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Arbuscular mycorrhizal inoculum potential: a mechanism promoting positive diversity–invasibility relationships in mountain beech forests in New Zealand?

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Abstract Mycorrhizal fungi are important symbionts for the majority of plant species, but their role in determining the susceptibility of habitat to plant invasion is poorly understood. *Hieracium lepidulum* is an arbuscular mycorrhizal herb, currently invading the understorey of ectomycorrhizal *Nothofagus solandri* var. *cliffortioides* (mountain beech) forest in New Zealand. Mountain beech is solely ectomycorrhizal, and other plant species within the understorey occur sporadically. *Hieracium* has been shown to establish preferentially in microsites with higher plant species richness at a scale of less than 1 m^2 within mountain beech forest, and we tested the hypothesis that more diverse microsites (<1 m^2) are associated with higher levels of arbuscular mycorrhizal fungal (AMF) inoculum. We found low levels of AMF inoculum across all microsites, and over a third of samples contained no inoculum at all. Higher vascular-plant species richness (but not biomass) was associated with higher AMF spore densities in field soil, and greater AMF colonization of *H. lepidulum* seedlings in a bioassay. Absence of AMF inoculum from much of the soil and the positive association of inoculum potential with species richness provide a potential mechanism for the establishment of a positive diversity–invasibility relationship in the mountain beech forest.

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Introduction

The influence of biodiversity on habitat susceptibility to invasion, the "diversity–invasibility relationship," is often regarded as paradoxical due to opposing patterns at different spatial scales (Fridley et al. 2007). At larger spatial scales (>10–100 m^2), diversity–invasibility relationships are often observed to be positive (e.g. Stohlgren et al. 1999, 2005; Brown and Peet 2003), a correlation that may be attributed to "biotic acceptance": some sites being intrinsically better for the establishment, growth, survival, and reproduction of plant species, both exotic and native, causing native species richness and exotic invasion success to be greater at such sites (Stohlgren et al. 2006). At small scales (<1 m^2), traditional niche theory predicts a negative relationship because higher diversity will lead to greater complementarity of resource use and hence greater resistance to the establishment of new species (Darwin 1859; Elton 1958; MacArthur and Wilson 1967). However, many observational studies reveal a positive diversity–invasibility relationship even at small scales (e.g. Wiser et al. 1998; Sax 2002; Keeley et al. 2003). This could arise indirectly from exotic and native species clustering at sites favorable for establishment or from facilitation of exotic establishment by resident biodiversity.

Mycorrhizal fungi are of great importance in nutrient acquisition for the majority of the world's flora (Smith and Read 1997) and are potential intermediaries of facilitative interactions between plants (Simard and Durall 2004). The compatibility of plant species with particular groups of mycorrhizal fungi (e.g., arbuscular (AMF),

ericoid) could influence their likelihood of invasion into different habitats. For example, local dominance of ectomycorrhizal fungi could exclude the establishment of arbuscular mycorrhizal plants: a mechanism that can contribute to tropical monodominance (Torti et al. 2001) and influence forest succession (Kovacic et al. 1984; Weber et al. 2005). Invader-induced changes to mycorrhizal fungal communities are increasingly implicated in positive feedback cycles that may promote plant invasion (e.g., Wolfe et al. 2008; Vogelsang and Bever 2009), but the role of resident arbuscular mycorrhiza in the exclusion and facilitation of exotic species colonization is largely unknown.

Mountain beech [*Nothofagus solandri* var. *cliffortioides* (Hook.f.) Poole] forests in New Zealand present an excellent system for the study of AMF communities within an ectomycorrhizal-dominated habitat because the tree is exclusively ectomycorrhizal (Baylis 1980) and naturally forms extensive monospecific stands (Wardle 2002). Mountain beech forests have low plant diversity with patchy vegetative cover in the understorey: many localities have an understorey comprised solely of mountain beech seedlings and saplings, while others have a more diverse assemblage. For example, measurements across 250 mountain beech forest plots (each of 400 m^2) revealed that 59% of the 6,000 subplots (0.75 m^2) within the plots have no species present, other than mountain beech itself. These forests are being invaded by *Hieracium lepidulum* (Stenstr.) *Omang* (Asteraceae), a functionally obligate AM-forming herb (Harley and Harley 1987). The presence of *H. lepidulum* shows a strong positive association with plant species richness in 0.75 m^2 plots within the forest, even after accounting for environmental variables such as altitude, soil nutrients, aspect, exposure, and distance to forest edge (Wiser et al. 1998). *H. lepidulum* and other *Hieracium* spp. are widespread, abundant, and pernicious weeds in the grassland habitats that surround the forest (Rose et al. 1995)—a predominantly arbuscular mycorrhizal habitat. We propose that *H. lepidulum* invasion into mountain beech forest is limited because it is unable to establish where little or no AMF inoculum exists. We test the hypotheses that higher species richness in understorey microsites (<1 m^2) is associated with greater AMF inoculum potential and that greater AMF inoculum potential promotes the establishment and growth of *Hieracium*, hence creating a positive diversity–invasibility relationship.

