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Reorganization of mycelial networks of *Phanerochaete velutina* in response to new woody resources and collembola (*Folsomia candida*) grazing

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ABSTRACT

Mycelial development of *Phanerochaete velutina* extending from wood inocula in 57 × 57 cm trays of non-sterile soil was characterized after adding: (1) collembola; (2) new wood resources; (3) both new wood resources and collembola; and (4) no new resources and no collembola. After 99 d, all systems had produced distinct mycelial cords, much of the diffuse mycelium and thinner cords that were produced early on having regressed. Systems to which new resources (but no collembola) had been added developed thick cords interconnecting inocula with new resources, and much of the non-connected mycelium regressed. Nonetheless, these systems had significantly greater hyphal coverage and mass fractal dimension than the other treatments, resulting from outgrowth from the new resources. Unexpectedly, morphology of grazed systems with no added resources was very similar to that of ungrazed systems with no added resources, apparently because the collembola grazed on senescing hyphae that would ultimately have regressed. Where new resources and collembola were added, there was proliferation of fine mycelium along connective cords and elsewhere, but this was not as extensive as in the new resource/no collembola systems, the fine mycelium apparently being grazed in patches. Fungus gnat (family *Sciaridae*) larvae contaminated eight (out of 14) trays with no added collembola, but none of the systems to which collembola had been added. They burrowed around the wood and caused cords to be severed.

Introduction

Saprotrophic cord-forming fungi are major agents of decomposition in forest ecosystems. Mycelia grow out from wood and other litter components in search of others, and interconnect these different dead organic resources. They grow predominantly at the soil–litter interface and form connected systems that can cover several square metres, and genets covering several to many hectares (Thompson & Rayner 1982; Smith *et al.* 1992; Ferguson *et al.* 2003; Cairney 2005), though the extent of interconnection within a genet is not known. These mycelial networks are constantly remodelled in response to local nutritional or environmental cues, interaction with other fungi, grazing by invertebrates or other disturbance events (Boddy 1999; Boddy & Jones 2006).

Studies in soil microcosms in controlled conditions have revealed remarkable patterns of reallocation of mycelial biomass and nutrients in response to encounters with new

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resources (Boddy 1999; Boddy & Jones 2006). Often, mycelium connecting new resources with inoculum forms thick cords, whilst elsewhere there is regression of non-connective mycelium, though the extent to which this occurs depends on species, and quantity and quality of resources. Grazing by invertebrates (particularly collembola) can result in dramatic changes to mycelial morphology and foraging patterns, but again these vary depending upon species of fungi, inoculum resource status, grazing intensity (density) and invertebrate species (Kampichler *et al.* 2004; Harold *et al.* 2005; Tordoff *et al.* 2006). However, the vast majority of these studies on factors effecting changes in mycelial morphology have concerned systems of 0.06 m^2 or less (an exception being Hughes & Boddy 1996). These provide a useful indication of responses of young, small mycelial systems but we now need to understand behaviour on a scale closer to that operating commonly in the field (Boddy 1993; Cairney 2005). Hence, in this paper we have employed soil microcosms having about six times the area (0.33 m^2) used previously, to investigate effects of the addition of new resources (four 4 cm^3 wood blocks) and collembola grazing, both singly and jointly, on *Phanerochaete velutina* mycelial system development (from 4 cm^3 wood inocula).

In the small microcosms used in the past, mycelial systems become more open (i.e. less space-filled, having a lower fractal dimension) with time (Donnelly *et al.* 1995) and a small inoculum (4 cm^3) will be able to support a limited amount of mycelial biomass. Therefore we hypothesized that with larger microcosms (57 × 57 cm): (1) in control systems (with no added collembola and no added resources) fine mycelia and thinner cords will eventually regress leaving systems comprising major cords, with much lower coverage than seen previously.

In studies of *P. velutina* mycelial systems in small trays to which new resources were added, development of major cords connecting inocula with the new resource followed by regression of non-connective mycelium only occurred when the new resource was considerably larger than the inoculum (Dowson *et al.* 1986; Bolton 1993). Nonetheless, we hypothesized that: (2) such thickening of interconnective cords and regression of non-connective mycelium will occur in the present experiment, as the inoculum has to support a very much larger mycelial system, and total new resource (as four separate supplies) is four times larger than the inoculum.

In small systems the collembolan *Folsomia candida* grazed on fine mycelium within the colony and hyphal tips at the growing margin, the main cords responding by fanning at the tips, and the lateral cords became much branched (Tordoff *et al.* 2006). We hypothesized that: (3) in the larger systems grazing will occur in similar locations. As there is likely to be a lot of regressing mycelium, and grazing of senescent mycelium of non-basidiomycetes has previously been suspected (Hanlon 1981) we hypothesized that: (4) senescent mycelium will also be grazed. (5) Hyphal coverage is hypothesized to be lower in grazed than in ungrazed systems.

In systems to which both new resources and collembola are added we hypothesized that: (6) major cords will interconnect inoculum with new resources, and non-connective mycelium will disappear more rapidly as it is likely to be grazed as soon as it starts regressing.

Materials and methods

Fungal isolates and inoculum preparation

Phanerochaete velutina (Cardiff University culture collection) was maintained on 2 % malt extract agar (MEA: 20 g l^{-1} Munton & Fison spray malt light, 15 g l^{-1} Lab M agar no. 2) in non-vented 9 cm diam. Petri dishes.

