Soil Microbial Dynamics in Costa Rica: Seasonal and Biogeochemical Constraints[1]

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ABSTRACT

The soil microbial biomass is largely responsible for the transformation of organic matter and the associated mineralization of important nutrients that regulate plant productivity. While the controls on soil microbial biomass and activity in temperate ecosystems have been well investigated, there is a paucity of such information from tropical rain forest ecosystems. Hence, we used a natural soil gradient (nutrient-poor oxisols and nutrient-rich mollisols) to investigate seasonal changes in the size and activity of the soil microbial community in two primary tropical rain forest sites in southwestern Costa Rica. In a nutrient-poor oxisol, microbial biomass ranged from 952 µg/g in the dry season (February) to 1967 µg/g in the wet season (June). The greater microbial biomass estimates corresponded to greater rates of microbial activity in the oxisol forest; however, the active fraction of the soil microbial biomass remained consistently low, regardless of soil C availability. In addition, while there was very little seasonal variability in microbial C:N ratios, we found extreme variations in seasonal microbial C:P ratios, with the highest microbial C:P ratio occurring when microbial biomass C was at a maximum. Our results suggest the importance of P availability in regulating soil microbial processes and that decomposition during the wet season (of litter accumulated in the previous dry season) may play an important role in driving seasonal changes in microbial biomass and activity.

RESUMEN

Las comunidades microbianas del suelo influencian en gran medida la descomposición de materia orgánica y la mineralización de los nutrientes necesarios para la producción primaria de plantas. Aunque los factores que gobiernan la actividad microbiana de suelos en la zona templada han sido investigados extensamente, carecemos de información sobre bosques tropicales húmedos. En este estudio, usamos sitios de fertilidades variables con el fin de investigar efectos estacionales en el tamaño y la actividad de las comunidades microbianas del suelo en dos bosques tropicales húmedos en el sudoeste de Costa Rica (Península de Osa). En los suelos infértiles (oxisoles), el tamaño de la comunidad microbiana se extendió de 952 µg/g en la estación seca (febrero) a 1967 µg/g en la estación lluviosa (junio). Además, las mayores estimaciones de la comunidad microbiana correspondieron a mayores tazas de cambio de la actividad microbiana. Sin embargo, la fracción microbiana activa se mantuvo siempre baja independientemente de la cantidad de carbono en el suelo. Además, aunque no hubo mucha variabilidad en las proporciones de carbono (C) y nitrógeno (N) en la masa microbiana con la estación del año, encontramos grandes cambios en las proporciones de C y de fósforo (P). En particular, observamos una proporción mas grande de C (sobre P) cuando el tamaño de la comunidad microbiana fue máximo. Nuestros resultados sugieren que la disponibilidad de P regula el tamaño y la actividad microbiana en este ecosistema, y que la descomposición de hojascara en la estación lluviosa (de hojas que se cayeron en la estación seca anterior) influencia en gran medida el tamaño y la actividad microbiana entre las estaciones del año.

Key words: C and P dynamics; microbial biomass; phosphorus; seasonal dynamics; tropical rain forest.

SOIL MICROBIAL BIOMASS, consisting largely of a consortium of bacteria and fungi, is important in regulating ecosystem processes such as decomposition, energy flow, carbon (C) storage, and trace gas fluxes (Swift *et al.* 1979, Paul & Clark 1997, Schlesinger 1997). In addition, soil microbial processes are responsible for the transformation of soil organic matter and the associated mineralization of important nutrients that regulate plant productivity. On an annual basis, most of the nutrient requirements of land plants are met via the mineralization of soil organic nutrients by the microbial community (Paul & Clark 1997); thus, soil microbial processes strongly regulate ecosystem net primary production.

The role of the soil microbial community in nutrient transformation may have additional importance in tropical rain forest ecosystems. Specifically, phosphorus (P) is generally believed to be the most limiting element in the majority of tropical forests on old, highly weathered soils (Walker & Syers 1976, Vitousek 1984, Vitousek & Sanford 1986). Although the biomass of soil microorganisms is typically less than that of vascular plants, its non-nitrogen mineral nutrient concentration may be as much as ten times greater (Cole *et al.* 1977, Paul & Clark 1997) and contain as much P per hectare as vegetation (Hayman 1975). Moreover, because there may be multiple generations of growth by diverse groups of soil microorganisms each year, their annual demand and uptake of nutrients often exceed those of vascular plants (Paul & Clark 1997). Hence, the strong P demands of the soil microbial community in an ecosystem, where P already limits primary productivity, may actually provide an important P retention mechanism by preserving P in actively cycling biological pools (*i.e.,* the microbial biomass) and attenuating losses to more permanent geochemical sinks (Uehara & Gillman 1981, Oberson *et al.* 1997).

