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Phosphorus Limitation of Microbial Processes in Moist Tropical Forests: Evidence from Short-term Laboratory Incubations and Field Studies

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Abstract

Although there is a widespread belief that phosphorus (P) limits basic ecosystem processes in moist tropical forests, direct tests of this supposition are rare. At the same time, it is generally believed that P does not limit soil microorganism respiration or growth in terrestrial ecosystems. We used natural gradients in P fertility created by soils of varying age underlying tropical rain forests in southwestern Costa Rica, combined with direct manipulations of carbon (C) and P supply, to test the effects of P availability on the decomposition of multiple forms of C, including dissolved organic carbon (DOC) and soil organic carbon (SOC). Results from a combination of laboratory and field experiments suggest that C decomposition in old, highly weathered oxisol soils is strongly constrained by P availability. In addition, P additions to these soils (no C added) also revealed that microbial utilization of at least labile fractions of SOC was also P limited. To our knowledge, this is the first direct evidence of P limitation of microbial processes in tropical rain forest soil. We suggest that P limitation of microbial decomposition may have profound implications for C cycling in moist tropical forests, including their potential response to increasing atmospheric carbon dioxide. Furthermore, this site is still relatively rich in P when compared to many other tropical forests on old soils; thus, we believe that P limitation of soil microorganisms throughout the humid tropics is a possibility.

Key words: decomposition; soil microorganisms; nutrient limitation; fertilization; tropical rain forest; phosphorus; carbon.

Introduction

In many terrestrial ecosystems, primary production and other basic ecosystem processes are constrained by low rates of nutrient supply. Although some exceptions do exist, substantial amounts of data from temperate and high latitude ecosystems demonstrate that in the absence of anthropogenic perturbation, nitrogen (N) frequently limits plant growth and organic matter storage (Aber and others 1991; Vitousek and Howarth 1991). This knowledge arises from a wide variety of data, including multiple direct manipulations of external nutrient supply.

In contrast, we lack a thorough understanding of the effects of low nutrient availability on basic ecosystem processes in moist tropical forest ecosys-

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tems, which present a unique set of problems related to nutrient cycling and limitation. For example, in contrast to most temperate systems, nitrogen appears to cycle in relative excess in many lowland tropical rain forests (Martinelli and others 1999). Moreover, many of these forests lie on extremely old, highly weathered soils that are depleted in "rock-derived" essential elements, including base cations (calcium [Ca], magnesium [Mg], potassium [K]) and phosphorus (P) (Uehara and Gillman 1981; Vitousek and Sanford 1986). Thus, where nutrients are a major constraint in tropical forests on old soils, this constraint likely comes from some combination of base cation and/or phosphorus availability (Vitousek and Sanford 1986; Cuevas and Medina 1988; Bruijnzeel 1991). Base cations are more mobile in soil solution than is P, and therefore one might expect their greater losses as soils weather to create the greatest nutrient shortage in old soils. However, the iron- and aluminum-rich clays typical of old tropical soils readily fix P into unavailable forms, and atmospheric inputs of base cations are much higher than for P, especially where sea salt aerosols are a major component of deposition inputs (Chadwick and others 1999). Hence, phosphorus is generally believed to be the most limiting element in the majority of tropical forests on older soils (Vitousek 1984; Jordan 1985; Herbert and Fownes 1995), and has been called the "master regulator" of biological activity (Walker and Syers 1976; Chadwick and others 1999).

However, direct, manipulative studies of nutrient limitation in lowland tropical rainforests are rare. Long-term fertilizations in Hawaiian rain forests have shown that production and decomposition on old soils are clearly limited by P availability (Vitousek and Farrington 1997; Hobbie and Vitousek 2000; Crews and others 1995). However, while the data from the Hawaiian studies are highly informative, these are isolated island ecosystems that contain a highly depauperate biota; therefore, the patterns seen here may or may not hold true in highly diverse—and often times far older—continental tropical forests. For the most part, the widespread belief that P is the most common limiting element in mainland tropical forests is largely based on indirect attempts to assess nutrient limitation, such as foliar element ratios (compare Vitousek 1984; Vitousek and others 1988). Again, such data strongly suggest that P is an important constraint, but direct tests of this hypothesis are scarce. Finally, most existing studies have focused primarily on plant responses to nutrient inputs. With few exceptions (for example, Nziguheba and others 1988), nearly nothing is known about links between P availability and microbial dynamics.

Microbial processes are clearly a key regulator of nutrient cycles in any system; most of the annual nutrient requirements of terrestrial plants are supplied via the decomposition of organic matter by soil fungi and bacteria (Swift and others 1979). However, while nutrient supply often limits plant growth, microbial communities in most ecosystems are typically thought to be more constrained by carbon availability than by nutrients (Gallardo and Schlesinger 1990; Paul and Clark 1997; Wardle 1992). In the case of nitrogen, additions of N to N-poor systems do not result in consistent effects upon decomposer communities; in some cases, N does appear to stimulate microbial growth, but in many others its addition has no effect, or sometimes even a negative effect (for example, Fog 1988; Söderström and others 1983). Nonetheless, although microbial biomass itself is typically less than that of higher plants, its nonnitrogen mineral nutrient content (in percent) may be as much as 10 times higher, and it may contain as much P per hectare as vegetation (Hayman 1975). Also, because there are multiple generations of growth by diverse groups of soil microorganisms each year, their annual demand and uptake of nutrients often exceeds that of higher plants (Paul and Clark 1997; Cole and others 1977). Thus, although microbial respiration and/or growth are usually C limited, the potential for strong constraints by P on the mineralization of C substrates clearly exists.

