Climate Change, Fungus–Invertebrate Interactions and Ecosystem Processes

A thesis submitted to Cardiff University for the degree of Doctor of Philosophy

by

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This work has not previously been accepted in substance for a degree and is not concurrently submitted in candidature for any degree.

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Summary

Saprotrophic fungi are the main agents of primary decomposition and nutrient cycling in woodland ecosystems. Powerful enzymatic capabilities enable then to break down the most recalcitrant components of wood and leaf litter, such as lignin and cellulose. Nutrients are retained by dynamic networks of mycelium, which are vulnerable to grazing by soil invertebrates. The studies reported in this thesis employed laboratory microcosm, mesocosm and field manipulations to further mechanistic understanding of climate change effects on basidiomycete fungal-dominated woodland decomposer community dynamics and ecosystem processes. Increased mycelial growth at elevated temperature can be prevented by collembola grazing in soil microcosms. The strength of this top-down effect varied with fungal palatability, which had a bottom-up effect on collembola populations and their responses to warming. A mesocosm multispecies collembola population was more strongly regulated by the bottom-up effect of inoculation with cord-forming fungi than climate change (warming, in combination with soil wetting or drying). Collembola can graze fungal cords, but thickness and chemical defences make them less palatable than soil microfungi, which are outcompeted by basidiomycete mycelia. In the absence of fungal biomass limitation by collembola, abiotic conditions regulated microbial community functioning. Warming stimulated fungal-mediated wood decomposition, particularly in drier soils. Moisture was the most important determinant of enzyme activity and displayed an interaction with temperature analogous to that for wood decay. Macro-invertebrates, such as woodlice, are better able to exploit nutritious, but thick and defensive, fungal cords. The consequences of macro-invertebrate grazing for fungal-dominated microbial community function were tested in a field manipulation of woodlouse (*Oniscus asellus*, Isopoda) population densities, predicted to increase due to climate warming. This provides the first evidence for bottom-up effects of fungal palatability on woodlouse populations. Body lipid analysis revealed fungi as a major component of the generalist woodlouse diet. Despite low population densities at the site, altered *O. asellus* abundance influenced aspects of microbial community functioning. The importance of biotic effects on decomposition may be more heterogeneous than abiotic influences, depending on microbial community dominance and the abundance of key macro-invertebrate taxa.

List of Publications

Publications associated with thesis

A'Bear AD, Boddy L, Jones TH (2012) Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola. *Global Change Biology*, **18**, 1823-1832.

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A'Bear AD, Crowther TW, Ashfield R, Chadwick DDA, Dempsey J, Meletiou L, Rees C, Jones TH, Boddy L (2013) Localised invertebrate grazing moderates the effect of warming on competitive fungal interactions. *Fungal Ecology*, **6**, 137-140.

A'Bear AD, Johnson SN, Jones TH (2013) Putting the 'upstairs–downstairs' into ecosystem service: what can aboveground–belowground ecology tell us? *Biological Control*, http://dx.doi.org/10.1016/j.biocontrol.2013.10.004.

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Crowther TW, Stanton D, Thomas SM, **A'Bear AD**, Hiscox J, Jones TH, Voříšková J, Baldrian P, Boddy L (2013) Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology*, **94**, 2518-2528.

Dray MW, Crowther TW, Thomas SM, **A'Bear AD**, Godbold DL, Ormerod SJ, Hartley SE, Jones TH (2014) Effects of elevated CO_2 on litter chemistry and subsequent invertebrate detritivore feeding responses. *PLoS ONE*, **9**, e86246.

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1 General introduction

1.1 The soil environment

The majority of terrestrial life, in terms of biomass and species diversity, exists below ground (Giller 1996). The activity of belowground biota (including, but not limited to, microbes, animals and plant roots) exerts strong influences on the physical (e.g. structure and moisture) and functional (e.g. carbon sequestration, greenhouse gas production and nutrient cycling) characteristics of soil, which supports all terrestrial life (Wardle 2002; Bardgett 2005). Inputs of dead plant material provide a major energy source for soil ecosystems and support highly diverse decomposer communities (Wolters *et al*. 2000; Wardle *et al*. 2004). Three quarters of the world's terrestrial carbon is contained within soil organic matter, the decomposition of which provides the nutrients required for continued primary productivity (Post *et al*. 1982; Wall *et al*. 2012).

Soil is fundamental to human civilisation (McNeill and Winiwarter 2004). The continued dependence of human populations on soil, for food and nutrition, is unquestionable. Despite the inherent reliance of terrestrial life on belowground systems, their complex and opaque nature have limited ecological understanding of soil environments and the organisms that maintain them. Consequently, soil has traditionally been considered by researchers as a 'black box' of decomposers, a single trophic level through which aboveground life is ultimately recycled (Sudgen *et al*. 2004). In recent decades, ecologists have begun unravelling the complexity of belowground communities. Significant advances have been made in scientific understanding of the processes affecting, and regulated by, soil organisms (Bardgett 2005; Wall *et al*. 2012). Soil ecology has direct relevance for natural and agricultural land management. A renewed appreciation for this is being driven by an increasing awareness of climate change. A thorough understanding of belowground interactions is vital as researchers strive to determine how the changing climate will affect soil ecosystem functioning and carbon cycle feedbacks.

1.2 Climate change and soil ecosystem function

The annual global carbon release to the atmosphere of 60 Pg, resulting from organic carbon decomposition by soil biota, is currently balanced by the approximately equal

quantity absorbed by primary productivity (Lal 2008). Climate change has the potential to drive a shift in this balance, with implications for ecosystem–climate feedback at regional and global scales (Heimann and Reichstein 2008). Atmospheric carbon dioxide (CO_2) concentration is predicted to reach 540-970 ppm by 2100, with globally differential temperature increases in the range 1.1-6.4 °C (IPCC 2013). Increases in precipitation, UV-B penetration and frequency of extreme events are also predicted, but with less certainty regarding magnitude.

The role of soil microbes and invertebrates in ecosystem regulation is well recognised (Wardle *et al.* 1998; Bradford *et al.* 2002), but detailed understanding of the impacts of climate change on these organisms is lacking (Bardgett *et al.* 2008). Soil respiration is thought to be more temperature-sensitive than primary production (Jenkinson *et al.* 1991); warming has potential to increase microbial breakdown of soil organic matter, promoting CO_2 efflux (Bardgett *et al.* 2008). This is complicated, however, by uncertainty regarding the differential sensitivity of microbial groups to elevated temperature (e.g. Kandeler *et al.* 1998; Bardgett *et al.* 1999) and their complex interactions with other soil biota (Wardle *et al.* 2004).

1.3 Forest soils

Forest vegetation contains 536 Pg carbon (Lal 2005). The majority of this vegetation escapes herbivory and enters the 704 Pg soil carbon pool. Woody plant material, from individual leaves to whole trees, is distributed heterogeneously across the litter horizon. Forest litter is heavily lignified and of low nutrient quality (Rayner and Boddy 1988). As a consequence, the number of taxa capable of degrading this resource is extremely limited. By virtue of their powerful enzymatic capabilities, basidiomycete fungi are an important group within a very limited numer of organisms capable of breaking down the more complex and recalcitrant components of organic material, such as lignin and cellulose. As a consequence, they are the primary agents of woody litter decomposition in forests (Boddy and Watkinson 1995). Saprotrophic basidiomycete fungi decay fresh resources sufficiently to enable exploitation by organisms reliant upon more labile nutrient sources, such as bacteria, lower fungi and invertebrates (Lindahl *et al.* 2002).

A major ecological grouping of basidiomycete fungi produce dynamic networks of mycelium which ramify at the soil–litter interface, linking resources and reallocating

nutrients over large distances (Boddy 1993, 1999). This non-resource-unit-restricted growth strategy enables cord-forming basidiomycete fungi to overcome the problems associated with resource heterogeneity and form systems that can cover very large areas of forest floor. These fungi are the dominant agents of primary decomposition in temperate woodlands (containing 292 Pg C; Lal 2005), making them a significant component of global decomposition patterns. The large biomass and high nutrient status (low C:N relative to organic matter) of mycelia make them vulnerable to grazing by soil invertebrates (Swift and Boddy 1984; Boddy and Jones 2008). Grazing can influence mycelial development and activity; the balance between mycelial growth and removal by grazing invertebrates determines decomposition rates and the ability of the fungus to acquire new resources (Tordoff *et al.* 2008; Crowther *et al.* 2011a).

1.4 Thesis aims

This research project aims to determine the effect of predicted climatic scenarios on the balance between mycelial development and removal by grazing invertebrates, and the resulting implications for decomposition. Elevated temperature and altered soil moisture (increased precipitation and drought) receive particular emphasis in empirical elements, as the major climate change factors directly affecting these organisms. These factors are considered individually and in combination; the temperature–moisture gradient is a key determinant of soil biota community composition (Briones *et al.* 1997).

Chapter 2 reviews the literature on ecophysiological relationships between saprotrophic basidiomycete mycelia and abiotic factors (e.g. temperature and water potential), and soil invertebrate responses to climate change. A general hypothesis is stimulated: increased fungal biomass and activity in temperate woodlands, due to climate change, could be moderated by a concomitant increase in grazing pressure from mycophagous invertebrates. This is explored in the experimental chapters that follow. A version of this review has been published in *Fungal Ecology* (A'Bear *et al*. 2013c).

Chapter 3 explores the influence of elevated temperature on interactions between fungal mycelia and collembola (used as 'model' fungal grazers) in two-species soil microcosms. Species-specific grazing preferences determine the importance of top-down and bottom-up mechanisms in regulating mycelial growth and grazer population

responses to warming. A version of this study has been published in *Global Change Biology* (A'Bear *et al.* 2012).

Woodland soil mesocosms are employed in Chapters 4 and 5 to 'bridge the gap' between reductionist microcosm experiments and the field. A natural soil community is subjected to controlled climate change (warming and soil wetting or drying) in the laboratory. *Chapter 4* investigates the importance of bottom-up (fungal species dominance) regulation of collembola community dynamics under climate change. A version of this study has been published in *Oecologia* (A'Bear *et al*. 2013a). *Chapter 5* explores the influence of the aforementioned interactions between climatic factors on fungal-dominated microbial community function (decomposition and enzyme activity). A version of this study has been published in *Soil Biology & Biochemistry* (A'Bear *et al*. 2014).

In *Chapter 6*, field populations of woodlice (Isopoda) are manipulated to investigate the influence of increased abundance of fungal-feeding macro-invertebrates, predicted due to climate warming in temperate regions, on decomposer community composition and function. A version of this study has been submitted to *Soil Biology & Biochemistry*.

Finally, *Chapter 7* brings together the salient points from the experimental chapters. The combination of microcosm, mesocosm and field experiments employed in this research project provides a unique opportunity to consider the extent to which mechanisms and concepts revealed in laboratory studies extrapolate to more complex systems.

2 Potential impacts of climate change on interactions among saprotrophic fungal mycelia and grazing soil invertebrates

2.1 Introduction

Organic carbon decomposition by soil biota generates an annual global release of 60 Pg carbon to the atmosphere (almost ten times that of fossil fuel emissions); this is balanced by the approximately equal quantity absorbed through primary production (Lal 2008). Shifts in this balance, mainly due to changes in ambient climate, have potentially far-reaching implications for CO_2 feedback and atmospheric gaseous composition. By 2100, atmospheric CO_2 concentration is predicted to reach 540-970 ppm, accompanied by globally differential temperature increases of 1.1-6.4 °C (IPCC 2013). Precipitation and the frequency of extreme events are also expected to increase, with less certainty regarding magnitude. Although key drivers of ecosystem processes (Wardle *et al*. 1998; Bradford *et al*. 2002), soil microbes and invertebrates are not explicitly considered in models predicting impacts of climate change on CO_2 feedbacks via, for example, decomposition and soil respiration (Cao and Woodward 1998; Cox *et al*. 2000; Wall *et al*. 2008). This is primarily a consequence of belowground food web complexity, the general neglect of the significance of soil interactions in climate feedback predictions, and the dearth of understanding of the direct and indirect effects of climate change in soil (Bardgett *et al*. 2008).

Impacts of projected climatic scenarios on aboveground communities and trophic interactions (e.g. plants and their insect herbivores) have been well-studied and thoroughly reviewed (e.g. Bezemer and Jones 1998; Bale *et al*. 2002; Harsch *et al*. 2009; Hooper *et al*. 2012). Although soil biotic activity exerts a strong influence on the composition, structure and functioning of aboveground communities (De Deyn *et al*. 2003; van der Heijden *et al*. 2008), relatively little is known about the impacts of climate change on belowground community activity and functioning. Any influence on decomposition, nutrient cycling and soil organic matter (SOM) dynamics will be of crucial importance in determining ecosystem-level responses to climate change at both regional and global scales (Heimann and Reichstein 2008).

Saprotrophic fungi, in particular, are important regulators of spatial and temporal variation in nutrient availability, SOM dynamics and the sensitivity of decomposition to

abiotic variables (Yuste *et al*. 2011). Basidiomycetes dominate primary decomposition in forest ecosystems (Hättenschwiler *et al*. 2005), a globally significant terrestrial carbon store (1240 Pg C; Lal 2005). A major ecological grouping of these fungi (saprotrophic cord-forming basidiomycetes) form extensive mycelial cord networks, mainly restricted to the woodland soil–litter interface, which link organic resources and conservatively retain and re-allocate nutrients (Boddy 1993, 1999). Decomposition rates are determined by fungal community composition, ecophysiological relationships with abiotic variables, and interactions with other biota.

Soil invertebrates exert the strongest influence on decomposition where fungi are the dominant component of the microbial community (Wardle *et al*. 2004). The low C:N ratios of fungal cords and hyphae relative to plant-derived organic matter make mycelia an attractive nutritional source for soil invertebrates (Boddy and Jones 2008). Mycelial development and function can be markedly affected by invertebrate grazers, including nematodes (Dyer *et al*. 1992; Crowther *et al*. 2011b), oribatid mites (A'Bear *et al*. 2010), collembola (Kampichler *et al*. 2004; Tordoff *et al*. 2008), enchytraeids (Hedlund and Augustsson 1995), millipedes and woodlice (Crowther *et al*. 2011b, c). Stimulation of mycelial growth can result from low intensity grazing by micro- and meso-invertebrates (Hedlund *et al*. 1991; Bretherton *et al*. 2006) but, more commonly, biomass is reduced, with macro-invertebrates often removing whole systems (Crowther *et al*. 2011b). Selective feeding on specific fungi can differentially affect the competitive abilities of interacting mycelia, influencing community composition (Newell 1984a, b; Crowther *et al*. 2011a).

Elevated temperature and high or low water availability have the potential to affect soil fungi and invertebrates both directly and indirectly. Given that CO_2 concentrations in soil are, at least, tenfold higher than in the atmosphere (Lamborg *et al*. 1983; Lal 2008), belowground impacts of elevated CO_2 are generally assumed to be indirect, mediated by plant growth, rhizo-deposition and litter chemistry. The chemical content of wood and leaf litter could be affected by all of the abiotic variables considered here, with implications for decomposition activity of cord-forming mycelial systems. Elevated CO_2, in particular, reduces the nitrogen content and increases the C:N ratio and structural (e.g. lignin) content of litter (Cotrufo *et al*. 1994, 1998; Coûteaux *et al*. 1999; Norby *et al*. 2001). These responses reduce resource quality, often slowing the rate of

decomposition, but could promote the dominance of lignocellulolytic cord-forming basidiomycetes due to their ability to decompose the structural components. Reduced quality of litter could further increase the relative palatability of nutritionally-conservative fungal mycelia to soil invertebrates, potentially increasing their influence on fungal-mediated decomposition. Such direct and indirect climate change impacts on saprotrophic cord-forming fungi and their soil invertebrate grazers will influence the interactions between these organisms and the ecosystem processes they facilitate.

This review aims to identify: (1) trends in the responses of saprotrophic cord-forming fungi and soil invertebrate taxa containing mycophagous members to experimentally manipulated abiotic variables; (2) implications of these responses for saprotrophic fungus–grazer interactions under climate change scenarios; and (3) future research priorities in terms of biotic and abiotic influences on saprotrophic fungal activity and functioning. The past 20 years has seen a body of literature emerge on the responses of potentially mycophagous soil micro- (nematodes) and meso- (collembola, mites and enchytraeids) invertebrates to experimental manipulation of temperature, CO_2, precipitation and drought. These data are synthesised using meta-analysis. This approach cannot be applied to saprotrophic fungi as they have rarely been partitioned from the rest of the fungal, or even microbial, biomass in studies on microbial responses to climate change. Ecophysiological relationships between saprotrophic cord-forming basidiomycetes and abiotic variables (e.g. temperature and water potential) have, however, been investigated and are considered. Other abiotic factors associated with climate change, such as increasing concentrations of methane (CH_4), ozone (O_3) and other gaseous pollutants (e.g. NO_x), will undoubtedly affect both fungi and fauna directly and indirectly, but as yet insufficient information is available in the literature to provide informative synthesis.

2.2 Impacts of climate change on saprotrophic cord-forming mycelia

Climate change effects on plant productivity are known to influence the composition and activity of soil microbial communities (Sadowsky and Schortemeyer 1997; Wolters *et al.* 2000). Elevated temperature (e.g. Zhang *et al.* 2005) and CO_2 concentration (e.g. Zak *et al.* 1993; Kandeler *et al.* 2008) have been reported to alter microbial community composition, favouring fungi. The extent to which this relates to the abundance and activity of cord-forming saprotrophs remains unclear. Climate-induced increases in

belowground allocation of photosynthetic carbon are known to stimulate root colonisation by mycorrhizal fungi (Klironomos *et al.* 1997; Olsrud *et al.* 2010; Fransson 2012), which could account for the observed fungal dominance. There do not, however, appear to be any studies that partition biomass between saprotrophic and ectomycorrhizal mycelium. The ability of saprotrophic fungi to retain nutrients and, in the case of cord-forming fungi, to translocate them to different regions (Boddy 1993) confers a competitive advantage over other microbes when nitrogen availability is limited by increased plant growth and nutrient uptake (Bardgett *et al*. 1999). This could increase fungal dominance when climate change stimulates plant productivity. That recent climate change is affecting fungal activity is evidenced by changes in fruiting phenology (Gange *et al*. 2007).

It is the production of lignin- and cellulose-decomposing extracellular enzymes by saprotrophic fungi that ultimately advances the breakdown of wood and litter resources (Valášková *et al.* 2007). The activity of enzymes is stimulated by warming and their diffusion through the soil is facilitated by water films between soil particles. At a global scale, activities of many of the commonly assayed soil enzymes, involved in carbon, nitrogen and phosphorus cycling, are correlated with mean annual temperature and precipitation (Sinsabaugh *et al.* 2008). Elevated temperature and altered patterns of precipitation have the potential to alter fungal growth, biomass and activity. Extension rates of mycelial cord-forming basidiomycetes generally increase with temperature up to optima of about 20-25 °C, but different species display contrasting sensitivities and patterns of response across a broad temperature range (Fig. 2.1; Boddy 1983a). For example, when compared with five other cord-formers, *Phanerochaete laevis* extended slowest at low temperatures, but was the most rapid at 20 and 25 °C (Dowson *et al*. 1989; Donnelly and Boddy 1997). In contrast, *Phallus impudicus* and *Hypholoma fasciculare* were stimulated to a lesser extent by warming to these temperatures (Fig. 2.1).

Fungal decomposition of wood increases with temperature up to optima similar to those for growth (Boddy 1986). Mycelial extension often correlates with resource decay (Bebber *et al*. 2011), but temperature sensitivity of these functions can differ (Dowson *et al*. 1989; Wells and Boddy 1995). For example, *P. laevis* extended rapidly at higher

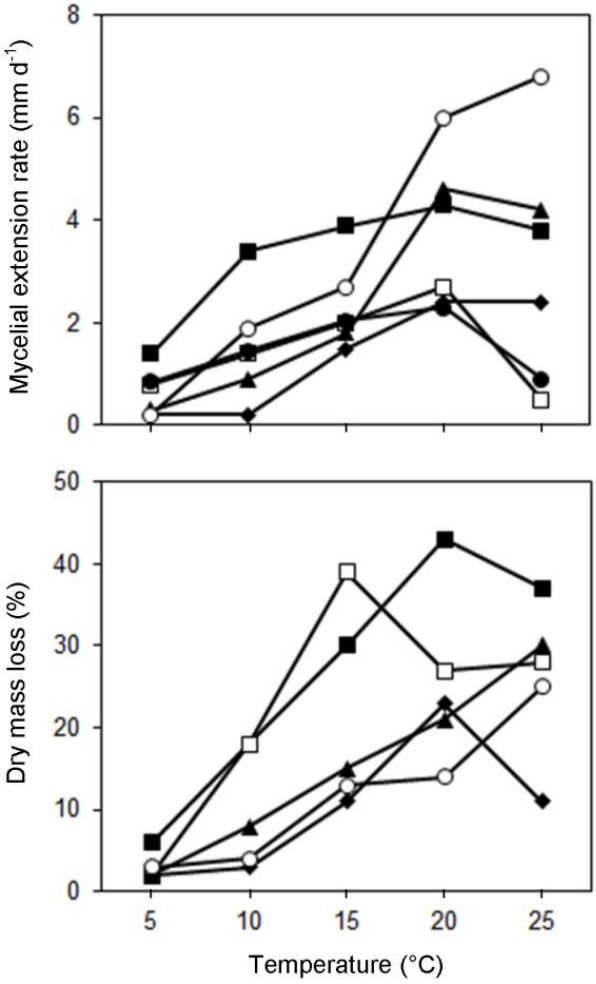


Fig. 2.1 Mycelial extension rate and beech (*Fagus sylvatica*) wood inoculum dry mass loss by *Phanerochaete velutina* (■), *Hypholoma fasciculare* (□), *Steccherinum fimbriatum* (▲), *Phallus impudicus* (♦), *Phanerochaete laevis* (○) and *Stropharia caerulea* (●) growing in soil at 5-25 °C. Redrawn from Dowson *et al.* (1989) and Donnelly and Boddy (1997).

temperatures, but was one of the slower decomposers of wood, and although *Phanerochaete velutina* and *H. fasciculare* did not display the greatest growth response to warming, they were among the most sensitive with respect to resource decay (Fig. 2.1). Given that soil temperatures are unlikely to exceed growth and decay optima in Northern European temperate forests, rarely reaching 20 °C (Boddy 1983b), warming has potential to increase the biomass and activity of saprotrophic cord-forming fungi. This may not, however, be true closer to the Equator.

Both low and high soil water contents can inhibit fungal growth and activity (Boddy 1986). At low water content, limitation is due to difficulty in obtaining water for cellular processes, basidiomycetes usually being unable to grow below -4 MPa (Boddy 1984). At high water content, limitation occurs when conditions are not sufficiently aerobic. Elevated temperature can exacerbate limitation of both low and high water contents. At already low water contents, physiological stress can be exacerbated by warming-induced soil moisture loss. At high water content, when temperature is

elevated towards the optimum for activity, limitation occurs because a diminished capacity for gaseous exchange with the atmosphere, due to water filling voids, cannot accommodate the more rapid diffusion of O_2 into, and CO_2 out of, soil and organic resources. Increased precipitation generally promotes fungal activity as long as conditions remain sufficiently aerobic. In naturally wet soils (e.g. bogs and tropical rainforest), moisture loss could relax anaerobic constraints on biological processes (Cleveland *et al*. 2010), increasing saprotrophic activity. Studies involving irrigation and drought manipulations have found fungal biomass and community composition to be fairly resilient to fluctuating moisture conditions (Yuste *et al*. 2011), displaying minimal responses relative to seasonal variation (Williams and Rice 2007; Hawkes *et al*. 2011). Impacts are likely to differ between fungal groups; responses of saprotrophic fungi have yet to receive any specific attention with respect to natural or experimental irrigation and drought.

Cord-forming basidiomycetes display species-specific sensitivity to low soil water potential, but commonly increase aggregation into cords (Dowson *et al*. 1989; Donnelly and Boddy 1997; Wells *et al*. 2001). Optima for mycelial extension and biomass production in soil tend to lie between -0.01 and -0.02 MPa, whereas decay optima are often lower, but generally not below -0.1 MPa (Dowson *et al*. 1989; Donnelly and Boddy 1997). Like other basidiomycetes, in agar culture, cord-forming saprotrophs cannot grow much below -4.4 MPa, many being even more sensitive (Boddy 1983a, 1984). Water can, however, be translocated through cords, allowing them potentially to grow from moist to drier regions; the dry rot fungus of buildings, *Serpula lacrimans*, being a classic example (Cairney 1992). Cord-forming fungi might also alter their growth location under different climatic scenarios. For example, in UK temperate forests extensive cord systems of *Megacollybia platyphylla* develop at the soil litter interface, whereas in the drier soils of Massachusetts they develop 5-10 cm below the surface litter (L Boddy *pers. obs.*). In addition to affects on growth, lowering of water potential also influences carbon utilization and nutrient translocation. When new woody resources were added to cord-systems in soil microcosms, colonisation had a significant carbon (energy) cost compared to controls, and phosphorous acquisition was reduced (Wells *et al.* 2001). Relatively minor fluctuations in soil moisture content are unlikely to prevent an increase in saprotrophic mycelial biomass and activity under elevated

temperature and CO_2. Less predictable extremes, such as prolonged periods of drought or precipitation, will have a more pronounced influence.

Climate change has the potential to alter the composition of saprotrophic cord-former communities by differentially influencing the growth and activity of individual mycelial systems, and altering the outcome of interspecific interactions. Combative mycelial interactions are major drivers of fungal community structure and development, both in organic substrata and in soil (Boddy 2000). When small mycelial systems of cord-forming basidiomycetes encounter each other in soil, a stronger combatant replaces a weaker one and becomes the main regulator of decomposition in a given area of forest floor (Dowson *et al*. 1988a, b). During interspecific mycelial interactions, enzyme production and nutrient loss from mycelia increase markedly (Wells and Boddy 2002; Šnajdr *et al*. 2011). Abiotic factors, such as temperature, water potential and gaseous regime, affect the rate of progression and outcome of interactions (Boddy *et al*. 1985; Griffith and Boddy 1991; Boddy 2000). For example, in agar culture, *H. fasciculare* does not replace *Phlebia radiata* at 20 ^{o}C, but it does at 25 ^{o}C. Differential sensitivity to temperature (Fig. 2.1) can either accelerate the progression of the dominant competitor through its opponent's mycelium, or reverse the outcome of the interaction by stimulating combative activity of the weaker competitor to the extent that it becomes dominant (Schoeman *et al*. 1996; Crowther *et al*. 2012). Competitive abilities of individual species tend to diminish towards the lower end of their water potential (Boddy 2000), and upper end of their CO_2 (Chapela *et al*. 1988), tolerance range. Interspecific variation in sensitivity of cord-forming basidiomycetes to climatic variables could, therefore, drive shifts in community structure and functioning. Species-specific extracellular enzyme production and fungal-mediated decay rates suggest that community composition will have a strong influence on decomposition.