In spite of the likely importance of the soil microbial biomass in maintaining nutrient fertility in the already P-poor soils of the humid tropics, very little is known about what regulates soil microbial biomass and activity in these ecosystems. In addition, tropical forests are currently undergoing rapid rates of land conversion and land use change. On a global basis, deforestation and subsequent land use in tropical ecosystems remove roughly 2 percent of the remaining forest cover per year (Williams & Chartres 1991, Houghton 1994, Nepstad 1999). The majority of cleared tropical forests are converted to cattle pasture (Fearnside 1996), a change that can have profound effects on the soil microbial community (and hence potentially serious effects on nutrient transformations in these nutrient-poor ecosystems). To understand how land use change is likely to affect the soil microbial community structure and function, and the associated biogeochemical consequences, it is critical that we first understand the factors that regulate soil microbial processes in natural tropical forest ecosystems.

Our objectives in this study were to determine seasonal changes in microbial biomass and a suite of microbial processes in two lowland tropical rain forest sites of southwestern Costa Rica. Like many tropical rain forests, our study sites experience a pronounced annual dry season from December through March. We hypothesized that both the structure and the function of the soil microbial community would fluctuate seasonally, with a larger, more active community characterizing the wet season (Scatena 2001). In addition, we hypothesized that changes in microbial community structure and function would have profound effects on both carbon and nutrient cycling in this ecosystem.

MATERIALS AND METHODS

STUDY AREA.—The study area was near the town of Agujitas on the Osa Peninsula in southwestern Costa Rica (Cleveland *et al.* 2002). This region lies within the tropical wet lowland forest bioclimate (Holdridge *et al.* 1971); annual rainfall is heavy, averaging *ca* 5000 mm/yr, but is also seasonally variable. The Osa Peninsula is dominated by the Pacific rainfall regime, with peak rainfall occurring during summer to early fall (June–October) and a pronounced dry season during the northern winter (December–April). While the dry season on the Osa is relatively short compared to many other tropical forests (Janzen 1983), the variation between wet and dry months in this area is noteworthy. Over a seven-year period (1980–1987), December–May rainfall averaged less than 250 mm/mo at the Sirena Biological Station, 25 km southwest of our sites. In contrast, over the same period, May–November rainfall averaged more than 500 mm/mo, and every month between May and November received more than 250 mm of rainfall (Sterrenberg 1990). Because overall precipitation is very high, however, nearly all forms of vegetation persist in an evergreen state throughout the year. Leaf senescence and litterfall reach a maximum during the dry season (Sterrenberg 1990).

The entire Osa Peninsula was formed in three large seafloor volcanic events between roughly from

TABLE 1. *Physical and biogeochemical characteristics of oxisol and mollisol (0–10 cm soil) forest sites. Error values are ±1 SE.*

	Oxisol	Mollisol
Soil texture	Clay	Loam
Sand (%)	26	42
Silt (%)	30	28
Clay (%)	44	31
pH (water)	5.4 ± 0.2***	6.0 ± 0.3***
Soil C (mg/g)	65.0 ± 3.0	68.0 ± 4.0
Soil N (mg/g)	6.0 ± 0.0	6.0 ± 0.0
Soil P (μg/g)	557.1 ± 18.5***	1051.4 ± 43.1***
Soil Ca (μg/g)	1418.4 ± 257.2***	4484.8 ± 687.3***
Soil Mg (μg/g)	285.4 ± 33.3***	546.2 ± 57.4***
Soil K (μg/g)	58.8 ± 7.1***	110.6 ± 10.2***
Bulk density	0.52 ± 0.02*	0.66 ± 0.02*
Total C (kg/m^2)	3.4	4.5
Total N (kg/m^2)	0.3	0.4
Total P (g/m^2)	28.9	69.4
C:N	11.6 ± 0.3	11.6 ± 0.4
C:P	117.0 ± 6.3**	69.9 ± 0.8**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

75 to 40×10^6 years ago, but some parts of the region were below sea level in more recent geologic eras (Berrange & Thorpe 1988). This created a wide range of parent material ages and subsequent soil types, from highly weathered more than 40×10^6-year-old oxisols to highly fertile alluvial mollisols of Quaternary origin. Forests in this region thus occur on three general soil types: (1) old, highly weathered oxisols on steeply dissected terrain that rarely exceeds a few hundred meters elevation; (2) much younger (2 to 4×10^6-year-old) ultisols on roughly similar upland terrain; and (3) the highly fertile mollisols found on the alluvial plains. Our sites were located near the transition between the old upland oxisols and the young lowland mollisols. We selected two forest sites representing each soil type, hereafter referred to as oxisol forest (OF) and mollisol forest (MF); the MF site was located on relatively young, nutrient-rich alluvial soils, while the OF site was on old, acidic, highly weathered, relatively nutrient-poor soils. The sites were all less than 500 m apart, and thus identical climatically (MAT = 26° C; MAP = *ca* 5100 mm/yr; Sterrenberg 1990). While the forests were all characterized by high plant diversity and were not dominated by any single species, they were floristically similar. For example, both sites were stratified, closed canopy primary forest sites, and both sites contained several common canopy species (*e.g., Brosimum utile, Caryocar costaricense, Hieronyma alchorneoides, Vantanea barbourii,* and *Schizolobium parahybum*) and understory species (*e.g., Bromelia* spp. and *Geonoma* spp.)

