Grazing by collembola affects the outcome of interspecific mycelial interactions of cord-forming basidiomycetes

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Abstract

While there is a plethora of studies on the effects of invertebrate grazing on mycelia, including several studies on saprotrophic cord-forming basidiomycetes, there is little information on the effects of grazing on mycelial interactions. The study compares the progress and outcomes of interspecific mycelial interactions between *Hypholoma fasciculare*, *Phallus impudicus*, *Phanaerochaete velutina* and *Resinicium bicolor* when grazed by the collembola *Folsomia candida* or *Protaphorura armata* in agar culture and trays (24 × 24 cm) of non-sterile soil. In ungrazed systems results were broadly consistent with previous studies, though there were few instances of deadlock, and a clear transitive (A > B > C) hierarchy could not be discerned. Instead, there was an intransitive hierarchy (i.e., A > B, B > C but C > A). Additionally, in agar culture, there were considerable differences in combative ability of four different strains of *H. fasciculare*. Collembola grazing had major effects on mycelial interactions. *F. candida* grazing altered both the outcome and progression of half of the fungal interactions studied, while the less active *P. armata* had almost no discernable effects on fungal interactions. Grazing by *F. candida* affected mycelial extension rate in five of the interaction combinations, some increasing, others decreasing. In grazed systems of *P. velutina* interacting with *H. fasciculare*, extension rate of the former was much more rapid over the opponent than over soil. Not only did grazing affect mycelial interactions, but interactions affected grazer activity; *H. fasciculare* was grazed in areas where it was interacting with *P. velutina* mycelium, but less so elsewhere.

By altering the outcome of mycelial interactions and mycelial extension rate, collembola grazing may alter the distribution of cord-forming fungi on the forest floor, and may also play a role in maintaining the diversity of fungal species. The differences in combative ability of different strains of a species imply an even more complex scenario in the natural world.

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Introduction

As many species of saprotrophic cord-forming fungi occupy similar spatial niches at the soil–litter interface, interactions are inevitable (Boddy 2000). Interspecific fungal interactions are highly aggressive and generally occur following gross mycelial contact (Boddy 2000). Interactions result in either deadlock or some form of replacement (i.e. partial, complete, or mutual); these outcomes are variable and can be substantially affected by both biotic and abiotic variables (Chapela et al. 1988; Woods et al. 2005). Intraspecific genetic variability may also be
important in determining fungal interaction progression and eventual outcome, although most studies investigating interactions of higher fungi have tended to concentrate on a single strain of each species. A few studies with *Trichoderma* species (Ascomycota) interacting with wood-decay fungi have involved multiple strains (see Philp et al. 1995; Wheatley et al. 1997) but most of these have focussed on strains of medical or biocontrol value (Walker et al. 1995; Vainio et al. 2001). Despite possible implications for both species abundance and diversity, the role played by fungal genetic variability in fungal fitness remains unknown.

As well as encountering other fungi, non-unit-resource-restricted fungi (*sensu* Cooke & Rayner 1984) growing out from resources are also more accessible to grazers. Various soil invertebrate taxa, including Nematoda, Collembola and oligochaeta, are known to graze on fungal mycelia and can substantially alter fungal morphology and physiology (Ruess et al. 2000; Harold et al. 2005; Tordoff et al. 2006; Boddy & Jones 2008). Such effects are likely to alter fungal fitness and, therefore, their competitiveness in interactions with other soil microorganisms, including fungi.

The visible changes in morphology and physiology during fungal interactions are associated with biochemical changes, such as increased enzymatic activity and production of diffusible (DOC) and volatile (VOC) organic compounds (Griffith et al. 1994; Baldrian 2004; Hynes et al. 2007; Evans et al. 2008). This enhanced activity may also lead to lysis of hyphal compartments and nutrient leakage into the surrounding environment (Wells & Boddy 2002). Numerous plant species use chemicals emitted as a result of insect–herbivore action to attract parasitoids to the vicinity (Dicke 1994; Tentelier & Fauvergue 2007), and a comparable process may occur with fungi. Mycophagous grazers may use chemical cues arising from interaction activity to locate high quality resource patches while fungi may actively recruit grazers to provide a competitive advantage over an opponent. Orientation to fungal odours by invertebrates has been well documented (Swift & Boddy 1984; Hedlund et al. 1995; Guevara et al. 2000) and anecdotal evidence exists of attraction of fungus gnats to fungal interaction zones (Boddy et al. 1983).

