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Are patterns in nutrient limitation belowground consistent with those aboveground: results from a 4 million year chronosequence

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Abstract Accurately predicting the effects of global change on net carbon (C) exchange between terrestrial ecosystems and the atmosphere requires a more complete understanding of how nutrient availability regulates both plant growth and heterotrophic soil respiration. Models of soil development suggest that the nature of nutrient limitation changes over the course of ecosystem development, transitioning from nitrogen (N) limitation in 'young' sites to phosphorus (P) limitation in 'old' sites. However, previous research has focused primarily on plant responses to added nutrients, and the applicability of nutrient limitation-soil development models to belowground processes has not been thoroughly investigated. Here, we assessed the effects of nutrients on soil C cycling in three different forests that occupy a 4 million year substrate age chronosequence where tree growth is N limited at the youngest site, co-limited by N and P at the intermediate-aged site, and P limited at the oldest site. Our goal was to use short-term laboratory soil C manipulations (using ^{14}C-labeled substrates) and longer-term intact soil core incubations to compare belowground responses to fertilization with aboveground patterns. When nutrients were applied with labile C (sucrose), patterns of microbial nutrient limitation were similar to plant patterns: microbial activity was limited more by N than by P in the young site, and P was more limiting than N in the old site. However, in the absence of C additions, increased respiration of native soil organic matter only occurred with simultaneous additions of N and P. Taken together, these data suggest that altered nutrient inputs into ecosystems could have dissimilar effects on C cycling above- and belowground, that nutrients may differentially affect of the fate of different soil C pools, and that future changes to the net C balance of terrestrial ecosystems will be partially regulated by soil nutrient status.

Keywords Carbon cycle · Hawai'i · Heterotrophic soil respiration · Fertilization · LSAG · Nitrogen · Nutrient limitation · Phosphorus · Soil development

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Introduction

Soil nutrient supply strongly regulates CO_2 exchange between the terrestrial biosphere and the atmosphere, and human alterations to nutrient cycles have important implications for global carbon (C) cycling

(Galloway et al. 2004; Billings et al. 2010; Vitousek et al. 2010). Nutrient limitation to plant production has received substantial recent attention (Elser et al. 2007; LeBauer and Treseder 2008), but net ecosystem C exchange also depends on how nutrient availability affects losses of C via heterotrophic respiration. Our understanding of nutrient controls over soil C losses remains relatively poor (Bradford et al. 2008a) and ecosystem models that integrate the combined effects of nutrient availability on both net primary production (NPP) and soil heterotrophic respiration are rare (van der Putten et al. 2009). Furthermore, the balance between C inputs and losses may shift as anthropogenic activities continue to alter global nitrogen (N) and phosphorus (P) cycles (Galloway et al. 2004; Cleveland and Townsend 2006; Thornton et al. 2009). However, we lack the ability to predict how such changes may affect above- and belowground processes differently, limiting our understanding of how net ecosystem C exchange will respond to changes in nutrient availability.

Here we took advantage of previous studies of nutrient limitation to aboveground tree growth (Vitousek and Farrington 1997; Harrington et al. 2001) and litter decomposition (Hobbie and Vitousek 2000) to explore how characteristics such as ecosystem developmental stage and soil nutrient availability regulate soil heterotrophic growth and respiration. The Hawai'i chronosequence represents a 'model system' for studying many processes across natural gradients (Vitousek 2004), and Vitousek and Farrington (1997) showed that along the $\sim$4 million year soil chronosequence, tree growth was N limited at the youngest site ($\sim$300 years old), co-limited by N and P at the intermediate-aged site ($\sim$20,000 years old), and P limited at the oldest site ($\sim 4.1 \times 10^6$ years old). This pattern of plant nutrient limitation corroborated a model of soil development suggesting that, as terrestrial ecosystems develop, increases in soil N availability occur in conjunction with overall decreases in soil P availability, as P-containing primary minerals (largely apatite) are weathered and gradually lost from ecosystems (Walker and Syers 1976). These studies provide insight into the nature of nutrient limitation aboveground, and offer a framework for testing how nutrient limitation to soil heterotrophic growth and respiration compare with limitation to NPP.

A number of studies have demonstrated that nutrient availability can limit both litter decomposition and soil respiration (Gallardo and Schlesinger 1994; Hobbie and Vitousek 2000; Valentini et al. 2000; Cleveland and Townsend 2006), though results are variable (Haynes and Gower 1995; Knorr et al. 2005; Olsson et al. 2005). Nonetheless, it remains unclear how patterns in heterotrophic nutrient limitation vary with soil nutrient availability, or how they compare with patterns of aboveground nutrient limitation. Plants and microbes vary in fundamental ways that could lead to differences in nutrient responses. For example, in addition to using different C sources, plants and heterotrophic soil microbes have different nutrient acquisition strategies, vary in their capacity for compositional shifts in response to environmental changes, and have distinct biological stoichiometries and nutrient demands (Sterner and Elser 2002; Cleveland et al. 2006; Cleveland and Liptzin 2007). Thus, there are multiple reasons why nutrient limitation may be experienced—and reflected—differently by plants and microbes, even within a single site.

However, while differences between plants and microbes may argue against the applicability of aboveground models of nutrient limitation belowground, there are also consistencies between plants and microbes that suggest these models may indeed apply to both. For example, in most terrestrial ecosystems both plants and soil microbes acquire C with C:nutrient ratios that are higher than stoichiometric requirements, and thus both require nutrients from the environment to balance C-source nutrient supply with metabolic nutrient demand. Similarly, the size and chemistry of soil nutrient pools often change in consistent ways over the course of soil development (Walker and Syers 1976; Vitousek 2004) and could exert broad controls over biological organisms in terrestrial ecosystems (Vitousek and Reiners 1975).

