

Soil organic phosphorus in lowland tropical rain forests

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Abstract Phosphorus is widely considered to constrain primary productivity in tropical rain forests, yet the chemistry of soil organic phosphorus in such ecosystems remains poorly understood. We assessed the composition of soil organic phosphorus in 19 contrasting soils under lowland tropical forest in the Republic of Panama using NaOH–EDTA extraction and solution ^{31}P nuclear magnetic resonance spectroscopy. The soils spanned a strong rainfall gradient (1730–3404 mm y^{-1}) and contained a wide range of chemical properties (pH 3.3–7.0; total carbon 2.8–10.4%; total phosphorus 74–1650 mg P kg^{-1}). Soil organic phosphorus concentrations ranged between 22 and 494 mg P kg^{-1} and were correlated positively with total soil phosphorus, pH, and total carbon, but not with annual rainfall. Organic phosphorus constituted $26 \pm 1\%$ (mean \pm STD error, $n = 19$) of the total phosphorus, suggesting that this represents a broad emergent property of tropical forest soils. Organic phosphorus occurred mainly as phosphate monoesters (68–96% of total organic phosphorus) with smaller concentrations of phosphate diesters in the

form of DNA (4–32% of total organic phosphorus). Phosphonates, which contain a direct carbon–phosphorus bond, were detected in only two soils (3% of the organic phosphorus), while pyrophosphate, an inorganic polyphosphate with a chain length of two, was detected in all soils at concentrations up to 13 mg P kg^{-1} (3–13% of extracted inorganic phosphorus). Phosphate monoesters were a greater proportion of the total organic phosphorus in neutral soils with high concentrations of phosphorus and organic matter, whereas the proportion of phosphate diesters was greater in very acidic soils low in phosphorus and organic matter. Most soils did not contain detectable concentrations of either *myo*- or *scyllo*-inositol hexakisphosphate, which is in marked contrast to many temperate mineral soils that contain abundant inositol phosphates. We conclude that soil properties exert a strong control on the amounts and forms of soil organic phosphorus in tropical rain forests, but that the proportion of the total phosphorus in organic forms is relatively insensitive to variation in climate and soil properties. Further work is now required to assess the contribution of soil organic phosphorus to the nutrition and diversity of plants in these species-rich ecosystems.

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Introduction

Phosphorus availability is widely considered to constrain productivity in lowland tropical forests (Vitousek and Sanford 1986; Tanner et al. 1998). In part, this is because strongly-weathered soils, which cover vast regions of the lowland tropics, contain low concentrations of readily exchangeable inorganic phosphate (Clinebell et al. 1995) and much of the total phosphorus occurs in sparingly-soluble crystalline or occluded forms associated with secondary minerals (Cross and Schlesinger 1995; Tiessen 1998). As occluded forms of phosphorus are of limited biological availability, it seems likely that phosphorus acquisition by plants growing on strongly-weathered tropical forest soils is regulated by the turnover of organic phosphorus compounds and the rapid recycling of phosphorus from litter fall (Johnson et al. 2003; Condon and Tiessen 2005). The dynamic nature of soil organic phosphorus in tropical forests was demonstrated recently by the relatively rapid changes that occurred following experimental addition or removal of leaf litter in large experimental plots of lowland forest in central Panama (Vincent et al. 2010).

Soil organic phosphorus occurs in a variety of chemical forms that differ markedly in their behavior and bioavailability in the soil environment (Condon et al. 2005) and that may influence the distribution and co-existence of plant species in lowland tropical forests (Turner 2008a). For example, organic phosphorus in most mineral soils occurs as a mixture of phosphate monoesters (e.g., mononucleotides, inositol phosphates) and phosphate diesters (mainly nucleic acids and phospholipids), with smaller amounts of phosphonates (compounds with a direct carbon–phosphorus bond) and organic polyphosphates (e.g., adenosine triphosphate). Phosphate diesters are often considered to be the most readily available forms of organic phosphorus to plants due to their relatively rapid turnover in soil (Bowman and Cole 1978; Harrison 1982). In contrast, phosphate monoesters such as the inositol phosphates are often considered to be of limited availability to plants, due to their strong association with soil minerals (Celi and Barberis 2007; Turner 2007).

The composition of soil organic phosphorus can be determined conveniently by solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy (Cade-Menun 2005), yet despite the widespread application

of this procedure in the last three decades only a few studies have examined lowland tropical forest soils (Forster and Zech 1993; Turner 2008b; Vincent et al. 2010). Of interest in the most recent studies was that inositol phosphates were not detected in any of the soils from the four sites examined (Turner 2008b; Vincent et al. 2010), despite being abundant in most mineral soils of temperate regions (Turner 2007). If widespread, this may indicate that inositol phosphates, conventionally considered to be recalcitrant in soil, are relatively unstable (and, therefore, biologically available) in lowland tropical forest.

Despite the importance of organic phosphorus in maintaining phosphorus availability in lowland tropical rain forests, its forms and dynamics in such ecosystems remain poorly understood. In particular, many of the data on the organic phosphorus content of tropical soils (reviewed in Harrison 1987) are likely to be overestimates (Condon et al. 1990), because inorganic phosphate in secondary minerals, abundant in strongly-weathered soils, is rendered acid-soluble by high temperature ignition (Williams and Walker 1967). To address this, we used solution ^{31}P NMR spectroscopy to determine the amounts and chemical nature of organic phosphorus in tropical forest soils with a range of chemical properties that occur across a strong rainfall gradient in central Panama. Our aim was to determine how the amounts and forms of organic phosphorus vary across a broad range of soils under lowland tropical forest.

Methods

Sites and soils

Soils were sampled from a series of 1 ha forest census plots located in the Panama Canal watershed, central Panama (Table 1, Fig. 1). All plots contained lowland tropical forest (elevation 10–410 m a.s.l.) and included secondary, mature secondary, and old growth primary forest (Pyke et al. 2001). The plots span a distinct rainfall gradient (annual rainfall ~ 1700 mm y^{-1} on the Pacific coast to ~ 3400 mm y^{-1} on the Caribbean coast) across the approximately 65 km width of the Isthmus, correlated with an increase in minimum gravimetric soil moisture in the dry season (10–57%; B.M.J. Engelbrecht, unpublished data) and a decrease in dry season length (147–115 days) (Pyke et al. 2001;

Table 1 Description of location, rainfall and geology of one hectare forest inventory plots in central Panama

Soil Ref.	Location	Plot code	Geology	Rainfall (mm y ⁻¹)	Taxonomic order
1.	Rio Paja	P26	Rhyolitic tuff ^a	2141	Ultisols
2.	Rio Paja	P25	Rhyolitic tuff ^a	2159	Ultisols
3.	Pipeline Road	P09	Basalt ^b	2349	Oxisols
4.	Fort Sherman	P02	Sandstone ^c	2873	Ultisols
5.	Pipeline Road	P08	Basalt ^b	2357	Oxisols
6.	Buena Vista	P12	Conglomerate ^d	2601	Inceptisols
7.	Santa Rita	P32	Basalt ^b	3404	Oxisols
8.	Pipeline Road	P15	Marine sediments ^e	2336	Alfisols
9.	Albrook	–	Marine sediments ^f	1860	Ultisols
10.	Pipeline Road	P17	Basalt ^b	2269	Oxisols
11.	Caritas	–	Marine sediments ^g	2027	Alfisols
12.	Cerro la Torre	–	Agglomerate ^h	1730	Inceptisols
13.	Las Cruces	P27	Agglomerate ⁱ	2033	Inceptisols
14.	Buena Vista	P13	Marine sediments ^j	2591	Inceptisols
15.	Fort Sherman	P01	Limestone ^k	2875	Alfisols
16.	Peña Blanca	P18	Marine sediments ^j	2628	Alfisols
17.	Gamboa	P24	Agglomerate ⁱ	2153	Alfisols
18.	Cerro Galera	–	Basalt ^l	1790	Inceptisols
19.	Campo Chagres	–	Calcareous sandstone ^m	2481	Alfisols

Plot codes are from Pyke et al. (2001) and geological information is from Stewart et al. (1980). Mean annual rainfall was estimated from location and elevation data as described in Engelbrecht et al. (2007). Plots are ranked in order of the total phosphorus concentration in surface soil (see Table 2). Taxonomic classes are based on US Soil Taxonomy (Soil Survey Staff 1999)

^a Mapped as pre-tertiary basalt in Stewart et al. (1980), but the plots are on fine-grained rhyolitic tuff

^b Altered basaltic and andesitic lavas and tuff, including dioritic and dacitic intrusive rocks; pre-Tertiary

^c Chagres Sandstone; massive, generally fine-grained sandstone; Late Miocene or Early Pliocene