2.3 Impacts of climate change on soil invertebrates

2.3.1 Meta-analysis

Data were collected from published studies reporting the effects of elevated temperature, CO_2 and precipitation, and drought on groups of soil micro- and meso-invertebrates containing mycophagous members: nematodes (phylum Nematoda), mites (subclass Acari), collembola (subclass Collembola) and enchytraeids (family Enchytraeidae). There are currently insufficient empirical data available on the

responses of mycophagous macro-invertebrate groups to climatic manipulation to make meta-analysis informative. Studies were identified by searching Web of Science databases, personal reference collections and literature cited therein. Measurements were all related to abundance (e.g. population density, number g^{-1} dry soil). Control and treatment means, errors and replication (*n*) were recorded for each measurement. In the source studies, elevated temperatures varied from 1.5-5 °C above summer ambient, elevated CO_2 from 200-350 ppm above ambient, and increased precipitation (where defined) from 10-40 % per month above ambient. There were sufficient data to analyse the responses of nematodes, Acari, collembola and enchytraeids to a range of climatic manipulations. Nematode feeding biology is well documented (e.g. Yeates *et al.* 1993) enabling responses of bacterial, fungal, plant and carnivorous feeding guilds to be considered separately within this taxon.

The natural log of the response ratio (lnR) was used as the effect size metric to reflect relative changes in soil invertebrate abundance:

$$\ln R = \ln(X_T/X_C),$$

where X_T and X_C are the mean abundance of the treatment and control groups, respectively. The logarithm linearises the metric so that deviations in the numerator are treated in the same way as deviations in the denominator; this normalises the distribution (Hedges *et al.* 1999). Positive values indicate an increase in the response variable with respect to the climatic factor. The mean, variance and bootstrapped 95 % confidence intervals of lnR were calculated in MetaWin 2.1 (Rosenberg *et al.* 2000) with *n* as the weighting function.

2.3.2 Responses to climate manipulations

Meta-analysis of data from published studies revealed that climate change impacts on soil invertebrate abundance vary according to climatic treatment, taxonomic group and, in the case of nematodes, feeding guild (Fig. 2.2). The lack of an effect of elevated temperature on collembola and mite abundance could appear counter-intuitive, until the close relationship between temperature and soil moisture is considered. Warming-induced moisture loss at the soil–litter interface, rather than a direct physiological response to temperature, is the most likely cause of this trend, due to the desiccation

sensitivity of soil invertebrates (Convey *et al.* 2002; Dollery *et al.* 2006; Day *et al.* 2009). Enchytraeids increased in abundance at elevated temperature (Fig. 2.2); they avoid adverse moisture conditions by migrating downwards within the soil profile (Maraldo *et al.* 2008). The influence of temperature on enchytraeid reproduction rate (Briones *et al.* 1997) often stimulates population growth deeper in the soil profile, or in naturally wet soils (e.g. peatlands; Briones *et al.* 2004; Carrera *et al.* 2009). Nematodes were not affected by temperature overall, but feeding guilds displayed differential responses; plant feeders decreased, whereas fungivores and carnivores increased in abundance (Fig. 2.2).

As moisture is a common limiting factor for soil invertebrate abundance and diversity (Briones *et al.* 1997; Lindberg *et al.* 2002), increased wetting and drying of the soil environment could potentially be one of the most important climate change factors in terms of direct effects on soil invertebrate communities. The moisture limitation imposed by drought reduced the abundance of collembola, mites, enchytraeids, and plant- and bacterial-feeding nematodes (Fig. 2.2). Enchytraeids and free-living nematodes are highly dependent on free water in soil for motility and survival (Briones *et al.* 1997; Kardol *et al.* 2010). Precipitation regimes are likely to have significant impacts on responses to other climatic factors, particularly elevated temperature. Increased precipitation alone only increased nematode abundance, but, in combination with elevated temperature, also increased the abundance of collembola and mites (Fig. 2.2). Warming accentuated the negative effect of drought on mites (Fig. 2.2).

Elevated CO_2 reduced mite (95 % CI just overlapping zero) and nematode (except plant-feeders) abundance (Fig. 2.2). The collembola, mites and enchytraeids that graze on fungi also contribute to litter transformation, providing a potential pathway for effects independent of the fungal food-chain. Decreased nitrogen and increased tannin concentrations in leaf litter grown under elevated CO_2 (Lindroth *et al.* 1995; King *et al.* 2001) are known to reduce micro-arthropod abundance (Loranger *et al.* 2004; Meehan *et al.* 2010). By reducing plant transpiration, elevated CO_2 could alleviate temperature-induced moisture loss from soil (Field *et al.* 1995), potentially explaining the positive interactive effect of warming and CO_2 on collembola and mites (Fig. 2.2).

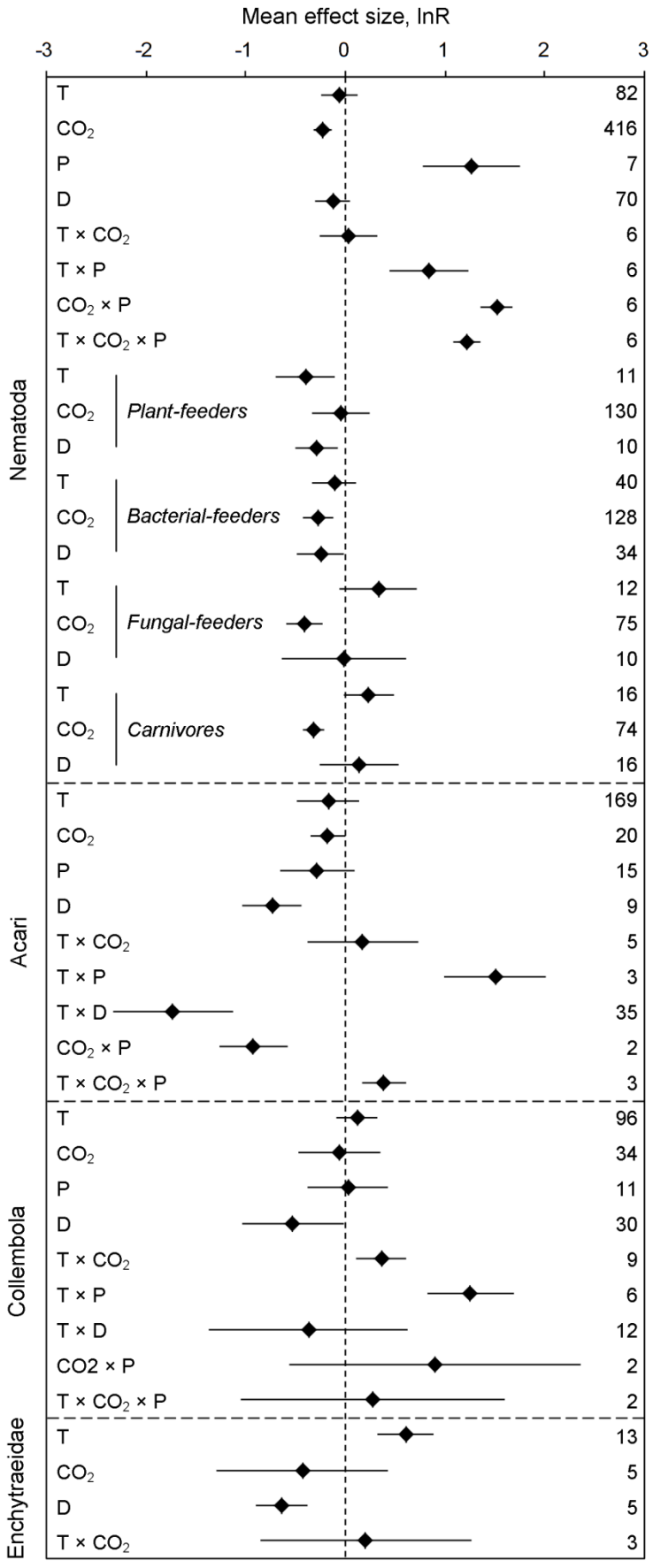


Fig. 2.2 Meta-analysis of published studies reporting climate change impacts on soil invertebrate populations. Effect sizes ($lnR = ln(X_T/X_C)$ ± bootstrapped 95 % confidence intervals, where X_T and X_C are mean abundance of the treatment and control groups, respectively) resulting from soil invertebrate abundance responses to elevated temperature (T), CO_2, and precipitation (P), and drought (D). Effects are significant ($P < 0.05$) where CIs do not overlap zero. Climatic factors (left) and the number of observations (right) are indicated for each effect size. Sources are provided in Appendix 1.

The role of soil invertebrates in decomposition is greatest where temperature and moisture constraints on biological activity are relaxed (Wall *et al.* 2008), increasing their abundance, as shown by the interactive positive effect of elevated temperature with CO_2, precipitation or both on all analysed taxa (Fig. 2.2). Regions becoming warmer and wetter due to climate change could experience accelerated decomposition rates as the abundance of soil invertebrates and their role in this process increase. It is less clear how indirect impacts of soil fauna on decomposition, mediated by interactions with the heterotrophic microbial community, will alter if their abundance and community composition change as predicted.

2.4 Implications for saprotrophic fungus–invertebrate interactions

Trophic interactions within the decomposer community, such as those between saprotrophic fungi and their invertebrate grazers, are potentially important determinants of ecosystem–atmosphere carbon feedbacks under climate change (Fig. 2.3). Abiotic conditions that stimulate saprotrophic fungal biomass production and activity are also likely to increase soil invertebrate abundance. As well as being influenced directly (temperature–moisture characteristics of soil) and indirectly (mediated by primary production) by climate change, these organisms are affected by interactions with each other (Fig. 2.3). Mycophagous soil invertebrates have the potential to drive, and respond to, changes in fungal community composition (Klironomos *et al.* 1992, 1997; Jones *et al.* 1998).

Temperate regions are predicted to get warmer and wetter due to climate change (IPCC 2013), potentially increasing basidiomycete mycelial biomass and invertebrate grazer abundance. Decomposition rates are determined, in part, by the balance between extra-resource mycelial growth (energetic exploitation of the resource, leading to the colonisation of new ones) and removal by grazing invertebrates. The direct stimulation of mycelial growth and activity due to climate change could be indirectly influenced by increased grazer abundance, with implications for the rate at which new resources are encountered and subsequently decomposed (Fig. 2.3). Grazing impacts on mycelial development and function are species-specific (Tordoff *et al.* 2008; Crowther *et al.* 2011b), reflecting feeding preferences of different invertebrates and differential susceptibility of fungi to grazing (Crowther *et al.* 2011a). Increased saprotophic fungal

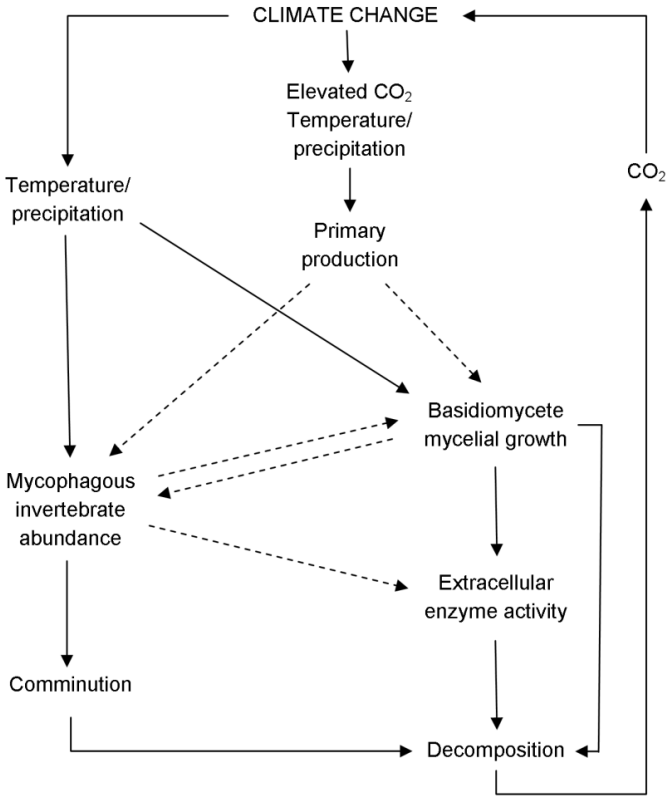


Fig. 2.3 Climate change effects on saprotrophic basidiomycetes and their soil invertebrate grazers and direct (closed arrows) and indirect (dashed arrows) feedback routes to CO_2 production, via decomposition. Direct effects include the influence of temperature and altered precipitation on the abundance and activity of these organisms; indirect effects are mediated by climate-driven changes in plant productivity, influencing soil physiochemical properties, and interactions between saprotrophic mycelia and invertebrate grazers.

biomass under elevated CO_2 has been correlated with increased collembola abundance (Klironomos *et al.* 1997), and counteracted by oribatid mite grazing (Allen *et al.* 2005). Further empirical study should investigate the extent to which soil invertebrate grazing is likely to mediate saprotrophic fungal responses to climate change, measuring decomposition rates as well as mycelial growth and grazer abundance.

Removal of mycelia by high intensity grazing has been shown to stimulate wood decomposition by *H. fasciculare* and *R. bicolor*, even though there is less extra-resource fungal biomass to support, as the fungus utilises more resource-derived energy to maintain explorative growth (Crowther *et al.* 2011b). Extracellular enzyme production (involved in carbon, nitrogen and phosphorus cycling) by mycelial systems is also influenced by grazing. Fungi display differential enzymatic responses to grazing; production by *R. bicolor* is reduced, and that by *H. fasciculare* and *P. velutina* increased, with grazer-specific impacts on different enzymes (Crowther *et al.* 2011d). Increased soil invertebrate abundance could stimulate both extracellular enzyme production and primary decomposition by basidiomycete mycelia, though this has not yet been investigated.

Mycophagous woodlice (e.g. *Oniscus asellus*) and millipedes (e.g. *Blaniulus guttulatus*) typically have stronger effects on mycelial growth (Crowther *et al.* 2011a; Crowther and A'Bear 2012) and extracellular enzyme production (Crowther *et al.* 2011d) than micro- and meso-invertebrates. Relatively few studies have investigated species and population level responses of these macro-invertebrates to climate change (e.g. Zimmer 2004; David and Gillon 2009). Warming is expected to influence population growth rates of some temperate woodlouse and millipede species positively, increasing their abundance and influence on decomposition (reviewed by David and Handa 2010). Selective grazing on specific fungi has the potential to influence community composition by differentially affecting the competitive abilities of interacting mycelia. By preferentially consuming the stronger competitor, collembola have been shown to increase the relative abundance of a weaker, but less palatable, species (Newell 1984a, b). Via the same mechanism, the stronger impact of woodlouse grazing can result in the complete replacement of the dominant fungus by an inferior competitor (Crowther *et al.* 2011a). In contrast, growth stimulation of the weaker species by low-intensity nematode grazing can also reverse the interaction outcome. There is potential for climate-driven changes in soil invertebrate abundance to influence the direct impact of abiotic factors on competitive interactions. Elevated temperature stimulated *R. bicolor* growth, enabling it to out-compete the formerly dominant *P. velutina* (Crowther *et al.* 2012). The concurrent increase in grazing pressure (increased collembola abundance) on *R. bicolor*, however, counteracted the impact of warming on the interaction outcome. Interactions between biotic and abiotic factors will therefore regulate decomposer community composition and function of saprotrophic cord-forming fungi under climate change.

2.5 Conclusions

Predicted climatic changes leading to a warmer and wetter environment are likely to stimulate the activity of saprotrophic cord-forming basidiomycetes and increase the abundance of mycophagous soil invertebrates. Although warming-induced increases in mycelial growth could, to some extent, be counteracted by grazing, there is potential for increased production and release of extracellular enzymes into the soil environment, and accelerated primary decomposition of colonised resources. To improve our mechanistic understanding of climate change impacts on decomposition, saprotrophic fungal activity needs to be partitioned from that of the general microbial biomass in

empirical investigations. The majority of studies have not done this, making functional implications of the overall trends difficult to identify. Future research should consider interactive effects of climate change factors on soil biota, particularly given the influence of elevated temperature on soil moisture properties. Understanding how biotic and abiotic factors interact to mediate saprotrophic fungal functioning is crucial in enhancing our predictive capabilities regarding primary decomposition and carbon feedback in a changing climate.

3 Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola

3.1 Introduction

The influence of climate on the biological mechanisms regulating carbon exchange between the biosphere and atmosphere has the potential to amplify or dampen the extent of regional and global environmental change through ecosystem–atmosphere feedback (Heimann and Reichstein 2008). The terrestrial carbon pool, comprising both pedologic and biotic pools, is estimated at approximately 2860 Pg (Lal 2008) and by releasing and absorbing greenhouse gases, these stores play a major role in such feedbacks (Woodward *et al.* 2009). The influence of climate change on soil carbon storage remains unclear (Bardgett *et al.* 2008; Bradford *et al.* 2008; Singh *et al.* 2010). The potential for warming to increase microbial decomposition of soil organic matter (SOM), stimulating CO_2 efflux (Cox *et al.* 2000), is a particularly pertinent issue. Soil respiration is generally thought to be more temperature-sensitive than primary production (Jenkinson *et al.* 1991), implying a positive soil–atmosphere carbon feedback due to warming (Cox *et al.* 2000; Allison *et al.* 2010).

While the role of temperature as the dominant regulator of soil carbon dynamics (Raich and Schlesinger 1992; Kirschbaum 1995; Trumbore *et al.* 1996) and related enzyme activity (Davidson and Janssens 2006) is well recognised, the potential feedback associated with the relationship between temperature and heterotrophic microbial respiration requires further investigation (Singh *et al.* 2010). This is complicated by uncertainty regarding the differential sensitivity of microbial groups to temperature change (Kandeler *et al.* 1998; Bardgett *et al.* 1999) and the complexity of interactions between microbes and other soil biota, such as invertebrate grazers (Wardle *et al.* 2004; Bardgett *et al.* 2008).

Fungi, in particular, exert a strong influence on spatial and temporal variation in SOM dynamics, and the temperature-sensitivity of decomposition (Yuste *et al.* 2011). In temperate woodland ecosystems, a globally significant terrestrial carbon store (292 Pg; Lal 2005), saprotrophic cord-forming basidiomycetes dominate primary decomposition (Boddy and Watkinson 1995; Hättenschwiler *et al.* 2005). The mycelia of these fungi grow out from colonised resources in search of new ones; dynamic networks are

formed that can cover many square metres at the soil–litter interface (Boddy 1993), and re-allocate nutrients across heterogeneous environments (Boddy 1999). The conservative nature of these networks confers a competitive advantage to fungi when nitrogen availability is limited by increased plant growth at elevated temperature, potentially increasing fungal dominance of the microbial community (Bardgett *et al.* 1999; Zhang *et al.* 2005). Long-term increases in temperature have resulted in increased activity of soil fungi, as indicated by fruit body production (Gange *et al.* 2007), with implications for decomposition and carbon feedback.

The role of soil invertebrates in decomposition is greatest in fungal-dominated systems (Wardle *et al.* 2004), due in part to the strong influence of nematodes (Dyer *et al.* 1992; Crowther *et al.* 2011a), collembola (Kampichler *et al.* 2004; Tordoff *et al.* 2006, 2008), oribatid mites (A'Bear *et al.* 2010), enchytraeids, millipedes and woodlice (Crowther *et al.* 2011b, c) on the development and function of mycelial networks. The low C:N ratio of fungal hyphae compared with organic matter (Swift and Boddy 1984; Boddy and Jones 2008) makes them an attractive source of nutrition for grazing invertebrates. Impacts vary from the stimulation of mycelial growth by micro- and meso-invertebrates (Hedlund *et al.* 1991; Kampichler *et al.* 2004; Bretherton *et al.* 2006) to the removal of whole systems by macro-invertebrates (Crowther and A'Bear 2012). Species-specific grazing preferences can reverse the outcome of competitive mycelial interactions, with implications for fungal community composition and the maintenance of fungal species diversity (Crowther *et al.* 2011a).

Soil invertebrate abundance and diversity are directly affected by temperature-moisture gradients (Briones *et al.* 1997). When elevated temperature causes moisture loss from the soil-litter interface, nematode (Bakonyi *et al.* 2007; Simmons *et al.* 2009), collembola (Convey *et al.* 2002; Day *et al.* 2009) and mite (Dollery *et al.* 2006) populations are often reduced. When moisture is not limiting, however, populations of these invertebrates often increase with elevated temperature (Day *et al.* 2009; Kardol *et al.* 2010). Changing abundance and community composition of soil invertebrate grazers will interact with the direct effect of elevated temperature on fungal-mediated decomposition (Chapter 2).

The study reported in this chapter investigates whether increased fungal growth and activity, or increased abundance of invertebrate grazers (potentially reducing fungal growth and activity), is the dominant factor in the control of primary decomposition rates under elevated temperature. The complex and opaque nature of belowground ecosystems can often make mechanistic understanding of observed trends difficult; a microcosm approach is, therefore, employed in an attempt to identify precise responses. Since basidiomycete mycelia ramify at the soil surface, below the litter layer, this experimental system is a good model of naturally occurring networks. *Folsomia candida* and *Protophorura armata* (Collembola) are used as 'model' invertebrate grazers to investigate the impact of elevated temperature on their interactions with five cord-forming basidiomycete fungi decaying beech (*Fagus sylvatica*) wood inocula. All species are common within temperate woodland soil (Boddy 1999, 2000; Hopkin 2007) and the impacts of these grazers on mycelial growth and development are known to be species-specific, with respect to both fungi and collembola (Tordoff *et al.* 2008). The study aims to determine: (1) the effects of elevated temperature on fungal growth and decay rate of colonised wood resources; and (2) the extent to which temperature-induced stimulation of fungal growth and decay is balanced by increased collembola abundance. Three specific hypotheses were tested: (1) elevated temperature will stimulate fungal growth and resource decay rates; (2) collembola abundance, and therefore grazing pressure, will be greater at elevated temperature; and (3) species-specific consequences for fungal growth will result from differential sensitivity to temperature and grazing.

3.2 Materials and methods

3.2.1 Experimental design

The effect of elevated temperature on the balance between decomposer basidiomycete mycelial growth and removal by grazing collembola was investigated in two species (one fungus and one collembola) compressed soil microcosms. Microcosms were incubated in darkness at ambient and elevated (ambient + 3 °C) temperature. Ambient temperature was 15 °C, based on late summer – autumn temperatures beneath the litter layer in UK temperate woodland (Boddy 1983b). A fully-factorial experimental design, with fungus- and collembola-only controls, was employed. All treatments were replicated four or five times.

3.2.2 Fungi

The cord-forming basidiomycete fungi *Coprinopsis picacea* (Bull.) Redhead, *Hypholoma fasciculare* (Huds.: Fr.) Kummer, *Megacollybia platyphylla* (Pers.: Fr.) Koutl & Pouzar, *Phallus impudicus* (L.) Pers. and *Resinicium bicolor* (Alb & Schwain.: Fr.) Parmasto were cultured on 2 % malt agar (MA). Freshly-felled beech (*Fagus sylvatica*) was cut into blocks (2 × 2 × 1 cm) and frozen (-18 °C) until required. Wood blocks were defrosted in de-ionised water (DH_2O) for 12 h before being heat-sealed within two layers of autoclave plastic and autoclaved (121 °C) three times at 24 h intervals. Sterile wood blocks were placed in 14 cm diameter Petri dishes containing MA, pre-colonised with the experimental fungi, and incubated at 16 ± 1 °C for 3 months.

3.2.3 Collembola

Folsomia candida and *Protophorura armata* (Cardiff University Culture Collection) cultures were maintained on a medium consisting of 95 % plaster of Paris (Minerva Dental, Cardiff, UK) and 5 % activated charcoal (Sigma, Poole, UK). Collembola were fed dried baker's yeast (*Saccharomyces*), and plaster was re-moistened with DH_2O, weekly. Cultures were stored in darkness at 20 ± 1 °C. Prior to experimental use, collembola were size-selected (200-400 μm body width) by allowing 'self-sorting' through a series of stacked sieves (Nickel-Electro Ltd, Weston-super-Mare, UK) for 5 min, and then deprived of food for 24 h on fresh plaster of Paris.

3.2.4 Microcosm preparation and inoculation

Topsoil collected from mixed deciduous woodland at Tintern (Wye Valley, UK, NGR 352800, 201800) was sieved through a 10 mm mesh on site and air-dried in the laboratory for 5 d. Dry soil was sieved through a 2 mm mesh and frozen for 24 h (to prevent population explosions of endogenous collembola) before mixing each kilogram with 340 ml DH_2O. Soil intended for inoculation with *C. picacea* was mixed with 0.15 M potassium hydroxide solution (instead of DH_2O), to reduce soil acidity from pH 4.5 to pH 6, which is more favourable for the growth of this species (Owen 1997). Microcosms consisted of 200 g of moist soil evenly compressed to a 5 mm depth within

lidded, clear plastic trays (24 × 24 cm, 2 cm deep; Nunc-Gibco, Paisley, UK). The water potential of compressed soil was -0.02 MPa.

Microcosms (other than the collembola-only controls) were inoculated centrally with a colonised wood block, from which adhering mycelium and agar were removed. Microcosms were weighed, and water loss was replaced by spraying with DH_2O to that weight every 7 d. When 50 % of replicates of each fungal species at each temperature had reached 8 cm diameter, 60 *F. candida* or 73 *P. armata* (based on mean body mass) were added to grazing treatments using an aspirator. As experimental protocol restricted the invertebrates to a two-dimensional habitat, collembola numbers were based on low estimates of field densities (783 m^{-2} and 953 m^{-2} for *F. candida* and *P. armata*, respectively; Petersen and Luxton 1982; Crowther and A'Bear 2012).

