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Emergent properties arising from spatial heterogeneity influence fungal community dynamics

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ABSTRACT

Community dynamics are mediated by species interactions, and within communities spatial heterogeneity and intransitive relationships promote coexistence. However, few experimental studies have assessed effects of heterogeneity on the interactions of competing individuals. Wood decay basidiomycete fungi are ideal for studying community structure and dynamics because they are easy to manipulate in laboratory microcosms, and communities resolve themselves rapidly. Most studies have only used simplistic pair-wise interactions in a 2-D plane, but here we investigate a three-species community in an environmentally realistic novel 3-dimensional system. We show how spatial heterogeneity and patch size dynamics are important for coexistence, and how competitive interactions change over different spatial dimensions. Emergent properties arose with increased spatial heterogeneity: the weakest competitor co-occurred with the community when its territory was less fragmented, and interactions became intransitive.

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1. Introduction

Spatial variation in natural ecosystems affects all aspects of ecology from the level of individual behaviour, to whole population and community dynamics (Holmes et al., 1994). Since interactions between individuals of a community play a pivotal role in shaping the structure of the community, understanding interactions in the context of spatial variation is critical to explaining community development and its contribution to the functioning of the wider ecosystem.

Spatial heterogeneity promotes coexistence by providing spatial refuges and reducing the encounter rate of individuals, hence reducing the risk of attack (Huffaker, 1958; Brockhurst et al., 2005). The classical study by Luckinbill (1973) demonstrated that the

unstable interaction between *Didinium nasutum* and *Paramecium aurelia* (where *P. aurelia* is driven to extinction by *D. nasutum*) was stabilised and coexistence occurred following an increase in viscosity of culture medium. More recently, theoretical models have predicted that the spatial distribution of individuals affects community stability such that greater dispersion of species will result in greater niche partitioning, which promotes coexistence over evolutionary time (Wang et al., 2015). Additionally, long-term investigations of habitat fragmentation show that patchiness has drastic negative effects on biodiversity (Lovejoy et al., 1986; Laurance et al., 2011). Little experimental work, however, has been conducted on the combined effects of spatial variability and patch size or quality on species interaction stabilisation (Daugherty, 2011).

Antagonism (interference competition; often called combat with fungi) between coexisting saprotrophic fungi causes spatial patterning of communities during decomposition (Hiscox et al., 2016b). During this process, wood decay fungi compete with one another in 3-dimensional space for resources whilst maintaining distinct individual territories (even different individuals of the

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same species do not merge). This system is ideal for studying community dynamics as the location of the decay fungi can be determined simply by culturing (Hiscox et al., 2017), and different spatial arrangements can be easily prepared and communities become resolved after a few months. Interference competition (combat) may result in deadlock (neither fungus gains territory held by another fungus), replacement (one fungus gains the territory held by another), partial replacement (one fungus partially gains territory of another) or even mutual replacement (both fungi partially gain territory held by the other) (Boddy, 2000). A hierarchy of combative ability is usually exhibited between specific species combinations where the K-selected characteristics of later stage resource colonisers frequently lend them greater competitive fitness than the earlier resource colonisers (Boddy, 2000), but interactions are sometimes intransitive and can alter depending on different environmental conditions (Woodward and Boddy, 2008).

Intransitivity is a key mechanism in maintaining species coexistence and biodiversity by satisfying an interaction relationship similar to that in a game of 'rock-paper-scissors' (Kerr et al., 2002; Reichenbach et al., 2007). Intransitive interactions often occur in species-rich communities and result in the survival of combatively weaker individuals (Gallien et al., 2017; Maynard et al., 2017a). A study which paired 18 basidiomycetes against each other to investigate the structure of their competitive network, found that diverse communities with high intransitivity showed a positive diversity-function relationship (Maynard et al., 2017b), which in the real world would translate to decomposition processes and carbon cycling.

Fungal community dynamics have been well studied in the past (Caruso et al., 2012; Hannula et al., 2013; Hiscox et al., 2015a; Persoh, 2015; Arnstadt et al., 2016; Van Der Wal et al., 2016), with some attention given to the effects of spatial patterns and individual patch size on interaction outcomes (White et al., 1998; Sturrock et al., 2002; Hiscox et al., 2017). All of these studies, however, have been conducted across essentially a 2-dimensional plane, yet in the real world decomposer communities operate in heterogeneous, 3-dimensional space, and at larger scales emergent properties may arise (Halley et al., 1996). The aim of the present study was to test the effect of spatial heterogeneity and patch size on community interactions in 3-dimensional space, and to determine the effect of such spatial variability on the ecosystem service provided by the community, namely resource decay rate. The decay of dead wood by fungi is crucial to forest ecosystem function and is pivotal to soil biogeochemical processes such as carbon cycling and nutrient release (Eriksson et al., 1990). The rate of decomposition, therefore, has been used as an indicator of fungal productivity and ecosystem service (Hiscox et al., 2015b, 2016a). Individuals compete for territory changing the structure of the community, different species cause different rates of decay and the interaction process itself can alter decay rate (Hiscox et al., 2016a), thus competition causes changes to the rate of wood decay and carbon and nutrient release in forest ecosystems.