The Hawai'i chronosequence offered the opportunity test this hypothesis across a soil nutrient gradient—while controlling for climate, plant community composition, parent material and topographic position—and to directly compare heterotrophic limitation patterns with known patterns of nutrient limitation to plant growth (Vitousek and Farrington 1997; Harrington et al. 2001) and litter decomposition (Hobbie and Vitousek 2000). Given the previously observed changes in the nature of nutrient limitation across the soil developmental gradient, we hypothesized that plants and soil heterotrophic

microbes would transition in concert from N limitation to P limitation across the chronosequence.

To address this hypothesis, we used two types of laboratory incubation experiments, a short-term (3 days) experiment using radiolabeled C (^{14}C) and nutrient (N × P) additions, which allowed us to investigate microbial growth and respiration responses to the addition of labile C (as sucrose) and nutrients, and a longer-term (65 days) intact soil core experiment to investigate the response of soil respiration to nutrients alone (i.e., no additional C substrates added). Together, these measurements allowed us to address the influences of nutrients on both the utilization of a common soil saccharide and of native soil organic matter (SOM).

Methods

Site description

We assessed nutrient controls over soil respiration and heterotrophic growth at three sites occupying the Long Substrate Age Gradient (LSAG) chronosequence (described by Crews et al. 1995). All sites have similar climate, plant species composition, topography and parent material, but vary substantially in parent material age and soil characteristics (Table 1; Crews et al. 1995; Herbert and Fownes 1995; Chadwick et al. 1999; Vitousek 2004). Mean annual temperature at each site is ~16°C and annual precipitation is ~2500 mm. All sites are located between 1130 and

Table 1 Physical and biological characteristics of *Metrosideros polymorpha* and of soil in Thurston ('young'), Laupahoehoe ('intermediate-aged'), and Koke'e ('old') tropical forests

Characteristics	Horizon	Thurston	Laupahoehoe	Koke'e
Substrate age (10^3 years)		0.3	20	4,100
Soil type		Lithic Hapludand	Hydric Hapludanad	Plinthic Kaniudox
Annual litter N inputs[a] (g/m^2/year)		1.7 ± 0.4	3.9 ± 0.3	1.2 ± 0.1
Annual litter P inputs[a] (g/m^2/year)		0.08 ± 0.02	0.21 ± 0.01	0.05 ± 0.01
Aboveground nutrient limitation[b]		N	N and P	P
Litter lignin:N ratios[c]		66.1	22.1	45.7
Resin bag NO_3^-–N[d] (μg/bag/day)		0.22 ± 0.12	4.25 ± 1.27	10.29 ± 4.91
Resin bag NH_4^+–N[d] (μg/bag/day)		3.09 ± 1.44	8.12 ± 2.05	4.12 ± 2.29
Resin bag PO_4^{3-}[d] (μg/bag/day)		0.20 ± 0.08	1.21 ± 0.28	0.41 ± 0.17
Total dissolved N (μg/g)	O***	27.01 ± 1.02^{A}	95.9 ± 4.89^{B}	53.0 ± 7.42^{C}
	A***	20.36 ± 1.35^{A}	64.5 ± 4.50^{B}	36.3 ± 11.15^{C}
Bray-extractable P (μg/g)	O***	5.13 ± 0.57^{A}	14.57 ± 1.17^{B}	9.64 ± 1.70^{C}
	A	2.29 ± 0.26	1.61 ± 0.37	2.15 ± 0.67
Microbial C (mg/g)	O*	4.54 ± 0.35^{A}	6.37 ± 0.40^{B}	$5.60 \pm 0.68^{A,B}$
	A*	2.44 ± 0.38^{A}	4.09 ± 0.46^{B}	2.33 ± 0.51^{A}
Microbial N (μg/g)	O**	458.67 ± 41.31^{A}	854.24 ± 76.07^{B}	$528.64 \pm 116.18^{A,B}$
	A*	$310.75 \pm 43.53^{A,B}$	556.29 ± 84.72^{A}	248.36 ± 71.78^{B}
Microbial P (μg/g)	O***	49.95 ± 3.80^{A}	177.38 ± 14.27^{B}	125.62 ± 32.28^{B}
	A	31.40 ± 4.03	33.63 ± 6.57	33.53 ± 23.08
Soil core total C** respired (μg C/g soil)		4272.8 ± 57.1^{A}	6169.7 ± 125.9^{B}	6219.5 ± 65.2^{B}

Soil measurements in this study were collected and analyzed for O and A horizons separately. Values represent means ± 1 SE. Significant differences among sites are indicated by asterisks at row headings (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) and significant Tukey's post hoc differences ($P < 0.05$) are depicted by differing uppercase letters within rows

[a] From Herbert and Fownes (1999)

[b] From Vitousek and Farrington (1997)

[c] From Hobbie and Vitousek (2000)

[d] From Crews et al. (1995)

1200 m above sea level, and all soils were derived from tephra deposits and are on the constructional surfaces of shield volcanoes. The youngest site (Thurston) is located in Hawai'i Volcanoes National Park, and its substrate was formed ~300 years ago. The intermediate-aged site (Laupahoehoe) is also located on Hawai'i and is within the Hilo Forest Reserve; its substrate formed ~20,000 years ago. The oldest site (Koke'e) is located in Koke'e State Park on the island of Kaua'i; its substrate formed $\sim 4.1 \times 10^6$ years ago. All three sites contain fertilization plots (N × P in a full-factorial design) in which both tree growth (Vitousek and Farrington 1997) and leaf litter decomposition (Hobbie and Vitousek 2000) have been measured.

