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# Root colonization dynamics of two ectomycorrhizal fungi of contrasting life history strategies are mediated by addition of organic nutrient patches

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## Summary

- Here we investigated whether root colonization dynamics of ectomycorrhizal fungi (EMF) of contrasting life history strategies (i.e. early vs late successional dominants) were affected by resource availability, as mediated either directly via the soil, or indirectly via host nutrition.
- In a two phase experiment, *Pinus muricata* seedlings were co-inoculated with spores of early (*Rhizopogon occidentalis*) and late (*Tomentella sublilacina*) successional dominant EMF, with or without squirrel faecal pellets added as a nutrient source, in single chambers (Phase A) subsequently converted to split-root chambers (Phase B).
- *R. occidentalis* colonized seedlings earlier than *T. sublilacina*. *R. occidentalis* root tip numbers peaked then declined in both treatments, but earlier in the minus pellet treatment than the plus. *T. sublilacina* increased steadily regardless of treatment. In the split-root treatment, we found no response by *R. occidentalis*, and a complex response by *T. sublilacina*, suggesting that plant nutrition may affect colonization dynamics.
- The strategy of *R. occidentalis* may be to colonize roots early in high resource environments; whereas that of *T. sublilacina* may be based upon slower colonization rates and greater competitive ability. The effect of nutrient additions on *R. occidentalis* may be highly dependent upon their timing.

**Key words:** life history strategy, ectomycorrhizas, organic nutrients, faecal pellets and fertilization, succession, co-inoculation, *Rhizopogon occidentalis*, *Tomentella sublilacina*, *Pinus muricata*.

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## Introduction

Ectomycorrhizal fungal (EMF) communities are quite diverse, often being composed of over 50 species in a single forest stand (Horton & Bruns, 2001). Understanding the causes and consequences of this diversity is a major challenge for ecologists (Bruns, 1995). One factor influencing stand level diversity could be variation in response to disturbance. Post-disturbance or post-planting successions of ectomycorrhizal fungal species have been demonstrated in a variety of systems (Fleming, 1985; Fleming *et al.*, 1986; Gibson & Deacon, 1988; Visser, 1995; Baar *et al.*, 1999; Taylor & Bruns, 1999).

Shifting dominance of EMF species in successions may be linked to variation in their life history strategies (Dighton & Mason, 1985; Deacon & Fleming, 1992). Aspects of life history strategy that could affect species dynamics include variation in the ability to colonize roots from spores after disturbance, to persist as propagules in the soil, and to persist on roots after colonization (Deacon *et al.*, 1983; Newton, 1992; Miller *et al.*, 1994; Taylor & Bruns, 1999). Colonization and persistence could also be affected by resource availability (Gibson & Deacon, 1990), and trade-offs might exist between ability to colonize roots and grow rapidly under high soil resource conditions, and to persist under low resource

conditions. It can be hypothesized that species that colonize roots effectively after disturbances such as fire might be effective at colonizing from spores under soil resource-rich conditions, but might not persist for long on those roots, as resource availability changes, and species adapted to lower resource availability colonize roots. This latter group would include species that dominate under mature forest conditions, and might be less effective at colonizing root systems rapidly from spores, especially under nutrient-rich conditions, but more effective at persisting on root systems.

Interspecific differences in the ability to colonize roots from spores have been demonstrated (Fox, 1986). In addition, high levels of mineral nutrients can suppress colonization of roots from spores or vegetative inoculum, but at least some fungi, such as *Rhizopogon vinicolor* and *Laccaria laccata*, can colonize roots in fertilized soils (Trappe, 1977; Castellano *et al.*, 1985). Shifts in resource availability caused by N deposition or fertilization have been shown to lead to below-ground changes in EMF community structure in mature forests (Kärén, 1997; Peter *et al.*, 2001; Lilleskov *et al.*, 2002), but the effect of resources on EMF community root colonization dynamics from spore inoculum has, to our knowledge, not been explored.

Also largely unexplored is the extent to which nutrient effects on EMF community dynamics are mediated directly by soil resource availability to the fungi vs indirectly by plant nutrition and its effect on fungal ability to colonize roots. Theodorou (1993) found that spore germination by *Rhizopogon luteolus* could be influenced by the nutrient status of the host plant alone, suggesting that plant nutrient status has the potential to affect EMF root colonization. However, the effect of plant nutrition, independent of soil resource availability, on EMF community dynamics has not been examined.

To explore the effect of nutrient availability on EMF communities, we examined the temporal dynamics of root colonization by two EMF species, *Rhizopogon occidentalis* Zeller & Dodge (referred to in earlier publications from our laboratory as *R. ochraceorubens*, but recently confirmed as *R. occidentalis*; L. Grubisha, personal communication) and *Tomentella subulilacina* (Ellis & Holw.) Wakef. Both occur in the spore bank and can colonize seedlings from spores (Taylor & Bruns, 1999), but they differ in the forest conditions in which they dominate and in their modes of dispersal. *R. occidentalis* dominates on seedling roots after stand-replacing disturbance (Horton *et al.*, 1998; Baar *et al.*, 1999), and it is dispersed via mammal mycophagy (Molina *et al.*, 1999). This dispersal mode means that its spores are initially deposited in the nutrient rich environment provided by faecal pellets of the mammals. By contrast, *T. subulilacina* is a dominant in mature forests (Gardes & Bruns, 1996a; Horton & Bruns, 1998; Taylor & Bruns, 1999), and is only a minor component of post-fire seedling communities (Baar *et al.*, 1999). It fruits on buried wood or litter below the soil surface, and is probably dispersed by movement of soil by wind, or microinvertebrates