This study aims to: (i) elucidate the effects of invertebrate grazing on the morphology and competitive abilities of saprotrophic cord-forming basidiomycetes when interacting interspecifically; (ii) determine whether physiological and chemical changes in the mycelium, such as pigment production, may affect invertebrate behaviour (e.g. with preferential invertebrate grazing at interaction zone); (iii) determine whether different fungal strains of the same species affect fungal combativeness and invertebrate grazing. One set of experiments was performed in agar culture, and another set in trays of soil. Though soil trays are more realistic, agar culture allows many more combinations to be performed.

Materials and methods

**Collembola culturing**

*Folsomia candida* and *P. armata* were cultured in 0.6 l plastic tubs with pierced lids for aeration. Each tub contained 9:1 plaster of Paris (Minerva Dental Ltd., Cardiff, UK): activated charcoal (Sigma, UK). Collembola were provided with dried baker’s yeast (*Saccharomyces cerevisiae*, Spice of Life Ltd., Cardiff, UK) weekly. Tub moisture was maintained with deionised water (DI).

Experimental *F. candida* were selected using a stacked sieving system with sieves of known pore size, the larger sieves being uppermost (Nickel-Electro Ltd., Weston-super-Mare, UK). Collembola were added to the top sieve and allowed to self-sort by moving through the sieves for 5 min. Those of body diameter 250–400 μm were placed in fresh culture pots and left without food for 24 hr to evacuate any gut contents. These were then removed to experimental plates using an electric “pooter”.

**Fungal isolates**

*H. fasciculare* (four fruit bodies isolates, from disparate locations; agar medium experiment only; Supplementary Table 1), *Phallus impudicus* (isolated from a cord), *P. velutina* (isolated from wood) and *Resinicium bicolor* (two wood isolates (1, 2) agar experiment only) were cultured on 2 % malt extract agar medium (MEA, 20 g l⁻¹ malt, Munton and Fison, UK; Lab M agar no. 2) and maintained in the dark at 20 °C. *H. fasciculare, P. impudicus* and *P. velutina* were from the Cardiff University Culture Collection and the *R. bicolor* isolate from Steve Woodward, Aberdeen University. The first three species are extremely common in temperate deciduous woodland; *R. bicolor* is common in coniferous woodland though infrequent in deciduous woodland. These species were selected for study because they cover a broad spectrum of foraging patterns (Boddy 1999), and their interaction outcomes (Boddy 2000) and effect of collembo grazing when growing alone (Boddy & Jones 2008) have been well characterised.

**Interactions in agar culture**

Fungi were paired opposite to each other in all of the 36 possible combinations. Inoculation was timed to ensure that when the two mycelia met each individual had colonised an equivalent area of agar medium. Three, 5 mm inoculum plugs were spaced equidistantly along a chord 2 cm from the edge of 2 % MEA plates (9 cm dia). Plates were incubated at 20 °C in the dark and maintained in cardboard boxes within black polythene bags to reduce water loss. Twenty collembo were added at two points at the edge of the Petri dish, 10 on each mycelium, to each of five replicates per combination when the interacting fungi in a minimum of 50 % of the replicates had made contact for 2 d or more. Twenty collembo are a realistic field density (Petersen & Luxton 1982) and have effects on fungal morphology and activity when growing alone (Boddy & Jones 2008).

Agar plate interactions were photographed with a Nikon® Coolpix™ 5700 digital camera mounted on a Kaiser RA1 camera stand (Kaiser, Germany) set at a height of 36.7 cm. Photography started on the day of collembo addition (*t₀*) and then every 2 d until 14 d (*t₄*), followed by every 4 d until 26 d (*t₆*).

**Determination of outcome of interactions in agar culture**

Outcome of interaction was classed as: (1) deadlock, where neither fungus gained territory of the other; (2) partial replacement, where one fungus extended at least 5 cm into
Grazing by collembola affects the outcome of mycelial interactions 3

In agar, outcomes were determined by visual inspection and confirmed by reisolation from the underside of the agar onto 2 % MEA. Relative combative ability of different H. fasciculare strains was compared by employing a scoring system, following Crockatt et al. (2008). Each replicate was scored as: 2, total replacement of opponent; 1, partial replacement of opponent; 0, deadlock; −1, partial replacement by opponent; −2, total replacement by opponent. A cumulative score was attributed to each strain providing a combative index. A strain showing total replacement in all five replicates scored 10, while a species totally replaced in all replicates scored −10.

