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

Kathleen K. Treseder\textsuperscript{1} and Michael F. Allen\textsuperscript{2}

\textsuperscript{1}Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA; \textsuperscript{2}Center for Conservation Biology, University of California, Riverside, CA 92521, USA

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 \textit{Scutellospora} was lower under N than under P fertilization, whereas the incidence of \textit{Glomus} was higher in the fertile site than the N-limited site. \textit{Gigaspora} and \textit{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 symbions 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 \textit{et al}., 1995; Karen & Nylund, 1997; Klironomos \textit{et al}., 1997; Lussenhop \textit{et al}., 1998; Eom \textit{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...
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 leptosichium, 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 increased 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 highest in the intermediate-aged (12.57 ± 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.

![Fig. 1 The interaction of plant and fungal nutrient limitation on the biomass of mycorrhizal fungi.](image)
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 occasionally observed ectomycorrhizal colonization of either N or P fertilization in the P-limited site, and the native shrub Cibotium glaucum has declined in density following N fertilization in the N-limited site. Of the major species, N fertilization tends to decrease densities 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 mycorrhizal status caused by changes in ecosystem-level mycorrhizal status. 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 (Vresedet & Vitousek, 2001b). We used 5-cm diameter soil cores 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 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 nonseparate, have irregular walls and display angular, unilateral branching (Boutinane-Fosbol, 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, Blosl & 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.

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 root length with AM biomass.
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 \( F_{\text{max}} \)-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 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, \( F_{2,4} = 2.16, P < 0.10 \)), 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
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. 4 Percentage of root length with arbuscular mycorrhizal (AM) hyphal (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 ($P < 0.02$).](image-url)
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.

Acknowledgements

We are grateful to: P. M. Vitousek for use of the chronosequence and comments on an earlier draft; the divisions of Forestry and Wildlife and State Parks of the State of Hawaii, Hawaii Volcanoes National Park, and the Joseph Souza Center at Kokee State Park for access to the field sites and logistical support; C. E. Dolanin, H. Farrington and D. Penn for field assistance; and K. Lamb, R. Lepe, T. Tennant and J. Lansing for laboratory help. This project was funded by a NSF Postdoctoral Fellowship in Biosciences Related to the Environment for KKT, and NSF grants (DEB 9996211 and DEB 9981548) to M. E. Allen. 

References

Allen MF. 1991. The ecology of mycorrhizae. Cambridge, UK: Cambridge University Press.
Allen MF, Egeston-Warburtan LM, Allen EB, Karen O. 1999. Mycorrhizae in *Adenostoma fasciculatum* Hook. & Arn.: a combination of unusual ecto- and endo-forms. *Mycorrhiza* 8: 223–228.
Arnebrand K, Soderstrom B. 1992. Effects of different fertilizer treatments on ectomycorrhizal colonization potential in two Scots pine forests in Sweden. *Forest Ecology and Management* 55: 77–89.
Bever JD, Schultz PA, Pringle A, Morton JB. 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *BioScience* 51: 925–931.
Blom AJ, Chapin FS III, Mooney HA. 1995. Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics* 16: 363–393.
Boddington CL, Dodd JC. 1999. Evidence that differences in phosphate metabolism in mycorrhizas formed by species of *Cladosporium* and *Gigaspora* might be related to their life-cycle strategies. *NewPhytoz* 142: 531–538.
Bonfante-Fasolo P. 1986. Anatomy and morphology of VA mycorrhizae. In: Powell C, Bagyaraj D, eds. *Evolutionary aspects of mycorrhizae*. Cambridge, UK: Cambridge University Press, 233–333.
Brandt M, Bougher N, Dell B, Grove T, Majeric N. 1996. Working with mycorrhizas in forestry and agriculture. ACAM monograph 32. Canberra, Australia: Australian Centre for International Agricultural Research.
Crews TE, Kitayama K, Fownes JH, Riley RH, Herbert DA, Mueller-Dombois D, Vitousek PM. 1995. Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology* 76: 1407–1424.
Dodd JC, Boddington CL, Rodriguez A, Gonzalez-Chavez C, Mansur L. 2000. Mycorrhizal types of arboreal mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant and Soil* 226: 131–151.
Egerston-Warburtan LM, Allen EB. 2000. Shifts in arboreal mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications* 10: 484–496.
Eom A-H, Hartnett DC, Wilson GWT, Figge DAH. 1999. The effect of fire, mowing and fertiliser amendment on arboreal mycorrhizas in tallgrass prairie. *American Midland Naturalist* 142: 55–70.
Fitter AH, Heinemeyer A, Suddath PL. 2000. The impact of elevated CO2 and global climate change on arboreal mycorrhizas: a mycoecosystem approach. *New Phytologist* 147: 179–187.
Ostertag R. 2001. Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. *Ecology* 82: 485–499.

Ostertag R, Verville JH. 2002. Fertilization with nitrogen and phosphorus increases abundance of non-native species in Hawaiian montane forests. *Plant Ecology*. (In press.)

Paul EA, Clark FE. 1996. *Soil Microbiology and Biochemistry*, 2nd edn. San Diego, CA, USA: Academic Press.

Read DJ. 1991. *Mycorrhizas in ecosystems – Nature’s response to the ‘Law of the minimum’*. In: Hawkesworth DL, ed. *Frontiers in mycology*. Wallingford, UK: CABI International, 101–130.

Rillig MC, Wright SF, Nichols KA, Schmidt WF, Torn MS. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant and Soil* 233: 167–177.

Smith SE, Read DJ. 1997. *Mycorrhizal Symbiosis*, 2nd edn. San Diego, CA, USA: Academic Press.

Sokal RR, Rohlf FJ. 1995. *Biometry*, 3rd edn. New York, NY, USA: W. H. Freeman.

SPSS. 2000. *Systat 10*. Chicago, IL, USA: SPSS.

Tisdall JM, Oades JM. 1982. Organic-matter and water-stable aggregates in soils. *Journal of Soil Science* 33: 141–163.

Treseder KK, Allen MF. 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO2 and nitrogen deposition. *New Phytologist* 147: 189–200.

Treseder KK, Vitousek PM. 2001a. Potential ecosystem-level effects of genetic variation among populations of *Metrosideros polymorpha* from a soil fertility gradient in Hawaii. *Oecologia* 126: 266–275.

Treseder KK, Vitousek PM. 2001b. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* 82: 946–954.

Treseder KK, Farrington H. 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. *Biogeochemistry* 37: 63–75.

Vitousek PM, Walker LR, Whitaker LD, Matson PA. 1993. Nutrient limitation to plant growth during primary succession in Hawaii Volcanoes National Park. *Ecology* 23: 197–215.

Vitousek PM, Farrington H. 1997. Nutrient limitation and soil development: experimental test of a biogeochemical theory. *Biogeochemistry* 37: 63–75.