3.2.5 Image capture and analysis

Digital images of experimental systems were captured at a height of 39.5 cm, under normal laboratory lighting, using a Nikon Coolpix 7500 digital camera. Images were captured prior to collembola addition, every 4 d until 24 d, every 8 d until 40 d, then every 10 d until termination of the experiment (100 d). Captured images were analysed using ImageJ (National Institute of Health, USA). Radial extension was measured as the mean of eight lines, at 45° intervals, from the centre of the wood inoculum to the outer mycelial margin. Extension rates of all fungi were linear; radial length measurement ceased before mycelia (in the 18 °C treatment) reached microcosm edges (12 d for *R. bicolor*, 16 d for *C. picacea*, *H. fasciculare* and *P. impudicus*, and 24 d for *M. platyphylla*). To determine hyphal area, images were converted to greyscale before bare soil and wood inocula were removed electronically, and the threshold was manually adjusted to show only mycelium as a black silhouette. The pixel count was then converted to area. Mass fractal dimension (D_m) was determined using the same image to quantify the extent to which hyphae branch to fill space (Boddy and Donnelly 2008). A D_m value of 1 indicates an un-branched line, while $D_m = 2$ indicates a highly branched mycelia entirely covering the two-dimensional area.

3.2.6 Collembola populations

At the end of the experiment (100 d), collembola populations were extracted into 100 % ethanol by transferring microcosm soil to a Tullgren funnel for 48 h. Extraction

efficiency was determined by adding 100 individuals to non-experimental microcosms (n = 5) and harvesting immediately. Extraction efficiencies of 54.8 % and 64.4 % for *F. candida* and *P. armata*, respectively, were applied as correction factors to final population numbers.

3.2.7 Wood decay rates

Wood block decay rates (mg $cm^{-3}d^{-1}$) were estimated from change in density (oven dry weight/fresh volume; mg cm^{-3}). Initial densities were determined from a random subsample (n = 5) of colonised wood blocks taken from the same Petri dishes as those used in experimental microcosms.

3.2.8 Statistical analysis

Radial extension rates at ambient and elevated temperature were compared for each grazing treatment using one-way Analysis of Covariance (ANCOVA; Minitab, release 15), with time (d) as the covariate. Hyphal coverage and fractal dimension were analysed using two-way repeated measures ANOVA and Tukey's pairwise comparisons (SPSS, release 16). Collembola abundances were analysed using two-way ANOVA and Tukey's pairwise comparisons, with temperature and fungal treatment (including controls) as factors. Wood inocula decay rates were analysed using three-way ANOVA and Tukey's pairwise comparisons, with temperature, fungus and grazing treatment (including controls) as factors. ANOVA was conducted in R (version 2.12; R Development Core Team 2012). All data were normally distributed.

3.3 Results

3.3.1 Radial extension

Mycelial extension rate of un-grazed *R. bicolor* ($F_{1, 32} = 4.2$, $P = 0.049$) and *P. impudicus* ($F_{1, 41} = 10.5$, $P = 0.002$) increased at elevated temperature, whereas the increase in that of *C. picacea* and *H. fasciculare* was only indicative ($P < 0.1$) of a trend (Fig. 3.1). *Megacollybia platyphylla* extension was unaffected by elevated temperature (Fig. 3.1). Grazing by *F. candida* and *P. armata* prevented a temperature-induced increase in *R. bicolor* extension (Fig. 3.1; Fig. 3.2 a, b, c). The increase in *P. impudicus* extension at elevated temperature remained with *F. candida* grazing ($F_{1, 46} = 9.2$, $P = 0.004$), but was not significant ($P \geq 0.05$) when grazed by *P. armata* (Fig. 3.1; Fig. 3.2

d, e, f). Elevated temperature stimulated *H. fasciculare* extension with *F. candida* ($F_{1, 46}$ = 6.0, P = 0.019), but not *P. armata* grazing (Fig. 3.1).

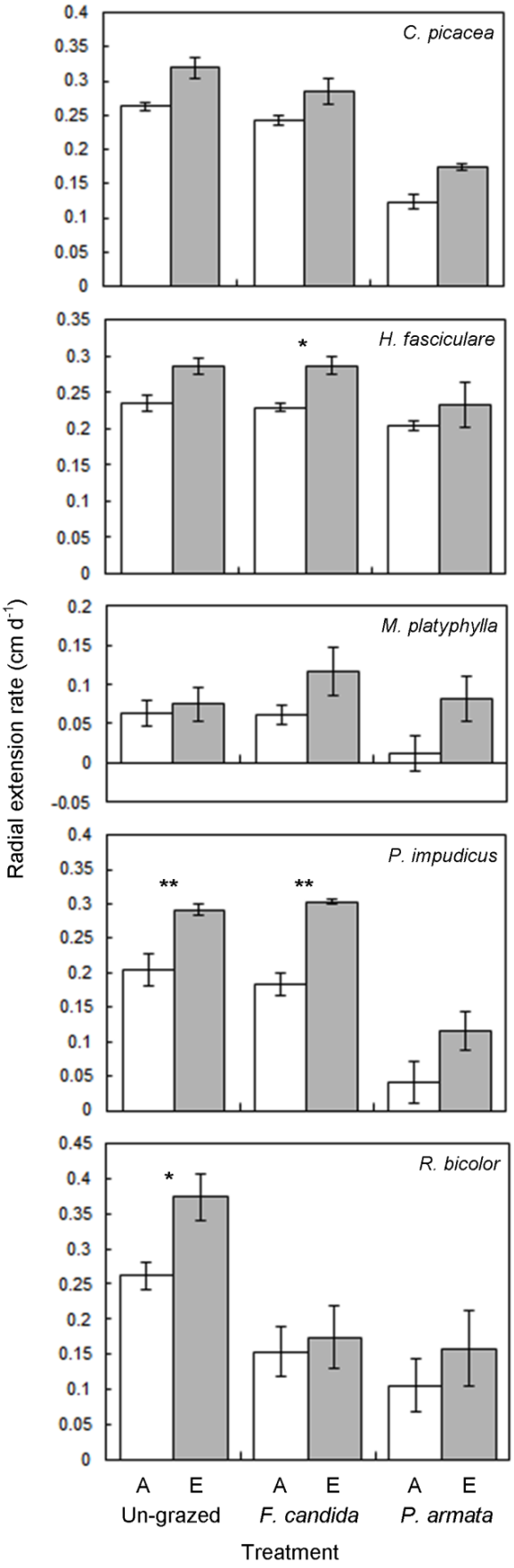


Fig. 3.1 Radial extension rate (± SEM) of *Coprinopsis picacea*, *Hypholoma fasciculare*, *Megacollybia platyphylla*, *Phallus impudicus* and *Resinicium bicolor* at ambient (A, unshaded bars) and elevated (E, shaded bars) temperature in un-grazed, *Folsomia candida* and *Protophorura armata* grazed systems. Within a grazing treatment, significant (* P < 0.05, ** P < 0.01) differences in extension between ambient and elevated temperature are indicated.

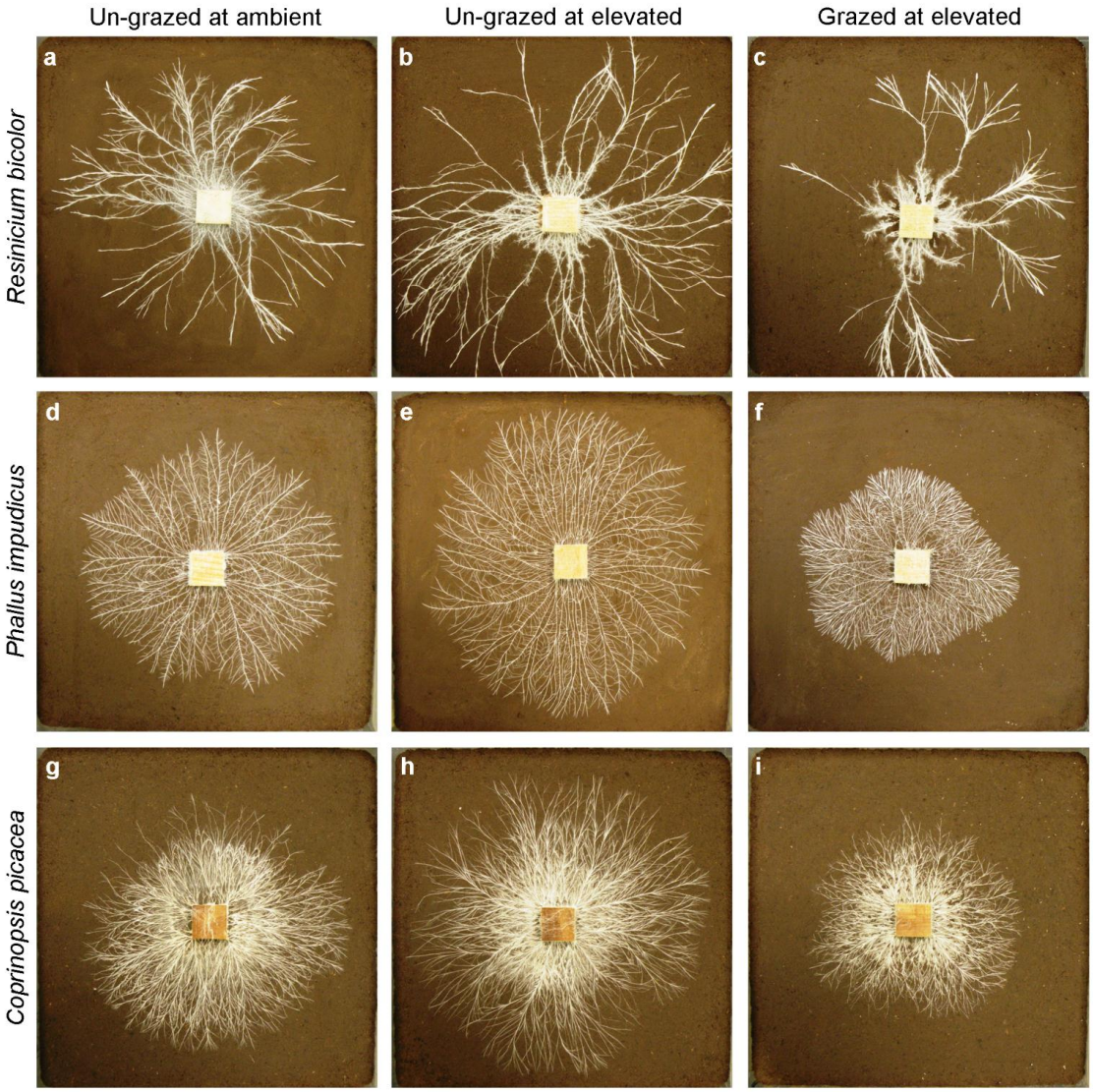


Fig. 3.2 Digital images of *Resinicium bicolor* (a-c), *Phallus impudicus* (d-f) and *Coprinopsis picacea* (g-i) growing from *Fagus sylvatica* wood blocks after 20 days; un-grazed at ambient (a, d, g) and elevated (b, e, h) temperature, and grazed at elevated temperature by *F. candida* (c) or *P. armata* (f, i). Wood block sides are 2 cm.

3.3.2 Hyphal coverage

Elevated temperature increased hyphal coverage of *C. picacea* (Fig. 3.2 g, h; Fig. 3.3; $F_{4, 103} = 9.1$, $P < 0.001$) and *M. platyphylla* (Fig. 3.3; $F_{2, 64} = 5.5$, $P < 0.001$). *Coprinopsis picacea* was also affected by grazing ($F_{8, 103} = 9.1$, $P < 0.001$); *P. armata* reduced hyphal coverage compared to un-grazed (Fig. 3.2 h, i; $P < 0.001$) and *F. candida*-grazed ($P = 0.008$) mycelia (Fig. 3.3). *Resinicium bicolor* coverage was not significantly ($P \geq 0.05$) affected by temperature, but reduced by both grazers (Fig. 3.3; $F_{6, 82} = 12.2$, $P < 0.001$). Hyphal coverage of *H. fasciculare* and *P. impudicus* was not

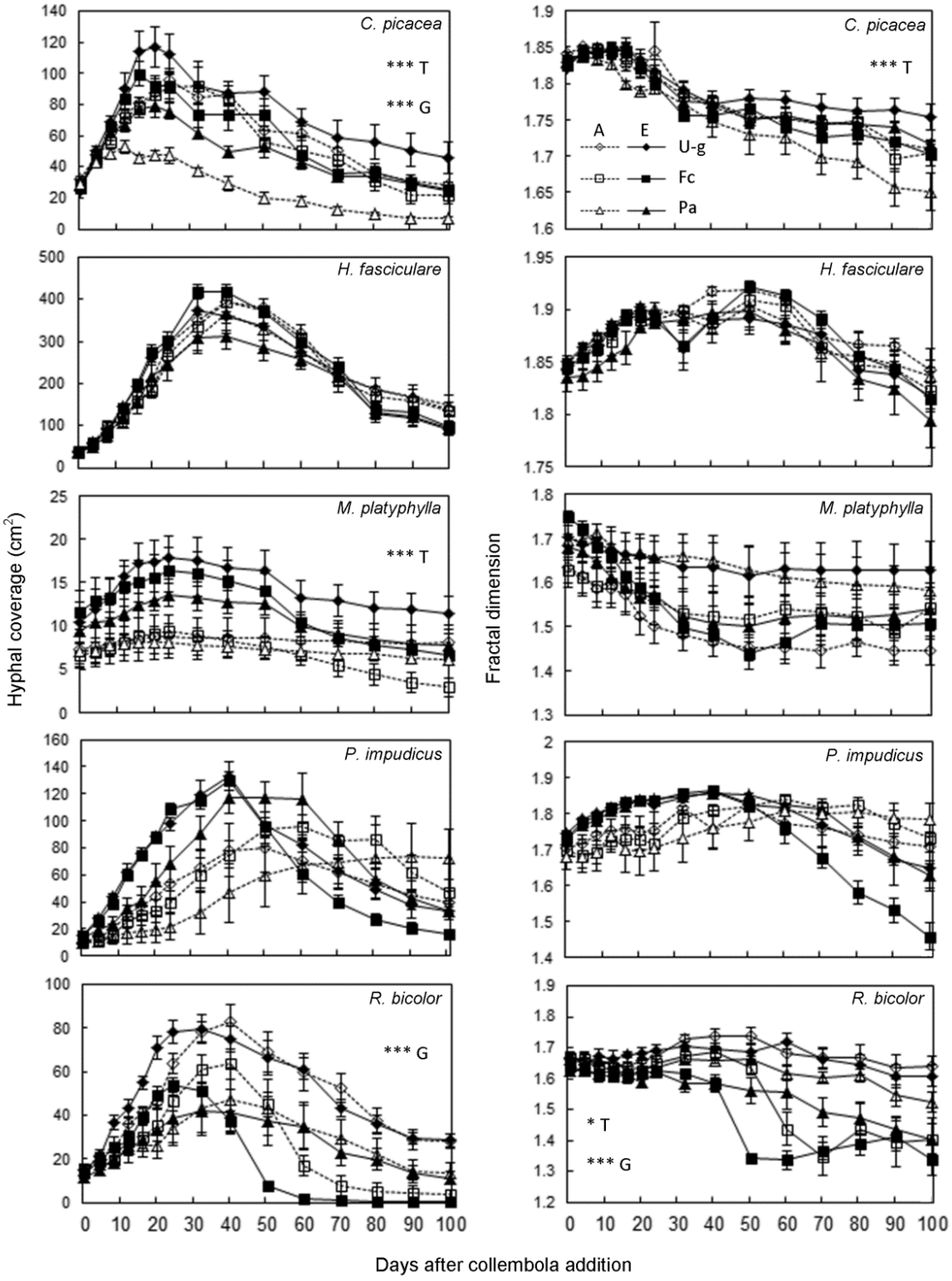


Fig. 3.3 Hyphal coverage (± SEM) and fractal dimension (± SEM) of *Coprinopsis picacea*, *Hypholoma fasciculare*, *Megacollybia platyphylla*, *Phallus impudicus* and *Resinicium bicolor* at ambient (A, dashed lines and open shapes) and elevated (E, solid lines and closed shapes) temperature in un-grazed (U-g, diamonds), *Folsomia candida*- (Fc, squares) and *Protophorura armata*- (Pa, triangles) grazed systems. Significant (* $P < 0.05$, *** $P < 0.001$) impacts of elevated temperature (T) and grazing (G) are indicated. Note that scales differ between Y axes.

significantly ($P \geq 0.05$) affected by temperature or grazing. The impact of grazing on hyphal coverage did not vary with temperature (grazer*temperature interaction; $P \geq 0.05$) in any combination.

3.3.3 Fractal dimension

Elevated temperature increased *C. picacea* ($F_{5, 120} = 5.2$, $P < 0.001$) and reduced *R. bicolor* ($F_{2, 57} = 3.5$, $P = 0.028$) fractal dimension (Fig. 3.3). Grazing also reduced that of *R. bicolor* ($F_{5, 57} = 11.2$, $P < 0.001$); the stronger impact of *F. candida* just missing significance ($P = 0.052$) compared with that of *P. armata* (Fig. 3.3). Fractal dimensions of *M. platyphylla*, *H. fasciculare* and *P. impudicus* were not affected by temperature or grazing. Impacts of grazing on fractal dimension did not vary with temperature (grazer*temperature interaction; $P \geq 0.05$) for any fungus.

3.3.4 Collembola populations

Final abundances of both collembola species were strongly affected by elevated temperature (*F. candida*: $F_{1, 54} = 15.9$, $P < 0.001$; *P. armata*: $F_{1, 55} = 8.9$, $P = 0.004$) and identity of the fungal resource (*F. candida*: $F_{6, 54} = 352.1$, $P < 0.001$; *P. armata*: $F_{6, 55} = 41.3$, $P < 0.001$), though direction and magnitude of change varied between fungus and invertebrate species (Table 3.1). Elevated temperature impacts on collembola were resource-specific (highly significant temperature*fungus interaction; *F. candida*: $F_{6, 54} = 18.4$, $P < 0.001$; *P. armata*: $F_{6, 55} = 4.9$, $P < 0.001$); *F. candida* and *P. armata* population size responded positively to elevated temperature when grazing on *R. bicolor* and *P. impudicus*, respectively ($P < 0.001$ for both; Table 3.1), and negatively, though not significantly ($P \geq 0.05$), with *H. fasciculare*.

3.3.5 Wood decay rates

Elevated temperature increased wood decay rate overall ($F_{1, 136} = 12.7$, $P < 0.001$). Decay rates differed between fungi ($F_{4, 136} = 108.2$, $P < 0.001$) as did the impact of elevated temperature (temperature*fungus interaction; $F_{4, 136} = 9.1$, $P < 0.001$). *Coprinopsis picacea*, *M. platyphylla* and *R. bicolor* decayed wood more rapidly at elevated temperature (Fig. 4; $P < 0.05$ in all cases), whereas decay by *H. fasciculare* and *P. impudicus* was stimulated at a significance level indicative of an effect ($P = 0.067$ and $P = 0.066$, respectively). In the case of *R. bicolor*, the overall effect arose from increased decay in grazed systems (Fig 3.4).

Table 3.1 Collembola populations (± SEM) at end of experiment (100 days).

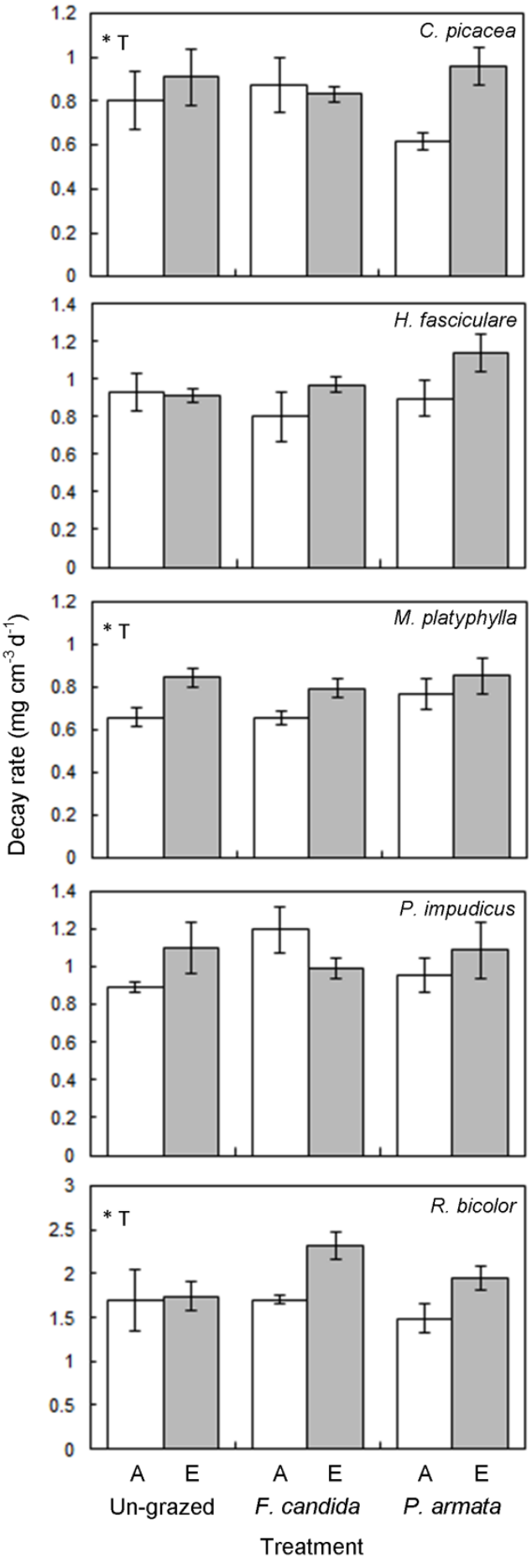
	F. candida			*P. armata*		
Temperature:	Ambient		Elevated	Ambient		Elevated
Control (pH 6)	11 (2.53)		21 (11.30)	18 (1.16)		29 (5.46)
C. picacea	25 (16.69)		18 (15.32)	137[a] (12.09)		150[b] (16.83)
Control (pH 4.5)	9 (2.37)		12 (3.67)	9 (3.72)		16 (2.75)
H. fasciculare	306 (82.01)		64 (26.87)	60 (7.52)		45 (16.51)
M. platyphylla	192 (26.22)		236 (28.04)	18 (3.64)		24 (4.19)
P. impudicus	382 (119.11)		650[a] (35.27)	99[a] (18.93)	***	201[b] (19.90)
R. bicolor	2304[a] (250.45)	***	3577[b] (131.80)	50 (9.96)		86[a] (25.38)

Numbers (±SEM) of *Folsomia candida* and *Protophorura armata* extracted from microcosms colonised by the fungi *Coprinopsis picacea*, *Hypholoma fasciculare*, *Megacollybia platyphylla*, *Phallus impudicus* and *Resinicium bicolor*. Significant (*** $P < 0.001$) differences in population size between ambient and elevated temperature are indicated for a given fungal resource. Different superscript letters indicate significant ($P < 0.05$) population differences on different fungi (i.e. within columns); populations with no letter are significantly ($P < 0.05$) different from those with a letter, but not significantly ($P \geq 0.05$) different from the relevant collembola-only control (Control - see methods: pH 4.5 for all fungi, except *C. picacea*, pH 6).

3.4 Discussion

Temperature elevated by 3 °C above ambient (15 °C) increased radial extension rate, hyphal coverage or hyphal branching (fractal dimension) of un-grazed mycelia of all fungi except *H. fasciculare*, potentially increasing the rate at which these basidiomycetes encounter and subsequently decompose new woody resources (supporting Hypothesis 1). The effect of elevated temperature on collembola population growth was highly dependent on the fungal resource, reflecting species-specific grazing preferences for different fungi (Newell 1984a, b; Crowther *et al.* 2011a). Although all of the fungi were utilised as a food source by the collembola, only certain fungus–grazer combinations promoted collembola population growth. Grazing pressure, therefore, increased at elevated temperature on specific fungi (partially supporting Hypothesis 2).

Fig. 3.4 Decay rate (±SEM) of *Fagus sylvatica* wood blocks colonised by *Coprinopsis picacea*, *Hypholoma fasciculare*, *Megacollybia platyphylla*, *Phallus impudicus* and *Resinicium bicolor* at ambient (A, unshaded bars) and elevated (E, shaded bars) temperature in un-grazed, *Folsomia candida* and *Protophorura armata* grazed systems. Significant (*P < 0.05) overall effects of temperature (T) are indicated. Note that scales differ between Y axes.



Differential sensitivity to elevated temperature and grazing resulted in species-specific consequences for fungal growth (supporting Hypothesis 3). The grazing pressure exerted by collembola was sufficient to counteract the stimulatory effect of elevated temperature on mycelial growth of some fungi. In contrast, grazing by *F. candida* amplified the warming-induced stimulation of *H. fasciculare* extension rate. The diversity of mycophagous soil invertebrates in the field, and their species-specific grazing preferences, could make grazing effects more even across fungal species, potentially preventing a temperature-induced increase in the rate at which decomposer fungi encounter and colonise new resources (Chapter 2). Even *H. fasciculare*, which produces sesquiterpenes that can deter mycophagy (Hynes *et al.* 2007), is selectively grazed by the diplopod *Blaniulus guttulatus*, despite being avoided by *F. candida* and the isopod *Oniscus asellus* (Crowther *et al.* 2011a, b).

The predicted increase in wood decay by un-grazed fungi at elevated temperature (Hypothesis 1) was only evident in a few cases. Energetic exploitation, and therefore decay, of a resource by a fungus is determined, in part, by the amount of extra-resource mycelial growth (Crowther *et al.* 2011b). The more extra-resource mycelium there is to support, the more rapid the decay of the resource. Although fungal growth often increased at elevated temperature, un-grazed mycelia of all fungi except *M. platyphylla* had reached their maximum size (limited by the dimensions of the experimental microcosms) well in advance of destructive harvest at 100 days. Had mycelia been able to continue radial growth for this entire period, temperature-induced growth stimulation would likely have been mirrored in increased resource decay by all species. Fungal decomposition of litter is promoted at elevated temperature, increasing CO_2 efflux from soil (Lin *et al.* 2001; Fierer *et al.* 2005). In long-term field studies, however, the initial increase in CO_2 loss due to respiration has been reported to diminish to control levels after a few years (Luo *et al.* 2001; Melillo *et al.* 2002; Bradford *et al.* 2008). This has been attributed to reduced carbon-use efficiency limiting microbial decomposer biomass (Allison *et al.* 2010), but whether this is a realistic microbial response to temperature elevation remains unclear.

