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Grazing by *Folsomia candida* (Collembola) differentially affects mycelial morphology of the cord-forming basidiomycetes *Hypholoma fasciculare*, *Phanerochaete velutina* and *Resinicium bicolor*

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ABSTRACT

Cord-forming basidiomycetes are important decomposers of dead wood in forest ecosystems but the impact of mycophagous soil invertebrates on their mycelia are little known. Here we investigate the effects of different grazing intensities of Collembola (*Folsomia candida*) on mycelial foraging patterns of the saprotrophic cord-forming basidiomycetes *Hypholoma fasciculare*, *Phanerochaete velutina* and *Resinicium bicolor* growing from beech (*Fagus sylvatica*) wood block inocula in dishes of non-sterile soil. Mycelial extension rate and hyphal coverage decreased with increased grazing intensity. *R. bicolor* was most affected, high grazing density resulting in only a few major cords remaining. Grazing of *H. fasciculare* often resulted in points of more rapid outgrowth as cords with a fanned margin. In grazed mycelia of *P. velutina* the main cords had fanned tips and lateral cords became branched. These results suggest that mycophagy by *Collembola* may hinder the growth of cord-forming fungi in woodlands, which might impact on the ability of these fungi to forage for and decompose dead organic material.

Introduction

Many woodland decomposer basidiomycetes that colonize discrete, patchily distributed woody resources produce large, persistent, but dynamic mycelial networks at the soil–litter interface (Thompson 1984; Smith *et al.* 1992; Boddy 1993, 1999). At the growing fronts these mycelia comprise individual hyphae, but elsewhere the hyphae are commonly aggregated into cords that allow translocation of water and mineral nutrients from one location to another, and provide resistance to antagonistic effects of soil microorganisms (Boddy 2000). Cord persistence indicates some resistance to grazing by soil mesofauna, but mycelia can be totally destroyed in soil microcosms if, for example, there are population explosions of contaminating Collembola (L. Boddy unpub.).

There are numerous studies on interactions between soil invertebrates and fungi (e.g. Booth & Anderson 1979; Bengtsson & Rundgren 1983; Moore *et al.* 1985; Leonard & Anderson 1991; Varga *et al.* 2002; Maraun *et al.* 2003), but little on the effects of grazing on spatial changes in mycelia, in terms of foraging patterns, enzyme production, translocation of energy, nutrients and water. Three studies demonstrate that spatial changes can be dramatic: grazing of *Mortierella isabellina* in culture by the collembolan *Protaphorura armata* (formerly *Onychiurus armatus*) resulted in a switch in mycelial morphology and enzyme production, but in locations away from grazed sites, indicating propagation of information through the mycelium (Hedlund *et al.* 1991). Grazing of the cord-forming basidiomycete *Hypholoma fasciculare* by the collembolan *Folsomia candida* in soil microcosms dramatically affected foraging

morphology, fractal geometry, mycelial extent and hyphal coverage, though grazing by two other species (*Proisotoma minuta* and *Hypogastrura cf. tullbergi*) had little effect (Kampichler et al. 2004; Harold et al. 2005). Effects were dependent on grazing density of *F. candida*, mycelial extent and hyphal coverage decreasing monotonously with increasing grazing density (Kampichler et al. 2004), and on inoculum resource quality and quantity (Harold et al. 2005).

It is not yet known whether: (1) there are different effects of grazing on different cord-forming species; (2) grazing occurs evenly within mycelia; and (3) grazing at initial stages of outgrowth has different effects to later grazing. These questions are addressed in this paper in two experiments investigating grazing by *F. candida* on the cord-forming basidiomycetes *H. fasciculare*, *Phanerochaete velutina* and *Resinicium bicolor* in soil microcosms. These fungal species are common in temperate woodlands and are likely to co-occur with *F. candida*, which is found in a range of habitats. Three specific hypotheses were tested: (1) as *H. fasciculare* has the least substantial cords and is the most responsive (to new resources, microclimate, etc.; Boddy 1999; Zakaria & Boddy 2002) of the three fungi, it will be more responsive to grazing than the longer-range foragers *P. velutina* and *R. bicolor*; (2) *F. candida* will graze preferentially on individual hyphae as they are relatively thin walled and without the protective rind of cords; and (3) mycelium newly emerging from resources will be more susceptible to grazing than established systems as it comprises a greater proportion of individual hyphae and no well-developed extra-resource mycelium to rely upon.

Materials and methods

Collembola culture and extraction

Folsomia candida (source: Centre for Ecology and Hydrology, Lancaster, UK) were cultured in 0.9 l plastic boxes containing a 9:1 plaster of Paris (Minerva Dental Ltd., Cardiff, UK): activated charcoal (Sigma, UK) mixture in their bases. Culture boxes were stored at laboratory temperature (approx. 18–22 °C) and lids were pierced to facilitate aeration. At weekly intervals the Collembola were supplied with dried baker's yeast (Spice of Life, Cardiff, UK) and the boxes were moistened with de-ionised (DI) water.

F. candida of body width 250–400 μm were extracted from cultures using a series of stacked metal sieves of known pore size (Nickel-Electro Ltd., Weston-super-Mare, UK). Collembola were added to the uppermost sieve and allowed to self-sort into size classes by moving through sieves of progressively smaller pore size for 5 min. Collembola of the desired body width were transferred into plastic boxes and the number required for inoculation of the experimental microcosms collected using an electrical Pooter (aspirator). This system resulted in negligible Collembola mortality.

Fungal isolates

Phanerochaete velutina and *Hypholoma fasciculare* (Cardiff University culture collection), and *Resinicium bicolor* (Steve

Woodward, University of Aberdeen) were routinely subcultured on 2% malt agar (MA; 20 g^{-1} Munton & Fison spray malt, 15 g^{-1} Lab M agar no. 2) in non-vented 9 cm diam. Petri dishes, and kept at 20 °C in the dark.

Preparation of colonized wood inocula

Wood inocula for experiments were colonized on 70 ml MA in 14 cm diam. Petri dishes (20 wood blocks per dish). The agar had been preinoculated with *H. fasciculare*, *P. velutina* or *R. bicolor* and incubated for 11 d at 20 °C in the dark. Beech (*Fagus sylvatica*) wood blocks (2 × 2 × 0.5 cm and 2 × 2× 1 cm in experiments 1 and 2, respectively) were placed on the agar, ensuring good contact with the medium, and incubated in darkness at 20 ± 1 °C for three months (experiment 1), six weeks (experiment 2) or five months (*R. bicolor* 11 cm diam. systems in experiment 2). The wood blocks had been obtained from freshly sawn and frozen planks, soaked overnight in DI water and then autoclaved twice at 24 h intervals, at 121 °C for 30 min for sterilization. Following colonization, and in readiness for experimental use, the inoculum blocks were removed and scraped free of adhering mycelium and agar.

