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# Development, persistence and regeneration of foraging ectomycorrhizal mycelial systems in soil microcosms

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**Abstract** Development of extraradical mycelia of two strains each of *Paxillus involutus* and *Suillus bovinus* in ectomycorrhizal association with *Pinus sylvestris* seedlings was studied in two dimensions in non-sterile soil microcosms. There were significant inter- and intraspecific differences in extraradical mycelial growth and morphology. The mycelial systems of both strains of *P. involutus* were diffuse and extended more rapidly than those of *S. bovinus*. Depending on the strain, *P. involutus* mycelia were either highly plane filled, with high mass fractal dimension (a measure of space filling) or sparse, low mass fractal dimension systems. Older mycelial systems persisted as linear cords interlinking ectomycorrhizal tips. *S. bovinus* produced either a mycelium with a mixture of mycelial cords and diffuse fans that rapidly filled explorable area, or a predominately corded mycelium of minimal area cover. In the soil microcosms, mass fractal dimension and mycelial cover tended to increase with time, mycelia encountering litter having significantly greater values. Results are discussed in terms of the ecology of these fungi, their foraging activities and functional importance in forest ecosystems.

**Keywords** Extraradical morphology · Fractal geometry · Litter resources · *Paxillus involutus* · *Suillus bovinus*

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## Introduction

The roots of most boreal and many temperate and tropical trees normally form ectomycorrhizal associations with appropriate fungi (Smith and Read, 1997). Many studies on ectomycorrhizal (EM) colonisation of plants have focused on the development and physiological activity of mycorrhizal root tips (Brun et al. 1995) but much less attention has been directed at the extraradical mycorrhizal mycelium. In most cases it is this mycelium that provides the main nutrient and water-absorbing interface between plants and soil, and it is often a dominant component of microbial biomass in forest soils. Fungal biomass in forest soils has been estimated at between 70 and 100 g (dry weight) $m^{-2}$ (Markkola et al. 1995), but this increases to over 800 g $m^{-2}$ in areas with particularly dense proliferation of mycorrhizal mycelium (Ingham et al. 1991), and estimates for the latter's contribution of 32% of the total microbial biomass are thought to be conservative (Högberg and Högberg 2002).

The extraradical ectomycorrhizal mycelium (ERM) is physiologically complex and morphologically dynamic (Cairney and Burke 1996). ERM can forage to encounter new roots to colonise (Read 1991), and forage for nutrients contained in leaf litter (Bending and Read 1995; Leake et al. 2001; Perez-Moreno and Read 2000), pollen (Perez-Moreno and Read 2001), wood ash (Mahmood et al. 2001) and dead seeds (Tibbett and Sanders 2002). Their mycelial systems appear to be directly involved in the mobilisation of nutrients (Bending and Read 1995; Finlay and Read 1986a; Finlay et al. 1988; Leake et al. 2001; Melin and Nilson 1950; Perez-Moreno and Read 2000, 2001) and can mediate transfer of nutrients to host trees interconnected by common mycelial networks (Finlay and Read 1986b; Simard et al. 1997).

Although the uptake of nutrients by ERM has been quantified in many studies, descriptions of ERM responses to resource patches and their patterns of growth while foraging have been largely qualitative. These studies have shown that there is wide variation in both the growth rates

and morphology of these mycelial systems (Bending and Read 1995; Mahmood et al. 2001; Unestam and Sun 1995), and that morphological changes can occur in response to resource encounter (Bending and Read 1995; Perez-Moreno and Read 2000, 2001) and upon transplant to new soils (Finlay and Read 1986b).

The few studies estimating amounts of extraradical mycelia have used destructive sampling to estimate hyphal lengths (Jones et al. 1990; Rousseau et al. 1994), mass (Colpaert et al. 1992) and surface area (Rousseau et al. 1994). Non-destructive measurement of these parameters and a quantitative descriptor of mycelial morphology are more desirable. Computerised image capture, image analysis and the use of fractal geometry has been used to great effect to quantify the foraging systems of saprotrophic mycelia in two-dimensional agar and soil-based microcosms (Boddy 1999; Boddy et al. 1999; Donnelly et al. 1995, 1999). The aim of this study was to adapt and apply these methods for the first time to provide a quantitative analysis of both growth and mycelial morphology in selected ERM in soil.