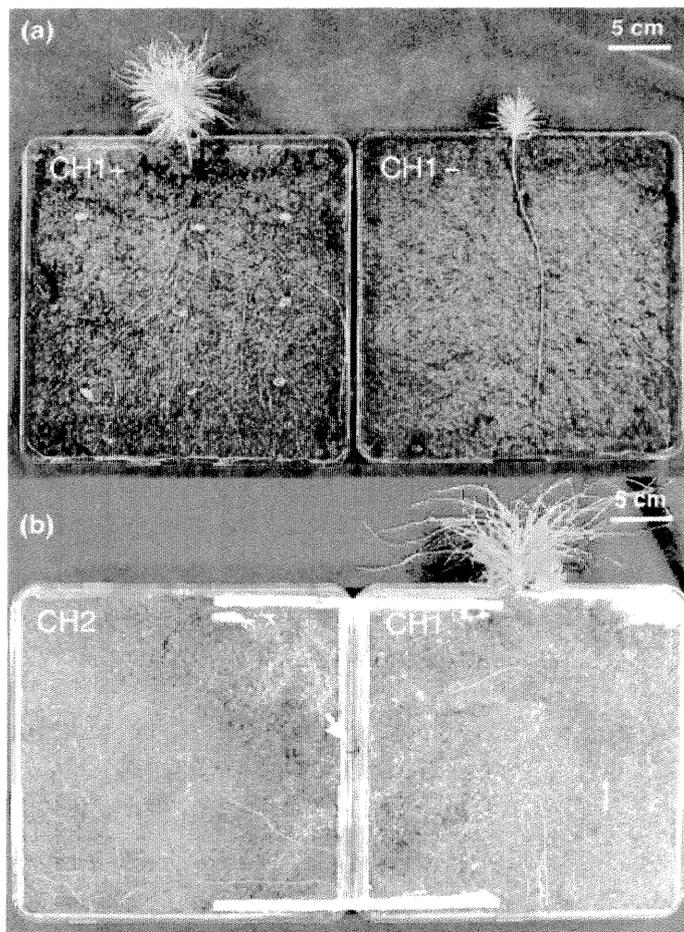
(E. A. Lilleskov & T. D. Bruns, unpublished). Neither of these modes would result in spores of *T. subulilacina* being deposited in large nutrient-enriched patches provided by mammal faecal pellets. We hypothesized that under our experimental conditions *R. occidentalis* would be the dominant root colonizer early in the growth period, but that colonization by *T. subulilacina* would increase relative to that of *R. occidentalis* over time. We also hypothesized that, because of its ability to colonize roots under high nutrient conditions, *R. occidentalis* would be favoured by addition of faecal pellets. Last, we examined whether the effect of nutrient addition on mycorrhizal colonization patterns was mediated by alteration of plant nutrient status alone, or in combination with alteration of soil resources.

## Materials and Methods

Bishop pine (*Pinus muricata* D. Don) seeds were collected at Salt Point State Park, Sonoma County, California, USA. Seeds were surface sterilized in 30% H<sub>2</sub>O<sub>2</sub> for 20 min, washed in sterile water, germinated on water agar and allowed to grow for 2 weeks. Sixteen experimental chambers were established, consisting of large flat Petri plates (243 × 243 × 18 mm, Nunc Brand Products, Naperville, IL, USA) filled with 500 ml of dry 2 mm-sieved unsterile peat (Premier Horticulture Inc., Red Hill, PA, USA). A slot was cut in the upper edge of the chamber to allow the shoot to emerge from the chamber. These chambers are referred to as chamber 1 (CH1), and are the same CH1 used in the second phase of the experiment (to be described later).

The experiment was established in two phases. In the first (Phase A), seedlings were grown in single chambers, either with or without an initial nutrient addition in the form of faecal pellets. In the second phase (Phase B), roots from these plates were divided and grown in a split root system.

**Phase A: initial faecal pellet treatment** Both EMF species were collected in bishop pine stands, either in Salt Point State Park (*T. subulilacina*), or on Santa Cruz Island (*R. occidentalis*, LG 286), California, USA. Fungal inoculum was added to the chambers as spores. *T. subulilacina* spores were isolated by scraping spores from resupinate crusts, whereas *R. occidentalis* spores were isolated from thin sections of a dried sporocarp. Sporocarp tissue was put in several ml of deionized water in a 15-ml falcon tube, broken up by hand with a plastic micropestle, and shaken at 200 r.p.m. on a shaker table for 30 min. This slurry was then filtered through a 50 µm Nytex mesh to remove sporocarp fragments. The filtrate was examined for spore density using a haemocytometer. Slurries were combined and diluted with deionized water to obtain a spore mixture of *c.* 333 000 spores ml<sup>-1</sup> for each species. Seedlings were placed on the surface of the peat, and 6 ml of the spore mixture was sprayed on using a manual pump sprayer, for a total of *c.* 2 million spores of each species per chamber. This



**Fig. 1** Experimental chambers: (a) Phase A, comparison of typical plus pellet (CH1+, left) and minus pellet (CH1-, right) chambers after 7 months growth. Pellets are visible as lighter patches. Note typical larger *Pinus muricata* seedlings in CH1+ than CH1-; (b) Phase B, split root chamber design, with CH1 (right, the original CH1 from Phase A) joined to CH2 (left, the chamber added for Phase B) via acrylic strips. Arrow indicates bridging root trained from CH1 across to CH2. Note typical root proliferation in CH2.

inoculation level was chosen based on previous successful *T. subulilacina* spore inoculations in an identical peat-based chamber system (E. A. Lilleskov & T. D. Bruns, unpublished).

