**Modest fertilization effects on litter decomposition in a tropical lowland wet forest in Costa Rica**

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Keywords: EFFEX, nutrient addition, wood decomposition, litter nutrient release, immobilization

### Abstract

*Background and aims* The decomposition of leaf litter and wood is crucial for nutrient cycling and the carbon balance of tropical forests, yet its relationship with soil fertility is unclear. We used a long-term fertilization experiment in Costa Rica to test the hypothesis that phosphorus (P) but not nitrogen (N) limits decomposition in a species rich, tropical lowland wet forest.

*Methods* We placed *Hyeronima alchorneoides* leaf litter and birch wood sticks in mesh bags and left to decompose in plots that had received +N, +P, +NP, and controls for 11 years, and harvested them five times over a 36 week-period. At each harvest time we estimated mass loss as well as N and P content of the remaining leaf litter.

*Results* Neither leaf litter mass loss nor *k* decay coefficients (y -1) were significantly affected by fertilization. In contrast, wood mass loss was inhibited in +NP plots relative to controls, and wood *k* coefficients were lower in plots that received P (+P, +NP) vs. plots that did not (+N, controls). Leaf litter N and P were always < 80% of the initial content indicating net release, and decreased with time in all treatments, but more so in +N plots than in controls. Finally, litter C:N ratios decreased, and N:P increased with time, and they were not influenced by fertilization.

*Conclusions* The lack of stimulation of fertilization on litter and wood decomposition, together with generalised N and P mineralization, suggest that decomposition is not nutrient limited at our study site, DEVELOP FURTHER/SAY SOMETHING ABOUT LITTER CHEMISTRY.

Keywords: nitrogen, phosphorus, leaf litter, wood sticks, mineralization, *Hyeronima alchorneoides*.

### Hypotheses

We hypothesize that leaf litter and wood decomposition are P limited. We predict that (1) leaf litter and wood mass loss will be faster in +P plots than in controls and +N plots, (2) decaying leaf litter will immobilize P, but not N, and this P immobilization will be reduced in +P plots, (3) CN RATIO HYPOTHESIS? IS IT WORTHWHILE?

### Introduction

Litter decomposition is important for ecosystem function, given its role in nutrient cycling, net ecosystem carbon balance, and the growth of soil organisms (Smith & Smith 2007, Capek et al. 2018). Although soil fauna, precipitation and substrate quality are known to strongly influence decomposition in species rich, tropical lowland forests (Aerts 1994, Barajas-Guzmán & Álvarez-Sánchez 2003, Powers et al. 2009, Waring 2012, Barantal et al. 2012), the role of nutrient availability is less clear, both due to a paucity of decomposition studies in large scale, long-term fertilization experiments (ref), which is the most direct way to test for nutrient limitation at the ecosystem scale (ref), and due to conflicting results (FIND BETTER REF Hobbie & Vitousek 2000). The goal of this study is to test nutrient limitation to litter and wood decomposition in a large-scale, long-term fertilization experiment in a species rich, lowland wet forest in Costa Rica.

Nutrient limitation is defined as XYZ. Nitrogen (N) and phosphorus (P) are macronutrients that limit primary producers in species rich, tropical lowland wet forests (e.g. Alvarez-Clare et al. 2013, Wright et al. 2018, Viana-Cunha et al. 2022). In contrast, the growth of soil microbial heterotrophs is carbon, not nutrient limited (Ekblad and Nordgren 2002, Demoling et al. 2007, Kamble and Bååth 2014, Soong et al. 2020). Still, stoichiometric nutrient ratios in microbial tissues are much narrower than those of plant tissue (Sterner and Elser 2002, Cleveland and Liptzin 2007, Manzoni et al. 2010, Yuan and Chen 2009), so that stoichiometric constraints can cause microbial limitation to growth on fresh leaf litter by nitrogen, phosphorus, or other nutrients (e.g. Elser et al. 2007, Kaspari and Yanoviak 2009, Fanin et al. 2014). Decomposer microorganisms take up nutrients from the soil, so that soil fertility may be an important determinant of microbial nutrient limitation (Camezind 2012).

Various field fertilization experiments show that N and P added singly do not alter decomposition significantly (Hobbie & Vitouseket al. 2000, McGroddy et al. 2004, Barantal et al. 2012). simultaneous N and P addition increase decomposition (Hobbie & Vitousek 2000, Barantal et al. 2012), with increases of up to 60% in mass loss relative to controls (Barantal et al. 2012). Other studies find ? But in other tropical forests, N addition alone reduced (Zhang et al. 2020), and P addition alone increased, leaf litter decomposition rate (Kaspari et al. 2008). A recent meta-analysis of tropical lowland forest N and P fertilization experiments shows widespread P limitation to several microbial processes, but not litter decomposition, in tropical lowland forests (Camezind et al. 2017).

Also lifted from camenzind: “On the other hand, the meta-analysis on factors controlling leaf litter decomposition in tropical forests conducted by Waring (2012) reported a large amount of unexplained variation and only little impact of litter nutrient concentrations on decomposition; this was possibly due to other factors such as soil fauna and secondary metabolites not accounted for in her analysis. Indeed, leaf litter decomposition in a lowland tropical forest of French Guiana was driven by larger soil fauna and correlated well with condensed tannin but not with P concentrations despite very low soil P concentrations (Coq et al. 2010).”

When organic matter decomposes, litter carbon is respired to CO2 and provides energy to microorganisms, while nutrient concentrations in the litter usually increase (Manzoni et al. 2008, Aber and Melillo 1982). The total content of nutrients in the decaying litter, i.e. litter nutrient concentration × remaining litter mass, can increase or decrease as decomposition proceeds. If the decomposer critical ratio of C:N or C:P is lower than the C:N or C:P ratio of the leaf litter, immobilization takes place, and if they are greater than litter ratios, mineralization proceeds (Bosatta & Staaf 1982, Manzoni et al. 2010). The critical carbon:nutrient ratios of the decomposers is a function of not only the C:nutrient ratios of the microbial biomass, but also of its carbon use efficiency (CUE), the rates of organic losses via leaching due to precipitation, and the preference of the decomposers for N and P rich vs. poor substrates (Manzoni et al. 2010). In experiments where a common leaf litter substrate with a set C:nutrient ratio is set to decompose on plots with different fertilization treatments, fertilization will presumably influence litter nutrient dynamics mainly via its effects on CUE (Ågren et al. 2001), the C: nutrient ratios of the decomposer biomass and the preferential decomposition of nutrient rich vs. nutrient poor substrates by the decomposers. Specifically, higher nutrient availability reduces CUE, thus increasing critical ratios (critical ratio = microbial biomass ratio/CUE, see Manzoni 2012 p.

As such, nutrient dynamics during decomposition are an indicator of the capacity of the decomposer sub-system to release nutrients form litter into the environment or to retain them (Hobbie & Vitousek 2000). As such, and is thus important to understand the role of the microbial biomass in nutrient availability to plants (CITE SOMETHING ABOUT COMPETITION). Some studies find that adding nutrients influences nutrient dynamics during decomposition. For example, +P and +NP addition can lead to an increase in P immobilization (McGroddy et al. 2004) sometimes reaching double the initial litter P concentration (Hobbie & Vitousek 2000). ADD MORE EXAMPLES HERE.

