

# Seasonal changes in soil organic matter after a decade of nutrient addition in a lowland tropical forest

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**Abstract** Soil organic matter is an important pool of carbon and nutrients in tropical forests. The majority of this pool is assumed to be relatively stable and to turn over slowly over decades to centuries, although changes in nutrient status can influence soil organic matter on shorter timescales. We measured carbon, nitrogen, and phosphorus concentrations in soil organic matter and leaf litter over an annual cycle in a long-term nutrient addition experiment in lowland tropical rain forest in the Republic of Panama. Total soil carbon was not affected by a decade of factorial combinations of nitrogen, phosphorus, or potassium. Nitrogen addition increased leaf litter nitrogen concentration by 7 % but did not affect total soil nitrogen. Phosphorus addition doubled the leaf litter phosphorus concentration and increased soil organic phosphorus by 50 %. Surprisingly, concentrations of carbon,

nitrogen, and phosphorus in soil organic matter declined markedly during the four-month dry season, and then recovered rapidly during the following wet season. Between the end of the wet season and the late dry season, total soil carbon declined by 16 %, total nitrogen by 9 %, and organic phosphorus by between 19 % in control plots and 25 % in phosphorus addition plots. The decline in carbon and nitrogen was too great to be explained by changes in litter fall, bulk density, or the soil microbial biomass. However, a major proportion of the dry-season decline in soil organic phosphorus was explained by a corresponding decline in the soil microbial biomass. These results have important implications for our understanding of the stability and turnover of organic matter in tropical forest soils, because they demonstrate that a considerable fraction of the soil organic matter is seasonally transient, despite the overall pool being relatively insensitive to long-term changes in nutrient status.

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## Introduction

Soil organic matter plays a fundamental role in the nutrition of tropical forests. It constitutes a major store of nitrogen (N), phosphorus (P), and sulfur (S), and its turnover supplies a considerable fraction of these

nutrients for plant uptake on an annual basis (Tiessen et al. 1994). Soil organic matter also represents an enormous reservoir of carbon (C), accounting for approximately half of the total C stock in tropical forests and almost 30 % of the total soil C stock in forests worldwide (Dixon et al. 1994; Pan et al. 2011). Information on the dynamics of soil organic matter in lowland tropical forests is therefore of considerable importance for understanding nutrient cycling and for predicting the fate of a globally important C pool.

Few studies have reported seasonal variation in soil C pools in tropical forests (e.g., García-Oliva et al. 2003), suggesting that soil organic matter is assumed to be relatively stable against short-term fluctuations such as seasonal drying. Protocols for quantifying soil C stocks do not explicitly state preferences for sampling times based on measured C concentrations, while the recommendation for consistent sampling of agricultural soils with respect to season is aimed specifically at avoiding confounding effects of crop residue inputs (Lal et al. 2001; Vladimir et al. 2005). In contrast, soil organic matter appears to be sensitive to changes in nutrient status, at least in the medium to long term. For example, P addition to a lowland forest in Costa Rica increased soil respiration at rates expected to manifest in a long-term decrease in soil C content (Cleveland and Townsend 2006). On the other hand, N addition is predicted to increase soil organic C by increasing forest productivity (Sayer et al. 2012) and reducing priming effects through a decline in the microbial demand for N from soil organic matter (Nottingham et al. 2012). Indeed, the addition of N for several years to tropical forests in Puerto Rico increased soil organic C stocks, primarily in the mineral-associated pool (Cusack et al. 2011). Such changes are of potential significance given the magnitude of the soil C stock in tropical forests, because even small changes in soil organic C could exert a considerable influence on atmospheric CO<sub>2</sub> concentrations and therefore future climate.

Although few studies have assessed seasonal variation in soil organic matter in tropical forests, three major inputs of organic matter to the soil undergo marked seasonal changes in magnitude that might be expected to influence soil C. First, soil microbial biomass undergoes strong seasonal variation in response to soil drying, declining by approximately 50 % during the dry season in seasonal forest in Panama (Turner and Wright 2014). Second, much of

the litter fall in seasonal tropical forests occurs during the dry season; in Panama, for example, approximately half of the annual leaf litter fall in lowland forests occurs during the four-month dry season, and almost all the annual litter fall has decomposed by the end of the following wet season (Wieder and Wright 1995). Decomposition occurs continuously throughout the year, although leaching of dissolved organic C from litter, including inputs from through fall, occurs predominantly in the wet season. Finally, root growth and turnover show strong seasonality, with much of the annual root growth occurring in the wet season or during seasonal transitions (i.e., wet-to-dry and dry-to-wet seasons) (Cavelier et al. 1999; Yavitt and Wright 2001). Root growth is often associated with C allocation to root exudates and mycorrhizal fungi, which together can account for a major proportion of primary production (Davidson et al. 2002). This belowground C allocation varies seasonally in parallel with variation in tree growth (Kaiser et al. 2011), although the majority turns over rapidly and is returned to the atmosphere as CO<sub>2</sub> within a year (Giardina et al. 2004). Overall, the strong seasonal variation in these three organic matter inputs to tropical forest soils suggest that the soil organic matter undergoes comparable seasonal fluctuations, even if the majority of it is relatively stable.

Given the uncertainties about (i) seasonal patterns of soil organic matter and (ii) the response of soil organic matter to long-term perturbations in nutrient cycles in lowland tropical forests, we examined how the concentrations of C, N, and P in leaf litter fall and soil organic matter varied throughout an annual cycle in a long-term fertilization experiment in lowland tropical forest. The experiment included factorial additions of N, P, and potassium (K), in addition to a separate treatment that receives cations and micronutrients. The study had two objectives. First, we aimed to assess seasonal changes in soil organic matter across an annual cycle. Second, we aimed to quantify the influence of a decade of nutrient addition on soil organic matter and nutrients in leaf litter fall.

## Methods

### The Gigante fertilization experiment

The study was conducted on the Gigante Peninsula, part of the Barro Colorado Nature Monument,

Republic of Panama. The fertilization experiment began in 1998 and is described in detail elsewhere (Yavitt et al. 2009; Wright et al. 2011). Briefly, fertilizer is added four times per year to  $40 \times 40$  m plots replicated four times for each treatment in a factorial  $N \times P \times K$  design. The experiment is replicated four times along a 36-m north–south topographic gradient and blocked within each replicate along the perpendicular east–west gradient to control spatial variation in soil properties. Fertilizer was added at the following times during the study period: 13–15 September 2006, 16–18 May 2007, 4–6 July 2007, 22–24 August 2007, and 10–12 October 2007. Fertilizer was added at the following annual rates: 125 kg N  $\text{ha}^{-1}$  as coated urea, 50 kg P  $\text{ha}^{-1}$  as triple super phosphate, and 50 kg K  $\text{ha}^{-1}$  as potassium chloride (KCl). A further treatment (four replicate plots) included calcium and magnesium (from dolomitic limestone) and micronutrients. Rates of N and K are similar to the annual amounts of these nutrients in litter fall (Sayer and Tanner 2010; Wright et al. 2011), but the rate of P addition is approximately tenfold greater than P in annual litter fall to overcome the inherent high P fixation capacity of the soil at this site (Schreeg et al. 2013).

