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REPORT

Terrestrial vertebrates promote arbuscular mycorrhizal fungal diversity and inoculum potential in a rain forest soil

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Abstract

We examined whether terrestrial vertebrates affected the arbuscular mycorrhizal fungal spore communities and mycorrhizal inoculum potential (MIP) of a tropical rain forest soil by comparing plots where terrestrial vertebrates had been excluded for 3 years to adjacent control plots. We extracted spores from soil using sucrose density gradient centrifugation and assayed MIP by growing seedlings of maize (*Zea mays*) and a rain forest tree (*Flindersia brayleana*) in intact soil cores from exclosure and control plots. Control plots had significantly higher spore abundance, species richness and diversity than exclosures. Spore community composition also differed significantly between exclosure and control plots. Seedlings of both plant species grown in control cores had significantly higher arbuscular-mycorrhizal colonization than those grown in exclosure cores. This study suggests that loss of vertebrates could alter rates of mycorrhizal colonization with consequences for community and ecosystem properties.

Keywords

Arbuscular mycorrhizal fungi, herbivory, mycophagy, mycorrhizal inoculum potential, rodent, rain forest, spore dispersal, terrestrial vertebrate.

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INTRODUCTION

Most terrestrial plant species form mutualistic associations with mycorrhizal fungi that enhance the uptake of mineral nutrients, frequently resulting in improved plant growth and survival (Smith & Read 1997). Variation in the dynamics of this symbiosis can also affect community and ecosystem properties such as plant competition (Allen & Allen 1984; Hetrick *et al.* 1989; Bever *et al.* 1997), plant community diversity (Grime *et al.* 1987; van der Heijden *et al.* 1998a,b; Hartnett & Wilson 1999), ecosystem productivity (Klironomos *et al.* 2000), and succession (Janos 1980). Despite the key roles that mycorrhizal fungi play in ecosystems, comparatively little is known about the biotic factors that affect mycorrhizal fungal abundance and community dynamics in natural systems.

Terrestrial vertebrates may directly or indirectly affect mycorrhizal fungi through their foraging activities. For example, mycophagous mammals could directly affect mycorrhizal fungal spore abundance and diversity by ingesting spores and passing them in their faeces (e.g. Maser *et al.* 1978; Claridge & May 1994; Janos *et al.* 1995; Johnson 1996; Reddell *et al.* 1997; Mangan & Adler 2000). Spores of two major types of mycorrhizal fungi, the arbuscular mycorrhizal fungi (AM) and the ectomycorrhizal fungi (EM), have been recorded in the faeces of mammals from Australia, North America, Central America and South America (e.g. Mangan & Adler 2000; Fogel & Trappe 1978; Maser *et al.* 1978; Claridge & May 1994; McGee & Baczocha 1994; Janos *et al.* 1995; Johnson 1996; Reddell *et al.* 1997). Many studies demonstrated that mycorrhizal fungal spores remained viable following passage through the mammalian gut (e.g. Trappe & Maser 1976; Rothwell & Holt 1978; Allen & MacMahon 1988; Claridge *et al.* 1992), and in some cases spore germination was enhanced (e.g. Cork & Kenagy 1989; Claridge *et al.* 1992). Mammals have also been demonstrated to be key vectors of mycorrhizal spores following extreme disturbance. For example, incidental ingestion of spores by elk was important for the reestablishment of mycorrhizal fungal propagules following the eruption of the Mt. Saint Helen's volcano (Allen 1987).

Alternatively, terrestrial vertebrates may alter spore production or mycorrhizal inoculum potential (MIP) through indirect effects on plants or soil nutrients. Herbivory by vertebrates could alter levels of mycorrhizal colonization on browsed or grazed plants (e.g. Rossow *et al.* 1997), and

thereby indirectly affect inoculum in the soil. Input of nitrogen via urine and faeces may increase soil nutrients, which could result in localized reductions in mycorrhizal colonization (Smith & Read 1997), while vertebrate foraging activities such as digging could alter inoculum distribution. Finally, selective herbivory could lead to shifts in host plant community composition that in turn alter mycorrhizal abundance and diversity. Ultimately, the importance of direct and indirect effects of vertebrates on mycorrhizal inoculum potential depends upon the magnitude of vertebrate effects relative to other factors that influence natural sources of inoculum (Janos *et al.* 1995; Johnson 1996).

