Soil phosphorus responses to chronic nutrient fertilisation and seasonal drought in a humid lowland forest, Panama

M. J. Mirabello, J. B. Yavitt, M. Garcia, K. E. Harms, B. L. Turner, and S. J. Wright

Abstract. We used the Hedley sequential fractionation scheme to assess phosphorus (P) chemistry of a strongly weathered soil from a humid lowland forest in Panama. Our analyses were part of a factorial experiment of nitrogen, P, and potassium addition, with nutrients added annually, i.e. a chronic input. The aim was to examine changes in soil P chemistry with 7 years of nutrient addition for soils collected in the wet season and the dry season. The majority of P occurred in fractions extracted by NaOH (24% of the total soil P) and hot concentrated HCl (58% of the total). Organic P (Pₒ) was ~54% of extractable P. Labile P, defined as Pₑ plus inorganic P (Pᵢ) extracted by NaHCO₃, was largely Pₑ (84% of the NaHCO₃-extractable P). Chronic P addition increased NaHCO₃-extractable Pₑ several-fold and NaOH-extractable Pᵢ two-fold. Seasonal variation occurred for labile P and NaOH-extractable P, whereas occluded P did not vary throughout the study period. Extractable P was ~15% higher in surface than subsurface soil. We added 350 kg P ha⁻¹ during the 7-year period and recovered ~55% by sequential extraction. According to biogeochemical theory, added P should show up in fractions with the shortest residence times, e.g. labile P. Our finding that added P accumulated in fractions with presumably long residence times, i.e. extracted by NaOH (bound) and hot concentrated HCl (occluded), suggests that greater attention be paid to the short-term dynamics of bound and occluded P in strongly weathered tropical forest soils.

Additional keywords: extractable phosphorus, Hedley fractionation, organic bound P, strongly weathered soil.

Received 12 July 2012, accepted 20 May 2013, published online 12 June 2013

Introduction

Over the lifetime of a soil, phosphorus (P) begins in primary minerals, is released via weathering as biologically available inorganic P (Pᵢ), then eventually cycles into organic forms (Pₒ), binds to calcium (Ca²⁺), aluminium (Al³⁺), or iron (Fe³⁺), or becomes physically protected (occluded P) (Walker and Syers 1976). Most P in strongly weathered soils occurs in occluded forms (Tiessen et al. 1984; Dieter et al. 2010), so the maintenance of P availability in sufficient quantities to support high forest productivity on strongly weathered tropical soils presents a conundrum (Vitousek et al. 2010). Sequential extraction is a common way to characterise the different forms of soil P (Chang and Jackson 1957; Condron and Newman 2011). The fractionation method developed by Hedley et al. (1982) is the most widely used procedure and involves a series of reagents designed to remove Pₑ and Pₒ bound with increasing chemical strength: NaHCO₃ for weakly adsorbed P; NaOH for P more strongly associated with Fe and Al hydrous oxides; HCl_wet for P associated with Ca in primary minerals; and finally a H₂SO₄–H₂O₂ digestion to determine occluded P. Subsequently, Tiessen and Moir (1993) introduced an additional step involving hot concentrated HCl (HCl_strong) in an attempt to obtain additional information for P associated with crystalline Fe and Al oxides, which are especially important in strongly weathered soils.

Here we examine seasonal dynamics of P in labile, bound, and occluded fractions in a strongly weathered soil. One reason is that the seasonal drought and soil drying that occur in many tropical regions can influence P fractions in several ways. For example, drying can disrupt organic matter coatings on mineral particles, exposing fresh surfaces for sorption and desorption of Pₑ and Pₒ (Haynes and Swift 1989). Drying also can alter mineral crystals and reduce the capacity for adsorption (McLaughlin et al. 1981). Although soil microbes can access P adsorbed to minerals during the dry season (Olander and Vitousek 2004), microbial cells desiccate during prolonged drought (Kieft et al. 1987), and cells can lyse upon rewetting, releasing soluble Pₒ (Turner and Haygarth 2001; Turner et al. 2003). On the other hand, plant demand for Pₑ should peak during the wet season and deplete labile forms of P (Vandecar et al. 2009). Therefore, movement of P among different fractions in relation to seasonal drought is unclear.

