SOIL PHOSPHORUS RESPONSES TO SEASONAL DROUGHT AND CHRONIC

NUTRIENT FERTILIZATION IN A HUMID LOWLAND FOREST, PANAMA

A Thesis

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by

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BIOGRAPHICAL SKETCH

Matthew Mirabello earned his Bachelor of Science in Chemistry and Biology from the State University of New York at Geneseo in 2003. In 2003 he joined the Master's program in the Department of Natural Resources at Cornell University. While pursuing his degree, Matthew worked as a research assistant and teaching assistant for the Department of Natural Resources and the Department of Chemistry. Matthew has been a coauthor of and contributor to multiple papers examining soil chemistry dynamics in lowland tropical rainforests. He is currently a full time science educator for the *Urban Advantage* science initiative based in the Gottesman Center for teaching and learning at the American Museum of Natural History in New York City.

Matthew's project, *Tropical Rain Forest Soil Chemistry*, was supervised by Dr. Joe Yavitt

DEDICATION

This is dedicated to my family & friends who have supported me all of these years in completing this phase of my education.

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Firstly I have to acknowledge my advisor, Joe Yavitt, for his patience and guidance through this long process. Many people have supported me over the years; I list them below in no particular order with a brief word about their support:

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vkk

ABSTRACT

We utilized a factorial nitrogen (N), phosphorus (P), and potassium (K) addition experiment, with nutrients added annually to a humid lowland forest in Panama, to study how the distribution of P in the strongly weathered soil changed 1) over a seven-year period, 2) seasonally between the wet season and dry season, and 3) in response to added nutrients. The sum of P extracted by $NaHCO_3$, $NaOH$, HCl, and H_2SO_4 from 0-15 cm depth interval was 259 ± 17 mg P kg^{-1} dry soil in 1997 versus 299 ± 28 mg P kg^{-1} dry soil in 2004 in plots without added nutrients. Inorganic P in the $NaHCO_3$- and $NaOH$-extractable fractions decreased during the 7-year interval, whereas organic P increased. Seasonal variations occurred for all fractions, except residual P. In particular, values for $NaOH$-extractable inorganic P and $NaHCO_3$-extractable organic P were elevated in the wet season, whereas values for $NaOH$-extractable organic P and $NaHCO_3$-extractable inorganic P were elevated in the dry season. Chronic P addition lessened the seasonal difference when peak values occurred in the wet season, but exacerbated the seasonal difference for dry season peak values. Subsoil P was predominantly organic, suggesting downward movement of organically bound P. These results indicate that P moves quickly between labile and aluminum and iron associated fractions, suggesting very short residence times for P, including organic P, which helps to alleviate poor soil P fertility.

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

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Abstract We utilized a factorial nitrogen (N), phosphorus (P), and potassium (K) addition experiment, with nutrients added annually to a humid lowland forest in Panama, to study how the distribution of P in the strongly weathered soil changed 1) over a seven-year period, 2) seasonally between the wet season and dry season, and 3) in response to added nutrients. The sum of P extracted by $NaHCO_3$, NaOH, HCl, and H_2SO_4 from 0-15 cm depth interval was 259 ± 17 mg P kg^{-1} dry soil in 1997 versus 299 ± 28 mg P kg^{-1} dry soil in 2004 in plots without added nutrients. Inorganic P in the $NaHCO_3$- and NaOH-extractable fractions decreased during the 7-year interval, whereas organic P increased. Seasonal variations occurred for all fractions, except residual P. In particular, values for NaOH-extractable inorganic P and $NaHCO_3$-extractable organic P were elevated in the wet season, whereas values for NaOH-extractable organic P and $NaHCO_3$-extractable inorganic P were elevated in the dry season. Chronic P addition lessened the seasonal difference when peak values occurred in the wet season, but exacerbated the seasonal difference for dry season peak values. Subsoil P was predominantly organic, suggesting downward movement of organically bound P. These results indicate that P moves quickly between labile and aluminum and iron associated fractions, suggesting very short residence times for P, including organic P, which helps to alleviate poor soil P fertility.

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

Introduction

Soil phosphorus (P) is borne in primary minerals, becomes available to organisms as weathering releases inorganic P (P_i), but eventually cycles into 1) organic forms (P_o), 2) binds to Ca^{2+}, Al^{3+}, or Fe^{3+}, or 3) becomes physically protected (occluded P) (Walker and Syers 1976). In strongly weathered soils, most P occurs in these occluded forms (Tiessen *et al.* 1984; Dieter *et al.* 2011). Therefore, the availability of P to maintain forest productivity on strongly weathered soils in the tropics presents an interesting conundrum (Vitousek *et al.* 2010), given that most of the P should be relatively unavailable to plants. Sequential extraction is a common way to characterize the different forms of soil P (Chang and Jackson 1957; Condron and Newman 2011), and the fractionation method developed by Hedley *et al.* (1982) uses a series of reagents designed to remove P_i and P_o bound with increasing strength: $NaHCO_3$ for labile P; NaOH for P bound to secondary minerals; HCl for P in primary minerals; and finally, H_2SO_4 and H_2O_2 for occluded P.