Understanding the role of nutrients in ecosystem processes is important to better adapt to global environmental change. The input of nitrogen and phosphorus (CHECK) in forest ecosystems increasing due to human activity (Matson et al. 1999, Smil 2000, Bennett et al. 2001, Hietz et al. 2011). Furthermore, since the beggining of the Green Revolution there has been an accumulation of P in agricultural soils due to the addition of mineral P fertilizers and the use of P-rich animal foods (Smil 2000, Bennett et al. 2001). This P could reach natural ecosystems via soil particles transported by wind or water, or P disolved in water from rivers or surface runoff. These types of processes are being enhanced by land use change, as well climate change, e.g. increased P transport via Saharan dust (Elser et al. 2007).

Large scale, long term fertilization experiments are crucial to investigate the role of nutrient availability as well as antropogenic changes in nutrient availability on ecological processes (Tanner et al. 1998, Wright et al. 2011, Álvarez-Clare et al. 2013). There are currently few active fertilization experiments in lowland tropical wet forests in the world. Using one such experiment, the Earth Forest Fertilization Experiment in Costa Rica, our goal is to test the role of nutrient availability on the decomposition of leaf litter and wood, as well as mineralization and immobilization. Nearly three years after the start of the experiment, the proportion of trees that grew was significantly higher in plots that received P (+P and +NP) relative to the controls, but the proportion of tree growth in the +N treatment was not significantly different from that in the control.

We hypothesize that 1) decomposition rates will be faster in plots that receive P than those that do not, 2) nutrient concentrations in remaining litter xyz, 3) nutrient content in remaining litter, 4) stoichiometric ratios… P immobilization rates will be higher than N immobilization rates, due to greater relative limitation by P than by N, leaf litter will decay faster than wood TERMINAR.

Barantal 2012: “As the naturally available tree leaf litter represents an unfavorable C:N:P stoichiometry for decomposers (Sterner and Elser 2002), we hypothesized that fertilization with N, and with P in particular, would decrease nutrient limitation to decomposers, and increase litter decomposition.”

### Methods

#### Study site

The study took place at the 900 ha “Escalera de Mono” forest reserve at EARTH University in Limón, Costa Rica (10°11'−10°15' N, 83°37' − 83°33' W) at 30 m.a.s.l. (Fig. 1). The forest is mature and has not been disturbed in over 25 years. Mean annual temperature is 25.1˚C, and mean annual precipitation is 3500 mm, distributed in a bimodal pattern with no months receiving < 100 mm of precipitation (Fig. 2). Soils are of volcanic origin and their texture is 50% clay, 30% sand and 20% loam, and there are Ultisols and Inceptisols (Sancho 1990). In the study plots 104 species of tree and palms have been identified, and vegetation is dominated by the tree *Pentaclethra macroloba* which constitutes 30% of the forest basal area, and the palm *Socratea exorrhiza* (Sancho, 1990). These vegetation traits are similar to those found at nearby La Selva biological station (Hartshorn and Hammel 1994).

#### Experimental design

This decomposition experiment took place within the EFFEX (EARTH Forest Fertilization EXperiment) large-scale, long-term fertilization experiment (Fig. 1), which started in 2007 (Álvarez-Clare et al. 2013). The experiment has four treatments: +N (100 kg N ha-1 y-1 as ammonium nitrate and urea), + P (50 kg P ha-1 y-1 as triple superphosphate from 2008−2014, and rock phosphate (30% P2O5, 40% CaO, 10% SiO2) thereafter), + NP (sum of the doses applied to individual N and P treatments) and control (CT) without fertilizer addition. Each treatment has six replicate plots, except for +N which only has four plots due to treefalls in 2009 which destroyed the majority of the vegetation in the plots. Due to resource constraints and in order to have a balanced design, we only used four of the six plots per treatment. After 2.7 years of fertilization some treatment effects had already been reported (Table 1), such as an increase in the proportion of trees that grew in plots that received P vs. those that did not (Álvarez-Clare et al. 2013). Furthermore, P additions resulted in doubled stem growth rates in small trees (5-10 cm DBH), and in an increase in seedling survival and in the proportion of seedlings that grew (Álvarez-Clare et al. 2013). Certain species such as *Socratea exorrhiza* responded to +P and +N+P fertilization with increases in foliar P concentration, whereas others like *Pentaclethra macroloba* responded to +N addition with increases in foliar N concentration (Álvarez-Clare & Mack 2015).

Every plot is 30 × 30 m, but we only used the internal 20 m × 20 m to minimize edge effects. Plots are located at least 50 m apart, in order to avoid nutrient transfer between treatments (Álvarez-Clare et al. 2013). We carried out a decomposition experiment using air-dried litterfall of the tree species *Hyeronima alchorneoides*, as well as wood craft sticks as a common substrate (Harmon et al. 1999). We chose *H. alchorneoides* litter because of its large leaves which facilitates collection in nets as well as the making of decomposition bags, and because it is a species native to the study area (Cardelus 2010). We collected *H. alchorneoides* leaves using nets in fixed locations in a forestry plantation on EARTH campus. Leaves were air dried HOW LONG, and homogenized HOW. We chose craft wood sticks as a standard substrate as they have commonly been used for wood decomposition experiments (Sinsabaugh et al. 1992; Hu, Yesilonis, and Szlavecz 2021).

Using the same substrates for all plots allowed us to test the effect of nutrient availability on organic matter decomposition via its influence on the decomposer community in soils and the litter layer. As such, this experiment did not test for fertilization effects on decomposition via induced changes in litter quality.

#### Litter bags

*Hyeronima alchorneoides* litterfall was collected in a forestry plantation (McGroddy et al. 2004) on the campus of EARTH university DATES, using mesh cloth traps suspended 1 m above the ground for one week. Leaf litter was collected with latex gloves and subsequently set to air dry for 1 week in a well-ventilated place (Harmon et al. 1999). The central vein and areas damaged by insects were removed from the air-dried litterfall. A 10 g subsample of *H. alchorneoides* litter was dried in an oven (48 h, 55°C) for determination of initial nutrient concentration (see below). Litter bags were sewn from fiber glass mesh (2 mm opening) in order to allow mesofauna in (Barajas-Guzmán & Álvarez-Sánchez 2003) given that mesofauna is critical for tropical leaf litter decomposition (Powers et al. 2009). Each bag was sewn with nylon string and labelled with a metal tag. Each bag received 7 g of air-dried litterfall and a wooden stick of known mass. The mass of each decomposition bag (sum of the masses of the mesh bag, *H. alchorneoides* litter, and wooden stick) was recorded, and bags were placed in the field on top of the litter layer in order to simulate the natural conditions that the litter would face upon falling on the forest floor (Harmon et al. 1999). Bags were placed in the field between August 3 and 5, 2015. In each of the 16 study plots (four each of control, +N, +P, +N+P), we placed 20 litter decomposition bags distributed in four locations (five bags per location), so as to collect one bag per location per each one of five collection moments (4 bags × 5 collection moments = 20 bags per plot). The first collection moment was four weeks after placing the bags in the field, so as to determine fast changes mainly in nutrient dynamics (Harmon et al. 1999). Subsequent collections were carried out after 12, 19, 24 and 32 weeks. In total we processed four bags per plot × 5 collection moments × 16 plots = 320 litter decomposition bags. The last collection was on April 22, 2016.

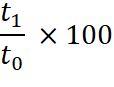
At each collection moment, litterfall bags were immediately placed in sealed polyethylene bags (Ziplock®) to avoid loss of material. In the laboratory, the surface of mesh bags was carefully cleaned using tweezers and a soft brush to remove stones, soil, and roots, and when clean, were dried in the oven until constant mass (72 h, 55oC), for dry weight determination. Oven-dried material was weighed and litterfall was subsequently pooled so as to yield one composite sample per plot and per collection time, in order to determine the N and P concentration (see below) (Harmon et al. 1999).

