Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test

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Summary

• Since mycorrhizal fungi constitute an important component of the soil–plant interface, their responses to changes in nutrient availability may mediate shifts in ecosystem function. We tested the hypothesis that initial soil nutrient availability may determine effects of nitrogen (N) and phosphorus (P) additions on the growth and community of arbuscular mycorrhizal (AM) fungi.

• Extraradical hyphal lengths and degree of root colonization of AM fungi were measured in control and fertilized plots along a soil fertility gradient in Hawaii. Responses of individual AM genera were assessed through immunofluorescent labeling.

• The AM biomass was increased by N and P additions in the N- and P-limited sites, respectively, and reduced by P fertilization in the fertile site only. The abundance of *Scutellospora* was lower under N than under P fertilization, whereas the incidence of *Glomus* was higher in the fertile site than the N-limited site. *Gigaspora* and *Acaulospora* did not vary among sites or treatments.

• Our results indicate that a decrease in AM abundance following nutrient additions cannot be assumed to occur and the effects may differ among AM genera and ecosystems with varying soil nutrients. Limitation of N and P may be one possible explanation.

Key words: arbuscular mycorrhizal fungi, community composition, extraradical hyphae, fertilization, Hawaii, immunofluorescent labeling, nitrogen, phosphorus

Introduction

Investigators of mycorrhizal fungi have long regarded plant nutrient status as a principle control over mycorrhizal abundance. Namely, it is hypothesized that since plants use mycorrhizal fungi to acquire nitrogen (N) and phosphorus (P), more carbon (C) should be allocated to mycorrhizal symbionts when plant growth is limited by soil nutrients than when it is not (Read, 1991; Smith & Read, 1997). However, N additions in field systems produce inconsistent effects on mycelial biomass of ectomycorrhizal and arbuscular mycorrhizal fungi; abundance is just as likely to increase, decline or remain constant (Tingey *et al.*, 1995; Karen & Nylund, 1997; Klironomos *et al.*, 1997; Lussenhop *et al.*, 1998; Eom *et al.*, 1999; Treseder & Allen, 2000). Given these discrepancies, we should consider that C supplies from plants are not the only nutritional requirement for fungi; N and P are also essential. Just as for plants, fungal growth may be limited when soil nutrient availability falls below a certain threshold. Under these circumstances, N or P additions may increase mycorrhizal growth. Because mycorrhizal fungi are more efficient scavengers for nutrients from the soil than are plant roots (Allen, 1991), the threshold for nutrient limitation may be lower for mycorrhizal fungi than for plants.

Given these interacting controls, mycorrhizal growth should be low under very low N- or P-availability and greatest where plant growth is still limited by N or P but fungal growth is not. Where plants are not nutrient-limited, fungal growth should become C-limited and decline owing to a drop in allocation of C by plants to the fungi (Fig. 1). Fertilization by N or P should increase mycorrhizal growth where mycorrhizal fungi are initially nutrient-limited, decrease mycorrhizal

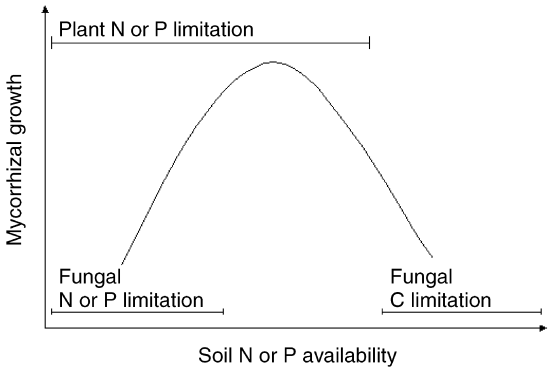

Fig. 1 The interaction of plant and fungal nutrient limitation on the biomass of mycorrhizal fungi. At high nutrient levels, fungi will receive little carbon from plants and will be C-limited. At lower nutrient levels, plants will be N- or phosphorus P-limited and will allocate C to mycorrhizal fungi. At the same time, if N or P concentrations are sufficient for fungal growth, mycorrhizal fungi will proliferate. At the lowest nutrient levels, both fungi and plants should be nutrient limited, and fungal biomass will be low regardless of C allocation to the fungi by plants.

growth where plants are nutrient-limited but fungi are not, and have no effect on fungi where neither organism is nutrient-limited.