The labile and bound fractions of P in strongly weathered soil might have shorter residence time than expected. One reason is that most tropical regions have seasonal drought, and soil drying 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 P_i and P_o (Haynes and Swift 1989). Drying also can alter mineral crystals and reduce the capacity for sorption (McLaughlin *et al.* 1981). Although, soil microbes can access P sorbed 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 P_o (Turner and Haygarth 2001; Turner *et al.* 2003). On the other hand plant demand for P_i during the wet season can deplete labile forms of P (Vandecar *et al.* 2009). Therefore, movement of P among different fractions in relation to seasonal drought is unclear.

Nutrient fertilization provides an investigative way to examine the biogeochemistry of P in soils. According to reservoir theory (Eriksson 1971) the amount of nutrient in a particular fraction in the soil will respond to a new chronic input value as a function of its residence time. Thus, a rapid increase in concentration indicates a very short residence time, whereas no immediate increase in magnitude with chronic input indicates a long residence time. We took advantage of a factorial nitrogen (N), P, and potassium (K) addition experiment, initiated in 1998 with nutrients added annually to a humid lowland forest in Panama (Wright *et al.* 2011). Here we examine changes in soil P fractions prior to the onset of nutrient addition in 1997 and again after seven years of chronic nutrient addition. We also examined seasonal patterns using soils collected in the late part of a 4-month dry season versus in the middle of the wet season. The temporal dynamics provide insight into the residence time of P in different fractions. For example, we expected that values for P in labile fractions would show considerable temporal variation reflecting plant uptake and microbial immobilization versus return to soil via litterfall. In contrast, we expected that P in bound fractions would show less temporal change. Combining temporal dynamics with the nutrient fertilization experiment allowed us to determine how quickly P cycles into organic, bound, and occluded fractions.

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 chronic fertilization experiment began in

1998 and has been described in detail elsewhere (Wright *et al.* 2011; Yavitt *et al.* 2011). 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

to 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. Average values across all plots (± one standard error, n=32) were: clay (<0.002

mm) = $73.7 \pm 2.2\%$, silt (0.002–0.05 mm) = $12.4 \pm 2.1\%$, sand (0.05–2.0 mm) = $13.9 \pm 1.3\%$

(determined by the pipette method following pre-treatment by H_2O_2 to destroy organic matter

and sodium dithionite to dissolve iron oxides (Gee and Or 2002).

We replicated the eight treatments of a $2 \times 2 \times 2$ factorial NPK experiment four times.

We placed the four replicates perpendicular to the 36-m topographic gradient because soil

properties (Yavitt *et al.* 2009) and tree distributions parallel the gradient. Within each replicate,

we blocked the N, P, K and NPK treatments vs. the NP, NK, PK, and control treatments. This

balanced incomplete-block design minimizes uncontrolled error associated with spatial

heterogeneity, enables evaluation of main effects and two-way interactions, but limits the power

to evaluate the three-way interaction (Winer 1971). The 32 treatment plots were 40 X 40 m with

a minimum distance of 40 m between plots, except for two plots were separated by 20 m and a 3

m deep stream channel.

Fertilizer treatments began in June 1998. Fertilizers 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 [$(NH_2)_2CO$] at an annual dose of 125 kg N ha^{-1} yr^{-1}; P is added as triple superphosphate [$Ca(H_2PO_4)_2.H_2O$] at an annual dosage of 50 kg P ha^{-1} yr^{-1}; and K is added as KCl at an annual dosage 50 kg K ha^{-1} yr^{-1}. Similar doses are used in forestry (Smethurst 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). Four additional plots received a micronutrient fertilizer (Scott's Soluble Trace Element Mix) consisting of HBO_2, $CuSO_4$, $FeSO_4$, $MnSO_4$, $ZnSO_4$ and $(NH_4)_6Mo_7O_{24}$ at 25 kg ha^{-1} yr^{-1} plus dolomitic limestone $CaMg(CO_3)_2$ at 230 kg ha^{-1} yr^{-1}.

Soil analysis

Soil samples were collected during the wet season in September 1997, prior to the first fertilizer application. We note that 1997 was the driest year in Panama, and we postponed the nutrient fertilization one year until 1998. A surface sample was collected with a hand trowel from 0–0.15 m depth, excluding litter and debris at the upper surface. Three scoops of soil from within 0.5 m of the sample point were bulked and a 300-g sample of this bulked soil was collected. A deeper soil sample (0.3–0.45 m depth was collected with a Dutch auger. Soils were resampled within one meter of the original locations in the wet season in July 2004 and in the dry season in April 2005 using a soil auger.

Soil samples were divided into portions for various analyses. Field-moist portions were used for soil moisture determination by quantifying the change in mass before and again after oven drying at 105°C for 48 hours.