^d Bohio formation; conglomerate, principally basaltic and graywacke sandstone; Early–Late Oligocene

^e Gatuncillo formation; mudstone, siltstone, quartz sandstone, algal and foraminiferal limestone; Middle–Late Eocene

^f La Boca formation; mudstone, siltstone, sandstone, tuff, and limestone; Early Miocene

^g Caraba formation; dacitic agglomerate, conglomerate, calcareous sandstone, and foraminiferal limestone; Late Oligocene

^h Panama formation; principally agglomerate, generally andesitic, in fine-grained tuff; Early–Late Oligocene

ⁱ Las Cascadas formation; agglomerate of tuffaceous siltstone, tuff, and foraminiferal limestone; Early Miocene

^j Caimito formation, marine facies; tuffaceous sandstone, tuffaceous siltstone, tuff, and foraminiferal limestone; Late Oligocene

^k Toro Limestone; basal member of Chagres Sandstone

^l Intrusive and extrusive basalt; Middle and Late Miocene

^m Upper member of the Alhajuella formation; tuffaceous sandstone, calcareous sandstone, and limestone; late Early Miocene

Engelbrecht et al. 2007). The mean annual temperature is 27°C and mean monthly temperature varies by <1°C during the year (Windsor 1990). The β -diversity (i.e., species turn-over in space) of the forests is exceptionally high (Condit et al. 2002) and rainfall exerts a strong influence on the floristic composition (Pyke et al. 2001; Engelbrecht et al. 2007).

The soils have developed on a range of tertiary and pre-tertiary geological substrates (Table 1; Woodring

1958; Stewart et al. 1980; Pyke et al. 2001), including material of volcanic (basalt, andesite, agglomerate, rhyolitic tuff) and marine sedimentary (limestone, calcareous sandstone, siltstone, mudstone) origin. Formations not described in Pyke et al. (2001) are the Alhajuella formation (upper member, including tuffaceous and calcareous sandstone and limestone; the plot is on calcareous sandstone), the Panama formation (principally agglomerate, generally andesitic, in

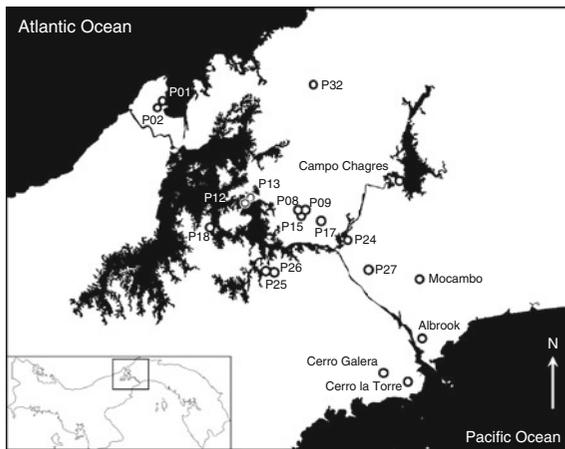


Fig. 1 Map of sampling locations in central Panama. Plot codes are from Pyke et al. (2001)

fine-grained tuff), and the Caraba formation (dacitic agglomerate, conglomerate, calcareous sandstone, and foraminiferous limestone) (Stewart et al. 1980).

No formal soil classification (e.g., FAO or US Soil Taxonomy) exists for the majority of the canal watershed, although a recent survey conducted on Barro Colorado Island (Baillie et al. 2007) described 23 soil classes developed on a variety of geological substrates, which are likely to be representative of many of the soils in central Panama. Soils developed on volcanic parent material include brown fine loams (Eutrudepts), pale swelling clays (dystrudertic variations of Udalfs and Udults), and deep, red light clays (Oxisols). The marine facies of the Caimito formation, which underlies two of the plots described here, also occurs in the west part of Barro Colorado Island, where soils are mapped as pale mottled heavy clays (Aquertic Hapludalfs) (Baillie et al. 2007). The volcanic facies of the Bohio formation that underlies one of the plots (Buena Vista, P12) also occurs in the northern part of Barro Colorado Island, where soils are mapped as brown shallow clays (Typic Eutrudepts). Soils of several plots are not represented by similar soils on BCI; soils on two plots (P25, P26) are developed on rhyolitic tuff but are sufficiently weathered to be Ultisols rather than Andisols, while two plots (P01 and Campo Chagres) are developed on calcareous parent material (mollic variations of Udalfs) and have a relatively shallow lithic contact (<1 m). In addition, two plots (Albrook, Cerro la Torre) are on steep slopes with gravelly topsoils and

appear to be undergoing a relatively rapid rate of erosion. Based on the BCI soil survey and preliminary results from a broad program of soil classification currently underway in the canal watershed, tentative soil orders are given in Table 1.

Soil sampling and preparation

Each 100 m × 100 m plot is marked on a 20 m × 20 m grid. A soil core (2.5 cm diameter) was taken to 10 cm in the center of each 20 m × 20 m square and bulked to form a single composite sample per plot (i.e., each sample consisted of 25 separate soil cores from the 1 ha plot). Samples were all taken within a 2-week period in the middle of the 8-month wet season. Although to our knowledge there is no information on seasonal changes in soil organic phosphorus in tropical forests, we assumed that such changes would be small and that sampling in the wet season would yield comparable results across the sites. Samples were returned to the laboratory and stored at 4°C for no more than 2 weeks. Samples were initially screened (<9 mm) to break up large aggregates, and stones and roots were removed by hand. Soils were then sieved again (<2 mm) to isolate the fine earth fraction and air-dried (~22°C, 10 days) to a constant weight. Subsamples were ground in a ball mill and stored in sealed plastic bags at ambient laboratory temperature and humidity (~22°C and 55%, respectively).

Determination of soil properties

Total carbon and nitrogen were determined by combustion and gas chromatography using a Thermo Flash NC1112 Soil Analyzer (CE Elantech, Lakewood, NJ). Soil pH was determined in a 1:2 soil to deionized water ratio using a glass electrode. Oxalate-extractable aluminum, iron, manganese, and phosphorus were determined by extraction in a solution containing ammonium oxalate and oxalic acid (Loeppert and Inskeep 1996) with detection by inductively-coupled plasma optical-emission spectrometry (ICP-OES) using an Optima 2100 (Perkin-Elmer Inc., Shelton, CT). Degree of phosphorus saturation (%) was calculated by oxalate P/[oxalate Al + Fe] * 100, using molar values. The concentrations

of sand (0.053–2.0 mm), silt (0.002–0.053 mm), and clay (<0.002 mm) sized particles were determined by the pipette method after pretreatment to remove salts (sodium acetate extraction) and organic matter (H_2O_2 oxidation), but not iron oxides (dithionite reduction) (Gee and Or 2002).

Total phosphorus was determined by ignition (550°C, 1 h) and extraction in 1 M H_2SO_4 (1:50 soil to solution ratio, 16 h), with orthophosphate detection in neutralized extracts by automated molybdate colorimetry at 880 nm using a Lachat Quickchem 8500 (Hach Ltd., Loveland, CO). This procedure gave quantitative recovery of total phosphorus from certified reference soil (Loam Soil D; High Purity Standards, Charleston, SC) and values for plot soils were virtually indistinguishable from those determined by a H_2O_2 – H_2SO_4 digestion procedure (Parkinson and Allen 1975) (natural log of total phosphorus by H_2O_2 – H_2SO_4 digestion = $0.998 \times$ natural log of total phosphorus by ignition; $p < 0.0001$; intercept forced through the origin).

NaOH–EDTA extraction and solution ^{31}P NMR spectroscopy

Phosphorus was extracted by shaking soil (1.50 ± 0.01 g) with 30 ml of a solution containing 0.25 M NaOH and 50 mM Na_2EDTA (ethylenediaminetetraacetate) for 4 h at 22°C (Bowman and Moir 1993; Cade-Menun and Preston 1996). Each extract was centrifuged at 8,000 g for 30 min. A 1 ml aliquot was neutralized using phenolphthalein indicator and 3 M H_2SO_4 and diluted to 20 ml with deionized water for determination of phosphorus by ICP–OES. We did not determine orthophosphate in the NaOH–EDTA extracts by molybdate colorimetry because previous reports have indicated considerable problems with this procedure (e.g., Turner et al. 2006). A 20 ml aliquot of each extract was spiked with 1 ml of 50 $\mu\text{g P ml}^{-1}$ methylene diphosphonic acid (MDPA) solution as an internal standard, frozen at -35°C , lyophilized (~ 48 h), and homogenized by gently crushing to a fine powder. This procedure was tested recently in detail for a soil under tropical forest taken from a site to the north of the Albrook census plot on gently sloping ground (Turner 2008b). Of note was that the concentration of organic phosphorus extracted from the soil was not increased by changing the conditions of the extraction procedure, including altering the

extraction time or solid/solution ratio, pre-treating the soil, or changing the concentration of NaOH or EDTA.

