Temporal and Spatial Patterns of Soil Nutrient Availability in a Wet Tropical Forest, Costa Rica

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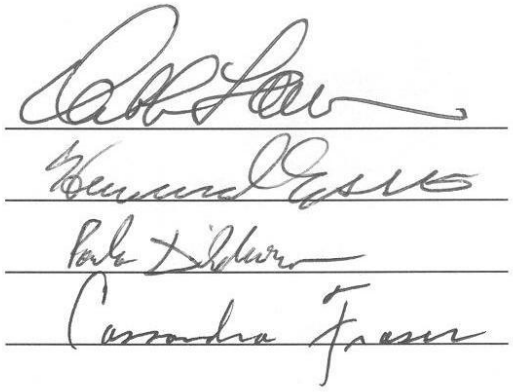

ABSTRACT

Tropical wet forests now cover approximately 13.7% of the earth's land surface (Melillo et al. 1993). Despite their relatively limited distribution, they support a disproportionately large percentage of the earth's biodiversity, and play a significant role in the global carbon budget (Brown & Lugo 1982, Melillo et al. 1993, Field et al. 1998, Clark et al. 2001). At present, human disturbance in the form of land use alteration is the primary driver of environmental change in tropical ecosystems, but changes in global atmospheric composition and subsequent climate changes may become ecologically significant in the future (Scholes and van Breeman 1997). Altered climatic conditions could affect the functioning of tropical ecosystems through the disruption of biogeochemical cycles. The limited temporal and spatial resolution of nutrient cycling studies in tropical ecosystems constrains our understanding of controls on nutrient availability in highly weathered soils.

This work presents a comprehensive investigation of temporal and spatial patterns of nutrient (in particular P and N) availability in a mature tropical rain forest on timescales of hours to years and spatial scales of meters to kilometers and explores the potential environmental and biological mechanisms driving these patterns. Chapter one presents the results from a field study to determine the biotic and abiotic controls on diurnal fluctuations in soil P availability of a wet tropical forest. Chapter two includes results from a field study in which anion exchange resin membranes were used to quantify variability in labile P over days to weeks. The influence of environmental conditions and vegetation characteristics on patterns of soil P availability were compared in two sites of contrasting P fertility. Chapter three presents the results of a three year

field study quantifying monthly soil nutrient (P and N) availability across a natural three-fold gradient in soil total P content. The influence of environmental conditions and vegetation characteristics on seasonal and interannual patterns of soil nutrient availability were assessed. Finally, Chapter four presents an investigation of potential links between P and N cycles as well as other soil chemical properties across a three-fold gradient in tropical forest soil P content.

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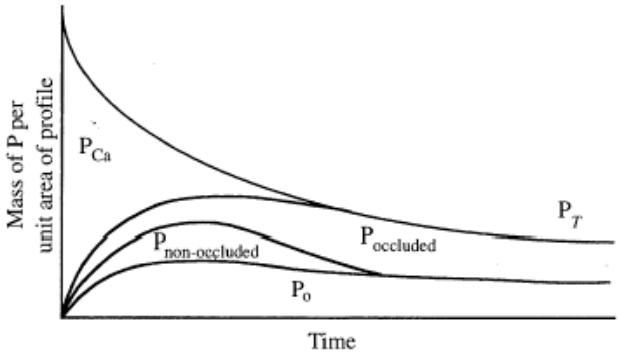
INTRODUCTION

Unlike N which can be fixed from the atmosphere, P is derived mainly from the weathering of primary minerals. Consequently, as soils age P tends to become limiting while N accumulates through biological fixation and deposition. Additionally, the strong P sorption capacity of iron and aluminum oxide clay minerals that dominate highly weathered soils competes with microbial and plant demand for the limited P supply (Vitousek and Sanford 1986, Sollins et al. 1988). Soil P may be partitioned into various chemical fractions of which only a small portion is available to plants. The four main P fractions are mineral P, organic P, occluded inorganic P and non-occluded inorganic P (Walker and Syers 1976, Tiessen and Moir 1993, Chapin et al. 2002).

Figure 1: Walker and Syers' (1976) model of P transformations through time.
P_T = total P, P_{Ca} = calcium phosphates, P_o = P bound to organic matter, $P_{non\text{-}occluded}$ = available, $P_{occluded}$ = unavailable .



Walker and Syers (1976) proposed a model describing transformations among P fractions throughout soil development (Figure 1). The model suggests that early in soil development most P is held in primary minerals. These gradually dissolve, releasing P for uptake by the biota or adsorption to soil minerals. Organic P can then re-enter the inorganic P pool through the decomposition of soil OM. The amount of P available for

biological uptake is strongly influenced by the partitioning of inorganic phosphorus between occluded and non-occluded forms. According to Walker and Syers (1976), the amount of occluded P increases with soil age while biologically available P decreases. Their model has been substantiated by a number of soil chronosequence studies (Crews et al. 1995, Cross and Schlesinger 1995, Johnson et al. 2003) and provides a useful theoretical framework for understanding the fundamental soil processes influencing P availability during pedogenesis.

Phosphorus Inputs and Outputs

On a biologically meaningful timescale, P availability depends on the relative rates of inputs, outputs and internal cycling of P between the biota and soil. In mature tropical forests, P inputs and losses tend to be low and internal P cycling is the primary means by which P availability is maintained. Parent material is often deeply buried beyond the reach of most plant roots minimizing inputs from primary mineral weathering. The most common estimated rates of P release from weathering to terrestrial ecosystems range from approximately 0.05 to 0.5 kg ha^{-1} yr^{-1} (Newman, 1995). However, Porder et al. (2006) suggested that weathering of primary P can contribute significant amounts of new inorganic P to tropical forest soils as a result of erosion and deposition or deep rooting by plants. Deposition of atmospheric dust provides an additional input of P that helps to sustain fertility in some tropical ecosystems (Swap et al. 1992, Chadwick et al. 1999, Okin et al. 2004).

Leaching losses of P from tropical forests tend to be low due to nutrient conservation mechanisms including rapid nutrient uptake by dense root mats, mychorrizae and other microbes, resorption of nutrients from senescing leaves,

sclerophylly, stabilization of P by association with OM and strong soil sorption capacity (Jordan and Herrera 1981, Vitousek 1984, Vitousek and Sanford 1986, Olander and Vitousek 2004). Runoff and erosion can cause significant losses of P following disturbance or where soil stability is low (Kauffman et al. 1993, Campo 2001).

Internal Phosphorus Cycling: Biotic and Abiotic Controls

Labile P, defined as P that is in soil solution or that can rapidly desorb from inorganic and organic soil components, is thought to be available for biological uptake in the short term (Tiessen and Moir 1993, Cross and Schlesinger 1995, Johnson et al. 2003). In tropical forest soils OM decomposition and subsequent nutrient mineralization are the dominant means of replenishing labile P (Tiessen et al. 1984, Stewart & Tiessen 1987, Tate and Salcedo 1988, Tiessen and Moir 1993, Johnson et al. 2003). Tiessen et al. (1984) found that 80% of the variability in labile P was explained by organic P forms.

Factors influencing decomposition rates include temperature, soil moisture, the composition and size of the microbial community, the quantity and quality of the substrate being decomposed and soil fertility (McGill and Cole 1981, Scholes and Breeman 1997). Decomposition is mediated by soil bacteria and fungi which convert OM into microbial biomass. Microbial biomass can account for up to 30% of the organic-P in soils (Chapin et al. 2002). This pool of labile SOM can act as a source (mineralization) or a sink (immobilization) for plant-available nutrients depending on environmental conditions and soil fertility. Microbial C:nutrient ratios are generally lower than those in decomposing material so microbes tend to immobilize nutrients in the early stages of the decomposition process (Chapin et al. 2002).

A tight linkage exists between microbial activity and the hydrological status of the soil (Lodge et al. 1994, Keith et al. 1997, Grierson et al. 1998). In the tropics where intra-annual temperature variability is low, rainfall patterns and soil moisture may be the primary drivers of belowground biological activity. Pulsed rainfall patterns that occur in the tropics can lead to drying and rewetting of soils. Strong fluctuations in soil moisture can lead to crashes in soil microbial populations, inducing pulses of nutrient mineralization due to lysis of microbial biomass (Vitousek 1984, Vitousek & Sanford 1986, Singh et al. 1989, Lodge et al. 1994, Grierson et al. 1998, McGrath et al. 2000). McGrath et al. (2000) found that labile P, in P deficient tropical soils, was highest at the start of the rainy season when wetting and drying cycles were initiated and litterfall was at its peak. The ability of microbes and plants to synchronize uptake with pulsed nutrient availability reduces the potential for loss of P to biologically unavailable pools (Tiessen 1989, Bolan 1991, Lodge et al. 1994, Olander and Vitousek 2004, 2005).

There is increasing evidence to suggest that biota may be able to access highly recalcitrant organic and inorganic P pools and reintroduce occluded P into the available pool (Barroso and Nahas 2005, Crews et al. 1995). Despite the strong affinity of tropical soils for P, Olander and Vitousek (2004) found that microbial demand rather than soil sorption capacity determined the partitioning of P into biological versus geochemical sinks in a tropical soil. In addition, they found that when P demand was increased by the addition of carbon (C), microbes were able to access tracer-P from the geochemically sorbed pool. When P supply is low soil microbes, as well as ectomycorrhizal fungi and plant roots, can produce extracellular phosphatases that cleave phosphates from soil minerals and OM (Barroso and Nahas 2005, Burns 1982, McGill and Cole 1981, Rojo et

al. 1990, Sinsabaugh 1994). Their findings suggest that biological demand for P is one of the main drivers of P availability in tropical soils.

Microbes are generally thought to out-compete plants for soil nutrients on short timescales, but on longer timescales plants may have a more significant impact on P availability (Cole et al. 1977, Schimel et al 1989, Silver 1998). Plant uptake, which is limited to the region around roots, can be enhanced by extensive root exploration and mycorrhizal associations (Bolan 1991). Soil moisture affects root growth and uptake as well as the diffusion of nutrients through the soil to roots. Transpiration-driven water flow and the associated passive uptake of nutrients by plants is an important regulator of soil nutrient availability (Novak and Vidovic 2003). Changes in solar radition, which drives active uptake of nutrients required for plant growth, is also likely to influence patterns of nutrient availability.

Global climate models not only predict increased temperatures in the tropics but also more intense and frequent ENSO events (Waylen et al. 1996, Trenberth and Hoar 1997) and in some cases reduced rainfall (Costa and Foley 2000). Changes in the length and timing of the dry season in tropical rain forests are likely to have a strong influence on biogeochemical cycling (Yavitt et al. 1993, Silver 1998, Campo et al. 2001). Seasonal rainfall patterns can influence P cycling and availability through effects on litter quality and quantity. Water stress can lead to premature leaf senescence (Silver et al. 2000, Wieder and Wright 1995, Wood et al. 2005). Wood et al. (2005) found that litter P concentrations were positively correlated with precipitation from the preceding two weeks. One of the proposed mechanisms explaining this increase in litter nutrient concentration was the impact of rainfall on nutrient availability in the soil which in turn

decreased nutrient resorption in senescing leaves. Wood et al. (2006) suggested that consistent temporal patterns in litter nutrient concentrations in soils of differing fertility were due to the influence of a common environmental factor such as precipitation or temperature on soil nutrients. Experimental tests of the relationship between climatic factors and nutrient availability in tropical forests are needed to substantiate these hypotheses.

Phosphorus and Nitrogen Cycle Interactions

A number of studies have found that soil P fertility plays a role in controlling C, N, and OM accumulation over geological timescales and it has been suggested that P is "the ultimate limiting nutrient" for terrestrial ecosystems (Walker and Adams 1958, Cole and Heil 1981, Tate and Salcedo1988, Crews 1995). There are a number of biological mechanisms that could contribute to P controls on N cycling: 1) P limitation of microbial activity, which can occur in highly weathered tropical soils (Amador and Jones 1993, Gallardo and Schlesinger 1994, Crews et al. 1995, Vitousek and Farrington 1997, Hobbie and Vitousek 2000, Cleveland et al. 2002, Stevenson 2004, Cleveland et al. 2006, Reed et al. 2007, Kaspari and Yanoviak 2008, Güsewell and Gessner 2009) could represent a rate limiting factor for decomposition and consequently N mineralization. 2) P availability can influence plant uptake of N and P limitation can lead to the production of low quality litter (high C:P and C:N ratios). Litter quality influences soil OM quality and consequently affects rates of decomposition and N mineralization. 3) P influences N accumulation through controls on N_2 fixation. Production of the nitrogenase enzyme which is responsible for fixing atmospheric N_2 into ammonia requires substantial quantities of P, Mo, and Fe (Hartwig 1998). 4) Finally, P solubilizing enzymes represent

a high N investment for plants and microbes and evidence suggests that phosphatase activity is enhanced by increasing N availability. Differences in the production of extracellular enzymes to mineralize P and N may be an important point of interaction between the two cycles. Further research is needed to elucidate short term interactions between P and N cycles in tropical forests.

Study Site

This research was conducted at La Selva Biological Station, 1,600 hectares of both mature and secondary wet tropical forest in the Caribbean lowlands of northern Costa Rica (10° 26' N, 83° 59' W; Organization for Tropical Studies). Elevation ranges from 35-137 meters above sea level. Mean annual temperature averages 25.8ºC with very little fluctuation from month to month. Mean annual rainfall is >4000 mm and is bimodally distributed throughout the year, with one short dry season from January to April and another short dry season from October to November (McDade et al. 1994). The seasonal variation in soil moisture at La Selva suggests that forests may experience periods of moisture stress during dry spells where rainfall does not exceed canopy interception (approximately 3 mm/day, McDade et al. 1994). The length of time between significant rain events may be a better indicator of whether forests are experiencing moisture related stress than monthly rainfall averages. The forests are evergreen but leaf flushes are often related to seasonal rainfall patterns and litterfall is quite sensitive to rainfall on even shorter timescales (Borchert 1973, Wood et al. 2005).

Soils at La Selva are quite variable and span a wide range of fertility from relatively fertile Inceptisols (fairly young fertile soils with poorly developed horizons) to acidic, P-poor Oxisols (highly weathered and infertile). Soils are of volcanic origin and

tend to have a low bulk density, high OM content, and are dominated by clay size particles allowing for a high water holding capacity. While soil nitrogen concentrations are generally high at La Selva, phosphorus (P) is sometimes in the deficient range and has the potential to limit plant growth (Denslow et al. 1987, Vitousek and Denslow 1987).

My research was primarily conducted in 18 0.5 ha plots in mature tropical forest, across La Selva's gradient in soil P fertility. Plots were established in 1996 for a long-term, landscape-scale project to investigate the effects of climatic factors on ecosystem carbon dynamics (the CARBONO plot network is described in Espeleta and Clark 2007). Plots were randomly located but stratified by topography and soil type using La Selva's GIS system. The 18 plots span a fertility gradient in total P from approximately 0.37 – 1.18 Mg/ha (0-10cm; Espelita and Clark 2007). Field experiments were also conducted in the vicinity of a carbon-exchange tower, located on upland soils with a total P content of approximately 2 Mg/ha (0-30cm).

CHAPTER 1: Biotic and abiotic controls on diurnal fluctuations in labile soil phosphorus of a wet tropical forest.

Abstract

The productivity of many tropical wet forests is generally limited by bio-available phosphorus (P). Microbial activity is a key regulator of P availability in that it determines both the supply of P through organic matter decomposition and the depletion of bio-available P through microbial uptake. Both microbial uptake and mineralization occur rapidly and their net effect on P availability varies with soil moisture, temperature, and soil organic matter quantity and quality. Exploring the mechanisms driving P availability at fine temporal scales can provide insight into the coupling of carbon, water, and nutrient cycles, and ultimately, the response of tropical forests to climate change. Despite the recognized importance of P cycling to the dynamics of wet tropical forests and their potential sensitivity to short term fluctuations in bio-available P, the diurnal pattern of P remains poorly understood. This study quantifies diurnal fluctuations in labile soil P and evaluates the importance of biotic and abiotic factors in driving these patterns. To this end, bi-hourly measurements of labile P were made in a Costa Rican wet tropical forest Oxisol. Spatial and temporal variation in Bray extractable P were investigated in relation to ecosystem carbon flux, soil CO_2 efflux, soil moisture, soil temperature, solar radiation and sap flow velocity. Spatially-averaged bi-hourly labile P ranged from 0.88-2.48 µg/g across days. The amplitude in labile P throughout the day was 0.61-0.82 µg/g (41-54% of mean P concentrations) and was characterized by a bimodal pattern with a decrease at midday. Labile P increased with soil CO_2 efflux and

soil temperature and declined with increasing sap flow and solar radiation. Together, soil CO_2 efflux, soil temperature and sap flow explained 86% of variation in labile P.

Introduction

In many tropical wet forests, primary productivity is constrained by low levels of bio-available phosphorus (P) (Walker and Syers 1976, Vitousek 1984). Iron and aluminum oxide minerals, abundant in highly weathered soils, can rapidly bind phosphate in insoluble complexes, making it less available to the biota (Schlesinger 1997). Labile P, defined as phosphate (PO_4^{3-}) that is in soil solution or that can rapidly desorb from inorganic and organic soil components, is the P fraction thought to be available for immediate biological uptake (Tiessen and Moir 1993, Cross and Schlesinger 1995). The size of this pool is strongly time dependent due to rapid geochemical and biologically mediated P transformations (Olander and Vitousek 2004, 2005). Lack of a widely accepted and reliable method for quantifying P fluxes through the bio-available pool complicates the investigation of soil P dynamics. The limited temporal and spatial resolution of P cycling studies constrains our understanding of the controls on P availability.

Microbial activity is undoubtedly a key regulator of phosphorus cycling in tropical forests; bio-available P is replenished in large part through decomposition of organic matter (Tiessen et al. 1994). Microbial biomass, which comprises up to 30% of the organic-P in soils (Chapin et al. 2002) can act as a source (mineralization) or a sink (immobilization) for plant-available nutrients. Microbial uptake of labile P occurs rapidly, within a matter of minutes (Olander and Vitousek 2004, 2005), but the net effect of the microbial community on P availability depends on abiotic factors such as soil

moisture and temperature, the quantity and quality of carbon (C) sources for microbial growth (Howard and Howard 1993, Yuste et al. 2007) as well as soil P status (Cleveland et al. 2002).

Despite the strong affinity of tropical soils for P, Olander and Vitousek (2004, 2005) found that microbial demand, rather than soil sorption capacity, determined the partitioning of newly added P into biological versus geochemical sinks. When nutrient availability was increased by long-term N and P fertilization the importance of microbial controls over P partitioning declined. In addition, when microbial demand for P was increased by the addition of carbon (C), microbes were able to access tracer-P from the geochemically sorbed pool (Olander and Vitousek 2004). When P supply is low, ectomycorrhizal fungi and plant roots can produce extracellular phosphatases that allow them to access even the most recalcitrant forms of P (McGill and Cole 1981, Dakora and Phillips 2002, Barroso and Nahas 2005). These findings suggest that, in P-deficient soils, there are strong biological controls on P availability.

Microbes are thought to out-compete plants for available P over short timescales due to the high P concentration of microbial biomass as well as its high surface area to volume ratio, rapid growth and high turnover rates (Cole et al. 1977, Schimel et al. 1989, Singh et al. 1989). Never the less, transpiration-driven water flow and the associated passive uptake of nutrients by plants is an important regulator of soil nutrient availability, although surprisingly little is known about nutrient uptake rates by roots in the field (Novak and Vidovic 2003, Lucash et al. 2007). Plant uptake, which is limited to the region around roots, can be enhanced by extensive root exploration, changes in root morphology (Lynch and Ho 2005, Wang et al. 2008), soil surface root mats (Luizao et al.

2007) and mycorrhizal associations (Bolan 1991). In addition, root exudation rates, which are coupled to rates of photosynthesis over a period of minutes to hours (Dilkes et al. 2004), may drive short term changes in microbial activity by providing a source of labile soil C. If plant uptake is a major control on labile soil P, local variation in the vegetation structure should affect diurnal P dynamics.

Both *in situ* studies of seasonal changes in soil P fractions and lab incubation studies have emphasized the importance of soil moisture in constraining P availability in tropical forest soils (Singh et al. 1989, Yavitt et al. 1993, Lodge 1994, Campo et al. 1998, Grierson et al. 1998, McGrath et al. 2000). P fluxes associated with seasonal wetting and drying cycles have been attributed to rapid turnover of the microbial biomass. However, the importance of seasonal drivers may or may not hold true at shorter timescales. Lab incubation studies, although useful for isolating variables of interest, do not adequately reflect environmental controls on P mineralization, plant uptake, and biogeochemical processes that influence adsorption. To our knowledge, no previous studies have investigated diurnal variability in labile P or possible driving mechanisms in a natural ecosystem. Given its potential to limit plant growth, the temporal variability in labile P is understudied.

The goals of this study were to describe diurnal fluctuations in labile P in a wet tropical forest and to elucidate the mechanisms driving these patterns. We expected to find a strong coupling between P dynamics and diurnal variation in environmental conditions that influence microbial and plant activity. Our main objectives were: 1) to quantify temporal variation in soil labile P at bi-hourly intervals in the surface horizon of a tropical Oxisol with high P-sorption capacity; 2) to compare this temporal variability

with spatial variability on the scale of meters; and 3) to evaluate how environmental conditions (soil moisture, soil temperature and solar radiation) and biological activity (ecosystem C flux, soil CO_2 efflux and sap flow velocity) influence labile P.

Methods

Study Site

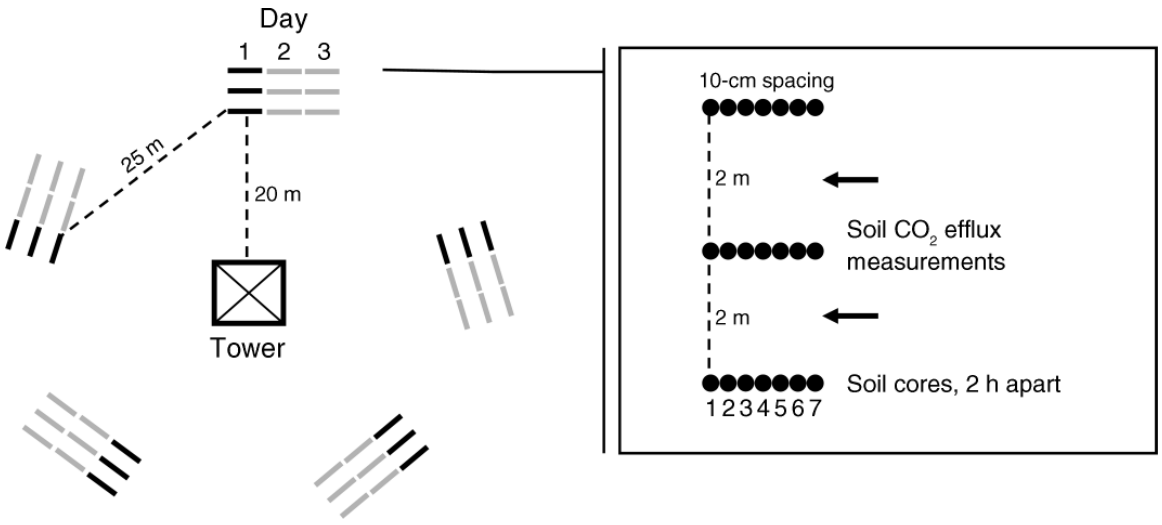
Our study was conducted in old-growth wet tropical forest at La Selva Biological Station in the Caribbean lowlands of northern Costa Rica (10° 26' N, 83° 59' W). Average annual rainfall is 4300 mm and average annual temperature of 25.8 ºC. Mean daily temperature fluctuates <3 ºC between months, while the average diurnal fluctuation in air temperature is 6-12 ºC (Organization for Tropical Studies meteorological data). Soil P can be in the deficient range and has the potential to limit plant growth (Denslow et al. 1987) and litter production (Wood et al. in press). Our study was conducted on an Oxisol with a total C, N and P content of 29.45, 2.54, and 0.62 Mg/ha (0-10 cm) respectively. These soils are characterized by low bulk density (0.63 g/cm^3) and low pH (4.3) (0-5 cm) (Espeleta and Clark 2007).

Soil Sampling and Chemical Analysis

Soil cores (2.5 cm diameter) were taken from the surface (0-10 cm) every two hours from 6am-6pm on three days approximately one week apart, during the rainy season of 2006 (July 22, 29 and August 8). Rainfall during the week prior to Day 1 was moderate, prior to Day 2 was low and prior to Day 3 was relatively high. Samples were collected in a nested design with five plots systematically arranged around a carbon-exchange tower (Figure 1). Within each plot, three 0.6 m long, parallel transects were positioned 2 m apart. During each sampling period, one soil core was collected from

each transect in randomized order. Samples were sieved (2 mm) immediately and extracted in the field. Five grams of field moist soil were shaken for one minute in 25 ml of a 0.03N NH_4F and 0.025N HCL solution (Bray and Kurtz 1945). Then, the extractant was filtered and taken to the laboratory for refrigeration. The P concentration of extracts was determined colorometrically using a molybdate blue methodology on an Alpkem Flow Solution IV Autoanalyzer (OI Analytical; College Station, Texas, USA). Sub-samples of each core were oven-dried at 105ºC for 24 hours to determine gravimetric soil moisture. All data are reported on a dry weight basis.

Figure 2: Field sampling design at La Selva Biological Station, Costa Rica.
Samples were collected in a nested design with five plots systematically arranged around a carbon-exchange tower. Day 1 transects are indicated in black while transects from Day 2 and Day 3 are indicated in light gray.