Beech (*Fagus sylvatica*) wood blocks (2 × 2 × 1 cm) were cut from a freshly felled tree and stored at –18 °C until required. Before use they were soaked overnight in de-ionized water to defrost and then autoclaved at 121 °C for 45 min in sealed autoclave bags, twice with a 24 h interval. Blocks were colonized by placing onto 30 d cultures of *P. velutina* on MEA in 14 cm diam. Petri dishes, and incubated at 20 °C in the dark for 15 weeks. The density (dry weight/fresh volume; g cm^{-3}) of a random sample of colonized wood blocks was determined at the start of the experiment, by oven drying at 70 °C for 1 week, as 0.51 ± 0.01 g cm^{-3}.

Collembola culturing

Folsomia candida (supplied by Centre for Ecology and Hydrology Lancaster, UK) were reared on a 9:1 plaster of Paris (Minerva Dental Ltd., Cardiff): activated charcoal (Sigma-Aldrich, Poole, UK) substrate in 0.9 l plastic containers with holes in the lid for aeration, at room temperature. Collembola were fed weekly with dried baker's yeast (Spice of Life, Cardiff, UK) and the substrate kept moist with de-ionized water.

Collembola, of known body size (250–400 μm), were extracted from cultures using a series of stacked metal sieves (Nickel-Electro Ltd., Weston-super-Mare, UK) of progressively smaller pore size. Those of the required size were then transferred to new culture boxes and starved for 1 d, to reduce the effect of previous food source on grazing activity. Individuals were then collected for introduction into each microcosm using an electrical suction pump aspirator, with any collembola visibly damaged during this process being removed and replaced.

Soil microcosms

Topsoil (to 20 cm depth) was collected from mixed deciduous woodland in the Coed Beddick Inclosure, Tintern, UK (National Grid ref. SO 528 018). Wood and leaf litter were removed and the soil sieved through 10 mm mesh. The soil was air-dried for 28 d, sieved through ≤4 mm mesh then through ≤2 mm mesh, and frozen (−18 °C) for 24 h to defaunate the soil. Soil was then thoroughly mixed with a known volume of de-ionized water to obtain a matric potential of – 0.012 MPa (determined by the filter paper method of Fawcett & Collis-George (1967).) Wet soil (1000 g) was added to 57 × 57 cm polythene trays (5 cm deep), and compacted evenly and smoothly to approximately 5 mm depth.

Forty microcosms were inoculated centrally with colonized wood blocks from which adhering mycelium and agar had been removed by scraping with a scalpel. Perspex blocks (2 × 2 × 4 cm) were placed in each corner of each tray, with the long side vertical, allowing trays to be stacked on one

another without damaging mycelium or touching wood blocks. Trays were stacked in groups of 10, with an empty tray on top of the stack, and a tray containing tap water at the bottom, to maintain high humidity. Each stack was wrapped in black plastic to provide darkness, maintain humidity and reduce any effect of air flow in the constant temperature (19 °C) room in which they were incubated. Trays were monitored weekly, moistened by evenly mist spraying de-ionized water onto uncolonized regions of soil, and they were rotated through 90° and their position in the stack randomly changed, to limit the effects of uneven soil drying (Wells *et al.* 1998).

Experimental design

After 36 d, when some mycelia had reached the sides of the trays, four sterile (2 × 2 × 1 cm, autoclaved as for inoculum preparation) beech wood blocks were added to 20 randomly selected trays, one block half way along each tray edge, 5 cm from the edge (Fig 1). At 49 d after inoculum addition 10 trays to which new resources were added and 10 with no additional resources were randomly selected, and to these 250 *F. candida* were added: 50 in each corner and 50 around the central inoculum. There were thus four treatments: (1) no additional resources and no collembola; (2) four new resources but no collembola; (3) four new resources and 250 collembola; and (4) no new resources but 250 collembola. Although the collembola populations increased by reproduction during the experiment, the height of the tray sides prevented all but a few collembola escaping. No collembola contaminated the treatments to which no collembola were assigned. Two hundred and fifty collembola per tray represents 769 m^{-2} soil, which is less than usual field densities (10^4–10^5 m^{-2}; Petersen & Luxton 1982); however, this is appropriate as in our experiments the collembola are restricted to two-dimensions whereas the field figures quoted are for three-dimensions.

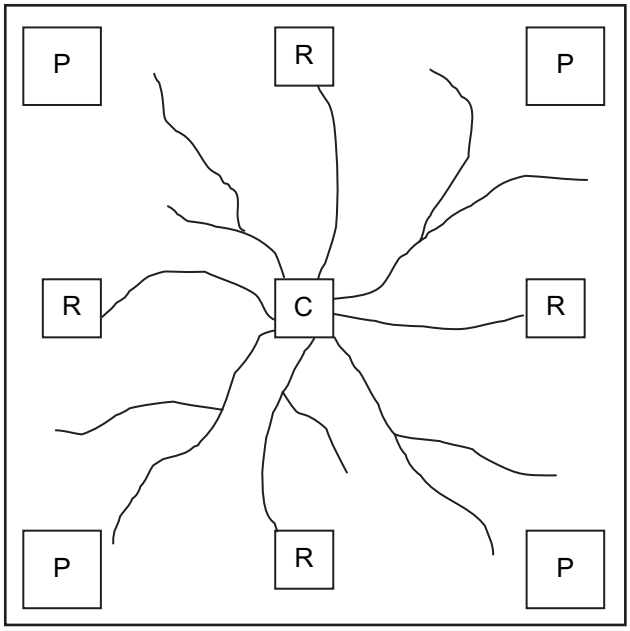

Fig 1 – Microcosm (57 × 57 cm) set up indicating position of central inoculum (C), new resources (R), Perspex blocks (P).

