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Soil fertility and fine root dynamics in response to 4 years of nutrient (N, P, K) fertilization in a lowland tropical moist forest, Panama

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Abstract The question of how tropical trees cope with infertile soils has been challenging to address, in part, because fine root dynamics must be studied *in situ*. We used annual fertilization with nitrogen (N as urea, 12.5 g N m^{-2} $year^{-1}$), phosphorus (P as superphosphate, 5 g P m^{-2} $year^{-1}$) and potassium (K as KCl, 5 g K m^{-2} $year^{-1}$) within 38 ha of old-growth lowland tropical moist forest in Panama and examined fine root dynamics with minirhizotron images. We expected that added P, above all, would (i) decrease fine root biomass but, (ii) have no impact on fine root turnover. Soil in the study area was moderately acidic (pH = 5.28), had moderate concentrations of exchangeable base cations (13.4 cmol kg^{-1}), low concentrations of Bray-extractable phosphate (PO_4 = 2.2 mg kg^{-1}), and modest concentrations of KCl-extractable nitrate (NO_3 = 5.0 mg kg^{-1}) and KCl-extractable ammonium (NH_4 = 15.5 mg kg^{-1}). Added N increased concentrations of KCl-extractable NO_3 and acidified the soil by one pH unit. Added P increased concentrations of Bray-extractable PO_4 and P in the labile fraction. Concentrations of exchangeable K were elevated in K addition plots but reduced by N additions. Fine root dynamics responded to added K rather than added P. After 2 years, added K decreased fine root biomass from 330 to 275 g m^{-2}. The turnover coefficient of fine roots <1 mm diameter ranged from 2.6 to 4.4 per year, and the largest values occurred in plots with added K. This study supported the view that biomass and dynamics of fine roots respond to soil nutrient availability in species-rich, lowland tropical moist forest. However, K rather than P elicited root responses. Fine roots smaller than 1 mm have a short lifetime (<140 days), and control of fine root production by nutrient availability in tropical forests deserves more study.

Key words: fine root biomass, fine root turnover, forest nutrient fertilization, minirhizotron, nutrient availability.

INTRODUCTION

How plants cope with infertile soils is an important question in the humid tropics. For example, 30% to 50% of Amazonian forests occur on infertile soils (Quesada *et al.* 2009). Dynamics of fine root growth are central to issues of soil fertility: (i) fine roots have intimate contact with soil and (ii) they are responsible for acquisition of water and soil nutrients. Our understanding of fine root dynamics has advanced with the implementation of minirhizotron technology that allows us to observe fine roots *in situ*. Minirhizotron studies have revealed properties of fine roots that are universally important for plant performance and ecosystem functioning. Among these, biomass and diameter, production and death, and turnover play crucial roles in functional ecology.

Fine root biomass represents the balance between constructing new roots and maintaining metabolically older roots. According to allocation theory, plants maintain a large biomass of fine roots when soil nutrients are scarce (Bloom *et al.* 1985), and studies have shown more fine root biomass in infertile than in fertile soils of the humid tropics (Gower 1987; Maycock & Congdon 2000; Powers *et al.* 2005; Espeleta & Clark 2007). The situation is especially acute for phosphorus (P) in highly weathered soils, as PO_4 is readily adsorbed to organic matter and Al- and Fe-oxides (Walker & Syers 1976), and Ca if it is available, which render P relatively unavailable for plant growth. Thus roots encounter P by growing through soil rather than having P delivered via diffusion or water flow. The same situation should apply to ammonium (NH_4), whereas nutrients such as nitrate (NO_3) and potassium (K) are more mobile in soils, and thus they should be delivered effectively by diffusion and mass flow.

The diameter of fine roots is taken to be <2 mm. However, studies have shown considerable variation in

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root behaviour within this group. For example, nutrient uptake increases with a decrease in fine root diameter, given that thinner roots maximize surface area per root volume, whereas fine root survivorship increases with increasing diameter (Eissenstat & Yanai 1997). In addition to diameter per se, the location of the root within branching architecture also influences longevity (cf. Guo *et al.* 2008). Root turnover is greater for distal roots than for proximal roots, even when diameter is the same, because distal first- and second-order roots are exposed to fluctuating soil resources (e.g., soil moisture) and are more likely to be eaten by herbivores.

Fine root longevity ranges from a few months to years (Pritchard & Strand 2008). Longevity is variable, in part, because making new roots to replace dead roots (a process called root turnover) is metabolically expensive and intimately tied to soil nutrient availability. Therefore, fine root turnover should be greater on fertile than infertile sites. The reasoning is that roots maintain elevated respiration rates in fertile soils in order to take up nutrients to sustain aboveground production, and elevated respiration leads to death at a young age. Alternatively, greater soil fertility could have no effect or decrease root turnover, either of which leads to less fine root production to account for less fine root biomass on fertile sites. Empirical and experimental evidence for fine root turnover as a function of soil fertility come for the most part from northern forests and in response to soil N availability (Brassard *et al.* 2009 and references cited therein). Relationships between soil fertility and fine root dynamics are particularly unclear for humid tropical soils.

Soil fertility is equally complex in tropical soils (cf. Ostertag 2001). Most research has focused on P (Walker & Syers 1976; Vitousek & Farrington 1997), given that P enters soil slowly from weathering of primary minerals, reaches maximum availability in mid-aged soils, followed by adsorption to secondary minerals and decline in availability in old highly weathered soils. However, a meta-analysis of experimental studies suggests that soil nitrogen (N) limitation is widespread globally (LeBauer & Treseder 2008), and very old soils do show N limitation in addition to P limitation (Lambers *et al.* 2008). There are also instances in which calcium (Ca) or K alone limit plant growth, or provide co-limitation with N and P (Cuevas & Medina 1988). Micronutrients required for plant growth also can bind tightly to soil minerals and organic matter, and could be unavailable in certain instances (Kaspari *et al.* 2008). A wide range of nutrients might be limiting in tropical soils. The precise way to assess soil fertility and plant growth is a nutrient fertilization study with multiple nutrient elements, added in a factorial experimental design, with replication (Sollins 1998). However, because of logistics and time constraints there are few examples from the tropics that last more then a few years (cf. Mirmanto *et al.* 1999).

To help understand relationships between plants and soil fertility, we initiated a plot scale nutrient fertilization experiment at the Barro Colorado National Monument (BCNM) in the Republic of Panama (Yavitt *et al.* 2009). The experiment involved treating 40×40 m plots of old-growth lowland tropical forest with N, P and K in a factorial experimental design. Based on allocation theory and empirical studies we predicted that (i) fine root biomass should decrease in response to increased fertility of the most limiting soil nutrient; and (ii) fine root turnover will show no response to increased soil fertility. The second prediction was based on the expectation that fine root turnover is already high in tropical forest soils (cf. Lauenroth & Gill 2003), and thus it is not likely that root longevity could decrease much.

MATERIALS AND METHODS

Study site description

The project area was a 38.4-ha rectangle of lowland, tropical moist forest located on the Gigante Peninsula, Republic of Panama, about 1 km inland from the shore of Gatun Lake, which forms the Panama Canal waterway (Fig. 1). The plot's centre is at 9°6′30.7″N, 79°50′36.9″W and *c.* 80 m above sea level. The northern border of the plot is 5 km due south of the southern border of the 50-ha Forest Dynamics Plot on Barro Colorado Island (BCI), the largest island within Gatun Lake. Gigante Peninsula and BCI form part of the BCNM, administered by the Smithsonian Tropical Research Institute (Leigh 1999).