#### Litterfall chemical analysis

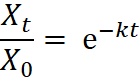
Litterfall nutrient concentration was determined on composite samples before the start of the experiment and at each one of the five collection moments. Total C and N were determined with an elemental analyzer (Vario MACRO cube, Elementar), at the Applied Tropical Ecology Laboratory (Laboratorio de Ecología Tropical Aplicada) at the Environmental Sciences School at the Universidad Nacional (UNA), Costa Rica. Total P was determined with an HCl extraction (HCl) followed by molibdate colorimetry (Díaz-Romeo & Hunter, 1982) in a flow injection autoanalizer (QuikChem 8500 Series 2 Lachat Instruments) at the Chemical Oceanography laboratory, Centro de Investigación en Ciencias del Mar y Limnología (CIMAR), at the Universidad de Costa Rica (UCR). The initial C concentration of *H. alchorneoides* litter was 35.4%, N was 1.33% and P 0.036%. This resulted in an initial litter C:N ratio of 26.7, N:P of 36.8, and C:P of 984 on a mass basis. The C, N and P concentrations in popsicle sticks (birch wood) were not determined.

#### Decomposition rate and nutrient release

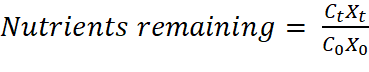
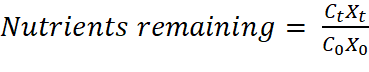
The percentage of remaining mass of leaf litter and wood was determined with the formula:



Where *t1* is the is the sample dry mass at collection moment *t1* and *t0* is the initial sample mass, and so on for all collection times (*t1*, *t2*, *t3 and t4*). We also calculated an exponential decay constant *k* (Swift et al. 1979) for both substrates using the formula for a single negative exponential model (Jenny et al. 1949, Olson 1963):



Where *Xt* is the substrate dry mass remaining at a specific time *t* (years), *X0* is the initial dry mass, *k* is the decomposition rate constant (year-1), *e* is the base of the natural logarithm (Harmon et al. 1999). This is useful to provide a standardized comparison of time-integrated rates of decomposition across sites and substrates (Vitousek 2004). We calculated the fraction of the initial nutrient content remaining at each collection time as the product of litter nutrient concentration by the mass remaining in the decomposition bags, and expressed it as a percentage of the original nutrient content remaining, using this formula:



where C0 is the mean initial nutrient concentration in leaf litter, Ct is the mean nutrient concentration at a given collection time (t), and X0 and Xt are the initial dry mass and mass remaining at time *t*, respectively. Hence, values greater than 100% indicate nutrient accumulation during decomposition, while values less than 80% represent nutrient release (Vitousek et al. 1994).

#### Statistical analysis

In order to test for the effect of fertilization treatment and time (predictors) on each of litter or wood mass loss (response) we used Bayesian generalized linear mixed models. All models were run in Stan (version 2.28.0; https://mc-stan.org/users/documentation/) through the R platform (http://www.R-project.org/) using the R package brms (Bürkner, 2017). We used a Bayesian rather than a frequentist approach because we have a small sample size relative to the high natural variability of our system (Townsend et al. 2008), and because the analysis we required was too complex for frequentist approaches given our data. For example we wanted to run a linear mixed model with plot as a random factor so as to be able to analyze each replicate litter bag per plot individually (and not use the plot-level average for litter mass remaining), however data were not normally distributed and neither various transformations nor using different distributions in GLMs led to model convergence. To test for the effect of fertilization treatment on *k* decay constants we also used Bayesian generalized linear mixed models, but looking at the overall effect of P addition (+P, +NP) vs. no P addition (+N, Controls), and the same for N addition (+N, +NP) vs. no N addition (+P, Control) (Wright et al. 2011).

All continuous predictors were zero-centered and transformed to unit variance to remove differences in magnitude and simplify interpretability. We use a beta distribution for error fitting models with proportions as response variable and gaussian distribution for all other models. We used weakly informed priors given the lack of previous information on this particular system (Table S1). Models were run with ‘adapt delta’ = 0.99 and ‘maximum tree depth’ = 15 to improve convergence. We present effect sizes as median posterior estimates and 95% credibility intervals (CI) as the highest posterior density interval (HDPI). Predictors were considered to have an effect on the responses when the 95% HPDI of their estimates did not cross zero and marginally significant when the 90% HPDI did not cross zero. All models were run on three chains for 30,000 iterations, following a burn-in of 5000 iterations. The effective sample size was kept above 10000 for all parameters. Performance was checked visually by plotting the trace and distribution of posterior estimates for all chains. We also plotted the autocorrelation of successive sampled values to evaluate the independence of posterior samples. The potential scale reduction factor was used to assess model convergence and kept below 1.05 for all parameter estimates.

### Results

#### Litter and wood mass loss

Leaf litter mass loss was constant through time, and after four weeks 25% of the initial mass had already been lost in all fertilization treatments (Fig. 3a). After 36 weeks, between 70 and 80% of all the litter had been lost. Wood had a slightly lower initial mass loss than litter, with 15% mass loss after the first four weeks of decay (Fig. 3b). Nevertheless, both substrates had a similar mass loss during the rest of the experiment (Fig. 3). Litter mass loss was not significantly influenced by fertilization treatment (Fig. 3a, Table 2), but wood mass loss was significantly slower in +NP plots relative to the controls (Fig. 3b, Table 2). Consistent with mass loss results, the decay constant *k* was not significantly different for leaf litter that decomposed in plots that received P (+P and +NP) versus those that did not (Control, +N) (Fig. 4a, effect size of P addition: −0.2, CI: −0.498, 0.094), but *k* values were lower for wood that decomposed in plots that received P (Fig. 4b, effect size of P addition: −0.497, CI: −0.893, −0.104), meaning that P addition inhibited wood decay. In contrast, no significant effects of N addition were observed on the decay constants for either litter (Fig. 4c, effect size of N addition: FALTA) or wood (Fig. 4d, effect size of N addition: 0.01, CI: −0.408, 0.434).

#### Litter nutrient dynamics

Litter N concentration significantly decreased with time whereas litter P concentration did not (Fig. S1), and neither was influenced by fertilization (Fig. S1, Table S1). Both litter N and P content were always < 80% and significantly decreased with time. Furthermore, N and P content were significantly and marginally significantly lower, respectively, in +N plots relative to the controls, indicating that while net N and P release occurred in all plots, this release was stimulated further by N fertilization (Fig. 5, Table 2). There were no significant interactions between time and fertilization for litter nutrient concentration or content (Table 2). Finally, the amount of N immobilized in leaf litter per unit mass loss (N concentration vs. remaining litter mass) increased significantly with time, and was lower in +N plots relative to the controls (Fig. S2a, Table S2). In turn, the amount of P immobilized in leaf litter per unit mass loss was not significantly affected by time or fertilization (Fig. S2B, Table S2)

#### Carbon-to-nutrient ratios

The initial C:N, C:P and N:P ratios of *H. alchorneoides* litter were approximately 26, 21, and 525 (Fig. S3). Litter C:N decreased, and N:P increased, significantly with time, but litter C:P was not significantly influenced by time (Fig. S3, Table S3). Neither ratio was significantly influenced by fertilization treatment.

