Effects of different canopy tree species on belowground biogeochemistry in a wet lowland tropical forest

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EFFECTS OF CANOPY TREE SPECIES DIVERSITY ON BELOWGROUND
BIOGEOCHEMISTRY IN A WET LOWLAND TROPICAL FOREST

By

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Thesis

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ABSTRACT

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Effects of different canopy tree species on belowground biogeochemistry in a wet lowland tropical forest

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Tropical rain forests are known for their tremendous biological diversity, but the effects of plant diversity on important ecosystem processes remain unclear. Interspecies differences in both the demand for nutrients and in foliar nutrient concentrations could drive differences in litter chemistry that affect both pools and fluxes of belowground resources. Yet, our understanding of the effects of aboveground biogeochemical heterogeneity on belowground ecosystems is poor, especially in the species-rich forests of the wet tropics. To investigate the effects of tree species diversity on belowground biogeochemical processes, I examined how carbon (C), nitrogen (N), and phosphorus (P) cycles vary under canopy tree species – including legume and non-legume species – that vary in foliar leaf nutrient concentrations in a wet tropical forest in southwestern Costa Rica. I found significant differences in belowground C, N and P cycling under different canopy tree species. Total C, N and P pools in standing litter varied by species, as did total soil and microbial C and N pools. Rates of soil extracellular acid phosphatase activity (Ptase) varied both by species and functional group, with higher rates of Ptase activity observed under legumes. In addition, Ptase activity was significantly negatively correlated to litter N/P, suggesting a tight coupling between N and P cycles belowground. I also conducted a laboratory incubation experiment in attempt to isolate the effects of litter chemistry on belowground biogeochemistry. Results showed a significant relationship between litter chemistry and cumulative C mineralization and inorganic N availability, but litter chemistry did not affect soil labile P pools or Ptase activity. Overall, my results suggest the importance of aboveground plant community composition in promoting belowground biogeochemical heterogeneity at small spatial scales.
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BACKGROUND AND RATIONALE

Tropical rain forests boast some of the greatest plant biodiversity of any biome on earth and are disproportionately important in global biogeochemical cycles (Field et al. 1998; Phillips et al. 1998). Previous research has highlighted the importance of linkages between biodiversity and biogeochemistry in tropical forests (Townsend et al. 2011), as aboveground biodiversity is reflected in high interspecific variation in foliar leaf nutrient concentration. For example, it has been shown that foliar nitrogen (N), foliar phosphorus (P) and foliar N/P ratios vary more among species in a given tropical forest site than across all temperate forests combined (Townsend et al. 2008). However, our understanding of the effects of such aboveground heterogeneity on key belowground biogeochemical properties in tropical forests remains poor.

Zinke (1962) was among the first to show that individual trees may exert a “sphere of influence” on soil properties and suggested that aboveground diversity may drive local patterns in soil heterogeneity. Since Zinke’s seminal paper, additional research has shown tree species may differentially affect leaf litter and soil biogeochemistry in multiple ways, thereby regulating fundamental biogeochemical processes (Hobbie 1992; Hobbie et al. 2006; Reed et al. 2008). Species effects on soil biogeochemistry are a result of both above- and belowground controls. First, interspecies variation in foliar nutrient concentration may translate to differences in litter chemistry that could both directly and indirectly affect pools and fluxes of belowground carbon (C) and nutrients (Binkley and Giardina 1998; Reed et al. 2008). For example, fine litterfall represents the dominant input of C and nutrients to forest soils, and species-specific variation in litterfall C and nutrient concentrations (and ratios) may drive differences in belowground C and
nutrient pools under different tree species (Binkley and Giardina 1998; Prescott 2002). Further, litter chemistry has been shown to strongly regulate rates of decomposition in a wide variety of ecosystems (Cornwell et al. 2008). Generally, litter lignin/N ratios are negatively related with decomposition rates (Melillo et al. 1982; Hobbie 1992), and in some cases other metrics of litter chemistry, such as the concentration of organic constituents (Hirobe et al. 2004), P (Wieder et al. 2009), or micronutrients (Hobbie et al. 2006; Kaspari et al. 2008) are also strong predictors of decomposition rates. Thus, interspecies variation in litter chemistry may drive differences in belowground C and nutrient availability both directly via litter C and nutrient concentrations and indirectly via controls over decomposition rates (Figure 1).

Second, species-specific variations in nutrient demand and acquisition may drive local heterogeneity in soil biogeochemistry (Figure 1). For example, species differences in fine root production, mycorrhizal fungal associations, and enzyme production, among others, may significantly alter belowground C and nutrient availability (Binkley et al. 2000; Rillig et al. 2001; Eviner and Chapin 2003; Finzi et al. 2007). Such effects may be more pronounced for certain element cycles compared to others. For example, differences in belowground C allocation among species could result in variable soil C dynamics (Russell et al. 2004) while species differences in root dynamics may have a stronger effect on P compared to N cycling, as P is highly immobile and plants typically must scavenge to obtain sufficient P (Clarkson 1985).

Species effects on P cycling may be particularly pronounced in lowland tropical forests where soil P availability is typically low (Walker and Syers 1976; Vitousek and Sanford 1986) and where P is often thought to limit ecosystem processes (Cleveland et al. 2002). One strategy used by both plants and microbes to acquire P is the production of extracellular phosphatase.
enzymes (Ptases) that convert organic P into plant-available inorganic P (P_i) by cleaving phosphate ester bonds. Ptase production requires N, and low N availability has been shown to limit Ptase activity (Olander and Vitousek 2000), suggesting a tight coupling between N and P belowground such that available N is allocated to Ptase production to increase soil P_i availability. Furthermore, Houlton et al. (2008) suggested this N/P interaction may help explain the abundance of putative N-fixing legumes in the relatively N-rich tropics where N-fixers may not be expected to have a competitive advantage. They proposed putative N-fixers (hereafter referred to as “legumes”) may have a competitive advantage in P-poor tropical forests due to their ability to allocate excess N to build Ptases to acquire P_i. While there are few data to support this hypothesis, it suggests legumes and non-legumes may differentially affect belowground biogeochemistry through important N/P interactions.