Climate at the BCNM is moist tropical with a 4-month dry season. Annual rainfall averages 2600 mm with 90% falling in the rainy season from May to mid-December. Monthly mean temperature is 27°C in April and 26°C in the other 11 months of the year. Relative humidity is >75%. Vegetation composition and stature (canopy heights > 35 m) are characteristic of old growth (>300 years) seasonally evergreen lowland tropical forest. Soil is an Endogleyic Cambisol (FAO classification; Koehler *et al.* 2009), which is highly weathered, moderately acidic, with high clay content. The underlying bedrock is a resistant andesite flow that overlies a series of nearly flat sedimentary rocks (Johnsson & Stallard 1989). These include volcanic and marine facies of Oligocene age material. The andesite has a maximum thickness of 85 m, and it is a nonvesicular, phenocrystic andesite with veins and vugs. The main minerals are plagioclase, clinopyroxene, orthopyroxene and magnetite; the veins and vugs contain quartz, calcite and zeolite. The regional terrain is highly varied, although the andesite is nearly flat and drained by small incised streams.

The 38.4-ha plot included a gradual 50-m elevation gradient from the southwestern to northeastern corners. Many soil properties changed along this gradient (Yavitt *et al.* 2009). Most important, soil pH increased from 4.5 in the southwestern corner to 7.5 in the northeastern corner. We measured (diameter at breast height or just above buttresses or deformations), mapped and identified all trees ≥20 cm Diameter at Breast Height (DBH) to species in the 38.4 ha

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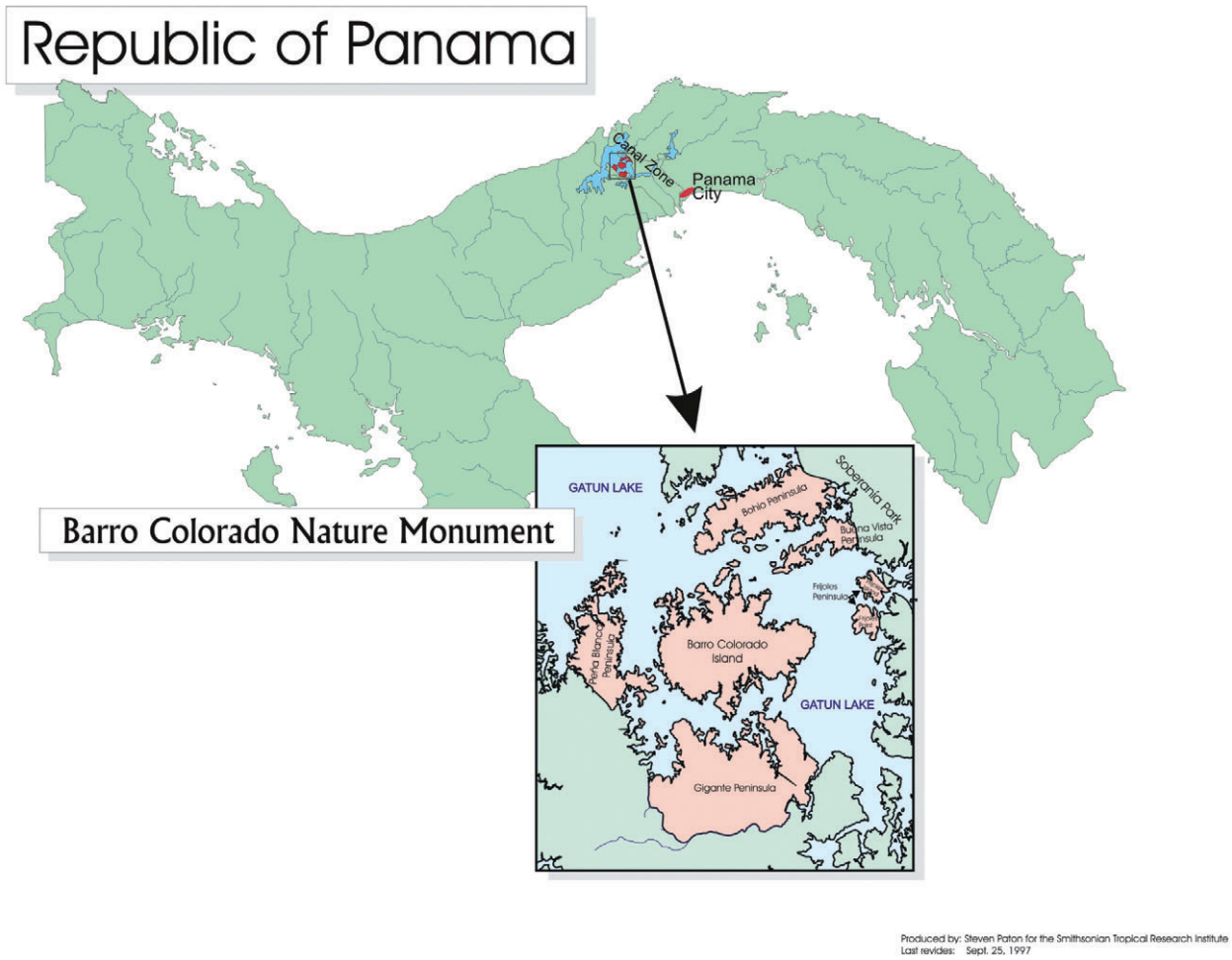

Fig. 1. Map of Panama showing the Barro Colorado Nature Monument.

plot. The distributions of many tree species also changed along the elevation gradient (unpubl. analyses, 1997). The experimental design attempted to control environmental variation associated with the elevation gradient.

Experimental design

The experiment consisted of nine treatments. Eight treatments comprised the factorial NPK experiment (control, +N, +P, +K, +NP, +NK, +PK, +NPK). The final micronutrient treatment added boron (B), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), sulfur (S) and zinc (Zn). Each treatment was replicated in four 40 × 40-m plots.

Basal area, forest age and inter-plot distance were used to locate the 36 1600-m^2 plots. Tree basal area and a forest age index, based on species composition and abundance, were calculated for every potential 40 × 40-m plot, where potential plots were oriented north-south and spaced at 10-m intervals. We eliminated potential plots whose basal areas were >1 standard deviation from the mean basal area for all potential plots, forest age indices indicated that the plots were composed mostly of pioneer tree species (suggesting recovery from recent tree falls), or nearest neighbour plot was <30 m distant. Of the 36 plots locations selected, two were 20 m apart, but a deeply incised, seasonal stream ran between them. All other plots were separated by at least 30 m from their nearest plot, and most were separated by at least 40 m.

The assignment of treatments to these 36 plots determined the experimental design. The four replicates were arrayed from north to south approximately perpendicular to the elevation gradient (Fig. 2). Substantial spatial variation in soil properties remained within the very large area required for each replicate of the factorial N-P-K experiment (eight 1600-m^2 plots separated from their nearest neighbours by 30 or 40 m) (Yavitt *et al.* 2009). We therefore nested two balanced blocks of four treatments (+N, +P. +K, and +NPK *vs.* Control, +NP, +NK, and +PK) within each replicate of the factorial N-P-K experiment. For each potential set of blocks, the within- and among-block variance was estimated for Principal Component Analysis (PCA) score 1 of the following soil variables: surface pH, N-mineralization rate at the surface and concentrations of PO_4 and K at the surface. The set of blocks that minimized the within-block variance was selected. Treatments were then assigned randomly to plots within blocks (Fig. 2). Winer *et al.* (1991) describe this experimental design in detail.

