Experimental investigation of the importance of litterfall in lowland semi-evergreen tropical forest nutrient cycling

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Summary

1. The cycling of nutrients in litterfall is considered a key mechanism in the maintenance of tropical forest fertility but its importance has rarely been quantified experimentally.

2. We carried out a long-term (5 years), large-scale litter manipulation experiment in lowland semi-evergreen tropical forest to determine how changes in litterfall affect forest nutrient cycling. We hypothesized that: (i) long-term litter removal would decrease the forest's nutrient supply; (ii) litter addition would increase the forest's nutrient supply; (iii) soil and foliar nutrient concentrations would change in response to litter manipulation and would eventually affect above-ground productivity.

3. To test our hypotheses, we measured trunk growth, litterfall, and nutrient concentrations in live leaves, litter and soil in plots where litter was removed once a month (L−), litter was added once a month (L+) and controls (CT).

4. After 5 years, the concentration of nitrate in the soil and soil stocks of inorganic nitrogen were higher in the L+ plots and lower in the L− plots compared to the controls. Ammonium concentrations in the soil were also lower in the L− plots. Nitrogen in leaves and litter and the annual nitrogen return by litter were higher in the L+ plots, while potassium return was lower in the L− plots. Surprisingly, our treatments had little effect on phosphorus in soil, leaves or litter, even though lowland tropical forests are generally thought to be largely phosphorus limited.

5. Trunk growth of large trees was not affected by litter manipulation but rainy season litterfall from 2003 to 2008 was 13% higher in the L+ plots compared to the controls.

6. *Synthesis.* Litter removal affected forest nutrient cycling and productivity less than expected, probably because the soil at our site is moderately fertile. However, litter addition increased litterfall indicating that some limitation of forest production was removed by litter addition. We expected strong effects of litter manipulation on phosphorus cycling; instead, we found a stronger effect on nitrogen cycling. Our results suggest that litter is an important source of nutrients, in particular nitrogen, to trees in this lowland semi-evergreen tropical forest.

Key-words: foliar nutrients, litter addition, litter manipulation, litter removal, litterfall seasonality, nitrogen, nutrient limitation, phosphorus, potassium, soil nutrients

Introduction

Litterfall is one of the most important and dynamic components of nutrient cycling in forest ecosystems (Attiwill & Adams 1993). In theory, when a forest is in steady state and if there is minimal loss of nutrients by leaching, then the nutrients in above-ground litter and throughfall will equal the amounts taken up to produce new above-ground growth. In temperate forests, the importance of litter in the forest nutrient cycle is well known from studies quantifying the effects of litter removal as a forest management practice, which depletes the soil of nutrients over the long term (see Sayer 2006 for a review). In tropical forests, the maintenance of fertility has long been attributed to the recycling of nutrients through decomposition of litterfall; in tropical forests on highly weathered soils a large proportion of available nutrients is tied up in the living biomass and recycled with the litter (e.g. Herrera *et al.* 1978; Richards 1996). Nutrient concentrations in leaves

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and litter may directly reflect soil fertility (Vitousek & Sanford 1986) and consequently, litterfall and litter nutrient concentrations have often been used as a measure of site fertility (Tanner, Vitousek & Cuevas 1998) or nutrient use efficiency (biomass production per unit of nutrient; Vitousek 1982, 1984). In seasonal tropical forests, the build-up of litter during the dry season represents an important component in the forest's nutrient cycle as nutrients are immobilized (Campo, Jaramillo & Maass 1998) and then released in a large pulse at the start of the rainy season, when the accumulated litter decomposes (Lodge, McDowell & McSwiney 1994).

Thus, there are various lines of evidence showing the importance of above-ground litterfall in the mineral nutrition of tropical forests, and litterfall is one of the most commonly measured processes at the ecosystem level (Vitousek & Sanford 1986). In spite of this, attempts to quantify the importance of leaf litter in the forest nutrient cycle rarely go beyond calculating the amounts of nutrients returned in the litter, and until recently there has been no experimental test of the role of litterfall in tropical forest nutrient cycling (but see Wood & Lawrence 2008; Wood et al. 2009).

Nitrogen and phosphorus are thought to be cycled mainly through litterfall (Vitousek 1982). Theoretically, at steady state, soil reserves should contribute < 10% to nitrogen cycling and < 20% to phosphorus cycling in forests; cycling of nitrogen and phosphorus should therefore be largely related to organic matter cycling (Attiwill & Adams 1993). In many lowland tropical forests, phosphorus availability is low and is thought to be limiting to growth, while nitrogen availability is relatively high (e.g. Vitousek 1984; Vitousek & Sanford 1986; Tanner, Vitousek & Cuevas 1998). Consequently, we would expect phosphorus cycling through litterfall to be more conservative and efficient than nitrogen cycling in lowland tropical forests.

We established a long-term, large-scale litter manipulation project to experimentally investigate the role of litterfall in nutrient cycling in a lowland semi-evergreen tropical forest in Panama, Central America. We hypothesized that:

1 If litterfall is the main pathway for nitrogen (N) and phosphorus (P) cycling, then continuous, long-term litter removal will disrupt the natural nutrient cycle and deplete the forest of these nutrients.
2 Litter addition will function as 'natural fertilizer' and increase the forest's nutrient supply, in particular of N and P.
3 Soil and foliar nutrient concentrations will change in response to litter manipulation and, eventually, above-ground productivity will be affected.

Materials and methods

STUDY SITE

The study site is located on the Gigante Peninsula (9°06′ N, 79°54′ W) in the Barro Colorado Nature Monument in Panama. The species composition and stature of the forest is characteristic of mature lowland semi-evergreen tropical forest. The soil is a moderately acidic oxisol (pH 5.0–5.5; Cavelier 1992). The topsoil (0–10 cm depth) has low concentrations of extractable phosphate (c. 2.2 mg kg^{-1}) and exchangeable potassium (c. 0.24 cmolc kg^{-1}), moderate concentrations of inorganic nitrogen (c. 20.5 mg kg^{-1}) and high concentrations of calcium (c. 1690 mg kg^{-1}) and magnesium (c. 553 mg kg^{-1}; Yavitt et al. 2009). Nearby Barro Colorado Island (c. 5 km from the study site) has a mean annual rainfall of 2600 mm with a strong dry season from January to April and an average temperature of 26 °C (Leigh 1999).

