European Journal of Soil Science

European Journal of Soil Science, February 2010, **61**, 48–57 doi: 10.1111/j.1365-2389.2009.01200.x

Soil organic phosphorus dynamics following perturbation of litter cycling in a tropical moist forest

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Summary

The productivity of tropical lowland moist forests is often considered to be limited by the availability of phosphorus. Organic phosphorus is often abundant in tropical soils, but its role in forest nutrition is largely unknown. We addressed this by using a large-scale litter manipulation experiment to investigate the stability of soil organic phosphorus in a tropical lowland forest in Central Panama. Three years of litter removal reduced the organic phosphorus concentration in the surface 2 cm of mineral soil by 23%, as determined by NaOH-EDTA extraction and ^{31}P-NMR spectroscopy; this included decreases in phosphate monoesters (20%) and DNA (30%). Three years of litter addition (equivalent to adding 6 kg P ha^{-1} per year) increased soil organic phosphorus by 16%, which included a 31% increase in DNA. We did not detect higher-order inositol phosphates, despite their abundance in mineral soils of temperate ecosystems. Our observed turnover rate suggests that even the 0–2-cm layer of the mineral soil contributes a fifth of the total phosphorus needed to sustain above-ground growth in this forest. Soil organic phosphorus is thus likely to make a more important contribution to the nutrition of semi-evergreen forest plants than has hitherto been acknowledged.

Introduction

Biological productivity in tropical lowland forests is considered conventionally to be limited by phosphorus availability (Tanner *et al.*, 1998), yet phosphorus limitation does not appear to be widespread. Of four experiments in lowland forest, three showed no effect of phosphorus fertilization (Newbery *et al.*, 2002; Davidson *et al.*, 2004; Kaspari *et al.*, 2008), although one in South East Asia showed increased litterfall in response to phosphorus fertilizer addition (Mirmanto *et al.*, 1999). In addition, many tropical lowland forests maintain a very large biomass despite small contents of leaf phosphorus (Kitayama, 2005). For example, in a South East Asian tropical forest growing on infertile soils, a large biomass of 65 kg m^{-2} occurs simultaneously with N:P ratios in leaf litter of >90 (Proctor *et al.*, 1983a,b). In regions of moderate tectonic uplift, such as parts of Central America, erosion may reduce phosphorus limitation through the continual supply of bedrock-derived phosphorus to the rooting zone (Porder *et al.*, 2007). However, there is evidence that phosphorus limitation, in at least parts of such areas, can be severe enough to constrain microbial oxidation of dissolved organic carbon (Cleveland *et al.*, 2002).

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Received 9 July 2009; revised version accepted 3 September 2009

Many of the world's tropical forests occur on strongly-weathered Ultisols and Oxisols, in which phosphorus is present mainly as organic phosphorus and 'occluded' inorganic forms of phosphate contained within the structure of secondary minerals (Walker & Syers, 1976; Tiessen *et al.*, 1984). Although there is some evidence that occluded phosphorus can be mobilized by roots (Gahoonia & Nielsen, 1992) or by reduction of iron-phosphate complexes during anaerobic conditions (Chacón *et al.*, 2006), it is usually considered to be of limited availability to organisms.

Given that most phosphorus in strongly-weathered tropical forest soils occurs as occluded phosphorus, the main supply of biologically available phosphorus (i.e. soluble inorganic phosphate ions) in such soils may be the turnover of organic phosphorus (Tiessen *et al.*, 1992; Johnson *et al.*, 2003). In forest ecosystems, organic phosphorus occurs in fresh organic matter inputs (e.g. leaf litter), microbial biomass and non-biomass (stable) soil organic phosphorus. It has been argued that the rapid decomposition of leaf litter (i.e. <1 year in the humid tropics; Sampaio *et al.*, 1993) provides a major fraction of the phosphorus supporting growth on strongly-weathered tropical soils. This 'direct nutrient cycling' (Stark & Jordan, 1978) can be interpreted as a process whereby biologically-available phosphate released during leaf litter decomposition is absorbed so quickly and so close to the decomposition sites by roots and mycorrhizae that almost no

phosphorus is lost via leaching or sorption to iron and aluminium oxides.

Although direct phosphorus recycling from litterfall occurs in tropical forests growing on white sands with extremely poor nutrient contents (Baillie, 1996), there is currently little information on the relative importance of this process compared with the release of soluble inorganic phosphate ions from soil organic phosphorus in the nutrition of trees growing on moderately fertile soils, which are abundant throughout the tropics (Cochrane *et al.*, 1985). Organic phosphorus is relatively abundant in tropical mineral soils (on average 29 $\pm$ 3% of the total phosphorus in 'equatorial zone' forests; Harrison, 1987) and can become available to plants following hydrolysis by extracellular and periplasmic phosphatase enzymes, which are produced by plants and soil microorganisms in response to the need for phosphorus. Organic phosphorus can also be released during the oxidation of organic carbon by soil organisms. These two mechanisms have been called 'biochemical' and 'biological' mineralization, respectively (McGill & Cole, 1981). Of particular significance is that organic phosphorus mineralization can occur independently of organic matter oxidation. The relative importance of these two processes can be assessed by examining carbon to organic phosphorus ratios.