SOIL SAMPLING.—Soils for bulk chemical analyses (Table 1) were sampled in June 2000, and soils for P and biological analyses were sampled in both June 2000 (wet season) and February 2001 (dry season). At both sites, 8×10 cm soil samples were extracted every 5 m from two randomly placed 25 m transects bisecting each site, for a total of ten samples per site. Soils were sampled to a depth of 10 cm, reflecting the region of greatest microbial density and activity observed commonly in the soil profile (Ekelund *et al.* 2001). Following collection, samples were double-bagged and chilled on ice, returned to the laboratory, and within 72 hours of collection, were coarsely sieved (4 mm) to remove rocks and plant material. A portion of each sample was then removed and air-dried for physical and chemical analyses. Fresh soil samples for microbial analyses were stored at 10°C, and all experiments were initiated within 96 hours of soil sampling to minimize artifacts incurred during long-term storage.

SOIL CHARACTERISTICS.—On air-dried soils, we measured pH on a soil:deionized water paste (1:1). Soil samples for organic C and nitrogen (N) were ground (0.5 mm) and analyzed using a Carlo Erba EA 1110 elemental analyzer (CE Elantech, Lakewood, New Jersey). Extractable N (NH_4^+/NO_3^-) was determined using a 2 M KCl solution, extracted for 24 hours and analyzed for NH_4^+ and NO_3^- colorimetrically on an Alpkem autoanalyzer (OI Analytical, College Station, Texas). Bulk density was determined using an excavation method (Par-

ent & Caron 1993). Exchangeable cations (calcium, Ca; magnesium, Mg, and potassium, K) were extracted in 55 ml of 1 M ammonium chloride (Silver *et al.* 2000), and were analyzed using an ARL 3410 inductively coupled plasma atomic emission spectrophotometer (ICP–AES).

P-ANALYSES.—A soil sample (1 g) 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 (resin P_i) to extract inorganic P, followed by a bicarbonate extraction (bicarb P_i). Organic bicarb extractable P (bicarb P_o) was determined as the difference between total bicarb extractable P (bicarb P_t) and bicarb P_i following digestion with ammonium persulfate and sulfuric acid (Tiessen & Moir 1993). These three fractions (resin P_i and bicarb P_i + P_o) are the most labile forms of P, and their sum is often taken as a proxy for readily available (labile) P. Bowman *et al.* (1978) and Levy and Schlesinger (1999) have shown that bicarb P (P_i + P_o) is well correlated with plant growth. Total available P (P_t) 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).

MICROBIAL BIOMASS.—Soil microbial biomass C and N were determined using the chloroform fumigation–extraction method (CFE; Brookes *et al.* 1985). Fumigated (5 day) and unfumigated samples (10 g dry weight; dw) were extracted with 0.5 M K_2SO_4, centrifuged for 5 minutes at 5400 rev/min, and filtered through pre-ashed 1.0 μm Gelman GF/F glass fiber filters. Organic C in extracts was analyzed using a Shimadzu TOC-5050A total organic C analyzer (Shimadzu Corporation, Kyoto, Japan). Microbial biomass C was determined as the difference between extractable organic C in fumigated and unfumigated samples using proportionality constant (Kc) of 0.45 (Vance *et al.* 1987). Filtered extracts were also analyzed for NH_4^+ and NO_3^- colorimetrically using an Alpkem autoanalyzer. Total N in soil extracts was determined following digestion with potassium persulfate (D'Elia *et al.* 1977). Microbial biomass N was determined as the difference between extractable N in fumigated and unfumigated samples using a correction factor (Kn) of 0.54 (Brookes *et al.* 1985).

Microbial P was determined using the fumigation–sorption method of Morel *et al.* (1996) and

modified for use with tropical soils by Oberson *et al.* (1997). Briefly, 10 g soil (dw) were fumigated with C-free chloroform liquid and vapor for 75 minutes. Following fumigation, deionized water (10 ml) was added and samples were allowed to equilibrate for 24 hours at 4°C. At the end of the equilibration, samples were extracted using an NH_4F extract (0.03 N NH_4F and 0.025 N HCl) using a solution to soil ratio of 10:1 (Bray & Kurtz 1945) for 1 minute, filtered, and stored for analysis. Concomitantly, parallel soil samples were spiked with different amounts of P (0, 2, 5, 10, 20, and 50 mg/kg) and extracted to determine the effect of sorption reactions over the course of the experiment. Solutions were analyzed for P by the ammonium molybdate ascorbic acid method (Kuo 1996). Microbial biomass estimates were obtained using the relationships described in Morel *et al.* (1996).