The Gigante Litter Manipulation Project consists of fifteen 45 × 45 m plots. During establishment (from 2000 to 2002) the plots were trenched to a depth of 0.5 m to minimize nutrient import via the root/mycorrhizal network, both sides of the trenches were lined with construction plastic and the trenches backfilled. A 7.5-m wide buffer was left around the inside of the trenches to eliminate trenching effects, resulting in a measurement plot size of 30 × 30 m. Treatments were assigned to plots according to pre-treatment (2002) litterfall in a stratified random design. Starting in January 2003, the litter in five plots was raked up once a month, resulting in low, but not entirely absent, litter standing crop (L− plots). The removed litter was immediately added to five plots, where it was spread as evenly as possible (L+ plots); the litter was transferred to the same, nearest, L+ plot each month; five plots were left undisturbed as controls (CT plots).

LITTERFALL AND LITTER STANDING CROP

Small litterfall (sensu Proctor 1983) was collected once a month in 0.76 × 0.76 m randomly placed traps with the rim c. 0.7 m above the soil surface. Five traps were set up in each plot in January 2002 and a further five traps per plot were added in August 2002. Litter collections started in February 2002. When large leaves (for example palm leaves) were found lying across the traps, the portion of the leaf lying within the frame area was included in the collection and the remainder was discarded. Woody debris with a diameter > 20 mm was discarded and the remaining small litter was oven-dried at 60 °C and weighed. Dry season litterfall was defined as litter collected from January to April, while rainy season litterfall was defined as litter collected from May to December for each year.

The litter moved between plots was measured at the start of treatments in January 2003 by weighing all the litter removed from two of the L− plots before it was added to the L+ plots; seven large subsamples were taken and the fresh-to-dry weight ratio was determined to calculate the total dry weight of the removed litter.

LITTER DECOMPOSITION

We measured litter decomposition in the L+ and CT plots at the end of the experiment in 2008 using mixed litter collected from the litter traps in the CT plots. Three grams of litter were placed in 1.2-mm mesh fibre-glass bags measuring 10 × 10 cm. Seven bags were placed at a random location in each plot in January 2008 and one bag was collected per plot and month until August 2008. The bags were carefully emptied; the litter was dried to constant weight at 60 °C and weighed.

TREE GROWTH

All trees in the plots with a diameter at breast height (d.b.h.) > 10 cm were tagged, mapped and identified in 2000. All trees were measured using a tape measure at breast height (1.3 m), unless buttresses or other irregularities were present, in which case the measurement was made above the irregularity. The point of measurement (POM) of each tree was clearly marked by painting a ring around the trunk. All

trees were remeasured annually from 2001 to 2005 and again in 2007 during the mid-rainy season (July and August) using a tape measure at the same POM. Trees with low measurement accuracy due to irregularities and palms with multiple stems were excluded from analyses. For all other trees within the 30×30 m measurement plots (300 individuals) growth was calculated as percentage initial girth for one pre-treatment period (2000–2003) and one post-treatment period (2004–2007).

NUTRIENTS AND PH IN THE MINERAL SOIL

Soil samples were collected from 2004 to 2007 at 0–2 and 0–10 cm depth at the end of the rainy season (November or December) of each year. In 2004, soil was collected at each of the four corners of the inner 20×20-m area of each plot. From 2005 to 2007, soil was collected at each of the previous four sampling sites and at an additional four randomly chosen sampling sites in the inner 20×20-m area of each plot, making a total of eight soil cores per plot. Samples were bulked to give one sample per depth and plot and mixed thoroughly. Subsamples were taken to determine gravimetric water content and all extracts for mineral nutrients were prepared from fresh soil within 48 h of returning from the field. Nitrate-N and ammonium-N were extracted in a 2 M KCl solution, but problems with contamination with ammonium obliged us to discard the results for ammonium-N at both depths in 2004 and for 0–10 cm in 2005. All other elements (P, K, Ca, Mg, Zn and B) were extracted from fresh soil in Mehlich III solution using a 1:10 ratio of soil to extraction solution. Soil pH was measured on a 1:3 soil solution in distilled water using a Corning 430 pH metre (Nova Analytics, Woburn, MA, USA). KCl-extractable ammonium and nitrate for 2004 to 2006 were analysed at the School of Biological Sciences, University of Aberdeen, UK, by flow injection at 590 and 540 nm, respectively. Mehlich III-extractable phosphorus and the cations for 2004 to 2005 were analysed at Cornell Nutrient Analysis Laboratories, Cornell University, USA, by ICP-OES (inductively coupled plasma optical emission spectrometry). All analyses for 2007 were performed at the Smithsonian Tropical Research Institute in Panama, where KCl-extractable ammonium and nitrate were analysed by automated colorimetry and Mehlich III-extractable phosphorus and cations were analysed by ICP-OES.

Soil nutrient stocks for the topsoil (0–10 cm) were estimated using the mean nutrient concentrations measured from 2005 to 2007 and the mean soil bulk density per plot measured in 2005 (E.J. Sayer & A.W. Cheesman, unpubl. data).

NUTRIENTS IN LEAVES AND LITTER

Live leaf samples were collected in November each year from 2004 to 2007 from individuals of five common tree species that occurred in all plots: *Alseis blackiana* Hemsl. (Rubiaceae) and *Tetragastris panamensis* Engl. (Burseraceae) are shade-tolerant canopy species; *Heisteria concinna* Standl. (Olacaceae) is a shade-tolerant understorey species; *Simarouba amara* Aubl. (Simaroubaceae) is a fast-growing canopy species that is associated with gaps in old growth forest; and *Virola sebifera* Aubl. (Myristicaceae) is a shade-tolerant midstorey tree (Uriarte *et al.* 2004). Individuals of these five common tree species made up *c.* 35% of all trees with d.b.h. > 10 cm in the plots and cover a fairly wide range in life-history characteristics; we therefore use the average nutrient concentrations of the five species pooled as a proxy for average forest foliar nutrients.

Leaves were collected from one individual per species and plot using a shotgun; wherever possible, sun leaves were shot down from the canopy. All leaf samples were oven-dried at 60 °C and finely

ground. For samples collected from 2004 to 2006, subsamples (20 mm³) of the five plots per treatment were combined to give one composite sample per species and treatment. In 2007, one sample per species and plot was analysed.

