



Revisiting nutrient cycling by litterfall—Insights from 15 years of litter manipulation in old-growth lowland tropical forest

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Abstract

The crucial role of tropical forests in the global carbon balance is underpinned by their extraordinarily high biomass and productivity, even though the majority of tropical forests grow on nutrient-poor soils. Nutrient cycling by litterfall has long been considered essential for maintaining high primary productivity in lowland tropical forests but few studies have tested this assumption experimentally. We review and synthesise findings from the Gigante Litter Manipulation Project (GLiMP), a long-term experiment in lowland tropical forest in Panama, Central America, in which litter has been removed from or added to large-scale plots for 15 years. We assessed changes in soil and litter nutrient concentrations in response to the experimental treatments and estimated nutrient return and nutrient use efficiency to indicate changes in nutrient cycling. The soil concentrations of most nutrients increased with litter addition and declined with litter removal. Litter removal altered nitrogen, potassium, manganese and zinc cycling, demonstrating the importance of litter inputs for maintaining the availability of these elements to plants. By contrast, litter addition only altered nitrogen cycling and, despite low concentrations of available soil phosphorus, the effects of litter manipulation on phosphorus cycling were inconsistent. We discuss potential mechanisms underlying the observed changes, and we emphasise the importance of decomposition processes in the forest floor for retaining nutrient elements, which partially decouples nutrient cycling from the mineral soil. Finally, by synthesising GLiMP studies conducted during 15 years of litter manipulation, we highlight key knowledge gaps and avenues for future research into tropical forest nutrient cycling.



1. Introduction

1.1 The dual role of litterfall in tropical forest nutrient cycling

The importance of tropical forests in global biogeochemical cycles is undisputed: tropical forests represent the largest store of terrestrial biomass carbon (Brown and Lugo, 1982), regulate continental scale hydrological cycles (Gloor et al., 2013) and contribute to global atmospheric circulation (Malhi et al., 2014). Unfortunately, our understanding of carbon (C) and nutrient dynamics in tropical forest ecosystems is deficient, which hampers our ability to predict the effects of global changes (Bonan, 2008; Wieder

et al., 2015). Long-term shifts in temperature (e.g. Corlett, 2011), precipitation (Meir and Woodward, 2010), and atmospheric deposition (Hietz et al., 2011) are likely to have major impacts on tropical forest ecosystem functioning by influencing tree growth and species composition (Wright, 2005) as well as elemental cycling. In this context, tropical forest nutrient cycling is of considerable interest because nutrient cycles regulate forest C storage (Finzi et al., 2011) and many tropical forests maintain extraordinarily high biomass and productivity, despite growing on nutrient-poor soils (Vitousek and Sanford, 1986).

Litterfall and litter decomposition play a central role in the cycling of C and nutrients in tropical forests (Sayer and Tanner, 2010). Although leaf litter only contains a small proportion of the nutrient content in above-ground forest biomass (typically <10%; Proctor, 1987), it dominates the intra-annual cycle of nutrients through rapid turnover (Schlesinger, 1991); litterfall therefore represents a major pathway for the transfer of mineral nutrients and organic matter from vegetation to soil (Vitousek and Sanford, 1986). Many lowland tropical forests are characterised by conservative or “tight” nutrient cycling with low losses of nutrients (Vitousek, 1984), which is facilitated by substantial direct uptake of macronutrients from decomposing leaf litter by plant roots (Medina and Cuevas, 1989; Tobón et al., 2004). The processes of microbial immobilisation and mineralisation of nutrients from organic material provide a “slow-release” mechanism of nutrients to plants, which limits nutrient losses in gaseous forms or via leaching (Sayer et al., 2012), and fine roots concentrated in organic horizons and the upper layers of the mineral soil intercept nutrients as they are released from decomposing litter (Attiwill and Adams, 1993; Yavitt et al., 2011). Indeed, due to the large amounts of nutrients contained in litter and other decomposing organic material on the soil surface (“forest floor”; Box 1), nutrient cycling between plants and soil can become partially decoupled from the mineral soil (Leuschner et al., 2001; Sayer et al., 2006a) and cycling of some nutrients in organic matter can meet much of the annual demand by plants (Attiwill and Adams, 1993). In addition to acting as a nutrient reservoir, the forest floor also intercepts nutrients in throughfall (Tobón et al., 2004) and can retain nutrients that would otherwise be lost through leaching or, in tropical soils with low base saturation and high aluminium mobility, through sorption to metal oxides (Medina and Cuevas, 1989; Tobón et al., 2004). Hence, litter inputs play a dual role in tropical forest nutrient cycling: as a primary source of nutrients and as an important mechanism for nutrient retention within the ecosystem.

BOX 1 Glossary—Guiding definitions of key terms used in this work.

Term	Definition and context
Direct cycling/uptake (of nutrients)	The capture and uptake of nutrients by roots and mycorrhizal fungi upon mineralisation and release from decomposing organic matter in the forest floor; regarded as an important nutrient conservation mechanism in highly weathered, infertile soils
Forest floor	The organic horizon on the surface of the mineral soil, comprising litter, roots, and organic material in various stages of decay (Sayer, 2006); often referred to as the "O Horizon"
Immobilisation (of nutrients)	Microbial conversion of nutrients from inorganic to organic forms (i.e. incorporation into cells), making them unavailable to plants; immobilisation is the reverse of mineralisation
Leaky nutrient cycle	Inefficient nutrient cycle characterised by high losses of a given element through leaching or in gaseous forms; nutrient cycling within an ecosystem can be characterised as leaky for one nutrient but as "tight" or efficient for another
Litter layer	The surface layer of the forest floor, which is not in an advanced stage of decomposition and comprises fallen leaves, needles, fruits, flowers, and twigs (Sayer, 2006); often referred to as the L or O _L horizon
Mineralisation (of nutrients)	Microbial conversion of nutrients in organic forms into soluble inorganic forms that may be available to plants; usually occurs during the decomposition of organic matter
Nutrient limitation	Nutrient limitation is inferred when additions of an essential element in biologically available forms cause an increase in the rate of an ecosystem process (sensu Tanner et al., 1998)
Nutrient return	The amount of a given nutrient cycled annually in litterfall, calculated as the product of litterfall mass and litter nutrient concentrations; also referred to as nutrient accession

BOX 1 Glossary—Guiding definitions of key terms used in this work.—cont'd

Nutrient use efficiency	A measure of how well plants use available mineral nutrients; defined for forests as the amount of organic matter lost from plants or permanently stored in plants per unit of nutrients lost or stored; calculated here as the inverse of nutrient concentrations in aboveground litterfall (sensu Vitousek, 1982)
Tight (conservative) nutrient cycle	Nutrient cycle characterised by high nutrient use efficiency and minimal losses of mineral nutrients, e.g. by rapid uptake by plants, mycorrhizas or decomposers (sensu Vitousek, 1984); also referred to as “efficient” or “conservative”

We know relatively little about nutrient and C cycling in tropical forests compared to temperate wooded systems (Townsend et al., 2011), in particular about the specific role of litterfall (Sayer, 2006). Fertilisation experiments in tropical forests have demonstrated that various ecosystem processes are limited by the availability of different nutrients (Cleveland and Townsend, 2006; Kaspari et al., 2008; Ostertag, 2010; Vitousek et al., 1993; Wright et al., 2011; Wright et al., 2018), but explicit consideration of nutrient return by litterfall is still crucial for understanding elemental cycling. Leaf litter can essentially be viewed as a natural complete fertiliser (Sayer et al., 2012) not only because it contains all the elements required for plant growth, but also because those nutrients are cycled with substantial amounts of C (*c.* 50% leaf dry mass). The stoichiometric balance of C and nutrients is critical to a large number of processes underpinning ecosystem C and nutrient cycling (Elser et al., 2010; Sardans et al., 2012; Sinsabaugh et al., 2008). Importantly, elemental stoichiometry drives the decomposition of organic material (Manzoni et al., 2010), because microbial decomposers rely on organic C as a source of energy, and usually process C and nutrients together (Finzi et al., 2011; Sinsabaugh et al., 2008). Carbon inputs also modify the rate at which nutrients are processed and released, as well as the forms in which nutrients are present in the soil (Palm et al., 1997), and there is evidence that nutrient availability can influence the storage or release of soil C in tropical forests (Cleveland et al. 2006; Nottingham et al., 2015; Wieder et al., 2015). Despite the clear importance of coupled

C and nutrient cycling by litterfall, we still lack an integrated understanding of nutrient pathways from leaf litter to decomposer organisms and soil through to re-uptake by roots. The gaps in our knowledge stem partly from the difficulties of accounting for the heterogeneity of tropical forest ecosystems (Townsend et al., 2011) and the extraordinarily high diversity of plants (Sayer and Banin, 2016), but also because experimental assessments of the role of leaf litter in tropical forest elemental dynamics are scarce.

1.2 Assessing the role of litterfall using manipulative experiments

Experimental manipulation of litter dates back to the 1800s and has provided key insights into the role of litterfall in forests (Sayer, 2006). Historically, studies have focussed on litter removal experiments to elucidate the importance of nutrients cycled in litterfall for maintaining forest productivity (Sayer, 2006). Recognition of important knowledge gaps in soil organic C dynamics has also given rise to numerous litter manipulation experiments, most notably the Detritus Input and Removal Treatments (DIRT) network (Nadelhoffer et al., 2004; Lajtha et al., 2018), which include litter removal and litter addition treatments, but also consider belowground carbon inputs using “no roots” and “no inputs” treatments. The DIRT network has focussed principally on C dynamics, demonstrating that aboveground litter inputs may be just as important for soil C sequestration as root inputs, and that site fertility plays a key role in the contribution of aboveground litter to soil C stocks (Lajtha et al., 2018). By contrast, there are few experimental studies of nutrient cycling by litterfall and most of our knowledge about the long-term effects of enhanced or reduced litter inputs on forest nutrient status is derived from temperate systems (Sayer, 2006). Although it seems reasonable to assume that leaf litter will play a similar role in temperate and tropical forest ecosystem C and nutrient dynamics, there are three crucial distinctions: (1) rapid decomposition in a warm humid tropical climate results in fast turnover of organic matter and high rates of element cycling; (2) in many tropical forests, growth occurs all year round and litterfall seasonality is much less pronounced than in temperate deciduous forests; (3) the vast majority of tropical forests are highly diverse, and the diversity of species contributing litter also influences decomposition processes by shaping soil fauna communities (Laird-Hopkins et al., 2017).

We aimed to provide a comprehensive overview of the role of litterfall in tropical forest nutrient cycling by establishing a large-scale, long-term litter manipulation experiment. The Gigante Litter Manipulation Project

(GLiMP) has been running continuously since 2003 and has afforded numerous insights into the role of leaf litter in tropical forest ecosystem processes. Here, we synthesise the results of 15 years of research within the GLiMP experimental plots and compare key findings with other studies in both temperate and tropical forests. We complement the results of published studies with new datasets to investigate the trajectory of change in nutrient cycles in response to long-term litter addition and removal treatments and assess the consequences for broader ecosystem functioning. Finally, we aim to motivate future research by identifying persisting and emerging knowledge gaps about the role of litterfall in lowland tropical forests (Box 2).



2. The Gigante Litter Manipulation Project (GLiMP)

2.1 Study site and experimental design

The long-term litter manipulation experiment is located on Gigante Peninsula (9° 06' N, 79° 54' W), part of the Barro Colorado Nature Monument in Panama, Central America. Mean annual rainfall is *c.* 2600 mm with a strong dry season from January to April and the mean annual temperature is 26 °C (Leigh, 1999). The soil is a moderately acidic Oxisol with pH *c.* 5.5, a total C content of *c.* 4%, and low concentrations of extractable phosphorus (*c.* 0.5 mg kg⁻¹) and potassium (*c.* 40 mg kg⁻¹) but high concentrations of calcium (*c.* 1500 mg kg⁻¹) and magnesium (*c.* 400 mg kg⁻¹) at 0–10 cm depth (Ashford et al., 2013; Sayer and Tanner, 2010; Sheldrake et al., 2017; Tanner et al., 2016; Yavitt et al., 2009).

Between 2000 and 2002, 15 plots were established within a 40-ha area of old-growth lowland tropical forest. Each plot measures 45-m × 45-m and is bordered by 0.5-m deep trenches to limit nutrient and water transfer; the trenches were double-lined with construction plastic and back-filled with soil (Sayer and Tanner, 2010). All measurements and samples were taken in the inner 30-m × 30-m of each plot to limit potential effects of trenching. Starting in January 2003, the litter has been removed every 1–2 months from five plots and added to five plots, where it is spread out as evenly as possible, leaving five plots as undisturbed controls (Fig. 1). The treatments were initially assigned to the plots in a stratified random design based on the annual litterfall in 2002 (Sayer and Tanner, 2010), but as the litter treatments were paired geographically for logistical reasons, each replicate block comprises one pair of litter removal (L–) and litter addition (L+) plots and the closest control plot.