We characterized P in soil using the sequential P fractionation described by Tiessen and Moir (1993) with minor modifications. The method extracts P with differing degrees of

availability held in a variety of inorganic and organic forms. We did not use anion exchange resin for the most labile P_i bound to soil surfaces; rather, we assume that the most labile P was included in the $NaHCO_3$ extract. We did the NaOH, HCl_{weak}, and HCl_{strong} extracts as prescribed. Inorganic P (P_i) was determined colorimetrically in $NaHCO_3$, NaOH, HCl_{weak}, and HCl_{strong} extracts (Murphy and Riley 1962). Total P in each extract, except the HCl_{weak}, was determined following digestion with ammonium persulfate and H_2SO_4, and organic bound P (P_o) was the difference between total P and Pi.

Statistical analysis

We used repeated measures analysis of variance (ANOVA) to evaluate differences among treatments (grouping factors), sampling dates (repeated measures factors) and their interactions for each P fraction. Between-subjects (henceforth, between-plot) effects evaluate responses over the entire treatment. Within-subject (henceforth, within-plot) effects evaluate variation among years and interactions among treatments and year. 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.

Results

The sum of P_i and P_o extracted from soil collected in the wet season in 2004 was considerably greater than total P determined by ignition at 550^oC for 1 hr followed by extraction in 1 M H_2SO_4, albeit, for soil collected in 2006 (Figure 1; Turner et al. unpublished data). We realize this compares extractable P to total P in soils collected in different years; however, it shows that summation of extracted P overestimates total P in our study site. Consequently we have excluded the HCl_{strong} fractions from further analyses.

No differences in the sum of extractable P were detected in surface soil and in subsurface soil across the 7-year time period for plots without added P (Table 1). In contrast, sum of extractable P was significantly greater in surface soil from plots with added P in 2004 versus the same plots in 1997. The significant time difference did not extend to the subsoil (Table 1), although in the 2004 collection the sum of extractable P was greater in plots with added P versus those that did not have P in the fertilizer ($p = 0.0393$). This effect, in part, reflects spatial heterogeneity of extractable P across the 38.4-ha study area.

Surface soils (Figure 2, Table 2)

We found several significant differences in values for soils collected in the wet season in 2004 versus the dry season in 2005. For plots that did not receive added P, values were greater in the dry season than in the wet season for $NaHCO_3$ P_i, NaOH P_o, and HCl_{weak} P_i, whereas values were greater in the wet season than in the dry season for $NaHCO_3$ P_o and NaOH P_i. Values did not vary seasonally for residual P.

Analysis of variance indicated that added N and K did not have a significant impact on values for P fractions. In contrast, added P had several significant impacts. Hence we compare

results separately for plots with added P versus without P addition. Phosphorus additions had much larger impacts on $NaHCO_3$ P_i, $NaHCO_3$ P_o and HCl_{weak} P_i in the dry season than in the wet season. In contrast, P additions increased values for NaOH P_i and NaOH P_o two- to three-fold in both the wet season and in the dry season.

Subsurface soil (Figure 3)

For plots that did not receive added P, values for the P_i fractions, $NaHCO_3$, NaOH, and HCl_{weak}, were not significantly different in soils collected in 1997 versus those collected in the wet season in 2004. In contrast, the P_o fractions showed large differences among years with $NaHCO_3$ P_o being larger in 2004 than in 1997, whereas the reverse was observed for the NaOH P_o fraction. We did not examine seasonal patterns for extractable P in subsoil, assuming that we would not be able to detect meaningful differences over such a short time period.

For the nutrient addition experiment, plots with added P had greater values compared to plots without added P for $NaHCO_3$ P_i ($F_{1,18} = 6.41$, $p = 0.021$), $NaHCO_3$ P_o ($F_{1,18} = 25.08$, $p < 0.001$), NaOH P_i ($F_{1,18} = 10.33$, $p = 0.005$), and HCl_{weak} ($F_{1,18} = 20.05$, $p < 0.001$).

Discussion

It is important to understand soil P availability in tropical forests, because strongly weathered soils can have less than adequate levels of readily available P to support maximum plant production. Phosphorus partitions between P_i and P_o forms as soils weather, and these forms can show differential sorption onto surfaces of secondary clay minerals (Shang *et al.* 1990; Berg and Joern 2006). The residence times of P in different fractions in strongly weathered soils have received limited study. Here we examined soil P fractions in a humid lowland forest in Panama in response to seasonal drought and with seven years of chronic nutrient fertilization. We can conclude that P_i and P_o in the $NaHCO_3$ and NaOH fractions have short residence times, which help to alleviate low P availability of the site.