### Discussion

#### Litter and wood mass loss

We hypothesized that leaf litter and wood decomposition are P limited in our study site, and thus that leaf litter and wood mass loss would be faster in +P plots than in controls and +N plots. However, we saw no stimulation of N and P addition on mass loss. This finding is consistent with Cleveland et al. (2006), who failed to find a significant effect of +P fertilization on leaf litter mass loss rate in mature tropical lowland evergreen wet (> 5000 mm precipitation y-1) forests in Costa Rica. Similar findings are reported for drier (2000 mm y-1) mature lowland evergreen forest in Brazil (McGroddy et al. 2004). A lack of a stimulating P effect on leaf litter decomposition is also reported for a seasonally dry secondary forest in tropical China (1700 mm rain) (Zhang et al. 2020). Our findings contrast with other studies in tropical mature forests who do find significant fertilization effects on litter decomposition. For instance both N and NP addition stimulated litter decomposition in montane forests in Hawaii (Hobbie and Vitousek 2000), NP addition stimulated it in tropical China (Chen et al. 2013), and P, K, and micronutrient additions stimulated litter decomposition in semideciduous lowland forest in Panama (Kaspari et al. 2008). In laboratory incubations, P and Zn additions stimulated the decomposition of leaf litter from mature dry forest tree species (Powers and Salute 2011), and N and P added together (but not singly) stimulated litter decomposition in a mature lowland moist forest in French Guiana (Barantal et al. 2012), Fertilization has also been shown to inhibit litter decomposition. For instance N addition has been found to inhibit leaf litter decomposition in mature tropical forest in China (Fang et al. 2007, Zhang et al. 2020), and so has N and P added singly (but NP together stimulated decomposition) (Chen et al. 2013). Given this diversity of responses, it is not surprising that a recent meta-analysis shows no significant influence of N or P addition on litter decomposition (Camezind et al. 2018).

A lack of fertilization effect on leaf litter mass loss suggest that (1) N and P availability do not limit litter decomposition at our study site, or (2) decomposition is limited by nutrients but at a later stage, in the form of dissolved organic matter (DOM) leached from leaf litter, assuming that litter mass loss is dominated by leaching of DOM due to high rainfall rates.

This occurred despite instantaneously available P concentrations being ca. 13 times higher, and microbial biomass P twice as high, in the soil of plots that received P vs. those that did not (Vincent et al., *unpublished*). It also occurred despite the fact that *H. alchorneoides* litter initial P concentration (0.036%) is in the range of values for litter P concentrations reported for other lowland wet forests (Vitousek et al. 1984, 0.01-0.08%). The initial C:P ratio of *H. alchorneoides* litter was 984, which is in the range of the 902 ± 506 reported for tropical regions (Aerts 1997), slightly lower than the 1,124 ± 109 reported for lowland moist and wet forests in Vitousek et al. (1984) (calculated by McGroddy et al. 2004), and much lower than the 1514 ± 65 reported for “Tropical forest” (Yuan and Chen, 2009). It is possible that the combination of high P availability in the litter we used with high total soil P availability in our study sites, lead to no P limitation to decomposition.

In another study in the Amazon, P fertiliztiaon did not stimulate litter decomposition rates either

Proximate causes could be that

Soil fertility is high

Mass loss is driven by leaching not by mineralization and CO2 evolution

Litter nutrient content is high

Some nutrient other than N or P does limit litter decomposition (Na, Mo, Ca, K, etc.).

The litter mass loss rates observed in this study are in the range of those reported for other tropical mature lowland wet forests with varying precipitation levels, in French Guyana (Hättenschswiler and Jørgensen 2010), Costa Rica (Cleveland, Reed and Townsend 2006; Wieder, Cleveland and Townsend 2009, both sites 5000 mm precipitation y-1, seasonal) and China (Fang et al. 2007, 1927 mm precipitation y-1, seasonal). Our standardized wood stick mass loss rates were similar to those reported for wood sticks in young forest stands in Maryland, USA (Hu, Yesilonis, and Szlavecz 2021), and slightly higher than those reported for a moist tropical forest in Panama (Gora, Lucas and Yanoviak 2019). Unsurprisingly, they were at least double the rates reported for wood of native species in dry forests (Harmon et al. 1986) and coarse woody debris of native species in a Panamanian moist forest (Gora et al. 2019).

Lack of P (or N) stimulus to mass loss

* We hypothesized that litter decomposition would be P-limited in this lowland wet forest, and thus expected litter and wood decomposition rates to be faster in +P and +NP plots than in controls. Instead, neither P nor N fertilization had any significant effects on litter or wood mass loss, and contrary to expectation, wood decay was *slower* in +NP plots than in controls. Our findings are consistent with with Cleveland et al. (2006) who found no effects of +P on decomposition rates.
* Cleveland 2006: High organic matter solubility throughout decomposition combined with high rainfall support a model of litter-layer decomposition in these rain forests in which rapid mass loss in the litter layer is dominated by leaching of dissolved organic matter (DOM) rather than direct CO2 mineralization. While P fertilization did not significantly affect mass loss in the litter layer, it did stimulate P immobilization in decomposing material, leading to increased P content and a lower C:P ratio in soluble DOM
  + So is it possible that we have to do a litter decomposition experiment with DOM instead of the solid litter? If mass loss is driven by rainfall (overlay mass loss with cumulative rainfall in the previous days!) then that, and not nutrients, will dominate mass loss patterns in the litter, and nutrient limitation to decomposition must be tested with DOM. the solution: doing a laboratory decomposition experiment, but actually soil fauna is super important in the tropics so I don´t think this would work either. Well it could, you just need to do a microbial mineralization incubation with DOM.
  + These results show that, while nutrients may not affect mass loss during decomposition in nutrient-poor, wet ecosystems, they may ultimately regulate CO2 losses (and hence C storage) by limiting microbial mineralization of DOM leached from the litter layer to soil
  + In addition, while estimates of decomposition are commonly reported as a single empirical constant representing mass loss rate (i.e., k in the expression e2kt; Olson 1963), decomposition includes two distinct mass loss vectors: CO2 mineralization in the litter layer and leaching (e.g., Yavitt and Fahey 1986, Currie and Aber 1997). The first of these, mineralization in the litter layer, represents a biologically mediated, chemical transformation of organic matter to CO2. In contrast, leaching does not transform organic material per se, but merely redistributes C from the litter layer to the soil. Thus, while ‘‘mass loss’’ is typically a single measured variable, it integrates two fundamentally different processes that may vary in their response to nutrients.
  + For example, in dry systems (where leaching is a relatively small mass loss vector) organic matter mass loss rates are most likely to be nutrient limited at the site of CO2 mineralization (i.e., in the litter layer). Alternatively, in wet systems (where leaching is a more dominant mass loss vector) nutrient availability may not limit mass loss rates themselves, but may ultimately limit microbial mineralization of transported dissolved organic matter (DOM) at the site of DOM delivery (i.e., in the soil)
  + “in systems with high precipitation and/or highly water-soluble litter, nutrient availability may not limit mass loss (B), but may ultimately regulate DOM mineralization in the soil” but how could this happen given that microbes are C limited? Did they study the stoichiometry of this DOM? Do you need to look at the CNP of single molecules dissolved in this DOM?
* Barantal 2012: As the naturally available tree leaf litter represents an unfavorable C:N:P stoichiometry for decomposers (Sterner and Elser 2002), we hypothesized that fertilization with N, and with P in particular, would decrease nutrient limitation to decomposers, and increase litter decomposition. In contrast to our hypothesis, fertilization with either N or P alone did not change litter mass loss regardless of whether or not fauna had access to decomposing litter.
* This lack of +P effects on mass loss could be due to several factors. First, nutrient availability in our study system could be naturally high and thus not limit organic matter decomposition. Second, the duration of the experiment was not long enough to generate an observable effect on decomposition. Third, nutrients other than N and P are more important (Li et al. 2006) and are limiting decomposition. Finally, high local precipitation lead to leaching being the most important driver of mass losss, as has been previously reported for a lowland wet forest in southern Costa Rica (Cleveland et al., 2006). Nevertheless, other tropical studies have indeed found a clear effect of adding N and P together on litter decomposition (Hobbie & Vitousek 2000, Barantal et al. 2012). It is clear that context-dependent conditions drive these observed differences in litter decomposition response to nutrients in all the sites studied to date.
* Another factor that could explain differences in the results found in this study and others is the type of leaf litter and wood used, given that this is one of the variables that differs most across studies investigating the effects of nutrient addition on litter decomposition rate in tropical wet forests (Barantal et al. 2012). For example, some studies use leaf litter of various species that differ greatly in chemical and structural properties (Kaspari 2008, Barantal et al. 2012). Other studies use leaf litter of only one species (Cleveland et al. 2006, Hobbie & Vitousek 2000, *this study*). A high variability in decomposition rates is obsered in tropical wet ecosystems with similar climatic conditions (Hӓttenschwiler et al. 2010).
* Vitousek book p. 62 has values for decomposition rates k and the values we found are in the range of those reported there in fig. 4.14.
* Hobbie and Vitousek 2000: they find that in Thurston, where NPP is very N limited, low soil N availability does not limit litter decomposition (nor do low litter N concentration). “The numerous studies showing little or no effect of N fertilization on decomposition (Staaf 1980, Gill and Lavender 1983, Pastor et al. 1987, Titus and Malcolm 1987, Fog 1988, Hunt et al. 1988, Theodorou and Bowen 1990, Van Vuuren and Van der Eerden 1992, Andren et al. 1993, O’Connell 1994, Prescott 1995, Downs et al. 1996), even in temperate ecosystems where N likely limits NPP, suggest that this uncoupling of factors that regulate litterfall and decomposition may be a general phenomenon at sites where N limits NPP”. Microbial ecologists would say that C limits? “At Kauai, on the other hand, both low soil nutrient availability and the resultant low litter P concentrations constrain litter decomposition rates, and probably contribute to the maintenance of nutrient limitation of ANPP at Kauai”
  + The interesting thing about this is that Silvia has found no evidence of nutrient limitation to NPP either. So in our case it´s not really a case of uncoupled factors that regulate litterfall and decomposition, just plain old no limitation. Really? What about N? N release despite
* McGroddy et al. 2004: differences in P availability did not affect decomposition rates, but P additions increased rates of P immobilization dramatically. Ourresultssuggestthat ratesof decompositionand net nutrient mineralization may be decoupled in ecosystemswith low nutrientavailability,providing an importantnutrient-retentionmechanism.