In this study, we tested experimentally the importance of terrestrial vertebrates to arbuscular mycorrhizal fungal spore abundance, spore community composition and the inoculum potential of an Australian rain forest soil by fencing field plots to exclude ground-dwelling vertebrates for 3 years. These plots were compared with control plots where vertebrates had unrestricted access. The extant, native mammalian fauna of Australia is depauperate compared to other tropical regions of the world, and is especially lacking in herbivorous mammals greater than 10 kg in size (Connell *et al.* in press). All of the mammal species and two of the bird species could potentially disperse spores of arbuscular mycorrhizal fungi (McGee & Baczocha 1994; Reddell *et al.* 1997). We tested three hypotheses: (1) fungal spore abundance and diversity will be lower in plots where terrestrial vertebrates have been excluded, (2) the mycorrhizal inoculum potential of soil from terrestrial vertebrate exclosure plots will be reduced relative to that of control plots, and (3) lower levels of mycorrhizal inoculum potential in soils where vertebrates have been excluded will result in reduced seedling growth.

MATERIALS AND METHODS

Study site

This study was conducted in 4 ha of primary rain forest in the Lamb Range of north-eastern Queensland, Australia (17°05′ S, 145°34′ E and approximately 850 m elevation) (Connell *et al.* 1984). Annual rainfall at the site averages ~3000 mm, most of which falls during a December–April wet season (Connell & Green 2000). Average annual maximum and minimum temperatures beneath the canopy are 22.1 °C and 15.2 °C, respectively (Connell & Green 2000). The forest canopy is 30–35 m tall, with emergent *Agathis atropurpurea* on the upper slope (Connell & Green 2000). The study site was set aside as an undisturbed research plot by the Queensland Department of Forestry and has thus been protected from logging (Volck 1968; Nicholson *et al.* 1988). The entire forested mountain range is currently protected as part of a larger World Heritage Area.

Soil at the study site is derived from deeply weathered medium to coarse grey biotite granite (Willmott *et al.* 1988). Soils derived from similar parent material in the area (Severin series) are well drained with dark brown sandy clay loam A1 horizons, and unbleached A2 horizons overlying yellowish brown sandy clay B horizons (Laffan 1988). The soils are acidic (mean pH = 4.06 ± 0.098 SE) and low in nutrients (0.47% ± 0.031 SE percentage total Kjehldahl N and 6.79 ± 1.028 mg P/kg soil using the methods of Rayment & Higginson 1992).

Vertebrate exclosures

In 1996, 16 vertebrate exclosures were constructed in the vicinity of a 1.7-ha long-term study plot where seedling and tree dynamics have been monitored by J.H. Connell and colleagues since 1963 (described in detail by Connell *et al.* (1984) and Connell & Green (2000)). Each exclosure was 6.5 by 7.0 m in size and paired with a nearby control plot of the same size that was fenced only along the up-slope side to control for the effects of litter washing downhill during heavy rains. Fencing was constructed of poultry netting supported by metal fence stakes to a height of 1 m, topped by a 0.3-m sheet of galvanized metal flashing. At the bottom of the fence, the poultry netting extended along the ground 0.5 m, and was held in place with metal pegs that effectively prevented vertebrates from burrowing beneath the fence. This exclosure design significantly reduced or eliminated access by ground-dwelling mammals as evidenced by live-trapping and track monitoring. The bush rat (*Rattus fuscipes*) and the long-nosed bandicoot (*Perameles nasuta*) were commonly captured in the control plots, but were never captured inside of the exclosures. The semi-arboreal fawn-footed melomys (*Melomys cervinipes*) and white-tailed rat (*Uromys caudimaculatus*) occasionally entered some exclosure plots, but their capture rates averaged seven-fold lower inside the exclosures than on control plots. The musky rat kangaroo (*Hypsiprymnodon moschatus*), and the red-legged pademelon (*Thylogale stigmatica*) were too large to be captured in our traps, but observation of tracks indicated that these animals did not enter the exclosure plots. Estimates of leaf litter disturbance and observations of tracks indicated that the exclosures also completely excluded the southern cassowary (*Casuarius casuarius*), and reduced access by two other ground-feeding bird species, the brush turkey (*Alectura lathami*), and the chowchilla (*Orthonyx spaldingii*).