BOX 2 Major knowledge gaps and future research directions in tropical forest nutrient cycling.

Collectively, the GLiMP studies demonstrate that ecosystem responses to declining nutrient availability involve multiple mechanisms, including plant physiological adaptations to reduce nutrient demand, shifts in the species composition of microbial communities, mining of organic compounds, and direct uptake of nutrients from the forest floor. Hence, future research to address the following major knowledge gaps will greatly advance our understanding of tropical forest nutrient and carbon dynamics.

Knowledge Gap 1: Plant adaptations to nutrient availability

Expanding current theory of nutrient limitation to account for plant adaptation to nutrient availability could greatly improve our understanding of tropical forest nutrient cycling and ecosystem responses to global change. To achieve this, we need to assess changes in plant productivity and foliar nutrient concentrations against a variety of other mechanisms that control nutrient demand. Studies of nutrient allocation to different structures and physiological or metabolic processes could be particularly informative for understanding plant responses to altered nutrient supply. A combination of gradient studies and controlled experiments would then link differences in plant productivity and nutrient allocation to adaptation mechanisms such as resorption, prolonged leaf retention, and shifts in biomass allocation.

Knowledge Gap 2: Micronutrient cycles

Micronutrients are key to understanding decomposition food-webs but we still know very little about how most trace elements are cycled through the ecosystem, or how their availability influences the dynamics of other elements. A more complete picture of trace elements in tropical forest functioning could be gained from broad studies and networks assessing the effects of micronutrient additions along a gradient of macronutrient availability, followed by targeted experiments to investigate specific processes or groups of organisms (Kaspari and Powers, 2016). Experiments assessing the impact of both macro- and micronutrient availability on microbial processes and plant-microbe interactions could reveal particularly important insights into ecosystem functioning.

Knowledge Gap 3: The relative contribution of "direct nutrient cycling" and nutrients stored in soil organic matter

We provide multiple lines of evidence for nutrient uptake from the forest floor and the potential for soil organic matter to act as a major alternative source of scarce nutrients. We still lack detailed studies tracking the flow of nutrients and carbon in litterfall through various stages of decomposition to re-uptake by plants, and a greater focus on the formation and turnover of soil organic matter in tropical forests could contribute to quantifying the relative importance of organic compounds as potential sources of nutrients. Molecular and isotopic techniques developed since the initial characterisation of direct nutrient uptake could facilitate a new wave of research into microbial processing and transfer of nutrients in organic materials at different stages of decomposition and by distinct functional groups.

BOX 2 Major knowledge gaps and future research directions in tropical forest nutrient cycling.—cont'd

Knowledge Gap 4: Links between plant and microbial nutrient requirements

Microbial mineralisation of nutrients in organic matter provides substantial amounts of nutrients for plant growth but microbial resource requirements vary widely among different functional groups (Camenzind et al., 2018). Our experiment provides an excellent platform for studies of microbial communities and functions linked to elemental cycling via litterfall, but very little work in this area has been achieved to date. The GLiMP studies demonstrate direct effects of litter quantity on heterotrophic respiration via the addition of fresh organic carbon (Sayer et al., 2007, 2011), and indirect effects on mycorrhizal associations via altered plant nutrient demand (Sheldrake et al., 2017, 2018). Shifts in microbial communities may also underpin many of the observed patterns of nutrient accumulation and release from litter, and it is conceivable that other crucial microbial processes, such as N-fixation, are also heavily influenced by changes in litterfall. New molecular techniques facilitate numerous avenues for research into microbial communities and processes (Camenzind et al., 2018), which could contribute to addressing the current knowledge gaps on micronutrient and forest floor processes.



Fig. 1 The Gigante Litter Manipulation Project (GLiMP), showing adjacent litter removal and control plots separated by plastic-lined trenches (A) during the first year of treatments in 2003; (B) the soil surface after the first application of treatments in February 2003; and (C) the soil surface after 5 years of treatments in 2008.

2.2 Field and laboratory measurements

Soil samples for analysis of nutrient concentrations and pH were taken annually from 2004 to 2007 and in 2009, 2010, 2012 and 2017 (see Table 1 for full details of analysis in original data sources). Each year, four to nine soil cores were collected from 0 to 10 cm depth in each plot; samples in 2004 were extracted individually but in all other years, the cores were mixed to give one composite sample per plot. Briefly, inorganic nitrogen

Table 1 Datasets used to analyse patterns of change in nutrient cycling in a lowland tropical forest in Panama, Central America, in response to 15 years of litter addition and litter removal treatments, giving the citation for previously published data, where N is nitrogen, N_{TOT} is total soil N, NO_3^- -N is soil nitrate-N, P is phosphorus, P_{EXTR} is extractable soil P, K is potassium, Ca is calcium, Mg is magnesium, B is boron, Mn is manganese, Na is sodium, Zn is zinc, pH is soil pH, SOC is soil organic carbon, C_{MIC} is microbial biomass carbon and N_{MIC} is microbial biomass N.

Source	Year(s)	Soil properties (0–10 cm depth)	Litter nutrients
Sayer et al. (2007)	2004, 2006	N_{TOT}^a , SOC, C_{MIC} , N_{MIC} ,	
Sayer and Tanner (2010)	2004–2007	NO_3^- -N, P_{EXTR} , K, Ca, Mg^b , B, Mn, Na ^b , Zn, pH	N, P, K, Ca, Mg, B, Mn, Na, Zn
Sayer et al. (2011)	2007	SOC	
Sayer et al. (2012)	2007	C_{MIC} , N_{MIC}	
Ashford et al. (2013)	2010	NO_3^- -N, P_{EXTR} , K, Ca, Mg, B, Mn, Na, pH	
Tanner et al. (2016)	2009	N_{TOT} , SOC, NO_3^- -N, P_{EXTR} , K, Ca, Mg, pH	
Sheldrake et al. (2017)	2012	N_{TOT} , SOC, NO_3^- -N, P_{EXTR} , K, Ca, Mg, Mn, Na, pH	
Previously unpublished data	2013		N, P, K, Ca, Mg, B, Mn, Na, Zn
	2016	N_{TOT} , SOC, C_{MIC} , N_{MIC} , NO_3^- -N, pH	
	2017		N, P, K, Ca, Mg, B, Mn, Na, Zn
	2018	P_{EXTR} , K, Ca, Mg, B, Mn, Zn	

^a2004 only.

^bNo data for 2006.

(N; ammonium-N and nitrate-N) was determined by colourimetry after extraction with potassium chloride. Soils were stored for 24 h before extraction from 2004 to 2007 and extracted immediately in 2010, 2012 and 2017. This difference in storage times can affect the relative concentrations of ammonium-N and nitrate-N (Turner and Romero, 2009). Issues with contamination affected the results for ammonium-N in several years, which precluded analysis of long-term trends and we therefore only present the results for nitrate-N. Phosphorus, potassium, magnesium, calcium and micro-nutrients were determined by inductively-coupled plasma spectrometry (ICP-MS) after Mehlich III extraction, except in 2010 and 2012, when extractable soil P (P_{EXTR}) was determined by resin extraction (Turner and Romero, 2009). Where relevant, values for soil boron were corrected for iron interference according to Turner et al. (2016). Soil pH was measured on fresh soils in deionised water. Total N was determined by Kjeldahl extraction in 2004, 2009, 2012 and 2016, total soil organic C was determined by combustion oxidation in 2004, 2007, 2009, 2012, 2015 and 2016, and microbial biomass C and N were assessed by chloroform fumigation in 2004, 2006–2008, and 2016.

Litter nutrients and nutrient return in litterfall were assessed using dried litter samples collected from 10 traps per plot in September or October annually from 2005 to 2007 (Table 1) and from 5 traps per plot in December 2013 and October 2017. The samples (excluding the woody fraction) were pooled by plot and year, shredded, mixed, and a subsample was finely ground for nutrient analysis. Nitrogen concentrations in the litter were determined by complete combustion gas chromatography and all other nutrient concentrations were determined by ICP-MS after nitric acid digest.

After 15 years of litter manipulation, we used ion exchange resins to assess short-term changes in nutrient exchange rates in the topsoil. Nutrient exchange rates during the rainy season were determined using plant root simulator probes (PRS probesTM, WesternAg, Canada), which consist of ion exchange resins held in plastic support frames. We installed four pairs of anion and cation exchange probes vertically at 0–10 cm in each plot in June 2017 (early rainy season) and November 2017 (late rainy season). The probes remained in place for 42 days on each occasion. After retrieval, the probes were cleaned with deionised water and returned to the manufacturer for analysis. Nitrate-N was determined by colourimetry and all remaining nutrient ions were measured by ICP-MS after acid digest. Nutrient exchange rates are based on the surface area of the resin and the exposure time and thus units are $\mu\text{g nutrient } 10 \text{ cm}^{-2} \text{ 42 d}^{-1}$; all data analyses were based on mean values per plot ($n = 5$ per treatment).

2.3 Data analysis and synthesis

We performed all analyses in R v.3.5.1 (R Development Core Team, 2018) using the lme4 package for linear mixed effects models (Bates et al., 2015) and the lmerTest package (Kuznetsova et al., 2017) to derive significance values for individual treatment levels, using Satterthwaite's approximation to estimate degrees of freedom. We estimated the annual nutrient return by litter for 2005–2007, 2013 and 2017 by multiplying annual total small litter mass per plot ($\text{g m}^{-2} \text{ year}^{-1}$) by the nutrient concentrations of the litter (mg kg^{-1}) collected in September and October (2005–2007 and 2017) or December (2013) each year. We then calculated nutrient use efficiency sensu Vitousek (1982) as the ratio of annual litterfall dry mass to the nutrient return by litter. We also estimated the mean and total amount of nutrients transferred between L– and L+ plots based on mean annual litterfall and nutrient concentrations per treatment, using plot-level data for 2005–2007, 2013 and 2017 (Table 1), and nutrient concentrations from one composite sample per treatment for 2003 and 2009–2012 (Sayer and Tanner, 2010; Rodtassana, 2016; Table 2).

To assess patterns of change in nutrient cycling in the GLiMP plots, we collated data on nutrient concentrations in topsoil and litter from 2004 to

Table 2 Estimated annual nutrient return in litterfall in control (CT) litter addition (L+) and litter removal (L–) treatments in lowland tropical forest in Panama, Central America, showing means and standard errors for $n = 15$ years, and the total amount of each nutrient transferred from L– to L+ plots over the duration of the experiment.

	Nutrient return in litterfall ($\text{kg ha}^{-1} \text{ year}^{-1}$)			Transferred (kg ha^{-1})
	CT	L+	L–	
Nitrogen (N)	191 ± 24	234 ± 28	149 ± 15	2246
Phosphorus (P)	6.30 ± 0.6	7.28 ± 0.7	5.14 ± 0.4	77
Potassium (K)	59.5 ± 8.3	73.0 ± 12	47.3 ± 7.2	710
Calcium (Ca)	207 ± 34	218 ± 33	169 ± 23	2532
Magnesium (Mg)	46.5 ± 6.9	50.7 ± 7.5	36.8 ± 4.7	553
Boron (B)	0.57 ± 0.1	0.65 ± 0.1	0.48 ± 0.07	7.2
Manganese (Mn)	4.05 ± 0.6	4.01 ± 0.4	2.45 ± 0.3	37
Sodium (Na)	6.40 ± 1.1	7.04 ± 1.2	5.3 ± 0.9	80
Zinc (Zn)	0.42 ± 0.06	0.31 ± 0.03	0.31 ± 0.03	4.6

2018. Published studies describe the datasets from 2004 through 2012 (Table 1) and are supplemented here with new data collected between 2013 and 2018 to evaluate longer-term trends and predictions made when the experiment began. There were small methodological differences among years (e.g. sampling month, sample processing and laboratory analyses) that could potentially confound trends over time. These methodological differences will have similar effects on all three treatments (L+, L- and control) and we therefore analysed log response ratios, which minimise variation associated with methodological differences among years by standardising values from the L+ and L- treatments by values from the control treatment. We calculated log response ratios (RR) for each variable, block and year as follows:

$$RR = \ln(R_X/R_{CT}) \quad (1)$$

where R_X is the measured value in treatment X and R_{CT} is the measured value in the control.

We first assessed the influence of litter manipulation on soil nutrients, litter nutrients, nutrient return in litterfall and nutrient use efficiency using linear mixed effects models (*lmer* function) with treatment as a fixed effect, and block and year as random effects. We then tested for trajectories in treatment responses (RR) over time using separate linear models for each treatment. We quantified nutrient exchange rates once in 2017, present their absolute values for each treatment and assess the effect of litter manipulation using linear mixed effects models, with treatment as a fixed effect, and block and sampling time as random effects.