Despite the abundance of organic phosphorus in most soils, its stability in undisturbed tropical lowland forests is unknown. This is partly because of the difficulty of measuring organic phosphorus mineralization by radioisotope procedures in strongly-weathered soils with a large phosphate fixation capacity (Bühler *et al.*, 2003). Indirect evidence suggests that organic phosphorus in cultivated tropical soils can be relatively labile (Nziguheba & Bünemann, 2005), but there are currently no estimates of organic phosphorus turnover in undisturbed tropical forest soils, despite the probable significance of this process for the phosphorus nutrition of plants growing in such ecosystems (Tiessen & Shang, 1998).

As an alternative method to estimate organic phosphorus turnover in soils with a large sorption capacity, we used a large-scale leaf litter manipulation experiment in central Panama together with solution ^{31}P NMR spectroscopy. The rationale behind this approach was that interrupting inputs of fresh organic matter to the soil would enable us to observe net changes in the concentration of soil organic phosphorus, the magnitude and direction of these changes being proportional to the degree of susceptibility to mineralization. In this way, if soil organic phosphorus is a dynamic pool it would degrade over time and its concentration would decrease, whereas a very stable pool would show little change. In addition, using NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy allowed us to investigate the dynamics of specific soil organic phosphorus groups following perturbation of litterfall. We hypothesized that short- to medium-term changes (over 3 years) in litterfall abundance on the forest floor would influence the quantity and structural composition of soil organic phosphorus. Knowledge of the stability of soil organic phosphorus is important not only to better understand plant nutrition and nutrient cycling in undisturbed forests, but also in tropical agroecosystems, where the mineralization of organic matter is often a crucial source of nutrients.

Materials and methods

Study area

The Gigante Litter Manipulation Project (GLiMP) is located on Gigante Peninsula, part of Barro Colorado Nature Monument (BCNM) (9°06′N, 79°54′W), Republic of Panama. This area of semi-deciduous forest has a tropical monsoon climate, with an average temperature of 26°C and a mean annual precipitation of 2620 mm (Windsor, 1990). Litterfall peaks early in the dry season during December, continues until the end of April, and then decreases by 50% in the wet season from May to November (Wright & Cornejo, 1990).

Soils on the northern part of Gigante Peninsula (and under our plots) are moderately acidic (pH 4.8–5.4) Oxisols (Cavelier, 1992) developed on basalt (Stewart *et al.*, 1980). The nutrient status of our study site is difficult to assess. On the one hand, the soils contain little 'plant-available' phosphorus (Cavelier, 1992; and our study: both determined using the procedure of Bray & Kurtz, 1945, hereafter called 'Bray-1') relative to values reported for other tropical lowland forests (e.g. Powers *et al.*, 2005; John *et al.*, 2007, both using Mehlich III; Phillips *et al.*, personal communication, using Bray-1). Furthermore, a long-term (11-year) fertilizer addition experiment close to our study site shows that phosphorus fertilizer treatment of large forest plots increased the phosphorus concentration and the decomposition rate of leaf litter (Kaspari *et al.*, 2008), also suggesting that phosphorus was in short supply. On the other hand, phosphorus concentrations in litterfall are relatively large in our study site compared with other tropical forests, indicating that phosphorus use efficiency is poor (*sensu* Vitousek, 1984), while phosphorus fertilization failed to increase leaf, twig and reproductive litterfall (i.e. fruits and flowers; Kaspari *et al.*, 2008): these two facts suggest that phosphorus may be in adequate supply.

Gigante Litter Manipulation Project

The Gigante Litter Manipulation Project (GLiMP) was established in 2000, and consists of 15 45 m $\times$ 45 m plots trenched to a depth of 50 cm. The trenches were lined on both sides with construction plastic sheets and back-filled with the original soil to reduce nutrient transport from outside the plots by roots and/or mycorrhizal hyphae. Litter manipulation started in January 2003 and there were three treatments: litter addition, litter removal and control. Each treatment has five replicate plots. The litter was removed from the five litter removal plots once a month by rake and hand, which resulted in almost no standing litter, and was then evenly distributed over the five litter-addition plots. Three years of litter addition (2003–2006) represent an approximate net addition of 6 kg P ha^{-1} per year. Annual phosphorus inputs via leaf litter on the Gigante Peninsula (6.4 kg P ha^{-1}; Kaspari *et al*., 2008) are relatively large compared with other mature lowland forests. For

example, Coomes (1997) reports an annual average for 11 forests growing on Ultisols and Oxisols of only 3.1 kg P ha^{-1}.