The sum of P_i and P_o extracted from surface and subsurface soils exceeded the amount of total P, albeit, for soils collected two years apart. We believe that the HCl_{strong} fraction accounts for the discrepancy. We analyzed this fraction following Tiessen and Moir (1993), which suggested that HCl_{strong} might provide more information on occluded forms of P_i and P_o residing within the structure of iron (Fe) and aluminum (Al) minerals. Certainly, such minerals exist in the study suite, but the large difference between the sum of extractable P and total P (Figure 1) cannot be ignored. It is not clear why the large difference occurred, but it might be a feature of strongly weathered soils (Blake *et al.* 2003). Benzing and Richardson (2005) provide an alternative explanation: P in the HCl_{strong} fraction could have been extracted from secondary minerals by NaOH, but it bound quickly to Ca^{2+} and was recovered subsequently as Ca-P in the HCl_{strong} fraction. This does not mean that all of the P recovered in the HCl_{strong} fraction should be added to the NaOH fraction, as silicon extracted by HCl_{strong} can interfere with the colorimetric analyses for P, resulting in larger than actual values (Aspila *et al.* 1976).

Tiessen and Moir (1993) suggested that some of the P_o in the HCl_{strong} fraction might be available to plants and soil microbes. We found P_o values of 225 ± 15 mg P kg^{-1} dry soil in the 0-15 cm depth interval and 181 ± 13 mg P kg^{-1} dry soil in the 30-45 cm depth interval. Although these values seem fairly large, there are few data from other strongly weathered soils for comparison. Nevertheless, we acknowledge that P_o might be lurking in the HCl_{strong} fraction in strongly weathered soils, which deserves further study.

Phosphorus fractions in the surface soils in our site show some distinctive features for an Oxisol (Yang and Post 2011). For example, the amount of labile P, defined as $NaHCO_3$ P_i plus $NaHCO_3$ P_o, equals 32 mg P kg^{-1} dry soil, which is a bit larger than a mean value of 20 (±19 SD) mg P kg^{-1} dry soil found in other Oxisols, We also note that labile P was 97% $NaHCO_3$ P_o compared to a typical value of 50% for labile P_o in other Oxisols. Residual P is typically the largest fraction in Oxisols, i.e., about 60% of the total, whereas residual P was 38% of the total in our study site. In contrast, organic P, defined as $NaHCO_3$ P_o plus NaOH P_o, was a larger percentage: 46% in our site versus 18% in other Oxisols. This percentage also is greater than an average value for soils in Panama (Turner and Engelbrechect 2011). A portion of our study site has somewhat poorly drained soils near drainage creeks, and it is plausible that the soil organic matter accumulation in these soils led to the somewhat larger percentage of P_o (Turner and Engelbrechect 2011). In contrast, secondary P_i, defined as NaOH Pi, was a smaller percentage: 4% in our site versus 15% in other Oxisols. Hence, the amount of extractable P in our site is similar to values in other strongly weathered Oxisols, but more of the extractable P is in organic-bound fractions than usual. Typically, predominance of P_o is associated with grassland soils (Negassa and Leinweber 2009).

A meta-analysis by Johnson *et al*. (2003) showed that availability of P to plants and microorganisms has several definitions in the literature. The most readily available is P_i that sorbs to anion-exchange membrane. However, several researchers have argued that plant-available P should include labile P_i that is weakly bound to charged surfaces and extractable by weak acid and / or salt solutions. One measure is Bray-extractable P (Bray and Kurtz 1945). Values for Bray-P are < 2 mg P kg^{-1} dry soil in our study area (Yavitt *et al*. 2009), which are low enough to limit plant growth in bioassays (Denslow *et al*. 1987). Although larger values for $NaHCO_3$-extractable P (see above) suggest better soil fertility, most of this fraction is P_o, and $NaHCO_3$ P_o might occur mostly within microbial cells with release tied to soil drying (Turner *et al*. 2003).

Soil P fractions had relatively homogeneous distribution across the 38.4-ha study area. Values for the Coefficient of Variation (CVs) for P in different fractions were 35 to 50% (N = 36 for soil collected in1997 prior to the nutrient fertilization treatments). This level of variation is quite similar to the variation that Heilmann *et al*. (2005) reported for a fairly similar sampling effort (N = 40) in a 6.25-ha study area in Germany, with CVs of 33%. It is notable that CV values for P fractions are towards the low end of values for other soil chemical properties in our site, where CVs are as large as 200% (Yavitt *et al*. 2009). One explanation is that P is used essentially the same way in all plants and microorganisms (Makino *et al*. 2003), and thus biotic activity tends to homogenize levels of P across a landscape.

For plots without added P, values for $NaHCO_3$ P_i and $NaHCO_3$ P_o were significantly different in soils collected in 1997 versus the wet season in 2004. In contrast, values for NaOH P_i, NaOH P_o, HCl_{weak} P_i, and residual P did not differ significantly in soils collected seven years apart. Different values for $NaHCO_3$ extractable P_i and P_o in surface soil in1997 versus 2004 are

neither surprising nor predictable. Undoubtedly, values for labile P swing widely in response to input, plant uptake and microbial immobilization of P_i, and mineralization of P_o to P_i.