Experimental design

The three LSAG sites vary in parent material age and soil nutrient concentrations (Crews et al. 1995; Table 1) providing unique opportunities to: (1) assess how nutrient availability regulates heterotrophic soil respiration and growth across the soil age gradient and (2) compare patterns in above- and belowground responses to experimental nutrient additions. We used two independent methods to address these questions. First, we used the microbiological Substrate Induced Growth Response method (SIGR; Colores et al. 1996 and modified by Cleveland et al. 2002) and a series of fertilization treatments to investigate how soil heterotrophic growth and respiration rates respond to sucrose additions across the gradient, and to assess how nutrient additions affected these responses. Next, we used a longer-term (65 days), intact soil core incubation experiment to investigate how nutrient additions affect soil respiration rates, isolating the effects of nutrient fertilization on the decomposition of native soil organic C (SOC) pools.

SIGR incubation

We used the modified SIGR technique to assess the effects of nutrient availability on sucrose respiration and heterotrophic microbial growth rates (Cleveland et al. 2002). A radiolabeled organic C source (^{14}C-sucrose) was added to soil (with and without nutrients) and respired $^{14}CO_2$ was captured using a liquid base trap and analyzed using a scintillation counter. SIGR has two main advantages over traditional substrate induced respiration (Anderson and Domsch 1978): First, the use of isotopically-labeled C allows quantitation of added C alone (e.g., eliminating issues related to priming; Kuzyakov et al. 2000); and second, this method allowed us to quantify both microbial growth rates as well as to evaluate patterns in soil respiration rates, facilitating the direct comparison of microbial growth with plant growth responses to fertilization. We chose to use sucrose as the C substrate for several reasons: A wide-range of heterotrophic organisms are capable of using sucrose as a C source; sucrose is the most prevalent disaccharide on Earth and is commonly found as a by-product of carbohydrate break-down during leaf senescence and decomposition; sucrose is commonly used in studies of microbial metabolism and nutrient limitation; and sucrose is a simple sugar that contains neither N nor P (Larcher 2001; Bowman et al. 2004; Meier and Bowman 2008).

Between February 10 and 12, 2009, we collected 14 samples from both the O horizon and A horizon in each site using an 8 × 10 cm soil bulb corer from unamended areas surrounding the fertilized plots used by Vitousek and Farrington (1997). We collected from the entire depth of each horizon, and A horizon samples were collected beneath associated O horizon samples (O and A horizon depths vary by site (Olander and Vitousek 2000)). Following collection, samples were kept in coolers with ice and shipped overnight to the laboratory at the University of Montana where they were stored at 4°C until analysis. All soil assays were initiated with 72 h of collection.

Prior to analysis, all samples were homogenized by hand and gently shaken through a 4 mm sieve to remove coarse fragments and roots. In line with SIGR methodology (Anderson and Domsch 1978; Colores et al. 1996; Lipson et al. 2000; Cleveland et al. 2002; Ley et al. 2004), individual samples from each horizon were composited to form a single homogenous sample (producing six homogenates: Thurston O and A horizons, Laupahoehoe O and A horizons, Koke'e O and A horizons). From each composite sample, 8 biometer sidearm flasks (Fisher Scientific, Pittsburgh, PA, USA) received 5 g (dry mass equivalent) of soil, with 1 ml of 0.5 M NaOH basetrap in the sidearm to collect respired CO_2 (Colores et al. 1996). The samples were split into four groups that

received either C alone (4000 μg/g C as sucrose), C + N (800 μg N/g dry soil as NH_4NO_3), C + P (800 μg P/g dry soil as KH_2PO_4) or C + N + P additions (800 μg N/g dry soil as NH_4NO_3 and 800 μg P/g dry soil as KH_2PO_4). The N and P concentrations were chosen to match the fertilization amounts used in the associated aboveground fertilization studies (100 kg/ha/year of each N and P; Vitousek and Farrington 1997). To each flask we added 4000 μg/g C dry soil (as sucrose), the amount of sucrose previously determined to induce maximal respiration in these tropical soils (data not shown). Each flask also received enough uniformly labeled ^{14}C sucrose to yield 5000 Bq per flask. The added C and label were dissolved in enough water to bring the soils to ~60% of field capacity and the solution was dripped over the surface of each soil and gently but thoroughly mixed into the soil using a metal spatula.

The ^{14}C-sucrose was universally labeled (meaning each C in the labeled sucrose molecule was ^{14}C), thus $^{14}CO_2$ respired from the decomposition of sucrose (and all potential sucrose derivatives) was detectable by liquid scintillation counting. For samples receiving nutrient additions, nutrients were dissolved in the ^{14}C solution before fertilization. Biometer flasks were sealed after fertilization and continuously sampled for ~72 h. Every 2–3 h the NaOH base trap was removed from each flask, flasks were vented to the atmosphere and then resealed, and the base trap renewed (Cleveland et al. 2002). Base trap (containing $^{14}CO_2$) was injected into 4 ml plastic vials, mixed with 2.5 ml of Scintiverse II scintillation cocktail (Fisher Scientific) and $^{14}CO_2$ activity was measured on a liquid scintillation counter (Packard now part of Perkin-Elmer, Waltham, MA, USA). Flasks were incubated at room temperature (25 ± 1°C) until soil respiration approached its basal rate (~3 days). Note that SIGR-based respiration data represent the respiration of added C.

Using the ^{14}C soil respiration data, maximum microbial growth rates (μ_{max}) were determined for each sample using KaleidaGraph software (Synergy Software, Reading, PA, USA) and equations derived by Colores et al. (1996). Briefly, soil respiration rates (μg CO_2/g dry soil/h) were plotted against time and a μ_{max} value was determined from the resultant curve. The SIGR estimate of growth rate (μ_{max}) is derived from the equation $dP/dt = \mu_{max}(X_1 \exp(\mu_{max}t))$ where P represents the amount of product produced (μg CO_2-C/g soil), t represents time, μ_{max} represents the microbial growth rate, and X_1 represents biomass in terms of product produced with the same units as the product (i.e., μg CO_2-C/g soil). As required for these analyses (Colores et al. 1996), only CO_2 data from the exponential growth phase were analyzed. Relative heterotrophic responses for N (RR_N), P (RR_P) and N + P (RR_{NP}) were calculated by dividing the measured μ_{max} value in the enriched treatment (C + N, C + P, or C + N + P) by the μ_{max} value in the control treatment (+C).