P inhibition of wood mass loss

* Berg 2003: Alternatively, a high level of a nutrient may be so high that it suppresses the decomposition process. Berg p. 221: Woody litter contains relatively small amounts of nutrients, especially when compared to foliar and root litter (Table 9.2)
* Hobbie and Vitousek 2000 introduction: N may actually inhibit the decomposition of the lignin fraction of litter either by inhibiting synthesis of lignolytic enzymes or by reacting with breakdown products of lignin degradation to form other compounds that resist decay (Berg 1986, Fog 1988). Such inhibition of lignin degradation could result in a neutral or negative effect of N on overall decomposition. DOES N ADDITION INHIBIT WOOD DECAY? MISSING ANALYSES, WRITE TO MAS. Alternatively, fertilization with N may directly inhibit the degradation of lignin, offsetting any stimulatory effect of N on decay of more labile carbon fractions (Fog 1988). Several studies in temperate systems have demonstrated such inhibition of lignin degradation by N in situ (Berg 1986, O’Connell 1994, Conn and Day 1996)
* Feng 2007: Fog (1998) concluded that N additions generally have either no effect or a negative one on litter decompo- sition in the long term. Our results are consistent with this conclusion: N additions had a negative effect on litter decomposition in pine and mature forest, but no effect in mixed forest. Fog K (1998) The effect of added nitrogen on the rate of decomposition of organic matter. Biol Rev 63:433–462. In the mature forest, N additions depressed litter decomposition further than that in the previous experiment (Mo et al. 2006). N itrogen addition did not reduce decomposition rate of C. chinensis leaf litter in the previous experiments but significantly educed in this experiment. The results for the mature forest and the difference between the two experiments may be explained by the followings. First, the mature forest is likely to be N saturated before N addition with the soil extractable inorganic N in the upper 10 cm mineral soils being 14.9 mg kg−1 compared with 5.9 mg kg−1 in pine forest and 6.4 mg kg−1 in mixed forest (Mo et al. 2006), so the earlier litter decomposition experiment showed no positive effect or some negative effect (Mo et al. 2006). Continuous N additions lowered the soil pH value from 3.76 in control plots to 3.70 in the high-N plots (data not published), and depressed the activity of microorgan- ism, thus further depressing litter decomposition.. Our results was supported by some study results showed that chronic N additions generally have either no effect or negative effect on litter decomposition in the long term (Fog 1998; Prescott 1995). The reasons might be that enhanced atmospheric N addition aggravates C-limitation for microbial degradation (Berg et al. 1998), depressed microbial activity (Fog 1998), depressed soil fauna activity (Xu et al. 2006), and more rapid formation of recalcitrant material (Ågren et al. 2001)
* Zhang et al. 2020: Here, we observed a negative effect of N addition on the litter decomposition, which was consistent with our hypothesis one and other N fertilization experiments carried out in tropical forests (Fang et al. 2007; Mo et al. 2008; Zhou et al. 2017). This result was also in agreement with our previous observation of slowed coarse woody debris (CWD) decomposition after N addition in the same nutrient manipulative experiment (Chen et al. 2016). Previous studies have shown that N deposition could decrease the soil microbial biomass and alter the microbial community composition (Li et al. 2015; Liu et al. 2013; Nilsson et al. 2007; Ramirez et al. 2012), and thus decrease the decomposition rate of organic matter (Pregitzer et al. 2008). In this site, our previous study (Li et al. 2015) found that N addition reduced soil bacterial biomass, which could partially explain the decreased decomposition rate as bacteriawere also important for the litter decomposition
* Barantal et al. 2012 say “Our results suggest that increased N availability is required for a positive P effect on decomposition in the studied P-poor tropical forest (0.1 mg P g-1 soil)”. N and P added alone had no effect on litter decomposition, but together they increased decomp by 33%.
* Barantal 2012: The effects of N fertilization on decomposition are more intensively studied in non-tropical biomes, and range from positive, neutral and negative responses depending mostly on initial leaf litter quality and site-specific ambient N-deposition levels (see Knorr and others
* 2005 for a review).
* Barantal et al. 2012 The NP fertilization effect increased with decreasing initial litter P concentration and increasing initial N:P and C:P ratios. This result highlights that nutrient fertilization effects on decomposition could strongly depend on initial leaf litter quality. THIS DOES NOT SUPPORT OUR WOOD DECOMP FINDINGS BC WE SAW SLOWER DECOMP WITH NP ADDITION.
* Chen et al. 2012 Since N and P addition alone decreased litter decomposition, we expected the combined NP-addition would have a severe negative effect. However, there was a significant interaction between N and P additions in the present study. We found that the k value in NP-addition plots was significantly higher than those in N-addition plots, while there were not significant differences between P-addition and NP-addition (Table 2). This indicated that P addition mitigated the negative effects of N addition on litter decomposition. Several studies have reported that the addition of either N or P alone did not change litter mass loss, but combined N and P addition significantly accelerated litter mass loss [28,32]. These studies implied that combined N and P addition may be more beneficial for litter decomposition compared to N addition alone, which partially supports our observation.
* Zhang et al. 2016 in pine plantations: “Because of macroinvertebrates, fertilization increased wood decomposition overall, despite significantly lower decomposition occurring in fertilized plots for sticks only decomposed by microbes”