The decrease in the magnitude of the $NaHCO_3$ P_i fraction of 3 mg P kg^{-1} dry soil between the dry season and wet season is consistent with plant uptake and microbial immobilization of labile P during the wet, growing season. For $NaHCO_3$ P_o, however, greater values in the wet season than in the dry season presents a conundrum. Most studies in the literature report that values for the $NaHCO_3$ P_o fraction peak in the non-growing season and decline during the growing season, driven by P_o mineralization 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 plant-growth season does correlate with the maximum rate of litter decomposition (Wieder and Wright 1995), suggesting litter decay is a proximate source for $NaHCO_3$ P_o in the mineral soil (Blair and Boland 1978; Vincent *et al.* 2010). This also suggests a very short residence time for $NaHCO_3$ P_o in the mineral soil, given that the amount appears to respond quickly to elevated input of P_o from litter decomposition.

The large seasonal change in the NaOH P_i fraction, 32 mg P kg^{-1} dry soil greater in the wet season than in the dry season, was unexpected. This fraction is associated with Fe and Al oxide minerals, and though potentially plant-available (Richter *et al.* 2006), it is not thought to be as labile as $NaHCO_3$ P_i. Assuming the residence time is longer for NaOH P_i than for $NaHCO_3$ P_i, the buildup of NaOH P_i during the wet plant-growth season might reflect a lag in its response to seasonal inputs and outputs. Alternatively, the binding ability of Fe and Al oxides for NaOH P_i might be greater in the wet season than in the dry season. For instance, Chacon *et al.* (2008) found that the concentration of the NaOH P_i fraction increased by about 13 mg P kg^{-1} dry soil upon soil flooding in tropical Venezuela, suggesting that P_i has better sorption to Fe and Al

oxides when soil is flooded. However, in that study, flooding caused the iron reduction from Fe^{3+} to Fe^{2+}, which provided better sorption. There is no large-scale evidence of seasonal iron reduction (gleying) in our study site, although it might occur in small pockets in the soil. The same seasonal dynamic of NaOH P_i occurs in tropical dry forests, which are characterized by a prolonged drought and a short wet season (Valdespino et al. 2009), implying that large-scale gleying is not necessary.

There is a general notion in the literature that seasonal dynamics for the NaOH P_o fraction parallel the $NaHCO_3$ P_o fraction (cf., Herr *et al.* 2007). However, this is not necessary so (cf., Fabre *et al.* 1996). Assuming that litter decomposition in the wet season is the proximate source of P_o for both the $NaHCO_3$ P_o and NaOH P_o fractions, Fe and Al oxides might be better suited for P_o sorption in the dry season than in the wet season, accounting for the large dry season values for NaOH P_o. A similar situation occurs in rice paddy soils, in which NaOH P_o levels are much greater in dry than in flooded soils (Darilek *et al.,* 2011). The explanation is that the redox status of Fe plays a large role in NaOH P_o dynamics in paddy soils, in particular, the reduction of Fe^{3+} to Fe^{2+} reduces the adsorption of NaOH P_o (Zhang *et al.* 1994). Clearly studies of potential P sorption with soils collected seasonally and at different soil moisture levels are needed to explain the complex patterns between P_i and P_o extracted by $NaHCO_3$ and NaOH observed here.

No seasonal differences in the HCl_{weak} P_i and Residual P fractions agrees with the long residence time of mineral bound and occluded fractions (Richter *et al.* 2006), and thus seasonal variations in values are not likely to occur. 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.

Another unexpected finding was that the sum of extractable P was 30% greater in the 30-45 cm depth interval compared to that in the 0-15 cm depth interval (paired t-test $p < 0.001$, n = 36). Typically, extractable P is concentrated at the soil surface. In our case, the pattern seemed to be driven by elevated values for P_o in the subsurface soil. Wagar *et al.* (1986) suggested that P_o is the predominant form of extractable P below 15 cm in soils, because P_o is more mobile than P_i, and thus P_o moves downward more readily than P_i. Somewhat surprising was the finding that $NaHCO_3$ P_o and NaOH P_o in subsurface soil showed different values in 1997 versus 2004. The very large decrease in NaOH P_o without a concomitant increase in the size of the NaOH P_i fraction suggests that either there has been a sharp decline in the deposition of P_o in the subsurface over the time period, or mineralization of NaOH P_o fueled plant uptake and microbial immobilization. We do know that plant roots and associated mycorrhizal fungi can access P_i in subsurface soil, if necessary, bringing it to the surface to support growth (Lynch and Ho 2005; Porder *et al.* 2006), where it then cycles between surface soil and litterfall.