Soil sampling and preparation

Soil was sampled on 21 January 2006 (i.e. in the first half of the dry season) in an 'X' design in the internal 30 m × 30 m area of each plot. Thirteen equidistant sampling points along each cross 'arm' were marked with flags prior to coring. Two samples 1 m apart were taken at each sampling point, giving a total of 52 individual cores per plot. Where the sampling point was <1.5 m from the base of a tree or palm clump, or if the corer encountered an air pocket or large root, a new site was chosen by moving 0.5 m in a N, S, E or W sequence until an adequate spot was found. The litter layer (Oi) was carefully removed by hand until no identifiable plant fragments remained and then samples were taken with a 2-cm diameter punch corer to 2-cm depth in the mineral soil (A horizon). In some places in litter-addition plots an incipient and thin fermentation layer (Oe) was encountered, which was also carefully removed by hand until the mineral soil was visible. We chose to sample to 2 cm depth because earlier soil analysis showed significant differences in extractable nutrients from 0 to 2 cm but not from 0 to 10 cm depth, suggesting that soil impoverishment was occurring from the surface downwards.

The cores from each plot were combined in the field to yield a single bulked sample per plot, sealed in plastic bags and transported back to the laboratory in Panama City on the same day. Samples were sieved (<4 mm) to remove stones and vegetative matter and a sub-sample was stored at 4°C until analysis (<48 h). The remaining portion was air-dried for 10 days at 24°C and milled to a fine powder.

Solution ^{31}P NMR spectroscopy

Phosphorus was extracted by shaking 1.5 g of dry and ground soil in 30 ml of a solution containing 0.25 M NaOH and 50 mM Na_2EDTA (ethylenediaminetetraacetate) for 16 hours (Cade-Menun & Preston, 1996). Extracts were centrifuged (30 minutes, 10 000 ***g***) and a 20 ml aliquot spiked with 1 ml of 50 μg P ml^{-1} methylene diphosphonic acid (MDPA) solution as an internal standard. The extracts were then frozen at −35°C, freeze-dried and ground. The NaOH-EDTA procedure extracts organic and inorganic phosphorus associated with aluminium and iron oxides (Turner, 2008) and is assumed to extract quantitatively organic phosphorus from soil, although this is impossible to verify as there is no procedure to determine soil organic phosphorus directly (Turner *et al.*, 2005). The ignition procedure, in which organic phosphorus is estimated as the difference in acid-extractable phosphate following high-temperature treatment, is known to markedly over-estimate the organic phosphorus in strongly-weathered tropical soils (Condron *et al.*, 1990). In contrast, the NaOH-EDTA procedure was recently reported to be suitable for the extraction of soil organic phosphorus from strongly-weathered soils under tropical moist forest (Turner, 2008).

Each freeze-dried extract (approximately 100 mg) was re-dissolved in 0.1 ml of deuterium oxide and 0.9 ml of a solution containing 1.0 M NaOH and 100 mM Na_2EDTA, and then transferred to a 5-mm NMR tube. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer (Bruker, Karlsruhe, Germany) operating at 202.456 MHz for ^{31}P. Samples were analysed using a 6 μs pulse (45°), a delay time of 2.0 s, an acquisition time of 0.4 s and broadband proton decoupling. Approximately 30 000 scans were acquired for all samples. Chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4. Spectra were plotted with a line broadening of 5 Hz and signals were assigned to phosphorus compounds on the basis of literature reports of model compounds spiked in NaOH-EDTA soil extracts (Turner *et al.*, 2003). Signal areas were calculated by integration and concentrations of phosphorus compounds (mg P kg^{-1} soil) were calculated from the integral value of the methylene diphosphonic acid spike. All spectral processing was done with NMR Utility Transform Software (NUTS) for Windows (Acorn NMR, Livermore, CA).

Other chemical analyses

Total carbon and nitrogen were determined on dried and ground samples by combustion and gas chromatography using a Thermo-Electron Flash 1112 CN analyser (CE Elantech, Lakewood, NJ). Total phosphorus was determined by ignition (550°C for 1 h) and acid extraction (1 M H_2SO_4, 1 : 50 soil to solution ratio for 16 h) (Saunders & Williams, 1955), with phosphate determined by automated molybdate colorimetry (Lachat Quickchem, Hach Ltd, Loveland, CO). Bray-1 phosphorus, widely used as an index of instantaneously 'available' phosphorus both in temperate agricultural (Page *et al.*, 1982) and in tropical soils (Sanchez, 1976), was extracted from fresh soils within 24 h of sieving using Bray-1 solution (30 mM NH_4F and 25 mM HCl) and a 1:10 soil-to-solution ratio (Bray & Kurtz, 1945), with phosphate determined by automated molybdate colorimetry. 'Strongly-bound' or 'residual' phosphorus was calculated by subtracting NaOH-EDTA-extractable phosphorus from the total soil phosphorus, assuming that calcium-bound phosphate in primary minerals (i.e. HCl-extractable phosphorus) is negligible in strongly-weathered soils such as those analysed here (Tiessen *et al.*, 1984). All concentrations are expressed on the basis of oven dry soil (105°C for 24 hours).

Statistical analysis

Results are presented as means of five replicate plots per litter-manipulation treatment ± 1 standard error of the mean. Differences between treatments were tested with one-way ANOVA and differences between a given treatment and the control with orthogonal treatment contrasts after a significant ANOVA. When data did not meet homogeneity of variances and normality assumptions (checked with visual inspection of quantile-quantile

plots) even after a transformation, a Kruskal-Wallis test was used as a non-parametric alternative, with differences between individual treatments and controls tested with Wilcoxon signed-rank tests (test statistic = W). All analyses were done with R version 2.2.0 (www.r-project.org). The stock of total organic phosphorus in the surface 2 cm of the soil was calculated from published soil bulk density values (Sayer *et al.*, 2006).