Seedling recruitment, growth, and survival were monitored at 6 month intervals for 3 years after exclosures were constructed (Theimer and Gehring, unpublished data.). During three of the semi-annual censuses, we recorded the number of plants experiencing leaf loss or leaf damage attributable to insects, vertebrates, and fungal pathogens. These measures allowed us to estimate the levels of

herbivory experienced by seedlings surviving on plots, but we could not attribute missing seedlings to any specific mortality agent. Missing seedlings may have died for several reasons, including herbivory or uprooting and burial by litter-disturbing birds and mammals. This indirect mortality caused by litter-disturbing animals is an important cause of mortality for seedlings on the study plot (Theimer & Gehring 1999).

Fungal spore abundance and diversity

To quantify arbuscular mycorrhizal fungal spore abundance and diversity, we collected two replicate soil cores from 13 exclosure-control pairs in June 2001. The two cores from each of the 26 plots were homogenized, and then a 25-g sample was removed for analysis. Spores were extracted from soil using wet sieving and sucrose density gradient centrifugation (Johnson *et al.* 1999). The extracted spores were observed under a compound microscope and identified to species where possible using current taxonomic criteria (Schenck & Pérez 1990, INVAM – http://invam.caf.wvu.edu/). The relative abundance of each species (number of spores per gram dry weight of soil) was estimated based on spore counts. Spore abundance, species richness (the number of species in a sample) and Brillouin's diversity (*H*) values for control and exclosure plots were compared using paired *t*-tests in SPSS version 8.0 (SPSS 1997). Brillouin's index of diversity was selected because it does not assume an equal probability of encounter of species, an important consideration given that the propensity of AM fungi to sporulate may vary among species exposed to the same environmental conditions (Eom *et al.* 2001). Spore community composition of exclosure and control plots was compared using Multi-Response Permutation Procedure (MRPP), a nonparametric discriminant analysis (McClune & Mefford 1999). This method was chosen to accommodate the nonparametric and interdependent nature of community response data. MRPP uses the species abundance data in each plot to calculate a measure of distance or dissimilarity between that plot and every other plot measured; re-sampling statistics are then employed to calculate a probability (*P*) that differences between groups of plots (in this case, control and exclosure) are due to chance alone (Zimmerman *et al.* 1985). Significant differences in community composition were followed by Indicator Species Analysis (McClune & Mefford 1999) to determine if differences in the frequency and relative abundance of certain species drive the differences between groups (Dufrene & Legendre 1997).

Mycorrhizal inoculum potential experiments

Two experiments were carried out to determine if terrestrial vertebrates influenced the mycorrhizal inoculum potential (MIP) of rain forest soils. The first experiment compared the MIP of a standard bioassay plant, *Zea mays*, grown at moderately high light in soil cores collected from exclosure and control plots. To determine whether differences observed in maize were relevant to natural rain forest conditions, a second experiment compared the MIP of a rain forest seedling, *Flindersia brayleana*, grown at understorey light conditions in soil cores collected from exclosure and control plots. In both experiments, the MIP of the soil was determined by growing bait plants in intact cores of soil and measuring the level of mycorrhizal colonization. We employed this method because it allows measurement of mycorrhizal colonization via hyphal fragments present in the soil core, as well as via spores (Brundrett *et al.* 1996). Other methods, such as most probable number assays using soil dilutions, measure primarily the potential for mycorrhizal colonization due to spores alone (Brundrett *et al.* 1996), and may not reflect the full inoculum potential of a soil.