For NMR spectroscopy, each lyophilized extract (~ 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, Germany) operating at 202.456 MHz for ^{31}P . Samples were analyzed 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 each sample. Spectra were plotted with a line broadening of 5 Hz and chemical shifts of signals were determined in parts per million (ppm) relative to an external standard of 85% H_3PO_4 . Signals were assigned to phosphorus compounds based on literature reports of model compounds spiked in NaOH–EDTA soil extracts (Turner et al. 2003a). Signal areas were calculated by integration and concentrations of phosphorus compounds were calculated from the integral value of the MDPA internal standard at 17.57 ± 0.06 ppm ($n = 19$). All spectral processing was done using NMR Utility Transform Software (NUTS) for Windows (Acorn NMR Inc., Livermore, CA).

It is difficult to estimate a detection limit for organic phosphorus compounds using the solution ^{31}P NMR spectroscopy procedure, as this varies among samples depending on parameters such as line broadening and the number of scans obtained. Here, pyrophosphate was detected at a concentration of 1 mg P kg^{-1} , so for detection of *myo*-inositol hexakisphosphate, based on the presence of the signal from the C-2 phosphate at approximately 5.9 ppm, we estimate a limit of detection of approximately 6 mg P kg^{-1} (the C-2 phosphate signal represents one-sixth of the phosphorus in *myo*-inositol hexakisphosphate). We did not obtain replicate spectra for each soil. However, error for replicate analyses of tropical forest soils, including extraction and NMR spectroscopy, were reported recently as approximately 2% for total organic phosphorus, 5% for phosphate monoesters, and 10% for phosphate diesters (Turner 2008b).

Note that we did not use the ignition procedure to determine soil organic phosphorus. Although used widely in the older literature, this method is now recognized as unsuitable for strongly-weathered soils (Condon et al. 1990). This is because organic phosphorus is overestimated due to solubilization of

inorganic phosphate in secondary minerals at high temperatures (e.g., Williams and Walker 1967) and inclusion of alkali-soluble inorganic phosphate in the organic phosphorus fraction (Turner et al. 2007).

Data analysis

All values are expressed on the basis of oven-dry soil weight (105°C, 24 h). Data were transformed by natural logarithm when not normally distributed (as determined by Kurtosis or Skew values significantly different from zero, $p < 0.05$). Pearson's product-moment correlations between soil properties (17 degrees of freedom) were determined using R software (www.r-project.org).

Results

Soil properties

Soils from the 19 plots spanned a wide range of physical and chemical properties (Table 2). Total carbon ranged between 2.81% for an Ultisol (soil 1, P26) on rhyolitic tuff and 10.4% for an Alfisol (soil 19, Campo Chagres) on calcareous sandstone (Table 2). Although some of the soils were developed in calcareous parent material, the contribution of carbonate to the total carbon values in surface horizons is negligible. Total nitrogen ranged between 0.21 and 0.89% for the same two soils, while the C:N ratio ranged between 9.7 and 15.4. Total soil phosphorus varied markedly across the plots, with values ranging between 74 and 1650 mg P kg⁻¹ (Table 2). It was notable that the lowest phosphorus concentrations were for the two Ultisols developed on rhyolitic tuff, while the highest concentrations were for Alfisols formed on calcareous parent material.

Soil pH in water ranged between 3.34 for an Ultisol (soil 9, Albrook) on marine sediments and 7.00 for an Alfisol (soil 19, Campo Chagres) on calcareous sandstone (Table 2). Most soils were clays, with the concentration of clay-sized particles ranging between 28 and 65%. Sand-sized particles ranged between 4 and 44%, while silt-sized particles ranged between 15 and 35% except for the two Ultisols developed on rhyolitic tuff (soils 1 and 2), which contained approximately 60% silt and were silty clay loams. It should be noted, however, that

such soils are usually not well dispersed in sodium hexametaphosphate (used here) due to the high concentration of allophanic minerals (Gee and Or 2002), so clay content may be underestimated in these samples.

Oxalate extractable metals (Table 3) were 1.47–3.34 g Al kg⁻¹, 2.43–9.53 g Fe kg⁻¹, and 0.03–6.07 g Mn kg⁻¹. Manganese concentrations were notably high for an Inceptisol (soil 12, Cerro la Torre) on volcanic agglomerate, and an Alfisol (soil 16, Peña Blanca) on marine sediments. Oxalate-extractable phosphorus, which is expected to include inorganic and organic phosphorus associated with amorphous metal oxides, ranged between <1 and 615 mg P kg⁻¹, or between <1 and 37% of the total soil phosphorus (Table 3). The degree of phosphorus saturation calculated using these values ranged between <0.1 and 10.3%, although all but one value was ≤3%. The lowest oxalate-extractable phosphorus (<1 mg P kg⁻¹) and degree of phosphorus saturation occurred on two Oxisols developed on basalt (soils 3 and 7; Table 3).

Phosphorus composition determined by NaOH–EDTA extraction and solution ³¹P NMR spectroscopy

The total phosphorus extracted in NaOH–EDTA and determined by solution ³¹P NMR spectroscopy using the internal standard ranged between 43 and 824 mg P kg⁻¹ (mean ± standard error 220 ± 40 mg P kg⁻¹), accounting for between 28 and 61% of the total soil phosphorus (Table 4). Values determined in the extracts by ICP–OES were similar to those determined by NMR spectroscopy, ranging between 41 and 883 mg P kg⁻¹ and accounting for between 27 and 56% of the total soil phosphorus (data not shown). A linear regression of log-transformed values (to correct for non-linearity), with the intercept forced through the origin, was described by the equation:

$$\log(\text{Total P}_{\text{ICP}}) = 1.002 \pm 0.002 * \log(\text{Total P}_{\text{NMR}}); \\ r^2 = 1.000, p < 0.0001; n = 19.$$

NaOH–EDTA extractable organic phosphorus (sum of phosphomonoesters, phosphodiester, and phosphonates) determined by solution ³¹P NMR spectroscopy ranged between 22 and 494 mg P kg⁻¹

Table 2 Chemical and physical properties of soils from one hectare forest inventory plots in central Panama. The soils are ranked in order of their total phosphorus concentration

	Location	Total elements			C:N ratio	pH	Topsoil texture	Sand (%)	Silt (%)	Clay (%)
		P (mg P kg ⁻¹)	C (%)	N (%)						
1.	Rio Paja (P26)	74	2.81	0.21	13.1	3.55	Silty clay loam	4	64	32
2.	Rio Paja (P25)	91	3.39	0.26	12.9	3.59	Silty clay loam	13	56	31
3.	Pipeline Road (P09)	175	5.20	0.44	11.8	4.86	Clay	13	15	72
4.	Fort Sherman (P02)	211	4.30	0.39	10.9	4.21	Clay	17	26	57
5.	Pipeline Road (P08)	278	4.01	0.35	11.5	4.71	Clay	23	29	48
6.	Buena Vista (P12)	280	4.65	0.40	11.7	4.22	Clay	15	33	52
7.	Santa Rita (P32)	283	4.72	0.47	10.0	5.06	Clay	15	33	52
8.	Pipeline Road (P15)	332	4.00	0.35	11.4	5.02	Clay loam	37	28	35
9.	Albrook	376	5.46	0.36	15.4	3.34	Clay	35	23	42
10.	Pipeline Road (P17)	471	3.93	0.36	10.9	5.35	Clay	25	34	41
11.	Caritas	505	5.66	0.45	12.5	6.42	Clay	28	31	41
12.	Cerro la Torre	518	5.25	0.40	13.0	5.04	Clay loam	45	27	28
13.	Las Cruces (P27)	532	3.66	0.31	11.9	5.46	Clay loam	31	31	38
14.	Buena Vista (P13)	574	4.86	0.47	10.3	5.72	Clay	33	27	40
15.	Fort Sherman (P01)	678	7.23	0.61	11.9	5.84	Clay	33	26	41
16.	Peña Blanca (P18)	688	6.60	0.68	9.7	5.48	Clay	10	31	59
17.	Gamboa (P24)	794	7.34	0.63	11.7	5.43	Clay	18	33	49
18.	Cerro Galera	833	5.84	0.43	13.5	6.49	Clay loam	34	31	35
19.	Campo Chagres	1650	10.40	0.89	11.6	7.00	Clay	29	27	44

(mean 125 ± 24 mg P kg⁻¹). This accounted for between 14 and 36% of the total soil phosphorus (mean $25.5 \pm 1.2\%$) (Table 4). Most of the organic phosphorus occurred as phosphate monoesters, which constituted between 16 and 474 mg P kg⁻¹ (mean 112 ± 24 mg P kg⁻¹), and accounted for between 68 and 96% (mean $86 \pm 2\%$) of the total organic phosphorus (Table 5).