The site supports mature moist semi-deciduous rain forest at least 200 years old (Wright et al. 2011). Soils in the upper part of the landscape on Gigante Peninsula are Oxisols developed on Miocene basalt and are morphologically similar to the Typic Eutrudox (AVA and Marron soil classes) on nearby Barro Colorado Island (Dieter et al. 2010). Soils on the lower part of the landscape are Inceptisols (Corre et al. 2010) and become seasonally waterlogged to relatively shallow depth. Topsoil texture is clay throughout the experiment, with no significant differences in sand, silt, or clay among treatments ( $p > 0.10$ ) (Turner et al. 2013). The experiment is remarkable in its duration and in the range of responses observed in forest productivity, with significant effects involving N, P, and K (Wright et al. 2011). There has also been a strong response of the microbial biomass and hydrolytic enzyme activities to P addition (Turner and Wright 2014) and of decomposition rates to P, K, and cations/micronutrients (Kaspari et al. 2008). Seasonal changes and treatment effects on inorganic nutrients during the study period were reported previously (Turner et al. 2013).

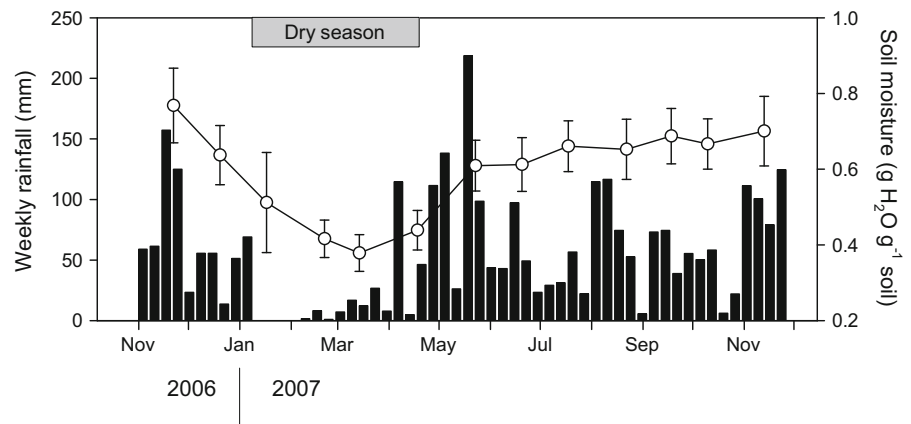
## Climate during the study period

On nearby Barro Colorado Island, about 6 km from the study site, the mean annual rainfall is 2,600 mm, with a four month dry season between late December and April and a mean annual temperature of 26 °C (Windsor 1990). During the study period (1 November 2006–31 October 2007), Barro Colorado Island received 2,604 mm of rain. The dry season began at the end of December 2006 and continued until late April 2007, although there were a few days of heavy rain in early April (Fig. 1). A total of 313 mm of rainfall (12 % of the annual total) was recorded between January and April (inclusive). Rainfall was recorded only once in January (<0.5 mm) and only 11 mm of rain was recorded in February (Fig. 1). Mean soil moisture content in the 16 plots in the  $N \times P$  factorial subset was lowest in March (0.38 g  $\text{H}_2\text{O g}^{-1}$  soil) and greatest in November 2006 ( $\sim 0.8$  g  $\text{H}_2\text{O g}^{-1}$  soil) when sampling occurred immediately following the highest rainfall event, but was otherwise relatively stable throughout the wet season (0.6–0.7 g  $\text{H}_2\text{O g}^{-1}$  soil) (Fig. 1).

## Soil sampling

Soils were sampled across an annual cycle between November 2006 and November 2007. On the third week of every calendar month, soils were sampled from a subset of 16 plots in an  $N \times P$  factorial design (control, +N, +P, +NP). Every four months all 36 plots were sampled, including 32 plots in the full  $N \times P \times K$  factorial design and the four base cation/micronutrient treatment plots. Soils were sampled to 10 cm depth using a 2.5 cm diameter corer at nine systematically distributed points in the center  $20 \times 20$  m quadrat of each plot. Samples from each plot were composited in the field and returned immediately to the laboratory, where stones, mesofauna, and coarse roots were removed manually. Fine roots were removed exhaustively using tweezers, although it is likely that some very fine roots remained. Subsamples of each soil were air-dried at ambient laboratory temperature ( $\sim 22$  °C, 55 % humidity) and milled to a fine powder for total element analysis. Total C and N were determined for all plots in samples taken every four months, in addition to analyses on the four control plots sampled monthly. Soil organic P was determined on monthly samples.

**Fig. 1** Weekly rainfall (filled bars) for Barro Colorado Island, Panama, between October 2006 and December 2007, and soil water content (open symbols) in experimental plots on Gigante Peninsula (mean of 16 plots in the NP factorial design). The approximate extent of the dry season is shown



We determined bulk density in the wet season (November–December 2012) and dry season (January–February 2013). On each occasion, four cores of constant volume (5 cm diameter × 10 cm depth) were taken in each plot and bulked. Roots and stones were removed by hand and the samples were dried at 105 °C and weighed. Bulk density was determined as oven-dry mass/core volume. Although the determination of bulk density by coring inevitably leads to a small degree of compaction, results were similar to values obtained in soil profile pits using the excavation method.

#### Litter fall sampling

Litter fall was collected in 0.58 m<sup>2</sup> traps, using three traps per plot from November 2006 to February 2007, and then five per plot from March to October 2007). Samples were dried at 60 °C for three days and separated into leaves, reproductive parts (flowers and fruits), branches, and dust. The fractions were weighed and a subsample was milled for elemental analysis. Leaves from November 2006, March 2007, July 2007, and November 2007 were analyzed for total C, N, and P. Concentrations of these elements varied seasonally (see below), so annual nutrient inputs were determined by summing monthly nutrient inputs calculated from monthly leaf litter weights multiplied by the four-monthly concentrations for the respective period.

#### Analytical

Dried and ground soil and litter samples were analyzed for total C and N by combustion and gas chromatography on a Flash 1112 analyzer (Thermo, Bremen,

Germany). Total P was determined by ignition at 550 °C × 1 h and extraction for 16 h in 1 M H<sub>2</sub>SO<sub>4</sub>, with detection by automated molybdate colorimetry at 880 nm using a Lachat Quikchem 8500 (Hach Ltd, Loveland, CO). Soil organic P was determined by the single-step NaOH–EDTA extraction procedure as described previously (Bowman and Moir 1993; Turner 2008). This procedure is assumed to extract soil organic P quantitatively, because it extracted similar concentrations as other methods considered to provide the most accurate measure of soil organic P (Bowman and Moir 1993; Turner et al. 2005). Briefly, P was extracted by shaking soil (1.50 ± 0.01 g) with 30 mL of a solution containing 0.25 M NaOH and 50 mM disodium ethylenediaminetetraacetate (Na<sub>2</sub>EDTA) for 16 h at 22 °C. Extracts were centrifuged (30 min, 8,000×g) and a 1 ml aliquot was neutralized using phenolphthalein indicator and 3 M H<sub>2</sub>SO<sub>4</sub> and diluted to 20 mL with deionized water. Total extracted P was determined by inductively-coupled plasma optical-emission spectrometry (ICP–OES) using an Optima 2100 DV (Perkin-Elmer Inc., Shelton, CT). Inorganic phosphate was determined by automated molybdate colorimetry as described above. Organic P was calculated as the difference between total P and inorganic phosphate. All nutrient concentrations are expressed on the basis of oven-dry soil (i.e., determined by drying for 24 h at 105 °C).