In order to interpret results of the nutrient fertilization treatment, our experimental design bears mention. We have maintained a factorial NPK addition experiment since 1998. Chronic addition of nutrients, broadcast across large plots (40 x 40 m in our study) are uncommon in natural forests (cf., Wright *et al.* 2011). The aim is to increase soil fertility at the ecosystem level rather than apply nutrients to individual trees, as is the goal in most forestry operations (Miller 1981). Nitrogen is added as urea, and urea mineralization has acidified the soil by one pH unit (Yavitt *et al.* 2011). This should increase P binding to Fe and Al oxides. However, we did not detect a treatment effect of added N on any of the soil P fractions. In contrast, trunk growth rates have responded to chronic N and K additions (Wright *et al.* 2011). Part of the explanation is that added K has reduced fine root biomass, suggesting that plants maintain fewer roots when K is

abundant in soil, and thus bole growth has responded. Added P has increased the concentration of P in leaves and in reproductive parts (flowers and fruits) that are returned to soil in litter fall and fuel P_o input into the mineral soil.

Although we apply nutrient fertilizers during the wet season, the largest responses in P fractions appeared in the dry season. We noticed two patterns in the responses. For $NaHCO_3$ P_i and $NaOH$ P_o, which have greater values in the dry season than in the wet season, P additions exacerbated the seasonal difference. This reinforces the notion that sorption of $NaHCO_3$ P_i and $NaOH$ P_o to soil colloids in the dry season largely controls the size and seasonal dynamics of these extractable fractions. In contrast, for $NaHCO_3$ P_o and $NaOH$ P_i, both of which have greater values in the wet season than in the dry season, added P lessened the seasonal differences. There might be multiple explanations for this pattern. For example, we argued above that P_o loss from decomposing litter controlled the seasonal dynamic in the $NaHCO_3$ P_o fraction. Therefore elevated values in the dry season in soil with added P might reflect early onset of decomposition in litter with added P (Kaspari *et al.* 2008) as well as more P coming from the litter in plots with added P. We also note that the dry season in 2005, when we made measurements, was about 27% wetter than normal (versus average dry season rainfall between 1994 to 2011). Therefore, rains in March and in April could have mobilized P_o from litter in plots with added P. For $NaOH$ P_i, we can only speculate that larger values occur when plant uptake and microbial immobilization are relaxed during the dry season. Hence, plant uptake and microbial immobilization might control dynamics of NaOH Pi to a greater extent than expected.

Research in agricultural soils has shown overwhelmingly that P fertilizers remain in surface soils. The main reason is 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). Therefore, our finding that added P increased values for fractions, except NaOH P_o, in subsurface soil suggests downward movement of added P. This might be an initial response to P saturation, much like that observed for chronic N additions (Corre *et al.* 2010).

Overall, we added 350 kg P ha^{-1} during the seven year study period. By comparison, we estimate that we recovered 57% from soil in extractable fractions: 118 kg P ha^{-1} in the 0-15 cm depth interval, 67 kg P ha^{-1} in the 15-30 cm depth interval, and 17 kg P ha^{-1} in the 30-45 cm depth interval. The amount in the 15-30 cm depth interval 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 nutrient cycle in leaf and reproductive litter (Wright *et al.* 2011), leaving a relatively large percentge unaccounted for. We suspect, at least, some of the missing P is residing in the HCl_{strong} fraction, presumably as P_o. Finding 40% of fertilizer P is a mystery that awaits further study.

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Table 1. Sum of extractable P (mg P kg-1 dry soil) for collects seven years apart, and test of statistical difference

Depth = cm; = pre-treatment value

Treatment	Depth	1997	2004	*P*
-P	0-15	259 + 17	299 + 28	0.2566
+P	0-15	243 + 18	430 + 64	0.0096
-P	30-45	340 + 12	310 + 20	0.4937
+P	30-45	356 + 17	369 + 17	0.4528

Table 2. Analysis of variance for soil P fractions (0-15 cm depth interval) in response to P addition and wet season versus dry season.

	Added P		Season	
Fraction	F	*P*	F	*P*
NaHCO$_3$ P$_i$	8.12	0.011	65.01	<0.001
NaHCO$_3$ P$_o$	14.71	0.001	6.22	0.023
NaOH P$_i$	61.85	<0.001	52.58	<0.001
NaOH P$_o$	6.47	0.020	34.71	<0.001
HClweak P$_i$	25.20	<0.001	33.39	<0.001

Figure Legends

Fig. 1. Sum of P in extractable fractions versus total P measured independently for surface soils in (a) plots without add P and (b) plots with added P.