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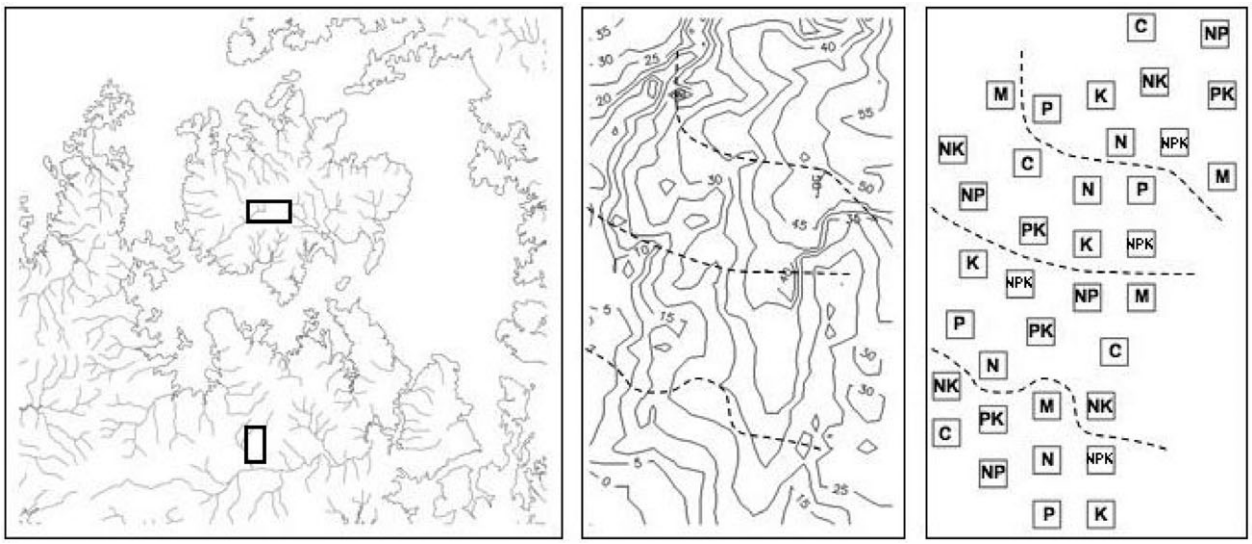

Fig. 2. Maps of the study area: (Left panel) Portions of the Barro Colorado Nature Monument (BCNM), oriented with North at the top; the northern rectangle is the 50-ha (1000 m × 500 m) Forest Dynamics Project Plot on Barro Colorado Island; the southern rectangle is the 38.4-ha (480 m × 800 m) Gigante Fertilization Project Plot on the Gigante Peninsula. (Centre panel) Gigante Fertilization Project Plot contour map; dashed lines outline four blocks. The plot slopes downward from the northeast to the southwest. (Right panel) Gigante Fertilization Project Plot, experimental design; dashed lines outline four blocks of nine sub-plots each. Each square subplot is 40 m × 40 m. Treatments appear as letter codes in each subplot: C = control (no nutrients added); M = micronutrient addition; N, P, K, NP, NK, PK, NPK = factorial additions of nitrogen, phosphorus and potassium.

Fertilizer additions

We added nutrients to plots four times each year beginning in 1998 and have continued annually to the present. Fertilizer was added during the wet season, during approximately the same four fortnights each year: May 15 to 30, July 1 to 15, September 1 to 15 and October 15 to 30. The rationale for dividing the fertilizer into four applications per year was to dampen the temporal pulse of exogenous nutrients into the plots. The start (15 May) and end (30 October) dates were chosen to be after the latest onset and before the latest end date to the wet season observed since 1923 in central Panama, respectively.

We used dry, granular fertilizers: N added as urea at the rate of 12.5 g N m^{-2} $year^{-1}$; P added as triple superphosphate at the rate of 5.0 g P m^{-2} $year^{-1}$; and, K added as KCl at the rate of 5.0 g K m^{-2} $year^{-1}$. Micronutrients were added as Soluble Trace Element Mix (STEM) from Scotts Company (Stock No. 91391) plus dolomitic limestone: STEM at the rate of 2.5 g m^{-2} $year^{-1}$; and, dolomitic limestone at the rate of 3.6 g m^{-2} $year^{-1}$. Application rates for N and K were approximately equal to the annual deposition of N and K in litterfall. Although the application rate for P was greater than that in litterfall, it was comparable to amounts used in forestry and in tropical montane experiments (see below in Discussion section).

Fertilizers were spread by walking systematically through the treatment plot and casting handfuls of N, P, K, or dolomitic limestone, or by spraying STEM diluted in nutrient-poor stream water from a handheld tank sprayer. A different systematic pattern was followed for each of the four applications to a given plot in a given year (North to South, East to West, South to North and West to East), which ensured as homogenous application of fertilizer as possible.

Soil sampling

Soils were sampled each year in October–November from the corners of the inner 20 × 20-m portion of each 40 × 40-m plot and always >1 m away from a previous soil sample. A surface sample was collected with a hand trowel from 0–10 cm depth, excluding litter and debris at the upper surface. Three scoops of soil from within 0.5 m of the sample point were bulked and a 300-g sample of this bulked soil was collected. A subsurface soil sample (30–40 cm depth) was collected with a Dutch auger. All soil samples were collected into plastic Whirl-pak bags for transportation from the study site to the laboratory on BCI or in Panama City. Transportation from the field to the laboratory never required >6 h. Bags were kept in a cold room for a maximum of 36 h before laboratory processing commenced.

Laboratory processing and chemical analyses of soils

Soil samples were divided into portions for various analyses. Field moist portions were used for soil moisture determination and extractions of inorganic N and PO_4. Portions air-dried at 50°C, pulverized and sieved through a 2-mm mesh screen to remove rocks were used for pH determination and exchangeable metals.

Percent soil moisture was estimated by the change in mass before and again after oven drying at 105°C for 48 h. Soil pH was measured by mixing a slurry of 4 g of air-dried soil with 8 mL distilled water (reported as pH_{H2O}), and also after adding 6 drops of 2 M $CaCl_2$ solution to the slurry (pH_{salt}). Soil pH_{H2O} measures the amount of free acidity, and pH_{salt} includes exchangeable acidity (Yavitt *et al.* 2009). Nitrogen

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was extracted from 2 g of fresh soil overnight in 20 mL of 2 M KCl.

Phosphorus availability was measured using two techniques. Phosphorus was extracted from 1 g of fresh soil by shaking vigorously for 1 min in 7 mL of Bray's P1 extraction solution (0.03 N NH_4F and 0.025 N HCl; Bray & Kurtz 1945) and centrifuged. Second, the Hedley fractionation procedure was applied (Tiessen & Moir 1993), which removes P sequentially using progressively stronger extracting solutions. Presumably plant-available P is removed in bicarbonate ($NaHCO_3$ inorganic Pi, and $NaHCO_3$ organic Po); less-readily-plant-available but non-occluded P bound to the outer surfaces of iron (Fe) and aluminum (Al) minerals is removed in hydroxide (NaOH inorganic Pi, NaOH organic Po); P in primary minerals is removed with dilute HCl (dilute HCl Pi); occluded P in hot HCl (concentrated HCl inorganic Pi, and concentrated organic Pi); and, finally residual P in H_2SO_4 and H_2O_2 (residual inorganic Pi). This method has been discussed extensively in the literature (e.g. Cross & Schlesinger 1995), and for brevity the major methods are omitted here. Importantly, an initial resin Pi extraction was skipped, and thus this fraction was recovered in the bicarbonate Pi pool.