Species can also indirectly affect belowground soil C and nutrient cycling through a variety of mechanisms. Species variation in organic acid root exudation, the ratio of cation versus anion uptake via roots, and litter chemistry can all affect soil pH, an important control on many biogeochemical processes (Finzi et al. 1998a; Eviner and Chapin 2003). Species also regulate soil hydrology via unique canopy and root architectures and physiologies that affect soil-atmosphere water dynamics and plant uptake (Herwitz 1985). Soil hydrology indirectly affects biogeochemical cycling by altering oxygen availability which affects various biological processes such as microbial activity and N mineralization that ultimately regulate soil nutrient concentrations. Although it is difficult to parse out the relative importance of direct versus indirect species effects on belowground biogeochemistry, there is clearly great potential for tree species to differentially affect soil C and nutrient cycling in multiple ways.
Field studies comparing soil biogeochemical properties under multiple different tree species offer an opportunity to assess the nature and magnitude of species effects in situ. There is ample evidence that species can exert detectable differences in belowground C and nutrient pools. For example, multiple studies have reported species effects on soil C and C/N ratios (e.g. Finzi et al. 1998b; Berger et al. 2002; Lovett et al. 2004), soil available N (Lovett et al. 2004; Ayres et al. 2009), and soil available P (Cross et al. 2010). In general, these studies do show some redundancy between species such that not every species has a unique soil biogeochemical imprint. Still, given the small number of species considered in each study, the detection of any effect among only a handful of species is noteworthy. However, other studies report no significant influence of species on belowground C, N and P pools. Boettcher et al. (1990) and Cross et al. (2010) both report no differences in total soil C and N in temperate forests. Cross et al. (2010) also found no difference in soil available N but did detect differences in soil available P, while Washburn et al. (2003) reported no species effect on either available N or P.

Although less well studied, species-specific differences on soil Ptase activity may relate to soil P availability. Ushio et al. (2010) reported a significant species effect on potential soil Ptase activity in a tropical montane forest characterized by low total soil P, while in a relatively P-rich temperate forest Ptase activity did not vary by tree species (Weand et al. 2010). These results suggest that in low soil P environments, species may differentially affect Ptase production: when P is readily available, species-specific strategies for procuring P no longer offer any competitive advantage, thus suppressing Ptase production and hence activity. However, neither of these studies explicitly investigated how variations between legume and non-legume functional groups relate to observed patterns in Ptase activity, and thus we still lack
an understanding of how legumes drive N/P interactions to differentially affect belowground biogeochemistry.

Here, I examined how soil C, N and P pools and fluxes vary under nine different tropical forest canopy tree species, including three putative N-fixing legumes and six non-legumes. Specifically, I hypothesized that different canopy tree species would drive differences in belowground C, N and P pools due to species-specific differences in litter chemistry, root activity and indirect species effects on multiple biotic and abiotic soil properties. Next, I hypothesized that soil Ptase activity would vary among species as well as by functional group due to important N/P interactions belowground, with higher Ptase activity under legume trees that allocate excess N resources to Ptase production.

MATERIALS AND METHODS

Study Area – This study was carried out in a wet lowland tropical forest on the Osa Peninsula in southwestern Costa Rica (8°24’ N 83°19’ W). Mean annual temperature is ~26°C and mean annual precipitation is 3450 mm, and the site experiences a relatively short dry season from December through April with heavy rains common throughout the rest of the year. The Nicoya Complex forms the basement geology of the region and is overlaid by the Osa Group, characterized by Pliocene greywacke-type sedimentary rock (Berrange and Thorpe 1988). Soils are highly weathered, nutrient-poor ultisols and yet support high plant productivity (Vitousek and Sanford, 1986; Sanchez-Azofeliza et al. 2002). The Osa Peninsula is one of the most biologically diverse ecosystems on the planet and is home to approximately 700 different tree species and more than 4000 vascular plant species (Sanchez-Azofeliza et al. 2002; Kapelle et al. 2003). Although specific phenology varies by species in this semi-deciduous tropical rain forest,
most tree species drop the majority of their leaves during the dry season and then leaf out again at the onset of the rainy season (Lobo et al. 2008).

Experimental Design – Nine common canopy tree species – including three putative N-fixing legumes and six non-legumes – were selected with 6-8 replicates per species for a total of 66 trees. Tree species included legume (*Dialium guianense, Inga alba* and *Tachigali versicolor*) and non-legume species (*Brosimum utile, Caryocar costaricense, Castilla tunu, Otoba novogranatensis, Pourouma bicolor* and *Socratea exorrhiza*). To investigate how variations in foliar and litter chemistry relate to differences in belowground processes, I chose common species representing a wide spectrum of foliar nutrient concentrations (G. Asner, unpublished data). Only trees with a diameter >10cm (Nardoto et al. 2008) and receiving full sun at some point during the day were included.

Soil and litter sampling – Soils were sampled under each tree twice, in April 2010 during the dry-to-wet season transition and in July 2010 during the peak of the wet season, to capture some seasonal variability. Four 0-10cm soil cores were taken with a bulb corer at cardinal directions within 1m of the base of each tree, capturing the most active region of belowground plant and microbial activity. Samples were bulked by tree, homogenized by hand in the field, and sorted to remove coarse roots and rocks. All analyses were performed on these composite samples, for a total of 66 samples. Recently fallen leaf litter (i.e., minimally decomposed standing litter composing the top-most litter layer) of each species was also collected under each tree in April.

