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Location, location, location: priority effects in wood decay communities may vary between sites

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Summary

Priority effects are known to have a major influence on fungal community development in decomposing wood, but it has not yet been established whether these effects are consistent between different geographical locations. Here, beech (*Fagus sylvatica*) wood disks that had been pre-colonized with three wood decay basidiomycetes were placed in seven woodland sites with similar characteristics for 12–24 months, and the successor communities profiled using culture-based techniques coupled with amplicon sequencing. On the majority of sites, assembly history differed as a result of primary versus secondary resource capture only (i.e. different communities developed in uncolonized control disks compared with those that had been pre-colonized), but on certain sites distinct successor communities followed each pre-colonizer species. This study provides preliminary evidence that differences in abiotic factors and species pools between sites can cause spatial variation in how priority effects influence wood decay communities.

Introduction

Understanding and predicting the establishment and development of decomposer communities is essential for modelling carbon cycling and other ecosystem functions (Bardgett *et al.*, 2008; Chapin *et al.*, 2009; McGuire and Treseder, 2010). However, decomposer community change is unpredictable, idiosyncratic and sensitive to environmental variation (Van der Wal *et al.*, 2014). Consequently, community structure is omitted from many models, despite its importance in ecosystem dynamics (Wardle,

2002; Deacon *et al.*, 2006; Hansen *et al.*, 2008; Heichmann and Reichstein, 2008). Wood decay communities are especially important since lignocellulolytic fungi are key determinants of forest carbon sequestration rates and nutrient cycling (Baldrian and Lindahl, 2011). Including fungal community dynamics could help bridge the gap between observed and predicted decay rates in wood decay models (Van der Wal *et al.*, 2014).

Community structure and function is largely determined by assembly history (the timing and sequence in which species join a community), a major driver of which are 'priority effects', where the resource's initial colonizers affect the colonization success of subsequent arrivals (Fukami *et al.*, 2010; Dickie *et al.*, 2012; Ottosson *et al.*, 2014; Hiscox *et al.*, 2015). Wood decay communities lend themselves well to studies of assembly history and priority effects. While the general order of colonization is well established for certain fungal species, there is large variation in the timing of species immigration and interactions (Boddy, 2001; Boddy and Heilmann-Clausen, 2008; Fukami *et al.*, 2010). Local dispersal sources of wood decay fungi, such as fruiting structures or mycelial cords and rhizomorphs (linear aggregations of hyphae; Boddy, 2001) strongly affect colonization patterns (Edman *et al.*, 2004).

Fungal community change is largely driven by antagonistic mycelial interactions, which are affected by local abiotic and biotic factors (Boddy and Heilmann-Clausen, 2008). Mycelial antagonism involves various morphological and biochemical responses (Boddy, 2000), resulting either in deadlock (no change in territory occupied by either combatant) or replacement (partial or complete) of one combatant by another, leading to community change (Boddy, 2000). The initial community is fairly characteristic of different angiosperm tree species, comprising endophytes latently present in functional sapwood and ruderal opportunists arriving as spores (Boddy *et al.*, 1989; Hendry *et al.*, 2002; Parfitt *et al.*, 2010). The initial community progressively changes as species are displaced by more aggressive colonizers (or, later on, stress-tolerant species), which arrive at the resource as spores or mycelial cords (Boddy, 2001; Boddy and Heilmann-Clausen, 2008; Fricker *et al.*, 2008; Boddy *et al.*, 2009).

Priority effects are determined not only through combative hierarchies/mechanisms, but by resource alteration

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through enzyme activity, mycelial growth and secondary metabolites (Boddy and Heilmann-Clausen, 2008; Allison, 2012). Species differ in their decay rates and/or biochemical processes, structurally and chemically altering the resource (Worrall *et al.*, 1997; Woodward and Boddy, 2008). Predecessor species may effectively select for successor species adapted to certain conditions. Several studies on wood decay communities demonstrated priority effects at the species level (Fukami *et al.*, 2010; Lindner *et al.*, 2011; Dickie *et al.*, 2012; Ottosson *et al.*, 2014; Hiscox *et al.*, 2015). Recently, community development following nine different single pre-colonizer species was studied in beech wood on the forest floor (Hiscox *et al.*, 2015). Distinct successor communities followed ascomycete versus basidiomycete pre-colonizers, and one of the basidiomycete pre-colonizers led to a unique successor community, indicating that individual species may have pivotal effects in assembly history.

Priority effects may account for much of the community structure variation in different localities with similar environmental conditions (Chase, 2010; Weslien *et al.*, 2011). However, it has not yet been tested whether priority effects are consistent across locations; previous studies were either undertaken in the laboratory or used a single field site. Assembly history within a resource is constrained by the pool of available successors, which differs between geographic areas (Edman *et al.*, 2004); the presence of potential replacing species varies through differences in local species abundance, fruiting phenology and species of wood available. For example, fungal assemblages within decaying *Picea abies* wood varied as much as 68% between sites (Olsson *et al.*, 2011). It is not yet known whether priority effects differ between similar forests in different locations.

Here, we assess the consistency of priority effects: do the same predecessor species result in convergent successor communities on different sites? Since abiotic conditions and hence community composition vary between woodlands (Ferris *et al.*, 2000; Nordén and Paltto, 2001; Iršénaitė and Kutorga, 2007), we hypothesize that successor communities will differ between multiple sites with similar characteristics. We test how priority effects determine fungal community composition in wood by pre-colonizing beech disks with species from different successional stages (*Vuilleminia comedens*, primary colonizer; *Trametes versicolor*, secondary colonizer; *Hypholoma fasciculare*, late secondary cord-forming colonizer) and blank, uncolonized control disks, and placing them on the floor of seven deciduous woodlands, followed by community characterization using culture-based approaches and high-throughput amplicon sequencing. We also test the hypothesis that time in the field has a greater effect on community structure than location.