Soil tray preparation

Soil (pH 4.5) collected from the top 5–10 cm mixed deciduous woodland in (S0517069) was air-dried for 7 d, and sieved through 4 mm then 2 mm mesh to remove large organic material and stones. The soil was then frozen for 24 hr to prevent population explosions of resident soil fauna (but minimising effects on microbial community) before rewetted with DI to attain a soil matric potential of ca. −0.012 MPa. The wet soil (200 g/tray) was evenly compacted to 4 mm depth into 24 × 24 × 2 cm non-vented lidded bioassay trays (Nunc—Gibco, Paisley, UK), which were then weighed to ±0.01 g and stored in stacks of 20, double-wrapped in black PVC bags to prevent desiccation (20 °C ± 1 °C, dark). Trays were used within 7 d of being made, and rewetted to original weight with DI every 7 d. During the experiment any contaminating flora were removed. No non-experimental fauna were observed.

Preparation of wood inoculum

Wood blocks were cut from freshly-felled beech (Fagus sylvatica) timber (Coed Cymru Hardwood Sawmill, Wentwood, UK) into 2 × 2 × 1 cm blocks. Blocks with discoloration and knots were discarded. Groups of 20 blocks were double-wrapped in heat-sealed biohazard bags and autoclaved for two separate 1 hr sessions each being 24 hr apart. Sterile wood blocks were added to cultures of one isolate each of H. fasciculare, P. impudicus, P. velutina and R. bicolor, on 2 % MEA and incubated in the dark at 20 °C for 3 months.

Inoculation of soil trays

All four fungal species were interacted in every possible interspecific combination, with 20 soil trays/interaction (total 120 soil trays). Mycelium and agar were scraped off each wood block inoculum before addition to soil trays, to prevent growth of soil microbes on the agar medium. To each tray a wood block of the slower growing of the two fungal species was added 9 cm away from a corner along a diagonal between corners. A wood block colonised by an opponent was added, 9 cm away from the opposite corner, timed such that the fungi met when the mycelia were approximately 8 cm dia. Trays were weighed and deionised water was added weekly to maintain that weight.

To half of the trays, collembola were added. For any given interaction, when 50 % or more of the fungi had met the opposing fungus, for a minimum of 2 d, 20 F. candida were added to each corner of the tray (80/tray). P. armata (80/tray) was used in two additional interactions; R. bicolor against H. fasciculare and P. impudicus against H. fasciculare. Methodology for culture and addition of P. armata was the same as for F. candida.

Images of soil trays were captured as for agar plates but with the camera at a height of 47 cm and artificial illumination provided by two 1 000 W spot flood lamps (Gamma 7 Al, Gamma, Chicago USA), each set 60 cm either side of the stand and 120 cm above it. Photography commenced on the day of collembola addition, t0, and continued every 2 d for the first 11 d (t30), then every 4 d until 23 d (t52), every 10 d until 43 d (t64) and finally at 85 d (t84). Outcome of interactions was determined t84 after collembola addition (see below).

Determination of outcome of interactions in soil microcosms

Extension rates of one mycelium growing over another were determined by linear regression analysis of extent by time from digital images. Mycelial extent was measured as the furthest visible extent of mycelium along four zones at equal angles through a 90° segment of the mycelium (Fig 1). Unless removed through grazing the mycelium at the two central measurements (30° and 60°) was invariably in contact with the opposing mycelium. Cords severed by grazing and not visibly

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connected to the wood block were not included in measurements.

At the end of the experiment wood blocks that had been reached by the mycelium of the opposing fungus were harvested. These blocks were cut in half, three wood chips taken from the freshly cut surface, and plated onto MEA and incubated for 7 d at 20°C, in the dark. If only the opposing species was re-isolated, the outcome was recorded as total replacement. If both fungal species were isolated the result was recorded as partial replacement. If only mycelium of the original inoculum was isolated the outcome was recorded as overgrowth by the opponent but not replacement. Results for each interaction were recorded as overgrowth, partial replacement or replacement, and expressed as a percentage of total reisolations. Where mycelium of one strain overgrew that of the opponent but did not reach the wood block, the outcome was reported as partial overgrowth.

Statistical analysis

Statistical comparisons made between mean extension rates were as follows: (1) the difference in mycelial extent between grazed and ungrazed trays across all four measurements at the final time point \( t_{\text{final}} \); the time when a mycelium reached the edge of the tray, which depended on species; (2) the difference in mycelial extent between grazed and ungrazed trays when growing toward (or over) the opponent mycelium (30° and 60° measurement) at the final time point \( t_{\text{final}} \); (3) the difference in mycelial extent over time between grazed and ungrazed trays when growing toward (or over) the opponent mycelium; (4) the difference in mycelial extension over time between grazed and ungrazed trays when growing over soil (0° and 90°); (5) the difference in mycelial extension over time when growing over soil compared to when growing over the opponent mycelium in grazed trays; and (6) the difference in mycelial extension over time when growing over soil compared to when growing over the opponent mycelium in ungrazed trays. This complete analysis was not always appropriate and a framework was developed to determine the suitable level of analysis for each species in each interaction (Fig 2).