Species and genera of mycorrhizal fungi appear to vary in responses to nutrient additions in field studies. Nitrogen deposition elicited a shift in communities of arbuscular mycorrhizal (AM) fungi toward *Glomus aggregatum*, *Glomus leptotichum*, and *Glomus geosporum* and away from *Scutellospora* and *Gigaspora* species in southern California coastal sage scrub (Egerton-Warburton & Allen, 2000). *Gigaspora gigantea* and *Glomus mossae* proliferated under N fertilization in a tallgrass prairie (Eom *et al.*, 1999). Nitrogen and P additions in the Cedar Creek Natural History Area in Minnesota decreased in *G. gigantea*, *Gigaspora margarita*, *Scutellospora calospora*, and *Glomus occultum*, and increases in *Glomus intraradices* (Johnson, 1993). In ectomycorrhizal fungi, changes in distributions among morphotypes or genotypes following N fertilization have been observed in plantations and forests (Taylor & Alexander, 1989; Arnebrant & Soderstrom, 1992; Karen & Nylund, 1997). This variation of response to fertilization or deposition may be partly related to different requirements of the fungi for C, N or P.

Mycorrhizal species can range from parasitic to mutualistic (Johnson *et al.*, 1997). Plants may exert some degree of control over fungal community composition to select more beneficial symbionts. Numerous pot studies have demonstrated variations in growth rate of plants inoculated with different arbuscular mycorrhizal species (Mosse, 1972; Bever *et al.*, 2001). In addition, in the Cedar Creek experiment, Johnson (1993) found that greenhouse plants inoculated with AM fungi from fertilized areas grew more slowly than plants inoculated with fungi from control areas. Both soil nutrient availability and plant controls may directly influence the composition (as well as the abundance) of the mycorrhizal community.

We tested the hypothesis that the initial nutrient status of an ecosystem determines the effects of N and P additions on the abundance and community composition of AM fungi. Mycorrhizal dynamics were assessed in control, N and P fertilized plots along a soil fertility gradient in Hawaii. This gradient included sites in which aboveground net primary productivity was limited by N, P or neither nutrient independently. The AM colonization and glomalin concentrations have been measured in this system (Rillig *et al.*, 2001; Treseder & Vitousek, 2001b). Compared with the mainland, Hawaiian angiosperms have a high incidence of mycotrophy (approx. 90%; Gemma & Koske, 1990; Koske *et al.*, 1992), although pteridophytes have a lower incidence (75%; Gemma *et al.*, 1992). Of those species that form relationships with mycorrhizal fungi, a strong majority (approx. 98%) are AM (Gemma *et al.*, 1992; Koske *et al.*, 1992), so this study focused on AM fungi. We predicted that: (1) AM fungi would be nutrient-limited at the endpoints of the gradient, so that AM hyphal length would increase in response to N fertilization in the N-limited site and to P fertilization in the P-limited site, while fertilization with either nutrient would reduce or have no effect on hyphal length in the fertile site; and (2) the relative abundance of the AM genera *Glomus*, *Gigaspora*, *Acaulospora* and *Scutellospora* would vary across sites and treatments.

Methods

Sites

We tested the relationship between nutrient availability and mycorrhizal dynamics in N and P fertilized plots in three rain forests in Hawaii. These sites are at different stages of soil development (300, 20 000, and 4 100 000 yr old) and therefore vary in soil nutrient availability. Phosphorus availability (as resin-P) is low in the youngest (0.20 ± 0.08 μg per bag d^{-1}) and oldest sites (0.41 ± 0.17 μg per bag d^{-1}) and greatest at the 20 000-yr-old site (1.21 ± 0.28 μg per bag d^{-1}) (Crews *et al.*, 1995). Nitrogen availability (as resin-ammonium plus nitrate) is lowest at the youngest site (3.31 ± 1.56 μg per bag d^{-1}) and higher in the intermediate-aged (12.37 ± 3.32 μg per bag d^{-1}) and oldest site (14.41 ± 7.20 μg per bag d^{-1}) (Crews *et al.*, 1995). Moreover, nutrient availability exerts a strong influence on plant growth across the sites. Long-term fertilization experiments have indicated that above-ground productivity is limited primarily by N in the youngest site (Vitousek *et al.*, 1993), P in the oldest site (Herbert & Fownes, 1995) and by neither N nor P independently in the relatively fertile 20 000-yr-old site (Vitousek & Farrington, 1997). Hereafter, we will refer to the youngest, intermediate-aged, and oldest sites as the 'N-limited', 'fertile', and 'P-limited' sites, respectively.