Dried litter samples, collected from the traps in September of each year from 2004 to 2007, were analysed for nutrient concentrations. The samples were pooled by plot and year and shredded. The shredded litter from each plot was mixed well and a subsample (excluding the woody fraction) was taken and finely ground for nutrient analysis. In 2004, subsamples of ground litter (20 mm³) from the five plots per treatment were combined to give one composite sample per treatment. For 2005–2007, one sample per plot and year was analysed.

Although litter nutrient concentrations can vary widely over the year, concentrations in the mid-rainy season are neither particularly high nor particularly low (Yavitt, Wright & Wieder 2004) and were therefore chosen as representative of the annual mean. Annual nutrient return by litterfall for 2005–2007 was thus estimated by multiplying annual total small litter mass per plot by the nutrient concentrations of the litter from the September collections.

Live leaf and litter samples were analysed by Waite Analytical Services, Adelaide, Australia. Phosphorus and cations were determined after acid digestion by Radial View ICP-OES and total nitrogen was determined by Complete Combustion Gas Chromatography.

DATA ANALYSIS

Treatment differences in small litterfall, decomposition rates, soil nutrients, litter nutrient concentrations and nutrient return in litterfall were investigated by repeated measures general linear models. The data from the decomposition experiment were split into dry season decomposition (January–April) and rainy season decomposition (May–August) for analysis. Foliar nutrient differences among treatments (live leaves collected in 2007) were investigated by one-way ANOVAs. The analyses were carried out in SPSS 16 for Mac (SPSS Inc., Chicago, IL, USA). When the main treatment effect was significant, each treatment was compared to the controls using two-sided Dunnett's *post hoc* tests. As the replication was low ($N = 5$ per treatment), we also report marginally significant treatment differences at $P < 0.1$.

Treatment differences in tree growth were analysed by comparing the percent differences between pre- and post-treatment relative growth in R http://www.r-project.org using a general linear model with tree species, treatment and their interaction as predictors; relative growth of −5% or lower was assumed to be a measurement error and was excluded from the analysis.

Results

LITTERFALL AND LITTER STANDING CROP

Litterfall showed a strong seasonal pattern in all plots, which corresponded to rainfall seasonality; the highest litterfall rates were 4–6 g m⁻² day⁻¹ during the dry season from January to March, and the lowest litterfall rates (< 2 g m⁻² day⁻¹) were at the beginning and towards the end of the rainy season from May to July and October to November, respectively (Fig. 1). Over all years, dry season litterfall contributed 53 ± 3% to total annual litterfall. The dry wt of the litter removed from the L− plots and added to the L+ plots at the start of the treatments in January 2003 was 950 ± 88 g m⁻².
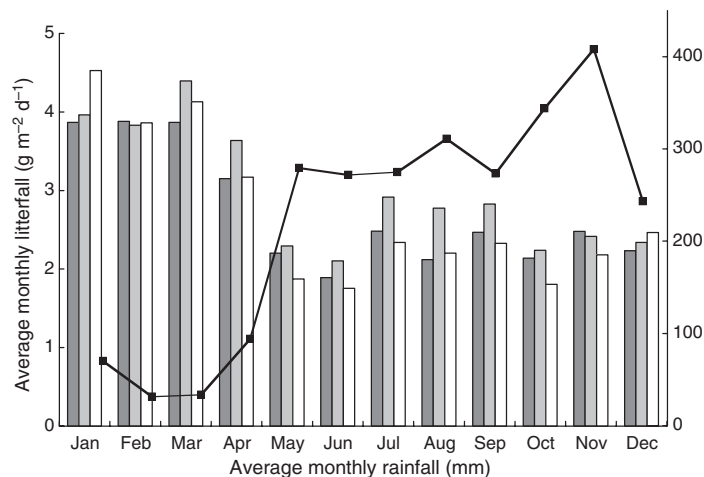
Fig. 1. Average monthly litterfall in litter manipulation plots in lowland semi-evergreen tropical forest in Panama, Central America, and long-term monthly averages for rainfall on Barro Colorado Island (BCI), 5 km from the study site; litterfall values are treatment means from 2003 to 2008, dark grey bars are controls, light grey bars are the litter addition treatment and white bars are the litter removal treatment; rainfall data are monthly means of precipitation from 1929 to 2005, courtesy of the Environmental Sciences Program, Smithsonian Tropical Research Institute, Republic of Panama.

Thereafter, the amount of litter removed from or added to the treatment plots was assumed to equal the amount collected from the litter traps, which was *c.* 991 ± 28 g m^{-2} year^{-1} averaged over all treatments and years.

Litterfall varied strongly among study years but total annual litterfall and dry season litterfall did not differ significantly between treatments (Fig. 2a,c). However, rainy season litterfall was 19% higher in the L+ plots compared to the controls in the first year of treatments (2003; main treatment effect: $P = 0.032$, $F_{2,12} = 4.67$; Dunnett's $P = 0.029$; Fig. 2b); and from 2003 to 2008 rainy season litterfall was 13% higher in the L+ plots compared to the controls (main treatment effect $P = 0.011$, $F_{2,12} = 6.70$; Dunnett's $P = 0.048$; Fig. 2b). There was no significant effect of litter removal on litterfall.

DECOMPOSITION

Mass loss from the litter bags during the dry season (January–April 2008) was significantly greater in the L+ plots compared to the controls ($P = 0.001$, $F_{1,8} = 23.1$), in particular during the first month of decomposition, where mass loss from the litter bags was $8.3 \pm 1.1\%$ in the L+ plots compared to $2.6 \pm 1.9\%$ in the CT plots (Fig. 3). There were no differences in mass loss between CT and L+ plots during the subsequent rainy season (May–August 2008; Fig. 3).