Fractal geometry provides quantitative measures of the degree of space filling exhibited by mycelial systems. Fractals are complex geometric shapes that do not possess the whole number dimensions of Euclidean shapes (e.g. a cube is three-dimensional, a flat surface has two dimensions, and lines one), but instead take intermediate values, i.e. have fractional or fractal dimensions. They are self-similar, i.e. when part of the structure is magnified, the magnified region looks similar to the structure as a whole. Mycelia, and many other biological entities, are self-similar, though over a finite range of length scales (Boddy et al. 1999). There are two fractal measurements appropriate to mycelial systems: (1) the border/surface fractal dimension ($D_S$)—a measure of the irregularity of the edges of the system, i.e. the boundary between the system and its surroundings; (2) the mass fractal dimension ($D_M$)—a measure of the extent to which a plane is filled by the system. When $D_S=D_M$ the mycelial system is said to be mass fractal, i.e. the whole system is fractal; when $D_S<D_M$ the system is said to be a border or surface fractal structure, i.e. only the edge of the colony is fractal.

This study was designed to quantify the morphology and growth of ERM. Two experiments were conducted. The first experiment aimed to: (1) compare the properties of young mycelial systems of two ectomycorrhizal species, *Paxillus involutus* and *Suillus bovinus*, grown in association with the same host plant species; and (2) determine intra- and inter-specific variation in ERM. The second experiment was designed to quantify the morphology and growth of (1) old, persistent mycelial systems and (2) ERM response to encounter with new resources.

## Materials and methods

### Fungal isolates

Ectomycorrhizal strains were obtained as described in Table 1, maintained on full-strength modified Melin-Norkrans medium (MNN) (Marx 1969) and incubated in the dark at 17°C. The fungi were routinely subcultured every 10–28 days depending on the strain, in order to maintain actively extending mycelial margins.

### Ectomycorrhizal synthesis

Scots Pine (*Pinus sylvestris* L.) tree seeds were surface-sterilised by two washes in 40% $H_2O_2$ on a vortex-mixer for 15 min. Seeds were rinsed in 500 ml sterile distilled water (DW) and aseptically plated onto DW agar (Lab M agar No. 2, 15 g $l^{-1}$; IDG, Bury, UK), in 9 cm Petri dishes, ten seeds per dish. Dishes were sealed with Parafilm and incubated vertically in a controlled environment room (15/20°C 8/16 h dark/light cycling, photon flux density of 100–150 $\mu$mol $m^{-2}$ $s^{-1}$).

In the first experiment, after 14 days, germinated seedlings were aseptically transferred to Petri dishes containing 34-day-old *P. involutus* or *S. bovinus* cultures growing in a medium consisting of 1:7:3 peat/vermiculite/MNN nutrient solution [modified with 1/10 N and P and 1 g $l^{-1}$ glucose (PVM)]. Individual seedlings were placed with shoots emerging from a slot cut in the Petri dish, and subsequently re-sealed with sterile lanolin (anhydrous; BDH, Poole, Dorset, UK). Dish edges were resealed with Parafilm and wrapped in foil to exclude light from the roots and ectomycorrhizal fungi. Dishes were incubated vertically, shoots uppermost, in propagators (10/15°C dark/light cycling and photon flux density as described above) and shoots were misted with DW every 2–5 days. After 114 days, the plants had well-developed ectomycorrhizal root tips and were transferred to peat microcosms as described below. Ten replicate chambers for each strain of *P. involutus* and *S. bovinus* were constructed.

In the second experiment, mycorrhizas were established as in the first experiment, except that *P. sylvestris* seedlings were transferred to 14-day-old *P. involutus* cultures growing in PVM, incubated for 76 days and then nine replicates were transferred to peat microcosms.

### Microcosm construction

Both experiments were performed in transparent Perspex chamber microcosms (20×20×0.5 cm or 30×24×0.5 cm). Chambers comprised a Perspex backing sheet covered with 0.2 cm thickness of compacted, air-dried peat, re-wetted to −0.02 MPa as determined by the filter paper method of Fawcett and Collis-George (1967). At the corners of the backing sheet, Perspex blocks (2×1×0.5 cm) were glued to support a sheet of Perspex identical to the backing sheet, which formed a lid with an air-space of 3 mm above the peat. The mycorrhizal pine seedlings growing in PVM were carefully

**Table 1** Sources of ectomycorrhizal fungal isolates

| Species | Strain | Location | Source | Habitat | Time in culture (years) |
|---|---|---|---|---|---|
| *Paxillus involutus* | 1 | Sheffield culture collection (87017) | Fruitbody | Birch wood | 13 |
| *P. involutus* | 2 | Hope Valley, UK | Fruitbody | Fallen *Pinus* sp. trunk | <1 |
| *Suillus bovinus* | 1 | Sheffield culture collection (JSB1) | Fruitbody | – | 1 |
| *S. bovinus* | 2 | Sheffield culture collection (096) | – | – | 17 |

removed from the dishes and the medium handled with care to minimise disruption of mycelial connections to roots. The thickness of the PVM was carefully pared down to 2–3 mm by removal of peat/vermiculite from the bottom. The plants, with a disc of PVM attached, were then each introduced to a peat chamber. Perspex top sheets were added, chambers were wrapped in foil, sealed in plastic bags with shoots protruding from a top slot and incubated as described above for 30 days.