Chambers initiated in Phase A are referred to as CH1 to distinguish them from the split-root chambers affixed to them in Phase B (to be described later). Eight of the chambers were untreated (i.e. they contained only peat, spores, and a pine seedling), and are referred to as CH1-. To eight of the chambers 6 g DW of air-dried faecal pellets were added at the beginning of Phase A, and are referred to as CH1+. The EMF-spore-free pellets were obtained from laboratory-reared golden-mantled ground squirrels (*Spermophilus lateralis* (Say)). These were added in a 3 × 3 equally spaced array (Fig. 1a). Oven dried (50°C) pellets had a percentage N of 3.1 (SE ± 0.1) and a C:N ratio of 10.5 (SE ± 0.36). All chambers were watered as required and placed in a lightproof box with a slot cut in the top for shoots. They were supported in the

vertical position by Styrofoam inserts. Chambers were additionally shielded from light by a layer of aluminium foil placed inside the box on top of the chambers. The box was placed in a growth chamber (Enconair model GC8-2H-SP, Winnipeg, Canada) with an irradiance of  $c. 350 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and 16 h at 18°C light/8 h at 16°C dark period. The box was tilted to encourage root growth on the back of the chambers. Seedlings were watered as required with deionized water, and their positions were randomly shuffled weekly.

The mycorrhizal status of seedlings was assessed at 4–8 week intervals by examining all roots on the upper and lower surfaces of the chamber using a 10–50× zoom stereomicroscope (Olympus SZ30, Olympus America, Inc., Melville, NY, USA). Numbers of root tips colonized by each target species, by contaminants, or uncolonized (defined by presence of root hairs or lack of mantle and any emanating hyphae) were quantified. The two EMF species used for inoculation are

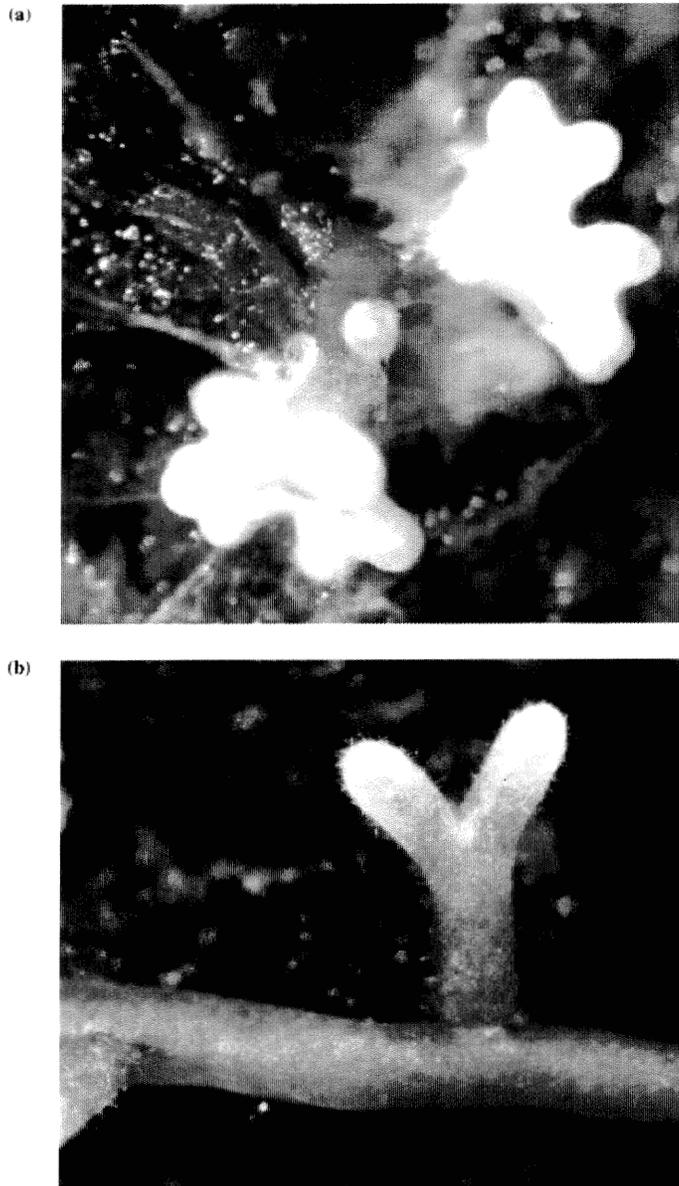


Fig. 2 Ectomycorrhizas formed by: (a) *Rhizopogon occidentalis* and (b) *Tomentella sublilacina*.

morphologically distinct, facilitating identification (Fig. 2). Over the course of the experiment, representative root tips were selected from each chamber and the identity of the EMF was checked by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) of the internal transcribed spacer (ITS) region of ribosomal DNA (Gardes & Bruns, 1996b). We counted only live mycorrhizal

tips, as determined by examining colour and turgor of tips and condition of the mantle (i.e. the fungal sheath around the root tip) and hyphae emanating from tips.