Image capture and analysis

Digital images of experimental systems were captured every 4–10 d with a Konica Minolta Revio KD-420Z digital still camera from a height of 82 cm, with natural illumination. Saved images (2272 × 1704 pixels) were processed using IMAGEJ (National Institute of Health, USA). Tray edges, inocula, additional resources and Perspex blocks were electronically removed from all images, before conversion to greyscale (8 bit), and images were adjusted to 1670 × 1670 pixels. Images were then subject to manual thresholding, to reduce the effect of any unevenness in soil colour: any pixels with a grey value less than the threshold were converted to black to represent soil, and pixels with values greater than the threshold were converted to white, representing mycelium. Hyphal coverage was determined as the number of white pixels in a binary image, converted by IMAGEJ to cm^2, after calibration. Fractal dimension provides a quantitative value that describes branching and space-filling (Donnelley *et al.* 1995, 1999; Boddy *et al.* 1999), and was determined in IMAGE J by the box-counting method. *P. velutina* is approximately mass fractal (i.e. the whole mycelium is fractal; Boddy *et al.* 1999) and hence only the mass fractal dimension (D_{BM}) was estimated.

Statistical analyses

Repeated measures analysis of variance (ANOVA) was applied to hyphal coverage and D_{BM} data, as the same trays were measured repeatedly. Assumptions of repeated measures ANOVA were met, data being normally distributed (Kolmogorov-Smirnov test), equal in variance (Levene's test) and displaying sphericity (Mauchly's test of sphericity). Significant results were further explored using the Tukey-Kramer *a posteriori* test to determine significant differences between means. Unless otherwise stated, data are presented with standard error of the mean.

Results

Phanerochaete velutina extended radially at 0.8 ± 0.1 cm d^{-1} until it approached the edges of the trays (three to four weeks). Though there was variation amongst trays, there was no significant difference between different treatments. There were dramatic between treatment differences in mycelial development (Fig 2).

Control systems: no additional resources, no collembola

Hyphal coverage and D_{BM} increased until the tray edge was contacted (Figs 3–4). At 43 d coverage was 537 ± 70 cm^2. Regression of fine mycelium around the wood blocks and of thinner cords (Fig 2a–d) resulted in a decrease in hyphal coverage and D_{BM} beginning 7–18 d after edge contact (Figs 3–4). Major cords thickened and did not regress during the experiment (Fig 2a–d).