For experiment 1, we collected two intact soil cores (7 × 20 cm) from each of 13 exclosure and 13 control plots in October 1999. The 13 pairs were chosen from the 16 available based on which exclosure plots had been most effective at excluding vertebrates as indicated by live-trap, track and litter disturbance estimates. Cores were transferred directly from the corer into an appropriately sized pot, labelled, and sealed in a plastic bag for transport to a glasshouse at the CSIRO Tropical Forest Research Centre in Atherton, Queensland. The following day, a single surface-sterilized (10% bleach for 30 min) organic maize seed was placed on top of each core and then covered with *c.* 3 cm of sterilized vermiculite to prevent drying. Pots were located at least 15 cm away from one another to reduce the possibility of cross-contamination. Light levels in the glasshouse were approximately 25% PAR and temperatures varied from a minimum of 14 °C to a maximum of 30 °C. Seedlings were watered daily using tap water.

Five weeks after germination, the maize seedlings were sacrificed, separated into shoots and roots and weighed to the nearest mg. Maize plants were grown for only 5 weeks because previous research has shown that the level of root colonization by mycorrhizal fungi is linearly related to soil densities of AM fungal propagules early in the colonization process (e.g. Smith & Walker 1981). A portion of the root system (*c.* 25%) of each plant was analysed for mycorrhizal colonization by clearing in 10% potassium hydroxide for 30 min at 90 °C, bleaching in alkaline hydrogen peroxide for 3 min and staining in trypan blue in lactoglycerol overnight. Roots were de-stained in 50% glycerol and 1 cm segments of root were viewed under a compound microscope at 200× magnification (McGonigle *et al.* 1990). Mycorrhizal colonization was estimated by scoring the presence or absence of mycorrhizal fungal structures at each intersection of root and reticle line for a minimum of 100

intersections per plant. An intersection was considered mycorrhizal if the reticle intersected an internal hypha, an intercellular coil, an arbuscule or a vesicle. Values obtained from the two replicate plants per exclosure or control were averaged prior to statistical analyses. Five maize seedlings were grown in steam-sterilized field soil (20 min at 120 °C) as a control for contamination by mycorrhizal inoculum present in the glasshouse.

For experiment 2, two replicate intact soil cores (7 × 20 cm) were collected in January 2000 as in experiment 1, but only 10 of the exclosure and control pairs were sampled. As before, cores were immediately transferred into an appropriately sized pot, labelled, bagged and returned to the lab. The following day, a single surface-sterilized *Flindersia brayleana* seed was placed on each core and allowed to germinate. *Flindersia brayleana* (Rutaceae) is a canopy tree found at a range of elevations in well-developed rain forests in Queensland (Hyland & Whiffin 1993), with small, wind-dispersed seeds (average dry mass in this study = 0.077 g). We chose this species because it forms AM associations under field and glasshouse conditions (C. Gehring, unpublished data). In contrast to the maize bioassay, which was intended to represent standard conditions used in studies of MIP, *F. brayleana* seedlings were grown in light conditions that mimicked the natural light intensities of the shaded understorey of the rain forest (3% PAR). This was accomplished by placing four seedlings, two from each control and exclosure pair, in individual shadecloth frames. Each frame was a single layer of 90% dark green shadecloth (Coolaroo Brand, Gale Pacific, Melbourne, Victoria, Australia) sewn onto a 70 × 45 × 30 cm wire support structure. The light intensity within each shadecloth frame was verified to average 3.5% PAR using a Decagon Model SF80 Sunfleck Ceptometer (Decagon Devices, Inc. Pullman, Washington, USA). Seedlings were rotated within the frames on a bi-weekly basis to reduce any position effects and always positioned at least 15 cm apart. As with maize, five *F. brayleana* seedlings were grown in steam-sterilized field soil as a control for contamination by mycorrhizal inoculum present in the glasshouse.

Because preliminary observations indicated that the formation of mycorrhizal associations takes longer in the low light conditions of the shaded understorey, *F. brayleana* seedlings were grown for 16 weeks prior to sacrifice for root and shoot biomass and mycorrhizal colonization measurements. *F. brayleana* seedlings were analysed as for maize with two exceptions. First, mycorrhizal colonization was quantified for the entire root system due to its small size. Second, time to clear and bleach the roots was increased (1 h in 10% potassium hydroxide and 10 min in alkaline hydrogen peroxide, respectively). Microscopic observations of root colonization by AM fungi were made as for the maize bioassay. Data on maize and *F. brayleana* mycorrhizal colonization and seedling shoot biomass were analysed using paired *t*-tests. Due to lack of normality, mycorrhizal colonization data were arcsine square root transformed and spore abundance data were natural log transformed prior to analysis. Non-transformed data are presented in the figures.