MICROBIAL ACTIVITY AND ACTIVE BIOMASS.—We also assessed soil microbial activity and active microbial biomass using the substrate-induced growth response (SIGR) method of Colores *et al.* (1996) and modified by Cleveland *et al.* (2002). The SIGR technique was used to test the response of the soil microbial community to added C, thus providing an independent estimate of microbial biomass. Whereas the chloroform fumigation method provides an estimate of total microbial biomass, the SIGR method provides an estimate of an active fraction of the total microbial biomass that is able to respond to specific C substrate additions (Colores *et al.* 1996). Briefly, 10–15 g dry mass equivalent of each soil was placed in a biometer flask and brought to 50 percent water holding capacity with deionized water. Amounts of C substrate determined previously to induce maximal respiration (*sensu* Colores *et al.* 1996) were added to each flask with the same uniformly labeled ^{14}C substrate to yield 2500 Bq (150,000 dis/min) per flask, and 1 ml 0.5 M NaOH in the sidearm to trap evolved CO_2 (Colores *et al.* 1996). The base trap was removed from each flask and refreshed at regular intervals and radioactivity was measured by liquid scintillation counting after mixing with Scintiverse II scintillation cocktail to determine respiration rate. Flasks were kept at 22°C until soil respiration returned to its basal rate (*ca* 56 h). Respiration data were analyzed with Kaleidagraph software using equations derived by Colores *et al.* (1996). To convert units of SIGR biomass (μg CO_2–C/g) to mg C-biomass/g, empirically derived yields (Y_c) of 0.50 for glutamic acid were used in the equation X_a =

X_1 (Y_c /1—Y_c), where X_a is the actual biomass in mg C-biomass/g, and X_1 is the biomass in units of mg CO_2–C/g (Colores *et al.* 1996, Lipson *et al.* 1999). Substrate-induced respiration (SIR) estimates represent the initial respiration rates (2–3 h) following substrate additions.

The SIGR method requires exponential growth by the soil microbial community following substrate additions, without any possible nutrient constraints (Colores *et al.* 1996). Our previous work, however, suggested strong constraints by P on the soil microbial community (Cleveland *et al.* 2002, 2003). Thus, our estimates of SIGR-biomass represent the effect of adding C substrates in combination with P.

MICROBIAL RESPONSE TO C AMENDMENTS.—Responses of microbial respiration following the addition of organic C were determined using SIGR (Cleveland et al. 2002). We utilized the amino acid glutamate (2.0 mg glutamate–C/g), a very labile C compound, to determine the physiological response of fast-growing heterotrophs to C additions. Glutamate (Glu) elicits greater microbial responses than other simple sugar compounds like glucose, and has a C:N ratio (5:1) that is sufficient to promote microbial growth (Lipson *et al.* 1999). To remove the effects of P limitation on soil microbial processes, we added labile, inorganic P in concert with the glutamate and salicylate additions in the SIGR incubations (*sensu* Cleveland *et al.* 2002). For the fertilizer treatments, P was added as KH_2PO_4 to water amendments to yield P additions of 200 μg KH_2PO_4/g. These P additions did not alter the pH of the +P solutions relative to the treatments without P (pH = 6.8).

PHOSPHATASE ACTIVITY.—Potential phosphatase activity was measured using a method in which enzyme activity releases p-nitrophenol (pNP) from added substrates, and concentrations of pNP are determined colorimetrically (Tabatabai & Bremner 1969). Briefly, 2–3 g (dw) of soil was combined with 150 ml of acetate buffer at pH 5. pNP–phosphatase substrate was added to the slurry and reacted at 25°C for 2 hours. Individual samples were centrifuged and the supernatant mixed with NaOH to halt enzyme activity and to color the samples. Amounts of pNP were measured at 410 nm absorbance using an Agilent 8453 benchtop spectrophotometer (Agilent Technologies, Palo Alto, California). Although this method is not sensitive enough to separate abiotic, extracellular, or intracellularly released enzymes, for enzyme activity to be detected using this assay, enzymes must cleave substrates that are too large to pass through cell membranes. Therefore, this assay effectively measures activity occurring outside of microbial cells and plant roots (Olander & Vitousek 2000). To test the effects of labile C and N additions on phosphatase activity, we added 5 mg glutamate/g to 25 g (dw) soil samples in 10 ml deionized water. We determined phosphatase activity in soil samples following reaction with added glutamate for 24 hours at 25°C.

STATISTICAL ANALYSES.—Differences in soil biogeochemical characteristics and microbial biomass between sites were tested with one-way ANOVA following tests for homoscedasticity (Levene's test) and verification that data were normally distributed (SPSS, Chicago, Illinois). Differences in microbial growth rates following substrate addition between the sites were determined with linear regression following log transformation of rate data during the exponential growth phase. Different regression lines to estimate homogeneity between growth rates were compared according to the methods described by Steel and Torrie (1960). Seasonal differences in microbial indices between the sites were analyzed by ANOVA with site and season as main factors. When there were significant site and date interactions, separate one-way analyses for the different sites and dates were performed. All results are reported as significant when $P < 0.05$.