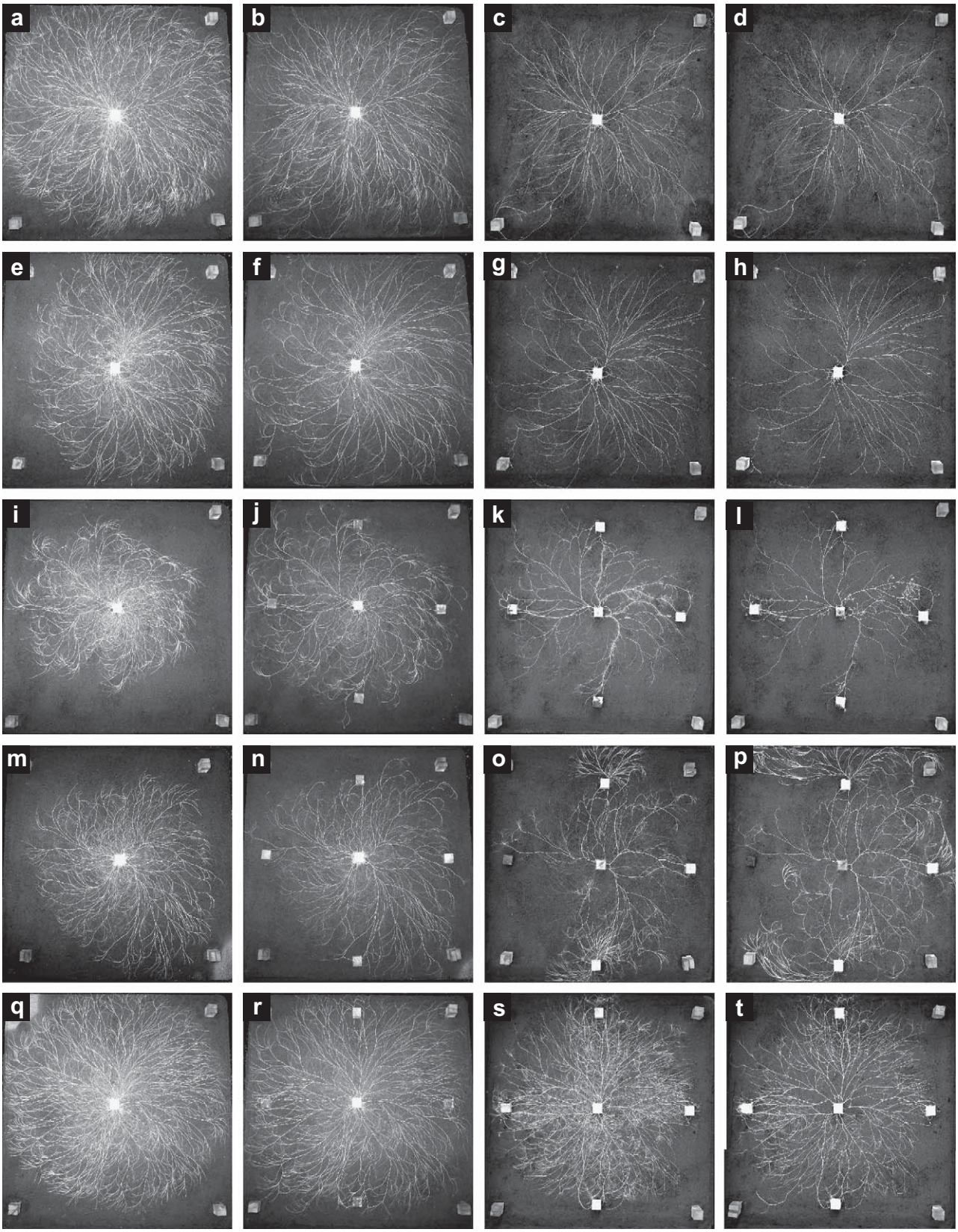

Fig 2 – Digital images of mycelial systems in 57 × 57 cm trays of compressed non-sterile soil, representative of each of the four treatments: a–d, no additional resources and no collembola; e–h, no new resources but 250 collembola; i–l, four new resources and 250 collembola; m–t, four new resources but no collembola. In ungrazed systems to which new resources were added morphological changes differed depending on whether the new resources were placed ahead of the mycelial margin (m–p) or on top of preformed mycelium (q–t). Images in each row are a time series: images in columns from left to right were obtained at 36, 49, 78 and 99 d after addition of wood inoculum. Sides of wood block are 2 cm.

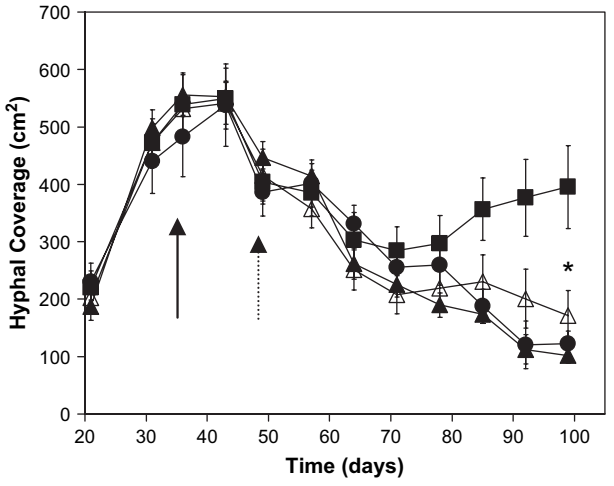

Fig 3 – Hyphal coverage over time in: ●, control (no added resources, no collembola); ■, ungrazed with new resources added; △, grazed with added wood resources; ▲, grazed with no added wood resources. Solid arrow indicates time of addition of extra resources, and dotted arrow indicates time of addition of collembola. Significant differences between treatments indicated by one-way ANOVA *, $P \leq 0.001$, at indicated time point.

Ungrazed, with additional resources

As soon as mycelium contacted the new resources, radial extension elsewhere ceased (Fig 2o). In some cases this prevented some of the new resources from being colonized.

Where the new resources were ahead of the mycelial margin (i.e. not added onto preformed mycelium) there was production of mycelial fans on and near to the new resource within 7 d of contact (Fig 5a), but those on soil regressed within 14 d of their formation. No such fanning was seen where the new resource was added directly onto preformed mycelium.

Irrespective of whether the new resource was ahead of the mycelium or on preformed mycelium, with time cords connecting the inoculum with the new resources thickened (Fig 2m–t). In some cases they actually changed location slightly, being pulled across the soil surface by a 'tightening up' of the cord to a point where some of the cords were no longer in contact with the soil surface (Fig 2d–e). Inoculum and new resources were usually connected by at least two cords, though this did not always represent the shortest route between the two (e.g. Fig 2k–l). Elsewhere there was regression of fine mycelium, thin cords (Fig 5e) and even of some of the larger non-connective cords (e.g. Fig 2k–l); as in the controls this resulted in decreased hyphal coverage and D_{BM} (Figs 3–4).

By 71–85 d, there were differences in systems to which new resources were added ahead of the mycelial margin compared with those in which the new resource was added on top of preformed mycelium. Where the new resource was ahead of the mycelial front, egression occurred from the new resource, at one or two points on its surface, and extended to the edge of the trays or back across other parts of the mycelium (Fig 5b–c). At the same time as egression, diffuse hyphal fans were produced near to the resources from cords not directly connecting the resource with the inoculum (Fig 5a). In some of the systems, following egression from new resources, foraging fronts began to grow again from previously stalled/regressing cords.