For all linear mixed effects models, we dropped terms sequentially to determine their significance, using AIC and *P*-values to check for model improvement (Pinheiro and Bates, 2000). We tested final models against corresponding null models using likelihood ratio tests and assessed the fit of each model with diagnostic plots. For all significant terms, we report the effect size *d* and significance at $P < 0.05$ in the text. We also report marginally significant trends for $P < 0.1$ and $|d| > 0.6$. Statistics for the best fit model for each response variable are given in Supplementary Table S1 in the online version at <https://doi.org/10.1016/bs.aecr.2020.01.002>.

We calculated Cohen's *d* effect size (Cohen, 1988) for each year with blocks pooled as follows:

$$d = (M_X - M_{CT})/SD_{\text{pooled}} \quad (2)$$

where M_X is the mean of a given treatment, M_{CT} is the mean of the control and SD_{pooled} is the pooled standard deviation:

$$SD_{pooled} = \sqrt{\left(\left(SD_X^2 + SD_{CT}^2\right)/2\right)} \quad (3)$$

where SD_X and SD_{CT} are the standard deviations of treatment and control, respectively. We present mean Cohen's d (\pm S.E.) across years for each response variable. Cohen's d represents standard deviations of difference, where $d \geq 0.8$ is a large effect (Cohen, 1988) and generally corresponded to significance at $P < 0.05$ in our analyses.



3. Macronutrient cycling in litter

Biogeochemical theory hypothesises that concentrations of rock-derived nutrients such as phosphorus (P) decline over geological time as a result of erosion and leaching, whereas nitrogen (N), which is derived mainly from biotic processes or atmospheric deposition, accumulates over time (Walker and Syers, 1976). Consequently, P is more likely to be limiting than N in old, highly weathered soils in the tropics, whereas N is more likely to be limiting in young soils in the temperate zone, but there are numerous lines of evidence for N and P co-limitation in many lowland tropical forests (see, e.g. Sayer and Banin, 2016; Townsend et al., 2011; Wright et al., 2018). Litterfall is the dominant pathway for N and P cycling (Schlesinger, 1991), and leaf litter also contains substantial amounts of potassium (K), calcium (Ca) and magnesium (Mg). Indeed, foliar concentrations of these nutrients often reflect soil fertility in tropical forests (Vitousek and Sanford, 1986) and hence 15 years of litter removal and addition treatments are likely to have a substantial impact on macronutrient cycling. We present and discuss the patterns for each nutrient in turn.

3.1 Nitrogen

Nitrogen (N) is the most extensively studied nutrient with respect to forest productivity and nutrient cycling. Foliar N is largely present in chlorophylls, proteins, and enzymes (Loomis, 1997), and is therefore the main nutrient element cycled in litterfall (Medina and Cuevas, 1989). Decades of research suggest that N is relatively more available to plants in the lowland tropics compared to temperate forests but there is also ample evidence for N-limitation of multiple ecosystem processes in tropical forests (e.g. Kaspari et al., 2008; Wright et al., 2018).

The forest soils in the Panama Canal area are relatively rich in N (Hietz et al., 2011; Santiago et al., 2005). High rates of N mineralisation at our study site (Yavitt, 2000) indicate rapid N cycling (Adams, 1986) and a “leaky” N-cycle characterised by substantial N losses in gaseous forms or via leaching (Corre et al., 2010; Erickson et al., 2001; Koehler et al., 2009). As the declines in soil N with litter removal in temperate forests were greater in soils with high initial N content (Sayer, 2006), we also expected substantial losses of N in our L – plots. Accordingly, during the first 5 years of treatments (2004–2007) soil inorganic N concentrations declined rapidly with litter removal, and there was a sizeable increase in nitrate-N with litter addition (Sayer and Tanner, 2010). A decade later (2016–2018), litter manipulation has clearly affected total N concentrations in the topsoil, as well as nitrate-N and litter N concentrations (Fig. 2A and C).

The largest and most rapid changes in response to litter manipulation were observed for nitrate-N concentrations in the soil (Fig. 2A). Within 2 years of the start of treatments, soil nitrate-N concentrations had declined with litter removal and increased with litter addition (Sayer and Tanner, 2010). After 7 years, nitrate-N had declined by 57% in L – plots, increased threefold in L + plots (Sayer et al., 2012) and the higher nitrate-N concentrations with litter addition were apparent to 15 cm depth (Tanner et al., 2016). The differences in soil nitrate-N concentrations between L – and L + plots have persisted throughout the experiment, with a greater overall effect of litter removal ($d = -2.38 \pm 0.34$, $P < 0.001$) compared to litter addition ($d = 1.83 \pm 0.31$, $P < 0.001$) and soil nitrate-N increased in the L + plots over time ($F_{1,38} = 8.1$; $P = 0.007$). After 15 years of treatments, the exchange rate of nitrate-N in L + plots was at least two-fold higher than in control plots in both the early and late rainy season ($P < 0.001$; Fig. 3A). We hypothesise that these changes in nitrate-N in response to litter manipulation occur because heterotrophic nitrification is stimulated by organic matter (Adams, 1986; Verstraete and Focht, 1977) and uses organic N compounds instead of ammonium as a substrate (Pedersen et al., 1999; Schimel et al., 1984).

Total soil N did not respond to litter manipulation during the first year of the experiment (Sayer et al., 2012) but concentrations of total N in the L – plots declined after 2009 ($F_{1,21} = 4.8$; $P = 0.04$; Fig. 2B), with a large effect size ($d = -2.21 \pm 0.31$, $P < 0.001$). We expected the total soil N pool to respond relatively slowly to litter manipulation because a large fraction of total soil N is bound in soil organic matter (Knicker et al., 1993). Long-term reductions in N inputs from leaf litter and decreased availability of inorganic

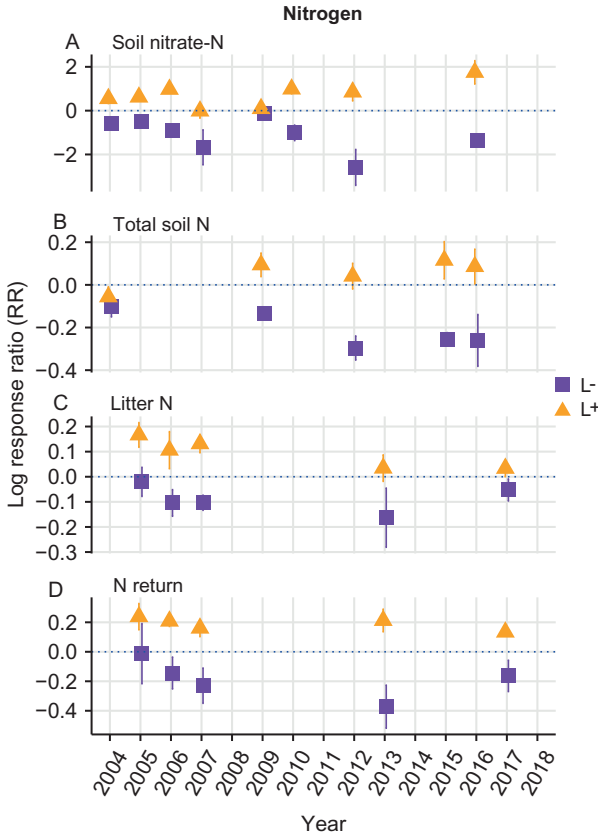


Fig. 2 Changes in nitrogen (N) cycling in response to 15 years of litter addition (orange triangles) and litter removal (purple squares) treatments in a lowland tropical forest in Panama, Central America. Differences in (A) nitrate-N and (B) total N concentrations in the soil at 0–10 cm depth, (C) N concentrations in litter (litter N) and (D) the estimated annual N return by litterfall (N return) are given as log response ratios, where the dashed blue line ($y=0$) indicates no difference relative to controls; dots and whiskers are means and standard errors for $n=5$.

N in soils are likely to have stimulated microbial degradation of soil organic matter as a source of N (Nottingham et al., 2012, 2015). Consistent with this hypothesis, the L– treatment reduced soil organic C concentrations ($d = -2.29 \pm 0.34$, $P < 0.001$; Fig. 4A), microbial biomass C ($d = -1.27 \pm 0.24$; $P = 0.025$; Fig. 4A) and microbial biomass N ($d = -1.28 \pm 0.24$; $P = 0.026$; Fig. 4C). The negligible effect of litter addition on total soil N concentrations and microbial biomass corresponds to the smaller increase in soil organic C in the L+ plots ($d = 0.75 \pm 0.20$, $P = 0.005$; Figs 3B and 4A–C).

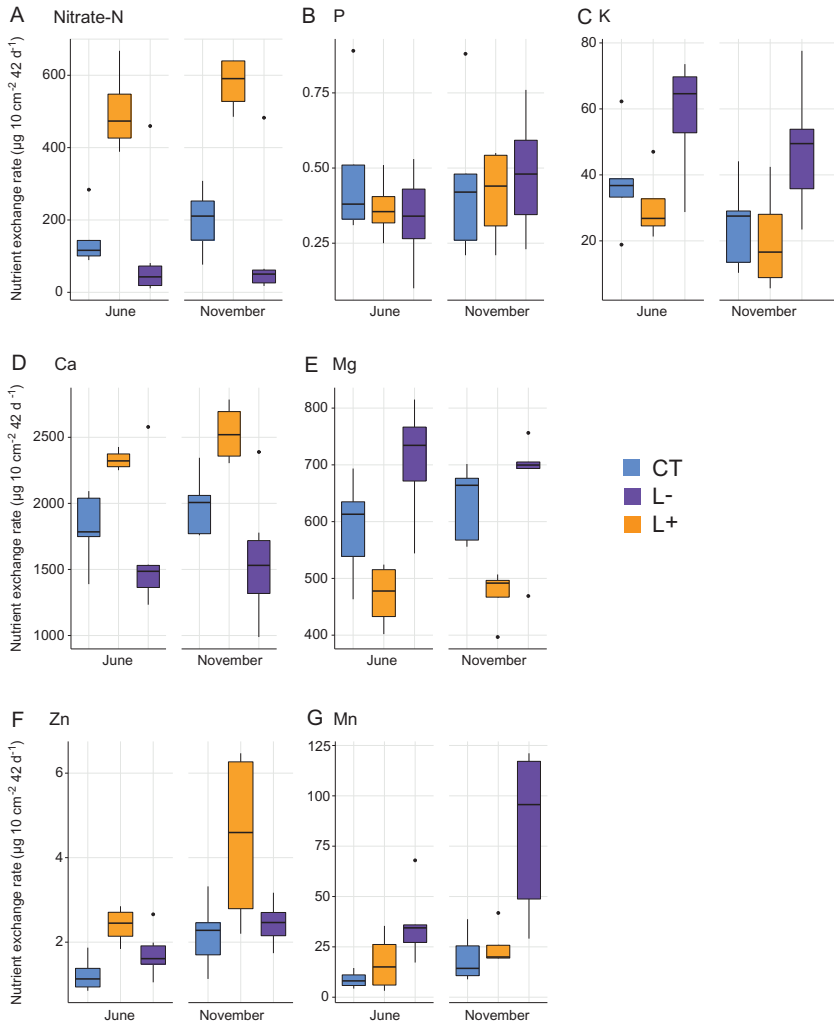


Fig. 3 Exchange rates of (A) soil nitrate-N, (B) phosphorus (P), (C) potassium (K), (D) calcium (Ca), (E) magnesium (Mg), (F) zinc (Zn), and (G) manganese (Mn) after 15 years of litter addition (L+, orange) and litter removal (L-, purple) treatments compared to controls (CT, blue) during the early rainy season (June) and towards the end of the rainy season (November) in a lowland tropical forest in Panama, Central America; nutrient exchange rates were measured by ion exchange resin strips at 0–10 cm depth during 42 days; boxes denote the 25th and 75th percentiles and median lines are given for $n = 5$, whiskers indicate values up to $1.5 \times$ the interquartile range, and dots indicate outliers.