Using wood-decay basidiomycetes as experimental organisms, the outcomes of multiple species competitive interactions were tested in both 2- and 3-dimensional systems. The effect of (1) the orientation of woody vessels (an aspect of resource structural complexity), (2) patch size and (3) spatial patterns on species interactions and decay rate were investigated, and the following hypotheses tested: (1) substratum orientation causes combative strength to change; (2) patch fragmentation negatively affects an individual's competitive success; and (3) spatial heterogeneity in a three-species community causes changes to the ecosystem function, in this case wood decay, provided by the community compared to dual-species competitive systems.

2. Methods

2.1. Fungal strains

Three fungal species were selected based on their ecological roles and successional order in the natural environment: a primary resource coloniser (*Stereum gausapatum*), and two early secondary colonisers (*Stereum hirsutum* and *Trametes versicolor*) (Supplementary Table 1). Specific strains were chosen based on their expected combative hierarchy: *T. versicolor* > *S. hirsutum* > *S. gausapatum* (Hiscox et al., 2015a, 2015b, 2016b).

2.2. Experimental design

Interactions were performed by combining precolonised wood blocks (three species, one per block) with different levels of complexity (Fig. 1): (1) 2 wood blocks; (2) 3 wood blocks; (3) a 3×3 (i.e. 9) matrix of blocks; (4) a $3 \times 3 \times 3$ (i.e. 27) matrix of blocks. (1) With pairs of blocks, all combinations of the three species were made, i.e. 3 combinations. (2) With triplicates, all three combinations of three species were made, each with a different species in the middle. (3) In the nine block matrix arrangement, 3 blocks precolonised with each species were arranged with the constraint

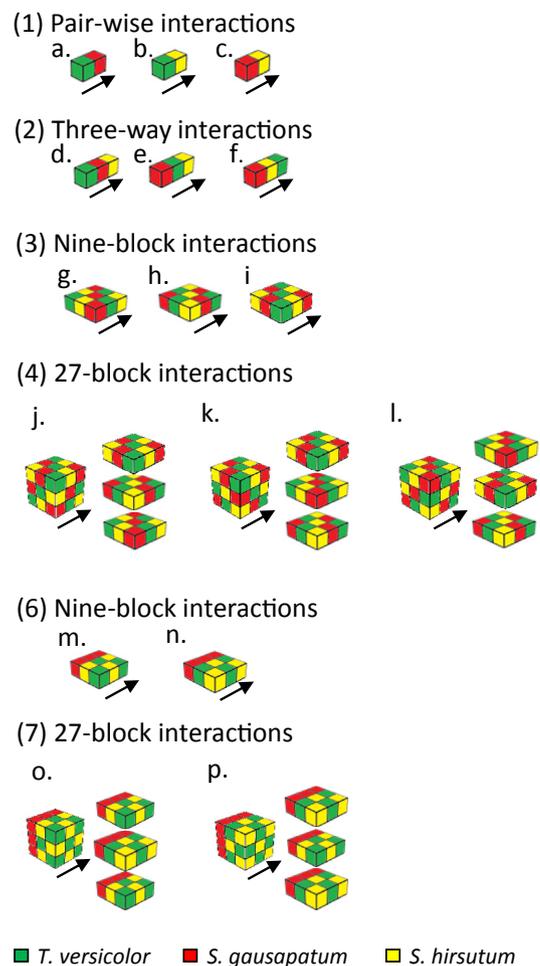


Fig. 1. Spatial distribution of species within the experimental design. Blocks coloured red indicate *S. gausapatum*, green indicates *T. versicolor* and yellow blocks are indicative of *S. hirsutum*. Dispersal patterns of nine-way and 27-block interactions were arranged such that blocks containing the same species do not have adjacent faces. Arrows indicate xylem directional flow.

that 2 blocks containing the same species never had faces adjacent to each other; 3 combinations were made. (4) In the 27-block cube, 9 blocks of each individual species were combined such that blocks containing the same species were not adjacent to one another. There are 9 possible combinations, but only 3, randomly selected combinations, were made. In (1) and (2) blocks were arranged with cut vessel ends touching (Fig. 2A). In (3) and (4) blocks were joined such that some cut vessel ends were touching but others were not, but all vessels were parallel. (5) Effect of vessel orientation was investigated with vessels laid parallel (Fig. 2B), and compared with outcomes of blocks joined with cut vessels touching in (1), (2) and (3).

The effect of one fungus (the primary coloniser *S. gausapatum*) occupying a larger adjacent volume of wood (but still a total volume equal to the other two species) was investigated in (6) 3×3 combinations and (7) $3 \times 3 \times 3$ cubes. In (6) *S. gausapatum* blocks were placed with cut vessel ends adjacent, and the other two species positioned in all combinations such that no two blocks containing the same species were adjacent, touching the *S. gausapatum* wood blocks with vessels lying parallel. In (7) a 3×3 “wall” was constructed with cut vessels adjacent, and vessels lying parallel, with the other two species arranged in both possible combinations with vessels lying parallel to those of *S. gausapatum*, but never with blocks of the same species (*T. versicolor* or *S. hirsutum*) touching each other.

