LETTER

Multiple nutrients limit litterfall and decomposition in a tropical forest

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Abstract

To explore the importance of 12 elements in litter production and decomposition, we fertilized 36 1600 m²-plots with combinations of N, P, K, or micronutrients (i.e. B, Ca, Cu, Fe, Mg, Mn, Mo, S, Zn) for 6 years in a lowland Panamanian forest. The 90% of litter falling as leaves and twigs failed to increase with fertilization, but reproductive litter (fruits and flowers) increased by 43% with N. K enhanced cellulose decomposition; one or more micronutrients enhanced leaf-litter decomposition; P enhanced both. 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. Multiple metallomic enzymes and cofactors likely create gradients in the break down of leaf litter. Selection favours individuals that make more propagules, and even in an N-rich forest, N is a non-substitutable resource for reproduction.

Keywords

Carbon cycle, decomposition, fungi, K, Liebig's Law of the Minimum, litter, Mg, micronutrients, N, P, reproduction, tropical forest, Zn.

INTRODUCTION

In forest ecosystems, most leaves fall uneaten to the forest floor (Coley & Barone 1996). There they are decomposed by fungi and bacteria, which in turn feed the rest of the brown food web (Swift *et al.* 1979). The trees and microbes that help drive the carbon cycle need perhaps 25 chemical elements to grow and reproduce (Sterner & Elser 2002). Despite the necessity of factorial experiments to sort out element control of ecosystem processes (Sollins 1998; Vitousek 2004) such experiments are rare (e.g. Tilman 1988; Scheu & Schaefer 1998; Hobbie & Vitousek 2000). Ecologists instead often wield Occam's Razor, in the form of Liebig's Law of the Minimum (Brock 2002), to focus on the most rate-limiting nutrient for a process or ecosystem.

Decomposer microbes are strong candidates for multiple-nutrient limitation. Leaf-litter decomposition requires the sequential breakdown of a variety of substrates (e.g. waxes, phenolics, lignins, celluloses) requiring a variety of enzymes produced by different microbes (Allison *et al.* 2000; Frausto da Silva & Williams 2001). This suggests that the decomposition of a leaf likely requires the collective synthesis of many metallomic enzymes. Moreover, tiny microbes without networks of roots or hyphae likely experience a fine-grained environment of nutrient shortfalls. Thus far, however, N and P limitation have been the focus of most decomposition studies. For example, many observational and a few experimental studies in boreal and temperate forests have shown nitrogen rich leaves decompose faster than nitrogen poor leaves (Berg & Laskowski 2006). In contrast, most lowland tropical forests lie on highly weathered soils that are relatively nitrogen rich but have been depleted with time of a variety of rock-derived chemical elements (Walker & Syers 1976; Wardle *et al.* 2004). Much circumstantial (Reich & Oleksyn 2004; Wardle *et al.* 2004) and some experimental (Hobbie & Vitousek 2000; Cleveland *et al.* 2006) evidence suggests that phosphorus plays a key role controlling leaf-litter decomposition in lowland tropical forests. However,

we know of no experiments testing multiple-nutrient control of leaf-litter decomposition by the other cations – potassium, magnesium, manganese among others – likely to be depleted on weathered soils (Chapin et al. 2002).

A further challenge is teasing apart the two complementary ways that nutrients shape decomposition rates (Hobbie & Vitousek 2000). First, fertilizer can be taken up directly by microbes, thus directly promoting a more active microbial community. Second, fertilizer when absorbed by trees can be used to build leaves that are more nutritious to herbivores and decomposers alike (Chapin 1980). This distinction may be less important in monospecific stands, where many fertilization experiments have been performed, as feedbacks between soil nutrient availability and leaf chemistry tend to homogenize nutrient availability in the soil and leaves (Fahey et al. 1998). However, in species-rich tropical forests, interspecific differences in, for example, investment in leaf defenses (Grime et al. 1996) may generate a mismatch between soil and leaf-mineral nutrients and subsequent heterogeneity in decomposition rates across the landscape.