**Phase B: split root treatments** In order to distinguish soil-mediated vs plant nutrition effects on EMF community dynamics, after 7 months of growth the initial treatments

**Table 1** Experimental design of the split-root dual inoculation experiment

Treatment ID	Phase A, Initial pellet addition to:	Phase B, split-root treatment pellet addition to:		<i>n</i> *
	CH1	CH1	CH2	
CH1A-, CH1B-, CH2B-	-	-	-	2
CH1A-, CH1B+, CH2B-	-	+	-	3
CH1A-, CH1B-, CH2B+	-	-	+	3
CH1A+, CH1B-, CH2B-	+	-	-	2
CH1A+, CH1B+, CH2B-	+	+	-	3
CH1A+, CH1B-, CH2B+	+	-	+	3

The eight replicates from each treatment in the initial phase of the experiment were divided among the three treatment combinations in the second phase of the experiment, for a total of six treatments. In the treatment ID, the symbol before the first comma represents plus (CH1A+) or minus (CH1A-) pellet addition to CH1 in Phase A of the experiment, the symbol before the second comma represents plus (CH1B+) or minus (CH1B-) pellet addition to CH1 in Phase B (split root experiment), and the symbol after the second comma represents plus (CH2B+) or minus (CH2B-) pellet addition to the newly added CH2 in Phase B (the split root experiment). *n* = the number of replicates for the treatment.

were converted into split-root treatments. To accomplish this, a second chamber (CH2) was affixed to the chamber from Phase A (CH1) by gluing two narrow acrylic strips across the backs of the two chambers (Fig. 2b). CH2 was filled with the same volume of peat as CH1. Next, a gap was cut in the walls of both chambers, to enable a root to be trained across a 1-cm air gap from CH1 to CH2. The exposed root section that passed between the chambers remained free of bridging hyphae.

The split-root phase of the experiment had a total of six treatments (Table 1). Of the eight chamber pairs in which in Phase A CH1 had minus pellet treatment, in Phase B two had no pellets added to either chamber (CH1A-, CH1B-, CH2B-), three had pellets added to CH1 (CH1A-, CH1B+, CH2-) and three had pellets added to the newly added CH2 (CH1A-, CH1B-, CH2B+). The same new treatments were applied to the eight chamber pairs in which in Phase A CH1 had a plus pellet treatment: in Phase B two had no pellets added to either chamber (CH1A+, CH1B-, CH2B-), three had pellets added to CH1 (CH1A+, CH1B+, CH2B-), and three had pellets added to the newly added CH2 (CH1A+ CH1B-, CH2B+). The same mass of faecal pellets that had been added to CH1 in Phase A was added in Phase B to the appropriate chamber.

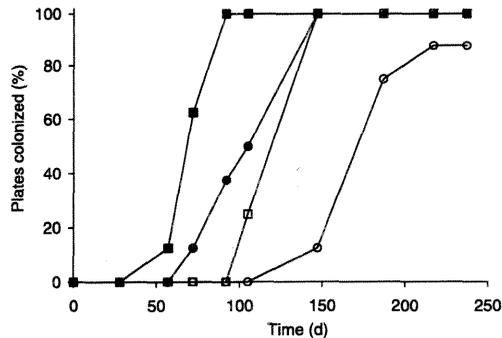
New lightproof boxes were constructed for this phase of the experiment. The split-root chambers were shuffled randomly among boxes at weekly intervals. To prevent root accumulation at the base of the split chambers, the chambers were oriented in a near-horizontal position, dipping slightly toward the base.

At the end of the experiment roots from each chamber half were harvested separately, washed, oven dried at 50°C, and weighed.

### Statistical analysis

To calculate whether roots were colonized significantly earlier in different treatments or for different species, we calculated Fisher's exact test (the equivalent of  $\chi^2$  test of independence used when expected cell values are small) using the online program SISA-Binomial (Uitenbroek, 1997). Tests were performed for the first date for which all seedlings had been colonized in one of the test pair of the species-treatment combinations (e.g. *R. occidentalis* minus pellet vs *R. occidentalis* plus pellet on day 92).

Other statistical analyses were performed using SPSS vs 10 (SPSS Inc., Chicago, USA). To examine the effect of the initial treatment on the time course of root colonization for both fungal species, we used doubly multivariate repeated measures analysis of variance (MANOVA). This approach is appropriate for designs in which more than one response variable (number of root tips of the two EMF species) is examined on the same individual (= chamber) over time (von Ende, 1993). We performed similar analyses for the number of nonmycorrhizal and contaminant mycorrhizal tips. To test the effect of the initial pellet addition, we used the five sampling dates before the split root treatment was applied for the response of *T. subulilacina* and *R. occidentalis*. For total, nonmycorrhizal and contaminant roots, no data were collected on the first sampling date, so only four dates were used. We examined some of the effects in more detail using repeated measures of analysis of variance (ANOVA). Mauchly's test of sphericity was used to examine whether the assumptions of repeated measures analysis of variance were met. If they were not, Greenhouse-Geisser, Huynh-Feldt, and Lower-bound epsilons were calculated to adjust the degrees of freedom of the test, leading to a more conservative significance test (SPSS, 1997). We examined the effect of pellet addition, time, and pellet



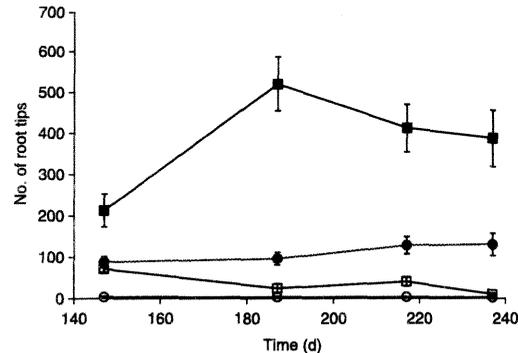
**Fig. 3** Percentage of *Pinus muricata* seedlings colonized over time in Phase A by each ectomycorrhizal fungal species in chambers with and without pellet addition. All chambers were inoculated with both species. Filled squares and filled circles represent *Rhizopogon occidentalis* and *Tomentella subulilacina*, respectively, in the minus-pellet chambers; open squares and open circles represent *Rhizopogon occidentalis* and *Tomentella subulilacina*, respectively, in the plus-pellet chambers.

addition  $\times$  time, on the number of root tips overall, and for the individual species. Examination of within-subject polynomial contrasts allowed us to determine whether there were significant linear or nonlinear trends in number of root tips for the individual species, and whether the shape of those trends depended on treatments. Root tip numbers were square root transformed to normalize and correct for heteroscedasticity. The data for contaminant mycorrhizal tips did not conform to the test assumptions, even after transformation. For these data, the nonparametric Mann–Whitney U-test was used.