2.3. Wood block colonisation and interaction assembly

Beech (*Fagus sylvatica*) blocks ($2 \times 2 \times 2$ cm) were precolonised by placing on agar (5 gL^{-1} malt extract, 15 gL^{-1} agar; Lab M, UK) cultures of the appropriate fungus (*S. gausapatum*, *S. hirsutum* and *T. versicolor*) and then incubated for 12 weeks at 20°C in the dark. Mycelia and agar were scraped from the surface of each wood block, using a sterile scalpel, 3 d prior to interaction set-up. Wood blocks were joined (by securing with elastic bands, which were removed after 7 d) and placed in polypropylene pots (70 ml, 340 ml, 340 ml, 500 ml for pair-wise, triplet, nine block and 27-block interactions respectively; Cater4You Ltd) containing perlite (20 ml, 40 ml, 60 ml and 85 ml respectively; medium grade, 3–6 mm). 2 ml, 3 ml, 6 ml and 12 ml of dH_2O was added to the perlite respectively. Each pot plus wood blocks was weighed and dH_2O added to the perlite every 14 d to maintain moisture. Each pot had a <1 mm hole covered with microporous tape for aeration. For pair-wise, three-way and nine-way interactions 10 replicates were set-up and 4 replicates of each 27-block cube, but a few replicates were lost due to contamination.

2.4. Determination of interaction outcome

Interacting wood block systems were incubated for 119 d. Then, they were deconstructed and each individual block split along the grain into thirds using a sterile (flamed in 70% ethanol) chisel. The middle third was used for density measurements (to estimate

decay rate), while 4 chips (approximately 3–5 mm) were taken from the inside-face of the remaining two thirds (8 chips in total per block) and were placed onto 2% (w/v) malt extract agar (MEA; 20 gL^{-1} malt extract, 15 gL^{-1} agar). Plates were incubated at 20°C for 6 d before morphological identification of the species finally present in each block.

2.5. Estimation of decay rate

The mean initial density (g cm^{-3}) of 10 blocks pre-colonised with each species individually was determined as dry weight (80°C for in excess of 72 h) per fresh volume (cm^3), measured with digital callipers. Following cube deconstruction at the end of the experiment, the density of a third of each individual block was determined to estimate rate of decay by comparing with density at the start.

2.6. Statistical analysis

All statistical analyses were conducted using R software (R Core Team, 2013). The rate of decay of wood in all configurations of the experimental set-up was compared using a one-way ANOVA followed by Tukey *Post hoc* tests.

For every interaction, each species was assigned a score of combative ability as a percentage of the total system colonised at the end of the experiment. Each competitor scored between 0 and 8 (since 8 regions were isolated) for each block within a system (0 indicates no recovery of a species from any isolation point, 8 indicates full recovery of a species from all 8 isolation points). Individual species scores were combined for all blocks within a system, normalised to the number of replicates performed and converted to a percentage of the blocks colonised by each species. The data were analysed using a GLM followed by Tukey *Post hoc* tests. The position of a given block within a system and, therefore, the number of faces of that block involved in direct combat was incorporated as a factor within the model. The effect of vessel orientation was tested by assigning each individual block a category based on the number of adjacent blocks whose vessels were touching, or laid parallel. For example, in pairs and triplets, blocks were either laid with vessel ends touching, or with vessels laid parallel to their neighbours. However, in nine-block systems where blocks were laid with vessel ends touching, blocks also had neighbours whose vessels were parallel, as did blocks in 27-block interactions. Vessel orientation was, therefore, a factor within the model. Water was always added to the perlite, as such, with 27-block interactions the layer laid on the perlite had greatest access to water, and the layer furthest from the perlite had least access to water. This was statistically accounted for by assigning each block to a category (greatest, moderate or least access to water), and was incorporated into the model.

3. Results

3.1. Combative ability in 2, 3, 9 and 27 block systems

Overall, *T. versicolor* consistently had the greatest score of combative ability ($F_{2, 42} = 89.84$, $P < 0.001$). *S. hirsutum* scored second highest, however, overall this was not significantly different from *S. gausapatum* ($P > 0.05$).

In pair-wise experiments (experiment 1), interactions concluded with complete replacement of *S. gausapatum* by *T. versicolor*, partial replacement (36% of territory) of *S. hirsutum* by *T. versicolor* (Table 1; Supplementary Table 2), and deadlock between *S. hirsutum* and *S. gausapatum* consistently across all replicates (Fig. 3A).

The position of a fungus (relative to the number of surrounding

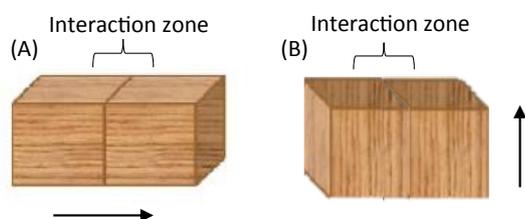


Fig. 2. Xylem vessel orientations of wood blocks. (A) Cut vessel ends touching; (B) vessels laid parallel. Arrows indicate xylem directional flow.