Preparation and inoculation of soil dishes

Soil was collected to a depth of 20 cm from deciduous woodland in the Coed Beddick Inclosure, Tintern, UK (National Grid Ref.: SO528018). After removal of surface litter, soil was sieved through a 10 mm mesh on site, and stored in lidded plastic bins. Before use in microcosms, soil was air-dried for 21 d in large plastic trays, frozen for 1 d to remove soil fauna, and then sieved through 4 and 2 mm metal mesh to remove organic material and stones. Dry soil was then thoroughly mixed with DI water to attain a soil matric potential of −0.012 MPa (determined by the filter paper method; Fawcett & Collis-George 1967), before compaction to 5 mm depth in 14 cm diam. Petri dishes (experiment 1; 85 g wet soil per dish) or 24 × 24 cm lidded bioassay dishes (Nunc-Gibco, Paisley, UK; experiment 2; 200 g wet soil per dish). Inoculum wood blocks were pushed firmly into the centre of each dish and incubated at 19 ± 1 °C in stacks in the dark. All treatments (see below) were randomly allocated within stacks. Soil dishes were weighed and rewetted back to the original weight with a fine mist of DI water every 7 d to maintain constant water potential. Soil dishes remained free of contaminating soil fauna for the duration of the experiment; although hyphae of soil fungi were periodically observed on the surface of the non-sterile soil these did not visibly impede the growth of the inoculated cord-former.

Image capture and analysis

Digital images of mycelia were captured using a Hitachi KP-M1 monochrome CCD video camera with a Canon TV macro-zoom lens, connected to a computer, and stored using a Synapse frame store (Synoptics, Cambridge, UK). Dishes were illuminated using a circular fluorescent bulb with opalescent diffuser fitted around the camera, which was mounted at 85 or 102 cm above the dishes in experiments 1 and 2, respectively. As the increased distance between camera and

dish in experiment 2 diminished the effect of the fluorescent lighting, a 60 W tungsten spot lamp was used to enhance light levels.

SEMPER 6 (Synoptics, Cambridge, UK) for Windows was used to pre-process and analyse images, using a method similar to that described by Boddy *et al.* (1999). After pre-processing, the surface hyphal coverage (cm^2) and radial extension (cm) of binary images were determined. A calibration line of known length [the internal diameter of a Petri dish: 13.6 cm (experiment 1) or the internal wall length of a bioassay dish: 22.6 cm (experiment 2)] was drawn electronically on the image, and pixel values were converted to area (cm^2) and line (cm) measurements. Hyphal coverage was given by the number of white pixels in a binary image, converted by SEMPER to square centimetre format, while radial extension was the mean length of eight lines drawn from the inoculum to the mycelial margin; lines radiated from a central point at 45° angles to each other, aligned on a grid overlaid by SEMPER.

Experiment 1: effect of grazing intensity on young extra-resource mycelia

H. fasciculare, *R. bicolor* and *P. velutina* were subjected factorially to three grazing densities (10, 20 and 40 individuals per dish) plus Collembola-free controls. Each of the 12 treatment combinations was replicated five times, giving a total of 60 soil microcosms. Before addition of Collembola, the fungi were allowed to grow out from wood inocula until 50% of the fungal mycelia of a particular species (i.e. 10 dishes) had reached a diam. of 8 cm; this was after 6, 10 and 16 d for *P. velutina*, *H. fasciculare* and *R. bicolor*, respectively. Colonies used were all initially fairly radially symmetric. Collembola were added to uncolonized soil evenly around the margin of each dish.

Individual dishes were then sealed in small polythene bags both to reduce moisture loss and to enable enumeration of any escaped Collembola. The number of escapees were minimal but any noted were replaced from culture to return numbers to their original levels. Images were captured (see above) immediately before addition of the Collembola, and then after 2, 4, 10 and 15 d for *P. velutina*, 2, 5 and 10 d for *H. fasciculare*, and 4 d for *R. bicolor*. No measurements were made after 15 d as mycelia had by then reached the edge of the dishes.

Experiment 2: effect of grazing prior to and during establishment of extra-resource mycelia

Folsomia candida (40 individuals per dish = constant treatment) were added to soil dishes at the same time as wood block inocula or when 50% of the mycelia of a particular species had reached 11 cm diam. This occurred 19 and 22 d after wood inocula addition for *R. bicolor* and *H. fasciculare* treatments, respectively. As mycelia of different species grow at different rates and fill space to different extents, Collembola density was also standardized across fungal species by adding one *F. candida* per square centimetre of area that the mycelium on each dish occupied (for *H. fasciculare* 80 ± 14 individuals; for *R. bicolor* 69 ± 5 individuals), and in another series of treatments by adding one *F. candida* per square centimetre of hyphal coverage (for *H. fasciculare* 83 ± 12 individuals; for

R. bicolor 16 ± 3 individuals). Hyphal coverage was determined by analysis of images captured immediately before Collembola addition. The area covered by mycelium was determined from images by electronically joining tips of the main mycelial cords, and determining the area of the entire space enclosed by this boundary line.

There were five replicates in the treatments with no previous outgrowth, and seven in the 11 cm diam. treatments of *H. fasciculare* and *R. bicolor*. However, due to erratic outgrowth of *P. velutina* Collembola were only added to no previous outgrowth treatments for this species. Images were captured immediately before Collembola addition and 1, 2, 4, 8, 12, 16 and 20 d after Collembola addition. After 20 d images were captured every 10 d until microcosms were harvested 80 d after Collembola addition.

Experiment 2: wood inoculum decay rate

At the time of addition of wood inocula to microcosms, the densities (oven dry weight/volume before drying; mg cm^{-3}) of a random subsample of inoculated wood blocks (five to 10 replicates) of each fungal species were determined. At the end of the experiment, the density of inocula used in the experiment was also determined. Wood inoculum decay rate (mg cm^{-3} d^{-1}) was then estimated as the change in density across the experiment (wood density at harvest subtracted from mean density for that species at the time of inoculation) divided by the experimental duration in days.

In all instances wood blocks were scraped free of adhering mycelium, frass and soil using a spatula, measured using callipers and then dried at 80 °C for 7 d before weighing.

Statistical analyses

The radial extension of mycelia of different grazing treatments was compared using analysis of covariance (ANCOVA; General Linear Model; Minitab Statistical Software, Release 13.31) with time (days after Collembola addition) as a covariate. Extension rates (i.e. the increase in radial extension over time for each replicate) were determined and significant time–treatment interactions investigated by performing one-way ANOVA and Tukey's pairwise comparisons.

Hyphal coverage of mycelia was analysed by performing repeated measures analysis of variance (ANOVA; SPSS, Release 11) with grazing treatment as the main effect and time (days after Collembola addition) as a sub-factor. Treatment data met the assumptions of repeated measures ANOVA, being normally distributed (Kolmogorov–Smirnov test), equal in variance (Levene's Test) and displaying sphericity (Mauchly's test of sphericity). Significant ($P \leq 0.05$) treatment and time–treatment interactions were investigated further by performing one-way ANOVA and Tukey's pairwise comparisons on individual time points.

In experiment 2, treatment means for wood decay rates were compared using one-way ANOVA (Minitab Statistical Software, Release 13.31); data were normally distributed (Anderson–Darling test) and variances were equal (Levene's test). Significant ($P \leq 0.05$) results were investigated using Tukey's pairwise comparisons.