Soil core incubation

From each site, 14 intact soil cores (5 cm diameter × 15 cm depth encased in PVC tubing and containing O and A horizon soil) were collected, and surface litter was removed. These cores were packaged to ensure stability, kept cool and returned to the laboratory at the University of Montana within 24 h of collection. In the laboratory, the bottom of each soil core was wrapped in gauze to prevent soil losses and placed atop glass wool in a 1 l jar. Cores were weighed each week and re-wetted using the appropriate fertilization treatments (control [H_2O], +N [83 μg N/cm^2/week as NH_4NO_3], +P [83 μg P/cm^2/week as KH_2PO_4/week] and N + P [83 μg/cm^2/week each of N and P]) with enough solution to maintain soil moisture at ~70% of field capacity: the quantity of added nutrients was kept constant across cores by varying the concentration of the solution. Nutrient additions were chosen to simulate the 100 kg/ha/year of N and P fertilization of associated aboveground studies (Vitousek and Farrington 1997; Harrington et al. 2001), and concentrations were adjusted to add nutrients over a 65 days period instead of over a year. Samples were allowed to equilibrate for 48 h after fertilization and the jars were sealed with lids fitted with septa. Soil cores were incubated for ~12 h, headspaces were mixed and sub-sampled with a gas-tight syringe, and headspace CO_2 concentrations were determined using a Shimadzu gas chromatograph (Shimadzu Inc., Kyoto, Japan) fitted with a Poropak N column (Supelco Inc., Bellefonte, PA, USA) equipped with a thermal conductivity detector. Following each sampling event, lids were removed from the jars, and between sampling events cores were kept in a well-aerated but humid container.

Soil analyses

The 14 O horizon and 14 A horizon soils collected from each site were composited to create seven samples per horizon per site (for a total of 42 samples). Composited samples were sub-sampled and analyzed for soil moisture, water holding capacity (WHC), total organic C (TOC), total dissolved N (TDN), Bray-extractable P, and microbial biomass C, N, and P concentrations. Each soil analysis was also performed on the composite soil sample used for the SIGR incubation. WHC was assessed by saturating ~8 g of soil with water, allowing samples to drain until no water drained from the bottom (~1 h), and then drying and reweighing samples. For TOC and TDN analyses, ~10 g of fresh soil was extracted with 0.5 M K_2SO_4. Samples were shaken for 1 h, filtered and analyzed on a Shimadzu TOC/TDN. To assess Bray-extractable P, ~6 g of soil were extracted with a 90% Bray (dilute HCl + NH_4F) solution and shaken for 1 min. Samples were filtered and P concentrations were measured colorimetrically using an ascorbic acid molybdate analysis (Kuo 1996) on an autoanalyzer (Bran + Luebbe, Norderstedt, Germany).

The chloroform fumigation method was used to assess microbial biomass C, N (Brookes et al. 1985; Vance et al. 1987), and P (Brookes et al. 1984). For microbial C and N, ~10 g of soil was sealed in a vacuum dessicator with a chloroform headspace for 5 days. Samples were then extracted with K_2SO_4 and analyzed for TOC and TDN concentrations as described above, and initial K_2SO_4 values were subtracted from fumigated values to determine microbial biomass C and N concentrations. The proportionality constant $K_c = 0.45$ was used for microbial C (Vance et al. 1987) and $K_n = 0.54$ for microbial N (Brookes et al. 1984) calculations. For microbial P, ~6 g of soil was placed in a dark environment with a chloroform headspace for 1 day, and was extracted with Bray and assessed for P concentrations as described above. Microbial biomass P was calculated for each sample by taking the difference between the chloroform-fumigated and initial PO_4^{3-} concentrations, and site-specific P sorption capacities were used to account for geochemical sorption of mineralized microbial P (Olander and Vitousek 2005).

Statistical analyses

All data were tested for normality and homeoscedasticity; if either assumption was violated, data were ln-transformed prior to statistical analyses. Horizon and site differences in soil biogeochemical characteristics were first tested with a multivariate general linear model, using Tukey's post hoc analyses to explore site differences. If significant interactions between site and horizon existed, one-way ANOVAs and Tukey's post hoc analyses were used to test site differences within a single horizon and horizon differences within a single site. All data were analyzed using SPSS (11.0.4, Chicago, IL, USA) and significance was determined at $\alpha = 0.05$.

Results

Site characteristics

Soil nutrient and microbial biomass concentrations varied significantly both between soil horizons (O and A) and among the three study sites (Table 1); patterns of variation in soil properties were generally consistent with earlier soil collections from these sites (Crews et al. 1995; Vitousek 2004; Olander and Vitousek 2005; Torn et al. 2005). For each site, TOC, TDN, and Bray-extractable inorganic P concentrations were significantly higher in O horizons compared with A horizon soils ($P < 0.001$, $P = 0.047$, $P < 0.001$, respectively; Table 1). Similarly, at each site soil microbial biomass C, N, and P concentrations were significantly higher in the O horizon than in the A horizon ($P < 0.001$ for each; Table 1).

There were also significant differences between sites (Table 1). Site variation within the O horizon showed a common trend in soil nutrient concentrations: Laupahoehoe contained the highest concentrations of N and P; Koke'e had significantly lower concentrations; and Thurston had the lowest concentrations of TDN and Bray-extractable P (Table 1). In the A horizon, TDN was also highest at Laupahoehoe, lower at Koke'e, and lowest at Thurston ($P < 0.001$), but no significant site variation was observed for A horizon Bray-extractable P (Table 1). As seen previously at these sites (Torn et al. 2005), microbial biomass concentrations varied significantly among sites (Table 1). For the O horizon,

Laupahoehoe soil had higher microbial biomass C and N concentrations than Thurston, and Koke'e soil microbial C and N concentrations were intermediate. O horizon microbial biomass P concentrations were also lowest at Thurston, and did not vary significantly between Laupahoehoe and Koke'e forests. Microbial biomass C was highest at Laupahoehoe in the A horizon, and not significantly different between Thurston and Koke'e. Microbial biomass N in the A horizon was higher at Laupahoehoe than Koke'e, and Thurston values were intermediate. There was no significant difference among sites for microbial biomass P values in the A horizon.

