available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/funeco

Antagonistic fungal interactions influence carbon dioxide evolution from decomposing wood

Jennifer HISCOX, Melanie SAVOURY, Ian P. VAUGHAN, Carsten T. MÜLLER, Lynne BODDY*

School of Biosciences, Cardiff University, Cardiff CF10 3AX, UK

ARTICLE INFO

Article history:
Received 16 June 2014
Revision received 23 October 2014
Accepted 1 November 2014
Available online
Corresponding editor:
Petr Baldrian

Keywords:
Antagonism
Basidiomycete
CO_2
Decomposition
Fungi
Interactions
Wood decay

ABSTRACT

Fungal species vary in the rate and way in which they decay wood. Thus, understanding fungal community dynamics within dead wood is crucial to understanding decomposition and carbon cycling. Mycelia compete for wood territory, by employing antagonistic mechanisms involving changes in morphology, and production of volatile and diffusible chemicals. This is metabolically costly, and may affect the rate of use of the resource. The metabolic rate during pairwise interactions between wood decay ascomycetes and basidiomycetes was determined by measuring CO_2 production. CO_2 evolution altered over time, but changes were combination-specific. In only two combinations – when the dominant competitor overgrew the opponent's territory as mycelia cords – did CO_2 evolution increase over the course of the whole interaction. In most interactions, CO_2 evolution increased only after complete replacement of one competitor, suggesting utilisation of the predecessor mycelium or differences in decay ability due to alteration of the resource by the predecessor. There was no relationship between rate of CO_2 evolution and combative ability nor outcome of interaction.

Introduction

Fungal community structure and development within wood is largely determined by interspecific interactions (Boddy and Heilmann-Clausen, 2008). Wood is a solid substratum, so competition for resources is effectively competition for space/territory, since when a fungus holds territory it potentially has access to all of the resources within that territory, provided that it has the enzymatic capacity to decompose lignocellulose (Boddy, 2000). Competition for territory, and hence changes in fungal community structure, is brought about by combative, antagonistic interactions whose ultimate outcome can be: (1) deadlock, where neither species gains headway; (2) replacement, where one species wrests territory from the other; (3) partial replacement, where one species captures some but not all of the antagonist's territory; or (4) mutual replacement, where one species takes some of the territory formerly occupied by the other and *vice versa* (Boddy, 2000).

Antagonistic interactions can be mediated at a distance and following contact (Boddy, 2000; Woodward and Boddy, 2008). Complex and varied morphological, physiological and biochemical changes occur during interactions, influenced by

* *Corresponding author*. Sir Martin Evans Building, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK. Tel.: +44 2920 875384.
E-mail addresses: evansja7@cf.ac.uk (J. Hiscox), savourym@cf.ac.uk (M. Savoury), vaughanip@cf.ac.uk (I.P. Vaughan), mullerc@cf.ac.uk (C.T. Müller), boddyl@cf.ac.uk (L. Boddy).
http://dx.doi.org/10.1016/j.funeco.2014.11.001

the species present and environmental conditions, including rapid cell division, branching, hyphal aggregation, aerial growth, autolysis, pigment production, release of volatile organic compounds (which also act as info-chemicals), diffusible enzymes, toxins and antifungal metabolites (Griffith et al., 1994; Boddy, 2000; Baldrian, 2004; Hynes et al., 2007; Evans et al., 2008; Woodward and Boddy, 2008). These physiological and biochemical changes determine the outcome of the interaction. Inevitably they have a metabolic cost, so it might be anticipated that interactions result in an increase in decay rate to provide the necessary carbon and energy.

Wood decay fungi can be broadly categorised into primary, secondary, late secondary and end stage colonisers depending on their position within the successional community. Primary colonisers generally arrive at the resources as spores; many of which will have been latently present in standing trees and attached branches as endophytes (Parfitt et al., 2010). Mycelia proliferate through the uncolonised wood, first rapidly utilising easily accessible nutrient sources and then more recalcitrant compounds (Boddy and Heilmann-Clausen, 2008). Early secondary colonisers subsequently arrive at the resource as spores, as do many later secondary and end stage colonisers, though some arrive as mycelium which has grown out of colonised resources in search of new ones, often forming linear aggregates of hyphae called cords (Boddy and Heilmann-Clausen, 2008). In general, there is a hierarchy of combative ability where late secondary colonisers > early secondary colonisers > primary colonisers, although these relationships are not always transitive (Boddy, 2000). End stage fungi and some secondary colonisers are not combative but succeed in some situations because they are tolerant of certain environmental stresses (Boddy and Heilmann-Clausen, 2008).

The aim of the present study was to investigate the time course of interspecific fungal interactions in wood and how these interactions might alter fungal metabolic rate, with potential implications for wood decay rate. We set out to determine the effects of interspecific antagonistic interactions on metabolic rate, using CO_2 as a surrogate, by testing the hypotheses that: (1) when fungal individuals interact in wood there is an increase in respiration, because these aggressive encounters are energetically expensive; (2) different interaction outcomes, e.g. deadlock and replacement, result in different costs which might occur if different mechanisms are employed; and (3) some predecessors will affect respiration by a succeeding fungus.