In systems where the new resource had been added to preformed mycelium, by 85 d, fans of mycelium began to proliferate along connective cords (Fig 6a–b). Subsequently, this proliferation of fine mycelium occurred throughout the system (Fig 2s) also developing from minor non-connective cords, and leading to an increase in hyphal coverage and D_{BM} (Figs 3–4). In some trays stalled/regressing cords began to extend again, at the same time as this general proliferation of fine mycelium. After 92 d the fine mycelium began to regress leaving the major cords (Fig 2t).

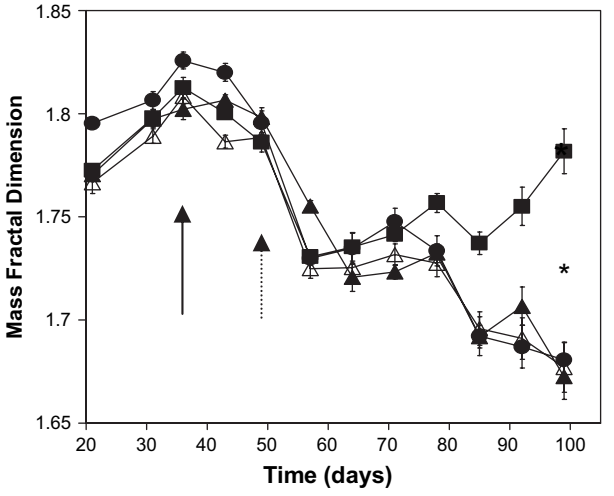

Fig 4 – Change in mass fractal dimension over time in: ●, control (no added resources, no collembola); ■, ungrazed with new resources added; △, grazed with added wood resources; ▲, grazed with no added wood resources. Solid arrow indicates time of addition of extra resources, and dotted arrow indicates time of addition of collembola. Significant differences between treatments indicated by one-way ANOVA *, $P \leq 0.001$, at indicated time point.

Grazed, no additional resources

The changes over time in overall morphology of grazed mycelia resembled that of ungrazed systems with no additional resources (Fig 2a–h). Hyphal coverage and D_{BM} were not significantly ($P \leq 0.05$) different from ungrazed systems with no new resources (Figs 3–4). Thus, regression of thinner cords was evident 7–18 d after reaching the edge of trays, hyphal coverage and D_{BM} reached a peak after 36–43 d and then declined, and diffuse mycelium around the inoculum disappeared by 64 d.

Nonetheless there was clear evidence of collembola grazing by 58–64 d (i.e. 9–15 d after addition). Fine mycelium around the inoculum was grazed from the inoculum outwards, which contrasted with regression from tips towards the inoculum in ungrazed controls. By 64 d grazing made senescing minor cords thinner, and by 71 d some were severed from the inoculum (Fig 7) and others completely grazed