Increased primary decomposition rates at elevated temperature are likely to occur, even if the abundance of specific grazers also increases. Although collembola grazing counteracted the increase in *R. bicolor* mycelial growth, it did not prevent more rapid

decomposition, at elevated temperature. Stimulation of *R. bicolor*- and *H. fasciculare*-mediated decomposition, by invertebrates that preferentially graze these fungi when given a choice of resource, has previously been reported (Crowther *et al.* 2011b). Extracellular enzyme production (involved in carbon, nitrogen and phosphorus cycling) by basidiomycete mycelia is also affected by grazing, impacts of which are species-specific (Crowther *et al.* 2011d). Macrofauna have the strongest effects, typically reducing enzyme production by *R. bicolor* (often by removing entire mycelial systems) and increasing that by *H. fasciculare* and *Phanerochaete velutina*. Temperature-driven increases in soil invertebrate abundance could stimulate both extracellular enzyme production and fungal-mediated wood decay (if more resource-derived nutrients are invested in mycelial growth) in response to increased grazing pressure, though this has not yet been investigated.

More empirical data are required for abiotic influences on soil biological interactions and processes to be incorporated into ecosystem–climate models, as currently they are too complex and species-specific to represent accurately (Cox *et al.* 2000; Wall *et al.* 2008; Wall *et al.* 2012). The results of the study reported in this chapter suggest that climate change impacts on decomposition are dependent on local composition of the decomposer community. Further investigation should incorporate interactive impacts of abiotic factors, particularly that between temperature and soil moisture, given the desiccation-sensitivity of most soil organisms. In the present study, soil moisture was carefully controlled, but in reality impacts of warming on soil moisture could influence the direct temperature impact (Briones *et al.* 1997). Understanding the interactive effects of biotic and abiotic factors on saprotrophic fungal distribution and function is key to our understanding of decomposition and carbon feedback in the context of climate change.

4 Bottom-up determination of soil collembola diversity and population dynamics in response to interactive climatic factors

4.1 Introduction

Soil invertebrates are key regulators of decomposition in terrestrial ecosystems (Wardle *et al.* 2004). The influence of soil mesofauna on decomposition is climate-dependent; regions becoming warmer and wetter are predicted to experience an increased contribution of soil fauna to carbon mineralisation (Wall *et al.* 2008). Limited understanding of climate change effects on soil invertebrate activity and population dynamics has, however, resulted in their omission from global models of organic mineralisation (e.g. Cox *et al.* 2000). The roles of biotic and abiotic factors in regulating soil invertebrate activity and population dynamics are difficult to disentangle, requiring more data from multi-factorial ecological studies.

Mycophagous collembola have the potential to be of considerable importance in regulating the influence of climate change on decomposition. This arises as a result of their ubiquitous distribution, large biomass and functional roles in the soil food-web (Petersen and Luxton 1982). As well as being important secondary decomposers, collembola exert a strong influence on ecosystem functioning by feeding on primary decomposers, particularly fungi. In temperate woodlands, basidiomycete fungi are dominant primary decomposers (Hättenschwiler *et al.* 2005). A major ecological grouping of these fungi, saprotrophic cord-formers, produce dynamic networks of foraging mycelium which are an attractive source of nutrition for soil invertebrates (Boddy and Jones 2008). At elevated temperature, direct mycelial grazing by collembola can prevent both the stimulation of saprotrophic fungal growth (Chapter 3) and the reversal of competitive interaction outcomes (Crowther *et al.* 2012). Such effects have major implications for fungal community composition and function. Temperature and soil moisture are key determinants of soil invertebrate population dynamics (Chapter 2; Blankinship *et al.* 2011), but the responses of mycophagous collembola abundance to these factors are strongly dependent on the fungal resource (Chapter 3).

Mechanistic understanding of abiotic influences on the interactions between these two major trophic and functional soil food-web components is based on microcosm

experiments at low levels of complexity (Chapter 2). The extent to which observations from these systems reflect the propagation of climate change impacts through a complex basidiomycete-dominated woodland decomposer system, in which alternative resources are available to grazers, has not been established. This study aims to determine the interactive influence of biotic (dominant component of the decomposer microbial community) and abiotic (temperature and soil moisture) factors on the population dynamics and species diversity of natural communities of mycophagous collembola. Three-factorial experimental designs are rare in soil ecology as the cost and time required often make them logistically unfeasible. To overcome this limitation and 'bridge the gap' between the simplicity of few-species microcosm experiments and natural conditions (Lawton 1995, 1996), soil mesocosms were extracted from temperate woodland and subjected a realistic biotic community to controlled climatic conditions. Three specific hypotheses were tested: (1) with respect to collembola abundance, elevated temperature will exert the greatest influence under wetting, and *vice versa*; (2) drying will reduce the abundance and community diversity of collembola; and (3) the identity of the dominant decomposer basidiomycete will influence collembola population responses to climatic factors.

4.2 Materials and methods

4.2.1 Experimental design

The interactive effects of elevated temperature, altered soil moisture (wetting or drying) and identity of the dominant basidiomycete fungus on collembola abundance and diversity was investigated in woodland soil mesocosms. Three sets of mesocosms (two inoculated with a different saprotrophic basidiomycete species and an un-inoculated control) were subjected to a fully-factorial combination of climate treatments: ambient moisture, wetting (ambient + 7.6 mm [10 % above monthly UK late summer – autumn precipitation]; Alexander and Jones 2001) or drying; and ambient or elevated (ambient + 3 °C) temperature. Ambient temperature was 15 °C, based on late summer – autumn temperatures beneath the litter layer in UK temperate woodland (Boddy 1983b). Six replicates were employed for all treatments.

4.2.2 Mesocosm preparation and harvesting

Soil turves (30 × 30 cm, 5 cm deep) were cut from mixed deciduous woodland (Tintern, Wye Valley, UK, NGR 352800, 201800) in October 2011, and immediately

transferred into lidded plastic boxes. Fresh litter was removed from the surface of the site prior to extraction; mesocosms consisted of humus and upper soil layers. The assemblage was realistic as extracted mesocosms contained the faunal and microbial communities occurring naturally at the site. The wetting and drying treatments were established during a 2-week acclimatisation period. Wetted mesocosms had 7.6 mm de-ionised water (DH_2O) added in 2 mm intervals every other day, using a watering can, and remained lidded to equilibrate. Drying was achieved by leaving boxes un-lidded for 12 h every other day with daily removal of condensation from lids. Mean soil moisture content of dried, ambient and wetted moisture treatments were 35 %, 39 % and 44 %, respectively (equivalent to -0.006, -0.004 and -0.002 MPa).

Beech (*Fagus sylvatica*) wood blocks (2 × 2 × 1 cm) were placed on 2 % malt agar colonised by the cord-forming basidiomycete fungi *Phanerochaete velutina* (DC.: Pers.) Parmasto or *Resinicium bicolor* (Alb and Schwain.: Fr.) Parmasto and incubated for 3 months at 16 ± 1 °C. Mesocosms (except un-inoculated controls) were inoculated centrally with a fungus-colonised wood block and incubated in darkness in Binder units (Binder GmbH, Tuttlingen, Germany). Mesocosms were aerated (lid removal) re-positioned at random within Binder units every other day. On termination of the experiment (100 d) collembola were extracted into 100 % ethanol by transferring three soil cores (5 cm diameter, taken at equal intervals across a diagonal transect) into a Tullgren funnel for 48 h. Collembola were identified using Hopkin (2007).

4.2.3 Statistical analyses

All statistical analyses were conducted in R (version 2.12; R Development Core Team, 2012). Collembola abundance and diversity (Simpson's reciprocal $D = 1/\sum_{i=1}^{n} P_i^2$, where P_i is the proportion of individuals in the i^{th} species and n is the total number of species) were analysed using three-way ANOVA and Tukey's pairwise comparisons, with fungus, temperature and moisture treatment as factors. Abundance data were log-transformed to achieve normality. Differences in collembola community composition across treatments were visualised through non-metric multidimensional scaling (NMDS), using the metaMDS function within the vegan package. This method of ordination reflects a matrix of dissimilarity calculated for all replicate points; the most similar replicate points are plotted closest together, and least similar furthest apart. The ordination ran for 1000 iterations, with a stress score of 0.081 for the final solution. A

stress score < 0.1 indicates that the ordinatrion can be very reliably visualised and interpreted in two dimensions. Treatment effects on NMDS groupings were assessed using permutational multivariate ANOVA (PERMANOVA) using the adonis function in vegan, based on 999 permutations. An overall (three-way) PERMANOVA was first used to investigate treatment effects on community composition, before pair-wise tests were used to assess differences between levels for significant treatment factors. Distance matrices for NMDS and PERMANOVA were constructed using the Bray-Curtis dissimilarity index as this metric can cope with zero-skewed community composition data (Clarke and Warwick 2001).

4.3 Results and discussion

Five species of common UK temperate woodland collembola were present in all treatments, the most abundant of which was *Friesea mirabilis* (Table 4.1). Abundance was strongly influenced by temperature ($F_{1, 90} = 9.1$, $P = 0.003$) and moisture ($F_{2, 90} = 5.9$, $P = 0.004$), being greatest under warming and wetting combined (Fig. 4.1; temperature*moisture: $F_{2, 90} = 3.7$, $P = 0.028$). Warming had the greatest effect on collembola abundance under wetting ($P = 0.006$), and *vice versa* ($P = 0.004$), supporting Hypothesis 1. The responses of individual species generally reflected that of the whole community, with the abundance of most being reduced by drying or increased by wetting (Table 4.1).

Soil moisture had a strong influence on community diversity ($F_{2, 90} = 35.3$, $P < 0.001$), which was reduced under drier conditions ($P < 0.001$), producing communities that differed significantly ($P < 0.05$) in composition from other moisture treatments (Fig. 4.2; Fig 4.3; $F_{1, 96} = 4.9$, $P < 0.001$). This supports Hypothesis 2 and was particularly evident in un-inoculated mesocosms, where *F. mirabilis* became even more dominant within the community (Fig. 4.2; temperature*moisture*fungus: $F_{4, 90} = 8.5$, $P < 0.001$). Within fungal treatments, collembola community diversity was consistently greater under wetting than drying, with those maintained at ambient moisture not being significantly different from either ($P \geq 0.05$; Table 4.1; Fig. 4.2).

The most striking result of this study is the highly significant influence of fungal treatment on collembola abundance (un-inoculated control > *P. velutina* > *R. bicolor*; $F_{2, 90} = 11.9$, $P < 0.001$) and the resulting bottom-up determination of population

Table 4.1 Final collembola populations [g^{-1} dry soil (± SEM)] and species diversity.

		Climatic treatment					
Fungus	Collembola	C	T	W	D	T W	T D
Un-inoculated	*F. candida*	0.260^{ab} (0.035)	0.226^{ab} (0.062)	0.128^{a} (0.042)	0.029^{a} (0.018)	0.515^{b} (0.140)	0.010^{a} (0.004)
	F. fimataria	0.189^{ab} (0.061)	0.195^{ab} (0.057)	0.143^{a} (0.034)	0.020^{a} (0.010)	0.401^{b} (0.109)	0.006^{a} (0.003)
	F. mirabilis	0.905^{a} (0.114)	0.645^{a} (0.066)	0.698^{a} (0.039)	0.545^{a} (0.168)	2.426^{b} (1.042)	0.300^{a} (0.175)
	I. virdis	0.323^{ab} (0.056)	0.209^{ab} (0.044)	0.323^{ab} (0.056)	0.006^{a} (0.003)	0.492^{b} (0.273)	0.004^{a} (0.002)
	P. armata	0.989^{abc} (0.272)	0.353^{a} (0.076)	0.431^{ab} (0.079)	0.073^{ab} (0.019)	1.807^{c} (0.727)	0.094^{b} (0.055)
	Total	2.665^{ab} (0.397)	1.628^{a} (0.222)	1.632^{ac} (0.129)	0.673^{ac} (0.196)	5.640^{b} (2.181)	0.414^{c} (0.229)
	Diversity	3.446^{a} (0.222)	3.659^{a} (0.265)	3.255^{a} (0.271)	1.527^{b} (0.120)	3.239^{a} (0.130)	1.842^{b} (0.119)
P. velutina	*F. candida*	0.019 (0.004)	0.083 (0.050)	0.102 (0.032)	0.021 (0.013)	0.113 (0.035)	0.097 (0.030)
	F. fimataria	0.042 (0.009)	0.110 (0.059)	0.062 (0.018)	0.022 (0.009)	0.062 (0.018)	0.063 (0.031)
	F. mirabilis	0.152 (0.047)	0.664 (0.386)	0.314 (0.064)	0.293 (0.110)	0.703 (0.234)	0.656 (0.126)
	I. virdis	0.028 (0.012)	0.062 (0.035)	0.081 (0.027)	0.029 (0.020)	0.045 (0.022)	0.065 (0.042)
	P. armata	0.085 (0.034)	0.162 (0.059)	0.243 (0.062)	0.088 (0.049)	0.476 (0.190)	0.281 (0.065)
	Total	0.327 (0.092)	1.080 (0.568)	0.802 (0.188)	0.453 (0.197)	1.399 (0.444)	1.163 (0.237)
	Diversity	2.943^{ab} (0.239)	2.536^{ab} (0.251)	3.469^{a} (0.178)	1.827^{b} (0.184)	2.516^{ab} (0.251)	2.457^{ab} (0.067)
R. bicolor	*F. candida*	0.051 (0.017)	0.100 (0.032)	0.093 (0.047)	0.031 (0.011)	0.345 (0.195)	0.037 (0.015)
	F. fimataria	0.028 (0.013)	0.034 (0.013)	0.007 (0.004)	0.026 (0.009)	0.187 (0.131)	0.021 (0.006)
	F. mirabilis	0.357 (0.220)	0.286 (0.089)	0.095 (0.057)	0.164 (0.080)	0.696 (0.440)	0.707 (0.260)
	I. virdis	0.033 (0.024)	0.033 (0.008)	0.037 (0.035)	0.022 (0.011)	0.201 (0.115)	0.019 (0.008)
	P. armata	0.145 (0.086)	0.103 (0.039)	0.042 (0.018)	0.083 (0.039)	0.452 (0.291)	0.230 (0.135)
	Total	0.615 (0.349)	0.556 (0.144)	0.273 (0.148)	0.326 (0.145)	1.881 (1.169)	1.083 (0.395)
	Diversity	2.458^{ab} (0.334)	2.847^{ab} (0.193)	2.410^{a} (0.325)	2.842^{ab} (0.240)	3.754^{b} (0.226)	2.152^{a} (0.230)

Abundance [number g^{-1} dry soil (±SEM)] of *Folsomia candida*, *Folsomia fimentaria*, *Friesea mirabilis*, *Isotoma virdis* and *Protophorura armata*, and species diversity (Simpson's D; see *Section 4.2.3*) in un-inoculated, *Phanerochaete velutina*- and *Resinicium bicolor*-inoculated mesocosms. Different superscript letters indicate significant ($P < 0.05$) differences across different climatic treatments (i.e. within rows; three-way ANOVA and Tukey's pairwise comparisons). Climatic factors are: control, C; elevated temperature, T; wetting, W; drying, D.

responses to warming (temperature*fungus: $F_{2, 90} = 9.5$, $P < 0.001$) and moisture (moisture*fungus: $F_{4, 90} = 4.8$, $P = 0.002$). Although collembola abundance increased with warming in *P. velutina*- ($P = 0.03$) and *R. bicolor*- ($P = 0.002$) inoculated mesocosms, significant ($P < 0.05$) pairwise differences between individual climate treatments were only evident in un-inoculated soil (Fig. 4.1; temperature*moisture* fungus: $F_{2, 90} = 2.6$, $P = 0.039$). This pattern was evident for individual species, as well as the whole community (Table 4.1). Fungal and moisture treatments interacted to determine collembola community composition ($F_{1, 96} = 3.6$, $P < 0.001$). This is reflected in their separation along different axes of the NMDS ordination (Fig. 4.3) and the constraints imposed independently by drying and inoculation with *P. velutina* or *R. bicolor* on collembola abundance and species diversity.

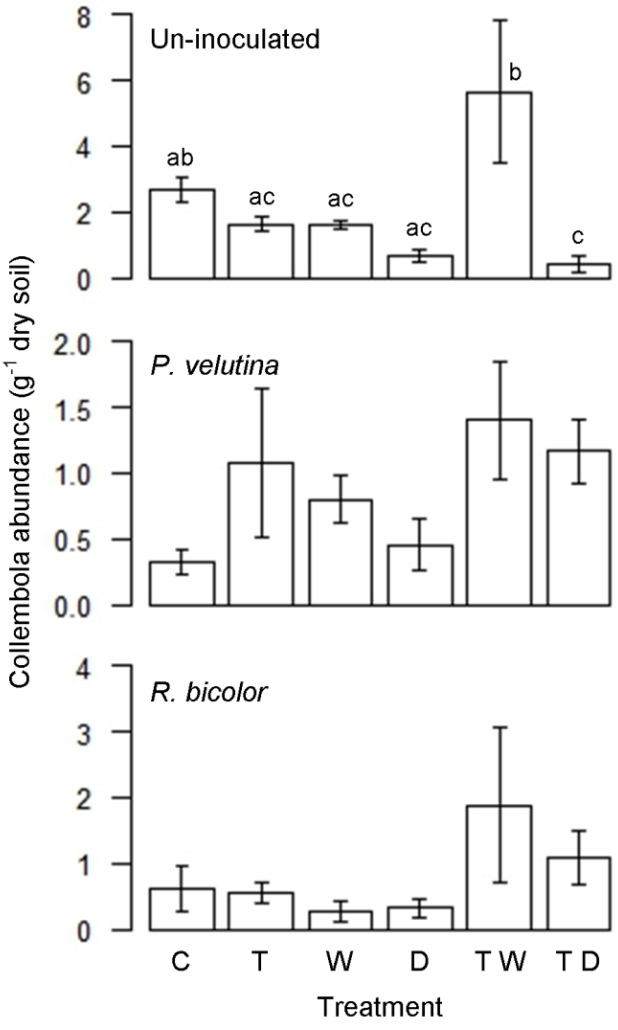


Fig. 4.1 Collembola abundance [number g^{-1} dry soil (±SEM)] in un-inoculated, *Phanerochaete velutina*- and *Resinicium bicolor*-inoculated mesocosms, under different climatic treatments. Different superscript letters indicate significant ($P < 0.05$) differences between climate treatments. Climatic factors are: control, C; elevated temperature, T; wetting, W; drying, D. Note that scales differ between Y axes.

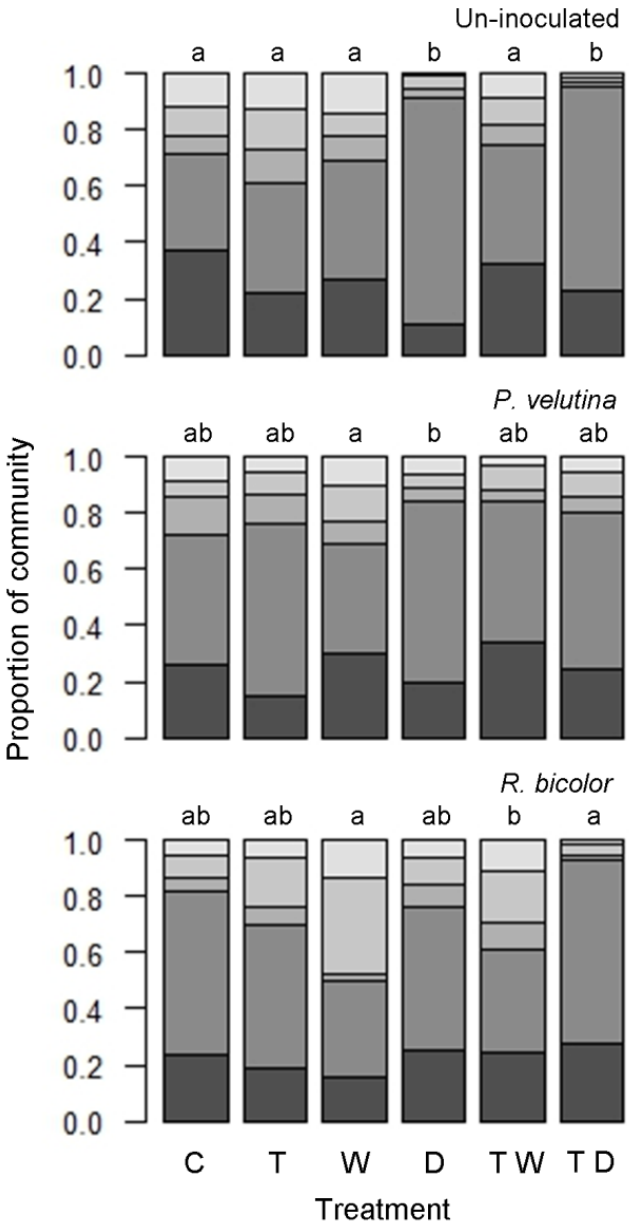


Fig. 4.2 Collembola community composition in un-inoculated, *Phanerochaete velutina*- and *Resinicium bicolor*-inoculated mesocosms, under different climatic treatments. Within bars, from top to bottom (light to dark shading), species are: *Folsomia candida*, *Folsomia fimentaria*, *Friesea mirabilis*, *Isotoma virdis* and *Protophorura armata*. Different letters indicate significant ($P < 0.05$) differences in species diversity (Simpson's reciprocal D; see *Section 4.2.3*) within fungal treatments. Climatic factors are: control, C; elevated temperature, T; wetting, W; drying, D.

The identity of the dominant decomposer basidiomycete fungus mediated collembola population responses to climate change, supporting Hypothesis 3. Collembola restricted to a single basidiomycete mycelial resource (in soil microcosms) have previously been differentially stimulated by warming, reflecting species-specific grazing preferences for different fungi (Chapter 3). *Resinicium bicolor* has been shown to be more palatable to a range of soil invertebrates than several other saprotrophic basidiomycetes (Crowther *et al.* 2011a), but the present study reports the lowest collembola abundance in the presence of this fungus. The mechanistic reasoning behind this is likely to reflect the palatability of basidiomycete mycelia relative to other soil fungi. The cords and hyphae of these fungi, although nutritious, may be difficult for collembola to exploit as a food

source, being thick and sclerotised. Cord-forming basidiomycetes are very competitive; their presence in woodland soil reduces the biomass and diversity of smaller, more easily ingested, soil fungi (Crowther *et al.* 2013), which appears to have limited the size of collembola populations.

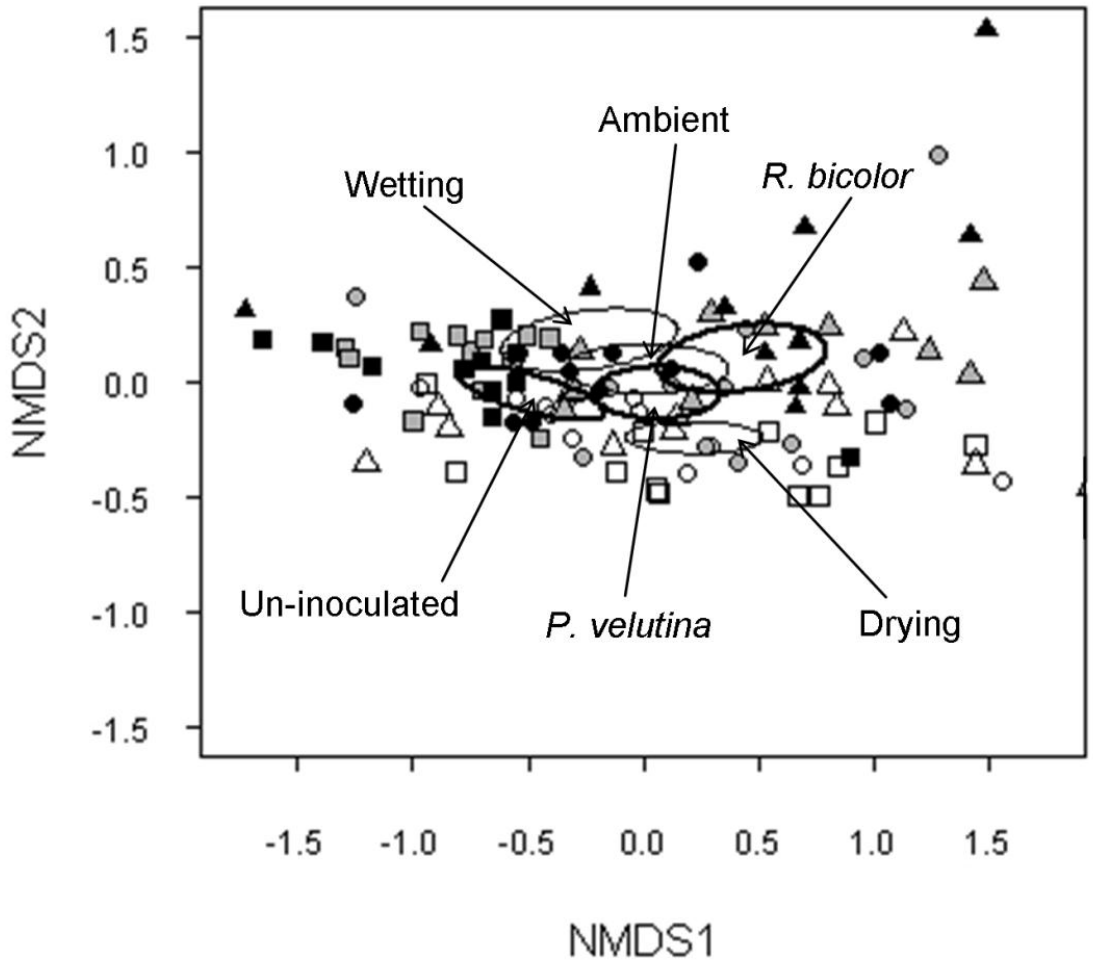


Fig. 4.3 Non-metric multidimensional scaling (NMDS) plot of moisture (thin-lined ellipses) and fungal (thick-lined ellipses) treatment effects on collembola community composition. Moisture treatments are: ambient (shaded shapes), drying (open shapes) and wetting (filled shapes); fungal treatments are: un-inoculated (squares), *Phanerochaete velutina*- (circles) and *Resinicium bicolor*- (triangles) inoculated. Ellipses indicate 95 % confidence intervals fitted into the spatial ordination. Non-overlapping ellipses of the same thickness indicate significant (PERMANOVA: $P < 0.05$) differences in collembola community composition between treatments.