Methods

Preparation of inocula

Inocula of eight native, beech (*Fagus sylvatica*)-inhabiting fungi (Table 1) from different stages of decay were prepared by incubating 2 × 2 × 2 cm beech wood blocks on colonised 0.5 % malt agar (MA: 5 g l^{-1} malt extract, 15 g l^{-1} agar; Lab M, Lancs, UK) at 20 °C in the dark (following A'Bear et al., 2012). Species were chosen to cover the main stages of fungal succession, as determined in previous studies (Rayner and Boddy, 1988; Boddy and Heilmann-Clausen, 2008). Blocks were left to colonise for 12 weeks, by which time growth had proliferated through the blocks, as determined by reisolating from diverse regions within the block onto agar. The initial decay state of colonised blocks was determined through measurement of wet volume/dry weight (15 replicates, destructively sampled). There were no significant differences between the density of blocks colonised with different species (0.54 g cm^{-3}), with the exception of *Trametes versicolor* (0.49 g cm^{-3}; $df = 89$, $F = 8.374$, $P < 0.001$).

Outcome of interspecific interactions

Pre-colonised blocks, scraped free of adhering mycelium using a scalpel, were paired with cut vessels touching and held together with a sterile rubber band which was removed after 5 d. Paired blocks were placed directly onto perlite (30 ml; a silicaceous rock that does not absorb CO_2) moistened with sterile distilled water to achieve a water potential of

Table 1 – Details of fungal species used

Ecological role	Species[a]	Strain	Source	Division	Class	Order	Family
Primary coloniser	*Hypoxylon fragiforme*	HpfFF1	Beech wood isolation	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae
	Biscogniauxia sp.	BxnFF1	Beech wood isolation	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae
	Vuilleminia comedens	VcWVJH1	Beech wood isolation	Basidiomycota	Agaricomycetes	Corticales	Corticariaceae
Early secondary coloniser	*Trametes versicolor*	TvCCJH1	Fruit body isolation	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae
	Stereum hirsutum	ShSS1	Fruit body isolation	Basidiomycota	Agaricomycetes	Russulales	Stereaceae
	Bjerkandera adusta	BaSS1	Fruit body isolation	Basidiomycota	Agaricomycetes	Polyporales	Meruliaceae
Late secondary/tertiary coloniser	*Hypholoma fasciculare*	HfDD3	Fruit body isolation	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae
	Phanerochaete velutina	Pv29	Beech wood isolation	Basidiomycota	Agaricomycetes	Polyporales	Phanerochaetaceae

a Species identities previously confirmed by ITS sequencing.

−0.012 MPa (determined by the method of Fawcett and Collins-George, 1967), in plastic 100 ml lidded deli pots (Cater4you, Bucks, UK). The pots were watered fortnightly to maintain water potential. Holes (4 × 1 mm diameter) covered in microporous surgical tape (3M, Bracknell, UK) allowed aeration. Interactions were set up in all combinations of species. Three replicate interactions were harvested for re-isolation at 7, 14, 21, 28, 42, 56 and 84 d after pairing. Blocks were separated and 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 20 °C until mycelium had emerged and could be identified morphologically. The proportion of the two blocks colonised by each species was estimated. The interactions were considered to be complete at 84 d and were recorded as deadlock, partial replacement or replacement, as defined below.

For each interaction, a combative ability score was calculated, as the sum of territory occupied by each competitor at each time point measured. For example, during a deadlock where both competitors maintain 50 % total territory at each of the 7 time points, the score for each competitor would be 350 for that particular interaction. Combative ability scores were assigned for an individual species as the sum of all scores from all interactions studied here.

Carbon loss during interactions

Pre-colonised wood blocks of *Biscogniauxia* sp., *Vuilleminia comedens*, *T. versicolor*, *Hypholoma fasciculare* and *Phanerochaete velutina* were paired in all combinations as described above, as well as controls of self-pairings and uncolonised sterile blocks, with five replicates. These combinations represented different interaction outcomes and included species that were primary, secondary and late secondary/tertiary colonisers. Pairings were housed in 120 ml glass jars (Richardsons of Leicester Ltd, UK) on moistened perlite, and incubated at 20 °C in the dark with loose lids (for aeration). Jars were watered weekly to maintain the water potential. At the end of the experiment (56 d), re-isolations were made as above, to confirm the outcome of the interaction. CO_2 evolution was measured 0, 7, 14, 28, 42, 56 d after blocks were paired. As *P. velutina* rapidly replaced *V. comedens*, additional measurements were made for this combination at 3 and 10 d. Prior to measurement, the jars were flushed by removing the lid for 5 min, and then closed with a lid housing a neoprene insert, covering a 1 mm hole drilled into the lid, to form an airtight seal. Jars were incubated at 20 °C for 1 hr to allow CO_2 accumulation. Headspace samples were taken using a gas-tight 2.5 ml plastic syringe (BD Plastipak, Oxford, UK), which had been previously flushed with nitrogen to ensure there was no CO_2 present. Samples were taken by inserting a 25 G needle through the neoprene insert. 2 ml of sample was injected though a non-thermal septum into a Natural Gas Analyser (NGA; Arnel Model 2101, PerkinElmer, MA, USA) and separated on a column using argon as a carrier gas at 32 ml min^{-1} at 110 °C. Detection of compounds was via a thermal conductivity detector (TCD; 110 °C), and the NGA was controlled using TotalChrom v.6.2.1 software (PerkinElmer, MA, USA). A natural gas calibration standard (Scott Speciality Gas, PA, USA; CO_2 content 40 000 ppm) was run at the start of each sampling day to ensure machine efficiency.