The objective of this study was to determine whether P limits microbial respiration (and decomposition) in a lowland continental rain forest on very old (44+ million years) soils. We used both natural gradients in P fertility created by soils of widely varying ages, combined with direct manipulations of P and C supply to the soils, to test the effects of P availability on the decomposition of multiple forms of carbon, including native dissolved organic C and soil organic C. Our overall hypothesis was that microbial utilization of carbon in P-poor soils would be constrained by P availability, potentially creating feedbacks to even greater P limitation to the plant communities, as well as very different storage times and fates for soil carbon than might be seen in more fertile sites.

Methods

Study Sites and Sampling Regime

We addressed the possibility of microbial P limitation in two adjacent primary tropical rainforests on

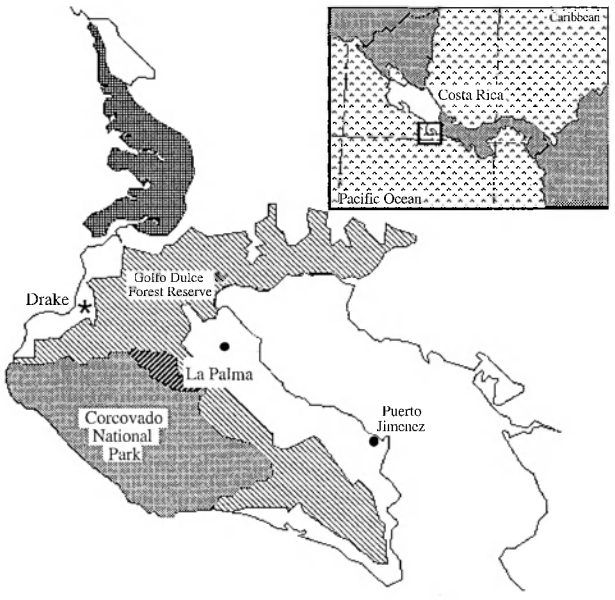

Figure 1. Map of the Osa Peninsula in southwestern Costa Rica, indicating the study area (Drake River Valley).

the Osa Peninsula in southwestern Costa Rica (Figure 1). This region lies within the tropical wet lowland forest bioclimate (Holdridge and others 1971); rainfall is heavy (more than 5000 mm/y) and peaks during summer to early fall. Like most tropical rainforests, those on the Osa Peninsula still experience a dry season; in this region, it occurs from January to March, with a second, less consistent dry period in late June and early July. These dry seasons are quite short in comparison to many other tropical forests, and overall rainfall is very high; thus, nearly all forms of vegetation persist in an evergreen state throughout the year (Tosi 1975).

The entire Osa Peninsula was formed in three large seafloor volcanic events between roughly 75 and 40 million years ago, but some parts of the region were below sea level in more recent geologic eras (Berrange and Thorpe 1988). This created a wide range in parent material ages and subsequent soil types, from highly weathered 40+ million-year-old oxisols to highly fertile alluvial mollisols of Quaternary origin. Forests in this region thus occur on three general soil types: (a) old, highly weathered oxisols on steeply dissected terrain that rarely exceeds a few hundred meters elevation, (b) much younger (2–4 million years old) ultisols on roughly similar upland terrain, and (c) the highly fertile mollisols found on the alluvial plains (Townsend and others, forthcoming; Berrange and Thorpe 1988). Our sites are located in the northwest corner of the Drake River Valley (8°43′N, 83°37′W), near the transition between the old upland oxisols and the young lowland mollisols. We selected sites representing each soil type, hereafter referred to as Oxisol Forest (OF) and Mollisol Forest (MF) sites. The sites are only a few hundred meters apart and are thus identical climatically (MAT = 26°C; MAP = around 5100 mm y^{-1}) and have similar species composition.

We sampled soils from both sites in June 2000. Within each site, 8 × 10 cm soil samples were extracted every 5 m from two randomly placed 25-m transects bisecting each site, for a total of 10 samples per site. Soils were sampled to 10 cm, reflecting the region of greatest microbial density and activity commonly observed in the soil profile (Cleveland and Yavitt 1998). Within 72 h of collection, soils were returned to the laboratory and coarsely sieved (4 mm) to remove plant material. A 50-g subsample was removed from each soil sampled and air-dried for physical and chemical analyses. Fresh soil samples were stored at 10°C until analysis. All incubation experiments were initiated within 96 h of soil sampling to avoid artifacts incurred during long-term storage.

Soil Physical and Chemical Analyses

We measured pH on air-dried soils using a soil: deionized water paste (1:1). Soil for organic C and N was ground to 40 mesh and analyzed using a Carlo Erba combustion-reduction elemental analyzer (CE Elantech, Lakewood, NJ, USA). Extractable N (NH_4^+/NO_3^-) was determined using a 2M KCl solution, extracted for 24 h and analyzed for NH_4^+ and NO_3^- colorimetrically on an Alpkem autoanalyzer. Soil bulk density at each site was determined using an excavation method (OI Analytical, College Station, TX, USA) (Parent and Caron 1993).

P Fraction Analyses

One gram of soil from each sample was analyzed for P fractions using the first two steps of the modified Hedley fractionation described by Tiessen and Moir (1993). Briefly, soil was subjected to a resin extraction (in water), followed by a bicarbonate extraction. A digest was also performed after the bicarbonate extraction, and organic P was determined by difference. These two fractions (resin and bicarbonate) are the most labile forms of P, and their sum is often taken as a proxy for readily available (labile) P; previous studies have shown that bicarbonate extractable P is well correlated with plant growth (Bowman and others 1978; Levy and Schlesinger 1999). Total P in soil samples was determined by digesting 5 g of sieved, air-dried soil in H_2SO_4 and

H_2O_2. Phosphate concentrations in all measured fractions were determined using the ammonium molybdate ascorbic acid method (Kuo 1996).

