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Mean annual temperature influences local fine root proliferation and arbuscular mycorrhizal colonization in a tropical wet forest

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Abstract

Mean annual temperature (MAT) is an influential climate factor affecting the bioavailability of growth-limiting nutrients nitrogen (N) and phosphorus (P). In tropical montane wet forests, warmer MAT drives higher N bioavailability, while patterns of P availability are inconsistent across MAT. Two important nutrient acquisition strategies, fine root proliferation into bulk soil and root association with arbuscular mycorrhizal fungi, are dependent on C availability to the plant via primary production. The case study presented here tests whether variation in bulk soil N bioavailability across a tropical montane wet forest elevation gradient (5.2°C MAT range) influences (a) morphology fine root proliferation into soil patches with elevated N, P, and N+P relative to background soil and (b) arbuscular mycorrhizal fungal (AMF) colonization of fine roots in patches. We created a fully factorial fertilized root ingrowth core design (N, P, N+P, unfertilized control) representing soil patches with elevated N and P bioavailability relative to background bulk soil. Our results show that percent AMF colonization of roots increased with MAT ($r^2 = .19$, $p = .004$), but did not respond to fertilization treatments. Fine root length (FRL), a proxy for root foraging, increased with MAT in N+P-fertilized patches only ($p = .02$), while other fine root morphological parameters did not respond to the gradient or fertilized patches. We conclude that in N-rich, fine root elongation into areas with elevated N and P declines while AMF abundance increases with MAT. These results indicate a tradeoff between P acquisition strategies occurring with changing N bioavailability, which may be influenced by higher C availability with warmer MAT.

KEYWORDS

arbuscular mycorrhizal fungi, fine roots, mean annual temperature, nitrogen availability, root proliferation, tropical montane wet forest

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1 | INTRODUCTION

Increasing air temperature resulting from greenhouse gas forcing is expected to affect terrestrial primary production (Luyssaert et al., 2007) and alter aboveground and belowground plant carbon (C) allocation (Litton & Giardina, 2008; Raich & Nadelhoffer, 1989). These temperature-driven changes in C fixation and allocation may induce feedbacks that alter the carbon dioxide (CO₂) balance of forest ecosystems (Vogel et al., 2008). Belowground C allocation comprises a significant and variable proportion of gross primary production (GPP) (Litton, Raich, & Ryan, 2007) supporting various belowground plant processes. The amount of C fixed during photosynthesis and the proportion of biomass C allocated to distinct belowground pools (e.g., fine roots, mycorrhizae) are ultimately important for the formation and decomposition rates of soil organic matter (SOM), in turn influence the rate of CO₂ efflux to the atmosphere (Kuzyakov & Schneckengerber, 2004; Schmidt et al., 2011).

The bioavailability of soil nutrients, in particular nitrogen (N) and phosphorus (P), has been tied to the allocation of C to root structures and symbioses (mycorrhizas, root nodules for N-fixation) that promote belowground nutrient acquisition (BassiriRad, 2000; Melillo et al., 2011; Pendall et al. 2004; Reich et al., 2014). Within the conceptual framework of nutrient acquisition strategy, plants adjust the proportion of net primary production (NPP) allocated to belowground components in order to overcome growth limitation (Bloom, Chapin, & Mooney, 1985; Treseder & Vitousek, 2001). As a result, we observe globally distributed patterns in nutrient acquisition strategies related to soil age and disturbance history (Lambers, Raven, Shaver, & Smith, 2008). While the relationship between lithology and soil nutrient acquisition is an important topic in ecosystems ecology (Walker & Syers, 1976), exactly how global climate changes, such as rising mean annual temperature (MAT), influence soil resource availability and plant nutrient acquisition under natural conditions remains poorly understood (Gill & Jackson, 2000).

Natural field observations (Ostertag, 2001), field fertilization experiments (Haynes & Gower 1995), and modeling exercises (Dybzinski, Farrior, Wolf, Reich, & Pacala, 2011) have all shown that increasing soil nutrient availability in forest ecosystems leads to reduced belowground C allocation and proportional increases in aboveground C allocation (Litton et al., 2007). Fertilization studies show that plants in nutrient-poor soils allocate a greater proportion of total plant C to fine roots, root exudates, and fungal symbionts, compared to those in nutrient-rich sites (Johnson et al. 2010; Treseder & Vitousek, 2001; Vicca et al., 2012). Phosphorus limits productivity in highly weathered soils typical of the global tropics and in volcanic soils where P availability is limited by biological and mineral occlusion (Olander & Vitousek, 2005), while N limits forest production in northern latitudes where younger, recently glaciated soils dominate (LeBauer & Treseder, 2008; Reich & Oleksyn, 2004). Fine root biomass (FRB) and fine root length (FRL) determine the root surface area available for soil exploration and nutrient uptake, and so provide indices of plant demand for nutrients (Berntson, Farnsworth, & Bazzaz, 1995; Powers et al., 2004). Fine root proliferation into

nutrient-enriched areas of bulk soil, described as “patches”, also depends on the ability of roots to elongate and proliferate in response to patchy distribution of soil resources (Adams, McCormack, & Eissenstat, 2013; Farley & Fitter, 1999).

Plant association with the major types of mycorrhizal fungi, variations in root/mycorrhizal morphology (Chen et al., 2016), and nutrient translocation within the root (Marschner & Dell, 1994) are also critical to the nutrition of terrestrial plants (Averill, Bhatnagar, Dietze, Pearse, & Kivlin, 2019). Arbuscular mycorrhizal fungi (AMF) support plant nutrient acquisition, particularly of P, via uptake by extraradical hyphae and translocation of nutrients in exchange for plant C within arbuscules and intracellular hyphal coils (Smith & Smith, 2011). Across tree species, nutrient foraging by AMF, inferred from extraradical hyphal length and biomass (mycelium) in nutrient patches, is less precise compared to foraging precision in ectomycorrhizal fungi, across AMF-associated tree species (Chen et al., 2016; Cheng et al., 2016), while the presence of intraradical AMF structures, an index of P translocation, is related to overall benefit to plant biomass across ecosystems (Treseder, 2013). Therefore, abundance of fungal structures in roots may indicate overall plant dependence on AMF more directly than extraradical AMF hyphae when not differentiating among AMF species (Chen et al., 2016; Hart & Reader, 2002). Plant colonization by AMF is more prevalent under conditions of nutrient limitation of plant growth, particularly in P-limited environments (Johnson, Wolf, & Koch, 2003). In soils with high N:P, root colonization by AMF, frequently quantified as percent root length colonized (Vierheilig et al., 2005), is increasingly advantageous and competition among mycorrhizal and nonmycorrhizal fungi for plant C favors AMF (Chagnon & Bradley, 2013; Johnson, Graham, & Smith, 1997; Johnson et al. 2010). These patterns illustrate the link between biogeochemical processes mediated by free-living soil microorganisms and the prevalence of the root/AMF symbiosis via soil N:P (Okiobe et al., 2019; Veresoglou et al., 2019). In addition to soil nutrient conditions, exchange of nutrients for C between AMF and plants is influenced by the availability of C in live biomass (Brzostek, Fisher, & Phillips, 2014; Peng et al., 1993). Temperature has been shown to directly limit the transfer of P from AMF to plants, potentially via effects on photosynthesis (Gavito et al. 2003; Hammer, Pallon, Wallander, & Olsson, 2011; Olsson et al., 2010). Therefore, temperature influences more than one of the ecological conditions which drive the extent of AMF colonization (Heinemeyer & Fitter, 2004), and stand-scale tests of the relationship between rising temperature, fine root proliferation, and AMF abundance are necessary to characterize the effects of climate warming on soil C and nutrient economies.