To examine the effect of the split-root treatments we used repeated measures MANOVA. The response variable was number of roots occupied by a species on each of the post-split sampling dates. To correct for the different starting conditions of the treatments, we re-expressed each species' root tip numbers for the split root experiment as percentages of the numbers at the beginning of the split-root treatment. We then used a square root transformation to normalize and correct for heteroscedasticity. For *R. occidentalis*, we separately analysed the data for the initially plus pellet vs the minus pellet treatments, because the large differences in the starting conditions (i.e. at the time of initiation of the split-root treatment) made comparisons between these two treatments suspect. For the test of effects on nonmycorrhizal root numbers, we used multivariate analysis of covariance (MANCOVA), with the number of root tips at the starting date as the covariate. Treatment effects on final root biomass were examined using ANOVA.

## Results

**Phase A: Initial pellet treatment effects** The inoculations were highly effective. All 16 chambers were colonized by both



**Fig. 4** Total (nonmycorrhizal + all mycorrhizal) and nonmycorrhizal *Pinus muricata* root tips (mean  $\pm$  se) in Phase A for chambers with and without pellets. Filled squares, total number of tips in plus-pellet chambers; filled circles, total number of tips in minus-pellet chamber; open squares, nonmycorrhizal tips in plus-pellet chambers; open circles, nonmycorrhizal tips in minus-pellet chambers.

EMF, except in the case of one plus pellet chamber that was only colonized by *R. occidentalis*. The time course of initial colonization of roots in chambers differed both for species and pellet addition (Fig. 3). The minus pellet chambers were colonized earlier than the plus pellet chambers by both species ( $P < 0.001$  for both species, Fig. 3), and *R. occidentalis* colonized seedlings earlier than *T. subulilacina* in both treatments ( $P = 0.013$  for minus pellets,  $P < 0.001$  for plus pellets, Fig. 3). After 92 and 147 days *R. occidentalis* had colonized all seedlings under minus and plus pellet conditions, respectively. By contrast, *T. subulilacina* took 147 days to colonize all seedlings in the minus pellet treatment, and had only colonized 7 of 8 seedlings in the plus pellet treatment by the end of the experiment (Fig. 3).

Pellet addition increased both seedling size (Fig. 1a) and the total number of root tips ( $P = 0.001$ ), as well as altering the time course of root production ( $P = 0.002$ ) (Fig. 4).

The two EMF species had dramatically different responses to pellet addition (Fig. 5). The overall response to pellet addition was a significant increase for *R. occidentalis* ( $P < 0.001$ , compare lines in Fig. 5a,b) but not *T. subulilacina* ( $P = 0.21$ , compare lines in Fig. 5a,b). In both pellet treatments, *T. subulilacina* tip numbers increased over time ( $P < 0.001$ ) in a predominantly linear fashion (within-subjects contrast,  $P < 0.001$  for linear component of time), with only a small, marginally significant difference in the temporal response between plus and minus pellet treatments ( $P = 0.04$  for time  $\times$  pellet addition, Fig. 5a,b).

In contrast, the pattern of root colonization by *R. occidentalis* exhibited distinct differences under plus and minus pellet conditions ( $P < 0.001$  for time  $\times$  pellet addition). Not only did *R. occidentalis* colonize roots later in the plus- than in the minus-pellet treatment, but also the number

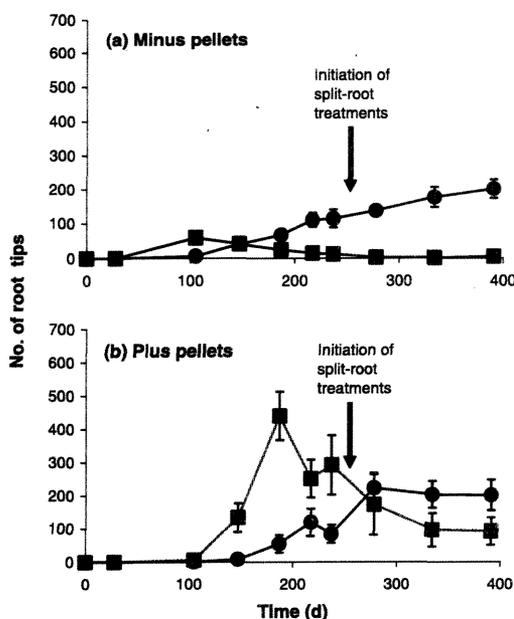


Fig. 5 Number of root tips (mean  $\pm$  SE) of *Tomentella subuilacina* (circles) and *Rhizopogon occidentalis* (squares) over time in chambers (a) without faecal pellets, and (b) with faecal pellets. Time course after initiation of split-root treatments (Phase B) is included to show continuation of general trends from Phase A of experiment. For response to split-root treatments, see Fig. 7.

of *R. occidentalis* root tips peaked later and at a 7-fold higher level (Fig. 5a,b). *R. occidentalis* tips exhibited a peak in both treatments ( $P < 0.001$  for the quadratic component of the time contrast). The number of *R. occidentalis* tips peaked 90 days later in the plus pellet treatment than in the minus pellet treatment, resulting in a significant quadratic component of the time  $\times$  pellet addition contrast ( $P < 0.001$ ).

