key mechanisms governing nutrient availability in many forest systems (Aber and Melillo, 2001) and helps determine long-term C balances (Berg and McClaugherty, 2007). Systems such as the subtropical mixed forests in southern and eastern China face growing human activity (Bruelheide et al., 2011), creating a potential for substantial alteration of nutrient cycling and dynamics. Nitrogen (N) and phosphorus (P) in particular have become much more available in many environments due to anthropogenic influences on their biogeochemical cycles (Falkowski et al., 2000; Vitousek et al., 1997). Improving our understanding of the processes that drive litter decomposition dynamics will augment our knowledge of the regulation of forest ecosystem function (Drewnik, 2006), and will be crucial for informing responses to future environmental perturbations (Cornwell et al., 2008; Knorr et al., 2005).

One of the key dynamics governing the fate of carbohydrates during decomposition is whether decomposers are limited by nutrients or energy. Decomposer biomass requires a relatively strict stoichiometry among C, N, and P, and the availability of specific nutrients can alter both the rate of decomposition and eventual composition of residual biomass (Aber and Melillo, 2001; Cherif and Loreau, 2007). For instance, changes to N availability in N-limited systems can influence decomposition of recalcitrant C, even if more accessible energy sources are present (Craine et al., 2007). These effects may be highly variable, however. Various studies have found the effects of N and P availability on litter decomposition to be neutral (Bridgham and Richardson, 1992; Coulson and Butterfield, 1978; Prescott, 1995), positive (Berg and Tamm, 1994; Hobbie, 2000), negative (Mcclaugherty and Berg, 1987; Mo et al., 2008), or specific to sites or species (Chen et al., 2012). Other studies have found the availability of energy, rather than nutrients, primarily limits decomposer activity (Hattenschwiler and Jorgensen, 2010), and that decomposer communities respond to the

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identity and accessibility of plant-derived C sources (Fontaine et al., 2007; Orwin et al., 2006). Manipulating the relative availabilities of key nutrients and energy sources can reveal system-specific nutrient controls on decomposition (Krashevska et al., 2010).

Soluble litter carbohydrates are the primary source of energy for decomposers, making the quality of litter composition a potential determinant of energy availability. Efforts to characterize litter composition quality, as defined by its influence on decomposition processes, are hampered by complicated measurement and multifarious features (Coq et al., 2010; McLeod et al., 2007). One challenge may be that typical quantifications of litter components such as lignin and carbohydrate polymers do not capture the properties of litter that are most important for decomposition (Newsham et al., 2001). One potentially effective alternative approach is the sequential extraction of structural components, which characterizes litter composition based on the cross-linkage of various constituents of the cell wall (Fry, 1986; Selvendran and Oneill, 1987). This method is effective at quantifying the relative availability of accessible energy-rich carbon compounds, and can reflect decomposition-relevant characteristics of decaying litter (McLeod et al., 2007). By tracking the abundance of functionally-defined carbohydrate fractions under a range of conditions, it may be possible to improve our understanding of decomposition constraints and dynamics in forest ecosystems.

In this study, we explored the effects of exogenous addition of different stoichiometries of labile C, N, and P on the in situ decomposition of leaf litter in the context of a subtropical mixed forest in China. We employed sequential extraction of plant structural components to track the changing composition of decomposing litter over a 540-day period. The aims of our study were to: 1) evaluate the effect of stoichiometrically balanced and unbalanced exogenous nutrient addition on litter decomposition rates and nutrient status; 2) track changes in extractable carbohydrate fractions during decomposition; and 3) evaluate the effects of exogenously applied nutrients on changes litter carbohydrate composition during decomposition. Our study improves our understanding of litter decomposition dynamics in an important and ecologically sensitive ecosystem, while also demonstrating the utility of an established but little-used method for functionally characterizing decomposing litter.

2. Materials and methods

2.1. Site description

Our study was conducted in Xinjian County (115°27′–116°35′E, 28°09–29°11′N), Jiangxi Province in southern China, described in detail in our recent publication (Chen et al., 2012). The area has a subtropical warm humid climate, with an annual mean air temperature of 17.5 °C, and mean annual precipitation of 1700 mm. Over 60% of the study area is covered by forest vegetation, with *Pinus massoniana* and *Liquidambar formosana* as the dominant coniferous and deciduous species respectively. Ultisols are the dominant soil order, with pH 4.4, 13.5 g organic C kg^{-1} soil, 1.15 g total N kg^{-1} soil, and 0.25 g total P kg^{-1} soil, on average (Zhan et al., 2009).