Table 1 Mycotrophic status and per cent canopy cover of major plant species

Species	Associated mycorrhizal fungi[1]	N-limited site[2]	Fertile site	P-limited site
Trees				
Metrosideros polymorpha	Arbuscular[3]	87.5	87.5	47.5
Cheirodendron trigynum	n.d.[4]	< 5	24.0	< 5
Ilex anomola	n.d.	< 5	9.0	< 5
Shrubs and tree ferns				
Cibotium chamissoi	Arbuscular	< 5	24.0	< 5
Cibotium glaucum	Arbuscular	62.5	45.5	< 5
Coprosma ochracea	Arbuscular	10.6	< 5	< 5
Pteridophytes				
Dicranopteris linearis	Arbuscular	6.0	< 5	< 5
Dryopteris wallichiana	Arbuscular	< 5	13.5	< 5
Elaphoglossum alatum	Arbuscular	< 5	< 5	43.0
Nephrolepis exaltata	Arbuscular	< 5	< 5	10.5
Nonnatives				
Hedychium gardnerianum	n.d.	57.5	< 5	< 5

[1]As reported by Koske *et al.* (1992), Gemma *et al.* (1992) and Gemma & Koske (1990) for Hawaiian plants. [2]Canopy cover data from Kitayama & Mueller-Dombois (1995). [3]Can associate with ectomycorrhizal fungi, although no ectomycorrhizal root tips were found in the study sites. [4]n.d., not determined.

The sites are described in detail by Crews *et al.* (1995). All sites are near 1200 m elevation and have a mean annual temperature of approximately 16°C. All receive about 2500 mm of rainfall annually, mostly from north-east Trade Winds. As such, precipitation is relatively evenly distributed throughout the year. Each site is located on the constructional surface of a shield volcano. Therefore, volcanic tephra is the parent material, and slopes are less than 2°. Soil classifications for the N-limited, fertile, and P-limited sites are Hydric Dystrandept, Typic Hydrandept, and Plinthic Acrudox, respectively. We have found little evidence of human disturbance in any site. Each field site covers approximately 1 km^2.

The vegetation at each site is comprised of native forest and is strongly dominated by the evergreen tree *Metrosideros polymorpha* (Crews *et al.*, 1995). This tree has been classified as an arbuscular mycorrhizal plant in Hawaii (Koske *et al.*, 1992). We have occasionally observed ectomycorrhizal colonization on *Metrosideros* roots, but never in these field sites. The abundance of major (> 5% cover) plant species in the sites are listed in Table 1, as reported by Kitayama & Mueller-Dombois (1995). Ostertag & Verville (2001) have measured shifts in plant community structure following N and P additions in the N- and P-limited sites. Of the major species, N fertilization in the N-limited site tends to decrease densities of *Hedychium gardnerianum* (non-native ginger). In contrast, the tree fern, *Cibotium glaucum*, tends to have higher densities in N fertilized plots in that site. Nonmajor species affected include *Rubis argutus*, a nonnative shrub, which has higher density following P fertilization in the P-limited site, and the native shrub *Vaccinium calycinum*, which has declined in density following N fertilization in the N-limited site. Ostertag & Verville (2001) did not characterize community structure in the fertile site. Mycotrophic status has been assessed by others (Gemma *et al.*, 1992; Koske *et al.*, 1992, and Gemma & Koske, 1990) for most major plant species found in these sites (Table 1). In each case, the plant species is consistently colonized by AM fungi when grown in Hawaii. At no time have ectomycorrhizal fungi been discovered in root samples (*Metrosideros* or otherwise) from these study sites. We expect that any shifts in ecosystem-level mycorrhizal status caused by changes in plant communities across sites or fertilization treatments will be minimal.

Fertilized plots received 100 kg ha^{-1} yr^{-1} of either N (half as ammonium nitrate and half as urea) or P (as triple superphosphate) in two applications per year (Vitousek & Farrington, 1997). Plots were 15 × 15 m in the N- and P-limited sites, and each encompassed several trees. In contrast, plots in the fertile site were each centered on a single adult *Metrosideros* individual and were 10 m in diameter. This difference is because *Metrosideros* trees in the fertile site were much larger and less dense than those in the other sites. We sampled from four replicate plots per treatment per in the N-limited and fertile sites, and from three replicate plots per treatment in the P-limited site. At the time of sampling, fertilization had been ongoing for 15, 7, and 9 yr in the N-limited, fertile and P-limited sites, respectively.

Sample collection

All measurements involved sampling roots and AM hyphae from control, N-fertilized, and P-fertilized plots in the three sites in March 2000. Seasonal variation in abundance of AM fungi in plant roots is minimal in these field sites (Treseder & Vitousek, 2001b). We used 5-cm diameter soil corers to collect soil (including roots) from the top 10-cm of the soil profile. Two cores were collected per plot at random locations.