RESULTS

Spore abundance and diversity

Mean spore abundance and spore species richness were significantly higher in soils from control plots than in soils from terrestrial vertebrate exclosure plots ($t = -3.008$, $P = 0.011$, d.f. = 12 for abundance, and $t = -3.606$, $P = 0.004$, d.f. = 12 for species richness) (Fig. 1). Brillouin's diversity (H) was also 30% higher in control plots than exclosure plots (mean $H \pm 1$ SE for controls = 1.003 ± 0.121 and for exclosures = 0.7120 ± 0.122, $t = 3.902$, $P = 0.002$, d.f. = 12). The species composition of the spore communities also varied between exclosure and control plots, when analysed using blocked MRPP ($P = 0.012$, control/exclosure pairs constitute blocks). *Glomus rubiforme* (Gerd. & Trappe) Almeida & Schenck, a sporocarpic species, was a significant indicator of treatment differences ($P = 0.007$). *Glomus rubiforme* was observed in all control plots but in only 7 of the 13 exclosure plots and was twice as abundant on control plots as on exclosure plots (Table 1).

Fourteen species of AM fungal spores were observed in the 26 samples, with three uncommon species exclusively found in exclosure or control plots (Table 1). Several species showed differences in frequency of occurrence in exclosure vs. control plots and/or differences in abundance within the two plot types. For example, an unidentified species in the genus *Glomus* (designated *Gl.*1) was observed in nearly twice as many control plots as exclosure plots, yet its average density was similar within exclosure and control plots (Table 1).

Maize bioassay

Levels of AM colonization of maize grown in cores collected from the control plots averaged 37% higher than that of maize grown in cores collected from exclosure plots (mean ± 1 SE for controls = 19.009 ± 1.805, and for exclosures = 11.903 ± 0.951, $t = 3.347$, $P = 0.0029$, d.f. = 12). Maize seedlings grown in sterilized field soil were not colonized by AM fungi, indicating the absence of greenhouse contamination. The difference in mycorrhizal colonization between control and exclosure seedlings suggests that exclusion of terrestrial vertebrates reduced the MIP of this rain forest soil.

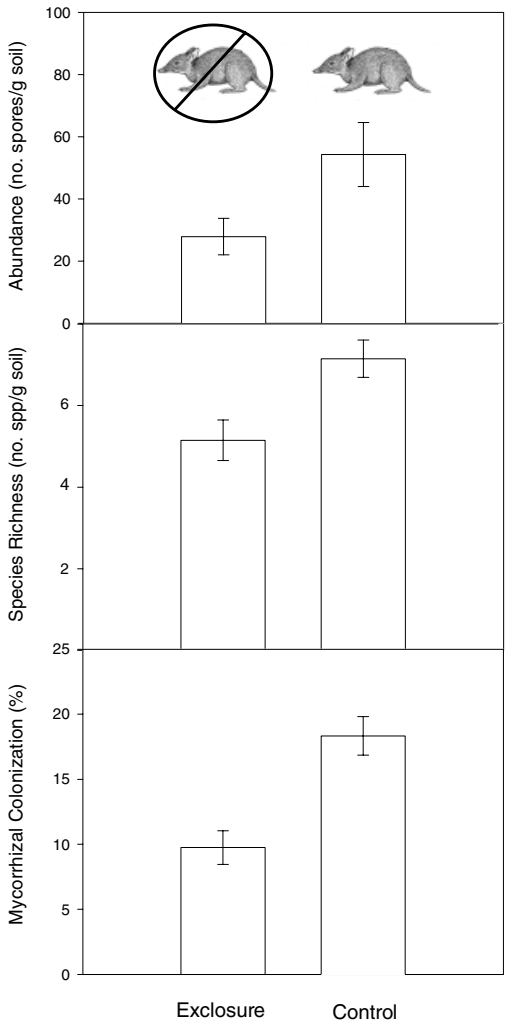

Figure 1 Arbuscular mycorrhizal fungal spore abundance (top panel) and species richness (centre panel) were significantly higher in plots where vertebrates had free access (controls) than in plots where vertebrates were excluded. These spore differences were associated with significantly higher mycorrhizal inoculum potential in soils from control plots (bottom panel). Mycorrhizal inoculum potential data are shown for a rain forest seedling (*Flindersia brayleana*), but similar results were obtained with maize, a standard bioassay plant (see text). Bars represent means ± 1 SE.