SIGR experiment

Total C respired, maximum soil respiration rates, and microbial growth rates from the SIGR incubation showed consistently higher values in O horizon relative to A horizon soils, and the O horizon communities took less time to reach maximum rates (Table 2). C loss, respiration, and microbial growth rates also varied by site. For the O horizon, Laupahoehoe soils had the highest maximum respiration and microbial growth rates and respired the largest quantities of C (Table 2). Koke'e and Thurston O horizon soils reached similar maximum respiration rates, but Koke'e soils respired more total C (Table 2) and had higher rates of growth than Thurston soils. For the A horizon soils, Laupahoehoe and Koke'e respired larger quantities of C and Laupahoehoe had higher respiration rates (Table 2). In the A horizon, Koke'e had lower maximum respiration rates than Laupahoehoe but higher rates than Thurston (Table 2).

When looking at the relative responses of nutrient additions for the three soil types, the addition of N and P together (N + P) always elicited a greater effect than the addition of either N or P alone (Fig. 1; Table 2). Nevertheless, the addition of single nutrients consistently resulted in a higher response than in the C-only samples (Fig. 1; Table 2) and the identity of the single nutrient that evoked the larger response was dependent upon site and horizon (Fig. 1; Table 2). For the O horizon, Thurston soils responded more to N additions than to P additions, and Laupahoehoe and Koke'e soil responses to N additions and to P additions were not different (Fig. 1; Table 2). For A horizon soils, Thurston soils again responded more to N additions than P additions, but both Laupahoehoe and Koke'e soils responded more to P additions (Fig. 1; Table 2).

Soil core experiment

Soil respiration rates in water-only (control) cores varied significantly among sites (Fig. 2) with Thurston soils respiring the least C over the course of the experiment (Table 1). There were also site differences in responses to nutrients. For both Thurston and Koke'e, soil that received N + P fertilization respired significantly more than water-only (control) cores ($P < 0.001$; $P < 0.05$, respectively). This was not true for Laupahoehoe, where none of the fertilization treatments significantly affected respiration rates (Fig. 2). Additions of N or P alone did not elicit

Table 2 Soil respiration measurements from the SIGR incubations for the O and A horizon soils from each of the three sites

	Total labile C respired (μg C/g soil)				Maximum respiration rate (μg C/g soil/h)				Time to maximum respiration rate (h)			
	Control	+N	+P	+NP	Control	+N	+P	+NP	Control	+N	+P	+NP
Thurston O	661.5^{a}	820.3^{b}	786.2^{c}	916.0^{d}	26.6^{a}	32.5^{b}	27.3^{c}	42.5^{d}	24	24	27	24
Thurston A	417.8^{a}	448.1^{b}	430.5^{c}	1266.5^{d}	10.3^{a}	14.4^{b}	11.7^{c}	59.6^{d}	35	43	43	39
Laupahoehoe O	748.2^{a}	790.7^{b}	773.5^{b}	949.5^{c}	35.9^{a}	35.6^{a}	38.7^{b}	48.9^{c}	6	9	12	18
Laupahoehoe A	498.2^{a}	990.7^{b}	1065.5^{c}	1106.8^{d}	26.0^{a}	35.4^{b}	42.0^{c}	63.9^{d}	35	35	23	23
Koke'e O	718.1^{a}	848.4^{b}	823.1^{b}	1042.0^{c}	26.2^{a}	30.5^{b}	29.4^{b}	72.5^{c}	12	21	24	21
Koke'e A	491.6^{a}	525.4^{b}	603.2^{c}	970.1^{d}	11.8^{a}	18.5^{b}	24.3^{c}	41.8^{d}	35	43	43	35

Data show the total labile C respired over the course of the SIGR incubations, the maximum respiration rate and the time when the maximum respiration rate was achieved. Data represent the means of two samples and significant differences among treatments within a row are depicted by different lowercase letters