Nutrient fertilisation provides a way to examine soil P biogeochemistry. According to reservoir theory (Eriksson 1971), the elemental content of a particular soil fraction will respond to an enhanced input level (i.e. chronic input) as a
function of its residence time. Thus, a rapid increase in concentration indicates a very short residence time, whereas no immediate increase with chronic input indicates a long residence time. We took advantage of a factorial experiment of nitrogen (N), P, and potassium (K) addition, initiated in 1998 with nutrients added annually to a humid lowland forest in Panama (Wright et al. 2011), to investigate soil P biogeochemistry. There are few long-term experimental studies with chronic nutrient addition to natural tropical forests for comparison (see Wright et al. 2011; and references cited therein). We hypothesised that labile P fractions would be smaller in the wet season than in the dry season, reflecting plant uptake and microbial immobilisation. We also expected that P in strongly bound fractions would increase after 7 years of chronic P addition.

Materials and methods

Study site

The study was conducted on the Gigante Peninsula (9°06′31″N, 79°50′37″W), part of the Barro Colorado Nature Monument, Republic of Panama. The site supports mature, semi-deciduous rain forest at least 200 years old (Wright et al. 2011). Annual precipitation averages 2600 mm, with <10% falling during a 4-month dry season (late December–late April). Soils on Gigante Peninsula are Oxisols developed on Miocene basalt and are morphologically similar to the Typic Eutrudox on nearby BCI (Dieter et al. 2010). Topsoil texture is clay.

The chronic fertilisation experiment began in 1998 and has been described in detail elsewhere (Wright et al. 2011; Yavitt et al. 2011). The experiment is remarkable for its size, duration, and consideration of responses in terms of forest productivity, species diversity, and soil properties (e.g. Yavitt et al. 2009; Wright et al. 2011). Briefly, we replicated four times the eight treatments of a 2 × 2 × 2 factorial NPK experiment. The 32 treatment plots are 40 m by 40 m with a minimum distance of 40 m between plots, except for two plots separated by 20 m and a 3-m-deep stream channel. Nutrient addition began in June 1998. Fertilisers are added by hand in four equal doses each wet season with 6–8 weeks between applications (15–30 May, 1–15 July, 1–15 September, and 15–30 October). Nitrogen is added as coated urea [(NH$_2$)$_2$CO] at an annual dose of 125 kg N ha$^{-1}$ year$^{-1}$; P is added as triple superphosphate [Ca(H$_2$PO$_4$)$_2$·H$_2$O] at an annual dose of 50 kg P ha$^{-1}$ year$^{-1}$; and K is added as KCl at an annual dose 50 kg K ha$^{-1}$ year$^{-1}$. Similar doses are used in forestry (Snethurst 2010) and have also been used in tropical montane studies (100–150 kg N, 50–65 kg P, 50 kg K, e.g. Tanner et al. 1992).

Soil analysis

Soil samples were collected during the wet season in September 1997 before the first fertiliser application. A surface sample was collected with a hand trowel from 0–0.15 m depth, excluding litter and debris at the upper surface, by combining three scoops of soil collected from within 0.5 m of the sample point to form a single 300-g sample. A deeper soil sample (0.3–0.45 m depth) was collected with a soil auger. Soils were resampled within 1 m of the original locations in the wet season in July 2004 and in the dry season in April 2005 using a soil auger. Field-moist soil was used for soil moisture determination by quantifying the change in mass before and again after oven-drying at 105°C for 48 h. Soil pH was determined in a 1:2 soil to solution ratio in distilled water.

Triplicate 0.5-g portions of air-dried soils were placed into 50-L screw-cap plastic centrifuge tubes and extracted according to the Hedley sequential extraction procedure (Tiessen and Moir 1993). A blank (no soil) was included with each run. We did not use anion exchange resin for the first extraction; rather, we assume that resin-extractable P was included in the NaHCO$_3$ extract. Inorganic P in each fraction was determined following neutralisation and automated molybdate colourimetry (Turner and Romero 2009). For extracts containing organic matter, a separate aliquot of neutralised solution was measured without molybdate reagent to correct for colour interference. Total P in each extract, except the HCl$_{\text{weak}}$ fraction, was determined by the same procedure following acid-persulfate digestion (Rowland and Haygarth 1997). Organic-bound P was the difference between total P and P$_t$.