Soil and litter analyses – Within 12 hours of collection, soil inorganic N (NH$_4^+$ and NO$_3^-$) was extracted from fresh soil samples for five hours using a 2M HCl solution. Extracts were frozen and transported back to the laboratory at the University of Montana for colorimetric analysis.
using a Synergy 2 Microplate Reader (BioTek, USA). Fresh soil samples were also transported to the laboratory for soil microbial biomass analysis within one week of field sampling. Microbial biomass C and N were determined using a chloroform fumigation-extraction method (Brookes et al. 1985) and K₂SO₄ extracts were analyzed using a Shimadzu TOC-V CPN/TNM-1 analyzer (Shimadzu Inc., Kyoto, Japan). Labile P and microbial biomass P were assessed using a NH₄F extraction (Bray and Kurtz 1945, Oberson et al. 1997) and analyzed colorimetrically, with labile P calculated as the P present in non-fumigated samples and microbial biomass P calculated as the difference between fumigated and non-fumigated samples and corrected for the efficiency of the digest (Morel et al. 1996). Soil dry weights and percent moisture were determined gravimetrically by oven drying soils for 48 h at 105°C. Total soil C and N were determined on dried and ground (using a mortar and pestle) samples using a CHNS-O elemental analyzer (CE Instruments EA 1110, Thermo Fisher, USA), and total soil P was determined using a nitric acid/hydrogen peroxide digest and analyzed colorimetrically. Species-specific litter samples were dried at 60°C for two days and ground using a Wiley-Mill (20-mesh screen). Litter was ground to a fine powder using a mortar and pestle for total C and N analysis. Total C, N and P of all litter samples were determined as described above.

Enzyme assays – Potential rates of Ptase activity were determined on subsamples of each composite soil sample that were frozen at -20°C prior to analysis. Ptase activity was measured with using a MUB-linked substrate following the methodology of Saiya-Cork et al. (2002). Microplates were incubated in the dark for 3 hours and NaOH was not added prior to reading due to the sensitivity of MUB fluorescence to NaOH over short time scales and the ability to acquire consistent results without NaOH addition (M. Weintraub, personal communication).
**Laboratory litter incubation experiment** – I conducted a laboratory incubation experiment to isolate the potential effects of species differences in litter chemistry on soil C, N and P pools, and rates of C mineralization and Ptase activity. Species-specific litter from six canopy tree species was incubated with a composite soil in the laboratory for 68 days. Specifically, recently fallen litter was collected in April under three legumes (*Dialium guianense*, *Inga alba* and *Tachigali versicolor*) and three non-legumes (*Brosimum utile*, *Castilla tunu*, and *Otoba novogranatensis*) species, with three trees per species for a total of 18 trees. Under each tree, litter was collected from four cardinal directions within 1m from the tree base. Litter was kept separate by individual tree, with three litter samples from the same species serving as field replicates for a total of six treatments. Initially, there were 12 replicates for each treatment, but on days 1, 19, 43, and 68 three replicates per treatment were destructively harvested for nutrient and potential enzyme activity analyses.

All litter samples were dried at 60°C for two days and ground using a Wiley-Mill. Fresh soil collected in July from each tree as described above was compositied using equal amounts of soil per tree (calculated by soil dry weight) to create a common bulk soil sample. In 50mL plastic centrifuge tubes, 0.24g of litter was mixed with 12g of composite soil using a metal spatula for a 1:50 soil to litter ratio. Samples were loosely covered with aluminum foil to allow for air flow and incubated in a dark cooler with moist paper towels at 20°C. Samples were maintained at field moisture by weighing tubes every seven days to determine moisture loss and adding sterilized deionized water with a micropipette as needed at least 24 hours prior to measurement.
Rates of C mineralization were determined eight times across the 68-day experiment using a static-incubation procedure (Fierer et al. 2003). Six hours prior to sampling, tubes were aerated and sealed with air-tight plastic capped fitted with rubber septa for gas-sampling. Headspaces were mixed with a syringe and a 10ml sample from each tube was removed. C mineralization was measured using a gas chromatograph (Shimadzu Inc, Kyoto, Japan) equipped with a thermal conductivity detector and rates were calculated as CO₂ produced per hour. On the same day C mineralization rates were measured, samples were destructively harvested and analyzed for inorganic N (as described above) and bicarbonate-extractable organic and inorganic P (Tiessen and Moir, 1993). Subsamples of each destructively harvested sample were stored at 4°C until analyzed for potential rates of Ptase activity as described above.

Statistical analyses – All data were tested for normality and variance homoscedasticity using Shapiro-Wilk and Levene’s tests, respectively. When assumptions of normality and variance homogeneity were not met, species differences were assessed using the Kruskal-Wallis non-parametric one-way analysis of variance test. Pairwise comparisons were performed using the Wilcoxon rank sum test with a Bonferroni correction. In cases with rank ties, exact p-values could not be calculated and approximate values are reported. Given that most of the data are non-normal, the median and a bootstrapped estimate of the standard error of the median (using 10,000 bootstrap samples) are provided along with the mean. A significance threshold of $P < 0.05$ was used for all analyses which were performed using the open-source R software (R v. 2.13.0).

RESULTS
Chemical characteristics of species-specific litter and soil below each canopy tree species are shown in Table 1. Recently fallen species-specific standing litter, collected in April 2010 during the dry to wet season transition, varied among species in leaf %N, %P and N/P ($P < 0.001$ for all; Figure 2-1 and Appendix 1). There were also functional group differences in %N and N/P, with legumes having higher %N and higher N/P ratios compared to non-legume species ($P < 0.001$ for both %N and N/P). Species-specific litter %P did not vary between functional groups.

There were significant differences in soil chemistry below different tree species but not between functional groups. Total soil organic C (TOC) and total soil N (TN; measured only in soils samples collected in April) varied significantly among species ($P < 0.001$ for both TOC and TN) but not between the two functional groups. Soil C/N varied among species ($P = 0.009$) and showed marginally significant differences between functional groups ($P = 0.05$) (Figure 2-1 and Appendix 1). Soil available N ($\text{NH}_4^+/\text{NO}_3^-$) pools and Bray’s extractable labile soil P concentrations did not vary significantly by species at either time point (Figure 2-2 and Appendix 2). Overall, soil available N concentrations were more than twice as large in April – during the dry to wet season transition – compared to the peak of the wet season in July.