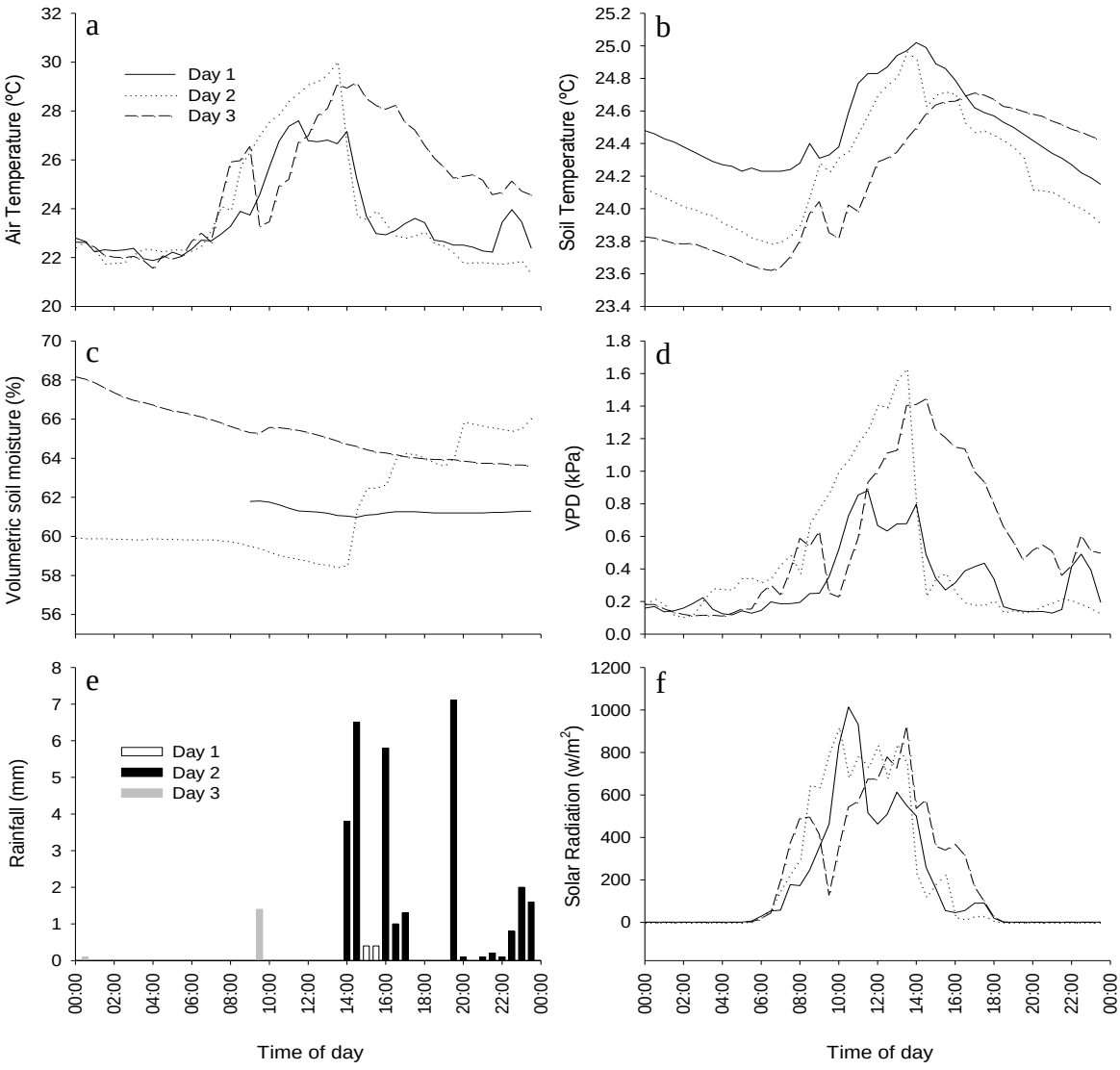
Environmental Variables & Indices of Biological Activity Derived From Them

We recorded ambient temperature, precipitation, solar radiation, and vapor pressure deficit (VPD) at half hour intervals atop a 42 m tower using a Campbell CR10X

data logger. We measured soil temperature with copper constantan thermocouples at 2.5 cm depth (n=3).

Figure 3: Environmental conditions during the three study days.

Environmental conditions measured at half hour intervals included a) air temperature, b) soil temperature, c) volumetric soil moisture as measured by Campbell CS615 (Campbell Scientific, Logan, Utah, USA) sensors (n=3, 0-30 cm) calibrated for La Selva soils (Veldkamp and O'Brien 2000), d) vapor pressor deficit, e) rainfall, and f) solar radiation.



Soil CO_2 Efflux Measurements

During each sampling period two CO_2 efflux measurements were made between transects within each plot (Figure 1). Plot order was randomized. We used a closed aluminum flux chamber that was pressed into the soil and allowed to equilibrate for approximately 6 minutes. Air was circulated at a flow rate of 0.6 L min^{-1} between an infrared CO_2 gas analyzer (LI-800; LI-COR; Inc., Lincoln, Nebraska USA) and the flux chamber. To prevent pressure differences between the chamber and the atmosphere, the chamber was vented to the atmosphere through a 0.25 m long stainless steel tube (1.5 mm inner diameter). CO_2 concentrations were recorded at 5 second intervals with a datalogger (Campbell CR510X; Campbell Scientific, Inc., Logan, Utah USA). CO_2 flux was calculated from a linear regression of CO_2 concentration within the chamber versus time. Due to failure of the data logger, measurements were only successfully recorded throughout the day on Day 1 and from 6am-12:00pm on Day 2.

Vegetation Structure and Composition

Stem density, basal area and species composition were tabulated in nested circular plots surrounding each of the five plots (Table 1). All individuals with DBH >10 cm were measured and identified in 10 m radius circular plots (314 m^2) around the center point of each of the five plots. All individuals with DBH > 5 cm and <10 cm were measured in concentric 5 m radius circular plots (79 m^2) within the larger plots.

Statistical Analyses

All data were analyzed using SAS Systems for Windows V9.1 (SAS Institute, Inc.). A repeated measures analysis of variance was used to detect significant differences in P through time and space on all three days. The Tukey-Kramer technique was used to

test for significant differences between sampling periods, plots and days. In order to compare the relative variability of labile P at the two spatial scales and at the two temporal scales variance components were generated using an ANOVA with time, day, plot, and transect as random effects. Linear regression was used to examine the relationship between soil labile P and possible drivers of labile P including ecosystem C flux, soil CO_2 efflux, sap flow velocity, soil temperature, and gravimetric soil moisture. Variables were tested based on mean or integrated values at the time of sampling and 30, 60 and 90 minutes prior to sampling using a 30 minute interval of data for each lag (thus 0-30 minutes prior = no lag, 30-60 minutes prior = 30 minute lag, etc.). Soil CO_2 efflux and gravimetric soil moisture were only tested based on values at the time of soil sampling and 120 minutes prior to sampling because measurements were not made at intervals less than 120 minutes. Regressions to test possible drivers included data from all sampling periods on all three days. Following visual inspection of the data, regressions were also run on specific parts of the day when relationships appeared stronger. Stepwise multiple regression was used to define a model explaining labile P based on a combination of factors, none of which had a variance inflation factor greater than 1.43. Finally, linear regression was used to explore the effect of plot-level plant basal area, stem density and palm density on average soil labile P.

Results

Temporal and Spatial Variation in Soil Labile P

Labile P varied significantly throughout the day ($F=5.57$, $p<0.0001$, $df=6$). The diurnal pattern was characterized by a bimodal curve with a decrease at midday (Figure 3). Labile P varied significantly across days with higher Bray P on Day 1 (2.0 µg/g) than

on Day 2 (1.3 µg/g; $F=48.67$, $p<0.0001$, $df=2$) and intermediate levels on Day 3 (1.5 µg/g). Variability between days was 2.4 times greater than variability between plots. Between-plot spatial variation (25-40 meters) in labile P ($F = 6.44$, $p = 0.0079$, $df=4$) was 2 times greater than temporal variability throughout the day. Between-transect spatial variation (2-4 meters) in labile P was 50% less than temporal variability throughout the day.

Biotic Controls on Soil Labile P

Labile P was positively correlated with soil CO_2 efflux 120 minutes prior to soil sampling ($r^2=0.56$, $F=10.25$, $p=0.0126$, $n=21$). This relationship was strongest from 8am-2pm ($r^2=0.75$, $F=17.50$, $p=0.0058$, $n=12$; Figure 4a). Labile P was negatively correlated with sap flow velocity 30 minutes prior to sampling from 8am-4pm ($r^2=0.26$, $F=4.67$, $p=0.0500$, $n=15$) with the strongest relationship from 8am-2pm ($r^2=0.36$, $F=5.58$, $p=0.0397$, $n=12$; Figure 4b). Labile P increased with ecosystem C flux at the time of soil sampling on all three days ($r^2=0.25$, $F=3.94$, $p=0.07$, $n=14$), however this relationship was only significant on Days 2 and 3 ($r^2=0.73$, $F=13.59$, $p=0.0142$, $n=7$; Figure 4c). Plot-level plant basal area was positively related to average soil labile P ($r^2=0.83$, $F=15.05$, $p=0.0303$, $n=5$; Figure 4d) while stem density and palm density were not.

Abiotic Controls on Soil Labile P

Labile P was positively related to soil temperature 90 minutes prior to sampling ($r^2=0.24$, $F=5.98$, $p=0.0243$, $n=21$). This relationship was strongest from 10am-6pm ($r^2=0.49$, $F=12.47$, $p=0.0037$, $n=15$; Figure 4e). Labile P declined with solar radiation 30 minutes prior to sampling from 8am-4pm ($r^2=0.36$, $F=7.42$, $p=0.0174$, $n=15$; Figure 4f). Labile P was not correlated with gravimetric soil moisture from 0-120 minutes prior.

Eighty-six percent of the variability in labile P was explained by a combination of CO_2 efflux, soil temperature and sap flow in a stepwise regression (r^2=0.86, F=12.64, p=0.0053, n=10).

Discussion

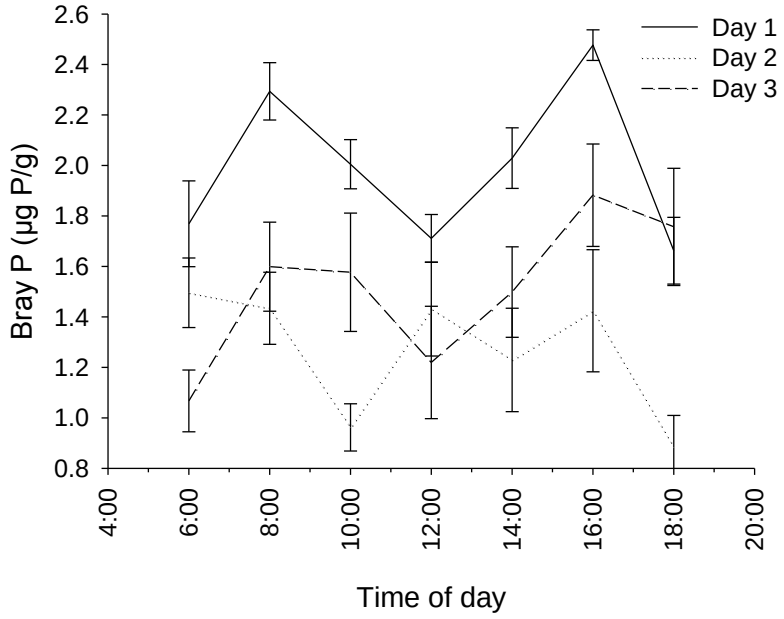
The bimodal diurnal pattern in P availability appears to be driven biologically, by the integrated response of the microbial and plant communities to environmental conditions, the relative influence of which varies throughout the day. Factors associated with enhanced microbial activity (soil temperature, soil CO_2 efflux) correspond with an increase in labile P; those associated with enhanced plant activity (sap flow velocity and solar radiation) correspond with a drawdown in labile P. Indicators of microbial activity are related at time lags of 90-120 minutes; indicators of plant activity are related at a time lag of 30 minutes. The delayed response of labile P to microbial drivers may reflect immobilization by a P limited microbial community in these P-deficient soils (Cleveland et al. 2002). If the microbial community is P rather than C limited this may explain why we did not find a positive correlation between solar radiation and labile P, which we expected as a result of the stimulatory effect of root exudates on microbial activity.

The bimodal pattern in labile P is quite clear on Day 1 (Figure 3). On Day 2 the drawdown in labile P occurs at 10am rather than 12pm. As expected if environmental constraints on plant activity are influencing labile P concentrations in the soil, VPD, solar radiation and air temperature also peak earlier in the day on Day 2 (Figure 2). Day 3 exhibits the same bimodal pattern as Day 1 absent the large decline in labile P at the end of the day. However, solar radiation, VPD, air temperature and soil temperature also peak later in the day than on the other study days (Figure 2). Future studies should

include a 24 hour investigation of P dynamics to clarify when and if labile P regularly returns to early morning levels.

Figure 4: Spatially-averaged labile P on the three study days.

Spatially-averaged Bray-extractable P (mean ± SE) throughout the day on each of the three study days, a) 22 July b) 29 July, and c) 8 August 2006.



Using a mass balance approach, we determined that the range in labile P throughout the day can not be explained entirely by the mineralization of P from new inputs of leaf litter. Annual litterfall P in our forest is ca. 6 kg/ha/yr (Wood et al. 2006). At this rate, 16.4 g/ha or 0.164 µg/cm^2 of P are added to the soil surface each day. Integrated over 10 cm, at a bulk density of 0.63, this is equivalent to approximately 0.03 µg/g. Assuming a similar magnitude of P inputs from belowground litter yields an estimate that is an order of magnitude smaller than the diurnal range in labile P, which varied from 0.61-0.82 µg/g. In nutrient poor systems, like the tropics, the biota have developed various nutrient conservation mechanisms that lead to tight internal cycling of limiting nutrients. The discrepancy between potential P sources and observed net fluxes

may be explained in part by the recycling of labile P within the microbial community. Microbial immobilization may represent an important mechanism for retaining P in biologically available pools.

In contrast to long-term field studies and lab incubations, soil moisture did not control diurnal P availability (Singh et al. 1989, Yavitt et al. 1993, Lodge 1994, Campo et al. 1998, Grierson et al. 1998, McGrath et al. 2000). Our study was conducted during the rainy season and gravimetric soil moisture content remained between 46 and 50%, a level unlikely to constrain microbial or plant activity (Howard and Howard 1993, Schwendenmann et al. 2003, Yuste et al. 2007). Soil moisture may only become an important regulator of P availability on a seasonal timescale, when moisture becomes limiting or soils experience wetting and drying cycles. Investigation of diurnal P dynamics during the dry season or in a dry tropical forest where soil moisture is more variable is an interesting avenue for further research.

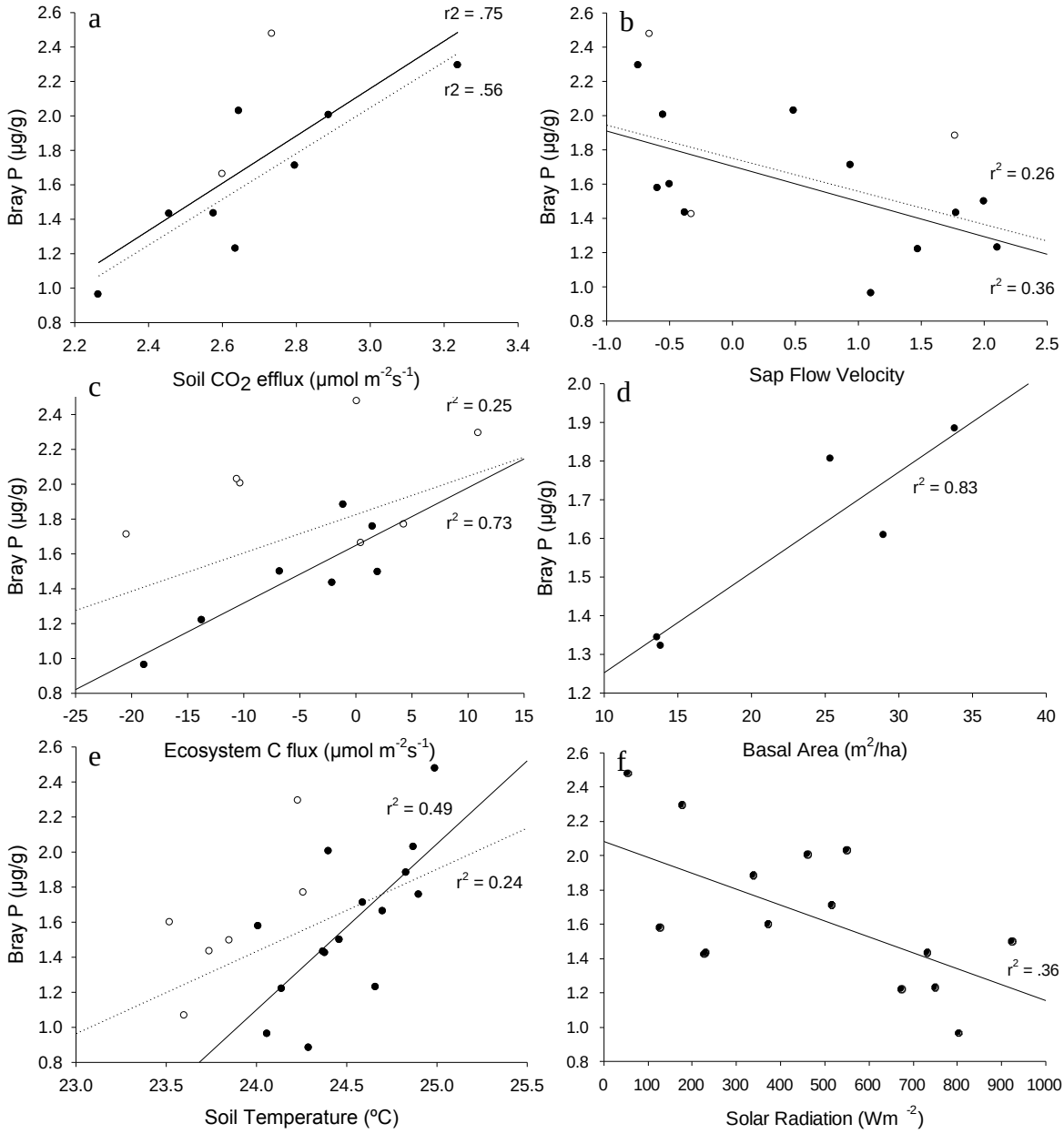
Soil temperature, one of the dominant drivers of microbial activity, was an important regulator of P availability on a diurnal timescale, particularly from 10am-6pm. This is not surprising, given the diurnal range in the temperature of the surface horizon. The 10am-6pm timeframe may coincide with a temperature threshold for increased microbial activity which is only reached by late morning (10am) in closed canopy tropical forests due to the time-lag between inputs of solar radiation and increases in soil temperature (Figure 2b vs. 2f).

As evidenced by the decline in labile P with solar radiation and sap flow velocity, plants played a significant role in structuring temporal variation in labile soil P. Plant uptake of P coupled with evapotranspiration and/or photosynthesis during times of peak

plant activity (8am-4pm) corresponded with the midday drawdown in labile P. In addition, spatial variation in labile P was strongly correlated with vegetation structure on the scale of tens of meters with higher basal area associated with higher labile P. Given the diurnal patterns, we might have expected greater basal area (and greater plant demand) to correspond with lower P availability, Instead, plant controls on mean P availability over longer timescales seem to differ substantially from controls on a diurnal timescale. Given that within-stand basal area correlates well with litter inputs at La Selva (Lawrence unpublished data) spatial variation in mean labile P over the three days may be explained by higher inputs of P through litterfall and belowground root turnover in areas with greater plant basal area.

Figure 5: Relationships between soil labile P and biotic and abiotic drivers.

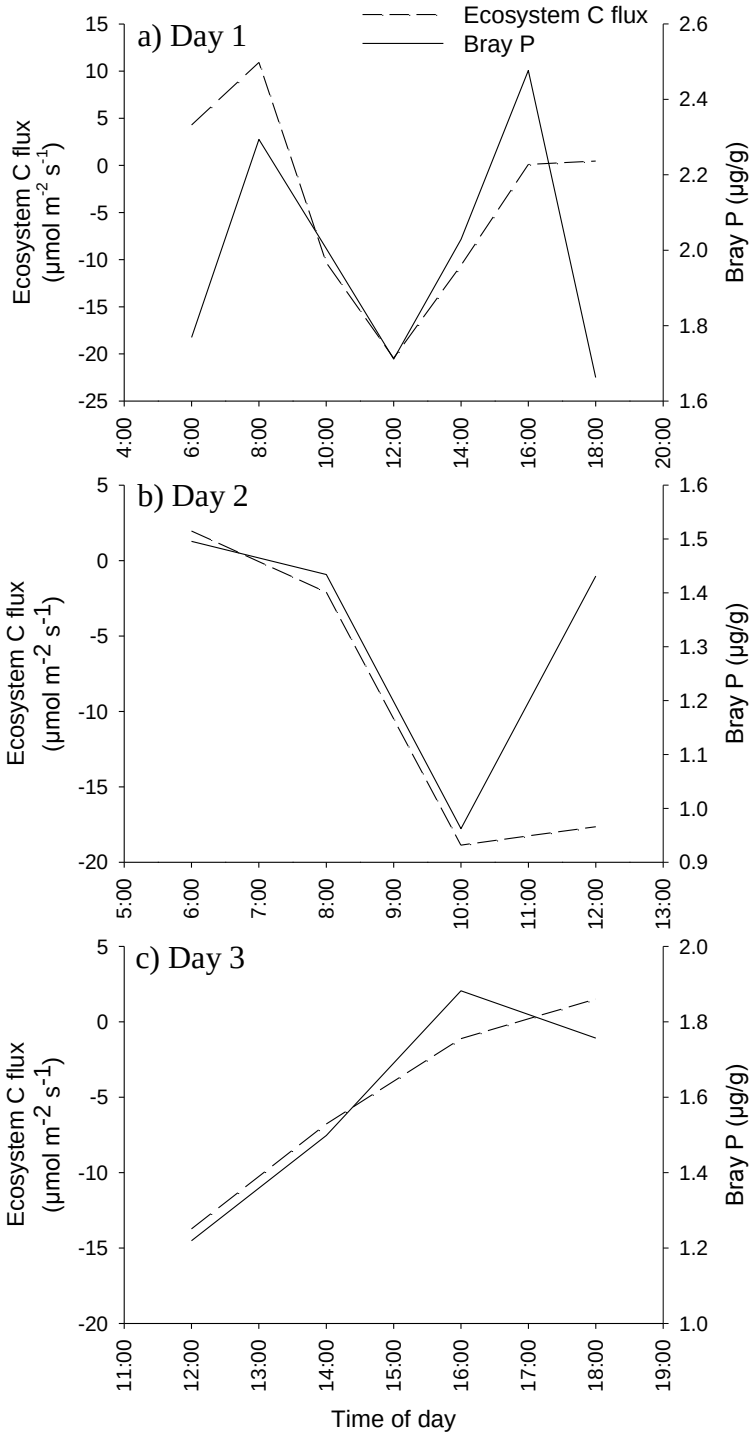
Soil labile P as a function of: a) soil CO_2 efflux 120 minutes prior to sampling from 08:00-18:00 (08:00-14:00 hours in solid black, which represents the strongest relathionship) b) sap flow velocity index 30 minutes prior to sampling from 08:00-14:00 hours in solid black, c) ecosystem C flux at the time of sampling from 06:00-18:00 hours (Days 2-3 in black) d) plot-level plant basal area, e) soil temperature 90 minutes prior to sampling from 06:00-18:00 (10:00-18:00 hours in solid black) f) solar radiation 30 minutes prior to sampling from 08:00-16:00 hours. The strongest relationships are represented by solid symbols and a solid regression line.



The factors that best captured temporal variability in labile P were the two integrated measures of plant and microbial activity; soil CO_2 efflux and ecosystem C flux (Figure 4a and 4c and Figure 5). As the product of both root respiration and heterotrophic respiration (Silver et al. 2005, Trumbore et al. 2006), soil CO_2 efflux represents the integrated response of the microbial and plant community to climatic conditions, although we have emphasized the microbial response. Ecosystem C flux integrates C assimilation by plants and ecosystem respiration. When C flux was positive (respiration exceeding uptake), labile P was high; when plant uptake dominated, labile P was low. It is clear that P dynamics in P-poor tropical soils are quite sensitive to environmental conditions and are closely coupled to important ecosystem processes such as soil respiration, net carbon exchange, and plant conductance over a matter of minutes to hours.

Figure 6: Ecosystem C flux on the three study days.

Ecosystem C flux at the time of sampling and spatially-averaged labile P on each of the study days: a) 22 July b) 29 July c) 8 August 2006. No noon C flux measurement was recorded on Day 2; the 11:45 measurement was used in its place.



This work highlights the need for careful attention to scale dependence in ecological research and has implications for the methodological design of long term P studies. Our findings suggest that for accurate estimation of labile P under field conditions, sampling integrated across time is equally as important as sampling integrated across space. Comparative studies measuring longer-term variation in soil P may incur errors if they do not account for the diurnal variation in P.

CHAPTER 2: Environmental controls on *in situ* soil phosphorus availability in tropical rain forest soils: Evidence from short-term anion exchange resin membrane incubations

Abstract

Bioavailable phosphorus (P) is recognized as a major constraint on productivity in many tropical rain forests. In highly weathered soils, replenishment of labile P is strongly dependent on environmental factors which influence the decomposition of organic matter on the forest floor. However, insufficient knowledge about short-term temporal patterns in soil labile P limits our understanding of the mechanisms controlling soil P supply in tropical rain forest soils. This study quantifies *in situ* labile P as measured by anion exchange resin membranes (AEMs) over various incubation periods (4, 8, 12, 14, 20 and 24 days) in the surface soil of two neighboring Costa Rican tropical rain forest Oxisols that are similar in most ways, but that differ in P content (total P: 0.46 vs. 1.18 Mg/ha, 0-10 cm). Using a nested design, we evaluated the importance of incubation length, environmental conditions (i.e. cumulative rainfall, mean temperature, and cumulative solar radiation during 1-24 days prior to AEM extraction from the soil), vegetation characteristics (i.e. basal area, stem density, and species composition), and total P status in driving spatial and temporal variability in AEM P. Spatially-averaged AEM P in the site with higher P content ranged from 0.26-1.38 µg/membrane across all incubation periods; on average 170% higher than AEM P in the more P-deficient site (0.11-0.77 µg/membrane). Although AEMs functioned as dynamic exchangers, they were able to detect fine-scale temporal and spatial variability in labile P associated with environmental conditions and within-stand vegetation characteristics in both sites.