Results

Isolation of fungi from wood disks

Retrieval rate of wood disks varied across sites (52.8–90%), but at least three replicates of each pre-colonizer/control were retrieved from every site (Table S1), excepting *H. fasciculare* on Wytham site 1 (set Wy1B), where disks could not be found, due to unexplained mammal activity (Hiscox *et al.*, 2015). The prevalence of attached cords varied between sites (12.5–100%; Fig. 1; Table S1). Fungal and/or bacterial outgrowth occurred from all wood isolation points, with multiple types of outgrowth per point being common. Unknown fungi (i.e. not pre-colonizer) grew out from 98.2% disks (corresponding to 77.1% isolation points), of which most (69.3% isolation points; Table S1) were considered to be opportunists that grew and sporulated rapidly on agar, but were not active in wood decay and probably only colonized disk surfaces. In only 0.7% cases (two disks) did all isolation points result in outgrowth of the same non-opportunistic invading fungus. The highest isolation occurrence of non-opportunistic invading fungi followed pre-colonization by *V. comedens* (71.1% disks), and the lowest following *H. fasciculare* (17.1% disks).

Vuilleminia comedens was not recovered from any disks by re-isolation, and while *T. versicolor* was recovered from 15% disks overall, it had been at least partially replaced within every disk (Fig. 1A; Table S1). Very little replacement of *H. fasciculare* occurred, with *H. fasciculare* re-isolated from 96.6% disks in the field for 12 months, and 57.1% disks after 24 months.

pH within disks at the end of the experiment was significantly lower at the Garth and Usk sites than the other sites ($F_{5,71} = 5.885$, $P = 0.00013$; Fig. S1A). Conversely, soil pH was significantly higher at the Garth site and Wytham site 2 compared with other sites ($F_{5,71} = 35.83$, $P < 0.0001$; Fig. S1B). There was no correlation between wood and soil pH (Fig. S1C).

Identification of unknown fungi from isolations and direct incubation

Isolation from wood chips, cords and incubation of disks, yielded 106 fungal strains that were not pre-colonizers, of which 75 were identified into 33 species by sequencing (Tables S1 and S2). The remaining fungi could not be identified due to insufficient material or inability to obtain pure cultures, and these were grouped into 23 morphospecies (Table S2).

Occurrence of pre-colonizer operational taxonomic units

Only three of 38 *V. comedens* disks contained operational taxonomic units (OTUs) corresponding to this species,