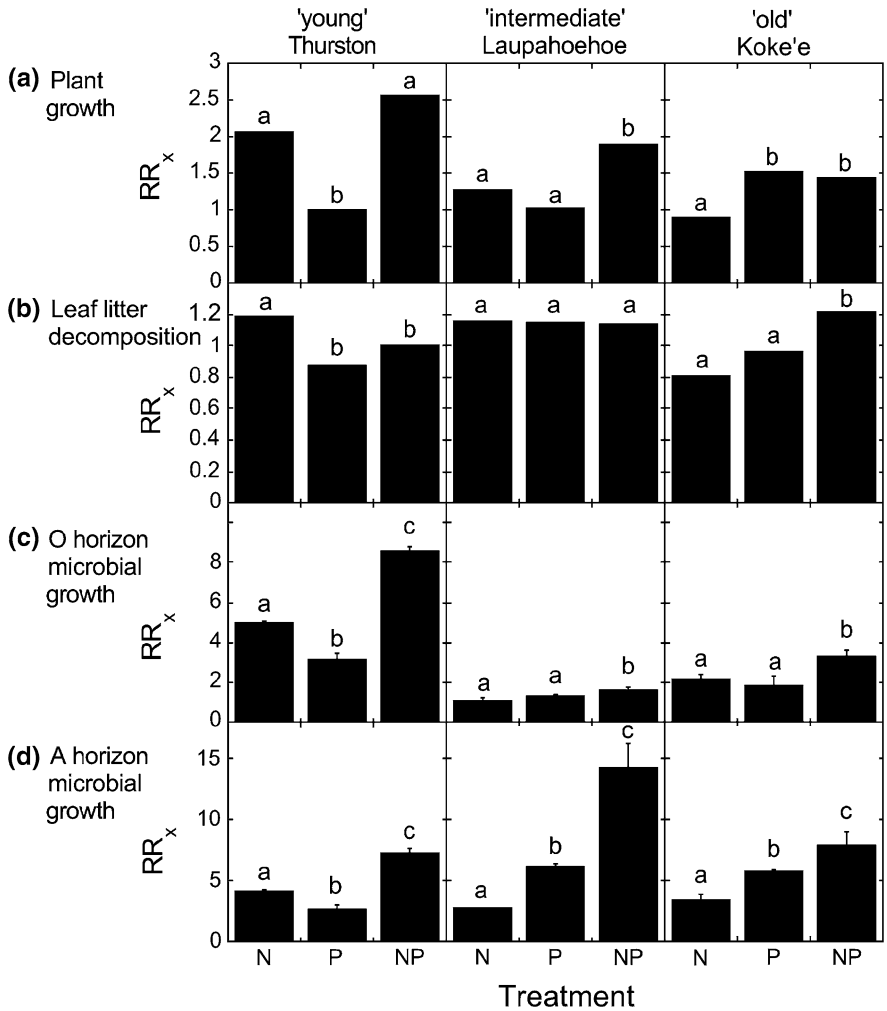

Fig. 1 Response ratios (RR_X) of plant growth, litter decomposition and soil heterotrophic growth rates to full-factorial N × P fertilization of Thurston ('young'), Laupahoehoe ('intermediate-aged') and Koke'e ('old') tropical forests. **a** Plant responses previously measured in the field as diameter increment trunk growth (mm/year; data from Vitousek and Farrington 1997), **b** common litter decomposition rate responses measured as percent mass loss (data from Hobbie and Vitousek 2000), and **c**, **d** indices of soil responses (μ_{max}) assessed in the laboratory by adding sucrose; samples were collected and analyzed separately from soil O horizons (**c**) and A horizons (**d**). All microbial growth responses were significantly higher compared with adding C alone. For all data, RR_X represents the measured value in the enriched treatment (+N, +P or N + P) divided by its value in the unfertilized treatment. Values represent means ± 1 SE and significant differences among RR_X's are shown by different *lowercase letters*. Note that the magnitudes of the *y*-axes are different for each group

significant responses in any of the soil cores, though patterns emerged as the experiment progressed (Fig. 2). In particular, data suggested that in both the Thurston and Koke'e soils the addition of N suppressed respiration relative to all other treatments. Also, in both Thurston and Koke'e soils, P-fertilized soils consistently maintained higher respiration rates relative to controls, though these differences were not statistically significant.

Discussion

Results from previous fertilization experiments investigating nutrient controls over plant growth (Vitousek and Farrington 1997) and ANPP (Harrington et al. 2001) in the LSAG sites were consistent with Walker and Syers' (1976) model of soil development: aboveground plant growth was limited by N at the youngest site, co-limited by N and P at the

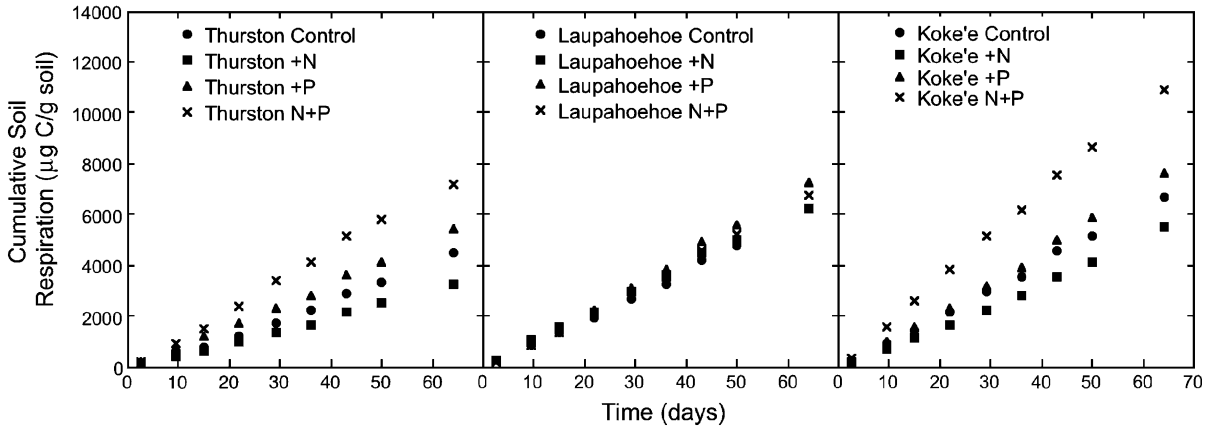

Fig. 2 Cumulative CO_2 evolution from intact soil core incubations for **a** Thurston ('young'), **b** Laupahoehoe ('intermediate-aged') and **c** Koke'e ('old') forests. Soil cores received H_2O (Control), +N, +P or N + P treatments and values represent means ($n = 3$). The N + P treatment is significantly higher than the unfertilized soils for Thurston and Koke'e sites but no treatment elicited significant effects in Laupahoehoe soils

intermediate-aged site, and P limited at the oldest site (Fig. 1a). Using the same plots, Hobbie and Vitousek (2000) found that decomposition rates of a common leaf litter were also N limited at the youngest site, unaffected by fertilization at the intermediate-aged site, and co-limited by N and P at the oldest site (Fig. 1b). Here, the results from the SIGR incubation were consistent with these observations in two important ways. First, nutrient fertilization drove increases in microbial growth (Figs. 1c, d, 3) and respiration (Table 2), depleting the pool of C added to soil relative to C-only additions. Second, there was a transition from greater N limitation to P limitation of labile C utilization with increasing substrate age;