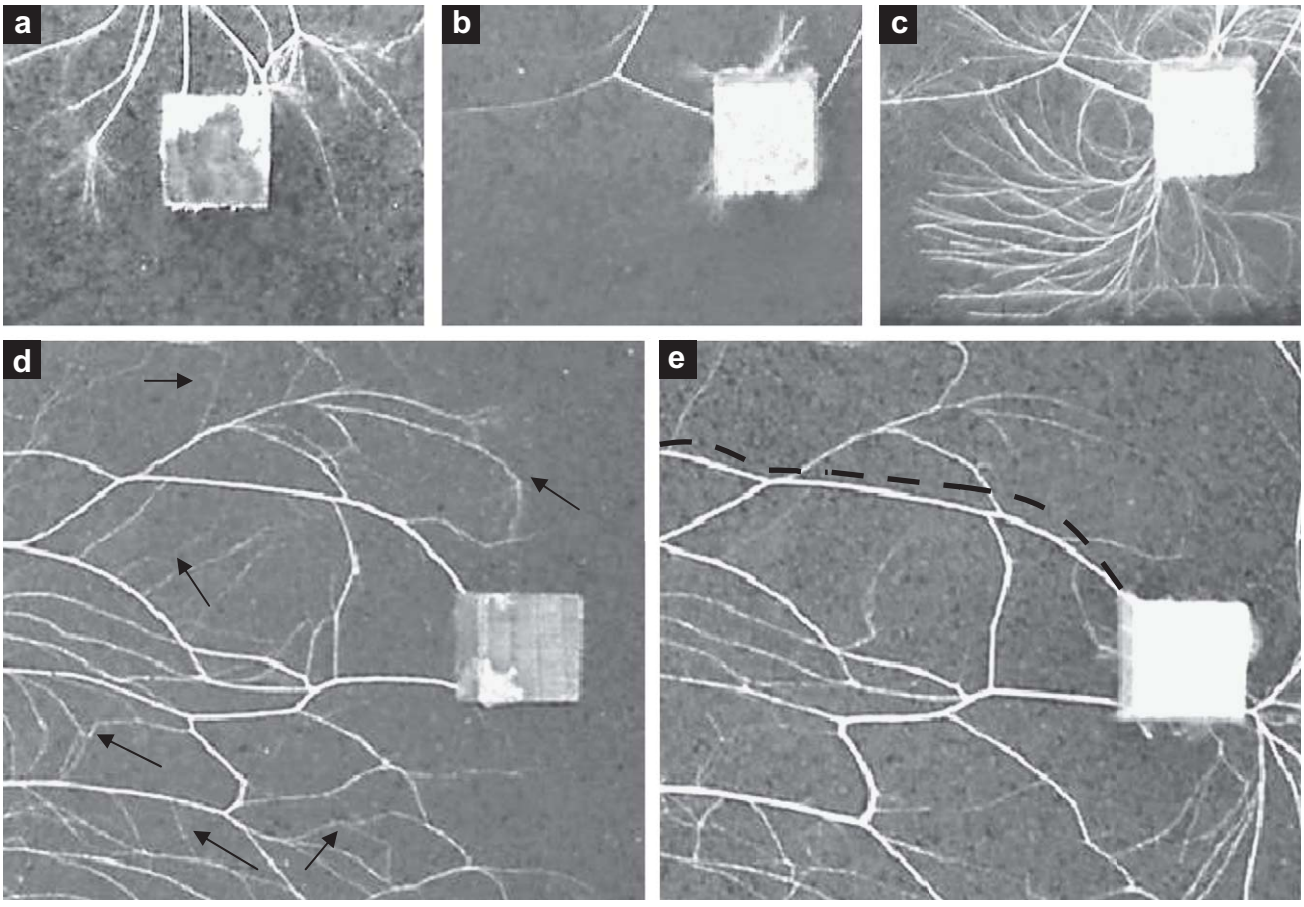

Fig 5 – Production of diffuse fans at mycelial fronts of *Phanerochaete velutina* after contact with new resources ahead of the mycelial margin in ungrazed systems (a). Egression of *P. velutina* from a newly colonized resource after 64 d (b) and 71 d (c). Movement of a *P. velutina* cord during system development 49 d (i.e. 10 d after addition of the new resource that is shown)(d) and 99 d after the central inoculum was added (e). In E the original position of the cord is indicated with a dashed line. Note that minor cords arrowed in d have regressed by 99 d (e). Scale is indicated by sides of wood blocks 2 cm.

away. Diffuse mycelia developed from the apices of some of the thicker cords from 71 d, but this was less than in the ungrazed controls, and was completely grazed away by 78 d. The damage caused by collembola grazing varied considerably between trays, apparently depending on how well developed cords were before collembola addition. In one tray all cord connections to the inoculum were severed by 78 d resulting in complete destruction of the system by 85 d. In all trays collembola frass was evident around cords, and eggs were present in small clumps near thick cords from 57 d.

Grazed, additional resources

Following collembola addition (49 d), the first signs of grazing were around the inoculum, diffuse mycelium and fine cords being grazed from the inoculum outwards, as in grazed systems to which no new resources had been added. After 78 d localized patches of mycelium proliferated as small fans along cords connecting inoculum with new resources (Fig 6c–d), but this was not as extensive and did not form effective networks as in the ungrazed systems to which new resources had been added (cf. Fig 6a–b); the degree of such proliferation was very variable between trays. These localized patches developed throughout the mycelium over time, but were often grazed. Regression and grazing of non-connective mycelium occurred as in ungrazed systems. After 99 d, systems generally consisted of thick connective cords, with a few non-connective cords, with localized fans located on both connective and non-connective cords. There was no mycelial egress from the new resources (Fig 2l). Hyphal coverage ($F_{3,24} = 11.4$, $P \leq 0.001$) and D_{BM} ($F_{3,4} = 2.86$, $P = 0.006$) were significantly less than in ungrazed systems to which new resources had been added, but not significantly different ($P > 0.05$) from other treatments (Figs 3–4). In one system there was complete severance of all cords near the inoculum by 78 d (Fig 8).

Throughout the experiment collembola were mostly located around the central inoculum and the new resources, though frass and small clumps of eggs were evident on or around thick cords from 57 d.

Fungus gnats

Fungus gnat larvae (*Diptera: Sciaridae*) were noted in eight of the trays (in two stacks), being first evident after 64 d. They were only seen in trays to which no collembola had been