Results

Experiment 1: effect of Collembola density on radial extension rate and hyphal coverage of mycelia 8 cm diam. at time of addition

The radial extent of mycelia increased linearly with time. With all fungal species there was a decrease in radial extension rate of colonies with increase in the number of Collembola added (Fig 1). With both *H. fasciculare* (Fig 1A) and *P. velutina* (Fig 1C) radial extension rate was significantly ($P \leq 0.05$) lower in the 40 Collembola treatment than in the ungrazed treatment, with 10 and 20 Collembola treatments having intermediate values. In the 40 Collembola treatment extension rates were 61.5 and 45.3% of those of ungrazed controls for *H. fasciculare* and *P. velutina*, respectively. There was no significant effect of grazing on the extension rate of *R. bicolor* ($P > 0.05$; Fig 1B).

With all fungal species (Fig 2), the greater the Collembola density the lower the hyphal coverage; though differences were not significant ($P > 0.05$) for *P. velutina*. There were significant ($P < 0.001$) time–treatment interaction effects on hyphal coverage of *H. fasciculare* and *R. bicolor*. After 10 d of grazing 20 and 40 Collembola treatments had lower hyphal coverage than the *H. fasciculare* control treatment (0 Collembola; Fig 2A). With *R. bicolor* differences between the treatments were significant with 40 and 20 Collembola after 4 d (Fig 2B). No measurements were made after 15 d as by then the presence of juveniles confounded the original densities of Collembola.

Experiment 1: effect of Collembola density on morphology of mycelia 8 cm diam. at time of addition

The morphology of mycelia was dramatically altered by grazing. With all three fungal species effects were dependent upon Collembola density; the highest densities of Collembola resulted in the most striking differences (Fig 3). Mycelial margins in grazed systems of *H. fasciculare* became progressively less even than in control dishes, with more rapid outgrowth and fanning at points around the margin (Fig 3A–C). Morphological changes were evident in *P. velutina* mycelia after 2 d: the tips of cords started fanning, and lateral cords became more branched. By 10 d all cord tips were well fanned in the highest density treatment, and aerial hyphae (in addition to those adpressed to the soil) developed towards the mycelial margins (Fig 3D–F). *R. bicolor*, which has a very open system comprising very linear cords but with fine hyphae around the wood inoculum, was intensively grazed leaving only a few often short, linear, major cords (Fig 3G–I).

Experiment 2: effect of grazing on radial extension rate and hyphal coverage of colonies 11 cm diam. or not emerged at time of Collembola addition

The extremely limited and/or unusual growth of mycelia when Collembola were added before outgrowth from wood inocula (see below) prevented analysis of measurements of radial extension and hyphal coverage.
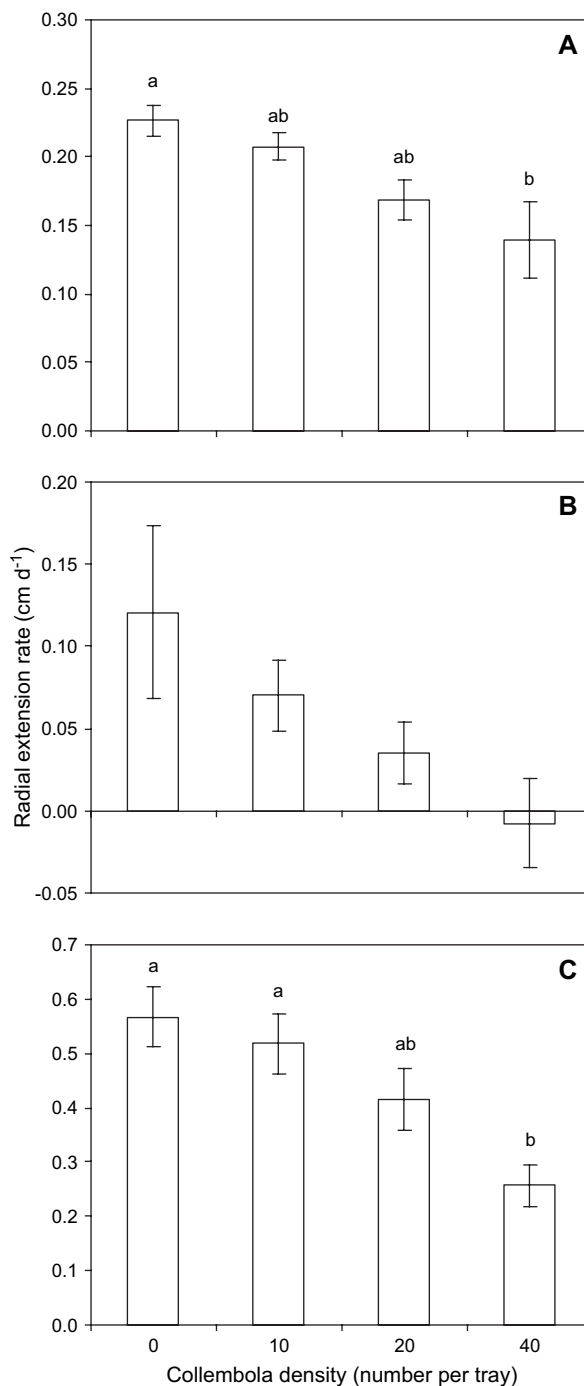

Fig 1 – Radial extension rates, respectively, of *Hypholoma fasciculare*, *Resinicium bicolor* and *Phanerochaete velutina* grazed by 0, 10, 20 or 40 *Folsomia candida* added to 8 cm diam. mycelia growing over compressed non-sterile soil from 2 cm³ beech wood inocula. Grazing treatment affected the extension rate of *H. fasciculare* (ANCOVA; $F_{3,72} = 5.73$; $P = 0.001$) and *P. velutina* ($F_{3,52} = 4.72$; $P = 0.005$), but not that of *R. bicolor* ($F_{3,32} = 0.98$; $P = 0.42$). Different letters above bars indicate significant differences between means (Tukey's pairwise comparisons; $P \leq 0.05$).
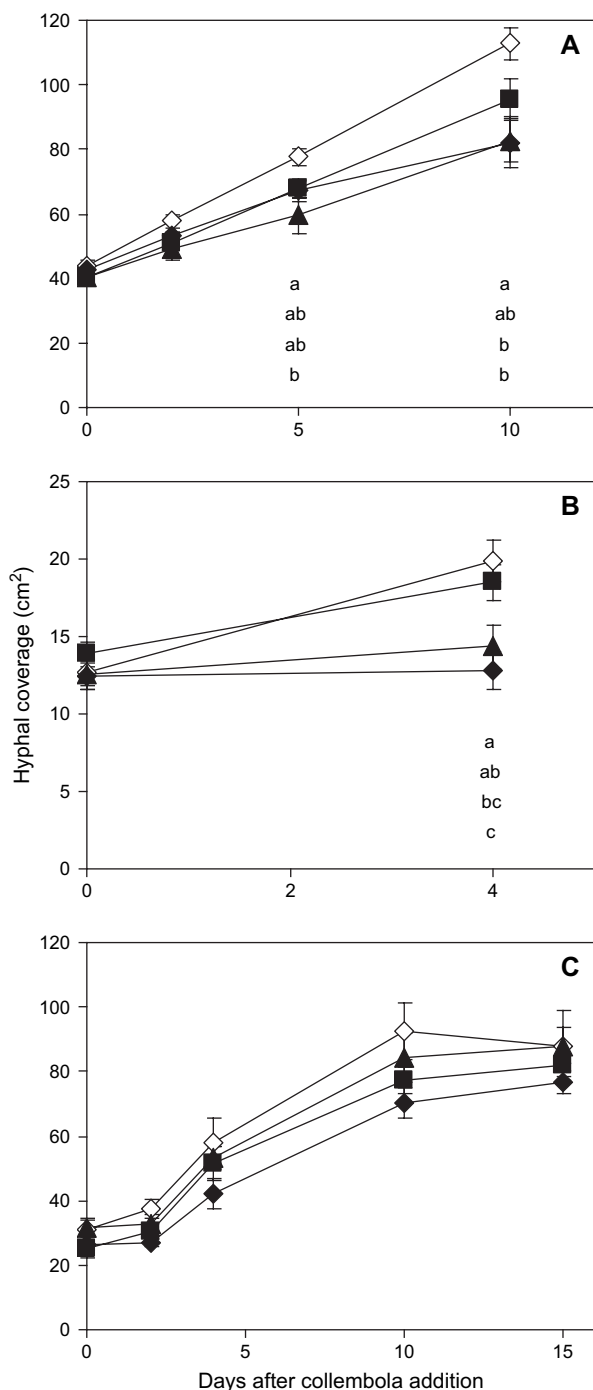
Fig 2 – Hyphal coverage, respectively, of *Hypholoma fasciculare*, *Resinicium bicolor* and *Phanerochaete velutina* grazed by 0 (◇), 10 (■), 20 (▲) or 40 (◆) *Folsomia candida* added to 8 cm diam. mycelia growing over compressed non-sterile soil from 2 cm³ beech wood inocula. Grazing treatment affected hyphal coverage of *H. fasciculare* (repeated measures ANOVA; $F_{9,48} = 4.84$; $P < 0.001$) and *R. bicolor* ($F_{3,16} = 17.5$; $P < 0.001$), but not that of *P. velutina* ($F_{12,64} = 0.97$; $P = 0.49$). Different letters below time points indicate significant differences between means at that time point (one-way ANOVA and Tukey's pairwise comparisons; $P \leq 0.05$). Letters are displayed in the same order as the treatment means.