Soil microbial biomass C concentrations varied significantly among species in April and in July ($P = 0.02$ for both) but not between functional groups. A similar pattern was observed for microbial biomass N, with a significant species effect in April ($P = 0.004$) and a marginally significant effect in July ($P = 0.05$), but there were no significant functional group differences in either April or July. There were no significant species effects on microbial C:N, microbial P or microbial N:P at either time point (Figure 2-3 and Appendix 2).
Soil Ptase activity varied both among species ($P = 0.02$) and functional groups ($P < 0.001$) in April and July ($P = 0.01$ for both species and functional group) (Figure 3). There were also significant relationships between litter chemistry (measured only on samples collected in April) and Ptase activity. Litter N/P correlated with Ptase activity at both time points ($r = 0.38$, $P = 0.002$ for both April and July; Figure 4). Litter N was also significantly related to potential enzyme activity in April ($r = 0.25$, $P = 0.043$) while litter P was negatively correlated with Ptase in July ($r = -0.29$, $P = 0.02$).

Soil pH (measured on soils collected in April only) varied significantly among species ($P < 0.001$) and between functional groups ($P = 0.002$) (Figure 2-4). Soil pH under legumes was generally lower than soil pH under non-legumes. Soil moisture varied significantly between April and July, with a marginally significant species effect in April ($P = 0.05$) and a stronger effect in July ($P = 0.02$) (Figure 2-4). No difference in soil moisture between functional groups was observed at either time point.

*Incubation experiment* – Species-specific litter varied significantly in C/N and N/P ratios ($P < 0.001$, $P = 0.05$, respectively), with legumes having consistently lower litter C/N ratios and higher N/P ratios compared to non-legumes. There were no significant differences in soil inorganic N concentrations between species on day 1 as all samples included a common soil. However, species differences in soil inorganic N concentrations were detected on days 19, 43, and 68 ($P < 0.001$ for all time points), and both litter C/N and N/P ratios were significantly related to inorganic N availability at these time points (for litter C/N: $r = -0.74$, $P < 0.001$; $r = -0.71$, $P = 0.002$; $r = -0.82$, $P < 0.001$ respectively for days 19, 43, and 68; for litter N/P: $r = 0.66$, $P = 0.006$; $r = -0.71$, $P = 0.002$; $r = -0.82$, $P < 0.001$ respectively for days 19, 43, and 68). Litter
N/P was also significantly related to inorganic N availability on day 1 ($r = 0.57, P = 0.02$).

Inorganic bicarbonate-extractable P ($P_i$) varied by species at all time points ($P = 0.02, P < 0.001, P = 0.027, P = 0.033$ on days 1, 19, 43, and 68 respectively). There were no significant relationships between litter chemistry and soil extractable $P_i$ at any time point.

Rates of C mineralization followed similar temporal patterns across all treatments. The highest rates of mineralization were observed at the beginning of the incubation, with rates decreasing over time. There was no significant species × time interaction, although there was a significant species effect on C mineralization rates during the initial stages of the incubation ($P = 0.01, P = 0.006, P = 0.02, P = 0.04$ on days 6, 8, 13, 19, respectively). This species effect disappeared until the final time point (day 68, $P = 0.02$), which also marked the only time during which a significant functional group effect ($P = 0.018$) was observed. Litter N/P ratio was the best predictor of cumulative C mineralization over the course of the experiment, with low litter N/P negatively related to cumulative C mineralization ($r = -0.56, P = 0.031$), although this relationship did not hold at specific time points.

Potential rates of Ptase activity increased from day 1 to day 43 when the highest mean activity among all treatments was measured. The lowest mean rate was measured on day 68. Litter P was the only significant predictor of soil Ptase activity on day 19 and there were no significant predictors of enzyme activity at any other time point. There were no significant species or functional group differences in soil Ptase activity at any time point (Figure 5).

**DISCUSSION**
Overall, my results indicate species have multiple effects on belowground biogeochemistry in this wet lowland tropical forest (Figures 2 and 3). In addition, my work is largely consistent with previous studies suggesting that individual trees create “spheres of influence” on soil properties (Zinke 1962), creating a species-specific patchwork of heterogeneous soil chemistry across the landscape. The species effects I observed appear to result from both the direct effects of litter and root inputs and indirect effects on biotic and abiotic properties such as microbial community composition, soil pH and soil moisture.

Although relatively few studies have explored individual tree species effects on soil properties in tropical forests, some of those that do exist report similar results. For example, Reed et al. (2008) found significant differences in litter and soil chemistry under six different canopy tree species in a similar lowland tropical rain forest also on the Osa Peninsula in Costa Rica. In a laboratory incubation experiment conducted using soils from that site, litter chemistry and its effects on soil respiration also varied significantly among tropical tree species, with variation in the C/P ratio of dissolved organic matter leached from species specific litter explaining more than half of all variation in cumulative C mineralization among species (Wieder et al. 2008). In a primary forest in the Brazilian Amazon, van Haren et al. (2010) found tree species identity best explained variation in N₂O soil gas fluxes across the landscape, possibly due to species differences in belowground C allocation.

The species-specific differences in soil biogeochemistry observed here likely reflect a combination of direct (e.g., litterfall and root activity) and indirect effects (e.g., soil pH and soil moisture). Litterfall chemistry, which varied significantly among species (Figure 2-1), may be particularly important in regulating belowground biogeochemistry in lowland tropical forests.
where fine litterfall may account for more than half of all aboveground net primary production (Clark et al. 2001) and complete litter turnover can occur over short time scales (Gholz et al. 2000; Cleveland et al. 2006). Moreover, the majority of nutrients in many nutrient-poor tropical forests are stored in the aboveground biomass. Thus, litterfall plays the dominant role in nutrient cycling across decadal or shorter time scales in these ecosystems (Vitousek 1982), implying that species differences in litter chemistry, as well as differences in resorption efficiency, could have a particularly strong effect on belowground nutrient heterogeneity and availability in lowland tropical forests.