Samples were placed on ice for transport to the field laboratory in Hawaii Volcanoes National Park on the Big Island of Hawaii, where they were frozen for transport to University of California, Riverside, CA, USA. Soil cores were stored there at −70°C prior to starting laboratory analyses in December 2000.

Hyphal extractions

As a proxy of AM biomass in soil, we extracted and quantified lengths of extraradical AM hyphae from two soil cores per plot (Sylvia, 1992). Hyphal lengths are often measured to calculate fungal biomass in soil (Paul & Clark, 1996), and under high magnification hyphae can be identified as AM or non-AM (Sylvia, 1992). Each soil core was passed through a 2-mm mesh sieve and the smaller particles were retained. Approximately 1.0 g sieved soil was dispersed in 12 ml deionized water, and then centrifuged for 10 min at 2000 r.p.m. The supernatant was discarded. Twelve milliliters of sucrose solution (500 g l^{-1} sucrose plus 50 g l^{-1} sodium hexametaphosphate) was added to the pellet, mixed, and centrifuged for 5 min at 2000 r.p.m. The supernatant was collected and passed through a 0.2-µm filter. The length of arbuscular mycorrhizal hyphae on the filter was quantified at ×200 using a phase-contrast microscope (Axioskop; Carl Zeiss Inc., Thornwood, NY, USA). We could distinguish AM hyphae from nonmycorrhizal hyphae by examining morphological structures, as arbuscular mycorrhizal hyphae are nonseptate, have irregular walls and display angular, unilateral branching (Bonfante-Fasolo, 1986). Results are reported as mm hyphae g^{-1} dry soil.

Community composition

The community distribution of external AM hyphae on roots was determined by direct immunofluorescence to identify fungi to genus level (Fig. 2). Antibodies were raised against each of the four major genera of AM fungi. Rabbits were immunized with whole-spore fractions of *Glomus deserticola* Trappe, Bloss & Menge, *Acaulospora laevis* (Nicol. & Gerd.), *Gigaspora margarita* (Becker & Hall), and *Scutellospora calospora* (Nicol. & Gerd.) Walker and Sanders as described in Egerton-Warburton & Allen (2000). Each antiserum was conjugated to fluorescein isothiocyanate, and its specificity was determined by evaluating immunoreactivity between all combinations of antisera and spores of each species; no cross-reactions were detected (Allen *et al.*, 1999). The antibody technique in our study is specific at the genus level, and only live hyphae fluoresce under this stain (Friese & Allen, 1991).

Live roots less than 2 mm diameter were selected (see 'Root colonization') and gently washed four times in deionized water. Roots were allocated to four subsamples. Subsamples were incubated in diluted antisera (1 : 1 antisera–water) of each genus in the dark for 24 h at 20°C. Roots were rinsed briefly in deionized water, mounted on glass slides, and examined at ×200 on a phase-contrast microscope equipped with epifluorescence (Axioskop; Carl Zeiss Inc.) (Egerton-Warburton & Allen, 2000). Per cent root length with immunoreactive hyphae was quantified using the magnified intersections method (McGonigle *et al.*, 1990). Results are reported as percentage root length with hyphae of individual genera.

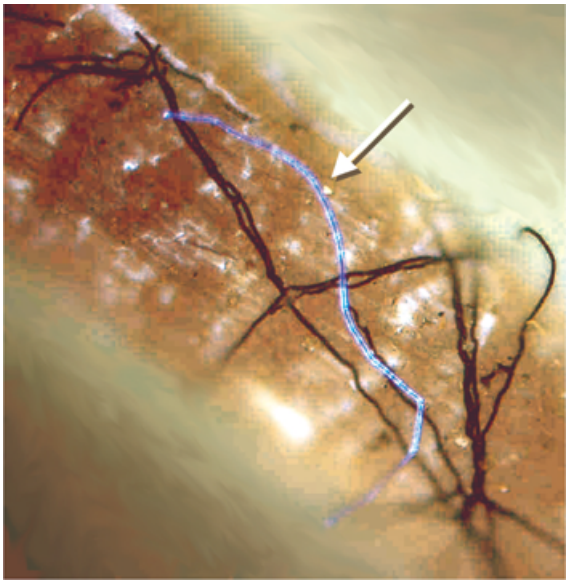

Fig. 2 Immunofluorescent reaction of an external hypha of *Acaulospora* (arrow) growing from a N fertilized tree root in the phosphorus-limited site. Dark nonreactive hyphae of other genera are also visible on the root.