Collembola grazing significantly ($P \leq 0.05$) affected extension rate of both *H. fasciculare* and *R. bicolor* 11 cm diam. mycelia (Fig 4). In *R. bicolor* all grazed systems had a significantly ($P \leq 0.05$) lower extension rate than ungrazed controls (Fig 4B), whereas in *H. fasciculare* the extension rate was only significantly ($P \leq 0.05$) lower than that of ungrazed controls when Collembola density was based on hyphal coverage or mycelial area (Fig 4A). In both species, the grazing treatment based on mycelial area had the lowest extension rate, 60.1 and 27.2% of that of ungrazed controls for *H. fasciculare* and *R. bicolor*, respectively (Fig 4).

The change in hyphal coverage of mycelia over time was also affected by Collembola grazing treatment (Fig 5), indicated by highly significant time–treatment interactions, in both *R. bicolor* ($P < 0.001$) and *H. fasciculare* ($P < 0.001$). With *R. bicolor*, there were significant ($P = 0.002$) differences between grazed and ungrazed treatments by 12 d (Fig 5B). By 30 d the treatment with density based on mycelial area had significantly ($P < 0.001$) lower hyphal coverage than the treatment with a constant number of Collembola or that based on hyphal coverage, although from 50–80 d the grazed treatments were not significantly different ($P > 0.05$; Fig 5B).

Differences in hyphal coverage between grazed and ungrazed *H. fasciculare* treatments were significant from 16 d ($P = 0.002$; Fig 5A), and by 30 d the treatment with a constant number of Collembola had significantly ($P \leq 0.05$) higher hyphal coverage than the other two grazing regimes (Fig 5A). From 40–80 d, however, the three grazed treatments did not differ significantly ($P > 0.05$) from each other in terms of hyphal coverage (Fig 5A).

Experiment 2: sites of Collembola grazing and reproduction, and changes in morphology of mycelia 11 cm diam. at time of Collembola addition

The morphology of mycelia was altered by grazing, with changes dependent upon grazing treatment in both *H. fasciculare* and *R. bicolor* (Fig 6A–I). There was evidence of grazing to the *H. fasciculare* systems 1 d after addition of *F. candida*. Fine white hyphae at the growing margin were heavily grazed in discrete patches. There was also some grazing of discrete patches in the interior of the systems adjacent to small areas of uncolonized soil. Fine diffuse mycelium and very narrow cords were grazed while thickened, yellowish cords remained wholly intact. These grazing effects became more accentuated with time from 1 to 12 d after Collembola addition. In ungrazed controls the mycelium had an even margin with only a few gaps close to the inoculum, whereas in grazed dishes relatively large spaces appeared close to the inoculum, and the margin was often uneven with large invaginations (cf. Fig 6G with 6H). Furthermore, there were differences between the different grazing treatments. In the treatments in which grazing intensity was determined based on hyphal coverage or mycelial area (i.e. at a density >80 Collembola per dish) grazing effects were pronounced across the mycelium, whereas at the lower density, effects were restricted more to the interior of the system, with the margin remaining relatively even.

