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Effects of pre-colonisation and temperature on interspecific fungal interactions in wood

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ABSTRACT

Understanding the effects of changing abiotic conditions on assembly history in wood decay communities is especially important with predicted environmental changes. Interspecific interactions drive community development, so it is important to understand how microclimatic environment affects outcomes of interactions between species from different successional stages in *natural* substrata. Interactions between eight wood decay fungi were performed in beech (*Fagus sylvatica*) wood at seven temperatures (12–30 °C), and in soil microcosms and wood that had been pre-colonised for different lengths of time. The hierarchy of combative ability could be altered by changes in temperature: at higher temperatures early secondary colonisers were able to outcompete usually later colonising cord-forming species. Length of pre-colonisation had a species-specific effect on combative ability, probably attributable to biochemical changes rather than the state of decay of the resource. Abiotic variables have clear effects on fungal interactions, underlining the importance of stochastic factors in fungal community succession.

1. Introduction

Saprotrophic decay fungi dominate primary wood decomposition in temperate woodlands, and are key determinants of carbon sequestration and nutrient cycling (Boddy and Watkinson, 1995; Hättenschwiler et al., 2005; Baldrian and Lindahl, 2011). Competition between mycelia for territory and the resources within is central to their ecology, and antagonistic interactions occur where there is overlap between the niches of different species or strains (Boddy, 2000). Apart from the very early and very late stages of decomposition, or under high environmental stress, community composition is determined by these antagonistic interactions. Antagonism is mediated through morphological changes such as the production of barrages and invasive cords, and metabolic changes such as the upregulation and secretion of antifungal toxins, metabolites, and oxidative enzymes (Boddy, 2000; Baldrian, 2004; Heilmann-Clausen and Boddy, 2005; Hiscox et al., 2010). Interactions either result in replacement of one mycelium by another, or deadlock, where neither mycelium can gain territory from the other, although a variety of outcomes can occur between these extremes (Boddy, 2000).

Depending on the timing of their development, the fungal community can be categorised into primary, secondary, late secondary, and end-stage colonisers. Primary colonisers are often ruderal or specialised opportunists which arrive as spores, many of which will have been latently present as endophytes within functional sapwood (Parfitt et al., 2010). The primary colonisers usually cause limited decay before they are replaced by early secondary colonisers, which likely arrive at the resource as spores and cause more extensive decomposition and utilisation of the resource (Boddy, 2000). These are in turn replaced by more combative 'later' secondary colonisers and end-stage colonisers, often arriving at the resource as mycelial cords, which are linear aggregations of hyphae

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that grow out of colonised resources, foraging for new ones (Holmer and Stenlid, 1993; Boddy and Heilmann-Clausen, 2008; Fricker and Bebber, 2008; Boddy et al., 2009). There is a general hierarchy of combative ability where primary colonisers are the least combative and late secondary colonisers the most, but these relationships are not always transitive, and certain species may outcompete others in some situations due to their tolerance of specific environmental stresses rather then through combative ability (Boddy, 2000; Boddy and Heilmann-Clausen, 2008).

Both biotic and abiotic factors have been shown to affect the progress and outcomes of interactions (e.g. invertebrate grazing, gaseous regime, water potential, substrate quality; Boddy et al., 1985; Griffith and Boddy, 1991; Crowther et al., 2014; Venugopal et al., 2016). Temperature changes can reverse outcomes of interactions between cord-forming fungi in soil (Crowther et al., 2012; A'Bear et al., 2012; A'Bear et al., 2013), because different species display contrasting sensitivities and patterns of response (Boddy, 1983a; A'Bear et al., 2013). Temperature has also been shown to alter fungal assembly history in mixed communities (Toljander et al., 2006). Temperature optima for wood decay basidiomycetes vary between species, although most are mesothermic with minimum, optimum, and maximum temperatures for growth around 5, 25, and 40 °C (Cartwright and Findlay, 1958; Magan, 2008). Fungal decomposition rates increase with temperature to similar optima (Boddy, 1986; A'Bear et al., 2012; Venugopal et al., 2016). Investigations into the effects of temperature on interactions have mostly used small shifts in temperature, and have not studied interactions between competitors from different successional stages.