Statistical analysis

All statistical analyses were performed in R (R Development Core Team, 2011) using the multcomp package (Hothorn et al., 2008), and graphs were generated using the R package ggplot2 (Wickham, 2009). The production of CO_2 during interactions was compared to the relevant self-pairing controls using a linear mixed-effects model with time as a fixed effect and biological replicate as a random effect (thus controlling for effects of repeated measurements). Tukey–Kramer *a posteriori* comparisons were performed to detect significant differences between treatments. For each interaction, an 'expected' value for CO_2 production at each measurement point could be estimated using the production by relevant self-pairings, scaled according to the proportion of total territory that each competitor occupied at that time point in the mixed species interactions (for example, at 28 d in the interaction BiPv the expected value is calculated as: [Bi: 19 % *944 ppm h^{-1}] + [Pv: 81 %*4 304 ppm h^{-1}] = 3 674 ppm h^{-1}). Statistical comparisons of actual and 'expected' values for CO_2 production are not valid; however in some interaction combinations one species had entirely replaced the other before the end of the experiment, so effectively the 'expected' values for CO_2 production by the interaction blocks became the same as the self-pairing of the dominant competitor. Therefore here we can indicate whether there was a significant difference between the data for the actual and 'expected' CO_2 production at that time point.

Results

Progression and outcomes of interactions

Interaction outcomes ranged from deadlock to complete replacement, with the speed of replacement varying between species combinations (Table 2). Outcomes were mostly consistent between replicates, but there was variability in the rate of progress of interactions between replicates; this can be seen as non-linear gain/loss of territory (Fig 1). In general, primary colonisers were replaced by early secondary colonisers, which were in turn replaced by late secondary/tertiary colonisers; this was supported by changes in comabtive ability scores based on territory acquisition/loss over time (Fig 1). The scores take the speed of replacement into account as well as eventual outcome. *Hypoxylon fragiforme* and *Biscogniauxia* sp. were both replaced in all interactions (except deadlock when paired with each other), but the progress of these replacements was much slower during interactions involving *Biscogniauxia* sp. which was reflected in a higher combative ability score (Fig 1). Different species exhibited different interaction 'strategies': *Stereum hirsutum*, for example, was not replaced in any of the combinations used, showing defensive abilities, but was relatively poor at replacing other species. *T. versicolor* had the same combative ability score overall as *S. hirsutum* (indicating that the overall territory occupied by both species was the same), but outcomes of interactions with opponents differed.

Table 2 – Outcomes of interspecific interactions. Interaction outcomes are defined as: R, complete replacement of one competitor by the other; PR, partial replacement of one competitor by the other; D, deadlock

Interaction	Outcome at 84 d	Time until complete replacement (d)
H. fragiforme vs. *Biscogniauxia* sp.	D	–
H. fragiforme vs. *V. comedens*	R by *V. comedens*	56
H. fragiforme vs. *T. versicolor*	R by *T. versicolor*	21
H. fragiforme vs. *S. hirsutum*	R by *S. hirsutum*	21
H. fragiforme vs. *B. adusta*	R by *B. adusta*	28
H. fragiforme vs. *H. fasciculare*	R by *H. fasciculare*	21
H. fragiforme vs. *P. velutina*	R by *P. velutina*	35
Biscogniauxia sp. vs. *V. comedens*	R by *V. comedens*	56
Biscogniauxia sp. vs. *T. versicolor*	R by *T. versicolor*	84
Biscogniauxia sp. vs. *S. hirsutum*	R by *S. hirsutum*	42
Biscogniauxia sp. vs. *B. adusta*	R by *B. adusta*	42
Biscogniauxia sp. vs. *H. fasciculare*	R by *H. fasciculare*	42
Biscogniauxia sp. vs. *P. velutina*	R by *P. velutina*	42
V. comedens vs. *T. versicolor*	R by *T. versicolor*	42
V. comedens vs. *S. hirsutum*	D	–
V. comedens vs. *B. adusta*	R by *B. adusta*	84
V. comedens vs. *H. fasciculare*	R by *H. fasciculare*	56
V. comedens vs. *P. velutina*	R by *P. velutina*	14
T. versicolor vs. *S. hirsutum*	D	–
T. versicolor vs. *B. adusta*	PR by *B. adusta*	–
T. versicolor vs. *H. fasciculare*	D	–
T. versicolor vs. *P. velutina*	R by *P. velutina*	84
S. hirsutum vs. *B. adusta*	D	–
S. hirsutum vs. *H. fasciculare*	R by *H. fasciculare*	56
S. hirsutum vs. *P. velutina*	D	–
B. adusta vs. *H. fasciculare*	R by *H. fasciculare*	56
B. adusta vs. *P. velutina*	R by *P. velutina*	28
H. fasciculare vs. *P. velutina*	R by *P. velutina*	42

Most of the interactions (24 out of 28), especially when both combatants were basidiomycetes, remained at deadlock for at least 7 d before replacement began, and in 2 of these pairings, deadlock was maintained for up to 28 d before replacement began. There was no correlation between the length of time a pairing spent in deadlock and the speed of subsequent replacement (Pearson's $r = 0.018$, df = 19, $P = 0.72$).