In experiment 2, chambers were established as described and then incubated for 69 days after which time Perspex top sheets and the corner spacer blocks were removed and the backing sheets were attached to larger (30×24×0.5 cm) peat chambers with silicon sealant, to form a continuous peat surface. New Perspex corner blocks and top sheets were added and chambers re-incubated as above. After a further 33 days, 0.5 g air-dried and re-wetted (300% oven dry wt) *P. sylvestris* needle litter (air-dried at room temperature and sieved <2 mm) was added as a square of approximately 9 $cm^2$ area to the peat surface at the ectomycorrhizal mycelial margin in three chambers and re-incubated for 42 days. Shoots were misted with DW at 2- to 3-day intervals and mycelial systems misted every 5–10 days.

### Photography and image analysis

Chambers were photographed at 7- to 10-day intervals from a height of 69 cm (Experiment 1) and, after transfer to larger chambers (experiment 2), at 11- to 22-day intervals, from a height of 94 cm, using a 35 mm Nikon SLR camera fitted with a 50 mm lens and Kodak EPY 35 mm colour film. Films were electronically scanned (Nikon 35 mm Coolscan) and imported into a PC running SEMPER (Synoptics, Cambridge, UK) image analysis system, as described in detail by Donnelly et al. (1995, 1999), modified to analyse images in full RGB mode. This modification enabled more accurate visual differentiation between host plant roots, ectomycorrhizal tips and extraradical mycelia. The 'windowing' option in SEMPER enabled selective removal of roots from the image, allowing extent, cover and fractal dimension of the extramatrical mycelial system alone to be determined.

### Quantification of extent, hyphal surface area covered, and fractal dimensions

For each image, extent was determined from linear measurements from the chamber top to the extending mycelial margin. Mycelial extension rate (mm $day^{-1}$) was estimated by linear regression of extent with time. Hyphal surface cover ($cm^2$) was determined from summed pixel area of the mycelium.

Fractal dimensions of extraradical mycelium were estimated by the box count method, described in detail elsewhere (Donnelly et al. 1995, 1999). Essentially, a series of grids of square boxes, of size 3–61 pixels, were positioned to overlay each electronic image. For a series of boxes of side length $s$ pixels, the number of boxes intersected by the set ($N$) was counted. Fractal structures obey the power law relation over a range of length scales such that:

$$N(s) = cs^{-D_B} \quad (1)$$

where $D_B$ is the box-counting fractal dimension, $N(s)$ is the total number of boxes of side length $s$ that intersect the mycelial image, and c is a constant. $D_{BS}$ (surface box fractal dimension) is estimated as the negative gradient of a regression line through the linear part of the plot of log $N(s)$ against log $s$, for a sequence of scales $s$: log $N(s)$ = log c + $(-D_B)$ log $s$.

However, since border boxes are not entirely filled, the area of mycelium is progressively overestimated by the larger box sizes, but an unbiased estimator of $D_{BM}$ (mass box fractal dimension) can be obtained by regression of log $[N(s) - \frac{1}{2} N_{border}(s)]$ against log $s$ (Donnelly et al. 1995)

Inter and intraspecific hyphal extension, cover and fractal dimensions were compared either by ANOVA followed by Scheffé test or where ANOVA criteria were not met, by Kruskal-Wallis followed by Mann-Whitney-U tests as indicated. Comparisons of hyphal cover and fractal dimensions between ectomycorrhizal control systems and litter-enriched systems were made by $t$ test.

## Results

### Experiment 1: inter- and intra- specific variation in morphology of young ectomycorrhizal mycelial systems

Both strains of both species produced extramatrical mycelium emanating from mycorrhizal root tips. Mycelium of *P. involutus* was buff-coloured (Fig. 1a, b) and that of *S. bovinus* bright white (Fig. 1c, d).

There were no differences ($P$>0.05) in mycelial extension rate between strains of the same species, but both *P. involutus* strains extended significantly ($P \leq 0.05$) faster (7–8 mm $day^{-1}$) than *S. bovinus* strains (5–6 mm $day^{-1}$; Fig. 2).

