ECOLOGY LETTERS

Ecology Letters, (2011) doi: 10.1111/j.1461-0248.2011.01682.x

LETTER

Outcomes of fungal interactions are determined by soil invertebrate grazers

Thomas W. Crowther, Lynne Boddy* and T. Hefin Jones
Cardiff School of Biosciences, Cardiff University, Museum Avenue, Cardiff, CF10 3AX, UK
**Correspondence: E-mail: boddyl@cardiff.ac.uk*

Abstract

Saprotrophic fungal community composition, determined by the outcome of competitive mycelial interactions, is one of the many key factors affecting soil nutrient mineralisation and decomposition rates. Fungal communities are not generally predicted to be regulated by top-down factors, such as predation, but rather by bottom-up factors, including resource availability. We show that invertebrate grazers can exert selective pressures on fungal decomposer communities in soil, reversing the outcomes of competitive interactions. By feeding selectively on the cord-forming fungus *Resinicium bicolor*, isopods prevented the competitive exclusion of *Hypholoma fasciculare* and *Phanerochaete velutina* in soil and wood. Nematode populations also reversed the outcomes of competitive interactions by stimulating growth of less competitive fungi. These represent two opposing mechanisms by which soil fauna may influence fungal community composition and diversity. Factors affecting soil invertebrate communities will have direct consequences for fungal-mediated nutrient cycling in woodland soils.

Keywords

Decomposer interactions, decomposition, ecosystem functioning, fungal community, grazing, mycophagy, nutrient cycling, soil biodiversity, soil fauna.

Ecology Letters (2011)

INTRODUCTION

The key influences of plant diversity and community composition on ecosystem processes are well established (Hooper & Vitousek 1997; Tilman *et al.* 1997) and as a result, the factors (biotic and abiotic) affecting them have been extensively explored (Clark & Tilman 2008; Yang *et al.* 2011). In contrast, despite the acknowledged contribution of belowground microbial communities to processes, including carbon and nutrient cycling (Wardle *et al.* 2004; Bardgett 2005; Van der Heijden *et al.* 2008), the biotic factors influencing their compositions are less well understood (Wardle 2006). Saprotrophic basidiomycete fungi are the primary decomposing agents in temperate woodland ecosystems (Hättenschwiler *et al.* 2005; Baldrian & Valášková 2008). Their filamentous mycelial networks grow throughout the soil-litter interface, forming systems which contribute significantly to the total ecosystem biomass and respiration (Post *et al.* 1982; Bardgett 2005). During mycelial extension, competitive interactions take place at a distance, via antagonistic volatile organic compound production, or following mycelial contact, and commonly result in the replacement of one fungus by another (Boddy 2000). The outcomes of these mycelial interactions determine fungal dominance and community composition in litter resources and soil (Boddy 2000). Species-specific fungal enzyme production and respiration rates suggest that factors affecting the outcomes of these competitive interactions are likely to have consequences for decomposition rates, nutrient mineralisation and the flux between terrestrial and atmospheric carbon pools (Hättenschwiler *et al.* 2005; Gessner *et al.* 2010).

The networks of interacting mycelia that pervade woodland soil represent the primary ecosystem nutrient pool for soil-dwelling animals (Pollierer *et al.* 2009). The effects of 'grazers' on fungal community structure are, however, relatively poorly understood. The Nutrient-enrichment Model (Moore *et al.* 2003) argues that, unlike bacteria, fungal communities are not regulated by top-down control (predation), but by bottom-up factors, including resource availability. By virtue of their large biomass and biochemical defences, it is predicted that fungi are relatively resistant to grazing (Wardle & Yeates 1993) and that nutrients are conserved within vast mycelial networks (Boddy 1999). This is supported by empirical studies suggesting that although mesofauna (Collembola and mites) feed selectively on specific fungi, the grazing pressures exerted are not strong enough to alter fungal community composition (Parkinson *et al.* 1979; Whittaker 1981; Kaneko *et al.* 1998). Newell (1984a, b) provided some evidence that Collembola may differentially influence the competitive abilities of interacting fungi. Selective grazing of the dominant fungus (*Marasmius androsaceus*) increased the relative abundance of a less palatable species (*Mycena galopus*) colonising Sitka spruce (*Picea sitchensis*) needles. Collembola have subsequently been found to stimulate the progression rates of mycelial interactions (Klironomos *et al.* 1992), but no study has shown that grazing can lead to the complete replacement of a formerly dominant fungus by a less competitive opponent (McLean *et al.* 1996; Wardle 2006).

More recent studies involving a wider range of invertebrate species suggest that macrofauna (including isopods and millipedes) consistently exert stronger grazing pressures on individual mycelial systems than smaller mesofauna (Crowther *et al.* 2011b, c). Effects vary dramatically between basidiomycete species; fungal palatability, based on mycelial biochemistry and morphology (Hiol Hiol *et al.* 1994), determines whether foraging systems are consumed entirely or ignored by grazers (Crowther *et al.* 2011a). Such high intensity grazing has been predicted to exert selective pressures on interacting fungi and drive changes in community composition (Kaneko *et al.* 1998). Moreover, the stimulatory effects of low intensity microfauna (nematode) grazing on the extension rates of selected fungi, analogous to the compensatory growth responses seen in plants during herbivory

(McNaughton 1983), highlight the need to investigate the effects of a broader range of invertebrate taxa on fungal community composition and functioning.

In the present study, the potential of invertebrate taxa representing the Isopoda, Myriapoda, Collembola and Nematoda to affect the outcomes of inter and intraspecific fungal interactions in soil and wood were explored. All fungi used are common within temperate woodland ecosystems (Boddy 1999, 2000) and were isolated originally from UK forest soil. A fully factorial microcosm experiment was used to test three hypotheses: (i) selective grazing can reverse the outcomes of competitive fungal interactions with subsequent shifts in fungal species composition; (ii) as a result of increased grazing intensity, macrofauna will exert greater selective pressures on fungal communities than meso and microfauna; and (iii) grazing will influence fungal-mediated wood decay.

MATERIALS AND METHOD

Experimental design

To test Hypotheses (i) and (ii), four cord-forming basidiomycete fungi were allowed to grow from wood blocks and interact (in pairwise combinations) within two-dimensional soil microcosms. A total of 150 microcosms provided a balanced, factorial design, with all six fungal combinations subject to five grazing treatments (collembola, nematode, isopod, millipede and ungrazed control), each replicated five times (i.e. 6 interactions × 5 treatments × 5 replicates). Wood decay rates were determined at the end of the experiment to test Hypothesis (iii).