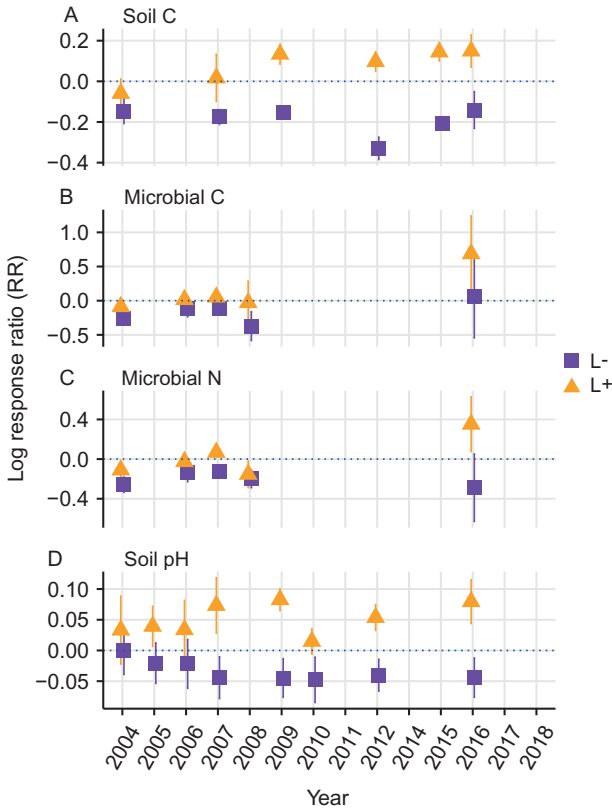


Fig. 4 Changes in (A) soil carbon concentrations (soil C), (B) microbial biomass carbon (microbial C), (C) microbial biomass nitrogen (microbial N) and (D) soil pH at 0–10 cm depth in response to 15 years of litter addition (orange triangles) and litter removal (purple squares) treatments in a lowland tropical forest in Panama, Central America. Differences are given as log response ratios, where the dashed blue line ($y=0$) indicates no difference relative to controls; dots and whiskers are means and standard errors for $n=5$.

The N concentrations in leaves (Sayer and Tanner, 2010), roots (Rodtassana and Tanner, 2018) and litter (Sayer et al., 2012) declined in the L– plots, with corresponding increases in the L+ plots and particularly rapid increases with litter addition in the first years of the experiment (Sayer and Tanner, 2010; Fig. 2C). However, the effect of litter addition declined over time ($F_{1,23}=5.0$; $P=0.034$) and by 2017, the mean effects on litter N concentrations were similar for litter addition ($d=1.13\pm 0.21$, $P<0.001$) and litter removal ($d=-0.90\pm 0.11$, $P<0.001$). The changes in litter N concentrations likely reflect the differences in nitrate-N

availability among treatments because microbial retention of nitrate-N is low, plants are better competitors for nitrate-N than microorganisms, and rates of N mineralisation are usually closely related to plant uptake (Attiwill and Adams, 1993). Lower availability of N in the L – plots could have contributed to lower abundance of arbuscular mycorrhizal (AM) fungi and the significant changes in AM fungal communities observed after 9 years of litter removal (Sheldrake et al., 2017). AM fungal hyphae have much higher N concentrations than plant tissues (Hodge et al., 2010) and hence the disruption of the N cycle by the removal of litter could have a negative impact on the abundance of some AM fungal taxa (Sheldrake et al., 2017).

Nitrogen return in litterfall also declined substantially with litter removal ($d = -1.72 \pm 0.44$, $P = 0.025$) and increased with litter addition ($d = 1.40 \pm 0.02$, $P < 0.001$). We transferred *c.* $149 \text{ kg N ha}^{-1} \text{ year}^{-1}$ from L – to L + plots (Table 2) and we initially predicted that higher N concentrations in plant material would create a positive feedback to litter addition by boosting photosynthetic capacity (Sayer and Tanner, 2010) and contributing to increased litter production in the L + plots (Sayer et al., 2012; Rodtassana, 2016; Rodtassana et al. unpublished data). However, there was no trajectory of change in N return by litterfall over time (Fig. 2D) and hence, the increasing levels of nitrate-N in the soil suggest that nitrate-N supply has exceeded plant demand in the L + plots. Indeed, high concentrations of nitrate-N (Fig. 2A) and increased nitrous oxide emissions from the soil in the L + plots (Welch et al., 2019) indicate an increasingly leaky N-cycle and the potential for substantial N losses from the system after 15 years of litter addition.

There is a strong relationship between N use efficiency and N availability across temperate and tropical forests (Vitousek, 1982). Accordingly, after 15 years of litter manipulation, N use efficiency had increased in the L – plots ($d = 0.90 \pm 0.16$; $P < 0.001$) and declined in the L + plots ($d = -1.16 \pm 0.27$; $P = 0.002$) relative to the controls (Fig. 5A). The N use efficiency we estimated for L – plots (79), L + plots (64), and controls (70) places our study site among N-rich tropical forests (NUE < 80; Vitousek, 1982), but the enhanced N use efficiency in the L – plots is perhaps the first sign of N-limitation after 15 years of litter removal. Hence, despite the initial moderate availability of soil N at our study site and local atmospheric N deposition of *c.* $9 \text{ kg ha}^{-1} \text{ year}^{-1}$ (Hietz et al., 2011), we observed substantial changes in N cycling in response to 15 years of litter manipulation, which illustrates the importance of N cycling by litterfall and N retention in organic matter.

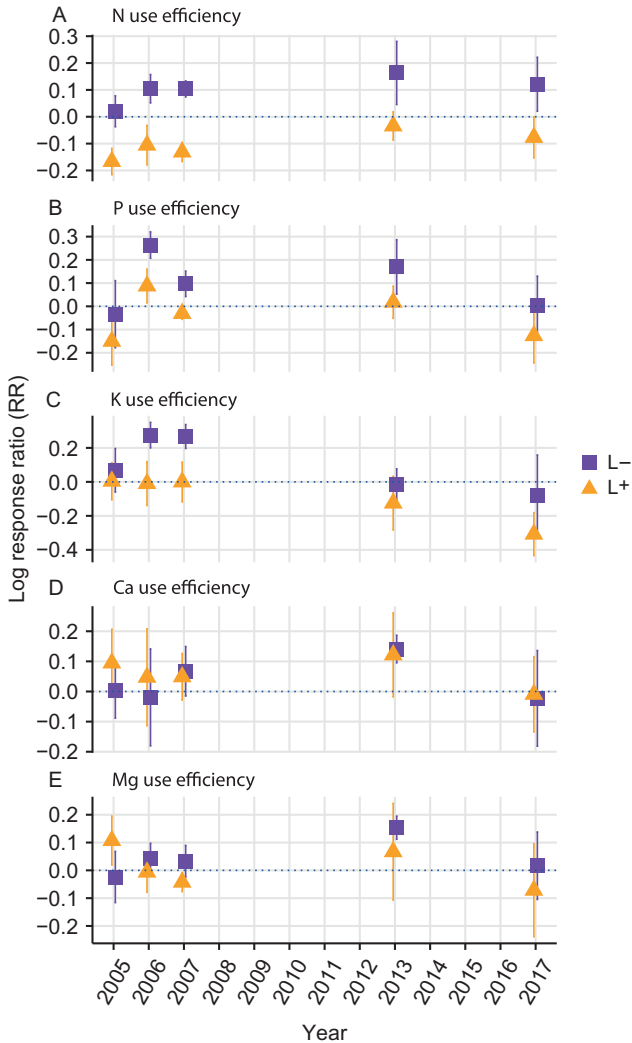


Fig. 5 Changes in nutrient use efficiency for (A) nitrogen (N), (B) phosphorus (P), (C) potassium (K), (D) calcium (Ca) and (E) magnesium (Mg) in response to 15 years of litter addition (orange triangles) and litter removal (purple squares) treatments in a lowland tropical forest in Panama, Central America. Differences are given as log response ratios, where the dashed blue line ($y=0$) indicates no difference relative to controls; dots and whiskers are means and standard errors for $n=5$.

The manifest changes in soil and litter N concentrations and N use efficiency during 15 years of litter manipulation not only highlight the importance of litterfall for N cycling but are also characteristic of an ecosystem adapted to high N availability. The rapid declines in soil and litter N concentrations in the L- plots indicate that conservation mechanisms

for N are lacking, and the changes in N use efficiency support [Wright's \(2019\)](#) hypothesis that tropical trees adapted to high levels of N cycling also have relatively high demands for N.

3.2 Phosphorus

Phosphorus (P) is a key component of numerous compounds in leaves, including cell walls, nucleic acids, ATP and co-enzymes. Unlike N, a large proportion of foliar P is present in inorganic forms ([Sinclair and Vadez, 2002](#)). Much of the demand for P in natural forests can be met by the cycling of P in organic matter ([Attiwill and Adams, 1993](#)), especially in old, highly weathered tropical soils with physical and chemical properties that promote strong P sorption ([Townsend et al., 2011](#); [Vitousek et al., 2010](#)). Hence, many lowland tropical forests have low soil concentrations of available P ([John et al., 2007](#); [Vitousek, 1984](#); [Vitousek et al., 2010](#)) and a large proportion of P cycling can be restricted to organic horizons in soils with high content of iron and aluminium oxides ([Attiwill and Adams, 1993](#)). Lowland tropical tree species have likely evolved strategies to adapt to low soil P availability ([Mo et al., 2019](#); [Zalamea et al., 2016](#)), resulting in a “tight” or highly efficient P cycle ([Vitousek, 1984](#)). Numerous lines of evidence indicate that P cycling at our study site is conservative and that various ecosystem processes are limited by P availability, including decomposition ([Kaspari et al., 2008](#)), seedling growth and photosynthesis ([Pasquini and Santiago, 2012](#); [Santiago et al., 2012](#)), and tree growth ([Wright et al., 2011](#)).

Litter inputs play a dual role in maintaining P cycling in soils with high sorption capacity. First, the P contained in litter is much more mobile than that held in the soil, as the latter is rapidly sorbed to iron, aluminium and calcium in soil minerals ([Yavitt, 2000](#)) and hence, litterfall provides much of the P required for plant growth ([Attiwill and Adams, 1993](#); [Vitousek, 1982](#)). Second, compounds released during litter decomposition can increase the overall availability of inorganic P in soils by forming complexes with aluminium and iron ([Ehrenfeld et al., 2005](#)) and reducing P sorption ([Palm et al., 1997](#)). Our litter manipulation treatments clearly have the capacity to influence both of these aspects of P cycling. Using a radioactive phosphate tracer (^{32}P), [Schreeg et al. \(2013\)](#) demonstrated lower P sorption in L+ soils and higher P sorption in L- soils compared to controls after 5 years of litter manipulation treatments. Hence, given the low relative availability of inorganic P in the soil, we expected that litter addition would enhance P availability, whereas P concentrations in soil and litter would

decline rapidly with litter removal (Sayer and Tanner, 2010), resulting in greater P use efficiency or reduced forest productivity.

The patterns of change we observed for P concentrations in soil and litter are at odds with our initial expectations. During the first 5 years of treatments, we measured lower soil extractable P (P_{EXTR}) concentrations in the L- plots compared to controls at 0–2 cm depth (Sayer and Tanner, 2010; Vincent et al., 2010), lower P_{EXTR} in the L- plots compared to L+ plots to 20-cm depth (Tanner et al., 2016), and lower litter P concentrations in the L- plots (Sayer and Tanner, 2010). We had expected to observe a gradual decline in soil and litter P concentrations in response to chronic removal of P with litter and it is surprising that there was no clear trajectory of change in soil or litter P concentrations in the L- plots over the 15 years of treatments (Fig. 6A and B). Despite a large overall effect of litter removal on P_{EXTR} ($d = -1.13 \pm 0.28$, $P = 0.008$), the effect varied widely from year to year (Fig. 6A). The difference between treatments can be explained by a small but more consistent trend towards increasing soil P_{EXTR} concentrations with litter addition ($P = 0.084$), which increased over time ($F_{1,38} = 5.0$, $P = 0.03$; $d = 0.68 \pm 0.29$; Fig. 6A). However, even after almost 15 years of treatments, phosphate exchange rates in the topsoil were unaffected by litter manipulation (Fig. 3B). The decrease in litter P concentrations was also smaller than expected ($d = -0.70 \pm 0.29$; $P = 0.06$; Fig. 6B), and although there was a significant treatment effect on P return by litterfall, neither treatment differed significantly from the controls.

Various studies in the GLIMP plots provide two plausible explanations for the apparent lack of substantial changes in P cycling with litter removal: (1) declining plant productivity has reduced the overall requirement for P uptake by plants and (2) plants have access to other sources of P. Although the growth of mature trees appears to be unaffected by litter manipulation to date (Sayer and Tanner, 2010; Edmund Tanner et al., unpublished data), lower annual litterfall in the L- plots (Rodtassana, 2016; C. Rodtassana et al., unpublished data) and lower P return in leaf litter (Fig. 6C) indicates that decreased leaf production has contributed to reduced plant P demand. Nonetheless, the slight decline in P return in litterfall only accounts for *c.* 13% of the P transferred annually from the L+ to L- plots (*c.* 5 kg P ha⁻¹; Table 2) and it is likely that alternative sources of P play a key role in maintaining plant P supply at our study site.