Mycelia of *P. involutus* strain 1 developing from individual mycorrhizal root tips typically merged with mycelia from adjacent tips to produce an almost uniform mycelial layer of high mass fractal dimension ($D_{BM}$=1.8±0.04; Fig. 3a), and this was maintained for the duration of the experiment, (29 days on peat). In contrast, mycelial edges became increasingly irregular with time as indicated by a rise in $D_{BS}$ (Fig. 3b). Mycelial cover was significantly ($P \leq 0.05$) greater for this strain than for all other isolates by 27 days (Fig. 3c).

The second strain of *P. involutus* produced a mycelium that developed from mycorrhizal root tips predominately as sparse fans supported by ≈1 mm thick, widely spaced linear cords (Fig. 1b, f). This mycelium became decreasingly ($P \leq 0.05$) plane filled (i.e. $D_{BM}$ declined) as it explored larger areas of soil (Fig. 3a), producing a mycelial cover only just over half (53%) of that of the first strain of this species at 27 days (Figs. 1e, f; 3c). As the mycelium became more open, mycelial edges became less branched as indicated by a fall in $D_{BS}$ (Fig. 3b).

*S. bovinus* characteristically produced mycelia that aggregated into cords (Fig. 1c, d), in contrast to *P. involutus* whose mycelium was more diffuse. *S. bovinus* strain 1 exhibited two growth forms in the same mycelial system: diffuse ensheathing mycelium, which extended primarily along roots, and aggregated cords supporting more discrete fans that extended radially from mycorrhizal root tips across soil (Fig. 1c, g). Exploratory cords encountering roots sometimes formed diffuse ensheathing mycelium from which cords re-emerged (Fig. 1c, g). *S. bovinus* strain 1 mycelium was initially significantly ($P \leq 0.01$) less plane filled than all other isolates ($D_{BM}$=1.5±0.08) (Fig. 3a), but plane filling increased to be not significantly different to that of *P. involutus* strain 2 by 27 days ($P$>0.05). However, mycelial coverage was minimal for this strain throughout the experiment and significantly ($P \leq 0.001$) less compared to all strains after 17 days, covering less than 13% of the available area