Statistical analyses

The NPK factorial design is replicated four times along a north–south topographic gradient and blocked within each replicate along the perpendicular east–west gradient to control spatial variation in soil properties (Wright et al. 2011). We performed repeated-measures analysis of variance (ANOVA) for each P fraction in surface soil with repeated-measures on season. We performed factorial ANOVAs for each P fraction in subsurface soil. Between-subject (or plot) effects evaluated responses over the entire treatment. Within-subject (or plot) effects evaluated variation among seasons and interactions among treatment and season. We used Levene’s test to determine whether variances were homogeneous for the eight NPK factorial treatments. If necessary, the data were transformed to achieve homogeneity. We performed correlation analysis (Pearson r) to evaluate relationships between P concentrations in surface and the associated subsurface soil.

Results and discussion

The coefficient of variation (CV) for each P fraction was 35–50% for the 36 surface soil samples (0–15 cm depth interval) and the 36 subsurface soils (30–45 cm depth interval) collected in 1997 before the nutrient fertilisation treatments. This suggests relatively little spatial variation for P fractions across the 38.4-ha study area, because CVs were 100–200% for other soil chemical properties in the site (Yavitt et al. 2009). One explanation for soil P showing less spatial variation across a large (38.4 ha) area is that P is used in essentially the same way in all plants and microorganisms (Makino et al. 2003), and presumably biotic activity will homogenise P concentrations spatially across a soil landscape.

Comparing concentrations of P for surface soil samples with the associated subsurface soil sample indicated relatively poor correlation, with Pearson r <0.35, for labile P (NaHCO$_3$-extractable) and for P in NaOH extracts. In contrast, correlation coefficients were >0.88 for occluded forms of P. Therefore, occluded forms of P have very similar concentrations across
the 0–45 cm depth interval of soil at any given place in the study area. In contrast, for labile fractions, concentrations in subsurface soils are a poor predictor for concentrations in surface soils and vice versa. Consequently, the more biologically active labile P fractions are redistributed between surface and subsurface soil.

**Differences among fractions**

Phosphorus extracted from surface soil is shown in Fig. 1, and P extracted from subsurface soil is shown in Fig. 2. We calculated P fractions as a proportion of the sum of extractable P (i.e. all extractable fractions plus the residual P fraction). Sodium bicarbonate extracted ~4% of the sum, with six-fold more P₀ than P₁ for surface soils, except for relatively large P₁ values during the 2005 dry season in plots with added P. Organic P was 100-fold greater than P₁ in the NaHCO₃ extract for subsurface soils. Phosphorus in the NaOH extract was ~26% of the sum, with about two-fold more P₀ than P₁ for surface soils and ~six-fold more P₀ than P₁ for subsurface soils. The HCl_strong extract had the largest amount of P, with 55% of the sum. In contrast to the previous two extracts, P₀ was only 10% greater than P₁ for surface soil and 25% greater for subsurface soil in the HCl_strong extract. The HCl_weak fraction contained a small amount of P₀, although somewhat larger values occurred for surface soils during the 2005 dry season in plots with added P. The residual fraction was 14% of the sum for both depth intervals.

It is important to understand P availability in strongly weathered soils that support tropical forests, because these soils can have inadequate levels of readily available P for maximum plant production. For instance, Bray-extractable P (Bray and Kurtz 1945) is commonly used to assess plant-available P, and values <2 mg P kg⁻¹ dry soil in our study area (Yavitt et al. 2009) are low enough to limit plant growth.

**Fig. 1.** Soil phosphorus fractions (mean ± standard error) determined by the Hedley sequential fractionation procedure for surface soils (0–15 cm depth interval) in plots with (+P) and without (−P) phosphorus in the nutrient addition experiment on Gigante Peninsula, Panama.
in bioassays (Denslow et al. 1987). On the other hand, labile P also has been defined as $P_i$ plus $P_o$ in the NaHCO$_3$ extract (Johnson et al. 2003), and our value of 32 mg P kg$^{-1}$ dry surface soil is toward the high end of values found in other Oxisols, i.e. a mean value of 20 (± 19 s.d.) mg P kg$^{-1}$ dry soil as reported in Yang and Post (2011). In our case, the NaHCO$_3$ extract was largely $P_o$, compared with a value of 50% reported by Yang and Post (2011) for other Oxisols. However, strongly weathered soils from near our study site also contain much greater concentrations of $P_o$ than $P_i$ in the NaHCO$_3$ extract (Dieter et al. 2010), suggesting that labile P is predominately $P_o$ in the region.