Mycelial cords were formed during interactions between *T. versicolor* vs. *H. fasciculare*, and *H. fasciculare* vs. *P. velutina*. Cords of *H. fasciculare* appeared on the outside of the *T. versicolor* blocks between 7 and 14 d after the start of the interaction (all replicates), and cords of *P. velutina* appeared on the outside of *H. fasciculare* blocks at approximately 7 d (all replicates). There was also a large amount of aerial mycelium produced by *P. velutina* during all interactions, from 7 d onwards.

Carbon loss during interactions

No CO_2 production was detected from uncolonised sterile wood blocks. Self-pairings raised the CO_2 concentration within the interaction chambers to between 3 and 10 times atmospheric concentration (Fig 2). Rate of CO_2 production was species-specific, with relatively little variation in production between replicates, and was not related to the position of a species within the succession community. After an initial peak, there was a general pattern of decline in production over the first 14–28 d, with fairly steady levels of production for the remainder of the experiment.

The production of CO_2 during interactions varied depending on the species combination, and was not directly related to interaction outcome (Fig 2). In six of the ten interactions studied the total production of CO_2 (six measurements over 56 days) was significantly higher than the expected production based on self-pairing controls (Fig 3). During interactions between *T. versicolor* vs. *H. fasciculare* (deadlock), and *H. fasciculare* vs. *P. velutina* (replacement of *H. fasciculare*), production of CO_2 was significantly higher than in self-pairing controls from 7 d onwards (with the exception of 14 d measurements where the increase was non-significant; Fig 2). For the interaction involving *H. fasciculare* and *P. velutina*, production of CO_2 increased almost two-fold by 7 d (df = 59, $t = 7.84$, $P < 0.001$), and decreased to starting levels by 56 d (df = 59, $t = -0.47$, $P = 0.64$). However, in the interaction between *T. versicolor* and *H. fasciculare* production remained significantly higher than in self-pairing controls throughout the 56 d measuring period.

During other interactions, neither deadlock nor active replacement were associated with increased CO_2 production. In three of the ten interaction combinations (*V. comedens* vs. *Biscogniauxia* sp., and *Biscogniauxia* sp. vs. *H. fasciculare* and vs. *P. velutina*) significant ($P < 0.05$) increases in CO_2 production relative to controls occurred only after replacement of one of the competitors. These increases lasted for the remainder of the experiment, at least 14 d after complete replacement of *Biscogniauxia* sp. by *H. fasciculare* or *P. velutina*.

In several combinations a reduction in CO_2 production relative to preceding and succeeding measurements