RESULTS

SITE CHARACTERISTICS.—Soil texture at the two sites were different, with clay dominating in the oxisol and sand in the mollisol (Table 1); however, in most respects, our soil data suggest similarities between the oxisol and the mollisol (Table 1). In June, concentrations of extractable N were nearly equal at the two sites, and N was nearly evenly distributed as NH_4^+ and NO_3^- (Table 2). N availability increased at both sites in February but was never significantly different between sites; nor was soil total N.

In addition, OF had (significantly) lower pH and bulk density but higher soil C:P ratios and consistently higher concentrations of soil-extractable C than MF (Table 1 and Fig. 1).

P fertility was markedly different between the two sites (Table 2). Specifically, values for P_t, resin P_i, and bicarb P_o at the mollisol forest were all nearly twice those measured at the oxisol forest, and bicarb P_i at the OF was less than 67 percent

TABLE 2.   *Soil N and P availability in the oxisol and mollisol soils in June (wet season) 2000 and February 2001 (dry season). Row values with different superscripts represent significant (P < 0.05) seasonal differences at one site. *, **, and *** indicate the level of significant difference between the two sites at P < 0.05, 0.01, and 0.001, respectively. Error values are ±1 SE.*

	Oxisol		Mollisol	
	February	June	February	June
Extractable N (mg/kg)	96.9 ± 6.8^a	42.8 ± 4.8^b	67.6 ± 11.1^c	45.7 ± 3.8^c
NO$_3$–N	13.4 ± 1.6^a	15.9 ± 0.9^a	24.2 ± 6.1^b	18.7 ± 1.3^b
NH$_4$–N	83.6 ± 6.3^{a***}	26.9 ± 4.5^b	43.5 ± 6.4^{c***}	27.0 ± 2.7^c
P Fraction				
Resin P$_i$ (μg/g)	6.6 ± 0.6^{a**}	4.2 ± 0.4^{b***}	11.5 ± 1.3^{c**}	8.9 ± 0.9^{c***}
Bicarb P$_i$ (μg/g)	3.6 ± 0.2^{a**}	3.4 ± 0.1^{a*}	5.4 ± 0.5^{c**}	5.1 ± 0.5^{c*}
Bicarb P$_o$ (μg/g)	14.5 ± 0.6^{a***}	13.7 ± .06^{a***}	28.8 ± 1.2^{c***}	27.6 ± 1.1^{c***}
P$_t$ (μg/g)	24.7 ± 1.1^{a***}	21.3 ± 0.9^{b***}	45.7 ± 1.5^{c***}	41.6 ± 1.4^{c***}

of that at MF. Seasonal differences in soil P availability were significantly different in the resin P$_i$ fraction ($P = 0.005$) and in the total labile P fraction (P$_t$; $P = 0.03$) in the oxisol (Table 2). In both the oxisol and the mollisol, other available P were generally, but not significantly, more abundant in February than in June.

Temperatures were quite stable at both sites; mean annual temperature varied between 25 and 26.5°C (<1.5°C range), and diurnal temperatures varied by less than 10°C (Sterrenberg 1990). Precipitation, however, did vary significantly between the seasons; in February, average precipitation reached an annual minimum of *ca* 95 mm, while in June, precipitation averaged more than 500 mm (Sterrenberg, 1990). In spite of strong seasonal differences in precipitation, however, soil moisture did not vary between sites or seasons (Fig. 1).

SOIL MICROBIAL BIOMASS.—Total soil microbial biomass showed significant spatial and temporal variability. In February (dry season), microbial C and N were not significantly different between the oxisol and mollisol sites, and biomass C and N were consistently lower than values observed in June (significant only in oxisol; Fig. 1). Microbial P, however, was significantly greater in the mollisol than in the oxisol in February ($P = 0.012$). In
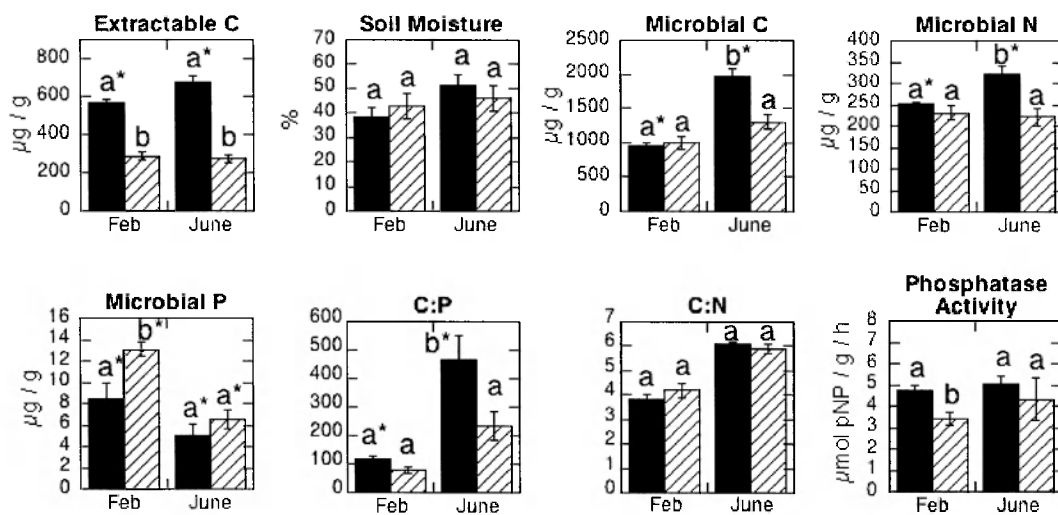