Base cations (Ca, Mg, Na, K) were extracted from 5 g of air-dried soil for 30 min on a shaker table in 100 mL 1 M NH_4Cl. Extraction solutions were collected into polyethylene sample bottles and stored in a cold room for no longer than 2 weeks prior to analysis. Concentrations of NH_4 and NO_3 were determined colorimetrically by continuous flow analyses. Concentrations of PO_4 were determined colorimetrically, and all metals were determined by inductively coupled plasma mass spectrometry. Nutrient concentrations for N and P are given in atomic units, i.e. mg N kg^{-1} and mg P kg^{-1}.

A 100-g air-dried portion of soil from each sampling point and depth (excluding N-incubations) has been archived in individual paper bags in an air-conditioned laboratory at the Smithsonian Tropical Research Institute in the Republic of Panama.

Fine root dynamics

Fine roots were sampled in 2000. Four replicate cores (5-cm diameter, 20-cm deep) were collected at randomly determined locations in each treatment and control plot. The cores were refrigerated until processed (<4 weeks). Roots were separated from soil by washing and picked from sieves until only tiny pieces remained. Live fine roots were sorted into two diameter classes: (i) fine roots <2 mm diameter, and (ii) small roots 2–5 cm diameter. Dead roots were distinguished by resilience, brittleness and colour. The sorted roots were dried at 65°C, weighed and a portion was ashed at 470°C to report mass on an ash-free basis.

Dynamics of fine roots were monitored using minirhizotrons. Two clear butyrate minirhizotron tubes (5 cm diameter) were installed at a 45° angle in each treatment and reference plot in 1998. Tubes were installed to a depth of 30 cm. Images were collected at three depths along each tube and along four axes filmed at about 45° from vertical along the upper and lower surfaces of each tube. For the first 3 years of the study, images were recorded at 2-month intervals. In the 4th year, we recorded images at 2- to 3-week intervals in order to evaluate short-term and seasonal (wet season *vs.* dry season) dynamics.

Images recorded in the first 3 years were analysed using RooTracker software (Duke University Phytotron, Durham, NC, USA). For each image the location, length, diameter and appearance of all new roots were recorded. Changes in size and morphology of each root were tracked through successive intervals. A root was classified as dead when it became faint or discontinuous with indistinct edges, shrivelled to a fraction of its previous width, or completely disappeared. Dead roots were tracked and returned to the live category if they later grew or appeared restored. Roots that became obstructed from view by condensation or shrinking and swelling of the soil were classified as obscured and watched for subsequent reappearance. In the 4th year, the large number of images precluded tracing of root length, and thus we counted each new root and existing roots in each image.

We evaluated fine root turnover by calculating a turnover coefficient (TC $year^{-1}$). TC equalled the ratio of the number of new roots divided by the average number of roots observed per camera angle, per depth, per tube in a calendar year (Tierney & Fahey 2007). In the 4th year of measurements, we estimated the TC separately for the wet season (June–December) and the dry season (January–May). TC is the inverse of average fine root longevity, and it is analogous to the fine root production method of Hendrick and Pregitzer (1992), except using root number rather than root length in the calculation (Crocker *et al.* 2003). The TC is not the supreme turnover value for the entire fine root system because it does not include the survivorship for every fine root, which typically shows a long-tailed distribution (Tierney & Fahey 2002). However, the large number of tubes (72) precluded determining complete survivorship for each fine root. Furthermore, we relied on minirhizotron images to track fine roots, and minirhizotron images favour first- and second-order roots over higher order roots, which often survive longer than lower-order roots (Guo *et al.* 2008). However, lower-order roots are more affected by soil fertility (King *et al.* 2002), which was our study objective.

Data analysis

We used repeated measures analysis of variance (ANOVA) to analyse the factorial NPK experiment. Response variables included mean values for soil resources and fine root dynamics calculated over the four replicate soil samples and two replicate minirhizotron tubes, respectively, for each 1600-m^2 plot. Repeated measures occurred on year (1998, 1999, 2000 and 2001).

We employed several post-hoc tests. We used Levene's test to determine whether variances were homogeneous for the eight N-P-K factorial treatments. If necessary, the data were transformed to achieve homogeneity. The most problematic assumption of repeated measures ANOVA is that the dependent variables have homogeneous variances and homogeneous covariances (the compound symmetry assumption). Therefore, we considered Greenhouse-Geisser and Huynh-Feldt corrections for violations of this problematic assumption across the 4 years of measurements.

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RESULTS

Soil fertility

Soil in the 0–10 cm depth interval was moderately acidic, and the sum of exchangeable base cations averaged 13.4 $cmol_c\ kg^{-1}$ (on a soil charge basis) consisting of Ca (62%) and Mg (34%) with minor contributions from K (2.5%) and Na (1.4%). KCl-extractable N was predominantly NH_4 (15.5 $mg\ kg^{-1}$) rather than NO_3 (5 $mg\ kg^{-1}$).

Concentrations of KCl-extractable NO_3 were significantly greater ($F_{1,\ 18} = 17.62$, $P = 0.001$) in plots with added N (Fig. 3). The addition of P or K along with the N addition did not affect the outcome, i.e. interactions among main effects were not significant (NP, $F_{1,\ 18} = 0.84$, $P = 0.371$; NK, $F_{1,\ 18} = 1.63$, $P = 0.218$). The concentration of KCl-extractable NO_3 varied significantly among years ($F_{3,\ 54} = 21.54$, $P < 0.001$) in all of the plots. All of the responses in NO_3 concentrations were consistent throughout the study area, i.e. replicate, block, and all interactions were not significant. In contrast, concentrations of KCl-extractable NH_4 were similar ($F_{1,\ 18} = 1.08$, $P = 0.312$) in plots with added N *versus* plots that did not receive added N. Concentrations of KCl-extractable NH_4 varied among years ($F_{3,\ 54} = 20.99$, $P < 0.001$), and the year-to-year variations in values were not affected by any of the fertilization treatments.