Root colonization

Percentage root length with AM colonization was determined using Trypan blue staining (Koske & Gemma, 1989). Roots were removed by sieving the soil core through a 2-mm mesh and examining the material retained for root pieces. About 10 mg live roots less than 2 mm diameter were selected and washed four times in deionized water. Color and texture were used to distinguish live roots from dead roots (dead roots are darker and more friable than live roots). Roots were not sorted by species and represent community-level biomass. Samples were cleared in 2.5% potassium hydroxide for 20 min at 90°C, then rinsed three times with deionized water. Next, roots were bleached in 0.525% sodium hypochlorite for 20 min, rinsed, and acidified in 1% hydrochloric acid overnight. The next day, samples were stained in acidic glycerol–trypan blue solution (50% glycerol (v : v), 1% hydrochloric acid (v : v), and 0.05% Trypan blue (w : v)) at 90°C for 20 min Roots were destained in acidic glycerol (50% glycerol (v : v), 1% hydrochloric acid (v : v)) and mounted on slides. Colonization by arbuscules, vesicles and internal hyphae were determined using the magnified intersections method (McGonigle *et al.*, 1990). Results are reported as percentage of root length with AM

vesicles, AM hyphae, and total AM structures (vesicles + hyphae). No arbuscules were observed in any samples.

Statistics

Statistical analyses were performed with SYSTAT 10 for Windows (SPSS, 2000). Data were log-transformed if necessary, and fully factorial analyses of variance (ANOVA) and Tukey *post hoc* tests were conducted with site and treatment as grouping variables. We tested for normal distribution of data by calculating standard deviates separately for each sample, pooling all deviates, then applying a Kolmogorov–Smirnov test for goodness of fit. An F_{max}-test was used to confirm homogeneity of variances (Sokal & Rohlf, 1995). Normality and homogeneity of variance could not be achieved in measures of per cent colonization by individual genera of by mycorrhizal structures. In these cases, ANOVAs and Tukey tests were conducted on ranked data. For all tests, differences were considered significant when $P < 0.05$ and marginally significant when $P < 0.10$ (Klironomos *et al.*, 1999).

Results

AM hyphal lengths in soil

The biomass of arbuscular mycorrhizal fungi in the soil, measured as hyphal length g^{-1} soil and determined by gross morphology, varied significantly across sites (Fig. 3; ANOVA, $F_{2,2} = 4.21$, $P < 0.03$). Specifically, hyphal biomass was significantly lower in the P-limited site (2800 mm g^{-1}) than in the fertile (4900 mm g^{-1}; Tukey, $P < 0.04$) or N-limited (4300 mm g^{-1}; Tukey, $P < 0.05$) sites. Fertilization treatment alone did not significantly affect hyphal lengths. However, there was a marginally significant site–treatment interaction (ANOVA, $F_{2,24} = 2.16$, $P < 0.10$), with N fertilization increasing biomass in the N-limited site, and P fertilization increasing biomass in the P-limited site. None of these responses was significant in a Tukey *post hoc* test.

Root colonization by AM hyphae

The fraction of root length colonized by AM fungi did not respond significantly to site, fertilization or their interaction (Fig. 4c). However, the formation of types of AM structures was affected. Specifically, per cent root length with AM vesicles varied significantly across sites (Fig. 4b; ANOVA, $F_{2,2} = 5.041$, $P < 0.019$) and was higher in the N-limited site (3.3%) than the fertile site (0.8%; Tukey, $P < 0.020$), while per cent root length with internal AM hyphae did not differ among sites (Fig. 4a). Fertilization had no significant effect on root colonization with hyphae or vesicles (Fig. 4a,b). No arbuscules were observed in any of our root samples.

Community composition of AM fungi

Two genera shifted significantly in abundance (Fig. 5): *Scutellospora* responded significantly to fertilization treatment (ANOVA, $F_{2,4} = 4.59$, $P < 0.02$), while *Glomus* varied significantly across sites (ANOVA, $F_{2,2} = 3.48$, $P < 0.05$). For *Scutellospora*, the N fertilized treatment had lower colonization than did the P fertilized treatment (N fertilized, 1.72%; P-fertilized, 6.78%; Tukey, $P < 0.02$), but neither differed significantly from the control (3.72%). *Glomus* abundance in the fertile site (6.33%) was significantly greater than that in the N-limited site (1.83%; Tukey, $P < 0.04$) but not the P-limited site (3.78%). *Gigaspora* and *Acaulospora* had no significant response to either factor.