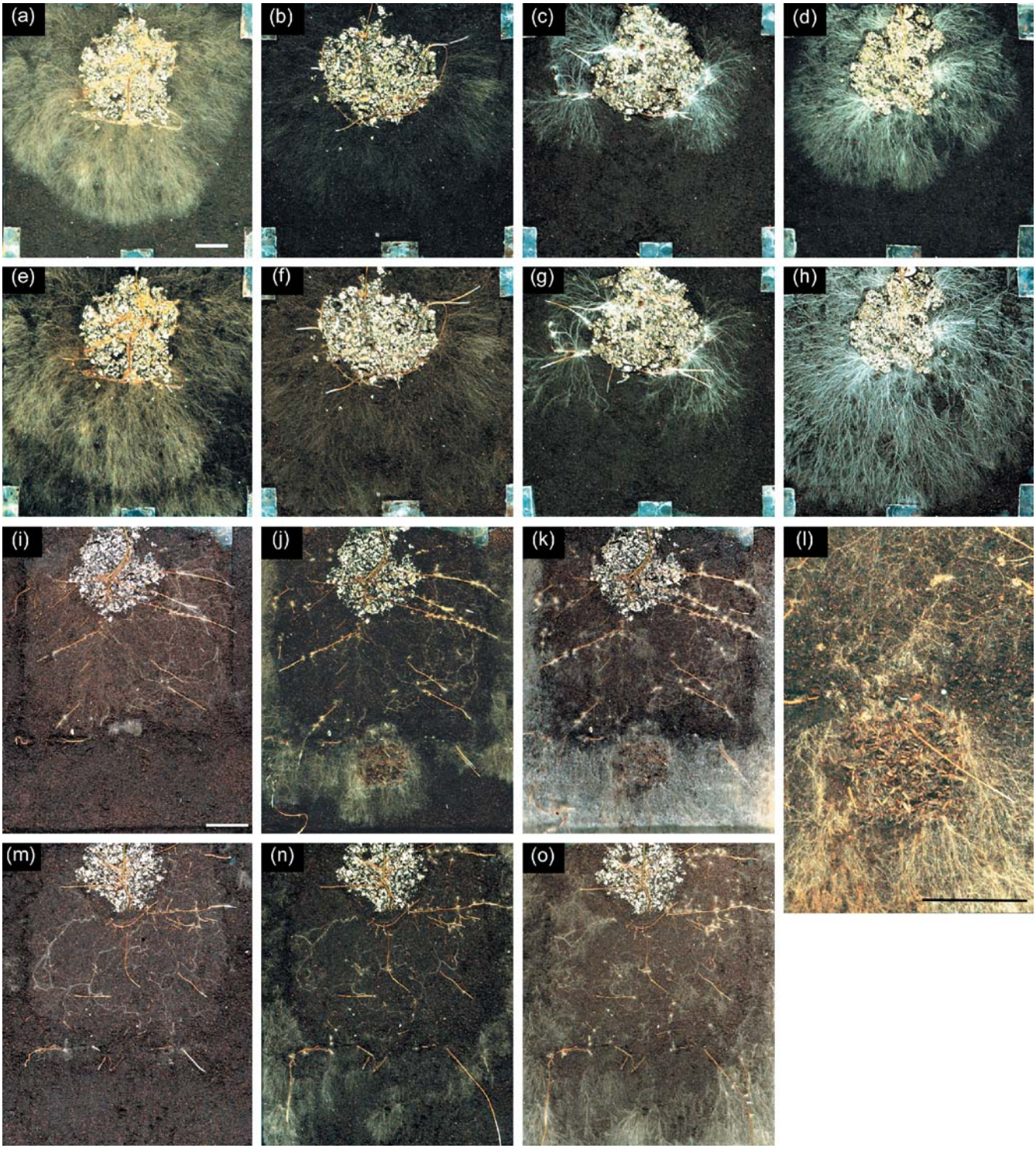


**Fig. 1 a–h** Ectomycorrhizal mycelial systems of two strains of *Paxillus involutus* (**a**, **e** strain 1; **b**, **f** strain 2) and *Suillus bovinus* (**c**, **g** strain 1; **d**, **h** strain 2) in peat microcosms after 10 days (**a–d**) and 17 days (**e–h**). *Bar* 2 cm. **i–o** Ectomycorrhizal mycelial systems of *Paxillus involutus* in peat microcosms with (**i–l**) and without (**m–o**) litter patch addition, 4 days, (**i**, **m**), 26 days (**j**, **n**) and 50 days (**k**, **o**) after transplant. *Pinus sylvestris* host shoots have been removed from images. **l** Detail of *Pinus sylvestris* litter colonised by *Paxillus involutus* after 75 days. Note increase in cover of mycelium exiting litter resource. *Bar* 3 cm

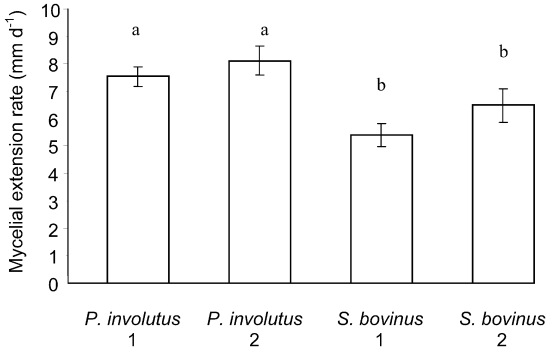


**Fig. 2** Extramatrical mycelium extension rates for two strains of *Paxillus involutus* and *S. bovinus* in peat microcosms, mycorrhizal with *Pinus sylvestris*. Error bars are ± SEM. Significant differences between means are indicated by different letters ($P \leq 0.05$)

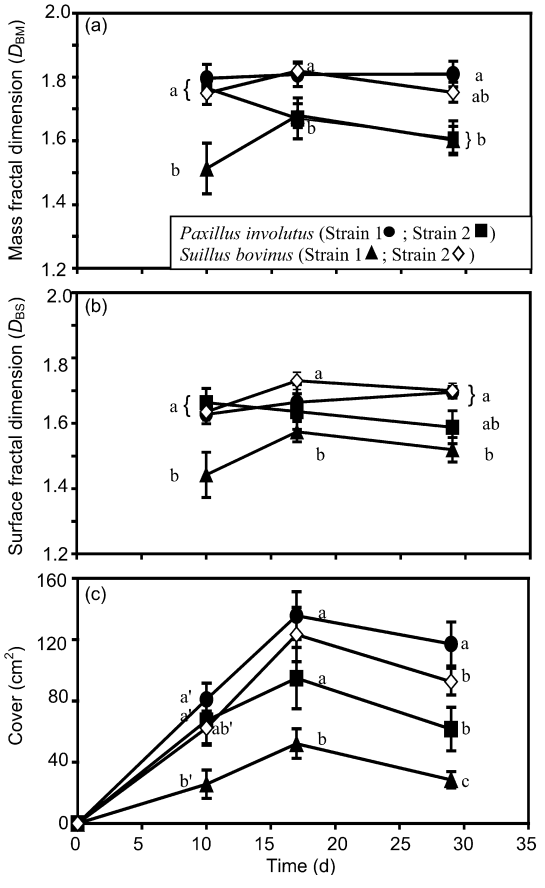


**Fig. 3** Extramatrical mycelium mass fractal dimension (**a**) surface fractal dimension (**b**) and mycelial cover (**c**) for two strains of *Paxillus involutus* and *S. bovinus* in peat microcosms, mycorrhizal with *Pinus sylvestris*. Error bars are +SEM. Significant differences between means are indicated by different letters ($P \leq 0.05$). *a′b′* Parametric, *ab* non-parametric comparisons

(Fig. 3c). *S. bovinus* strain 1 also had significantly ($P \leq 0.05$) less branched mycelial edges (i.e. lower $D_{BS}$) being composed predominantly of linear cords (Figs. 3b), compared to all other isolates to 17 days.