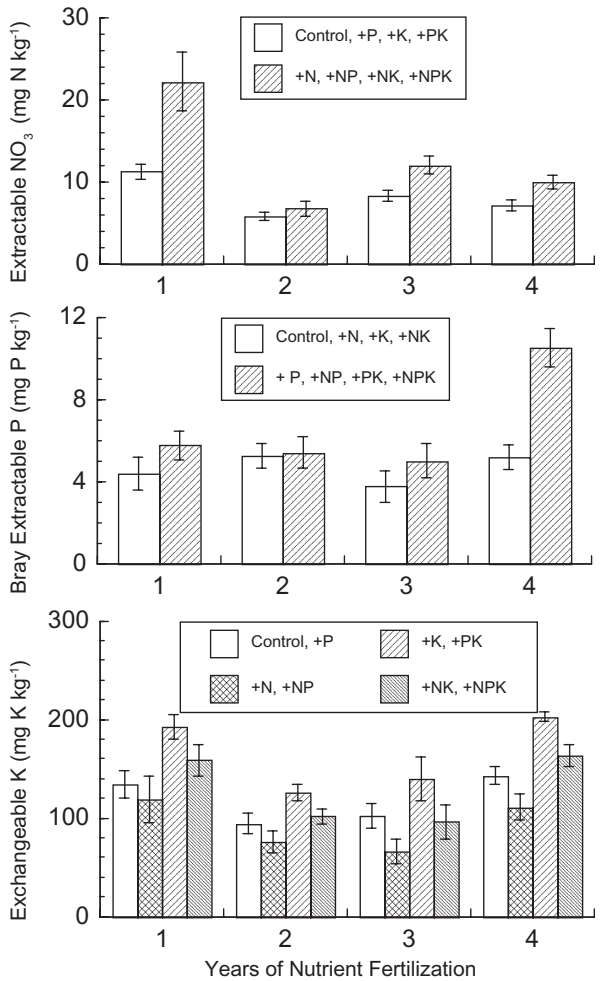

Fig. 3. Concentrations of KCl-extractable NO_3, Bray-extractable PO_4 and exchangeable K in soil from the 0–10 cm depth interval at Gigante, Panama, during 4 years of nutrient fertilization. Legends indicate plots that received different nutrients. Values are means ($n = 16$ for NO_3, PO_4; $n = 8$ for exchangeable K); error bars are 1 SE.

Concentrations of Bray-extractable P were significantly greater ($F_{1,\ 18} = 27.72$, $P < 0.001$) in plots with added P *versus* plots that did not receive additional P (Fig. 3), and the addition of N or K along with the P addition did not affect the outcome, i.e. interactions among main effects were not significant (NP, $F_{1,\ 18} = 1.90$, $P = 0.185$; PK, $F_{1,\ 18} = 2.00$, $P = 0.174$). There was a significant year effect ($F_{3,\ 54} = 15.68$, $P < 0.001$) on Bray-extractable P concentrations, which was most evident in the plots with added P (Year × P, $F_{3,\ 54} = 10.64$, $P < 0.001$). Neither of the spatial variability effects (replicate, block) nor any interactions were significant.

Added P also led to significant increases (Table 1) in $NaHCO_3$ Pi ($F_{1,\ 18} = 8.11$, $P = 0.011$), NaOH Pi ($F_{1,\ 18} = 40.41$, $P = 0.001$) and weak HCl Pi ($F_{1,\ 18} = 18.18$, $P = 0.001$). For P in organic fractions, there was a significant interaction with N, such that the +NP treatment had increased $NaHCO_3$ Po ($F_{1,\ 18} = 6.67$, $P = 0.019$) and increased NaOH Po ($F_{1,\ 18} = 6.47$, $P = 0.020$). There were non-significant differences in P in the occluded and resistant fractions.

Concentrations of exchangeable K were significantly greater ($F_{1,\ 18} = 15.04$, $P = 0.001$) in plots with added K than in plots without added K (Fig. 3). Concentrations of exchangeable K also were consistently less in plots with added N ($F_{1,\ 18} = 8.51$, $P = 0.009$). The concentration of exchangeable K varied significantly from year to year ($F_{3,\ 54} = 54.93$, $P < 0.001$), but it did not vary among years in one of the replicates (Year × Replicate, $F_{3,\ 54} = 8.51$, $P < 0.001$). In addition, the concentration of exchangeable K in deeper soils (30–40 cm depth) was statistically similar ($F_{1,\ 18} = 1.11$, $P = 0.382$) in plots with added K *versus* plots without added K (data not shown).

There were no consistent effects of nutrient fertilization on other soil resources, except for soil pH (Fig. 4). Added N acidified pH_{H2O} of the soil ($F_{1,\ 18} = 10.42$, $P < 0.001$), and the effect was much more pronounced in year 2 and year 4 (Year, $F_{3,\ 54} = 28.40$, $P < 0.001$; Year × N, $F_{3,\ 54} = 10.85$, $P < 0.001$). This finding was robust across the +NP and +NK treatment combinations. Furthermore, the acidification soil by added N was evident in the pH_{salt} data (Year,

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Table 1. Means and SE for concentrations of P in different fractions of soil from two depth intervals in plots without added P (Control) and plots with added P

	Control		Added P	
Fraction	Mean	SE	Mean	SE
Inorganic 0.5 M $NaHCO_3$ Pi				
0–0.15 m	0.01	0.01	4.31	1.50
0.15–0.30 m	0.01	0.0	0.41	0.16
Organic 0.5 M $NaHCO_3$ Po				
0–0.15 m	36.3	5.8	44.4	5.8
0.15–0.30 m	20.7	1.5	43.0	3.8
Inorganic 0.1 M NaOH Pi				
0–0.15 m	45.6	3.6	106.7	9.3
0.15–0.30 m	21.3	0.7	27.8	1.8
Organic 0.1 M NaOH Po				
0–0.15 m	68.7	18.9	146.8	60.0
0.15–0.30 m	66.2	21.7	62.9	24.6
1 M HCl Pi				
0–0.15 m	3.90	0.21	5.92	0.4
0.15–0.30 m	2.88	0.16	4.23	0.29
Inorganic concentrated HCl Pi				
0–0.15 m	178	21	182	26
0.15–0.30 m	135	19	132	15
Organic concentrated HCl Po				
0–0.15 m	51.3	7.1	59.1	8.4
0.15–0.30 m	54.4	12.1	44.6	5.4
Residual P				
0–0.15 m	101	5.7	113	7.9
0.15–0.30 m	100		112	
Total				
0–0.15 m	484	33	662	71
0.15–0.30 m	402	34	428	32

Fractions are based on different chemical extractions. The forms in each fraction are ionic, inorganic PO_4 (Pi) and P bound to organic matter (Po).

$F_{3, 54} = 392.08$, $P < 0.001$; Year × N, $F_{3, 54} = 76.03$, $P < 0.001$). The acidification by added N led to only one significant effect on concentrations of exchangeable metals, a twofold increase in exchangeable Ni (data not shown).

Fine root biomass

After 2 years of nutrient fertilization, the biomass of fine roots (<2 mm diameter) was significantly less ($F_{1, 18} = 9.42$, $P < 0.001$) in plots with added K, including those that also had added N and P (Fig. 5), *versus* that in plots without added K. About 50% of the fine roots occurred in the 0–5 cm depth interval, and 75% occurred in the top 10 cm of the soil. The depth distribution was unaffected by nutrient fertilization ($P = 0.70$). We also examined the biomass of small roots (2–5 mm diameter) and found significantly greater amounts ($F_{1, 18} = 7.75$, $P < 0.012$) in plots with added P than in plots without added P: the P effect was marginally significant at the shallowest and deepest depths and highly significant at the 5–10- and 10–15-cm depth intervals. The analysis for small roots also revealed a significant depth-N-K interaction ($F_{3, 12} = 4.30$, $P < 0.009$): the interaction was significant because added N increased small root biomass in plots without added K at the 0–5-cm depth intervals and in plots with added K at the 5–10- and 10–15-cm depth intervals.