*R. occidentalis* exhibited a striking growth response to the presence of pellets: its rhizomorphs were observed directly colonizing the decomposing pellets (Fig. 6). Similar growth patterns were not observed with *T. subuilacina*.

The number of nonmycorrhizal tips was greater in the plus pellet treatment ( $P < 0.001$ , Fig. 4) and was quite low in the minus pellet treatment. Initially, numbers of nonmycorrhizal roots were much greater in the plus pellet treatment, but these declined over time, leading to convergence of the nonmycorrhizal tip numbers in the two treatments as time passed (time  $\times$  pellet addition,  $P < 0.001$ , Fig. 4).

There was only one contaminant species evident in the chambers, an ectendomycorrhizal species with dark emanating hyphae. Number of tips of this species was low in both treatments, the mean never exceeding 3% of total tips, but was significantly higher in the low nutrient treatment at the

first ( $P = 0.011$ ) and second ( $P = 0.034$ ), but not the third and fourth sampling dates (data not shown).

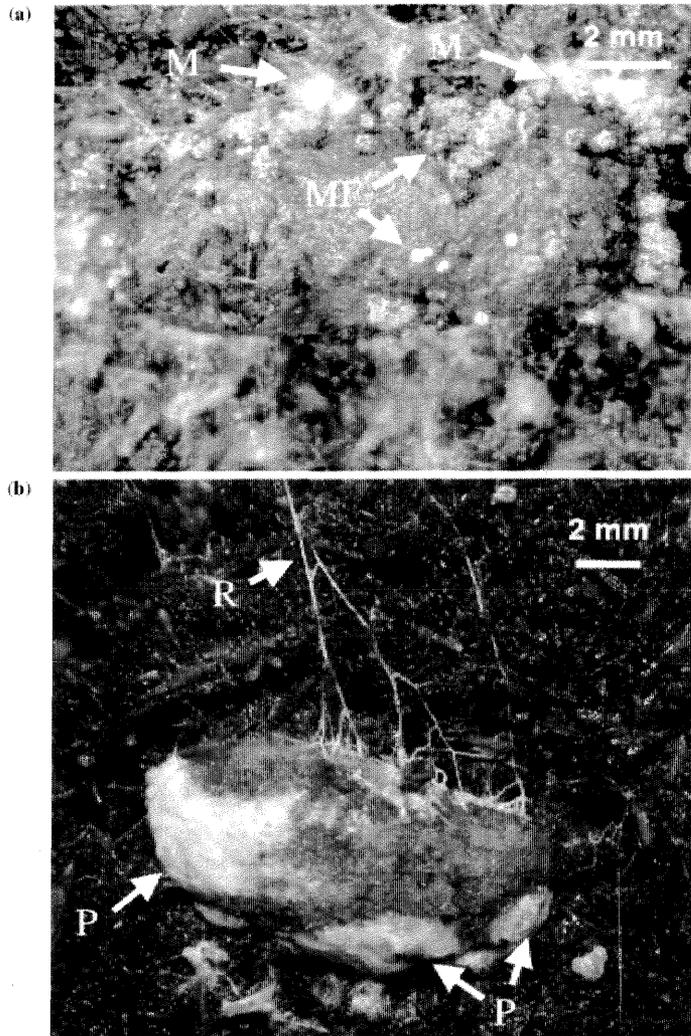
**Phase B: Split-root treatment effects** Although roots colonized both chambers well (e.g. Fig. 1b), only roots in CH1 of the split-root chambers were examined for response to the treatments during Phase B. Our initial intent was to examine EMF community dynamics in both CH1 and CH2. However, inoculations of CH2, carried out as for CH1 in Phase A, did not result in successful colonization by *R. occidentalis*, perhaps because older dried sporocarps were used for inoculation. Therefore, we only quantified and report on the community dynamics in CH1 (i.e. in the same chamber examined over the first 7 months). In this chamber, the treatments where pellets were added to CH1 in Phase B should alter both soil nutrient pools and plant nutrition in CH1, whereas the treatments where pellets were added to CH2 (Table 1) should only alter plant nutrition without directly altering soil nutrients available to the EMF in CH1.

Overall, responses to the second pellet addition were weaker, whether pellets were added to CH1 or CH2. There were no significant effects on the number of nonmycorrhizal and ectendomycorrhizal root tips, and no or weak effects on the number of root tips of the two EMF species. Both species continued their general trends from the initial phase of the experiment (i.e. continued decline for *R. occidentalis*, and increase for *T. subuilacina* (Fig. 5)).

By contrast with the first phase of the experiment, the second pellet addition had little effect on root tip colonization by *R. occidentalis*, regardless of initial treatment, or where the pellets were added (Fig. 7a).

In the case of *T. subuilacina* there was no significant treatment main effect, but there was a significant time  $\times$  initial pellet treatment  $\times$  second pellet treatment interaction ( $P = 0.01$ , Fig. 7b), that can be interpreted as follows. In the treatments for which CH1 was minus pellets in Phase A, there was no difference in *T. subuilacina* root numbers among treatments applied during Phase B. In contrast, in the chambers that received the plus pellet treatment in Phase A, the response of *T. subuilacina* in Phase B depended on whether or not pellets were added to CH2. When pellets were added to CH2, *T. subuilacina* tip numbers exhibited a slow steady increase. When no pellets were added to CH2, *T. subuilacina* tip numbers rose rapidly then somewhat declined (Fig. 7b).