Natural elevation gradients provide an ideal setting to test the role of environmental variation in ecological processes in ways that more accurately represent real ecosystems than manipulation studies that often produce transient effects and experimental artifacts (Fukami & Wardle, 2005; Malhi et al., 2010; Sundqvist et al., 2013). Giardina, Litton, Crow, and Asner (2014) used a natural but highly constrained elevation gradient on the island of Hawaii to show that MAT is positively related to the total flux of autotrophic C

belowground, as well as soil CO₂ efflux (Litton, Giardina, Albano, Long, & Asner, 2011). Across the same tropical montane wet forest elevation gradient as in Giardina et al. (2014), we previously showed that soil N bioavailability increases with MAT, which suggests that increasing C fluxes and N bioavailability may be linked to warming MAT through soil microbial mechanisms (Pierre et al., 2017). Previous research has suggested that increases in N cycling and availability with warming could contribute to higher forest productivity with climate change (Cleveland et al., 2011; LeBauer & Treseder, 2008; Rustad et al., 2001).

In the present study, we leveraged the permanent plots located along a MAT/elevation gradient on Hawaii to investigate the effects of MAT and local soil nutrient bioavailability on fine root proliferation within nutrient-fertilized patches across a 5.2°C range of MAT (Litton et al., 2011). Ecological variables other than temperature (soil moisture, plant community composition, successional stage, soil type, lithology) are constant across temperature gradient (Giardina et al., 2014; Litton et al., 2011; Selmants et al., 2014). We determined how fine root proliferation into N- and P-fertilized patches (defined here as ~5.25 cm³ of soil) respond to increasing MAT and site fertility in situ. We hypothesized that the increasing magnitude of ecosystem C fluxes with MAT would drive an overall increase in fine root proliferation and root mycorrhizal colonization at the plot level, while fine root proliferation into N-fertilized patches would decline with declining native soil N bioavailability. Conversely, we hypothesized that fine root proliferation within P-fertilized patches would increase with MAT and soil N bioavailability, as increasing soil N bioavailability with warming would increase soil N:P and drive greater P demand (Hendricks, Nadelhoffer, & Aber, 1993). We also determined the abundance of AMF within ingrowth fine roots as % root length colonized, an indication of plant community P status (Treseder, 2013), and anticipated that all ingrowth roots across treatments would reflect a shift toward P acquisition via increased abundance of AMF structures with increasing MAT and soil N bioavailability. Higher root colonization by AMF with increasing MAT would indicate a

compensatory mechanism for plant P acquisition in response to increasing bioavailability of mineral N with warmer MAT (Pierre et al., 2017).

2 | METHODS

2.1 | Study site

This study was conducted along an 800 m elevation gradient on the northeastern slope of the Mauna Kea Volcano on the Island of Hawaii (Litton et al., 2011). Nine permanent research plots (20 × 20 m) located in native-dominant, mature tropical montane wet forest were included this study (Table 1). The seven lower-elevation permanent plots are located in the Hawai'i Experimental Tropical Forest (HETF; 19°56'41.3"N, 155°15'44.2"W; 600–1,800 m.a.s.l.) and the two higher-elevation plots in the adjacent Hakalau Forest National Wildlife Refuge (HFNR; 19°50'31.3"N, 155°17'35.2"W; 600–2,000 m.a.s.l.). All plots are characterized as *Metrosideros polymorpha* Gaudich. – *Acacia koa* A. Gray forests. *M. polymorpha* and *Cheirodendron trigynum* (Gaudich.) A. Heller dominate the canopy and midstory, respectively, across all plots (84%–97% of basal area excluding tree ferns). Additionally, three species of tree ferns (*Cibotium* spp.; midstory) make up an average 46% of stand basal area in these plots (Litton et al., 2011). Arbuscular mycorrhizal fungi associate with >90% of plant species endemic to the Hawaiian islands and are therefore the focal mycorrhizal type in this study (Koske, Gemma, & Flynn, 1992).

Plots along the elevation gradient vary in MAT from 13°C at the highest elevation plot (1,600 m.a.s.l.) to 18.2°C at the lowest elevation plot (800 m.a.s.l.), thus forming a 5.2°C MAT gradient (Table 1). Soil water balance is relatively constant across all plots due to a concomitant decline in mean annual precipitation with increasing elevation (Litton et al., 2011; Selmants et al., 2014). Substrate in all plots is derived from ~20 ky (14–65 ky) weathered tephra, and soils are

TABLE 1 Climate and stand data for 9 permanent plots located in a tropical wet montane forest on Mauna Kea, Hawaii, USA

Elevation (m.a.s.l.)	Plot name	MAT (°C) ^b	MAP (mm) ^a	Soil temperature (°C) ^b	Soil VWC (%) ^b	Total stand BA (m ² /ha) ^b	Stand density (individuals/ha) ^b
800	SPE800	18.2	4,204	18.15	53.81	116	4,225
934	SPE934	17.3	4,133	16.98	55.84	100	3,300
1,024	SPE1024	16.7	4,043	16.33	54.19	97	3,750
1,116	SPE1116	16.1	3,988	15.80	51.19	155	4,275
1,116	WPL1116	16.1	3,714	15.20	33.94	109	5,875
1,204	WPL1204	15.5	3,521	15.59	30.66	102	3,900
1,274	WPL1274	15.1	3,448	15.01	32.06	81	4,375
1,468	HKL1468	13.8	3,488	14.09	50.66	54	13,200
1,600	HKL1600	13.00	3,195	13.18	53.59	66	16,400

Note: Plots comprise a 5.2°C mean annual temperature (MAT) gradient.

^aMean annual precipitation (MAP) data from Giambelluca et al. (1986).

^bData from Litton et al. (2011).

moderate to well-drained hydrous, ferrihydritic/amorphous, isothermic/isomesic Acrudoxic Hydrudands of the Akaka, Honokaa, Maile, and Pihihouna soil series (Soil Survey Staff 2010). Mean soil pH is 3.9 and base saturation and estimated mean cation exchange capacity are 32.4% and 11.9 cmol/kg, respectively (Litton et al., 2011). Mean soil (0–10 cm) bulk density and carbon content across the MAT gradient are 0.21 g/cm³ and 14%, respectively. Soil nitrate (NO₃⁻) bioavailability in these plots, measured using ion exchange resins (Western Ag, Saskatoon, SK, Canada), increased linearly with MAT ($r^2 = .79$, $p = .003$; Pierre et al., 2017).

2.2 | Fertilized root ingrowth core construction and placement

Cores were constructed of polyvinyl mesh netting with 1 mm × 1.5 mm holes (open area = 50%), 10 cm in height and 7.5 cm in diameter, and sewn closed using nylon thread. Each core was filled with ~25 g (~5.25 cm³) of a mixture of a calcined clay pellets (Turface®) and vermiculite matching the average bulk density of 0.21 g/cm³ of native soils across the gradient. This mixture was selected for the absence of background N and P in calcined clay, and the modulation of bulk density by the vermiculite fraction (Raich et al., 1994). For fertilization, we approximated the fertilization rate for N and P (40 g/m²) achieved by previous root ingrowth core studies in very similar tropical montane wet forests on the island of Hawaii (Raich et al., 1994). Cores received a one-time dose of 100 ml of deionized water (control), 6.016 g/L urea (CH₄N₂O) (N treatment), 21.69 g/L Na₃PO₄·H₂O (P treatment), or a combined solution of both solutes at the same concentrations (N+P treatment) Riley and Vitousek 1995. Each treatment was applied with a needle and syringe by evenly injecting and releasing small aliquots totaling 100 ml throughout the media (Raich et al., 1994). Cores were covered and air-dried at laboratory temperature overnight, and then weighed and stored in plastic bags at room temperature until burial. Based on previous studies, fertilization treatments were assumed to be largely retained by the calcined clay media within the cores across the study period (Raich et al., 1994). Because a standard amount of each fertilization treatment was added to cores, the contrast between naturally available soil N and N added in ingrowth cores varied across the gradient with the natural change in bulk N (Table 2), but differences in N contrast among plots were assumed to be negligible compared to the difference in N availability between the ingrowth core and the background soil.

Between August 25–29, 2015, replicates of each treatment (Control, +N, +P, N+P) were buried to 10 cm depth in each of the nine plots in clusters spaced ~30 cm apart. Five replicate clusters of fertilized cores were buried per plot (total of 5 treatment replicates within 9 permanent plots; $n = 180$). Replicate clusters were placed at the corners of each 20 × 20 m plot, with the fifth cluster placed equidistant between two of the corners on the perimeter of the plot. All cores were collected between November 1–11, 2015, for an average burial time of 75 days. Three cores were lost in the field, for a total of $n = 177$ cores returned to the laboratory.