Plant elemental analyses (K, Ca, Mg) were performed on bulk (green) litter collected along established soil transects at each site. Plant material was dried and ground to 40 mesh, and 1 g of material was ashed at 550°C for 6 h. Following ashing, 5 ml HCl (50%) was added, and samples were diluted to 250 ml with deionized water. Nutrient content of plant tissue samples was determined using inductive coupled plasma emission spectroscopy (ICP) on a TJA-975 ICP (Thermo Elemental, Franklin, MA, USA).

P Limitation of Substrate-induced Growth

We used radiolabeled carbon substrate additions to assess the constraints of phosphorus availability on the mineralization of added C substrate to the microbial biomass. This method was has been used successfully to estimate microbial biomass and activity. It has been conclusively shown to estimate microbial growth rates and avoids complications due to enzyme induction and other lags. A detailed description of the method can be found in Colores and others (1996); but, briefly, 10–15 g dry mass equivalent of each soil was placed in a biometer flask with 1 ml NaOH in the sidearm to trap carbon dioxide (CO_2) (Colores and others 1996). Amounts of carbon substrate (that is 2 mg glutamate-C g^{-1} or 0.2 mg salicylate C g^{-1}) previously determined to induce maximal respiration (Colores and others 1996) were added to each flask with the same uniformly labeled ^{14}C substrate to yield 2500 Bq (150,000 dpm) per flask and enough water to bring the soils to 50% of field capacity. Incubations were conducted with both glutamate (Glu) (5:1 C:N ratio; a labile form of C) and salicylate (Sal) (a more recalcitrant carbon compound mineralized by the same general pathways as breakdown products from lignin and detrital polyphenols) to reconcile the possible confounding effects of N in glutamate; salicylate does not contain N. For the fertilizer treatments, P was added as KH_2PO_4 to water amendments to yield P additions of 200 μg g^{-1}. The base trap was removed from each flask at regular intervals (3 h) and radioactivity was measured by liquid scintillation counting after mixing with Scintiverse II scintillation cocktail (Fisher Scientific, Pittsburgh, PA, USA) to determine respiration rate. All flasks were incubated at 22°C until soil respiration returned to its basal rate.

P Limitation of In Situ Growth

To assess the constraints of P limitation on the mineralization of added labile C in situ, we used similar C and P additions to our laboratory incubation study, but in a field setting. At each site, 24–15 cm × 5 cm PVC collars were inserted into the ground to a depth of 10 cm and allowed to equilibrate for 24 h. Following equilibration, equal numbers of collars received one of four treatments, each dissolved in 10 ml water: (a) 50 mg C as L-glutamic acid; (b) 30 mg P as KH_2PO_4; (c) both C and P, in amounts described above; or (d) 10 ml water (as control). Collars were capped with sampling chambers at regular intervals over 12 h, and respiration (as CO_2) was measured using a PP Systems EGM-3 environmental gas monitor (PP Systems, Haverhill, MA, USA).

P Constraints on Decomposition of Native Dissolved Organic Carbon

To determine the effect of P limitation on the decomposition of native dissolved organic carbon (DOC) of leaflitter origin, we conducted another incubation experiment using carbon extracted from recently fallen litter. Recently fallen (senesced) leaflitter was collected from a 50 × 50 m parcel from the oxisol site, air-dried in paper bags, and returned to the laboratory. The litter was then dried at 40°C for 72 h, and known quantities were extracted in deionized water for 24 h. Following extraction, the leachate was filtered to 0.45 μm, and DOC concentration of the leachate was measured using a Shimadzu TOC 5050A total organic carbon analyzer (Shimadzu Corporation, Kyoto, Japan). Fifteen-gram (dry weight [dw]) soil samples were place in 1-l glass jars and received one of three treatments: 5 ml 305 μg DOC-C g^{-1}); 5 ml DOC + P (305 μg DOC-C g^{-1}, 250 μg P g^{-1} as KH_2PO_4); or 5 ml water (as control). Following treatment additions, jars were capped with lids equipped with a rubber septum for gas sampling, and jars were sampled for CO_2 at 0.5, 1, 2, 3, 4, 6, 8, 10 14, 24, and 32 h using glass gastight syringes. The CO_2 concentration in each syringe was determined immediately using a LICOR-6252 gas analyzer (LICOR, Inc., Lincoln, NE, USA).

P Constraints on Decomposition of Native SOM

Ten 25-g samples of soil (dw) from each site received one of two treatments: 10 ml P (250 μg P g^{-1} soil as KH_2PO_4), or 10 ml water as control. Soil samples were incubated at 25°C, and evolved CO_2 was measured on days 3, 7, 14, and 30 following

Table 1. Soil Physical, Chemical, and Biogeochemical Characteristics

Site Characteristic	Oxisol Forest	Mollisol Forest	Tropical Average	*n*	*P*
Primary substrate age (y)	44–70 × 10^6	< 1.0 × 10^6			
Elevation (m)	120	100			
Mean annual precipitation (1980–94) (mm)	5179	5179			
Soil bulk density (0–10 cm) (g cm^{-3})	0.52	0.65		3	0.011
Soil pH (0–10 cm)	5.42	6.00		7	0.001
Soil carbon (%)	11.39 ± 0.98	11.723 ± 1.19		5	0.65 (NS)
Soil nitrogen (%)	0.56 ± 0.06	0.59 ± 0.11		5	0.34 (NS)
KCl extractable NO_3−N + NH_4−N (kg ha^{-1})	22.30 ± 7.55	29.93 ± 6.92		5	0.05
Labile soil P (μg g^{-1} soil)	21.3 ± 3.4	41.6 ± 6.5	19.3 ± 21.1[b]	8	<0.001
Total soil P (μg g^{-1} soil)	557.0 ± 45.1	1051.0 ± 96.3	237 ± 148[b]	8	<0.001
Litter P (%)[a]	0.06	0.13	0.027 ± 0.013[c]		
Litter Ca (%)[a]	1.86	2.64			
Litter Mg (%)[a]	0.38	0.29			
Litter K (%)[a]	1.27	1.63			