Discussion

The initial nutrient status of ecosystems may determine responses of AM fungi to fertilization, and this factor may

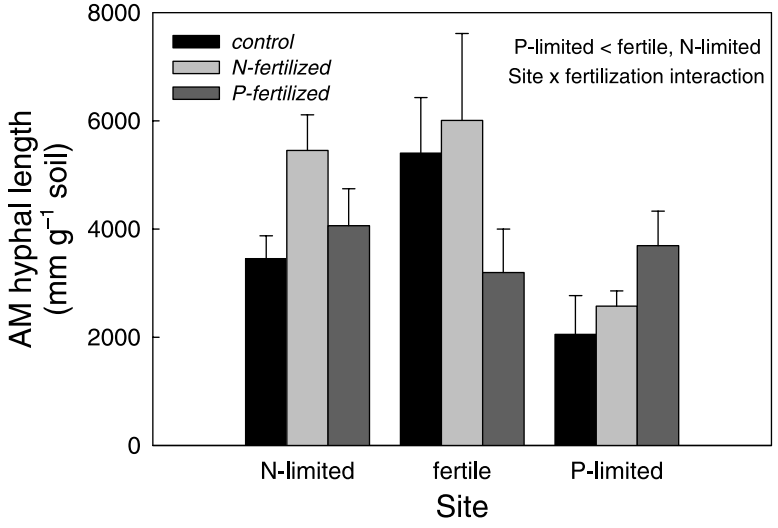

Fig. 3 Standing crop of external arbuscular mycorrhizal (AM) hyphae in soil, as determined by hyphal extractions and identification by gross morphology. Bars, means of three or four plots ±1 SE. Hyphal biomass was lower in the phosphorus (P)-limited site than in the fertile ($P < 0.04$) or N-limited ($P < 0.05$) sites, and there was a marginally significant site–fertilization interaction ($P < 0.10$).

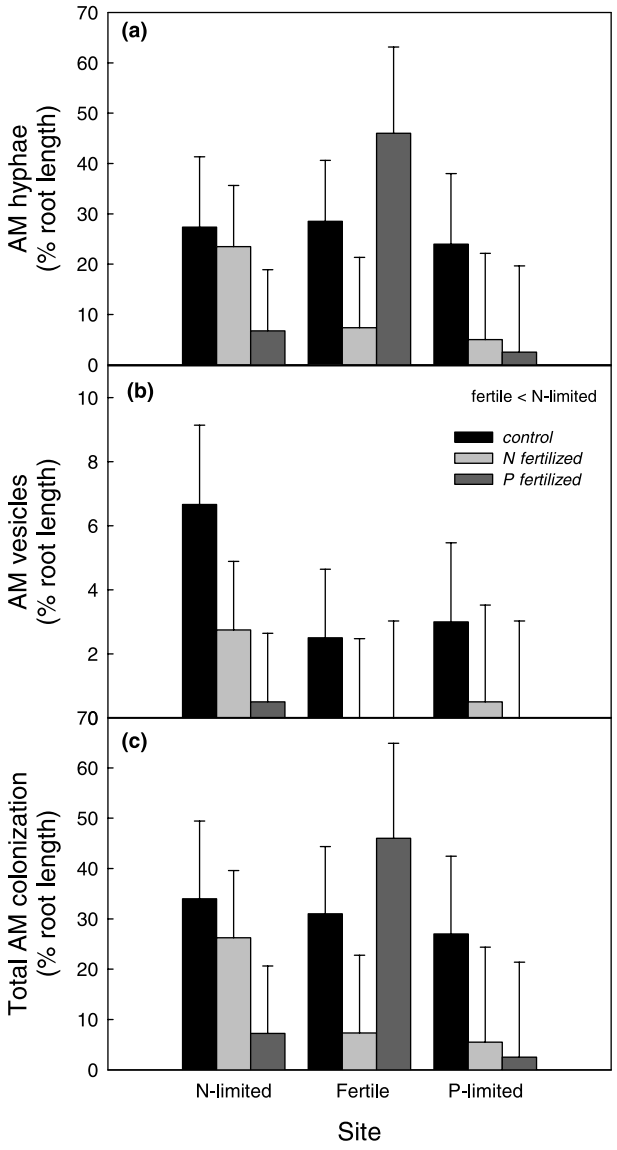

Fig. 4 Percentage of root length with arbuscular mycorrhizal (AM) hyphae (a), AM vesicles (b) and all AM structures combined (c), determined by staining with Trypan blue. No arbuscules were observed in any sample. Bars, means of three or four plots plus ±1 SE. Vesicle abundance was higher in the N-limited site than in the fertile site ($P < 0.02$).