Here we use a factorial N, P, K fertilization of 40 × 40-m plots in a tropical forest, plus an additional micronutrient treatment (i.e. B, Ca, Cu, Fe, Mg, Mn, Mo, S, Zn) to evaluate nutrient control of litter production and decomposition in lowland tropical forest. We document changes in leaf, twig and reproductive litterfall and leaf-litter chemistry after 6 years and contrast decomposition of cellulose and leaf litter from fertilized plots in situ and in a common garden. We test the Walker & Syers (1976) hypothesis that P (and, by extension, K), but not N, limits production and decomposition in this weathered soil. We also evaluate the hypothesis that the decomposition of the more complex leaf-litter substrate requires a larger suite of metallomic enzymes and, by extension, a greater array of minerals than the decomposition of cellulose. Finally, we use a common garden, and the cellulose standard, to evaluate the relative importance of soil nutrients (i.e. direct microbial subsidy) vs. leaf nutrients on decomposition rate. We conclude that (i) tree reproduction, but not leaf production, is N-limited, and (ii) at least three elements (P, K, and a micronutrient) contribute to spatial variation in decomposition, likely through changes in the microbial community and not through changes in leaf-litter chemistry.

METHODS

This study spans 1998–2004 on the Gigante Peninsula (9°06′31′N, 79°50′37′W) of the Barro Colorado Nature Monument (BCNM) in the Republic of Panama. The species composition and stature (canopy heights > 35 m) of this forest are characteristic of an old (> 300 years) seasonal primary forest (Leigh et al. 1996). The soil is an Oxisol and low P and K availabilities (PO_4^{3-} < 0.5 ppm; K^+ < 0.8 meq 100 g m^{-1}) reflect low bedrock concentrations (Yavitt & Wieder 1988; Cavelier 1992).

A factorial NPK fertilization (i.e. N, P, K, NP, NK, PK, NPK, control) with four replicates was laid out across 32 plots in a stratified random design (see Figure S1). The four replicate strata stepped down a 36 m topographic gradient (25–61 m a.s.l.) and were initially treated as statistical blocks, as nutrient availability and other soil factors can vary over relatively small topographic gradients (Espeleta & Clark 2007). Fertilizer treatments, applied four times a year during the wet season, began in June 1998. Fertilizers were applied to reach the following total doses for each year's four applications: 125 kg N ha^{-1} year^{-1} (as coated urea [(NH$_2$)$_2$CO]), 50 kg P ha^{-1} year^{-1} (as triple superphosphate [Ca(H$_2$PO$_4$)$_2$.H$_2$O]), and 50 kg K ha^{-1} year^{-1} (as KCl). Similar doses are used in forestry (Binkley 1986) and have also been used in tropical montane studies (100–150 kg N, 50–65 kg P, 50 kg K, e.g. Tanner et al. 1992; Vitousek et al. 1995). An additional four '+M' plots were dosed with a micronutrient fertilizer (Scott's S.T.E.M) consisting of HBO$_2$, CuSO$_4$, FeSO$_4$, MnSO$_4$, ZnSO$_4$ and (NH$_4$)$_6$Mo$_7$O$_{24}$ at 25 kg ha^{-1} year^{-1} plus dolomitic limestone CaMg(CO$_3$)$_2$ (36.8 kg year^{-1}) at 230 kg ha^{-1} year^{-1}.

Litter chemistry and litterfall rates were monitored via three 0.76 × 0.76 m (0.58 m^2) litter traps on each plot, which were emptied monthly beginning in September 1997. Three traps were determined sufficient to quantify litterfall variation based on power analysis of trap data from a long-term experiment in a forest 3 km away (Cavelier et al. 1999). The traps were randomly located within the inner 30 × 30 m core of each plot, as long as each was > 10 m from the nearest neighbouring trap. Trap contents were oven dried to constant mass (60°C). Starting in 1999 litter was pre-sorted into leaves, flowers and fruits, wood ≤ 2 cm diameter (or, 'twigs') and then dried and weighed. Litterfall rates of these components were calculated as g m^{-2} year^{-1}. Starting in 1998, each September + October's leaf-litter samples were chemically analysed for Ca, Cu, Fe, K, Mg, N, P, S, Zn, and neutral detergent fibre in an assay aimed at elements potentially limiting consumers (OSU 2006). We thus analyse litter chemistry for 6 years post-fertilization (1998–2003) and annual litter fall (divided into leaf litter, reproductive litter and twigs) for five years post-fertilization (1999–2003).