TABLE 2 Soil characteristics for 9 permanent plots located in tropical montane wet forest on Mauna Kea, Hawaii, USA

Plot elevation (m.a.s.l.)	Soil series ^a	Soil pH ^b	Bulk density (g/ cm ³)	Soil C stock (0–10 cm; g C m ⁻²) ^c	Soil N stock (0–10 cm; g N m ⁻²) ^d	Soil bioavailable NO ₃ ⁻ (mg-N m ⁻² d ⁻¹) ^d	Soil bioavailable NH ₄ ⁺ (mg-N m ⁻² d ⁻¹) ^d
800	Akaka	4.1	0.21	4,066.7	162.6	2.159	0.1073
934	Akaka	4.2	0.19	4,400.1	291.2	0.816	0.1277
1,024	Akaka	3.7	0.19	2,562.4	152.5	1.234	0.07711
1,116	Akaka	3.8	0.20	2,662.8	157.3	1.389	0.5905
1,116	Honokaa	3.6	0.26	-	-	-	-
1,204	Honokaa	3.7	0.23	4,139.4	192.1	0.6003	0.1986
1,274	Maile	3.9	0.22	3,674.2	29.0	0.6766	0.7173
1,468	Akaka	4.2	0.18	2,958.3	151.6	0.01587	0.06333
1,600	Piihouna	4.1	0.23	5,666.0	311.9	0.05214	0.5883

Note: Plots comprise a 5.2°C mean annual temperature (MAT) gradient.

^aSoils in all plots are classified as hydrous, ferrihydritic/amorphous, isothermic/isomesic Acrudoxic Hydrudands (Litton et al., 2011).

^bQuantified on fresh soils ($n = 10$ soil cores plot⁻¹ to 10 cm depth) (Litton et al., 2011).

^cFrom Litton et al. (2011).

^dFrom Pierre et al. (2017).

Cores were stored in plastic zip-lock bags and placed in insulated containers for immediate transport to the laboratory, where they were stored at $\sim 1.5^{\circ}\text{C}$ until root sampling approximately 10 days later. Fine roots ($< 2\text{ mm}$) were removed from the media using a fine ($< 1\text{ mm}$) sieve and forceps. After rinsing with deionized water, the fresh fine root sample was weighed for fine root biomass. The average length of a single intact (*i.e.*, not damaged or fragmented) root found inside an ingrowth core was $\sim 5 \pm 1\text{ cm}$, and this length was chosen as the standard subsample length to allow for comparison of AMF colonization between plots. A subsample of $\sim 5 \pm 1\text{ cm}$ of root length was taken from the total fresh root sample in each ingrowth core to store in 50% ethanol until clearing and staining for mycorrhizal colonization (Grace & Stribley, 1991). The remaining fine roots in each sample were weighed, dried at 60°C for 72 hr, and reweighed to calculate moisture content (Figure S1). The subsamples used for quantifying mycorrhizal colonization were similarly weighed and dried following visual quantification $200\times$ magnification under microscope. The total dried root sample was then scanned using a digital scanner, and FRL was measured using the WinRHIZO software (Régent Inc.), and specific root length ($\text{SRL} = \text{FRB}/\text{FRL}$; g/cm) was determined from these measurements Ostonen et al. 2007. Drying roots prior to scanning is a methodological limitation of this study, as drying may have affected root length and because fine roots of plant species within plots may have different drying effects. For our analyses, we assume equivalent laboratory drying effects on fine roots of species within and between plots similarly.

2.3 | Mycorrhizal colonization

The extent of root colonization by AMF was estimated using the percent root length colonization method (Biermann & Linderman, 1981; McGonigle et al., 1990; Toth et al., 1991). This is the standard method of quantifying AMF abundance within plants (Vierheilig et al., 2005) through standardized observations of the proportion of root length containing intraradical AMF structures (arbuscules, intracellular hyphae, and vesicles) (Biermann & Linderman, 1981; Treseder, 2013). From the total ingrowth cores collected from the field ($n = 177$) collected from the field, a subset contained $\geq 5\text{ cm}$ total fine root length ($n = 108$) and a further subset ($n = 37$) contained $\geq 5\text{ cm}$ of fine roots that could be chemically cleared in a reasonable amount of time ($< 24\text{ hr}$) for quantifying intraradical AMF structures. Fine root subsamples for mycorrhizal colonization were cleared and stained with vinegar and ink following the methods of Brundrett and Abbott (1994), reviewed by Vierheilig et al. (2005). Briefly, roots were cleared in 10% KOH solution and autoclaved on a liquid cycle in 10-min intervals until roots were cleared of all pigment (maximum 24 hr), with KOH solution changed between each autoclave cycle. Roots were then stained in 0.05% Parker Quink black ink (Parking Pen Products) in a 1:1 solution of glycerol and vinegar for $\sim 24\text{ hr}$. Roots were cut with scissors into 2 cm segments and mounted on glass slides parallel to one another. Roots were observed at $200\times$ magnification, with two lateral visual scans from one end of the

microscope slide to the other across the upper and lower halves of the slide. Five equally spaced lines were marked on the slide demarking points for observation, which produced a total of 10 observation points per slide. When a point was met in a visual scan, an observation was made for the presence or absence of fungal structures (arbuscules and intercellular hyphae). Total root mycorrhizal colonization was then quantified as the percentage of observation points where mycorrhizal structures were present. Observations were made by three different individuals to minimize observer bias, and percent colonization is an average of three values per sample. A total of 37 root samples were scored, the remainder being too small to make these measurements.

2.4 | Statistical analysis

Fine root length, FRB, SRL, and AMF abundance were determined to be non-normally distributed by a Shapiro–Wilk test and were log transformed to meet assumptions of normality for subsequent statistical tests. The responses of these root variables to MAT and natural soil NO_3^- bioavailability were then explored by testing linear models of for additive and interactive effects, and significant responses were determined using *t* tests. Third, linear mixed effects (LME) regression models were used to determine the significance of individual and interacting fixed (*i.e.*, observed or manipulated) variables while accounting for random (*i.e.*, uncontrolled or unobserved) effects in this study. Fixed effects in the LME model were MAT (Table 1), the natural soil NO_3^- bioavailability (Table 2), and fertilization treatment. Random effects included in the LME were the plot and ingrowth core clusters within plots. Reduced and full (*i.e.*, including observed and treatment variables) LME models were compared by Akaike's information criterion (AIC). Estimated marginal means (EM means) (*i.e.*, frequency-adjusted), rather than arithmetic means, were then determined from the selected LME model, to adjust for unbalanced quantities of root ingrowth cores collected from plots, as some ingrowth cores were lost in the field (Harrison et al., 2018; Searle et al., 1980). Estimated marginal means were also calculated in order to determine the significance of the three-way interaction of MAT, soil NO_3^- bioavailability, and nutrient patch availability. Post hoc contrasts of fine root responses to fertilization treatments at every observed value of MAT and soil NO_3^- bioavailability were conducted by ANOVA using Tukey's method and a 95% confidence interval. The significant interaction effects on the EM means derived from the selected LME model were visualized as interaction plots using the *emmeans* function in the *emmeans* package (R Core Team, 2016). The LME model estimates visualized in the interaction plot were simplified by specifying only three observed levels of NO_3^- bioavailability (Low = 0.25 mg N m^{-2} , Mid = 0.75 mg N m^{-2} , High = 1.25 mg N m^{-2}) in the LME model, rather than plotting all 9 observed values of soil NO_3^- bioavailability (see Table 1). Finally, percent root length colonized by AMF was measured for 37 ingrowth cores, which had unbalance sample sizes among treatments and plots. Response of AMF root colonization

to MAT, NO_3^- bioavailability, and fertilization treatment was determined through ANOVA and a post hoc Tukey's honest significant difference test to account for unequal samples between plots and treatments in the AMF colonization subsample. All statistical analyses were performed in R (R Core Team, 2016).