Fine root dynamics

There was a marginally significant ($F_{1, 18} = 3.34$, $P < 0.084$) effect of added K on the standing crop of fine root length (Table 2). Less standing crop was evident in plots with added K across all 4 years of measurements. During the study period, the standing crop of fine root length showed a significant increase from year to year ($F_{3, 54} = 159.0$, $P < 0.001$) with an especially large increase between year 3 and year 4. In contrast, the diameter of fine roots showed a concomitant, significant decrease ($F_{3, 54} = 175.0$, $P < 0.001$). Although added nutrients did not have a significant impact on fine root diameter ($P > 0.30$), fine roots were notably thinner in plots with added K (0.28 ± 0.08 mm) and added PK (0.33 ± 0.10 mm) in the first year of measurements.

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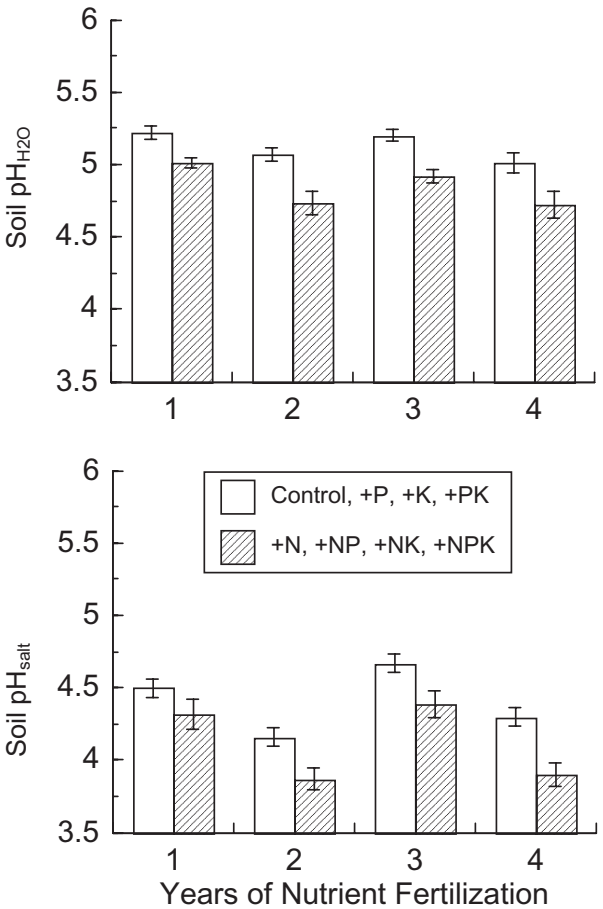

Fig. 4. pH in water and in salt solution in soil from the 0–10 cm depth interval at Gigante, Panama, during 4 years of nutrient fertilization. Legends indicate plots that received different nutrients. Values are means (n = 16); error bars are 1 SE.

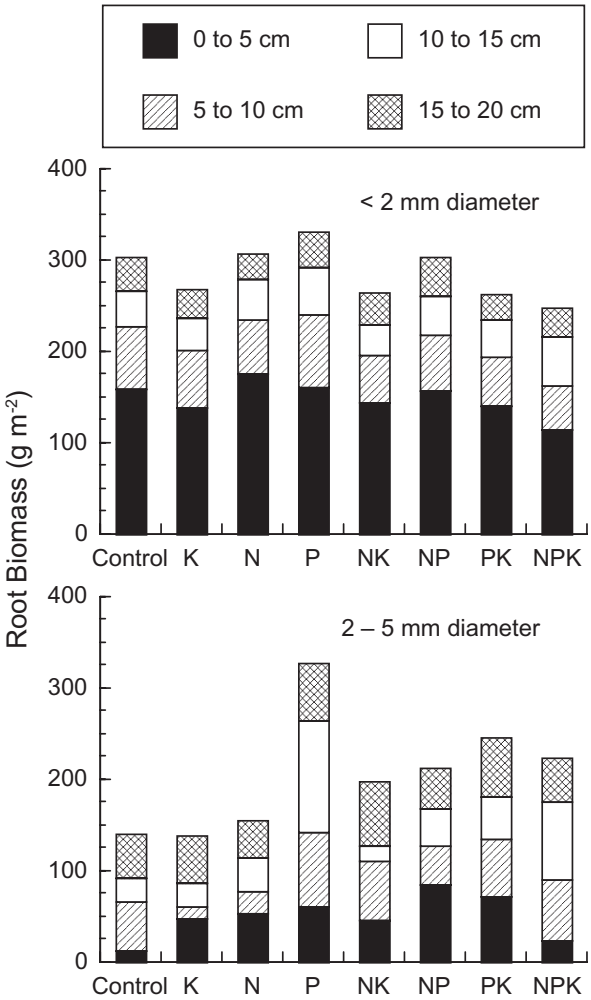

Fig. 5. Biomass of fine roots (<2 mm diameter) and small roots (2–5 mm diameter) in soil from four depth intervals after 2 years of nutrient fertilization (treatments on y-axis) at Gigante, Panama.

Added nutrients, except N alone and P alone, increased the TC of fine roots (<1 mm), but only for roots in the 0–5-cm depth interval (Table 3). The increase was only marginally significant for added K ($F_{1,\ 18} = 2.38$, $P = 0.140$) and for added NP ($F_{1,\ 18} = 3.35$, $P = 0.085$), but it was highly significant for added NK ($F_{1,\ 18} = 7.10$, $P = 0.016$) and for added PK ($F_{1,\ 18} = 6.40$, $P = 0.021$). Added nutrients did not affect the TC of fine roots at the two deeper depth intervals; there the TC averaged 2.9 ± 0.1 year^{-1} across all treatments, both depths, and 3 years. In the 4th year of nutrient fertilization, minirhizotron images were taken at 3-week intervals. Although there was variation in the TC (Table 3), we found greater values during the wet season (mean = 0.45 ± 0.04 season^{-1}) than in the dry season (mean = 0.18 ± 0.02 season^{-1}). Some of the effects of added nutrients on fine root turnover were still evident per season, i.e. the significant effect of added NK ($F_{1,\ 18} = 6.13$, $P = 0.023$) and added PK ($F_{1,\ 18} = 8.91$, $P = 0.008$).

DISCUSSION

Soil fertility

We sampled soils at the same time and place each year. This experimental design provides a robust assessment of the effects of nutrient fertilization on soil chemical properties *versus* that in non-fertilized controls. Although soil nutrient availability does vary temporally in tropical moist forests in Panama (Yavitt & Wright 1996), it does not show consistent seasonal or year-to-year patterns (Yavitt & Wright 1996). Rather, temporal variability is driven by seasonal leaf fall, decomposition and nutrient release from litter on the soil surface (Yavitt *et al.* 2004). For these reasons, the application rates for N and K in fertilizer were approximately equal to the annual deposition of N and K in litterfall (Yavitt *et al.* 2004). The application rate for P was

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Table 2. Average standing crop of fine root length and average fine root diameter (mean ± SE)

	Years of nutrient fertilization			
Plot	1	2	3	4
Fine root length (m of root length per m^2 of minirhizotron viewing surface)				
No added K	8.8 ± 2.1	12.7 ± 1.9	17.2 ± 2.6	38.8 ± 3.4
+K	5.8 ± 1.3	9.3 ± 1.4	13.1 ± 2.4	33.4 ± 3.4
Fine root diameter (mm)	0.46 ± 0.04	0.33 ± 0.02	0.30 ± 0.02	0.23 ± 0.01

Table 3. Average fine root turnover for plots with added nutrients and the control plots

Plot	Annual ($year^{-1}$)	Dry season ($season^{-1}$)	Wet season ($season^{-1}$)
Control	2.7 ± 0.3	0.13 ± 0.03	0.31 ± 0.11
+N	2.9 ± 0.2	0.12 ± 0.03	0.40 ± 0.20
+P	2.9 ± 0.3	0.30 ± 0.17	0.55 ± 0.15
+K	4.2 ± 0.4	0.17 ± 0.05	0.23 ± 0.14
+NP	3.9 ± 0.3	0.18 ± 0.07	0.22 ± 0.08
+NK	3.2 ± 0.4	0.16 ± 0.11	0.85 ± 0.12
+PK	3.2 ± 0.4	0.16 ± 0.08	0.40 ± 0.12
+NPK	3.1 ± 0.3	0.20 ± 0.05	0.68 ± 0.36

greater than that in litterfall, but comparable to amounts used in forestry (Fisher & Binkley 2000), and in tropical montane experiments (Tanner *et al*. 1998).