Temporal variability in AEM P was best explained by rainfall. Despite consistently high volumetric soil moisture (50-66%), increasing rainfall was associated with greater P availability in both sites. AEM P was also associated with temperature and solar radiation, however, this relationship was positive in the site with lower P content and negative in the site with higher P content. The timing of AEM P responses to environmental signals differed as a function of total P status, with the less fertile soil responding more rapidly to environmental cues. Mean P availability increased with plant basal area and basal area of legumes.

Introduction

Despite the recognized importance of P cycling to the productivity of tropical rain forests (Vitousek 1984), fine temporal and spatial scale soil P dynamics remain poorly understood. Replenishment of bioavailable P, particularly in P-deficient soils, is controlled in large part by the biologically mediated process of decomposition and the relative rates of microbial mineralization and immobilization (Chapin et al. 2002). Whether microbial decomposers act as a source or a sink for plant-available nutrients is directly affected by environmental factors such as moisture and temperature, the quantity and quality of carbon (C) sources for microbial growth (Swift et al. 1979) and soil P status (Crews et al. 1995, Vitousek and Farrington 1997, Hobbie and Vitousek 2000, Cleveland et al. 2002, - 2006). Microbial biomass responds strongly and rapidly to changing environmental conditions, particularly moisture availability (Sparling et al. 1987, Yang and Insam 1991, Srivastava 1992, Luizao et al. 1992, Grierson et al. 1998, Pandey and Srivastava 2009). However, the coarse temporal resolution of conventional *in situ* P cycling studies limits our understanding of transient fluctuations in nutrient

availability associated with environmental conditions. The goal of this study was to evaluate the relationship between potential environmental drivers of labile P on a timescale of days to weeks in tropical soils of contrasting P fertility. Our main objectives were: 1. to quantify *in situ* anion exchange resin membrane (AEM) P over various incubation lengths in two Oxisols of differing P content in order to improve our understanding of AEM dynamics in tropical soils; 2. to evaluate the influence of environmental conditions (precipitation, temperature and solar radiation) on soil labile P dynamics and determine time lags between environmental signals and P response; and 3. to explore links between local vegetation characteristics (basal area, stem density, and species composition) and mean AEM P.

Previous work investigating climatic controls on P supply in tropical soils has focused on seasonal variation and has emphasized the importance of soil moisture in regulating P availability (Singh et al. 1989, Yavitt et al. 1993, Yavitt and Wright 1996, Lodge 1994, Campo et al. 1998, McGrath et al. 2000). Strong pulses in nutrient availability have been observed at the onset of the rainy season in a number of tropical forests (Singh et al. 1989, Davidson 1993, Lodge et al. 1994, McGrath et al. 2000) but not in others (Yavitt and Wright 1996, Matson et al. 1987, Vitousek and Denslow 1986). Seasonal nutrient pulses have been attributed to the lysis of microbial biomass due to osmotic stress as well as leaching of nutrients from litter accumulated during the dry season (Sparling et al. 1987, Singh et al. 1989, Lodge et al. 1994, Grierson et al. 1998, McGrath et al. 2000). Seasonality in nutrient availability has been documented predominantly in soils at the dry end of the tropical forest precipitation spectrum where seasonal variability in rainfall and litterfall is more pronounced. It is unclear whether the

effects of rainfall on P dynamics observed in drier tropical forests remain valid in the wettest regions of the tropics where precipitation is rarely limiting. Soil saturation can lead to anaerobic conditions that hinder decomposition and nutrient mineralization from organic matter (Schuur 2001). However, anoxic conditions also cause changes in soil redox potential that can lead to the solubilization of P through iron reduction (Miller et al. 2001, Wright et al. 2001, Peretyazhko and Sposito 2005, Chacon et al. 2006, -2008, Pandy and Srivastava 2009). Clearly, moisture availability has strong effects on processes controlling P cycling, however, there is a general lack of information on the magnitude and timing of P responses to rainfall in wetter regions of the tropics.

The size of the bioavailable P pool is strongly time dependent in P-deficient soils as a result of rapid biological and geochemical P transformations (McKean and Warren 1996, Olander and Vitousek 2004, -2005, Vandecar et al. 2009). Rainfall can induce rapid changes in labile P through influences on P diffusion rates, geochemical reaction rates, leaching of P from the litter layer, decomposition rates, microbial biomass and activity, and plant uptake (Lodge et al. 1994, Chapin et al. 2002, Austin and Vitousek 2000). Laboratory incubation studies on seasonal tropical and subtropical forest soils indicate that P responses to moisture are not always linear and that wetting can drive pulses in P availability on the scale of days to weeks (Campo et al. 1998, Grierson et al. 1998, DeLonge 2007). Additionally, antecedent conditions and the level of wetting appear to modulate the magnitude and timing of P fluctuations, with heavy rain events leading to greater and more prolonged pulses in labile P (Campo et al. 1998, Grierson et al. 1998, Delonge 2007). Rain-induced pulses in P availability may be short-lived and the timing of sampling in relation to heavy rainfall events may have a significant impact

on P estimates. Infrequent sampling in long term nutrient cycling studies may not adequately capture rapid environmentally-driven fluctuations in labile P.

In wet tropical forests where moisture availability remains high, litter chemistry and soil fertility drive local-scale variation in decomposition and have the potential to influence P availability through a variety of processes (Ewel 1976, Vitousek 1982, Hobbie 1992, Crews et al. 1995, Scrowcroft et al. 2000, Hobbie and Vitousek 2000, Xuluc-Tolosa 2003, Vasnocelos and Laurance 2005, Wieder et al. 2008, - 2009). Low fertility tropical forests tend to produce similar quantities of litter, but lower quality litter than forests on more fertile soil (Edwards 1982, Vitousek 1984, Vitousek and Sanford 1986, Crews 1995, Aerts and Chapin 2000, McDonald and Healy 2000, Wood et al. 2006). The P content of litter, lignin:P ratios, and the leaching of dissolved organic P from litter, can vary greatly between species and can be even more important than rainfall in determining rates of decomposition (Wieder et al. 2009). Furthermore, low quality litter can act as a sink for soil labile P with immobilization of P in leaf litter varying from a month to several years in tropical forests where soil P is limiting (Montagnini et al. 1993, Cornejo et al. 1994, Songwe et al. 1995, Hobbie and Vitousek 2000, McGrath et al. 2000). Labile P in more fertile sites may respond to environmental change more rapidly than in less fertile sites due to the buffering of microbial immobilization.

Conventional P extractions and lab incubations are not sensitive to the on-site processes that influence P release from decomposing material. AEMs used *in situ* may provide a more accurate estimate of P bioavailability. They are thought to be functionally similar to plant roots, acting as sinks for P ions made available by both biological and geochemical release (Menon et al. 1990, Skogley et al. 1990, Yang and

Jacobsen 1990, Walbridge 1991). Numerous studies have demonstrated that AEM P is highly correlated with soil solution P, even at low solution concentrations (0-2mgP/L), and is closely related to measures of plant uptake in various soil conditions (Menon et al. 1990, Schoenau and Huang 1991, Walbridge 1991, Abrams and Jarrell 1992, Qian et al. 1992, Cooperband and Logan 1994, Fernandes and Coutinho 1997, Weih 1998, Turrion et al. 1999, Ziadi 2000, Bissani et al. 2002, Mallarino and Attia 2005, Shirvani et al. 2005). Resin filled bags, and more recently AEMs, have been used successfully *in situ* to evaluate nutrient bioavailability at fine temporal and spatial scales in a variety of arid, temperate and arctic ecosystems (Sibbesen 1977, 1978, Hart and Binkley 1985, Gibson et al. 1986, Krause and Ramlal 1987, Lajtha 1988, Lajtha and Schelsinger 1988, Skogley et al. 1990, Walbridge 1991, Giblin et al. 1994, Huang and Schoenau 1996, Turrion et al. 1997, Weigh 1998, Wright et al. 2001, Drohan et al. 2005, Gill et al. 2006). However, their use in tropical forest ecosystems has been more limited and there has been some debate about interpretation of AEM dynamics in tropical soils (Cooperband and Logan 1994, Crews et al. 1995, Yavitt and Wright 1996, McGrath et al. 2000, Idol et al. 2007, Meason and Idol 2008). Evidence suggests that in soils with low solution P and high sorption capacity, AEMs act as dynamic exchangers rather than infinite sinks for P due to competition with microbes, plants, and soil geochemical processes (Krause and Ramlal 1987, Cooperband and Logan 1994, McGrath et al. 2000, Drohan et al. 2005, Meason and Idol 2008). AEMs adsorb anions from solution until an equilibrium is reached, after which they are thought to provide a composite measure of soil labile P that is dependent on soil P sorption capacity, microbial P demand, and the level of plant available P (Cooperband and Logan 1994, McGrath et al. 2000, Qian and Schoenau 2002, Drohan et

al. 2005, Meason and Idol 2008). The aim of this study was to provide a more comprehensive understanding of AEM dynamics in tropical soils and to explore the response of labile P to short-term variation in environmental conditions and vegetation characteristics in soils of contrasting P fertility.

Methods

Study Site and Field Sampling

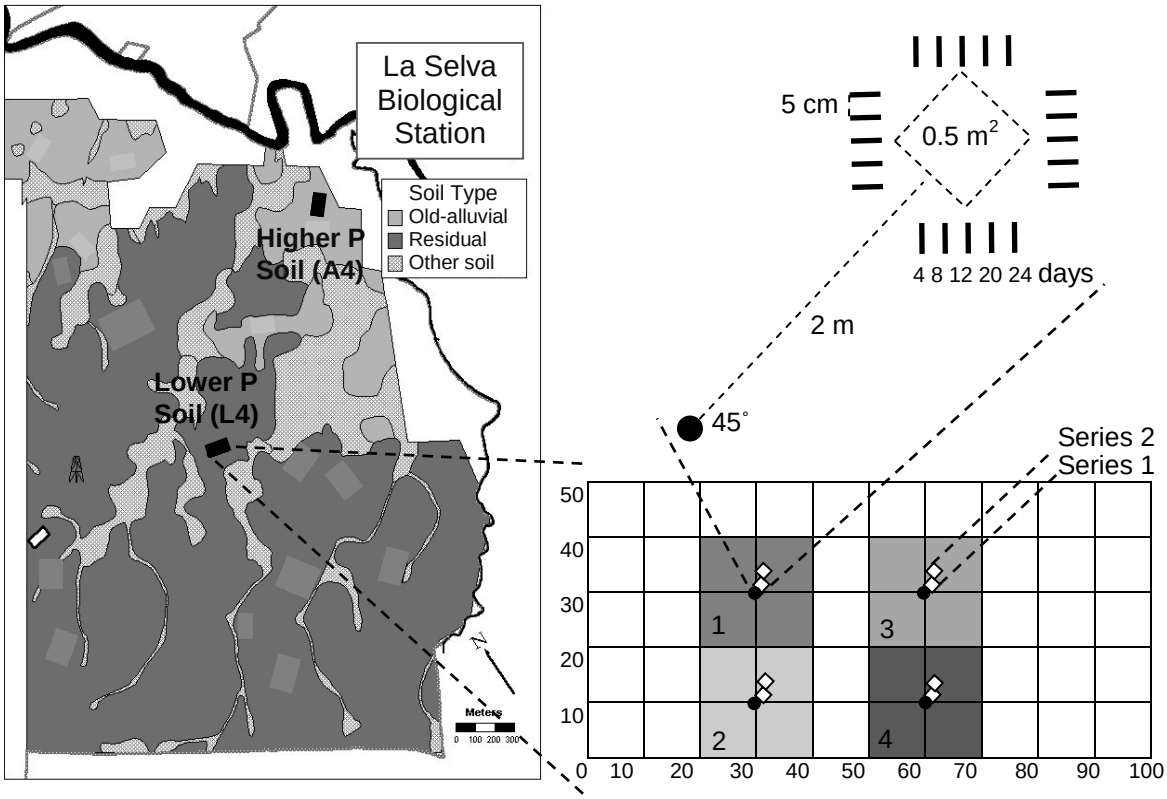
The study was conducted in mature tropical rain forest at La Selva Biological Station in the Caribbean lowlands of northern Costa Rica (10° 26' N, 83° 59' W; Organization for Tropical Studies). La Selva has a mean annual precipitation of 4300 mm (Organization for Tropical Studies, *unpublished data [available online]*), with the driest period occurring between late January and April (McDade et al. 1994). Weekly rainfall ranges from 10 mm to 147 mm. Mean annual temperature is 25.8 ºC, with a much greater diurnal range in temperature (6-12 ºC) than the range in mean monthly temperature (<3ºC, McDade et al. 1994). La Selva is characterized by deep, acidic, clay-rich soils that are volcanic in origin. They span a wide range of P fertility (Espeleta & Clark 2007); soil P is sometimes in the deficient range and has the potential to limit plant growth (Denslow et al. 1987, Vitousek and Denslow 1987) and litter production (Wood et al. 2009). The forest is predominantly evergreen (McDade et al. 1994) and is dominated by a N-fixing legume *Pentaclethra macroloba* , which accounts for as much as 36% of the basal area and 13% of stems (McDade et al. 1994, Lieberman et al. 1996, Clark and Clark 2000).

Two sites on Oxisols of differing P content, approximately 1.2 km from one another (Figure 7), were chosen for this study: CARBONO plots A4 and L4 (total soil P:

1.18 Mg/ha and 0.46 Mg/ha (0-10 cm), respectively; Table 2; the CARBONO plot network is described in Espeleta & Clark 2007). Concentrations of extractable P similar to those in the site with lower P content (Bray P 3 µg/g) have been associated with reduced plant growth in bioassays at La Selva (Vitousek and Denslow 1987). In each 50 x 100 m (0.5 ha) plot, a 20 x 20 m subplot sampling area was established at each of four standardized locations within the plot (Figure 7), in order to capture within-site variability. During the rainy season of 2005 (beginning on June 23rd), a set of 5 AEM replicates at approximately 5 cm spacing was placed at each of the four corners of a 0.5 m x 0.5 m square within each subplot (Figure 7). One AEM from each of the four corners in each subplot was collected after 4, 8, 12, 20 and 24 days of incubation. After four days, a second series, consisting of three AEMs at each of the four corners of a 0.5 m^2 square, was installed within a few meters of the first series in each subplot. For the second series one AEM was collected from each of the 4 corners in each subplot after 4, 8, and 20 days of incubation. Two and four days following insertion of the second series another set of four AEMs was placed nearby in the same configuration and incubated for 12 and 14 days respectively. This staggered deployment allowed us to collect the two series during the same visit to the field. Volumetric soil moisture was measured in the center of each plot by a Campbell CS615 (Campbell Scientific, Logan, Utah, USA) sensor (0-30 cm) calibrated for La Selva soils (Veldkamp & O'Brien 2000) on three dates; before, during and after the study.

Figure 7: Location of study plots and field sampling design.

Samples were collected in a nested design with four 20 x 20 m subplots, numbered 1-4, at standardized locations within each plot. On June 23rd of 2005 a set of 5 AEMs at approximately 5 cm spacing were placed at the corners of a 0.5 m^2 sampling areas near the center of each subplot. One AEM from each of the four corners of the sampling area was collected after 4, 8, 12, 20, and 24 days. A second set of 3 AEMs were placed in the same configuration around a second 0.5 m^2 sampling area and collected after 4, 8, and 20 days. Two and four days following insertion of the second series another set of four AEMs were placed in the same configuration and collected after 12 and 14 days.



AEM preparation and chemical analysis

AEMs were cut into 2.5 by 5.0 cm strips; the equivalent of 1 g of dry resin (GE Infrastructure Sensing, Inc. Part Number A R204SZRA; formerly, Ionics part number 204-U-435). Each AEM contains approximately 1 g of dry resin and has the potential to accumulate 272 mg of P. Bright nylon fishing line was sewn onto each membrane so that it could be easily recovered in the field. AEM strips were rinsed with de-ionized water

(DIW) and pre-saturated with Cl^- using a 5% NaCl solution for at least 24 hours before use. Cl^- was chosen as the counter-ion rather than HCO_3^- in order to minimize effects on soil pH (Cooperband and Logan 1994, Myers et al. 2005). Membranes were then rinsed with DIW and placed in widemouth bottles filled with DIW just before transportation to the field. Membranes were placed in the soil by making a vertical slit with a knife and then sliding the membrane in, so that it stood vertically just below the soil surface (0-5 cm depth). The soil was pressed down and inward around the membrane to insure maximal contact on both sides. When membranes were recovered they were rinsed in the field with DIW and placed in individual sample bags filled with DIW and brought back to the laboratory prior to extraction. AEMs were placed in 50 ml centrifuge tubes and shaken for 2 hours with 20 ml of 0.5M HCl solution. Extractants were frozen in scintillation vials for later analysis. The P concentration of extracts was determined colorometrically using a molybdate blue methodology on an Alpkem Flow Solution IV Autoanalyzer (OI Analytical; College Station, Texas, USA).

Vegetation Structure and Composition

Basal area, stem density and species composition were tabulated in September-October 2005 for all live stems with a diameter at breast height (DBH) greater than 10 cm within each 400 m^2 subplot (Table 3).

Statistical analyses

All data were analyzed using SAS Systems for Windows V9.1 (SAS Institute, Inc.). AEM P values greater than two standard deviations above the mean of all samples (n = 260) were considered outliers (n = 10) and were not included in any analyses. Residuals of all analyses were checked for normality and homogeneity of variance. Data

were log transformed when appropriate to meet the assumptions of analysis of variance (ANOVA). ANOVA was used to determine the effect of incubation length and soil total P content (site) on AEM P. Tukey-Kramer multiple comparison tests were used to determine significant differences between incubation lengths. When a significant effect of soil total P content (site) was found, a separate ANOVA was run for each site to determine the effect of subplot and incubation length on AEM P. To evaluate how P accumulation rate varied with incubation length, a t-test was used to compare P estimates from one long incubation to the sum of P estimates from a series of shorter incubations covering the same time period (ie. the sum of AEM P for 4 day incubations in series 1 and 2 were compared to AEM P for the 8 day incubation in series 1, see Figure 8a; five such comparisons were made for each site). The coefficient of variation among replicates within a site ($11 \leq n \leq 16$; up to 4 replicates per subplot x 4 subplots) was calculated for each incubation length in order to compare spatial variability among sites. Some AEM replicates were not analyzed due to damage or drying in the field, however, each incubation length was represented by at least 11 AEMs and each subplot within an incubation was always represented by at least 2 AEMs.

Daily meteorological station data, including daily cumulative rainfall, mean temperature, and cumulative solar radiation were collected by the Organization for Tropical Studies (OTS) (http://www.ots.ac.cr/en/laselva/metereological.shtml). Spatially-averaged AEM P ($11 \leq n \leq 16$; up to 4 replicates per subplot x 4 subplots) was determined for each incubation length by site. This value was regressed against cumulative rainfall, mean temperature, and cumulative solar radiation during 1 up to 24 days prior to AEM removal from the soil (see Figure 8a for removal dates). In other

words, the climate conditions on the day prior to removal of AEMs from the soil were regressed against mean AEM P for each respective incubation length. Then climate conditions during the two days prior to AEM removal were summed or averaged and regressed against mean AEM P for each incubation length, and so on, up to 24 days prior to AEM removal. This resulted in 10 climate-AEM P pairs for each site (series 1: 4,8,12, 20 and 24 days; series 2: 4, 8, and 20 days, with an additional 12 and 14 day incubation during series 2, Figure 8a) that were used to test for differences in the timing, magnitude and direction of P response to environmental signals as a function of total P content. In this case, the incubation length was disregarded as a factor, and the samples were treated simply as moments in time. Stepwise multiple regression was performed to determine if a combination of environmental drivers (rainfall, temperature and solar radiation) could be used to predict AEM P during 1 up to 24 days prior to AEM removal from the soil.

Linear regression was used to explore the effect of subplot-level plant basal area, basal area of legumes, basal area of palms, and stem density on minimum, maximum, and average subplot AEM P (average of AEMs from all incubation lengths within a subplot), and range in AEM P over all incubation lengths. Regressions were then run again with average subplot AEM P normalized by site mean AEM P (a *z*-score) so the two plots could be combined in one analysis.

Results

Soil Total P Fertility and Incubation Length Effects

Over the 24 days, mean AEM P among all incubation lengths ranged from 0.26-1.38 µg/membrane in the site with higher P content; on average 170% higher than the site with lower P content (0.11-0.77 µg/membrane, Figure 8d). The range in AEM P values

across all incubation periods, once outliers were removed, was 0-3.87 µg/membrane in the more P-deficient site and 0-4.37 µg/membrane in the site with higher P content. AEM P varied significantly by incubation length ($F = 10.17$, $p < 0.0001$) and by total P content (site) ($F = 75.97$, $p < 0.0001$). When sites were tested independently for subplot and incubation length effects, incubation length was a significant driver in both sites (A4: $F = 6.36$, $p < 0.0001$, L4: $F = 5.29$, $p = 0.0002$), but subplot was not. In the site with higher P content (A4) AEM P increased consistently with incubation length, while in the site with lower P content AEM P was lowest for 8 day incubations, peaked at 14 day incubations and then declined with incubation length. The coefficient of variation (CV) between replicates within an incubation period ($n = 16$) was on average 110% higher in the site with lower P content. Longer incubations yielded significantly lower estimates of P availability than a series of shorter deployments covering the same time period in the site with higher P content ($t = 2.66$, $p = .0281$, $df = 4$). However, in the more P-deficient site there was no consistent pattern between P estimated from one long incubation and P estimated from the sum of a series of shorter incubations.

Environmental Controls on AEM P

In the site with higher P content, AEM P was positively related to cumulative rainfall during the 6 days prior to AEM removal and up to 13 days prior (though not significant for 11 days prior), as well as 18 and 19 days prior (Figure 10a). This relationship was strongest for cumulative rainfall during the 6 days prior to AEM removal ($r^2 = 0.54$, $F = 9.52$, $p = 0.0150$, Figure 9a). In the site with lower P content AEM P was positively related to cumulative rainfall during the 2 days and up to 5 days

prior to AEM removal (Figure 10a). This relationship was strongest for rainfall during the 2 days prior to AEM removal ($r^2 = 0.48$, $F = 7.49$, $p = 0.0256$, Figure 9b).

In the site with higher P content, AEM P was negatively related to mean temperature during the 18 days and up to 24 days prior to AEM removal (though not significant for 22 days prior, Figure 10b), with the strongest relationship for 24 days prior ($r^2 = 0.55$, $F = 9.66$, $p = 0.0145$, Figure 11a). AEM P was negatively related to cumulative solar radiation during the 23 days prior to removal ($r^2 = 0.42$, $F = 5.86$, $p = 0.0417$). AEM P in the more P-deficient site was positively related to mean temperature during the 5-8 days prior to AEM removal (though not significant for 6 days prior, Figure 10b), with the strongest relationship during the 5 days prior ($r^2 = 0.43$, $F = 6.03$, $p = 0.0396$, Figure 11b). AEM P was positively related to cumulative solar radiation during the 7-8 days prior to AEM removal with the strongest relationship during the 8 days prior ($r^2 = 0.44$, $F = 6.38$, $p = 0.0355$, Figure 10c). Mean temperature and cumulative solar radiation were correlated with one another during the study period of June 23 through July 17 ($r^2 = 0.75$, $F = 67.48$, $p < 0.0001$, Figure 8b).

In a stepwise multiple regression, eighty-three percent of temporal variability in mean AEM P for the site with higher P content was explained by a combination of cumulative rainfall (positively related) and cumulative solar radiation (negatively related) during the 19 days prior to AEM removal ($r^2 = 0.83$, $F = 16.73$, $p = 0.0022$). Considering those factors during 18 rather than 19 days prior lowered the variance explained to seventy-seven percent ($r^2 = 0.77$, $F = 11.78$, $p = 0.0058$). In the more P-deficient site no combination of factors explained more of the variation than the single factors (cumulative rainfall, mean temperature, and cumulative solar radiation) in the univariate regressions.

Vegetation Structure and Composition Effects

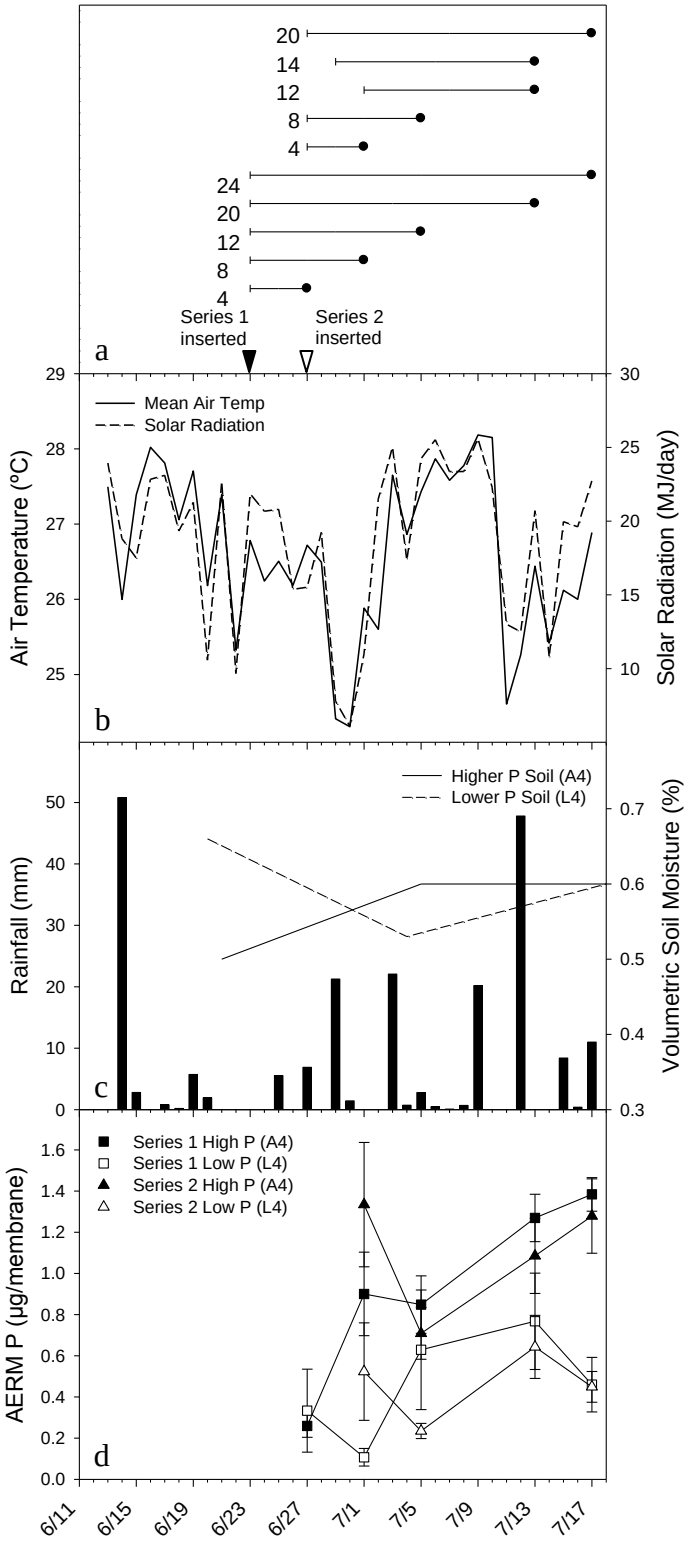
Subplot-level plant basal area and basal area of legumes, which were correlated with one another ($r^2 = 0.88$, $F = 43.10$, $p = 0.0006$), were positively related to average AEM P within each site, although due to the small sample size (four subplots) the relationship was only significant for basal area of legumes in the site with lower P content ($r^2 = 0.97$, $F = 61.01$, $p = 0.0160$). When subplot average AEM P values were normalized for site mean AEM P (*z*-scores), sub-plot level total basal area explained 74 percent ($r^2 = 0.74$, $F = 17.13$, $p = 0.0061$, Figure 12a) and basal area of legumes explained 89 percent ($r^2 = 0.89$, $F = 46.48$, $p = 0.0005$, Figure 12b) of the variation in normalized AEM P across sites. Stem density and basal area of palms were not significantly related to average AEM P or normalized AEM P. Minimum and maximum AEM P, and range in AEM P over all incubation periods was not related to subplot-level total basal area, basal area of legumes, basal area of palms or stem density.