2.2. Experimental design and treatments

To track decomposition over time, we employed the litter nylon net bag method (Berg and Staaf, 1980; Chen et al., 2012). Briefly, we collected *P. massoniana* and *L. formosana* foliar litter from three 20 × 20 m plots, composited equal dry weights from both species, and placed 5 g of the litter mixture in 15 × 15 cm nylon bags with a 1 mm mesh. In each plot from which we sampled the litter, we arbitrarily placed 9 subplots measuring 2 × 2 m and separated by >5 m. In each subplot, we placed 12 bags on the soil surface below the litter layer, for 324 bags in total. Nutrient addition was carried out at the subplot level, with each subplot receiving one of nine treatments including: 75 or 150 g sucrose-C m^{-2} $year^{-1}$, 7.5 or 15 g NH_4NO_3-N m^{-2} $year^{-1}$, 3.75 or 7.50 g NaH_2PO_4-P m^{-2} $year^{-1}$, all three nutrients together at their respective high or low levels, and a no nutrient control (Table 1). All nutrients were delivered as an aqueous solution at 30-day intervals, with the control receiving an equal volume of water. The lower level of N addition was matched to observed rates of N deposition in south-central China (Lü and Tian, 2007). Carbon and P rates selected to achieve the C:N and N:P ratios of 10 and 2 commonly found in soils (Zhan et al., 2009), so that treatments receiving all nutrients would be stoichiometrically balanced with standard soil conditions.

2.3. Sampling and chemical analysis

At 90-day intervals for 6 time points, 2 bags were sampled from each subplot, cleaned of roots and attached soil, washed with deionized water, dried at 45 °C, then weighed, composited and ground to 0.25 mm for chemical analysis. Litter organic C content was determined by the Walkley–Black wet oxidation method following acid removal of carbonates (Allen, 1989). Nitrogen and phosphorus concentrations were determined by the Kjeldahl method and phosphomolybdic acid blue color method after the samples were digested with 18.4 M H_2SO_4 solution (Allen, 1989).

We quantified extractable carbohydrate fractions at days 90, 270, and 450 using a seven-step serial fractionation that classified carbohydrates based on their linkages within cell wall architecture (McLeod et al., 2007): (1) free sugars and polysaccharides were extracted with 10% (v/v) formic acid, which also denatured enzymes and scavenged hydroxyl radicals; (2) weakly-bound polysaccharides and pectins were extracted with phosphate buffer (200 mM NaH_2PO_4, 0.5% w/v chlorobutanol, 10 mM $Na_2S_2O_3$, final pH adjusted to 7.0 with NaOH); (3) strongly-bound polysaccharides and pectins were extracted by removal of calcium and solubilization by CDTA (50 mM w/v *trans*-1,2-diaminocyclohexane-N, N, N′, N′-tetraacetic acid, 0.5% w/v chlorobutanol at pH 7.5); (4) bonds between hemicellulose and cellulose were broken by extraction with urea (8 M H_2NCONH_2, 50 mM N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid at pH 7.5) leading to extraction of some hemicelluloses; (5) otherwise inaccessible sugars were extracted by low temperature ester hydrolysis with mildly alkaline sodium carbonate (200 mM Na_2CO_3, at 5 °C); (6) remaining hemicelluloses were extracted with strongly alkaline sodium hydroxide with $NaBH_4$ to protect carbohydrates from oxidation (6 M NaOH, 1% w/v $NaBH_4$ at 37 °C); (7) lignocellulose released by removal of previous fractions was solubilized in 5% (v/v) formic acid. Carbohydrates remaining after the seventh extraction were assumed to be structural cellulose (Fry, 2000). Extractions consisted of 3 replicates of 0.3 g ground litter

Table 1
Nutrient addition treatments and their associated decay constants.

Treatment	Nutrient level	Decay constant (*k*)		
Control (CK)		-9.4×10^{-4}	*A*	*a*
Carbon (+C)	75 g-C m^{-2} $year^{-1}$	-9.7×10^{-4}	*AB*	
(as glucose)	150 g-C m^{-2} $year^{-1}$	-10.3×10^{-4}	*AB*	
	Combined	-10.0×10^{-4}		*ab*
Nitrogen (+N)	7.5 g-N m^{-2} $year^{-1}$	-9.9×10^{-4}	*AB*	
(as NH_4NO_3)	15 g-N m^{-2} $year^{-1}$	-10.8×10^{-4}	*AB*	
	Combined	-10.4×10^{-4}		*ab*
Phosphorus (+P)	3.75 g-P m^{-2} $year^{-1}$	-10.3×10^{-4}	*AB*	
(as NaH_2PO_4)	7.5 g-P m^{-2} $year^{-1}$	-11.2×10^{-4}	*AB*	
	Combined	-10.7×10^{-4}		*b*
Combined nutrients	Low	-11.4×10^{-4}	*B*	
(+CNP)	High	-11.5×10^{-4}	*B*	
	Combined	-11.5×10^{-4}		*b*

Treatments sharing a letter did not have significantly different decay constants ($P < 0.05$, based on Tukey's multiple comparison correction). Uppercase letters correspond to treatments distinguishing among nutrient levels, lowercase letters correspond to combined addition levels. Low and High levels for +CNP correspond to the low and high levels for individual nutrients.