Decomposition experiments

We used two experiments to explore how inorganic fertilizers enhanced decomposition rates of a simple (cellulose) and complex (leaf litter) substrate. The first experiment ran July–August 2003. Each plot received 12 nylon litterbags, 20 × 20 cm with 2-mm mesh. Six were

filled with 4 g of cellulose (Fisherbrand P8 coarse filter paper Fisher Scientific, Pittsburg, PA, USA). Six were filled with 4 g of leaf litter collected from litter traps on the plot of origin in June 2003, dried at 50° C, and homogenized. These litterbags represented the collective response of the trees, lianas, palms and epiphytes in the plot of origin.

Two litterbags of each substrate (cellulose versus leaves) were placed within 1 m of each of three litter traps on each plot. After 4 and 8 weeks, three bags of both substrates were collected, cleaned and dried at 50°C as per Harmon *et al.* (1999). Litter decomposition rates were expressed as *k*, where the fraction of remaining litter $= e^{-kt}$; '*k*' was calculated by regressing the median ln(%-remaining litter) of a sample against the fractions of a year the litter was in the field, with the value at $t = 0$ set at ln(100) (Harmon *et al.* 1999).

Decomposition responses to fertilization could arise because the inorganic fertilizers feed decomposer microbes and/or because these microbes preferentially use leaf litter whose quality has been enhanced by the fertilizer (Hobbie & Vitousek 2000). To remove the effect of long-term microbial subsidies and to extend the time over which decomposition occurred, we performed a common-garden experiment in June 2004 in the Allee watershed 6 km away. Like the Gigante plots, it is an old forest with an ca 35 m canopy on an ultisol, with a mild slope and an elevation 35 m a.s.l. (Yavitt & Wieder 1988; Cavelier 1992). Leaf litter was collected and prepared as before. Litterbags were laid out along six transects ($n = 36$ litterbags per transect, litterbags separated by 1 m, transects separated by 3 m). Litterbags were collected after 6 and 15 weeks and analysed as before.

Statistics

Annual leaf-litter chemistry and annual litterfall was monitored from 1999 to 2003. The NPK factorial experiment, with main effects of N, P, and K, yearly measures, and blocking on topographic strata, was analysed using Repeated Measures ANOVA. To evaluate micronutrient effects we compared means for four +M plots to four control plots in a separate Repeated Measures ANOVA.

We analysed data from decomposition experiments in two steps. First we used a factorial ANOVA to explore how *k* varied with N, P, and K fertilizers, blocking on elevational strata (Winer *et al.* 1991). No N–P–K interaction was included. If the NP, NK, PK, or blocking effects were not significant ($P > 0.05$) they were deleted from the model and the analysis run again. A Wilcoxon test was used to compare means between Control and Micronutrient (+M) plots.

Given our underlying hypothesis of nutrient limitation, we used one-tailed tests to evaluate the hypothesis that enhancing N, P, K or micronutrients increased decompo-

sition and litterfall. We used two-tailed tests for changes in leaf chemistry and for all topographic effects.

RESULTS

Litterfall productivity and chemistry

Litterfall averaged 1065 g m^{-2} year^{-1} across the plots, of which 65% fell as leaves, 25% as twigs and 10% as fruit and flowers (Table 1). Leaf litterfall and twig litterfall did not increase on +N (leaf: $P = 0.16$, twig: $P = 0.19$), +P (leaf: $P = 0.15$, twig: $P = 0.28$), and +K plots (leaf: $P = 0.34$, twig: $P = 0.41$, Table 1, see Appendices S1–S4), although both varied with strata (leaf $P < 0.001$, twig $P < 0.004$), showing a strong increase between blocks 2 and 3 (*c.* 40 and 55 m a.s.l. respectively). In contrast, that part of litterfall that reflected investment in reproduction, although highly variable from year to year (year effect $P < 0.0001$), was 43% higher on +N plots ($P < 0.05$) and trended 32% higher on +P plots ($P < 0.10$). Reproductive litterfall was invariant across strata. Micronutrients had no detectable effect on litterfall.