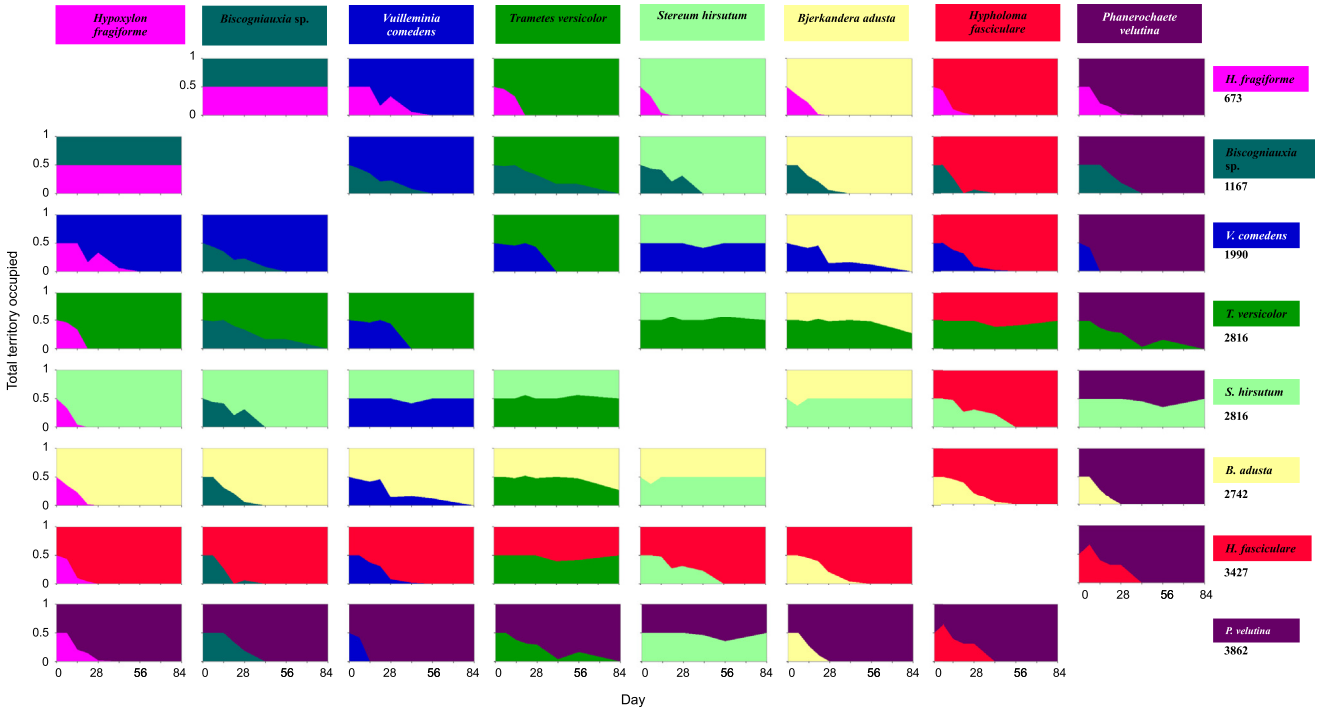

Fig 1 – Progression of interspecific interactions over 84 d. The total territory occupied by each competitor is represented by different colours. Each competitor begins with equal territory (one wood block, 50 %) at 0 d. Combative scores for each species are given underneath their label on the right hand side; see text for details of calculation. The graphs are replicated in mirror-image along the diagonal split for ease of viewing.

occurred. In interactions involving *Biscogniauxia* sp. vs. *T. versicolor*, and *H. fasciculare* vs. *P. velutina* this dip in CO_2 production occurred at 14 d; however in *V. comedens* vs. *P. velutina*, and *Biscogniauxia* sp. vs. *H. fasciculare* the dip occurred at 7 d. The dip was concurrent with a shift from deadlock to replacement in two of the four interactions affected (*Biscogniauxia* sp. vs. *T. versicolor*, and *Biscogniauxia* sp. vs. *H. fasciculare*).

No significant relationship was found between the relative combative ability of a pair of competitors (quantified as the ratio of combative scores of the pair of competitors) and the total amount of CO_2 produced over the course of the interaction. Nor was there a significant relationship between combative ability ratios and the difference between actual and expected production of CO_2.

Discussion

Progression and outcomes of interactions

This study, which is the first to follow the time course of interspecific interactions in wood, revealed that when replacement of the mycelium of one species by that of another occurs there is a lag before replacement starts. This time lag was correlated with the combative ability of the fungus, thus time lag before replacement began was shorter with the most competitive fungus – *P. velutina*, than with the next best competitor – *H. fasciculare*, which was in turn more rapid than with *Bjerkandera adusta* and *T. versicolor*. The reason for this lag is not yet known, but presumably relates to the interplay between offensive chemicals and the ability of the opponent to break them down or in some other way to defend against them. Or it may take some fungi longer than others to produce the necessary offensive chemicals.

The study also clearly indicated that some species are better at attack and others better at defence: the early secondary coloniser *T. versicolor* was able to replace all primary colonisers, and was replaced by late secondary colonisers. In contrast, *S. hirsutum*, also an early secondary coloniser, was not able to replace all primary colonisers, yet could resist replacement by the late stage, highly combative, *P. velutina*. This highlights an inadequacy in the numerical system used to score combative ability in that it could not differentiate between *T. versicolor* and *S. hirsutum*, despite clear differences in their combative strategies. Nonetheless, it is valuable for making general comparisons between species, though it should not be used alone for interpretation of ecological abilities. This study also revealed that intransitive interactions (i.e. A > B, B > C, but C > A) occur in wood, not just on artificial media (Boddy, 2000): *P. velutina* > *H. fasciculare*; *H. fasciculare* > *S.hirsutum*; *P. velutina* = *S. hirsutum*. These sorts of intransitive interactions probably result from differences in the antagonistic mechanisms employed by different species, and/or differential ability to cope with various mechanisms of attack.