#### Litter nutrient dynamics

As part of our P limitation to decomposition hypothesis we also predicted that decaying leaf litter would immobilize P, but not N, and that this P immobilization would be alleviated in +P plots given a reduction in C:P and C:N critical ratios.

Contrary to our expectations, we saw no P immobilization in litter with P addition. Instead, there was net N and P release throughout the experiment, and the release of both elements was enhanced by N addition. Why?

Although N and P tend to be immobilized at the start of decomposition in boreal and temperate climates (Aber and Melillo, 1980), in tropical areas nitrogen is usually released, and P may be immobilized or released, despite typically low litter P concentrations (Manzoni et al. 2010). This net N release has been shown to occur despite an increase in N concentrations in the remaining litter, which is associated with initial litter C:N and C:P ratios being lower than the corresponding decomposer critical C:N and C:P ratios, and net N and P mineralization occurs from the start of decomposition. The initial C:N and C:P ratios of H. alchorneoides were 26.7 and 984 respectively,

Increase in litter N concentration with time

* Manzoni et al. 2010: At the beginning of decomposition, nitrogen and phosphorus tend to be immobilized in boreal and temperate climates (i.e., both C:N and C:P critical ratios are lower than the initial ratios) (Aber and Melillo, 1980), while in tropical areas nitrogen is generally released and phosphorus may be either immobilized or released, regardless of the typically low phosphorus concentrations. IS THIS BECAUSE OF STRONGER RAINFALL AND LOSS OF NUTRIENTS FROM LITTER THROUGH LEACHING?
* Most decomposition studies find increases in N concentration in litter as decomposition proceeds. Aber & Melillo (1980).
* **Litter N concentration can increase even if there is a net release of N (page 94 of Manzoni).** How do they explain it? They ascribe it to scenario D in Figure 2, (D) “The initial C:N and C:P ratios are both lower than the corresponding critical ratios and net nitrogen and phosphorus mineralization occurs from the start of decomposition, with the trajectories remaining below one. This limiting regime may occur even in low-nutrient litters when decomposer carbon-use efficiency is also very low (black curves in Fig. 2). In fact, when e is low enough, the decomposer nutrient demand is decreased, and immobilization is less necessary. Under these conditions nutrients typically accumulate without net immobilization (i.e., the concentration in the litter increases even with net nutrient release) .remember that the critical ratio of the decomposer community is decreased by a low CUE and a high leaching rate? Conditions at EARTH?
* Townsend et al. 2006: While nutrient fertilization had no significant effect on mass loss during decomposition, it did alter litter nutrient dynamics through time, with potentially profound effects on leached DOM decomposition in soil. In particular, material decomposed in plots amended with P or N 1 P had significantly higher P concentrations than plots with no P amendments, suggesting microbial immobilization of P during decomposition in the P-amended plots (Fig. 2). Previous work has shown that fertilization can induce microbial nutrient uptake without simultaneous effects on litter mass loss rates (McGroddy et al. 2004)
  + I am not sure what should be interpreted as immobilization, increases in net content (concentration x mass remaining) or just concentration?

Net N release

* **V**itousek et al. 1984: The P concentration of *H. alchorneoides* litter (0.036%) is within the the range of values reported in moist and wet lowland tropical forests (Vitousek et al. 1984, Fig. 3, 0.01˗0.08% P)
* Critical nutrient ratios are basically the C: nutrient ratios of the microbial biomass, but modulated by three terms: CUE of the microbes, leaching rates of C, and to what extent that particular microbe prefers degrading nutrient rich substrates (e.g. bacteria vs. fungi?). they are like a more realistic indicator of the stoichiometric demand of the microbial community. When the litter has C:nutrient ratios higher than the critical ratio, microbes immobilize nutrients, and vice versa. Normally what happens is that fresh litter has C:nutrient ratios much higher than the critical nutrient ratios, so initially there is immobilization, but as C loss from litter proceeds the ratios of litter decrease and may become lower that the critical ratio, and so mineralization occurs. The thing is, in tropical environments we have a lot of rain, and high leaching rates increase the critical ratio (look at equation 4 in Manzoni: it leads to lower values in the denominator). This means that in order for immobilization to occur, you would need really really high C: nutrient ratios in litter (because microbes critical ratios are already so high). This is my hypothesis and I am leaving out other terms of the equation, like preference for high nutrient substrates and CUE. Manzoni et al 2010 say in p. 93, whilst commenting on fig 2, that a black line scenario (like my case) of N and P mineralization throughout, could also happen if CUE of the microbes is super low, again because this means critical ratios will be high, and a medium fertile litter like hyeronyma will have a ratio that is lower than the critical ratio, thereby leading to mineralization. When decomposer CUE is low, the decomposer nutrient demand is decreased, and immobilization is less necessary. What is CUE of tropical lowland forests? **Under these conditions nutrients typically accumulate without net immobilization (i.e. the concentration of the litter increases even with net nutrient release) THIS IS WHAT I SAW AND I MUST TALK ABOUT IT.**
* Manzoni 2008 “In general, the lower the (decomposer) critical ratio is, the earlier N release occurs, even in N-poor residues. Moreover, when critical ratio < initial litter C/N ratio, net release occurs from the beginning of decomposition. Conversely, if rCR is high, large amounts of miner-a!N have to be immobilized to increase the litter N concentration to its critical value, Woody residues have the lowest nutrient concentrations (Manzoni 2010)
* Manzoni 2008: On the basis of 55 litter types classified by initialN concentrations ranging from 0.03% to 3% (13), we show that the carbon-use efficiency tends to increase with higher initial substrate N:C ratio, which means that Npoor substrates (i.e., litterwith low N concentration and low N:C) have a more-efficient nitrogen use and a less-efficient carbon use.
  + This means that low litter C:N (tons of N available) is associated with high CUE (tons of C incorporated into microbial biomass per unit C absorbed) and vice versa, i.e. litter with low N have low CUE. In other words, they found a pattern of declining CUE Why?
* . In tum, low carbon-use efficiencies
* allow net mineralization to occur early during decomposition, even in relatively N-poor residues
* In Manzoni 2010, chuyong 2002 report net release of both N and P”
* In my case, release of both nutrients indicates that the critical ratios of N and P in microbes is higher than the ratios in litter, hence release occurs (o sea, un ratio alto implica que necesita poco nutriente en relación a C, entonces una hojarasca con ratio menor i.e. más rica en nutrientes, satisface esa necesidad
* What controls critical nutrient ratios of decomposers?
* McGroddy et al found continuos N mineralization during an 11 month decomposition experiment in brazil (ours was 8) but mineralization then after 6 months, immobilization of litter P. N content also was mineralized up until month 9 and then immobilised. At the end of the experiment, litter P content of control plot litter was 30% lower than initial litter, in our case it was 80% lower.
* In Hawaii, Thompson and Vitousek do show some N and P immobilization at a young, infertile 300 y old site (infertile? Is this P rich but N poor?) but all in litter harvested less than 1 month after having set them, and our first harvest was 3 months after first placing the bags so perhaps we missed a brief period of immobilization (assuming our site is equivalent in soil fertility to their 300-year old site). At the 20K site in that study, N immobilization happened during most of the decomposition period, but P immobilization only half-way through this 150 day experiment. They say that the most N poor litter immobilized more N than N-rich litter, when both were placed in a fertile site. Same was observed for P. So perhaps in my experiment we see tons of mineralization because hyeronima litter is super N and P rich? Compare with manzonis critical ratios.