The differences in MIP between exclosure and control sites were correlated with differences in the shoot biomass of maize plants. Maize shoot biomass was significantly greater in the control plots than the exclosure plots (mean ± 1 SE for controls = 0.951 g ± 0.062 and for exclosures = 0.780 ± 0.062, $t = 2.458$, $P = 0.015$, d.f. = 12) suggesting that the differences in inoculum potential between the two site types may have influenced growth.

Flindersia brayleana bioassay

Consistent with our findings in maize, *F. brayleana* seedlings grown in cores from control plots had significantly higher levels of mycorrhizal colonization than seedlings grown in cores from exclosure plots (Fig. 1). As with maize, *F. brayleana* seedlings grown in sterilized field soil were not colonized by AM fungi, indicating the absence of significant greenhouse contamination. The difference between the two treatments was more dramatic for this species, with levels of mycorrhizal colonization nearly twice as high in the control plots than the exclosure plots ($t = –5.252$, $P = 0.00026$, d.f. = 9). These results indicate that the inoculum potential differences we observed in maize, a standard bioassay plant, were also found in a rain forest seedling grown at light intensities typical of the forest floor environment where seedlings establish. Despite large differences in mycorrhizal colonization, *F. brayleana* seedlings grown in soil from both exclosure and control plots were similar in shoot biomass (mean ± 1 SE for controls = 0.1289 g ± 0.011 and for exclosures = 0.1186 ± 0.011, $t = –1.064$, $P = 0.1573$, d.f. = 9).

DISCUSSION

Our results demonstrate that terrestrial vertebrates increase AM fungal spore diversity, abundance and inoculum potential. These patterns could be caused by either direct or indirect effects. Vertebrates could directly alter spore abundance and diversity through consumption of sporocarps (mycophagy) and subsequent defecation of ingested spores, or dispersal of spores through other activities including incidental ingestion, transport on feet or body, or soil disturbance. Previous studies demonstrated that terrestrial vertebrates were important vectors of mycorrhizal spores in habitats experiencing large-scale disturbance (e.g. elk (*Cervus elaphus roosevelti*) and pocket gophers (*Thomomys talpoides*) after the eruption of Mount St. Helens (Allen 1987); red-backed voles (*Clethrionomys gapperi*) into long-abandoned beaver meadows (Terwilliger & Pastor 1999)). Our findings suggest that vertebrates may help maintain fungal spore abundance and diversity in undisturbed rain forest areas as well.

Many of the mammal species excluded from our plots have the potential to disperse fungal spores in their faeces. Arbuscular mycorrhizal fungal spores were recorded in 25–61% of the scats examined from the three most common native rodent species, *M. cervinipes* (61%), *R. fuscipes* (54%), and *U. caudimaculatus* (25%) (Reddell *et al.* 1997). Spores extracted from these faeces also were effective in inoculating bioassay plants with mycorrhizal fungi (33–67% success) (Reddell *et al.* 1997). The majority of scats collected from the musky rat kangaroo (*H. moschatus*) and the long-nosed bandicoot (*P. nasuta*) also contained arbuscular mycorrhizal

Table 1 Size, density, relative abundance and frequency of AM fungal spores extracted from exclosure and control plots