#### Statistical analysis

We performed repeated measures analyses of variance (ANOVA) for each response variable to evaluate temporal variation. Between subject (or plot) effects

evaluate treatment responses over the full annual cycle. Within subject (or plot) effects evaluate seasonal variation and interactions among treatments and month (for monthly samples from the subset of plots that comprise the subset of plots in the factorial  $N \times P$  design) or season (for four-monthly samples from the full factorial  $N \times P \times K$  experiment). Repeated measures ANOVA assumes compound symmetry of the variance–covariance matrix if there are more than two repeated measures. We therefore used the conservative Greenhouse-Geisser correction for violations of the compound symmetry assumption. All analyses were performed with SYSTAT© 11.0 (Richmond, CA). Throughout the results we report treatment effects and seasonal effects and their interactions for the seasonal samples from the full factorial  $N \times P \times K$  experiment. A one-way analysis of variance (ANOVA) was used to contrast the micronutrient and control treatments without blocking. There were no significant differences between dolomite/micronutrient plots compared to control plots for any of the soil properties measured here and these plots are not discussed further.

## Results

### Carbon, nitrogen, and phosphorus in soil organic matter

Total soil C concentration was unaffected by nutrient addition ( $p > 0.10$ ; Fig. 2a), but varied seasonally ( $F_{3,54} = 29.3$ ,  $p < 0.001$ ; Fig. 2b). Soil C concentrations were greatest in the wet season and lowest in the dry season, with values declining by 16 % from  $4.93 \pm 0.16$  % in November 2006 to  $4.12 \pm 0.12$  % in March 2007. Similarly, total soil N concentration was unaffected by nutrient addition ( $p > 0.10$ ; Fig. 2c), but varied seasonally ( $F_{3,54} = 40.4$ ,  $p < 0.001$ ; Fig. 2d). Total soil N concentrations were lowest in the dry season, declining by 9.3 % from  $0.43 \pm 0.01$  % in November 2006 to  $0.39 \pm 0.01$  % in March 2007.

Soil organic P concentration was increased significantly by P addition ( $F_{1,18} = 59.8$ ,  $p < 0.001$ ; Fig. 2e), but not by N or K addition. Mean organic P concentrations were  $98.5 \pm 3.1$  mg P kg<sup>-1</sup> in no-P plots and  $143.8 \pm 4.2$  mg P kg<sup>-1</sup> in P addition plots, an increase of 46 %. Soil organic P varied seasonally

( $F_{3,54} = 21.5$ ,  $p < 0.001$ ), with lowest values in the dry season (Fig. 2f). In plots that did not receive P addition, soil organic P declined by 18.6 %, from 106.3 mg P kg<sup>-1</sup> in November 2006 to 86.5 mg P kg<sup>-1</sup> in March 2007. In P addition plots, soil organic P declined by 25.1 %, from 142.3 mg P kg<sup>-1</sup> in November 2006 to 117.2 mg P kg<sup>-1</sup> in March 2007, before increasing again to 158.8 mg P kg<sup>-1</sup> in August 2007 (Table 1).

### Nutrient stoichiometry in soil organic matter

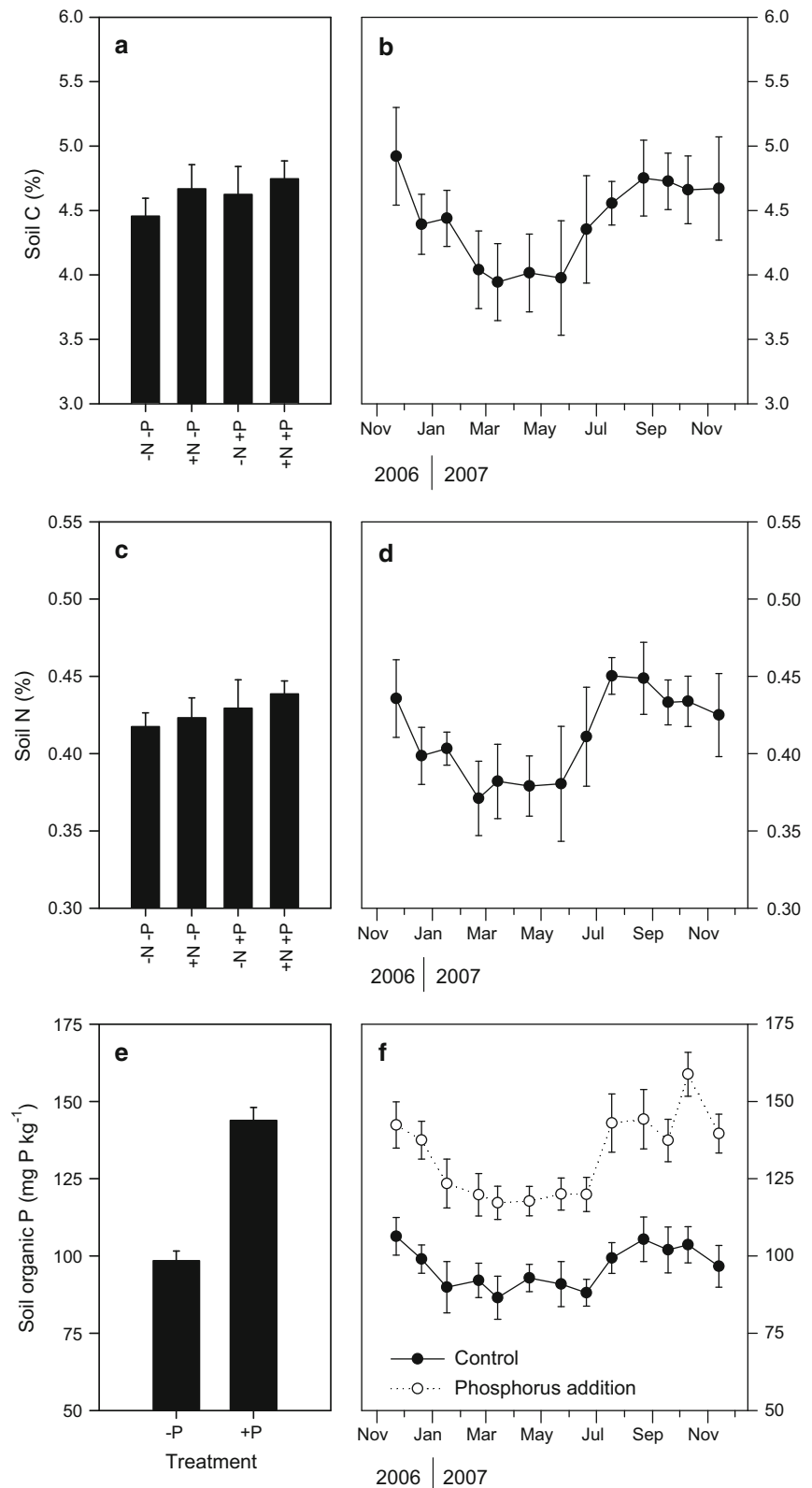
The soil C:N ratio was unaffected by nutrient addition ( $p > 0.10$ ), but varied seasonally ( $F_{3,54} = 40.3$ ,  $p < 0.001$ ; Fig. 3a). The magnitude of the seasonal variation was small, however, declining from  $11.4 \pm 0.16$  in the late wet season (November 2006) to  $10.6 \pm 0.09$  in the dry season (March). The soil C to organic P ratio decreased significantly following P addition ( $F_{1,18} = 26.5$ ,  $p < 0.001$ ) (Fig. 3b), but there was no significant effect of N or K addition. The C to organic P ratio did not vary seasonally ( $p > 0.10$ ; Fig. 3b). Mean C to organic P ratios in the NPK factorial design were  $474 \pm 27$  in no-P plots and  $331 \pm 12$  in P addition plots (standard error of four sampling events between November 2006 and November 2007, each the mean of 16 plots), a decline of 30 % with P addition.