3 | RESULTS

Data and statistical models show MAT ($p = .041$), natural soil NO_3^- bioavailability ($p = .019$), (from here on referred to as "bioavailable NO_3^- "), and their interaction ($p = .017$) positively correlate with FRL in ingrowth cores (Table 2, Model 2). Inclusion of fertilization treatments in the linear mixed effects model showed a negative trend for FRL response to fertilized ingrowth cores with increasing MAT and soil bioavailable NO_3^- (Table 3, Model 1). This decline in FRL across MAT was only significant for N+P fertilized cores ($p = .02$; Figure 1). Fine root biomass and SRL showed no response to the MAT gradient, NO_3^- bioavailability, or fertilization. Fine root moisture was invariant across MAT (Figure S1). These results suggest the direction of the FRL response to MAT and NO_3^- bioavailability is sensitive to the combination of elevated N and P in patches. Figure 1 visualizes the FRL response to N+P fertilization as an estimated marginal means interaction plot for Model 1 (Table 3), showing that the interaction between MAT and NO_3^- bioavailability significantly influences the slope of the FRL response to the N+P treatment.

Arbuscules and intercellular hyphae were observed in fine roots collected from fertilized root ingrowth cores. Arbuscular mycorrhizal colonization of fine roots increased linearly with MAT ($p = .0022$, Figure 2), with a mean increase of 0.074% (SE $\pm$ 0.022) per 1°C increase in MAT. Fine root AMF colonization did not respond to NO_3^- bioavailability ($p = .25$). No fertilization treatments influenced the percent mycorrhizal colonization observed in fine root ingrowth, and no significant difference was shown for between fertilization treatments ($p > .05$).

4 | DISCUSSION

4.1 | Fine root responses to nutrient availability and MAT

Fine root proliferation into fertilized patches responded positively to warming (MAT) and the concomitant increases soil NO_3^- bioavailability along this tropical montane wet forest MAT gradient. As these environmental drivers of fine root proliferation (discussed here in terms of FRL) increase, they also show a statistical interaction that specifically reduces fine root proliferation into soil patches (ingrowth cores) with elevated N and P concentrations. These divergent results suggest two distinct influences of MAT and NO_3^- bioavailability on fine root proliferation into nutrient-enriched patches. First, increasing temperature and resultant increases in bulk NO_3^- bioavailability (Pierre et al., 2017) appear to drive increasing total belowground C flux across this MAT gradient (Giardina et al., 2014), which appears to be related to increasing FRL within ingrowth cores observed in this study (Table 3, Model 2). Second, the negative relationship among FRL within N+P-fertilized cores, MAT, and NO_3^- bioavailability suggests that the length of fine root proliferation into patches with elevated N+P is largely temperature-dependent and bulk soil N availability ecosystem scale (Figure 1). Additionally, fine root proliferation into soil patches (*i.e.*, at the scale of the ingrowth core) with elevated N and P was significant across the gradient compared to other treatments, suggesting that at the plot scale, vegetation prioritizes localized acquisition of both N and P, but that this requirement significantly declines based on the environmental conditions of MAT on bulk soil NO_3^- bioavailability. The two primary findings of this study highlight the important differences between ecosystem scale (plot) and local-scale (root ingrowth core) drivers of root morphological response to temperature and nutrient conditions, and the potential dual influence of MAT on root growth via ecosystem C fixation and via modulation of soil N bioavailability and cycling (Giardina et al., 2014; Pierre et al., 2017).

	Model 1	Model 2	Model 3
MAT	0.057 (0.359)	0.558** (0.264)	-0.039 (0.192)
Bioavailable nitrate	10.425 (13.270)	23.619** (9.756)	
+N	-6.443 (5.729)		
+N+P	-13.663*** (5.838)		
+P	-10.794* (5.716)		
MAT:Bioavailable nitrate	-0.706 (0.856)	-1.567** (0.630)	
AIC	735.631	732.344	739.474

Note: Random effects included in the models (not shown) include within-plot ingrowth core placement and plot effect not accounted for by experimental design. Measurements were made in 9 permanent plots located in tropical montane wet forest on Mauna Kea, Hawaii, USA. Plots comprise a 5.2°C mean annual temperature (MAT) gradient.

*** $p < .01$.

** $p < .05$.

* $p < .1$.

TABLE 3 Three linear mixed effects models comparing inclusion of observed variables only (natural soil nitrate (NO_3^-) bioavailability, mean annual temperature (MAT); Model 2, Model 3) and including both observed and manipulated variables (phosphorus (+P) and nitrogen (+N), and combined nitrogen (N) and P (N+P) root ingrowth core fertilization; Model 1) for estimating fine root ingrowth length (cm)

The design of this study featured conditions that should be considered in the interpretation of the root proliferation response to MAT, N bioavailability, and fertilized ingrowth cores. The one-time fertilization treatment necessitated a shorter ingrowth duration (75 days), and therefore captured short-term fine root responses to fertilized patches, and therefore cannot be directly compared to studies with longer ingrowth periods. The N fertilization treatment was added in the form of a urea solution and is assumed to have been converted to ammonium (NH_4^+) after burial by soil microorganisms (Burton & Prosser, 2001). This may have created a contrast between the N species inside ingrowth cores and other mineral and organic N species occurring naturally in surrounding soils, which could have implications for root proliferation if plant N foraging shows discrimination among forms of N.

Co-limitation by N and P has been observed across many terrestrial and aquatic ecosystems, where a simultaneous addition of both nutrients increases primary production above enrichments of either nutrient alone (Elser et al., 2007; Vitousek & Farrington, 1997). Observed increases in primary production in response to N+P additions reflect the stoichiometry of photosynthesis and plant growth, but these observations do not shed light on the dynamics of internal plant C partitioning for N and P acquisition. Roots respond dynamically to heterogeneous supplies of potentially growth-limiting resources through internal signaling pathways induced by internal and external cues (Forde & Lorenzo, 2001; Hutchings & de Kroon, 1994). While diverse root responses to nutrient-rich patches under different background nutrient conditions have been shown in model plant systems (Drew, Saker, & Ashley, 1973; Zhang & Forde, 1998), a limited number of studies have evaluated their ecological causes or consequences (Callaway, Pennings, & Richards, 2003; Chen et al., 2016; Cheng et al., 2016). Primary root initiation and elongation are linked to the immediate presence of these nutrients through root elongation gene expression (Zhang & Forde, 1998) and hormonal pathways (Drew et al., 1973; Le Deunff, Lecourt, & Malagoli, 2016). Root proliferation responses to external cues are species-specific and seasonally dependent (Eissenstat & Caldwell, 1988; Kembel & Cahill, 2005). In the present study, these sources of variation were minimized by uniform vegetation composition, parent material, oil moisture availability across the MAT gradient and by the short-term nature of this study. The dependence of root proliferation into N+P-enriched patches upon both N bioavailability and MAT suggests complex interactions with environment and plant nutritional status, independent of variation in vegetation composition, soil characteristics, and water availability. Moreover, our observations illustrate the interplay between plant nutrition, fine root behavior, and environmental conditions at the stand scale (de Kroon, Visser, Huber, Mommer, & Hutchings, 2009). Because roots within ingrowth cores were not separated by species, results may not proportionately reflect the root proliferation response to N+P fertilization of all dominant plant species in the plot. If one plot-dominant species exerts a disproportionate influence on fine root proliferation within plots, the results of this study can still be interpreted to reflect stand-scale processes in response to MAT and N bioavailability, which may have

implications for forest nutrient acquisition and associated C costs of these functions.