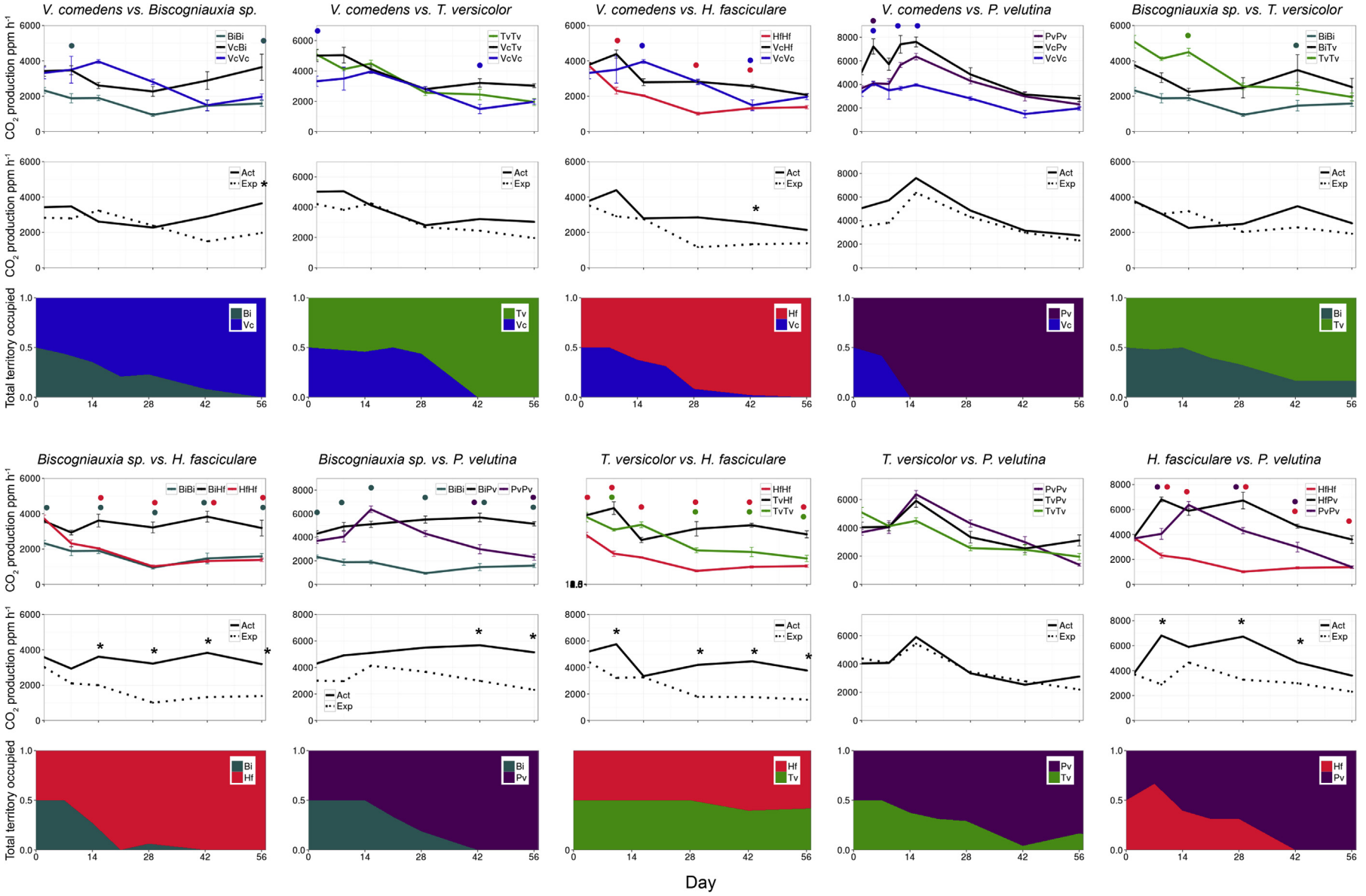

Fig 2 – CO_2 production and territory capture during interactions. Vc, *Vuilleminia comedens*; Bi, *Biscogniauxia* sp.; Tv, *T. versicolor*; Hf, *H. fasciculare*; Pv, *P. velutina*. Self-pairings are indicated by repetition of the code (e.g. VcVc is the *V. comedens* self-pairing), whilst interspecific interactions are indicated by pairing different codes (e.g. VcBi is the interaction between *V. comedens* and *Biscgniauxia* sp.). Rows 1 and 4 show the CO_2 production during interactions compared to the relevant self-pairings, data points are the mean ± standard error of the mean. Significant differences in production by self-pairings compared to the interaction are indicated using a circle, the colour of the circle indicates which of the self-pairings is different. Rows 2 and 5 compare the expected CO_2 production to the actual production during interactions. * represents an inferred significant difference ($P < 0.05$) between actual and expected production. Rows 3 and 6 show the progression of the interactions over 56 d. Each competitor occupied 50 % of the total territory (one block) at 0 d (see Fig 1).

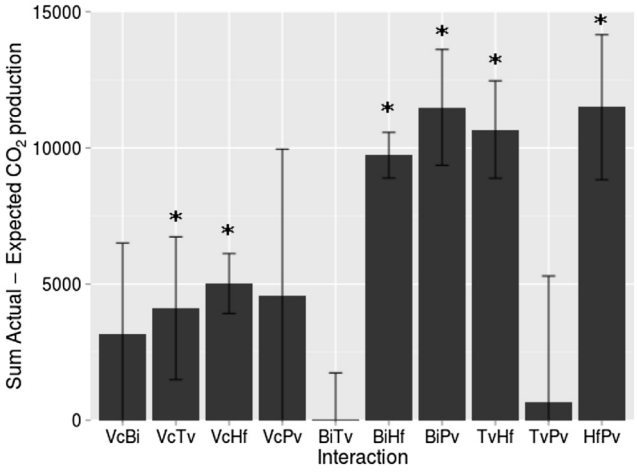

Fig 3 – Differences between the total actual (measured) production of CO_2 during interactions and the total expected (calculated) CO_2 production. Bars show the mean difference between cumulative actual and expected CO_2 production over 56 d, ±95 % confidence intervals. * indicates where the difference is significantly ($P < 0.05$) greater than zero. See Fig 2 for species name abbreviations.

CO_2 evolution changes during time course of interactions

Our results show that CO_2 evolution from decomposing wood alters during interspecific antagonistic fungal interactions and during the time course of an interaction, though these effects were combination-specific. Our first hypothesis that interactions lead to an increase in CO_2 evolution, was partly supported: 60 % of the pairings studied resulted in increased total CO_2 production over the whole experiment relative to controls. However, in only a few cases (20 % of total) did the initial confrontation itself lead to increased respiration, indicated by elevated CO_2 evolution very early in the interaction. In these pairings, increased CO_2 production occurred over the whole course of the interactions, correlated with the production of mycelial cords (linear aggregations of hyphae) by one or both competitors. Mycelial cords are largely produced in the natural environment by some of the aggressive later stage colonisers, forming extensive (many m^2), long-lived (many years) dynamic systems at the soil-litter interface, connecting disjointed resources and reallocating nutrients across heterogeneous environments (Boddy, 1993, 1999; Fricker et al., 2008; Boddy et al., 2009). Production of cords is energetically expensive, and may contribute to the observed increases in CO_2 evolution.