Results

Outcome of interactions in agar culture

H. fasciculare: It was combative, several strains replacing R. bicolor, and being replaced only occasionally by P. velutina and R. bicolor. H. fasciculare 4 was, however, sometimes replaced by R. bicolor and P. velutina though not P. impudicus. There were marked differences between different strains of the same species. H. fasciculare 3, for example, replaced R. bicolor 1 whereas H. fasciculare 4 did not (Table 1). H. fasciculare 1 was the most combative of the four isolates, and H. fasciculare 4 was the weakest (Tables 1 and 2). Grazing increased combativeness in two isolates (H. fasciculare 2 and H. fasciculare 3) and reduced it in the others (Table 2). H. fasciculare grew as dense mycelium from the inoculum and overgrew opponents with the formation of cords (Supplementary Table 2), occasionally emerging through the interaction zone from several discrete points (Fig 3A). H. fasciculare 4 often produced non-linear, apparently disordered, cords when overgrowing other species (Fig 3I). All H. fasciculare isolates produced yellow pigment although H. fasciculare 1 produced pigmentation (Fig 3A and B) only when overgrowing another species. All new growth of H. fasciculare isolates was white, with the exception of the cords of H. fasciculare 4 when interacting with P. impudicus, which developed a yellow colour (Fig 3I).

P. impudicus: Although it was overgrown to varying degrees by H. fasciculare 1, 2 and 3, P. impudicus was strongly combative partially replacing, mutually replacing or deadlockng with all other species and strains (Table 1). In the presence of other species, the morphology of P. impudicus changed before contact occurred - dense cords originated from up to 1 cm behind the growing front (Fig 3K; Supplementary Table 2). This morphological response also occurred in self-pairings; when P. impudicus was substantially overgrown it occasionally broke through the opposing mycelium at the interaction zone, producing fast growing plumes (Fig 3G).

P. impudicus mycelia darkened in some interactions but did not produce strong pigmentation (Supplementary Table 2). During interactions with R. bicolor, a zone of lysis was produced in the interaction zone as P. impudicus replaced R. bicolor (Supplementary Table 2; Fig 3K and O). Collembola burrowed within the lytic zones (Fig 3N). Grazing slightly increased the overall combativeness of P. impudicus (Table 2).

P. velutina: It was less combative than H. fasciculare and P. impudicus though it occasionally replaced H. fasciculare 2 and 3 (Table 1). When overgrowing another mycelium, P. velutina formed finely branched cords often emerging from a narrow point of the interaction zone (Fig 3I). Combativeness of P. velutina increased slightly when grazed (Table 1), and rate of overgrowth increased when grazed cords of P. velutina and P. impudicus overgrew each other (Supplementary Table 2). A dark pigment permeated the medium when P. velutina was overgrown and when interacting with itself (Supplementary Table 2).

R. bicolor was less combative than H. fasciculare and P. impudicus. R. bicolor 1 was more combative than R. bicolor 2. Combative ability of R. bicolor 1 was reduced when grazed whereas that of R. bicolor 2 increased slightly (Table 1). Following contact with mycelium of an opposing species, R. bicolor 1 rapidly produced dense aerial hyphae up to about 1 cm behind the interacting front (Fig 3L), but this was either less marked or absent in R. bicolor 2 (Supplementary Table 2). R. bicolor overgrew as dense, tightly packed, unbranched linear cords (e.g. Fig 3L). When interacting with H. fasciculare and P. velutina (Fig 3M), R. bicolor produced deep red pigment (Fig 3E, F and H), which was more pronounced in R. bicolor 1 than in R. bicolor 2 (Fig 3E, F but see H).

Outcome of interactions between extra-resource mycelia on soil

In ungrazed systems there was never evidence of one mycelium replacing another on the surface of soil, but there was often overgrowth especially by P. velutina and P. impudicus (Table 3; Fig 4), to the extent that the wood block inoculum of an opponent was sometimes reached and interactions then took place within the wood (see below). In interactions...
between R. bicolor and P. velutina, but not other combinations, cords of the latter grew to both sides of the R. bicolor wood block forming a complete ring comprising a thick cord in ungrazed systems (Fig 4A and B).

In some interactions fungi produced pigments and exudates at the point of contact with the opponent (Fig 4J and K). R. bicolor produced a red pigment along the interaction line when interacting with H. fasciculare and small globules of exudate were visible where R. bicolor cords met those of P. velutina (Fig 4J and K). There was evidence of preferential grazing by F. candida at the interaction zone, for example with H. fasciculare when interacting with either P. velutina or P. impudicus (Fig 4F, G and I).