Organic P (P_{ORG}) is a likely alternative source of P for plants because it is abundant in tropical mineral soils (Harrison, 1987; Turner and Engelbrecht, 2011) and turnover of P_{ORG} could supply substantial amounts

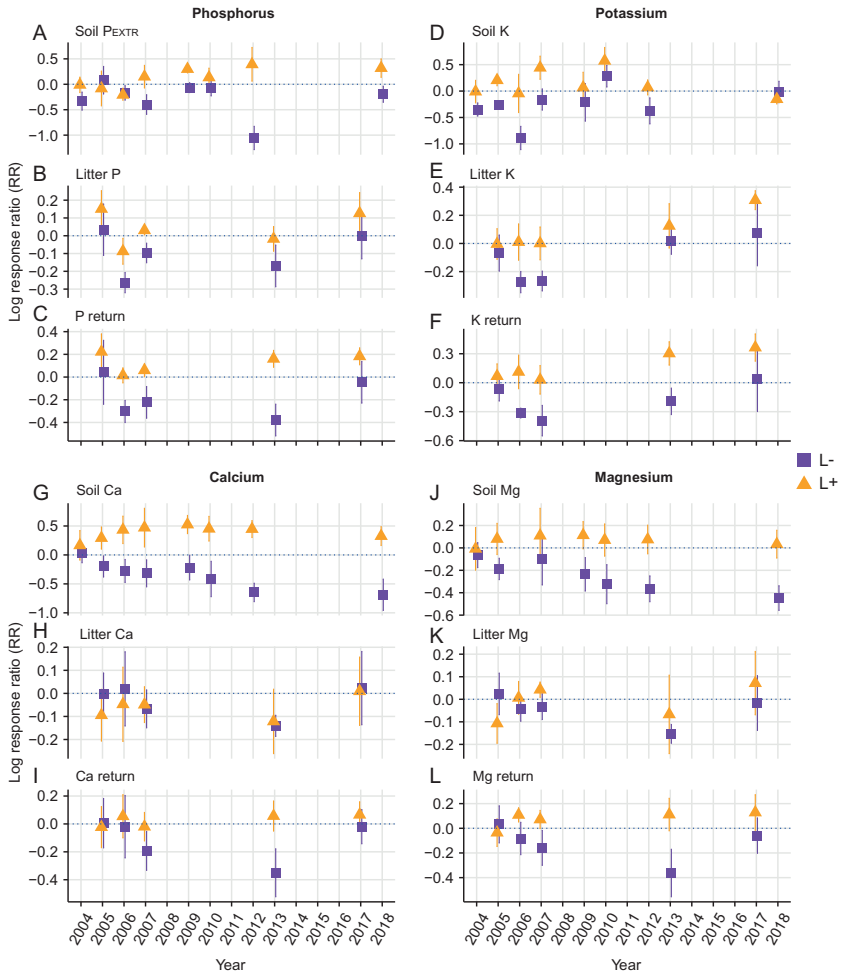


Fig. 6 Changes in (A–C) phosphorus (P), (D–F) potassium (K), (G–I) calcium (Ca) and (J–L) magnesium (Mg) cycling in response to 15 years of litter addition (orange triangles) and litter removal (purple squares) treatments in a lowland tropical forest in Panama, Central America. Differences in soil extractable nutrient concentrations at 0–10 cm depth (A, D, G, J), litter nutrient concentrations (B, E, H, K) and the estimated annual nutrient return by litterfall (C, F, I, L) are given as log response ratios, where the dashed blue line ($y=0$) indicates no difference relative to controls; dots and whiskers are means and standard errors for $n=5$.

of bioavailable P (Tiessen et al., 1992). In 2006, after 3 years of treatments, P_{ORG} in the surface soils of the L– plots had already declined by 23% relative to the controls and increased turnover of P_{ORG} provided an estimated $\approx 1.4 \text{ kg P ha}^{-1} \text{ year}^{-1}$ in the L– treatment (Vincent et al., 2010),

representing almost a third of the P removed with litter. After 9 years of litter removal, P_{ORG} was *c.* 18% lower in the L– plots than the controls and microbial P had declined by two thirds (Sheldrake et al., 2017). Hence, increased turnover of P_{ORG} has likely compensated for the lower availability of inorganic P to some extent (Vincent et al., 2010) but it is uncertain how long this alternative supply can meet demands. It will be a matter of great interest to monitor changes in distinct P pools to understand the potential for P_{ORG} to sustain forest P–status in future.

Phosphorus availability is also influenced by AM fungi, as they represent a crucial mechanism for the uptake of P and its transport to plants (Smith and Read, 2010). Sheldrake et al. (2017, 2018) observed shifts in AM fungal communities in the L– plots that potentially signal a change in P acquisition strategies, and which could help plants to maintain their P status. Although both litter manipulation treatments altered AM fungal communities in the soil, only litter removal altered fungal communities in roots, indicating a potential change in plant–mycorrhizal relationships (Sheldrake et al., 2018). In addition, AM fungal community composition in the L– plots was similar to adjacent N-fertilised plots (Sheldrake et al., 2018), which is noteworthy because greater availability of N can create or exacerbate P-limitation (Vitousek et al., 2010). Hence, the similarity of AM fungal communities in the L– and N-fertilised plots in our study forest indicates a potentially important role for AM fungi in plant P-acquisition when the demand for readily available P outstrips the supply (Sheldrake et al., 2018).

It is conceivable that, despite the removal of large amounts of P, declining litter production reduced the demand for P in the L– plots, while increased turnover of P_{ORG} replenished soil supplies, and greater P-acquisition by altered mycorrhizal associations helped meet plant P requirements (Sheldrake et al., 2018). Although we cannot rule out other mechanisms of P supply, such as the transport of P reserves from subsoil horizons to the topsoil by deep roots (Grubb, 1989), it is nonetheless surprising that the annual removal of P (77 kg P ha^{-1} in total over 15 years; Table 2) has not resulted in more pronounced changes in P cycling after 15 years. Given the substantial decline in organic P reserves after 9 years of litter removal (Sheldrake et al., 2017), it is possible that the P status of trees in the L– plots will eventually decline to levels that substantially impede productivity.

The effects of litter addition on P cycling were also minor and inconsistent. Although there was no discernible change in soil P concentrations with

litter addition for several years, this coincided with increasing litter production during the rainy season (Sayer et al., 2012; Sayer and Tanner, 2010), which indicates that the extra nutrients added with the litter were used to boost productivity. If this was the case, soil P_{EXTR} only started to increase once litter production had reached a new equilibrium and the nutrient demands for greater litter production were met. However, we also observed no consistent increase in litter P concentrations and the trend towards greater P return by litterfall in the L+ plots ($d = 0.88 \pm 0.16$, $P = 0.05$; Fig. 6C) can be attributed to greater litter production (Sayer et al., 2012; Rodtassana et al. unpublished data).

Some of the temporal variation in P cycling with litter addition might be due to the first application of the treatment in January 2003, which comprised the entire litter standing crop from the L- plots. Based on nutrient concentrations in the litter standing crop of the control plots in 2005 (Sayer et al., 2010), the first application of litter to the L+ plots would have doubled the amount of P in the litter standing crop (*c.* 11 kg P ha⁻¹; Sayer et al., 2010), which is twice the mean amount of P returned annually with litterfall (Table 2). The large increase in P return in litterfall in the L+ plots in 2005 could therefore signal enhanced litter production and higher foliar P concentrations in response to the large pulse of nutrients added with litter at the start of the experiment. A similar substantial and rapid change to litter P inputs, with no corresponding change in soil P concentrations, was observed after a one-off fourfold increase in litter inputs in a wet tropical forest in Costa Rica (Wood et al., 2009). Hence, much of the P cycled in litterfall is likely to be taken up by plants (Sayer et al., 2012). Furthermore, higher soil P_{ORG} concentrations at the surface after 3 years of litter addition (Vincent et al., 2010) and a *c.* 11% increase in P_{ORG} in top-soil after 9 years of litter addition (Sheldrake et al., 2017) indicate that above-ground litter also plays a role in maintaining P_{ORG} stocks. This seems likely because litter is a source of organic compounds such as DNA (Turner and Engelbrecht, 2011) and because more readily accessible P inputs from litter would reduce the turnover rates for P_{ORG} in the soil (Vincent et al., 2010).

The P use efficiency at our study site does not indicate severe P-limitation (Vitousek, 1982), but we nonetheless observed slightly higher P use efficiency in L- plots compared to the controls ($d = 0.79 \pm 0.45$; $P = 0.036$), although there was no consistent trajectory of change in P use efficiency over time (Fig. 5B). Taken together, GLiMP studies support the concept of tight P cycling in lowland tropical forest, but we found little evidence for P-limitation with litter removal. Although it is possible that

alternative sources of P are able to compensate for the substantial removal of nutrients with litter, it is also conceivable that co-limitation of different biological processes by multiple nutrients (Kaspari et al., 2008; Wright et al., 2011) has masked changes in P cycling. The balance of nutrient elements in plant tissues is largely determined by the requirements of essential biological processes, as well as adaptation to local conditions, and is thus less flexible than previously thought (Elser et al., 2010). Based on the premise that the N-cycle in our forest is leaky, but P-cycling is conservative, rapid and substantial losses of N with litter removal could have reduced P requirements, which would explain the contrasting results for shifts in N and P cycling in response to litter removal. Relatively constant litter N:P ratios in both treatments provide some support for stoichiometric constraints of plant responses to the litter manipulation treatments. Indeed, the simultaneous removal of multiple nutrients with the litter could constrain the cycles of individual elements and their potential responses to the L- treatment.

All things considered, the surprisingly minor response of P cycling to long-term litter removal can be attributed to a combination of P conservation mechanisms, as well as exploitation of alternative P sources and reduced P demand with the removal of multiple other elements, in particular N. New research to determine the relative importance of different processes in maintaining tropical forest P status could provide valuable information for tropical forest restoration on P-poor soils.

3.3 Potassium

Potassium regulates stomatal activity, enzyme activation, and the transport of water in plants (Prajapati and Modi, 2012) but as K is rapidly leached from the canopy (Likens et al., 1994), throughfall is the major pathway for K cycling in tropical forests (Vitousek and Sanford, 1986). Nonetheless, we transferred *c.* 47 kg K ha⁻¹ year⁻¹ between L- and L+ plots (Table 2), which is 3/4 of the K inputs from throughfall at the study site (Vitousek and Sanford, 1986). In addition, there are strong biotic controls on K cycling (Tripler et al., 2006) and the forest floor contributes substantially to the retention of K (Tobón et al., 2004).

The concentrations of K in the soils at our study site (84–131 mg kg⁻¹) are similar to soils across Mesoamerica (Barthold et al., 2008; Wright et al., 2011) but concentrations of exchangeable K are lower than expected (Yavitt et al., 2009) and previous work in the study forest has demonstrated K-limitation of plant growth (Santiago et al., 2012; Wright et al., 2011;

Yavitt et al., 2011) and decomposition processes (Kaspari et al., 2008). We expected that availability of K to plants in the L+ plots would be enhanced by greater K retention in the forest floor, indicated by substantially reduced K release from litter in the L+ plots during the early stages of decomposition (Sayer et al., 2006b). We further expected that the impact of litter removal on K availability would be amplified by higher leaching losses (Sayer et al., 2012). It is thus surprising that the initial strong responses of soil K concentrations to both litter manipulation treatments appear to have attenuated over time (Fig. 6D). We initially measured a rapid and sizeable decline in soil K concentrations in the L- plots between 2004 and 2006, but although soil K concentrations were lower in the L- plots compared to the controls across all years, the effects were moderate ($d = -0.76 \pm 0.15$, $P = 0.041$) and inconsistent (Fig. 6D). In the L+ plots, soil K concentrations increased markedly in 2005 and 2007, and although soil K generally increased in response to litter addition ($P = 0.039$) the pattern over time was so inconsistent that the mean effect across all years was negligible ($d = 0.38 \pm 0.15$; Fig. 6D).

High temporal variability in leaching losses of K provides one explanation for the lack of a clear trajectory of change in soil K concentrations over time. Potassium is highly soluble and readily leached from litter (Schreeg et al., 2013) and soil (Likens et al., 1994); hence, even small shifts in sampling times combined with variable inter- and intra-annual precipitation patterns are likely to influence our results. We sampled soils at the end of the rainy season each year from 2004 to 2007 (Sayer et al., 2012; Sayer and Tanner, 2010) but during or at the end of the dry season in 2009, 2010, 2012 and 2018 (Ashford et al., 2013; Sheldrake et al., 2017; Tanner et al., 2016), which complicates the interpretation of long-term patterns. Previous work at our study site, and nearby, demonstrated accumulation of K in soils during the dry season (Yavitt and Wright, 1996) and rapid release of K from decomposing litter at the start of the rainy season (Cornejo et al., 1994; Sayer et al., 2006b). Accordingly, our measurements of nutrient exchange rates using resin probes showed a clear decline in K supply over the course of the rainy season. Greater exchange rates of K in the L- plots ($P = 0.037$; Fig. 3C) could suggest that more K is reaching the mineral soil via throughfall and greater movement of K through the soil in the absence of a litter layer (Sayer et al., 2012).

Treatment differences for litter K concentrations are less variable than those for soil because we determined nutrients in litter samples collected at the same time each year. Although K leaches rapidly from leaves after

abscission (Schreeg et al., 2013), and substantial losses of K can occur from litter traps when samples are collected monthly (Cuevas and Medina, 1986), it is reasonable to assume that relative losses of K from litter-traps were the same across all three treatments.