TREE GROWTH

There were no differences in trunk growth among any of the treatments neither pre-treatment (2000–2003) nor post-treatment (2004–2007). Mean pre-treatment d.b.h. growth per individual was 2.4 ± 0.3 mm year^{-1} in the CT plots, 2.8 ± 0.5 mm year^{-1} in the L+ plots and 2.7 ± 0.8 mm year^{-1} in the L− plots. Mean post-treatment growth was 3.4 ± 0.6 in the CT plots, 3.4 ± 0.4 in the L+ plots and 3.9 ± 0.7 mm year^{-1} in the L− plots. However, there was a
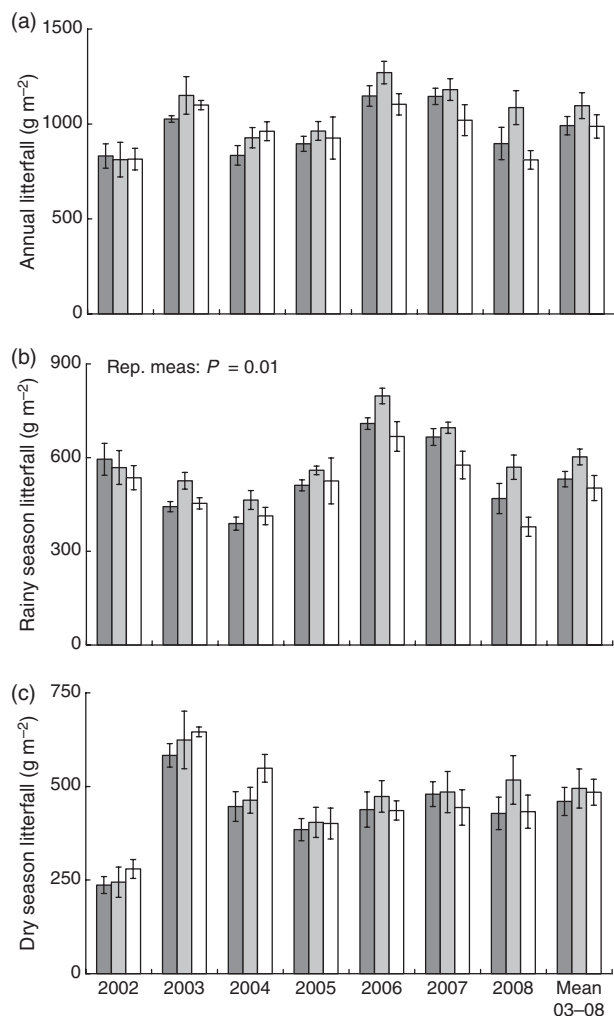
significant treatment × species interaction ($P < 0.001$, $F_{52,135} = 2.24$).

SOIL NUTRIENTS AND PH

The concentrations of nitrate-N in the soil from 2004 to 2007 were greater in the L+ plots and lower in the L− plots relative to the controls at 0–2 cm ($P < 0.001$, $F_{2,12} = 38.2$; Dunnett's $P = 0.01$ and $P = 0.04$, respectively) and at 0–10 cm depth ($P < 0.001$, $F_{2,12} = 35.2$; Dunnett's $P = 0.001$ and $P = 0.01$, respectively; Fig. 4). Treatment differences increased over time at both depths (time × treatment interaction 0–2 cm: $P = 0.013$, $F_{6,36} = 38.2$; 0–10 cm: $P < 0.001$, $F_{6,36} = 6.71$). The concentrations of ammonium-N in the soil (2005–2007) were lower in the L− plots than in the controls at 0–2 cm depth ($P = 0.008$, $F_{2,12} = 7.47$; Dunnett's $P = 0.01$) and the main treatment effect was marginally significant at 0–10 cm depth ($P = 0.079$, $F_{2,12} = 3.15$; Fig. 4).

Soil P concentrations from 2004 to 2007 were affected by litter manipulation at 0–2 cm depth ($P = 0.023$, $F_{2,12} = 5.45$) and the treatment differences increased over time (time × treatment interaction: $P = 0.016$, $F_{4,22} = 5.35$). Soil P was lower in the L− plots compared to the controls in 2004 and higher in the L+ plots in 2007, but *post hoc* comparisons of the repeated measures showed that, overall, neither L+ nor L− differed significantly from the controls. There were no treatment effects on P concentrations at 0–10 cm depth (Fig. 4).

Soil K and Ca concentrations from 2004 to 2007 differed among treatments at 0–2 cm ($P = 0.021$, $F_{2,12} = 5.4$ and $P < 0.001$, $F_{2,12} = 23.1$, respectively). Soil K at 0–2 cm depth was higher in the L+ plots compared to the controls (Dunnett's $P = 0.011$), while Ca concentrations were higher in the L+ plots and lower in the L− plots compared to the controls (Dunnett's $P = 0.007$ and $P = 0.035$, respectively). Calcium concentrations also differed among treatments at 0–10 cm depth
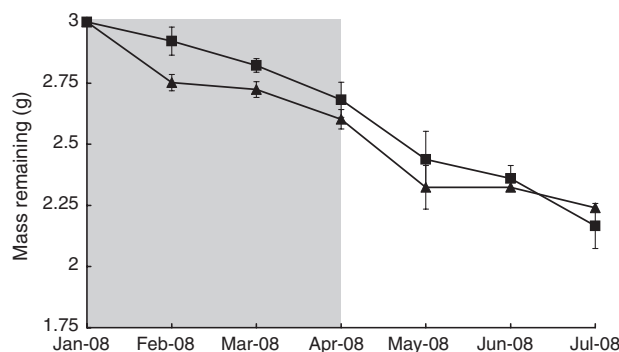
Fig. 3. Mass loss of mixed litter in litterbags during decomposition from January to August 2008 in litter manipulation plots in lowland semi-evergreen tropical forest in Panama, Central America; squares are control, triangles are litter addition; error bars are standard errors of means for $N = 5$; grey shading indicates the dry season.

Fig. 2. (a) Total annual litterfall, (b) rainy season litterfall and (c) dry season litterfall from 2002 to 2008 in litter manipulation plots in lowland semi-evergreen tropical forest in Panama, Central America; dark grey bars are controls, light grey bars are the litter addition treatment and white bars are the litter removal treatment; error bars are standard errors of means for $N = 5$.

($P = 0.041$, $F_{2,12} = 4.24$), but although there were differences between treatments and controls in individual years, *post hoc* comparisons of the repeated measures showed that neither the L+ nor L− plots differed significantly from the controls (Fig. 4). For both K and Ca, the differences among treatments increased over time at both depths (time × treatment interaction for K at 0–2 cm: $P = 0.003$, $F_{4,23} = 6.79$ and at 0–10 cm: $P = 0.014$, $F_{4,23} = 3.95$; time × treatment interaction for Ca at 0–2 cm: $P < 0.001$, $F_{4,23} = 11.76$ and at 0–10 cm: $P = 0.008$, $F_{4,23} = 4.82$).

Soil Mg concentrations at 0–2 cm depth were lower in the L− plots compared to the CT plots from 2004 to 2007 ($P = 0.004$, $F_{2,12} = 8.94$; Dunnett's $P = 0.025$), but there were no effects of litter manipulation on soil Mg concentrations at 0–10 cm depth (Fig. 4).