Discussion

The range in spatially-averaged AEM P over our 24 day study (0.11 – 1.38 mg kg^{-1} resin) was comparable to levels reported for monthly 10-day *in situ* AEM incubations in a P-poor Amazonian agroforest Ultisol over a 14 month period (0.014 – 1.62 mg kg^{-1} resin, McGrath et al. 2000). Multiple regression analysis revealed that up to 83% of the temporal variation in labile P could be explained by environmental variability over days to weeks. However, the timing and in some cases the direction of labile P responses to environmental conditions differed as a function of total P status.

Figure 8: Incubation lengths, environmental conditions, and labile P.

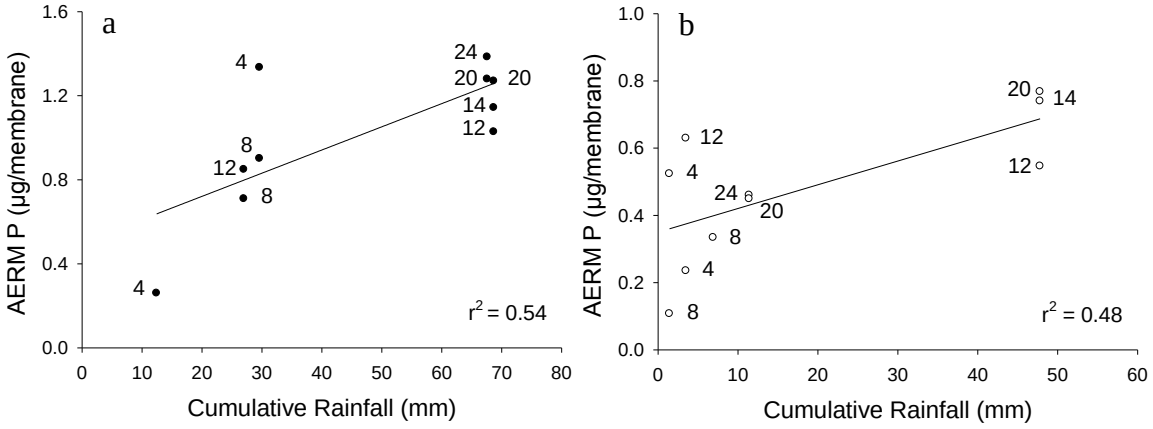
a) Incubation lengths for series 1 and 2 (solid circles represent removal dates). b) Temperature and solar radiation, c) rainfall and volumetric soil moisture. d) Spatially-averaged AEM-P (error bars represent ± 1 SE). 12 and 14 day AEM P values for series 2 were averaged and plotted on 7/13 when both were extracted (a).



Despite consistently high soil moisture (50-66%) throughout the study, the factor that best explained variability in AEM P was rainfall, which was positively associated with labile P in both sites. Although, soil saturation can lead to anoxic conditions that slow decomposition (Schuur 2001), recent evidence suggests that leaching of dissolved organic matter from litter and potentially nutrient mineralization may increase with rainfall up to very high levels of precipitation (Cleveland et al. 2006, Wieder et al. 2009). Additionally, P solubilization associated with iron reduction in anoxic microsites may contribute to greater availability of P with increasing rainfall. Our results demonstrate that rainfall patterns in wet tropical forests generate variability in labile P on the timescale of days to weeks, however, further research is needed to determine how the mechanisms driving this relationship differ from those in drier tropical soils.

Figure 9: Relationship between AEM P and rainfall.

Spatially-averaged AEM P for each incubation length as a function of cumulative rainfall during: a) the 6 days prior to AEM removal in the site with higher P content (solid circles) and b) the 2 days prior to AEM removal in the site with lower P content (open circles). Number labels indicate incubation length.

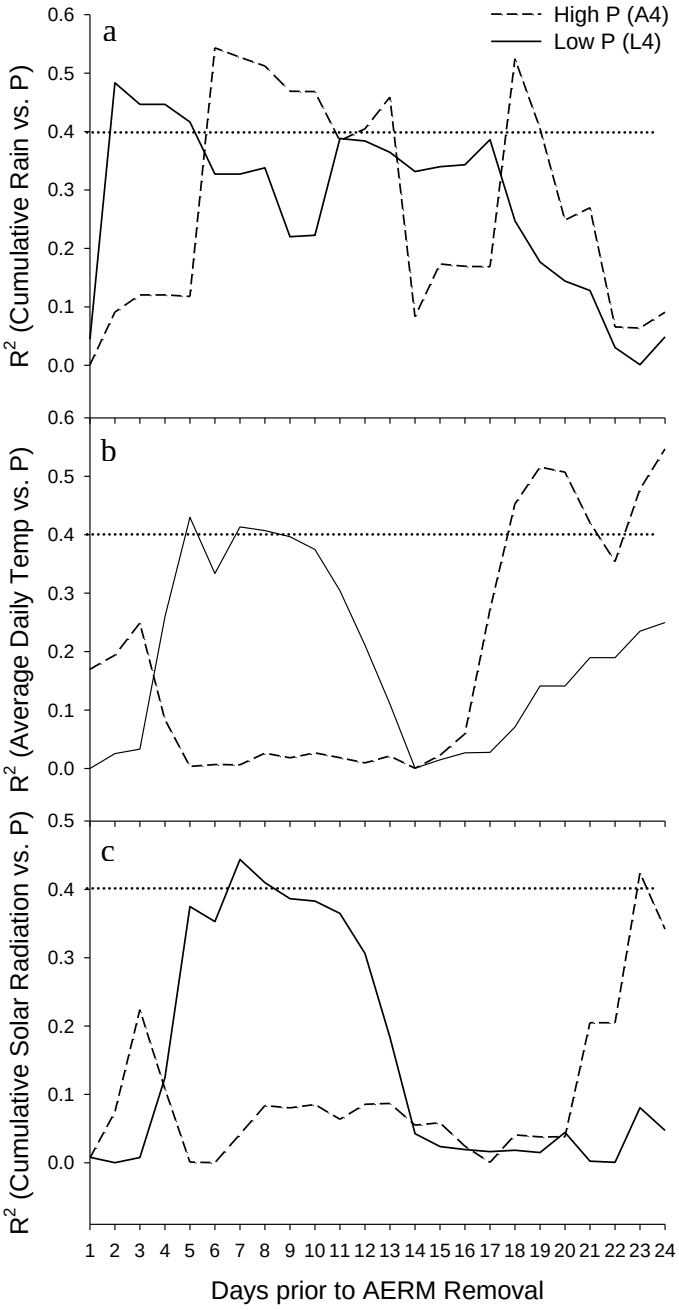


Across a range in rainfall conditions (five different removal dates, see Figure 8a), we found that the less fertile soil exhibited a more immediate response to rainfall, with

the strongest coupling between rainfall and AEM P in the range of 2-5 days prior to AEM removal from the soil as opposed to 6-13 and 18-19 days in the site with higher P content. Given that our sites experienced similar environmental conditions during the study, differences in the timing of P responses to rainfall are likely the result of biological controls. The more rapid response in the P-poor soil is at odds with our hypothesis that microbial immobilization may lead to a delayed response to environmental drivers in less fertile soils. An alternative explanation is that greater microbial and plant production of phosphatase in the P-poor soil may lead to more rapid release of P from soil organic material as soil moisture increases. An influence of soil moisture on the production and the activity of phosphatase enzymes has been reported in a number of ecosystems, however, these results are quite variable (Chen et al. 2003, Criquet et al. 2004, Yavit et al. 2004, Sowerby et al. 2005).

Figure 10: Strength of the relationship between AEM P and climate through time.

r^2 values from regressions of spatially-averaged AEM P for each incubation length and a) cumulative rainfall, b) mean temperature, and c) cumulative solar radiation, during 1 up to 24 days prior to AEM removal from the soil in both study sites. Dotted lines mark the cutoff for r^2 values that are significant at a p value of < 0.05.



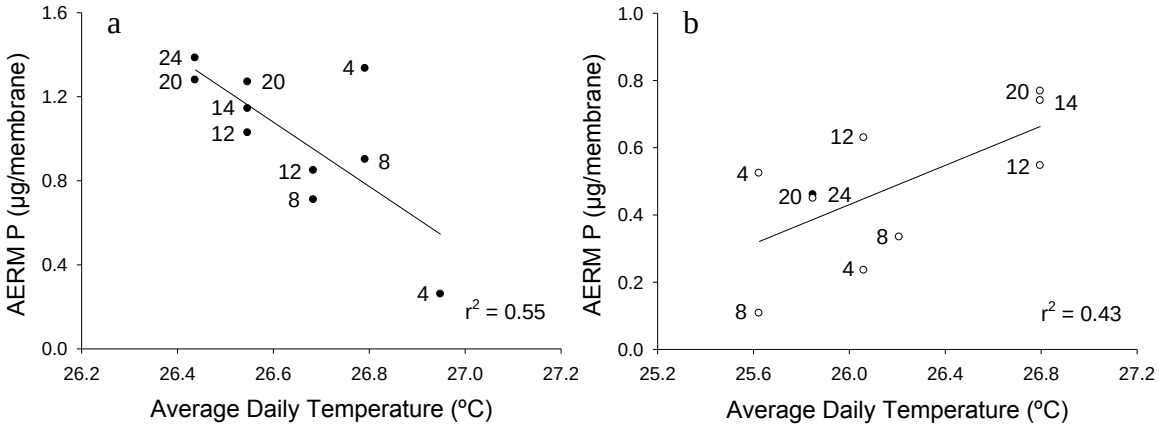
The size and intensity of rainfall events has a strong influence on subsequent microbial contributions to labile P (Yang and Insam 1991, Lodge 1994, Campo et al.

1998). Campo et al. (1998), using a lab incubation of dry tropical forest soils collected during the wet season, found that microbial immobilization of P was stimulated by a 30 mm simulated rainfall event but not a 10 mm event. The one very large rain event (47.8 mm on 7/12) that occurred during our study marked a divergence in P patterns in our two sites. This event was followed by a temporary increase in AEM P (captured in both the series 1- 20 day incubation and the series 2 - 12 and 14 day incubations) in both sites, followed by a decline in labile P (captured in both the series 1 - 24 day incubation and the series 2 - 20 day incubation) in the more P-poor site. No corresponding decrease in AEM P was observed in the site with higher P content. Given that the importance of biological controls on labile P dynamics increases as P availability declines (McGill and Cole 1981, Walbridge 1991, Dakora and Phillips 2002, Olander and Vitousek 2004, 2005, Barroso and Nahas 2005) greater biological demand in the more P deficient site may explain this observation.

Temperature, one of the primary regulators of microbial activity, is known to influence many processes relating to nutrient availability and plant uptake. Under field conditions and in lab incubation studies positive effects of temperature on labile P have been observed, although they tend to be less important than moisture effects (Yang et al. 1991, McKean and Warren 1996, Vandecar et al. 2009). We found that labile P responded positively to temperature (and solar radiation) in the more P-deficient site, during the 5-8 days prior to AEM removal. However, labile P declined with temperature (and solar radiation) in the site with higher P content, during the 18-24 days prior to AEM removal.

Figure 11: Relationship between AEM P and temperature.

Spatially-averaged AEM P for each incubation length as a function of mean temperature during a) the 24 days prior to AEM removal in the site with higher P content and b) the 5 days prior to AEM removal in the site with lower P content. Number labels indicate incubation lengths.



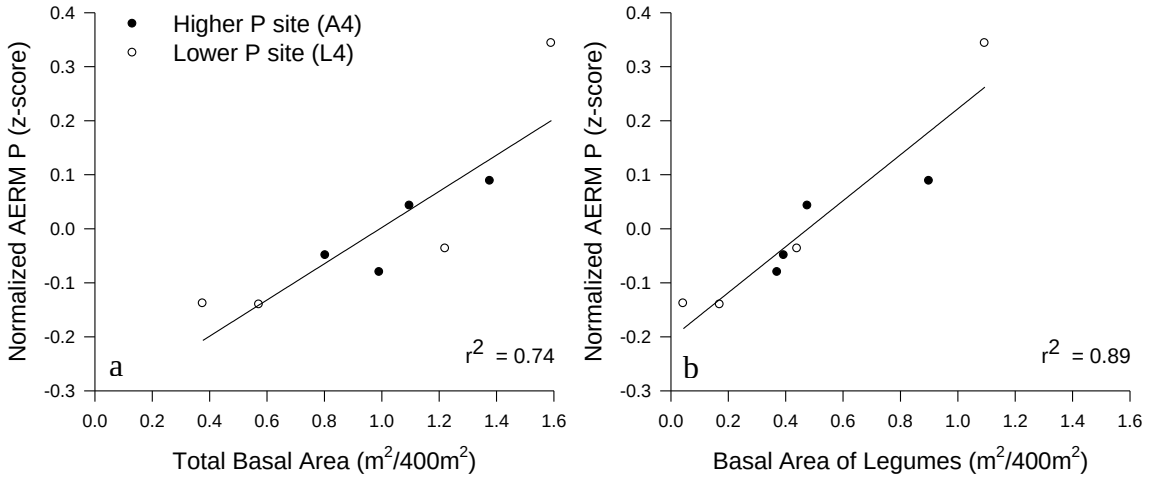
Given that temperature and solar radiation covaried during the study, our ability to distinguish their relative influences on P patterns is limited. It is possible that under similar environmental conditions site-specific differences in microbial and plant demand for P could potentially lead to differences in patterns of P release or uptake. Greater microbial controls over P dynamics in the more P-deficient site is consistent with the positive and more immediate response to temperature on AEM P patterns observed there.

Vegetation structure at the subplot-scale appeared to play a role in driving spatial heterogeneity in mean soil labile P, such that higher basal area was associated with higher mean P availability in both sites. These results should be viewed with caution given the small sample size (8 subplots). However, this relationship has been reported previously, on a similar spatial scale (tens of meters) at a different site in this forest (Vandecar et al. 2009). Vegetation-driven local P enrichment may be attributed to higher inputs of litter and belowground root turnover in areas of greater plant basal area. Litterfall inputs,

which correlate well with within-stand basal area at La Selva, (Lawrence unpublished data), represent one of the primary sources of plant available P. Legume species common in tropical forests may also contribute to local P enrichment by releasing more phosphatase into the soil than non-legume species in order to support N-fixation (Houlten et al. 2008). Basal area of legumes explained 89 percent of the variation in mean AEM P among subplots, however, given that basal area of legumes was correlated with total basal area we cannot make any inferences about their relative contribution to P availability.

Figure 12: Relationships between vegetation characteristics and AEM P.

Relationships between subplot-level a) total plant basal area and b) basal area of legumes and normalized mean subplot AEM P across all incubation lengths for plot A4 (solid) and L4 (open).



This work has implications for the methodological design of *in situ* AEM incubation studies. There is no standard length for AEM incubations and those reported in the literature range from hours to longer than 6 months. Our study design of deploying two overlapping series of incubations in soils of contrasting P fertility allowed us to address a number of questions about the effects of incubation length on AEM P estimates

in tropical forest soils. Congruent with previous investigations of AEM P dynamics under field conditions in P-deficient soils, our overlapping series of incubations revealed nonlinear AEM accumulation (Drohan et al. 2005, Meason and Idol 2008). Although AEMs in the site with higher P content appeared to accumulate ions throughout the 24 day study, the rate of P accumulation declined such that a series of short incubations always yielded higher P estimates than one long incubation covering the same time period. In contrast, AEMs in the more P-deficient site did not continue to accumulate P throughout the study, but peaked at 14 days and subsequently declined, indicating AEMs were acting as dynamic exchangers. Our findings support the interpretation of *in situ* AEM P as an equilibrium estimate of labile P that integrates soil sorption capacity and biological demand, rather than an estimate of gross P flux through the available pool during the incubation. The time needed for AEMs to equilibrate with soil labile nutrient pools appears to be site-specific, ranging from 2 weeks to possibly longer than 24 days in our soils. Given that incubation length can affect the detection and magnitude of treatment effects (Meason and Idol 2008), optimum periods of burial should be chosen carefully based on preliminary testing of the soils under study. Despite site-specific differences in incubation length effects, AEMs were able to detect fine-scale temporal and spatial variation in *in situ* P availability associated with environmental conditions and vegetation characteristics. On the whole, our results demonstrate that labile P in tropical soils is quite sensitive to environmental conditions on a sub-monthly timescale. Consequently, infrequent soil P measurements used in long term nutrient cycling studies may not adequately capture environmentally-driven variability in labile P.

CHAPTER 3: Temporal patterns of soil nutrient availability across a tropical forest landscape

Abstract

Phosphorus (P) availability in tropical soils is strongly dependent on environmental conditions that drive the recycling of organic forms of P through decomposition. The goal of this study was to improve our understanding of seasonal and inter-annual patterns of nutrient availability in tropical rain forest soils spanning a wide range in total P (TP) fertility and to evaluate the importance of environmental conditions (rainfall, soil moisture, temperature), and vegetation indicators (basal area, stem density, and species composition) in driving these patterns. To this end, we quantified monthly soil labile P over a three year period, as well as labile nitrogen (N) over a two year period, in tropical rain forest soils of northern Costa Rica that span a three-fold gradient in TP content (0.37 – 1.18 Mg/ha, 0-10 cm). Across 18 sites, P (mean labile P: 1.32 -7.35 µg/g), and N (mean labile N: 28.52 - 46.72 µg/g) availability varied both seasonally and interannually. P availability was lower during the dry season (2.33 µg/g) than the wet season (3.04 µg/g), while N availability was higher during the dry season (56.41 µg/g) than the wet season (34.81 µg/g). A remarkable degree of synchrony in soil P availability across a tropical forest landscape suggests a strong climatic driver, yet we found no evidence of a common driver at timescales of weeks to months. No environmental indicator, or combination of indicators, that we tested could explain monthly patterns in P availability. N availability was negatively associated with cumulative rainfall during the 2, 3, 4, and 5 months prior to soil sampling. Spatial variation in vegetation structure and composition was associated with P, but not N availability.

Introduction

Tropical soils are often depleted of rock-derived minerals and are characterized by iron- and aluminum-oxides that bind phosphorus making it less available to the biota (Schlesinger 1997). Although it is widely recognized that productivity in many tropical forests is constrained by low available phosphorus (Walker and Syers 1976, Vitousek 1984), seasonal and inter-annual patterns of soil P supply have not been well characterized. Previously, attention has been given to the importance of pulsed nutrient release in tropical ecosystems, and the role it plays in maintaining forest productivity by reducing competition between microbes and plants for limiting resources (Singh et al. 1989, Srivastava 1992, Lodge et al. 1994, Diaz-Ravina et al. 1995). Pulsed nutrient dynamics have been attributed to environmental variability, particularly drying and wetting cycles caused by seasonal shifts in rainfall (Lodge et al. 1994). However, research investigating climatic controls on P availability has been conducted predominantly in soils at the dry end of the tropical forest precipitation spectrum where seasonal variability in rainfall is more pronounced. The goals of this study were to (1) quantify seasonal and inter-annual patterns of soil nutrient availability in soils at the wet end of the tropical forest precipitation spectrum (mean annual precipitation (MAP) > 4,000 mm), and spanning a wide range in soil P fertility, (2) evaluate the influence of environmental conditions (i. e. rainfall, soil moisture, and temperature), and site fertility (i. e. TP content) on the magnitude and timing of these patterns, and (3) determine the relationship between spatial variation in soil nutrient availability and vegetation characteristics (basal area, stem density, and species composition). By exploring links between nutrient dynamics and current climate conditions we may be able to better

predict responses of nutrient cycling in tropical ecosystems to future environmental change.

Much of our understanding of the role of soil moisture in determining nutrient availability in wet tropical forest soils is based on comparisons of wet and dry season soil nutrient pools (Campo et al. 1998, Yavitt et al. 1993, Cleveland et al. 2004, Chacon et al. 2008). In dry tropical forests, significant pulses of nutrient mineralization observed during the dry to wet season transition have been attributed to lysis of the microbial biomass and leaching from litter accumulated during the dry season (Kieft et al. 1987, Singh et al. 1989, Lodge et al. 1994, Campo et al. 1998, Grierson et al. 1998). In wet tropical forests soil moisture fluctuates less widely and links between soil moisture and nutrient availability are less clear. Some evidence suggests a lack of seasonal patterns in soil nutrient availability in tropical wet forests (Yavitt and Wright 1996, Matson et al. 1987, Vitousek and Denslow 1986). However, this finding does not appear to be universal; McGrath et al. (2000) found that in a P-deficient Amazonian agroforest soil, labile P was highest at the start of the rainy season when litterfall was at its peak and wetting and drying cycles were initiated (MAP: 2000 mm). The few studies that have monitored *in situ* nutrient availability throughout an entire year in tropical wet forests, have reported disparate results, potentially due to differences in site-specific characteristics such as MAP, soil fertility, or land use (Vitousek and Denslow 1986, Yavitt and Wright 1996, McGrath et al. 2000). More intensive multi-year studies of nutrient dynamics are needed to reconcile these findings and to improve our understanding of the biotic and abiotic mechanisms driving seasonal and interannual variability in nutrient availability.

Climatic conditions, particularly precipitation, have the potential to influence nutrient cycling in tropical rain forests through a variety of processes occurring at various timescales. The quantity and intensity of precipitation has immediate impacts on the rate at which nutrients are leached from the litter layer, the rate of nutrient diffusion through the soil, and the rate of nutrient uptake by microbes and plants (Linn and Doran 1984, Skopp et al. 1990, Lodge et al. 1994, Chapin et al. 2002, Porporato et al. 2003). Laboratory studies indicate that labile nutrient pools tend to increase upon soil wetting, particularly when soils are initially dry and responses vary with the length and intensity of the drying period and the level of wetting (Linn and Doran 1984, Yavitt et al. 1993, Lodge 1994, Campo et al. 1998, Grierson et al. 1998, Delonge 2007). On longer timescales, rainfall patterns influence both the quantity and quality of organic matter inputs to the forest floor and the ability of the microbial community to decompose them. The biomass and activity of microbial decomposers is quite sensitive to available moisture in tropical soils (Kieft et al. 1987, Singh et al. 1989, Luizao et al. 1992, Davidson et al. 1993, Lodge 1994, Wardle 1998) and experimental irrigation studies have demonstrated that moisture seasonality controls forest floor decomposition (Wieder and Wright 1995).

Seasonality in precipitation also influences the timing, the quantity, and the quality of litter inputs to the forest floor through regulation of the phenological patterns of tropical tree species. Wet-dry seasonality induces pulses of litterfall at La Selva with peak litterfall most often occurring toward the end of the dry season (Frankie et al. 1974, D. A. Clark unpublished data). Short dry spells (2-4 weeks) or heavy rains can lead to premature senescense of leaves and sudden large inputs of high quality litter to the forest

floor (Frangi and Lugo 1991, Lodge et al. 1991, Veneklaas 1991, Cuevas and Lugo 1998, Wood et al. 2005). It is clear that moisture availability has strong effects on processes controlling nutrient availability, however, responses to wetting can be nonlinear and are strongly time dependent.

Soil fertility, which drives variation in vegetation structure, species composition and associated differences in litter chemistry and quantity, has the potential to influence the magnitude and timing of fluctuations in soil nutrient availability (Chapin et al. 2002). Oxisols and ultisols, the most common soil types found in the tropics, span a wide range in TP content (Vitousek and Sanford 1986). Low fertility forests tend to produce similar quantities of litter, but lower quality litter than forests on more fertile soil (Vitousek 1984, Vitousek and Sanford 1986, Wood et al. 2006). When litter quality is poor, soil microbes immobilize nutrients from the soil to support growth, effectively reducing soil nutrient availability to plants. Immobilization of P in leaf litter varies at scales ranging from a month to several years in tropical forests where soil P is limiting (Montagnini et al. 1993, Cornejo et al. 1994, Songwe et al. 1995, Hobbie and Vitousek 2000, McGrath et al. 2000). An experimental litter addition in Costa Rican tropical rain forests stimulated litter production 2-6 months following litter addition, presumably due to litter-derived soil nutrient pulses that were then taken up by plants (Wood et al. 2009). The rapid response of vegetation to litter addition illustrates the tight link between decomposition of organic inputs to the forest floor and plant productivity. Differences in biological demand and decomposition rates across a gradient in soil P content could lead to variation in temporal patterns of nutrient availability.