Further, although I did not specifically test the effects of variation in roots dynamics on soil biogeochemistry in this study, differences in root activity and rates of nutrient uptake likely also contribute to local soil heterogeneity. Variations in fine root biomass and decomposition, as well as other nutrient acquisition strategies such as arbuscular and ectomycorrhizal associations, cluster roots, and root enzyme exudates, have all been shown to affect belowground biogeochemistry (Rillig et al. 2001; Silver et al. 2005; Lambers et al. 2007). Nutrient acquisition strategies, including specific mycorrhizal associations, can vary by species (Johnson et al. 1992; Helgason et al. 1998; Helgason et al. 1999) and such variation could play a significant role in determining species effects on soil biogeochemistry, especially given that a small net change in nutrient concentration can represent a significant fraction of the total available nutrient pool in these relatively infertile soils.

The litter incubation experiment was designed to isolate the potential effect of litter chemistry from belowground effects on soil C, N and P pools and fluxes. Results from this laboratory experiment showed species differences in litter chemistry drove significant
differences in cumulative C mineralization, inorganic N availability, and Ptase activity. Species effects on C mineralization have been reported across multiple forest types (Wieder et al. 2008; Ayres et al. 2009; Yohannes et al. 2011) and could represent an important species effect on ecosystem functioning as C mineralization rates reflect decomposition dynamics which influence both C and nutrient cycles (Wieder et al. 2008).

In the field, I also detected species effects on soil pH and soil moisture, edaphic factors that have been shown to indirectly affect belowground biogeochemistry by altering rates of decomposition, weathering and nutrient cycling, among others (Chapin et al. 2002). For example, in an analysis of soils across the western hemisphere, Fierer and Jackson (2006) found that soil pH was a strong predictor of microbial community composition which can affect rates of decomposition and nutrient cycling (Strickland et al. 2009; McGuire and Treseder 2010). This result persisted at small scales, with soils from sites similar in climate and vegetation but varying in pH exhibiting different microbial communities. Specifically, more acidic soils were characterized by lower microbial diversity and richness. Differences in microbial community composition can affect rates of decomposition as well as the retention of nutrients in the soil (Schimel 2001; Zak et al. 2003). Similarly, soil moisture affects rates of decomposition and nutrient cycling, with low soil moisture restricting diffusion of C and nutrients to microbes and high soil moisture limiting oxygen diffusion (Chapin et al. 2002). In both cases, microbial activity is reduced and decomposition and nutrient cycling slows. Thus, indirect effects of species on soil properties may also contribute to species-driven variation in belowground biogeochemistry.
However, detectable species effects on soil biogeochemistry are not ubiquitous across tropical forest landscapes. For example, Powers et al. (2004) found no significant difference in soil chemistry, including total soil C, N, extractable nutrients and pH, under four different tree species compared to a common baseline species (*Pentaclethra macroloba*) in a wet lowland tropical forest. Further, although my results did show some significant species effects on soil properties, this result was not universal across all measured soil variables. There were no detectable species effects on soil inorganic N, labile P or microbial biomass P pools in the field, although litter chemistry and inorganic N concentrations were directly related in the laboratory incubation experiment. These conflicting results may be due in part to rapid plant nutrient uptake. Litter chemistry not only reflects belowground inputs but also plant nutrient demand. Thus, for example, while larger available N pools may be expected under species with high litter N, these species also have a high N demand, resulting in greater N uptake and no observable difference in belowground N availability compared to species with low litter N. The inconsistent results between the field and laboratory studies also suggest the importance of various homogenizing agents in the field as described above, such as litter mixing and the subcanopy plant and soil faunal communities (Figure 1).

The lack of differences in soil labile P or microbial biomass P under different species in the field (Table 2), in spite of species differences in litter P (Figure 2-1), is not necessarily surprising given the relatively low P content of these highly weathered tropical soils (Bern et al. 2005; Townsend et al. 2002). The lack of a significant relationship between litter P and inorganic labile P in both the incubation experiment and the field study implies litter chemistry may not be a strong direct control on plant-available soil P. Further, much of the aboveground P
input via litterfall may be rapidly occluded due to high rates of P adsorption common in such soils (Cleveland et al. 2002). The low concentrations of soil available P and microbial biomass P measured in this study resemble those from a similar Costa Rican lowland tropical rain forest where P was found to be the primary nutrient limiting microbial processes (Cleveland et al. 2002). Thus, low soil P availability likely plays an important role in regulating ecosystem processes in this study site, and may have contributed to the lack of species-specific differences in soil P metrics.

Conversely, low soil P availability in this site may help explain the difference in Ptase activity between legumes and non-legumes. Low P availability may stimulate the allocation of excess available N to Ptase production (Houlton et al. 2008). My data showing higher soil Ptase activity under legumes compared to non-legumes in both the field and laboratory experiment supports the hypothesis proposed by Houlton et al. (2008) that legumes may allocate excess available N to Ptase production belowground – either directly from root-derived enzymes or indirectly from microbial Ptases. Further, the legume species in my study had significantly higher litter N and litter N/P ratios compared to the non-legumes and this difference in litter chemistry drove significant differences in soil Ptase activity in the field. Litter N/P ratios most strongly predicted Ptase activity in both April and July (Figure 4) while the relationship between litter N and P concentrations alone and enzyme activity were season dependent.