### Soil bulk density

Mean bulk density averaged across the 32 plots in the NPK design was  $0.793 \pm 0.079$  g cm<sup>-3</sup> (mean  $\pm$  standard deviation of 32 plots) in the wet season and  $0.826 \pm 0.090$  g cm<sup>-3</sup> in the dry season, an increase of 4 % from wet to dry season. There were no significant differences in bulk density among treatments ( $p > 0.10$ ), but the seasonal effect was significant ( $F_{1,18} = 5.2$ ,  $p = 0.035$ ).

Despite the small seasonal change in soil bulk density, we can evaluate its role in the seasonal changes in soil organic matter. Using data from a soil profile pit at the Gigante experimental site (11–22 cm depth; dry season bulk density 0.87 g cm<sup>-3</sup>, total C 2.71 %) and the 4 % increase in soil bulk density between wet and dry season samples (=4 mm increase in effective soil depth sampled during the dry season), we calculate that the change in bulk density alone would lead to a 1.9 % decrease in the soil organic C

**Fig. 2** Seasonal changes and treatment effects on total soil carbon (**a, b**), total soil nitrogen (**c, d**), and soil organic phosphorus (**e, f**) in nutrient addition plots on Gigante Peninsula, Panama, between November 2006 and November 2007. **a** and **c** show the non-significant effects of nitrogen and phosphorus addition, with means and standard errors of the eight replicate plots in each treatment sampled every four months between November 2006 and November 2007. (Note that the eight replicates include those involving potassium addition along with the respective nutrient). **e** shows the significant effect of phosphorus addition on soil organic phosphorus, with means and standard errors of sixteen replicate plots in each treatment sampled four times between November 2006 and November 2007. **b, d, and f** show the significant seasonal variation in total soil carbon, total soil nitrogen, and soil organic phosphorus, with **b** and **d** showing means and standard errors of four replicate control plots, and **f** showing the means and standard errors of 16 replicate plots in each treatment



**Table 1** Summary of seasonal and treatment effects for litter fall components and total elements in leaf litter fall and soil

Parameter	Treatment ( $F_{1,18}$ )	Season ( $F_{3,54}$ )	Time $\times$ nutrient interaction ( $F_{3,54}$ )
Total soil C (%)	ns	Lowest in dry season ( $F = 29.3^{***}$ )	ns
Total soil N (%)	ns	Lowest in dry season ( $F = 40.4^{***}$ )	ns
Soil $P_o$ (mg P kg <sup>-1</sup> )	Increased by P ( $F = 59.8^{***}$ )	Lowest in dry season ( $F = 21.5^{***}$ )	ns
Non-microbial soil $P_o$ (mg P kg <sup>-1</sup> )	Increased by P ( $F = 21.9^{***}$ )	No clear pattern ( $F = 5.4^{***}$ )	ns
Soil C:N	ns	Lowest in dry season ( $F = 40.3^{***}$ )	ns
Soil C: $P_o$	Reduced by P ( $F = 26.5^{***}$ )	ns	ns
Leaf litter C (%)	ns	Highest in early wet season ( $F = 10.3^{***}$ )	ns
Leaf litter N (%)	Increased by N ( $F = 11.6^{**}$ )	Highest in late wet season ( $F = 44.4^{***}$ )	Time $\times$ K interaction ( $F = 3.3^*$ )
Leaf litter P (mg P kg <sup>-1</sup> )	Increased by P ( $F = 80.9^{***}$ ) N $\times$ P interaction ( $F = 5.2^*$ )	Modest changes ( $F = 4.1^*$ )	Time $\times$ N interaction ( $F = 8.0^{**}$ )
Leaf litter C:N	Reduced by N ( $F = 10.9^{**}$ )	Highest in dry season ( $F = 41.2^{***}$ )	ns
Leaf litter C:P	Reduced by P ( $F = 79.5^{***}$ )	Highest in dry season ( $F = 5.1^{**}$ )	Time $\times$ N interaction ( $F = 3.6^*$ )
Leaf litter N:P	Reduced by P ( $F = 89.8^{***}$ ) Increased by N ( $F = 6.1^*$ )	ns	ns

Not shown are the non-significant effects of nutrient addition on annual litter fall or its components, or mass of C, N, and P in leaf litter fall

All effects were evaluated in the NPK factorial design.  $F$  values are shown in parentheses, with significance indicated by \*, \*\*, and \*\*\*, representing probability at the 5, 1, and 0.1 % levels, respectively

ns not significant,  $P_o$  soil organic phosphorus

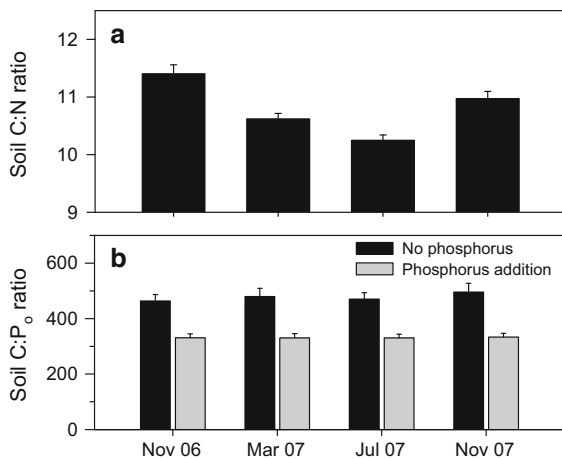
concentration. This is probably a slight overestimation, as the bulk density will be slightly lower, and the total C slightly higher, at the upper part of the 11–22 cm horizon.