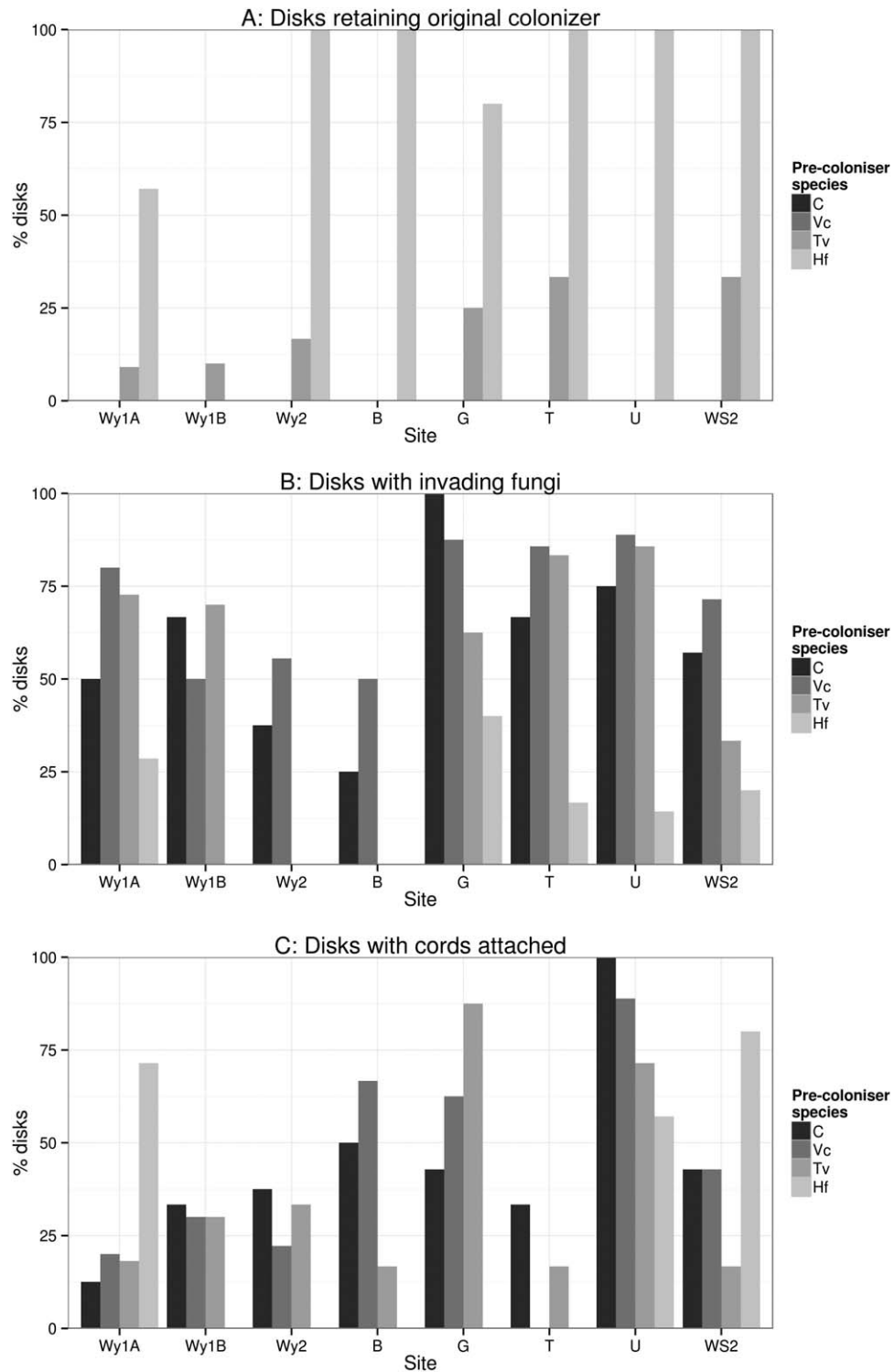


Fig. 1. Changes in community composition within disks that had been left in the field for 12 or 24 (Wy1A only) months. Disks were either initially uncolonized controls (C), or had been pre-colonized with either *V. comedens* (Vc), *T. versicolor* (Tv) or *H. fasciculare* (Hf). Plots show the percentage of disks that: A, retained the original pre-colonizer; B, contained invading fungi; and C, had mycelial cords attached. See Table 1 for site name abbreviations. No data were available for Hf in site Wy1B as disks could not be found.

Table 1. Locations and characteristics of sites used.

Site	Location			Ground cover			Soil pH	Soil type ^a
	Abbrev	County	Grid ref	Tree species present	%	Species		
Wytham site #1	Wy1	Oxfordshire	51.775912, -1.342048	Predominantly beech, some oak and hazel	80	Bluebell, sedge	-	Shallow lime-rich soil over chalk or limestone
Wytham site #2	Wy2	Oxfordshire	51.768902, -1.344022	Predominantly beech, some hazel	< 5	Sedge, moss	6.58	Shallow lime-rich soil over chalk or limestone
Bagley	B	Oxfordshire	51.720134, -1.266750	Beech, hazel, holly	25	Fern, bramble, sedge	4.52	Slowly permeable, seasonally wet, acid loamy and clayey soil
Hazelgrove, Tintern	T	Monmouthshire	51.711529, -2.684246	Predominantly oak, some beech and holly	95	Bluebells, bramble, ferns, sedge	5.67	Freely draining, slightly acid, loamy soil
Whitestone	WS	Monmouthshire	51.725378, -2.953690	Beech, oak, sycamore	75	Bluebells, ivy, ferns, bramble, ivy, wood anemone	4.95	Freely draining, slightly acid, loamy soil
Coedy Paen, Usk	U	Monmouthshire	51.672524, -2.953690	Predominantly beech, some ash and sycamore	< 5	Woodrush, fern, ivy	4.68	Freely draining, slightly acid, loamy soil
Garth, Cardiff	G	Cardiff	51.535784, -3.277605	Beech, oak, holly	< 5	Fern, sedge, bramble	4.70	Freely draining, slightly acid but base-rich soil

a. Soil type information from Cranfield University 2015. *The Soils Guide*. Available: www.landis.org.uk. Cranfield University, UK. Last accessed 09/06/2015.

and at low levels (mean 3.1% OTU counts/sample where present; Table S3). *Trametes versicolor* maintained a greater foothold, and was detected in nine out of 38 disks (mean 37.4% OTU counts/sample where present), although in two samples it dominated the OTU profile with > 80% counts (Table S3). Very little replacement of *H. fasciculare* occurred (mean 95.9% OTU counts/sample; Table S3).

Overall differences in composition between sites

Community composition was significantly different following different pre-colonizers, and on different sites, and there was a significant interaction between pre-colonizer and site ($F_{14,110} = 1.7244$, $P < 0.001$; Figs 2 and 3). These highly significant differences were maintained when the decomposer communities ($F_{14,110} = 1.4992$, $P < 0.001$) and co-colonizer communities ($F_{14,110} = 1.7749$, $P < 0.001$) were considered separately.

Differences in profiles of invading species between sites

Overall variability of OTU profiles did not significantly differ ($P > 0.05$) between sites (Table S4). Significant differences ($P < 0.05$) in species diversity and community evenness were detected between Usk and all other sites (Table S5). There were more Ascomycota OTUs detected on each site than Basidiomycota (Fig. 4A; Table S5). However, in terms of OTU counts (i.e. occurrence), roughly equal numbers of Ascomycota and Basidiomycota were detected in disks from Wytham site 1 (Wy1A&B), Bagley, Tintern and Whitestone, while Basidiomycota outnumbered Ascomycota at Usk, Garth and Wytham site 2 (Table S5). Each site had a different dominant replacing OTU, averaging 26.5% OTU counts on any one site. On five of the seven sites, the dominant replacing species were cord-forming Basidiomycota (Fig. 4C; Table S5).

Similar pools of invading species were detected on certain sites: e.g. there were no significant differences in community composition in disks from the Whitestone (WS2) and Bagley (B) sites, regardless of the pre-colonizer treatment (Fig. 2; Table S6). However, on other sites invading species were affected by different pre-colonizers: e.g. communities within disks from Tintern, Bagley and Whitestone were not significantly different in disks pre-colonized with *V. comedens* (Fig. 2). Conversely, disks from the Usk site had unique successor communities in controls and following *V. comedens* pre-colonization, but not following *T. versicolor* pre-colonization (Fig. 2).