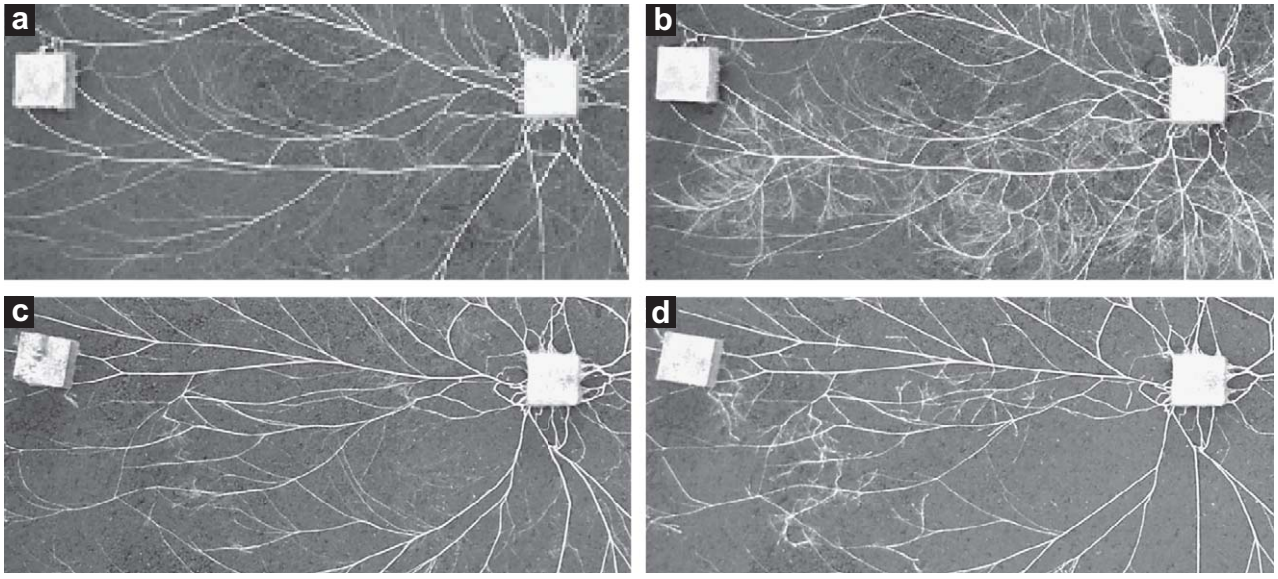

Fig 6 – Mycelial proliferation along cords of *Phanerochaete velutina* connecting the inoculum with a new resource that had been placed on preformed mycelium, in systems which were ungrazed (a–b) or grazed by *Folsomia candida* (c–d). Hyphal coverage in a is 40.63 cm^2 (71 d) and in b 96.78 cm^2 (78 d; 138 % increase between a and b). Hyphal coverage in c is 23.39 cm^2 (85 d) and in d 30.18 cm^2 (99 d; 29 % increase between c and d). Scale is indicated by sides of wood blocks 2 cm.

added (six with added resources, two with just inocula). Soil was disturbed by burrowing around inocula and new resources, and in six of the systems mycelial cords were severed by their grazing. Grazing did not result in any obvious morphological changes.

Discussion

Patterns of mycelial development in the large (0.33 m^2) soil microcosms exhibited some similarities to, but also some major differences from, previous studies in smaller microcosms. As hypothesized (1), in controls with no added collembola and no added resources, much of the diffuse mycelium and thinner cords produced early on had regressed by the time the mycelium had reached the edge of the trays, leaving major cords. Hypothesis 2, that in systems to which new resources (but no collembola) were added there would be considerable regression of mycelium apart from that connected to new resources, is also accepted. As soon as mycelium started to grow out from the new resources, however, there was an increase in coverage (due to this new outgrowth) of these systems, such that by 99 d systems with additional resources but no collembola had significantly higher coverage than the other three treatments.

Production of diffuse mycelial fans at growth fronts around the mycelial perimeter, following contact with new resources in the present experiment, has also been reported for smaller systems where several new resources had been encountered (Bolton 1993; Boddy 1993). While clearly indicating relaying of messages within the mycelium, this response was previously interpreted as possibly indicating that the mycelium ''anticipated'' new resources across a widespread area. However, a different or additional reason for proliferation might be that the mycelium needs access to more nutrients so as to produce the enzymes necessary for colonization of the new resources. In small microcosms in which new resources were added to mature mycelia there was extensive production of small patches of fine hyphae throughout the mycelium, which were sites of uptake of ^{32}P from soil (Wells *et al.* 1997). A further indication that this might be the case is the extensive proliferation of diffuse mycelium along the cord interconnecting the inoculum with the new resource, when the new resource was added

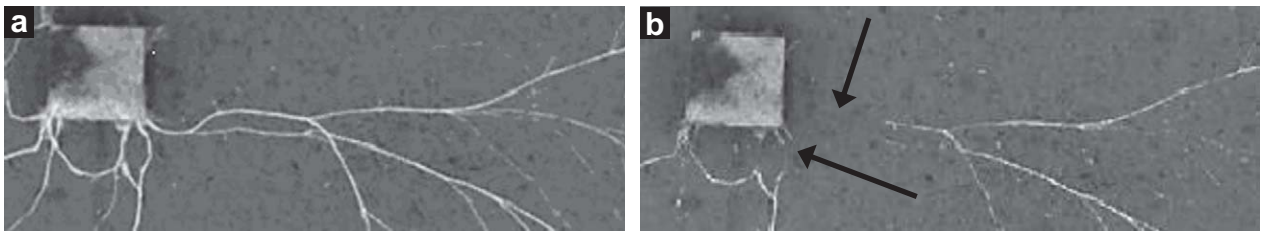

Fig 7 – Severance (indicated by arrows) of *Phanerochaete velutina* cords from inoculum by *Folsomia candida* grazing after 64 d (a) and 71 d (b). Scale is indicated by sides of wood blocks 2 cm.

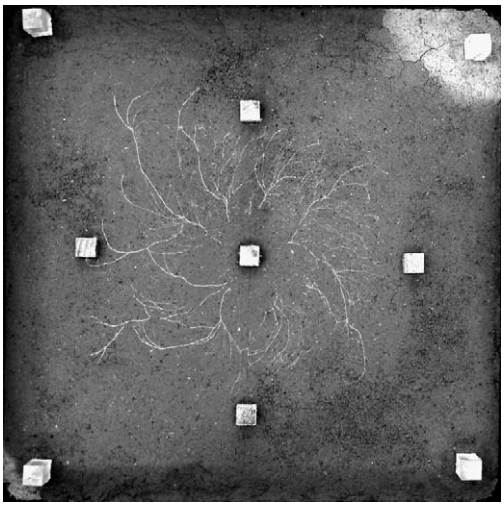

Fig 8 – Complete severance of all cords around the inoculum after 78 d (i.e. 29 d after addition of collembola) in systems with new resources. Scale is indicated by sides of wood blocks 2 cm.

to preformed mycelium in the present study. The patches in the earlier study (Wells *et al.* 1997) were ephemeral, as were the fanned tips and fine proliferations along the interconnecting cords in the present study.