The nutrient content of leaf litterfall varied with fertilizers and across the topographic strata (Table 1, see Appendix S1 and S2). Leaf litter N averaged 1.5% of dry weight and was enhanced by 7% ($P < 0.008$) on +N plots. Leaf litter P averaged 0.06% and was enhanced by 27% ($P < 0.0001$) on +P plots. Leaf litter K averaged 0.27%, and was enhanced by 16% on both +K plots ($P < 0.015$) and +N plots ($P < 0.015$). Among the other elements measured, only leaf litter S changed with fertilization, increasing 5% on +P plots ($P = 0.046$). P was the only macronutrient in litterfall to vary across the four topographic strata ($P = 0.03$) with stratum 2 (*c.* 40 m a.s.l.) yielding P concentrations 13% higher than the experimental mean (Table 1, Appendix S1). S varied topographically in a similar fashion as P ($P = 0.013$). These results should be interpreted conservatively, given the numerous tests conducted. Micronutrient additions increased only leaf litter N, by 9% N ($P = 0.048$, Table 1, Appendix S2).

Decomposition on NPK plots

After 8 weeks, an average of 87% of the cellulose was missing from litterbags. The cellulose decomposition rate varied sevenfold ($k = 4.3$–30.1) across the +N, +P and +K plots. The full-model ANOVA yielded one significant pairwise interaction (N × P, $P = 0.04$) and no block effects ($P > 0.9$). In the simplified model (Table 2, see Appendix S5), cellulose decomposed 49% faster on +P plots (Fig. 1, Table 2, $P = 0.01$) and 30% faster on +K plots ($P = 0.04$) and failed to vary significantly with nitrogen fertilization. The N–P interaction ($P = 0.02$) revealed that decomposition

Table 1 Fertilization effects on chemistry and quantity of litterfall in a lowland Panamanian forest

	Annual Mean	+N	+P	+K	Stratum 1	Stratum 2	Stratum 3	Stratum 4	+Micron.
	CV	*Pr > F*							
%C	46.0	0.46	−0.37	0.44	1.0	−0.5	−0.4	0.0	0.29
	2	*0.363*	*0.459*	*0.388*	*0.157*				*0.752*
%N	1.47	**6.86**	−1.88	2.07	−2.16	4.16	−1.68	−0.33	**8.57**
	10	***0.008***	*0.417*	*0.381*	*0.221*				***0.048***
%P	0.06	−2.86	**27.04**	4.89	**−6.41**	**12.74**	**−2.61**	**−3.73**	16
	28	*0.538*	***0.000***	*0.314*	***0.031***				*0.108*
%K	0.27	**15.52**	−1.83	**15.55**	−4.51	1.66	−0.75	3.60	24.42
	27	***0.015***	*0.741*	***0.015***	*0.750*				*0.086*
%Ca	1.37	−7.40	14.25	4.76	−13.27	−0.51	11.93	1.85	5.55
	23	*0.255*	*0.055*	*0.487*	*0.085*				*0.550*
%Mg	0.26	8.83	9.67	0.50	−9.61	6.21	4.44	−1.03	12.28
	20	*0.135*	*0.104*	*0.929*	*0.195*				*0.213*
%S	0.16	−0.33	**4.72**	−0.89	**−0.18**	**6.40**	**−1.87**	**−4.36**	6.98
	11	*0.881*	***0.046***	*0.687*	***0.013***				*0.160*
Fe ppm	81.0	3.30	5.10	−4.30	13.9	−9.0	−2.2	−2.7	−4.24
	60	*0.650*	*0.488*	*0.540*	*0.154*				*0.832*
Cu ppm	11.5	−8.51	−4.45	−1.36	−8.5	9.3	−4.7	4.0	544.42
	82	*0.481*	*0.717*	*0.913*	*0.738*				*0.151*
Zn ppm	39.7	0.40	−6.25	−13.4	6.7	12.9	−15.7	−4.0	−9.43
	32	*0.960*	*0.420*	*0.080*	*0.079*				*0.650*
%Fibre	53.9	1.82	0.42	0.09	0.7	0.4	−0.8	−0.2	2.72
	5	*0.082*	*0.681*	*0.932*	*0.710*				*0.166*
Leaf fall g m^{-2} year^{-1}	689	5.9	6.3	−2.0	**−3.1**	**−22.5**	**20.8**	**4.7**	11.5
	23	*0.164*	*0.148*	*0.344*	***0.0002***				*0.227*
Repr fall g m^{-2} year^{-1}	108	**42.9**	32.3	−9.3	−2.5	−13.7	2.9	11.7	37.0
	73	***0.048***	*0.093*	*0.314*	*0.841*				*0.168*
Twigs g m^{-2} year^{-1}	268	13.9	8.98	−3.8	**−22.6**	**−34.9**	**20.6**	**37.6**	30.1
	55	*0.187*	*0.281*	*0.405*	***0.004***				*0.190*