Comparing priority effects between sites

The effect of pre-colonizers on community development differed between sites. On two sites (Wy1B and B) each

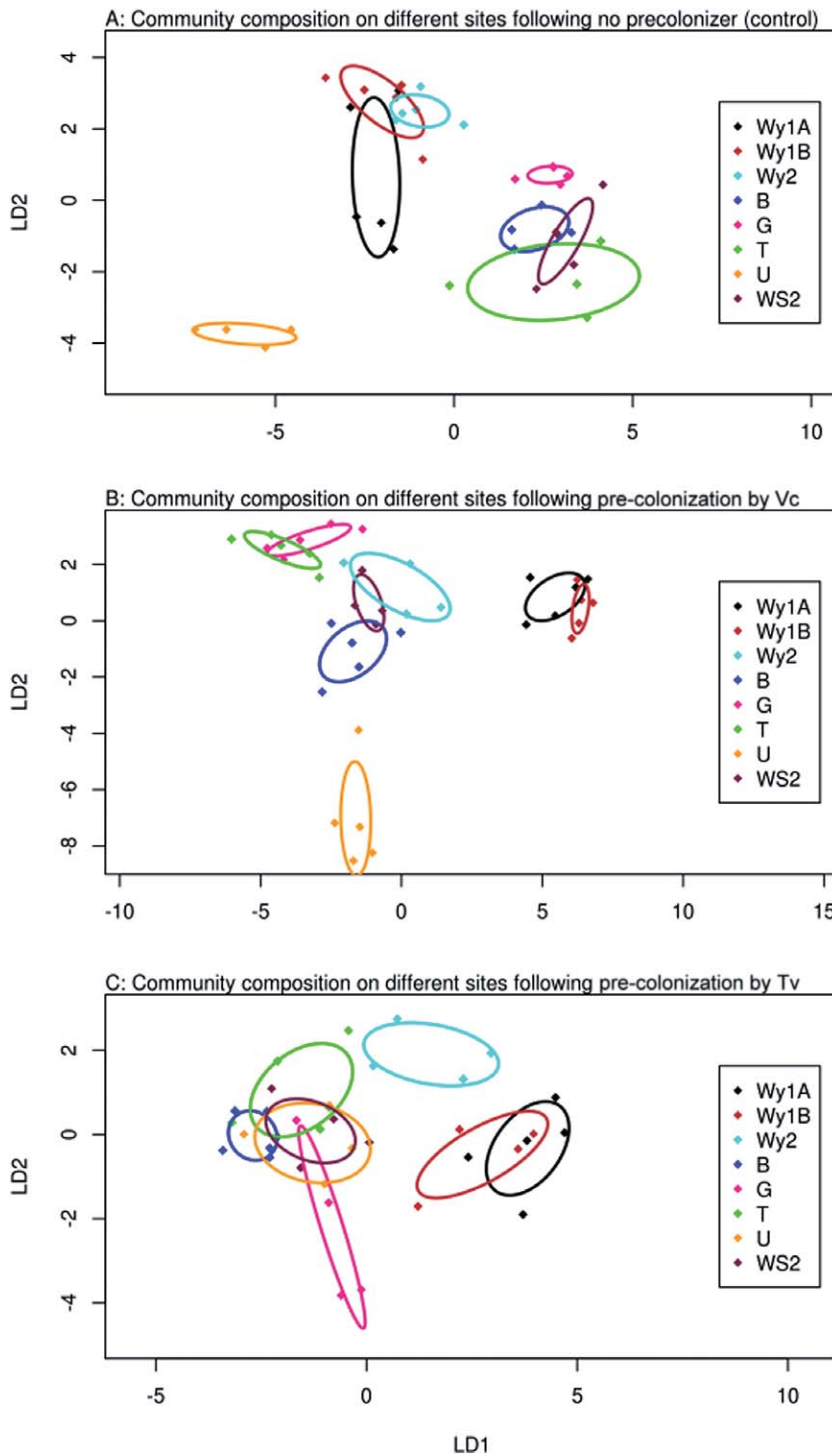


Fig. 2. Fungal community composition after 12 or 24 months in disks pre-colonized with different species at different field sites. CAPdiscrim plots of fungal OTU composition in A, initially uncolonized (control) disks; B, disks pre-colonized with *V. comedens* (Vc); and C, disks pre-colonized with *T. versicolor* (Tv). Ordination was based on the Bray–Curtis metric of dissimilarity. Points represent individual samples and ellipses indicate treatment (i.e. site) means with 95% confidence intervals fitted onto the spatial ordination. See Table 1 for site name abbreviations.

pre-colonizer led to a significantly ($P < 0.05$) distinct community (Fig. 3; Table 2). However, on five sites, there were no significant differences ($P > 0.05$) between communities following pre-colonization with *V. comedens* or *T. versicolor*, but a significantly distinct community followed the controls (Fig. 3; Table 2).