*S. bovinus* 2 mycelium developed as a mixture of both cords and more diffuse mycelium (Fig. 1d, h). As the diffuse marginal foraging front extended, supporting cords became thickened (Fig. 1d, h), and both tip-to-tip and tip-to-side fusions occurred throughout the mycelium. *S. bovinus* 2 mycelium was more dynamic than all other isolates, achieving both similar $D_{BM}$ and coverage as *P. involutus* 1 mycelium by 17 days, but having significantly ($P \leq 0.05$) less mycelia cover by 27 days (Fig. 3c). Both $D_{BM}$ and $D_{BS}$ values for *S. bovinus* 2 mycelium were equivalent and in decline by the end of the experiment (Fig. 3a, b) indicating an 'opening' mass fractal mycelium.

## Experiment 2: morphology and growth of persistent mycelial systems and response to encounter with new resources

Older mycelial systems of *P. involutus* persisted as a network of fine (0.56 mm ± 0.03 mm), sparsely branched, linear ($D_{BM} = D_{BS} = 1.2$) cords that interlinked widely separated EM tips and mycelial-ensheathed root areas (Fig. 1i, m).

After transfer to larger peat-filled chambers, *P. involutus* mycelia extended across the peat surface and did so with increasing plane filling in all systems (Figs. 1i–o, 4a). ERM systems within chambers to which litter had been added, rapidly colonised the resource patches as diffuse mycelia (Fig. 1l). Following litter colonisation, mycelium directly growing from litter patches (after 5–7 days), and mycelium distant from the litter patch, became significantly ($P \leq 0.01$) more plane filled (Figs. 1j, k; 4a) compared to mycelium in control microcosms without litter (Figs. 1n, o; 4a). However, there was no significant ($P \geq 0.05$) effect of litter addition on the regularity of edges of the mycelial systems as determined by surface fractal dimension; all system margins became increasingly irregular with time (Fig. 4b).

Although there were no significant ($P \geq 0.05$) differences between mycelial cover in the chambers prior to litter addition (Fig. 4c), litter resources significantly ($P \leq 0.01$) stimulated *P. involutus* mycelial cover to over 230% that of control systems (Figs. 1k, o; 4c) and it remained consistently greater throughout the remainder of the experiment (Fig. 4c). The majority of increase occurred in the foraging front extending over new peat; however, there was some increase in the density of mycelium behind the foraging front, which developed from new mycorrhizal root tips.

The pattern of development of *P. involutus* mycelium following transfer to the larger chambers, to which litter was then added, mirrored the development in the smaller chambers (Fig. 5), with both rapid coverage rise and then fall once all soil surfaces had been covered.

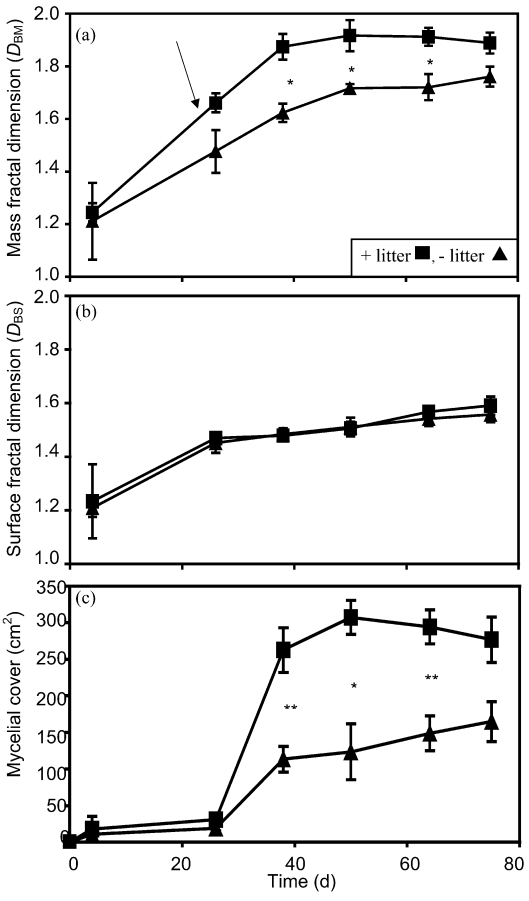


**Fig. 4a–c** *Paxillus involutus* extramatrical mycelium in peat microcosms, mycorrhizal with *Pinus sylvestris*. **a** Mass fractal dimension, **b** surface fractal dimension, **c** mycelial cover, with and without litter patch addition. *Arrow* Time point of addition of litter. Error bars are ±SEM. Significant differences between means are indicated by asterisks (* $P \leq 0.05$, ** $P \leq 0.01$)