Table 1

Total occupancy of systems at the end of the experiment. Individual occupancy is expressed as the percentage of a system occupied by an individual and is taken from the mean of each replicate set. Patterns are coded as per Fig. 1. *Denotes interactions where vessels are laid parallel.

Pattern	Total system occupancy (%)		
	<i>S. gausapatum</i>	<i>S. hirsutum</i>	<i>T. versicolor</i>
a	0.00	–	100.00
a*	12.50	–	87.50
b	–	14.00	86.00
b*	–	40.00	60.00
c	50.00	50.00	–
c*	50.00	50.00	–
d	0.00	27.00	73.00
d*	0.00	29.00	71.00
e	0.00	3.33	96.67
e*	0.00	30.00	70.00
f	33.33	27.00	39.67
f*	28.33	26.67	45.00
g	5.22	10.67	84.11
g*	9.67	20.22	70.11
h	3.00	11.11	85.89
h*	11.00	20.33	68.67
i	0.00	10.56	89.44
i*	0.00	13.17	86.83
j	5.59	6.37	88.07
k	5.48	5.70	88.82
l	3.67	0.00	96.33
m	33.33	10.56	56.11
n	30.00	12.11	57.89
o	33.33	1.67	65.00
p	33.15	3.41	63.44

competitor held blocks) within 3-way interactions (experiment 2) affected the territory held in a manner that was specific to individual fungi. *T. versicolor* obtained more territory after 119 d when placed between its competitors: when placed centrally it occupied 100% of *S. gausapatum*'s territory and 90% of *S. hirsutum*'s territory, whereas when placed on the edge next to *S. gausapatum* it occupied 100% of *S. gausapatum*'s territory but only 19% of *S. hirsutum*'s territory, and when placed on the edge next to *S. hirsutum* it captured none of *S. gausapatum*'s territory and only 19% of *S. hirsutum*'s territory (Table 1). *S. gausapatum* was more successful (100% territory retention in every replicate) when placed on the edge of an interaction next to *S. hirsutum* (arrangement pattern f; Fig. 1) with whom it was in deadlock with (neither fungus captured territory held by the other), however, when positioned next to *T. versicolor* (as in arrangements d and e; Fig. 1), *S. gausapatum* was constantly replaced by *T. versicolor* (Fig. 3B). *S. hirsutum* lost 19% of its territory to *T. versicolor* when both positioned between competitors (pattern f; Fig. 1) and when placed on the edge next to *S. gausapatum* (pattern d; Fig. 1) but it only managed to retain 10% of its original territory when placed on the edge next to *T. versicolor* (pattern 3; Fig. 1; Table 1).

The extent of replacement of individuals in nine-block systems (experiment 3) was rather more variable across replicates although an overall hierarchy could still be discerned: *T. versicolor* > *S. hirsutum* > *S. gausapatum* (Fig. 3C). After 119 d, *T. versicolor* occupied an average of 84% of the total system in arrangement g (Fig. 1), 86% in arrangement h and 89% in arrangement i. *S. hirsutum* lost 68% of its original territory to *T. versicolor* in arrangement g, 67% in arrangement h and 68% in arrangement i, and *S. gausapatum* lost 84%, 91% and 100% of its original territory