Patterns of climate variability, such as ENSO, contribute to significant inter-annual rainfall fluctuations in tropical rain forests. ENSO events lead to the intensification of seasonality (i.e. a wetter wet season and a drier dry season) in northeastern Costa Rica (Waylen et al. 1996). Decreased growth and increased mortality experienced by tropical trees during hot, dry years has been attributed to their lack of adaptations for managing significant moisture stress (Clark 2004, Chazdon et al. 2005) but may also be related to decreased nutrient availability. Despite evidence of the importance of ENSO events for vegetation-soil feedbacks, multi-year nutrient cycling studies that capture these phenomena are rare. The increasing frequency and severity of ENSO events predicted for tropical regions have the potential to disrupt conservative nutrient cycling by uncoupling the tight balance between nutrient supply and biological demand. Understanding current nutrient dynamics in tropical ecosystems and how they are linked to climate variability may help us predict responses of tropical forests to future climatic conditions.

Methods

Study Site

The study was conducted in old-growth tropical rain forest at La Selva Biological Station in the Caribbean lowlands of northern Costa Rica (10° 26' N, 83° 59' W; Organization for Tropical Studies). La Selva receives an average annual rainfall of 4300 mm (Organization for Tropical Studies, unpublished data [available online]), with the driest period occurring between late January and April (McDade et al. 1994). Mean annual temperature is 25.8 ºC (McDade et al. 1994). Soils at La Selva are volcanic in origin and are characterized as deep, well-drained, acidic, and clay-rich (McDade et al.

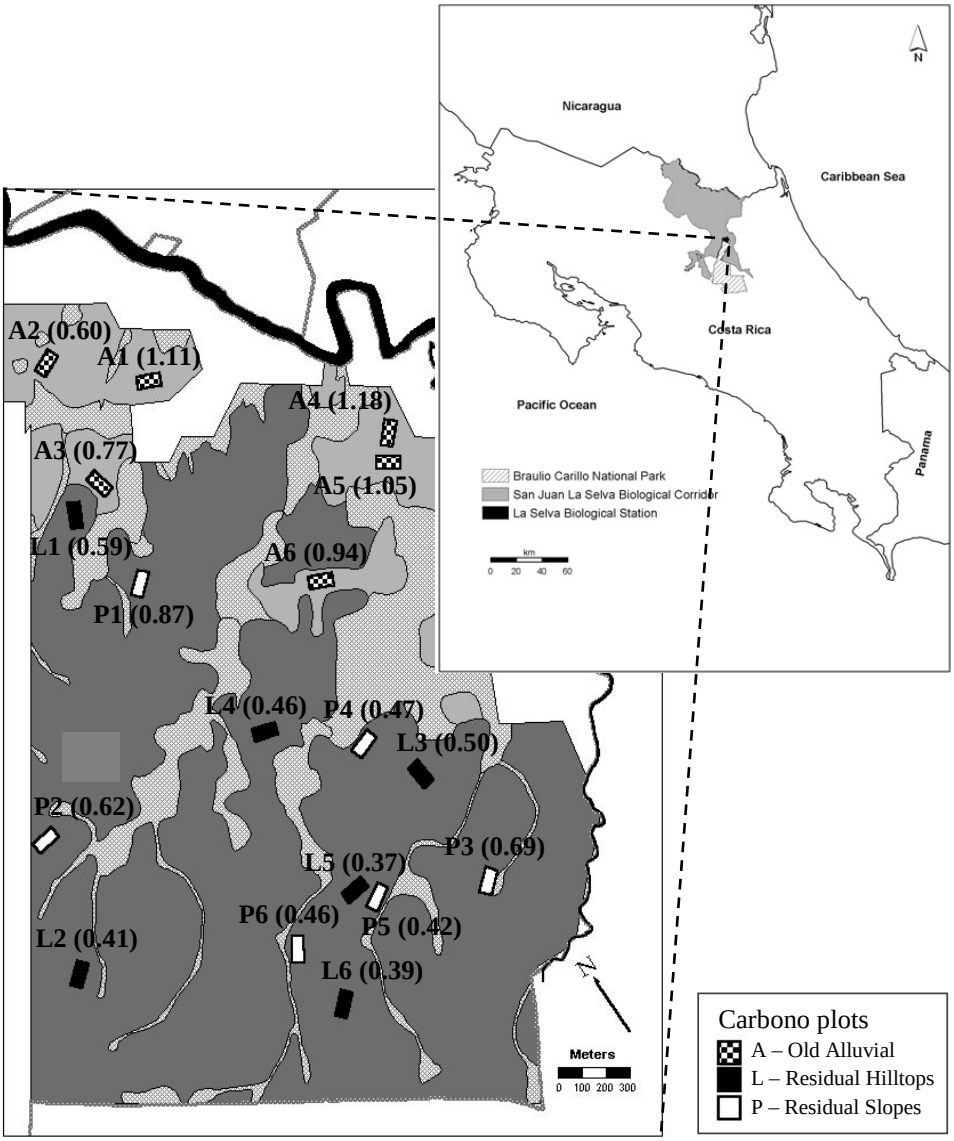
1994). Soils span a wide range of fertility (Espeleta & Clark 2007); P is sometimes in the deficient range and has the potential to limit plant growth (Denslow et al. 1987, Vitousek and Denslow 1987) and litter production (Wood et al. 2009). The forest is predominantly evergreen and is dominated by a N-fixing legume *Pentaclethra macroloba* , that accounts for as much as 36% of the basal area and 13% of stems (McDade et al. 1994, Lieberman et al. 1996, Clark and Clark 2000).

Soil Sampling and Chemical Analysis

18 sites on Oxisols of differing P content, within a 5.5 km^2 area (Figure 13), were chosen for this study: CARBONO plots A1-6, L1-6, and P1-6 (Figure 13; the CARBONO plot network is described in Espeleta & Clark 2007).

Figure 13: Map of Costa Rica and inset of La Selva Biological Station.

CARBONO plots A1-6 are indicated by checkered rectangles, plots L1-6 by solid black rectangles, and plots P1-6 by open rectangles. Total P content (0-10 cm) is listed in parentheses following the plot ID.



In each 50x100 m (0.5 ha) plot, soil samples (0-10 cm depth) were collected from 16 locations systematically arrayed across each plot, every month between September 2005 and August 2008. Soil samples were composited and sieved through a 2 mm mesh screen. Gravimetric soil moisture was determined on a 10 g sub-sample dried at 105

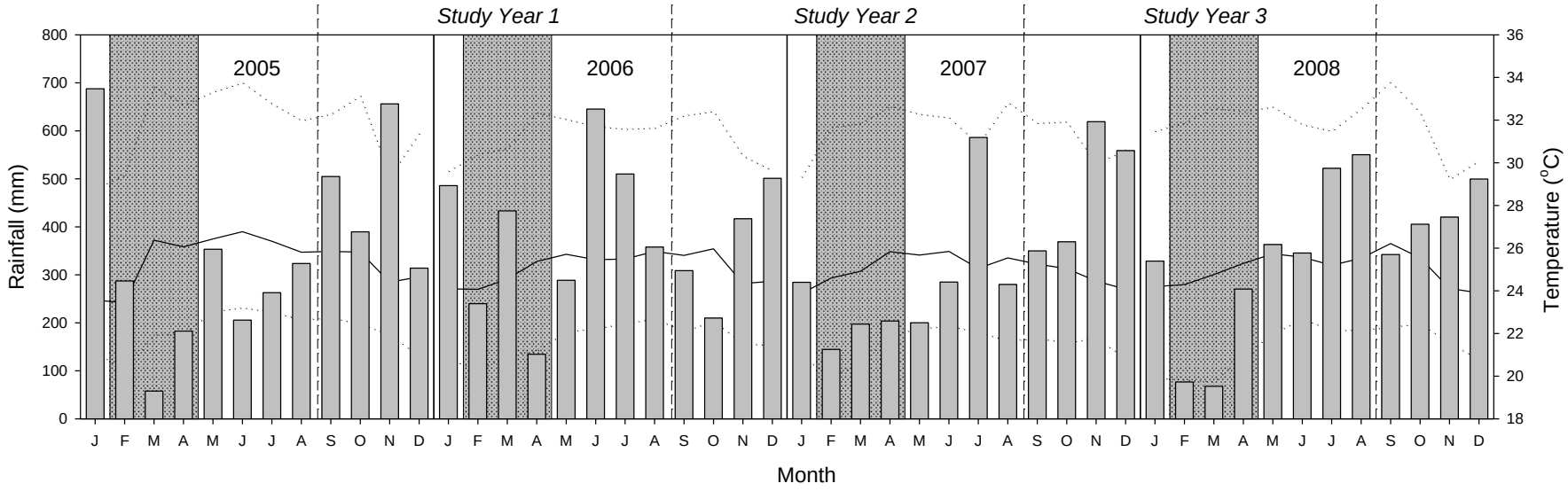
degrees Celsius for 24 hours. We chose an acid fluoride extraction that is commonly used for tropical soils and measures P that is considered readily available for uptake by both plants and microbes (Cross and Schlesinger 1995). P in the form of phosphate was extracted from 5 grams of fresh soil by shaking for one minute in 25 ml of a 0.03M NH_4F and 0.025M HCL solution (Bray and Kurtz 1945). The extractant was filtered and the P concentration of extract solutions was determined using molybdate blue colorimetric reactions measured on an Alpkem Flow Solution IV 250 Auto Analyzer (OI Analytical; College Station, Texas, USA). A 10 g sample of sieved field-moist soil from each of the 18 sites was extracted for ammonium (NH_4-N) and nitrate (NO_3-N) by shaking for one hour with 50 ml of 2M KCl solution. Extracts were passed through filters that were pre-extracted with KCl and were analyzed colorimetrically on a Lachat QuikChem 8500 (Hach Co., Loveland, CO).

Environmental Variables

Daily meteorological station data were collected by the Organization for Tropical Studies (OTS), including cumulative rainfall, and minimum, maximum and average temperature (http://www.ots.ac.cr/en/laselva/metereological.shtml; data were screened and gap-filled by D. A. Clark; Figures 14).

Figure 14. Climate conditions at La Selva Biological Station, Costa Rica.

Monthly cumulative rainfall and average (solid line), minimum and maximum (dotted lines) temperature during the three study years (September 2005 – August 2008). Shaded regions indicate the dry season (February through April).



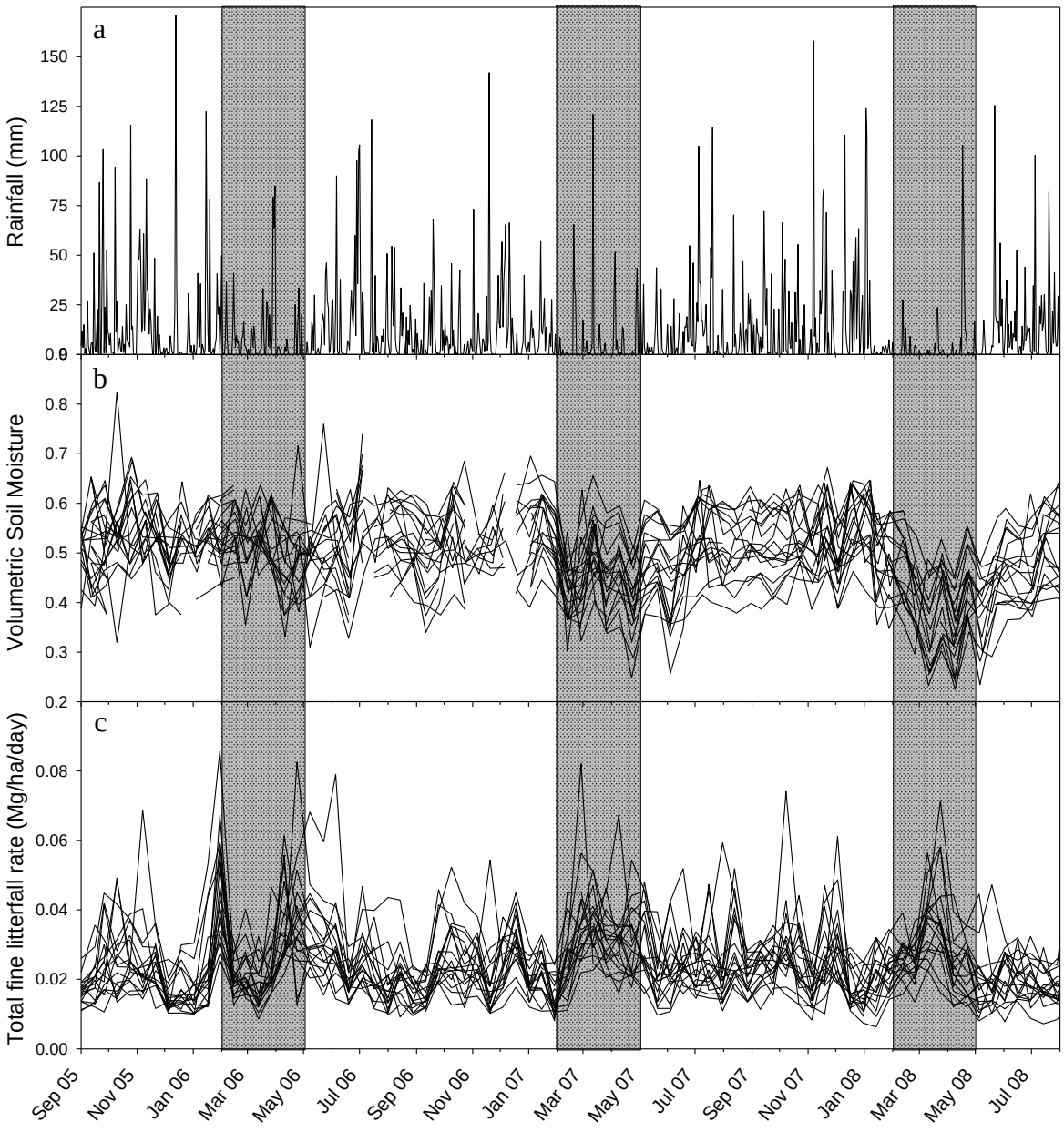
Volumetric soil moisture was measured in the center of each plot by a Campbell CS615 (Campbell Scientific, Logan, Utah, USA) sensor (0-30 cm; calibrated for La Selva soils as per Veldkamp & O'Brien 2000) every two weeks throughout the study (Figure 15b; D. A. Clark and S. F. Oberbauer unpublished data).

Litterfall

Litter sampling was conducted every two weeks. Nine standing litter traps (each 0.25 m^2 in collecting area, with the trap opening maintained level and 80 cm above the ground) and nine ground-level litter/fine wood traps (vertically-projected area = 0.25 m^2 per trap) were set out in all 18 plots in a standard grid. Material from the 9 standing traps was sorted into three categories: small leaves, woody litter and plant reproductive parts. All four litter components were then dried at 65 degrees Celsius until they weighed the same on two consecutive daily weighings (Figure 15c; D. A. Clark unpublished data)

Figure 15: Rainfall, soil moisture, and fine litterfall rate.

Environmental conditions including a) daily rainfall collected by the Organization for Tropical Studies (OTS), (http://www.ots.ac.cr/en/laselva/metereological.shtml; screened and gap-filled by D. A. Clark), and b) volumetric soil moisture measured in the center of each site by a Campbell CS615 (Campbell Scientific, Logan, Utah, USA) sensor (0-30 cm; calibrated for La Selva soils as per Veldkamp & O'Brien 2000) every two weeks throughout the study (D. A. Clark and S. F. Oberbauer unpublished data). c) Total fine litterfall was collected every two weeks from 9 standing and 9 ground-level litter traps in each site (D. A. Clark unpublished data)



Vegetation Structure and Composition

Basal area, stem density, median DBH and species composition were tabulated in September-October 2006 for all live stems with a diameter at breast height (DBH) greater than 10 cm within each 0.5 ha plot (Table 4, D. A. Clark unpublished data).

Statistical Analyses

All data were analyzed using SAS Systems for Windows V9.1 (SAS Institute, Inc.). Residuals of all analyses were checked for normality and homogeneity of variance. Sites were grouped into three TP levels (low, intermediate and high; n = 6 per group), each group having a mean TP content significantly different from all the others (t-test, $p<0.05$). Analysis of variance (ANOVA) and a Tukey-Kramer multiple comparison test were used to detect significant differences in P (18 sites x 34 months) and N availability (18 sites x 23 months) between seasons (wet season: May – January, dry season: February – April), years, and TP levels and total N (TN) content. The coefficient of variation (CV) among labile P measurements taken within a site over the three years (n = 34) was calculated and an ANOVA was used to determine the relationship between temporal variability (as indicated by the CV) in labile P and TP status. Monthly soil labile N:P ratios were calculated for each site.

Univariate linear regression and stepwise multiple regression were performed to investigate the relationship between climatic factors and soil P and N availability. We considered the following factors: cumulative rainfall, and minimum, maximum, and average temperature. Samples were collected from all 18 sites over a period of 5 days (and up to two weeks) each month. Each environmental variable was tested based on daily values summed or averaged over 1, 2, 3, and 4 weeks as well as 2, 3, 4, and 5

months prior to each sampling date. The number of dry days (days receiving less than 7 mm of rainfall) for the 4 weeks prior to sampling were calculated and regressed against labile P and N. Regression analysis was used to determine the relationship between gravimetric soil moisture at the time of soil sampling and labile P (18 sites x 34 months) and N (18 sites x 23 months). ANOVA and a Tukey-Kramer multiple comparison test were used to detect significant differences in bi-monthly volumetric soil moisture between seasons (wet season: May – January, dry season: February – April), years, and TP levels (18 sites x 72 bi-monthly measurements).

Previous work has demonstrated that labile P in more P-deficient soils responds more rapidly to rainfall (increasing within 2-5 days) than in soils with higher P content (increasing within 6-13 and 18-19 days). In order to test this hypothesis with our three year dataset of monthly P concentrations we determined which soil collection dates were preceded within 5 days by a large (> 30 mm) rainfall event and which were preceded within 6-13 days by a large rainfall event. Within each P fertility group (low, intermediate, and high TP) a t-test was used to determine whether soil labile P varied significantly between sampling dates that did and did not experience a large rain event during the 5 days prior to soil P measurement. A second t-test was used to determine whether soil labile P varied significantly between sampling dates that did and did not experience a large rain event during the 6-13 days prior to soil P measurement.

An ANOVA and a Tukey-Kramer multiple comparison test were used to detect significant differences in total fine litterfall rate and reproductive litterfall rate between seasons (wet season: May – January, dry season: February – April), years, and TP levels (18 sites x 72 bi-monthly measurements).

Linear regression was used to explore the relationship between vegetation characteristics (i. e. total stem density and basal area, the stem density and basal area of legumes, and the proportion of total stems and basal area comprised of legumes) and P availability (i. e. minimum, maximum, and average site labile P, and range in labile P over all collection periods), as well as TP content. An ANOVA was used to compare the median DBH among TP fertility groups.

Results

Soil P availability and environmental indicators

Over three years, within-site mean labile P ranged from 1.3 to 7.4 µg/g, with monthly labile P ranging from 0 to 18.4 µg/g among the 18 sites. Annual amplitude in monthly labile P ranged from 3.0 to17.0 µg/g, more than two times mean P concentrations. Mean labile P over the course of the study was well correlated with TP content ($r^2 = .74$, $F = 45.8$, $p < 0.0001$). Labile P varied significantly by season, year, and P fertility level, with lower labile P during the dry season (2.33 µg/g) than the wet season (3.04 µg/g; $F = 15.13$, $p = 0.0001$), lower labile P during the third year of the study (2.0 µg/g) than the first (3.1 µg/g), or second year (2.9 µg/g; $F = 16.23$, $p < 0.0001$), and higher labile P in sites with high TP content (4.8 µg/g) than sites of intermediate (1.8 µg/g) or low TP content (1.5 µg/g; $F = 124.75$, $p < 0.001$; Figure 16a). The CV of monthly labile P for sites of high TP content was significantly lower than for sites of low and intermediate TP content ($F = 8.07$, $p = 0.0042$).

No single climatic indicator or combination of indicators could explain the monthly pattern in P availability. Labile P increased with gravimetric soil moisture ($r^2 = .07$, $F = 46.04$, $p < 0.0001$). P availability was not significantly related to cumulative

rainfall, or minimum, maximum, or average daily temperature over any of the time lags tested (1, 2, 3, or 4 weeks and 2, 3, 4, or 5 months prior to sample collection). Labile P was also unrelated to the number of dry days during the 4 weeks prior to sample collection. Heavy rainfall events (> 30 mm) during the five days prior to soil sampling were associated with higher labile P concentrations in sites of low and intermediate P content but not soils of high P content (low TP: $t = 3.73$, $p = .0003$; intermediate TP: $t = 2.76$, $p = .0064$). Heavy rainfall events during the 6-13 days prior to soil sampling were associated with higher labile P in sites of high TP content but not in soils of low or intermediate TP content (high TP: $t = 4.83$, $p < .0001$).

Soil N availability and environmental indicators

Over two years, within-site mean labile N ranged from 28.5 to 46.7 µg/g, with monthly N availability (NH_4 plus NO_3) ranging from 3.7 – 103.2 µg/g among the 18 sites. Annual amplitude in monthly labile N ranged from 62.1 to 91.7 µg/g, as with P, about twice the level of the mean. Mean labile N was correlated with TN content ($r^2 = 0.47$, $F = 14.47$, $p = 0.0016$). Monthly Labile N varied significantly by season, and year, but not by TN content. Labile N was higher during the dry season (56.2 µg/g) than the wet season (34.5 µg/g; $F = 88.61$, $p < 0.0001$), and was higher during the second year (53.6 µg/g) than the third year (37.1 µg/g; $F = 50.54$, $p < 0.0001$; Figure 16b) of the study.

The gravimetric soil moisture of soil samples explained only 6% of the variation in labile N, with N availability declining with increasing soil moisture ($r^2 = .06$, $F = 26.16$, $p < 0.0001$). Cumulative rainfall during the 2, 3, 4, and 5 months preceding soil sampling was inversely related to N availability and explained between one quarter and

one third of the variation in N availability ($r^2 = 0.24$, $F = 125.18$, $p < 0.0001$; $r^2 = 0.30$, $F = 164.52$, $p < 0.0001$; $r^2 = 0.35$; Figure 17, $F = 209.94$, $p < 0.0001$; $r^2 = 0.30$, $F = 159.13$, $p < 0.0001$; respectively). N availability was not significantly related to minimum, maximum, or average daily temperature over any of the time lags tested (1, 2, 3, or 4 weeks and 2, 3, 4, or 5 months prior to sample collection). Labile N was also unrelated to the number of dry days during the 4 weeks prior to sample collection. Labile N:P ratios were more constant in sites of high TP content (1-188), than in sites of intermediate (2-1515), or low (3-723) TP content (Figure 16c).

Volumetric Soil Moisture

Bi-monthly volumetric soil moisture varied significantly by season and year with lower soil moisture during the dry season (0.46 Mg/ha/day) than the wet season (0.51; $F = 134.19$, $p < 0.0001$, Figure 15b). Volumetric soil moisture declined from the first year of the study (0.52), to the second (0.48), and the third (0.45; $F = 68.59$, $p < 0.0001$).

Total fine litterfall and reproductive litterfall

Total fine litterfall rate varied significantly by season, year, and TP fertility level, with higher fine litterfall rates during the dry season (0.029 Mg/ha/day) than the wet season (0.023 Mg/ha/day; $F = 77.45$, $p < 0.0001$), higher fine litterfall rates during the second year of the study (0.028 Mg/ha/day) than the first (0.025 Mg/ha/day), or third year (0.025 Mg/ha/day; $F = 7.55$, $p = 0.0005$), and lower fine litterfall rates in sites with intermediate TP content (0.024 Mg/ha/day) than sites of high (0.027 Mg/ha/day) or low TP content (0.026 Mg/ha/day; $F = 8.41$, $p = 0.0002$; Figure 15c). Reproductive litterfall rates varied by TP fertility level, with higher reproductive litterfall rates in sites with high TP content (0.006 Mg/ha/day) than sites of intermediate (0.003 Mg/ha/day) or low TP

content (0.003 Mg/ha/day; $F = 55.69$, $p < 0.0001$). Reproductive litterfall rates did not vary by season or year.

Relationships between vegetation characteristics and soil nutrient status

Total stem density of trees greater than 10 cm ranged from 154-311 (stems/0.5 ha) and total basal area ranged from 9.4 – 14.1 (m^2/0.5 ha). Legumes comprised 9-25% of the total stem density and 20-57% of the basal area. Total stem density was not related to legume stem density across the 18 plots. However, total basal area was positively related to legume basal area ($r^2 = 0.53$, $F = 17.79$, $p = 0.0007$). Stand stem density was negatively related to labile P ($r^2 = 0.40$, $F = 10.61$, $p = 0.0049$) and TP ($r^2 = 0.61$, $F = 24.82$, $p < 0.0001$; Figure 18a and b). Total basal area and basal area of legumes was not significantly related to mean labile P or TP. Median DBH in the high TP group (17.1 cm) was significantly higher than in the intermediate (15.7) or low (15.5 cm) TP groups ($F = 8.22$, $p = 0.0039$). However, the percentage of total stems in the legume family was positively related to labile P ($r^2 = 0.45$, $F = 12.99$, $p = 0.0024$) and TP ($r^2 = 0.49$, $F = 15.42$, $p = 0.0012$; Figure 18c and d). No measure of vegetation structure or composition tested was related to minimum, maximum, or range of labile P over all collection periods, or N availability or TN.