Fig. 2. Soil P fractions for surface soils.
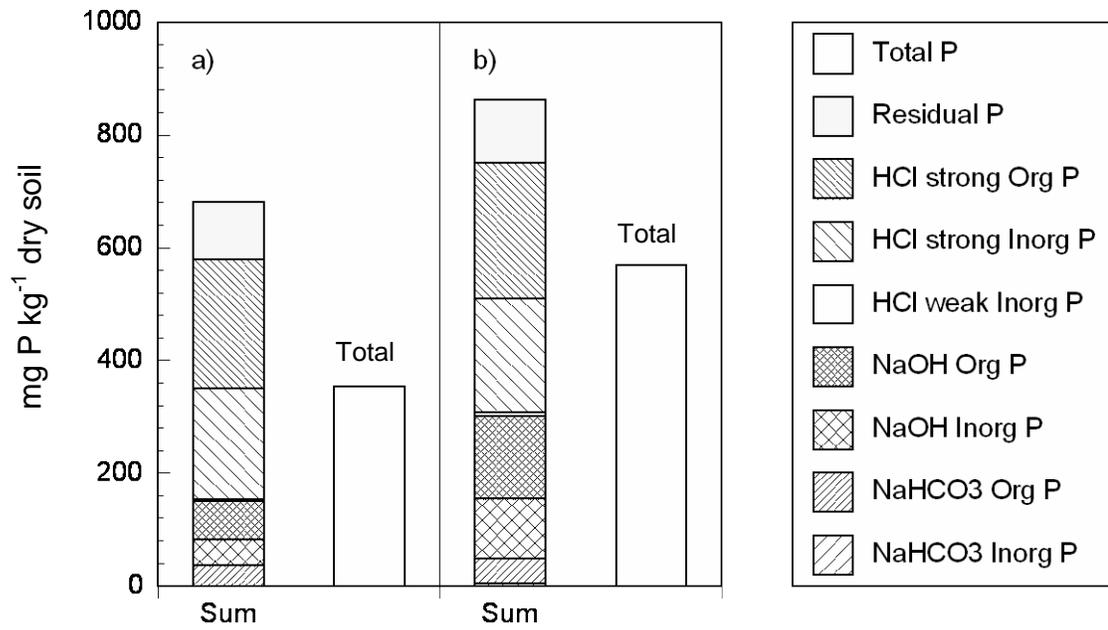
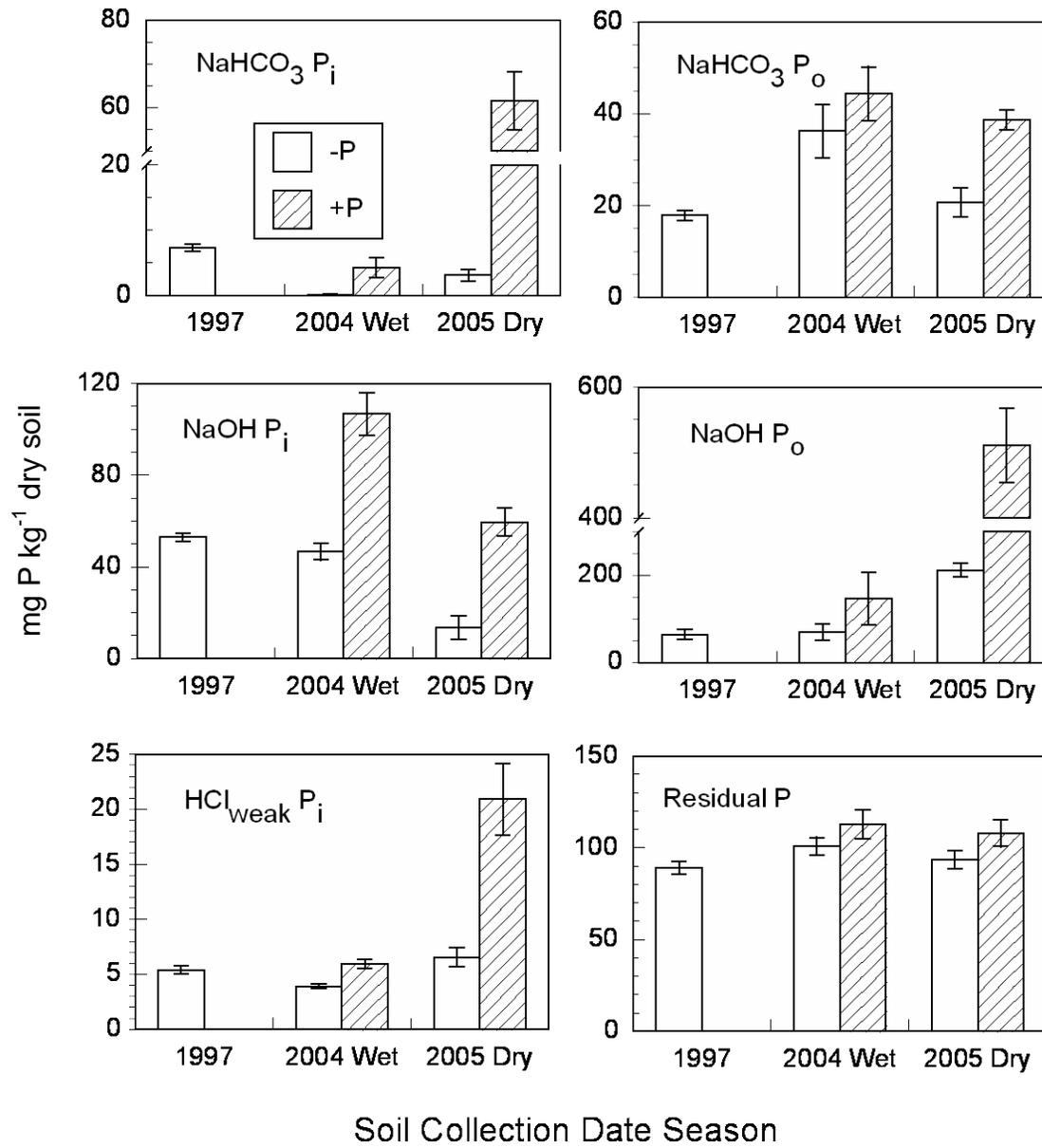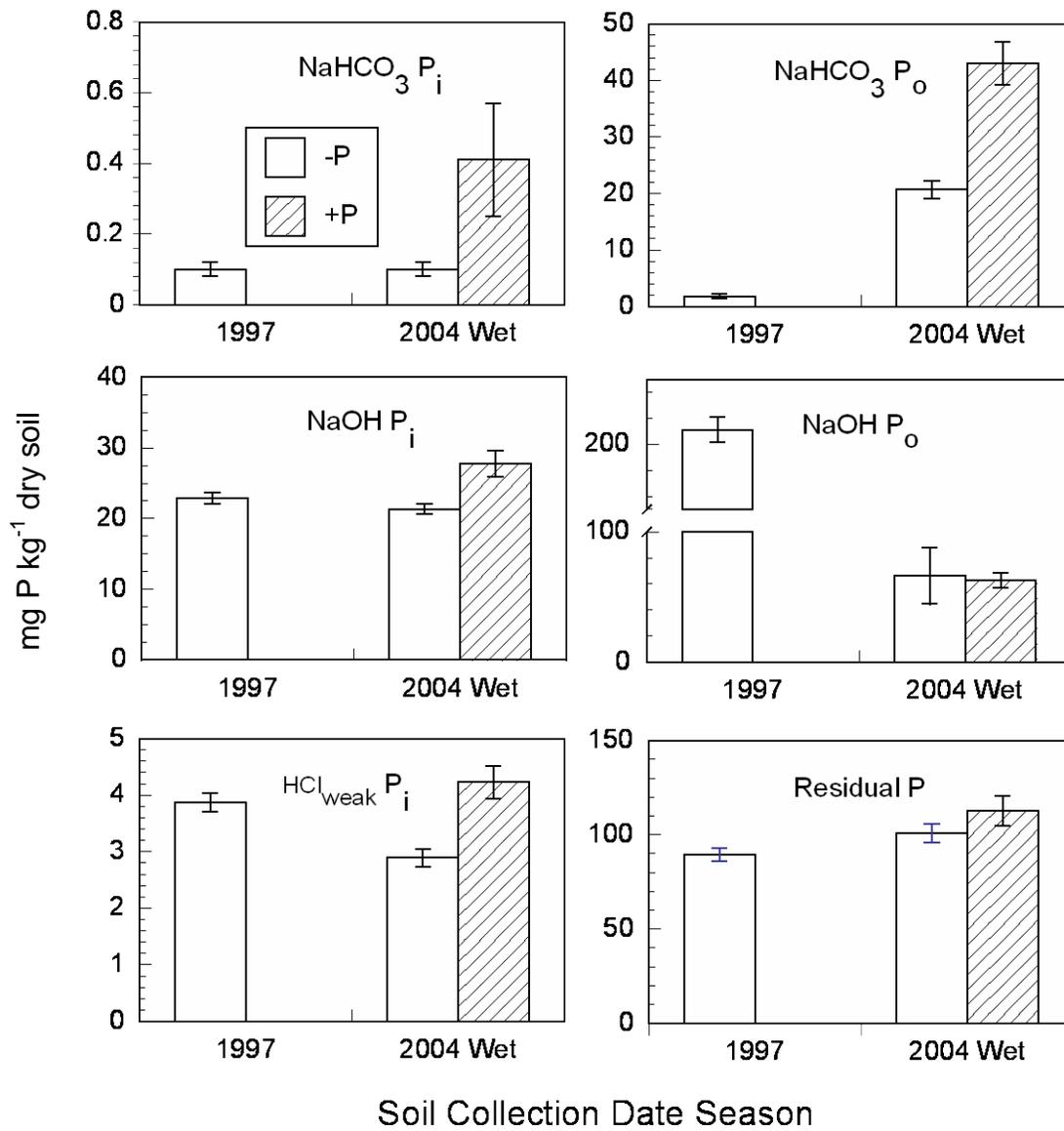**Fig. 3.** Soil P fractions for subsurface soils.

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Fig. 1

Fig. 2.

Fig. 3.