Fungal culturing and inoculum preparation

Fungal isolates, *Hypholoma fasciculare* (strains DD3 and JH) (Huds.: Fr.), *Phanerochaete velutina* (DC.: Pers.) and *Resinicium bicolor* (Abertini and Schwein.: Fr.) (Cardiff University Fungal Genetic Source Collection), were subcultured in non-vented 9-cm diameter Petri dishes on 2% malt extract agar (MEA; 15 g L^{-1} Lab M agar no. 2, 20 g L^{-1} Munton and Fiston malt). Freshly cut beech (*Fagus sylvatica*) wood blocks (2 × 2 × 1 cm) were stored at −18 °C, and autoclaved at 121 °C for 20 min prior to use. Sterilised wood blocks were then added to fungal cultures. Petri dishes were sealed with Nescofilm® (Nescofilm, Bando Chemical IND. LTD., Kobe, Japan), and incubated in the dark at a constant temperature of 20 °C for 3 months prior to experimental use.

Invertebrate collection and culturing

Folsomia candida Willem 1902 (Collembola) (Cardiff University Culture) were cultured in 0.8 L containers on a medium of 95% plaster of Paris (Minerva Dental, Cardiff, UK) and 5% activated charcoal (Sigma, Poole, UK). Cultures were fed weekly on dried baker's yeast. *Blaniulus guttulatus* (Fabricius 1798) (Myriapoda) and *Oniscus asellus* Linnaeus 1758 (Isopoda) [collected from Coopers Field, Bute Park, Cardiff, UK (ST 17819 76785, 51°29′ 20.4″ N, 3°11′ 20.4″ W)] were kept in 2 L plastic pots containing compost. All containers were stored in the dark at 20 °C and moistened weekly using deionised water (DH_2O). Before introduction into experimental microcosms, all the three species were starved for 24 h in pots with fresh plaster of Paris.

Panagrellus redivivus (Linnaeus 1767) (Nematoda) cultures (supplied by UK Parasitology Group, Aberystwyth University, Aberystwyth, UK) were maintained in 500 mL jars on a medium of porridge oats (45 g) and DH_2O (75 mL) which had been autoclaved (121 °C for 20 min) prior to nematode addition. Before introduction into experimental microcosms, nematode suspensions were extracted using wet-funnel extraction (Southwood & Henderson 2000). Worms were then washed in a solution of 30 p.p.m. chlorotetracycline and 5 p.p.m. benomyl to reduce bacterial and fungal contamination associated with the culture medium (Dyer *et al.* 1992). A final funnel extraction was performed to acquire suspensions of free-living *P. redivivus* (1000 worms mL^{-1}).

In addition to being good model species, the invertebrates used are common representatives of their respective taxa in temperate European soils (Dyer *et al.* 1992; Jones & Hopkin 1996; Bradford *et al.* 2002; Fountain & Hopkin 2005). All have been shown to influence the growth and physiology of saprotrophic basidiomycete fungi in soil (Crowther *et al.* 2011a, c).

Microcosm preparation, inoculation and running

Loamy soil was collected from deciduous woodland [Coed Beddick Enclosure, Tintern, UK (NGR 352800, 201800; 51°41′ 48.37″ N, 2°40′ 53.11″ W)] to a depth of 20 cm and sieved on site through a 10 mm mesh. This was air-dried in plastic trays and sieved again through 2-mm mesh before being frozen overnight at −20 °C to kill any remaining fauna. Prior to use, soil was re-wetted with DH_2O (340 mL kg $soil^{-1}$) giving a final water potential of −0.012 MPa. A quantity of 200 g moistened soil was then compacted and smoothed to a depth of 5 mm into 24 × 24 cm bio-assay dishes.

Fungus-colonised wood blocks were removed from agar cultures. Densities (dry weight/fresh volume; g cm^{-3}) of seven blocks colonised by each fungus were determined at 0 day. The remaining blocks were cleaned of surface mycelia before being added to soil trays; they were placed 9 cm from opposing corners on a diagonal line ensuring a gap of 8 cm between wood blocks of interacting fungi. Wood block addition dates varied depending on the species-specific mycelial extension rates of the fungi (Tordoff *et al.* 2008). This was done to ensure that emerging mycelia met after 4 cm growth. Once opposing mycelia in 50% of the trays for each fungal interaction had met for 2 days, invertebrates were introduced onto uncolonised regions of soil. As grazers were restricted to a 2D environment, population numbers added to microcosms represented low estimates of field densities of each taxon (Petersen & Luxton 1982). *Panagrellus redivivus*, *F. candida*, *B. guttulatus* and *O. asellus* were added at densities of 16.6×10^3, 783, 83 and 83 m^{-2}, respectively, following Crowther *et al.* (2011a). Trays were then stacked in polythene bags to reduce water loss and stored at 20 °C and 70% relative humidity. Microcosms were re-wetted weekly to a constant weight with DH_2O. The experiment was concluded after 63 days, and each wood block was then cut in half; one half was used for re-isolation of colonising fungi and the second half was used to determine wood block density enabling the estimation of wood decay rate (g cm^{-3} d^{-1}).

Image analysis

Digital images were captured after 0, 3, 7, 14, 21, 35, 49 and 63 days using a Nikon Coolpix 57000 camera, mounted on a stand at a height of 39.5 cm. These were subsequently analysed using IMAGEJ (National Institutes of Health, USA). A 5-cm line was drawn against a ruler for

calibration. The extents of mycelia growing through a 90° angle were estimated using the mean length of four lines (30° apart) drawn from the centre of each wood block to hyphal tips. Extension rates (cm d^{-1}) were determined for each interaction until mycelia from any replicate reached the opposing wood block.

Determination of interaction outcomes

Interaction outcomes on the soil were determined by observing which mycelia had reached the opposing wood block after 63 days. Outcomes were classified as: (1) replacement, where mycelia of one fungus was killed and replaced by its opponent; (2) overgrowth, where one fungus had grown over another and caused the cessation of growth without killing its opponent; (3) mutual replacement, where mycelia from both opponents had grown into the opponent's territory and (4) deadlock, where neither fungus had gained territory of the other (Fig. 1). Overgrowth and replacement were recorded as being a 'win' if the aggressor reached the opposing wood block. Mutual replacement and deadlock were recorded as a 'draw' as both, or neither, wood block had been reached.

Wood chips taken from the freshly cut surfaces of halved wood blocks were placed onto 9-cm Petri dishes containing 2% MEA. These were incubated in the dark for 7 days at 20 °C. Emerging mycelia were identified by visual inspection. A 'win' was recorded if both wood blocks from a single microcosm had been colonised by one fungus (i.e. if one fungus had replaced its opponent and successfully defended its own resource). If the two wood blocks were colonised by different fungi (i.e. neither or both fungi had been replaced), the interaction was recorded as a 'draw'.