In grazed systems R. bicolor mycelia were always completely grazed away by F. candida (e.g. Fig 4D) whereas P. armata apparently had little impact (Fig 5E). P. velutina was sometimes heavily grazed, but not to extinction, especially in

Fig 2 – The framework applied to refine application of statistical tests in analysis of overall extension change during grazed and ungrazed interactions. Where data violated normality a Mann–Whitney U-test (instead of the t-test) or a two-way ANOVA on ranked data (instead of the RM ANOVA) was used. When assumptions were violated a Scheirer Ray Hare test replaced two-way ANOVA.
Table 1 – Outcome of interactions 12 weeks after addition of *Folsomia candida* as grazing treatment

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Outcomes were mutual replacement (MR), partial replacement (PR), total replacement (R) and deadlock (D). For outcomes designated PR and R, upper case signifies that replacement was by the fungus listed at the head of the column whereas lowercase signifies replacement by the fungus listed in the row. Numbers refer to percentage of replicates exhibiting a given response. There were 4–10 replicates/interaction. Hf = *H. fasciculare*, Pi = *P. impudicus*, Pv = *P. velutina*, Rb = *R. bicolor*. 

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interactions with R. bicolor (Fig 5A). H. fasciculare was usually only sparsely grazed, except when interacting with P. velutina where it was completely removed in grazed systems by 64 d (Fig 5G). In three (of six) interaction combinations, grazing by F. candida altered the extent of overgrowth by mycelia on the soil surface (Table 3). For example, the mutual overgrowth of P. velutina and R. bicolor in ungrazed systems shifted to complete overgrowth by 64 d in grazed systems (Table 3; Fig 5A and B). There was greater growth of P. impudicus over R. bicolor when grazed than ungrazed. Interactions involving H. fasciculare were little changed when grazed except when interacting with P. velutina, where F. candida grazing shifted the balance in favour of the latter (Table 3). There were no differences in mycelial overgrowth between ungrazed systems and those grazed by P. armata (Table 3).

Extension rate of mycelial opponents in soil microcosms

In five interaction combinations the change in mycelial radial extent was significantly different between grazed and ungrazed treatments (Table 4, Figs 5 and 6). Of these, in three combinations, R. bicolor against H. fasciculare, P. impudicus and P. velutina, the presence of F. candida resulted in a decrease in mycelial extent of R. bicolor (Fig 5). In the other two interactions, F. candida grazing of P. velutina interacting with either H. fasciculare or P. impudicus, both grazed and ungrazed mycelia increased in size over time (Fig 6). The radial extension of P. velutina (for all four extension measurements taken per replicate) was significantly (P < 0.05) less in grazed than in ungrazed treatments when interacting with P. impudicus but not significantly (P > 0.05) different when interacting with H. fasciculare (Fig 6A and B). P. velutina growth over H. fasciculare was slightly but not significantly (P > 0.05) faster in grazed systems than in ungrazed systems (Fig 6C). When over soil and interacting with H. fasciculare, however, mycelial extension of P. velutina was significantly (P < 0.05) faster in ungrazed trays (Fig 6E).

Outcome of interactions within wood inocula in soil microcosms

H. fasciculare often replaced or partially replaced R. bicolor in wood blocks, though it was itself often replaced by P. velutina (Table 5). P. velutina also sometimes replaced R. bicolor. In other interactions there was no evidence of replacement even if mycelial overgrowth on soil had occurred. There was some evidence of changes in the outcome of interaction in wood blocks following grazing: F. candida grazing enhanced the ability of P. velutina to replace H. fasciculare. In the presence of P. armata, H. fasciculare was less able to replace R. bicolor (Table 5).

Discussion

The outcomes of ungrazed interactions in agar and soil were similar to those reported in previous studies of the same species (Dowson et al. 1988). Importantly, outcomes on agar varied depending on strain (only single strains were studied on soil), H. fasciculare 1 being more combative than the other three strains. Such intraspecific variation in combativeness has also recently been reported in the non-cord-forming basidiomycete, Hericium coralloides (Crockatt et al. 2008). Thus, care must be taken when extrapolating to species based on results for single strains.

This study provides the first report of effects of invertebrate grazing on the outcome of mycelial interactions. Effects, however, depended on grazer, F. candida altering both the outcome and progression of half of the mycelial interactions studied, while P. armata had almost no discernable effects on fungal interactions. Thus, outcomes of interactions in the field, where there are many different species of collembola and other invertebrates, may differ considerably from those in the simplified laboratory microcosms.