Signals from phosphate monoesters were detected at chemical shift values between 4.0 and 7.0 ppm, with prominent signals at 6.88 ± 0.01 ppm ($n = 7$), 5.79 ± 0.01 ppm ($n = 10$), 5.53 ± 0.02 ppm ($n = 3$), 5.31 ± 0.01 ppm ($n = 19$), 5.15 ± 0.03 ppm ($n = 9$), 4.96 ± 0.03 ppm ($n = 19$), 4.74 ± 0.01 ppm ($n = 11$), 4.60 ± 0.03 ppm ($n = 14$), 4.26 ± 0.01 ppm ($n = 18$) (Fig. 2). However, signals that could be assigned to higher-order inositol phosphates, including *myo*- and *scyllo*-inositol hexakisphosphate, were not identified clearly in any spectrum, even when spectra were plotted with 1 Hz line broadening to enhance resolution. The signals at 6.88 ppm may, however, represent unidentified inositol phosphates,

due to their resistance to hypobromite oxidation in a previous study (Turner and Richardson 2004).

The remaining organic phosphorus in most soils was detected as DNA (chemical shift -0.14 ± 0.03 ppm; $n = 19$), which constituted between 6 and 20 mg P kg⁻¹ (mean 13 ± 2 mg P kg⁻¹) and accounted for between 4 and 32% (mean $14 \pm 2\%$) of the total organic phosphorus. Phosphonates were detected in two soils (chemical shift 20.65 ± 0.02 ppm; $n = 2$) at 3 mg P kg⁻¹ (3% of the total organic phosphorus), both of which contained relatively high total phosphorus concentrations and were developed on the same geological formation (Caimito marine facies; see Table 1). Traces of phospholipids and/or RNA were detected in the 0.5–2 ppm region of some spectra, but were not quantified (Fig. 2). Most of these compounds degrade rapidly in the alkaline conditions of the NaOH–EDTA extraction and are detected as phospho-monoesters (Turner et al. 2003a).

NaOH–EDTA extractable inorganic phosphorus (sum of orthophosphate and pyrophosphate) determined by solution ³¹P NMR spectroscopy ranged

Table 3 Oxalate-extractable metals and phosphorus in soils from one hectare forest inventory plots in central Panama

	Location	Al (g kg ⁻¹)	Fe (g kg ⁻¹)	Mn (g kg ⁻¹)	P ^a (mg P kg ⁻¹)	DPS ^b
1.	Rio Paja (P26)	1.90	2.43	0.12	17 (23)	0.5
2.	Rio Paja (P25)	2.21	3.02	0.03	14 (16)	0.3
3.	Pipeline Road (P09)	2.68	9.53	1.80	<1 (<1)	<0.1
4.	Fort Sherman (P02)	3.89	7.48	0.47	13 (6)	0.1
5.	Pipeline Road (P08)	1.80	5.14	1.79	19 (7)	0.4
6.	Buena Vista (P12)	2.99	7.55	0.25	21 (7)	0.3
7.	Santa Rita (P32)	3.22	9.09	1.40	1 (<1)	<0.1
8.	Pipeline Road (P15)	1.47	5.92	1.05	35 (11)	0.7
9.	Albrook	2.05	4.01	0.03	39 (10)	0.8
10.	Pipeline Road (P17)	2.01	6.85	0.95	55 (12)	0.9
11.	Caritas	2.41	4.98	0.67	95 (19)	1.7
12.	Cerro la Torre	1.42	2.67	2.82	83 (16)	2.7
13.	Las Cruces (P27)	2.38	6.84	0.62	114 (21)	1.7
14.	Buena Vista (P13)	3.04	5.93	0.83	99 (17)	1.5
15.	Fort Sherman (P01)	2.38	5.07	0.72	161 (24)	2.9
16.	Peña Blanca (P18)	3.28	7.69	6.07	163 (24)	2.0
17.	Gamboa (P24)	2.53	7.53	1.72	194 (24)	2.7
18.	Cerro Galera	1.80	5.12	1.16	148 (18)	3.0
19.	Campo Chagres	3.34	3.88	0.58	615 (37)	10.3

^a Values in parentheses are the proportion (%) of the total soil phosphorus

^b Degree of phosphorus saturation (oxalate P/[oxalate Al + Fe] × 100) calculated from molar values

between 18 and 330 mg P kg⁻¹ (mean 94 ± 16 mg P kg⁻¹), which accounted for between 14 and 25% (mean 20 ± 1%) of the total soil phosphorus (Table 4). The dominant compound was inorganic orthophosphate (chemical shift 6.33 ± 0.04 ppm; *n* = 19), which occurred at concentrations of between 17 and 319 mg P kg⁻¹ and accounted for between 83 and 97% of the NaOH–EDTA extractable inorganic phosphorus (Table 5). The remainder was pyrophosphate (chemical shift -4.06 ± 0.12 ppm; *n* = 19), which was detected in all soils at concentrations up to 13 mg P kg⁻¹ (mean 7.4 ± 0.8 mg P kg⁻¹) and between 3 and 17% (mean 9.1 ± 0.8%) of the NaOH–EDTA extractable inorganic phosphorus (Table 5). Long-chain inorganic polyphosphate, which gives signals at approximately -3 and -20 ppm, was not detected in any soil.

Carbon to organic phosphorus ratios

Assuming that the NaOH–EDTA extractable organic phosphorus approximates the total soil organic

phosphorus (Bowman and Moir 1993; Turner et al. 2005), we calculated the ratios of C:organic P and N:organic P (Table 5). The C:organic P ratios ranged between 211 and 1544 (mean 595 ± 80), while N:organic P ratios varied between 18 and 120 (mean 50 ± 6). Values were lowest in an Alfisol (soil 19, Campo Chagres) developed on calcareous sandstone and highest in Ultisols developed on rhyolitic tuff (soils 25 and 26).

Relationships between phosphorus forms and soil properties

Total carbon and total nitrogen were correlated strongly and positively with each other (*r* = 0.95, *p* < 0.0001; correlations involving nitrogen are not discussed further) and with soil pH (for total carbon *r* = 0.67; *p* = 0.0017). Clay concentration and the sum of oxalate-extractable aluminum and iron were strongly correlated with each other (*r* = 0.85, *p* < 0.0001), but not with other soil properties. Mean annual rainfall was correlated positively with clay

Table 4 Phosphorus fractions in NaOH–EDTA extracts of soils from one hectare forest inventory plots in central Panama, determined by solution ^{31}P NMR spectroscopy

	Location	NaOH–EDTA extraction			C:organic P	N:organic P
		Total P (mg P kg ⁻¹)	Inorganic P ^a (mg P kg ⁻¹)	Organic P ^b (mg P kg ⁻¹)		
1.	Rio Paja (P26)	45 (61)	18 (25)	27 (36)	1050	80
2.	Rio Paja (P25)	43 (47)	21 (23)	22 (24)	1544	120
3.	Pipeline Road (P09)	85 (49)	29 (17)	56 (32)	930	79
4.	Fort Sherman (P02)	93 (44)	45 (21)	48 (23)	901	82
5.	Pipeline Road (P08)	133 (48)	48 (17)	85 (31)	470	41
6.	Buena Vista (P12)	143 (51)	64 (23)	79 (28)	587	50
7.	Santa Rita (P32)	125 (44)	58 (21)	67 (24)	709	71
8.	Pipeline Road (P15)	163 (49)	77 (23)	86 (26)	465	41
9.	Albrook	106 (28)	53 (14)	53 (14)	1039	68
10.	Pipeline Road (P17)	190 (40)	81 (17)	110 (23)	358	33
11.	Caritas	238 (47)	104 (21)	134 (27)	421	34
12.	Cerro la Torre	185 (36)	90 (17)	95 (18)	552	42
13.	Las Cruces (P27)	215 (40)	100 (19)	114 (21)	320	27
14.	Buena Vista (P13)	259 (45)	115 (20)	144 (25)	336	33
15.	Fort Sherman (P01)	333 (49)	128 (19)	206 (30)	352	29
16.	Peña Blanca (P18)	283 (41)	116 (17)	167 (24)	395	41
17.	Gamboa (P24)	361 (45)	159 (20)	202 (25)	363	31
18.	Cerro Galera	349 (42)	154 (18)	195 (23)	299	22
19.	Campo Chagres	824 (50)	330 (20)	494 (30)	211	18

Values in parentheses are the proportion (%) of the total soil phosphorus, and element ratios are mass based

^a Sum of orthophosphate and pyrophosphate

^b Sum of phosphate monoesters, phosphate diesters (DNA), and phosphonates

($r = 0.51$, $p = 0.026$) and the sum of oxalate-extractable aluminum and iron ($r = 0.70$, $p = 0.001$), and negatively with the C:N ratio ($r = -0.73$, $p = 0.0004$).