While I measured only bulk soil Ptase activity and did not isolate root enzyme activity, I would expect root Ptase activity to follow a similar – if not stronger – pattern of higher activity under legumes compared to non-legumes because root-derived Ptases are responding directly to the tree-specific environment (Helal 1990; Barret et al. 1998). Interestingly, while Ptase activity
was the only belowground pool or flux measured in the field that showed a significant functional group difference in both April and July, no functional group differences were observed in the laboratory incubation. This may be due to a high proportion of root-derived Ptase production, as roots were excluded from the soil in the laboratory experiment. Although it is difficult to assess the relative contributions of plant versus microbe derived Ptase, other studies have suggested roots contribute a large fraction of total soil Ptase activity (Tarafdar and Jungk 1987; Reed et al. 2011). Also, potential rates of Ptase activity in roots have been shown to be more sensitive to nutrient availability compared to enzyme activity in mineral soils and functional group differences in nutrient availability may be more pronounced at the root compared to the bulk soil (Marklein and Houlton in press).

Thus, data from my field study provide some evidence suggesting that Ptase activity is higher under relatively N-rich legumes compared to relatively N-poor non-legumes (Houlton et al. 2008), although the specific mechanism remains unclear. My results are also consistent with a recent hypothesis suggesting that in P-poor tropical soils, N-fixers may allocate increased N resources to the production of N-rich Ptase enzymes, thereby increasing the ability of N-fixing species to acquire scarce P (Houlton et al. 2008). However, although I did not rigorously assess nodulation of the legumes (putative N-fixers), I found no evidence of nodulation in any of the soil cores extracted under the legumes trees in this study. More, recent work suggests that nodulation of even putatively N-fixing species may be uncommon in closed canopy tropical forests (Barron et al. 2010). If so, this would suggest that rates of symbiotic N-fixation are likely quite small in this mature, closed canopy forest and any N/P interactions driving the observed functional group differences in Ptase activity may actually be driven primarily by differences in
litter chemistry and/or plant nutrient demand rather than profound differences in N inputs via symbiotic N fixation.

For example, in a greenhouse study using herbaceous plants, Venterink et al. (2011) found Ptase activity to generally be higher under legumes compared to non-legumes and yet root Ptase activity was not dependent on nodulation. Further, they found differences in Ptase activity between legumes and non-legumes to be most pronounced under low N and P conditions. Although this study was conducted in a much different system, it does lend support to the idea that putative N-fixers and non N-fixers differentially affect rates of Ptase activity in response to nutrient availability, particularly in nutrient poor soils such as those found in this study and many other lowland tropical forests.

Potential soil Ptase activity was the only belowground pool or flux measured in this study to show a significant direct relationship with litter chemistry. While there were species differences on belowground biogeochemistry, there were not direct relationships between species-specific litter chemistry and belowground inorganic or total C, N and P pools – even though litter inputs represent a substantial and critical component to belowground nutrient cycling in warm, wet and nutrient-poor systems such as this one (Clark et al. 2001). These results differ from other studies showing a strong relationship between litter and soil N (Stump and Binkley 1993; van Cleve et al. 1993; Ferrari 1999) and P concentrations (Reed et al. 2008). However, the majority of these studies were carried out in low diversity, cool and relatively dry systems compared to our study site. Aboveground-belowground biogeochemical linkages likely play out much differently in a wet lowland tropical forest, and multiple factors may explain the lack of a direct relationship between litter and soil C and nutrient concentrations.
First, high rates of decomposition (Cleveland *et al.* 2006) and high nutrient demand by plants in highly productive tropical forests may result in rapid turnover of soil nutrients, limiting the build-up of available nutrient pools and masking direct relationships between litter and soil available nutrient concentrations (Vitousek 1986). Although gross fluxes of nutrients into the soil via litter decomposition may be high, rates of nutrient uptake and immobilization may also be high, resulting in low net fluxes of available soil nutrients. The consistently low available nutrient pools I observed across all tree species support this idea (Table 2). Across a temperate forest soil fertility gradient, Cross *et al.* (2010) detected larger species effects on soil nutrient pools at high fertility sites. Thus, if a similar pattern holds for tropical forests, we may expect to find more pronounced species effects in higher fertility tropical forests compared to my study site.

Soil nutrient pools not only varied spatially, but across seasonal timescales as well. I observed a small seasonal shift in soil nutrient fertility, with larger inorganic N and P pools measured in April (marking the beginning of the wet season) compared to July (Figure 2-2). These results are consistent with previous research in a nearby tropical forest that showed a large pulse of nutrients is delivered from the litter layer – built up over the course of the dry season – to the mineral soil at the onset of the rainy season in seasonal wet tropical forests when nutrients are leached into the soil and microbial decomposition accelerates (Cleveland and Townsend 2006). However, observed species effects on given soil biogeochemical properties were not dependent on season as might be expected if litter nutrient inputs, which vary across seasons, were the dominant drivers of such species effects.
Second, the high plant diversity characteristic of this study site and many other wet lowland tropical forests adds complexity to the system that likely limits potential direct litter-soil relationships. For example, while I measured species-specific litter chemistry to assess species-specific effects on soil biogeochemistry, litterfall below each tree consists of a mixture of litter from multiple species, potentially obscuring species effects on soil biogeochemistry. Further, the relative importance of litterfall homogenization may vary by species due to factors such as phenological variability (Wright and Cornejo 1990), litterfall mass (Cuevas and Lugo 1998) and/or canopy architecture (e.g., litterfall from one species may be concentrated at the base of the tree while litter from a different species may predominately fall near the canopy drip line). Similarly, subcanopy and understory plant communities may also influence both litterfall and soil biogeochemistry and obscure potential canopy tree species effects. In particular, many tropical forests have a high density of lianas, whose unique structural characteristics may contribute to their role as important homogenizing agents across the diverse forest landscape (Powers et al. 2004). Unlike the typical close cycling between a tree’s litterfall and root system, lianas often exhibit spatially disconnected litterfall and root nutrient uptake, thereby influencing C and nutrient cycling across a wide spatial area (Putz 1984). Finally, soil fauna can play an important role in mixing soil both laterally and vertically (Lavelle et al. 1992).