Warming stimulates the growth and activity of decomposer fungi, but it has previously been hypothesised that this could be prevented by a concurrent increase in mycpohagous collembola abundance (Chapter 2; Chapter 3). The study reported in this chapter shows how, in a realistic woodland soil decomposer community, the influence of experimental climatic manipulation on soil collembola population dynamics and diversity is regulated by bottom-up mechanisms. The communities produced by the interaction between biotic and abiotic factors could exert differential influences on ecosystem processes. In low diversity collembola communities, composition, rather than species number or diversity, is the most important determinant of contributions to decomposition and nutrient mineralisation (Cragg and Bardgett 2001; Eisenhauer *et al.* 2011). Whilst mesocosm experiments can provide deeper insights into otherwise inaccessible and complex ecological interactions, caution must be exercised in extrapolation to the natural situation in which the complexity of abiotic changes and biotic variation are far greater. The reductionist approach employed in the present study has revealed a potential mechanism requiring further evaluation under natural conditions.

5 Interactive effects of temperature and soil moisture on fungal-mediated decomposition and extracellular enzyme activity

5.1 Introduction

Climate change has the potential to influence carbon exchange between the biosphere and the atmosphere. Soil respiration is thought to be more temperature-sensitive than primary production (Jenkinson *et al.* 1991); this implies that warming may lead to a net release of greenhouse gas from the terrestrial carbon pool (estimated at 2860 Pg; Lal 2008). The influence of climate change on soil carbon storage, however, remains unclear (Bardgett *et al.* 2008; Singh *et al.* 2010), due mainly to poor resolution of the responses of different microbial groups to abiotic factors (Chapter 2; Kandeler *et al.* 1998; Bardgett *et al.* 1999). Elevated temperature and changing precipitation patterns (increasing incidence of prolonged precipitation or drought), through their direct physiological influences on soil organisms (Chapter 2; Blankinship *et al.* 2011), will be the most important determinants of soil biotic activity under climate change scenarios.

Heterotrophic microbes, in producing extracellular enzymes capable of breaking down the most recalcitrant components of plant material, such as lignin and cellulose, regulate the rate-limiting step of soil organic matter (SOM) decomposition (German *et al.* 2012). Slight temperature elevation increases physiological and metabolic processes rates in microbes, including enzyme production. The temperature sensitivity of soil extracellular enzymes (Koch *et al.* 2007; Sinsabaugh *et al.* 2008; German *et al.* 2012; Stone *et al.* 2012) implies that stimulation of respiratory CO_2 efflux from soil is likely to occur with warming, up to a maximum typically much higher than ambient conditions (Davidson and Janssens 2006). Changing precipitation patterns (increasing frequency of prolonged periods of precipitation and drought) will alter soil moisture properties; as well as the enzyme producers themselves (Chapter 2), this will also affect substrate diffusion and metabolism by extracellular enzymes. Prolonged soil wetting or drying, therefore, has considerable potential to influence decomposition processes and modify the effect of elevated temperature.

Fungi exert a particularly strong influence on SOM dynamics, and on the sensitivity of decomposition to warming and altered patterns of precipitation (Yuste *et al.* 2011). While both temperature and soil moisture are known to influence microbial community

composition, in general (Zhang *et al.* 2005; Castro *et al.* 2010), uncertainty remains regarding the differential responses of fungal functional groups. Saprotrophic basidiomycetes, as important producers of extracellular enzymes regulating the breakdown of complex lignocellulose substrata, are major agents of decomposition in temperate woodlands (Hättenschwiler *et al.* 2005). Decomposer fungi have rarely been partitioned from the general fungal, or even microbial, biomass in climate manipulation studies, making functional implications difficult to identify. An important ecological grouping of saprotrophic fungi, the cord-forming basidiomycetes, form dynamic networks of mycelium which ramify at the soil–litter interface, linking lignocellulose resources (e.g. wood) and releasing extracellular enzymes into the soil (Boddy 1993, 1999). Mycelial growth and decomposition of colonised wood resources are stimulated by temperature elevation representing climate change predictions for temperate regions (Chapter 3). Moisture also has the potential to influence the response of fungal growth and functioning to warming; mycelial development can be inhibited if soil is too wet or too dry (Donnelly and Boddy 1997). Species-specific sensitivity of fungal growth and activity to temperature and soil moisture indicate that functional responses to abiotic factors could differ depending on the identity of the locally dominant decomposer basidiomycetes (Chapter 2). The fungal-mediated influence of abiotic conditions on the soil decomposer system represents a significant regulatory component of the balance of carbon uptake and release from temperate forest soil (Bardgett *et al.* 2008; Singh *et al.* 2010).

Using woodland soil mesocosms, the study reported in this chapter investigates the influence of temperature and soil moisture on basidiomycete-dominated microbial community functioning. Grazing by mycophagous collembola, when used as model grazers in two-species (fungus and grazing collembola) interactions in soil microcosms, can, in some cases, counteract the warming-induced stimulation of fungal colony expansion (Chapter 3) and influence mycelial enzyme production (Crowther *et al.* 2011d). Recent evidence from mesocosm experiments has, however, revealed that, in a realistic woodland decomposer community, competitive suppression of soil microfungi by cord-forming basidiomycetes limits the size of natural collembola populations (Chapter 4; Crowther *et al*. 2013). This reflects a reduction in the availability of fine microfungal hyphae, which are more palatable to collembola than thick cords (Crowther *et al.* 2013). Bottom-up limitation also restricted collembola population

responses to experimental climate change, indicating they are unlikely to influence the biomass and function of cord-forming basidiomycetes in this system (Chapter 4). The direct and interactive influences of temperature and moisture are, therefore, expected to be the main factors regulating the functioning of microbial communities dominated by these decomposer macrofungi.

This study aims to determine the interactive effects of elevated temperature and altered moisture (wetting or drying) on fungal biomass, wood decomposition and the potential activities (i.e. when substrate availability is not limiting) of soil extracellular enzymes involved in the decomposition of organic matter. Woodland soil mesocosms were employed (Chapter 4) to test three specific hypotheses: (1) warming will increase fungal biomass, enzyme activity and decomposition; (2) fungal biomass and enzyme activity will be increased by irrigation and reduced by drying; and (3) moisture limitation imposed by drying will prevent the warming-induced stimulation of fungal biomass, enzyme activity and decomposition. Fungal-mediated decomposition of colonised wood can be measured directly whereas, in soil, extracellular enzyme activity is an indicator of microbial community function and the potential for decomposition (Sinsabaugh *et al.* 2008; Henry 2012). The activities of hydrolytic (β-glucosidase, cellobiohydrolase, β-xylosidase, *N*-acetyl-glucosaminidase, acid phosphatase and leucine aminopeptidase) and oxidative (peroxidase and phenoloxidase) enzymes were determined to provide an insight into the decay of organic substrates of differing rates of turnover. Hydrolytic enzymes regulate the decay of organic substrates with relatively rapid turnover times (e.g. carbohydrates, chitin) and oxidative enzymes break down substrates with relatively longer residence times in soil (e.g. lignin).

5.2 Materials and methods

5.2.1 Mesocosm preparation and harvesting

The design of the experiment and methods for mesocosm preparation and treatment establishment are described in *Sections 4.2.1* and *4.2.2*, respectively. This achieved three distinct moisture contents (Table 5.1). After 100 d, 30 g soil samples (comprising four subsamples taken from half way between the centre and each mesocosm corner) were extracted, sieved through a 2 mm mesh and frozen (-18 °C) for later analysis of ergosterol content and enzyme activities.

Table 5.1 Organic C, total N, C:N ratio and soil moisture content (± SEM) of mesocosms.

Treatment	Organic C (µg g^{-1} soil)		Total N (µg g^{-1} soil)		C:N		Soil moisture (% oven dry weight)	
C	93.44	(6.96)	6.03	(0.38)	15.36	(0.28)	38.5[a]	(1.3)
T	94.53	(6.41)	6.11	(0.36)	15.41	(0.22)	40.8[a]	(1.5)
W	101.83	(6.24)	6.50	(0.35)	15.59	(0.23)	47.7[b]	(1.1)
D	95.16	(6.19)	6.00	(0.34)	15.75	(0.21)	33.2[c]	(1.6)
T W	95.41	(5.32)	6.11	(0.27)	15.53	(0.28)	46.9[b]	(1.5)
T D	105.63	(8.60)	6.28	(0.39)	15.74	(0.25)	34.1[c]	(1.9)

Different superscript letters indicate significant ($P < 0.05$) differences between climate treatments. Climatic factors are: control, C; elevated temperature, T; wetting, W; drying, D.

Soil organic carbon and total nitrogen were determined by dry combustion in a LECO 2000 CNS elemental analyser (Table 5.1). Wood block decay rates (mg $cm^{-3}d^{-1}$) were estimated from change in density (oven dry weight/fresh volume; mg cm^{-3}). Initial wood block densities were determined from a random subsample (n = 8) of colonised wood blocks from the same Petri dishes as those added to mesocosms.

5.2.2 Ergosterol

Ergosterol is commonly assayed as an indicator of fungal biomass as it is the dominant sterol of many fungi, including ascomycetes and basidiomycetes, and does not occur in plants. Soil ergosterol content was determined following the method of Djajakirana *et al.* (1996). Briefly, 0.5 g (soils inoculated with a fungus-colonised wood block) or 1 g (un-inoculated soils) of soil was dispersed in 25 ml of ethanol on a horizontal shaker for 30 min and centrifuged for 30 min at 34.9 g. A 10 ml aliquot was evaporated to dryness using a rotary vacuum concentrator (Martin Christ GmbH, Osterode, Germany). The ergosterol residue was dissolved in 1 ml methanol and transferred, through 0.45 µm cellulose-acetate filters, into 2 ml brown glass high performance liquid chromatography (HPLC) vials. Ergosterol concentration was quantified using HPLC analysis (Beckmann Coulter, System Gold 166 UV-detector, Fullerton, CA, USA). Calibration standards consisted of ergosterol (Sigma-Aldrich, St. Louis, USA) dissolved in ethanol and diluted to provide final concentrations of 0.2, 0.5, 1, 2 and 5 µg ml^{-1}.

5.2.3 Extracellular enzyme assays

All enzyme measurements are of potential activity. To ensure that assays were conducted under optimal conditions for each enzyme, substrate concentration, buffer

Table 5.2. Components of soil organic matter and the measured extracellular enzymes which contribute to their breakdown.

Component	Enzyme(s)	Reference
Cellulose	Cellobiohydrolase β-glucosidase	Sinsabaugh *et al.* 2008
Chitin	*N*-acetyl-glucosaminidase	Sinsabaugh *et al.* 2005
Lignin	Peroxidase Phenoloxidase	Sinsabaugh, 2010
Phosphoesters	Acid phosphatase	Turner *et al.* 2002
Polypeptides	Leucine aminopeptidase	Sinsabaugh *et al.* 2008
Xylan	β-xylosidase	Collins *et al.* 2005

pH, soil to solution ratio, temperature, and incubation time were standardised (Burns *et al.* 2013). The activities of the hydrolytic enzymes β-glucosidase, cellobiohydrolase, β-xylosidase, *N*-acetyl-glucosaminidase, acid phosphatase and leucine aminopeptidase (Table 5.2) were measured according to Marx *et al.* (2001). The substrates, containing 4-methylunbelliferone (4-MUF) and 7-amino-4-methyl coumarin (7-AMC) fluorescent compounds, standards and buffers (MES-buffer (pH 6.1, 0.1 M), 2-[*N*-morpholino]-ethanesulphonic acid; Trizma-buffer (pH 7.8, 0.05 M), mixture of α-tris-[hydroxymethyl]-methylamin and tris-[hydroxymethyl]aminomethane hydrochloride), were from Sigma-Aldrich (St. Louis, USA). Substrate stock solutions (10 mM) were obtained by dissolving substrates in 300 µl dimethyl sulphoxide and making up to 10 ml with autoclaved de-ionised water (DH_2O). Stock solutions were diluted with autoclaved buffer (MES for MUF substrates [all except leucine aminopeptidase]; Trizma buffer for leucine aminopeptidase [AMC substrate]) to obtain 1 mM working solutions. Substrate concentrations of 500 µM are saturating for a range of temperate soils (grassland, agricultural and forest), whereas concentrations higher than 1 mM can cause enzyme inhibition (Giacometti *et al*. submitted). MUF and AMC standards were dissolved in methanol and autoclaved DH_2O (v:v; 1:1), before being diluted to a final concentration of 1 µM with the appropriate buffer.

For MUF and AMC enzymes, 1 g (fresh weight) of soil was dispersed in 50 ml of autoclaved DH_2O using an ultrasonic disaggregator (50 J s^{-1} for 120 s). Microplate wells (black 96 well microplate, Greiner Bio-one GmbH, Frickenhausen, Germany) received 50 µl of soil suspension, 50 µl of autoclaved buffer and 100 µl of substrate solution. Standards were mixed with buffer and soil suspension to obtain final

concentrations of 0, 0.5, 1, 2.5, 4 and 6 µM. Microplates were incubated at 30 °C and fluorescence was measured after 30, 60, 120 and 180 min (excitation at 360 nm, emission at 460 nm; Microplate Fluorescence reader, FLX 800, Bio-Tek Instruments Inc., USA). The use of black microplates prevented interference between samples and the use of a separate standard curve for each sample accounts for any possible interference from soil particles (Poll *et al.* 2006; Kramer *et al.* 2013).

For assays of the oxidative enzymes peroxidase and phenoloxidase (Table 5.2), the substrate tetramethylbenzidine (TMB) was dissolved in dimethylsulphoxide, before adding autoclaved DH_2O (v:v; 1:1) to obtain a 60 mM stock solution (Johnsen and Jacobsen 2008; modified by Kramer *et al.* 2013). The TMB stock solution was mixed with autoclaved sodium acetate buffer (50 mM, adjusted to pH 5 using 12 % acetic acid) to obtain a 12 mM working solution. 0.4 g of soil was dispersed in 50 ml of autoclaved sodium acetate buffer, as above. Microtitre plate wells received 200 µl soil suspension and 50 µl of TMB working solution. 10 µl of hydrogen peroxide solution (0.3 %) was added to assay wells for peroxidase, the activity of which was calculated by subtracting phenoloxidase absorbance from the total absorbance. Blank and negative control wells received acetate buffer instead of TMB working solution and soil suspension, respectively. Microplates were incubated in darkness at 25 °C. Enzyme activities were spectrophotometrically determined by measuring absorbance at 630 nm (Absorbance Microplate Reader, EL 808, BioTek Instruments Inc., USA) 0, 3, 6, 9 and 12 min after TMB addition to microtitre plate wells. Immediately prior to each measurement, microplates were shaken at 250 rpm for 3 s to prevent soil from collecting at the bottom of the microplate wells. Three technical replicates were employed for all enzyme assays.

5.2.6 Statistical analyses

All statistical analyses were conducted in R (version 2.15; R Development Core Team 2012). Following removal of clear outliers from biochemical data sets, 4-6 replicates remained for all treatments. Ergosterol content, enzyme activities, wood block decay rate, soil moisture content, organic carbon, total nitrogen, C:N and enzyme:ergosterol ratios were analysed using three-way ANOVA and Tukey's pairwise comparisons, with fungus, temperature and moisture treatment as factors. Where the fungal treatment did not affect the response variable, these data were pooled by removing fungus from the

ANOVA (unless the significance of a higher order interaction prevented this). Associations between the activities of different enzymes, as well as with soil moisture, ergosterol, organic carbon and total nitrogen were explored using Pearson's correlation. To achieve data normality: β-glucosidase, β-xylosidase, *N*-acetyl-glucosaminidase, leucine aminopeptidase, peroxidase and phenoloxidase activities were log-transformed; cellobiohydrolase activity and ergosterol content were square root-transformed.

Differences in the activity of the overall enzyme complement between climate treatments were visualised through non-metric multidimensional scaling (NMDS), using the metaMDS function within the vegan package. The global stress score of 0.041 was generated after the ordination had run for 206 iterations and was sufficiently low to enable reliable data interpretation in two dimensions. Differences between treatment groupings shown in NMDS plots were assessed using permutational multivariate analysis of variance (PERMANOVA). An overall PERMANOVA was first used to confirm a treatment effect, before testing pairwise differences between groups. PERMANOVA was conducted using the Adonis function in vegan, based on 999 permutations. Distance matrices for NMDS and PERMANOVA were constructed using the Bray-Curtis dissimilarity index.

5.3 Results

5.3.1 Fungal biomass

Inoculated fungi established in the mesocosms; mycelia emerged from the woodblocks and extended across the soil surface, and into the humus and upper soil layer. At ambient moisture, fungal biomass, as indicated by soil ergosterol content, was greater in *R. bicolor*-, compared with *P. velutina*- ($P = 0.023$) and un-inoculated ($P = 0.001$) soils (Fig. 5.1; moisture*fungus; Table 5.3). Both wetting ($P = 0.024$) and drying ($P = 0.017$) reduced fungal biomass in *R. bicolor*-inoculated soils to a level that did not differ significantly from the other fungal treatments (Fig 5.1). Empirical climate modification did not significantly ($P \geq 0.05$) influence fungal biomass in *P. velutina*- and un-inoculated soils (Fig. 5.1). Fungal biomass was positively correlated overall with soil moisture content, carbon and nitrogen (Table 5.4), and was not significantly ($P \geq 0.05$) affected by elevated temperature (Fig. 5.1).

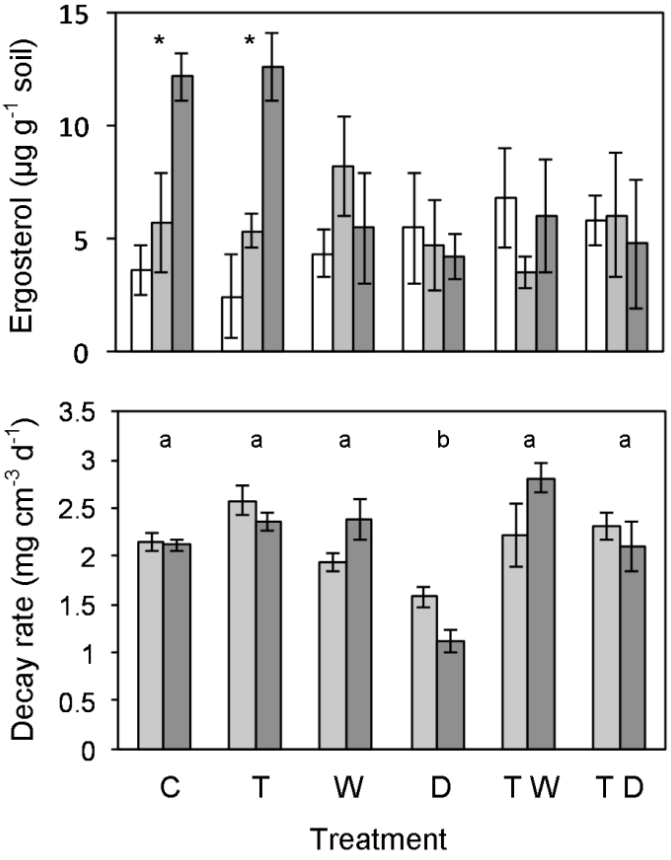


Figure 5.1 Ergosterol content (± SEM) of soil and decay rate (± SEM) of *Fagus sylvatica* wood blocks in un-inoculated (open bars), *Phanerochaete velutina*- (light shading) and *Resinicium bicolor*-inoculated (dark shading) mesocosms, under different climate treatments. Different letters indicate significant ($P < 0.05$) differences between climate treatments. * indicates a significant ($P < 0.05$) difference between fungal treatments. Climatic factors are: control, C; elevated temperature, T; wetting, W; drying, D. Note that units and scales differ between axes.

5.3.2 Wood decay rates

Overall, wood decay was increased by warming (27 %) and reduced by drying (23 %) ($P < 0.001$ in both cases; Table 5.3). Elevated temperature had the strongest influence on wood decay under drying (temperature*moisture; Table 5.3), where the only significant ($P < 0.05$) warming-induced stimulation of decay within a given moisture treatment was evident, for both fungi (Fig. 5.1). Moisture conditions differentially affected decomposition mediated by the two fungi (moisture*fungus; Table 5.3); *R. bicolor* decayed wood more rapidly than *P. velutina* under wetting ($P = 0.030$).

5.3.3 Extracellular enzyme activities

All enzymes, except leucine aminopeptidase, were significantly affected by moisture treatment ($P < 0.05$; Table 5.3). Activities of β-glucosidase (26 %; $P = 0.022$), cellobiohydrolase (27 %; $P = 0.009$), β-xylosidase (31 %; $P = 0.005$), *N*-acetyl-glucosaminidase (30 %; $P = 0.014$), acid phosphatase (20 %; $P = 0.001$), peroxidase (15 %; $P = 0.018$) and phenoloxidase (25 %; $P = 0.018$) were all lower under drying than wetting overall, across both temperatures. Peroxidase activity was increased by

Table 5.3 Overall effects (three-way ANOVA: $F_{\text{degrees of freedom}}$ and P values) of temperature (T), moisture (M) and fungal (F) treatments, and interactions, on fungal biomass (ergosterol, µg g^{-1} soil), beech (*Fagus sylvatica*) wood block decay rate (mg cm^{-3} d^{-1}) and extracellular enzyme potential activities.

	Ergosterol		Decay		BG		XYL		CBH		NAG		AP		PER		POX	
Factor(s)	$F_{1, 60}$	P	$F_{1, 60}$	P	$F_{1, 90}$	P	$F_{1, 87}$	P	$F_{1, 91}$	P	$F_{1, 84}$	P	$F_{1, 81}$	P	$F_{1, 92}$	P	$F_{1, 90}$	P
T			28.4	<0.001														
M			13.9	<0.001	3.9	0.024	5.4	0.007	4.6	0.013	4.4	0.015	7.0	0.002	7.6	0.001	9.2	<0.001
F																		
T × M			3.2	0.048	3.5	0.036	3.3	0.046	3.8	0.026	3.2	0.040	7.3	0.001	3.1	0.049	3.4	0.048
T × F											4.1	0.020						
M × F	4.8	0.002	7.1	0.002														
T × M × F													3.3	0.015				

Non-significant ($P \geq 0.05$) results are omitted for clarity. Enzymes are: β-glucosidase, BG; β-xylosidase, XYL; cellobiohydrolase, CBH; *N*-acetyl-glucosaminidase, NAG; acid phosphatase, AP; peroxidase, PER; phenoloxidase, POX.

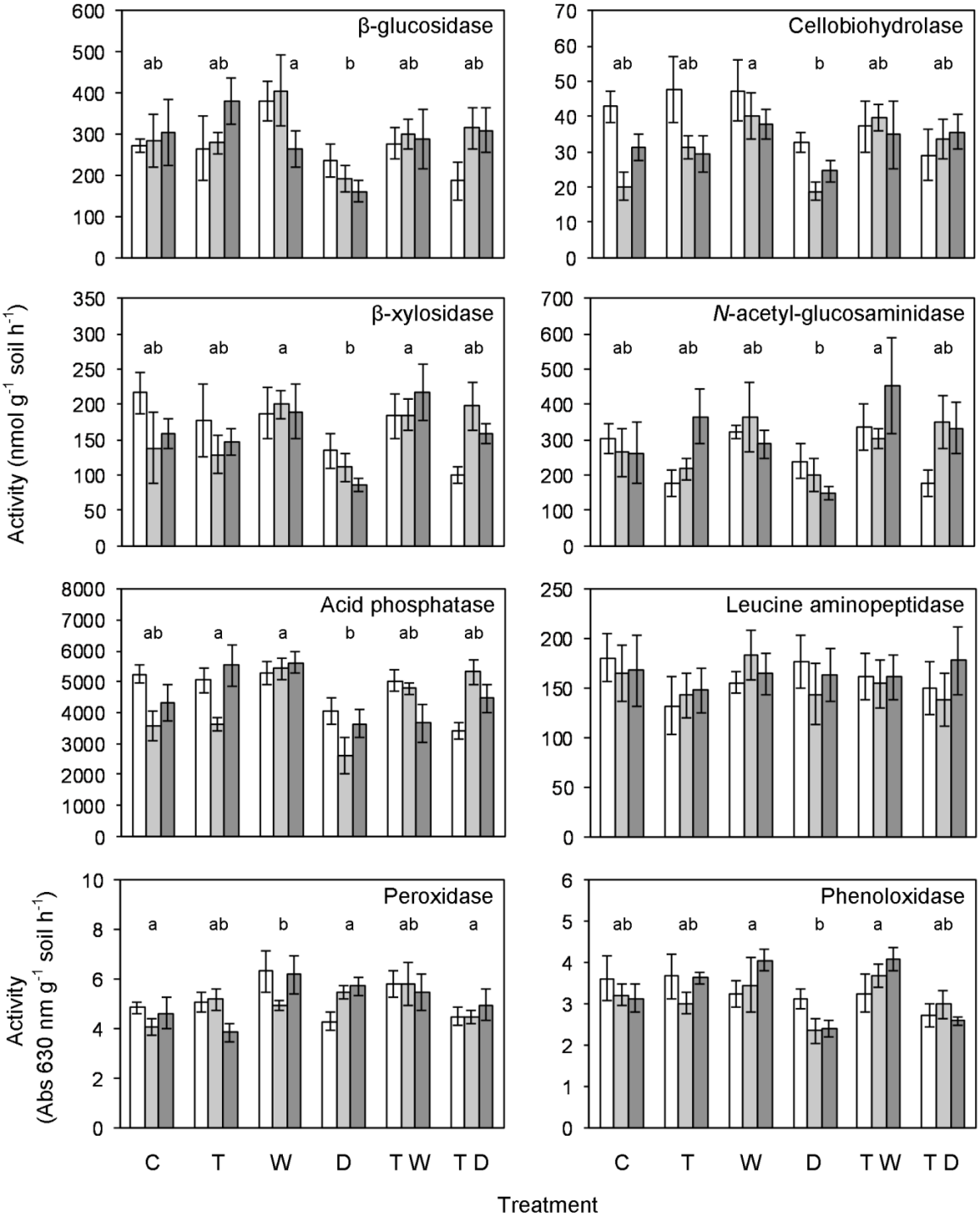


Fig. 5.2 Enzyme activities (± SEM) in un-inoculated (open bars), *Phanerochaete velutina*-(light shading) and *Resinicium bicolor*-inoculated (dark shading) mesocosms, under different climate treatments. Different letters indicate significant ($P < 0.05$) differences between climate treatments. Climatic factors are: control, C; elevated temperature, T; wetting, W; drying, D. Note that units and scales differ between Y axes.

wetting (P = 0.001) compared with the control, but not drying treatment, at ambient temperature (Fig. 5.2). At elevated temperature, however, there was no significant difference ($P \geq 0.05$) between moisture treatments for any enzyme (Fig. 5.2). This result is also evident from the NMDS of enzyme data, which shows a marked separation of the wetting treatment from control ($F_{1, 22} = 16.0$, P = 0.015) and drying ($F_{1, 24} = 54.8$, P = 0.001), being distinctly reduced and rendered non-significant ($P \geq 0.05$) in combination with elevated temperature, with a much wider spread of replicate points (Fig. 5.3).