Most inter-site differences were driven by variation in colonization rate by the dominant replacing species

(Table 2). For example, on Wytham site 2, *V. comedens* and *T. versicolor* disks had high occurrences of *Coprinus lagopus*, whereas control disks had higher occurrence of *Coprinus cortinarius* and *L. hispidus* (Table 2). On Wytham site 1 (Wy1B), dominant invading cord-forming species differed depending on pre-colonizer: control disks were dominated by *Coprinellus impatiens*, *V. comedens* disks were dominated by *Phanerochaete* sp., and *T. versicolor*

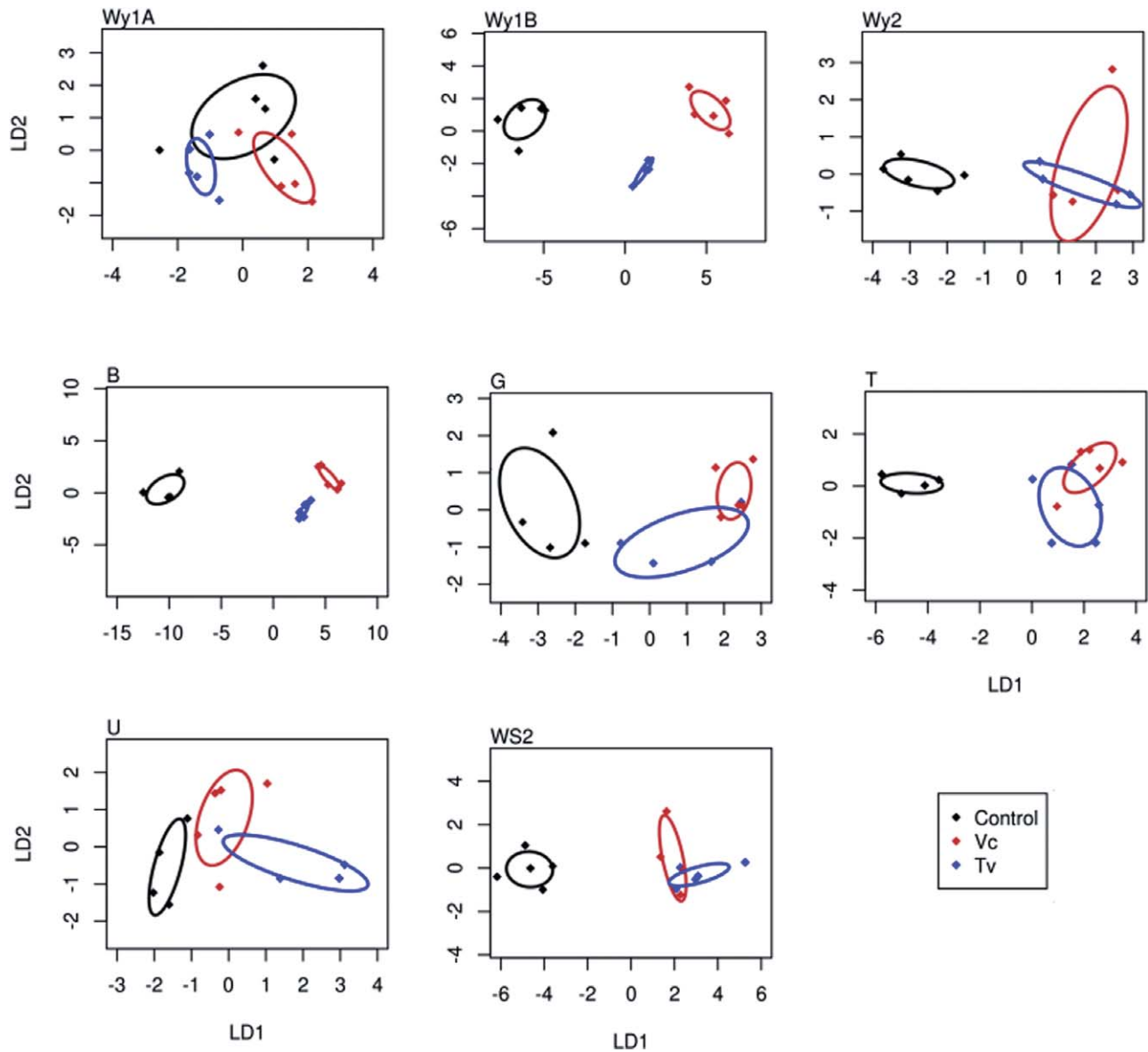


Fig. 3. Fungal community composition in disks following different predecessors on different field sites. CAPdiscrim plots of fungal OTU composition in uncolonized (Control) disks, and in disks pre-colonized with either *V. comedens* (Vc) or *T. versicolor* (Tv), after 12 months at field sites Wy1A, Wy2, B, G, T, U and WS2, and after 24 months at Wy1B. Ordination was based on the Bray–Curtis metric of dissimilarity. Points represent individual samples and ellipses indicate treatment (i.e. pre-colonizer) means with 95% confidence intervals fitted onto the spatial ordination.

disks were dominated by *Xenamatella vaga* (Table 2). On the Usk site, differences in extent of replacement by *Megacollybia platyphylla* and *Resinicium bicolor* drove most of the variation in community composition following different pre-colonizers (Table 2).

Ascomycota and Basidiomycota occurrence, in terms of OTU counts, were similar following all pre-colonizers (75–77% and 22–24% OTU counts, respectively; Table S3). The dominant replacing species was an ascomycete (*L. hispidia*) in the control disks, whereas a basidiomycete (*Scopuloides hydnoides*) dominated

the replacing community in disks pre-colonized by *V. comedens* and *T. versicolor* (Table S3). *Lasiosphaeria hispidia* and *S. hydnoides* were detected at all sites (Table S7).

Effect of time in the field

There were no significant differences in overall community composition in disks on the same site for 12 (Wy1B) and 24 (Wy1A) months (Fig. 2; Table S6). However, there were different patterns of priority effects (Fig. 3; Table 2).