Proximate and ultimate limiting conditions (sensu Vitousek et al., 2010) are important to distinguish in order to characterize ecosystem responses to abiotic change. Vitousek et al. (2010) define proximate and ultimate limiting nutrients as those which influence individual biological processes and structure ecosystems, respectively. While this definition serves to contrast short versus long-term responses to additions of different macronutrients, it should also invoke the role of abiotic conditions in determining the availability of nutrients leading to limitation (Raich et al., 1997; Vitousek & Farrington, 1997; Vitousek et al., 2010). By using an environmental gradient where environmental factors other than MAT were constrained, we were able to show the relationship between the bioavailability of an ultimate limiting nutrient, N, and an ultimate limiting condition, MAT (Pierre et al., 2017). Our results suggest that increasing N availability due to rising MAT could have consequences for fine root proliferation in soils. Decreasing fine root proliferation in N+P-fertilized ingrowth cores with increasing MAT may indicate how tropical montane wet forests prioritize nutrient acquisition as N becomes less limiting. Unless at least one environmental condition, such as MAT, is strongly limiting, plot fertilization studies may confound the roles of first-order variables such as temperature,

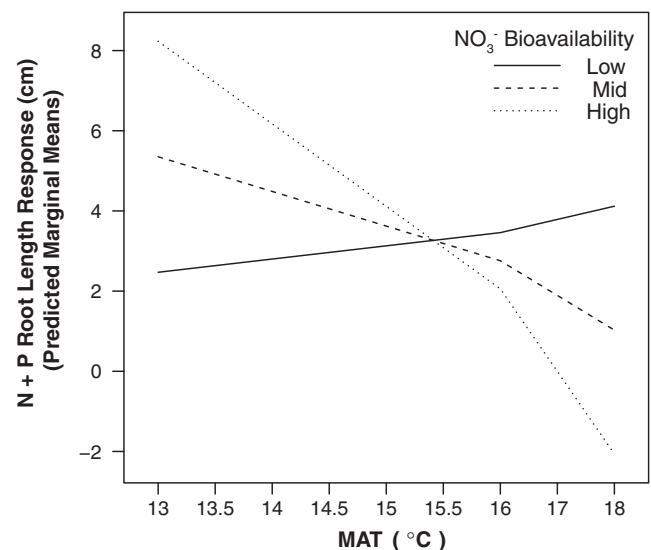


FIGURE 1 A plot of estimated marginal mean length (cm; log scale) of fine root ingrowth within nitrogen and phosphorus (N+P)-fertilized root ingrowth cores across a MAT gradient (x axis) at three observed levels of NO_3^- bioavailability (lines; Low = 0.25 mg N m^{-2} , Mid = 0.75 mg N m^{-2} , High = 1.25 mg N m^{-2}). Measurements were made in 9 permanent plots located in tropical montane wet forest on Mauna Kea, Hawaii, USA. Plots comprise a 5.2°C mean annual temperature (MAT) gradient. The y-axis shows the difference in FRL from a null response, for which FRL response equals zero, predicted by a linear mixed effects regression model. The root length response to the N+P treatment was significantly different from other treatments ($p = .023$) and the root length response to N+P at High, Mid, and Low NO_3^- bioavailability significantly responded to MAT ($p = .02$)

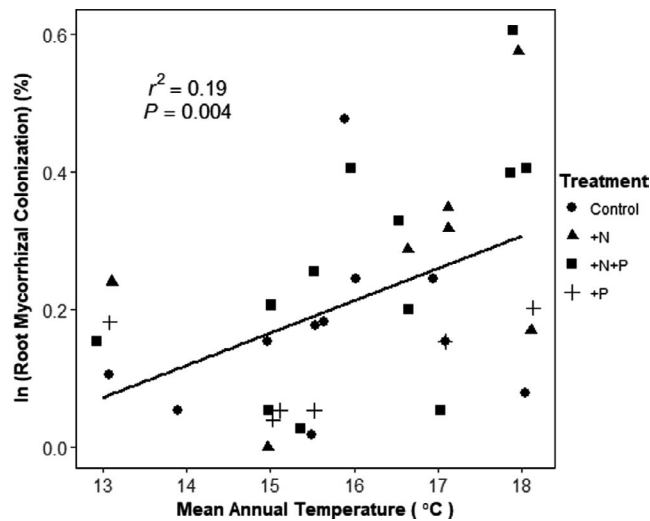


FIGURE 2 Arbuscular mycorrhizal fungal (AMF) colonization (% of root length colonized, log scale) of fine roots in fertilized root ingrowth cores measured in 9 permanent plots located in tropical montane wet forest on Mauna Kea, Hawaii, USA. Plots comprise a 5.2°C mean annual temperature (MAT) gradient. AMF colonization increases linearly with mean annual temperature (MAT) ($r^2 = .43$; $p = .0039$). Each point represents % mycorrhizal colonization of roots subsampled from a core. Mycorrhizal colonization did not differ among fertilization treatments

pH, substrate age, and precipitation, which influence nutrient limitation of forest growth (Lambers et al., 2008; Reed et al., 2011; Vitousek, 1984).

While prior research has shown that temperature influences fine root growth and turnover (Gill & Jackson, 2000; Norby & Jackson, 2000; Pregitzer et al., 2000), and that NO_3^- bioavailability drives fine root production and turnover (Pregitzer et al., 1993; Robinson et al., 1999), few studies have described their interactive influences (BassiriRad, Caldwell, & Bilbrough, 1993; Leppälammikujansuu, Salemaa, Kleja, Linder, & Helmsaari, 2014; Vogt et al., 1995). In our study, MAT and N bioavailability both decrease with increasing elevation, complicating the interpretation of root foraging responses to nutrient-enriched patches. Moreover, C availability for fine root foraging in enriched patches would be expected to increase with temperature due to concomitant increases in C cycling rates (Giardina et al., 2014; Litton et al., 2011). However, the results of our statistical model indicated that fine root length in N+P-enriched patches increased with increasing MAT given low bulk soil N availability, while the converse was true given high bulk soil N availability ($p = .023$, Figure 1). One interpretation of this unexpected result is that root response to enriched patches is co-dependent on C availability and background soil fertility (Robinson, 2001); in warmer, more productive climates, trees may be better able to widely explore for limiting nutrients in response to overall soil nutrient conditions (Lynch & Ho, 2005). If this interpretation is correct, it would imply that with increasing temperature and constant moisture availability, trees may be better able to overcome nutrient limitation with concomitant increases in root proliferation for nutrient acquisition as a result of overall increased productivity.

4.2 | Mycorrhizal colonization across MAT

Through symbioses with mycorrhizal fungi, plants can compensate for soil nutrient limitation (Johnson, 2010; Rillig, 2004; Treseder, 2004) at the cost of fixed C to support the association (Hodge, 2010; Lynch & Ho, 2005). The degree of symbiosis with arbuscular mycorrhizal fungi (AMF) is a straightforward proxy for the degree of plant P limitation, though it can also reflect plant productivity (Fellbaum et al., 2012; Hawkes, Hartley, Ineson, & Fitter, 2008). We observed increasing root colonization by AMF with increasing MAT (Figure 2), which may reflect an increase in P limitation at higher N bioavailability at warmer sites (Pierre et al., 2017). Arbuscular mycorrhizae generally enhance P acquisition (Lambers et al., 2008; Rillig, 2004) and provide an advantage to plants in high- NO_3^- environments where P demand is typically higher (Bradley, Drijber, & Knops, 2006; Egerton-Warburton & Allen, 2000). Increasing AMF colonization with MAT suggests that MAT could impact the ecological stoichiometry of this tropical montane wet forest by proportionally changing N bioavailability relative to P bioavailability. While we did not measure soil bioavailable P, we can infer from increasing percent AMF colonization that C allocation to mycorrhizae is increasingly prioritized at higher MAT, as plants can invest between 4% and 20% of total C budget in the AMF mutualism (Eissenstat, Graham, Syvertsen, & Drouillard, 1993; Peng et al., 1993; Watkins et al., 1996).

Colonization by AMF did not respond to any of the fertilized ingrowth core treatments, suggesting that the AMF mutualism across the gradient is driven by an interaction between productivity and bulk soil fertility, rather than the availability of nutrient-enriched soil patches (Treseder & Allen, 2002). Plants may be able to supply more carbohydrates to support their AM symbionts at higher MAT, diverting C that would otherwise go to the root apoplast (Fitter, 2006). Our results show that AMF symbioses are more responsive to MAT than to nutrient-rich patches, which is in contrast with our observations for fine root proliferation. Taken together, the differing patterns of fine root patch foraging and fine root AMF colonization along the MAT gradient suggest that fine roots forage in nutrient-rich patches under N-limited conditions at a lower C cost to the plant, while AMF are more abundant and forage more broadly under N-rich conditions at a greater C cost to plants (Johnson et al., 1997). Increasing primary production with MAT, coupled with increasing soil N bioavailability in moist forests, may favor increased AMF colonization to maintain N and P co-limitation.