Labile soil P is defined as the sum of resin and bicarbonate (organic + inorganic) extractable P fractions from a modified Hedley soil P fractionation (Tiessen and Moir 1993).
Tropical average values are for tropical sites on ultisols and oxisols; they are adapted from the literature.
Values vary in their coverage and number of samples represented, and tropical averages are meant solely for comparative purposes to our data.
Errors are ± 1 SE.
[a]*Values are preliminary data representing the mean of three bulk litter analyses from each site.*
[b]*From Cross and Schlesinger 1995*
[c]*From Vitousek and Sanford 1986*

the start of the incubation. For each soil sample, we drilled several holes in the bottom of a 50-ml polystyrene beaker, placed a glass fiber filter in the bottom of each beaker, and added 15 g of soil on top of the filter. Each beaker containing soil was then stacked into a second beaker used to capture leached water. Fertilizer solution or water was added as one 10-ml dose to provide the desired treatment and to bring the soils roughly to field capacity. Beakers were then placed in 1-l glass jars. Evolved CO_2 was measured by trapping it in 1 N NaOH and titrating with HCl and BaCl (Zibilske 1994); eight blanks (no soil) were used to estimate CO_2 not due to respiration. Soil moisture was kept at field capacity, and soils were retreated with water or fertilizer 2 weeks after the experiment began. The lids of the jars were removed for approximately 1 h at each sampling interval to prevent anoxia in the jars.

Data Analyses

Differences in soil biogeochemical characteristics between sites were tested with a one-way analysis of variance (ANOVA) (SPSS, Chicago, IL). In time-course incubations, fluxes were log-transformed before ANOVA and post hoc Tukey analyses. All reported differences were significant at $P \leq 0.05$.

Results

Site Biogeochemistry

Soil characteristics are presented in Table 1. Although many of the physical and chemical properties of the MF and OF sites are similar, the biogeochemistry of the two sites is strikingly different with respect to P availability. Labile soil P, total soil P, and litterfall P at MF are all more than double the values observed at the OF site (Table 1). The difference in P fertility likely reflects the differences in soil type and the age of the parent material from which they are derived. However, although P availability clearly varies between the sites, evidence from plant litter analyses suggests that the sites are relatively rich in other major critical plant elements. For example, not only are soil extractable and litter N values high in each site (litter-N values not shown), but Ca, Mg, and K concentrations in litterfall at both sites are significantly higher than average values for other tropical rain forests ultisols and oxisols (Table 1). Assuming that plant nutrient concentrations reflect (in a relative way) soil nutrient availability (*sensu* Vitousek 1984), our data suggest that both the oxisol and mollisol are relatively cation rich (Vitousek and Sanford 1986). The high base cat-

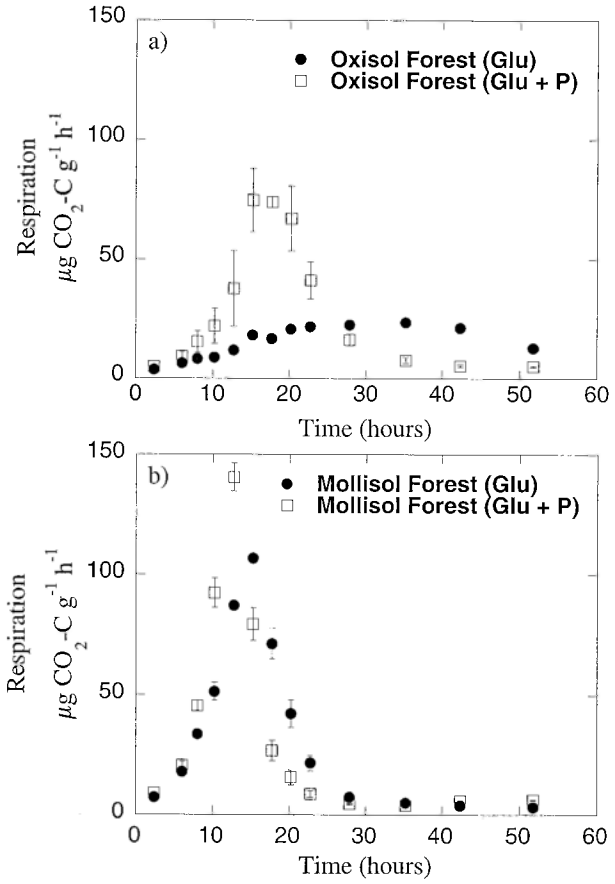

Figure 2. Radiolabeled substrate (glutamate, Glu) addition response of laboratory incubations (with and without P fertilization) in tropical rain forest soil from a the P-poor oxisol forest and b P-rich mollisol forest. Error bars are ± 1 SE.

ion values in litter from the oxisol site probably reflect high rainfall inputs of these elements from the nearby ocean (compare Chadwick and others 1999). The data shown in Table 1 suggest that if ecosystem processes in our sites are indeed nutrient limited, phosphorus is the most likely candidate, at least in the oxisols.