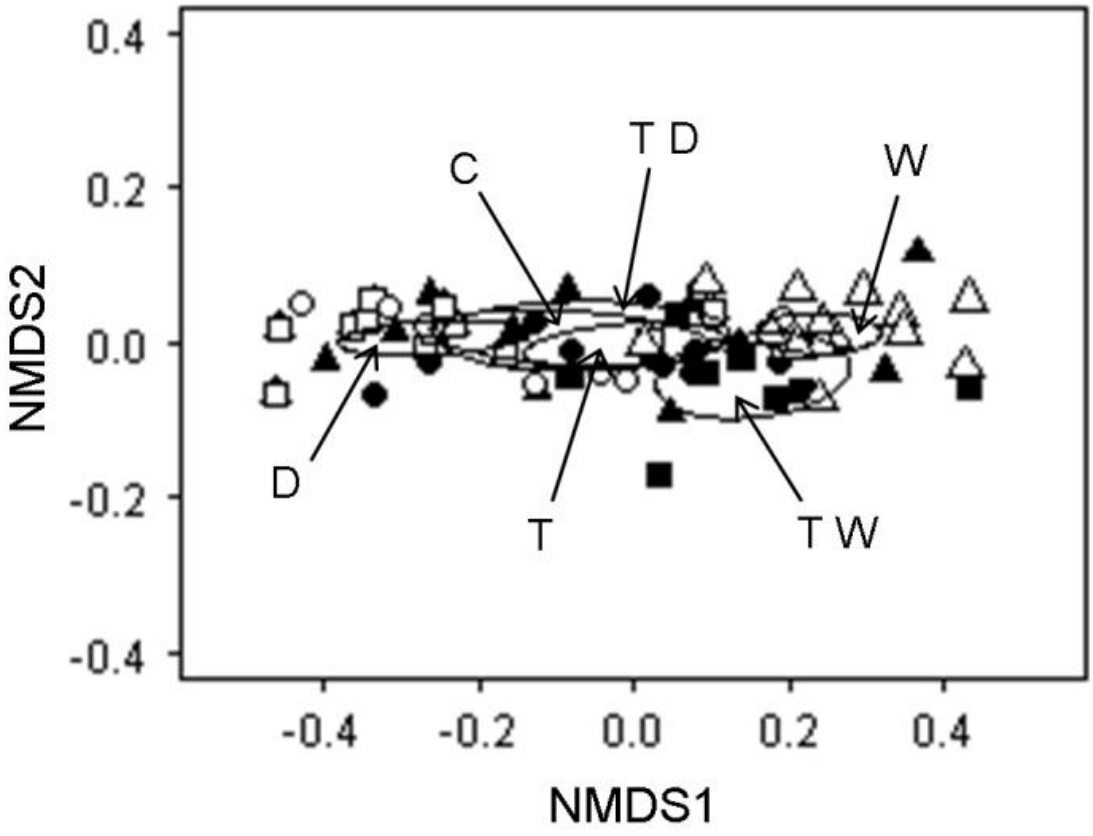


Fig. 5.3 Non-metric multidimensional scaling (NMDS) plot of enzyme activity under different climatic treatments: control, C (○); elevated temperature, T (●); wetting, W (Δ); drying, D (□); elevated temperature × wetting, T W (▲); elevated temperature × drying, T D (■). Ellipses indicate climate treatment 95 % confidence intervals fitted onto the spatial ordination. Non-overlapping ellipses indicate significant (P < 0.05) differences between treatments.

No enzyme activity was significantly affected by warming, overall (Table 5.3; Fig. 5.2). *N*-acetyl-glucosaminidase activity was differentially influenced by elevated temperature across fungal treatments (temperature*fungus; Table 5.3), only being stimulated in *R. bicolor*-inoculated soil ($P = 0.048$). The interactive influence of warming and altered moisture on acid phosphatase activity also differed between fungal treatments (temperature*moisture*fungus; Table 5.3), being stimulated by warming only in dry *P. velutina*-inoculated soil ($P = 0.007$).

The potential activities of all enzymes, except leucine aminopeptidase, were significantly ($P < 0.05$) positively correlated with soil moisture content, and all enzymes, except peroxidase and phenoloxidase, were positively correlated with ergosterol (Table 5.4). The ratios of enzyme activity to ergosterol content were not significantly ($P \geq 0.05$) affected by abiotic factors for any enzyme. β-glucosidase, β-xylosidase, *N*-acetyl-glucosaminidase and acid phosphatase activities were all significantly ($P \geq 0.05$) positively correlated with soil organic C and total N (Table 5.4). As most enzymes displayed similar associations (though varying in magnitude) with biotic and abiotic variables, their activities were often correlated (Table 5.4).

Table 5.4 Correlation matrix of enzyme activities, fungal biomass (ergosterol, μg g^{-1} soil), soil moisture (% oven dry weight), organic C (μg g^{-1} soil) and total N (μg g^{-1} soil).

	BG	XYL	CBH	NAG	AP	L	PER	POX	Ergosterol
BG		**0.751**	**0.698**	**0.693**	**0.426**	**0.270**	-0.003	**0.317**	**0.520**
XYL			**0.697**	**0.762**	**0.677**	**0.253**	-0.020	**0.399**	**0.395**
CBH				**0.558**	**0.466**	**0.384**	0.152	**0.324**	**0.302**
NAG					**0.437**	**0.242**	0.028	**0.358**	**0.457**
AP						0.173	0.068	**0.331**	**0.315**
L							0.127	0.144	**0.254**
PER								-0.180	-0.036
POX									0.029
Soil moisture	**0.411**	**0.352**	**0.380**	**0.260**	**0.263**	0.027	**0.197**	**0.376**	**0.405**
Organic C	**0.378**	**0.241**	0.163	**0.296**	**0.200**	0.076	0.082	0.087	**0.228**
Total N	**0.383**	**0.241**	0.161	**0.320**	**0.222**	0.099	0.069	0.101	**0.223**

Bold correlation coefficients are significant ($P < 0.05$). Enzymes are: β-glucosidase, BG; β-xylosidase, XYL; cellobiohydrolase, CBH; *N*-acetyl-glucosaminidase, NAG; acid phosphatase, AP; leucine aminopeptidase, L; peroxidase, PER; phenoloxidase, POX.

5.4 Discussion

The interaction between elevated temperature and altered soil moisture (wetting and drying) regulated the functioning of a saprotrophic basidiomycete-dominated woodland decomposer system. Interactions between environmental factors were non-additive, generating effects that would not have been predicted from consideration of the individual abiotic variables. One overarching trend was revealed; the drying-induced decreases in extracellular enzyme activity (compared to the wetting treatment) and primary decomposition rates were altered by elevated temperature. Warming did not affect fungal biomass, but did increase the decomposition of wood colonised by the inoculated fungal species, whereas soil enzyme activity was influenced by elevated temperature only in specific interactions with moisture and fungal treatments (partially supporting Hypothesis 1). The contrasting influences of soil wetting and drying on fungal biomass and extracellular enzyme activities were not entirely consistent; soil ergosterol was correlated with soil moisture but did not differ between specific moisture treatments (partially supporting Hypothesis 2). Warming alleviated the negative influence of drying on soil enzyme activities and primary decomposition of fungal-colonised wood (in contrast to Hypothesis 3).

The inoculated fungi (both of which became established in mesocosms) have previously been shown, using next generation sequencing, to reduce the diversity and relative abundance of non-cord-forming soil and litter fungi markedly, and become the dominant component of the fungal biomass (Crowther *et al.* 2013). This shift in fungal community had a functional consequence, as hydrolytic enzyme activity increased. In the present study, however, microbial community functioning did not differ between fungal treatments. This could reflect the fact that Crowther *et al.* (2013) inoculated mesocosms with both saprotrophic cord-former species simultaneously; extracellular enzyme production and nutrient loss by mycelia are known to increase during interspecific combative interactions (Wells and Boddy 2002; Šnajdr *et al.* 2011). The establishment of a single additional mycelial system in the mesocosms employed in the present study, therefore, likely altered fungal community composition, but not enzyme activity and decomposition. Enzymes were mainly derived from fungi, as indicated by their positive correlations with ergosterol concentration. This is expected in temperate woodland soil (even in the absence of standardised inoculation with a cord-forming

basidiomycete), where the microbial biomass is predominantly fungal (Markkola *et al.* 1996; Wells and Boddy 2002).

The absence of a fungal biomass response to warming does not preclude increased mycelial growth rates; ergosterol was measured at the end of the experiment, reflecting a maximum biomass determined by the dimensions of the experimental systems (Chapter 3). Temperature is predicted to increase extracellular enzyme activity, as long as moisture is not limiting (Henry 2012; Baldrian *et al.* 2013a). This prediction is not supported by the influence of warming alone, or in combination with wetting, reported in the present study. Indirect effects of elevated temperature decreasing fungal biomass and enzyme production by reducing soil moisture (Allison and Treseder 2008) would not explain this result, as moisture was maintained at the established treatment level. While *in situ* enzyme kinetics (substrate diffusion and binding to enzymes) could be promoted by warming (Henry 2012), enzyme production and release from the microbial biomass was not. The potential activity of enzymes analysed in soil does not reflect their activity in wood, where *in situ* decomposition was stimulated by warming (Sinsabaugh *et al.* 1993). Stimulation of the *R. bicolor*-mediated *N*-acetyl-glucosaminidase pool under warming could reflect increased chitin availability, as collembola abundance was markedly reduced in systems dominated by this species (Chapter 4).

The generally neutral response of extracellular enzyme activity to warming may reflect the fact that fungal biomass did not increase. Alternatively, it is possible that the warming increment (3 °C) employed in the present study, with an ambient temperature representative of a cooler time of the year, would have had a stronger effect. Enzymes involved in the cycling of different components of organic matter differ in their temperature sensitivity; that of many soil hydrolytic enzymes increases as environmental temperature decreases, with winter measurements being more sensitive than summer measurements (Koch *et al.* 2007; Wallenstein *et al.* 2009). Enzymes at higher latitudes are, therefore, hypothesised to display greater sensitivity to temperature than those from lower latitudes. Local adaptation of microbial extracellular enzyme kinetics to temperature is an important consideration in predicting carbon cycle responses to global change (German *et al.* 2012). Initial increases in CO_2 efflux from soil in field studies, due to temperature-induced stimulation of microbial respiration,

have returned to control levels after a few years (Luo *et al.* 2001; Melillo *et al.* 2002; Bradford *et al.* 2008). This observation has been attributed to decomposer microbial biomass limitation by reduced carbon-use efficiency (Allison *et al.* 2010). Thermal acclimation of fungal growth and respiration has been demonstrated for the decomposer basidiomycete species employed in the present study (Crowther and Bradford 2013).

The correlation between ergosterol and soil moisture is independent of the moisture treatment categories, where the detection of an effect is most likely precluded by variation in pre-treatment soil moisture content causing overlap between mesocosm moisture treatments. Contrasting responses of fungal biomass to wetting and drying in *R. bicolor*- and *P. velutina*-dominated systems reflect species-specific sensitivity of fungal mycelia to altered soil moisture (Chapter 2; Donnelly and Boddy 1997). Marked deviations from moisture optima for mycelial growth had a negative effect on *R. bicolor* mycelial biomass, whereas *P. velutina* appears more robust against such changes. Optima for mycelial extension differ from those for decay (Dowson *et al.* 1989); the negative influence of wetting on *R. bicolor* mycelial biomass did not prevent it from decomposing wood more rapidly than *P. velutina*. Further, the influence of soil moisture on extracellular enzyme activities was largely consistent across fungal treatments, despite differing fungal biomass responses to wetting and drying. Moisture is an important determinant of forest soil and litter hydrolytic and oxidative enzyme activity (Criquet *et al.* 2000, 2002, 2004; Sardans and Penuelas 2005). Enzyme potential activities increase with soil moisture (Baldrian *et al.* 2010, 2013a) and have been correlated, at a global scale, with mean annual precipitation (Sinsabaugh *et al.* 2008). Moisture levels in the drying treatment, although substantially reduced, were not sufficiently limiting to prevent any influence of warming. Had mesocosm soil moisture levels been lower pre-treatment, or reduced to a greater extent by drying, it is unlikely that warming would have compensated for the decrease in enzyme potential activity relative to wetting, because moisture stress would have been the limiting factor (Manzoni *et al.* 2012).

Positive correlations between several hydrolytic enzymes and soil chemical properties (organic carbon and total nitrogen) indicate that natural variation in soils, as well as the experimental manipulations, affected enzyme activities. The link between the enzyme producers (indicated by ergosterol) and enzyme activity is stronger for hydrolases than

oxidases. Measurements of oxidase activity are interpreted with caution due to uncertainty regarding the potential influences of other soil properties (see Sinsabaugh 2010). Hydrolases are mainly bound to particulate organic matter, which is broken down relatively rapidly and the enzymes released and also decomposed. In contrast, oxidases are mainly stabilised onto mineral surfaces, so remain in the environment for longer (Allison and Jastrow 2006). Soluble substrates are used to measure oxidase activity, but environmental substrates are often insoluble or mineral-bound. Potential activity measurements may not accurately reflect *in situ* peroxidase and phenoloxidase activities, due to inefficient enzyme–substrate interactions caused by stabilisation (Allison 2006). Further, oxidases exhibit greater spatiotemporal variation than hydrolases, obscuring their association with environmental variables (Sinsabaugh 2010). Peroxidase and phenoloxidase activities are strongly associated with pH (optima of pH 8 ± 1) and the lignin content of organic material (Sinsabaugh *et al.* 2008).

The differential sensitivity to experimental climate change of the dominant component of the fungal biomass highlights the potential importance of microbial community composition in regulating SOM decomposition. Nevertheless, the study reported in this chapter has revealed clear overall trends in the regulation of fungal-mediated decomposition and the extracellular enzyme pool in basidiomycete-dominated woodland decomposer communities. The potential for climate warming to moderate the influence of fluctuating moisture conditions on microbial community functioning is important and warrants further empirical investigation. Considering interactions between abiotic factors has revealed that the woodland soil decomposer system could be more functionally robust against climate change than is implied by the manipulation of single factors.

6 Effects of isopod population density on woodland decomposer microbial community function

6.1 Introduction

Terrestrial carbon and nutrient cycling is primarily regulated by heterotrophic soil microbes. Soil microbial and invertebrate faunal populations are linked; these soil biotic components influence one another via top-down and bottom-up mechanisms (Wardle 2006). Soil mixing and litter shredding by invertebrates alters resource availability for microbial decomposers, influencing their community composition (Hättenschwiler *et al.* 2005; Wardle 2006). Bottom-up influences of microbes on invertebrate abundance and diversity arise due to differential palatability of microbial taxa to microbivores (e.g. collembola; Chapter 4; Jones *et al.* 1998). Top-down regulation of the relative abundance of different microbial species, due to selective grazing by soil invertebrates, is an important mechanism structuring fungal (A'Bear *et al.* 2013d; Crowther *et al.* 2013) and bacterial (Rønn *et al.* 2002) communities.

Temperate forests are a major global store of terrestrial carbon (292 Pg; Lal, 2005). The production of extracellular enzymes responsible for the breakdown of lignin and cellulose makes basidiomycete fungi the main agents of primary decomposition in these ecosystems (Hättenschwiler *et al.* 2005; Baldrian and Valášková 2008). Some of these fungi, which form mycelial cords, extend from colonised woody resources and form large networks of mycelium which retain and translocate nutrients (Boddy 1999; Fricker *et al.* 2008). The higher nutrient status (low C:N) of fungal mycelium, relative to organic matter, makes it an attractive source of nutrition to soil invertebrates (Boddy and Jones 2008). As a consequence of the large size and biochemical defences of fungal mycelia, the capacity for grazers to influence their biomass and activity usually reflects invertebrate body size, population density and species-specific feeding preferences (Kaneko *et al.* 1998; Crowther *et al.* 2011a; Crowther and A'Bear 2012).

Competitive dominance of decomposer basidiomycete mycelium reduces soil microfungal abundance and diversity (Crowther *et al.* 2013). This, in turn, limits mycophagous soil mesofauna (e.g. collembola) abundance because the size and biochemical defences of basidiomycete mycelium may make them less palatable than soil microfungi (Chapter 4). Macrofauna (e.g. woodlice), with their larger body size

and greater metabolic requirements, are more able to exploit basidiomycete fungal mycelia. As a consequence of their longer lifecycles, bottom-up effects of fungal palatability on macrofauna have not been assessed by short-term grazing studies. Taxon-specific population responses to field carbon and nutrient amendment have been reported, none of which mirrored microbial biomass change (Scheu and Schaefer 1998). Woodlice, even at low density, consistently reduce mycelial biomass and exert selective pressures strong enough to alter the outcomes of competitive interactions (Crowther *et al.* 2011a; A'Bear *et al.* 2013b, d). Extensive mycelial ingestion by a widespread woodlouse species (*Oniscus asellus*, Isopoda) has been shown to reduce soil extracellular enzyme activities and increase collembola abundance by releasing the more easily ingested microfungi from competitive suppression (Crowther *et al.* 2013). Their capacity to maintain high fungal diversity and mycophagous mesofaunal abundance has led to woodlice being suggested as keystone grazers in temperate woodland soil (Crowther *et al.* 2013). As the rate of fungal utilisation of colonised wood depends, at least in part, on extra-resource biomass (Bebber *et al.* 2011), extensive mycelial ingestion may reduce resource decay as well as the potential for decomposition (enzyme activities) in soil.

Mechanistic understanding of top-down and bottom-up interactions occurring between decomposer cord-forming fungi and mycophagous soil fauna has been based on mainly microcosm (few-species interactions) and occasionally mesocosm (natural communities subjected to simple biotic manipulations) studies, always at relatively small spatial scales, under controlled abiotic conditions (Chapter 3; Chapter 4; Tordoff *et al.* 2008; Crowther *et al.* 2011a, b, 2013). Whether the strengths of interactions revealed in these systems can be extrapolated to the greater spatial and temporal scales, and abiotic variability of field conditions remains unexplored. Populations of mycophagous macrofauna, including woodlice, are predicted to increase in temperate regions as they become warmer and wetter due to climate change (David and Handa 2010). The capacity for field woodlouse populations to regulate decomposer community structure and function will determine their potential to moderate climate-induced stimulation of decomposition and CO_2 efflux from temperate forest soil (Chapter 2).

The study reported in this chapter investigates the influence of woodlouse (*O. asellus*) population density on the biomass, composition and functioning of soil microbial

communities, and the abundance and diversity of mycophagous micro-arthropods (collembola and oribatid mites), in a saprotrophic basidiomycete-dominated woodland decomposer community. A field experiment was established to test four specific hypotheses: (1) Woodlouse population density and diet will be affected by fungal species dominance in the decomposer community. Invertebrate fatty acids reveal dietary composition, providing a means of assessing feeding habits that cannot be observed directly (Ruess *et al.* 2005; Ruess and Chamberlain 2010). Increasing woodlouse population density will: (2) reduce fungal biomass and the ratio of fungi to bacteria; (3) increase mycophagous micro-arthropod abundance and diversity; and (4) reduce extracellular enzyme activity and fungal-mediated beech (*Fagus sylvatica*) wood decomposition.

6.2 Materials and methods

6.2.1 Experimental design

The influence of woodlouse (*O. asellus*) population density on decomposer community dynamics and functioning, in a saprotrophic basidiomycete-dominated temperate woodland soil, was investigated in a field experiment. Nine treatments were derived from the factorial crossing of three fungal treatments (un-inoculated, or inoculated with *Hypholoma fasciculare* (Huds.: Fr.) Kummer or *Phanerochaete velutina* (DC.: Pers.) Parmasto) with three woodlouse population density ('reduced', 'current' or 'double') treatments. Eight replicate blocks contained nine plots to which treatments were assigned at random. Woodlouse diet, microbial biomass and community composition, mycophagous micro-arthropod (collembola and oribatid mites) populations, extracellular enzyme activities and *in situ* decomposition of fungus-colonised beech (*Fagus sylvatica*) wood were assessed.

6.2.2 Field site and sampling

The experiment was established in a beech stand within Wytham Woods (Savill *et al.* 2010; Oxfordshire, UK; NGR 445355, 208612; 51.774, -1.344) in July 2012. For the most part, temperature at the site fell within the long-term (100 year) average maxima and minima for the area, but an unusually cold March and early April delayed the onset of spring (Fig. 6.1). Autumn and early winter at the site were wetter than the long-term average (Fig. 6.1). The soil was silty loam, classified as a typical brown earth, and had a pH of 5.3 (measured in H_2O). Replicate blocks were delimited by 30 cm deep

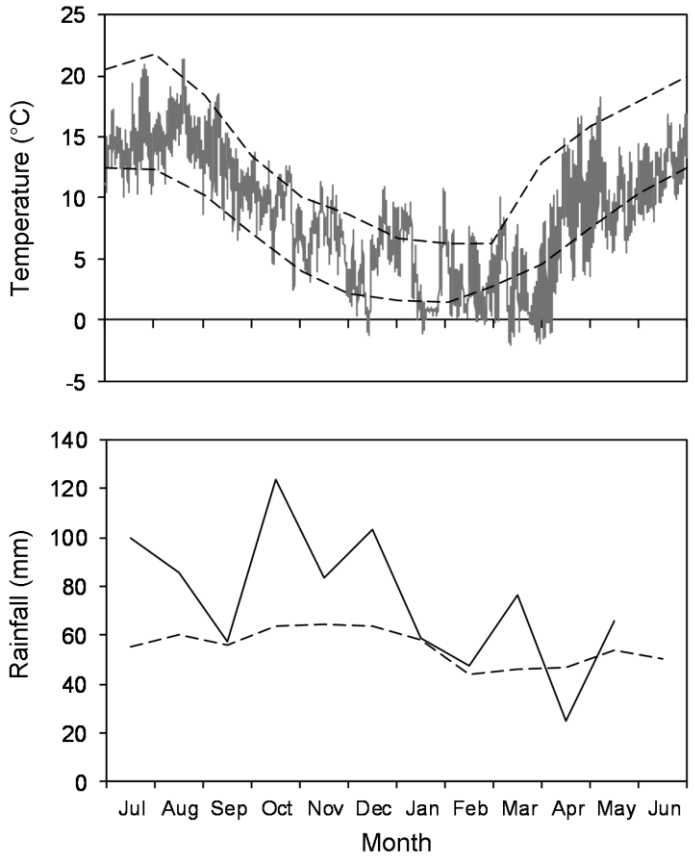


Fig. 6.1 Hourly temperature and monthly rainfall at the field site (Wytham Woods, Oxfordshire) during the experimental period (July 2012 – June 2013). Dashed lines represent the 100 year monthly averages; maximum and minimum for temperature (data from Meteorological Office).

polycarbonate sheets (10 and 20 cm below- and above-ground, respectively), screwed together to create gap-free joins and sunk into trenches, which were back-filled with soil and compressed to ensure inter-plot woodlouse dispersal did not occur (Scheu and Schaefer 1998). Plastic sticky traps (Oecos, Kimpton, UK) affixed internally around the top of the plot sides confirmed that woodlice did not climb out of individual plots. Litter was removed from all plots and replaced after sifting by hand in the laboratory to remove woodlice. Population densities ranged from 1-100 m^{-2}, with a mean of 28 m^{-2}. *Oniscus asellus* was the most abundant species at the site. Woodlice population density treatments were established by replacing adult *O. asellus* in plots at three densities: zero ('reduced'), mean ('current') or twice the mean ('double') population density.

Wood discs (6-9 cm diameter, approximately 1.5 cm deep) from freshly-cut beech branches were heat-sealed in two layers of autoclave plastic and autoclaved (121 °C) three times at 24 h intervals. Sterile discs were placed into 'deli' pots (12 cm diameter, 400 ml; Cater for You, High Wycombe, UK) containing 2 % malt agar pre-colonised with one of the two cord-forming basidiomycete fungi, *Hypholoma fasciculare* and *Phanerochaete velutina*, and incubated in darkness at 16 ± 1 °C for 3 months. Thick

cords were removed from the soil surface to standardise and each plot (except un-inoculated treatments) was inoculated centrally with two beech discs pre-colonised with either *H. fasciculare* or *P. velutina*. Initial density (oven dry weight/ fresh volume; mg cm^{-3}) of fungus-colonised wood discs was determined (n = 10).

Sampling occurred after 6 (January 2013) and 12 (July 2013) months. At each sampling point, one wood disc was removed from each fungal-inoculated plot and decay rate (mg cm^{-3} d^{-1}) estimated from change in density. Samples of the top 2 cm of soil (30 g, comprising four subsamples taken from half way between the centre and the outer margin of mycelial extension in the direction of each plot corner) were extracted, sieved through a 2 mm mesh and stored (-18 °C) for analysis of ergosterol, phospholipid fatty acids (PLFAs) and extracellular enzyme activities. Soil organic carbon and total nitrogen were determined by dry combustion (LECO 2000 CNS elemental analyser). Collembola and oribatid mites were extracted into 100 % ethanol by transferring two soil cores (5 cm diameter, 5 cm deep; taken from within the mycelium-colonised area) into a Tullgren funnel for 48 h. At the final sampling period, litter was removed from all plots and sifted by hand to remove woodlice, which were stored (-18 °C) for analysis of neutral lipid fatty acids (NLFAs) and PLFAs. Three replicates of potential woodlouse dietary sources were collected for analysis of total lipid fatty acids (TLFAs): humus, beech leaf litter, beech bark, mycelium from both inoculated fungal species and *P. velutina* cords (this species forms thick cords which are easily extracted from the surface of soil, as well as the finer soil-penetrating hyphae characteristic of *H. fasciculare*).

6.2.3 Ergosterol

Soil ergosterol content, a fungal biomass indicator, was measured as described in *Section 5.2.2*, except that 0.5 g of soil was used for all treatments.