The initial decline in litter K concentrations in the L – plots largely mirrors the patterns for soil K, with substantial declines in 2006 and 2007 but only a minor and inconsistent effect across all years (litter: $P=0.036$, $d=-0.54\pm 0.26$; Fig. 6E). The overall decline in K return by litterfall ($d=-1.11\pm 0.38$, $P=0.005$) suggests that the declining K supply in the L – plots is unable to meet plant demands (Fig. 6F). However, interannual variability was high and in 2017, after 15 years of litter removal, we measured similar litter K concentrations and K return by litterfall to the controls (Fig. 6E and F). By contrast, although litter K concentrations and K return by litter were unaffected by litter addition in the first 5 years of the experiment, litter K concentrations increased over time in response to litter addition ($F_{1,23}=5.3$, $P=0.03$) and K return was higher in later years ($F_{1,23}=4.4$, $P=0.048$; Fig. 6F). Direct uptake of K by roots from decomposing litter (Burghouts et al., 1992) in the L+ plots could explain the higher litter K concentrations and increased K return by litterfall in 2013 and 2017. We measured greater K stocks in the forest floor (Sayer et al., 2010) as well as higher fine root biomass in the forest floor within 2 years of litter addition (Sayer et al., 2006a), and the latter persisted even after 10 years of treatments (Rodtassana and Tanner, 2018). However, given the high interannual variability in soil and litter K concentrations, it remains to be seen if this trend persists in future.

Surprisingly, there was some indication that litter manipulation altered K use efficiency (treatment effect: $P=0.008$, Fig. 5C), although neither treatment differed significantly from the controls. Nonetheless, differences in K use efficiency between litter removal and addition treatments support various lines of evidence that processes at our study site are limited by low K availability (Kaspari et al., 2008; Wright et al., 2011; Wright et al., 2018). Hence, despite the importance of K inputs by throughfall and the high variability in soil and litter K concentrations among years, the GLiMP studies nonetheless demonstrate that litterfall contributes substantially to K cycling in our study forest. Importantly, litter inputs also appear to play a role in reducing leaching losses of K via retention in the forest floor and possible direct uptake by roots from decomposing organic matter.

3.4 Calcium and magnesium

Calcium (Ca) and magnesium (Mg) are two of the most abundant elements in litter (Tobón et al., 2004) and we transferred $c. 169 \text{ kg Ca ha}^{-1} \text{ year}^{-1}$ and $c. 37 \text{ kg Mg ha}^{-1} \text{ year}^{-1}$ between L- and L+ plots (Table 2). Calcium is one of the main constituents of cell walls in leaves (Kirkby and Pilbeam, 1984), whereas Mg plays a key role in photosynthesis (Cakmak and Kirkby, 2008). Litterfall can contribute 80–90% of the Ca and 65–85% of the Mg being cycled in tropical forests (Campo et al., 2000; Parker, 1983) and although Ca or Mg can be limiting to productivity in some tropical forests (Cuevas and Medina, 1988), the soils at our study site are relatively rich in exchangeable Mg (553 mg kg^{-1}) and particularly rich in Ca (1690 mg kg^{-1}) compared to other highly weathered tropical soils (Yavitt et al., 2009).

Despite high Ca concentrations in the topsoil, we observed sizeable and rapid changes in soil Ca (Ca^{2+}) to litter manipulation (Sayer and Tanner, 2010). As substantial amounts of Ca cycle through litterfall (Table 2), soil Ca concentrations increased with litter addition ($d = 1.28 \pm 0.12$, $P < 0.001$), decreased with litter removal ($d = -1.21 \pm 0.31$, $P < 0.001$), and the effect of litter removal became more pronounced over time ($F_{1,37} = 7.6$, $P = 0.009$; Fig. 6G). Our study of nutrient exchange rates also demonstrates the importance of litterfall and decomposition for Ca cycling. As Ca is a structural component of leaves and is not soluble (Schreeg et al., 2013), its release during decomposition strongly mirrors mass loss (Gosz et al., 1976) and thus Ca exchange rates were higher in the L+ plots ($P = 0.034$), especially towards the end of the rainy season, but remained unchanged in the L- plots (Fig. 3D). Litter Ca concentrations did not change significantly with either treatment, but this is not surprising given the high soil Ca concentrations at our study site (Yavitt et al., 2009), which likely represent the main source of Ca for plants. Thus, although the concentrations of exchangeable Ca in the soil were altered by litter manipulation, litter Ca concentrations and Ca return by litter were unaffected by litter manipulation (Fig. 6H and I).

The patterns of change for Mg in soil and litter are broadly similar to those for Ca (Fig. 6J–L), and differences in the cycling of the two elements can largely be explained by the greater mobility of Mg and its crucial role in various processes involved in photosynthesis (Cakmak and Kirkby, 2008). Both high and low concentrations of Mg in plant cells can have adverse effects on photosynthesis (Shaul, 2002) and the maintenance of Mg homeostasis in plant tissues could explain why litter Mg concentrations remained

relatively constant, even though soil Mg concentrations were significantly lower in L- plots compared to controls ($d = -1.09 \pm 0.16$, $P < 0.001$) and declined over time ($F_{1,32} = 5.7$, $P = 0.02$; Fig. 6J). Although litter addition had no discernible effect on soil Mg concentrations, Mg exchange rates in the topsoil were lower in L+ plots compared to the controls ($P < 0.001$), especially in the late growing season, and there was a non-significant trend towards higher Mg exchange rates in the L- plots ($P = 0.12$; Fig. 3E). However, although Mg is relatively mobile and prone to leaching, it is also retained in organic matter in the forest floor (Gosz et al., 1973; Gransee and Fühns, 2013). We hypothesise that the slight increase in Mg exchange rates in the L- plots reflects greater leaching losses with litter removal and the lower exchange rates of Mg with litter addition reflect accumulation of Mg in the thick forest floor of the L+ plots (Sayer et al., 2010) and slower release of Mg during decomposition (Sayer et al., 2006b).

Calcium and Mg are important for exchange reactions that buffer acidity in many soils, so the observed changes in soil Ca and Mg concentrations probably resulted in the sizeable increase in soil pH with litter addition ($d = 1.12 \pm 0.22$, $P < 0.001$) and the parallel decline with litter removal ($d = -0.83 \pm 0.15$, $P = 0.004$; Fig. 4D). Although soil acidification with increased nitrification is a common issue in fertilisation experiments (Wright, 2019), it is unlikely to explain the changes in pH in response to litter manipulation because we would expect lower rates of nitrification in the L- plots. High spatial variation in forest soil pH is associated with the concentrations of exchangeable Ca and Mg (Finzi et al., 1998) and a litter manipulation study in temperate forest observed a strong relationship between changes in Ca and Mg concentrations and soil pH (Tóth et al., 2011). At our study site, the response of soil pH to litter addition and removal treatments was strongly related to the magnitude of change in the combined concentrations of Ca and Mg ($R^2 = 0.73$, $P < 0.001$; Fig. 7). The initial rapid changes in soil pH reflect the low buffering capacity of the soil at our study site (Yavitt et al., 2009) and the considerable influence of organic matter inputs on soil pH (McFee et al., 1977). Hence, nutrient shortages with litter removal could be exacerbated by the combination of lower soil pH and declining soil organic C concentrations.

Collectively, the GLiMP studies demonstrate that litterfall plays an important role in maintaining soil Ca and Mg concentrations and regulating soil pH, despite high availability of Ca and Mg in the soils at our study site. The marked changes in soil Ca, Mg and pH we observed with litter removal in particular suggest that litterfall may be crucial for buffering pH changes and maintaining the availability Ca and Mg in the majority of tropical forests.

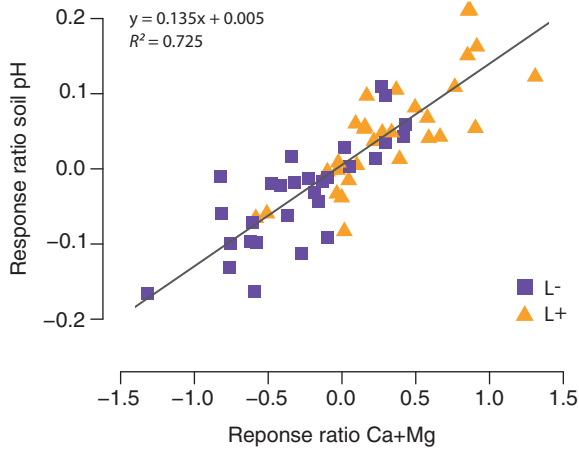


Fig. 7 The relationship between changes in soil calcium and magnesium concentrations (Ca + Mg) and changes in soil pH at 0–10 cm depth during 15 years of litter addition (yellow triangles) and litter removal (purple squares) treatments in a lowland tropical forest in Panama, Central America. Changes relative to the controls are given as log response ratios calculated for each of $n = 5$ replicate blocks and $n = 6$ years (2004, 2005, 2007, 2009, 2010, 2012).



4. Micronutrients and decomposition processes

Our knowledge of micronutrient cycling in natural ecosystems is limited and the roles of biological processes in the availability of trace elements in soils has yet to be fully established (Li et al., 2008). There is a particular dearth of information about trace element cycling in tropical forest ecosystems, even though they are essential for biological processes from the cellular level to the ecosystem scale (Kaspari and Powers, 2016). The role of trace elements as enzyme co-factors makes them particularly crucial for nutrient cycling, as well as decomposition (Kaspari et al., 2008, 2014; Powers and Salute, 2011) and soil food webs (Kaspari et al., 2017) in tropical forests. Here, we present the evidence for biotic cycling of four micronutrients and discuss the specific links to decomposition processes and the nutrient requirements of decomposer organisms.

4.1 Boron, sodium, zinc and manganese

We measured concentrations of five micronutrients (boron, iron, manganese, sodium and zinc) in multiple years. The concentrations of boron

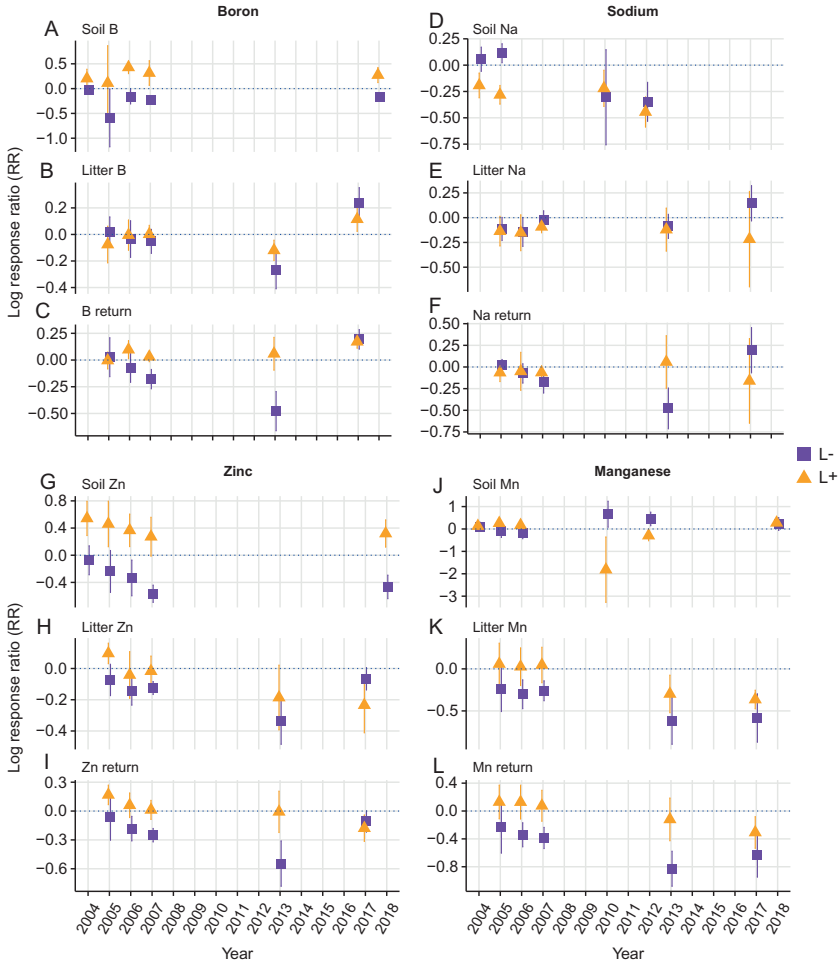


Fig. 8 Changes in (A–C) boron (B), (D–F) sodium (Na), (G–I) zinc (Zn) and (J–L) manganese (Mn) cycling in response to 15 years of litter addition (orange triangles) and litter removal (purple squares) treatments in a lowland tropical forest in Panama, Central America. Differences in soil extractable nutrient concentrations at 0–10 cm depth (A, D, G, J), litter nutrient concentrations (B, E, H, K) and the estimated annual nutrient return by litterfall (C, E, I, L) are given as log response ratios, where the dashed blue line ($y=0$) indicates no difference relative to controls; dots and whiskers are means and standard errors for $n=5$.