In the present study fanning at the mycelial margin only occurred when the new resource had been placed ahead of the mycelial growing front but not when added to preformed mycelium. By contrast diffuse hyphal proliferation only occurred along cords interconnecting resources when the new resource was added to preformed mycelium. The latter may be analogous to the Wells *et al.* (1997) study in which diffuse hyphal patches proliferated in systems to which new resources had been added to mature mycelium. The different sites of proliferation probably reflect slightly different solutions to the same problem of nutrient shortage, but differing due to differences in location of resources relative to the growth front.

The third hypothesis that two of the main locations of grazing in larger systems will be similar to that in smaller systems (i.e. fine mycelium within the colony and hyphal tips), was also accepted. This extended to grazing of the localized patches of fine mycelium that developed in response to addition of new resources, and of the diffuse mycelia that developed from the apices of some of the thicker cords in systems to which no resources were added. In systems with no additional resources, grazed and ungrazed mycelia had very similar morphological and quantitative (hyphal coverage and D_{BM}) characteristics (rejecting hypothesis 5) at least for the first ten weeks. This implies that grazing was often on senescing hyphae that would have regressed anyway (hypothesis 4). However, although mycelial morphology may be similar, nutrient relations may have changed: thus grazing may release nutrients from hyphae to the soil, whereas in ungrazed systems nutrients from senescing hyphae may be relocated elsewhere within the mycelium. Despite the forgoing, grazing of thicker cords did sometimes occur, and occasionally these were completely severed. This emphasizes the benefit, to the mycelium, of considerable tangential networking and the formation of several thick cords interconnecting between inoculum and new resources. Some of these interconnecting cords do not provide the shortest route for translocation between one resource and another but make the system more resistant to damage: thus even if one translocation route is removed, others are still available. Even with the multiple interconnections between inoculum and new resources all cords were completely severed from the inoculum in two systems, effectively removing this nutrient source from the mycelial system.

Hypothesis 6 was also accepted: there was significantly less hyphal coverage in grazed than ungrazed systems to which new resources had been added. Presumably many mycelial fans that proliferated along connective cords were grazed. Further there was no new mycelial egress evident from the new resources, either because hyphae failed to grow out, or they were grazed as soon as they did so.

This study has revealed several previously unreported phenomena. When new resources were added they were always placed at a constant distance from the inoculum, but as mycelial extension in different trays and in different parts of the same tray were not always identical this led to the new resources sometimes being positioned ahead of the growing front and at other times on mycelium that had already developed. There was some evidence of difference in subsequent mycelial development depending on relative positioning of the new resources. For example, when wood resources were added to preformed mycelium, fans of mycelium proliferated along connective cords. Though previous studies have described morphological effects of adding new resources directly onto mycelium, which include proliferation of fine mycelium (Wells *et al.* 1997; Harris & Boddy 2005), no experiments have been conducted to compare directly effects of position of added new resources. This needs to be done before further explanations can be presented.

The physical movement, over several millimetres, of major cords interconnecting resources has not been reported previously. It is reminiscent of contraction of the fibres in string or damp cotton on drying, but what is actually happening here is not known. One result is shortening of the major cord, which may or may not shorten the actual internal route of translocation. Although spectacular, this phenomenon may have relatively little impact on mycelial functioning.

Even though a wide range of soil invertebrates are mycophagous (Walter 1987), few studies have focused on grazing of saprotrophic basidiomycetes apart from by collembola. One exception showed that nematodes in *P. velutina* cultures on agar resulted in differences in enzyme production (Dyer *et al.* 1992). The ability of fungus gnats to sever cords seen in the present study indicates that they may also be an important group of invertebrates mycophagous on saprotrophic basidiomycetes. Fungus gnats (*Sciaridae*) are well-known fungal grazers, sometimes causing serious damage during commercial cultivation of *Agaricus bisporus* (Sheepmaker *et al.* 1996). The effects of fungus gnats, nematodes and other invertebrate groups, including enchytraeid worms and earthworms, on basidiomycete mycelial development and functioning needs exploring.

Using larger microcosms that more closely approximate the spatial scales over which mycelia operate in the field is clearly appropriate. Increasing the duration of experiments is also likely to be revealing. Immediate priorities for study in these larger microcosms are: effects of site of encounter of new resources, movement of large cords due to ''tightening'', and understanding the effect of mycelial network architecture on the ability to maintain translocation pathways around the mycelium and the ability to reconnect following grazing and other mechanical damage. Maintenance of translocation pathways in mycelial systems and the ability to reconnect when broken is obviously crucial to mycelial functioning in nature. The use of mathematical networking/graph theory (Strogatz 2001; Dorogovtsev & Mendes 2002; Amaral & Ottino 2004) is a promising approach for such analysis in the future (Bebber *et al.* 2006; Fricker *et al.*, in press).

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