After 12 d, when juvenile Collembola started to increase the grazing density in all microcosms, there was a rapid
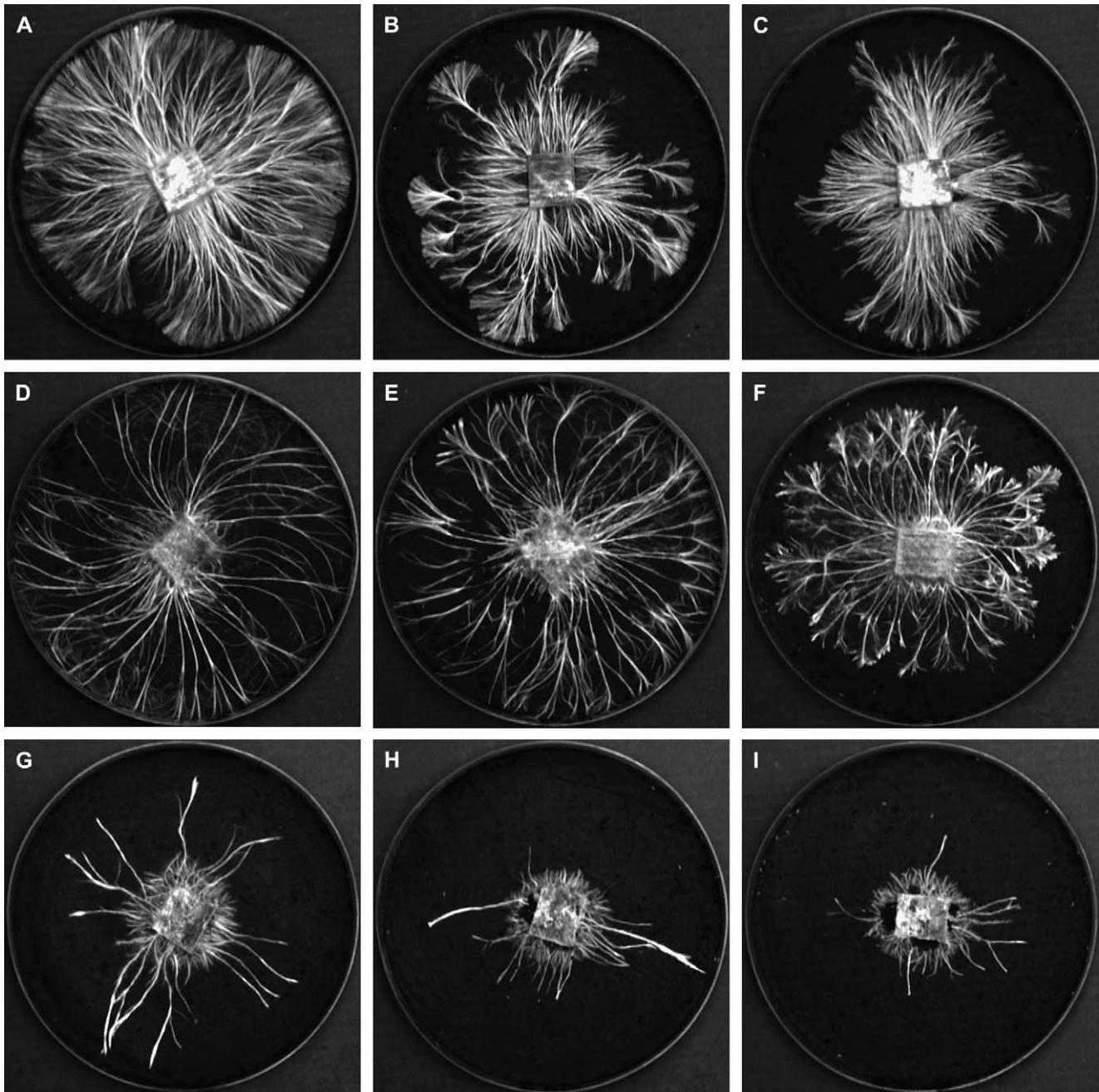
Fig 3 – Digital images showing effects of grazing intensity by *Folsomia candida* on mycelial systems (8 cm diam. at time of addition of Collembola) of *Hypholoma fasciculare* (A–C), *Phanerochaete velutina* (D–F) and *Resinicium bicolor* (G–I) 10 d, 10 d and 4 d, respectively, after addition of Collembola. A, D and G, 0 Collembola; B, E and H, 20 Collembola; and C, F and I, 40 Collembola per dish. Dishes were 14 cm diam. and contained non-sterile, compressed soil.

increase in the magnitude of effects, with most fine white mycelium being grazed away in high *F. candida* density microcosms to leave mycelia composed of thick yellowed cords. From 16–40 d, two broad types of grazed mycelial pattern developed: (1) systems with similar extension from all sides of wood inocula, but with only thick yellowed cords remaining (e.g. Fig 6B); and (2) uneven systems with little mycelium in one section of microcosm, but with the other sections relatively intact, with advancing white mycelial growth fronts still present (e.g. Fig 6C). After 40 d the amount of young white

mycelium decreased in all grazed dishes, and ungrazed controls also began to regress. By 80 d all systems were more or less reduced to thick yellowed cords only.

With *R. bicolor*, there was only slight evidence of grazing effects during the first 8 d. This consisted chiefly of small discrete grazed patches close to the wood inocula, and occasionally cord tips were grazed and finer cords severed. By 20 d, grazing effects were much more striking and obviously dependent on grazing treatment. In the treatment where density was calculated based on hyphal coverage (mean: 16 *F.*
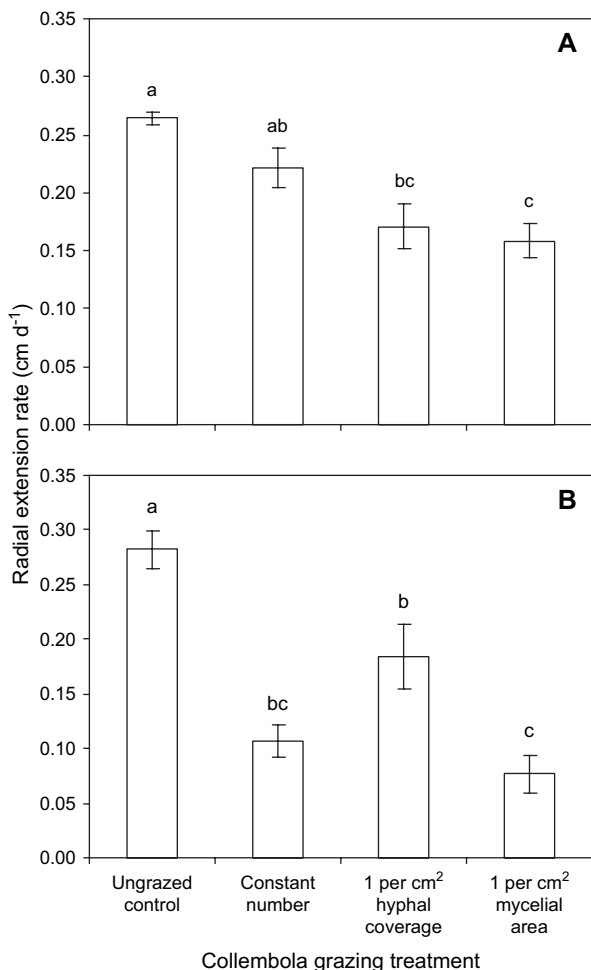
Fig 4 – Radial extension rates, respectively, of *Hypholoma fasciculare* and *Resinicium bicolor* grazed by *Folsomia candida* added to 11 cm diam. mycelia growing over compressed non-sterile soil from 4 cm^3 beech wood inocula. Grazing treatment affected the extension rate of *H. fasciculare* (ANCOVA; $F_{3,188} = 3.83$; $P = 0.011$) and *R. bicolor* ($F_{3,160} = 9.34$; $P < 0.001$). Different letters above bars indicate significant differences between means (Tukey's pairwise comparisons; $P \leq 0.05$).
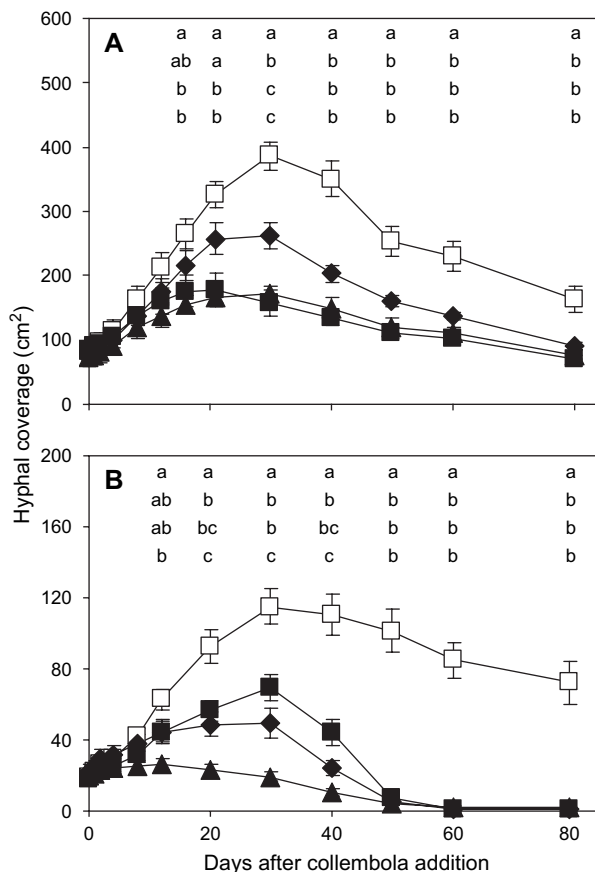