P Limitation of Respiration and Decomposition

In the substrate addition incubations, the two forest sites displayed strikingly different responses to P fertilization (Figure 2a and b). Respiration rates in soil from the P-poor (OF) site increased linearly ($r^2 = 0.96$) following additions of Glu alone (Figure 2a). Typically, dividing populations during microbial growth result in exponential CO_2 response curves (Colores and others 1996); thus, the linear increase in CO_2 following Glu addition suggests that microbial growth in this site is not solely limited by carbon, as is most common in the microbial community (Wardle 1992). In contrast, OF soil amended with both Glu and P responded much more rapidly (Figure 2a), and CO_2 evolution rates increased exponentially ($r^2 = 0.99$). Fluxes of CO_2 in the Glu-only additions reached a maximum of 23.52 ± 0.05 μg CO_2-C g^{-1} h^{-1} (± 1 Se) and occurred 32 h after the substrate addition; whereas OF soil fertilized with Glu + P reached maximum CO_2 production rates of 74.73 ± 13.13 μg CO_2-C g^{-1} h^{-1} and occurred 20 h after the experiment began (Figure 2a). Furthermore, 28 h after the experiment began, cumulative CO_2 respired in the C + P treatment was 9931.77 ± 292.89 μg CO_2-C. This amount represents approximately 50% of the C added, consistent with an exponentially growing population with an expected respiration: growth ratio of approximately 1:1 (Colores and others 1996). In contrast, cumulative CO_2 respired in the Glu-only treatment was less than half (4680.70 ± 39.07 μg CO_2-C) of that observed with both C and P over the same time period.

In the P-rich site (MF), substrate induced growth respiration following additions of Glu alone and of Glu + P was nearly identical in samples from each treatment (Figure 2b). Although the C + P addition caused a significantly higher maximum flux of CO_2 14 h after the incubation began ($F = 110.52$, $P < 0.01$), (Figure 2b), exponential responses were elicited in both the Glu-only treatment ($r^2 = 0.99$) and the Glu + P treatment ($r^2 = 0.97$). Similarly, cumulative CO_2 evolved after 28 h was not significantly different in the C-only treatment (11528.39 ± 198.76 μg CO_2-C) and the Glu + P treatment (11251.87 ± 10.78 μg CO_2-C), suggesting that P constraints on the microbial community in this site are much less pronounced than in the P poor OF site, if they are present at all.

To assess the constraints P has on the decomposition of a carbon substrate that did not contain N, we conducted a separate set of incubations using salicylate as the C source. Our results with salicylate show a similar pattern to the data obtained with glutamate (Figure 3a and b). Specifically, in the oxisol forest, samples treated with Sal alone showed suppressed growth rates and respiration rates relative to samples treated with both Sal and P (Figure 3a). With Sal alone, respiration response was again linear ($r^2 = 0.98$) and achieved maximum respiration rates of 2.34 μg CO_2-C g^{-1} h^{-1}. However, with the addition of P, respiration rates increased exponentially ($r^2 = 0.88$) and reached higher maximum rates (3.62 μg CO_2-C g^{-1} h^{-1}). In the mollisol site, both Sal and Sal + P treatments induced exponential increases in respiration follow-

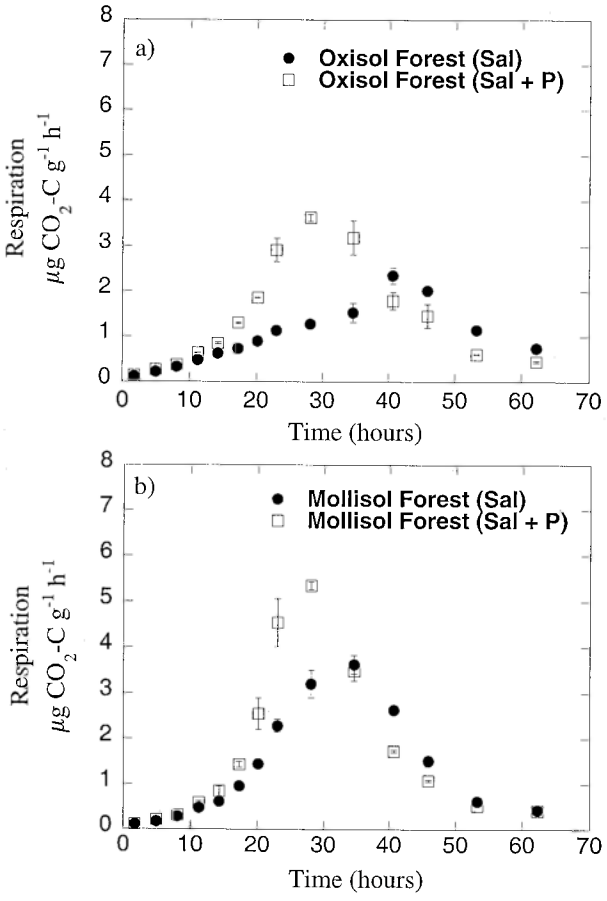

Figure 3. Radiolabeled substrate (salicylate, Sal) addition response of laboratory incubations (with and without P fertilization) in tropical rain forest soil from the (a) P-poor oxisol forest and (b) P-rich mollisol forest. Error bars are ± 1 SE.