The annual mean is reported with its Co-efficient of Variation (across plots and years) in italics below. N, P, and K and elevational strata effects are reported as % – change in the mean (*P*-values from repeated measures ANOVA in italics below). For example, N fertilization did not change leaf litter %C but did enhance %N by 6.86%; C content of the leaf litter did not vary with strata but P content did, with only stratum 2 deviating positively from the mean. Results from +Micronutrient plots are at far right – only leaf % – N changed significantly on +M plots. *P*-values <0.05 in bold. See Appendices S1 and S2 for full ANOVA tables

rates on +P plots (*k* = 26) approached +N rates (*k* = 15.8) on +NP plots (*k* = 16.7), suggesting that nitrogen inhibited the phosphorus effect.

After 8 weeks, an average of 33% of the leaf-litter mass was missing from litterbags. The leaf-litter decomposition rate varied 21-fold (*k* = 0.32–6.6) across the NPK plots. Like cellulose, decomposition was uniform across topographic strata (*P* = 0.98) but, unlike cellulose, no pairwise interactions of N, P, or K arose in the initial ANOVA (NP: *P* = 0.58, NK: *P* = 0.88, PK: *P* = 0.75). In the simplified model, leaf litter on +P plots decomposed 30% faster (*P* = 0.04, Fig. 2). In contrast, leaf litter from +K and +N plots did not decompose faster (although both trended toward a 20% increase in decomposition rates, *P* = 0.12).

We used a common garden to test if this enhanced decomposition was because of plot effects vs. enhanced litter quality. An average of 52% of leaf-litter mass was lost after 15 weeks and decomposition rates varied fourfold (*k* = 1.4–6.1) within the smaller spatial extent of the common garden. As before, the ANOVA was simplified after finding no significant pairwise interactions (*P* > 0.16) or block effects (*P* > 0.05). In this ANOVA (Table 2), leaf litter from plots fertilized with N, P and K did not decompose faster relative to controls (*P* = 0.31, 0.17, 0.12, respectively, Fig. 1).

Decomposition on micronutrient plots

We used a Kruskal–Wallis test to compare mean decomposition rates on +M plots (*n* = 4) with control plots (*n* = 4). Cellulose did not decompose faster on plots with added B, Ca, Cu, Fe, Mg, Mn, Mo, S and Zn (Kruskal–Wallis χ^2 = 0.33, *P* = 0.28, Fig. 2, see Appendix S5). In

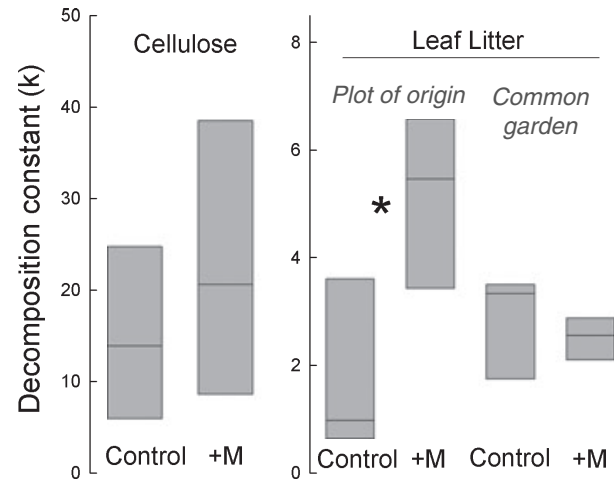**Table 2** Effects of N, P, K fertilization on decomposition rate of cellulose and litter

Source	d.f.	SS	F	Pr > F
Cellulose				
On fertilized plots				
N	1	101.6	1.93	0.17
P	1	402.3	7.64	0.01
K	1	174.4	3031	0.04
N*P	1	300.5	5.71	0.02
Error	27	2400.1		
Leaf litter harvested from traps on fertilized plots				
On fertilized plots				
N	1	1.61	1.61	0.12
P	1	3.49	3.49	0.04
K	1	1.60	1.61	0.12
Error	28	30.64		
In common garden				
N	1	0.18	0.26	0.31
P	1	0.65	0.95	0.17
K	1	1.00	1.46	0.12
Error	28	20.89		