6.2.4 Fatty acid analyses

Soil PLFAs were extracted and identified using the method of Frostegard *et al.* (1993). Briefly, Bligh and Dyer reagent (chloroform: methanol: citrate buffer, 1:2:0.8; pH 4) was used as the solvent to extract lipids from 2 g of soil. Silica acid columns (Varian Medical Systems, Palo Alto, CA) were employed for lipid fractionation and lipids were eluted using chloroform (NLFA), acetone (glycolipids) and methanol (PLFA).

Methanolic potassium hydroxide (0.2 M) was used for methanolysis of PLFAs and isooctane as a solvent for analysis. Methylnondecanoate (19:0) was used as an internal standard for quantitative analyses. Fatty acid methyl esters (FAMEs) were identified by comparing chromatographic retention times with a standard FAME mixture (range from C11 to C24; Sigma-Aldrich, St Louis). Analysis was performed by gas chromatography (GC) using an Auto System XL (Perkin Elmer Corporation, Norwalk) containing an HP-5 capillary column (50 m length, 0.2 mm internal diameter, 0.3 μm film thickness). The temperature started at 70 °C (hold time 2 min) and increased by 30 °C min^{-1} to 160 °C, then by 3 °C min^{-1} to 280 °C (hold time 15 min). The injection temperature was 260 °C with helium as the carrier gas. The soil microbial community was assessed using the PLFA biomarkers for fungi (18:2ω6), Gram positive (i15:0, a15:0, i16:0 and i17:0), Gram negative (cy17:0 and cy19:0) and total (16:1ω7 in addition) bacteria (Frostegård and Bååth 1996; Zelles 1999).

Oniscus asellus samples consisted of 4-7 individuals. Fatty acids were extracted and identified using the method of Ruess *et al*. (2004). Briefly, samples were shaken over night in extraction solvent (chloroform: methanol: phosphate buffer, 1:2:0.8, pH 7.4) and lipids gained after phase separation in the chloroform fraction. Lipids were fractionated by silica acid columns, as for soils. The NLFA and PLFA fractions from isopods and the TLFAs of potential dietary sources were saponified and methylated following the procedures outlined for the Sherlock microbial identification system (MIDI, Newark, Del.). Saponification was conducted in a sodium hydroxide-methanol solution at 100 °C; acid methanolysis in hydrochloric acid-methanol at 80 °C. FAMEs were extracted into hexane/methyl tertiary butyl ether (1:1) and washed with aqueous sodium hydroxide. FAMEs were identified and quantified according to their chromatographic retention times using the Sherlock microbial identification system (Agilent 7890 gas chromatograph equipped with a flame ionization detector and a HP Ultra 2 capillary column [25 m x 0.2 mm i.d., film thickness 0.33 μm]) and associated Sherlock pattern recognition computer software (MIDI®). The oven temperature programme started with 170 °C and increased by 28 °C min^{-1} to 288 °C, followed by 60 °C min^{-1} to 310 °C. FAME identification (chain length and saturation) was verified by analysing a range of TLFA, NLFA and PLFA samples by GC-mass spectrometry (Agilent Series 7890 GC system and Mass Selective Detector, Agilent 7000 Triple quadruple, equipped with HP5MS capillary column [30 m x 0.25 mm i.d., film

thickness 0.25 µm]). The temperature started at 60 °C and increased by 15 °C min^{-1} to 280 °C, then by 20 °C min^{-1} to 300 °C (hold time 1 min). A mass range of 40-400 m/z was monitored in Scan mode.

6.2.5 Extracellular enzyme assays

Enzyme activities were measured as described in *Chapter 5.2.3*.

6.2.6 Statistical analyses

All statistical analyses were conducted in R (version 3.0.2; R Development Core Team 2013). For soil biochemical data sets, 6-8 replicates remained for all treatments after the removal of clear outliers. Final woodlouse populations, ergosterol, PLFAs, enzyme activities, wood disc decay rates, collembola and oribatid mite abundance and diversity (Simpson's reciprocal $D = 1/\sum_{i=1}^{n} P_i^2$, where P_i is the proportion of individuals in the i^{th} species and n is the total number of species) were analysed using linear mixed-effects models (*lmer* package), with fungal-inoculation, initial woodlouse population density and season as fixed factors, and soil moisture content (as a biologically meaningful indicator of variation between replicate blocks) as a random factor. Non-significant ($P \geq 0.05$) interactions between fixed factors were sequentially removed from models. Planned contrasts were used to explore significant ($P < 0.05$) higher order interactions. Associations between the activities of different enzymes, as well as fungal biomass, soil moisture, carbon and nitrogen were assessed using Pearson's correlation. To achieve data normality: oribatid mite abundance, ergosterol, β-glucosidase, cellobiohydrolase, β-xylosidase, *N*-acetyl-glucosaminidase, acid phosphatase, leucine aminopeptidase, carbon and nitrogen were log-transformed; collembola abundance and diversity, all soil PLFA groups, peroxidase and phenoloxidase were square root-transformed.

Differences in the composition of NLFAs and PLFAs from woodlice, TLFAs of potential dietary sources, soil PLFAs and the activity of the overall enzyme complement between treatments were assessed using permutational multivariate ANOVA (PERMANOVA), conducted using the Adonis function in vegan, based on 999 permutations. Differences between treatment groupings were visualised through non-metric multidimensional scaling (NMDS), using the metaMDS function within the vegan package. The global stress scores (< 0.1 for all ordinations) were sufficiently low

to enable reliable data interpretation in two dimensions. Distance matrices for NMDS and PERMANOVA were constructed using the Euclidean dissimilarity index.

6.3 Results

6.3.1 Woodlice

Woodlouse (*Oniscus asellus*) abundances after 12 months were lower than the standardised 'current' (28 m^{-2}) and 'double' (56 m^{-2}) population densities (Fig. 6.2). Although *O. asellus* abundance reflected initial population density, overall (density: $F_{2, 54} = 11.2$, $P < 0.001$), the persistence of different woodlouse population density levels was strongly affected by fungal inoculation treatment (Fig. 6.2; fungus*density: $F_{4, 54} = 4.9$, $P = 0.002$). Un-inoculated plots had higher populations in 'current' ($P < 0.001$) and 'double' ($P = 0.010$) than in 'reduced' density treatments. *Phanerochaete velutina*-inoculated plots had higher populations in 'double' than in 'reduced' ($P < 0.001$) and 'current' ($P = 0.002$) density treatments. No significant ($P \geq 0.05$) differences remained between population density treatments in plots inoculated with *Hypholoma fasciculare*.

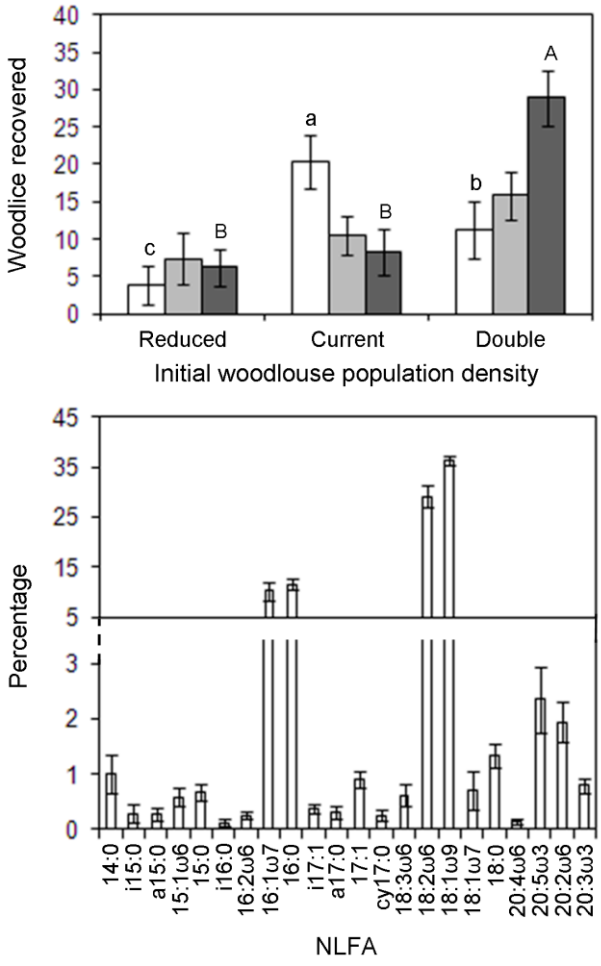


Fig. 6.2 Numbers of woodlice (*Oniscus asellus*) (± SEM) recovered from un-inoculated (open bars), *Hypholoma fasciculare*- (light shading) and *Phanerochaete velutina*- (dark shading) inoculated field plots at the end of the study. Lower- and upper-case letters indicate significant ($P < 0.05$) differences between woodlouse density treatments for un-inoculated and *P. velutina*-inoculated plots, respectively. NLFA composition (± SD) of recovered *O. asellus* specimens.

The NLFA (Fig. 6.2) and PLFA (Appendix 2) composition of *O. asellus* was not affected by fungal dominance (PERMANOVA: $P \geq 0.05$). *Oniscus asellus* was confirmed as a true generalist feeder, containing dietary markers from plants (18:3ω6, 18:1ω9), higher (or saprotrophic/ectomycorrhizal) fungi (18:2ω6), bacteria (i, a [iso/anteiso] in C14-C18, cy17:0, monounsaturated ω7) (Ruess and Chamberlain 2010 and references cited therein) and algae (16:2ω6; Buse *et al.* 2013) (Fig. 6.2). Potential dietary sources differed significantly in their TLFA composition (Fig. 6.3; PERMANOVA: $F_{5, 17} = 14.8$, $P < 0.001$). The most prominent plant marker, oleic acid (18:1ω9; 36.5 %), in *O. asellus* NLFAs is abundant in leaves, humus and bark (Fig. 6.3). Another major diet source was fungi, as indicated by the trophic marker linoleic acid (18:2ω6; 29.0 %). The prominence of both markers in the storage lipids reflects direct fungal and plant feeding, as well as coincidental ingestion of these materials. Some of the bacteria-specific fatty acids could have come from soil adhering to fungal mycelium (*P. velutina* mycelium vs. cords), or humus and plant material (Fig. 6.3). The overall proportion of bacteria-derived fatty acids (12.4 %), however, indicates a considerable assimilation of bacterial biomass.

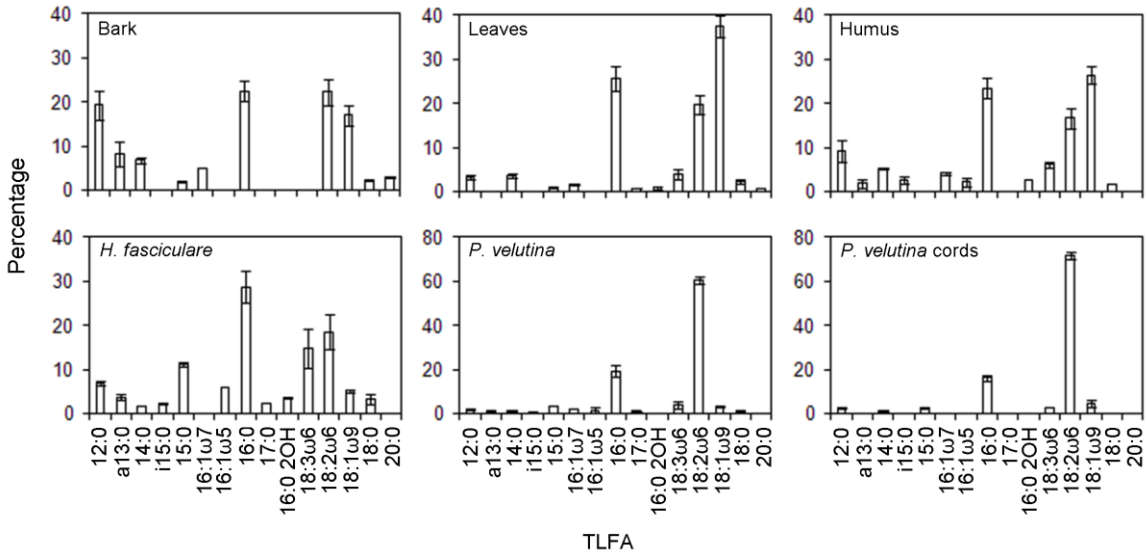


Fig. 6.3 TLFA composition (± SD) of woodlouse (*Oniscus asellus*) dietary sources: beech (*Fagus sylvatica*) bark and leaves, humus, *Hypholoma fasciculare* mycelium, *Phanerochaete velutina* mycelium and cords. Note that scales differ between Y axes.

6.3.2 Microbial community

Soil ergosterol content was not significantly ($P \geq 0.05$) affected by fungal inoculation or woodlouse population density, but was higher in winter than summer (Table 6.1). Ergosterol was correlated positively with soil moisture, carbon and nitrogen, and negatively with pH (Table 6.2). PLFAs revealed greater biomass of all microbial groups in *H. fasciculare*-inoculated treatments (Table 6.1; Appendix 3). This effect of fungal inoculation was reflected in PLFA composition (PERMANOVA: $F_{2, 132} = 3.4$, $P = 0.007$), although visualisation of this effect using NMDS revealed that the *H. fasciculare* treatment did not completely separate from the other fungal treatments (Fig. 6.4). Fungal, bacterial (but not gram positive or gram negative individually) and total PLFAs were lower in summer than winter (Table 6.2; Appendix 3). Season had a strong effect on PLFA composition (Fig. 6.4; PERMANOVA: $F_{1, 132} = 69.3$, $P = 0.001$). Ratios of fungi to bacteria, and Gram positive to Gram negative bacteria were not significantly ($P \geq 0.05$) affected by the experimental treatments (Appendix 3). Ergosterol and fungal PLFA abundances were not correlated ($P \geq 0.05$).

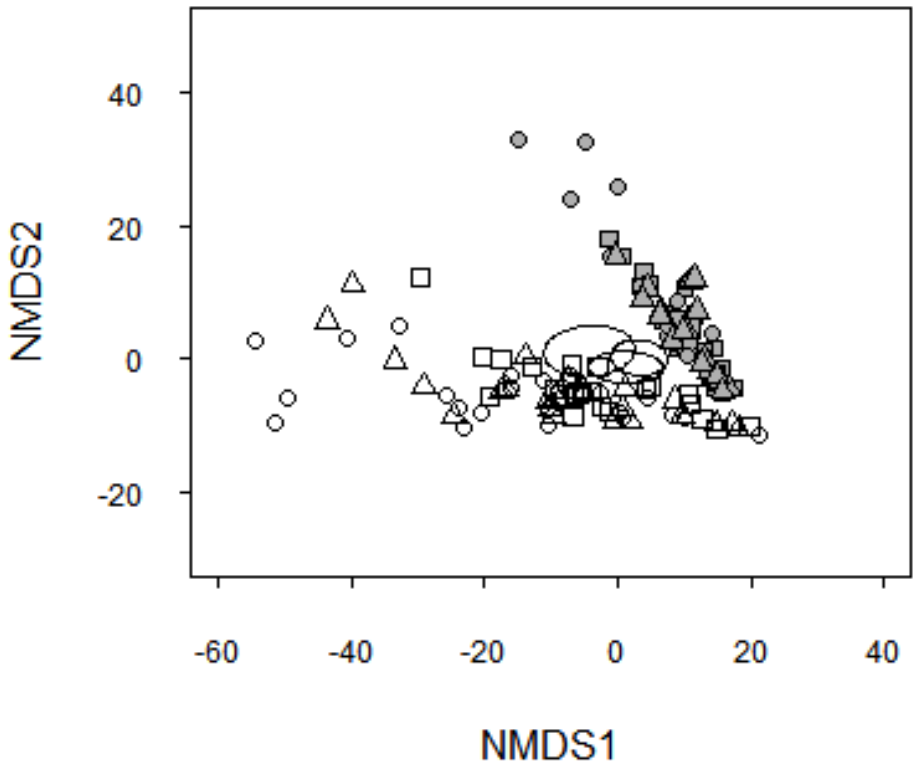


Fig. 6.4 Non-metric multidimensional scaling plot of soil PLFA composition in un-inoculated (squares), *Hypholoma fasciculare*- (circles), and *Phanerochaete velutina*- (triangles) inoculated plots in winter (shaded shapes) and summer (open shapes). Ellipses indicate 95 % confidence intervals for fungal inoculation treatments.

Table 6.1 Main effects (linear mixed effects: $F_{\text{degrees of freedom}}$ and P values) of fungal inoculation treatment and season on microbial biomass indicators and mycophagous micro-arthropod communities.

Parameter	Fungus		Season	
	$F_{1, 132}$	P	$F_{1, 132}$	P
Ergosterol (μg g^{-1} soil)			10.9	0.001
PLFA (nmol g^{-1} soil):				
Total	5.0	0.008	26.5	< 0.001
Fungi	5.5	0.005	41.5	< 0.001
Bacteria	4.6	0.011	25.0	< 0.001
Gram positive bacteria	3.5	0.032		
Gram negative bacteria	4.9	0.009		
Collembola abundance (g^{-1} soil)			40.4	< 0.001
Collembola diversity*			76.8	< 0.001
Oribatid mite abundance (g^{-1} soil)				
Oribatid mite diversity*			18.6	< 0.001

Non-significant ($P \geq 0.05$) results are omitted for clarity.

* Simpson's reciprocal D (see *Section 6.2.6*)

6.3.3 Wood decay rates

Hypholoma fasciculare decomposed wood at a greater rate than *P. velutina* (fungus: $F_{1, 76} = 7.6$, $P = 0.007$); this was driven by the effects of 'current' and 'double' woodlouse densities in winter, and 'reduced' densities in summer (Fig. 6.5; fungus*density* season: $F_{2, 76} = 4.9$, $P = 0.010$). Decay by *P. velutina* was stimulated by the 'double', compared with the 'reduced' woodlouse density treatment during the 6 months leading up to the summer sampling (Fig. 6.5). Fungal-mediated wood decay was more rapid overall during the latter half of the study (Fig. 6.5; season: $F_{1, 76} = 28.2$, $P < 0.001$).

6.3.4 Extracellular enzyme activities

Only leucine aminopeptidase and peroxidase activities were affected by woodlouse population density and fungal inoculation treatments. For leucine aminopeptidase, treatment effects were only evident in winter (Fig. 6.5; fungus*density*season: $F_{4, 117} = 2.77$, $P = 0.030$). Doubling woodlouse population density increased and decreased leucine aminopeptidase activity in *P. velutina*- and un-inoculated plots, respectively, compared with 'reduced' and 'current' densities (Fig. 6.5). Peroxidase activity was reduced by woodlice in un-inoculated plots (Fig. 6.5; fungus*density: $F_{4, 102} = 5.2$, $P = 0.001$), reflecting final population density. 'Reduced' density treatments had the highest peroxidase activity, whereas 'current' density treatments

(highest woodlouse abundance and the end of the study; Fig. 6.2) had the lowest activity. The activities of several of the hydrolytic enzymes were reduced in summer (Appendix 4); only season affected the activity of the overall enzyme complement (PERMANOVA: $F_{1, 101} = 8.8$, $P = 0.002$). Seasonal effects reflected the strong associations of the hydrolytic enzymes and phenoloxidase with ergosterol and soil moisture (Table 6.2). Peroxidase activity was strongly associated with soil pH (Table 6.2).

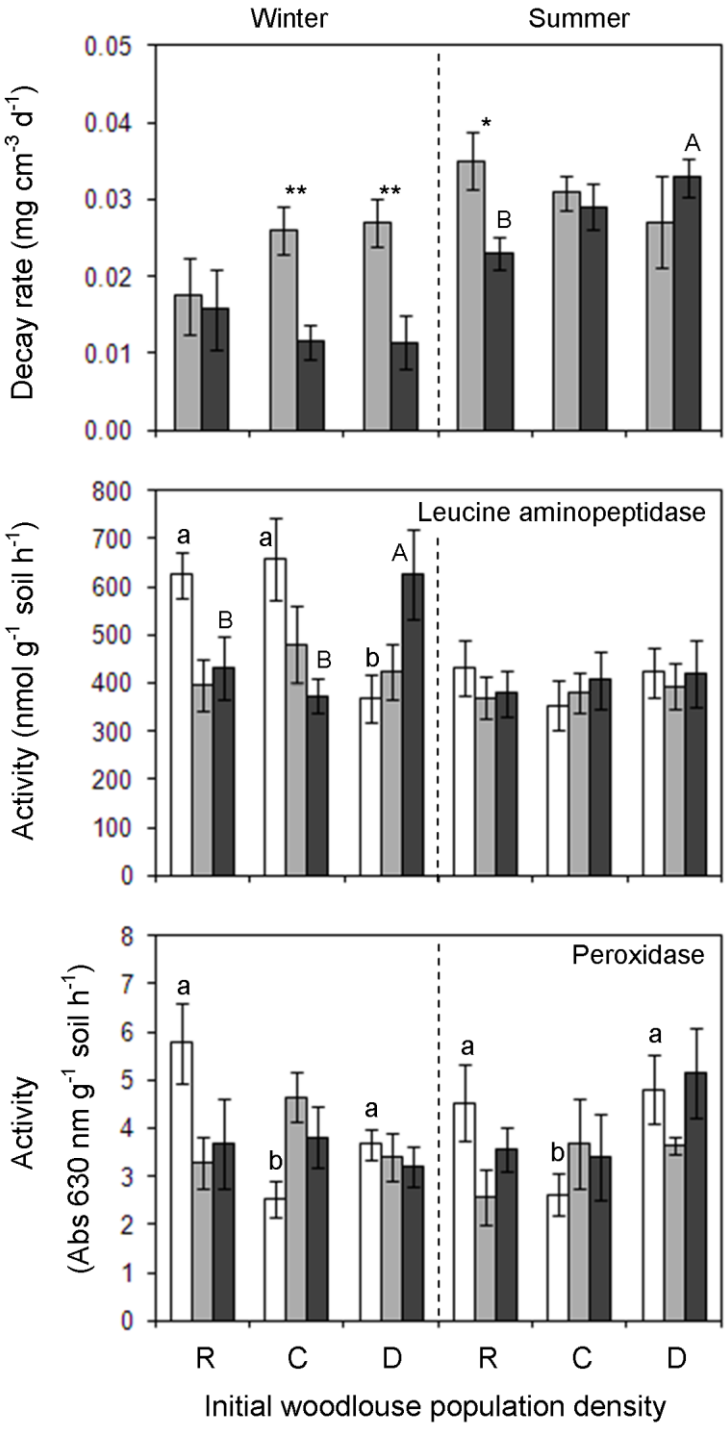


Fig. 6.5 Beech (*Fagus sylvatica*) wood decay rate (± SEM) and soil extracellular enzyme activities (± SEM) in un-inoculated (open bars), *Hypholoma fasciculare*- (light shading) and *Phanerochaete velutina*- (dark shading) inoculated field plots, with reduced (R), current (C) and double (D) woodlouse (*Oniscus asellus*) population densities. Significant (* $P < 0.05$, ** $P < 0.01$) differences in decay mediated by the two fungal species, at a given woodlouse density, are indicated. Lower- and upper-case letters indicate significant ($P < 0.05$) differences between woodlouse density treatments for un-inoculated and *P. velutina*-inoculated plots, respectively, within a given season. Note that units and scales differ between Y axes.

Table 6.2 Correlation matrix of enzyme activities, fungal biomass (ergosterol, μg g^{-1} soil), soil moisture (% oven dry soil weight), organic C (μg g^{-1} soil), total N (μg g^{-1} soil) and pH.

	BG	CBH	XYL	NAG	AP	LAP	PER	POX	Ergosterol
BG		**0.800**	**0.872**	**0.713**	**0.652**	**0.730**	-0.198	0.215	**0.732**
CBH			**0.796**	**0.661**	**0.588**	**0.622**		*0.228*	**0.657**
XYL				**0.727**	**0.829**	**0.718**	*-0.282*	*0.273*	**0.853**
NAG					**0.533**	**0.677**	-0.210	0.172	**0.580**
AP						**0.521**	**-0.361**	*0.272*	**0.835**
LAP							-0.189	*0.252*	**0.628**
PER								*-0.282*	*-0.303*
POX									**0.306**
Soil moisture	**0.729**	**0.607**	**0.838**	**0.640**	**0.821**	**0.636**	*-0.259*	**0.316**	**0.881**
Organic C	**0.621**	**0.588**	**0.791**	**0.490**	**0.894**	**0.528**	**-0.364**	*0.233*	**0.866**
Total N	**0.598**	**0.577**	**0.776**	**0.480**	**0.890**	**0.528**	**-0.359**	*0.239*	**0.843**
pH	**-0.418**	**-0.412**	**-0.614**	**-0.284**	**-0.775**	**-0.365**	*0.283*	*-0.252*	**-0.747**

Significance levels for correlation co-efficients are indicated by font style (normal: $P < 0.05$; italicised: $P < 0.01$; bold: $P < 0.001$. Enzymes are: β-glucosidase, BG; β-xylosidase, XYL; cellobiohydrolase, CBH; *N*-acetyl-glucosaminidase, NAG; acid phosphatase, AP; leucine aminopeptidase, LAP; peroxidase, PER; phenoloxidase, POX.

6.3.5 Soil micro-arthropods

The abundance and diversity of collembola and oribatid mites were not significantly ($P \geq 0.05$) affected by fungal inoculation or woodlouse population density. Collembola abundance was higher and diversity lower (reflecting increased dominance of *Friesea mirabilis*; data not shown) in summer than winter (Table 6.1; Appendix 5). Oribatid mite diversity was higher in summer than winter and abundance was unaffected (Table 6.1; Appendix 5).

6.4 Discussion

Woodlouse (*Oniscus asellus*) population densities were manipulated in beech woodland, simulating changes in abundance predicted for temperate regions as a consequence of climate warming (David and Handa 2010). Having recently been suggested as keystone grazers in fungal-dominated woodland decomposer communities (Crowther *et al.* 2013), changes in *O. asellus* abundance were expected to have important consequences for the composition and function of the wider decomposer community. This is the first study to report bottom-up effects of fungal species dominance on woodlouse abundance (supporting Hypothesis 1). Analogous effects on

collembola (more specialist fungal-feeders) have been demonstrated in short-term laboratory manipulations (Chapter 3; Chapter 4; Crowther *et al.* 2013). Saprotrophic fungi were a major and consistent component of the diet of *O. asellus*, even in *H. fasciculare*-dominated microbial communities where no differences remained between population density treatments. Thus the results do not indicate a diet switch of isopods in *H. fasciculare*-inoculated plots, even though this fungus is considered unpalatable to most soil invertebrates (an exception is the diplopod, *Blaniulus guttulatus*; Crowther *et al.* 2011a, b); it produces sesquiterpenes which likely deter mycophagy and may even be toxic to grazers (Hynes *et al.* 2007). The effect of fungal dominance on the persistence of distinct woodlouse densities does not, therefore, reflect a change in dietary composition (in contrast to Hypothesis 1).