Overall, my data show plant community composition can drive important differences in belowground biogeochemistry. While there exists redundancy in species effects on soil properties, this study revealed significant differences in soil properties between nine canopy tree species. Thus, aboveground heterogeneity clearly influences belowground variability at small
spatial scales and, given the high plant diversity at this site, it is likely my results underestimate the role of species on belowground heterogeneity in this system.

Such local variability and the importance of plant community composition on belowground processes should be considered when extrapolating plot-level measurements to larger scales. Currently, few ecosystem models account for species composition (e.g. Parton 1996; White et al. 2000; but see Post and Pastor 1996; He et al. 1999) and yet my results suggest some of the implications of ignoring aboveground heterogeneity in biogeochemical models. If species differentially affect belowground biogeochemical cycles as my results show, the exclusion of aboveground community structure in ecosystem models could impair our ability to appropriately model and predict C and nutrient cycling. Further, while this study showed there is some redundancy in the effect of individual species on belowground biogeochemistry, it also suggests some species push the system significantly away from the mean, creating local biogeochemical patchiness belowground. This is important to consider when attempting to predict how these forests will respond to future climate change (Pastor and Post 1988; Prentice et al. 1993). Shifts in aboveground plant community composition as a result of environmental changes, such as the predicted increase in drought across much of the tropics (Li et al. 2008; Malhi et al. 2009) or increased atmospheric N deposition (Galloway et al. 2004), may affect ecosystem processes in variable, non-random ways depending on the nature of the shift. Finally, as evidenced by the strong species and functional group differences in Ptase activity observed in this study, belowground N/P interactions are driven to a large extent by aboveground community composition. Developing a mechanistic understanding of these interactions is critical to broadly
predicting aboveground-belowground linkages and the effects of environmental change on ecosystem processes.

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REFERENCES


Multiple nutrients limit litterfall and decomposition in a tropical forest. Ecology Letters
11:35-43.

Lambers, H. and M. W. Shane. 2007. Phosphorus nutrition of Australian Proteaceae and
Cyperaceae: A strategy on old landscapes with prolonged oceanically buffered climates.

Reorganization in Casts of the Geophagous Tropical Earthworm Pontoscolex-Corethrurus
(Glossoscolecidae). Biology and Fertility of Soils 14:49-53.

Li, W., R. Fu, R. I. N. Juárez, and K. Fernandes. 2008. Observed change of the standardized
precipitation index, its potential cause and implications to future climate change in the
Amazon region. Philosophical Transactions of the Royal Society B: Biological Sciences.
363:1767-1772

Peninsula and Golfo Dulce region, Costa Rica. Stapfia 88, zugleich Kataloge der
oberösterreichischen Landesmuseen Neus Serie 80: 547-555.

hardwood forest: Do species matter? Biogeochemistry. 67:289-308

Malhi, Y., L. E. O. C. Aragao, D. Galbraith, C. Huntingford, R. Fisher, P. Zelazowski, S. Sitch,
C. McSweeney, and P. Meir. 2009. Exploring the likelihood and mechanism of a climate-
change-induced dieback of the Amazon rainforest. Proceedings of the National Academy
of Sciences of the United States of America 106:20610-20615.


Climatic Change 34:253-261.

of Climate Change on Forest Landscapes. Ecological Modeling 65:51-70.

Prescott, C. E. 2002. The influence of the forest canopy on nutrient cycling. Tree Physiology 
22:1193-1200.

65:1713-1724.

Reed, S., C. Cleveland, and A. Townsend. 2008. Tree species control rates of free-living nitrogen 
fixation in a tropical rain forest. Ecology. 89:2924-2934

Reed, S., A. Townsend, P. Taylor, and C. Cleveland. 2011. Phosphorus cycling in tropical forests 
growing on highly-weathered soils. Pages 339-369 in E. Buneman, A. Oberson, and E. 
Frossard, editors. Phosphorus in Action--Biological Processes in Soil Phosphorus 
Cycling. Springer Publishing Co.

Rillig, M. C., S. F. Wright, K. A. Nichols, W. F. Schmidt, and M. S. Torn. 2001. Large 
contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. 
Plant and Soil 233:167-177.

form diversity effects on soil carbon in experimental tropical ecosystems. Ecological 
Applications 14:47-60.

deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil 
Biology and Biochemistry 34:1309-1315.


Venterink, H. O. 2011. Legumes have a higher root phosphatase activity than other forbs, particularly under low inorganic P and N supply. Plant and Soil 347:137-146.


FIGURES

Figure 1.
Figure 2-1

- **Figure 2-1a**: Lithium % N
- **Figure 2-1b**: Lithium % P
- **Figure 2-1c**: Lithium % N\(\times\)P
- **Figure 2-1d**: Soil pH
- **Figure 2-1e**: Soil organic C (mg/g)
- **Figure 2-1f**: Soil total N (mg/g)
- **Figure 2-1g**: Soil total C:N (mg/g)
Figure 2-2.
Figure 2-3.
Figure 2-4.
Figure 3-1.

Figure 3-2.
Figure 4

The graph shows the relationship between soil acid phosphatase activity (nmol h\(^{-1}\) g\(^{-1}\)) and litter N:P ratio. The data points are categorized into Legume (●) and Non Legume (○) categories. The correlation coefficient (r) is 0.38, and the p-value is 0.002.
Figure 5.

![Graph showing soil acid phosphatase activity (nmol h\(^{-1}\) g\(^{-1}\)) by genus over different sampling days.](image)

**Genus**

- Dialium
- Inga
- Tachigali
- Brosimun
- Castilla
- Otoba

**Sampling Day**
- Day 1
- Day 19
- Day 43
- Day 68
**Figure Captions**

**Figure 1.** Simplified conceptual diagram of how different tree species (Species A, black; Species B, grey) may differentially affect C, N, P cycling. Differences in foliar C, N, and P ratios between species may drive species differences in litterfall chemistry that, in turn, may affect soil C, N, and P pools. Species differences in plant nutrient uptake can also affect belowground C, N, and P pools. Alternatively, homogenizing agents such as canopy overlap among species, extensive root systems, and faunal activity may limit the importance of individual tree “spheres of influence” and effectively wash out potential species effects.