The stocks of inorganic N in the topsoil (0–10 cm) after 5 years of treatments were 46% higher in the L+ plots than in the CT plots (main treatment effect: $P < 0.001$, $F_{2,12} = 36.2$;

Dunnett's $P = 0.001$) and 40% lower in the L− plots relative to the controls (Dunnett's $P = 0.004$; data not shown). There were no differences among treatments for any other nutrient stocks but there were significant time × treatment interactions for soil K and Ca stocks ($P = 0.03$; $F_{4,22} = 3.26$ and $P = 0.023$, $F_{4,22} = 3.53$, respectively).

Litter manipulation affected soil pH at 0–2 cm depth (main treatment effect: $P = 0.014$, $F_{2,12} = 9.53$) but not at 0–10 cm. After 5 years of treatments, soil $pH_{0–2}$ was 5.5 ± 0.2 in the L+ plots, which was marginally higher than in the controls (4.8 ± 0.2; Dunnett's $P = 0.051$), but there was no effect of litter removal ($pH_{0–2} = 4.8 \pm 0.1$).

NUTRIENTS IN LEAVES AND LITTER

By 2007, after 5 years of litter manipulation, foliar N concentrations were marginally higher in the L+ plots and marginally lower in the L− plots compared to the controls (main treatment effect: $P = 0.002$, $F_{2,12} = 10.3$; Dunnett's $P = 0.074$ for both comparisons; Fig. 5). Foliar P concentrations were affected by litter manipulation (main treatment effect: $P = 0.045$, $F_{2,12} = 4.07$), but *post hoc* tests showed no differences between either treatment compared to the controls. There were no significant effects of litter manipulation on any other foliar nutrients (Fig. 5).

The concentrations of N in litter from 2005 to 2007 were 16% higher in the L+ plots compared to the controls ($P < 0.001$, $F_{2,12} = 15.6$; Dunnett's $P = 0.005$; Fig. 6a), but there was no significant effect of litter removal on litter N concentrations. The concentrations of P and K in the litter differed marginally among treatments ($P = 0.05$, $F_{2,12} = 3.90$ and $P = 0.085$, $F_{2,12} = 3.06$, respectively; Fig. 6b,c), and there was a marginally significant time × treatment interaction for K concentrations ($P = 0.093$, $F_{4,24} = 2.25$). There were no effects of litter manipulation on Ca or Mg concentrations in litter (Fig. 6d,e).

The estimated annual return of N by litterfall was greater in the L+ plots compared to the controls (main treatment effect: $P = 0.003$, $F_{2,12} = 7.84$; Dunnett's $P = 0.038$; data not
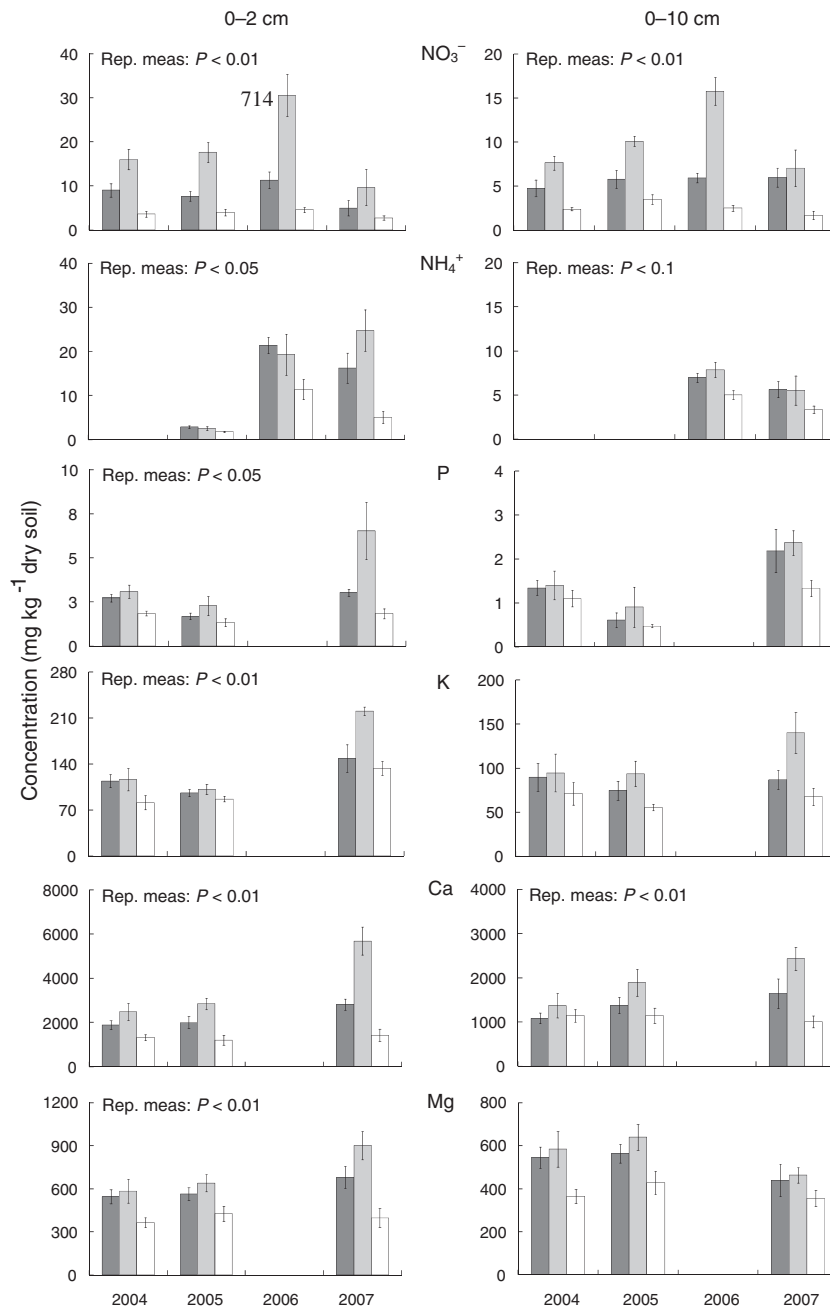
Fig. 4. Concentrations of soil nutrients at 0–2 and 0–10 cm depth in litter manipulation plots in lowland semi-evergreen tropical forest in Panama, Central America; dark grey bars are controls, light grey bars are the litter addition treatment and white bars are the litter removal treatment; error bars are standard errors of means for $N = 5$; concentrations of NO_3^- and NH_4^+ are given as NO_3^--N and NH_4^+-N.

shown). The estimated annual K return by litterfall in the L− plots was marginally lower than in the controls (main treatment effect: $P = 0.032$, $F_{2,12} = 4.67$; Dunnett's $P = 0.067$; data not shown), and the differences between treatments increased over time (time × treatment interaction: $P = 0.03$, $F_{4,24} = 3.23$). There were no significant effects of litter manipulation on the estimated annual return of P, Ca or Mg by litter.