P. armata is less active than F. candida, with a longer generation time. Thus, whilst an equivalent biomass of the two collembola species was used, the extent of grazer activity may have been lower with P. armata. Frequently the most abundant collembola species under natural conditions (Petersen & Luxton 1982), P. armata affects mycelium of other species of fungi (Hedlund et al. 1991), and may affect the basidiomycetes in the field, if density of grazers is higher.

Previous studies have revealed effects of collembola grazing on extension rates of individual mycelia including both reduction and increase of extension rate (e.g. Bengtsson & Rundgren 1983; Kampichler et al. 2004; Bretherton et al. 2006;}

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Fig 3 – Morphological and pigment changes during interspecific mycelial interactions in agar, either ungrazed or grazed by Folsomia candida. Hf, Hypholoma fasciculare; Pi, Phallus impudicus; Pv, Phanerochaete velutina; Rb, Resinicium bicolor. Numbers discriminate different strains. Images A–M are 9 cm diam, while in N and O the interaction zone is magnified (2 cm diam).

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Tordoff et al. 2006, 2008). In the present study, there were several examples of increases and one example of decrease in extension rate in grazed systems of interspecifically interacting mycelia. The effect varied depending on where the mycelium was growing (i.e. across soil or over the opponent mycelium). For example, grazing by F. candida accelerated P. velutina extension rate when the mycelium was growing over H. fasciculare, compared to growing over soil. Differences in extension rate in different mycelial regions have previously been observed: Mortierella isabellina, for example, exhibited accelerated growth away from the region of grazing in areas where collembola (P. armata) were excluded (Hedlund et al. 1991).

The accelerated growth of mycelium across an opponent mycelium only occurred in P. velutina, and only when interacting with H. fasciculare. This suggests that the interacting fungi respond differentially to one another and that grazing is a further complicating factor. In contrast, grazing by F. candida on R. bicolor had a negative impact on mycelial extension rate. The frequent severing of R. bicolor cords by F. candida in all interactions suggests that R. bicolor was a preferred food source. How R. bicolor survives in the field when it is so heavily damaged by F. candida in microcosm studies remains an unanswered question. The microcosms lacked the presence of collembola predators but, in the field, high population densities of F. candida may attract predators such as Acarina, Coleoptera, such as Staphylinidae and Carabidae, thus limiting mycelial damage through grazer population control (Hedlund et al. 1991).

Such complete removal has not been seen with H. fasciculare growing alone (Kampichler et al. 2004; Harold et al. 2005) suggesting that the interaction with P. velutina may have altered the palatability of H. fasciculare. Initially, H. fasciculare was only grazed in areas where P. velutina had overgrown it. This further suggests that interacting with P. velutina increased the palatability of H. fasciculare but, as grazing took place through P. velutina mycelium, that there was active searching for H. fasciculare mycelium by the collembola. Collembola prefer cords of low vitality in some fungi (Kaneda & Kaneko 2004), although preference for actively growing hyphae has also been reported in other species (Moore et al. 1985).

Though intense grazing of mycelia might be expected to impact negatively on the outcome of interspecific mycelial interactions, R. bicolor retained possession of more wood block resources when extra-resource mycelia were destroyed than when ungrazed during interactions with both H. fasciculare and P. velutina. This may, however, reflect reduced competitiveness of the opponent when grazed rather than a directly positive effect of grazing on R. bicolor.

Grazing on one interacting species may also have an indirect effect on the competitiveness of the opponent fungus. For example, the accelerated growth of P. velutina when grazed limited the ability of H. fasciculare to overgrow the P. velutina mycelium. With the exception of the interaction with P. velutina, H. fasciculare was generally tolerant of F. candida grazing. This may provide a niche advantage, allowing H. fasciculare to proliferate in areas of high mycophagy, where more palatable species would be unable to compete. Collembola densities in soil can be very high (Hopkin 1997) but H. fasciculare is ubiquitous in forest soils (Rayner & Boddy 1988). Whether the abundance of H. fasciculare can be attributed, even in part, to grazing tolerance is yet to be confirmed.