4.3 | Implications for ecosystem biogeochemistry

Across these tropical montane wet forest plots, a significant root proliferation response to N plus P-enriched patches suggests that fine root foraging is most strongly influenced by the availability of both N and P. Within a stand, roots appear to be directed and elongated into patches of available N and P when background nutrient availability is low under cooler climatic conditions. The effect of background N fertility of a site appears to be temperature-dependent in

the absence of other ecosystem variation, suggesting that localized fine root growth depends on the interaction between temperature and N bioavailability. Our results suggest that increasing AM fungal colonization with MAT may be related to previously observed increasing primary production with MAT across this gradient (Giardina et al., 2014). Increasing primary production with warming may interact with temperature-driven N availability (Pierre et al., 2017) to affect the relative C cost of mycorrhizal associations (Treseder & Allen, 2002). We hypothesize that more productive lowland tropical plant communities under warmer climatic conditions increase AMF colonization for nutrient acquisition. Further study of the dynamics of total fine root biomass and turnover across gradients of MAT paired with whole stand fertilization experiments and N availability analyses will help to determine how influential these conditions are to fine root contributions to soil C. These results add to the growing body of research demonstrating the interconnections among increasing MAT, soil nutrient availability, and plant C allocation strategies (Johnson et al. 2010; Ostonen et al., 2011; Reich et al., 2014).

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CONFLICT OF INTEREST

We declare no competing personal, financial, or institutional competing interests.

AUTHOR CONTRIBUTIONS

Suzanne Pierre: Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); resources (lead);

software (lead); supervision (lead); validation (lead); visualization (lead); writing – original draft (lead); writing – review & editing (lead). **Creighton M. Litton:** Conceptualization (supporting); methodology (supporting); resources (supporting); supervision (supporting); writing – review & editing (supporting). **Christian Giardina:** Conceptualization (supporting); resources (supporting); writing – review & editing (supporting). **Jed P. Sparks:** Conceptualization (supporting); methodology (supporting); resources (supporting). **Timothy J. Fahey:** Conceptualization (supporting); formal analysis (supporting); methodology (supporting); supervision (lead); validation (supporting); writing – original draft (supporting); writing – review & editing (supporting).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available Dryad at <https://doi.org/10.6078/D14X4B>.

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REFERENCES

- Adams, T. S., McCormack, M. L., & Eissenstat, D. M. (2013). Foraging strategies in trees of different root morphology: The role of root lifespan. *Tree Physiology*, 33(9), 940–948. <https://doi.org/10.1093/treephys/tpt067>
- Averill, C., Bhatnagar, J. M., Dietze, M. C., Pearse, W. D., & Kivlin, S. N. (2019). Global imprint of mycorrhizal fungi on whole-plant nutrient economics. *Proceedings of the National Academy of Sciences of the United States of America*, 116, 23163–23168.
- BassiriRad, H. (2000). Kinetics of nutrient uptake by roots: Responses to global change. *New Phytologist*, 147, 155–169. <https://doi.org/10.1046/j.1469-8137.2000.00682.x>
- BassiriRad, H., Caldwell, M. M., & Bilbrough, C. (1993). Effects of soil temperature and nitrogen status on kinetics of $^{15}\text{NO}_3^-$ uptake by roots of field-grown *Agropyron desertorum* (Fisch. ex Link) Schult. *New Phytologist*, 123, 485–489.
- Berntson, G. M., Farnsworth, E. J., & Bazzaz, F. A. (1995). Allocation, within and between organs, and the dynamics of root length changes in two birch species. *Oecologia*, 101, 439–447. <https://doi.org/10.1007/BF00329422>
- Biermann, B., & Linderman, R. G. (1981). Quantifying vesicular-arbuscular mycorrhizae: A proposed method towards. Source. *The New Phytologist*, 87, 63–67.
- Bloom, A., Chapin, F., & Mooney, H. (1985). Resource limitation in plants—An economic analogy. *Annual Review of Ecology and Systematics*, 16(1), 363–392. <https://doi.org/10.1146/annurev.es.16.110185.002051>
- Bradley, K., Drijber, R. A., & Knops, J. (2006). Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. *Soil Biology & Biochemistry*, 38, 1583–1595.
- Brundrett, M. C., & Abbott, L. K. (1994). Mycorrhizal fungus propagules in the Jarrah forest. I. Seasonal study of inoculum levels. *New Phytologist*, 127, 539–546.
- Brzostek, E. R., Fisher, J. B., & Phillips, R. P. (2014). Modeling the carbon cost of plant nitrogen acquisition: Mycorrhizal trade-offs and multipath resistance uptake improve predictions of retranslocation. *Journal of Geophysical Research-Biogeosciences*, 119, 1684–1697.