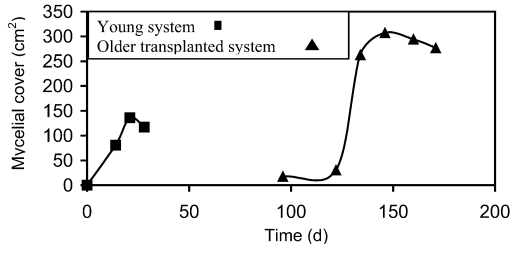


**Fig. 5** Area cover of *Paxillus involutus* strain 1 extramatrical mycelium, 0–28 days and 96–171 days. Note similar growth curves, rapid cover of soil surface followed by similarly steep cover reduction through mycelial thinning

## Discussion

This is the first study to non-destructively quantify, using image analysis, the morphology of ERM of ectomycorrhizal fungi when grown on non-sterile soil in natural association with host trees. We have shown differences both between species and within species (between strains) in growth and morphology of young foraging mycelial systems and, for the first time, quantified changes to older, persistent *P. involutus* mycelium when encountering new litter resources.

ERM development corresponded with systems previously described for these species (Bending and Read 1995; Finlay 1989; Finlay and Read 1986b). The increase in mycelial cover and fractal dimension to a peak followed by a decrease, seen here with *S. bovinus* and to a lesser extent with *P. involutus*, is also common with saprotrophic mycelia extending into soil (see e.g. Boddy 1999). The increase in mycelial cover was correlated here with merging of the mycelial outgrowth from mycorrhizal hyphal tips to form a dense foraging front. This front has been shown (Leake et al. 2001, 2002) to be a sink for host-plant-derived carbon in *P. involutus*.

Decrease in mycelial cover once chambers were fully colonised, resulted from thinning of diffuse mycelium and increased aggregation of mycelia cords rather than reduction in mycelial extent, as indicated by the corresponding reduction in $D_{BM}$, this change being most marked in *S. bovinus*. Reduction in external hyphal development in older cords has been observed previously in *S. bovinus* (Finlay and Read 1986b). Mycelial aggregation in many ectomycorrhizal mycelia is through establishment of founder hyphae around which parallel hyphae anastomose (Raidl 1997). Increased mycelial aggregation and localised autolysis of diffuse hyphae is a widespread phenomenon in mycelial systems of migratory wood decomposers (Donnelly and Boddy 1997; Dowson et al. 1986) and as quantified here also occurs in ectomycorrhizal mycelial systems. This morphological shift may be a more energetically efficient means of supporting an increasingly extended mycelium over large areas, and may involve recycling and redistribution of limited internal resources.

Extensive cord formation has been reported in root chambers with old ectomycorrhizal systems of *Suillus luteus*, *P. involutus* and *Scleroderma citrinum* (Colpaert et al. 1992). Indeed, in the present study in the older microcosms, mycelial systems had thinned considerably, persisting as mycelial cords interlinking widely separate mycorrhizal colonised root regions, from which new fans of mycelium emerged on transfer to larger chambers with fresh peat. Aggregation of mycelium into cords may enable maintenance of a mycelial presence in an explored/exploited region, where new nutrient sources may become available, e.g. through seasonal inputs, and as new root tips form they become available for mycorrhizal colonisation. Regression and interlinking of mycorrhizal tips by mycelial systems that have explored available areas allows rapid redevelopment of foraging fronts once environmental stresses have been alleviated, or new resources become available.

Cords also form behind the foraging front of developing mycelial systems, as in the present study and others (Ek 1997; Finlay and Read 1986b). Cord aggregation may provide greater robustness and efficiency of translocate supply, providing functional links, supplying water (Read and Boyd 1986) and nutrients in ectomycorrhizal (Bend-

ing and Read 1995; Ek 1997; Finlay 1989; Finlay and Read 1986b; Jentschke et al. 2001; Skinner and Bowen 1974) systems. Persistent ectomycorrhizal networks have a major role in plant ecology, being implicated in establishing new mycorrhizal colonisations and supplying carbon to seedlings (Onguene and Kuyper 2002) or suppressed plants (Finlay and Read 1986b).