Feeding study

A further 126 soil trays were prepared (as above) to observe the distribution of *F. candida*, *B. guttulatus* and *O. asellus* in relation to the position of two opposing fungal resources. Seven replicates were established of each of six fungal combinations (*R. bicolor*, *P. velutina* or *H. fasciculare* DD3 paired against itself and against a different species in a fully factorial design) for each invertebrate species. Once mycelia had extended to 2 cm from the centre of each wood block, two isopods, two millipedes and 10 Collembola were introduced into

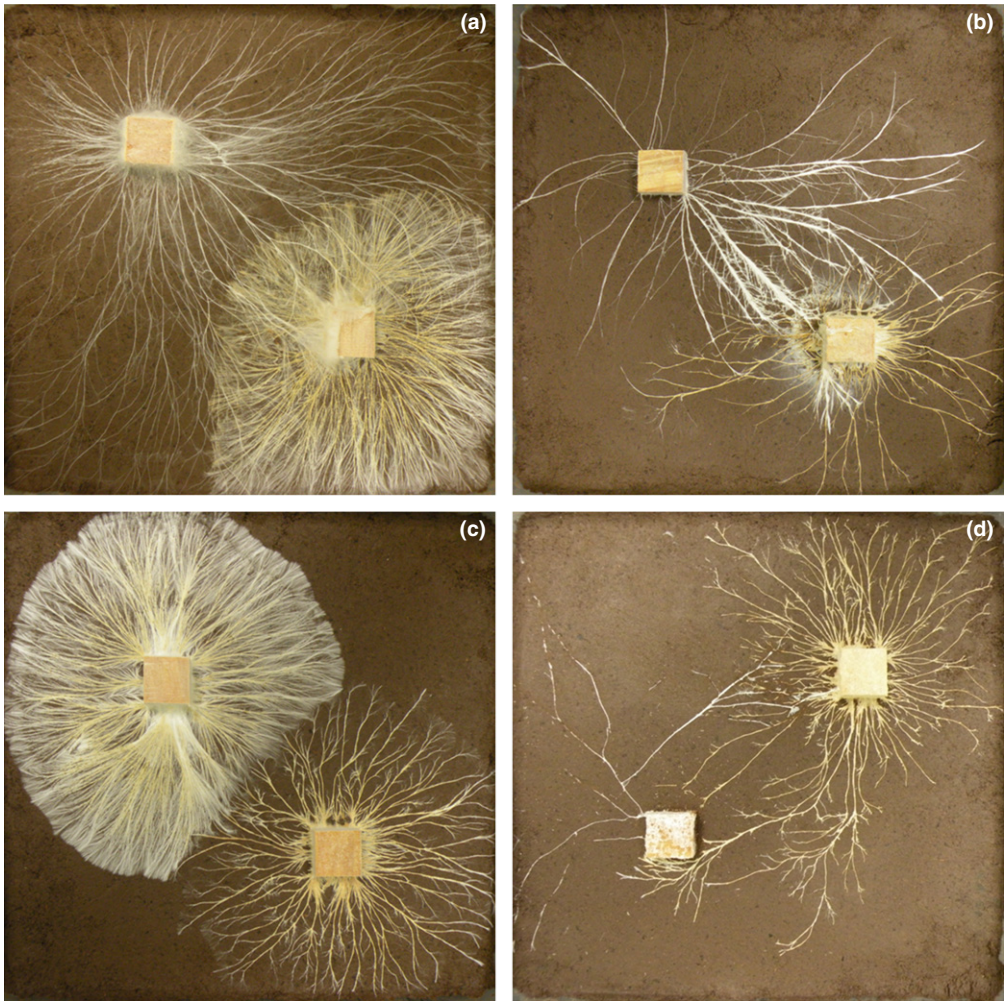

Figure 1 Digital images showing examples of the four possible outcomes of mycelial interactions in soil: overgrowth (a) of *Hypholoma fasciculare* DD3 (right) by *Phanerochaete velutina* (left), replacement (b) of *H. fasciculare* JH (right) by *Resinicium bicolor* (left), deadlock (c) between *H. fasciculare* JH (right) and DD3 (left), and mutual replacement (d) between *H. fasciculare* JH (right) and *Resinicium bicolor* (left).

separate microcosms. These were then maintained in the dark at 20 °C and 70% relative humidity. Over the following 12 h, the number of invertebrates grazing within a 4-cm diameter circle around each wood block was recorded every hour.

Statistical analysis

Multinomial logistic regression (Minitab 15) was used to compare frequencies of three possible interaction outcomes (win for fungus 'a', win for fungus 'b' or draw) in different grazer treatments in soil and wood. When only two outcomes were recorded for any given comparison, binary logistic regression was used.

The general relationship between extension rates of competing fungi in ungrazed interactions was investigated using Pearson's Correlation (Minitab 15). Mycelial extension rates of both fungi in each mycelial interaction were then compared across invertebrate treatments using analysis of covariance (ANCOVA; General Linear Model; Minitab 15) with time (days after invertebrate addition) as a covariate; data not meeting assumptions of linearity were log transformed, if necessary. Chi-squared tests were used to compare the numbers of invertebrates recorded grazing on each fungus during the feeding study.

The general relationship between fungal wood decay and extension rate was investigated using Pearson's correlation. Wood decay rates of each fungal strain were then compared across invertebrate and competing fungus treatments using two-way analysis of variance (two-way ANOVA). All data were normally distributed (Anderson-Darling test) and variances equal (Levene's test) and individual treatments were compared using the Tukey pairwise comparison.

RESULTS

Interaction outcomes in soil

All mycelial interactions were completed within 63 days. In the absence of grazers, extension rates of interacting fungi were negatively correlated with each other ($r = -0.601$, $n = 30$, $P < 0.001$); the growth of one competitor generally restricted development of its opponent. In ungrazed control trays, there was a clear hierarchy of fungal dominance (*H. fasciculare* < *P. velutina* < *R. bicolor*). There was no difference between the competitive abilities of the two *H. fasciculare* strains with all replicates resulting in deadlock (Fig. 1). Both were consistently overgrown by *P. velutina*, and *R. bicolor* replaced all the three competitors in the absence of grazing (Fig. 2).

Although invertebrates had no effect on the outcome of interactions between *H. fasciculare* and *P. velutina* (*P. velutina* overgrew both *H. fasciculare* strains in every treatment), all other interactions were significantly (logistic regression; $P \leq 0.05$) affected (Fig. 2). Isopods