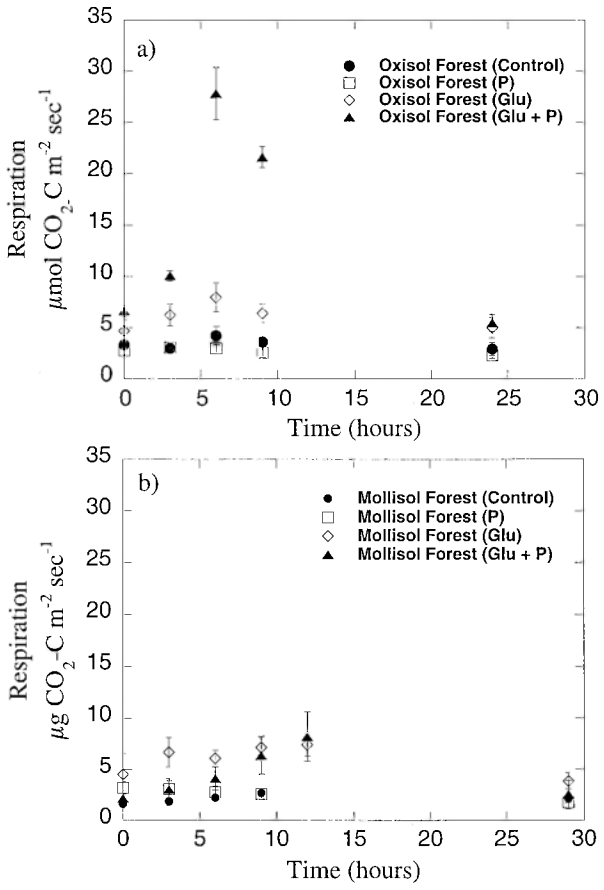

Figure 4. In situ soil respiration rates following Glu, P, or Glu + P fertilization in both (a) P-poor oxisol forest and (b) P-rich mollisol forest (b). Error bars are ± 1 SE.

ing substrate addition (Figure 3b). However, the Sal + P treatment reached higher maximum rates than the Sal-only treatment, even at MF.

Phosphorus limitation of microbial activity in situ was also observed when P was added in combination with labile C (Figure 4a and b). Both Glu and Glu + P additions increased soil respiration as compared to controls in the OF site, but respiration in the Glu-only treatment reached a maximum of 7.95 ± 1.40 μmol CO_2 m^{-2} sec^{-1} 6 h after the C addition, while respiration in the Glu + P treatment reached a significantly higher maximum rate of 27.81 ± 2.55 μmol CO_2 m^{-2} sec^{-1} 6 h after the Glu + P addition ($F = 40.72$; $P < 0.001$) (Figure 2a). This evidence suggests an interaction between C and P in their influence on microbial activity, where the degradation and fate of labile C is clearly linked to P availability. In the P-rich MF site, responses to both Glu and Glu + P were more muted, and significant increases above control rates were only observed in the C-only treatment 9 h after C additions ($F = 3.82$; $P < 0.05$), perhaps reflecting the greater P availability at this site (Figure 4b).

The observation that P availability limits the ability of the microbial community to grow following additions of glutamate suggests P constraints on decomposition in this system, but a direct test of this hypothesis requires the use of native C. Thus, we conducted a similar incubation experiment using native DOC extracted from leaflitter and soil from the P-poor, oxisol forest (Figure 5). Following additions of C alone to soil, cumulative respired CO_2 increased to a maximum of 5391 μmol CO_2 mol^{-1}. In contrast, cumulative respired CO_2 in samples amended with both DOC and P reached 6141 μmol mol^{-1} in OF 31 h after the experiment began. Cumulative respired CO_2 was significantly higher in the DOC + P samples than in the DOC-only samples at all sampling times ($P < 0.01$). Rates of respiration were consistently higher in the DOC + P

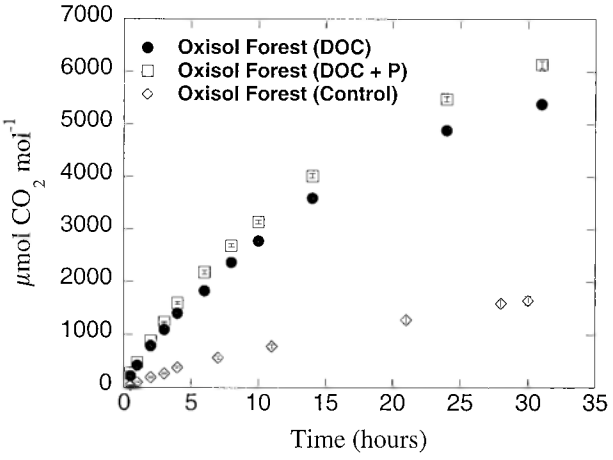

Figure 5. Cumulative CO_2 production following addition of leached DOC to oxisol forest soil, with and without P fertilization. Error bars are ± 1 SE. All differences are significant ($P \leq 0.05$).

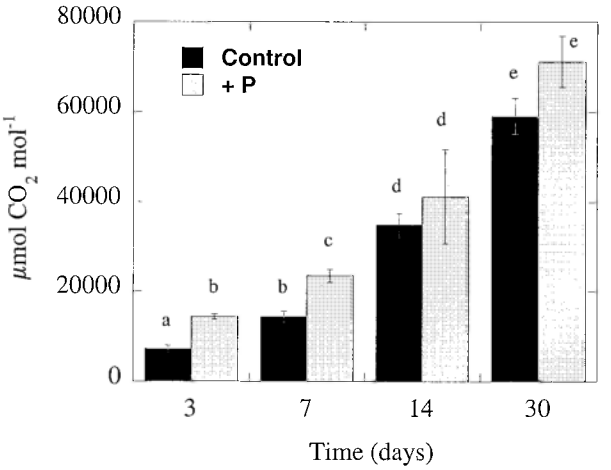

Figure 6. Cumulative CO_2 respired in oxisol forest soil samples with and without added P. Values labeled with dissimilar letters are significantly different ($P \leq 0.05$). Error bars are ± 1 SE.

treatment, with maximum rates (503.13 μg CO_2-C g^{-1} h^{-1}) occurring 30 min after the substrate/fertilizer additions (Figure 5). However, respired CO_2 in the DOC-only samples reached a maximum of 376.42 μg CO_2-C g^{-1} h^{-1} and were also highest 30 min after the DOC addition. Rates of respiration were significantly higher in the DOC + P treatment at all sampling times except 1, 2, and 8 h ($P \leq 0.05$). Cumulative respired CO_2 in control samples reached a maximum of 1641 μmol mol^{-1}, and maximum rates of respiration reached 138.22 μg CO_2-C g^{-1} h^{-1} 30 min after the experiment began but remained roughly at this level for 4 h before declining to approximately 58 μg CO_2-C g^{-1} h^{-1}.