Results

Phosphorus fractions

Total phosphorus in surface soil of control plots was 399 ± 9 mg P kg^{-1}, and is somewhat smaller than the 550 mg kg^{-1} reported by Köhler *et al.* (2009) for 0–5 cm in a nearby forest; our value is well within the range of values (218–557 mg P kg^{-1}) reported for Oxisols under tropical lowland forest (Cleveland *et al*., 2002; Johnson *et al*., 2003). Total phosphorus was significantly affected by litter manipulation ($F = 7.18$, $P = 0.009$). The NaOH-EDTA extractable phosphorus in control plots was 179 ± 9 mg kg^{-1}, equivalent to 45 ± 7% of the total soil phosphorus (Table 1). Litter removal reduced NaOH-EDTA extractable phosphorus by 17%. In contrast, strongly bound phosphorus (i.e. total phosphorus minus NaOH-EDTA extractable phosphorus), which represented 53–59% of the total phosphorus, did not differ between treatments (Table 1). Bray-1 phosphate in surface soil of control plots was 0.59 ± 0.04 mg P kg^{-1} and represented ≤ 0.3% of the total soil phosphorus in all plots. Our values are small relative to Olsen-extractable phosphorus values for Oxisols under lowland semi-deciduous forest in adjacent Barro Colorado Island (Yavitt, 2000), and small relative to Mehlich III values for Barro Colorado Island and lowland evergreen forest Ultisols in Yasuni, Ecuador (John *et al.,* 2007). They are also small relative to Mehlich III values for rainforest Oxisols in La Selva, Costa Rica, and Cocha Cashu, Peru (Powers *et al*., 2005). Litter removal reduced the mean concentration of Bray-1 phosphorus in our study site by 27%, whereas litter addition increased it by 103% (Table 1).

Phosphorus composition determined by solution ^{31}P NMR spectroscopy

Representative solution ^{31}P NMR spectra of the three treatments are shown in Figure 1. Apart from the internal standard (methylene diphosphonic acid) at 17.5 ppm, the main signals were assigned to inorganic orthophosphate at approximately 6.3 ppm, phosphate monoesters between 3.5 and 6 ppm, DNA at approximately 0 ppm, and pyrophosphate at approximately −4 ppm. The latter compound is a condensed inorganic phosphate of chain length $n = 2$, which must be hydrolysed prior to plant uptake. Intact phospholipids, which occur between 0.5 and 2.0 ppm, were detected in unquantifiable trace amounts. Neither phosphonates (usually detected at approximately 20 ppm), nor long-chain polyphosphates (observed at approximately −20 ppm) were detected.

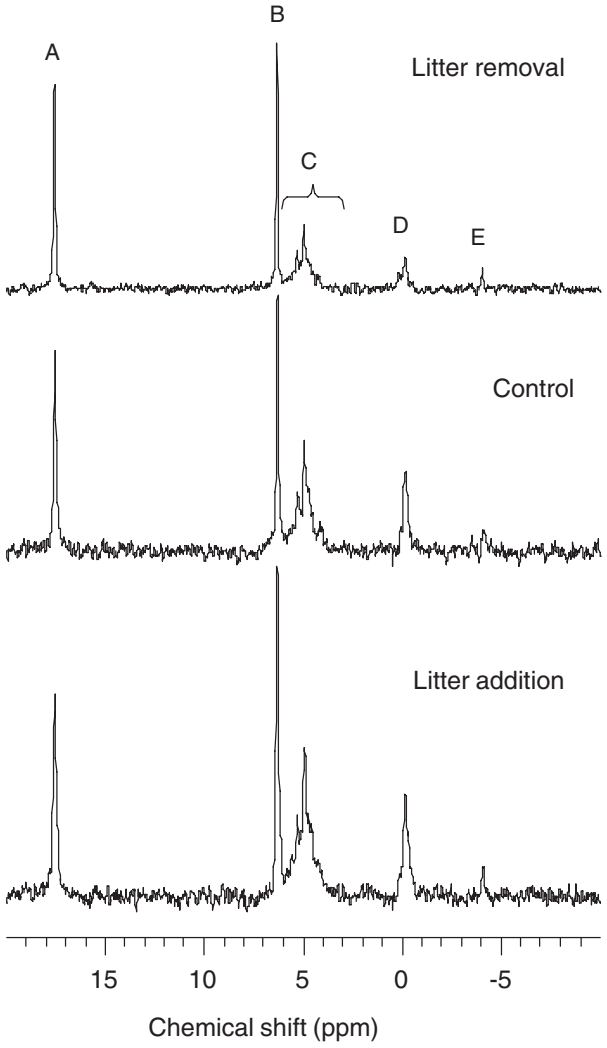

Figure 1 Representative solution ^{31}P NMR spectra of NaOH-EDTA extracts of surface soil from litter removal, control and litter addition plots in a tropical moist forest on the Gigante Peninsula, central Panama. Signals were assigned as follows: A, methylene diphosphonic acid (MDPA) internal standard; B, phosphate; C, phosphate monoesters; D, DNA; E, pyrophosphate. Trace concentrations of phospholipids occurred between 0.5 and 2.0 ppm but were not quantified. Spectra were adjusted to the phosphate signal at 6.3 ppm and scaled to the height of the MDPA signal.