Fig 5 – Hyphal coverage, respectively, of *Hypholoma fasciculare* and *Resinicium bicolor* grazed by *Folsomia candida* added to 11 cm diam. mycelia growing over compressed non-sterile soil from 4 cm^3 beech wood inocula. Treatments were: ungrazed control (□); constant number per dish (♦); standardized as 1 per cm^2 hyphal coverage (■); standardized as 1 per cm^2 mycelial area (▲). Grazing treatment affected hyphal coverage of *H. fasciculare* (Repeated Measures ANOVA; $F_{36,288} = 7.08$; $P < 0.001$) and *R. bicolor* ($F_{33,264} = 30.4$; $P < 0.001$). Different letters above time points indicate significant differences between means at that time point (one-way ANOVA and Tukey's pairwise comparisons; $P \leq 0.05$). Letters are displayed in the same order as the treatment means.

candida; Fig 6E), systems were more or less intact, resembling the ungrazed control dishes (Fig 6D), but with a few severed cords and a more sparse mycelium. Where a constant number (40) were added irrespective of hyphal coverage or mycelial area, more damage was evident. In treatments where density was based on mycelial area (Fig 6E; where Collembola occurred at a high density—mean 69), mycelial damage was greater still with many cords being severed (Fig 6I). Some fanning of hyphae was noted in the latter treatment, at both the cord tips and at grazed patches along the cords. Grazing damage increased slowly over time from 20 to 40 d, and was followed by a rapid increase in damage by 50 d. By this time most of the grazed systems were reduced to just a few thick cords, and these were often severed from the wood inocula. By 80 d all grazed microcosms contained very little mycelium; ungrazed control mycelia were beginning to regress.

Large egg masses were seen in treatments with all fungal species as early as 1 d after adding *F. candida*. These were generally situated on soil up to 4 cm beyond the mycelial margin, or actually on the mycelium itself. Juvenile *F. candida* began to emerge from these egg masses after 12 d, and within a few days brought about a dramatic increase in the number of *F. candida* present within microcosms. Only a few dead *F. candida* were observed during the experiment; these were consumed by surviving Collembola.

Experiment 2: effect of grazing on morphology of mycelia that had not emerged at time of Collembola addition

Collembola grazed on mycelium of all species emerging from the wood inoculum, but much more frequently on *R. bicolor*.
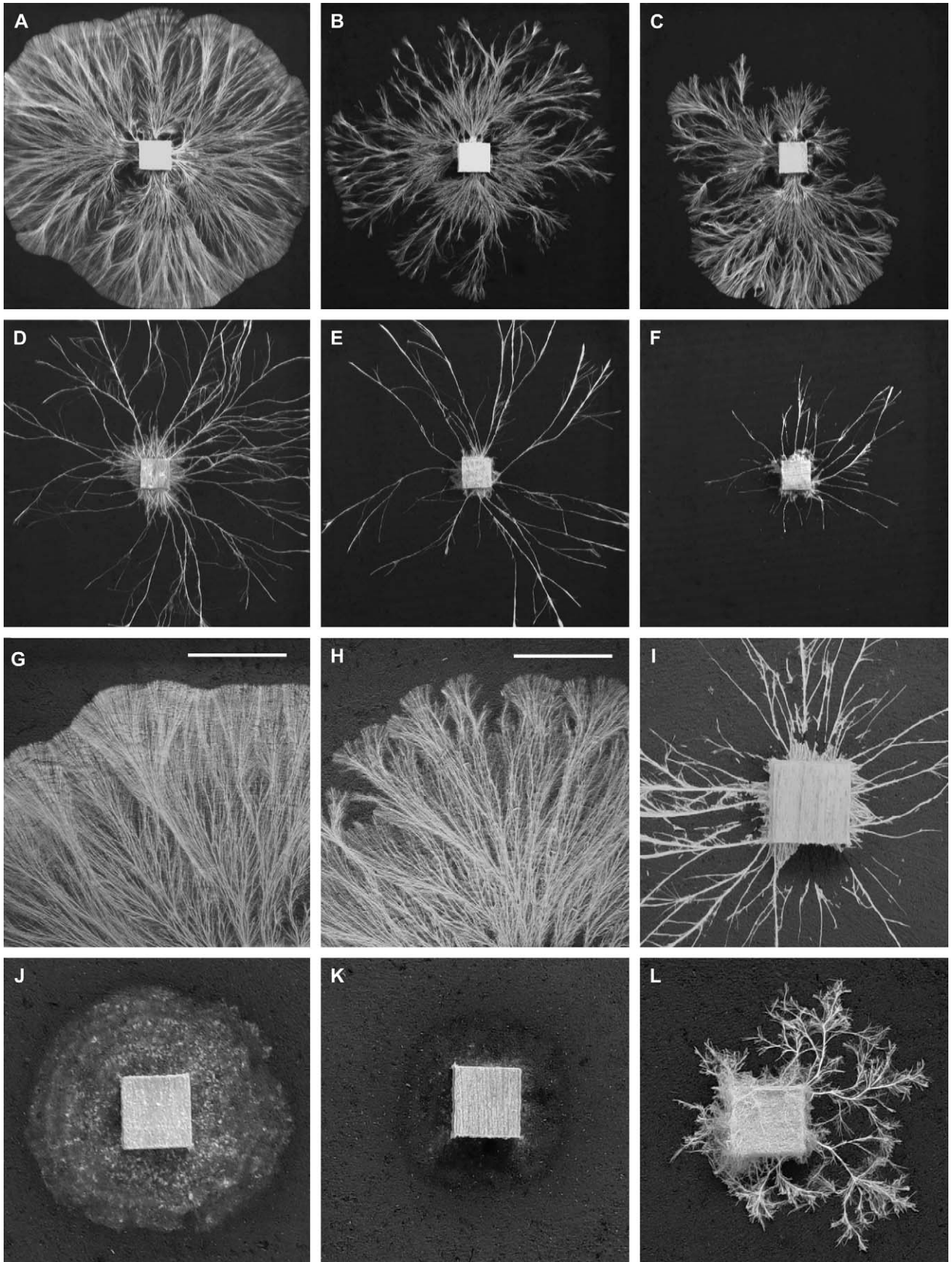
This resulted in more frass being present on *R. bicolor* inocula by the end of the experiments. The effect of grazing varied depending on fungal species. With *H. fasciculare* and *R. bicolor* the presence of Collembola prevented the formation of cords. Instead, systems consisted of diffuse white mycelium that grew through a dense ring of Collembola faeces around the wood inocula. In *H. fasciculare* the mycelium developed as a slowly enlarging circle, which reached a diameter of approximately 8 cm after 50 d (Fig 6J) and then remained stable until 80 d. A similar pattern of growth occurred in *R. bicolor*, although the mycelium was much more sparse and attained a diameter of only 6 cm (Fig 6K). In this species very little hyphal growth was visible on the wood inoculum, whereas in *H. fasciculare* the wood had a fine covering of hyphae. In *P. velutina* mycelial cords did form, although there was considerable fanning and aerial mycelium (Fig 6L), but there was evidence of cords being grazed, particularly in necrotic regions (experiment 1). Extension from inocula was slow but continued until harvest at 80 d.