Finally, the results of P additions to soils in the absence of any simultaneous C addition are depicted in Figure 6. These data also suggest that the decomposition of native soil organic carbon (SOC) is P limited. Specifically, total respired CO_2 in samples amended with P was significantly higher than control samples on days 3 ($F = 25.66$; $P < 0.001$) and 7 ($F = 22.60$; $P < 0.001$). However, total CO_2 was not significantly higher in + P-amended samples than controls on days 14 ($F = 1.07$; $P > 0.1$) and 30 ($F = 3.05$; $P > 0.1$), suggesting that the effects of P were very important immediately following P additions and the effects diminished as the experiment proceeded.

Discussion

Multiple lines of evidence from our data all suggest that microbial respiration was strongly limited by available C, but that the use of that C was further limited by P availability in the oxisols but not the more fertile mollisols. In the (relatively) P-rich mollisol forest, substrate addition incubations treated with Glu and Glu + P responded in a very similar manner and showed only subtle signs of P limitation. Both Glu and Glu + P treated samples showed respiration patterns consistent with growth of the microbial community, suggesting that P was not colimiting in the MF site. However, in the OF site, the effects of P limitation on the decomposition of labile C are striking. Additions of Glu alone produced linear increases in respiration, suggesting that C was being respired, but that rapid microbial growth was not stimulated. In contrast, respiration in samples amended with both Glu and P increased exponentially, suggesting that the combination of both carbon and phosphorus was necessary to induce rapid, exponential microbial growth rates in the OF site.

Our results were in stark contrast to results using glutamate as a C substrate for microbial growth in soil from other ecosystems. For example, Lipson and others (1999) found that microbial respiration responded exponentially following glutamate addition in an alpine soil, with no indication that the utilization of glutamate was limited by P. Ley and others (2000) found a similar result, and P additions actually caused an inhibitory effect on microbial activity in soil from the Colorado alpine (R. Ley unpublished). However, even after adding C and N at levels that have been shown to induce microbial growth in other studies, at our sites microbial respiration occurred at much lower levels than when carbon substrates were added in combination with

P. This suggests that P is the critical element limiting the utilization of this substrate in our tropical soil. This was also observed following salicylate addition. However, the fact that salicylate lacks N suggests that the clear P limitation on the utilization of C was not induced by simultaneous N enrichment in the glutamate treatments. In addition, salicylate is more chemically complex than glutamate (resulting in the lower respiration rates in Figure 3 relative to Figure 2), and it is mineralized by the same general pathways as breakdown products from lignin and detrital polyphenols. Again, our data suggest that while the ability of the microbial community at OF to mineralize this complex substrate is also limited by available P, the P responses we observed were not unique to a single carbon compound.

The between-site differences in the responses of the microbial community to additions of labile C were also observed in our field fertilization experiment. In the P-rich mollisol forest site, respiration rates in the Glu and Glu + P samples were significantly higher than control and + P treatments, but they were never significantly different from each other. This finding again likely reflects the relative P-rich status of the mollisol forest, which appears likely to contain labile P in amounts sufficient to meet the demands of the microbial community. In the P-poor oxisol site, the addition of labile C increased respiration above levels observed in the control or + P treatments, demonstrating clear C limitation of the microbial community in the oxisol soil. However, samples amended with Glu + P were significantly higher than all other treatments at 3, 6 and 9 h, again suggesting that C utilization at OF was further limited by P availability.

One might expect that additions of a highly labile compound such as glutamate would be more likely to produce a P response, by alleviating any C limitation and focusing the constraints upon nutrient supply. However, our data show that the decomposition of native ecosystem carbon was also clearly limited by available P. To determine the net response of soil respiration stimulated by the addition of DOC, rates of respiration in control samples were subtracted from both DOC and DOC + P treatments, and respired CO_2 was calculated as a percentage of total DOC added to each treatment (Figure 6). In the DOC-only treatment, 32 h after substrate additions C accumulation reached an asymptote, and data suggested that at this time 10.72% of the total DOC added to the samples had been respired as CO_2-C. In the DOC + P samples, cumulative net CO_2 reached a higher asymptote than that observed in the C-only samples, with data suggesting that in the presence of excess labile P,

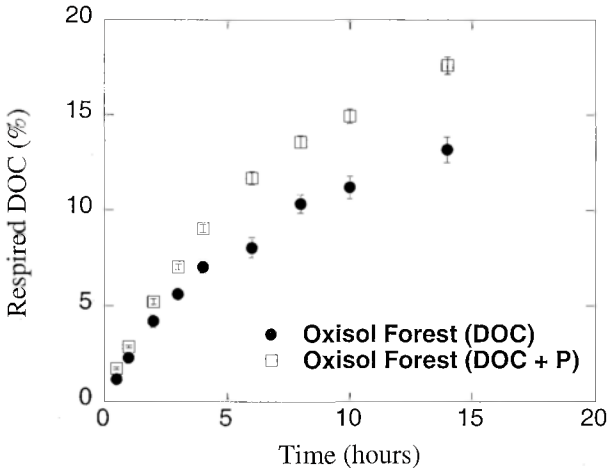

Figure 7. Percent added DOC respired in oxisol forest soil samples following glutamate (Glu) additions, with and without added P. Error bars are ± 1 SE. All differences are significant ($P \leq 0.05$).