Closer inspection of the phosphate monoester region of the spectra revealed that the main signals occurred at 5.3 and 4.9 ppm (Figure 2), indicating the presence of compounds derived from the hydrolysis of phospholipids, notably phosphatidyl choline, in alkaline solution (Makarov *et al*., 2002; Turner *et al.*, 2003). Additional phosphate monoester signals between 4.2 and 4.9 ppm (Figure 2) were probably mononucleotide degradation products of RNA in alkaline solution (Makarov *et al*., 2002; Turner *et al.*, 2003). Inositol phosphates, most readily identified by a signal at 5.9 ppm from the C-2 phosphate of *myo*-inositol hexakisphosphate (Turner *et al.*, 2003) and a signal at 4.2 ppm from *scyllo*-inositol hexakisphosphate were not detected in any of the spectra, although

Table 1 The mean concentrations of phosphorus (P) compounds in mineral soil (0–2 cm) from litter manipulation plots in the Gigante Peninsula, central Panama

	Litter removal	Control	Litter addition	Litter removal	Control	Litter addition
P fraction	Concentration / mg P kg^{-1}			Proportion of total P (%)		
Total P	368 (11)	399 (9)	433 (16)	–	–	–
Strongly-bound P	219 (12)	220 (14)	228 (14)	59 (2)	55 (3)	53 (2)
NaOH-EDTA Extractable P	149* (6)	179 (9)	205 (12)	41 (4)	45 (7)	47 (5)
Bray-1 P	0.43* (0.04)	0.59 (0.04)	1.30* (0.2)	0.12 (0.01)	0.15 (0.01)	0.3 (0.05)

*Values are means followed (in parentheses) by one standard error of five replicate plots. Asterisks denote statistical differences ($P < 0.05$) for a particular phosphorus fraction between a litter manipulation treatment and controls after a significant ANOVA. All phosphorus fractions below were analysed with ANOVA except for Bray-1 phosphorus, analysed with a Kruskal Wallis rank sum test.

the relatively poor signal-to-noise ratios mean that the presence of small concentrations of inositol phosphates cannot be ruled out (Turner & Richardson, 2004). However, on the basis of the identified signals (see earlier) it seems probable that most of the phosphate monoesters detected here occurred as phosphate diesters prior to extraction.

Surface soil of control plots contained 120 ± 5 mg P kg^{-1} as organic phosphorus (Figure 3a), equivalent to $67.0 \pm 1.3\%$ of the NaOH-EDTA extractable phosphorus and $30.1 \pm 3.2\%$ of the total soil phosphorus. Of this, 74% was phosphate monoesters and 26% was DNA (Figure 3c,e). The concentration of total inorganic phosphorus was 59 ± 4 mg P kg^{-1} (Figure 3b), equivalent to $33.0 \pm 1.3\%$ of the NaOH-EDTA extractable phosphorus and $14.9 \pm 1.2\%$ of the total soil phosphorus (Table 1). Of this, 86% was inorganic orthophosphate (Figure 3d) and 14% was pyrophosphate (Figure 3f).

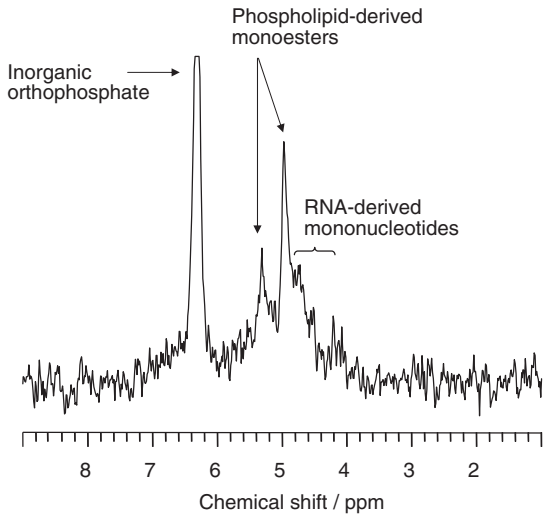

Figure 2 Signals in the phosphate monoester region of a solution ^{31}P NMR spectrum of a NaOH-EDTA extract of mineral soil (0–2 cm) from a control plot in the Gigante Litter Manipulation Project, central Panama.

Litter manipulation significantly affected total soil organic phosphorus ($F = 18.34$, $P < 0.001$). Litter removal reduced total organic phosphorus by 23% (Figure 3a). DNA was reduced by a greater proportion (30%) than phosphate monoesters (20%) (Figure 3c,e). Litter addition increased total organic phosphorus by 16% and the change was mainly accounted for by an increase in the concentration of DNA (31%) because the increase in phosphate monoesters (11%) was not significantly different from the control (Figure 3). Pyrophosphate and inorganic orthophosphate were not significantly changed by litter manipulation (Figure 3).