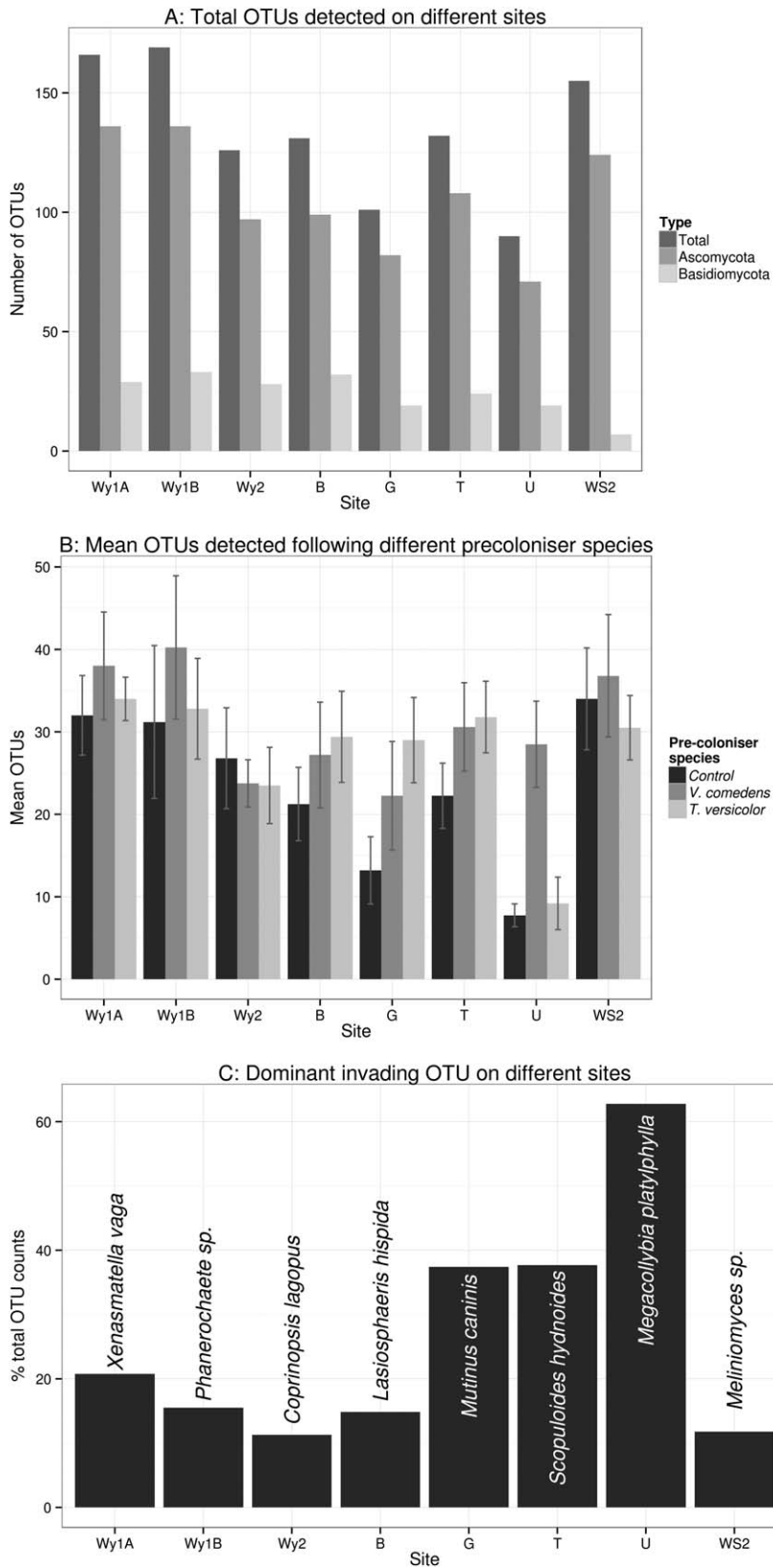


Fig. 4. Composition of invading communities following different pre-colonizers. A. The number of OTUs identified on each site, and the number of Ascomycota or Basidiomycota. B. The mean number of OTUs detected following different pre-colonizer species (five replicates, \pm 95% confidence interval). C. The dominant invading OTU detected on each site, expressed as a per cent of total OTU counts from each site. For site abbreviations, see Table 1.

Table 2. Operational taxonomic units responsible for the top 50% variability between pre-colonizer species.