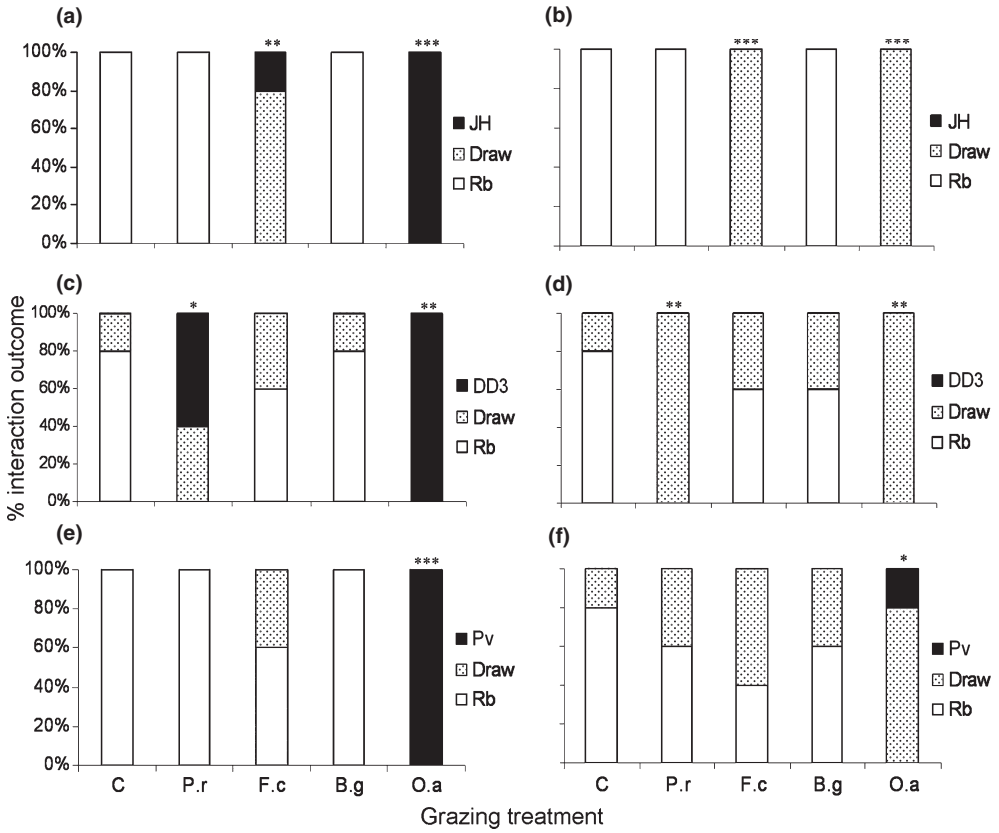

Figure 2 Percentage outcomes of competitive fungal interactions with *Resinicium bicolor* (Rb) against *Hypholoma fasciculare* JH (Hf JH), *Hypholoma fasciculare* DD3 (Hf DD3) and *Phanerochaete velutina* (Pv) in soil (a, c, e) and wood blocks (b, d, f) during control (C), *Folsomia candida* (Fc), *Oniscus asellus* (Oa), *Blaniulus guttulatus* (Bg) and *Panagrellus redivivus* (Pr) grazing treatments. Stars indicate significant differences (logistic regression) compared to ungrazed controls (***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$). All other fungal interactions are not included, as *P. velutina* out-competed both *H. fasciculare* strains in 100% of each grazing treatment and every interaction between the two *H. fasciculare* strains resulted in a draw.

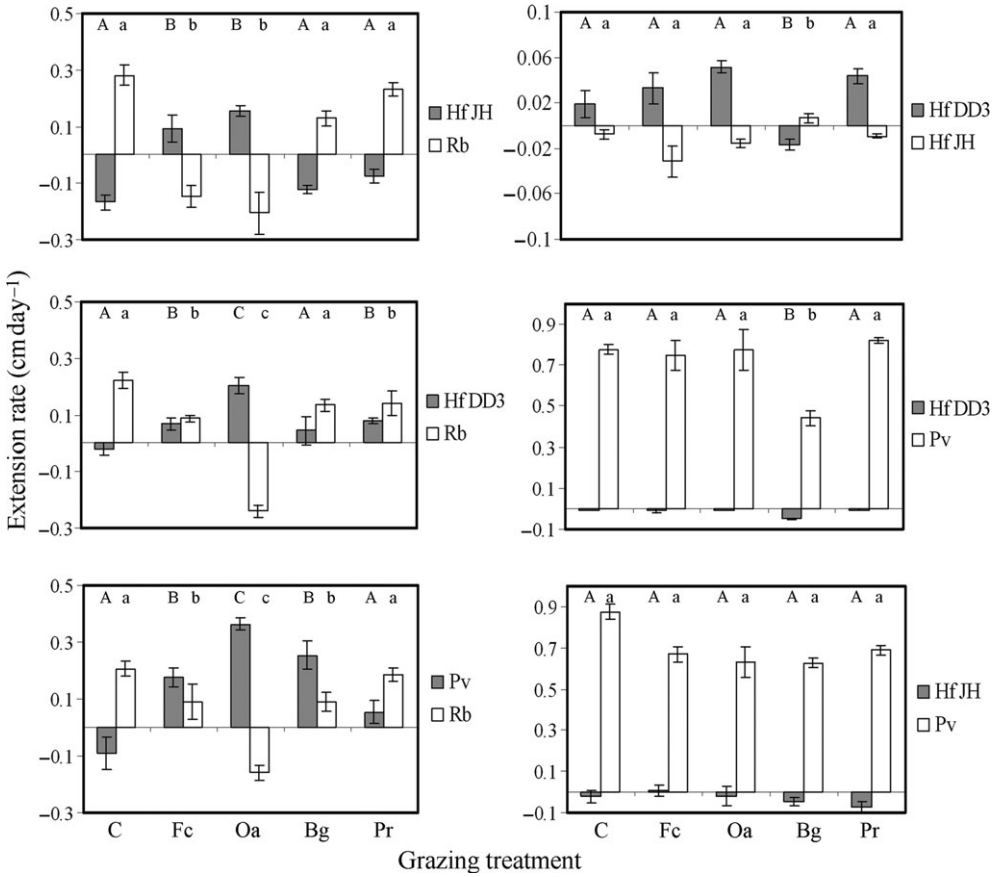

Figure 3 Extension rates of *Resinicium bicolor* (Rb), *Phanerochaete velutina* (Pv), *Hypholoma fasciculare* DD3 (Hf DD3) and *Hypholoma fasciculare* JH (Hf JH) growing towards one another in control (C), *Folsomia candida* (Fc), *Oniscus asellus* (Oa), *Blaniulus guttulatus* (Bg) and *Panagrellus redivivus* (Pr) grazing treatments. Negative values indicate that mycelia are retreating due to grazing or competitive interactions. Different letters indicate significantly ($P \leq 0.05$; ANCOVA; Minitab 15) different extension rates. Upper and lower case letters refer to different fungi and were analysed separately. *Y*-axis scales vary between graphs.