Discussion

Our estimates of monthly P availability, which ranged from 0 to 18.42 µg/g, are comparable to those previously reported for Costa Rica (Vitousek and Denslow 1986, Vitousek and Denslow 1987, Cleveland et al. 2004). Although we detected weak seasonality in P availability, characterized by lower labile P during the dry season months, this variability was much less marked than seasonality in nutrient availability

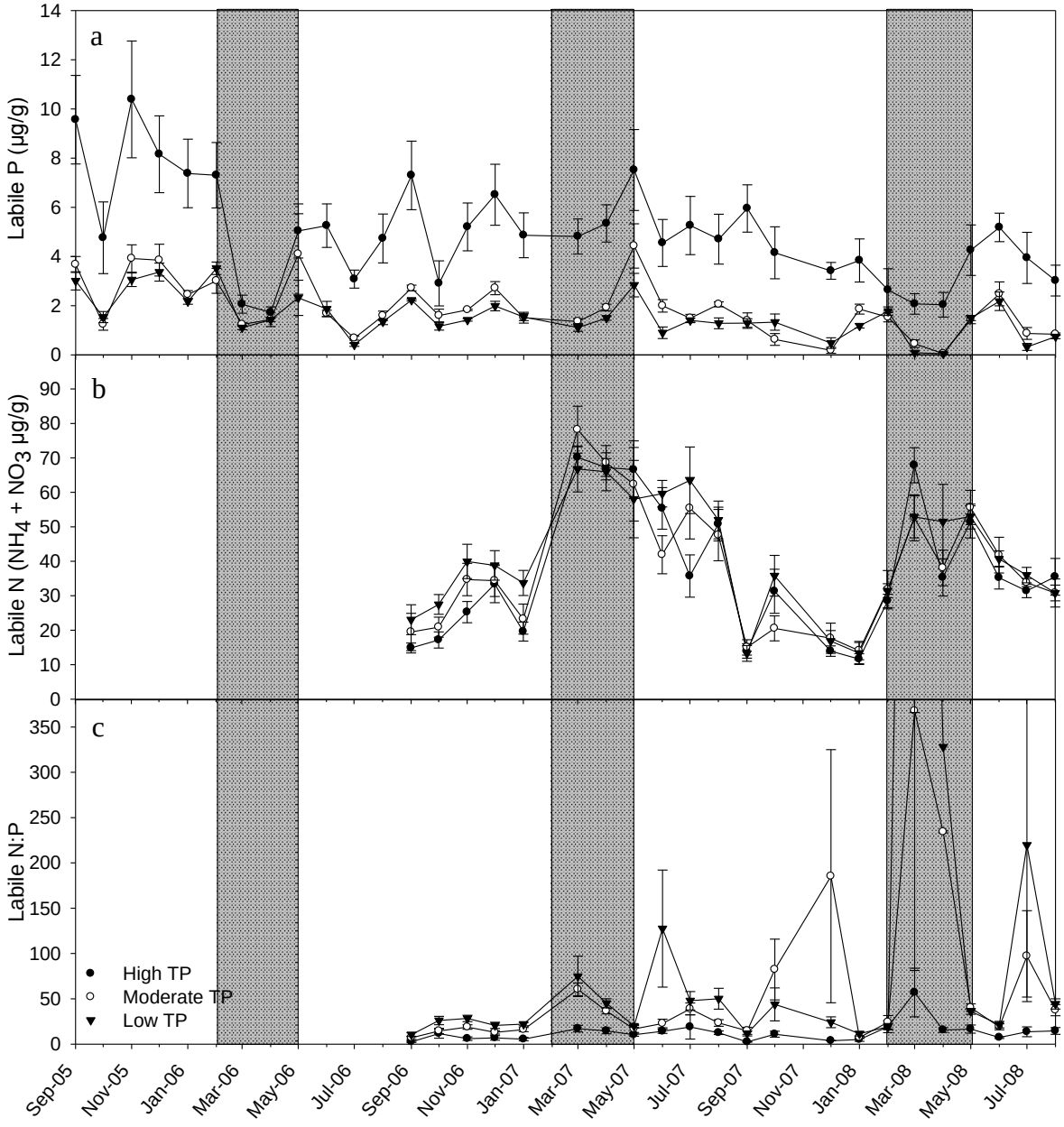
reported in dry tropical forests (Kieft et al. 1987, Singh et al. 1989, Lodge et al. 1994, Campo et al. 1998, Grierson et al. 1998). A number of studies in tropical wet forests, have failed to detect strong seasonality in nutrient availability (Vitousek and Denslow 1986, Matson et al. 1987, Yavitt and Wright 1996). Vitousek and Denslow (1986) observed very little variation in soil P concentrations among four sampling dates throughout the year at La Selva. Similarly, in a wet forest in Panama (MAP: 2600 mm), Yavitt and Wright (1996) found no seasonal pattern in soil labile P sampled every other month over approximately three years. In contrast, McGrath et al. (2000) found higher P concentrations at the onset of the rainy season in an Amazonian agroforest soil (MAP: 2000 mm). The mechanisms driving soil P supply may differ in forests of differing MAP and TP content. Site-specific differences in the distribution of rainfall throughout the year, particularly the length and intensity of the dry season may have a strong influence on fluctuations in nutrient availability.

More noteworthy was the high degree of synchrony in temporal patterns of soil P availability across a three-fold gradient in soil TP content. Although local factors, namely soil P fertility, determined the magnitude of labile P concentrations, fluctuations throughout the year were the same across the fertility gradient. P availability increased and decreased together, two fold, across the landscape. Similarly, Wood et al. (2006), found that, while soil type influenced the magnitude of leaf litter nutrient concentrations at La Selva, temporal patterns in litter P concentrations did not differ across soil types. Temporal synchrony in soil nutrient and litter nutrient concentrations indicate a common environmental cue. However, temporal patterns in soil P availability could not be explained by any single environmental factor that we tested. Lower P availability

during the dry season, particularly the extreme drought of 2008, suggests a link between soil moisture and labile P, however soil moisture at the time of soil sampling was only able to explain seven percent of the variation in labile P.

Figure 16: Soil nutrient availability in sites of differing TP content.

Monthly a) soil labile P, b) soil labile N (NH_4 plus NO_3), d., and c) soil labile N:P ratio in sites by TP fertility level (low, intermediate, and high; n = 6 per group. Monthly mean nutrient availability (± 1 SE) by fertility group represents the mean of samples collected over a 5 day period (up to 2 weeks).



High rates of decomposition and rapid turnover of the forest floor in the tropics indicate a tight link between litter inputs and soil nutrient availability. Given that forest floor turnover times range from 3-7 months at La Selva (Wood et al. 2009), litter-derived soil nutrient pulses should have been detectable within the timeframe of this study. We expected soil fertility and associated differences in litter quality to affect the timing of fluctuations in P availability, however, on a monthly timescale temporal patterns in labile P were consistent across the fertility gradient. On shorter timescales, our results did support previous findings that labile P in more P-deficient soils responds more rapidly to environmental conditions than it does in sites with higher P content. Higher labile P was associated with large rainfall events during the preceding 5 days in sites of low and intermediate TP, while labile P in sites of high TP increased in response to large rainfall events during the preceding 6-13 days. This result is at odds with the idea that microbial immobilization in more P-deficient soils should slow nutrient release from decomposing organic matter. A possible explanation for the more rapid response of labile P to environmental change in P-poor soils is that higher production of extracellular phosphatase enzymes, associated with greater biological demand for P, may lead to more rapid conversion of organic P to inorganic P upon soil wetting. A positive influence of soil moisture on the activity of phosphatase enzymes has been reported in a number of ecosystems, however, these results are quite variable (Chen et al. 2003, Criquet et al. 2004, Yavit et al. 2004, Sowerby et al. 2005).

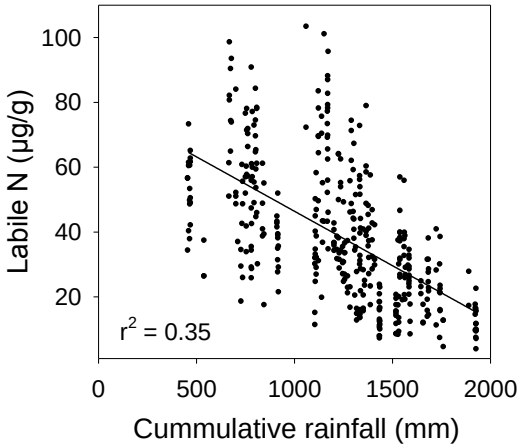
Lower labile P concentrations during the dry season, the time when live fine root biomass is lowest at La Selva (Espeleta and Clark 2007), indicates that P availability is not controlled by plant demand, but likely by factors that control P supply. Microbial

activity and biomass, which integrate the effects of environmental conditions and variation in organic matter quantity and quality on nutrient mineralization, may be better predictors of soil labile P. Soil P availability was positively associated with soil CO_2 efflux, an indicator of microbial activity, on a diel timescale (Vandecar et al. 2009), however, the variability in labile P over several days is much less than the seasonal or inter-annual variability measured here. The fundamental mechanisms driving variation at these timescales may be different. Links between microbial activity and P availability warrant further study and may help to elucidate the ecological processes underlying temporal variability in labile P in tropical soils.

N availability was high across sites at La Selva, as is the case in many tropical wet forests (Vitousek and Sanford 1986), however, unlike P, N availability displayed a fairly strong seasonal pattern, characterized by higher N availability during the dry season. Also, unlike P, both the timing and magnitude of variation in N were similar across the fertility gradient. Inorganic N accumulation during the dry season has been reported in a number of tropical forests (Luizao et al. 1992, Davidson et al. 1993, Cleveland et al. 2004). Given that N mineralization rates did not exhibit a seasonal pattern (Figure 23) at La Selva, these findings suggest that N availability is not controlled by factors influencing N supply but rather by factors that influence biological demand for N (microbial and plant uptake), factors limiting microbial metabolism, or ecosystem N losses (denitrification and leaching). We found that N availability was related to cumulative rainfall during the 2, 3, 4, and 5 months preceding soil sampling (Figure 17).

Figure 17: Relationship between N availability and rainfall.

N availability as a function of cumulative rainfall during the 4 months prior to soil sampling.



Lower live fine root biomass during the dry season at La Selva (Espeleta and Clark 2007) and an associated reduction in plant uptake of N is consistent with the observed pattern of higher N availability during the dry season. Additionally, greater losses of N through leaching during the wet season may contribute to the seasonal pattern we observed. Higher denitrification and nitrous oxide fluxes have been reported during the wet season in secondary tropical forests in Costa Rica (Matson et al. 1987, Keller and Reiners 1994), although overall rates of denitrification were low.

Our three year dataset of soil nutrient availability demonstrates that large interannual variations in P availability can occur in tropical wet forests. Although our study did not include an El Niño year, we did capture drought conditions typical of El Niño dry seasons in northeast Costa Rica (Waylen et al. 1996) during the dry season of 2008. During February and March of 2008, very low concentrations of labile P were found, in comparison to February and March of 2006 and 2007. Soil N:P ratios peaked during the dry season months of 2008 suggesting a shift to greater P limitation relative to

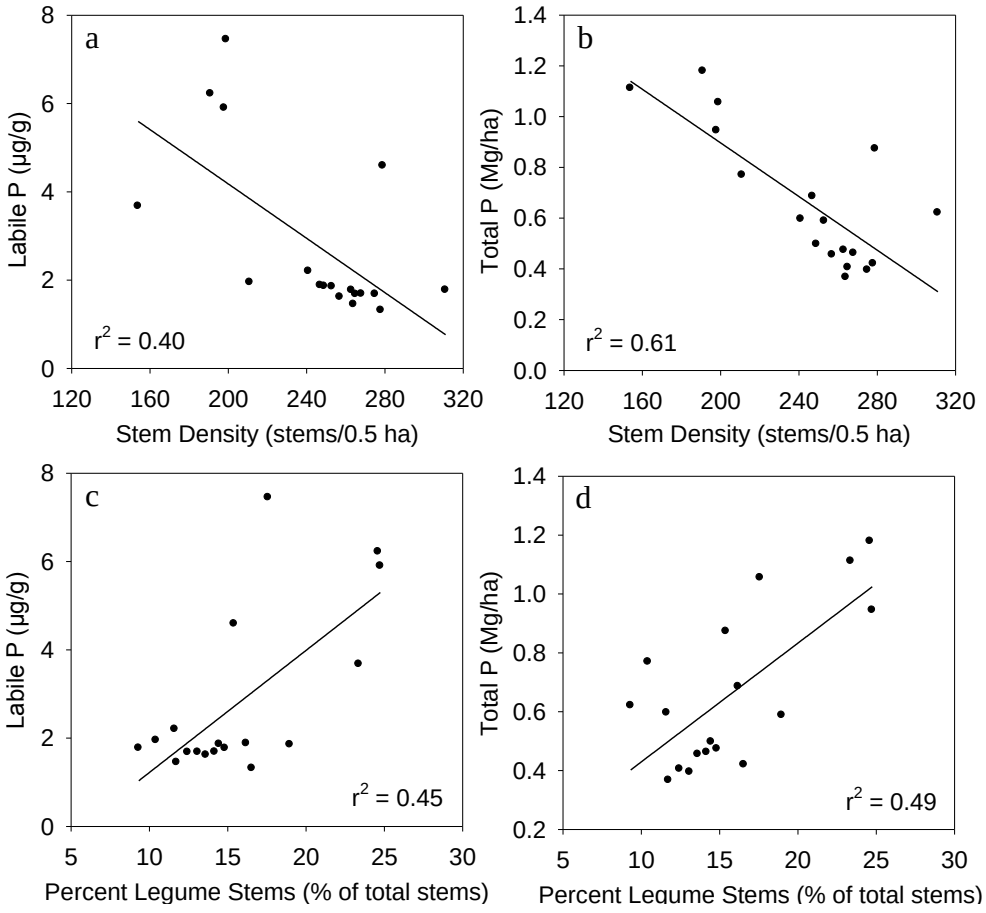
N during this period. The wider fluctuations in N:P ratios in soils of lower P content, particularly during the drought of 2008, suggests that more P-deficient soils may respond more strongly to inter-annual climate variability characteristic of ENSO events (Figure 16c). Predicted increases in the frequency and severity of El Niño events (Waylen et al. 1996), and the associated intensification of seasonality in tropical forests could lead to major shifts in nutrient cycling pathways, with implications for the future productivity and resilience of these systems. Understanding current patterns of nutrient cycling in mature tropical forests is key to predicting how they will respond to future climatic conditions. Despite three years of monthly data with substantial variation in key climatic factors and a high degree of synchrony in soil nutrient dynamics across a tropical forest landscape, we were unable to identify the critical drivers. Additional research is clearly needed to help elucidate the complex interactions between local and regional environmental factors and tropical forest nutrient dynamics.

Soil P status, but not N availability, appears to be strongly linked to vegetation characteristics at La Selva. We found that, across all stands, total stem density but not basal area declined with increasing soil P availability. Previously, at smaller spatial scales, we found that higher basal area is associated with higher labile P. On a local scale, plants may concentrate actively cycling forms of P in the upper soil horizon by accessing P from deeper soil layers and depositing it on the forest floor in litter. On larger spatial scales, stand-level differences in topographic conditions and gap formation rates may mask the influence of fertility on vegetation characteristics. Sites located on slopes had higher stem densities and generally lower P availability, suggesting that the negative association between stem density and labile P may not be causal. Species

composition, namely the distribution of N-fixing legume species, is consistently linked with P availability at La Selva. In accord with our findings at smaller spatial scales, the percentage of stems in the legume family across the 18 sites was positively associated with P availability.

Figure 18. Relationships between vegetation characteristics and soil labile P.

Relationships between a) soil labile P and total stem density, b) soil TP and total stem density, c) soil labile P and percent of stems in the legume family, and d) soil TP and percent of stems in the legume family across the 18 sites.



Higher soil P fertility may be necessary to support greater numbers of N-fixing species due to their relatively high demand for P. In addition, N-fixing species may augment

local P supplies by releasing more phosphatase into the soil than non-legume species in order to support N-fixation (Houlten et al. 2008). Zou et al. (1995) found that phosphatase activity as well as P availability increased in plantations of N-fixing trees. The strong coupling between labile P and vegetation characteristics in this forest indicate the potential for P constraints on plant productivity and ecosystem C balance and underscores the need for a better understanding of the mechanisms that drive P availability in tropical ecosystems.

CHAPTER 4: Assessment of phosphorus constraints on nitrogen cycling across a gradient in tropical rain forest soil P content

Abstract

It has been suggested that declining phosphorus (P) availability in highly weathered soils can ultimately constrain soil microbial processes and restrict nitrogen (N) cycling. Theoretically, there are a number of biological mechanisms that could contribute to P controls on N cycling, however, empirical evidence of such interactions on biologically relevant timescales is scarce. We investigated the potential link between P availability and N cycling in tropical rain forest soils similar in N content (2.2 – 3.9 Mg/ha; 0-10 cm), but spanning a three-fold gradient in TP content (0.39 – 1.18 Mg/ha; 0-10 cm). Vegetation structure and species composition were characterized across the gradient in soil P content. For two years, surface soils (0-10 cm) in 18 sites were sampled monthly for labile inorganic P (P_i), labile organic P (P_o) and labile inorganic N concentrations, and were incubated aerobically for one week to determine N mineralization potential (N_{minp}). We found that soil total N and total carbon (C) content were positively related to mean soil labile P_o. Mean nitrogen availability was strongly linked to both mean soil labile P_i and P_o in the most P-deficient sites. The soil C:N ratio, an indicator of SOM quality, was inversely related to both TP and mean labile P_i. During the two years, monthly N_{minp} was negatively related to the soil labile N:P_i ratio in eight sites. A multiple regression model including soil moisture and labile N:P_i was able to explain up to 49% of the variation in N_{minp}. Across all stands, the percentage of stems in the legume family was positively related to all measured P fractions, signifying the importance of soil P status for N-fixing species. Soil P availability appears to play an

important role in N transformation rates and vegetation dynamics in these N-fixing forests. Predicted changes in the moisture regimes of tropical ecosystems have the potential to disrupt the coupling of N and P cycles with important implications for carbon accumulation in tropical forests.

Introduction

P supply often constrains net primary productivity in highly weathered soils and replenishment of plant available P is strongly dependent on biological processes that recycle organic forms of P (Walker and Syers 1976, Hedin et al. 2003). Although plants and microbes can promote solubilization of occluded P by releasing extracellular phosphatase enzymes when P becomes limiting, there is no biological mechanism analogous to N fixation by which biota can radically increase P inputs to terrestrial ecosystems. Walker and Syers (1976) argued that soil P content ultimately becomes the most limiting nutrient to productivity in terrestrial ecosystems. Evidence from a number of chronosequence studies have provided support for this hypothesis, demonstrating that over geological timescales the accumulation of C, N, and SOM is regulated by the pool of available P (Walker and Adams 1958, Syers et al. 1970, Walker and Syers 1976, Cole and Heil 1981, Tate and Salcedo 1988, Crews et al. 1995). Theoretically, there are a number of biological mechanisms that could contribute to P controls on N cycling, however, empirical evidence of such interactions on biologically relevant timescales is scarce. N and P availability interact through a number of ecosystem processes that could potentially lead to P controls on N cycling:

1. Low soil P availability can limit soil microbial activity impacting decomposition and N transformation rates

2. P limitation can lead to the production of low quality litter which feeds back to negatively influence rates of N cycling and productivity
3. Synthesis of the nitrogenase enzyme for N fixation requires substantial amounts of P, molybdenum, and iron.
4. Plants and microbes must invest N in the production of phosphatase enzymes when soil P is low

P limitation of microbial processes

The mineralization of N, which is directly bound to C in OM, is dependent on the microbial breakdown of C through decomposition. In contrast, P which is indirectly bound to OM-C through ester linkages, can be mineralized enzymatically, independent of decomposition (McGill and Cole 1981). Consequently, N turnover is tightly coupled to C turnover and microbial energy demands, while P turnover is dependent on soil P supply and biological demand for P. Cole and Heil (1981) proposed that P availability exerts controls on N cycling through its central role in microbial metabolic reactions, (i.e. the high energy cost of microbial growth) and consequently N transformation rates. It is increasingly recognized that P availability, as well as the N:P supply ratio can constrain microbial activity and decomposition in P-deficient soils (Amador and Jones 1993, Gallardo and Schlesinger 1994, Crews et al. 1995, Vitousek and Farrington 1997, Hobbie and Vitousek 2000, Cleveland et al. 2002, Stevenson 2004, Cleveland et al. 2006, Reed et al. 2007, Kaspari and Yanoviak 2008, Güsewell and Gessner 2009). P availability thus, could represent a rate limiting factor for C turnover, N mineralization (Munevar and Wollum 1977, Nommik 1978, Pastor et al. 1984, Tate and Salcedo 1988, Hossain et al. 1995, Saggar et al. 2000, White and Reddy 2000, Carlyle and Nambiar 2001, Kranabetter

et al. 2005) and nitrification in some ecosystems (Purchase 1974, Pastor et al. 1984). Short-term interactions between N and P cycles driven by P limitation of microbial metabolic processes should be evident in the amounts of N and P that are bioavailable. Kranabetter et al. (2005) found that organic P concentrations in the forest floor and mineral soil of temperate forests on the north coast of British Columbia were positively correlated with extractable inorganic N concentrations. Additionally, P fertilization increased the availability of inorganic N, particularly in more P-deficient soils. Positive effects of P fertilization on rates of N and C mineralization have been reported in a range of forest soils (Munevar and Wollum 1977, Nommik 1978, Haynes and Swift 1988, Amador and Jones 1993, White and Reddy 2000, Cleveland et al. 2002, Kranabetter et al. 2005). These findings suggest that under P limitation microbial biomass may be less efficient in utilizing SOM substrates for energy.

P controls on N uptake and litter quality

Soil P limitation can result in the production of lower quality litter (higher C:P and C:N ratio) (McGroddy et al. 2004, Parfitt et al. 2005). In a chronosequence across the Hawaiian islands, Crews et al. (1995) found that foliar N content was lowest in the oldest site despite high available N, indicating that something other than N availability was limiting N uptake by plants, potentially P. Parfitt et al. (2005) proposed that lower quality litter associated with P limitation increased the C:N ratio of SOM. This hypothesis was supported in a subsequent study across precipitation and temperature gradients in Hawaii, in which Idol et al. (2007) found that soil C:N ratios were negatively correlated with soil P availability and the mineral P content of the soil and concluded that soil P availability was one of the principal determinants of SOM quantity and quality.

P controls on nitrogen fixation

Biological N-fixation represents one of the primary N inputs to ecosystems (Cleveland et al. 1999, Galloway et al. 2004). N-fixing organisms should have a competitive advantage over non-N-fixers in N-deficient soils, however, evidence suggests that low N availability is not the only control on biological N fixation (Vitousek and Howarth 1991, Vitousek and Field 1999). Converting atmospheric N_2 into ammonium (NH_4^+) is an energetically expensive process relative to uptake of ammonium or nitrate from soils (Hartwig 1998, Vitousek and Field 1999). N fixation requires three to six grams of carbon per gram of N fixed and also requires substantial amounts of P-containing compounds including ATP (Chapin et al. 2002). Nitrogenase, the enzyme responsible for fixing atmospheric N_2 into ammonia is rich in P, Fe, and Mo (Hartwig 1998). Although C limitation in temperate forests appears to determine the distribution of N-fixing plants, limitation by nutrients such as P and Mo, may secondarily constrain N fixation rates in early successional and tropical systems (Vitousek and Field 1999, Vitousek and Hobbie 2000, Rastetter et al. 2001, Barron et al. 2009, Finzi & Rogers 2009).

Several lines of evidence suggest that soil P availability or the ratio of N:P in soils may regulate symbiotic and asymbiotic N fixation (Ekblad and Huss-Danell 1995, Crews et al. 2000, Uliassi et al. 2000, Finzi and Rodgers 2009). Finzi and Rogers (2009) found that the abundance, growth, and reproductive output of N_2 fixing species, as well as the fraction of plant-N derived from N_2 fixation in temperate, old-field ecosystems was constrained by the availability of P. P fertilization studies have demonstrated positive effects of P on the abundance (Kirkham et al. 1996, Martiniello 1998), growth (Crews

1993, Uliassi et al. 2000), and nodule biomass (Gates 1974, Grove and Maljczuk 1992, Ekbald and Huss-Danell 1995, Uliassi et al. 2000) of N-fixers. Low foliar N:P ratios have also been associated with higher N-fixation rates (Matzek and Vitousek 2003).

Production of nutrient mineralizing enzymes

Enzyme production is one of the ways in which plants and microbes regulate nutrient mineralization in soils. When soil nutrient supply is low, ectomycorrhizal fungi and plant roots produce extracellular enzymes that allow them to access organically bound nutrients (McGill and Cole 1981, Olander and Vitousek 2000, Dakora and Phillips 2002, Barroso and Nahas 2005). High nutrient availability, on the other hand, can have inhibitory effects on enzyme production leading to a negative feedback mechanism, which has been observed in some soils for the P solubilizing phosphatase enzyme (Juma and Tabatabai 1978, Spiers and McGill 1979, Clarholm 1993, Sinsabaugh et al. 1993, Tadano et al. 1993). The supply of one nutrient in the soil could also influence the mineralization of other nutrients through constraints on enzyme production. P solubilizing enzymes represent a substantial N investment for plants and microbes and a large body of research demonstrates that phosphatase activity is enhanced by increasing N availability (Dick et al. 1988, Zou et al. 1995, Olander and Vitousek 2000, Treseder and Vitousek 2001, Hrynkiewicz et al. 2009, Kritzler and Johnson 2010, Naples and Fisk 2010). Treseder and Vitousek (2001) found that N fertilization increased extracellular phosphatase activity across a chronosequence in Hawaii, while P additions decreased phosphatase activity, mycorrhizal colonization, and P uptake capacity. Increased phosphotase activity has also been associated with higher litter N:P ratios (Güsewell and Freeman 2005). However, P availability may not have a reciprocal influence on the

production of enzymes involved in N mineralization, given that P is not an important component of enzymes such as chitenase. Additionally, the production of N mineralizing enzymes may not be tightly linked to N demand because there are other pathways for N mineralization (Olander and Vitousek 2000). Olander and Vitousek (2000) found that phosphatase activity was high across a chronosequence in Hawaii and was suppressed by P additions at all sites and enhanced by N addition in sites where N availability was low. Alternatively, they found that chitanase activity decreased as N availability increased across the chronosequence, was suppressed by N additions only in the most N limited site, and was not influenced by P additions. Differences in enzyme production may be an important point of interaction between N and P cycles and may help to explain variation in observed patterns of N and P availability. N_2 fixing plant species common in tropical forests may hold an advantage in P acquisition by allocating excess N to N-rich P acquiring enzymes (Houlten et al. 2008).