In most combinations (40 % of total), increases in CO_2 production only occurred when the replacing fungus was growing within the territory of its dispossessed competitor. This shows that occupation of the wood by certain predecessors resulted in elevated CO_2 production (hypothesis 3). In all cases, the replaced fungus was a primary coloniser. Elevated CO_2 production following replacement of another mycelium could imply utilization of that mycelium by the replacer. Another possibility is that the previous coloniser may have altered the wood structure in a way that increases the ability of a successor to effect rapid decay, due to resource partitioning and variability in enzyme production. For example, prior colonisation by *Marasmius androsaceus* was found to alter *Pinus sylvestris* litter such that the later colonising community broke it down more efficiently, probably due to alterations to the lignocellulose structure (Cox et al., 2001). Similarly, mixtures of fungi inhabiting the same resource, or in sequence in the same resource, utilise the substrates differently, which results in higher levels of decomposition (Deacon, 1985).

The general trend of decreased CO_2 production over time, may occur as certain nutrients become limiting, or, in the case of replacement, when the mycelium of the weaker antagonist has been metabolised by the victor. Also, initial high levels may be attributable to the mycelial damage that would have occurred when setting up the experiment, or by hyphal death in the interaction region associated with initial antagonism. Interestingly, the break of deadlock after several weeks, discussed above, was associated with a transient dip in CO_2 production in some combinations. This might occur because the weaker competitor had become dead or dysfunctional in the region adjacent to the interaction zone, but the more aggressive mycelium had not yet proliferated into the territory of the opponent, hence the total respiring biomass was lower.

Interspecific and interaction-specific variation in CO_2 evolution

CO_2 production varied between species, but this was not a surprise since it is known that different fungi often decay wood at different rates under similar conditions (Rayner and Boddy, 1988; Worrall et al., 1997), which reflects different utilisation strategies.

Different antagonistic mechanisms are employed by different species (Boddy, 2000), and these are likely to differ in their metabolic 'cost'. Our results suggest that production of mycelial cords is sufficiently costly to cause an observable increase in respiration. Changes in CO_2 production were not, however, associated with a particular interaction outcome during active antagonism (while both competitors were still present; hypothesis 2), i.e. deadlock was no more energetically costly than replacement or *vice versa*, and any changes relative to controls occurred on an interaction-specific basis. Similarly, there was no relationship between the relative combative ability of the competitors and changes in CO_2 production, nor with successional stage of the fungus and CO_2 production.

Implications for decay rate

Rate of CO_2 production has often been used as a proxy for decomposition rate of wood, and is useful because it is non-destructive (Yoneda, 1980; Boddy, 1983; Chambers et al., 2000, 2001; Progar et al., 2000; Bond-Lamberty et al., 2002; Mackensen et al., 2003; Liu et al., 2006; Jomura et al., 2008; Hérault et al., 2010). Usually, in established fungal communities, CO_2 is likely to be a reasonable proxy, and indeed better than change in weight, because the latter does not take into account fungal material present. However, in the current study elevated CO_2 cannot be taken to imply an increase in

wood decay rate, as the fungi could be metabolising storage compounds or, in the case of replacement interactions, the mycelium of the fungus being replaced. Where CO_2 evolution remained elevated for many days this may imply that wood decay rate had increased to supply increased metabolic costs.

Certainly, the species composition of a wood decay community will affect the overall dynamics of decomposition because different fungi decompose wood at different rates. The present study sheds no light on whether or not antagonistic interactions *per se* alter wood decay rate, for the reasons indicated above. Determining weight loss in small systems only during the time over which the interaction is active is not feasible, so at present the problem is intractable. Predicting rates of decomposition for different community assemblages remains challenging at best.

Conclusions

Our results show that different species decompose wood at different rates, that metabolic changes associated with antagonism are species- and combination-specific, that metabolism often increased in tissues where one coloniser had replaced another, and that production of mycelial cords by some species increased the metabolic rate. These metabolic responses to antagonism may reflect utilization of stored compounds within mycelium, use of an opponent's mycelium and/or utilization of the resource which would result in increased wood decay rate. Any increases in metabolism occurring during active interactions were almost certainly due to processes involved in antagonism. We have no evidence of complementarity effects, where resource partitioning or facilitative interactions occur between species to increase decomposition.

Acknowledgements

This work was funded by the Natural Environment Research Council grant NE/I01117X/1. Thanks to the Cardiff University Department of Earth Sciences for the use of their NGA, especially Dr H Sass and Mrs X Tang, and to the Cardiff Council Parks Department for providing us with freshly felled beech wood. LB was a Miller Visiting Professor at the University of California, Berkeley, when this manuscript was revised.

REFERENCES

A'Bear, A.D., Boddy, L., Hefin Jones, T., 2012. Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola. *Global Change Biology* 18, 1823–1832.