Nutrient fertilization should increase concentrations in the soil provided that a given nutrient element has a short residence time, and thus availability responds quickly to the added input (Eriksson 1971). No response has two explanations: either (i) the nutrient element has a long residence time leading to a delayed response to additional input, or (ii) rapid removal of the added nutrients, such as via plant uptake, microbial immobilization or enhanced leaching loss. Here added N increased the concentration of KCl-extractable NO_3; added P increased concentrations of Bray-extractable and labile P; and, added K increased concentrations of exchangeable K, suggesting short residence times for these nutrients.

We added N as urea. Urea decomposes to NH_4, which is subsequently oxidized to NO_3 and protons (H^+). Hence, the significant increase in KCl-extractable NO_3 and acidification of the soil confirm that urea contributed to soil N cycling. The increase in concentration of KCl-extractable NO_3 concentrations reflects the very short residence time for NO_3 and NH_4 in most soils (Booth *et al*. 2005). Soil acidification by urea is well known (cf. Tamm & Popovic 1995; Fox 2004), although mechanisms vary. One reason is that nitrification of NH_4 produces H^+ (Killham 1990). Other explanations include: (i) leaching loss of base cations with mobile NO_3 that effectively concentrates H^+ and exchangeable Al in the soil, or (ii) increased plant uptake of base cations and retention in biomass (Mitchell & Smethurst 2004). In our study acidification occurred without statistically significant loss of exchangeable base cations or an increase in exchangeable Al, suggesting direct acidification by nitrification and little buffering of the additional H^+.

The significant increase in exchangeable Ni with added N is likely a result of acidification and increase in Ni solubility at acidic pH. Concentrations of several micronutrient metals are sensitive to soil pH, and increasing levels depend on the degree of acidification (Harapiak *et al*. 2000). Because Ni is intermediate in solubility among the metals we investigated, we suggest that it also has a shorter residence time than the other metal micronutrients.

Concentrations of total P in the control soils were within the range reported by Quesada *et al*. (2009) for Cambisols in Amazonian tropical forests. The concentration of Bray-extractable P is much less than values reported by Sharpley *et al*. (1987) for a broad range of soils; however, labile P from the Hedley fractionation was typical for Cambisols (Johnson *et al*. 2003). Therefore, soil test P (Bray-extractable P) indicates a P limited soil, but rapid turnover of labile P might relax this constraint. Consistent with other Cambisols, soil in our study area has a much larger proportion of P in the HCl-extractable fraction (47%) compared with P residing largely in the residual fraction in highly weather tropical soils (McGroddy *et al*. 2008). Concentrated HCl extracts P associated with Ca, which is consistent with the high soil Ca concentrations in our study area.

We added P as triple superphosphate. Although added P led to a statistically significant increase in the concentration of Bray-extractable PO_4, the increase was only modest. For example, Olander and Vitousek (2004) found a 400-fold increase in Bray-extractable PO_4 after 15 years of P fertilization in a montane

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tropical forest. Our results also show a large increase in labile $NaHCO_3$ Pi and labile $NaHCO_3$ Po in response to added P, suggesting these forms of P have a short residence time in the soil. Other studies have shown that added P is not always directly responsible for elevated concentrations of labile P. Often labile P concentrations remain elevated for several years after P fertilization as well as in experiments with a single application (Fransson & Bergkvist 2000). One explanation is that added P stimulates mineralization of organic P, which decreases concentrations of Po and fuels labile P (Olander & Vitousek 2004). However, we did not observe a net decrease in any of the organic P pools. Rather we observed an increase in the concentration of NaOH Po with added P, indicating that added P is being incorporated into organic matter and subsequently sequestered in this relatively stable organic fraction. Phosphorus in NaOH extractable fractions is thought to be less labile than $NaHCO_3$-extractable P (Cross & Schlesinger 1995), and thus it cycles more slowly. Nevertheless our results suggest that NaOH Po has a fairly short residence time. In contrast, continuous application of P to agricultural soils rarely affects the NaOH Po pool (cf. Vu *et al.* 2008), although soil with crops often have less clay content and more basic pH values than the soils in our study. No change in the acid extractable and residual pools with added P was expected as these represent mineral fractions with very long residence times.

Overall, soil in the treatment plots contained, on average, 81% of the P added during the previous 7 years of the study. This is a fairly large percentage of added P to reside in the soil. For example, Beck and Sanchez (1994) added P for 13 years to tropical agricultural soils and recovered 43% in the soil *versus* 25% to 43% in plant biomass. McGroddy *et al.* (2008) added P for 1 year and recovered 33% to 69% in the soil. Nevertheless, the large amounts in organic fractions in our study indicate that added P contributes to plant-soil cycling, but that P eventually resides in the soil.

The increase in exchangeable K in surface soil with added K was somewhat unexpected. Potassium is very soluble and readily leached from soil with little retention on cation exchange sites that preferentially adsorb divalent and trivalent cations. Despite soil K mobility, added K can increase concentrations of exchangeable K in soil if the added K causes greater concentrations in foliage, or more foliage, and subsequent foliage decomposition on the soil surface leads to a new equilibrium level with elevated soil concentrations (Stone & Kszystyniak 1977; Shepard & Mitchell 1990). Paré *et al.* (1993) found that added K resulted in elevated concentrations of exchangeable K in an acid forest soil, but the increase occurred in soil >15 cm deep and not at shallower soil depths. Potassium accumulation in deeper portions of the soil profile has been linked to less competition with Al, Ca and Mg for exchange sites (Romanowicz *et al.* 1996). We did not find greater concentrations of K in soil 30 to 40 cm below the surface, although K might be accumulating deeper in the soil profile. Furthermore, our finding that added N offset the increase in exchangeable K, suggests that the mobile NO_3 is the anion that pairs with K to facilitate leaching loss.

Fine root dynamics

Less fine root biomass with 4 years of added K is consistent with predictions made on the basis of carbon allocation theory (Bloom *et al.* 1985), i.e. plants maintain less fine root biomass when growing on fertile soils. A biomass response to soil fertility has been observed in many temperate forests, primarily, with N availability (Brassard *et al.* 2009), whereas the prevailing paradigm for tropical forests is that fine root biomass varies inversely with soil P availability (Espeleta & Clark 2007; references cited therein). Relationships between fine root biomass and base cation fertility have been reported (cf. Gower 1987; Cuevas & Medina 1988). However, the situation for base cations might be more complicated. Despite predictions from biogeochemical theory that only one nutrient element controls growth of organisms at any given time, i.e. Leibig's Law, ratios between N, P, base cations, and other nutrient elements might control plant biomass allocation (cf. Danger *et al.* 2008).