In this study, we exploited the natural gradient in P content across tropical rain forest soils at La Selva Biological Station in northern Costa Rica to investigate potential links between P and N cycling as well as other soil chemical properties. Specifically we addressed the questions: (1) How does soil P status (i.e. labile P_i and P_o and TP) relate to measures of N and C accumulation and cycling (i.e. mean N availability, mean N mineralization potential (N_{minp}), total soil N, total soil C, soil C:N ratio) across a three-fold gradient in soil TP content. (2) Does within-site N availability or N_{minp} track labile P dynamics over time? Does this relationship differ along the gradient in soil TP? (3) Does variation in vegetation structure (basal area and stem density) and composition, particularly the distribution of N-fixing legumes, correspond to the gradient in soil TP?

Does it affect the relationship between N and P in soils? We expected to find stronger coupling of N and P cycles in soils on the more P-deficient end of the TP gradient.

Methods

Study Site

The study was conducted in mature tropical rain forest at La Selva Biological Station in the Caribbean lowlands of northern Costa Rica (10° 26' N, 83° 59' W; Organization for Tropical Studies). La Selva has a mean annual precipitation of 4300 mm (Organization for Tropical Studies, *unpublished data [available online]*), with the driest period occurring between late January and April (McDade et al. 1994). Mean annual temperature is 25.8 ºC (McDade et al. 1994). The soil parent material at La Selva is volcanic in origin, however, some soils have formed on old alluvial deposits while others are residual soils that have formed *in situ* on old lava flows. Soils at La Selva are similar in most ways, being characterized as deep, well-drained, acidic, and clay-rich, however they differ markedly in P fertility (Espeleta & Clark 2007). Soil P is sometimes in the deficient range and has the potential to limit plant growth (Denslow et al. 1987, Vitousek and Denslow 1987) and litter production (Wood et al. 2009). The forest is predominantly evergreen (McDade et al. 1994) and is dominated by a N-fixing legume *Pentaclethra macroloba* , which accounts for as much as 36% of the basal area and 13% of stems (McDade et al. 1994, Lieberman et al. 1996, Clark and Clark 2000).

Soil Sampling and Chemical Analysis

18 sites on Oxisols of differing P content (Figure 13), were chosen for this study: CARBONO plots A1-6, L1-6, and P1-6 (Table 4; the CARBONO plot network is described in Espeleta & Clark 2007). In each 50 x 100 m (0.5 ha) plot, soil samples (0-

10 cm depth) were collected from 16 locations systematically arrayed across each plot, every month between September 2006 and September 2008. Soil samples were composited and sieved through a 2 mm mesh screen. Gravimetric soil moisture was determined on a 10 g sub-sample oven-dried at 105 degrees Celsius for 24 hours. Volumetric soil moisture was measured in the center of each plot by a Campbell CS615 (Campbell Scientific, Logan, Utah, USA) sensor (0-30 cm; calibrated for La Selva soils as per Veldkamp & O'Brien 2000) every two weeks during the study. Using the Olsen extraction technique, P was extracted from 4 grams of fresh soil by shaking for 20 hours in 30 ml of 0.5M $NaHCO_3$ (Olsen et al. 1954). The extractant was filtered and the P concentration of extract solutions was determined using molybdate blue colorimetric reactions measured on an Alpkem Flow Solution IV 250 Auto Analyzer (OI Analytical; College Station, Texas, USA). The bicarbonate solution is thought to extract P loosely bound to soil surfaces as well as some P associated with the microbial pools and is considered readily available for uptake by both plants and microbes (Cross and Schlesinger 1995). TP in the extracts was determined by persulfate digestion. Samples were digested in an autoclave for 60 minutes with ammonium persulfate and sulfuric acid to convert all P to orthophosphate. The P concentration of the digested sample was determined using the molybdate blue method and organic P in the bicarbonate extracts was calculated as the difference between TP and P_i. Bicarbonate-extractable P_o is considered highly labile and is related to plant available P (Bowman and Cole 1978, Cross and Schlesinger 1995).

A 10 g sample of sieved field-moist soil from each of the 18 sites was extracted for ammonium (NH_4-N) and nitrate (NO_3-N) by shaking for one hour with 50 ml of 2M

KCl solution. Extracts were passed through filters that were pre-extracted with KCl. Extracts were analyzed colorimetrically on a Lachat QuikChem 8500 (Hach Co., Loveland, CO). A second 10 g sample of sieved field-moist soil from each of the 18 sites was placed in a 120 ml specimen cup for determination of N_{minp} during a one week aerobic incubation procedure (at room temperature, ~24°C) described by Hart et al. (1994). Cups were partially closed to allow gas exchange while minimizing evaporation loss. Ammonium (NH_4-N) and nitrate (NO_3-N) were extracted and analyzed as described above. N_{minp} was estimated as the difference between inorganic N at the beginning and end of the incubation.

Vegetation Structure and Composition

Basal area, stem density and species composition were tabulated in September-October 2006 for all live stems with a diameter at breast height (DBH) greater than 10 cm within each 0.5 ha plot (Table 4).

Statistical Analyses

Regression analysis was used to determine the relationship between soil P pools (mean labile P_i, mean labile P_o, and TP) and soil total N, mean labile N, mean N_{minp}, soil total C, and soil C:N ratio across the gradient in soil TP content. All plot-level mean values were calculated based on monthly values measured between September 2006 and September 2008. We tested for within-site relationships between P and N cycles by regressing monthly labile P_i, labile P_o and the labile N: P_i ratio with monthly labile N and N_{minp} in each of the 18 sites over two years. For sites with a significant relationship between labile N:P ratio and N_{minp}, the slope of the relationship for each site was regressed against soil TP content. A t-test was used to compare the soil properties in sites

that exhibited this relationship and those that did not. Stepwise multiple regression was performed to determine whether a combination of soil moisture and labile N:P ratio could be used to better predict temporal patterns in N_{minp} within each site.

Linear regression was used to explore the relationship of stand-level stem density and basal area, stem density and basal area of legumes, and the percentage of total stem density and total basal area comprised by legumes, to mean soil labile P_i and P_o, labile N, TN and soil total N:P. Measures of vegetation structure were also regressed against the soil total N:P ratio and the mean labile N:P_i ratio across sites.

Results

Relationships between soil P fractions and other soil chemical properties

The bicarbonate-extractable P_i pool made up less than 1% of the soil TP (0.37-0.84%) in all 18 sites. The bicarbonate-extractable P_o pool was positively correlated with ($r^2 = 0.30$, $F = 6.80$, $p = 0.0190$), but 3-12 fold greater than the P_i pool across the 18 sites (Figure 19). Labile P_i and labile P_o were positively related to soil TP ($r^2 = 0.78$, $F = 56.89$, $p < 0.0001$; $r^2 = 0.23$, $F = 4.86$, $p = 0.0425$, respectively). The ratio of labile P_o to P_i was inversely related to the TP content of the soil ($r^2 = 0.40$, $F = 10.60$, $p = 0.0050$, Figure 20).

Soil labile P_o was positively related to total C ($r^2 = 0.24$, $F = 5.01$, $p = 0.0397$) and total N ($r^2 = 0.32$, $F = 7.46$, $p = 0.0148$; Figure 21a and b). Soil TP and labile P_i were negatively related to the soil C:N ratio ($r^2 = 0.41$, $F = 11.17$, $p = 0.0041$, $r^2 = 0.38$, $F = 9.94$, $p = 0.0062$; Figure 21c and d). Soil labile P_i and TP were inversely related to the soil C:labile P_o ratio ($r^2 = 0.49$, $F = 15.20$, $p = 0.0013$; $r^2 = 0.50$, $F = 15.74$, $p = 0.0011$, respectively). Mean N availability was not significantly related to P_o across all sites.

However, when sites were grouped into three TP levels (low, intermediate and high TP), each group having a mean TP content significantly different from all the others (t-test, $p<0.05$), N availability was positively related to labile P_i and P_o in sites with lower TP ($r^2 = 0.80$, $F = 16.34$, $p = 0.0156$; $r^2 = 0.74$, $F = 11.23$, $p = 0.0285$, respectively; Figure 22). When site P3 was excluded from the analysis (P3 had a P_o:P_i ratio that was more than two standard deviations above the mean site P_o:P_i ratio) the relationship between P_o and N availability was positive for each of the TP levels (high TP: $r^2 = 0.66$, $F = 7.61$, $p = 0.0509$; intermediate TP: $r^2 = 0.76$, $F = 9.66$, $p = 0.0530$; low TP: $r^2 = 0.74$, $F = 11.23$, $p = 0.0285$), although only marginally significant in the intermediate and high TP groups. The slope of the relationship increased from high to intermediate to low TP sites when P3 was excluded (Figure 22).

Relathionships between soil N, N_{minp}, and other soil chemical properties

Mean KCl-extractable N (27.2-44.7 µg/g, Figure 23) was positively related to soil total N ($r^2 = 0.49$, $F = 15.63$, $p = 0.0011$). Mean N_{minp} was not related to soil total N. N mineralization potential (0.5-1.7 µg/g/day) did not vary consistently among sites over the two year study, with the exception of May 2007 when a peak in N mineralization rates was observed in all 18 sites (Figure 23). Monthly N_{minp} was negatively related to the ratio of labile N (KCl-extractable N) to labile P (P_i) in the soil in eight sites (soil labile N:P_i; Table 2; Figure 24). The labile N:P_i ratio explained one quarter to one third of the variability in N_{minp}. The slope of the regression relationships and the intercepts varied among sites, with slope inversely related to TP content ($r^2 = 0.59$, $F = 8.72$, $p = 0.0255$). Mean site soil moisture was significantly higher in the eight sites that exhibited this relationship ($t = 2.37$, $p = 0.0321$), however soil TP content was not significantly higher

than in sites with no relationship. A stepwise multiple regression model including soil moisture (negatively related) and labile N:P ratio (negatively related) was able to explain a greater percentage of the variation in N_{minp} in four of the sites that exhibited this relationship (L3: r^2=0.49, F=8.99, p=0.0018; P1: r^2=0.44, F=7.47, p=0.0040; P3: r^2=0.41, F=6.48, p=0.0072; P6: r^2=0.35, F=4.51, p=0.0268). Monthly N_{minp} was positively related to monthly labile P_i in four of the 18 sites, however this relationship was driven by the peak in N_{minp} and labile P_i during May of 2007. Monthly N availability was not related to labile P_o. N_{minp} was not related to N availability during the two years, with the exception of site L2 where they were inversely related (r^2 = 0.21, F = 5.58, p = 0.0278).

Relationships between vegetation characteristics and soil nutrient status

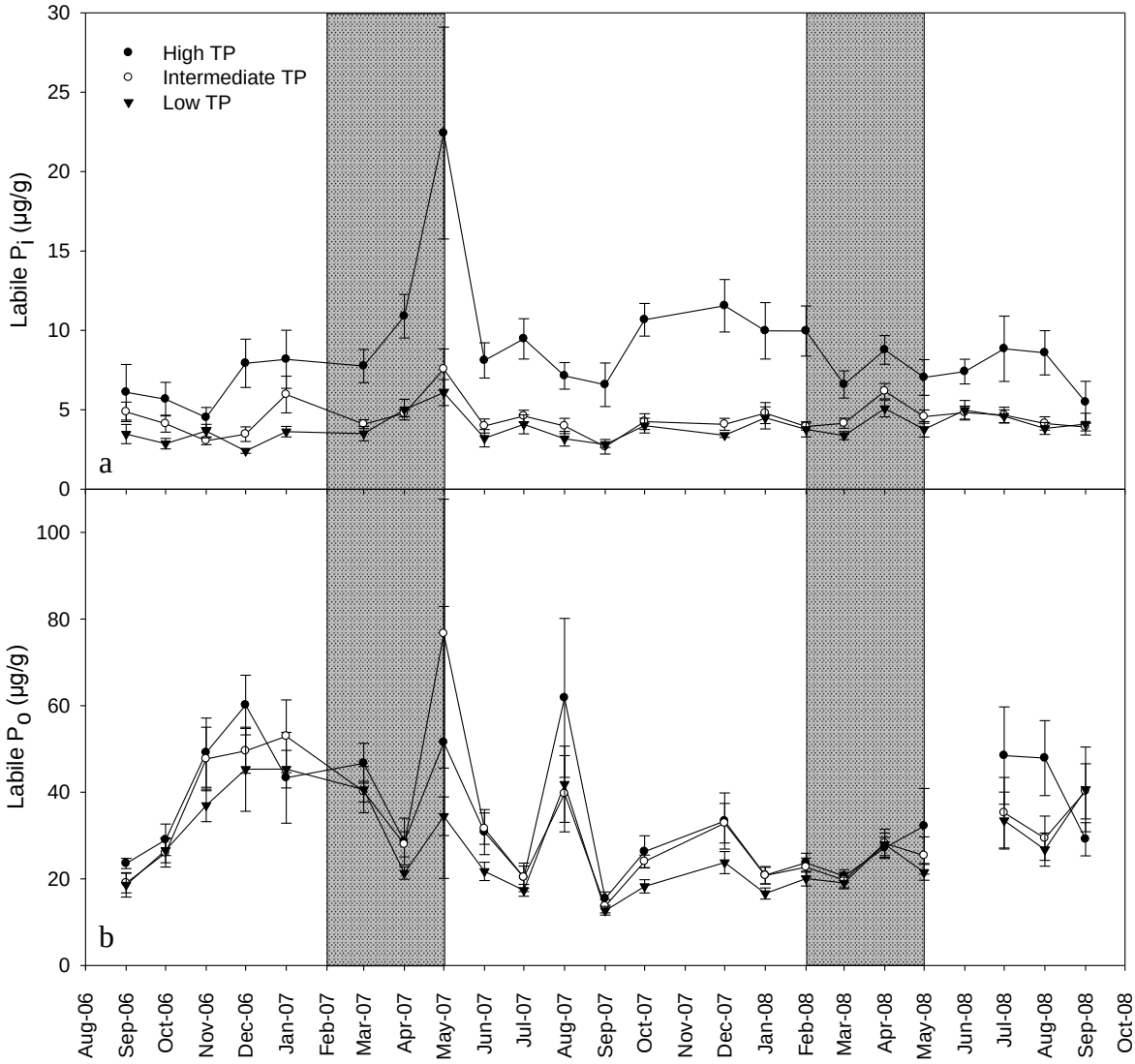
Total stem density of trees greater than 10 cm ranged from 154-311 (stems/0.5 ha) and total basal area ranged from 9.4 – 14.1 (m^2/0.5 ha). Legumes comprised 9-25% of the total stem density and 20-57% of the basal area. Stand stem density declined with increasing labile P_i (r^2 = 0.63, F = 26.99, p < 0.0001) and labile P_o (r^2 = 0.42, F = 11.56, p = 0.0037). Stand stem density increased with increasing soil total N:P (r^2 = 0.31, F = 7.25, p = 0.0160). The percentage of total stems in the legume family was positively related to labile P_i (r^2 = 0.41, F = 11.12, p = 0.0042), labile P_o (r^2 = 0.35, F = 8.62, p = 0.0097; Figure 25). The percentage of total stems in the legume family was negatively related to soil total N:P (r^2 = 0.25, F = 5.18, p = 0.0369). Total basal area and basal area of legumes was not related to labile P_i, P_o, TP, or N availability. Total stem density was not related to legume stem density across the 18 plots. However, total basal area was positively related to legume basal area (r^2 = 0.53, F = 17.79, p = 0.0007).

Discussion

As is the case in many mature tropical wet forests, soil N availability was consistently high among soils at La Selva (Vitousek 1984). Contrastingly, labile P_i made up a very small fraction of the TP pool, and varied widely in concert with TP. Although these sites are centered in one region of the Neotropics, the three-fold gradient in TP captures a large portion of the variation in soil P status of tropical forests worldwide (Vitousek and Sanford 1986). Bioavailable phosphorus (P) is recognized as a major constraint on productivity in many tropical rain forests, and low P availability has been associated with reduced plant growth in the more P-deficient soils at La Selva (Denslow et al. 1987, Vitousek and Denslow 1987). Thus, this group of sites provides a useful context for comparative studies of the effects of P availability on diverse ecosystem processes.

Figure 19: Labile P_i and P_o in sites of differing TP content.

Monthly a) soil labile P_i and b) soil labile P_o between September 2006 and September 2008. Sites are grouped by TP fertility (n = 6 per group). Monthly mean P availability (± 1 SE) by fertility group represents the mean of samples collected over a 5 day period (up to 2 weeks). The shaded region indicates the dry season (February – April; < 270 mm rainfall per month)

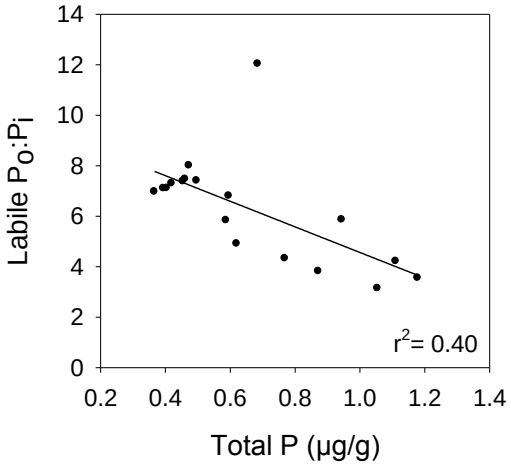


Although geochemical processes dominate changes in P distribution over geological timescales, biological processes drive short term variation in the small but dynamic labile P pool. The importance of organic P mineralization in supplying plant available P, particularly in old tropical soils, has been well established (Tiessen et al. 1984, Johnson

et al. 2003). We found that labile P_o made up a much larger percentage of TP than labile P_i (0.5 vs. 4% respectively) and that its relative importance increased as TP content declined.

Figure 20: Relationship between the soil labile P_o: P_i ratio and TP.

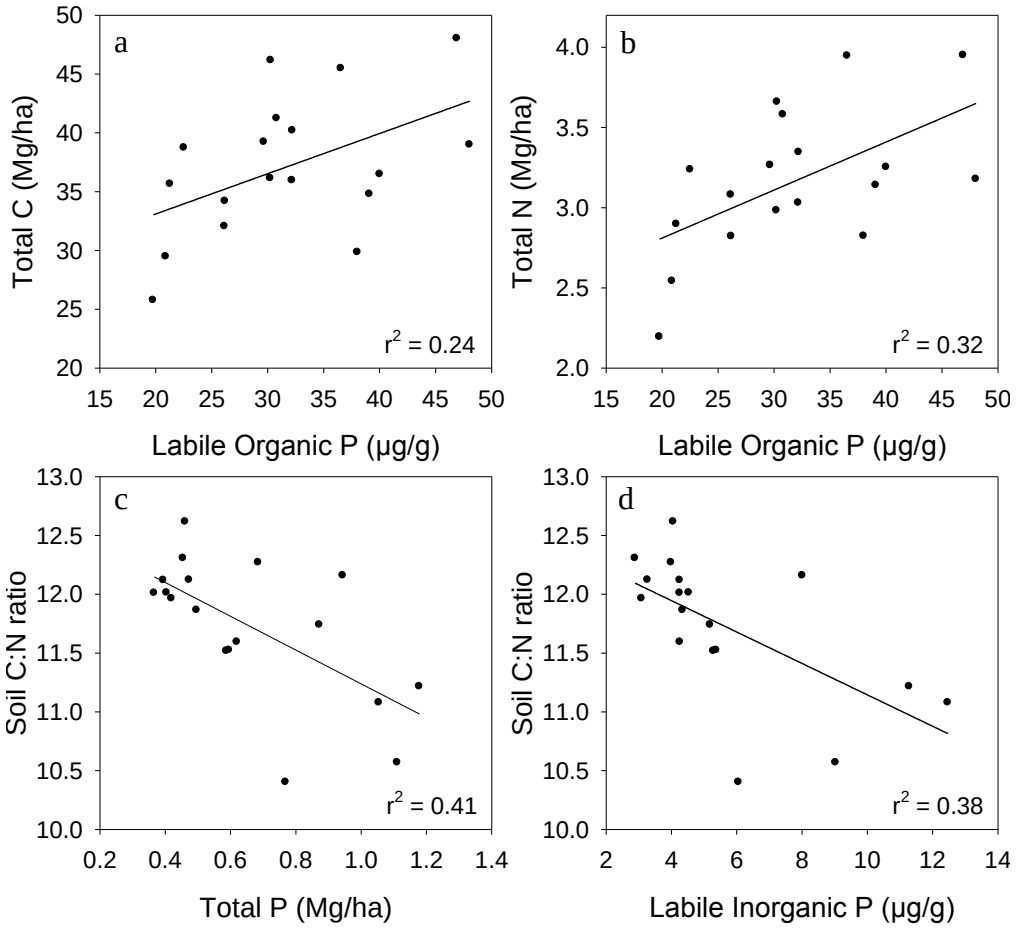
The outlier is site P3 which had a P_o: P_i ratio greater than 2 standard deviations above the mean P_o: P_i ratio.



The inverse relationship between the labile P_o: P_i ratio and TP may be the result of rapid uptake of labile inorganic P associated with greater biological demand in the more P deficient sites. A number of chronosequence studies (Crews et al. 1995) and a global meta-analysis of soil stoichiometry by Cleveland and Liptzen (2007) demonstrate the importance of soil organic P content in regulating the accumulation of C and N in tropical soils. Similarly, we found that the total C and N content of our soils was related to soil labile P_o concentrations (which contributes to labile P_i on short-timescales).

Figure 21: Relationships between soil P and total soil C, N, and C:N ratio.

Relationships between a) soil labile P_o and soil total N b) soil labile P_o and soil total C content, c) soil P_i and soil C:N ratio, and d) soil and TP and soil C:N ratio across the 18 sites.

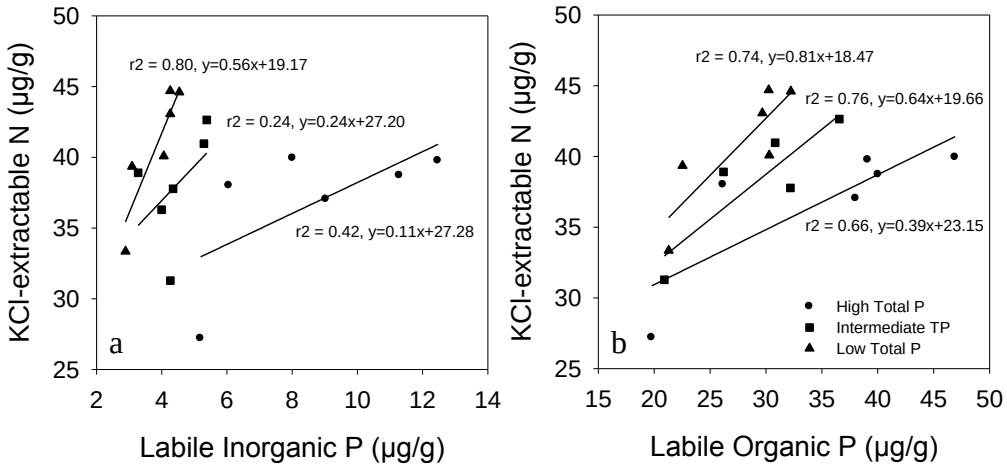


Parallel changes in total soil C, N and labile P_o are consistent with the idea that C and N accumulation are dependent on biologically available P, which cycles more conservatively than C and N in tropical soils (Walker and Adams 1958, Walker and Syers 1976, Cole and Heil 1981, Tate and Salcedo 1988, Crews et al. 1995). We also found that biologically active N closely tracked labile P_i and P_o dynamics, particularly in the more P deficient sites, as would be expected if biologically active P regulates N accumulation and transformation rates (Cole and Heil 1981). The slope of the relationship between labile P_o and N availability was inversely related to TP content,

suggesting that P availability may have a stronger influence on labile N in the more P-deficient soils.

Figure 22: Relationships between N availability and labile P_i and P_o.

Relationships between a) soil labile P_i and soil N availability, and b) soil labile P_o and soil N availability (site P3 excluded) across the 18 sites.



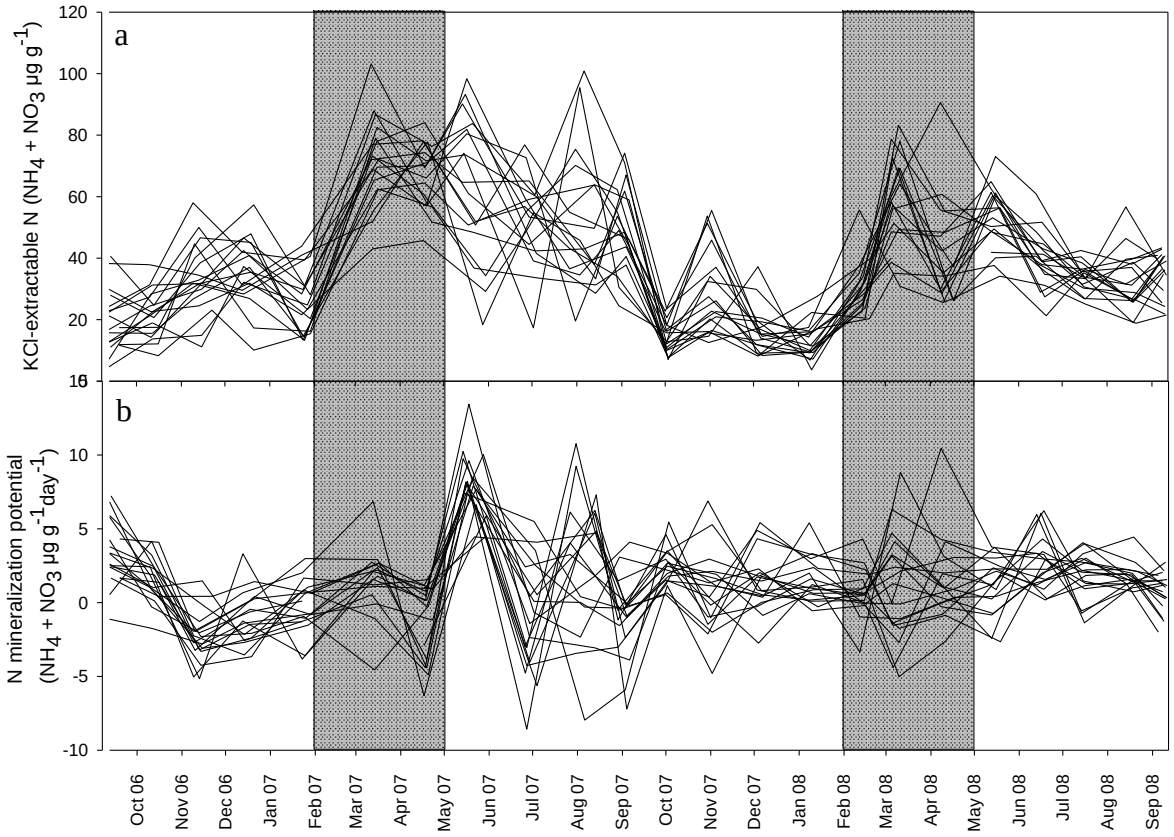
Plants that are P limited tend to produce litter with high C:P ratios which leads to increases in SOM C: P_o ratios (McGroddy et al. 2004, Parfitt et al. 2005). We found that the C:labile P_o ratio grew wider as soil TP declined. P limitation can also result in higher litter C:N ratios (Walker and Adams 1957). The C:N ratio, an indicator of SOM quality, was highest in low P sites, and declined with increasing labile P_i and TP in our soils. This relationship was also reported in regenerating montane forests, across temperature and precipitation gradients in Hawaii (Idol et al. 2007), and in leguminous pastures along chronosequences in New Zealand (Parfitt et al. 2005). Parfitt et al. (2005) suggested that P limitation led to the production of low quality litter which in turn increased the soil C:N ratio.