Baldrian, P., 2004. Increase of laccase activity during interspecific interactions of white-rot fungi. *FEMS Microbiology Ecology* 50, 245–253.

Boddy, L., 1983. Microclimate and moisture dynamics of wood decomposing in terrestrial ecosystems. *Soil Biology Biochemistry* 15, 149–157.

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

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

Boddy, L., 2000. Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS Micriobiology Ecology* 31, 185–194.

Boddy, L., Heilmann-Clausen, J., 2008. Basidiomycete community development in temperate angiosperm wood. In: Boddy, L., Frankland, J.C., van West, P. (Eds.), Ecology of Saprotrophic Basidiomycetes. Academic Press, pp. 211–237.

Boddy, L., Hynes, J., Bebber, D.P., Fricker, M.D., 2009. Saprotrophic cord systems dispersal mechanisms in space and time. *Mycoscience* 50, 9–19.

Bond-Lamberty, B., Wang, C., Gower, S.T., 2002. Annual carbon flux from woody debris for a boreal black spruce fire chronosequence. *Journal Geophysical Research* 108, 8220–8230.

Chambers, J.Q., Higuchi, N., Schimel, J.P., Ferreira, L.V., Melack, J.M., 2000. Decomposition and carbon cycling of dead trees in tropical forests of the central Amazon. *Oecologia* 122, 380–388.

Chambers, J.Q., Schimel, J.P., Nobre, A.D., 2001. Respiration from coarse wood litter in central Amazon forests. *Biogeochemistry* 52, 115–131.

Cox, P., Wilkinson, S.P., Anderson, J.M., 2001. Effects of fungal inocula on the decomposition of lignin and structural polysaccharides in *Pinus sylvestris* litter. *Biology Fertility of Soils* 33, 246–251.

Deacon, J.W., 1985. Decomposition of filter paper cellulose by thermophilic fungi acting singly, in combination, and in sequence. *Transactions of the British Mycological Society* 85, 663–669.

Evans, J.A., Eyre, C.A., Rogers, H.J., Boddy, L., Müller, C.T., 2008. Changes in volatile production during interspecific interactions between four wood rotting fungi growing in artificial media. *Fungal Ecology* 1, 57–68.

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

Fricker, M.D., Boddy, L., Bebber, D.P., 2008. The interplay between topology and function in fungal mycelial networks. *Topologica* 1, 004.

Griffith, G., Rayner, A., Wildman, H., 1994. Interspecific interactions, mycelial morphogenesis and extracellular metabolite production in *Phlebia radiata* (Aphyllophorales). *Nova Hedwigia* 59, 311–334.

Hérault, B., Beauchêne, J., Muller, F., Wagner, F., Baraloto, C., Blanc, L., Martin, J.-M., 2010. Modeling decay rates of dead wood in a neotropical forest. *Oecologia* 164, 243–251.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50, 346–363.

Hynes, J., Müller, C.T., Jones, T.H., Boddy, L., 2007. Changes in volatile production during the course of fungal mycelial interactions between *Hypholoma fasciculare* and *Resinicium bicolor*. *Journal of Chemical Ecology* 33, 43–57.

Jomura, M., Kominami, Y., Dannoura, M., Kanazawa, Y., 2008. Spatial variation in respiration from coarse woody debris in a temperate secondary broad-leaved forest in Japan. *Forest Ecology Management* 255, 149–155.

Liu, W.H., Bryant, D.M., Hutyra, L.R., Saleska, S.R., Hammond-Pyle, E., Curran, D., Wofsy, S.C., 2006. Woody debris contribution to the carbon budget of selectively logged and maturing mid-latitude forests. *Oecologia* 148, 108–117.

Mackensen, J., Bauhus, J., Webber, E., 2003. Decomposition rates of coarse woody debris—A review with particular emphasis on Australian tree species. *Australian Journal of Botany* 51, 27.
Parfitt, D., Hunt, J., Dockrell, D., Rogers, H.J., Boddy, L., 2010. Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of wide range of angiosperm tree. *Fungal Ecology* 3, 338–346.
Progar, R.A., Schowalter, T.D., Freitag, C.M., Morrell, J.J., 2000. Respiration from coarse woody debris as affected by moisture and saprotroph functional diversity in Western Oregon. *Oecologia* 124, 426–431.
R Development Core Team, 2011. R, A Language and Environment for Statistical Computing.
Rayner, A., Boddy, L., 1988. Fungal Decomposition of Wood, its Biology and Ecology. Wiley, Chicester.
Wickham, H., 2009. ggplot2, Elegant Graphics for Data Analysis. Springer, New York.
Woodward, S., Boddy, L., 2008. Ecology of Saprotrophic Basidiomycetes. In: Boddy, L., Frankland, J.C., van West, P. (Eds.), Ecology of Saprotrophic Basidiomycetes. Elsevier, Oxford, pp. 125–142.
Worrall, J.J., Anagnost, S.E., Zabel, R.A., 1997. Comparison of wood decay among diverse lignicolous fungi. *Mycologia* 89, 199–219.
Yoneda, T., 1980. Studies on the rate of decay of wood litter on the forest floor. III. Effect of moisture content on CO_2 evolution from decaying wood. *Japanese Journal of Ecology* 30, 55–62.