- Burton, S. A. Q., & Prosser, J. I. (2001). Autotrophic ammonia oxidation at low pH through urea hydrolysis. *Applied and Environment Microbiology*, 67, 2952–2957.
- Callaway, R. M., Pennings, S. C., & Richards, C. L. (2003). Phenotypic plasticity and interactions among plants. *Ecology*, 84, 1115–1128.
- Chagnon, P. L., & Bradley, R. L. (2013). Evidence that soil nutrient stoichiometry controls the competitive abilities of arbuscular mycorrhizal vs. root-borne non-mycorrhizal fungi. *Fungal Ecology*, 6, 557–560.
- Chen, W., Koide, R. T., Adams, T. S., DeForest, J. L., Cheng, L., & Eissenstat, D. M. (2016). Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 8741–8746.
- Cheng, L., Chen, W., Adams, T. S., Wei, X., Li, L., McCormack, M. L., ... Eissenstat, D. M. (2016). Mycorrhizal fungi and roots are complementary in foraging within nutrient patches. *Ecology*, 97, 2815–2823.
- Cleveland, C. C., Townsend, A. R., Taylor, P., Alvarez-Clares, S., Bustamante, M. M. C., Chuyong, G., ... Wieder, W. R. (2011). Relationships among net primary productivity, nutrients and climate in tropical rain forest: A pan-tropical analysis. *Ecology Letters*, 14, 939–947.
- de Kroon, H., Visser, E. J. W., Huber, H., Mommer, L., & Hutchings, M. J. (2009). A modular concept of plant foraging behaviour: The interplay between local responses and systemic control. *Plant, Cell and Environment*, 32, 704–712.
- Drew, M. C., Saker, L. R., & Ashley, T. W. (1973). Nutrient supply and the growth of the seminal root system in barley. *Journal of Experimental Botany*, 24, 1189–1202.
- Dybzinski, R., Farrior, C., Wolf, A., Reich, P. B., & Pacala, S. W. (2011). Evolutionarily stable strategy carbon allocation to foliage, wood, and fine roots in trees competing for light and nitrogen: An analytically tractable, individual-based model and quantitative comparisons to data. *American Naturalist*, 177, 153–166.
- Egerton-Warburton, L. M., & Allen, E. B. (2000). Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications*, 10, 484–496.
- Eissenstat, D. M., & Caldwell, M. M. (1988). Seasonal timing of root growth in favorable microsites. *Ecology*, 69, 870–873. <https://doi.org/10.2307/1941037>
- Eissenstat, D., Graham, J. H., Syvertsen, J. P., & Drouillard, D. L. (1993). Carbon economy of sour orange in relation to mycorrhizal colonization and phosphorus status. *Annals of Botany*, 71, 1–10.
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., ... Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10, 1135–1142.
- Farley, R. A., & Fitter, A. H. (1999). The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches. *Journal of Ecology*, 87, 849–859.
- Fellbaum, C. R., Gachomo, E. W., Beesetty, Y., Choudhary, S., Strahan, G. D., Pfeiffer, P. E., ... Bücking, H. (2012). Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 2666–2671.
- Fitter, A. H. (2006). What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist*, 172, 3–6. <https://doi.org/10.1111/j.1469-8137.2006.01861.x>
- Forde, B., & Lorenzo, H. (2001). The nutritional control of root development. *Plant and Soil*, 232, 51–68.
- Fukami, T., & Wardle, D. A. (2005). Long-term ecological dynamics: Reciprocal insights from natural and anthropogenic gradients. *Proceedings. Biological Sciences*, 272, 2105–2115.
- Gavito, M. E., & Olsson, P. A. (2003). Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. *FEMS Microbiol. Ecol.*, 45, 181–187.
- Giambelluca W., T. Rainfall atlas of Hawaii : State of Hawaii. Dep. L. Nat. Resour. Rep. 267, (1986).
- Giardina, C. P., Litton, C. M., Crow, S. E., & Asner, G. P. (2014). Warming-related increases in soil CO₂ efflux are explained by increased below-ground carbon flux. *Nature Climate Change*, 4, 822–827.
- Gill, R. A., & Jackson, R. B. (2000). Global patterns of root turnover for terrestrial ecosystems. *New Phytologist*, 147, 13–31.
- Grace, C., & Stribley, D. P. (1991). A safer procedure for routine staining of vesicular-arbuscular mycorrhizal fungi. *Mycological Research*, 95, 1160–1162.
- Hammer, E. C., Pallon, J., Wallander, H., & Olsson, P. A. (2011). Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiology Ecology*, 76, 236–244.
- Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E. D., ... Inger, R. (2018). A brief introduction to mixed effects modelling and multi-model inference in ecology. *PeerJ*, 2018, e4794. <https://doi.org/10.7717/peerj.4794>
- Hart, M. M., & Reader, R. J. (2002). Does percent root length colonization and soil hyphal length reflect the extent of colonization for all AMF? *Mycorrhiza*, 12, 297–301.
- Haynes, B. E., & Gower, S. T. (1995). Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. *Tree Physiol.*, 15, 317–325.
- Hawkes, C. V., Hartley, I. P., Ineson, P., & Fitter, A. H. (2008). Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus. *Global Change Biology*, 14, 1181–1190.
- Heinemeyer, A., & Fitter, A. H. (2004). Impact of temperature on the arbuscular mycorrhizal (AM) symbiosis: Growth responses of the host plant and its AM fungal partner. *Journal of Experimental Botany*, 55, 525–534.
- Hendricks, J. J., Nadelhoffer, K. J., & Aber, J. D. (1993). Assessing the role of fine roots in carbon and nutrient cycling. *Trends in Ecology & Evolution*, 8, 174–178.
- Hodge, A. R. (2010). The acquisition of water and nutrients from the heterogeneous soil environment. In W. LütBeyschlag & B. F. D. Büdel (Eds.), *Roots: The acquisition of water and nutrients from the heterogeneous soil environment* (pp. 307–337). Berlin, Heidelberg: Springer.
- Hutchings, M. J., & de Kroon, H. (1994). Foraging in plants: The Role of morphological plasticity in resource acquisition. *Advances in Ecological Research*, 25, 159–238. [https://doi.org/10.1016/S0065-2504\(08\)60215-9](https://doi.org/10.1016/S0065-2504(08)60215-9)
- Johnson, N. C. (2010). Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist*, 185, 631–647. <https://doi.org/10.1111/j.1469-8137.2009.03110.x>
- Johnson, N. C., Graham, J. H., & Smith, F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, 135, 575–585.
- Johnson, N. C., Wolf, J., & Koch, G. W. (2003). Interactions among mycorrhizae, atmospheric CO₂ and soil N impact plant community composition. *Ecology Letters*, 6, 532–540.
- Johnson, N. C., Wilson, G. W. T., Bowker, M. A., Wilson, J. A., & Miller, R. M. (2010). Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc. Natl. Acad. Sci. U. S. A.*, 107, 2093–8.
- Kembel, S. W., & Cahill, J. F. (2005). Plant phenotypic plasticity belowground: A phylogenetic perspective on root foraging trade-offs. *American Naturalist*, 166, 216–230.
- Koske, R. E., Gemma, J. N., & Flynn, T. (1992). Mycorrhizae in Hawaiian angiosperms: A survey with implications for the origin of the native flora. *American Journal of Botany*, 79, 853–862.
- Kuzakov, Y., & Schneckenberger, K. (2004). Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation. *Archives of Agronomy and Soil Science*, 50, 115–132.

- Lambers, H., Raven, J., Shaver, G., & Smith, S. (2008). Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution*, 23, 95–103.
- Le Deunff, E., Lecourt, J., & Malagoli, P. (2016). Fine-tuning of root elongation by ethylene: A tool to study dynamic structure–function relationships between root architecture and nitrate absorption. *Annals of Botany*, 118, 607–620.
- LeBauer, D. S., & Treseder, K. K. (2008). Nitrogen limitation of net primary production in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371–379.
- Leppälampi-Kujansuu, J., Salemaa, M., Kleja, D. B., Linder, S., & Helmisaari, H.-S. (2014). Fine root turnover and litter production of Norway spruce in a long-term temperature and nutrient manipulation experiment. *Plant and Soil*, 374, 73–88. <https://doi.org/10.1007/s11104-013-1853-3>
- Litton, C. M., & Giardina, C. P. (2008). Below-ground carbon flux and partitioning: Global patterns and response to temperature. *Functional Ecology*, 22, 941–954.
- Litton, C. M., Giardina, C. P., Albano, J. K., Long, M. S., & Asner, G. P. (2011). The magnitude and variability of soil-surface CO₂ efflux increase with mean annual temperature in Hawaiian tropical montane wet forests. *Soil Biology and Biochemistry*, 43(11), 2315–2323. <https://doi.org/10.1016/j.soilbio.2011.08.004>.
- Litton, C. M., Raich, J. W., & Ryan, M. G. (2007). Carbon allocation in forest ecosystems. *Global Change Biology*, 13, 2089–2109.
- Luyssaert, S., Inglima, I., Jung, M., Richardson, A. D., Reichstein, M., Papale, D., ... Janssens, I. A. (2007). CO₂ balance of boreal, temperate, and tropical forests derived from a global database. *Global Change Biology*, 13, 2509–2537.
- Lynch, J. P., Ho, M. D., & phosphorus, L. (2005). Rhizoeconomics: Carbon costs of phosphorus acquisition. *Plant and Soil*, 269, 45–56. <https://doi.org/10.1007/s11104-004-1096-4>
- Malhi, Y., Silman, M., Salinas, N., Bush, M., Meir, P., & Saatchi, S. (2010). Introduction: Elevation gradients in the tropics: Laboratories for ecosystem ecology and global change research. *Global Change Biology*, 16, 3171–3175.
- Marschner, H., & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil*, 159, 89–102. <https://doi.org/10.1007/BF00000098>
- McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., & Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, 115, 495–501. <https://doi.org/10.1111/j.1469-8137.1990.tb00476.x>
- Melillo, J. M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., ... Tang, J. (2011). Soil warming, carbon-nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 9508–9512.
- Norby, R. J., & Jackson, R. B. (2000). Root dynamics and global change: Seeking an ecosystem perspective. *New Phytologist*, 147, 3–12.
- Okiobe, S. T., Augustin, J., Mansour, I., & Veresoglou, S. D. (2019). Disentangling direct and indirect effects of mycorrhiza on nitrous oxide activity and denitrification. *Soil Biology & Biochemistry*, 134, 142–151.
- Olander, L. P., & Vitousek, P. M. (2005). Short-term controls over inorganic phosphorus during soil and ecosystem development. *Soil Biology & Biochemistry*, 37, 651–659.
- Olsson, P. A., Rahm, J., & Aliasgharzad, N. (2010). Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiology Ecology*, 72, 125–131.
- Ostertag, R. (2001). Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. *Ecology*, 82, 485–499. [https://doi.org/10.1890/0012-9658\(2001\)082\[0485:EONAP A\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0485:EONAP A]2.0.CO;2)
- Ostonen, I., Helmisaari, H.-S., Borken, W., Tedersoo, L., Kukumägi, M., Bahram, M., ... Lohmus, K. (2011). Fine root foraging strategies in Norway spruce forests across a European climate gradient. *Global Change Biology*, 17, 3620–3632.
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lohmus, K., ... Brunner, I. (2007). Specific root length as an indicator of environmental change. *Plant Biosystems - an International Journal Dealing with All Aspects of Plant Biology*, 141, 426–442.
- Pendall, E., Bridgman, S., Hanson, P. J., Hungate, B., Kicklighter, D. W., Johnson, D. W., Law, B. E., Luo, Y., ... Wan, S. (2004). Below-ground process responses to elevated CO₂ and temperature: a discussion of observations, measurement methods, and models. *New Phytol.* 162, 311–322.
- Peng, S., Eissenstat, D. M., Graham, J. H., Williams, K., & Hodge, N. C. (1993). Growth depression in mycorrhizal citrus at high-phosphorus supply (analysis of carbon costs). *Plant Physiology*, 101(3), 1063–1071. <https://doi.org/10.1104/pp.101.3.1063>
- Pierre, S., Hewson, I., Sparks, J. P., Litton, C. M., Giardina, C., Groffman, P. M., & Fahey, T. J. (2017). Ammonia oxidizer populations vary with nitrogen cycling across a tropical montane mean annual temperature gradient. *Ecology*, 98(7), 1896–1907. <https://doi.org/10.1002/ecy.1863>
- Powers, J. S., Treseder, K. K., & Lerdau, M. T. (2004). Fine roots, arbuscular mycorrhizal hyphae and soil nutrients in four neotropical rain forests: Patterns across large geographic distances. *New Phytologist*, 165, 913–921.
- Pregitzer, K. S., Hendrick, R. L., & Fogel, R. (1993). The demography of fine roots in response to patches of water and nitrogen. *New Phytologist*, 125, 575–580.
- Pregitzer, K. S., King, J. S., Burton, A. J., & Brown, S. E. (2000). Responses of tree fine roots to temperature. *New Phytologist*, 147, 105–115.
- R Core Team (2016). *A language and environment for statistical computing*.
- Raich, J. W., & Nadelhoffer, K. J. (1989). Belowground carbon allocation in forest ecosystems: Global trends. *Ecology*, 70, 1346–1354. <https://doi.org/10.2307/1938194>
- Raich, J. W., Riley, R. H., & Vitousek, P. M. (1994). Use of root-ingrowth cores to assess nutrient limitations in forest ecosystems. *Canadian Journal of Forest Research*, 24, 2135–2138.
- Raich, J. W., Russell, A. E., & Vitousek, P. M. (1997). Primary productivity and ecosystem development along an elevational gradient on Mauna Loa, Hawai'i. *Ecology*, 78, 707–721.
- Reed, S. C., Vitousek, P. M., & Cleveland, C. C. (2011). Are patterns in nutrient limitation belowground consistent with those aboveground: Results from a 4 million year chronosequence. *Biogeochemistry*, 106, 323–336. <https://doi.org/10.1007/s10533-010-9522-6>
- Reich, P. B., Luo, Y., Bradford, J. B., Poorter, H., Perry, C. H., & Oleksyn, J. (2014). Temperature drives global patterns in forest biomass distribution in leaves, stems, and roots. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 13721–13726.
- Reich, P. B., & Oleksyn, J. (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 11001–11006.
- Riley, R. H., & Vitousek, P. M. (1995). Nutrient dynamics and nitrogen trace gas flux during ecosystem development in montane rain forest. *Ecology*, 76, 292–304. <https://doi.org/10.2307/1940650>
- Rillig, M. C. (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters*, 7, 740–754. <https://doi.org/10.1111/j.1461-0248.2004.00620.x>
- Robinson, D. (2001). Root proliferation, nitrate inflow and their carbon costs during nitrogen capture by competing plants in patchy soil. *Plant and Soil*, 232, 41–50.
- Robinson, D., Hodge, A., Griffiths, B. S., & Fitter, A. H. (1999). Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 266(1418), 431–435. <https://doi.org/10.1098/rspb.1999.0656>
- Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., ... Gurevitch, J. (2001). A meta-analysis of the response of soil

- respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 126, 543–562. <https://doi.org/10.1007/s004420000544>
- Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., ... Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, 478(7367), 49–56. <https://doi.org/10.1038/nature10386>
- Searle, S. R., Speed, F. M., & Milliken, G. A. (1980). Population marginal means in the linear model: An alternative to least squares means. *Am. Stat.*, 34, 216–221.
- Selmants, P. C., Litton, C. M., Giardina, C. P., & Asner, G. P. (2014). Ecosystem carbon storage does not vary with mean annual temperature in Hawaiian tropical montane wet forests. *Global Change Biology*, 20, 2927–2937.
- Smith, S. E., & Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: New paradigms from cellular to ecosystem scales. *Annual Review of Plant Biology*, 62, 227–250.
- Sundqvist, M. K., Sanders, N. J., & Wardle, D. A. (2013). Community and ecosystem responses to elevational gradients: Processes, mechanisms, and insights for global change. *Annual Review of Ecology Evolution and Systematics*, 44, 261–280.
- Toth, R., Miller, R. M., Jarstfer, A. G., Alexander, T., & Bennett, E. L. (1991). The calculation of intraradical fungal biomass from percent colonization in vesicular-arbuscular mycorrhizae. *Mycologia*, 83, 553–558. <https://doi.org/10.1080/00275514.1991.12026054>
- Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO₂ in field studies. *New Phytologist*, 164, 347–355. <https://doi.org/10.1111/j.1469-8137.2004.01159.x>
- Treseder, K. K. (2013). The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil*, 371, 1–13. <https://doi.org/10.1007/s11104-013-1681-5>
- Treseder, K. K., & Allen, M. F. (2002). Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: A model and field test. *New Phytologist*, 155, 507–515.
- Treseder, K. K., & Vitousek, P. M. (2001). Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology*, 82, 946–954. [https://doi.org/10.1890/0012-9658\(2001\)082\[0946:EOSNAO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0946:EOSNAO]2.0.CO;2)
- Veresoglou, S. D., Verbruggen, E., Makarova, O., Mansour, I., Sen, R., & Rillig, M. C. (2019). Arbuscular mycorrhizal fungi alter the community structure of ammonia oxidizers at high fertility via competition for soil NH₄⁺. *Microbial Ecology*, 78, 147–158. <https://doi.org/10.1007/s00248-018-1281-2>
- Vicca, S., Luyssaert, S., Peñuelas, J., Campioli, M., Chapin, F. S., Ciais, P., ... Janssens, I. A. (2012). Fertile forests produce biomass more efficiently. *Ecology Letters*, 15, 520–526.
- Vierheilig, H., Schweiger, P., & Brundrett, M. (2005). An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum*, 125, 393–404. <https://doi.org/10.1111/j.1399-3054.2005.00564.x>
- Vitousek, P. M. (1984). Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology*, 65, 285–298. <https://doi.org/10.2307/1939481>
- Vitousek, P. M., & Farrington, H. (1997). Nutrient limitation and soil development: Experimental test of a biogeochemical theory. *Biogeochemistry*, 37, 63–75.
- Vitousek, P. M., Porder, S., Houlton, B. Z., & Chadwick, O. A. (2010). Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen–phosphorus interactions. *Ecological Applications*, 20, 5–15.
- Vogel, J. G., Bond-Lamberty, B., Schuur, E. A. G., Gower, S. T., Mack, M. C., O'Connell, K. E. B., ... Ruess, R. W. (2008). Carbon allocation in boreal black spruce forests across regions varying in soil temperature and precipitation. *Global Change Biology*, 14, 1503–1516.
- Vogt, K. A., Vogt, D. J., Palmiotto, P. A., Boon, P., O'Hara, J., & Asbjornsen, H. (1995). Review of root dynamics in forest ecosystems grouped by climate, climatic forest type and species. *Plant and Soil*, 187, 159–219. <https://doi.org/10.1007/BF00017088>
- Walker, T. W., & Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma*, 15, 1–19. [https://doi.org/10.1016/0016-7061\(76\)90066-5](https://doi.org/10.1016/0016-7061(76)90066-5)
- Watkins, N. K., Fitter, A. H., Graves, J. D., & Robinson, D. (1996). Carbon transfer between C3 and C4 plants linked by a common mycorrhizal network, quantified using stable carbon isotopes. *Soil Biology & Biochemistry*, 28, 471–477.
- Zhang, H., & Forde, B. G. (1998). Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. *Science* (80-), 279(5349), 407–409. <https://doi.org/10.1126/science.279.5349.407>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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