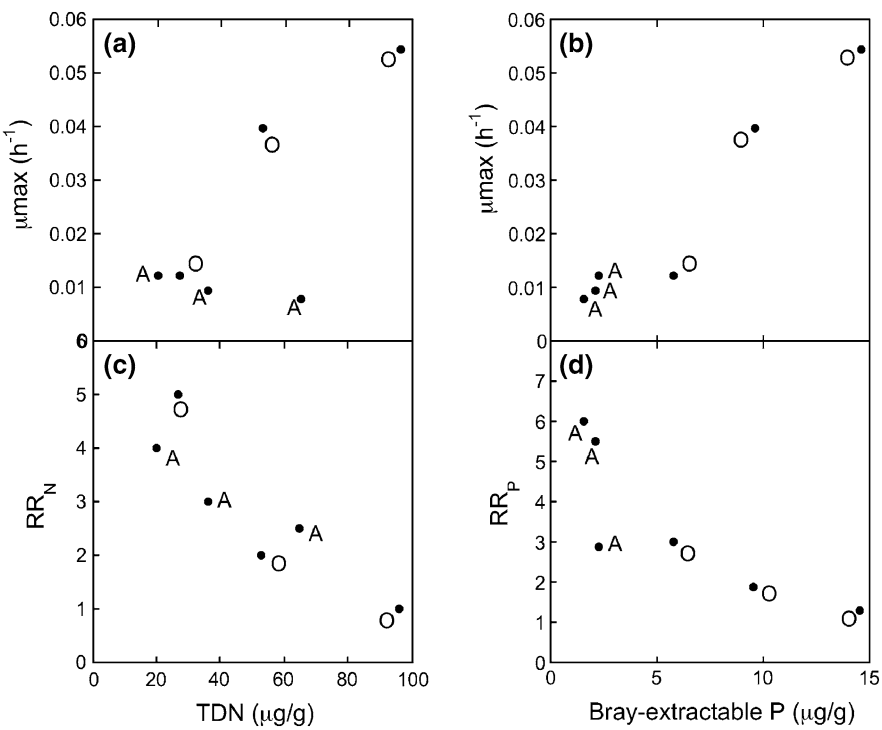

Fig. 3 Relationships between soil nutrient availability, soil responses to labile C additions, and nutrient limitation to heterotrophic growth. For O and A horizons, the top panels (**a**, **b**) show heterotrophic growth rate (μ_{max}) responses to labile C additions (y-axis) versus soil nutrient availability (x-axis) at the time of collection for TDN and Bray-extractable P (**a**, **b**, respectively). The bottom panels show the relative responses of soils to nutrient additions (relative to the additions of C alone) versus soil TDN and Bray extractable P (**c**, **d**, respectively) for both horizons

following sucrose additions, belowground heterotrophic growth (Fig. 1c, d) and respiration (Table 2) responded more to N than to P additions at the youngest site and more to P than to N additions at the oldest site (although all soils responded most to N + P).

The observed nutrient constraints on microbial responses to sucrose additions in the SIGR assays suggested that nutrient availability may strongly regulate the fate of episodic dissolved organic C (DOC) inputs along the LSAG. For example, data from Hawai'i and elsewhere (e.g., Schimel et al. 1994; Townsend et al. 1997) suggest that labile C inputs may fuel 70–80% of heterotrophic respiration. Thus, understanding how soil nutrient availability regulates the fate of soil C inputs is critical for predicting net ecosystem C balance under changing nutrient regimes. To that end, the SIGR experiment is meant to mimic microbial responses to soluble (and labile) C fluxes entering the soil as leachate from the litter layer. There are obvious and important differences between these SIGR incubations and natural litter leachate inputs. For example, litter leachate is a mix of carbohydrates (including simple sugars such as sucrose) but is not limited to sucrose, the SIGR incubations necessitate soil homogenization, and, although leachate typically has much higher C:nutrient ratios than soil microbial biomass (Reiners 1986), leached C also contains other elements. Nevertheless, the SIGR responses provide information on microbial C utilization rates, and offer insight about the effects of nutrient availability on soil C dynamics.

Data from other tropical sites support the SIGR data in suggesting that elevated nutrient availability increases the proportion of leached DOC that is respired from the soil (Ilstedt and Singh 2005; Wieder et al. 2008). For example, Cleveland and Townsend (2006) found that additions of N and P significantly increased soil respiration rates in a Costa Rican rain forest, and that this fertilization effect was strongest at the beginning of the wet season when leached DOC amounts were at their peak. However, while heterotrophic soil respiration represents a loss of soil C, the ultimate fate of C entering microbial biomass is more complex and poorly understood (Bradford et al. 2008b). Another study at these Hawaiian sites also suggested links between nutrients and rates of soil C turnover. Torn et al. (2005) found that high quality litter (from relatively N and P rich sites; Hobbie and Vitousek 2000) was associated with SOM that was more quickly decomposed than SOM from relatively nutrient poor sites, further suggesting that increases in nutrient availability could result in faster decomposition of soil C, and larger net CO_2 losses to the atmosphere.

While some aspects of microbial responses to nutrient additions in this study were generally consistent with previous data describing fertilization effects on the C cycle along the LSAG, there were also several important differences. First, in the SIGR assays, soils amended with labile C always showed strong positive responses to either added nutrient (Fig. 1). However, simultaneous additions of C + N + P consistently elicited the largest increases in microbial activity, while maximum plant growth most often occurred after the addition of a single nutrient (Fig. 1; Table 2; Vitousek and Farrington 1997). For example, at the youngest site, heterotrophic growth rates in the samples receiving C + N + P were double rates in samples that received C + N or C + P in the O horizon and A horizons (Fig. 1). Similarly, in the intact soil cores, N + P additions drove increases in soil respiration rates, while single nutrient additions (i.e., either N or P alone) to the intact cores had variable, and sometimes negative effects on soil respiration (Fig. 2). This is consistent with other studies showing non-linear responses of soil respiration to single versus multiple nutrient additions (e.g., Bradford et al. 2008b).