The stock of total organic phosphorus in soil from control plots was 17.2 ± 1.5 kg P ha^{-1} (0–2 cm), which was reduced by 4.2 ± 1.6 kg P ha^{-1} after 3 years of litter removal, which was equivalent to an organic phosphorus turnover rate of 1.4 kg P ha^{-1} per year.

Total soil carbon and nitrogen

Surface soil (0–2 cm) in control plots contained 82 ± 2 g C kg^{-1} soil and 7.1 ± 0.1 g N kg^{-1} (Table 2). Litter removal significantly reduced the total carbon concentration by 26% and the total nitrogen concentration by 20%. Litter addition tended to increase these values by 36% ($W = 6$, $P = 0.22$) and 23% ($W = 8$, $P = 0.42$) for carbon and nitrogen, respectively, although the values were not statistically significantly different, probably because of the greater variability observed in litter-addition plots (Table 2). The carbon-to-nitrogen ratio in surface soil of control plots was 11.5 ± 0.2 and was significantly affected by litter manipulation ($F = 11.43$, $P = 0.002$). The ratio of carbon to organic phosphorus in control plots was 690 ± 20 and was not significantly changed by litter manipulation (Kruskal-Wallis, $\chi^2 = 1.37$, $P = 0.51$). Similarly, the ratio of nitrogen to organic phosphorus in control plots was 60 ± 2.4 and was not significantly changed by litter manipulation ($F = 0.21$, $P = 0.81$).

Discussion

Organic phosphorus mineralization is often cited as being important in supplying phosphorus to vegetation (Johnson *et al.*,