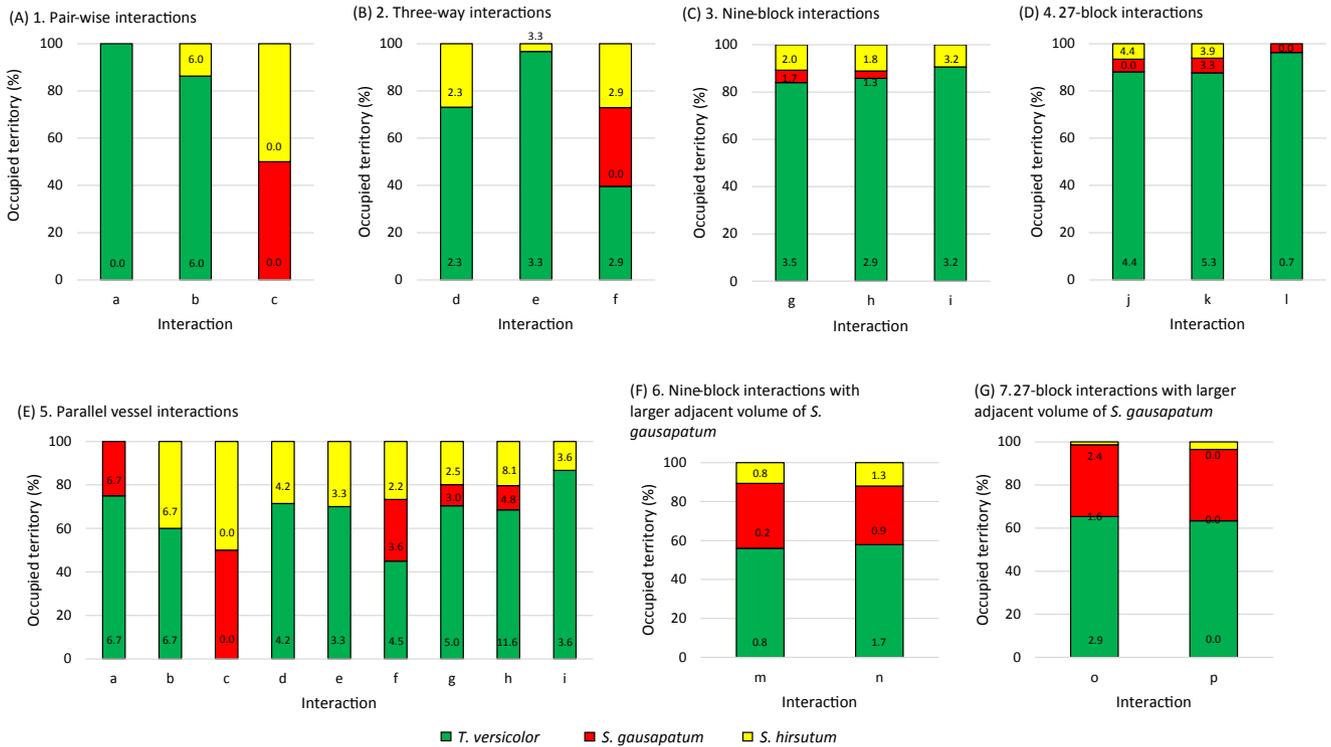


Fig. 3. Combative ability scores of fungi during pair-wise (1), three-way (2), nine-block (3), 27-block (4) interactions, interactions with a parallel vessel alignment (5) and interactions with a larger adjacent volume of *S. gausapatum* in nine-block (6) and 27-block (7) systems. Colours represent combative ability scores of each species per each interaction type and are expressed as a percentage of each interaction system occupied by a species at the end of the experiment, normalised to the replicate number. Numbers within bars are \pm SEM. Numbers in brackets refer to treatment number indicated in Fig. 1. N.B. blocks in A, B, C, D and F are laid with vessel ends touching. For significant differences ($P < 0.05$) between groups see Supplementary Fig. 1.

respectively to *T. versicolor* (Table 1). Any given block could be positioned centrally and therefore have 4 faces directly engaged in combat, on a corner so that 2 faces were engaged in combat, or on the edge of a system between two corner blocks (3 faces engaged in combat). The position that a block occupied within the system, however, had no significant effect on the combative fitness of individuals ($F_{21, 42} = 0.389$, $P > 0.05$).

For 27-block interactions (experiment 4; Fig. 1), a given block could have all 6 faces engaged in combat if positioned centrally, 3 if positioned on a corner, 4 if on the edge between two corner blocks, or 5 if positioned in the centre of an outside layer. The position of blocks within the system had no significant effect ($F_{21, 42} = 0.389$, $P > 0.05$) on the interaction outcome. *T. versicolor* consistently replaced its competitors, finally occupying 88, 89 and 96% of the system in patterns j, k and l (Fig. 1) respectively, regardless of relative position (Table 1). The amount of territory occupied by *S. gausapatum* and *S. hirsutum* was not significantly different from each other in patterns j, k and l (Fig. 1), both always occupy <6%. **Effect of vessel orientation on outcome of interaction.**

The orientation of vessels sometimes affected combative success (experiment 5: effect of vessel orientation). When *T. versicolor* was paired with *S. gausapatum* (pattern a; Fig. 1), the latter defended 25% of its territory when vessels were laid parallel to those of *T. versicolor*, but was fully replaced when cut vessel ends were touching. Combative ability of *S. hirsutum* was greater ($F_{14, 1665} = 3.29$, $P < 0.001$) against *T. versicolor* when in wood with vessels laid parallel compared to when vessel ends touched (Fig. 3E). For example, in the three-block interaction arrangement e (Fig. 1), *S. hirsutum* lost 90% of its territory to *T. versicolor* when cut vessel ends were touching, but only lost 10% of its original territory when vessels were laid parallel (Table 1).

3.2. Effect of patch size on outcome of interactions

Consistently, *S. gausapatum* was far more combative when its initial adjacent patch size was greater than that of competitors, despite total initial volume being identical (experiments 6 and 7). In nine-block systems where competitors were evenly dispersed (experiment 3), *S. gausapatum*'s maximum final occupancy was 11% (arrangement i; Fig. 1) and it was fully replaced in arrangement h (Fig. 3C). However, when *S. gausapatum* was arranged such that it occupied a larger adjacent volume, but with the same total volume as its competitors (experiment 6), it managed to retain 100% ($F_{24, 1682} = 87.85$, $P < 0.001$) of its original territory (33% of the entire system) (arrangement m; Fig. 3F). Similarly, in evenly dispersed 27 cube systems (experiment 4) the maximum amount of territory held by *S. gausapatum* at the end of the experiment was 6% (arrangement k; Fig. 3D), but when *S. gausapatum* occupied a larger initial adjacent volume (experiment 7), it retained 100% of its original territory (Fig. 3G) ($F_{24, 1682} = 87.85$, $P < 0.001$).