Total soil phosphorus was correlated strongly and positively with soil pH and total carbon (for both relationships $r = 0.83$, $p < 0.0001$; Fig. 3), NaOH–EDTA total phosphorus ($r = 0.98$, $p < 0.0001$), and oxalate-extractable phosphorus ($r = 0.72$, $p = 0.0005$). NaOH–EDTA total phosphorus and oxalate-extractable phosphorus were correlated positively ($r = 0.73$, $p = 0.0003$), although neither was correlated with mean annual rainfall or the sum of oxalate-extractable aluminum and iron.

Total organic phosphorus (i.e., extracted in NaOH–EDTA and determined by solution ^{31}P NMR spectroscopy) was correlated most strongly with total soil phosphorus, soil pH, and total carbon (Table 6,

Fig. 4). Similar relationships occurred for NaOH–EDTA extractable inorganic phosphorus (not shown). Organic phosphorus was also correlated positively with oxalate-extractable phosphorus, the degree of phosphorus saturation, and oxalate-extractable manganese, but not with the sum of oxalate-extractable aluminum and iron (Table 6). When expressed as a percentage of the total soil phosphorus, organic phosphorus was not correlated with any soil property, due apparently to the relatively constant percentage across soils (mean \pm standard error $26 \pm 1\%$ of the total phosphorus; see above).

As for total organic phosphorus, the concentrations of phosphate monoesters and pyrophosphate were all correlated strongly with total soil phosphorus, total carbon, soil pH (Table 6, Fig. 4), and with oxalate-extractable phosphorus, the degree of phosphorus saturation, and oxalate-extractable manganese

Table 5 Phosphorus compounds determined by solution ^{31}P NMR spectroscopy in NaOH–EDTA extracts of soils from one hectare forest inventory plots in central Panama

Location	Organic P ^a			Inorganic P ^b	
	Phosphate monoesters (mg P kg ⁻¹)	DNA (mg P kg ⁻¹)	Phosphonate (mg P kg ⁻¹)	Ortho-phosphate (mg P kg ⁻¹)	Pyrophosphate (mg P kg ⁻¹)
1. Rio Paja (P26)	18 (68)	8 (32)	nd	17 (93)	1 (7)
2. Rio Paja (P25)	16 (71)	6 (29)	nd	20 (96)	< 1 (4)
3. Pipeline Road (P09)	46 (83)	10 (17)	nd	24 (83)	5 (17)
4. Fort Sherman (P02)	41 (85)	7 (15)	nd	42 (93)	3 (7)
5. Pipeline Road (P08)	67 (78)	19 (22)	nd	40 (83)	8 (17)
6. Buena Vista (P12)	66 (83)	14 (17)	nd	58 (90)	6 (10)
7. Santa Rita (P32)	57 (86)	9 (14)	nd	51 (88)	7 (12)
8. Pipeline Road (P15)	78 (91)	8 (9)	nd	68 (88)	9 (12)
9. Albrook	40 (77)	12 (23)	nd	49 (92)	4 (8)
10. Pipeline Road (P17)	98 (90)	11 (10)	nd	74 (92)	7 (8)
11. Caritas	120 (89)	14 (10)	nd	95 (91)	9 (9)
12. Cerro la Torre	82 (87)	12 (13)	nd	78 (87)	12 (13)
13. Las Cruces (P27)	105 (92)	10 (8)	nd	94 (93)	7 (7)
14. Buena Vista (P13)	128 (88)	14 (10)	3 (3)	102 (89)	13 (11)
15. Fort Sherman (P01)	189 (92)	17 (8)	nd	117 (92)	11 (8)
16. Peña Blanca (P18)	143 (86)	20 (12)	3 (3)	107 (92)	9 (8)
17. Gamboa (P24)	183 (91)	19 (9)	nd	150 (94)	9 (6)
18. Cerro Galera	181 (93)	14 (7)	nd	143 (93)	11 (7)
19. Campo Chagres	474 (96)	20 (4)	nd	319 (97)	11 (3)

nd not detected

^a Values in parentheses are the proportion (%) of the NaOH–EDTA extractable organic phosphorus

^b Values in parentheses are the proportion (%) of the NaOH–EDTA extractable inorganic phosphorus

(Table 6). Correlations between DNA and these properties were generally weaker, and not significant for oxalate extractable manganese (Table 6). Organic phosphorus fractions were not significantly correlated with clay concentration, the sum of oxalate-extractable aluminum and iron, or mean annual rainfall (Table 6).

When expressed as a proportion (%) of the total organic phosphorus, phosphate monoesters were correlated positively with total soil phosphorus ($r = 0.87$, $p < 0.0001$), soil pH ($r = 0.86$, $p < 0.0001$), and total carbon ($r = 0.66$, $p = 0.002$), while DNA was correlated negatively with these properties ($r = -0.88$ and -0.87 , $p < 0.0001$; and -0.67 , $p = 0.002$, respectively) (Fig. 5). In other words, DNA was a greater proportion of the organic phosphorus in acidic soils with low phosphorus

concentrations, while phosphate monoesters were a greater proportion of the organic phosphorus in neutral soils with high phosphorus concentrations.

The C:organic P ratio was correlated negatively with total soil phosphorus, soil pH, and total carbon (Table 6, Fig. 6). It was also correlated positively with DNA expressed as a proportion (%) of the total organic phosphorus ($r = 0.88$, $p < 0.0001$) and negatively with phosphate monoesters expressed as a proportion (%) of the total organic phosphorus ($r = -0.87$, $p < 0.0001$). Thus, ratios were lowest in acidic soils with low phosphorus concentrations and greatest in neutral soils with high phosphorus concentrations. The degree of phosphorus saturation was correlated negatively with the C:organic P ratio ($r = -0.65$, $p = 0.003$) and clay concentration ($r = -0.59$, $p = 0.008$).

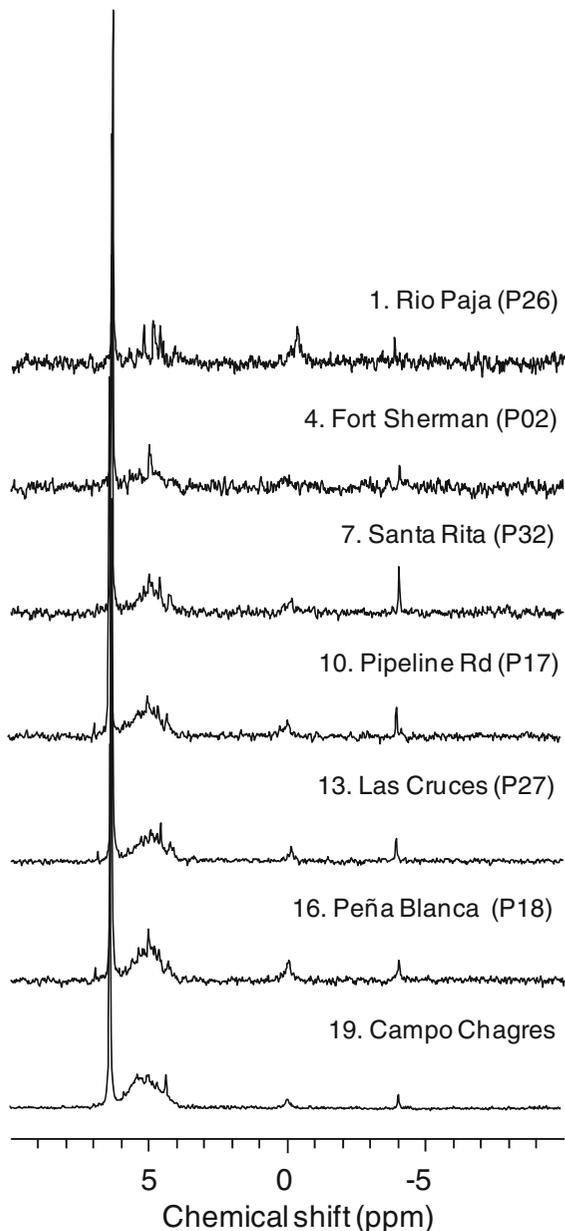


Fig. 2 Selected solution ^{31}P NMR spectra of NaOH-EDTA extracts of soils under lowland tropical rain forest in central Panama. The spectra are presented in order of ascending total phosphorus concentration (Table 2) and are scaled to the height of the orthophosphate peak (adjusted to 6.30 ppm)

Discussion

Total soil phosphorus concentrations in the soils studied here varied >20-fold, which is surprising given their relatively small geographical range. Based

on soil-forming factors (Jenny 1941), total soil phosphorus can be considered as a function of climate (which influences the rate of weathering, biological productivity, leaching, etc.), vegetation (here lowland tropical forest), topography or the degree of soil disturbance (e.g., stable vs. actively eroding surfaces; Porder et al. 2007), parent material (phosphorus content of bedrock), and time (strongly-weathered soils usually contain less total phosphorus than younger soils developed on the same geology; Walker and Syers 1976). In addition, total phosphorus can be influenced by external inputs, including dust and volcanic ash (e.g., Okin et al. 2004).