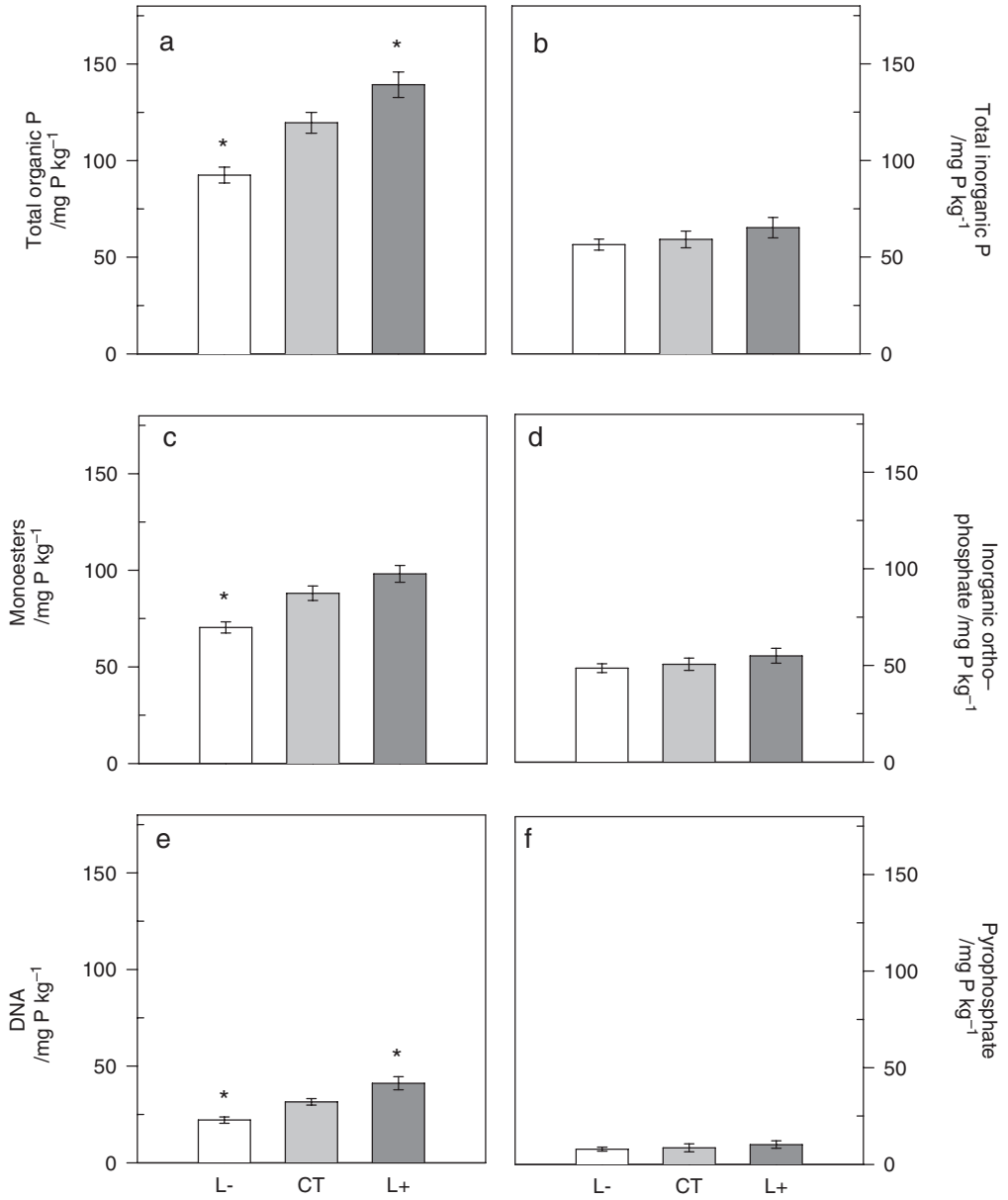

Figure 3 Concentration of phosphorus compounds in NaOH-EDTA extracts of surface mineral soil (0–2 cm) from litter manipulation plots on the Gigante Peninsula, central Panama, determined by solution ^{31}P NMR spectroscopy. Bars are the mean ± one standard error of five replicate plots. L–, litter removal; CT, control; L+, litter addition. (a) Total organic phosphorus (the sum of (c) phosphate monoesters and (e) DNA); (b) total inorganic phosphorus (the sum of (d) inorganic orthophosphate and (f) pyrophosphate). *Indicates significant differences between a treatment and the control at $P < 0.05$, determined with orthogonal treatment contrasts after a significant ANOVA, except for total organic phosphorus where $P < 0.06$.

2003), particularly for vegetation growing specifically on old, strongly-weathered soils (Tiessen *et al.*, 1992; Tiessen & Shang, 1998). To our knowledge, however, there are no estimates of turnover rates of soil organic phosphorus in undisturbed tropical forests. In our study site, a tropical lowland forest growing on an Oxisol, 3 years of litter removal caused a decline in soil organic phosphorus, estimated at 1.4 kg P ha^{-1} per year for the surface 2 cm of soil. From our leaf litter measurements at the study site, the annual plant uptake of phosphorus for above-ground growth was estimated at 6.4 kg P ha^{-1} (Kaspari *et al.*, 2008). Therefore our observed turnover rate suggests that even the 0–2-cm layer of the mineral soil can contribute a fifth of the total phosphorus needed to sustain above-ground growth in this forest. The decline in soil organic phosphorus in litter removal plots was mirrored by an accumulation following litter addition, confirming the dynamic nature of the soil organic phosphorus pool in this forest. The fact that we observed differences in the organic phosphorus fractions after 3 years of litter manipulation, but did not find differences

Table 2 The mean concentration of organic carbon and total nitrogen in mineral soil (0–2 cm) from litter manipulation plots in the Gigante Peninsula, central Panama

Treatment	C	N	C:N ratio
	/g kg^{-1}		
Litter removal	60 (3)*	5.7 (0.2)*	10.7 (0.3)
Control	82 (2)	7.1 (0.1)	11.5 (0.2)
Litter addition	112 (13)	8.7 (0.8)	12.6 (0.4)*

*Values are means followed (in parentheses) by one standard error of five replicate plots. Asterisks denote statistical differences ($P < 0.05$) for a particular element between a litter manipulation treatment and controls, determined with orthogonal treatment contrasts after a significant ANOVA.

in the strongly-bound phosphorus fraction suggests that, at least over 3 years, organic phosphorus mineralization contributed more to plant and microbial phosphorus availability than phosphorus in strongly bound inorganic pools.

Although it seems likely that the decline in organic phosphorus following litter removal resulted from mineralization and uptake by vegetation, it could also result in part from the loss of phosphorus from the soil in leachate, or the transformation of mineralized organic phosphorus into strongly-bound inorganic forms. Transfer from the plots in leachate is unlikely given that the loss of both inorganic and organic phosphorus by leaching is negligible in strongly-weathered soils, including those under tropical forest (e.g. Vitousek, 2004). Transformation into strongly-bound inorganic phosphorus was also unlikely, because the strongly-bound phosphorus pool (defined here as total phosphorus minus NaOH-EDTA extractable phosphorus) did not differ significantly among treatments. We therefore conclude that soil organic phosphorus in this forest is relatively labile and can contribute to the nutrition of plants.

The reduction in soil organic phosphorus measured here is comparable to the marked decline in soil organic phosphorus that occurs after the cultivation of tropical soils. For example, soil organic phosphorus in surface soil (0–20 cm) declined by 12–57% after the conversion of native tropical vegetation to agriculture (reviewed in Nziguheba & Bünemann, 2005). These sites, which did not receive mineral phosphate fertilizer and varied in the time since the onset of agriculture (3–45 years), indicate that the mineralization of organic phosphorus can occur relatively rapidly. For example, cultivation of *caatinga* land in semi-arid northeastern Brazil reduced the soil organic phosphorus content by 30% after only 6 years (Tiessen *et al.*, 1992). Our 23% reduction in soil organic phosphorus after removing litterfall only, that is without tillage or canopy removal, confirms that there is a great risk of rapid losses of potentially plant-available soil phosphorus with agriculture in this part the world, given fast mineralization rates of organic phosphorus and subsequent transformation of the released phosphate into strongly-bound inorganic forms. The development and implementation of sustainable agricultural practices for organic phosphorus management should thus be a high priority, given that this is an important phosphorus source for crops, as has been shown in a number of studies (e.g. Tiessen *et al.,* 1992).