Organic resources can dramatically affect mycelial characteristics even in older aggregated systems that no longer possess a defined foraging front, as indicated in the present study by the significant increase in mycelial plane filling and cover of the entire mycelium following encounter with litter patches. In previous studies, encounter with resources invariably led to mainly local increase in hyphal density as small resources became colonised by the extending mycelial margin (Bending and Read 1995; Dowson et al. 1986; Mahmood et al. 2001; Perez-Moreno and Read 2000, 2001) and corresponding allocation of C to these areas (Bending and Read 1995; Leake et al. 2001). The more widespread mycelial increase in this study may reflect differences in amount and distribution of organic resources: here a single large litter patch was used whereas previous studies used smaller multiple resources, which were either simultaneously or sequentially colonised depending upon the regularity of the foraging front (Bending and Read 1995; Leake et al. 2001).

Uptake of nutrients from the litter patches, whilst not measured in the present study, almost certainly increased the ability of the plants to fix more carbon and thus provides a positive-feed-back mechanism stimulating mycelial production. Similarly, quantity, quality and distribution of organic resources have all been shown to play a significant role in determining the morphological (Boddy et al. 1999) and nutrient allocation (Boddy 1999) responses of foraging mycelial systems of many decomposer basidiomycetes on soil.

Although litter patches induced localised ERM proliferation in *S. bovinus* (Bending and Read 1995), localised additions of inorganic N to peat did not lead to localised proliferation by *S. bovinus* (Read 1991). However, *P. involutus* has been shown to actively forage for localised inorganic N in sand when only N was limiting (Jentschke et al. 2001). N additions to soil tend to reduce ERM production (Arnebrant 1994; Nilsson and Wallander 2003; Wallander and Nylund 1992) and may indicate a shift of carbon allocation from vegetative growth to N assimilation (Bidartondo et al. 2001; Wallander 1995). *P. involutus* is regarded as 'N-tolerant' (Bidartondo et al. 2001) and *S. bovinus* as 'N-sensitive' (Arnebrant 1994) and differences in mycelial growth rate, biomass production and respiration have recently been observed between *P. involutus* and four suilloid species colonising N- or P-fertilised soil areas (Bidartondo et al. 2001). Thus, differences in response to soil N may have a major impact on interspecific ERM development and morphology.

Development of mycelial aggregation in saprotrophs is related primarily to nitrogen economy (Watkinson 1983), and is expressed in interspecific differences in foraging patterns. Species foraging for abundant resources spread as a more diffuse mycelium with high probability of encountering utilisable resources, whereas species with more specific resource requirements, which may be more widely dispersed, may be more efficiently incorporated into the mycelial network through a more aggregated mycelium (e.g. Boddy 1999; Boddy et al. 1999). This is also likely for extraradical mycelium of ectomycorrhizal fungi. *P. involutus* has a broad host range including coniferous and deciduous species, and therefore will have a high probability of contacting compatible roots for colonisation. It also has a high degree of saprotrophic ability (Erland and Söderström 1991), and therefore may be able to utilise a wide range of both saprotrophic and biotrophic resources. *S. bovinus*, on the other hand, has a narrower host range of coniferous species and, although able to utilise litter patches, is unable to forage entirely saprotrophically (Erland and Söderström 1991).

Under a recent classification based upon mycelial exploration strategies (Agerer 2001), the species in this study are considered long distance rhizomorph producers, in contrast to medium and short explorers and species exploring via direct contact between organic resources and mycorrhizal tips. A consequence of longer range foraging is to extend sites of uptake away from the mantle. It is suggested (Agerer 2001) that functional differences in enzyme production, utilisation of soil organic matter and supply of nutrients to host plants may be related to differences in mycelial exploration type.

The phenotypic plasticity of mycorrhizal mycelium and responses to localised resources, together with the greater surface areas for absorption provided by hyphae compared with roots (Smith and Read 1997), provides mycorrhizally colonised plants with more efficient mechanisms for nutrient capture far beyond the nutrient depletion zone of the root system. Recent microcosm studies (Bending and Read 1995; Leake et al. 2001; Mahmood et al. 2001; Perez-Moreno and Read 2000, 2001) have revealed the degree to which some ectomycorrhizal fungi can modify mycelial morphology of the growing front in response to heterogeneous soil conditions. A range of soil factors, including pH, can influence mycelial morphology, degree of mycelial fanning density and growth rate in ectomycorrhizal species (Ek et al. 1994; Erland et al. 1991). A combination of image analysis and fractal geometry will allow quantitative analysis of responses of ectomycorrhizal mycelium (whole systems and selected parts) to such biotic and abiotic conditions in soil.

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