FIGURE 1.   Seasonal extractable C, soil moisture, microbial C, N, P, microbial C:P, microbial C:N, and phosphatase activity in the oxisol (solid bars) and mollisol (dashed bars) soils. Microbial C, N, and P were determined using chloroform fumigation-extraction. Bars with different superscripts depict significant ($P < 0.05$) differences between the oxisol and mollisol within a sampling date. * indicates significant ($P < 0.05$) differences in the same variable between the seasons. Error values are ±1 SE.

TABLE 3.   *Seasonal microbial biomass and activity in oxisol and mollisol forest sites. Substrate-induced respiration (SIR) values represent average respiration rate 2 h after substrate addition; SIGR is biomass calculated using the substrate-induced growth response method; μ_{max} is the exponential growth rate following substrate addition; V_{max} is the maximum rate of respiration achieved following substrate addition. Row values with different superscripts represent significant (P < 0.05) seasonal differences at one site. * Indicates a significant difference (P < 0.05) between the sites. Error values are ±1 SE.*

		Oxisol		Mollisol	
Substrate	Variable	February, 2001	June, 2001	February, 2001	June, 2001
Glutamate	SIR (µg C/g/h)	4.9 ± 0.5[a]*	8.1 ± 0.6[b]*	8.9 ± 0.4[a]*	15.0 ± 0.8[b]*
	SIGR: Biomass (µg C/g)	14.5 ± 1.2[a]	44.6 ± 6.5[b]*	30.0 ± 9.5[a]	120.0 ± 24.7[b]*
	SIGR: μ_{max} (per hour)	0.20 ± 0.01[a]	0.15 ± 0.01[a]	0.23 ± 0.03[a]	0.13 ± 0.01[b]
	Vmax (µg C/g/h)	74.7 ± 13.1[a]*	73.7 ± 0.9[a]	140.5 ± 5.9[a]*	86.3 ± 0.3[b]

June, microbial C in the oxisol was significantly greater than in the mollisol ($P = 0.003$); microbial N followed the same general pattern (Fig. 1). Amounts of microbial P were not significantly different between the two sites in June, however, and were higher in February at both sites. Microbial C:P ratios were higher in February than in June, but the difference was significant only for the oxisol. C:N ratios were fairly constant (and low) regardless of site or season (Fig. 1).

SOIL MICROBIAL ACTIVITY.—Phosphatase activity was consistently low at both sites (Fig. 1). Phosphatase activity, however, was significantly higher in the oxisol in February than in the mollisol sites ($P < 0.001$). In June, phosphatase activities in the OF and MF were not significantly different. There was no significant effect of labile C and N additions on phosphatase activity (data not shown).

The SIGR model suggested considerable seasonal variability in the magnitude of the active microbial community. In the oxisol, SIGR estimates of biomass following additions of Glu revealed that biomass ranged from 14.5 ± 1.2 in February to 44.6 ± 6.5 in June. In the mollisol, SIGR estimates revealed that biomass ranges from 30.0 ± 9.5 µg C/g in February to 120.0 ± 24.7 µg C/g in June. Furthermore, the observed growth rates of the communities responding to C enrichments were inversely related to community size at both sites (Table 3). SIR values showed similar seasonal variability. In the oxisol, following Glu additions, SIR values ranged from 4.9 ± 0.5 µg C/g/h in February to 8.1 ± 0.6 µg C/g/h in June, and in the mollisol, ranged from 8.95 ± 0.42 µg C/g/h in February to 15.02 ± 0.81 µg C/g/h in June (Table 3). While the size of the communities varied considerably with season (Table 3), maximum growth rates (μ_{max}) and maximum respiration rates (V_{max})

of the communities responding to C additions did not vary consistently between seasons. In the oxisol site, despite increases in biomass from February to June, neither μ_{max} nor V_{max} were significantly different between seasons. In the mollisol site, however, significant increases in microbial biomass from February to June were accompanied by significant decreases in μ_{max} and V_{max} (Table 3).

DISCUSSION

MICROBIAL BIOMASS.—Singh *et al.* (1991), Luizao *et al.* (1992), Srivastava (1992), and Henrot and Robertson (1994) have shown that the soil microbial biomass displays pronounced seasonal variation in tropical soils. We also found that total soil microbial biomass and active biomass varied with season, but our results also suggest the importance of P availability in regulating microbial activity in tropical rain forest soils. In general, soil microbial biomass and activity were high in June and low in February. In the oxisol, elevated microbial biomass C in June relative to February corresponded to a decrease in soil resin P_i (and no significant changes in other P fractions), suggesting that the resin P_i fraction of soil P is an important source of P for the soil microbial biomass. Although microbial biomass C in the mollisol was elevated in June relative to February, P_t values were not significantly different, suggesting that in contrast to the oxisol, higher amounts of labile P in the mollisol alleviate P limitations on the microbial community. Microbial biomass C values observed in both sites fell within the range commonly observed in tropical soils (Singh *et al.* 1991, Luizao *et al.* 1992, Feigl *et al.* 1995, Groffman *et al.* 2001).