(*O. asellus*) preferentially grazed *R. bicolor* over *P. velutina* ($\chi_1^2 = 8.9$, $P = 0.003$) and *H. fasciculare* ($\chi_1^2 = 13.32$, $P < 0.001$). Selective grazing significantly ($P \leq 0.001$) reduced extension rates of *R. bicolor* compared to those in ungrazed controls (*R. bicolor* vs. *H. fasciculare* JH: $F_{1,46} = 32.5$, $P < 0.001$; *H. fasciculare* DD3: $F_{1,46} = 103.59$, $P < 0.001$; *P. velutina*: $F_{1,46} = 30.39$, $P < 0.001$; Fig. 3), leading to its replacement in all *O. asellus* microcosms (Fig. 4). No other invertebrate species altered the outcome of interactions between *R. bicolor* and *P. velutina* (Fig. 2), but competition with *H. fasciculare* DD3 and *H. fasciculare* JH was significantly ($P \leq 0.05$) affected by *P. redivivus* and *F. candida*, respectively. The nematodes (*P. redivivus*) had no significant ($F_{1,46} = 3.25$, $P = 0.078$) effect on extension rates of *R. bicolor*, but stimulated growth ($F_{1,46} = 5.05$ $P = 0.029$) and branching of *H. fasciculare* DD3 (Figs 3 and 4). This enabled the latter to overgrow its opponent in 60% of *P. redivivus* microcosms and reversed the interaction outcome ($G = 10.044$, $P = 0.007$; Fig. 2). Despite showing a clear preference for *R. bicolor* over *H. fasciculare* ($\chi_1^2 = 21.33$, $P < 0.001$), collembola (*F. candida*) grazed extensively at the interaction zone between the two. This prevented either *R. bicolor* or *H. fasciculare* JH from reaching the opposing wood block in 80% of microcosms (Figs 2 and 4). As neither fungus was removed, *F. candida* effectively ensured the survival of both species, whereas *H. fasciculare* was replaced in all ungrazed microcosms. Millipedes (*B. guttulatus*) preferentially grazed *H. fasciculare* over *R. bicolor* ($\chi_1^2 = 5.4$, $P = 0.02$) and showed no preferences between the latter and *P. velutina* ($\chi_1^2 = 0.476$, $P = 0.49$). As *H. fasciculare* was the least competitive fungal species, *B. guttulatus* grazing did not significantly ($P > 0.05$) affect the outcomes of any fungal interactions (Fig. 2).

Colonisation of wood blocks

The hierarchy of fungal dominance observed in soil was maintained in wood block colonisation. In the absence of grazing, *P. velutina* replaced *H. fasciculare* DD3 and JH, whereas all the three were replaced by *R. bicolor* (Fig. 2).

Invertebrates did not affect the colonisation of *H. fasciculare* (DD3 and JH) wood blocks by *P. velutina*; the former were replaced in every replicate. Grazing significantly ($P \leq 0.05$) affected wood block colonisation during *R. bicolor* interactions, but unlike in soil, the interaction outcomes were not reversed (Fig. 2). *Oniscus asellus*, for example, grazed *R. bicolor* mycelia from the soil, but could not access hyphae within wood blocks. Opposing fungi were, therefore, able to dominate the soil and encounter *R. bicolor* wood blocks, but were unable to displace the dominant competitor from its original resource. As a result, *O. asellus* grazing caused a shift from *R. bicolor*-dominated microcosms (both wood blocks colonised by *R. bicolor*) to those in which both fungi survived (Fig. 2). *Panagrellus redivivus* had the same effect during interactions between *R. bicolor* and

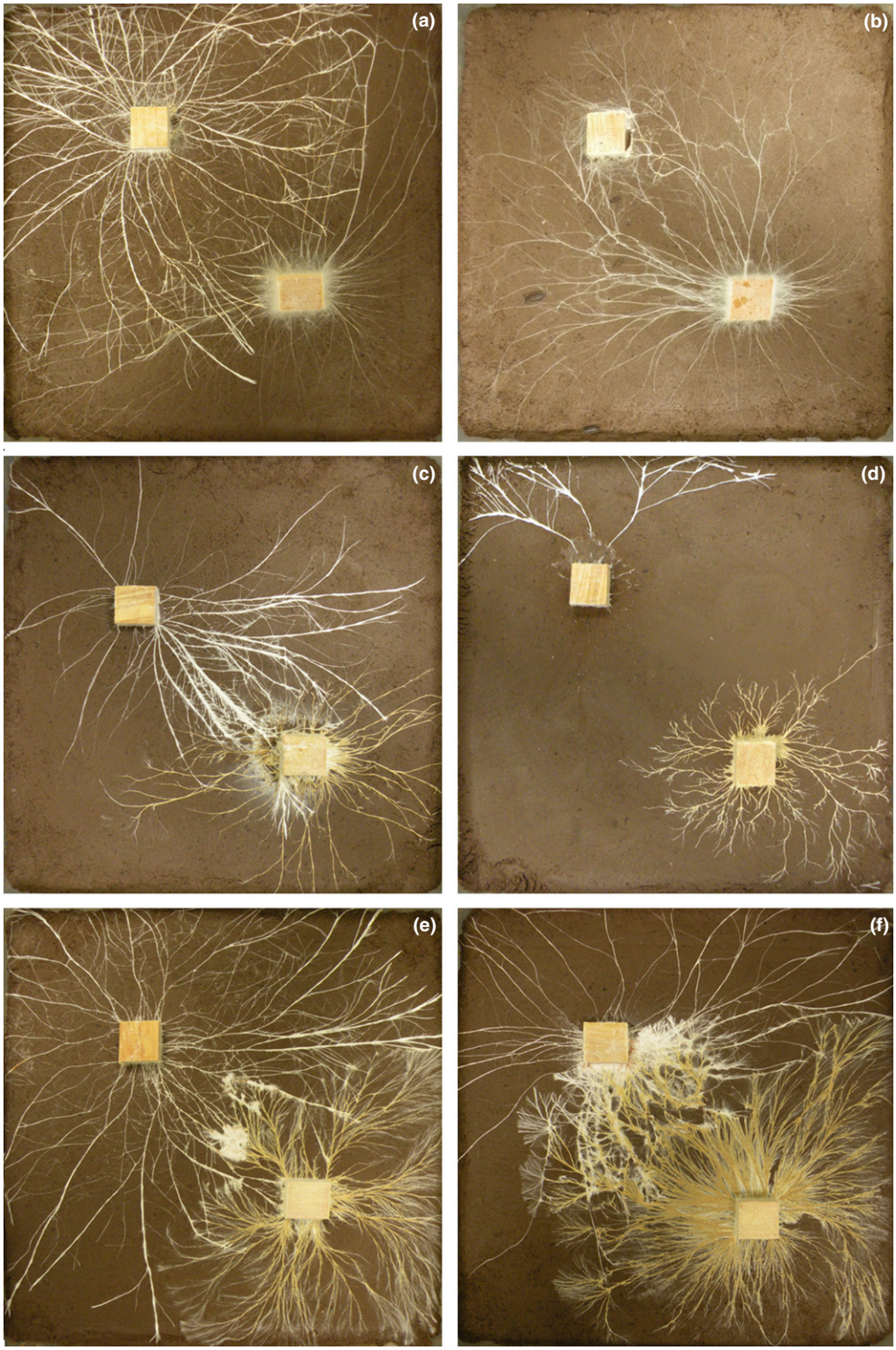

Figure 4 Digital images showing the outcomes of mycelial interactions with *Resinicium bicolor* against *Phanerochaete velutina* (a, b), *Hypholoma fasciculare* JH (c, d) and *Hypholoma fasciculare* DD3 (e, f) in ungrazed control (a, c, e) and grazed [*Oniscus asellus* (b), *Folsomia candida* (d), *Panagrellus redivivus* (f)] treatments. Competing mycelia extended from 2 × 2 × 1 cm wood blocks across 24 × 24 cm soil trays.