The territory finally occupied by the other competitors was consequently reduced when *S. gausapatum* occupied a larger adjacent patch size in comparison to when all three species were evenly dispersed (Fig. 3F and G). The average final occupancy of *T. versicolor* in nine-block systems across all spatial arrangements dropped significantly from 81% when evenly dispersed (experiment 3) to 57% ($F_{24, 1682} = 50.36$, $P < 0.001$) when *S. gausapatum* occupied a larger adjacent volume (experiment 6; Fig. 3F), and in 27 cube systems it fell from 91% when evenly dispersed (experiment 4) to 64% ($F_{24, 1682} = 50.36$, $P < 0.001$) when *S. gausapatum*'s initial adjacent patch size was greater (experiment 7; Fig. 3G). The allocation of water within 27-block interactions had no effect (*T. versicolor*: $F_{2, 1665} = 2.32$, $P > 0.05$; *S. hirsutum*: $F_{2, 1665} = 1.28$, $P > 0.05$; *S. gausapatum*: $F_{2, 1665} = 1.24$, $P > 0.05$) on outcome of interaction.

3.3. Effect of community composition on decay

Wood colonised by all species decayed significantly during the experiment. *T. versicolor* and *S. hirsutum* decayed wood at a similar rate (18 and 13% total weight loss respectively), and blocks originally colonised by *S. gausapatum* had a significantly faster rate of decay than both *T. versicolor* and *S. hirsutum* (Supplementary Fig. 2). The pair-wise permutation of *S. gausapatum* and *S. hirsutum* (treatment c) in both wood vessel orientations resulted in a rate of decay that was significantly slower (12 and 14% weight loss for each vessel orientation respectively; $F_{25, 1752} = 8.85$, $P < 0.01$; Supplementary Table 3) than any other arrangement (Fig. 4). Neither the number of blocks interacting nor systems with larger adjacent *S. gausapatum* volume significantly affected the rate of decay.

4. Discussion

4.1. Patch size, fragmentation and emergent properties

The study showed, for the first time, that extent of fragmentation of fungal territory, and substratum complexity influenced community dynamics of wood decay fungi. In macroecology, patch fragmentation, or "patchiness" has a profound effect on species interactions and subsequent community structure and ecosystem functioning (Kareiva, 1987; Didham, 2010), and as hypothesised in the present study patchiness negatively impacted combative ability (hypothesis 2). Increased patch size, but unchanged total territory, of the weakest competitor, *S. gausapatum*, resulted in a dynamic shift in the competitive hierarchy of this simple community (Fig. 3F and G). The ability of *T. versicolor* to invade and capture the territory of *S. gausapatum* was inhibited, resulting in *S. gausapatum* retaining more of its territory. Anastomosis, or hyphal fusion, of genetically identical hyphae (i.e. that of the same individual) occurs following self-recognition and results in enhanced communication and resource pooling throughout the mycelium (Rayner, 1991). Emergent properties have been demonstrated to arise in forest plots, e.g. mycorrhizal networks facilitate greater seedling establishment of Douglas-fir (*Pseudotsuga menziesii*) (Teste et al., 2009), and increased combativeness by *S. gausapatum* and resulting coexistence with its competitors when occupying a greater adjacent volume (Fig. 3F and G) is a clear emergent property in the present study. Relative combative ability increases when larger volumes of wood are occupied (Holmer and Stenlid, 1993), however, total volume remained the same in the present study. Exactly which mechanisms lead to this emergent property are unclear; the overall perimeter to defend is lower in large patch sizes than with the distributed blocks and the availability of extra resources that can be translocated from elsewhere in its territory to the antagonistic mycelial front is likely to be important (Lindahl and Olsson, 2004; Hiscox and Boddy, 2017). The production of extracellular enzymes, diffusible and volatile organic compounds (VOCs) and non-diffusible metabolites are mediators of antagonistic interactions (Hiscox et al., 2010; El Arieibi et al., 2016), and changes in these need further study.

While *S. gausapatum* maintained a greater proportion of its territory when occupying a greater adjacent volume (experiments 6 and 7; Fig. 3F and G), concomitantly *S. hirsutum* lost more territory to *T. versicolor* suggesting a faster rate of replacement of *S. hirsutum* by *T. versicolor* which was not the case when *S. gausapatum* was less combative (i.e. *S. gausapatum* was replaced first when fungi were dispersed). This same phenomenon of initial replacement of the weakest competitor before all others by the stronger was seen previously in an experiment when the aggressive *Phanerocheate velutina* grew from wood across the surface of soil to