Given that all the plots studied here were under lowland tropical forest (although there is variation in tree species along the rainfall gradient; Pyke et al. 2001) and that total soil phosphorus was not correlated with annual rainfall, the wide range in total soil phosphorus concentrations appears to be determined primarily by lithology rather than vegetation or climate. This presumably reflects differences in the phosphorus content of parent materials, which have a marked effect on soil properties in lowland tropical forests (Baillie 1989). Of the soils studied here, two Ultisols developed on rhyolitic tuff contained the lowest total phosphorus concentrations, similar to those reported on volcanic ash in El Salvador (70 mg P kg^{-1} ; Dahnke et al. 1964). In contrast, two Alfisols developed on calcareous parent material contained the highest phosphorus concentrations; this is unusual given that calcareous soils tend to be relatively poor in phosphorus (Harrison 1987), and is presumably due to relatively high phosphorus concentrations in the bedrock.

Soil organic phosphorus concentrations measured here by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy were within the range of those determined previously for surface soils in tropical forests. Harrison (1987) reported that the mean organic phosphorus concentration in 76 soils from 'equatorial zone forests' (defined as sites between the 40° parallels, excluding New Zealand, but including Japan), including values determined by both ignition and extraction procedures, was $153 \pm 28 \text{ mg P kg}^{-1}$. This represented $28.7 \pm 2.5\%$ of the total soil phosphorus, although there were differences between Oxisols ($24.9 \pm 2.8\%$) and Ultisols ($33 \pm 3.3\%$). In another study involving the analysis by sequential fractionation of 120 uncultivated soils from eight soil

Fig. 3 Scatter plots showing the relationship between total soil phosphorus and **a** soil pH and **b** total carbon for soils under lowland tropical rain forest in central Panama

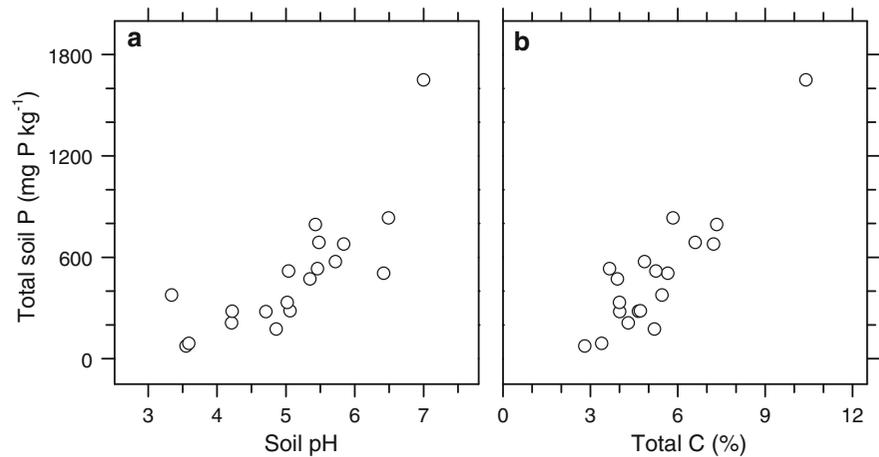


Table 6 Pearson's correlation coefficients for relationships between phosphorus forms, soil properties, and mean annual rainfall, for soils from one hectare forest inventory plots in central Panama

	Organic P (mg P kg ⁻¹)	Phosphate monoesters (mg P kg ⁻¹)	DNA (mg P kg ⁻¹)	Pyrophosphate (mg P kg ⁻¹)	C:organic P
Total P (mg P kg ⁻¹)	0.96***	0.96***	0.71***	0.84***	-0.90***
Total C (%)	0.83***	0.81***	0.75***	0.65**	-0.61**
Soil pH	0.89***	0.90***	0.57*	0.79***	-0.90***
Clay (%)	ns	ns	ns	ns	ns
Oxalate Al + Fe (g kg ⁻¹)	ns	ns	ns	ns	ns
Oxalate Mn (g kg ⁻¹)	0.54*	0.57*	ns	0.66**	-0.56*
Oxalate P (mg P kg ⁻¹)	0.70***	0.68**	0.59**	0.57*	-0.72***
Degree of P saturation (%)	0.62**	0.60*	0.54*	0.53*	-0.65**
Rainfall (mm y ⁻¹)	ns	ns	ns	ns	ns

ns not significant

Significance at the 5%, 1%, and 0.1% levels denoted by *, **, and ***, respectively

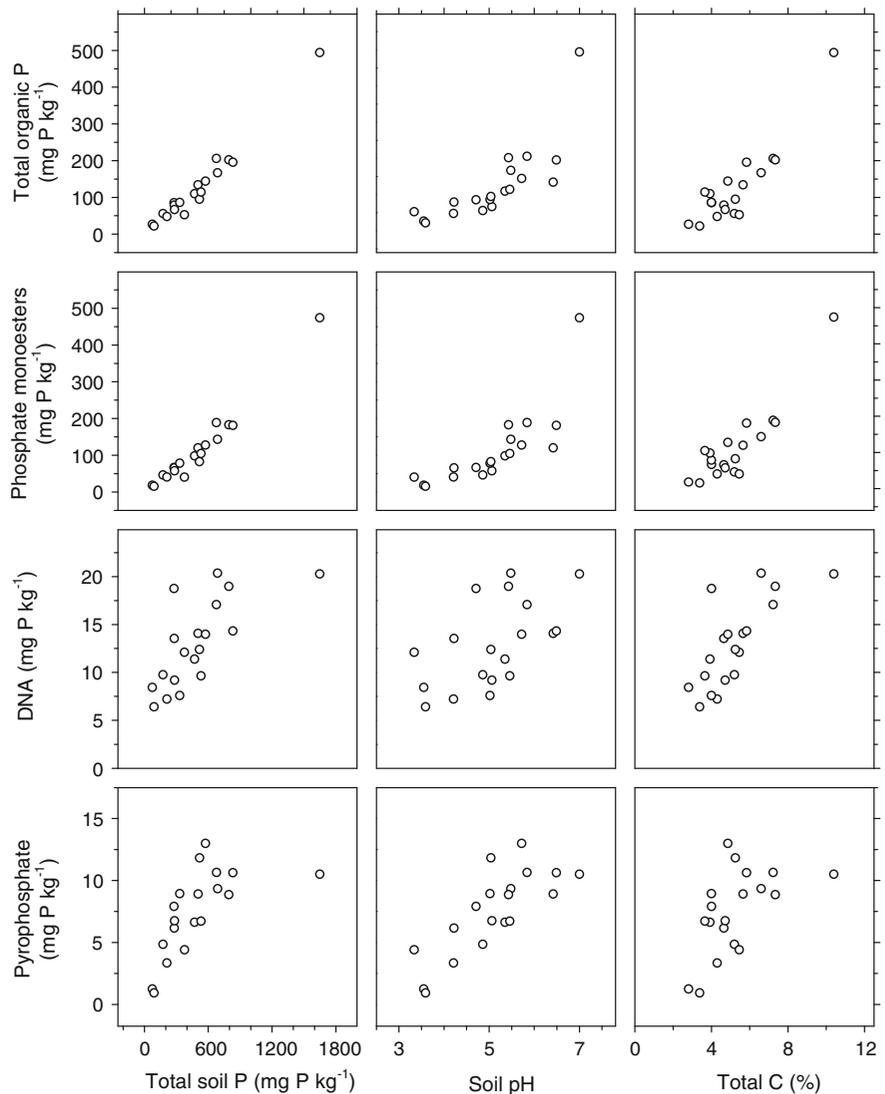
orders, organic phosphorus in strongly-weathered soils represented an average of 26% of the total soil phosphorus (Sharpley et al. 1987). These values compare well with those reported here (mean 125 ± 24 mg P kg⁻¹ and $25.5 \pm 1.2\%$ of the total soil phosphorus) for a series of soils under lowland tropical rain forest in central Panama.