Discussion

Our study confirms the importance of litterfall in tropical forest nutrient cycling. The soils at our study site can be classed as

'moderately fertile' compared to other lowland tropical forests (Vitousek & Sanford 1986; Table 1). Despite low extractable P in the soil (Cavelier 1992; Yavitt *et al.* 2009), nutrient use efficiency for both P and N are relatively low and yet litter manipulation affected nutrient cycling and forest litterfall productivity within only a few years of the start of treatments.

Contrary to expectations, litter addition had a greater effect on forest nutrient cycling than did litter removal. Furthermore, our litter manipulation treatments mainly affected the forest N cycle, with lesser effects for P and Ca, although litter is thought to be a major source for all three nutrients in lowland tropical forest (Vitousek 1982). Surprisingly, litter removal did not
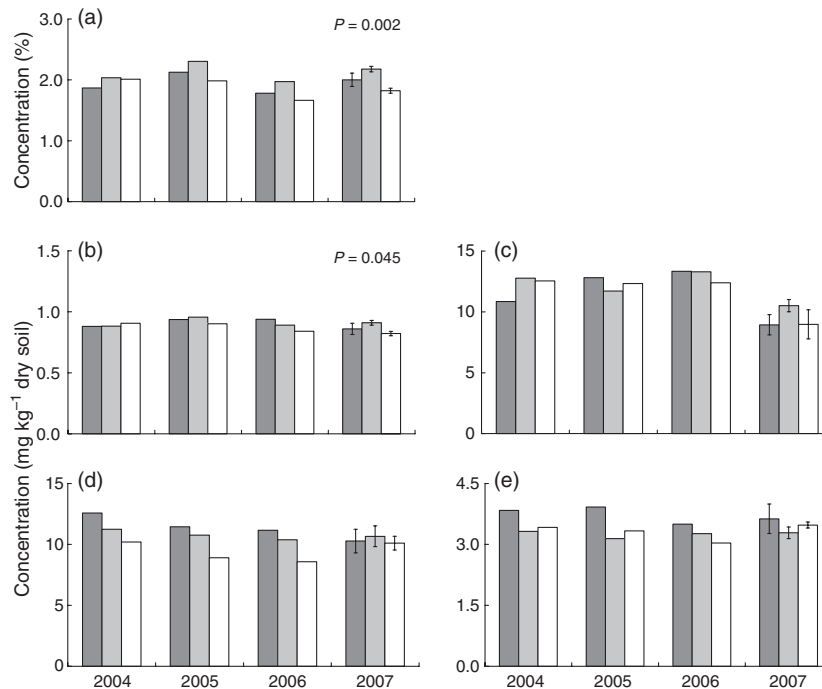
Fig. 5. Foliar nutrient concentrations from 2004 to 2007 collected from the five most common species in litter manipulation plots in lowland semi-evergreen tropical forest in Panama, Central America; (a) nitrogen, (b) phosphorus, (c) potassium, (d) calcium, and (e) magnesium; dark grey bars are controls, light grey bars are the litter addition treatment and white bars are the litter removal treatment; values for 2004–2006 are for one composite sample per treatment, error bars for 2007 are standard errors of means for $N = 5$.

have a great effect on P cycling, even though productivity in lowland tropical forests is generally thought to be P-limited (Vitousek 1984; Vitousek & Sanford 1986) and the amount of P we removed each year with the litter (*c*. 5 kg ha^{-1}) was likely to have been a large proportion of the P required for growth. Exchangeable soil Ca in our study forest was very high (Yavitt & Wieder 1988; Cavelier 1992; Yavitt *et al.* 2009) and consequently we did not expect it to be affected by litter manipulation.

LITTERFALL AND DECOMPOSITION

Despite high interannual and seasonal variability in litterfall, we measured an increase in litterfall during the rainy season in response to our litter addition treatments (Fig. 2b). The first litter addition treatment in January 2003 included the entire litter standing crop from the L− plots, which was almost equivalent in mass to a full year's litterfall, and we measured a large (19%) increase in rainy season litterfall in the L+ plots within 7–8 months of the start of treatments (Fig. 2b). In a litter manipulation experiment in a tropical lowland forest in Costa Rica, a single fourfold litter addition treatment produced a similar transient increase in litterfall. The increase coincided with the release of P from the litter and was strongly correlated with the amount of P added with the litter (Wood *et al.* 2009). In our study, repeated measures analyses for each month over the study period showed that the increase in rainy season litterfall was driven mainly by higher rates of litterfall in July, August and Octo-

ber, which generally coincides with the release of N and P after initial immobilization during the dry season (Sayer, Tanner & Lacey 2006).

We suggest that the measured increase in rainy season litterfall constitutes an increase in leaf turnover rather than an increase in total leaf production, as dry season litterfall was not affected by litter manipulation. During the main growth period in the rainy season, the increased nutrient supply in the L+ plots most likely led to an increase in leaf exchange, where old leaves were replaced by new, more efficient, ones (Wood *et al.* 2009). Leaf shedding during the dry season is less likely to be affected by changes nutrient availability as it is thought to be triggered by water stress (e.g. Reich & Borchert 1984) or environmental cues associated with low humidity (Wright & Cornejo 1990).