partly account for inconsistencies found among other field experiments. In our study, N and P additions raised AM biomass in the N and P limited sites, respectively, while P fertilization reduced total hyphal lengths in the fertile site only. In addition, patterns across the nutrient gradient were consistent with responses to N and P fertilization: hyphal lengths were highest in soil of the fertile site. These trends follow our predictions and lend support to our model of AM growth in response to nutrient availability (Fig. 1), although the site–fertilization interaction is only marginally significant ($P < 0.10$). Heterogeneity is often much higher for soil-related traits than for plant-related traits, so low *P*-values can be difficult to obtain in experiments designed for ecosystem- or vegetation-level measurements (Klironomos *et al.*, 1999). The observed responses of AM hyphal length to fertilization in the N- and P-limited sites are in the opposite direction to those expected if plants were the sole control over mycorrhizal growth. If direct nutrient limitation were not a factor, we would expect mycorrhizal biomass to decrease in response to N and P fertilization in all sites because plants would allocate carbohydrates elsewhere. In addition, standing hyphal length does not track standing root stocks. In these forests, fine root lengths are significantly greater in the P-limited site than the others and do not change significantly with N or P fertilization (Ostertag, 2001).

When genera of AM fungi were examined individually, they displayed varied responses to soil fertility across sites and fertilization treatments. The relative abundance of *Glomus* on plant roots was significantly higher in the fertile site than in the N-limited site. This finding is consistent with studies demonstrating that *Glomus* species increase following N deposition or fertilization in California coastal sage scrub (Egerton-Warburton & Allen, 2000) and tallgrass prairie (Eom *et al.*, 1999). After N and P fertilization in a Minnesota field study, *G. intraradices* proliferated, although *G. occultum* declined. Notably, fertilization itself did not significantly affect *Glomus* abundance in our study. *Glomus* populations in Hawaii may respond differently to long-term (i.e. site) vs short-term (i.e. fertilization) shifts in soil fertility. Alternately, *Glomus* species may have been affected by some other factor that changes across sites, such as genetic variation in *M. polymorpha*, the dominant tree (Treseder & Vitousek, 2001a). By contrast, *Scutellospora* varied among fertilization treatments but not sites, being more abundant in P fertilized plots than in N fertilized plots. Nitrogen additions have also reduced abundance of *Scutellospora* spores in coastal sage scrub (Egerton-Warburton & Allen, 2000). *Glomus* and *Scutellospora* may occupy separate niches with respect to soil fertility, either because of direct influences of N or P availability, or controls via plant hosts.

While relatively unknown, life history characteristics of AM fungi have been suggested by Hart *et al.* (2001) to vary among species or genera. Physiological traits that may promote rapid colonization of soil during early ecosystem succession include prolific spore production, short spore dormancy, rapid spore germination, many infection points, large infection units, high per cent root colonization, and high nutrient requirement. By contrast, mycorrhizal fungi that dominate at later stages of succession may require lower nutrient availability and may engage in interference competition with other mycorrhizal species through induced host resistance or chemical allelopathy (Hart *et al.*, 2001). While these traits have yet to be comprehensively assessed for individual genera, spore morphology can vary dramatically among groups and may be related to one or more of the above-mentioned traits. Any potential