In general, soil microbial N co-varied directly with soil microbial C; C:N ratios maintained a very narrow range and were low relative to those in oth-

er ecosystems (3–6:1). Most studies suggest that microbial C:N ratios vary between 5 and 12 (Hassink 1993, Gallardo & Schlesinger 1994, Paul & Clark 1997, Wardle 1998); however, we observed that microbial C:N ratios stayed constant at both sites (irrespective of season), suggesting that the N status of the soil is sufficient at both sites and during both seasons to meet the N demands of the soil microbial community. The fact that C:N ratios did not vary considerably between the seasons in spite of profound increases in total microbial biomass from February to June would suggest that perhaps N availability does not exert as large a control on the microbial community in this N-rich tropical ecosystem as it may in more N-limited temperate ecosystems in which N availability largely regulates soil microbial processes. Both MF and OF sites contain a number of leguminous, potential N-fixing tree species in the canopy (*e.g., S. parahybum, Hymenaea courbaril* and *Mora megistosperma*), and both soil and litter N fixation rates are high at both sites (S. Reed, pers. comm.). The abundance of both potential symbiotic N-fixing species and free-living N-fixing species may contribute to the narrow microbial C:N ratios we observed, and suggest that these sites are relatively N-rich.

PHOSPHATASE ACTIVITY.—Phosphatase activity was consistently low in both soil types. While estimates of phosphatase activity in tropical forests are extremely rare, Olander and Vitousek (2000) recently reported rates of 7.8 to 22.7 μmol pNP/g/h in three tropical forests spanning a soil-age gradient in Hawaii. Their results also revealed an inverse relationship between phosphatase activity and soil P availability (Olander & Vitousek 2000). McGill and Cole (1981) suggested a model implying that biotic demand for P drives phosphatase production, and hence P mineralization. In general, our phosphatase results support this model; however, in spite of the relationship observed in previous studies between P availability and phosphatase activity (McGill & Cole 1981, Olander & Vitousek 2000), there are other factors that may influence phosphatase activity levels in our soil that cannot be accounted for by a simple model based on P availability. For example, enzymatic production and activity are influenced by physicochemical factors, such as soil temperature, soil moisture, and soil N availability (Paul & Clark 1997). Moreover, biotic constraints, including temporal and spatial variation in organic P availability (the substrate upon which phosphatase enzymes act) and rapid micro-

bial degradation of extracellular phosphatase enzyme molecules in soil, may preclude accumulation of phosphatase concentrations necessary to stimulate high enzyme activity.

Due to the high energetic cost and high nitrogen demand of microbial enzyme production (Paul & Clark 1997, Treseder & Vitousek 2001), we hypothesized that the low phosphatase activity we observed was related to low soil labile C and/or N availability. Thus, we expected that inputs of C and N would increase microbial demand for P, leading to increases in phosphatase production and activity. In our laboratory experiment, however, additions of both labile C and N (as glutamate) did not induce an increase in phosphatase activity. We concede that our short incubation time may have been insufficient to allow increased phosphatase production and that C and/or N availability may still ultimately control the production of phosphatase in these soils. It is also possible that rapid soil physical sorption or microbial degradation of newly produced phosphatase contributed to the neutral response we observed. Nonetheless, our results suggest that something other than C or N availability may limit phosphatase production and activity in both soil types. Furthermore, both our biogeochemical and microbial data suggest that the strong constraints imposed by P availability on C utilization and the strong demand for P by the microbial community is not satisfied via phosphatase production. The fact that phosphatase activity rates were higher in June than in February at both sites is consistent with the model of McGill and Cole (1981). Based on this model, one would predict that biotic demand for P would be higher in June than in February, as this marks the onset of the plant growing season in these ecosystems and thus the time of highest P demand by the plant community. Our results showed just such a pattern (Fig. 1).

MICROBIAL ACTIVITY.—Similar to the pattern observed for total soil microbial biomass, active biomass (responding to substrate additions) at both sites was higher in June than in February (Table 3). Soil moisture values, however, did not vary significantly between seasons, suggesting that the seasonal changes in microbial activity we observed were not driven by soil moisture. We hypothesize that the increase in total and active microbial biomass from the dry season (February) to the wet season (June) may reflect the outcome of intense precipitation events leaching C from large standing pools of biomass in these ecosystems in June. Lit-

terfall accumulates during the dry season at these sites (Sterrenberg 1990), but concurrent low precipitation inputs prevent rapid delivery of soluble C to the soil microbial community. The sudden onset of heavy precipitation in late May and June, however, may result in large, sudden fluxes of highly soluble, low molecular weight organic matter through litter into surface soils (Neff & Asner 2001). Organic C originating as litter leachate is composed of highly biologically available, decomposable carbon that may be rapidly mineralized and incorporated into new microbial biomass (Jandl & Sollins 1997, Neff & Asner 2001, Cleveland *et al.* in press). Such C fluxes may have led to the observed increase in activity and suggest the importance of litterfall dynamics in driving these patterns. Similar patterns have been noted in soil from other tropical forests in which nutrient pulses caused by seasonal cycles of soil wetting and drying strongly regulate nutrient cycling, and studies have shown that such pulses can have both positive and negative effects on the soil microbial biomass (Singh *et al.* 1989, Luizao *et al.* 1992).