We showed greater mass loss of decomposing litter in the L+ plots compared to the CT plots during the dry season (Fig. 3). We suggest that litter decomposition during the dry season may be stimulated by greater water content of the thicker litter layer in the L+ plots rather than increased nutrient availability, as there were no treatment differences in mass loss during the rainy season. In previous decomposition experiments conducted in the litter manipulation plots, the maximum mass loss in the CT plots during the first month of decomposition was 5.6% of the total dry weight of freshly fallen litter (Sayer, Tanner & Lacey 2006). Thus, by collecting litter from the traps only once a month, we have probably underestimated annual litterfall by < 5% due to decomposition of the litter between collections.
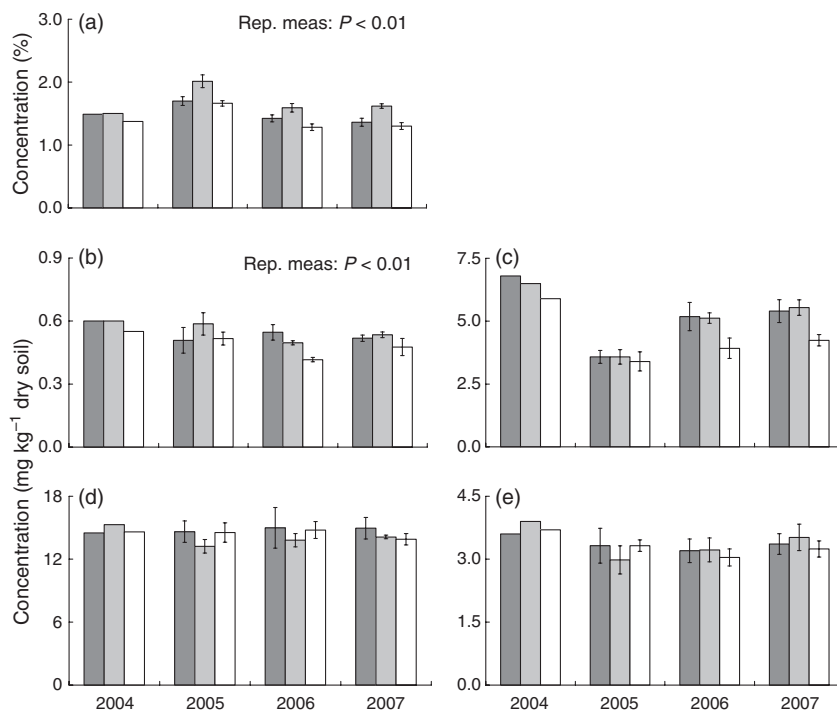
Fig. 6. Nutrient concentrations in mixed litter collected from traps from 2004 to 2007 in litter manipulation plots in lowland semi-evergreen tropical forest in Panama, Central America; (a) nitrogen, (b) phosphorus, (c) potassium, (d) calcium, and (e) magnesium; dark grey bars are controls, light grey bars are the litter addition treatment and white bars are the litter removal treatment; values for 2004 are for one composite sample per treatment, error bars for 2005–2007 are standard errors of means for $N = 5$.

Table 1. Annual small litterfall and nutrient concentrations of litter in selected mature lowland tropical forests; selection criteria were: annual precipitation 1700–3500 mm, and elevation < 150 m a.s.l

Litterfall (t ha^{-1} year^{-1})	N (%)	P (mg kg^{-1})	Ca (mg kg^{-1})	Source
Infertile soils				
5.8	1.05	138	1379	Jordan & Herrera (1981)
6.4	1.16	219	3125	Franken (1979)*
7.3	1.45	288	2466	Klinge & Rodrigues (1968)*
7.6	0.86	280	3600	Coomes (1997)
8.7	1.18	391	14 253	Folster & de la Salas (1976)*
8.9	1.12	315	7865	Lim (1978)*
Moderately fertile soils				
9	1.88	644	9778	Ewel (1976)*
9.6	1.49	600	14 500	This study
10.5	–	257	10 095	Golley *et al.* (1975)*
11.1	1.76	1351	19 099	Haines & Foster (1977)*
11.4	–	825	22 456	Golley *et al.* (1975)*

*Data from Vitousek (1984).

TREE GROWTH

We found no effect of litter manipulation on tree growth over the 5-year study period. Litter removal has often led to a reduction in growth in temperate forests over 10–15 years (Sayer 2006) and we expected a more rapid response in the tropics because growth is year-round. Furthermore, various fertilization experiments in montane tropical forests have shown that nutrient augmentation can lead to increases in

trunk growth within 3 years of the first application (e.g. Tanner, Kapos & Franco 1992; Tanner, Vitousek & Cuevas 1998). However, most studies in temperate forests include only three to four tree species, whereas we measured growth rates for 127 different tree species, which will display a wide range of responses to changes in their environment (Wright & Cornejo 1990). The significant species × treatment interaction indicates that growth responses to the experimental treatments were species-specific. Species-specific responses to changes in

the species' environment may be particularly relevant in the litter manipulation plots, as litter removal can also lead to greater fluctuations in soil temperature and water content, increased soil compaction and greater surface run-off (Sayer 2006).

SOIL AND LITTER NUTRIENTS

Many of the observed effects of litter manipulation on soil nutrient concentrations appeared first at the soil surface (0–2 cm depth), and later extended to 0–10 cm depth (e.g. nitrate-N and Ca). We found significant time × treatment interactions for nitrate-N, P, K and Ca at one or both depths, indicating that the differences between treatments are increasing over time. Thus, the changes we observed at 0–2 cm depth in the soil serve as a first indicator of treatment effects and we would expect to see differences in soil P and K concentrations at 0–10 cm within the next few years.

Nitrogen

Despite moderate concentrations of extractable inorganic N in the soil at the study site (> 10 mg kg^{-1} in the topsoil in the CT plots), we saw changes in soil nitrate-N concentrations within 2 years of the start of litter manipulation (Fig. 4) and changes in litter N concentrations by the end of the third year (2005; Fig. 6a). Concentrations of nitrate in the soil can be used as a measure of N availability in forests (Robertson 1982). Thus, the higher concentrations of nitrate-N in the soil in the L+ plots indicate that N availability has been greatly increased by the litter addition treatments. Conversely, both nitrate-N and ammonium-N decreased in the soil in the L− plots (Fig. 4). By the end of the study, total inorganic N in the soil had decreased by *c*. 50%, but litter N concentrations remained unaffected (Fig. 6a), though there was a trend towards lower foliar N concentrations towards the end of the study (Fig. 5a). We interpret these results from the L− plots to mean that a large proportion of the plants' annual requirement for N is normally cycled in the litter and that litter removal resulted in greater plant uptake of N from soil stocks. We predict that N concentrations in leaves and litter in the L− plots will decline in the future as N stocks in the soil are depleted. In the L+ plots, increasing foliar N concentrations may create a positive feedback over time, as greater photosynthetic capacity can be predicted in response to the higher foliar N concentrations (Harrington, Fownes & Vitousek 2001).