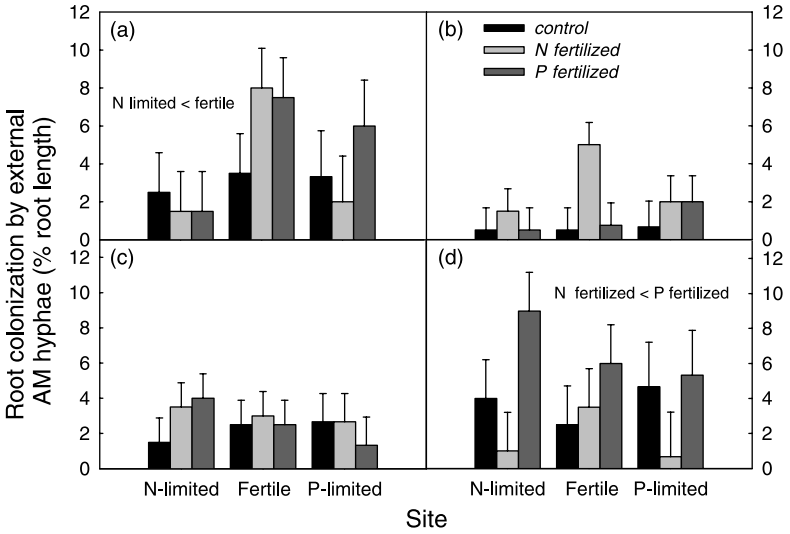

Fig. 5 Percentage of root length colonized by live arbuscular mycorrhizal (AM) hyphae of each genus (a, *Glomus*; b, *Acaulospora*; c, *Gigaspora*; d, *Scutellospora*) as determined by direct immunofluorescence. Bars, means of three or four plots plus ±1 SE. *Glomus* abundance was significantly greater in the fertile site than the N-limited site ($P < 0.04$). *Scutellospora* abundance was higher in P fertilized sites than in N fertilized sites ($P < 0.02$).

differences in life history characteristics among genera may also influence shifts in the AM community across the soil fertility gradient.

Changes in the community composition of AM fungi following fertilization may have altered their function in these ecosystems. The AM species vary in their effects on plant growth (Mosse, 1972; Mosse, 1973; Smith & Read, 1997), and shifts in biodiversity of AM fungi can influence plant diversity, growth and nutrient status (van der Heijden *et al.*, 1998). Morphological and physiological differences among genera of AM fungi may also produce variation in their direct influences on soil dynamics (Boddington & Dodd, 1999; Dodd *et al.*, 2000). Unlike *Glomus* species, *Gigaspora* and *Scutellospora* species tend to have well-developed networks of external hyphae (Dodd *et al.*, 2000). In addition, hyphae of *Gigaspora* species can have higher concentrations of glomalin than those of *Glomus* species (Wright *et al.*, 1996). Soil macroaggregate formation is positively correlated with external hyphal lengths (Tisdall & Oades, 1982; Oades, 1984; Miller & Jastrow, 1990; Oades & Waters, 1991; Jastrow *et al.*, 1998; Miller & Jastrow, 2000) and glomalin (Wright *et al.*, 1999; Wright & Anderson, 2000), and glomalin itself may be a significant carbon sink in the soil (Treseder & Allen, 2000; Rillig *et al.*, 2001). Shifts in community composition away from *Scutellospora* with N fertilization and toward *Glomus* in the fertile site may be one influence on the physical structure and C turnover of soils along the Hawaiian fertility gradient.

Root colonization is a function of both standing root length and abundance of the AM symbionts. This variable did not change across sites or fertilization treatments, although P fertilization tended to decrease colonization levels in the N-limited and P-limited sites. These results are similar in pattern and magnitude to those reported by Treseder & Vitousek (2001b) for samples collected from the same plots across four dates in 1996 and 1997. In this study, even though the quantity of mycorrhizal structures did not change, the quality did. Vesicles were most apparent in the N-limited site and least apparent in the fertile site, while internal hyphae were consistent across sites. Vesicles are thought to be a storage structure for lipids and other energy reserves (Smith & Read, 1997). Fungi in the N-limited site may receive a greater excess of carbohydrates from their plant symbionts than do fungi in the fertile site, although this response is not accompanied by an increase in external hyphal lengths or root colonization by internal hyphae. In the N-limited site, lack of N, rather than lack of C from plants, may inhibit growth. Alternately, changes in vesicle abundance may be caused by shifts in the AM community, as species of *Scutellospora* and *Gigaspora* do not form vesicles, whereas *Glomus* and *Acaulospora* do (Brundrett *et al.*, 1996).

Conclusions

We present here a model detailing the interaction of two controls on mycorrhizal abundance in ecosystems: direct N- or P-limitation of AM fungi when soil nutrient availability is very low, and C-limitation of AM fungi when plants are not nutrient-limited. As predicted by the model, responses of AM fungi to fertilization appeared to be influenced to some extent by the initial nutrient status of ecosystems, although only marginally significantly. We also found that soil fertility varied in its effect on different genera of AM fungi. *Glomus* and *Scutellospora*, in particular, appeared to have distinct responses to nutrient availability, either directly or indirectly via plant controls. Our results suggest that mycorrhizal fungi should not be viewed simply as mechanisms for N and P uptake by plants. These fungi also require soil nutrients and should allocate them according to economic principles, just as plants should (Bloom *et al.*, 1985). As attested by Fitter *et al.* (2000), a more 'mycocentric' view of plant–mycorrhizal relationships

may improve our ability to predict consequences of shifts in environmental parameters such as N deposition and other aspects of global change.

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