#### Litter fall mass and components

Annual input of total litter fall averaged across all 32 plots in the NPK factorial design (i.e., excluding the micronutrient treatment) between November 2006 and October 2007 (inclusive) was  $11.80 \pm 0.52$  Mg ha<sup>-1</sup>. Of this,  $57.5 \pm 1.4$  % was leaves,  $23.6 \pm 1.2$  % was branches,  $9.4 \pm 0.8$  % was reproductive structures (fruits and flowers), and  $9.7 \pm 0.4$  % was other material (dust, insect frass, unidentifiable fragments, etc.). Phosphorus addition was recently reported to have increased total litter fall by  $\sim 25$  % over 11 years in the Gigante experiment (Wright et al. 2011) and during the 2006–2007 annual cycle P addition plots received  $12.65 \pm 0.84$  Mg ha<sup>-1</sup> of total litter fall compared to  $10.95 \pm 0.87$  Mg ha<sup>-1</sup> in no-P plots. This one-year 15.5 % increase with P addition was marginally non-significant ( $F_{1,18} = 3.6$ ,  $p = 0.076$ ). Annual input of

fruits and flowers in litter fall between Nov 2006 and Oct 2007 (inclusive) averaged across all 32 plots in the NPK factorial design was  $1.10 \pm 0.10$  Mg ha<sup>-1</sup> and did not vary significantly ( $p > 0.10$ ) with P addition (no-P plots =  $1.08 \pm 0.13$  Mg ha<sup>-1</sup>, P addition plots =  $1.11 \pm 0.15$  Mg ha<sup>-1</sup>). There was a large input of branches and leaves in November 2006, presumably linked to a major storm that yielded 146 mm rain on BCI in late November.

The annual input of leaf litter averaged across the 32 plots in the NPK factorial design between November 2006 and October 2007 (inclusive) was  $6.70 \pm 0.28$  Mg ha<sup>-1</sup>. The amount in no-P plots was  $6.26 \pm 0.48$  Mg ha<sup>-1</sup>, while P addition plots received  $7.14 \pm 0.45$  Mg ha<sup>-1</sup> (Fig. 4a). Although more leaf litter fell in the P addition plots in every month except April, the overall 14.0 % increase in leaf litter fall with P addition was not significant ( $p > 0.10$ ) (Fig. 4b). In no-P plots, monthly leaf litter fall in the dry season between January and March was  $\sim 900$  kg ha<sup>-1</sup>, falling slightly in April (Fig. 4b). In comparison, monthly amounts in the same plots between May and October were 200–400 kg ha<sup>-1</sup>. Thus, around half the



**Fig. 3** Seasonal changes in the soil total element ratios in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007. **a** shows the significant seasonal effect on soil carbon to nitrogen (C:N) ratio (no treatment effect), while **b** shows the significant treatment effect on the soil carbon to organic phosphorus (C:P<sub>o</sub>) ratio (no seasonal effect). Values are means of all 32 plots in the full factorial design (**a**) or 16 plots in each treatment (**b**)

leaf litter fell in the four-month dry season, and half in the eight-month wet season.

#### Leaf litter fall nutrient concentrations and mass

The C concentration in leaf litter was unaffected by nutrient addition ( $p > 0.10$ ), but varied significantly among months ( $F_{3,54} = 10.3$ ,  $p < 0.001$ ), with the highest values in July (Fig. 5a). The mean C concentration in leaf litter fall for the 32 plots in the NPK factorial design averaged across the four sampling dates was  $45.3 \pm 0.2$  %. The total mass of C in leaf litter fall for no-P plots was  $2.86 \pm 0.15$  Mg C ha<sup>-1</sup>, while P addition plots received  $3.22 \pm 0.21$  Mg C ha<sup>-1</sup>. However, this 12.6 % increase in C mass in response to P addition was not significant ( $p > 0.10$ ).

The concentration of N in leaf litter was increased by N addition ( $F_{1,18} = 11.6$ ,  $p = 0.003$ ) and varied significantly among months ( $F_{3,54} = 44.4$ ,  $p < 0.001$ ), with highest values in the late wet season (Fig. 5b). There was also a significant time  $\times$  K interaction ( $F_{3,54} = 3.3$ ,  $p = 0.031$ ), due to a greater decline in leaf litter N in the dry season in K addition plots compared to no K plots (not shown). Mean leaf litter N concentrations over the annual cycle were  $1.55 \pm 0.03$  % in no-N plots and  $1.67 \pm 0.03$  % in N addition

plots, an increase of 7.7 % with N addition. The total mass of N in leaf litter over the annual cycle from November 2006 to October 2007 inclusive was  $95.8 \pm 4.9$  kg N ha<sup>-1</sup> in no-N plots and  $110.9 \pm 6.9$  kg N ha<sup>-1</sup> in N addition plots. This 15.8 % increase in leaf litter N mass was marginally non-significant ( $F_{1,18} = 3.3$ ,  $p = 0.084$ ). For P addition, N mass in leaf litter was  $96.6 \pm 7.5$  kg N ha<sup>-1</sup> in no-P plots and  $111.1 \pm 6.9$  kg N ha<sup>-1</sup> in P addition plots. Again, this 15.0 % increase in N mass in leaf litter was marginally non-significant ( $F_{1,18} = 4.1$ ,  $p = 0.058$ ).

Phosphorus addition increased the P concentration in leaf litter fall substantially ( $F_{1,18} = 80.9$ ,  $p < 0.001$ ; Fig. 5c). Seasonal changes were modest but significant ( $F_{3,54} = 4.1$ ,  $p = 0.011$ ). The mean leaf litter P concentration was  $533 \pm 17$  mg P kg<sup>-1</sup> in no-P plots and  $809 \pm 29$  mg P kg<sup>-1</sup> in P addition plots, an increase of 52 % with P addition. There was also a significant time  $\times$  N interaction ( $F_{3,54} = 8.0$ ,  $p = 0.001$ ), because leaf litter P was increased by N in November 2006, but decreased in the other months (not shown). The total mass of P in leaf litter over the annual cycle from November 2006 to October 2007 inclusive was  $3.19 \pm 0.18$  kg P ha<sup>-1</sup> in no-P plots and  $5.61 \pm 0.36$  kg P ha<sup>-1</sup> in P addition plots, a significant 76 % increase with P addition ( $F_{1,18} = 43.9$ ,  $p < 0.001$ ).

#### Nutrient stoichiometry in leaf litter fall

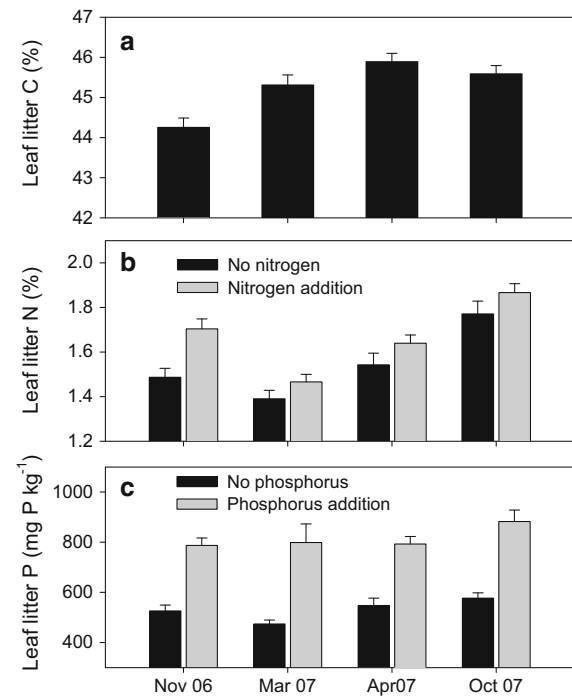
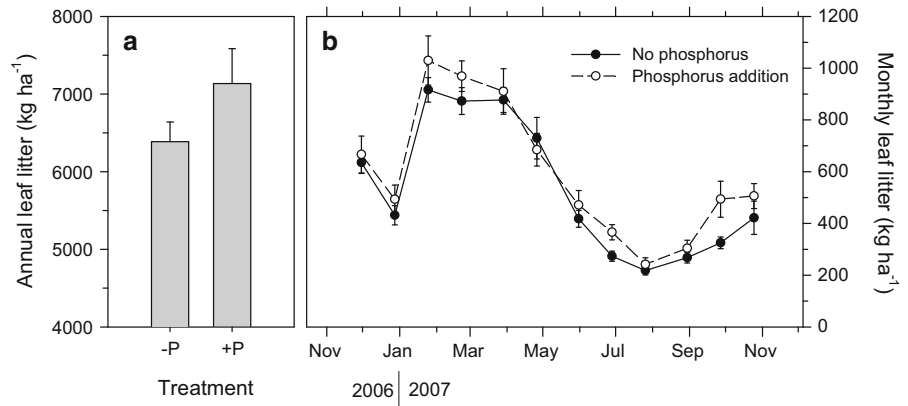
The leaf litter C:N ratio was reduced by N addition ( $F_{1,18} = 10.9$ ,  $p = 0.004$ ) and varied seasonally ( $F_{3,54} = 41.2$ ,  $p < 0.001$ ), being highest in the dry season and lowest in the late wet season (Fig. 6a). The average C:N ratio of leaf litter fall in no-N plots was  $29.7 \pm 0.6$ , compared to  $27.7 \pm 0.4$  in N addition plots, a modest 6.7 % decrease with N addition.