There was a significant overall treatment effect on root biomass at the end of Phase B ( $P = 0.002$ ), arising from higher biomass in chambers that received the plus pellet treatment in Phase A than in those that were minus pellet ( $P = 0.0005$ ) (Fig. 8) and a nonsignificant trend toward higher root biomass when, in Phase B, pellets were added to CH2 vs when pellets were added to CH1 or to neither chamber ( $P = 0.08$ , Fig. 8). At the end of Phase B, there was no significant overall treatment effect on root biomass allocation between the CH1 and CH2 ( $P = 0.21$ ), and a weak nonsignificant trend toward



**Fig. 6** Colonization of faecal pellets by *Rhizopogon occidentalis*: (a) Early stage of pellet colonization. Microfungi (MF) are visible on the recently added pellet, and *Rhizopogon occidentalis* mycelium (M) is proliferating at the margin of the pellet. (b) Later stage of pellet colonization. Mycelial patches (P) of *Rhizopogon occidentalis* are visible on surface of pellet, and rhizomorphs (R) are visible extending upward from the pellet.

a shift in root biomass allocation away from CH2 when nutrients were added to the CH1 ( $P = 0.11$ , data not shown).

## Discussion

**Phase A, initial experiment** The observed interspecific difference in the time course of root colonization is consistent with our knowledge of the life history strategies of the two EMF species. The ability of the post-disturbance dominant, *R. occidentalis*, to colonize roots rapidly from spores, and its early peak in abundance, contrasted with later root colonization and the slow and steady increase in abundance on

root tips seen in the case of the mature forest dominant, *T. subulilacina*. Furthermore, the shift in dominance from *R. occidentalis* to *T. subulilacina* took longer under high nutrient conditions. This suggests that the differences in life history strategy may be associated with differences in ability to colonize roots rapidly from spores under high nutrient availability, and to persist on those roots as nutrient availability declines. This is consistent with hypothetical trade-offs between post-disturbance colonization and persistence.

Whereas most mature forest-dominant EMF fail to colonize roots readily from spores, clearly *T. subulilacina* has this capability. While this had not been previously demonstrated,

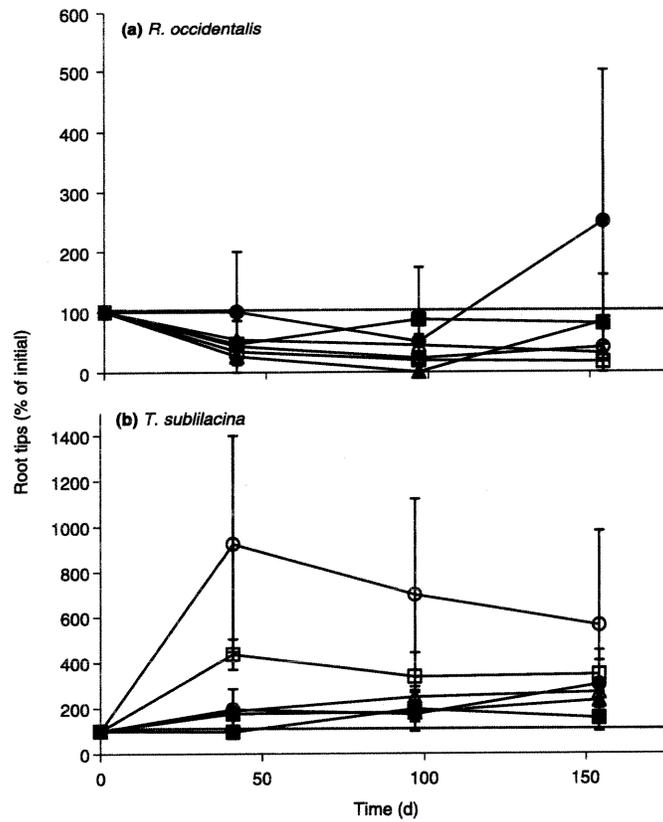


Fig. 7 Number of root tips colonized by: (a) *Rhizopogon occidentalis*, and (b) *Tomentella subulilacina* in the split root experiment, as a percent of those present at the end of Phase A. Closed symbols, Phase A, minus pellets; open symbols, Phase A, plus pellets; circles, Phase B, minus pellets in both CH1 and CH2; squares, Phase B, plus pellets in CH1 only; triangles, Phase B, plus pellets in CH2 only.

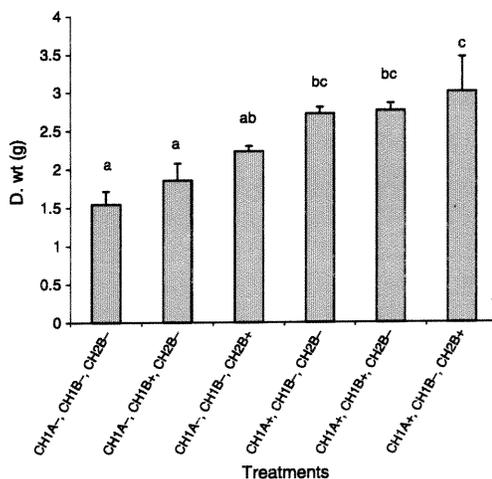


Fig. 8 Combined effects of initial (Phase A) and split-root (Phase B) treatments on Bishop pine total root weight. Different letters above treatments indicate treatments differ significantly,  $P < 0.05$ .

it was predictable on the basis of positive bioassays results (Baar *et al.*, 1999; Taylor & Bruns, 1999) and from the presence of this ability in related species, *Tomentella crinalis* (Köljalg, 1992) and *Thelephora terrestris* (Birraux & Fries, 1981). This contrasts with earlier studies indicating that mature forest dominants rarely colonize seedlings from spores in unsterile soils (Deacon *et al.*, 1983; Fox, 1983). It thus appears that *T. subulilacina* may follow a different strategy from that seen in many other mature forest dominants: a combination of spore dormancy, early establishment from spores after disturbance, and slow clonal expansion leading to mature forest dominance.