Materials and methods

Soil was collected over 1 week in November 2006 from ten sites along creeks within mountain beech forest in the Craigieburn Forest Park, eastern Southern Alps, New Zealand (43°09″S, 171°42″E, elevation 850–1,150 m). The sites were selected randomly as a random distance along each of ten randomly selected creeks that fell within forested habitat in the study area. Each site was 10×10 m and ran parallel to, and 5 m from, the creek edge. We sampled along creek edges, as these represent the most frequent habitats for *Hieracium* invasion in *Nothofagus* forest. Within each site, we sampled soil from four 1 m^2 plots differing in species richness (0, 1–2, 3–5, or >5 vascular plant species other than mountain beech) within each site. We also sampled a fifth 1 m^2 plot at random distances between 40 and 55 m into the forest from the creek; this was to provide a soil sample well away from the creek—a region that might have elevated AMF inoculum levels due to dispersal by water (Gemma and Koske 1992). A 10×10×10 cm cube of soil was removed from the center of each plot and placed intact into a zip-lock bag. Trowels were cleaned between collections using 90% ethanol. Soil was stored at 4°C within 8 h. The following variables were measured for each plot: vascular plant species richness; above-ground plant biomass of shoots associated with the cube of collected soil; distance to creek; soil water content; and soil pH (2:1 water:soil extraction). Four different measures of species richness were taken: total or AM-forming only (Brundrett 2009), within 0.03 or 0.75 m^2 of the soil collection point; and two different measures of above-ground plant biomass: total and AM-forming only within 0.03 m^2. The main reason for quantifying above-ground plant biomass in only the smaller area was to minimize destruction to the understorey plant communities in the area.

Arbuscular mycorrhizal inoculum potential of soils was quantified in two complementary ways: (1) as spore density in field soil and (2) as colonization levels of *H. lepidulum* seedlings in an ex situ bioassay. Live AMF spores were extracted from 20-g subsamples of field soil using a standard wet-sieving and sucrose density centrifugation technique (Daniels et al. 1982): the soil, including roots, was initially blended, the sediment allowed to settle, and then the liquid poured through a sieve stack with mesh sizes 500, 150, and 53 μm. Material from the 150 and 53 μm sieves was collected separately for centrifugation and subsequent spore extraction. Viable spores were isolated and counted on a grid-lined dish under a stereo microscope (Leica MZ12.5), spores were deemed "viable" if they were shiny, smooth, translucent, and had no obvious signs of parasitization.

For the bioassay, a sterile seedling was planted into field soil (60 ml) from each plot, within 24 h of soil collection. The soil had been homogenized by hand-mixing and chopping all roots into fragments smaller than 1 cm, and the seedling was grown for 60 days before harvesting

Harvested roots were cleared and stained using a standard method (Phillips and Hayman 1970), and percentage of root length colonized by AMF (total and arbuscules and vesicles only) was quantified using a modified gridline intersect method (McGonigle et al. 1990) at ×200 magnification (Leica DM 1000). To test our second hypothesis, we measured the biomass and foliar N and P concentrations of harvested seedlings to assess *H. lepidulum* growth response to mycorrhizal colonization.

The factors affecting AMF inoculum potential were investigated using mixed-effects regression modelling. Eleven alternative maximal models were compared using AIC_c and Akaike weights, w_i, with explanatory variable structure having either a measure of both plant species richness (total or AM-forming in 0.03 or 0.75 m^2) and above-ground plant biomass (total or AM-forming in 0.03 m^2) or only one, or neither of these (see Table 1), along with the other plot-level environmental variables. Site was included as a random effect with plot nested within site, but was not found to have any significant effect on the three response variables. The best fitting model was simplified by stepwise removal of variables until those remaining all explained significant variation in the response. Response variables were spore density [$\log_{10}(y+1)$ transformed, with normal errors] and AMF colonization of seedlings from the bioassay (total colonization and arbuscules and vesicles only, both modelled with binomial errors). Stepwise regression was repeated to ascertain the factors explaining significant variation in *H. lepidulum* seedling growth response to AMF colonization. All analyses were conducted using the lme4 library (Bates and Maechler 2009) of R (R Development Core Team 2009).