AM fungal morphospecies	Mean spore size (μm)[1]	Mean density (spores/g soil)		Relative abundance[3] (%)		Frequency[4]	
		Exclosure	Control	Exclosure	Control	Exclosure	Control
Acaulospora morrowiae-like	105	2.70	5.20	9.76	9.61	11	13
A. myriocarpa-like	45	17.67	33.30	63.82	61.54	12	13
A. scrobiculata Trappe	120	0.04	0.07	0.15	0.13	1	2
A. spinosa Walker & Trappe	170	0.01	0.00	0.03	0.00	1	0
Archaeospora trappei Ames & Linderman	55	1.72	5.47	6.19	10.12	10	13
Gigaspora sp.	250	0.26	0.32	0.94	0.59	7	8
Glomus aggregatum Schenck & Smith	90	0.23	0.28	0.81	0.53	3	4
Gl. fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	80[2]	1.34	3.44	4.83	6.35	6	8
Gl. rubiforme (Gerd. & Trappe) Almeida &Schenck	110 × 180[2]	1.45	3.78	5.23	6.99	7	13
Gl. sp. 1	90	1.90	1.68	6.87	3.11	6	10
Gl. sp. 2	100	0.05	0.09	0.19	0.17	1	1
Gl. sp. 3	150	0.02	0.00	0.08	0.00	1	0
Scutellospora calospora-like	200	0.30	0.41	1.10	0.75	2	5
S. erythropa (Koske & Walker) Walker & Sanders	100 × 250	0.00	0.07	0.00	0.12	0	4

[1]Mean diameter (for spherical spores) or dimensions (of non-spherical spores) observed in this study.
[2]Denotes sporocarpic species.
[3]Relative abundance = (number of spores of the species/total spore numbers) ×100.
[4]Frequency = number of plots where species was observed (maximum = 13).

fungal spores, but those collected from *Perameles* were ineffective as mycorrhizal inoculum. Inoculation tests were not performed on scats from *Hypsiprymnodon* (Reddell *et al.* 1997).

Mammals such as these could alter MIP either by increasing the total amount of inoculum present, or by selectively dispersing the spores of a subset of fungal species into areas where they may not arrive via hyphae or other means. In our study, spores of a mycorrhizal species that forms macroscopic sporocarps showed striking differences in both frequency of occurrence and abundance across treatments, consistent with a hypothesis of selective dispersal. The faeces of rodents in the neotropics tend to be dominated by species of AM fungi that produce sporocarps (Janos *et al.* 1995; Mangan & Adler 2000), probably because these fungal species represent a more concentrated food resource. Sporocarpic fragments were occasionally or frequently observed in the faeces of the five species of Australian rain forest mammals common at the study site (Reddell *et al.* 1997). If fungal species favoured by mammals are significantly better at colonizing roots or affecting plant growth, this selective dispersal could have contributed to the inoculum potential differences between exclosure and control plots. Recent studies in both temperate (van der Heijden *et al.* 1998b) and tropical systems (Kiers *et al.* 2000) demonstrated that growth responses to mycorrhizal colonization varied depending upon which species of AM fungus was present.

Although mammals may be the most likely agents of spore dispersal, three species of birds (the southern cassowary, the brush turkey and the chowchilla) were also excluded from our exclosure plots. The southern cassowary is largely frugivorous, whereas the brush turkey and chowchilla feed on invertebrates, small vertebrates and other material gleaned from leaf litter. Spores of arbuscular mycorrhizal fungi were found in the faeces of cassowaries and brush turkeys (Reddell *et al.* 1997), and spores could potentially be carried on the feet of all three species, suggesting that they may move spores incidentally during their foraging activities.

The indirect effect of vertebrate exclusion on plant performance could also alter the inoculum potential of the soil. Grazing and browsing by vertebrates could alter mycorrhizal colonization levels or the propensity of fungi to sporulate. However, large, terrestrial herbivorous mammals are absent from the native Australian rain forest fauna, and only one species, the red-legged pademelon, is an

important seedling herbivore at our study site. These small kangaroos were relatively rare and during 3 years of study only 5% of seedlings on control plots showed leaf damage consistent with vertebrate browsing. Our results indicate that even in a system lacking large grazing or browsing herbivores, terrestrial vertebrates can affect the mycorrhizal inoculum potential of the soil.