Previous studies have noted that site of grazing within the mycelium depends on fungal species (Tordoff et al. 2006). With interacting mycelia additional ‘types’ of grazing site are available. In particular, the interaction zone comprises dead and dying hyphae, leaked hyphal content, pigments, volatile and diffusible compounds, and mycelium with different morphology (e.g. Boddy 2000; Wells & Boddy 2002; Evans et al. 2008; Woodward & Boddy 2008). Such sites may be nutrient rich (Wells & Boddy 2002) and contain attractive or inhibitory compounds. Grazing of H. fasciculare beneath P. velutina has already been noted. There was also concentrated grazing in the interaction zone with P. impudicus though this did not alter the

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>H. fasciculare (Hf1)</th>
<th>H. fasciculare (Hf1)</th>
<th>P. impudicus</th>
<th>P. velutina</th>
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<tbody>
<tr>
<td></td>
<td>Ungrazed</td>
<td>Grazed by F. candida</td>
<td>Ungrazed</td>
<td>Grazed by P. armata</td>
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<tr>
<td>R. bicolor (Rb1)</td>
<td>P 100</td>
<td>P 100</td>
<td>P 100</td>
<td>P 100</td>
</tr>
<tr>
<td>P. velutina</td>
<td>o 70</td>
<td>o 100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P. impudicus</td>
<td>o 90</td>
<td>o 100</td>
<td>–</td>
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Table 3 – Outcomes of interactions between extra-resource mycelia on the surface of soil after 50–56 d

Outcomes with upper case letters were mutual overgrowth (M), partial overgrowth (P), total overgrowth (O), by the fungus at the head of the column, whereas lowercase signifies overgrowth by the fungus listed in the row. Numbers refer to percentage of replicates exhibiting a given response. There were 8–20 replicates/interaction.
outcome of any interaction. The apparent increased density of hyphae and networking (Rotheray et al. 2008) at the mycelial margin in *P. impudicus* mycelium does, however, indicate that it was not entirely unaffected. The resilience of *P. impudicus* to *F. candida* grazing when growing alone has been established (Tordoff et al. 2006) although, unlike *H. fasciculare*, *P. impudicus* always remained resilient when interacting with other mycelia. Deadlock in all interactions indicates that, relative to the

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**Fig 4** — Mycelial morphology on soil during interspecific interactions in (B, F, J, K) ungrazed systems, systems grazed by (A, C, E, G, H, I) *Folsomia candida* or by (D) *Protophorura armata*. Hf, *Hypholoma fasciculare*; Pi, *Phallus impudicus*; Pv, *Phanerochaete velutina*; Rb, *Resinicium bicolor*. Scale bar (10 cm) on image H applies to images A–H. Scale bar (2 cm) on image I applies to image I alone. Images J and K show pigment and exudate production respectively during interactions.
Fig 5 — Mycelial radial extent over time on soil for Resinicium bicolor interacting with (A, B) Hypholoma fasciculare, (C, D) Phallus impudicus and (E, F) Phanerochaete velutina, in ungrazed systems (closed symbols) and systems grazed by Folsomia candida (closed symbols). (A, C, E) are mean ± standard error of the mean of all four (0, 30, 60, 90) measurements (Fig 1), and (B, D, F) are the mean of 30' and 60' measurements. Resinicium bicolor interacting with H. fasciculare (A) Scheirer Ray Hare $\chi^2 = 0.999$, $P < 0.001$; (B) Two-way ANOVA on ranked data, $F_{3,88} = 15.55$, $P < 0.001$; R. bicolor interacting with P. impudicus (C) RM ANOVA $F_{2.859,74.345} = 4.803$, $P = 0.005$; (D) RM ANOVA $F_{3.102,36.154} = 6.461$, $P < 0.001$; R. bicolor interacting with P. velutina, (E) SRH $\chi^2 = 0.762$, $P = 0.238$; (F) Two-way ANOVA on ranked data, $F_{3,104} = 2.14$, $P = 0.1$; critical values are for time × treatment interaction.

Table 4 — Analysis of mycelial extent in grazed and ungrazed systems. Data are presented for each interaction combination with comparisons carried out for each species in all interactions

<table>
<thead>
<tr>
<th>Species in column</th>
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<tr>
<td>H. fasciculare (Hf1)</td>
<td>H. fasciculare (Hf1)</td>
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<td>P. impudicus</td>
<td>P. impudicus</td>
<td>P. velutina</td>
<td>P. velutina</td>
</tr>
<tr>
<td>R. bicolor (Rb 1)</td>
<td>$t_{35} = 1.08$</td>
<td>$t_{35} = -6.14$</td>
<td>$t_{36} = 0.61$</td>
<td>W = 200</td>
<td>$t_{35} = 1.08$</td>
<td>$t_{35} = -3.61$</td>
<td>$t_{36} = -3.12$</td>
</tr>
<tr>
<td>P = 0.291</td>
<td>$P &lt; 0.001$</td>
<td>$P = 0.549$</td>
<td>$P = 0.9085$</td>
<td>$P = 0.291$</td>
<td>$P = 0.004$</td>
<td>$P = 0.1$</td>
<td>$P = 0.005$</td>
</tr>
<tr>
<td>P. armata</td>
<td>$t_{42} = 2.16$</td>
<td>$t_{42} = -2.16$</td>
<td>$t_{42} = 0.74$</td>
<td>W = 85</td>
<td>$t_{35} = 0.74$</td>
<td>$t_{35} = -4.65$</td>
<td>$t_{36} = 0.463$</td>
</tr>
<tr>
<td>W = 375</td>
<td>$P = 0.351$</td>
<td>$P = 0.037$</td>
<td>$P = 0.463$</td>
<td>$P &lt; 0.001$</td>
<td>$P = 0.55$</td>
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</table>