Results

AMF spore density ranged from 0 to 69.2 spores/g of dry soil but was generally low (2.1±1.4, mean ± SE, n=50), and 38% of samples harbored no viable spores. The best supported alternative model of spore density contained total plant species richness within 0.03 m^2 as a positive predictor, but not plant biomass (Table 1; Fig. 1a), and species richness was the only variable retained in the minimal adequate model (Table 2).

Total AMF colonization of *H. lepidulum* seedlings in the bioassay ranged from 0 to 30% of root length (5.7±1.1, n=48), and 38% of seedlings showed no signs of colonization. Two alternative models of total AMF colonization were supported equally and contained, as a positive predictor, AM-forming plant species richness within 0.75 m^2 either with, or without, AM-forming plant biomass (Table 1; Fig. 1b). However, plant biomass was not retained in the minimal adequate model, in which higher colonization levels were also significantly associated with lower pH, and lower soil water content (Table 2). Occurrence of arbuscules and vesicles in harvested seedlings ranged from 0 to 18% of root length (0.87±0.4, n=48). The best supported alternative model of arbuscule–vesicle levels contained AM-forming plant species richness within 0.75 m^2 and AM-forming plant biomass (Table 1) as positive predictors. These variables were retained in the minimal adequate model in which higher arbuscule and vesicle levels were also significantly associated with lower soil water content (Table 2). There was no correlation between spore density and AMF colonization levels.

Biomass of harvested seedlings in the bioassay was not influenced by any measure of mycorrhizal inoculum

Table 1 Strength of evidence (ΔAIC_c and w_i) for alternative models fitted to the three measures of mycorrhizal inoculum potential

Plant species richness measure	Above-ground plant biomass measure	Spore density		AMF colonization (%)		Arbuscules and vesicles (%)	
		ΔAIC_c	w_i	ΔAIC_c	w_i	ΔAIC_c	w_i
Total in 0.03 m^2	Total in 0.03 m^2	1.4	0.25	41	0	12	0
Total in 0.75 m^2	Total in 0.03 m^2	9	0.01	33	0	4	0.1
AM-forming in 0.03 m^2	AM-forming in 0.03 m^2	4	0.07	16	0	12	0
AM-forming in 0.75 m^2	AM-forming in 0.03 m^2	8	0.01	0.4	0.46	0	0.88
Total in 0.03 m^2	None	0	0.49	53	0	45	0
Total in 0.75 m^2	None	7	0.02	38	0	33	0
AM-forming in 0.03 m^2	None	3	0.12	20	0	45	0
AM-forming in 0.75 m^2	None	5	0.04	0	0.54	20	0
None	Total in 0.03 m^2	15	0	41	0	10	0.01
None	AM-forming in 0.03 m^2	15	0	40	0	10	0.01
None	None	12	0	57	0	45	0

The best supported models for each measure of mycorrhizal inoculum potential are underlined

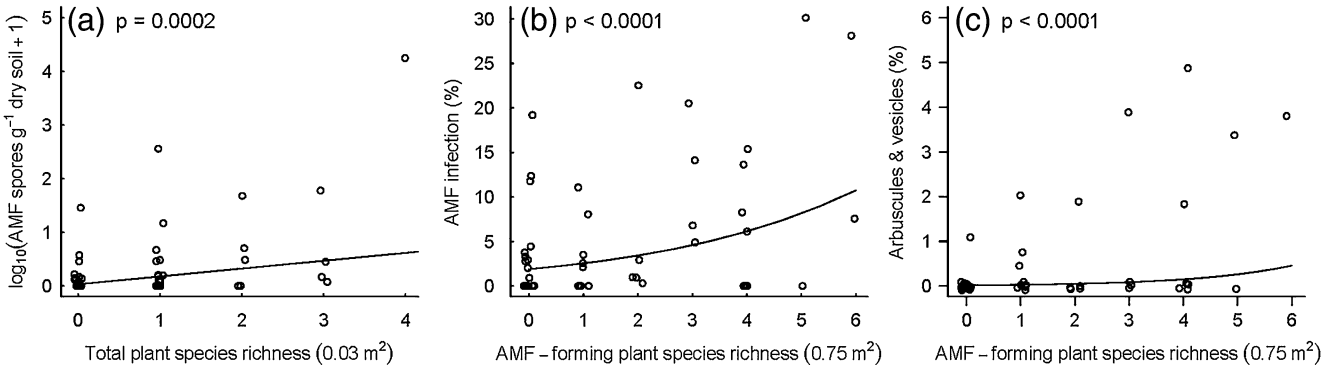

Fig. 1 Relationship with plant species richness in best-supported model for **a** spore density of field soil, **b** AMF colonization, and **c** arbuscule and vesicle occurrence in bioassay seedlings. A small amount of noise has been added to the measurement to separate overlapping data points

potential, but was positively associated with larger initial seedling weight (t=2.4, P=0.015) and lower soil water content (t=2.8, P=0.006). Foliar N concentrations were not significantly related to any measured variable; however, foliar P concentrations were negatively correlated with the degree of arbuscule and vesicle colonization of roots (t=2.2, P=0.028).