Although it is widely accepted that P availability plays a key role in many ecosystem processes, including soil microbial metabolism and forest productivity, few

attempts have been made to measure short-term P constraints on N transformation rates in tropical ecosystems. Vitousek and Denslow (1987) found a weak positive correlation (r^2 = 0.25-0.31) between net N mineralization and P_i availability within four soil types at La Selva, however this relationship was not significant when examined across soil types. Given that the mass based C:P ratio of our soils ranged from 27 in the site with the highest P content to 107 in the most P-deficient site, while the C:P ratio of bacteria ranges from 20:1 to 30:1 (Paul and Clark 1989), we expected P limitation to constrain microbially-mediated N mineralization in the more P-deficient sites. We found that the relative availability of N to P was more important than the absolute concentration of P in the soil in determining N_{minp}. Given the fairly well constrained N:P stoichiometry of microbial biomass (Cleveland and Liptzen 2007), it is not surprising that soil labile N:P ratios would influence microbial utilization of C substrates and consequently N mineralization rates.

Figure 23: N availability and N mineralization potential.

Monthly a) N availability and b) N mineralization potential in each of the 18 sites between September 2006 and September 2008. The shaded region indicates the dry season (February – April; < 270 mm rainfall per month).

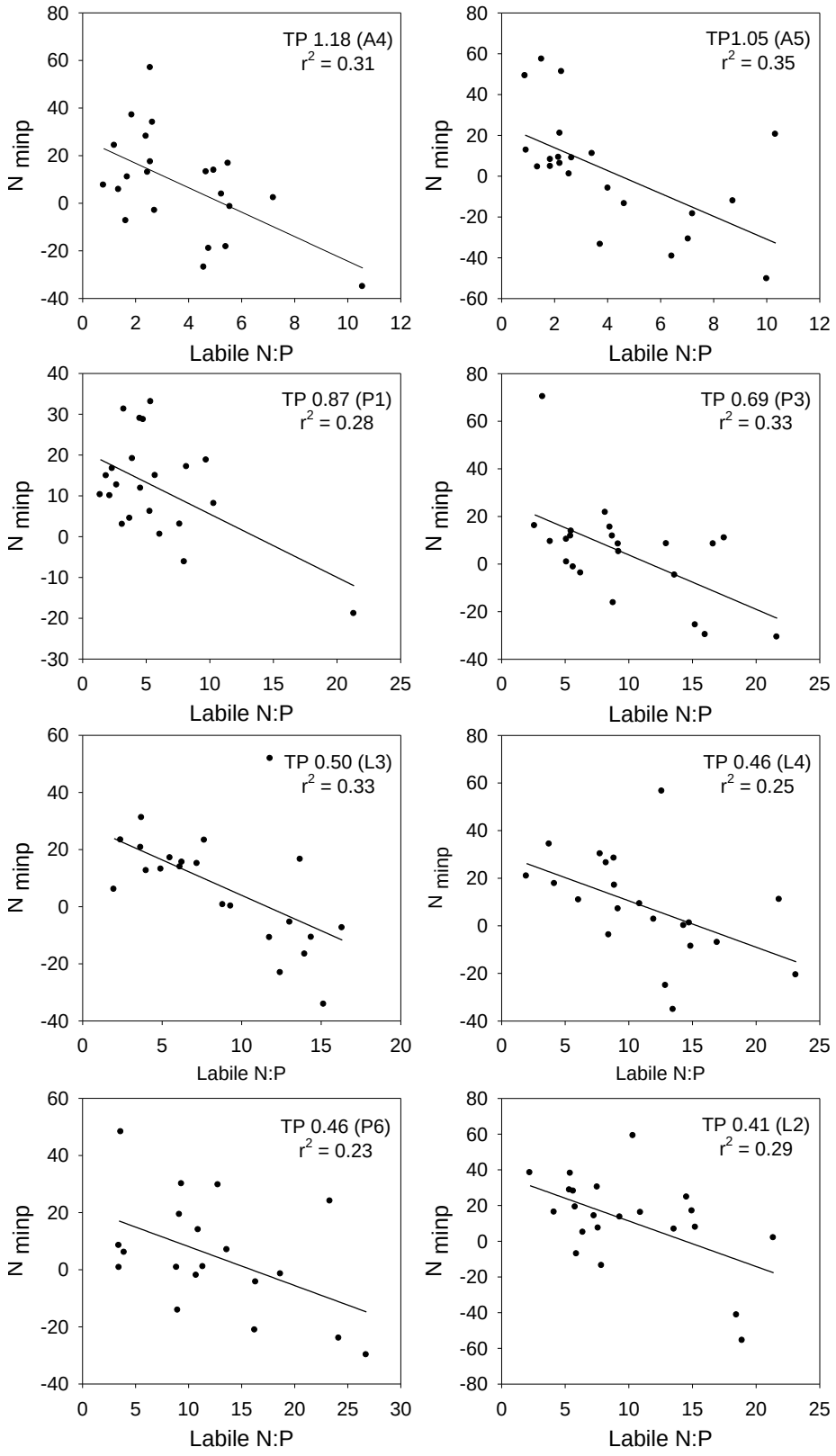


N-mineralization potential was influenced by the soil labile N:P_i ratio in eight study sites spanning the gradient in TP content. Congruent with our hypothesis that N and P cycles are more tightly coupled in soils of lower P content, the slope of this relationship was inversely related to TP content indicating that changes in the labile N:P ratio may have a stronger impact on N mineralization in more P-deficient soils. However, in relation to previous reports of the influence of P availability on N mineralization in P-deficient soils (Kranabetter et al. 2005, Carlyle and Nambiar 2001), we found the labile N:P ratio to be a weak predictor of N_{minp}. Carlyle and Nambiar (2001) found that the soil N: P_o ratio explained 82% of the variaition in N mineralization

among pine plantations in Australia. In our study, labile N:P ratio explained approximately one quarter to one third of the variation in N_{minp}, and up to half of the variation when soil moisture was considered, and only in approximately half of the sites tested, suggesting that P availability was not the predominant control on N_{minp}. Soil moisture appeared to influence which sites exhibited this relationship, with higher mean soil moisture in sites that did exhibit this relationship than those that did not.

Figure 24: Relationships between N_{pmin} and the soil labile N:P_i ratio.

Relationships between N_{pmin} and the ratio of labile N to labile P in the soil (soil labile N:P_i ratio) in eight sites. Total phosphorus content followed by site ID in parentheses is listed in the upper right corner of each graph.

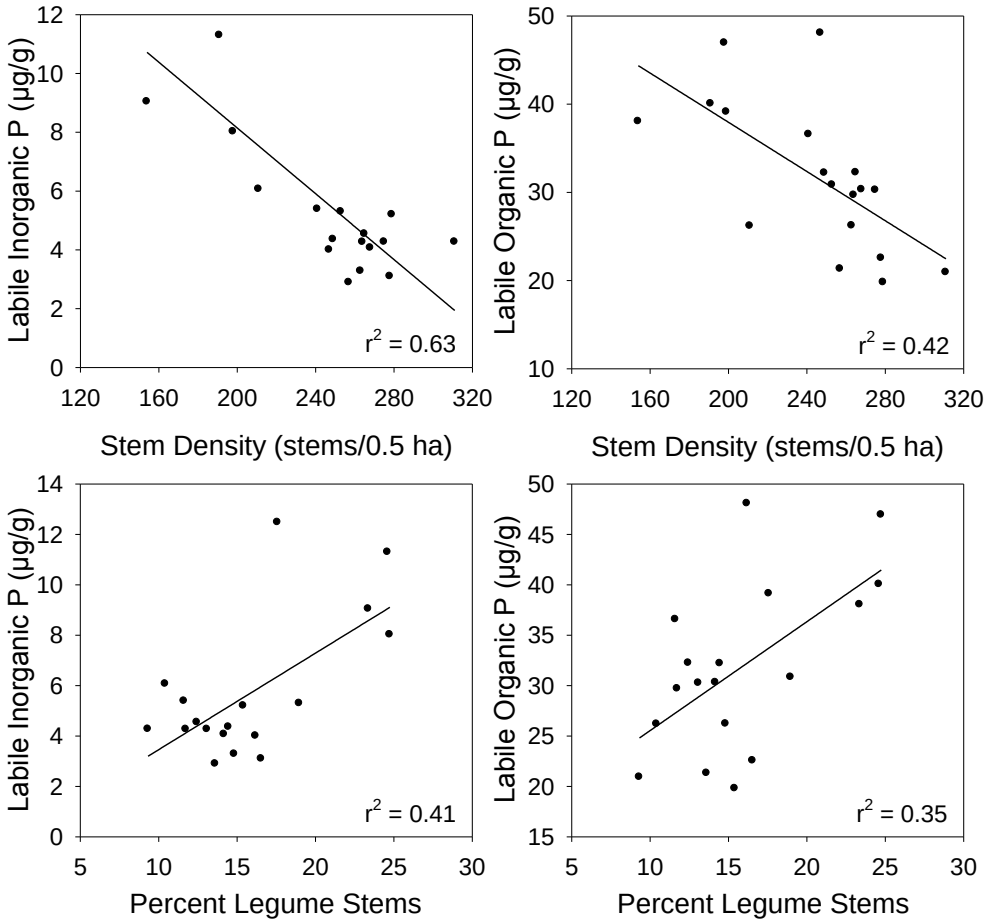


By investigating a narrow range of soil types; oxisols of similar N content and pH but differing P status, we hoped to isolate the influence of P availability on N cycling processes and minimize the confounding effects of other soil properties. However, some of the studies that have reported strong effects of P availability on N mineralization examined a much wider range of soils and looked at spatial variability rather than temporal variability (Carlyle and Nambiar 2001). Relationships between P and N cycling may vary through time in association with environmental fluctuations, making it difficult to detect strong relationships based on a time series of measurements. In addition, although we were interested in P constraints on N cycling at natural background levels of nutrient availability, a P fertilization experiment may have demonstrated a more consistent pattern of P limitation to N cycling across sites.

P availability in these soils appears to play a role in stand-level vegetation structure, such that higher stem density was found in soils with lower labile P_i, labile P_o and TP. Spatial variation in P availability could result from differences in plant uptake of P, however, the negative relationship between TP and stem density suggests that soil P fertility or some other associated soil property is influencing vegetation structure rather than vice versa. In agreement with our previous findings, the percentage of stems in the legume family was positively related to labile P_i and P_o and TP content.

Figure 25: Relationships between vegetation characteristics and soil labile P.

Relationships between a) soil labile P_i and total stem density, b) soil labile P_o and total stem density, c) soil labile P_i and percent stems in the legume family, and d) soil labile P_o and percent stems in the legume family across the 18 CARBONO plots.



Due to the high P requirement of N fixation it is possible that more P-rich soils are needed to support a greater density of legumes. P fertilization has long been used as a means of promoting the establishment and growth of N-fixing species to enhance N fertility (Walker et al. 1959). Alternatively, legume species may contribute to local P enrichment by releasing more phosphatase into the soil than non-legume species in order to support N-fixation (Houlten et al. 2008). Zou et al. (1995) found that phosphatase activity as well as P availability increased in plantations of N-fixing trees.

Our results demonstrate that soil P availability is an important regulator of soil nutrient and vegetation dynamics in tropical forests. Within a landscape, we found relationships between P supply and C and N accumulation that parallel findings from soil chronosequence studies (Walker and Adams 1958, Syers et al. 1970, Walker and Syers 1976, Cole and Heil 1981, Tate and Salcedo 1988, Crews et al. 1995). Our findings provide some support for the hypothesis that P constrains microbially-mediated N transformations in tropical soils. However, P availability does not appear to be the principal control on nitrogen mineralization in this N-rich tropical forest. Altered P availability associated with future climate scenarios, particularly changes in rainfall regimes, have important implications for N cycling, forest productivity and C balance in these forests.

SUMMARY AND CONCLUSIONS

Tropical forests account for more than one-third of global primary productivity and consequently play an important role in carbon exchange between the biosphere and the atmosphere (Melillo et al. 1993, Field et al. 1998). Changes in global atmospheric composition and subsequent altered climatic conditions could affect the functioning of tropical ecosystems through the disruption of biogeochemical cycles. Understanding current patterns of nutrient cycling in mature tropical forests is key to understanding linkages between carbon, nutrients, and water in these systems and to predicting ecosystem responses to future climate scenarios.

While the spatial heterogeneity of soil properties and processes is widely recognized, the temporal variability in soil nutrient availability is an aspect of tropical biogeochemistry that is not well understood. This work represents the first quantification of diurnal variability in labile P under field conditions. Our findings demonstrate that labile P in tropical soils is quite sensitive to environmental conditions on an hourly to daily timescale. Despite strong geochemical controls on P dynamics in tropical soils, biological processes played a primary role in determining diurnal fluctuations in labile P. We found that CO_2 efflux, an integrated measure of microbial and plant activity, and modeled sap flow velocity, were two of the key regulators of P availability, as well as soil temperature (one of the primary drivers of microbial activity) and solar radiation (one of the primary drivers of plant activity). Evidence of the dynamic nature of the labile soil P pool highlights the need for careful attention to scale dependence in ecological research.

Results from the AEM incubation study demonstrate that the primary controls on P availability are timescale dependent. Rainfall and soil moisture were not important

drivers of labile P on a diel timescale, however, over days to weeks, rainfall was the dominant driver of fluctuations in labile P. Investigation of P dynamics at this intermediate time-scale (days to weeks) revealed that the timing and in some cases the direction of environmentally-driven fluctuations in labile P can vary as a function of inherent site fertility (i.e. total soil P content). Across a range of environmental conditions, the low fertility site responded more rapidly to variability in rainfall, temperature, and solar radiation. Evidence from our long-term dataset of nutrient availability also indicated a more rapid response of labile P to rainfall in less fertile soils on a sub-monthly timescale.

This work represents one of the few multi-year data sets of monthly soil nutrient availability in a tropical wet forest. Congruent with the common paradigm of tropical wet forests as aseasonal ecosystems, long-term monthly monitoring did not demonstrate strong seasonality in labile P. Marked synchrony in the timing of P fluctuations across a three-fold gradient in soil total P content suggests a common environmental driver. Despite three years of monthly data with substantial variation in key climatic factors, we were unable to identify the critical drivers. The lack of clear climatic controls on monthly P availability may be due to fairly constant biological demand and rapid uptake of P as it enters the labile pool. N, the less limiting nutrient, did exhibit clear seasonal trends, with higher N availability during the dry season. Moving forward, research investigating the influence of integrated measures of microbial and plant activity on labile P, may help to elucidate the mechanisms underlying long-term P dynamics in wet tropical forests.

Long-term patterns in P availability demonstrated significant interannual variability in labile P. A trend of decreasing P availability over three years paralleled a pattern of decreasing soil moisture, and soil P concentrations were at their lowest during the drought conditions of February and March 2008. Additionally, wider fluctuations in the ratio of soil labile N to soil labile P in soils of lower P content, particularly during the dry season of 2008, suggest that more P-deficient soils may respond more strongly to inter-annual climate variability characteristic of ENSO events. These observations highlight the need for long-term soil nutrient studies to determine the influence of major climatic phenomena, such as the El Niño Southern Oscillation, in tropical regions.

Soil P fertility, but not N availability, appears to be strongly linked to vegetation characteristics at La Selva. However, relationships between P availability and vegetation structure differed at local and landscape-scales. Local P-enrichment of surface soils by vegetation was evident in both fine-scale studies, however, the opposite pattern was observed at larger spatial scales, with lower P associated with higher stem density. At broader spatial scales, phenomena such as topographic position and variation in gap formation rates may obscure small-scale relationships between nutrient availability and vegetation characteristics. Across spatial scales, legume species were strongly and positively linked to soil P status in these forests. The high P demand of nitrogen fixation may inhibit the establishment of legumes in the most P-deficient soils.

P availability is clearly one of the primary determinants of ecosystem properties and processes in tropical forests. On geological timescales, there is evidence that P controls on N, C, and OM accumulation. However, the lack of empirical evidence of such interactions on biologically relevant timescales prompted my interest in investigating

potential short-term links between P and N cycles. Within a landscape, I found relationships between soil P status and C and N accumulation that coincide with findings from soil chronosequence studies. However, P availability does not appear to be the primary control on microbially-mediated N transformations in this N-rich tropical forest. A two year soil incubation study revealed a negative relationship between the ratio of soil labile N to labile P ($N:P_i$ ratio) and nitrogen mineralization potential in approximately half of the sites studied. In combination with soil moisture the soil labile $N:P_i$ ratio was able to explain up to 49% of the variability in N mineralization potential, however this finding was not consistent across sites. Further investigations in more P-deficient sites or more controlled fertilization experiments may yet reveal evidence of short-term P controls on N cycling in highly weather tropical forest soils.

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APPENDIX

Table 1: Plot characteristics including vegetation structure and composition.

Characteristic	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Average labile P (µg/g)	1.88	1.80	1.61	1.32	1.34
Stem density >10 cm (no. trees per plot)	15	21	19	10	14
Basal area (m^2/ha) (dbh>5 cm)	33.8	25.3	29.0	13.8	13.6
Heliconia sp. density	35	27	30	40	41
Dominant species with dbh >5 cm †					
Casearia arborea				29(*4.0*)	
Conceviba pleistemona	18(*3.2*)				
Iriartea deltoidea			9(2.6)		
Laetia procera		18(*4.6*)			
Pentaclethera macroloba	48(*16.3*)	27(*6.8*)	48(*13.9*)	22(*3.1*)	29(*4.0*)
Welfia regia					7(*1.0*)

Notes: Each circular plot had a radius of 10 m for a total area of 314 m^2. We measured all trees with a dbh > 5 cm and < 10 cm in a circular plot with a 5-m radius nested within the larger cirle that had a 10-m radius where we measured all trees with a dbh > 10 cm. The basal areas from each of these circular plots was scaled up to one hectare and pooled to give a total basal area in m^2/ha. We measured the number of *Heliconia* sp. plants within each 10 m radius circular plot as they were the most abundant plant on the forest floor.

† Values are basal area in m^2 with basal area percentage in parentheses

Table 2: Soil characteristics for sites of contrasting P fertility.

Surface soil (0-10 cm depth) chemical and physical properties (from Appendix L, Espeleta and Clark 2007).

Characteristic	High P (A4)	Low P Site (L4)
pH in H_2O of dry soil	4.21	4.19
Bulk Density (g/cm3)	0.71	0.63
Effective cation exchange capacity (mmol(+)/kg)	88.32	101.12
C stocks (Mg/ha)	36.46	46.14
N stocks (Mg/ha)	3.25	3.66
P stocks (Mg/ha)	1.18	0.46
Bray P (µg/g) (monthly mean over 3 yrs)	7.67	3.12
Fe (kg/ha)	36.20	44.81
Al (kg/ha)	461.75	455.72

Table 3: Subplot characteristics including vegetation structure and composition.

	High P Fertility (A4)				Low P Fertility (L4)			
Characteristic	1	2	3	4	1	2	3	4
Stem density (no. trees per subplot)	10	14	16	20	25	20	28	22
Total basal area (m^2/ha)	20.1	27.4	24.8	34.4	14.3	9.4	39.8	30.5
Basal area of legumes (m^2/ha)	9.9	11.9	9.3	22.5	4.3	1.1	27.4	11.0
Basal area of palms (m^2/ha)	1.4	1.0	0.0	4.4	2.6	1.9	3.6	1.9
Dominant species (*% of total basal area*)								
Ampelocera macrocarpa Forero & A.H. Gentry (Ulmaceae)					8			
Balizia elegans (Ducke) Barneby & J.W. Grimes (Mimosoideae)							41	
Carapa nicaraguensis Audl. (Meliaceae)					11			
Dussia sp.(Fabaceae)				22				
Goethalsia meiantha (Donn. Sm.) Burret (Tiliaceae)	32		45					
Inga thibaudiana (DC.) (Fabaceae)					12			
Neea elegans P.H. Allen -vel aff. (Nyctaginaceae)					8			
Pentaclethra macroloba (Willd.) Kuntze (Fabaceae)	47	36	18	39	14		21	33
Protium panamense (Rose) I.M. Johnston (Burseraceae)						12		
Simarouba amara Aubl. (Simaroubaceae)		19						
Tapirira guianensis Aubl. (Anacardiaceae)								10
Trichilia septentrionalis C. DC. (Meliaceae)						12		
Virola koschnyi Warb. (Myristicaceae)						17		
Virola sebifera Aubl. (Myristicaceae)						14		
Xylopia sericophylla Standl. & L.O. Williams (Annonaceae)								16

Notes: We measured all trees with a dbh of ≥ 10 cm. Each subplot had a total area of 400 m^2. The basal area from each subplot was sc
one hectare to give units of m^2/ha.

Table 4: Plot soil and vegetation characteristics.

Surface soil (0-10 cm depth) chemical and physical properties and vegetation characteristics of the 18 CARBONO plots (from Appendix L, Espeleta and Clark 2007)

	Low P Fertility					Intermediate P Fertility							High P Fertility					
Characteristic	L5	L6	L2	P5	P6	L4	P4	L3	L1	A2	P2	P3	A3	P1	A6	A5	A1	A4
pH in H2O of dry soil	4.2	4.1	4.0	4.2	4.2	4.2	4.3	4.2	4.0	4.2	4.3	4.2	4.5	4.4	4.1	4.4	4.4	4.2
Bulk density (g/cm3)	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.71	0.63	0.63	0.71	0.63	0.71	0.71	0.71	0.71
ECEC (mmol(+)/kg)	5.2	5.4	3.2	5.8	5.9	7.8	4.1	3.2	5.0	3.6	5.1	3.2	10.0	4.1	5.0	5.8	4.6	5.4
C stocks (Mg/ha)	39.2	36.1	40.2	38.7	35.6	46.1	34.2	35.9	41.2	45.5	29.5	39.0	32.0	25.7	48.0	34.8	29.8	36.5
N stocks (Mg/ha)	3.3	3.0	3.3	3.2	2.9	3.7	2.8	3.0	3.6	3.9	2.5	3.2	3.1	2.2	3.9	3.1	2.8	3.3
P stocks (Mg/ha)	0.37	0.39	0.41	0.42	0.46	0.46	0.47	0.50	0.59	0.60	0.62	0.69	0.77	0.87	0.94	1.05	1.11	1.18
K (kg/ha)	46.8	44.8	46.3	51.1	41.3	55.7	46.1	43.3	54.4	79.0	53.0	45.7	58.2	56.0	73.5	83.2	79.0	99.5
Ca (kg/ha)	65.8	68.4	40.5	73.4	74.3	98.7	52.3	40.5	63.3	51.3	65.0	40.5	142	51.5	71.3	82.7	51.3	77.0
Mg (kg/ha)	32.7	31.4	27.6	32.7	33.8	38.0	28.9	22.0	41.3	46.5	32.7	28.8	38.8	28.8	29.4	35.6	46.5	29.4
Fe (kg/ha)	32.7	36.2	8.9	34.0	34.9	44.8	35.7	36.2	47.9	53.3	10.0	43.5	1.4	8.6	50.7	18.0	1.7	36.2
Al (kg/ha)	395	397	448	412	340	455	389	410	450	629	308	401	285	292	581	456	327	461
Bray P (µg/g)	1.5	1.7	1.7	1.3	1.6	1.7	1.8	1.9	1.9	2.2	1.8	1.9	2.0	4.6	5.9	7.5	3.7	6.2
Olsen P_i (µg/g)	4.3	4.3	4.5	3.1	2.9	4.1	3.3	4.4	5.3	5.4	4.3	4.0	6.1	5.2	8.0	12.5	9.0	11.3
Olsen P_o (µg/g)	29.7	30.2	32.2	22.5	21.3	30.3	26.2	32.2	30.8	36.6	20.9	48.1	26.2	19.8	46.9	39.1	38.0	40.0
Labile N(µg/g)	43.1	44.7	44.6	39.3	33.3	40.1	38.9	37.8	41.0	42.6	31.3	36.3	38.0	27.2	39.9	39.7	37.0	38.7
N mineralization (µg/g/day)	1.3	1.6	1.7	0.9	1.0	1.7	1.0	1.0	1.7	1.2	1.1	1.0	0.6	1.7	1.0	0.5	1.5	1.1
Volumetric Soil Moisture	0.48	0.45	0.47	0.48	0.49	0.55	0.44	0.48	0.54	0.52	0.49	0.55	0.48	0.52	0.51	0.56	0.41	0.58
Stem density (in 0.5 ha)	264	275	265	278	257	268	263	249	253	241	311	247	211	279	198	199	154	191
Basal area (m^2/0.5 ha)	11.3	12.8	9.6	14.1	11.1	9.5	13.5	12.4	9.4	10.8	11.4	12.2	11.3	10.1	12.8	13.9	11.9	12.6
Legume stem density	31	36	33	46	35	38	39	36	48	28	29	40	22	43	49	35	36	47
Legume basal area	3.3	4.2	2.0	7.1	3.1	3.4	6.7	4.4	4.1	3.6	2.6	4.6	3.5	2.9	7.3	4.8	6.4	7.0