Experiment 2: wood inoculum decay rate

Inoculum wood decay was slower in all grazed systems than controls, except for the treatment with 40 Collembola grazing on *H. fasciculare* prior to outgrowth (Table 1). However, the only significant ($P \leq 0.05$) reduction in decay rate was with Collembola density based on hyphal coverage of 11 cm diam. *H. fasciculare* mycelia.

Discussion

Mycelial morphology was affected by grazing in all fungi, and was broadly consistent across the two experiments. The largest effects were associated with the highest density of Collembola, decreasing extension rate and hyphal coverage occurring with increasing number of Collembola, supporting the results of a previous study on *H. fasciculare* (Kampichler *et al.* 2004). In experiment 2 the lack of significant grazing density effects on hyphal coverage beyond 40–50 d was probably due the fact that the Collembola populations had increased to such an extent that there were effectively no differences in population density.

There were, however, considerable differences between fungal species. For example, while both the radial extension rate and hyphal coverage were significantly reduced by grazing of *H. fasciculare*, in *P. velutina* only the extension rate was reduced. *R. bicolor* was most dramatically affected, with often only a few major cords remaining. Results of grazing experiments should, however, be treated cautiously, as adding an equal number of Collembola to similarly aged systems (as in experiment 1) does not necessarily imply equivalent grazing pressure. Adding Collembola based on the area occupied by the mycelium or hyphal coverage (in the second experiment) provided equivalent grazing pressure to mycelia that extend at different rates and fill space to different extents. The importance of this is seen in interpreting grazing effects on *R. bicolor*. In experiment 1 the extension rate of *R. bicolor* mycelia was reduced to 58% of the control with only 10 Collembola added whereas that of *H. fasciculare* and *P. velutina* decreased by less than 9%. In experiment 2 percentage reduction in the extension rate of *R. bicolor* was similar to that of *H. fasciculare* when Collembola addition was standardized on hyphal coverage.

R. bicolor, with its large diameter cords and coating of calcium oxalate crystals (Connolly & Jellison 1995), which possibly act as a deterrent to consumption by grazers, might have been expected to be unpalatable. In fact it appeared to be the most palatable species in this study. In contrast, *H. fasciculare*, with its profuse space-filling mycelium comprising many fine hyphae as well as cords might have been expected to be the most palatable, but appeared to be the least palatable species. Though the relationship between interspecific differences in hyphal/cord morphology and apparent palatability is not self-evident, intra-mycelial differences were more obvious. Thus, with *H. fasciculare* fine white hyphae at the mycelial margin were most heavily grazed; hyphae within the colony were sometimes grazed, in which case it was always the diffuse mycelium or very narrow cords that were consumed, not the yellowish thicker cords. Extensive grazing of fine cords was also evident in the other two species, as hypothesized.

There was some evidence that more mature systems are more resistant to grazing, at least for *R. bicolor* though not necessarily *H. fasciculare*, however, inferences must be tentative as comparisons of mycelial size are being made across experiments. The decreased effects during early grazing on the larger *R. bicolor* systems may be because the cords are less palatable, due to increase in rind thickness and/or possible production of encrusting crystals and secondary chemicals.

The morphology of a mycelium that has been grazed not only changes as a result of loss of biomass, but also by producing new growth with different characteristics. This is particularly evident at the margins of *H. fasciculare* and *P. velutina* systems grazed by 40 Collembola in experiment 1, where cord tips were fanned compared with ungrazed systems (Fig 3C, F). This is comparable with the growth patterns of plants grazed by insect herbivores, where feeding can cause the development of multiple leading shoots after the removal of terminal buds (Carne 1969). Modified growth of grazed fungal

Fig 6 – A–L Digital images showing effects of *Folsomia candida* when added to 11 cm diam. mycelial systems (A–I) or before outgrowth of systems (J–L). Figs A–C, *Hypholoma fasciculare* 20 d after addition of Collembola; Figs D–F, *Resinicium bicolor* 20 d after addition of Collembola. Treatments are ungrazed controls (A, D), or with Collembola density determined by hyphal coverage (B, E) or mycelial area (C, F). Margins of *H. fasciculare* systems were more regular in ungrazed (G) than in grazed (H) systems 8 d after Collembola addition. (I), *R. bicolor* system with Collembola density determined by mycelial area, showing grazed patches close to wood inoculum 12 d after *F. candida* addition. (J–L), *H. fasciculare*, *R. bicolor* and *P. velutina*, respectively, after 54 d of grazing when Collembola were added before mycelial outgrowth. Dishes were 24 × 24 cm and contained non-sterile, compressed soil. Wood inocula (2 × 2 cm) indicate scale except in (G) and (H) where scale bar is 2 cm.

Table 1 – Mean decay rates (mg cm^{-3} d^{-1} ± standard error) of wood inocula in experiment 2, where Collembola were added before mycelial outgrowth or to 11 cm diam. mycelia

	Phanerochaete velutina		Hypholoma fasciculare		Resinicium bicolor	
	Prior to mycelial outgrowth	11 cm diam. mycelium	Prior to mycelial outgrowth	11 cm diam. mycelium	Prior to mycelial outgrowth	11 cm diam. mycelium[a]
Ungrazed control	1.68 ± 0.24[b]	—	1.76 ± 0.13[b]	1.63 ± 0.12[b]	1.85 ± 0.29[b]	1.36 ± 0.07[b]
Constant number per dish	1.19 ± 0.19[b]	—	2.16 ± 0.17[b]	1.38 ± 0.06[bc]	1.59 ± 0.33[b]	1.26 ± 0.09[b]
Standardized as 1 per cm^2 hyphal coverage	—	—	—	1.14 ± 0.09[c]	—	1.34 ± 0.09[b]
Standardized as 1 per cm^2 mycelial area	—	—	—	1.39 ± 0.10[bc]	—	1.14 ± 0.13[b]

a R. bicolor 11 cm diam. mycelium wood inocula were incubated for longer than other treatment inocula (see methods).
Figures in the same column followed by the same letter are not significantly different (one-way ANOVA and Tukey tests; $P > 0.05$). Dashes indicate treatment combinations not performed.

mycelia has also been reported for a soil zygomycete (Hedlund et al. 1991). Our hypothesis suggested that, as a short range, responsive forager, the mycelium of H. fasciculare would be most affected, and that of P. velutina, a long range and generally less responsive forager less affected. In fact, the morphology of P. velutina did change dramatically (Fig 3F) suggesting that how a mycelium responds to grazing may relate more to the sites where grazing predominates rather than to foraging strategy and general responsiveness to abiotic and biotic cues.