Although we assessed a number of contrasting soils under similar vegetation across a strong rainfall gradient, the study can provide only limited insight into the extent to which the five soil forming factors influence soil organic phosphorus. Conclusive evidence would require constraint of all except a single soil-forming factor; for example, soil chronosequences include sites that differ only in the time since the onset of pedogenesis, which allows isolation of the effect of time on soil organic phosphorus (e.g.,

Walker and Syers 1976; Crews et al. 1995; Turner et al. 2007). Despite this, our results do provide strong evidence for the importance of total phosphorus in regulating soil organic phosphorus in tropical forests. Harrison (1987) reported that 59% of the variation in the organic phosphorus concentration of surface soils worldwide was explained by total phosphorus concentration, yet in the soils studied here total soil phosphorus explained 92% of the variation in soil organic phosphorus. It therefore seems that total phosphorus is the primary control on organic phosphorus concentrations in lowland tropical rain forests.

The fact that about one quarter of the total phosphorus occurred in organic forms in this and other studies of tropical forests (see above) was surprising given the range in mean annual rainfall and

Fig. 4 Scatter plots showing the relationship between concentrations of soil phosphorus fractions determined by solution ^{31}P NMR spectroscopy and chemical properties (total phosphorus, soil pH, total carbon) of soils under lowland tropical rain forest in central Panama. All correlations are statistically significant ($p < 0.05$; Table 6)



soil properties. Thus, while total phosphorus is the primary control on the *concentration* of soil organic phosphorus in the soils studied here, the *percentage* of the total phosphorus in organic form appears to be a function of the ecosystem, and may represent a broad emergent property of lowland tropical forests. The percentage of organic phosphorus is greater in other natural forest ecosystems; for example, organic phosphorus determined by the same procedure used here constituted $62 \pm 3\%$ (mean \pm STD error, $n = 12$) of the total phosphorus in birch forest and tundra soils of the Scandinavian subarctic (Turner et al. 2004) and $52 \pm 2\%$ ($n = 7$) of the total phosphorus in temperate rain forest soils along the

Franz Josef chronosequence in New Zealand (excluding two young sites; Turner et al. 2007).

Although the rainfall gradient studied here is sufficient to exert a strong influence on floristic composition (Pyke et al. 2001; Engelbrecht et al. 2007), as well as some soil properties (clay concentration, amorphous aluminum and iron concentrations, and C:N ratio), it did not appear to influence the forms or concentrations of soil organic phosphorus. The rainfall gradient is not as wide as in other studies where significant effects of rainfall have been shown. For example, a strong influence of rainfall on soil organic carbon was detected across a rainfall gradient from 2200 to $>5000 \text{ mm y}^{-1}$ in montane forest on

Fig. 5 Scatter plots showing the relationship between soil properties (total phosphorus, pH, total carbon) and the proportion (%) of the soil organic phosphorus occurring as phosphate monoesters and phosphate diesters in soils under lowland tropical rain forest in central Panama

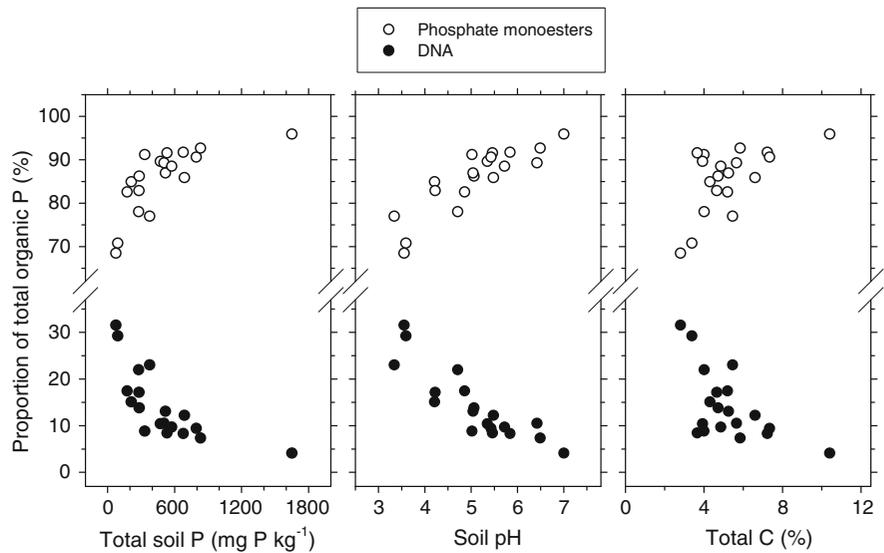
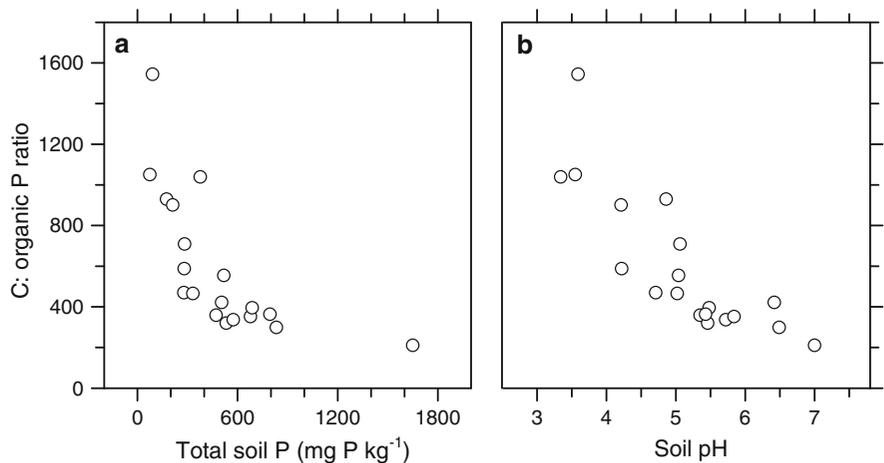


Fig. 6 Scatter plots showing the relationship between the carbon to organic phosphorus ratio and **a** total soil phosphorus and **b** pH for soils under lowland tropical rain forest in central Panama



the Hawaiian Islands (Schuur et al. 2001). In general, the influence of mean annual precipitation on soil organic phosphorus appears to vary among climates and ecosystems (e.g., Tate and Newman 1982; Harrison 1987; Sumann et al. 1998; Turner et al. 2003c), although no studies appear to have isolated the effect of rainfall from other soil forming factors.

In addition to total phosphorus, soil organic phosphorus was correlated strongly and positively with soil pH and total soil carbon. The correlation with pH contrasts markedly with most previous studies of temperate ecosystems, which have indicated that organic phosphorus accumulates in more acidic soils (e.g., Hawkes et al. 1984; Turner et al. 2003c). However, Harrison (1987) reported that only

a small proportion of the variation in organic phosphorus concentrations in surface soils worldwide was accounted for by soil pH. It therefore seems likely that the correlation between organic phosphorus and pH found here is a product of the influence of the parent material on both pH and total soil phosphorus.

Organic phosphorus and organic carbon tend to be correlated strongly (e.g., Turner et al. 2003c). Harrison (1987) reported that organic carbon explained 44% of the variation in organic phosphorus concentrations in surface soils worldwide, but noted that the relationship did not seem to hold for tropical soils. This was perhaps due to the widespread use of the ignition procedure to determine soil organic

phosphorus in the older literature (e.g., Bates and Baker 1960; Dahnke et al. 1964; Uriyo and Kesseba 1975). This method is no longer used for strongly-weathered soils, because it overestimates organic phosphorus in such soils by increasing the solubility of inorganic phosphate contained within secondary minerals following high temperature ignition (Williams and Walker 1967; Condon et al. 1990) and by including alkali-labile inorganic phosphate in the organic phosphorus fraction (Turner et al. 2007).

It is commonly assumed that organic phosphorus, especially in the form of phosphate monoesters, is stabilized in soils by association with amorphous metal oxides (Harrison 1987; Celi and Barberis 2007) and there are several reports of strong correlations between soil organic phosphorus and oxalate-extractable aluminum and iron in temperate systems (e.g., Harrison 1987; Murphy et al. 2010). There is less information for manganese oxides, although amorphous manganese, rather than aluminum and iron, was most strongly correlated with organic phosphorus in the soils studied here. Amorphous manganese was also correlated strongly with pyrophosphate, as reported previously for semi-arid arable soils (Turner et al. 2003c). Interaction of organic phosphorus and amorphous manganese would most likely occur via ligand exchange between the phosphate groups on the organic phosphorus molecule and hydroxyl ions on the mineral surface (Celi and Barberis 2007). This warrants further study given the potential importance of redox fluctuations in maintaining phosphorus availability in lowland tropical forests (Chacón et al. 2006).