H. fasciculare DD3. By preventing either fungus from encountering opposing wood blocks, *F. candida* also shifted the balance between *R. bicolor* and *H. fasciculare* JH, allowing both fungi to co-exist in wood resources (Fig. 2).

Decay rates

In the absence of grazing, the decay rates of *P. velutina*-colonised wood blocks were influenced by the competing fungal species (Fig. 5).

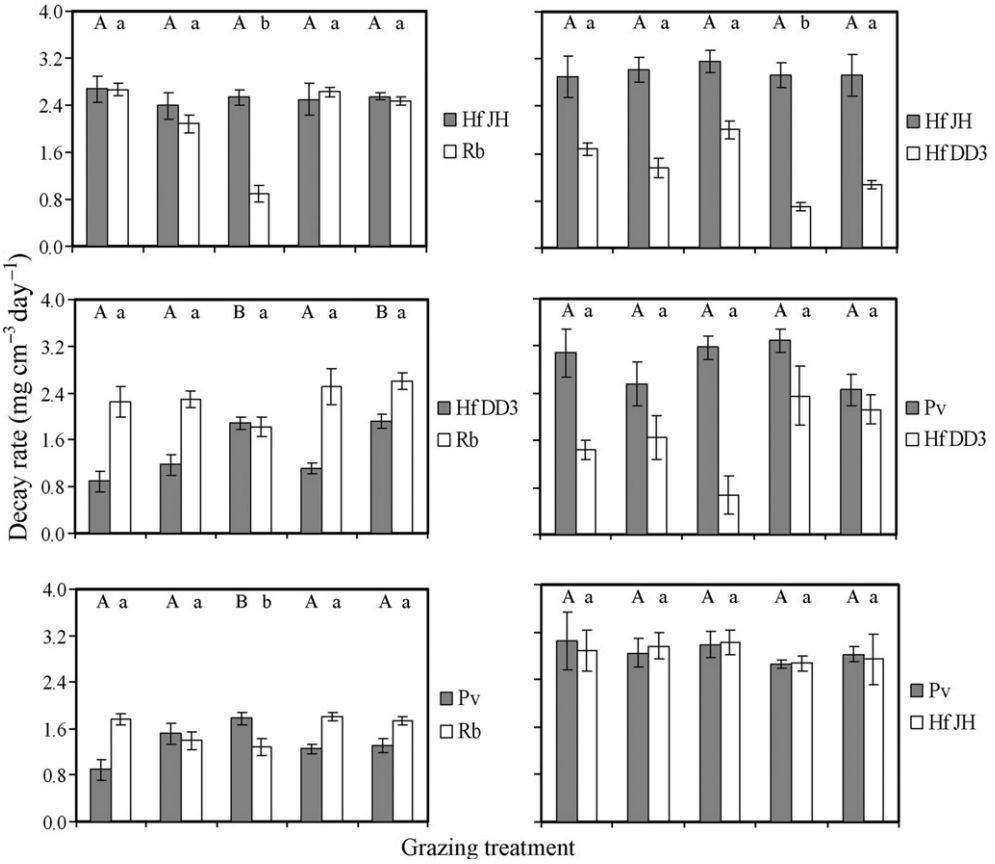

Figure 5 Decay rates of wood blocks colonised originally by *Resinicium bicolor* (Rb) against *Hypholoma fasciculare* JH (Hf JH), *Hypholoma fasciculare* DD3 (Hf DD3) and *Phanerochaete velutina* (Pv) during competitive mycelial interactions against one another in control (C), *Folsomia candida* (Fc), *Oniscus asellus* (Oa), *Blaniulus guttulatus* (Bg) and *Panagrellus redivivus* (Pr) grazing treatments. Different letters indicate significantly ($P \leq 0.05$; two-way ANOVA; Minitab 15) different decay rates. Upper and lower case letters refer to different fungi and were analysed separately.

Decay rates were significantly ($F_{2,12} = 10.22$, $P = 0.003$) faster during interactions with *H. fasciculare* JH and DD3 than when competing with *R. bicolor*. Decay rates of wood blocks colonised by the three remaining fungi were not significantly ($P > 0.05$) influenced by any opposing fungal strain.

The presence of grazers affected the rate of decay of wood blocks colonised by *P. velutina*, *R. bicolor* and *H. fasciculare* DD3 (Fig. 5). Significant (two-way ANOVA; fungus × invertebrate interaction: $P \leq 0.05$) interactive effects of competing fungus and grazing treatments suggested that fungal opponents determined the potential of grazers to affect wood decay rates. Overall decay rates were, however, positively correlated ($r = 0.521$, $n = 300$, $P < 0.001$) with mycelial extension rates of the colonising fungi. This suggests that any reduction in wood decay was the result of reduced mycelial growth, following intensive grazing or replacement by an opponent.

During interactions between *R. bicolor* and *P. velutina*, decay rates of wood blocks colonised by the former were significantly ($F_{6,68} = 10.95$, $P = 0.014$) reduced by *O. asellus* (Fig. 5). This also led to significantly ($F_{6,68} = 17.94$, $P = 0.003$) increased decay rates of *P. velutina* wood blocks during the same interaction (Fig. 5). Decay rates of *R. bicolor* wood blocks were also decreased by *O. asellus* during interactions with *H. fasciculare* JH, but unlike *P. velutina*, *H. fasciculare* JH decay rates were not significantly ($F_{6,68} = 24.21$, $P = 0.001$) increased (Fig. 5). While competing against *R. bicolor*, decay rates of *H. fasciculare* DD3 wood blocks were significantly ($P \leq 0.05$) increased by *O. asellus* ($F_{6,68} = 10.19$, $P = 0.013$) and *P. redivivus* ($F_{6,68} = 11.82$, $P = 0.009$) grazing; the same two species which stimulated *H. fasciculare* DD3 extension across soil (Fig. 2). In contrast, *B. guttulatus* reduced *H. fasciculare* DD3 wood decay rates during intraspecific interactions with *H. fasciculare* JH ($F_{6,68} = 11.93$, $P = 0.01$; Fig. 5). This is the only confirmed direct influence of grazers on decay rate; as neither opponent reached the opposing wood block, the decreased decay of *H. fasciculare* DD3 was the direct result of reduced fungal biomass during *B. guttulatus* grazing.