Added nutrients did not change the depth distribution of fine root biomass, at least, across the top 20 cm of the soil profile. There are suggestions in the literature that fine roots permeate to great depths in tropical forest soils (Jackson *et al.* 1997). However, an earlier study in Panama revealed little fine root biomass between 20 and 1.5 m depth (Yavitt & Wright 2001), suggesting that we captured most of the fine root biomass in the present study. The amount of fine root biomass in the top 20 cm of the soil is less than the median value reported in the literature for tropical forests (Jackson *et al.* 1997; Powers *et al.* 2005); however, the amount is greater than that reported for lowland tropical forests in neighbouring Costa Rica (Espeleta & Clark 2007). Therefore, despite the small amount of fine root biomass, the packing of fine roots close to the soil surface implies competition among plant species for nutrients released from decomposing litter on the soil surface.

Greater biomass of small root (2–5 cm diameter) with added P and with added NK was not expected. We did not track birth and death dynamics of small roots, and thus we can only speculate about explanations for the biomass response. However, it seems likely that small roots live longer and respond to added nutrients the same way as biomass aboveground; that is, added nutrients increase growth and lead to greater biomass of permanent tissue at a younger age (cf. Miller 1981).

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The standing crop of fine root length is an important aspect of root growth for several reasons. For example, installing the viewing tubes dislocates roots adjacent to the tubes, and steady state in the standing crop among a time series of images implies recovery from the disturbance (Joslin & Wolfe 1999). We installed the viewing tubes 1 year before recording images, and thus we contend that our findings are not merely an artefact of recovery from disturbance. Moreover, significantly less standing crop of fine root length with added K agrees with allocation theory that roots need less length to locate nutrients in a fertile soil. We do not have an explanation for the large increase in standing crop in year four, i.e. 5 years after the tubes were installed, but the observation highlights the dynamic nature of fine roots in this forest.

The mean diameter of roots in the minirhizotron images was quite thin, less than 0.3 mm diameter. Thin roots are thought to be an adaptation to nutrient poor conditions, thereby increasing nutrient uptake ability with a large surface area to volume ratio (Eissenstat 1992). Our findings that fine root diameter decreased through time are surprising. However, morphological patterns of fine roots in tropical forests is unclear, and the high species diversity and, presumably, intense competition for soil resources make general conclusions difficult (cf. Graefe *et al.* 2008).

It is well established that root turnover is greater for thin roots (Eissenstat & Yanai 1997; Wells & Eissenstat 2001). For instance, thin roots have high metabolic activity in order to take up nutrients, resulting in a shorter lifetime (Withington *et al.* 2006). Also, very-fine diameter roots are more likely to succumb to root herbivory and pathogen attack (Wells *et al.* 2002), which further increases turnover. The overall turnover value of 3.0 per year in this study means that fine roots live about 120 days.

Furthermore, fine root turnover increased with added K. Somewhat surprisingly there is no consensus in the literature on whether soil fertility promotes, reduces, or has no effect on fine root turnover (Brassard *et al.* 2009); albeit, most of the research has been done in temperate forest soils in response to soil N fertility. Lauenroth and Gill (2003) concluded that fine root turnover is greater in tropical forests than in temperate forests as a result of higher temperatures, longer growing seasons and more pathogens in tropical forests. We acknowledge that our data apply to only a portion of the fine roots present, given that minirhizotrons have a small viewing window and overemphasize first- and second-order roots, which have greater turnover than higher-order roots (Pritchard & Strand 2008). It is well recognized in the literature that one must apply multiple, complementary methods to determine turnover for the whole fine root system (Tierney & Fahey 2007).

Low-order roots also are colonization sites for mycorrhizal fungi. In this forest, arbuscular mycorrhizal fungi are common and associated with most of the plant species (Mangan *et al.* 2004). Although mycorrhizae are thought to decrease fine root turnover (King *et al.* 2002), the situation is unclear for low-order roots, as turnover for colonized and non-colonized roots may vary with root age (Guo *et al.* 2008). We do not know how mycorrhizae might have influenced findings here, as arbuscular mycorrhizae are not readily apparent on minirhizotron images.

Notwithstanding these limitations, the product of fine root turnover and fine root biomass provides a generalized estimate of fine root production ($g\ m^{-2}\ year^{-1}$). For added K, the greater turnover appears to merely offset less biomass resulting in similar amounts of fine root production compared with that in the control plots. Because the smallest diameter roots show the largest response to added nutrients (King *et al.* 2002), we advance a cautious conclusion that added nutrients did not result in more C allocated belowground. However, further research is need to estimate belowground C allocation accurately for the entire system of small and fine roots.

Fine root turnover occurred throughout the year, and with greater turnover in the wet season than in the dry season. On the other hand, on a monthly basis, turnover was similar among seasons. Root growth and turnover during the dry season are not so surprising as some species show leaf and shoot growth in response to increased light conditions in the dry season *versus* cloudy skies during the wet season (Barone 1998). This implies that the cue for root growth might reside in the aboveground biomass rather than in the soil. However, the addition of soil nutrients did influence fine root turnover in both seasons, pointing to a soil nutrient cue. Despite considerable research, we still have incomplete understanding of the extent that exogenous and endogenous factors control fine root growth in most forest ecosystems (cf. Tierney *et al.* 2003).

Results from the literature suggest that turnover of fine roots is greater for plants adapted to nutrient-rich rather than nutrient-poor soils (Eissenstat & Yanai 1997). The reason is that construction costs for new roots are less than maintenance costs when soil nutrient resources are plentiful. The theory also predicts that plants adapted to nutrient-rich soils are able to increase root turnover in response to increased nutrient availability, in part, because nutrient pulses are more common in nutrient-rich than in nutrient-poor soils (Espeleta & Donovan 2002). In our study, however, there was no relationship between turnover and fine root biomass measured in the same treatment or control plot (Pearson correlation = 0.07). Moreover, there were only weak relationships between fine root turnover and our assessments of nutrient (N, P, and K) availability for a given plot in a given year (Pearson correlation = –0.20 to 0.20).

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We know surprisingly little about the ways to describe soil fertility in natural forests, especially, in tropical forests. Large-scale experiments with factorial experimental design are needed (cf. Mirmanto *et al*. 1999, this study) because the results have widespread implications for ecosystem ecology. For instance, we know that soil fertility varies across the tropical climate zone, much like that in better-studied temperate and boreal forest. While biogeochemical and ecosystem research has focused mostly on roles for N and P limiting plant production, our results show that base cations (in particular K) can play an equally important functional role. Fine roots can account for 50% of the Net Primary Production (NPP) in mature forest ecosystems, and thus relationships and feedbacks between soil fertility and fine roots are crucial to understanding soil carbon cycling. In our study, fine root biomass was very sensitive to soil K fertility, but biomass alone does not mean less productivity belowground. Fine root production can increase even with less biomass, resulting in maintenance of carbon allocation belowground. Studying and quantifying fine root dynamics are tedious and time-consuming, but it is absolutely necessary in order to provide a better mechanistic understanding of controls on forest productivity.

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