The strong responses to N + P additions in both the SIGR and soil core experiment are also consistent with data showing that N + P co-limitation of tree growth may be common in these sites over the long term (Fig. 1; Vitousek and Farrington 1997; Elser et al. 2007). For example, while short-term plant responses suggested single nutrient limitation (Vitousek and Farrington 1997), after 6–11 years of fertilization on these same LSAG sites, Harrington et al. (2001) found N + P limitation in the youngest and oldest sites, suggesting N + P co-limitation existed over longer timescales. Similarly, while common litter decomposed in fertilized plots at these sites showed variable responses to fertilization (Fig. 1b), decomposition rates of litter produced and decomposed within fertilized plots (assessed >3 years after fertilization began, and suggested here to represent longer-term decomposition responses to fertilization) consistently decomposed most rapidly with N + P additions

(Hobbie and Vitousek 2000). Thus, at these sites, plant growth and litter decomposition may also respond substantially to concurrent N and P fertilization in the long-term.

Next, in contrast to the SIGR incubations with labile C additions, soil respiration in the intact cores did not significantly increase in response to adding N or P alone, nor did fertilization in the field consistently increase the rates of SOC turnover (Torn et al. 2005). Further, while N additions consistently drove increases in soil respiration when added with labile C in the SIGR assays, N additions caused relative declines in soil respiration in the intact soil cores (Figs. 1, 2). N suppression of soil respiration following N-only additions is not uncommon (e.g., Fogg 1988; Burton et al. 2004; Pregitzer et al. 2008; Zak et al. 2008), but the mechanisms driving this response are unclear. However, N toxicity (Treseder 2008), suppression of decomposition via decreased oxidative enzyme production (DeForest et al. 2004), and/or N-driven changes in microbial growth versus waste respiration (Schimel and Weintraub 2003) may all contribute to the response. In any case, the variable soil respiration responses to nutrient additions in the SIGR and soil core experiments suggest that SOC chemistry may interact with nutrient availability to control heterotrophic responses to fertilization. Methodological differences may hinder our ability to directly compare the effects of N in SIGR and intact core incubations, yet other data also suggest that discrete fractions of SOC respond differently to fertilization. For example, Neff et al. (2003) observed that N fertilization increased the decomposition of 'light', more rapidly cycling C fractions while further stabilizing the 'heavy' more slowly cycling C pools, a result analogous to our observation of N increasing the mineralization and uptake of labile C (sucrose) but lowering the mineralization of extant SOC. In addition, the nature of N deposition could affect these patterns: when N is delivered alone it may suppress SOC decomposition (DeForest et al. 2004; Zak et al. 2008), but when delivered in conjunction with P it could stimulate C mineralization (Figs. 1, 2).

Third, patterns of nutrient limitation in the O and A horizons were neither identical to one another nor to observed patterns of nutrient limitation to plant growth or litter decomposition (Fig. 1). Heterotrophic growth and soil respiration of sucrose in both the O and A horizons were more N limited in the youngest site soils; however, at the intermediate-aged and oldest soils O horizon growth and respiration rates were close to equally N and P limited. These patterns match those observed for leaf litter mass loss (Fig. 1; Hobbie and Vitousek 2000), suggesting some continuity within the forest floor. The litter layer and O horizon are connected physically and by organic matter chemical similarities, and the analogous nature of nutrient limitation between these two forest floor components makes sense. In turn, rates in the A horizon soils were relatively more P limited (Fig. 1c, d; Table 2) and observed variations in soil nutrient availability help explain these patterns. For example, A horizon soils at all sites had significantly lower P concentrations and higher available N:P ratios (Table 1) than O horizon soils. Lower absolute and relative P availability in the A horizon could promote greater P limitation there. In addition, at the time the soils were collected, relative N availability in the O horizon at the oldest site (Koke'e) was significantly lower than in the intermediate-aged soils (Table 1), thus the observed response to N in the older site is consistent with the soil TDN values. Moreover, the relatively large response of A horizon soils to nutrient additions (Fig. 1d) suggests that nutrient limitation could help to maintain the slower C turnover rates previously observed in the mineral A horizon (Torn et al. 2005).

Taken together, these data suggest that the nature of the nutrient input (e.g., N versus P versus N + P) as well as the SOC chemistry and the soil nutrient status of an ecosystem interact to regulate the magnitude of the soil heterotrophic activity, and offer insight into how anthropogenic changes to N and P cycling could alter the balance between terrestrial C inputs and losses. In light of ongoing changes to both the global N and P cycles (Compton et al. 2000; Filippelli 2002; Galloway et al. 2004), simultaneous increases in ecosystem N and P deposition seem likely. For example, data suggest that in the coming decades both N (Matson et al. 1999) and P deposition (Okin et al. 2004) will increase in tropical forests, biomes that exchange more CO_2 with the atmosphere than any other (Phillips et al. 1998; Grace et al. 2001). If simultaneous deposition of N and P results in soil CO_2 effluxes that outpace C incorporation into biomass via primary production, this could fundamentally alter the C balance of tropical forests and perhaps modify their ability to sequester CO_2.

Additional research investigating links between above- and belowground responses over both the short- and long-term is needed, as predicting the nature and extent of soil nutrient controls will require both a better knowledge of spatial and temporal changes in soil N and P availability and a better mechanistic understanding of their effects on soil C turnover over a range of ecosystems and timescales. However, our results suggest some general patterns of nutrient limitation and offer hope for the development of conceptual and predictive models that effectively link above- and belowground C cycle responses to variations in nutrient availability (van der Putten et al. 2009).

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