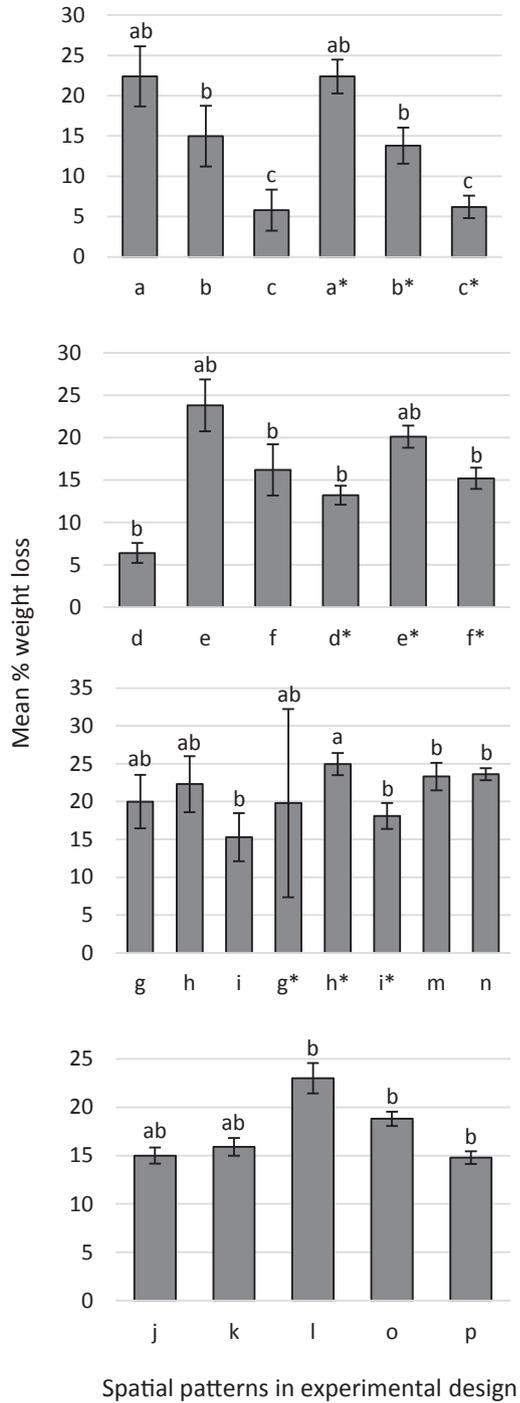


Fig. 4. Mean % weight loss after 119 d of all blocks within each interaction type. Error bars denote SEM and statistical difference between group means is shown. Spatial patterns are coded as per Fig. 1. * Denotes parallel vessel alignment.

three wood blocks each colonised by a different species: the weaker combatant of the three was attacked and replaced first (Abdalla & Boddy 1996).

4.2. Relative positioning of individuals affects outcomes

The pair-wise outcomes were transitive (*T. versicolor* > *S. hirsutum* > *S. gausapatum*; Fig. 3A), as in earlier studies (Hiscox et al., 2015b, 2016a) and reflects the life history

strategies (Andrews, 1992) of the species: secondary colonisers > primary coloniser. If fed into a community model, this would likely result in fast replacement of *S. gausapatum* by *T. versicolor*, followed by later replacement of *S. hirsutum* and collapse of the community to a single species. When spatial heterogeneity was increased in 3-dimensional structures and patchiness decreased (i.e. when *S. gausapatum* occupied a larger adjacent volume), however, the community network loop closed, which is characteristic of intransitivity and promotes co-existence (Laird and Schamp, 2006), with *T. versicolor* successfully attacking *S. hirsutum*, and *S. gausapatum* deadlocking with both of the other competitors. The survival rate of less competitive species is thought to increase with environmental heterogeneity due to the provision of permanent spatial refuges (Brockhurst et al., 2005). Given a longer interaction time and following resource depletion, it is probable that the relationships in the 3-D cube would de-stabilise and the relative volume of territory occupied by each species would change, with *S. hirsutum* likely becoming extinct from the community and *T. versicolor* establishing dominance within it. The latter, most ecologically dominant species, would then occupy even more territory, and ultimate replacement of *S. gausapatum* would be predicted. While the outcomes of interactions in three-species 2-D systems were largely reflective of those in pairs (Fig. 3A,B,C), scaling up of spatial dynamics into larger systems and altering the patchiness of species distributions, resulted in outcomes that were not expected. Changes in community structure are commonly predicted from pair-wise experimental data (Veech, 2012), however, the current study indicates why caution should be taken when making such extrapolations.

The position of individual fungi relative to their competitors affected outcomes in three-block interactions (Fig. 3B), but this was not evident in more complex nine-block and 27-block interactions (Fig. 3C and D). *T. versicolor* was most combative when positioned between its competitors, *S. gausapatum* fared better when it was not positioned next to *T. versicolor* (i.e. on the edge next to *S. hirsutum*), and *S. hirsutum* was equally combative when placed next to *S. gausapatum* in both arrangements (edge position next to *S. gausapatum* and when placed centrally between the two) but lost the majority of its territory when placed on the edge next to *T. versicolor* (Fig. 3B). The greater success of *T. versicolor* when placed centrally probably relates to its ability to rapidly extend its territory by capturing that of *S. gausapatum*, making it then relatively more combative than *S. hirsutum* by virtue of the large resource to which it then had access. This aligns with the reason for the greater success of *S. gausapatum* when territory was not fragmented, increased competitive advantage when larger territory is occupied (Holmer and Stenlid, 1993) and to a previous study (Hiscox et al., 2017) with combinations of three beech wood blocks that found fungi were most successful when positioned next to an uncolonised resource, and thus able easily to obtain additional territory.