Site	Pairwise comparison	Adonis outcomes			Adonissimper results					
		F	P (adj)	Ratio	Av. % A OTUs	Av. % B OTUs	Cumulative variation	OTU Identification	Co-colonizer or decomposer?	Taxonomy
Wy1A	Control – <i>V. comedens</i>	1.419	0.152	0.90	371.2	214	0.1912	<i>Xenasmattella vaga</i>	D	Basidiomycota
				0.67	101.6	242.2	0.3239	<i>Coprinellus impatiens</i>	D	Basidiomycota
				0.67	248.8	36.6	0.4242	<i>Phialocephala dimorphospora</i>	C	Ascomycota
				0.71	8.6	221	0.5231	uncultured fungus/Chalara	C	Ascomycota
	Control – <i>T. versicolor</i>	1.412	0.152	0.90	371.2	215.6	0.2018	<i>Xenasmattella vaga</i>	D	Basidiomycota
				0.97	248.8	212	0.3275	<i>Phialocephala dimorphospora</i>	C	Ascomycota
				0.52	164.2	9.8	0.4064	<i>Lasiosphaeris hispida</i>	C	Ascomycota
				0.81	1.2	155.8	0.4786	<i>Mycena galopus</i>	D	Basidiomycota
	<i>V. comedens</i> – <i>T. versicolor</i>	1.929	0.051	0.62	101.6	31.4	0.535	<i>Coprinellus impatiens</i>	D	Basidiomycota
				0.67	214	215.6	0.1512	<i>Xenasmattella vaga</i>	D	Basidiomycota
				0.55	242.2	31.4	0.2651	<i>Coprinellus impatiens</i>	D	Basidiomycota
				0.76	221	29.2	0.3662	uncultured fungus/Chalara	C	Ascomycota
Wy1B	Control – <i>V. comedens</i>	4.301	0.007	0.51	187	2.8	0.449	<i>Hypocrea pachybasioides</i>	C	Ascomycota
				0.98	36.6	212	0.5264	<i>Phialocephala dimorphospora</i>	C	Ascomycota
				0.87	406	154	0.1784	<i>Phanerochaete</i>	D	Basidiomycota
				2.10	0.4	283.8	0.2941	<i>Hyphodiscus hymeniophilus</i>	C	Ascomycota
	Control – <i>T. versicolor</i>	2.491	0.007	0.50	2.8	235	0.3907	<i>Xenasmattella vaga</i>	D	Basidiomycota
				0.72	222	0.2	0.4813	<i>Coprinellus impatiens</i>	D	Basidiomycota
				1.14	0	134	0.5362	<i>Helotiales sp SGSg25</i>	C	Ascomycota
				0.75	406	0.5	0.1655	<i>Phanerochaete</i>	D	Basidiomycota
	<i>V. comedens</i> – <i>T. versicolor</i>	2.779	0.003	1.12	92.6	322.25	0.2985	<i>Lasiosphaeris hispida</i>	C	Ascomycota
				0.57	2.8	277.5	0.4132	<i>Xenasmattella vaga</i>	D	Basidiomycota
				0.76	222	19.5	0.5063	<i>Coprinellus impatiens</i>	D	Basidiomycota
				0.73	235	277.5	0.1695	<i>Xenasmattella vaga</i>	D	Basidiomycota
Wy2	Control – <i>V. comedens</i>	2.307	0.003	1.02	0.4	322.25	0.3057	<i>Lasiosphaeris hispida</i>	C	Ascomycota
				2.09	283.8	1	0.4254	<i>Hyphodiscus hymeniophilus</i>	C	Ascomycota
				0.49	154	0.5	0.4905	<i>Phanerochaete</i>	D	Basidiomycota
				1.11	134	4.5	0.5458	<i>Helotiales sp SGSg25</i>	C	Ascomycota
	Control – <i>T. versicolor</i>	2.496	0.021	0.56	0.2	289.75	0.1175	uncultured Cantharellales	D	Basidiomycota
				0.92	228.8	104.5	0.219	<i>Psathyrella microrhiza</i>	D	Basidiomycota
				0.49	245.8	0	0.3185	<i>Coprinus cortinatus</i>	D	Basidiomycota
				0.49	229.2	0	0.4115	<i>Lasiosphaeris hispida</i>	C	Ascomycota
	<i>V. comedens</i> – <i>T. versicolor</i>	1.088	0.386	0.56	0	205	0.4953	<i>Ceriporia reticulata</i>	D	Basidiomycota
				0.86	170	0	0.5644	<i>Mycena galopus</i>	D	Basidiomycota
				1.62	36.4	390.25	0.1544	<i>Coprinopsis lagopus</i>	D	Basidiomycota
				1.38	1.6	249.25	0.2572	<i>Chalara sp PRM915970</i>	C	Ascomycota
B	Control – <i>V. comedens</i>	6.599	0.006	0.50	245.8	4.5	0.3595	<i>Coprinus cortinatus</i>	D	Basidiomycota
				0.75	104.4	184.25	0.4577	<i>Scopuloides hydnooides</i>	D	Basidiomycota
				0.49	229.2	0.25	0.5524	<i>Lasiosphaeris hispida</i>	C	Ascomycota
				1.58	43.25	390.25	0.1582	<i>Coprinopsis lagopus</i>	D	Basidiomycota
	Control – <i>T. versicolor</i>	3.915	0.003	0.56	289.75	0	0.2819	uncultured Cantharellales	D	Basidiomycota
				0.56	205	0	0.37	<i>Ceriporia reticulata</i>	D	Basidiomycota
				1.25	72.75	249.25	0.458	<i>Chalara sp PRM915970</i>	C	Ascomycota
				0.68	40.5	184.25	0.5455	<i>Scopuloides hydnooides</i>	D	Basidiomycota
	<i>V. comedens</i> – <i>T. versicolor</i>	3.009	0.005	1.46	681.25	0	0.273	<i>Lasiosphaeris hispida</i>	C	Ascomycota
				0.76	332.25	187.2	0.4419	<i>Phallus impudicus</i>	D	Basidiomycota
				2.13	0	210.2	0.5261	uncultured Chalara	C	Ascomycota
				1.46	681.25	0.2	0.2641	<i>Lasiosphaeris hispida</i>	C	Ascomycota
G	Control – <i>V. comedens</i>	6.599	0.006	1.25	0	466.8	0.445	uncultured Chaetosphaeria	C	Ascomycota
				0.58	332.25	0	0.5736	<i>Phallus impudicus</i>	D	Basidiomycota
				1.26	1	466.8	0.1949	uncultured Chaetosphaeria	C	Ascomycota
				0.50	0.6	265.8	0.3056	<i>Psathyrella fibrillosa</i>	D	Basidiomycota
	Control – <i>T. versicolor</i>	3.915	0.003	2.02	210.2	8.4	0.3899	uncultured Chalara	C	Ascomycota
				0.49	187.2	0	0.468	<i>Phallus impudicus</i>	D	Basidiomycota
				0.56	172.6	4.6	0.5399	uncultured Basidiomycota	D	Basidiomycota
				1.18	716.4	0.2	0.3023	<i>Mutinus caninus</i>	D	Basidiomycota
	<i>V. comedens</i> – <i>T. versicolor</i>	1.979	0.087	1.21	223.8	586	0.5456	<i>Scopuloides hydnooides</i>	D	Basidiomycota
				1.17	716.4	818.5	0.3913	<i>Mutinus caninus</i>	D	Basidiomycota
				0.72	223.8	296.25	0.6484	<i>Scopuloides hydnooides</i>	D	Basidiomycota
				1.49	0.2	818.5	0.3659	<i>Mutinus caninus</i>	D	Basidiomycota
Control – <i>T. versicolor</i>	2.243	0.038	1.23	586	296.25	0.6291	<i>Scopuloides hydnooides</i>	D	Basidiomycota	