Leaf litter was collected from traps, dried, and replaced on plot of origin. This leaf litter was also transferred to a common garden. An initial ANOVA (Type III sum of squares) included interactions, which were retained in the model only if they accounted for significant variation.
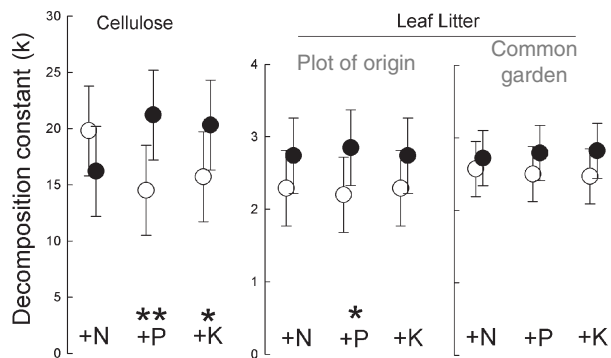
Figure 1 Decomposition rates (least square means ± 2 SE) from a factorial-NPK fertilization experiment in a tropical rain forest. Two substrates – cellulose and leaf litter that was harvested, dried, and placed on plots of origin or in a common garden – were decomposed in litterbags. Decomposition rates, k, were calculated as the fraction of remaining litter mass = e^{-kt}, where t is a proportion of 1 year. Dark circles represent plots receiving N, P or K, white circles the controls failing to receive N, P, and K respectively. *Significance at $P < 0.05$.

Figure 2 Decomposition rates (bar and whisker plots) of cellulose and leaf litter from plots receiving a micronutrient fertilizer (B, Ca, Cu, Fe, Mg, Mn, Mo, S, Zn) vs. control plots in a tropical rain forest. Two substrates – cellulose and leaf litter that was harvested, dried, and placed on plots of origin or in a common garden – were decomposed in litterbags. Decomposition rates, k, were calculated as the fraction of remaining litter mass = e^{-kt}, where t is a proportion of 1 year. *Significance at $P < 0.05$ via a Kruskal–Wallis test. Only leaf litter showed significant increases in decomposition, and only on plots of origin.

contrast leaf litter decomposed 81% faster on +M plots relative to controls (Kruskal–Wallis $\chi^2 = 4.08$, $P = 0.021$ $k = 3.2$ vs. 1.8, Fig. 2). In the common-garden experiment, this effect disappeared and the trend was in the opposite direction (Kruskal–Wallis $\chi^2 = 0.75$, two-way $P = 0.39$, Fig. 2).

DISCUSSION

The warm, humid climate of lowland tropical moist to wet forests facilitates tree and microbial growth (Meetemeyer 1978). This in turn fosters the immobilization of essential mineral resources that are themselves patchy at local scales (John *et al.* 2007). Here a large-scale, long-term factorial fertilization experiment suggests that a variety of nutrients influence population and ecosystem rates from tree reproduction (N) to decomposition of cellulose (P, K) to decomposition of leaf litter (P, at least one micronutrient). A particular surprise was the potential role of micronutrients in shaping decomposition: leaf litter decomposed 81% faster with micronutrient augmentation vs. 33% faster with P augmentation. We conclude that it may be necessary to cast a wider net than suggested by Liebig's Law of the Minimum, which postulates the existence of a primary limiting nutrient underlying population and ecosystem processes (Chapin *et al.* 2002).

Tree responses

Our two results on tree responses at Gigante have implications for both ecosystem and population definitions

of limitation. For the 90% of litter production reflecting growth and maintenance, NPK enhanced leaf-litter nutrient concentration, not production. Natural nutrient gradients do not always translate to changing litterfall rates in tropical forests (Baillie *et al.* 2006), but the latter result contrasts to numerous studies showing enhanced litterfall when nutrients are added experimentally: NPK fertilization enhanced litterfall by 55% in a young eucalypt forest (Giardina *et al.* 2003); NP fertilization enhanced litterfall by 41% in a sandy, nutrient poor dipterocarp forest (Mirmanto *et al.* 1999) and frequently did so in a series of studies in Hawai'i (see review in Vitousek 2004). The failure of this component of NPP to increase with fertilization suggests at least two working hypotheses. First, old forests like Gigante have achieved a higher Leaf Area Index (Hirose *et al.* 1997) relative to the young (Giardina *et al.* 2003), nutrient poor (Mirmanto *et al.* 1999) or monodominant (Vitousek 2004) forests cited above. This remains to be tested. A second possibility is that any extra carbon fixed on NPK plots, especially in leaves, was consumed by herbivores. The functional significance of the increased nutrient concentrations on fertilized plots – as defences, more photosynthetic machinery, or as storage – deserves further study.