The leaf litter C:P ratio was reduced by P addition ( $F_{1,18} = 79.5$ ,  $p < 0.001$ ) and varied seasonally ( $F_{3,54} = 5.1$ ,  $p = 0.005$ ), being highest in the dry season and lowest in the late wet season (Fig. 6b). The average C:P ratio of leaf litter in no-P plots was  $888 \pm 27$ , compared to  $586 \pm 19$  in P addition plots. There was a significant time  $\times$  N interaction ( $F_{3,54} = 3.6$ ,  $p = 0.022$ ), because the C:P ratio was increased strongly by N addition in the dry season but not in the wet season (not shown).

The leaf litter N to P ratio was reduced strongly by P addition ( $F_{1,18} = 89.8$ ,  $p < 0.001$ ) and increased



**Fig. 4** Leaf litter fall on experiment plots on Gigante Peninsula, Panama, between November 2006 and October 2007 (inclusive), showing **a** the non-significant effect of phosphorus addition on total annual leaf litter, and **b** seasonal variation in monthly leaf litter



**Fig. 5** Seasonal changes in leaf litter carbon (a), nitrogen (b), and phosphorus (c) in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007. The significant effects of nitrogen and phosphorus addition on the respective nutrients in leaf litter are shown

modestly by N addition ( $F_{1,18} = 6.1, p = 0.024$ ), but did not vary seasonally ( $p > 0.10$ ) (Fig. 6c). Mean values were  $29.8 \pm 1.1$  in plots that received neither N nor P,  $31.3 \pm 1.3$  in plots receiving N but not P,  $19.0 \pm 0.8$  in plots receiving P but not N, and  $22.7 \pm 0.9$  in plots receiving both N and P.

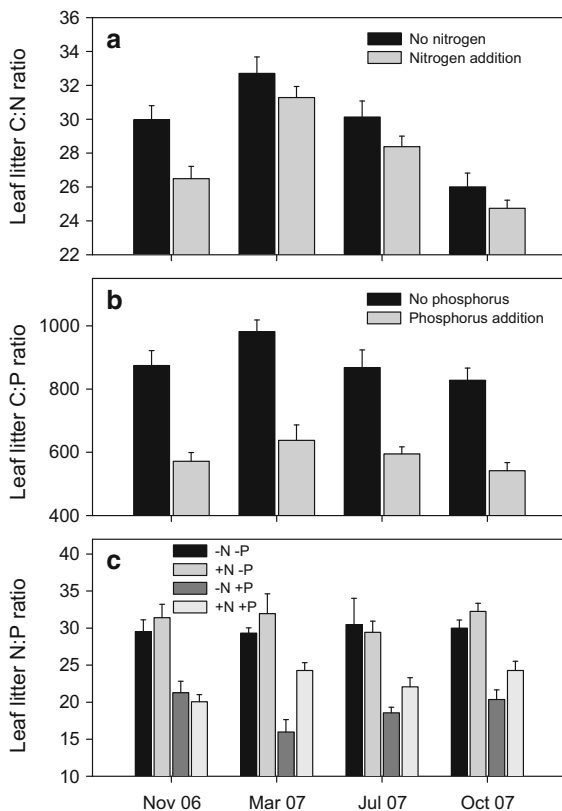
## Discussion

### Treatment effects on soil organic matter

#### Carbon and nitrogen concentrations

Nutrient addition to tropical forest soils can influence soil organic matter concentrations, CO<sub>2</sub> fluxes, and priming effects (Cleveland et al. 2006; Cusack et al. 2011; Nottingham et al. 2012), yet we found no significant effects of a decade of N, P, K, or micronutrient additions on C and N concentrations in soil organic matter. This is despite significant effects of P addition on litter fall and microbial biomass, of K addition on root growth, of N plus K addition on trunk growth, and of K, P, and dolomite/micronutrient addition on litter decomposition (Kaspari et al. 2008; Turner and Wright 2014; Wright et al. 2011; Yavitt et al. 2011). We therefore conclude that sustained changes in nutrient status, such as via atmospheric N deposition, are unlikely to influence soil C stocks in the medium term in lowland tropical forests.

The absence of a detectable change in soil organic matter concentrations in response to P addition was unexpected, given that P availability is widely assumed to limit primary productivity in the lowland tropics, and that there are significant effects of P addition on microbial biomass and decomposition on Gigante Peninsula and elsewhere in the neotropics (Cleveland et al. 2006; Kaspari et al. 2008; Fanin et al. 2014; Turner and Wright 2014). In contrast, the absence of a response to N addition is perhaps less surprising, given that N is assumed to be abundant in the lowland tropics, including on Gigante Peninsula



**Fig. 6** Seasonal changes in leaf litter carbon to nitrogen (C:N) ratio (a), carbon to phosphorus (C:P) ratio (b), and nitrogen to phosphorus (N:P) ratio (c) in fertilized plots on Gigante Peninsula, Panama, between November 2006 and November 2007. The significant effects of nitrogen and phosphorus addition on the respective ratio to carbon are shown in a and b, while the significant effects of nitrogen and phosphorus on N:P ratio are shown separately in c. There are significant seasonal changes in a and b, but not c

(Koehler et al. 2009; Hietz et al. 2011). However, N addition increased soil organic C in tropical forests in Puerto Rico despite no change in primary productivity (Cusack et al. 2011), indicating that soil organic matter can be sensitive to N addition even at sites that appear to be relatively N rich.

In a litter manipulation experiment adjacent to the nutrient addition experiment studied here, Sayer and Tanner (2010) reported greater soil respiration ( $\text{CO}_2$  production) from plots receiving double litter than would be expected from the litter addition alone. They attributed this to a priming effect, whereby labile C from the extra litter promoted the mineralization of stable soil organic C. Here, we find no change in soil organic C in response to the approximately 25 %

increase in litter fall in P addition plots (measured across the entire duration of the experiment; Wright et al. 2011). Presumably the greater rate of litter decomposition in response to P addition (Kaspari et al. 2008) negates the effect of the greater input of litter on the soil organic C stock (Giardina et al. 2004).