DISCUSSION

By differentially affecting the competitive abilities of interacting fungi, grazing invertebrates exerted selective pressures on fungal communities [supporting Hypothesis (i)]. The potential for selective grazing to alter fungal community composition has been recognised since the 1970s (Parkinson *et al.* 1977, 1979) but has never been shown empirically. Although grazers are known to influence the relative abundances of litter fungi (Newell 1984a, b), no previous study has shown that grazing can reverse the outcomes of competitive mycelial interactions in soil. Grazers can determine fungal dominance via two opposing mechanisms: (1) restriction of the more competitive fungus or (2) stimulation of the less competitive species. Here, extensive, selective grazing of the dominant fungus (*R. bicolor*) by *O. asellus* populations prevented the competitive exclusion of

opponents, while the stimulated, 'compensatory' growth of *H. fasciculare* during *P. redivivus* feeding (recorded previously in this species and probably the result of mobilisation of storage compounds and increased nutrient uptake from wood resources to counteract the negative effects of grazing; Crowther *et al.* 2011a) enabled it to overcome a more competitive opponent by a process of gross mycelial contact (Boddy 2000). Their ability to alter fungal dominance suggests that along with litter type and soil quality (Bardgett 2005), grazing invertebrates represent a key factor determining the community composition of saprotrophic fungi in soil and wood resources. This will have direct implications for nutrient mineralisation and cycling. *Phanerochaete velutina*, for example, produces more cellulytic enzymes (Crowther *et al.* 2011c), and decomposes wood at a faster rate (Tordoff *et al.* 2008) than *R. bicolor* when growing alone. The potential of *O. asellus* to shift fungal communities in favour of the former may therefore lead to increased nutrient mineralisation and wood decomposition rates.

Top-down determination of community composition has been well documented in aboveground terrestrial and aquatic ecosystems, as well as belowground bacterial-based communities (Wardle & Yeates 1993; Walker & Jones 2001; Veen *et al.* 2010). These effects are commonly associated with high intensity grazing, exerting strong selective pressures. Prior to this study, the lack of evidence supporting this process in fungal communities may have been due to absence of macrofauna in empirical studies; previous work on the effects of grazers on fungal community structure have been limited almost exclusively to micro and mesofauna (Nematoda, Collembola and Oribatida). In the present study, *F. candida* and *P. redivivus* affected the development of specific fungal interactions but *O. asellus* exerted the strongest selective pressures on competing fungi. This supports Hypothesis (ii) and highlights the Isopoda as a particularly important group of decomposers, not only through the communition (shredding and digestion) of litter but also through their modification of fungal activity (Bradford *et al.* 2002; Hättenschwiler *et al.* 2005). Along with *Porcellio scaber*, *O. asellus* is one of the most abundant terrestrial isopods in temperate ecosystem soils (Jones & Hopkin 1996). Even at low density, both species are capable of selectively removing entire mycelial cord systems from soil microcosms (Crowther *et al.* 2011a, b), highlighting their potential capacity to regulate fungal abundance, community composition and diversity in natural and agricultural systems (Mitschunas *et al.* 2006).

Even within invertebrate size groups, grazers varied in their effects on interacting fungi. Unlike isopods, millipede (*B. guttulatus*) populations selectively grazed the least competitive fungi (*H. fasciculare* JH and DD3), and as a result, had no effect on the outcomes of fungal interactions. Contrasting feeding preferences among mycophagous soil fauna have rarely been recorded; most studies report similar preferences for palatable or nutritious fungal resources (e.g. dark-pigmented fungi; Maraun *et al.* 2003 and references cited therein). *Hypholoma fasciculare* and *P. velutina* are generally considered unpalatable resources due to their production of sesquiterpenes – secondary metabolites, commonly used in defence against fungivores (Hynes *et al.* 2007). These fungi were avoided by *O. asellus* and *F. candida*, but the apparent tolerance of *B. guttulatus* may represent an example of resource partitioning (Maraun *et al.* 2003; Setälä *et al.* 2005), enabling the coexistence of various decomposer invertebrate species within similar soil environments. Species-specific feeding behaviour suggests that predicted changes to soil invertebrate diversity and taxonomic make-up brought about by global climate change (Jones *et al.* 1998; Wolters *et al.* 2000) will have direct consequences for fungal community composition. While abiotic climate change parameters (e.g. elevated temperature and rainfall) directly affect microbial activity (Gange *et al.* 2007) and community composition (Fierer *et al.* 2003; He *et al.* 2010), the indirect effects, mediated through changes in the biological community, may be equally important for the functioning of the decomposer subsystem (Gessner *et al.* 2010).

The aforementioned changes in fungal community structure are likely to influence litter decomposition rates (i.e. if a slow decomposer fungus is replaced by a more rapid species, overall decay rates will be increased). The changes in wood decay rates highlight that the impacts of grazing may not be quite as straightforward. For example, decay rates by *R. bicolor* were reduced during extra-resource mycelial grazing by *O. asellus*. This effect has been recorded previously (Tordoff *et al.* 2008; Crowther *et al.* 2011b, c), but in the present study, the removal of *R. bicolor* also stimulated mycelial growth and decomposition rates by *P. velutina*. Resulting decay rates were increased not only via the change in fungal community composition but also by the stimulated growth and activity of the promoted fungal species during grazing. This suggests that by modifying the development of one mycelial system, high intensity grazing events may alter the activities of all interacting species within the local fungal community. In conclusion, selective grazing by soil fauna will affect rates of nutrient turnover, both by altering fungal species composition, and also by modifying the relative capacities of interacting fungi to decompose wood.

ACKNOWLEDGEMENTS

The authors thank Jade Relf for assistance in the invertebrate feeding study. The work was funded by the Natural Environment Research Council (NE/G523420/1).

REFERENCES

Baldrian, P. & Valášková, V. (2008). Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiol. Rev.*, 32, 501–521.

Bardgett, R.D. (2005). *The Biology of Soil*. Oxford University Press, Oxford.

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 Microbiol. Ecol.*, 31, 43–56.

Bradford, M.A., Jones, T.H., Bardgett, R.D., Black, H.I.J., Boag, B., Bonkowski, M. *et al.* (2002). Impacts of soil faunal community composition on model grassland ecosystems. *Science*, 298, 615–617.

Clark, C.M. & Tilman, D. (2008). Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, 451, 712–715.

Crowther, T.W., Boddy, L. & Jones, T.H. (2011a). Species-specific effects of soil fauna on fungal foraging and decomposition. *Oecologia*, DOI: 10.1007/s00442-011-2005-1.

Crowther, T.W., Jones, T.H. & Boddy, L. (2011b). Species-specific effects of grazing invertebrates on mycelial emergence and growth from woody resources into soil. *Fungal Ecol.*, 5, 333–341.

Crowther, T.W., Jones, T.H., Boddy, L. & Baldrian, P. (2011c). Invertebrate grazing determines enzyme production by basidiomycete fungi. *Soil Biol. Biochem.*, 43, 2060–2068.

Dyer, H.C., Boddy, L. & Preston-Meek, C.M. (1992). Effect of the nematode *Panagrellus redivivus* on growth and enzyme production by *Phanerochaete velutina* and *Stereum hirsutum*. *Myc. Res.*, 96, 1019–1028.

Fierer, N., Schimel, J.P. & Holden, P.A. (2003). Influence of drying-rewetting frequency on soil bacterial community structure. *Microbial Ecol.*, 45, 63–71.

Fountain, M.T. & Hopkin, S.P. (2005). *Folsomia candida* (Collembola): a "standard" soil arthropod. *Annu. Rev. Entomol.*, 50, 201–222.