The composition of organic phosphorus in the lowland tropical rain forest soils studied here was similar to most mineral soils in that it contained mainly phosphate monoesters and smaller concentrations of DNA (Condon et al. 2005). Phosphonate was detected in only two soils, both of which were on the same geological substrate (Caimito marine facies) and relatively rich in phosphorus. Phosphonates originate from a variety of organisms, but little is known about their behavior in soil (Condon et al. 2005). All soils contained pyrophosphate, but none contained long-chain polyphosphate. These compounds are inorganic, but they can be considered functionally similar to organic phosphorus because they have a biological origin and require hydrolysis by phosphatase enzymes prior to plant uptake.

Polyphosphates in mineral soils appears to originate from fungi, notably ectomycorrhizal fungi (Koukol et al. 2008), but these are not common root symbionts in lowland forests in Panama or elsewhere (Alexander 1989). Both phosphonate and polyphosphate tend to occur in abundance only in soils where decomposition is slowed, including cold and wet soils high in organic matter (e.g., Cade-Menun et al. 2000; Turner et al. 2004).

Apart from recent studies in central Panama (Turner 2008b; Vincent et al. 2010), the only previous study of lowland tropical rain forest soils by solution ^{31}P NMR spectroscopy was by Forster and Zech (1993), who assessed soil organic phosphorus along a catena (pH in surface soils 3.9–5.0; total carbon 1.1–3.2%) under evergreen forest in Liberia. Organic phosphorus constituted between 46 and 65% of the phosphorus extracted in 0.5 M NaOH (1:20 solid to solution ratio with repeated sonication at 0°C), of which between 74 and 85% was phosphate monoesters and the remainder DNA. These values are therefore similar to those reported here. Other solution ^{31}P NMR spectroscopy studies of tropical forest soils involved montane sites in Thailand and Ethiopia (Möller et al. 2000; Solomon et al. 2002) and did not include any lowland forests.

The chemical nature of soil organic phosphorus was influenced strongly by total soil phosphorus and soil pH, with a greater proportion of phosphate diesters in acidic soils low in total phosphorus. Phosphate diesters are often considered to be labile in soil due to their relatively rapid turnover (e.g., Bowman and Cole 1978). However, their degradation may be slowed in acidic soils due to the relatively strong stabilization of compounds such as DNA at $\text{pH} < 5$, which protects them from enzymatic attack (Condon et al. 2005). This, along with their occlusion within large humic molecules, may allow them to accumulate to form a considerable proportion of the soil organic phosphorus, as reported recently for a chronosequence of soils under temperate rain forest in New Zealand (Turner et al. 2007).

A striking feature of the NMR spectra here, and the greatest contrast to most previous reports of temperate mineral soils, was the absence of clear signals from inositol hexakisphosphate in the majority of soils. This confirms previous evidence indicating the absence of inositol phosphates from a limited number of tropical forest soils in the central Panama region (Turner

2008b; Vincent et al. 2010). Where present in sufficient concentrations, inositol hexakisphosphate can be identified by a signal at approximately 4.2 ppm (*scyllo*-inositol hexakisphosphate; Turner and Richardson 2004) and a series of four signals at approximately 5.9, 4.9, 4.6 and 4.5 ppm (*myo*-inositol hexakisphosphate; Turner et al. 2003a, b). At low concentrations, spiking experiments may be required to detect *myo*-inositol hexakisphosphate (Smernik and Dougherty 2007), although its presence can usually be verified by a signal from the C-2 phosphate group at approximately 5.9 ppm, slightly downfield of the large signal from inorganic phosphate.

In the samples analyzed here, a signal at 5.9 ppm was not identified clearly in any spectrum, while a signal at 4.2 ppm, which would indicate *scyllo*-inositol hexakisphosphate, was identified clearly only in an Alfisol developed on calcareous parent material at Campo Chagres (Fig. 2). Small but clear signals at 6.8 ppm were present in spectra of several of the phosphorus-rich soils (Fig. 2), notably those containing >100 mg P kg⁻¹ of phosphate monoesters. These signals probably represent inositol phosphates, as they resist hypobromite oxidation, a procedure that destroys all organic phosphorus except the higher-order inositol phosphates (Turner and Richardson 2004). It therefore seems likely that inositol phosphates were present at low concentrations in at least some soils that were relatively rich in phosphorus.

The absence of inositol hexakisphosphate in many of the soils studied here is in marked contrast to results from temperate mineral soils, which typically contain a relatively large fraction of their soil organic phosphorus in the form of higher-order inositol phosphates (Cosgrove 1980; Turner 2007). Their accumulation in mineral soils is assumed conventionally to be due to the strong interaction of higher-order inositol phosphates with soil components (clays, metal oxide surfaces, etc.), leading to their stabilization and protection from biological attack (Celi and Barberis 2007). However, there is evidence that inositol hexakisphosphate interacts relatively weakly with clay surfaces in strongly-weathered soils, due to a reduction in the number of phosphate groups involved in its stabilization on crystalline mineral surfaces (Giaveno et al. 2008). Indeed, studies of soil chronosequences in New Zealand indicated that inositol phosphate concentrations decline in old, strongly-weathered soils in parallel

with a reduction in the concentration of amorphous metal oxides (McDowell et al. 2007; Turner et al. 2007). Inositol hexakisphosphate was also reported to occur in low concentrations in strongly-weathered soils from South Australia (Smernik and Dougherty 2007) and in a series of Oxisols in Madagascar (Turner 2006).

Weak sorption to crystalline minerals in strongly-weathered soils cannot completely explain the absence of detectable inositol phosphate concentrations here, however, because several soils are undergoing active development (Inceptisols and soils with relatively rapid rates of erosion) and most soils contained abundant amorphous metal oxides. Alternative explanations are that (1) limited phosphorus availability, which seems likely in at least some of the soils (see below), reduces the accumulation of inositol phosphates by favoring microbes that have the capacity to synthesize phytase, the enzyme responsible for releasing phosphate from the inositol ring (Hill and Richardson 2007); and (2) inputs of inositol phosphates, which are abundant in seeds and pollen (Raboy 2007), are lower in lowland tropical forests compared to temperate ecosystems. The latter seems possible, because wind pollination is extremely rare in lowland tropical forests (Bawa 1990), but common in temperate forests and grasslands where high concentrations of inositol phosphates have been recorded (Turner 2007). Clearly, additional studies are required on the cycle of inositol phosphates in tropical forests.

The absence of inositol phosphates suggests that at least some of the phosphate monoester pool in these soils probably occurred as alkali-labile phosphate diesters (e.g., RNA and some phospholipids), which degrade to phosphate monoesters during extraction and analysis (Makarov et al. 2002; Turner et al. 2003a). Similar NMR spectra, with phosphate monoester signals corresponding to degradation products of alkali-labile phosphate diesters, were also reported for soils from Gigante Peninsula, a lowland forest site in the Panama Canal watershed with intermediate rainfall (Vincent et al. 2010). This indicates the importance of phosphate diesters and, therefore, phosphodiesterase activity in regulating the turnover of organic phosphorus in these lowland tropical forests.

Smekc (1985) predicted that C:organic P ratios >200 in soil organic matter indicate a shortage of

biologically-available phosphorus. Although this has not been assessed in lowland tropical forests, it suggests that all the soils studied here are low in available phosphate and represent phosphorus-limiting environments for plants and soil organisms involved in organic matter decomposition (e.g., Cleveland et al. 2002). This seems unlikely given the high phosphorus concentrations in some soils (e.g., those developed on calcareous parent material) and the fact that not all stages of the decomposition process appear to be limited by the same nutrient (Kaspari et al. 2008). Nevertheless, the marked variation in C:organic P ratios across the Panamanian Isthmus raises questions about the influence of nutrients on forest community composition and productivity in the region. The distribution of tree species across the isthmus is linked strongly to drought tolerance (Engelbrecht et al. 2007), although a large proportion of the variation remains unexplained. Given that soil phosphorus concentrations vary >20-fold among the plots, it seems probable that much of the remaining variation may be explained by soil nutrients.

Conclusions

Soil organic phosphorus concentrations in lowland tropical rain forest soils of central Panama were correlated strongly with total phosphorus, pH, and total carbon. Concentrations were lowest in acidic soils with low phosphorus concentrations and highest in neutral soils with high phosphorus concentrations. However, the percentage of the total phosphorus in organic forms remained relatively constant, despite considerable variation in climate and soil properties. Soil organic phosphorus consisted mainly of phosphate monoesters with smaller concentrations of DNA. The latter constituted a greater proportion of the organic phosphorus in low phosphorus, acidic soils. Remarkably, inositol phosphates did not constitute a major fraction of the phosphate monoesters in any of the soils, despite often occurring in abundance in mineral soils of temperate ecosystems. Further work is now required to assess the contribution of soil organic phosphorus to the nutrition and diversity of plants in these species-rich ecosystems.

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