Other effects of vertebrates on plant performance could alter mycorrhizal inoculum potential. In undisturbed habitats, the most important sources of inoculum may be those associated with living and dead roots (Janos *et al.* 1995). The rain forest we studied is characterized by a thick surface root mat (5–7 cm thick) that could provide abundant inoculum. However, an on-going study on our plot has demonstrated that mycorrhizal colonization did not differ between seedlings in field plots where the root mat had been removed compared to seedlings grown in an intact root mat. In fact, colonization was highest when the severed, dead root mat was replaced, suggesting that severed roots and hyphae act as greater sources of inoculum than living roots (Green and Gehring, unpublished data). If vertebrates increased the number of dead seedlings on control plots, or if vertebrate herbivory caused increased root mortality, control plot cores could have been better sources of inoculum for reasons independent of spore production and dispersal.

The effects of terrestrial vertebrates on plant community composition could also indirectly affect mycorrhizal spore communities and MIP. In our study, the exclusion of vertebrates led to a two-fold increase in seedling abundance, but no difference in seedling diversity between control and exclosure plots (Connell *et al.* in press; Theimer and Gehring, unpublished data). The main cause of the difference in seedling abundance was seed predation by rodents and early seedling mortality caused by litter-disturbing birds and omnivorous mammals (Theimer & Gehring 1999). Higher rates of seedling recruitment on exclosure plots could lead to increased inoculum from living plants. However, we documented the opposite pattern, with reduced inoculum potential on the exclosure plots despite higher seedling abundance. One important caveat of our study is that MIP was measured in plants growing in soil cores where inoculation by hyphae attached to living roots was not possible.

Vertebrates may have altered nutrient dynamics through input of faeces and urine, resulting in reduced mycorrhizal colonization and inoculum in the exclosures. The exclosures significantly reduced access by ground dwelling birds and omnivorous mammals like native rats and bandicoots, as well as herbivorous pademelons. However, arboreal mammals (possums) were unaffected by the exclosures and would have provided animal-generated nutrient inputs to both exclosure and control plots. These animals are arboreal folivores that rarely venture to the ground, and therefore would be unlikely to ingest fungal spores or carry them in their faeces. Preliminary analyses also showed no differences in nitrogen and phosphate levels in soil samples from control and exclosure plots (mean ± 1 SE total Kjehldahl nitrogen (%) in exclosures = 0.481 ± 0.003 and controls = 0.456 ± 0.005, $t = -0.366$, $P = 0.727$, d.f. = 7; mean ± 1 SE bicarbonate phosphate (mg/kg) in exclosures = 6.00 + 0.976 and controls = 7.57 + 1.849, $t = 1.658$, $P = 0.148$, d.f. = 7), suggesting that this mechanism may be less important than others.

The differences we observed in MIP were correlated with growth differences in the maize bioassay, but not in the *Flindersia* bioassay. Although the lack of an effect on short-term growth of *F. brayleana* could suggest that the differences in inoculum potential have no consequence for rain forest seedlings, we caution against this interpretation for two reasons. First, mycorrhizae may provide benefits to seedlings other than enhanced growth. For example, the ability of mycorrhizae to enhance resistance to root-borne pathogens can be more important than improved growth (e.g. Newsham *et al.* 1995). Second, growth responses to mycorrhizal colonization can appear slowly under low light conditions and longer studies may be necessary to examine the effect of mycorrhizal fungi on growth in rain forest plants.

Regardless of the specific mechanism, this study provides experimental evidence that the exclusion of terrestrial vertebrates reduced mycorrhizal fungal spore abundance, spore diversity and the inoculum potential of a rain forest soil. As rain forests become increasingly fragmented due to human activities, native vertebrates are among the first taxa to decline or disappear. For example, populations of *H. moschatus* and *O. spaldingii* were reduced or absent in smaller rain forest fragments in Australia (Laurance 1994; Warburton 1997). The impact of these declines on forest processes has often focused on the role of vertebrates as seed dispersers, seed predators and seedling herbivores (e.g. Dirzo & Miranda 1990; Wright *et al.* 2000), but this study suggests they can have important impacts on below-ground processes as well. Although native fauna may be replaced by non-native counterparts in many ecosystems, whether non-native animals would have the same effects are unknown. Also, given the small scale of these experiments in both space and time, the relatively large effects of vertebrate exclosure on spore abundance and diversity and mycorrhizal inoculum potential suggests that loss of vertebrates could have substantial effects on the mycorrhizal symbiosis.

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