**Figure 2-1.** Box-whisker plot showing species variation in total concentrations of litter and soil C, N and P and soil pH (measured in April). Plots show sample minimum, lower quartile, median, upper quartile, and sample maximum; outliers are depicted as open circles. Letters correspond to individual tree species on the x-axis as follows: *Brosimum utile* (A), *Caryocar costaricensis* (B), *Castilla tunu* (C), *Dialium guianensis* (D), *Inga alba* (E), *Otoba novagranatensis* (F), *Pourouma bicolor* (G), *Socratea exorrhiza* (H), *Tachigali versicolor* (I). Light grey boxes correspond to non-legume species and dark grey boxes correspond to putative N-fixing species.

**Figure 2-2.** Species variation in soil available N and P pools in April (Figure 2-3a) and July (Figure 2-3b). Plots show sample minimum, lower quartile, median, upper quartile, and sample maximum; outliers are depicted as open circles. Letters correspond to individual tree species on the x-axis as follows: *Brosimum utile* (A), *Caryocar costaricensis* (B), *Castilla tunu* (C), *Dialium guianensis* (D), *Inga alba* (E), *Otoba novagranatensis* (F), *Pourouma bicolor* (G), *Socratea*
exorrhiza (H), Tachigali versicolor (I). Light grey boxes correspond to non-legume species and dark grey boxes correspond to putative N-fixing species.

**Figure 2-3.** Species variation in microbial biomass C, N and P pools in April (Figure 2-4a) and July (Figure 2-4b). Plots show sample minimum, lower quartile, median, upper quartile, and sample maximum; outliers are depicted as open circles. Letters correspond to individual tree species on the x-axis as follows: Brosimum utile (A), Caryocar costaricensis (B), Castilla tunu (C), Dialium guianensis (D), Inga alba (E), Otoba novagranatensis (F), Pourouma bicolor (G), Socratea exorrhiza (H), Tachigali versicolor (I). Light grey boxes correspond to non-legume species and dark grey boxes correspond to putative N-fixing species.

**Figure 2-4.** Species variation in soil moisture, measured in April (Figure 2-2a) and July (Figure 2-2b). Plots show sample minimum, lower quartile, median, upper quartile, and sample maximum; outliers are depicted as open circles. Letters correspond to individual tree species on the x-axis as follows: Brosimum utile (A), Caryocar costaricensis (B), Castilla tunu (C), Dialium guianensis (D), Inga alba (E), Otoba novagranatensis (F), Pourouma bicolor (G), Socratea exorrhiza (H), Tachigali versicolor (I). Light grey boxes correspond to non-legume species and dark grey boxes correspond to putative N-fixing species.

**Figure 3-1.** Comparison of potential rates of soil acid phosphatase activity below nine different canopy tree species in April (Figure 3-1a) and July (Figure 3-1b). Bars represent means and error bars depict standard deviations. Putative N-fixing species are denoted by dark grey bars while light grey bars indicate non-N-fixers. No significant differences were found between individual species.
**Figure 3-2.** Comparison of potential rates of soil acid phosphatase activity under legumes and non-legumes in April (Figure 3-2a) and July (Figure 3-2b). Bars represent means and error bars depict standard deviations.

**Figure 4.** Relationship between species-specific litter N:P and potential rates of soil acid phosphatase activity in April. Each point represents litter and soil enzyme data from under an individual tree. Putative N-fixing species are represented with black circles and non-legumes are shown with open circles. Significance and the correlation coefficient (r) is shown.

**Figure 5.** Effects of species-specific litter on soil acid phosphatase activity in a laboratory incubation experiment. Bars represent means and error bars depict standard deviations. No significant species or functional group effect on enzyme activity was observed at any time point.
APPENDICES

Appendix 1-1

<table>
<thead>
<tr>
<th>Litter</th>
<th>Brosimum</th>
<th>Caryocar</th>
<th>Castilla</th>
<th>Dialium</th>
<th>Inga</th>
<th>Otoba</th>
<th>Pourouma</th>
<th>Socratea</th>
<th>Tachigali</th>
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* p<0.05  
** p<0.001

Appendix 1-1. Chemical characteristics (median and bootstrapped standard error with mean in parentheses) of species-specific litter and bulk soil under nine canopy tree species (measured in April). Significant differences between species (p<0.05) are denoted with different lowercase letters. Only the genus of each species is shown to ease readability but refers to specific species.
Appendix 2-1

<table>
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<tr>
<th></th>
<th>Brosimum</th>
<th>Caryocar</th>
<th>Castilla</th>
<th>Dialium</th>
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<td>8.89</td>
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* p<0.05
### Appendix 2.2

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<th>Socratea</th>
<th>Tachigali</th>
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<td>July</td>
<td>April</td>
<td>July</td>
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<td>39.57</td>
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<td>3.45, 1.92</td>
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<td>(3.42)</td>
<td>(1.48)</td>
<td>(1.92)</td>
<td>(1.54)</td>
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<tr>
<td>(3.42)</td>
<td>(1.48)</td>
<td>(1.92)</td>
<td>(1.54)</td>
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<td>(42.9)</td>
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<td>(59.14)</td>
<td>(53.92)</td>
<td>(42.74)</td>
<td>(30.90)</td>
<td>(42.9)</td>
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</tbody>
</table>

* p<0.05

Appendix 2. Soil moisture and chemical characteristics (median and bootstrapped standard error with mean in parentheses) of inorganic available nutrients and microbial biomass in the soil under nine canopy tree species measured in April and July. Significant
differences between species (p<0.05) are denoted with different lowercase letters. Appendices 2-1 and 2-2 were divided and only the
genus of each species is shown to ease readability but the genus refers to specific species.