Different species of fungi decompose wood at different rates and in different ways. An extreme example of this is the difference between white and brown rot fungi, which use different enzymatic processes to attack lignin to access bound cellulose and hemicellulose (Eastwood et al., 2011). However, even between white rot species, the relative proportion and location of substrates used will vary, due to differences in production and specificity of oxidative enzymes (Tuor et al., 1995; Worrall et al., 1997). Further, production and deposition of secondary metabolites differs between species, or different species may maintain a specific water potential or pH within the resource (Heilmann-Clausen and Boddy, 2005; Woodward and Boddy, 2008). Within a decaying woody resource, there would be patches of the resource in different states of structural and chemical modification, due to historical occupancy by different species (Pyle and Brown, 1999; Kubartova et al., 2012). These alterations of the resource may affect the ability of a competitor to invade the resource, and are thought to be partly responsible for determining succession and assembly history within decomposing wood (Hiscox et al., 2015). In theory, the longer a mycelium has inhabited a resource, the greater the alteration of the resource and the more difficult it would be for a competitor to invade. For example, it was found that increasing the duration of colonisation of resources by *Gloeophyllum trabeum* increased its ability to outcompete the more combative *Irpex lacteus* (Song et al., 2015). Conversely, nutrients within the resource will be depleted with increasing colonisation time, making the resident mycelium less able to mount metabolically costly antagonistic mechanisms to resist invasion or capture new territory.

The aim of this work was to investigate the effect of ambient temperature and length of colonisation on the combative abilities of eight wood decay fungi in natural substrata. Interactions were set up in beech wood blocks, to simulate conditions within a piece of decaying wood where two mycelia from adjacent regions encounter each other. Interactions were also set up in soil microcosms (colonisation length experiment only), to simulate the interactions between mycelial cords and remote resources. Firstly, the effect of temperature was investigated by incubating interacting blocks at seven temperatures spanning 12–30°C; we hypothesise that different species will vary in their temperature optima, and their ability to tolerate temperature stress, which will lead to changes in interaction outcomes at different temperatures. Secondly, interactions were set up between blocks that had been pre-colonised for short (2 or 3 months) or long (9 or 12 months) periods; we hypothesise that the longer a species has inhabited a resource, the more it will have depleted the nutrients within, so that combative ability decreases as colonisation time increases.

2. Methods

2.1. Preparation of inocula

Eight native, beech (*Fagus sylvatica*)-inhabiting fungi (Table 1) from different stages of decay were used to colonise beech wood blocks. Blocks were either 20 × 20 × 10 mm (temperature experiment; colonisation length soil interactions experiment) or 20 × 20 × 20 mm (colonisation length wood interactions experiment; Fig. 1). Blocks were sterilised by autoclaving 3 times over 72 h, then placed onto cultures of single species on 0.5% malt agar (MA: 5 g l^{-1} malt extract, 15 g l^{-1} agar; Lab M, Lancs, UK) and incubated at 20 °C in the dark, following Hiscox et al. (2010a). Blocks were pre-colonised for either 3 or 12 months for wood block interaction experiments where blocks were paired by joining together (temperature experiment; colonisation length wood interactions experiment), or for either 2 or 9 months for soil microcosm experiments. Block densities, used as an indication of amount of decay, were determined destructively at the start of experiments as dry weight/fresh volume (g cm^{-3}; 15 replicates). Radial extension rates were determined for all species at all temperatures on 2% MA (5 replicates), by inoculating a 6 mm plug of agar plus mycelium centrally and measuring two diameters perpendicular to each other over 1–6 d.

2.2. Interactions between colonised blocks

Pre-colonised blocks were scraped free of adhering mycelium and paired with cut vessels touching (wood grain running in the same direction; Fig. 1). Blocks were held together using a sterile rubber band which was removed after 5 d. Paired blocks were placed directly onto perlite (25 ml; Homebase, UK) moistened with sterile distilled water to achieve a water potential of −0.012 kPa (determined by the method of Fawcett and Collins-George, 1967), in plastic 100 ml lidded pots (Cater4you, UK). The pots were incubated at 20 °C in the dark and watered fortnightly to maintain the water potential. A hole in the pot wall (1 × 2 mm diameter) covered in microporous surgical tape (3M, UK) allowed aeration. Interaction outcomes were determined by reisolation (length of interactions varied between experiments; Fig. 1). Blocks were split in half using a sterile chisel, perpendicular to the point of contact. Pieces of wood (2 mm^3) were excised approximately 2, 7, 12 and 17 mm from the point of contact, inoculated onto 2% MA and incubated at 21 °C until mycelia had emerged and could be identified morphologically. The proportion of the two blocks colonised by each species was estimated, and interaction outcomes recorded as deadlock, partial replacement or complete replacement. Final block densities were determined from the other half of the block as dry weight/fresh volume.

Interactions between wood blocks at different temperatures were established by pairing together blocks that had been colonised for 3 months (5 replicates per temperature), and incubating at 12, 15, 18, 21, 24, 27, or 30 °C in the dark (Fig. 1A). Interaction durations varied between temperatures, and were calculated using