Grazing at initial stages of outgrowth has different effects to grazing on more mature extra-resource mycelium. The inability of H. fasciculare and the ability of P. velutina to produce cords, albeit to a limited extent, correlates with the former's tendency to plane-fill and the latter's tendency to form more open systems with distinct mycelial cords (Boddy 1999). R. bicolor, however, when ungrazed formed open systems with distinct cords, but was unable to do so when grazed before an extra-resource mycelium had established, presumably reflecting its palatability. Outgrowth of all species was very slow in the presence of Collembola and, with H. fasciculare and R. bicolor, continued for the duration of the experiment, effectively restricting the fungus to the inoculum. Grazing may, thus, be one factor that determines whether a decomposer fungus is resource-unit restricted or non-restricted. Given that Collembola occur at a high density in temperate woodlands (Petersen & Luxton 1982) and that other soil invertebrate groups, particularly oribatid mites (Kaneko et al. 1995; Schneider & Maraun 2005), also graze fungal hyphae, how do extensive extra-resource mycelia develop? A possible explanation is that these fungi exploit situations when grazing intensity is low. Soil faunal population densities are variable both spatially and temporally (Usher 1969), and extra-resource mycelia may be best able to develop in patches of low faunal density. It is also possible that fungi growing in forest soils are less accessible to Collembola than they are in the laboratory, and that Collembola may selectively feed on other fungi.

Finally, grazing may affect wood decomposition; inoculum decay of H. fasciculare was reduced by grazing. Interspecific fungal interactions also sometimes result in a decreased decay rate, though equally there are examples of increases occurring (Owens 1989; Boddy 2001). More generally, interspecific interactions between saprotrophic fungi and soil invertebrates are likely to have consequences for decomposition, through nutrient mobilisation from leaf litter and senescent fungal material (Anderson et al. 1983; Hanlon & Anderson 1979). Each fungal species used in the present study appears to be palatable to Collembola, and displays distinctly modified growth as a result of their grazing. It is therefore probable that Collembola, and other mycophagous soil invertebrates, affect the spatial organization of these fungi and hence their ability to forage for and decompose dead organic matter. Extrapolation from microcosm studies such as these must, however, be undertaken cautiously; feeding by soil invertebrates on cord-forming fungi has not been investigated in the field.

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R E F E R E N C E S

Anderson JM, Ineson P, Huish SA, 1983. Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodlands. Soil Biology and Biochemistry 15: 463–467.

Bengtsson G, Rundgren S, 1983. Respiration and growth of a fungus, Mortierella isabellina, in response to grazing by Onychiurus armatus (Collembola). Soil Biology and Biochemistry 15: 469–473.

Boddy L, 1993. Cord-forming fungi: warfare strategies and other ecological aspects. Mycological Research 97: 641–655.

Boddy L, 1999. Saprotrophic cord-forming fungi: meeting the challenge of heterogeneous environments. Mycologia 91: 13–32.

Boddy L, 2000. Interspecific combative interactions between wood-decaying basidiomycetes—a review. *FEMS Microbiology Ecology* **31**: 185–194.

Boddy L, 2001. Fungal community ecology and wood decomposition processes: from standing tree to complete decay of coarse woody debris. In: Jonsson BG, Kruys N (eds), *Ecology of Woody Debris in Boreal Forests Ecological Bulletins*, **49**, pp. 43–56.

Boddy L, Wells JM, Culshaw C, Donnelly DP, 1999. Fractal analysis in studies of mycelium in soil. *Geoderma* **88**: 301–328.

Booth RG, Anderson JM, 1979. The influence of fungal food quality on the growth and fecundity of *Folsomia candida* (Collembola: Isotomidae). *Oecologia* **38**: 317–323.

Carne PB, 1969. On the population dynamics of the eucalypt-defoliating sawfly *Perga affinis affinis* Kirby (Hymenoptera). *Australian Journal of Zoology* **17**: 113–141.

Connelly JH, Jellison J, 1995. Calcium translocation, calcium oxalate accumulation, and hyphal sheath morphology in the white-rot fungus *Resinicium bicolor*. *Canadian Journal of Botany* **73**: 927–936.

Fawcett RG, Collis-George N, 1967. A filter-paper method for determining the moisture characteristics of soil. *Australian Journal of Experimental Agriculture and Animal Husbandry* **7**: 162–167.

Hanlon RDG, Anderson JM, 1979. The effects of *Collembola* grazing on microbial activity in decomposing leaf litter. *Oecologia* **38**: 93–99.

Harold S, Tordoff GM, Jones TH, Boddy L, 2005. Mycelial responses of *Hypholoma fasciculare* to Collembola grazing: effect of inoculum age, nutrient status and resource quality. *Mycological Research* **109**: 927–935.

Hedlund K, Boddy L, Preston CM, 1991. Mycelial responses of the soil fungus, *Mortierella isabellina*, to grazing by *Onychiurus armatus* (Collembola). *Soil Biology and Biochemistry* **23**: 361–366.

Kampichler C, Rolschewski J, Donnelly DP, Boddy L, 2004. Collembolan grazing affects the growth strategy of the cord-forming fungus *Hypholoma fasciculare*. *Soil Biology and Biochemistry* **36**: 591–599.

Kaneko N, McLean MA, Parkinson D, 1995. Grazing preferences of *Onychiurus subtenuis* (Collembola) and *Oppiella nova* (Oribatei) for fungal species inoculated on pine needles. *Pedobiologia* **39**: 538–546.

Leonard MJ, Anderson JM, 1991. Grazing interactions between a collembolan and fungi in a leaf litter matrix. *Pedobiologia* **35**: 239–246.

Maraun M, Martens H, Migge S, Theenhaus A, Scheu S, 2003. Adding to "the enigma of soil animal diversity": fungal feeders and saprophagous soil invertebrates prefer similar food substrates. *European Journal of Soil Biology* **39**: 85–95.

Moore JC, St. John TV, Coleman DC, 1985. Ingestion of vesicular–arbuscular mycorrhizal hyphae and spores by soil microarthropods. *Ecology* **66**: 1979–1981.

Owens EM, 1989. Fungal community structure and functioning in decomposing wood. *PhD thesis, University of Wales, Cardiff, UK*.

Petersen H, Luxton M, 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos* **39**: 287–388.

Schneider K, Maraun M, 2005. Feeding preferences among dark pigmented fungal taxa ("Dematiacea") indicate limited trophic niche differentiation of oribatid mites (Oribatida, Acari). *Pedobiologia* **49**: 61–67.

Smith ML, Bruhn JN, Anderson JB, 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* **356**: 428–431.

Thompson W, 1984. Distribution, development and functioning of mycelial cord systems of decomposer basidiomycetes of the deciduous woodland floor. In: Jennings DH, Rayner ADM (eds), *The Ecology and Physiology of the Fungal Mycelium*. Cambridge University Press, Cambridge, UK, pp. 185–215.

Usher MB, 1969. Some properties of the aggregation of soil arthropods: Collembola. *Journal of Animal Ecology* **38**: 607–622.

Varga J, Naar Z, Dobolyi C, 2002. Selective feeding of collembolan species *Tomocerus longicornis* (Mull.) and *Orchesella cincta* (L.) on moss inhabiting fungi. *Pedobiologia* **46**: 526–538.

Zakaria AJ, Boddy L, 2002. Mycelial foraging by *Resinicium bicolor*: interactive effects of resource quantity, quality and soil composition. *FEMS Microbiology Ecology* **40**: 135–142.