(B) sodium (Na), and zinc (Zn) in the soil, and the concentration of manganese (Mn) in litter responded to at least one litter treatment (Fig. 8), suggesting that the cycles of these elements are influenced by litterfall—most likely via decomposition processes.

Boron (B) is critical for biological N cycling because it is involved in various processes that mediate interactions between legumes and rhizobia (Bolaños et al., 2004). Boron is only present in trace amounts in the soil and litterfall, and hence we only transferred $c. 0.5 \text{ kg B ha}^{-1} \text{ year}^{-1}$ between L- and L+ plots (Table 2). Nonetheless, the increase in soil B concentrations with litter addition indicate that litterfall could be important for maintaining B availability at our study site ($d = 1.0 \pm 0.14$, $P < 0.001$; Fig. 8A). However, the differences in soil B among treatments did not increase over time and were not reflected in litter B concentrations, B return or B use efficiency (Fig. 8B and C and 9A).

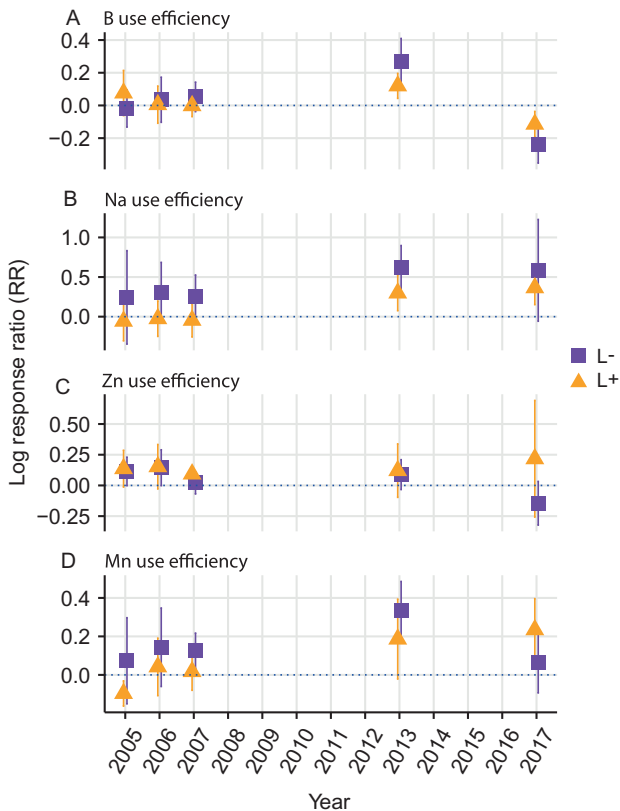


Fig. 9 Changes in nutrient use efficiency for (A) boron (B), (B) sodium (Na), (C) zinc (Zn), and (D) manganese (Mn) in response to 15 years of litter addition (orange triangles) and litter removal (purple squares) treatments in a lowland tropical forest in Panama, Central America. Differences are given as log response ratios, where the dashed blue line ($y = 0$) indicates no difference relative to controls; dots and whiskers are means and standard errors for $n = 5$.

Low variation in foliar B concentrations is expected because the range of B concentrations that is beneficial to plants is extremely narrow (Goldberg, 1997).

Sodium plays an important role in decomposition food-webs because it is essential to numerous arthropod taxa (Kaspari et al., 2014) and has been linked to arthropod abundance in the forest floor at our study site (Sayer et al., 2010). We transferred $c. 5 \text{ kg Na ha}^{-1} \text{ year}^{-1}$ between L- and L+ plots (Table 2) but we did not expect soil or litter Na concentrations to be affected by litter manipulation because our site is located within 30 km of the coast and likely receives sufficient Na inputs from marine deposition (Kaspari et al., 2014). Accordingly, litter removal did not affect soil Na concentrations (Fig. 8D) and neither treatment influenced litter Na concentrations, Na return or Na use efficiency (Figs 8E and F and 9B) but surprisingly, soil Na concentrations were lower in the L+ plots ($d = -1.41 \pm 0.30$, $P < 0.001$, Fig. 8D). It is likely that lower Na concentrations in the mineral soil with litter addition are the result of effective retention of mobile nutrients by the thick forest floor because Na concentrations increased with forest floor dry mass in the L+ plots (Sayer et al., 2010).

Zinc is an essential component of thousands of plant proteins but around 90% of the Zn in soils is insoluble and unavailable for plant uptake (Broadley et al., 2007). We only transferred $c. 0.3 \text{ kg Zn ha}^{-1} \text{ year}^{-1}$ between L- and L+ plots (Table 2), but there was a consistent pattern of higher Zn concentrations in the soil with litter addition ($d = 0.85 \pm 0.06$, $P < 0.001$), and lower concentrations with litter removal ($d = 0.76 \pm 0.16$, $P = 0.03$, Fig. 8G). We also measured greater Zn exchange rates in the L+ plots ($P = 0.005$), particularly at the end of the rainy season (Fig. 3F). Lower litter Zn concentrations in the L- plots ($d = 0.70 \pm 0.11$, $P = 0.004$; Fig. 8H) also resulted in lower Zn return by litter ($d = -0.81 \pm 0.24$, $P = 0.004$; Fig. 8I) and greater Zn use efficiency in response to litter removal ($d = 0.63 \pm 0.23$, $P = 0.012$; Fig. 9C).

Although chemical and physical processes are thought to be more important for Na and Zn cycling than biological processes (Laskowski et al., 1995), the changes in soil Na and Zn with litter manipulation are most likely linked to nutrient retention during decomposition processes in the forest floor. Litter decomposition can be enhanced by addition of Na (Kaspari et al., 2014) and Zn (Powers and Salute, 2011), which is likely related to high decomposer requirements for these elements (Gosz et al., 1976). The concentration of Na in arthropod biomass can be much higher than in the surrounding forest floor (Cromack et al., 1977), and tropical forest fungi

accumulate both nutrients (Stark, 1972). Interestingly, Na concentrations in decomposing boles were also higher in the L+ plots compared to the controls (Gora et al., 2018) and hence, the lower Na concentrations in the mineral soil could indicate active transport of Na by fungi into decomposing woody litter. The higher Zn supply in the L+ plots at the end of the wet season indicates substantial release of Zn during the later stages of decomposition (Fig. 3F). Hence, accumulation of Na and Zn in the forest floor is likely to accelerate litter turnover, whereas lower Zn availability in the L- plots could contribute to slower rates of decomposition (Sayer et al., 2006b).

Manganese (Mn) cycling is of particular interest because of the strong relationship between litter Mn concentrations and decomposition rates in multiple ecosystems (Keiluweit et al., 2015) and the effect of Mn in reducing tree growth on nearby Barro Colorado Island (Zemunik et al., 2018). Leaf litter is a major source of Mn (Marschner, 1986) and we transferred *c.* 2.5 kg Mn ha⁻¹ year⁻¹ between L- and L+ plots (Table 2), so it is somewhat surprising that the concentrations of Mn in the soil showed no clear response to litter manipulation (Fig. 8J). However, Mn^{3+/4+} oxides in the mineral soil are insoluble and unavailable for plant uptake (Keiluweit et al., 2015), so their concentrations are less likely to be affected by litter manipulation. By contrast, the higher exchange rates of Mn with litter removal, especially during the rainy season, ($P=0.002$; Fig. 3G) indicate greater amounts of water-soluble Mn in the soil of the L- plots, possibly as a result of lower pH (Laskowski et al., 1995; Page, 1962). Increased leaching of water-soluble Mn could explain the lower concentrations of Mn in litter in the L- plots after the first 4 years of treatments ($d = -0.94 \pm 0.06$; $P=0.003$; Fig. 8K), and the decline in Mn return in litter ($d = -1.02 \pm 0.17$; $P=0.002$; Fig. 8L). Greater Mn use efficiency with litter removal ($d = 0.97 \pm 0.10$, $P < 0.001$; Fig. 9D) suggests tight cycling of Mn in leaf litter, and the decline in litter but not soil Mn concentrations in the L- plots could indicate the importance of Mn cycling with organic matter and its direct uptake by roots from the forest floor. Decomposer fungi actively cycle Mn from plant material, maintaining it in forms available for plant uptake (Keiluweit et al., 2015). Hence, fungal accumulation of Mn in the forest floor would reduce leaching losses of water-soluble Mn and might also explain why we observed no increase in soil Mn with litter addition. Indeed, given the central role of Mn in fungal decomposition of organic material, greater concentrations of Mn in the forest floor could explain higher decay rates of fine woody litter (Sayer et al., 2006b) and leaf litter (Sayer and Tanner, 2010) in the L+ plots.

Overall, our findings reveal potentially important links between decomposer nutrient requirements and the observed changes in Mn, Na, and Zn with litter manipulation, and hence that litterfall and decomposition processes are crucial for the retention and cycling of multiple micronutrients in tropical forests. Given the combined influence of site fertility and litter inputs on soil C dynamics in forest ecosystems (Lajtha et al., 2018), further research into the links between micronutrient availability and decomposition processes could potentially improve our understanding of soil C storage in tropical forests.

4.2 Links between nutrients, decomposition, and organisms in the soil and forest floor

The GLiMP treatments demonstrate a key role of litterfall in the cycling or retention of most of the nutrients we investigated (Fig. 10). However, just as litterfall is one of the main pathways for the transfer of C and nutrients between plants and soil, decomposer organisms make those mineral elements available to plants. The nutrient requirements of decomposers contribute substantially to nutrient cycling but are often overlooked. Hence, the response of decomposition processes to altered litter inputs is likely to be influenced by the abundance and community composition of arthropods in litter and soil, as well as the availability of nutrients. As such, the effects of litter manipulation on organisms in the soil and forest floor deserve a separate mention.

Decaying litter on the forest floor not only represents a source of nutrients and substrate to decomposers, but also buffers variation in humidity and temperature (Sayer, 2006) and provides habitat space for a wide range of organisms (Gill, 1969; Poser, 1990; Sayer et al., 2010). It is therefore not surprising that changes in the abundance and diversity of decomposer organisms and arthropods are generally greater in response to litter removal than litter addition (Sayer, 2006). At our study site, arthropod abundance in both the litter and the mineral soil was best explained by the depth and mass of the forest floor (Ashford et al., 2013; Sayer et al., 2010) and differences in litter decomposition rates were at least partially explained by mesoarthropod abundance (Sayer et al., 2006b). Arthropod community composition was strongly related to P, Ca and Na concentrations in the forest floor (Sayer et al., 2010) and to P availability in the mineral soil (Ashford et al., 2013). Decreased arthropod abundance and lower soil nutrients likely contributed to lower decay rates of leaf litter in the L – plots (Rodtassana, 2016; Sayer et al., 2006b). By contrast, greater retention of nutrients (particularly K,

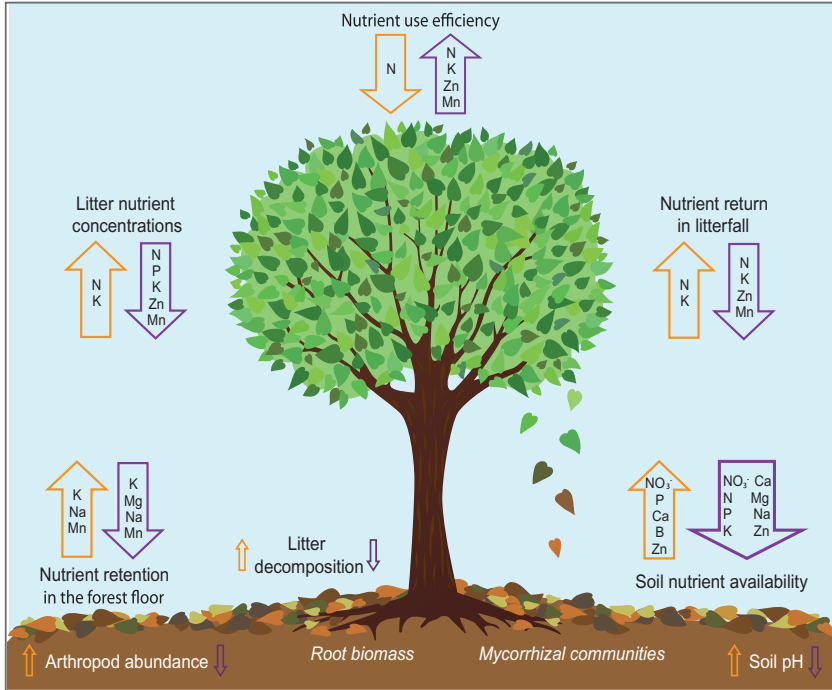


Fig. 10 Conceptual diagram illustrating the effects of litter addition (orange arrows) and litter removal (purple arrows) on different components of the forest nutrient cycle, where upward and downward arrows denote an increase or decrease, respectively, in nutrient concentrations in soil and litter, nutrient retention by the forest floor, stand-level nutrient use efficiency, soil pH, arthropod abundance or litter decomposition rates. Abbreviations inside arrows indicate nutrient elements significantly affected by litter manipulation treatments, following standard chemical nomenclature; labels in italics indicate other variables influenced by litter manipulation without a clear directional change.