Phosphorus

At our study site, extractable P in the mineral soil is close to the level that limits plant growth (Yavitt *et al.* 2009), and in P-poor soils, the cycling of P can be more or less restricted to the organic horizons (Attiwill & Adams 1993). The direct transfer of P from decomposing organic matter to roots via mycorrhizae has been demonstrated in tropical forests on highly infertile soils (Herrera *et al.* 1978; Stark & Jordan 1978), and even a relatively thin layer of litter on the soil surface has a high

capacity to retain P (Tobón, Sevink & Verstraten 2004). In our litter addition treatment, the continual additional input of fresh leaf litter may act as a sink for P being released from older decomposing organic matter (Piatek & Allen 2001). Furthermore, higher fine root biomass in the organic horizons in the L+ plots (Sayer, Tanner & Cheesman 2006) will have facilitated the direct uptake of P (Stark & Jordan 1978). It is therefore likely that P was transferred from the decomposing litter to plants as soon as it was mineralized and before reaching the mineral soil (Herrera *et al.* 1978; Stark & Jordan 1978; Tobón, Sevink & Verstraten 2004). This is supported by our measurements of soil P concentrations: P inputs from litter were effectively doubled by the litter addition treatment, but the concentrations of extractable P in the mineral soil (0–10 cm) did not increase significantly relative to the controls over the 5-year study period (Fig. 4). This suggests that much of the additional P from the litter had been cycled directly before reaching the mineral soil. The lack of increased concentrations of P in leaves and litter in the L+ plots may be a consequence of the 'dilution effect' (Tanner, Kapos & Franco 1992), where the additional P taken up by plants in the litter addition treatments was sufficient to support the increased leaf turnover during the rainy season, but not enough to significantly increase foliar P concentrations as well.

Surprisingly, 5 years of litter removal did not significantly decrease inorganic P in the soil or foliar P concentrations. There was a trend towards lower P concentrations in litter towards the end of the study (2006–2007; Fig. 6b), which may be a first indication of increased P resorption in response to nutrient depletion, but overall, the effects of litter removal were much smaller than expected. We measured a large (32%) decrease in extractable soil P at the soil surface (0–2 cm depth) in the L− plots in 2004 (Fig. 4). This suggests that the litter standing crop was a major source of P to plants and when it was removed at the start of the treatments in 2003, the plants took up P from the mineral soil instead. However, the lack of further decreases in soil and foliar P over the study period implies that plants have an alternative source of P. Organic P had decreased by 23% at 0–2 cm depth in the mineral soil after 3 years of litter removal and was therefore suggested as a source of P to plants. However, this decrease would only amount to around a fifth of the P needed for above-ground litter production each year (Vincent, Turner & Tanner 2010). Thus, although the direct transfer of P from decomposing litter appears to constitute a major source of P for above-ground production, plants at our site appear to be able to switch to other P sources when litter is removed, at least for the first 5 years of treatments. While these alternative sources of P remain to be identified, there is currently little evidence for P limitation of above-ground production at our study site.

Potassium

Throughfall, rather than litterfall, is usually the main pathway for the transfer of K from the canopy to the soil in tropical forests (Vitousek & Sanford 1986). Inputs in throughfall of 63 kg K ha^{-1} year^{-1} have been reported for lowland forest on

a moderately fertile soil in Panama (Golley *et al.* 1975), which is a little more than our estimated K return by litterfall (*c.* 50 kg ha^{-1} year^{-1}); consequently, litter removal only halved K inputs in the L− plots. Potassium plays a major role in many physiological processes in plants and the demand for K at the ecosystem level can be high (Tripler *et al.* 2006). At our study site, the availability of soil K was low (Yavitt *et al.* 2009) and 5 years of litter removal decreased both the concentrations of K in the soil (Fig. 4) and K return by litterfall. However, foliar K was not reduced in the L− plots (Fig. 5c), which suggests that soil K stores, and increased retranslocation of K from senescing leaves, allowed trees to maintain foliar K levels during the 5 years of treatments. Indeed, the significant time × treatment interaction for soil K concentrations indicates that soil stocks are gradually being depleted by litter removal. Although K is highly mobile and readily leached from litter, the litter layer retains a proportion of the K inputs in throughfall, where it is taken up rapidly by plants and soil organisms (Tobón, Sevink & Verstraten 2004); a decomposition experiment in the treatment plots showed greater retention of K in decomposing litter in the thick litter layer of the L+ plots (Sayer, Tanner & Lacey 2006). Thus, in addition to halving annual K inputs to the L− plots, the removal of the litter layer probably also exacerbated leaching of K through the soil profile.

Calcium and Magnesium

We had not expected Ca and Mg to respond to the litter manipulation treatments, as both nutrients are present in the bedrock and in marine aerosol inputs at high concentrations (Yavitt & Wieder 1988; Cavelier 1992). Instantaneously exchangeable soil stocks of Ca and Mg were over ten times higher than the annual input by litterfall at our study site (Yavitt *et al.* 2009), but in spite of this, litter manipulation affected Ca and Mg concentrations at the soil surface within 2 years of the start of treatments (Fig. 4). However, there were no effects on concentrations of Ca or Mg in live leaves (Fig. 5d,e) or litter (Fig. 6d,e). This suggests that although the inputs of Ca and Mg in litter are substantial, the availability of these nutrients in the soil is well in excess of the amount required by the vegetation.

Conclusions

Litterfall plays a major role in the nutrient cycling of this lowland semi-evergreen tropical rain forest and is an important source of nutrients, in particular of nitrogen, to plants. Although productivity in the study forest is not obviously nitrogen limited, nitrogen cycling is relatively conservative; decreases in litter standing crop rapidly affected the availability of nitrogen to plants, while prolonged addition of litter increased nitrogen concentrations in leaves and litter. Contrary to expectations, we found little evidence in support of the widely accepted hypothesis that phosphorus is the main limiting element in lowland tropical forests. Despite removing a large proportion of phosphorus being cycled with organic mat-

ter, we saw only minor changes in soil or foliar phosphorus concentrations over the 5 years of the study and no decrease in forest productivity. We suggest that litter is a major source of the nitrogen required for plant growth and that the plants in the study forest have access to alternative sources of phosphorus and cations at least over a 5-year period.

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