Where data were not normal, a Mann–Whitney U-test (W) was applied. The change in mycelial extent was measured from the time of completion of the interaction ($t_{reach}$) to the point at which one of the interacting myelia reached the wood block of the opposing fungus ($t_{reach}$). Figures in bold are significant values at $P \leq 0.05$. 

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Fig 6 – Mycelial radial extent over time on soil for Phanerochaete velutina interacting with (A, C, E, G, I) Hypholoma fasciculare and (B, D, F, H, J) Phallus impudicus. Measurements (see Fig 1) are mean ± standard error of the mean of: (A, B) mycelial extent in grazed and ungrazed systems across all four extension measurements (Fig 1) (A, F, 2.443, 92.850, 2.147, P = 0.112; B, F, 2.651, 151.785, 2.147, P < 0.001); (C, D) mycelial extent in grazed and ungrazed systems over opponent (C, F, 2.443, 92.850, 2.147, P = 0.112; D, F, 2.125, 84.981, 5.611, P = 0.004); (E, F) mycelial extent in grazed and ungrazed systems over soil (E, F, 2.140, 212.849, P < 0.001; F, F, 2.534, 73.490, 25.626, P < 0.001); (G, H) mycelial extent in the soil and over the opposing mycelium within grazed systems (G, RM ANOVA F, 2.601, 46.819, 22.264, P < 0.001; H, F, 3.51, 3.651, P = 0.018); (I, J) mycelial extent over soil and over the opposing mycelium within ungrazed systems (I, F, 3.141, 56.541, 32.797, P < 0.001; J, F, 1.751, 26.262, 9.835, P < 0.001). All reported critical values are for RM ANOVA time × treatment interaction.
species against which it was paired, P. impudicus was effective in defence but not in attack. This contrasts with a previous study (Dowson et al. 1988) where P. impudicus was a poor competitor against a similar range of species. The species and strains used in the present study were, however, different, the microcosms larger and the experimental period shorter.

Conclusions

Saprotrophic fungi play a critical role in ecosystem function making recalcitrant nutrients available for continued plant primary productivity. These nutrients tend to be retained in mycelia, but are probably released during mycelial interactions (J.M. Wells & L. Boddy unpub.). Further, invertebrate grazing of the microbial communities (including fungi) can increase the rate of carbon mineralisation (Bardgett et al. 1993; Cole et al. 2000). The present study indicated that collembola may also play a role in determining fungal species combativekeness during interactions with potential effects for species assemblages and diversity, and consequently may also affect decomposition processes. The fungal response to collembola grazing during interspecific interactions appears to vary depending on the combination of grazer species and the identity of the interacting fungi. Grazing clearly adds another variable affecting the outcome of interspecific mycelial interactions to those already known, for example, abiotic environmental conditions (Boddy 2000), inoculum size (Holmer & Sterlilid 1993, 1997) and species composition (Holmer & Sterlilid 1993; Boddy 2000; Wald et al. 2004).

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.funeco.2010.09.001.

Table 5 – Outcomes of interactions after 80–84 d from wood block reisolations

<table>
<thead>
<tr>
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</tr>
<tr>
<td>P. impudicus</td>
<td>o 100</td>
<td>o 100</td>
<td>o 100</td>
<td>o 100</td>
</tr>
<tr>
<td>R. bicolor (Rb1)</td>
<td>P 40</td>
<td>P 20</td>
<td>R 42</td>
<td>R 29</td>
</tr>
<tr>
<td></td>
<td>O 60</td>
<td>O 80</td>
<td>O 29</td>
<td>o 71</td>
</tr>
<tr>
<td>P. velutina</td>
<td>m 70</td>
<td>m 100</td>
<td>—</td>
<td>—</td>
</tr>
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</table>

Outcomes were mutual overgrowth (M), partial replacement (P), total replacement (R) and overgrowth (O). For outcomes designated PR, R and O, uppercase signifies replacement was by the fungus listed at the head of the column whereas lowercase signifies replacement by the fungus listed in the row. Numbers refer to percentage of replicates exhibiting a given response. There were four to 10 replicates per interaction treatment.

References