In the oxisol, the total (chloroform extractable) and active (SIGR) biomass varied seasonally, while the proportion of the total biomass that is active (SIGR:CFE) did not and stayed relatively low (*ca* 3%). In the mollisol, however, the proportion of the total microbial biomass that is active was higher in the wet season (10%) than in the dry season (3%). This may also suggest that the fraction of active biomass that can respond to C inputs is tightly constrained by available P. In the mollisol, where P fertility was relatively high, the soil microbial community appeared to be able to utilize this available P and to grow following inputs of C in the rainy season.

Carbon decomposition in soil depends not only on the total biomass of the microbial community but also on its physiological activity, a reflection of functional group composition. The SIGR assay provides a valuable glimpse of the functional group composition of the soil microbial community (Colores *et al.* 1996). Despite clear seasonal changes in the size of the active microbial biomass at OF, represented by the increase in CFE, SIR, and SIGR biomass values at OF from February to June, microbial community maximum growth rate (μ_{max}) and maximum respiration rate (V_{max}) did not show significant changes (Table 3), an indication that there were no significant seasonal changes in the physiology of the community responding to glutamate additions. At the MF site, however, increases in microbial biomass from Feb-

ruary to June were also accompanied by profound seasonal differences in μ_{max} and V_{max} (June < February), a clear indication that the physiology of the mollisol's microbial community had changed. We hypothesize that the observed changes in the functional composition observed at MF may be the result of changes in actual species composition of the microbial community, and that these changes may be related to differences in P availability between the two sites.

MICROBIAL DYNAMICS AND NUTRIENT CYCLING.—A number of studies have shown that soil microbial biomass and processes are closely linked to soil moisture. In general, these studies have indicated that soil moisture strongly correlates with soil microbial biomass (Luizao *et al.* 1992, Srivastava 1992, Wardle 1992); however, the studies also reveal that the effects of increased soil moisture may be positive or negative (Wardle 1998). Other factors that vary seasonally (which may or may not directly relate to soil moisture) may better explain the patterns and activity of the soil microbial biomass we observed. In particular, it is noteworthy that in June, when soil microbial biomass was high, soil-extractable C was inversely related to available P (P_t) at OF; extractable organic C was strongly inversely correlated with resin P_i ($r = -0.93$), bicarb P_i ($r = -0.88$), and bicarb P_o ($r = -0.89$). In addition, in the oxisol, extractable organic C was 428.6 μg C/g in February and 674.5 μg C/g in June; in the mollisol, extractable DOC remained relatively low and constant (Fig. 1). The SIGR experiments with substrate or substrate +P suggest that the relative increase in extractable organic C in the oxisol relative to the mollisol may result from the constraints P availability poses on the decomposition of C, and this is most pronounced in the P-poor oxisol.

C:P ratios of the soil microbial community at each site also varied considerably with season, and the large response of the soil microbial community (via increases in community size following substrate addition, SIR, and active biomass) in June following the onset of the rainy season corresponded with the most strikingly high C:P ratios. In addition, while chloroform-extractable C roughly doubled from February to June, microbial P actually declined in the oxisol. This suggests either seasonal shifts in soil microbial composition from a relatively small microbial community rich in P to a large microbial community with a lower P demand, or a relatively "plastic" microbial consortium that tolerated very different amounts of labile P and

that could remain active even at extremely high C:P ratios. Strong demand for P by forest plants during the rainy season may decrease the soil available P pool, and accelerated P uptake by plants at this time may further limit P available for microbes and lead to the large C:P ratios we observed in June (Srivistava 1992). In the mollisol, microbial C:P ratios remained relatively constant between the seasons, perhaps reflecting the overall P richness of the mollisol relative to the oxisol. C:N ratios were fairly constant (and low) regardless of site or season, suggesting that N may exert less control on soil microbial biomass and activity in this tropical rain forest ecosystem than it may in temperate ecosystems.

We hypothesize that the high microbial biomass and activity observed in the wet season is driven by precipitation providing a vehicle for the movement of leached soluble organic carbon through a litter layer that accumulates in the dry season, and that this labile carbon source in turn fuels increases in microbial biomass and activity. Although our limited sampling regime necessitates that our results and interpretations be viewed with some caution, our data further suggest that the magnitude and nature of the response to new carbon subsidies is a function of P availability.

ACKNOWLEDGMENTS

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