Mn, Na and Zn) by decomposer organisms in the forest floor and rapid turnover of the litter standing crop (Rodtassana, 2016) likely accelerated the cycling of nutrients from litter while minimising losses from the system in the L+ plots (Gosz et al., 1976; Tobón et al., 2004). Termites contribute to the break-down of woody debris and their abundance is strongly linked to the availability of Na (Kaspari et al., 2014), but their distribution is patchy (Salick et al., 1983) and we observed no consistent differences among treatments in the abundance of termites in the forest floor or soil (Ashford et al., 2013; Sayer et al., 2010).

We currently lack detailed studies into microbial decomposers in the litter manipulation plots, but changes in fungal communities is an intriguing avenue for future research. Fungi are crucial to the decomposition of lignified material (Rayner and Boddy, 1988) and fungal accumulation of K, Na, Zn and Mn could explain some of the patterns we observed in response to litter manipulation. In addition, the slower decay of boles in the L – plots was attributed to reduced availability of N, P and K for decomposer fungi (Gora et al., 2018).

The distinct effects of the litter treatments on nutrient exchange rates compared to soil nutrient concentrations demonstrate the importance of decomposition processes in the forest floor for retaining nutrients. The exchange of nitrate-N, Ca and Zn mirror the concentrations of these nutrients in the soil because the treatments removed or added substantial amounts of N and Ca, and likely affected a large proportion of soluble Zn. The particularly large differences in the exchange rates of these nutrients between L + and L – plots towards the end of the rainy season (Fig. 3A, D and F) can be attributed to the initial immobilisation of N in the early stages of decomposition (Sayer et al., 2006b) and the release of Ca and Zn during the late stages of decomposition (Laskowski et al., 1995) in the L + plots. By contrast, the higher exchange rates of the mobile elements K, Mg and Mn in the L – plots (Fig. 3C, E and G), despite lower or unchanged concentrations in the soil, indicate increased leaching of mobile elements in the absence of an intact litter layer. Hence, more explicit consideration of the links between nutrient availability, decomposer organisms and the turnover of organic matter in the forest floor could shed light on many open questions about tropical forest nutrient cycling.



5. Synthesis and conclusions

The Gigante Litter Manipulation Project has contributed greatly to our understanding of nutrient cycling by litterfall. By disrupting or augmenting nutrient inputs in litter, the experimental treatments allow us to tease apart the relative importance of nutrient supply in the soil or litter, and test some of the basic principles of tropical forest nutrition and nutrient use efficiency (Fig. 10). Fifteen years of continuous litter removal and addition treatments in large experimental plots have expanded the mechanistic basis for the efficient use of several nutrients and highlighted numerous avenues for future research (Box 2).

5.1 Nutrient availability and nutrient use efficiency

We assessed overall changes in forest nutrient cycling in response to long-term litter manipulation treatments by calculating nutrient use efficiency, which provides an ecosystem-level measure of nutrient availability to plants (Vitousek, 1982). Nutrient use efficiency can be regulated by the allocation of a given element to different tissues or functions, or via resorption of foliar nutrients prior to leaf abscission (Vitousek, 1982). Hence, changes in nutrient use efficiency in response to litter manipulation underscore the importance of nutrient cycling by litterfall. Although we observed no clear trajectory of change in nutrient use efficiency over time in our experimental plots, the continuous addition or removal of nutrients with litter still had an overall effect on N, P, K, Mn and Zn cycling, as well as Ca, Mg and B concentrations in the soil (Fig. 10).

The extent of changes in N and P status of litter and soils at our study site conforms to the widely held view of a leaky N but tight P cycle. Nonetheless, we found that cycling of N in litter is critical for sustaining N availability to plants, and that N return in litterfall helps maintain the N-status of the forest, even on soils with moderate or high N-availability. By contrast, the maintenance of plant P-status despite 15 years of litter removal suggests that plants are less dependent on P cycling by litterfall than previously presumed. Many tree species at our study site occur on low-P soils across central Panama (Condit et al., 2013; Wright et al., 2018) and might maintain their P status by accessing alternative sources of P (Liu et al., 2018; Steidinger et al., 2015), via increased resorption (Chapin, 1980) or other physiological adaptations to reduce their P-demand (Mo et al., 2019). Multiple conservation strategies for P, but not N, would explain why the relationship between soil nutrient availability and litter nutrient concentrations is much stronger for N than for P (Vitousek, 1982; Wright, 2019). It is possible that plants adapted to high levels of N cycling also have relatively high demands for N (Wright, 2019), which would explain why we observed rapid changes in N but not P use efficiency in response to litter removal (Fig. 5B). Increased Mn and Zn use efficiency in response to litter removal (Fig. 9C and D), as well as the potential shift in K use efficiency indicate strong biotic controls on these elemental cycles via decomposition processes in the forest floor. Indeed, major declines in nutrient availability in the mineral soil in response to litter removal were largely restricted to the most abundant elements (N, Ca, Mg), which provides strong support for the biotic retention and cycling of scarce and potentially limiting nutrients in lowland tropical forests (Box 2; Fig. 10).

Clearly, extractable nutrients in soil samples only provide a snapshot of potential nutrient availability to plants, and many of the discrepancies we observed in the responses of soil and litter nutrient concentrations to the litter manipulation treatments can be explained by the retention of nutrients in the forest floor. Our study of nutrient exchange rates gives a good indication of how decomposing plant matter in the forest floor influences measurements of nutrient availability from the mineral soil. Higher exchange rates of nitrate-N, Ca, and Zn in the mineral soil of the L+ plots compared to the L- plots (Fig. 3A, D and F) suggest that large amounts of these nutrients are being released from the forest floor to the mineral soil before they can be taken up by plants. By contrast, higher exchange rates of mobile nutrients such as K, Mg and Mn with litter removal compared to litter addition indicates increased leaching of those elements in the absence of the forest floor (Fig. 3C, E and G).

Of particular note are the changes we observed in trace element cycling. The building blocks of life involve at least 25 different elements, but we know relatively little about the role of micronutrients in ecosystem function (Kaspari and Powers, 2016). Our data only included measurements of five trace elements, but we present strong evidence for changes in four micronutrients (B, Na, Mn, Zn) in response to litter manipulation and an increase in nutrient use efficiency for Mn and Zn. There is clearly much more work to be done to characterise micronutrient cycling and the relative importance of different trace elements in tropical ecosystem function (Box 2). The GLiMP studies indicate that a more comprehensive view of tropical forest nutrient cycling would need to consider specific plant adaptations to shortages of different elements, alternative sources of scarce nutrients, and detailed patterns of nutrient retention and release from decomposing organic matter.

5.2 The importance of forest floor processes

As decomposition is rapid in moist lowland tropical forests, the depth of the superficial organic horizons can vary seasonally at our study site, from a thick layer of fresh litter and decomposing organic material at the end of the dry season to a thin and patchy covering of partially decomposed leaves towards the end of the rainy season (Wieder and Wright, 1995). The dynamic nature of the tropical forest floor requires high spatial and temporal resolution for process studies. Many key steps of multiple elemental cycles appear to bypass the mineral soil, and although direct nutrient uptake from the forest floor

was demonstrated over 40 years ago (Herrera et al., 1978; Stark and Jordan, 1978), the relevant processes are still relatively poorly characterised (Box 2).

Collectively, our results give a strong indication that a substantial proportion of multiple nutrient cycles occur within the forest floor (Fig. 10). First, altered fine root distribution and greater proliferation of fine roots (Rodtassana and Tanner, 2018; Sayer et al., 2006a) and fungal hyphae (Sheldrake et al., 2017) into the thick forest floor in the L+ plots demonstrate that plants perceive the added litter as a “nutrient hotspot”, where nutrients can be taken up as they are mineralised and released from the litter. Second, slower release of K and Mg during decomposition with litter addition (Sayer et al., 2006b) and contrasting mobility of these elements in the mineral soil in the L+ and L− plots (Fig. 3C and E) point towards substantial retention of K and Mg in the forest floor. Third, the shifts in AM fungal communities and depletion of organic P in the L− plots signal substantial changes in P-acquisition and cycling with litter removal, demonstrating the importance of organic matter for maintaining P-status in the forest. Finally, the increase in microbial biomass in the mineral soil of L+ plots was surprisingly small, despite substantial inputs of C and nutrients with the added litter (Fig. 4B and C). Given the cumulative evidence for nutrient retention and uptake in the forest floor, we propose that measurements of processes in the mineral soil only represent a small part of the picture. It follows that explicit consideration of processes in the forest floor could greatly improve our understanding of nutrient and C dynamics in tropical forests (Box 2).

5.3 Microbial pathways of nutrient cycling

We could begin to address many of the open questions about long-term effects of altered litter inputs with in-depth assessments of microbial communities (Box 2). As our results demonstrate that some processes in the litter layer and mineral soil are at least partly decoupled, current discrepancies may be resolved by linking changes in microbial functional groups to specific substrates and processes. Substantially higher rates of soil respiration in the L+ plots are a strong indication that microbial activity in the mineral soil is influenced by aboveground litter inputs (Sayer et al., 2007, 2011). However, the effects of litter addition on soil microbial biomass and soil C were much smaller than expected (Fig. 4B and C) and a recent assessment of broad microbial groups represented by phospho-lipid fatty acid biomarkers revealed only minor effects of both litter treatments on microbial

community composition and the abundance of bacterial functional groups in the mineral soil (E. Sayer et al., unpublished data).

Although others have investigated the responses of soil microbial communities to litter manipulation, their findings vary widely. Several studies noted only limited responses in temperate (Leitner et al., 2016; Wang et al., 2017) and tropical forests (Cantrell et al., 2014; Chen et al., 2014), whereas others noted increased abundance of Acidobacteria in response to litter removal, and greater abundance of Alphaproteobacteria with litter addition in a tropical forest, which were related to changes in soil C and N pools (Nemergut et al., 2010). Analysis of phospho-lipid fatty acid biomarkers has also revealed shifts in the relative abundances of broad microbial functional groups, with greater abundance of stress-tolerant Gram-positive bacteria in response to litter removal (Pisani et al., 2016; Wang et al., 2013) and increased abundance of actinomycetes, Gram-positive, and Gram-negative bacteria in response to litter addition (Pisani et al., 2016). However, changes in microbial function (Leff et al., 2012) and extracellular activity (Weintraub et al., 2013; Zhao et al., 2017) with litter manipulation are not always consistent, even within individual studies. Hence, there is still a dearth of information on microbial pathways of nutrient cycling in tropical forests, especially over the long-term and at the ecosystem scale.

The results from studies of AM fungi in the GLiMP plots demonstrate that aboveground litter inputs can shape belowground microbial communities via changes in nutrient availability (Sheldrake et al., 2017, 2018). Although AM fungi are not directly involved in decomposition processes, substantially greater AM fungal colonisation of roots in the forest floor in L+ and control plots indicate that AM fungi nonetheless play an important role in plant uptake of nutrients from organic matter (Sheldrake et al., 2017). Consequently, research into interactions between roots, AM fungi and saprobes in the forest floor could shed new light on tropical nutrient cycling and the concept of direct nutrient uptake. Indeed, fungal decomposition of litter might be particularly crucial to the cycling and retention of several elements in the forest floor. Fungi actively transport and accumulate N, K, Mn and Zn during decomposition (Cromack et al., 1975; Stark, 1972), and these processes are severely disrupted by the litter removal treatments. It is therefore noteworthy that the availability of these nutrients to plants in the L – plots declined to such an extent that nutrient use efficiency increased (Figs. 5 and 9). Shifts in the composition and activity of other microbial groups are likely to elucidate some of the mechanisms underpinning the observed changes in nutrient cycling with litter manipulation, and we call for future work using high-resolution

techniques to assess changes in microbial community composition and function in response to altered litter inputs (Box 2).

Our review demonstrates the need for a more holistic view of elemental cycling and nutrient co-limitation. Our insights into tropical forest nutrient cycling arise from studies spanning the molecular to the ecosystem scale, and incorporate aspects of plant, soil, and microbial ecology with biogeochemistry (Fig. 10). Hence, the GLiMP studies not only provide novel insights into the importance of litterfall in tropical forest nutrient cycling, they also collectively demonstrate the importance of “integrative biogeochemical ecology” (Kaspari and Powers, 2016) via the wide range of different processes involved in elemental cycling and the timescales within which they operate.

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