the microbial community respired 16.96% of the added DOC (Figure 7). This result suggests that a large fraction of the DOC flux from litter into soils in tropical forests is labile and rapidly utilized by microbial populations. In addition, the fact that the percent of DOC respired in + P samples reached higher values than in DOC-only samples can be explained in one of two ways. First, the addition of excess P may enable the microbial community to utilize more recalcitrant forms of the soluble C added to the samples. Alternatively, excess P may facilitate growth of the microbial biomass above that observed in DOC-only samples, and the turnover of this relatively larger biomass pool may result in increased CO_2 production over the course of the experiment.

These data suggest that rapid decomposition of episodic inputs of labile, soluble C may be limited by P availability. In addition, our data suggest that the response of the microbial community to inputs of labile C is extremely rapid, and even following relatively large fluxes of carbon into the soil, respiration rates return to "background" rates less than 24 h after the input event. This is noteworthy given that multiple soil respiration studies have shown that 70%–80% of heterotrophic respiration is derived from rapidly turning over soil C pools that account for less than 5% of the total soil C reservoir (Townsend and others 1997). Principal sources of such labile C can include turnover of the microbial biomass itself (Paul and Clark 1997), exudation from roots (Biondini and others 1988), and soluble fluxes of organic compounds through litterfall and into the soil (McDowell and Likens 1988). In tem-

perate systems, the latter can amount to 1%–19% of the total litterfall C flux and 1%–5% of net primary production (NPP) (Neff and Asner 2001). We suggest that during periods of high C availability, colimitation by P could constrain the total respiratory flux of CO_2 from tropical soils below values that would be expected if P were not limiting and cause some fraction of labile C flushes to remain within the ecosystem for longer periods of time. An increase in residence time for such C due to P constraints may increase the odds for its stabilization in soils (Neff and Asner 2001) and/or its transport to aquatic systems.

To this point, we have focused on the effects of P limitation on the response of the microbial community to inputs of exogenous carbon, much of which is labile; these data clearly demonstrate colimitation of the microbial community by both C and P. However, our longer-term incubation experiment addressed the effects of P availability on the decomposition of native SOC. The fact that cumulative CO_2 reached significantly higher levels than in control samples suggests that the microbial community is clearly P limited even in the absence of more labile C inputs, at least with respect to the decomposition of soil carbon. After 3 days, CO_2 respired in + P treatments was approximately 50% higher than in control samples; after 7 days, it was approximately 40% higher (Figure 6). The effect of P on long-term soil C decomposition dynamics merits further investigation.

To our knowledge, these data offer the first clear evidence of direct P limitation to the activity of the soil microbial community in a moist tropical forest and have important implications for our understanding of basic processes in these systems. First, because microbes have high substrate affinities, rapid growth rates, and high surface-to-volume ratios, they are thought to outcompete plant roots for nutrients (Rosswall 1982; Chapin and others 1986; Jackson and others 1989; Stark and Hart 1997). If true, P immobilization by a P-limited microbial community may exacerbate P limitation to primary productivity in tropical forests. At long time scales, however, a P-limited microbial community may actually help retain more labile forms of P in actively cycling biological pools than they otherwise would (if P was not limiting), by lowering the rates at which labile inorganic P is lost to more permanent geochemical sinks in these high P-sorbing tropical systems (Morel and others 1996).

Second, in the tropics the combination of intense but episodic precipitation events and a large standing pool of carbon make it likely that microbial populations experience frequent inputs of high-quality C as DOC flushes through surface soils (Jandl and Sollins 1997). As well, exudation of labile C from roots can be a significant carbon flux in many ecosystems (Biondini and others 1988), and recent reports from elevated CO_2 experiments have shown substantial increases in labile C inputs to soils following a doubling of atmospheric CO_2 (Hungate and others 1997). If similar responses occur in tropical systems with P-limited microbial communities, the fate of this excess C may be very different from that seen in ecosystems where microbial activity is primarily constrained by C alone. Our data suggest a somewhat counterintuitive hypothesis, in which we would predict higher rates of belowground C storage in response to rising CO_2 levels in more nutrient-poor ecosystems. This prediction is opposite to that for plant responses, in which higher C storage is predicted for more nutrient-rich environments (compare Lloyd and Farquhar 2000). We believe a key unknown for tropical forests is how plants on phosphorus-poor soils may respond to higher levels of CO_2. Given the high levels of NPP (Phillips and others 1998; Field and others 1998) seen in forests on these soils (despite the low nutrient levels) and the fact that N is often relatively abundant, we believe significant increases in labile photosynthate production and transport to the rhizosphere are quite possible in these forests. Again, our data suggest that if such increases occur, the more P-poor forests may display longer-term soil storage of the new carbon inputs.

Finally, while our results suggesting P limitation to microbial processes in a tropical rain forest are novel, we suggest that the phenomenon may be prevalent in other tropical rain forest sites, and the possibility merits further investigation. For example, although our data suggest that the OF site is P poor relative to many temperate forests, it is actually P rich compared to most tropical forests on older soils (Table 1). Values for labile soil P, total soil P, and litter P in our "P-poor" OF site are all still higher than average values for a range of other tropical forests on ultisols and oxisols (Table 1). Roughly 60% of all tropical moist forests are underlain by P-poor ultisol or oxisol soils; thus, P limitation to microbial processes in these ecosystems may be widespread.

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