Discussion

Low levels of arbuscular mycorrhizal inoculum were found in this study, indicating that ectomycorrhizal dominance of mountain beech may suppress the development of AMF communities, as has been observed in other forest ecosystems (Kovacic et al. 1984; Weber et al. 2005). Our data indicate AMF inoculum is spatially variable not only in quantity but also in presence, as over a third of samples had no inoculum when measured either as spore density or mycorrhizal colonization of roots in the bioassay. Hence, the establishment of obligate arbuscular mycorrhizal plants within the mountain beech forest could be limited to the subset of sites with AMF inoculum present, analogous to the facilitation of plant establishment by discrete foci of ectomycorrhizal fungi at a small spatial scale (Baylis 1980; Dickie et al. 2005).

The data support our hypothesis that higher AMF inoculum potential is associated with higher plant species richness at a small spatial scale (<1 m^2). Our observed positive correlation between spore density and plant species richness in the field is similar to those of a number of studies in artificial communities (Johnson et al. 2005). Further, we show that this influences mycorrhizal colonization of bioassay seedlings. This could arise through positive feedback between diversities of plant and mycorrhizal communities (van der Heijden et al. 1998a; Johnson et al. 2005), and with a more diverse community of fungi present, the probability of an invasive plant encountering compatible symbionts is likely to increase. Alternatively, there could be a "sampling effect," the reverse of that usually cited in experiments of the diversity–invasibility relationship (Wardle 2001) such that particular plant species may have a disproportionate effect in increasing local inoculum potential, and the probability of these strong facilitators being present is higher in more diverse plant communities. AM-forming plant species richness is a better predictor than total species richness of root colonization levels in our bioassay, showing that plant species identity has a significant influence on inoculum potential.

There was no observable effect of AMF colonization on the growth of seedlings in our bioassay. *H. lepidulum* is a

Table 2 Parameter estimates and standard errors for explanatory variables in the minimal adequate models fitted to three measures of arbuscular mycorrhizal inoculum potential: spore density [back transformation: 10^x-1]; AMF colonization; and arbuscule and vesicle occurrence [back transformation: $(1+e^{-x})^{-1}$]

Explanatory variable	Spore density	AMF colonization (%)	Arbuscules and vesicles (%)
Plant species richness (total in 0.03 m^2)	0.15±0.04***		
Plant species richness (AM-forming in 0.75 m^2)		0.33±0.03***	0.56±0.12***
Above-ground plant biomass (AM-forming in 0.03 m^2)			0.88±0.18***
pH		−0.41±0.16*	
Soil water content		−0.14±0.05**	−1.46±0.32***
Intercept	0.03±0.07	−3.98±0.37	−8.29±0.73

Significance levels: *p=0.05–0.01; **p=0.01–0.001, ***p<0.001

slow-growing perennial herb, and nutrient levels in mountain beech forest soils are low. Therefore, the absence of a growth response is likely to be due to the short growth period of 60 days—necessary to accurately reflect mycorrhizal inoculum potential of the field soil (Brundrett 1991). Support for this comes from the positive correlation between harvested biomass and initial seedling weight. Foliar P concentrations were significantly lower in plants with a greater occurrence of arbuscules and vesicles, which may reflect the cost of establishing the symbiosis. A "growth depression" may be a precursor to the positive effect of growth caused by AMF colonization (Buwalda and Goh 1982; Koide 1985), and significantly lower P concentrations in AM-colonized plants, relative to uncolonized plants, has been observed for a period of 7 weeks in soybean (Bethlenfalvay et al. 1982). Therefore, our results are inconclusive as regards the second hypothesis. However, there is evidence from literature for a strong mycorrhizal dependency for other species in the *Hieracium* genus (Grime et al. 1987; van der Heijden et al. 1998a, b) and significantly greater establishment rates, leaf cover, and biomass of *H. lepidulum* plants grown with AMF inoculum relative to those grown without AMF inoculum (Downs and Radford 2005).

In conclusion, we show significant variation in levels of AMF inoculum within mountain beech forest soil at a scale of <1 m^2, and microsites with higher species richness have significantly higher AMF inoculum potential. Although further experimentation is necessary to establish the growth response of *H. lepidulum* to AMF colonization in mountain beech forest soils, this provides a potential mechanism for the establishment of a positive diversity–invasibility relationship for those exotic species, including *H. lepidulum*, that are highly dependent on AMF symbionts.

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