The sum of $P_o$ in the NaHCO$_3$, NaOH, and HCl$_{strong}$ extracts was 53% of extractable P. This value is much larger than 18% $P_o$ for Oxisols reported in Yang and Post (2011); however, Harrison (1987) reported a value of 30% for Oxisols in an extensive literature review. Moreover, Turner and Engelbrecht (2011) and Vincent et al. (2010) reported a value of 25% for soils in central Panama based on NaOH-EDTA extraction, which includes NaHCO$_3$- and NaOH-extractable P. Thus, the large value in the present study is likely caused by $P_o$ in the HCl$_{strong}$ fraction. In contrast, secondary $P_{i,d}$, defined as NaOH $P_i$, was a smaller percentage, i.e. 4% in our site vs. 15% in other Oxisols (Yang and Post 2011).

Walker and Syers (1976) argued that occluded forms of P should predominate over $P_o$ in strongly weathered soils derived from relatively silica-poor bedrock, such as the basalts in the study area. However, our finding of a large amount of $P_o$ needs to be interpreted with caution. Most of the $P_o$ occurred in the HCl$_{strong}$ extract, which is technically an occluded form of P. It

Fig. 2. Soil phosphorus fractions (mean ± standard error) determined by the Hedley sequential fractionation procedure for subsurface soils (30–45 cm depth interval) in plots with (+P) and without (−P) phosphorus in the nutrient addition experiment on Gigante Peninsula, Panama.
is possible that we overestimated this fraction, perhaps through silica interference in phosphate detection by molybdate colourimetry. For example, Agbenin and Tiessen (1995) reported up to 30% more P by sequential fractionation compared with an independent measurement of total soil P. Here, total soil P determined by ignition (550°C for 16 h) gave total soil P concentrations of 340 mg P kg⁻¹ dry soil in control plots and 560 mg P kg⁻¹ dry soil in P-addition treatments, for samples taken in 2006. These values are considerably less than the sums of soils from central Panama to the H₂O₂ total P, although it yielded similar total P concentrations in a range of soils tested. We have been adding N as urea, and urea mineralisation has been observed. In contrast, added P led to significant increase in P. Here, the HClstrong fraction was a much larger percentage of occluded P (HClstrong P plus residual P = 88% of the total P in surface soil) than the values of 36–55% reported for other strongly weathered soils (Lilienfein et al. 2000; Xavier et al. 2011). We therefore suspect that the HClstrong extract overestimated P in that fraction.

Impact of chronic nutrient addition

The ANOVA for the nutrient fertilisation experiment indicated that additions of N and K, added alone and in combinations, did not have a significant impact on values for any of the P fractions. We have been adding N as urea, and urea mineralisation has acidified the soil: pH 5.3 for plots with no N addition v. pH 4.6 for plots with N addition. However, plots with added N and added P had slightly less acidification, with pH 4.9. Acidification could increase P binding to Fe and Al oxides, but this was not observed. In contrast, added P led to significant effect on several of the P fractions (Table 1).

Although P₂ in the NaHCO₃ extract showed large values in surface soils in plots with added P, the dry season response was likely transient, as the increase was one-tenth lower in the wet season. In contrast, added P led to constant values for P₂ in NaHCO₃ extract among seasons, compared with seasonal fluctuation in the plots with no added P, suggesting, perhaps, a new steady-state value for this fraction with added P. Added P also had a large effect on P₁ in the NaOH extract, with a consistent two-fold increase. The only other large impact of added P in surface soils was a transient increase in the HClweak-extractable fraction. However, even a transient increase in P in the HClweak extract might be an artefact of the procedure. For example, Benzing and Richardson (2005) found that P recovered in HClweak extract might have been extracted from secondary minerals by NaOH, but it bound quickly to Ca²⁺ and was recovered subsequently as Ca-P in the HClweak extract.

In subsurface soils, there was evidence that added P led to a significant increase in P₀ in the NaHCO₃ extract. Although P₀ in the NaOH extract and HClweak-extractable P also showed significant increases with added P (Table 1), the amounts were not large. Research in agricultural soils has shown overwhelmingly that P fertilisers remain in surface soils. The main reason is the very high phosphate fixation capacity of soil materials, and thus there are few examples in which subsurface soils show a response to chronic P additions (cf. Wang et al. 2007; Rivaie et al. 2008). However, our data agree with Wagar et al. (1986) that P₀ is more mobile than P₁, and thus P₀ in the labile fraction with added P moves downward more readily than P₁.