4.3. Xylem vessel orientation influences community structure

S. hirsutum and *S. gausapatum* were less successful when they met the most combative opponent, *T. versicolor*, ‘head on’ growing towards one another along vessels whose cut ends were in contact (Fig. 3A and B), than when they were in parallel xylem alignments (Fig. 3E), showing effects of resource structural complexity (hypothesis 1). This probably relates to the different combative mechanisms that fungi employ simultaneously, and how these are affected by wood architecture. Interference competition (combat) can occur at a distance from the antagonist, as a result of VOCs and/or diffusible organic compounds (DOCs) and enzyme activity, and following hyphal contact with these chemicals and other

mechanisms (Boddy, 2000; Boddy and Hiscox, 2016). When mycelia meet 'head on', all mechanisms can be brought to bear, and in large quantity. When mycelia are in columns of decay parallel to one another, antagonistic chemicals and hyphae themselves can only reach the target opponent hyphae via natural pores in wood cell walls or decay cavities produced by fungal breakdown of the wood cell wall, and breaching of any barriers (such as pseudosclerotial plates) formed by opponents. This presumably allows a smaller 'invasive force', at least at early stages of decay. When traversing parallel decay columns, spatial refuges may occur which may account for the greater competitive strength of *Stereum* spp. When interacting in this orientation, *T. versicolor* was unaffected by substratum orientation. Its dominance in real-world communities, before the arrival of cord-forming species, may be partially explained by its ability to compete efficiently regardless of substratum complexity as natural resources often exhibit non-uniformity in the form of wounds, burrs and knots.

4.4. Effect of interactions and community dynamics on community productivity

The rate of wood decomposition often increases during interactions, as evidenced by weight loss after several months (Hiscox et al., 2016a) and 'instantaneous' CO₂ evolution (Hiscox et al., 2015b) presumably reflecting increased metabolic cost of the production of enzymes, volatiles, reactive oxygen species etc., required for attack and defence (Woodward and Boddy, 2008). In the present study the rate of decomposition varied under different scenarios, but notably the decay rate of wood colonised by both *S. hirsutum* and *S. gausapatum* paired together (Fig. 4C), was much slower than in other combinations. This interaction resulted in deadlock with no further metabolic demand for attack and defence chemicals. Hiscox et al. (2017) similarly reported a lack of change in the rate of decay when outcomes were deadlock. When *T. versicolor* interacted with *Bjerkandera adusta* decay rate varied depending on whether the fungus had access to an extra, uncolonised resource.

It was hypothesised (hypothesis 3) that spatial heterogeneity in a multi-species community would cause changes to community performance due to species complementarity and resource partitioning (Wagg et al., 2015; Hiscox et al., 2015b). Although the dimensionality of interactions and the effect of the primary coloniser's larger adjacent volume were not of direct significance to the rate of decomposition, spatial structure caused changes to community dynamics. Since different species decay wood at different rates, community composition has a major impact on decay rate. In the real-world where decay communities are made up of many more species and individuals of the same species, the rate of productivity, therefore, is likely to change depending on the specific suite of species comprising the community. Additionally, as spatial heterogeneity and reduction in patch fragmentation lead to intransitive relationship networks which support biodiversity, over a longer period, i.e. sufficient time for decomposition to progress, community productivity should be influenced (Maynard et al., 2017b).

5. Conclusions

This study is the first to assess the combative interactions of multiple fungi in wood with comparisons between 2- and 3-dimensional experimental systems. Our results provide an insight into the mechanisms of species coexistence, and how interactions change over different spatial scales. Competitive outcomes observed in 3-D systems were not reflective of the transitive interactions (*T. versicolor* > *S. hirsutum* > *S. gausapatum*) exhibited in 2-D systems. This highlights the importance of 3-dimensional

decay systems with multi-species interactions for ecologically pertinent study of community dynamics. Spatial heterogeneity was important for coexistence of all individuals within the community, while the weakest individuals were outcompeted in more fragmented, and spatially more simple systems. The exact mechanisms responsible for this change in coexistence potential are unclear, and investigation into VOCs, extracellular enzyme and metabolite production during 2- and 3-dimensional community interactions of differing spatial dynamics should be conducted. Whether fungi met and interacted with each other 'head on' growing towards each other along xylem vessels or whether decay columns were parallel with each other (i.e. complexity of the resource) affected the outcome of interactions such that an increase in the weaker competitor's combativeness was positively correlated with substratum complexity. Dimensionality did not have a direct effect on the rate of decomposition, however, spatial heterogeneity altered the dynamics of the community, which in the real-world is likely to be reflected by changes to decomposition and nutrient cycling.

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Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.funeco.2018.02.001>.

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