McGill & Cole (1981) proposed that the 'biochemical' mineralization of organic phosphorus occurs independently of the 'biological' mineralization of carbon-bonded nitrogen and sulphur, and is driven solely by a demand for phosphorus. These mechanisms were suggested to account for observed differences in C:N:P:S ratios among soils. Although phosphatase enzymes are known to be important in controlling organic phosphorus (i.e. 'biochemical') mineralization in the rhizosphere, it is increasingly evident that the dynamics of carbon and organic phosphorus can be tightly coupled in forest systems (i.e. 'biological' mineralization, Gressel *et al*., 1996; Möller *et al.*, 2000). For example, inorganic phosphorus and carbon in the surface horizons of a temperate forest soil are strongly correlated, suggesting that phosphorus mineralization is coupled with the decomposition of organic matter (Gressel *et al*., 1996). Biological mineralization has additionally been proposed to affect the accessibility of organic phosphorus, thus influencing the rate of biochemical hydrolysis by extracellular phosphatase enzymes (Condron *et al.*, 2005). There is at present little information on the relative importance of biochemical compared with biological processes on organic phosphorus mineralization in undisturbed tropical forests over time-scales of a few years. Our findings support the hypothesis that biological mineralization is the dominant driver of organic phosphorus turnover in this forest, because litter manipulation did not significantly alter the ratio of carbon (or nitrogen) to organic phosphorus in soil. This agrees with many studies that report consistent carbon to organic phosphorus ratios during cultivation of tropical soils (Nziguheba & Bünemann, 2005) and suggests a 'non-discriminating' biological mineralization of soil organic matter.

Information on the chemical nature of soil organic phosphorus can provide insight into the mechanisms regulating its behaviour and biological availability (Condron *et al.*, 2005). One of the most interesting results from our study is that organic phosphorus in the surface 2 cm of the mineral soil was dominated by phosphate diesters, i.e. DNA and the monoester breakdown products of phospholipids and RNA, probably formed during extraction and analysis (Makarov *et al*., 2002; Turner *et al*., 2003; Doolette *et al*., 2009). A similar phosphorus composition was recently reported for subtropical wetlands (Turner & Newman, 2005) as well as northern forest soils (e.g. Cade-Menun *et al.*, 2000; Preston & Trofymow, 2000), but was unexpected here because organic phosphorus in most mineral soils (our site has mineral soils) is dominated by higher-order inositol phosphates, notably salts of *myo*-inositol hexakisphosphate (phytate) (Turner, 2007). These compounds, which constitute the major phosphorus compounds in plant seeds (Lott *et al.*, 2000), are stabilized in soil by precipitation and sorption reactions and therefore usually accumulate to a greater extent than other organic phosphorus compounds (Celi & Barberis, 2005). There have been no broad studies of inositol phosphates in tropical forest soils, although it was recently reported that concentrations of higher-order inositol phosphates

declined markedly in old, strongly-weathered soils supporting temperate rainforest (Turner *et al.*, 2007). The absence of inositol phosphates may explain, in part, the apparent dominance of biological mineralization, because the decoupling of organic phosphorus and organic carbon mineralization, expected to occur during biochemical mineralization, may only be detectable when soils contain a relatively large concentration of organic phosphorus stabilized on mineral surfaces (i.e. inositol phosphates).

Given that our data include only the surface 2 cm of the soil we cannot establish with certainty the total depletion of organic phosphorus in the soil rooting depth. However, the organic phosphorus in this 0–2 cm layer is clearly labile, because its concentration declined by 23% in only 3 years. Furthermore, this layer can provide one-fifth of the phosphorus needed to sustain above-ground growth. Our results demonstrate that a large proportion of phosphorus necessary for plant growth can be obtained from the soil and need not come directly from litterfall, as is generally assumed. We also found that the total soil nitrogen was 20% less in litter removal plots after 3 years of litter removal; this shows that organic nitrogen could supply 50% of the nitrogen needed for above-ground growth, at least when litter is removed. We now need to establish to what extent mineralization of soil organic matter normally supplies phosphorus and nitrogen for tree growth. Our research site has semi-evergreen rainforest, a forest type that covers extensive areas in the tropics, so our results should be of general importance, but more experiments in different places are necessary to confirm this.

Acknowledgements

We thank Tania Romero (STRI) for help with laboratory analysis, Dr O. Phillips (University of Leeds, UK) and collaborators for access to their dataset on soil nutrient concentrations for South American forests and Drs Charles Shand (Macaulay Institute, Scotland) and David Coomes (University of Cambridge, UK) for valuable comments on the manuscript. Dr Emma Sayer (STRI) was the principal manager of the litter manipulation experiment. This work was funded by a Smithsonian Tropical Research Institute (STRI) fellowship to AGV and a Mellon Foundation grant to EVJT. This work was conducted at STRI in Panama, to whom we are grateful for logistical support.

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