Because we separated litter into its functional categories, we were able to detect an increase in a parameter of interest to population biologists – investment in reproduction – which increased 43% on +N plots. Accounting for only 10% of litterfall, the signal provided by flowers and fruits is dwarfed by leaf and twig fall (and likely underestimated, given that much of this carbon is consumed by nectarivores and frugivores). Intriguingly, an NPK + micronutrients fertilizer applied to a Puerto Rican wet tropical forest (3500 mm annual precipitation) increased both leaf fall and 'miscellaneous' (non-leaf, non-branch) litter production (Li *et al.* 2006), suggesting another reproductive response. Reproductive structures are built from 2% to 10%N, representing the largest investment in nitrogen on average (leaves are second, Bazzaz *et al.* 1987); investment in reproduction in one tropical tree comes at the cost of diminished photosynthetic capacity (Wheelright & Logan 2004). Ours is the first study to our knowledge to suggest that even in comparatively N-rich tropical soils, nitrogen can limit a small, but evolutionarily key fraction of litter fall.

In sum, the nutrients that limit ecosystem properties like carbon fixation may not always coincide with those limiting population parameters like reproduction. Early in life, fitness and NPP likely covary as competition for light, and the avoidance of overtopping, determine a sapling's future. Mature trees, in contrast, at maximum height and LAI, may find their fitness better correlated to the ability to invest in relatively carbon poor but nutrient rich offspring. At the same time, the balance of nutrients limiting growth versus reproduction may further shape the secondary production

and fitness of the herbivores, frugivores, and pollinators of tropical forests (Leigh *et al.* 1996). If so, mapping plant recruitment and consumer abundance onto N-gradients in tropical forests should be fruitful.

Breaking down common, energy-rich cellulose

Cellulose is the main constituent by weight of leaf tissue, and the main store of energy for the brown food web. We found carbon-rich cellulose decayed 50% faster on +P plots ($P = 0.01$, see also Cleveland *et al.* 2006) and 30% faster on +K plots. Given its relative lability (contrast the average k of cellulose, 18, with that of leaf litter, 2.5) a new source of cellulose in the litter likely engenders a race among competing fungi in the aerobic litter environment (Wood & Garcia-Campayo 1990). This puts a premium on the availability of P as a rate-limiting nutrient in the synthesis of cellulolytic enzymes (Sterner & Elser 2002) and on K, key to the cytoplasmic streaming needed for fungi to colonize and deplete local resources. This demand, combined with the paucity of both P and K in the weathered soils of Gigante (Walker & Syers 1976; Yavitt & Wieder 1988), creates a powerful and independent (P–K interaction $P > 0.05$) need for these two elements by the microbial community. This is the first evidence of K-limitation of microbial decomposition we know of; however, it is also the first experimental addition of K to test for this effect.

Interactions among macronutrients in this study were scarce (a strength of the factorial design). The only such case was when N suppressed the +P increase in cellulose decomposition (Table 2). While N can suppress lignin breakdown (Carreiro *et al.* 2000), this is the first evidence for such an effect for cellulose. The potentially complex interactions among minerals in the decomposition process bears further study.

Finally, a second factor underlying P limitation of decomposition occurs at the next trophic level. Microbivores in the tropics can enhance decomposition, through some combination of shredding substrate and grazing senescent hyphae (Milton & Kaspari 2007). Increases of microbivore biomass on +P plots (Kaspari in preparation), suggest that P fertilization triggers a positive feedback between microbes and microbivores, accelerating the already rapid tropical decomposition.