There are two caveats to the conclusion that nutrient addition has not affected soil organic matter concentrations in the experiment. First, we sampled only the upper 10 cm of mineral soil, but considerable C stocks can occur in the subsoil of lowland tropical forests (e.g. Silver et al. 2000). It is therefore possible that we did not detect treatment effects manifesting as changes in deep soil C. However, the strongest effects of nutrient additions on soil C stocks are detected at the soil surface (e.g. Cusack et al. 2011), suggesting that the absence of detectable treatment effects in the surface soil of the Gigante experiment likely reflects the response of the entire soil profile.

Second, the statistically insignificant effect of nutrient addition on soil organic C and N does not preclude the possibility of relatively slow changes that are not yet reflected in total C concentrations, or of changes in the dynamics, solubility, or chemical nature of C within the total organic matter pool. For example, N addition to a temperate grassland increased turnover of the light fraction of soil organic matter but further stabilized the heavy fraction (i.e. associated with mineral material), resulting in no net change in soil C (Neff et al. 2002). Similarly,  $\text{CO}_2$  addition to a loblolly pine plantation increased litter fall but also increased litter decomposition, resulting in no net change in soil organic matter (Schlesinger and Lichter 2001). In a tropical forest in Puerto Rico, N addition did not change soil organic C, but did increase mineral-associated C (Li et al. 2006), a process that eventually resulted in a detectable change in soil organic C concentrations and stocks (Cusack et al. 2011).

#### *Organic phosphorus concentrations*

Organic P is abundant in tropical forest soils (Turner and Engelbrecht 2011), yet it is sensitive to perturbation, for example as a result of changing inputs of leaf litter (Vincent et al. 2010). Here, a decade of P addition caused a substantial increase in soil organic P. This increase corresponds with a parallel decline in phosphatase enzyme activity (i.e., the enzymes that hydrolyse organic P to release inorganic phosphate; Turner

and Wright 2014) and a marked increase in readily-extractable soil phosphate (Turner et al. 2013), suggesting that the increase in soil organic P occurred in response to a strong reduction in biological P demand. However, P addition also increased soil microbial P by 49 %, from 64 to 96 mg P kg<sup>-1</sup> (Turner and Wright 2014). Comparison of these values with the soil organic P concentrations detected here (99 mg P kg<sup>-1</sup> in no-P plots and 144 mg P kg<sup>-1</sup> in P addition plots) indicates that the soil microbial biomass accounted for two thirds of the soil organic P and 70 % of the increase in soil organic P with P addition.

Notwithstanding potential errors associated with the quantification of microbial P in soils (e.g. Brookes et al. 1982), it therefore seems likely that a large proportion of the soil organic P in these soils occurs in live microbes. In temperate rain forest soils on the west coast of the South Island of New Zealand, microbial biomass also accounted for a considerable fraction of the soil organic P, and for approximately two thirds of the total biomass P in the ecosystem (i.e., plant plus microbial) (Turner and Condron 2013). Given the significance of soil organic P in the nutrition of tropical forests, and the need to accurately portray biologically available soil P pools in biogeochemical models (Yang et al. 2013), a priority must be to quantify the extent to which live microbial cells contribute to soil organic P in tropical forests.

#### Seasonal dynamics of soil organic matter

We found significant seasonal changes in soil organic matter, with a strong decline in C, N, and organic P during the course of the dry season, followed by a rapid recovery at the onset of the wet season. Few studies have examined seasonal variation in soil organic matter in tropical forests, although a study of Mexican tropical dry forest on Entisols developed on rhyolite reported no seasonal pattern in C, N, or P concentrations in soil organic matter (García-Oliva et al. 2003). Possible explanations for the marked dry season decline in organic matter found here include (i) a transient increase in bulk density, (ii) a decline in microbial biomass, (iii) an increase in the rate of soil organic matter decomposition, and (iv) a reduction in soluble organic matter inputs from leaf litter, root exudates, or root turnover.

An increase in bulk density would be expected to decrease the concentration of organic matter in dry

season samples, assuming that organic matter concentrations decline with depth and that a 10 cm deep dry-season core would sample ‘deeper’ soil than a 10 cm deep wet-season core, effectively diluting the organic matter concentration of the sample. However, this can account for only a small proportion of the observed change, because bulk density was only 4 % greater in the dry season, which we calculate would lead to a small (<2 %) decline in soil organic C. This is considerably less than the 16, 9, and 19–25 % dry-season declines in C, N, and organic P determined here.

The seasonal decline in organic matter nutrients parallels the previously reported declines in microbial biomass nutrients during the dry season (Turner and Wright 2014). However, microbial C and N represent only a small fraction of the total soil C and N, indicating that the decline in microbial biomass *per se* can explain only a small proportion of the change in soil C and N. For example, although microbial C declined by 36 % from 1,321 µg C g<sup>-1</sup> in the wet season to 751 µg C g<sup>-1</sup> in the dry season (in plots that did not receive P), this 0.57 mg C g<sup>-1</sup> decline accounts for only 1 % of the decline in total C (or for 7 % of the 8.1 mg C g<sup>-1</sup> decline in total C from the wet to the dry season). Moreover, this assumes that lost microbial biomass is mineralized completely to CO<sub>2</sub> and inorganic N, whereas much of the organic matter originating from microbial turnover contributes to the stable soil organic matter pool (Miltner et al. 2009).

In contrast, changes in microbial biomass P can explain much of the seasonal pattern in soil organic P, because approximately two thirds of the soil organic P appears to be contained in live microbial biomass (see above). The strong seasonal variation in microbial P (Turner and Wright 2014) must therefore account for a considerable proportion of the seasonal pattern in soil organic P. When soil organic P was corrected to remove the microbial contribution, it varied among sampling dates ( $F_{3,54} = 5.4$ ,  $p = 0.0002$ ) but showed no clear seasonal pattern. This suggests that extracellular soil organic P is relatively stable, despite seasonal changes affecting soil organic C and N.

The decomposition rate of soil organic matter is not greater during the dry season, because although soil CO<sub>2</sub> emissions continue through the dry season (Sayer et al. 2007), the rate is reduced compared to the wet season. For example, a study in the fertilization experiment reported that 26 % of the annual soil CO<sub>2</sub> flux occurred during the four-month dry season

(Koehler et al. 2009). Nottingham et al. (2010) partitioned soil respiration from a lowland tropical forest in Panama, close to the current study site, into fine root, mycelial, and microbial sources. Root and associated mycorrhizal mycelial respiration varied little between dry and wet seasons, although respiration from microbial heterotrophs declined markedly during the dry season.