Lack of nutrient immobilization

* We saw no immobilization, suggesting the system is super fertile (but see Thompson and Vitousek, they found more immobilization in more fertile sites).
* Hobbie and Vitousek 2000 Sources of immobilized nutrients during decomposition include translocation from soil or litter via fungal hyphae, throughfall, and fixation (for N) (Aber and Melillo 1991). Translocation may be more limited for P than for N because of the relatively slow diffusion of P through soil to fungal hyphae. Furthermore, the discrepancy between microbial and litter nutrient concentrations is greater for P than it is for N, resulting in relatively high microbial demand for P (Paul and Clark 1996). Thus, low supply and high demand may make low P availability more profoundly limiting to decomposition than low N availability AND HENCE P MORE LIKE TO BE IMMOBILIZED? OR LITTER DECOMP MORE LIKELY TO RESPOND TO P ADDITION?
* N dynamics usually follow a 2-phase pattern, with an initial increase in N content (% remaining) and then mineralization (Melillo et al. 1989, fig. 3) Melillo et al. 1989: Patterns of N accumulation and release, and factors controlling them have been identified for the early stages of decomposition (Aber and Melillo, 1980; 1982; Berg and Staaf,  
  1981),

Gráfico, Gráfico de dispersión

Descripción generada automáticamente

Citar a LI et al. 2006 puerto rico, ellos hicieron un estudio de descomposición.

Powers y salute tienen una lista de los mecanismos específicos a través de los cuales los nutrientes afectan la descomposicíon, me gusta porque va al grano> “addition may affect litter decomposition and soil processes, including biological effects on decomposer community composition (Compton et al. 2004), abundance (Demoling et al. 2008; Wallenstein et al. 2006), activity (Galicia and Garcia-Oliva 2004; Cleveland et al. 2002), and extracellular enzyme production and activity (Allison and Vitousek 2005; Sinsabaugh et al. 2005; Carreiro et al. 2000; Tyler 1974), and/or through abiotic interactions with organic matter or microbial degradation products (Dail et al. 2001; Hobbie 2008). It is possible that both stimulation of the microbial community and abiotic interactions explain our results” NO LO COPIE. Talk about the role of soil fauna, which seems to have a disproportionate role in the tropics (barantal et al. 2012, waring 2012)

NP inhibit wood decay

#### Litter nutrient dynamics

Fertilization affected litter nutrient dynamics to a greater extent than it did decomposition, and we saw an uptake of N by decomposers that was not always accompanied by changes in decomposition rates. We hypothesized that P and N addition would increase P and N immobilization in litter, respectively, and that P immobilization would be stronger than N immobilization, responding to a hypothesized stronger P limitation in this study system.

Litter N and P concentration

Wild fluctuations in P concentration we saw were also observed by Zhang et al. 2020. Why is this? Doesn’t happen with N.

Zhang et al. 2020: “During litter decomposition, the nutrient release usually goes through three stages, which are leaching, aggregation, and release (Blair 1988a). Generally, N concentration

in litters increases continuously during decomposition, especially in the initial stage (Aerts 1997)…. early N accumulation was a necessary condition to meet the nutritional needs of the decomposers. “The observed pattern of initial N accumulation shifted into a net N release after

15 months, suggesting that immobilization of N was needed in this forest until a critical concentration for decomposition was reached. Previous studies observed an initial immobilization of N, which was then reversed due to the increased availability of N by rapid microbial turnover (Fisk and Fahey 2001; Jacobson and Bustamante 2014) in temperate frosts. However, in our site, excessive N has already caused a lower decomposition rate and lower microorganism activities (Li et al. 2015) which was less likely to reverse the immobilization of N. Besides that, inorganic N compounds can condense with carbohydrates, causing the formation of melanoidins, which may subsequently increase the polymerization of polyphenol, and eventually, resulted in N immobilization (Treseder 2008).”

“The pattern of P releasing from leaf litter in the control plots was different among species. Species with lower P concentration went through an aggregation process in the first three months, then started to release P while species with higher P concentration started P releasing immediately at the beginning of decomposition. Experiments on P-limited tropical soils

have shown that added P either supplied internally through elevated litter concentrations or externally by soil fertilization could increase decomposition rates of litter and soil organic matter directly (Hobbie and Vitousek 2000; Vitousek and Farrington 1997) or indirectly via effects on the quality of DOM leached into mineral soil (Cleveland et al. 2006). **Immobilized P in litters of P addition plots compared to the control indicated that additional P supply was immobilized in this P limited ecosystems (Cleveland et al. 2006; Olander and Vitousek 2004).”**

AV- a lack of P immobilization points to lack of P limitation? Why does P addition increase microbial P then? Perhaps N is lost from litter due to leaching, but P is not so mobile (is this true in litter?) thus P is not leached as much as N? why is N immobilized? Wait, is N immobilised or mineralized? Make sure you understand what the metrics mean. N concentration increases with time, but in increasingly fewer “bits” of litter. So the total content of N in litter decreases because litter mass decreases inside the bags – and presumable the increase in litter N concentration is not high enough to make up for mass loss. So although there is a loss of N content, this is driven by mass loss, because per unit mass, there is immobilization – this is shown in the aber and melillo graph. Why is the same not observed for P? how do others explain this?

Litter N and P content (% initial)

McGroddy et al. 2004 Fig 2. Show that N content (% initial) decreases up until month 9 and then increases. In contrast, they show that P content (% initial) fluctuates with time but clearly increases (becomes immobilised) towards the end of the study in the fertilized plots. How do they interpret this immobilization in +P plots? I would assume mineralization increased with P fertilization…

REVISE ALL THIS.

Our results are partially consistent with this hypothesis, because we did find a greater P immobilization in +P plots, reflected in lower N:P and C:P ratios in the remaining leaf litter in +P plots than in the rest of the fertilization treatments. However, we did not see a significant N immobilization increase in +N plots, which could indicate that N is not as limiting to the microbial community as P. A lack of N immobilization could be related with basic N biogeochemistry, given that it can become volatilized into nitrous oxides, and also has a greater solubility in soil than P (Güsewell, 2004). NO ENTIENDO. QUIERE DECIR QUE LOS MICROORGANISMOS NO LO PUEDEN INMOVILIZAR PORQUE EL N SE VUELVE GAS O SE LIXIVIA ANTES QUE LOS MICROBIOS LO ABSORBAn?