The delay in root colonization in both EMF species seen under the high nutrient conditions provided by pellet addition suggests that either roots are more receptive, or spores are more effective as colonists, under low nutrient conditions. Reduced root colonization has been commonly observed under high nutrient (especially high N) conditions (Smith & Read, 1997).

Since *Rhizopogon* sporocarps are typically consumed by and dispersed in the faecal pellets of small mammals (Molina *et al.*, 1999), it is likely that the mycelia growing after spore

germination should be adapted to the enriched nutrient conditions and altered microbial communities found in the presence of these faecal pellets. The pellet colonization pattern of its mycelium, and increased *R. occidentalis* abundance in the plus pellet treatment, support this idea. In addition, the mycelial colonization of pellets suggests that *R. occidentalis* may provide host plants better access to this source of nutrients. By contrast, there is neither evidence of rodent consumption of the thin resupinate crusts formed by *T. subulilacina*, nor a positive effect of pellet addition on root tip colonization by *T. subulilacina*.

The dynamics evident in this system make it clear that the results of soil bioassays for EMF inoculum potential could be highly dependent upon the timing of bioassay harvests and the nutrient conditions in the bioassays. Many bioassays are harvested after a set time interval, so will only record a snapshot of an EMF community that may be rapidly changing. Although we did not directly measure EM root tip turnover rates, they appeared to be quite high in the present study, especially for *R. occidentalis*, so capturing these dynamics will require intensive sampling.

**Phase B, split-root experiment** *R. occidentalis* did not respond to the later pellet additions in the same way as it did to the initial addition. There are several possible explanations for the observed difference. First, it is possible that *R. occidentalis* is more effective at colonizing roots rapidly in the absence of competition. The increasing number of *T. subulilacina* ectomycorrhizas and the low number of nonmycorrhizal tips at the start of the split-root treatment would lead to increased competition for space on root tips compared with the conditions at the beginning of the first phase. This interpretation is consistent with the dominance of *R. occidentalis* early in succession (Horton *et al.*, 1998; Baar *et al.*, 1999) and in bioassays (Taylor & Bruns, 1999). Under these conditions nutrient availability is typically high, and competing vegetative inoculum is likely to have been greatly reduced or eliminated. The positive response of *R. occidentalis* to nutrients and its ability to colonize roots more rapidly than competitors would allow it to establish early dominance after disturbance, but may be less beneficial when root tips are fully colonized.

Second, because seedlings were much larger at the initiation of the split-root treatments, later pellet additions might have differed in their effect on soil nutrient availability, plant nutrition and/or carbon availability to fungi. Given the potentially greater nutrient sink and the larger established root system of the larger plants, nutrients from pellets are likely to be mobilized and moved to the seedling much more rapidly than at the time of the initial pellet addition treatment. These nutrients would be diluted in the larger plant biomass, and soil resources would be drawn down more rapidly, leading to a different set of plant nutritional and soil environmental conditions in the two treatments, despite identical pellet additions.

Finally, pellet additions may have nonnutritional effects on the fungi. For example, some *Trichoderma* species can function as fungal antagonists via competition, antibiosis and mycoparasitism (Tronsmo & Hjeljord, 1998). We observed *Trichoderma* sp. commonly sporulating in rings around the added faecal pellets, as well as among and on root tips of both species. Root tips usually appeared unhealthy when this occurred, suggesting either virulence to the roots or fungus, or secondary colonization of moribund root tips. It is possible that some of the difference in *R. occidentalis* root colonization patterns between the initial and later faecal pellet addition was mediated by enhancement of *Trichoderma* or other antagonistic microorganisms over time, as inoculum built up in response to repeated faecal pellet addition, and turnover of roots and mycorrhizal fungi.

The effect of pellet addition to the NS chamber on root colonization by *T. subulilacina* suggests that there might be a host plant nutrition-mediated effect on colonization by that species. Theodorou (1993) found that *Rhizopogon luteolus* spore germination and germ tube length were reduced under high nutrient conditions. This depression was evident even when seedlings were grown in a high nutrient medium, and then shifted to low nutrient medium at the time of inoculation. This suggests a host-mediated effect on spore germination and growth. However, in the split root phase of the present study mycorrhizal communities were already established, so patterns of vegetative growth rather than spore germination probably drove community responses. One possibility is that seedling C allocation was shifted to the side of the chamber where the nutrients were added, leading to a negative effect on C supply to roots in the low nutrient chamber half. The trend in the distribution of root biomass between the chambers, with relatively more biomass in the plus pellet chambers, is consistent with this interpretation.

In conclusion, the potential for organic nutrient additions to mediate interspecific interactions of EMF has been demonstrated for the initial seedling colonization phase. The nutrient mediation of interspecific interactions has implications for our basic understanding of mycorrhizal fungal community dynamics in response to changing resource availability, and could also be used in managing the community of fungi on roots of seedling stock. Future experiments should attempt to elucidate the significance of these community dynamics for the plant hosts, by comparing plant growth and nutritional response to these EMF species singly and in combination, at both low and high nutrient availability.

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