Breaking down complex leaf litter

One key similarity between cellulose and leaf litter was that both decomposed more with P fertilizer, albeit less so for leaf litter (30% vs. 49% in cellulose). In contrast, both K and N failed to enhance leaf-litter decomposition in this lowland tropical forest ($P = 0.12$ in both cases, Fig. 2, Table 2). Similar NP-based fertilization in Hawai'i montane forests

enhanced leaf-litter decay (Hobbie & Vitousek 2000; Vitousek & Hobbie 2000).

Micronutrients had an even bigger effect than P on leaf litter decomposition (although none on cellulose), increasing decomposition rates by 82%. Experiments are currently underway to test which elements – B, Ca, Cu, Fe, Mg, Mn, Mo, S, and/or Zn – combine to create this effect.

The lack of increased decomposition in our common garden – despite 7%, 27%, and up to 30% increases in leaf litter N, P and K suggests decomposition at Gigante is enhanced primarily through direct fertilization of the microbial community (Li *et al.* 1998; Hobbie & Vitousek 2000; Hobbie *et al.* 2006). This inference is further strengthened by the accelerated decay of a chemically homogenous cellulose standard on +P and +K plots. The failure of changes in average leaf quality to enhance decomposition is intriguing, especially when numerous studies suggest the importance of leaf traits on decomposition (Cadisch & Giller 1997). One big difference, in this case, is that our enhanced leaf litter was a mixed, random sample from a 40 × 40-m plot's trees, palms, lianas, and epiphytes. Such a heterogeneous collection of leaf litter, representing numerous, species specific responses to fertilization (e.g. storage vs. photosynthesis vs. defence) can alter the effects of higher overall nutrient concentration (Gartner & Cardon 2004). In this light, our differences with a similar study in Hawai'i (Hobbie & Vitousek 2000), where P fertilization of monodominant *Metrosideros polymorpha* litter carried over to enhance decomposition in a common garden, is more easily reconciled. Using species level traits to predict decomposition rates may be increasingly challenging as stand diversity increases (Gartner & Cardon 2004).

CONCLUSION

We propose two working hypotheses. First, while the production of leaf carbon in mature lowland tropical forests is unlikely to change with nutrient addition, the production of more N-rich plant propagules is likely under constant selection. N limitation of reproduction may thus be a more common population phenomena, even in relatively N-rich ecosystems (for a similar comparison of ecosystem and population phenomena, see Tilman 1996). Testing this further will require understanding allocation decisions of trees along multiple nutrient gradients, experimental and natural (Espeleta & Clark 2007).

Second, P and K limit the mass-production of cellulases needed to break down earth's most abundant macromolecule (Scheu & Schaefer 1998; Cleveland *et al.* 2002). However, *access* to cellulose, bound up in lignins, pectins and waxes, comes only when these compounds are stripped away (Sinsabaugh *et al.* 2002; Allison & Vitousek 2005). In sum, these results push leaf-litter decomposition away from

a Liebig worldview ('one most-limiting factor') toward one that accounts for the many enzymatic functions, their differing composition, and the complex intra- and extra-cellular environment in which they act. In the short term, experiments must identify *which* elements contributed to the above results. In the longer term, understanding *why* these elements shape decomposition will require study of the identity, biochemistry and proteomics of decomposers in the soil (Sinsabaugh *et al.* 2002; Schulze 2004).

Not surprisingly, these results have implications for global ecology, as nutrient deposition and availability varies at the global scale (Chadwick *et al.* 1999; Hedin *et al.* 2003; McGroddy *et al.* 2004). Even small increases in global decomposition rates can accelerate global warming (Chapin *et al.* 2002). Furthermore, biogeochemical gradients of availability can also transfer up the green and brown food webs (Weiser 1966; Reich *et al.* 2005). Maps of biogeochemistry, combined with an understanding of how this chemistry generates patterns of growth and reproduction, should be powerful tools in the prediction of global change.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1 Effects of NPK fertilization on leaf chemistry and litterfall in a lowland Panamanian forest.

Appendix S2 Raw data for litter chemistry.

Appendix S3 Monthly variables of litterfall captured in 0.58 m² traps, three traps per plot, on 36 fertilization plots.

Appendix S4 Effects of micronutrient fertilization on leaf chemistry and litterfall.

Appendix S5 Raw data for decomposition in Gigante plot and Common Garden experiments.

Figure S1 The Gigante fertilization experiment, an NPK factorial fertilization in a lowland Panama rainforest.

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