A greater P immobilization in +P plots is consistent with previous studies. For instance, McGroddy et al. (2004) documented increases in P immobilization in decaying litter after +P and +N+P addition, of up to double the initial P concentration. In the same vein, Hobbie & Vitousek (2000) in a premontane forest in Hawaii found that +P caused the greatest P immobilization, consistent with what we found, and despite no fertilizer effects on litter decomposition rates, concluding that fertilization affected nutrient dynamics more strongly than it did decomposition rates. Cleveland et al. 2006 also detected greater P immobilization in litter in +P plots Nevertheless, Chen et al. (2013) found that adding N did encontraron que la adición de N sí tuvo un efecto en el contenido remanente tanto de N como de P, y que por su parte el tratamiento de adición de P solo generó efectos (mayor inmovilización de P) en uno de los sustratos utilizados.

It is worth noting that in this study, significant effects related with P dynamics, N:P and C:P yielded contrasting results in +N vs. +P treatments, which could be due to antagonistic effects of these elements. For example, N addition tends to lower pH whereas P to increase it (Lu et. al 2014); N stimulates the growth of certain fungi whereas P stimulates bacteria (Fanin et al. 2015). Both effects of pH and N vs. P availability could cause changes in the composition of the decomposer community, as has been reported in other fertilization studies (Chen 2013). Nevertheless, additional information on soil community structure and diversity are needed to understand this further.

In spite of our finding of no direct effect of N and P fertilization on litter and wood decomposition rate, A pesar de que no se encontró un efecto directo de la fertilización sobre la pérdida de biomasa, no se debe dejar de lado la importancia que tiene la disponibilidad de nutrientes sobre los procesos de liberación de CO2 a la atmósfera o bien de fijación en los suelos de estos ecosistemas. Si bien es cierto estos procesos no regulan directamente la pérdida de biomasa, si pueden regular la velocidad con la que la materia orgánica disuelta es devuelta al ambiente en forma de CO2 (Cleveland et al. 2006). Esto, aunado a que los bosques lluviosos tropicales de bajuras están entre los ecosistemas en los que se descompone mayor cantidad de material orgánico (Wieder 2009), los cambios en las tasas de descomposición, aun cuando estas sean pequeñas, deben ser prioritariamente estudiadas ya que pueden tener un gran efecto sobre fenómenos a nivel global como el calentamiento global (Chapin et al. 2011).

#### Things to discuss

Microbial N mining hypothesis

From Camezind VitousekandHobbie(2000b) reportedthathigherN

and Puptakebymicrobesdidnotalwaystranslateinto

increased decompositionrates.Thus,despitethefact

thatmicrobeswereapparentlynutrientlimited,itdid

not changethemeasuredmicrobialprocess.Thisappar-

ent paradoxisaddressedinthemicrobialNmining

hypothesis,whichstatesthatanincreasedavailabilityof

mineral Nrequireslessinvestmentintothebreakdown

of organicmaterialforNacquisition(Craineetal.2007,

Condronetal.2010).Thismayevenbeanexplanation

forsomenegativeeffectsobservedfollowingNadditions

(Hagedornetal.2003,HartmanandRichardson2013),

though thereisnoindicationforthisphenomenonin

our datasetwhenconsideringoveralleffects(Fig.5).In

the caseofP,thereisnoevidenceforPminingeffectsso

far (SinsabaughandMoorhead1994,Craineetal.

2007). Thesefindingsunderlinethenecessitytounder-

stand geochemicalandbiologicalimpactsoftheadded

element anditsrelationtothemeasuredresponsevari-

ablesinmoredetail.

Non-Liebig world

Kaspari et al. 2008 Our results suggest tropical forests are a non-Liebig world of multiple nutrient limitations, with at least four elements shaping rates of litterfall and decomposition.

Powers and Salute 2011 found positive effects of K and negative effects of Mg on litter decomposition rates.

Barantal et al. 2012: Further stimulation of decomposition by C amendment through priming indicates energy limitation of decomposers that is co-determined by nutrient availability. The demonstrated intricate control of the key resources C, N, and P on decomposition calls for an intensified research effort on multiple resource limitation on key processes in tropical

forests and how they change under multiple human impacts.

Camenzind et al. 2018 has a great section on this.

Fauna

Barantal et al. 0212 Soil fauna strongly stimulated litter mass loss and enhanced nutrient fertilization effects. Sol Moreover, nutrient effects on decomposition increased with additional C fertilization in the presence of fauna. They say soil fauna was the most important factor. Fertilization was the second.

Initial stoichiometry

Possible reason to explain very different results across fertilization studies.

Barantal et al. 2012 say This result highlights that nutrient fertilization effects on decomposition

could strongly depend on initial leaf litter qualityof our litter vs. that of litter form studies that did find a fert effect

|  |  |  |  |
| --- | --- | --- | --- |
|  | CP | CN | NP |
| This study | 984 | 26.7 | 36.8 |
| Barantal et al. 2012 | 900-2770 |  | 20-80 |
| Hobbie & Vitousek 2000 |  |  |  |
| Cleveland et al. 2006 | 344-512 |  | 14-26 |
| Chen et al. 2013 | 551 | 29.2, 32.9 | 16.7 |
|  |  |  |  |

Variation across decomposition studies

Barantal et al. 2012 also discuss Otherwise, it is likely that part of the divergent results of fertilization effects on decomposition among different studies is related to the distinct types of humid tropical forests that have been studied. For example, N and P cycling have been shown to vary as a function of topography, temperature temperature, precipitation, and soil types in the tropical biome (Houlton and others 2006; Nardoto and others 2008; Quesada and others 2010). Perhaps our study site was more N and P limited than other sites as might be suggested by the lower total soil N and P concentrations at our site (Fanin and others 2011) compared to other sites (Hobbie and Vitousek 2000; Cleveland and others 2006; Kaspari and others 2008; see Appendix 2 in Supplementary material for a comparison of soil N and P concentrations in these studies) which may in part explain the strong NP fertilization effect observed here.

Leaching vs. direct heterotrophic respiration decomp model

Zhang et al. 2020 say that because their site is rainy, it’s possible that leaching is a more important driver of decomposition than P fertilization. mass loss during litter decomposition includes two distinct processes: direct mineralization of organic matter to CO2 in the litter layer, and leaching and transport of soluble organic material from the litter layer to the soil (Currie and Aber 1997). Our site has a mean annual temperature of 23 °C and annual precipitation of 1700–2000 mm Therefore, despite the biochemical process, there was a great chance that the leaf litter decomposition in our site was also influenced by leachingwhich could cause the P fertilization effect not significant.

### notes

Harmon p. 234



Aber melillo 1980



Vitousek et al. 2004 has a figure 8.11 with NP ratios in decaying litter. They are around 15, much lower than ours (40-90)

Vitousek fig 4.15 has fraction of N or P remaining after x amount of tiem of decomposition. You can show this as a bar plot for our data. The scale of this is in 0-100%, and you can plot both of them on the same graph. How do you calculate it?

Hobbie 2000

The N:P ratios in the forest floor on the water- shed which we studied were 16.5:1, 17:1, and 15:1 for the L-, F-, and H-layers, respectively. After 12 months on the forest floor the N:P ratios of yellow birch, sugar mapl e, and beech leaves were 16.5: 1, 15.6: 1, and 15.4: 1, respectively. These similar ratios indicate a correlation between the N and P in decaying litter in decaying litter. The correlations between N and P concentrations in decaying leaf litter after the 1-month leaching period were highly significant (P < 0.01) for all three species at the 550- and 670-m elevations. A test for homogeneity indicated that the correlation coefficients were homogeneous and estimated a common parametric value of 0.973. Acknowledgements

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