The decline in organic matter during the dry season does not correspond with a decline in litter inputs, which were much greater during the dry season than the wet season, with approximately half the annual leaf litter falling during the four month dry season. Litter continues to decompose during the dry season, but at a slower rate than during the wet season (Wieder and Wright 1995). Net decomposition is therefore relatively constant throughout the year. However, inputs of dissolved organic C to the forest floor in litter leachate and throughfall are reduced during the dry season, particularly during periods of very low rainfall (e.g., January to February during the current study year, when <12 mm of rain fell in two months). Inputs of DOC from litter leaching can be considerable and can make an important contribution to organic matter in mineral soil (Fujii et al. 2009, 2011). For example, litter leachate contributed 277 kg C ha<sup>-1</sup> y<sup>-1</sup> to the soil in a tropical forest under a perudic moisture regime in Costa Rica, much of which occurred in stable high-molecular weight compounds that were retained in the soil through sorption to mineral surfaces (Schwendenmann and Veldkamp 2005). It therefore seems likely that changes in dissolved organic matter leaching from leaf litter contributes at least in part to the seasonal pattern in soil C and N concentrations observed here.

Allocation of C to support the activity of roots and mycorrhizas represents a considerable proportion of the primary production in forests worldwide (Davidson et al. 2002; Drigo et al. 2010). Although much of the belowground allocation returns to the atmosphere relatively rapidly (80 % was lost as CO<sub>2</sub> within one year in a plantation in Hawaii; Giardina et al. 2004), the belowground C flux can make a considerable contribution to the soil organic matter in some ecosystems (e.g., Clemmensen et al. 2013). Root growth occurs mainly in the wet season in seasonal tropical forests, particularly during the transitions between seasons (Cavelier et al. 1999; Kho et al. 2013; Yavitt and Wright 2001), while fine root mortality occurs mainly

during the dry season (Kummerow et al. 1990). A study on Barro Colorado Island reported that dead fine roots disappear quickly (Yavitt and Wright 2001), so it is possible that death and decomposition of very fine roots and associated mycorrhizal fungi might contribute to the dry season decline in soil organic matter. This would be exacerbated if some of the very fine roots were not removed during the processing of wet-season samples and were therefore included in the measurements of soil C and N. However, fine root biomass in the fertilization plots is 233 g m<sup>-2</sup> for the upper 10 cm of soil (average of measurements made in 2008 and 2009; Wright et al. 2011), equivalent to only 2.9 % of the soil organic C (assuming that roots are 50 % C, wet season soil C is 5 %, and bulk density is 0.8 g cm<sup>-3</sup>). Thus, even if all fine roots were included in the wet-season samples and disappeared during the dry season, this would make a only a small contribution to the observed decline in soil organic matter.

Root exudates vary seasonally in response to patterns in productivity, peaking in the latter part of the growing season in temperate and boreal forests (Högberg et al. 2010; Horwath et al. 1994; Kagawa et al. 2006), although comparable evidence for tropical forests is scarce. Root exudates degrade rapidly upon release into the soil (Butler et al. 2004). However, they might contribute to the seasonal pattern in soil organic matter reported here, given that exudation presumably declines in response to the forest-wide decline in primary productivity during the dry season when many species shed their leaves, and then increases again at the onset of the wet season during following the corresponding leaf flush. A caveat is that the C flux through roots and mycorrhizas can be reduced by nutrient addition (Giardina et al. 2004), yet we observed no significant differences in soil C or N following a decade of nutrient addition. It therefore seems likely that root exudates could make an important contribution to the seasonal pattern in soil organic matter, but additional studies are required to evaluate the contribution of exudates to the soil organic matter pool during wet season conditions.

Overall, we conclude that the most likely explanation for the dry season decline in soil organic C and N concentrations observed here is a reduced input of labile organic matter to the soil from leaf litter leachate and root exudates, coupled with continuous (albeit relatively low) rates of organic matter decomposition as the dry season proceeds. The small increase in bulk

density and the decline in microbial biomass during the dry season together account for only a small proportion of the change in total soil C and N. However, seasonal changes in soil organic P appear to primarily reflect seasonal changes in the microbial biomass, which contains approximately two thirds of the soil organic P and undergoes a marked decline during the dry season.

#### Seasonal and treatment effects on leaf litter fall and nutrient concentrations

Nutrient concentrations in leaf litter fall are widely used to infer the nutrient status of tropical trees (Townsend et al. 2007; Vitousek 1984). Previous studies in the Gigante experiment have reported treatment effects on litter N and P (Kaspari et al. 2008; Sayer et al. 2012; Mayor et al. 2014) but have not examined seasonal variation.

Here we detected treatment effects broadly consistent with previous studies. Across the full duration of the experiment (i.e. since 1998), litter fall has increased significantly with P addition (Wright et al. 2011). In the current study, we found marginally non-significant increases in litterfall and its components in response to P addition for the 2006–2007 annual cycle, which was presumably due to the reduced power of data from a single annual cycle. For leaf litter collected in the first six years of the experiment, the P concentration was 27 % greater than controls (Kaspari et al. 2008). Our results demonstrate that leaf litter P continued to increase in response to P addition in the tenth year of the experiment, to almost double the concentration found in litter from the no-P plots. This parallels the continued increase in soil available P, which was 30 to 50 times greater with P addition during the period of the current study (Turner et al. 2013).

In contrast, the 7 % increase in leaf litter N after a decade of fertilization is identical to the increase observed in the first six years (Kaspari et al. 2008), suggesting that continued N addition does not further increase leaf litter N. Leaf litter N concentrations varied seasonally, with highest concentrations in the late wet season, but there were no seasonal differences for P. Greater N concentrations in wet season leaf litter were also reported for a nearby forest on Barro Colorado Island (Yavitt et al. 2004), as well as in a variety of other tropical forests (e.g., Chuyong et al. 2000; Cuevas and Lugo 1998), suggesting that it is a

widespread phenomenon. Seasonal variation in leaf litter P appears to be slightly more muted compared to the variation in N, as was also reported for an African forest dominated by ectomycorrhizal trees (Chuyong et al. 2000).

The seasonal patterns in leaf litter nutrients are difficult to interpret given the number of tree species contributing litter to the traps at different times during the year and in different plots (e.g., the relative proportion of leaves from legumes vs non-legumes, etc.) (Cuevas and Lugo 1998). Indeed, only four species out of the hundreds at the site occur in all plots, and those four species vary markedly in their litter nutrient concentrations (Mayor et al. 2014). However, the change in leaf litter N might indicate reduced resorption of N during leaf senescence in the late wet season, or the contribution of non-senesced leaves (with higher N and a lower C:N ratio) in this period of greatest rainfall and frequent storms (Cuevas and Lugo 1998). This latter possibility seems unlikely, however, given the absence of parallel variation in leaf litter P.

#### Summary and conclusions

A decade of nutrient addition caused no significant changes in total soil C or N concentrations, although P addition increased soil organic P by approximately 50 %. Much of the increase in soil organic P appears to reflect a change in the soil microbial biomass. The concentrations of C, N and P in soil organic matter undergo marked seasonal variation, declining strongly in the dry season. For C and N, this presumably reflects reduced inputs of labile organic matter from leaf litter leachate and root exudates during the dry season, with minor contributions from the decline in microbial biomass and small increase in bulk density. In contrast, the seasonal pattern in soil organic P appears to be driven by the decline in microbial P during the dry season. The finding that soil organic matter varies seasonally will facilitate the development and evaluation of ecosystem models and has important implications for future soil C stocks, given the predicted changes in precipitation and dry season length in the lowland tropics (Collins et al. 2013).

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