Table 1. Analysis of variance for soil phosphorus (P) fractions in response to P addition (0–15 and 30–45 cm depth) and wet season v. dry season (surface soils only)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Added P</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>0–15 cm depth interval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaHCO₃ P₁</td>
<td>8.12</td>
<td>0.011</td>
</tr>
<tr>
<td>NaHCO₃ P₀</td>
<td>14.71</td>
<td>0.001</td>
</tr>
<tr>
<td>NaOH P₁</td>
<td>61.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NaOH P₀</td>
<td>6.47</td>
<td>0.020</td>
</tr>
<tr>
<td>HClweak P₁</td>
<td>25.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30–45 cm depth interval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaHCO₃ P₁</td>
<td>6.41</td>
<td>0.021</td>
</tr>
<tr>
<td>NaHCO₃ P₀</td>
<td>25.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NaOH P₁</td>
<td>10.33</td>
<td>0.005</td>
</tr>
<tr>
<td>NaOH P₀</td>
<td>0.97</td>
<td>0.763</td>
</tr>
<tr>
<td>HClweak P₁</td>
<td>20.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Seasonal patterns in surface soils

We found significantly less P₀ in the NaHCO₃ extract for dry season soil than for wet season soil. However, this seasonal pattern must be regarded with caution. Most studies report that P₀ in the NaHCO₃ extract is less during the wet, growing season, driven by P₀ mineralisation and plant demand for available P (cf. Perrott et al. 1990; Chen et al. 2003). In our case, however, the peak value during the wet season correlates with the maximum rate of litter decomposition (Wieder and Wright 1995), and perhaps litter decay is a proximate source of NaHCO₃ P₀ in the mineral soil (Blair and Boland 1978; Vincent et al. 2010).

The NaOH-extractable fraction is associated with Fe and Al oxide minerals, and although potentially plant-available (Richter et al. 2006), it is thought to be less labile than the NaHCO₃-extractable fraction. Our findings for the NaOH P₁ fraction agree with the pattern for other regions with seasonal drought, in which values peak during the wet season (cf. Chacón et al. 2008; Valdespino et al. 2009). The interpretation is that the binding ability of Fe and Al oxides for NaOH P₁ is greater in wet than in dry soil. Although there is a general notion that seasonal dynamics of the NaOH P₁ fraction parallel the NaHCO₃ P₀ fraction (Herr et al. 2007), this is not necessarily so (Fabre et al. 1996). For instance, Fe and Al oxides might sorb P₀ more strongly in the dry season than in the wet season, accounting for the large dry season values for NaOH P₀. A similar situation occurs in rice paddy soils, in which NaOH P₀ levels are much greater in dry than in flooded soils (Darilek et al. 2011).

The finding of no seasonal difference for P in the HClstrong and HClweak Extracts and in the residual fraction agrees with the long residence time of mineral-bound and occluded fractions (Richter et al. 2006), and thus seasonal variations in values are unlikely. On the other hand, McGroddy et al. (2008) found seasonal fluctuation in the amount of residual P in a tropical soil
in Brazil, and thus the residence time of this fraction might be more variable than expected.

**Recovery of phosphorus fertiliser**

The data presented here allow us to assess the amount of added P that we could recover in extractable fractions. We added 350 kg P ha\(^{-1}\) during the 7-year study period, but recovered only 57% from soil in extractable fractions (excluding the HCl\(_{\text{strong}}\) fraction): 118 kg P ha\(^{-1}\) at 0–15 cm depth, 67 kg P ha\(^{-1}\) at 15–30 cm depth, and 17 kg P ha\(^{-1}\) at 30–45 cm depth. The amount at 15–30 cm depth is a linear extrapolation of measured values in the depth intervals above and below. We know that an additional 1.8 kg P ha\(^{-1}\) has been added to the ecosystem nutrient cycle in leaf and reproductive litter (Wright et al., 2011), leaving a relatively large percentage unaccounted for. We suspect at least some of the missing P is residing in the HCl\(_{\text{strong}}\) fraction, presumably as P\(_2\)O. Although most studies of soil P fractions skip the HCl\(_{\text{strong}}\) fraction, results here suggest that it is an important fraction in highly weathered soils and more dynamic than expected.

**Acknowledgements**

Rufino Gonzalez and Omar Hernandez have added nutrients since 1998. Funding was provided by grants from the Scholarly Studies program of the Smithsonian Institution and the Andrew W. Mellon Foundation to S. J. Wright.

**References**