The compositions of the wider decomposer microbial and mycophagous micro-arthropod communities were not affected by woodlouse population density (in contrast to Hypotheses 2 and 3). Woodlouse densities at the study site were lower than those in forest floor populations previously reported (e.g. 35-130 m^{-2}, Topp *et al.* 2006; 200 m^{-2}, Zimmer and Topp, 2000), and used as low estimates of field density in laboratory studies (Crowther *et al.* 2013). Excess soil moisture due to heavy autumn and early winter precipitation, and a prolonged period of sub-zero temperatures during early spring could have caused high *O. asellus* mortalities (Zimmer 2004). This indicates that climate extremes may be important moderators of isopod abundance and should be considered when making predictions for population responses to climate warming. As microbial biomass was not affected by the number of isopods, the *O. asellus* densities employed in the present study were likely to have been too low to exert a grazing pressure strong enough to reduce mycelial growth. Increased microbial biomass in *H. fasciculare*-inoculated treatments suggests positive associations with bacterial taxa. Whilst bacterial abundance in beech wood is reduced by *H. fasciculare* activity (e.g. acidification; de Boer *et al.* 2010), it can be stimulated in soil adhering to exploratory hyphae (Gramss *et al.* 1999; Folman *et al.* 2008).

Despite the low *O. asellus* field densities, altered population density did elicit some process-specific effects on microbial community functioning. These effects were dependent on fungal dominance in the microbial community. The activities of leucine aminopeptidase (in winter only) and lignin-degrading peroxidase, in un-inoculated

plots, were lowest where final *O. asellus* abundance was highest (partially supporting Hypothesis 4). In contrast, certain functional aspects (leucine aminopeptidase activity in winter and wood decay during the latter 6 months of the study) of *P. velutina*-dominated communities increased with woodlouse population density. Hydrolytic enzyme (β-glucosidase, cellobiohydrolase, *N*-acetyl-glucosaminidase, acid phosphatase) production by *P. velutina* was stimulated by *O. asellus* grazing in a short-term (10 days) microcosm study (Crowther *et al.* 2011c), but reduced due to extensive mycelial removal after three months in more realistic woodland soil mesocosms (Crowther *et al.* 2013). Increased activity of the fungal biomass could reflect enzyme leakage from damaged mycelia or the stimulation of enzyme production as substrate availability is increased by grazers liberating hyphal cell contents (Wells and Boddy 2002). Stimulation of β-glucosidase activity has previously been suggested as a consequence of increased glucose requirements for energetically expensive mycelial repair and growth responses to grazing (Burns 1982; Hedlund *et al.* 1991; Crowther *et al.* 2011d). As fresh hyphal tips are often preferentially grazed over dense mycelium or thick cords (Tordoff *et al.* 2006, 2008), increased production of specific extracellular enzymes by fungi as a stress response to grazing is the most likely mechanism explaining the reported alterations to microbial activity. Such responses could indirectly affect the mineralisation of soil nutrients.

Despite the handful of enzyme-specific responses to woodlice and fungal inoculation, seasonal influences on decomposer community composition and function were much stronger. Organic matter quality, temperature and diffusion of substrates (influenced by moisture) are well known constraints to enzyme activities (Burns *et al.* 2013). Reduced soil moisture in the summer was more important than warmer temperatures in regulating microbial biomass and enzyme production (Chapter 5), particularly that of hydrolases, which are correlated with mean annual precipitation at a global scale (Sinsabaugh *et al.* 2008). Soil pH likely constrained peroxidase and phenoloxidase activities (optimum pH 8; Sinsabaugh 2010).

Saprotrophic fungi were confirmed as a major component of the woodlouse diet, with the bottom-up effect of fungal community dominance regulating field population size. Although relatively low woodlouse field densities and poor survival, potentially due to climate extremes, resulted in no influence on microbial community composition,

fungal-feeding had consequences for certain aspects of microbial community functioning. Stronger effects on fungal biomass and activity are likely at microsites of woodlouse aggregation, such as beneath logs (200-630 m^{-2}; Topp *et al.* 2006), and where forest floor population densities are higher.

7 General discussion

The studies reported in this thesis employed a combination of laboratory microcosm and mesocosm, and field manipulations to further mechanistic understanding of potential climate change effects on community dynamics and ecosystem processes, in a cord-forming basidiomycete fungal-dominated woodland soil decomposer system. A review of the literature on ecophysiological relationships between saprotrophic basidiomycetes and abiotic factors (e.g. temperature and water potential), and meta-analysis of soil invertebrate responses to climate change, stimulated the general hypothesis that increased fungal biomass and activity in temperate woodlands, due to climate change, could be moderated by a concomitant increase in grazing pressure from mycophagous invertebrates (Chapter 2). This hypothesis was tested with a microcosm-based exploration of the influence of a 3 °C increase in ambient temperature on the balance between mycelial growth and removal by grazing collembola (Chapter 3). Interactions between five fungus and two collembola species revealed that species-specific grazing preferences and differential fungal palatability regulated the strength of top-down and bottom-up effects (Chapter 3). Collembola abundance markedly increased at elevated temperature only when grazing particular fungi (bottom-up), resulting in increased grazing pressure and the prevention of warming-induced mycelial expansion (top-down). Limitation of a fungal growth response to warming by grazing, where this occurred, did not prevent increased decomposition of colonised wood at elevated temperature. Increased resource decay due to warming could reflect increased enzyme activity (Davidson and Janssens 2006; Sinsabaugh *et al*. 2008; Wallenstein *et al*. 2009) and/or production by mycelia. Wood may have been decayed more rapidly by fungi to provide the carbon, energy and nutrients required to produce more mycelium, in order to maintain explorative growth as grazing pressure increased (Crowther *et al*. 2011d).

The use of microcosms enables the study of complex and opaque systems (Lawton 1995, 1996). They are not intended to be completely representative of the natural situation, but they can provide insights into otherwise inaccessible interactions and processes. Laboratory studies have long been used in ecology to identify salient questions for exploration in more realistic systems. With regards to the woodland decomposer system that is the subject of this current research, the use of collembola as model fungal grazers enabled assessment of bottom-up influences on mycophagous

invertebrate populations (due to their short generation times) as well as the potential for top-down limitation of fungal responses to climate change.

The relative importance of top-down and bottom-up determinants of decomposer community dynamics and functional (fungal-colonised wood decay and extracellular enzyme activities) consequences were assessed further in woodland soil mesocosms; a realistic biotic community subjected to controlled climate change (warming and drying or increased precipitation). Although elevated temperature increased abundance in a multi-species collembola community, their populations were more strongly regulated by the bottom-up effect of mesocosm inoculation with cord-forming fungi (Chapter 4). In contrast with the expectation that inoculated mycelia would be grazed, limiting their growth, collembola abundance was lower and displayed diminished responses to climate in these systems. Collembola are capable of grazing basidiomycete mycelia, but the cord thickness and chemical defences are likely to make them less palatable than soil microfungi. As a consequence of the competitive dominance of these fungi, mycelial establishment in mesocosms has been shown to reduce the biomass of other fungal taxa and overall community diversity (Crowther *et al*. 2013). This imposed a resource limitation on collembola population density. Bottom-up effects reported in microcosms were, therefore, important in regulating collembola population dynamics in mesocosms.

In the absence of any capacity for top-down control of fungal biomass by collembola, in mesocosms, the direct effect of climate regulated the functioning of the microbial community (Chapter 5). Drying decreased fungal-mediated wood decomposition rate, whereas warming resulted in an increased rate and compensated for the negative effect of drying. Moisture regulated the extracellular enzyme pool; potential activities were significantly greater under wetting than drying. Again, elevated temperature consistently compensated for the negative effect of drying, but did not increase enzyme activity, alone or in combination with wetting. This reflected microbial production (fungal biomass was not increased under these conditions) rather than *in situ* effects on enzyme kinetics. The patterns of response of a range of hydrolytic and oxidative enzymes were similar. This suggests that fungal-mediated carbon and nutrient cycling will be very responsive to climate change and demonstrates that interactions between abiotic factors are not always predictable from the manipulation of single variables.

Although collembola themselves did not limit mycelial biomass and activity in more complex systems, the concepts suggested by the microcosm observations have gained support for other taxa. Macro-invertebrates, such as woodlice and millipedes, consistently exert stronger effects than collembola on the growth of individual mycelia and interspecific interaction outcomes (Crowther *et al*. 2011a, b, 2013; A'Bear *et al*. 2013d). Their larger size and greater metabolic requirements make them better able to exploit nutritious, but thick and defensive, basidiomycete cords. The top-down effects of collembola in two-species microcosm interactions appear more representative of macro-invertebrates, such as woodlice, in mesocosms. Extensive removal of mycelia from the surface of mesocosm soil by woodlice (*Oniscus asellus*) has been shown to prevent the aforementioned competitive suppression of other fungal taxa (and consequent limitation on collembola abundance) and reduce extracellular enzyme activities (Crowther *et al*. 2013).

The ubiquity of woodlice and basidiomycete fungi in temperate woodland ecosystems, coupled with predictions for increased abundance of the former as their regional climate becomes warmer and wetter (David and Handa 2010), suggest that such top-down community control could be of widespread significance for carbon and nutrient cycling under climate change. Woodlice are generalist detritivores; it is not clear whether extensive fungal ingestion in laboratory studies is a reflection of palatability, or conspicuousness due to the large size and overall biomass of individual mycelia. The multi-trophic and functional consequences of woodlouse grazing were tested in a field manipulation of *O. asellus* population densities. This year-long study provides the first evidence that bottom-up effects of fungal palatability, revealed as being important in regulating collembola population densities, also affected woodlice (Chapter 6).

The presence of saprotrophic fungal markers in *O. asellus* body lipids revealed direct mycelial grazing as a major and consistent component of their generalist diet (Chapter 6). Even though woodlouse population densities at the field site were relatively low (and may have been further reduced by extremes of climate during the winter and early spring), altered *O. asellus* abundance did influence certain aspects of microbial community functioning. Wood decay and the activity of one enzyme (leucine aminopeptidase) in *Phanerochaete velutina*-dominated plots were stimulated, rather

than reduced; a typical consequence of low intensity or short-term grazing (Crowther *et al*. 2011d). Effects of grazing on enzyme activity and decomposition depend on the extent of mycelial damage. Fungi exhibit mycelial growth responses to short-term or low intensity grazing; these are energetically expensive, so enzyme activity and resource decay increase (Burns 1982). Extensive mycelial removal, as a consequence of high intensity or long-term grazing, reduces enzyme activities (Crowther *et al*. 2013). The latter may be most important at microsites of macro-invertebrate aggregation, such as under large woody debris, where they may also seek refuge from extremes of climate (prolonged cold, wet or dry conditions) that could cause mortality (Chapter 6).

7.1 Conclusions

The presence, and to some extent identity, of a saprotrophic cord-forming basidiomycete influenced invertebrate abundance. Although collembola can graze fungal cords (Chapter 3), they appeared to avoid them in realistic soil communities containing other microbial food sources (Chapter 4). Macro-invertebrates, particularly woodlice, are better able to exploit cords as a food source and can have dramatic effects on mycelial growth and functioning. Woodlice do, however, also appear to be affected by the palatability of basidiomycete mycelia (Chapter 6). The true reason for the apparent unpalatibility of the study fungi to collembola and, in some cases, woodlice, is a tantilising prospect for future research. Potential defences include secondary metabolites, such as sesquiterpenes, and the presence of oxalate crystals on the surface of cords, but it is not know if such defence responses are important in deterring mycophagy or upregulated in response to grazing.

The influence of variation in soil moisture, as a consequence of targeted treatments or natural seasonal fluctuations, on fungal biomass and enzyme activity was consistent between laboratory and field. Climate extremes may be more important than overall trends in regulating decomposer community functioning (Reichstein *et al*. 2013). Drought has particular potential to affect soil biota negatively, which could moderate the influence of general warming trends and consequent temperature-driven increases in soil CO_2 efflux. Woodland soils, however, do not reach extremes of moisture limitation as readily as other more exposed systems (e.g. grasslands), and cord-forming fungi are relatively resistant to fluctuating moisture conditions (Chapter 2). Differential responses of *in situ* wood decay and potential enzyme activities to warming (Chapter 5)

indicate that, while climatic extremes may prevent a sustained increase in the size of enzyme pools, their activity in decomposition will likely be stimulated by elevated temperature. Further field experimentation, in which elevated temperature and rainfall extremes are simulated, would shed further light on decomposer community responses to climate change and the consequences for ecosystem processes. The importance and direction of biotic effects on decomposition may be more heterogeneous than abiotic influences, depending on microbial community dominance and the density of key macro-invertebrate taxa.

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Appendix 1

Meta-analysis of the response (lnR [=ln(X_T/X_C), where X_T and X_C are the treatment and control means, respectively] and bootstrapped 95% CI) of soil invertebrate abundance to elevated temperature (T), elevated CO_2, increased precipitation (P), and drought (D) (*Chapter 2*).

	Factor(s)	n	lnR	Bootstrapped CI	Source
Nematoda	T	82	-0.0589	-0.1764 to 0.1235	1–4
	CO_2	416	-0.2252	-0.3253 to -0.1326*	2, 5–14
	P	7	1.2709	0.6009 to 1.7591*	2, 15
	D	70	-0.1267	-0.3024 to 0.0509	3, 14
	T × CO_2	6	0.0308	-0.1597 to 0.3152	2
	T × P	6	0.8361	0.5700 to 1.2276*	2
	CO_2 × P	6	1.5174	0.3250 to 1.6730*	2
	T × CO_2 × P	6	1.2226	0.7427 to 1.3570*	2
Plant-feeders	T	11	-0.4036	-0.7678 to -0.1120*	2, 3
	CO_2	130	-0.0465	-0.3085 to 0.2406	2, 8–14
	D	10	-0.2859	-0.5176 to -0.0746*	3, 14
Bacterial-feeders	T	40	-0.1116	-0.4069 to 0.1075	1–4
	CO_2	128	-0.2768	-0.4425 to -0.1290*	2, 8, 10–14
	D	34	-0.2494	-0.4781 to -0.0181*	3, 14
Fungal-feeders	T	12	0.3280	0.0542 to 0.7178*	1–3
	CO_2	75	-0.4081	-0.5862 to -0.2278*	2, 8, 10–14
	D	10	-0.0128	-0.6234 to 0.6133	3, 14
Carnivores	T	16	0.2344	0.0371 to 0.4822*	2, 3
	CO_2	74	-0.3205	-0.4406 to -0.2111*	2, 8–14
	D	16	0.1406	-0.2620 to 0.5378	3, 14
Acari	T	169	-0.1749	-1.7182 to 0.1392	16–25
	CO_2	20	-0.1785	-0.3979 to 0.0006	5, 6, 14, 16, 18, 26–28
	P	15	-0.2831	-0.4030 to 0.0962	16, 29–33
	D	9	-0.7392	-1.5572 to -0.4464*	14, 26, 32, 34
	T × CO_2	5	0.1709	0.0091 to 0.7298*	16, 19
	T × P	3	1.5019	0.4520 to 2.0149*	16
	T × D	35	-1.7348	-2.2566 to -1.1331*	25
	CO_2 × P	2	-0.9255	-1.6964 to -0.5798*	16
	T × CO_2 × P	3	0.3868	-0.0513 to 0.6098	16
Collembola	T	96	0.1163	-0.1489 to 0.3276	16–21, 23, 24, 35–38
	CO_2	34	-0.0665	-0.3455 to 0.3451	5, 6, 14, 16, 19, 26–28, 39–41
	P	11	0.0275	-0.3306 to 0.4336	16, 30–32, 36
	D	30	-0.5273	-0.9641 to -0.0145*	14, 31, 33, 34, 38
	T × CO_2	9	0.3612	0.0490 to 0.6114*	16, 19
	T × P	6	1.2525	0.8396 to 1.6868*	16, 36
	T × D	12	-0.3694	-2.0515 to 0.6314	25
	CO_2 × P	2	0.9023	-0.5596 to 2.3643	16
	T × CO_2 × P	2	0.2795	-0.5596 to 1.6078	16
Enchytraeidae	T	13	0.6064	0.2134 to 0.8853*	19, 20, 42–46
	CO_2	5	-0.4327	-1.9082 to 0.4252	5, 19, 43
	D	5	-0.6406	-1.8075 to -0.3815*	43, 47, 48
	T × CO_2	3	0.2025	0.0206 to 1.2657*	19, 43

n = number of observations; * bootstrapped 95 % CIs does not overlap zero.

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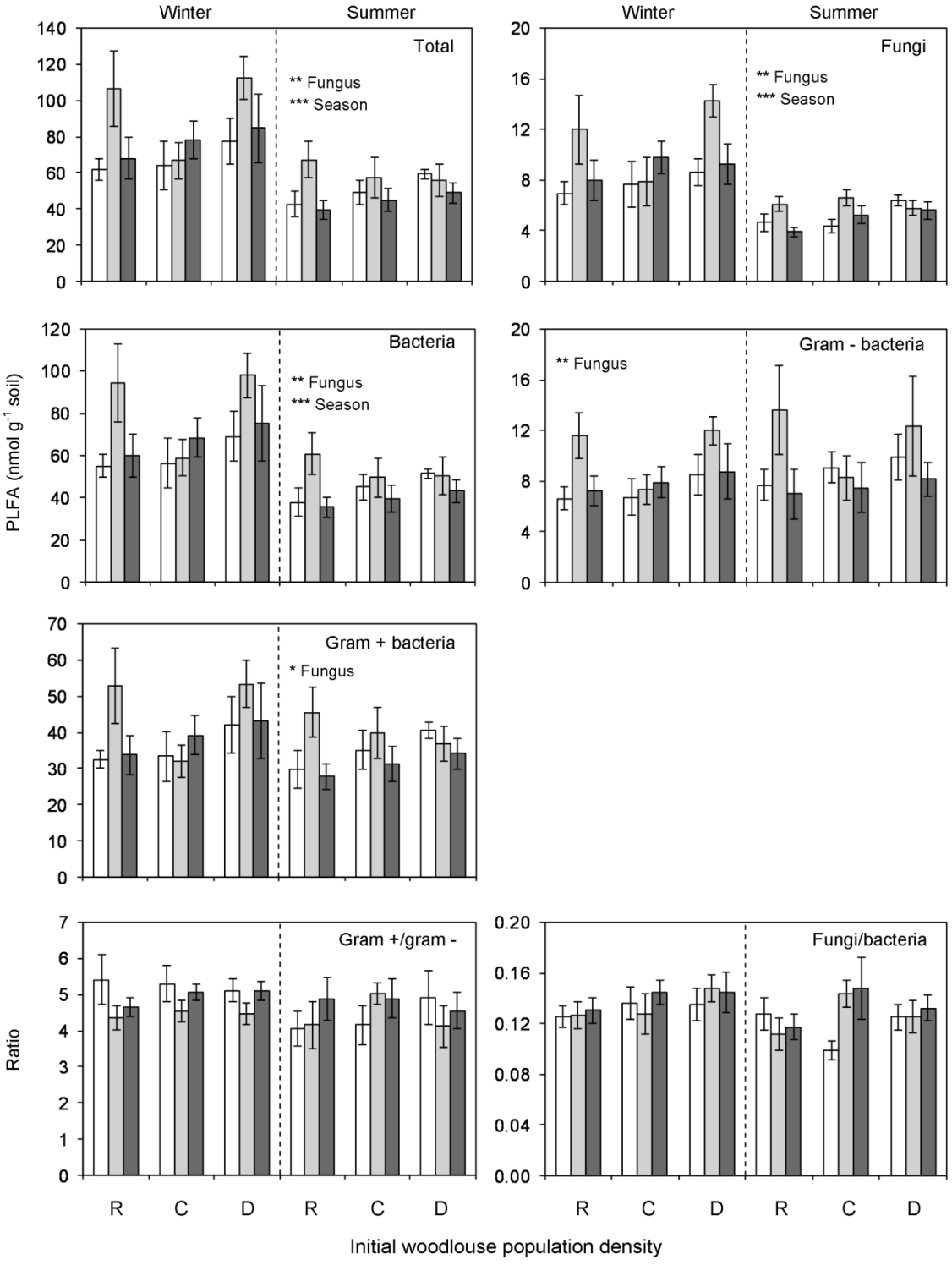
Appendix 2

PLFA composition of recovered *Oniscus asellus* (*Chapter 6*).

PLFA	Percentage	Standard deviation
14:0	0.22	0.08
15:0	0.52	0.11
16:1ω7	2.57	0.73
16:0	12.23	1.22
i17:1	0.74	0.29
a17:0	0.35	0.38
17:1ω8	0.56	0.15
cy17:0	0.34	0.14
18:3ω6	0.20	0.05
18:2ω6	27.56	2.00
18:1ω9	33.86	1.32
18:0	6.58	1.14
19:1ω11	1.39	0.29
20:4ω6	7.02	1.02
20:5ω3	2.81	0.42
20:3ω6	0.30	0.14
20:2ω6	1.40	0.21
20:3ω3	0.25	0.05
20:0	0.77	0.20
22:1ω9	1.46	0.70
22:0	0.28	0.08

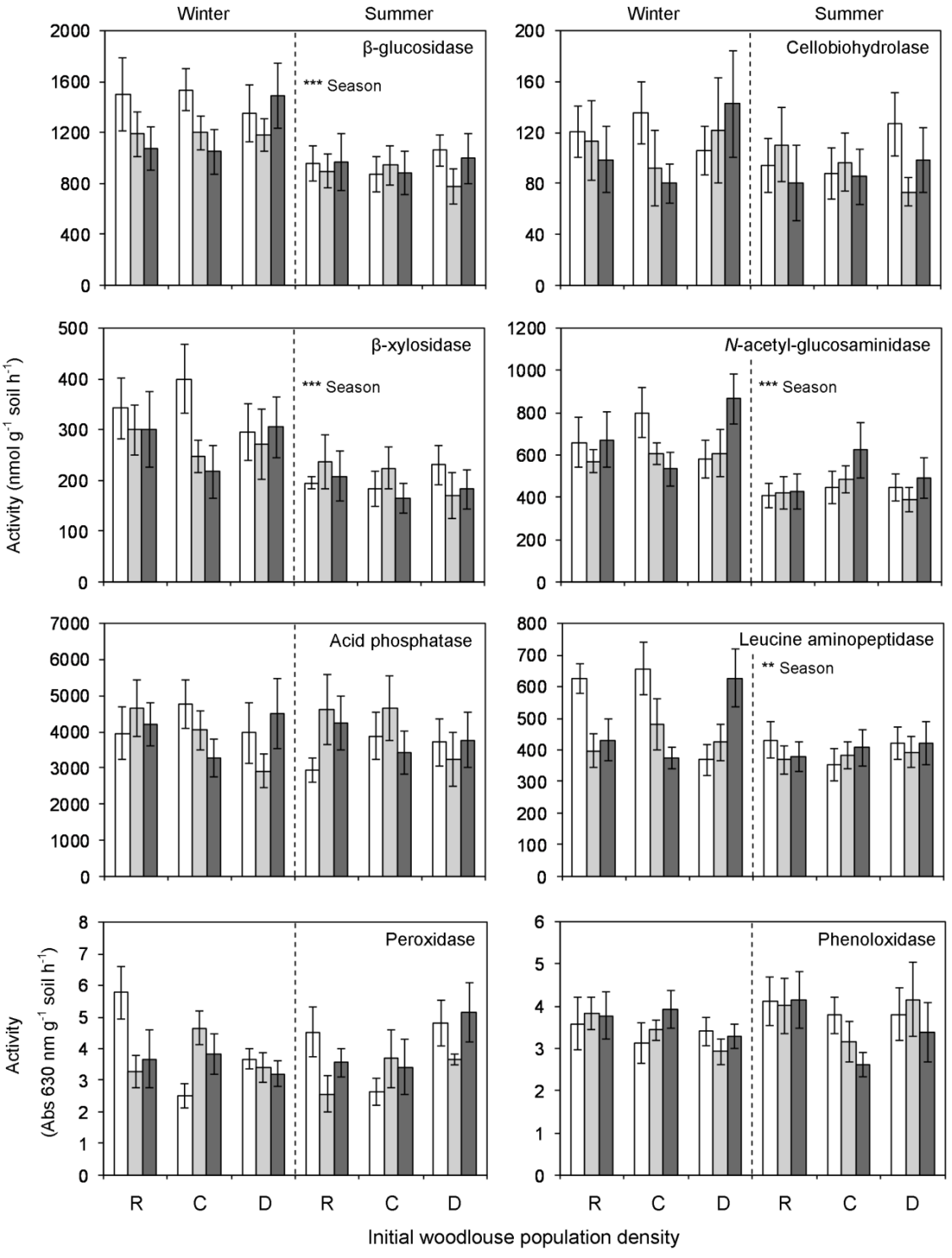
Appendix 3

Soil PLFA content (± SEM) for different microbial groups in un-inoculated (open bars), *Hypholoma fasciculare*- (light shading) and *Phanerochaete velutina*- (dark shading) inoculated field plots, at reduced (R), current (C) and double (D) woodlouse (*Oniscus asellus*) population densities (*Chapter 6*). Significant (* P < 0.05, ** P < 0.01, *** P < 0.001) effects of fungal inoculation treatment and season are indicated. Note that scales differ between Y axes.



Appendix 4

Soil extracellular enzyme activities (± SEM) in un-inoculated (open bars), *Hypholoma fasciculare*- (light shading) and *Phanerochaete velutina*- (dark shading) inoculated field plots, at reduced (R), current (C) and double (D) woodlouse (*Oniscus asellus*) population densities (*Chapter 6*). Significant (** P < 0.01, *** P < 0.001) seasonal differences are indicated. Note that units and scales differ between Y axes.



Appendix 5

Abundance (± SEM) and species diversity (Simpson's reciprocal D = $1/\sum_{i=1}^{n} P_i^2$, where P_i is the proportion of individuals in the ith species and n is the total number of species) (± SEM) of collembola and oribatid mites in un-inoculated (open bars), *Hypholoma fasciculare*- (light shading) and *Phanerochaete velutina*- (dark shading) inoculated field plots, at reduced (R), current (C) and double (D) woodlouse (*Oniscus asellus*) population densities (*Chapter 6*). Significant (*** P < 0.001) seasonal differences are indicated. Note that units and scales differ between Y axes.