Gange, A.C., Gange, E.G., Sparks, T.H. & Boddy, L. (2007). Rapid and recent changes in fungal fruiting patterns. *Science*, 306, 71.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. *et al.* (2010). Diversity meets decomposition. *Trends Ecol. Evol.*, 25, 372–380.

Hättenschwiler, S., Tiunov, A.V. & Scheu, S. (2005). Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. Syst.*, 36, 191–218.

He, Z., Xu, M., Deng, Y., Kang, S., Kellogg, L., Wu, L. *et al.* (2010). Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. *Ecol. Lett.*, 13, 564–575.

Hiol Hiol, F.H., Dixon, R.K. & Curl, E.A. (1994). The feeding preference of mycophagous collembola varies with the ectomycorrhizal symbiont. *Mycorrhiza*, 5, 99–103.

Hooper, D.U. & Vitousek, P.M. (1997). The effects of plant composition and diversity on ecosystem processes. *Science*, 277, 1302–1305.

Hynes, J., Muller, C.T., Jones, T.H. & Boddy, L. (2007). Changes in volatile production during the course of fungal mycelial interactions between *Hypholoma fasciculare* and *Resinicium bicolor*. *J. Chem. Ecol.*, 33, 43–57.

Jones, D.T. & Hopkin, S.P. (1996). Reproductive allocation in the terrestrial isopods *Porcellio scaber* and *Oniscus asellus* in a metal polluted environment. *Funct. Ecol.*, 10, 741–750.

Jones, T.H., Thompson, L.J., Lawton, J.H., Bezemer, T.M., Bardgett, R.D., Blackburn, T.M. *et al.* (1998). Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. *Science*, 280, 441–443.

Kaneko, N., McLean, M.A. & Parkinson, D. (1998). Do mites and Collembola affect pine litter fungal biomass and microbial respiration? *Appl. Soil Ecol.*, 9, 209–213.

Klironomos, J.N., Widden, P. & Deslandes, I. (1992). Feeding preferences of the collembolan, *Folsomia candida*, in relation to microfungal successions on decaying litter. *Soil Biol. Biochem.*, 24, 685–692.

Maraun, M., Martens, H., Migge, S., Theenhaus, A. & Scheu, S. (2003). Adding to 'the enigma of soil animal diversity': fungal feeders and saprotrophic soil invertebrates prefer similar food substrates. *Eur. J. Soil Biol.*, 39, 85–95.

McLean, M.A., Kaneko, N. & Parkinson, D. (1996). Does selective grazing by mites and collembola affect litter fungal community structure? *Pedobiologia*, 40, 97–105.

McNaughton, S.J. (1983). Compensatory plant growth as a response to herbivory. *Oikos*, 40, 329–336.

Mitschunas, N., Wagner, M. & Filser, J. (2006). Evidence for a positive influence of fungivorous soil invertebrates on the seed bank persistence of grassland species. *J. Ecol.*, 94, 791–800.

Moore, J.C., Mccann, K., Setälä, H. & De Ruiter, P.C. (2003). Top-down is bottom up: does predation in the rhizisphere regulate aboveground dynamics? *Ecology*, 84, 846–857.

Newell, K. (1984a). Interaction between two decomposer Basidiomycetes and a collembolan under Sitka spruce: grazing and its potential effects on fungal distribution and litter decomposition. *Soil Biol. Biochem.*, 16, 235–239.

Newell, K. (1984b). Interaction between two decomposer Basidiomycetes and a collembolan under Sitka spruce: distribution, abundance and selective grazing. *Soil Biol. Biochem.*, 16, 223–233.

Parkinson, D., Visser, S. & Whittaker, J.B. (1977). Effects of collembolan grazing on fungal colonization of leaf litter. In: *Soil Organisms as Components of Ecosystems* (eds Lohm, U. & Persson, T.). *Ecological Bulletins*, 25, 75–79.

Parkinson, D., Visser, S. & Whittaker, J.B. (1979). Effects of collembolan grazing on fungal colonization of leaf litter. *Soil Biol. Biochem.*, 11, 529–535.

Petersen, H. & Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in the decomposition process. *Oikos*, 39, 287–388.

Pollierer, M.M., Langel, R., Scheu, S. & Maraun, M. (2009). Compartmentalization of the soil animal food web as indicated by dual analysis of stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$). *Soil Biol. Biochem.*, 41, 1221–1226.

Post, W.W., Emanual, W., Zinke, P.J. & Stangenberger, A.G. (1982). Soil carbon pools and world life zones. *Nature*, 298, 156–159.

Setälä, H., Berg, P.M. & Jones, T.H. (2005). Trophic structure and functional redundancy in soil communities. In: *Biological Diversity and Function in Soils* (eds Bardgett, R.D., Usher, M.B. & Hopkins, D.W.). Cambridge University Press, Cambridge, pp. 236–249.

Southwood, T.R.E. & Henderson, P.A. (2000). *Ecological Methods*, 3rd edn. Blackwell, Oxford.

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. *Science*, 277, 1300–1302.

Tordoff, G.M., Boddy, L. & Jones, T.H. (2008). Species-specific impacts of collembola grazing on fungal foraging ecology. *Soil Biol. Biochem.*, 40, 434–442.

Van der Heijden, M.G.A., Bardgett, R. & Van Straalen, N.M. (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.*, 11, 296–310.

Veen, G.F., Olff, H., Duyts, H. & Van der Putten, W.H. (2010). Vertebrate herbivores influence soil nematodes by modifying plant communities. *Ecology*, 91, 828–835.

Walker, M. & Jones, T.H. (2001). Relative roles of top-down and bottom-up forces in terrestrial tritrophic plant-insect herbivore-natural enemy systems. *Oikos*, 93, 177–187.

Wardle, D.A. (2006). The influence of biotic interactions on soil biodiversity. *Ecol. Lett.*, 9, 870–886.

Wardle, D.A. & Yeates, G.W. (1993). The dual importance of competition and predation as regulatory forces in terrestrial ecosystems: evidence from decomposer food-webs. *Oecologia*, 93, 303–306.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H. & Wall, D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, 304, 1629–1633.

Whittaker, J.B. (1981). Feeding of *Onychiurus subteniuis* (Collembola) at snow melt in aspen litter in the Canadian Rocky Mountains. *Oikos*, 36, 203–206.

Wolters, V., Silver, W.L., Bignell, D.E., Coleman, D.C., Lavelle, P., Van Der Putten, W.H. *et al.* (2000). Effects of global changes on above- and belowground biodiversity in terrestrial ecosystems: implications for ecosystem functioning. *Bioscience*, 50, 1089–1098.

Yang, H.J., Wu, M.Y., Liu, W.X., Zhang, Z., Zhang, N. & Wan, S. (2011). Community structure and composition in response to climate change in a temperate steppe. *Glob. Change Biol.*, 17, 452–465.

Editor, Wim van der Putten
Manuscript received 25 May 2011
First decision made 27 June 2011
Second decision made 7 August 2011
Manuscript accepted 16 August 2011