Table 2. cont.

Site	Pairwise comparison	Adonis outcomes				Adonis-Simper results				
		F	P (adj)	Ratio	Av. % A OTUs	Av. % B OTUs	Cumulative variation	OTU Identification	Co-colonizer or decomposer?	Taxonomy
T	Control –	3.633	0.002	1.94	42.5	767.4	0.2954	<i>Scopulooides hydnooides</i>	D	Basidiomycota
	<i>V. comedens</i>			1.52	548	2.6	0.5179	<i>Chalara sp PRM915970</i>	C	Ascomycota
	Control –	2.651	0.002	1.10	42.5	577.4	0.2319	<i>Scopulooides hydnooides</i>	D	Basidiomycota
	<i>T. versicolor</i>			1.52	548	27	0.4487	<i>Chalara sp PRM915970</i>	C	Ascomycota
				0.56	321.25	0	0.5798	<i>Tubaria sp TU110249</i>	D	Basidiomycota
	<i>V. comedens</i> –			1.46	767.4	577.4	0.3312	<i>Scopulooides hydnooides</i>	D	Basidiomycota
<i>T. versicolor</i>	0.49	0	171.2	0.4337	<i>Psathyrella fibrillosa</i>	D	Basidiomycota			
	1.11	171.2	14	0.53	<i>Phialocephala dimorphospora</i>	C	Ascomycota			
U	Control –	1.529	0.166	1.77	763.5	1221.2	0.4496	<i>Megacollybia platyphylla</i>	D	Basidiomycota
	<i>V. comedens</i>			0.97	358	0	0.7716	<i>Resinicium bicolor</i>	D	Basidiomycota
	Control –	3.267	0.101	1.87	763.5	390.75	0.3174	<i>Megacollybia platyphylla</i>	D	Basidiomycota
	<i>T. versicolor</i>			0.97	358	0	0.4938	<i>Resinicium bicolor</i>	D	Basidiomycota
				0.77	182.25	163	0.6236	<i>Tubaria sp TU110249</i>	D	Basidiomycota
	<i>V. comedens</i> –			2.921	0.101	1.75	1221.2	390.75	0.4763	<i>Megacollybia platyphylla</i>
<i>T. versicolor</i>	0.56	0	247.25	0.6107	<i>Ceriporia reticulata</i>	D	Basidiomycota			
WS2	Control –	1.864	0.023	0.56	0	269.5	0.1093	<i>Phallus impudicus</i>	D	Basidiomycota
	<i>V. comedens</i>			0.96	227.2	0	0.2024	uncultured <i>Sebacina</i>	D	Basidiomycota
				0.49	215.6	0	0.2895	<i>Phanerochaete</i>	D	Basidiomycota
				0.56	0	191	0.3677	<i>Crepidotus lundellii</i>	D	Basidiomycota
				0.57	0	181.75	0.4416	uncultured <i>Atractiellales</i>	D	Basidiomycota
				0.96	150.2	0	0.503	uncultured <i>Chloridium</i>	C	Ascomycota
	Control –	3.570	0.003	1.02	52.6	284	0.1023	<i>fungal sp</i>	C	Ascomycota
	<i>T. versicolor</i>							<i>mh34632</i> Meliniomyces		
				1.01	0	239.8	0.2029	uncultured <i>Chaetosphaeria</i>	C	Ascomycota
				0.94	227.2	33.6	0.293	uncultured <i>Sebacina</i>	D	Basidiomycota
				0.49	215.6	0.2	0.383	<i>Phanerochaete</i>	D	Basidiomycota
				0.97	150.2	0.2	0.4464	uncultured <i>Chloridium</i>	D	Ascomycota
	1.05	144.4	0	0.5073	<i>Mycena metata</i>	C	Basidiomycota			
<i>V. comedens</i> –	1.076	0.329	0.56	269.5	0	0.1174	<i>Phallus impudicus</i>	D	Basidiomycota	
<i>T. versicolor</i>			1.08	115.5	284	0.2207	<i>fungal sp</i>	C	Ascomycota	
							<i>mh34632</i> Meliniomyces			
			0.98	8.25	239.8	0.3224	uncultured <i>Chaetosphaeria</i>	C	Ascomycota	
			0.56	191	0	0.4064	<i>Crepidotus lundellii</i>	D	Basidiomycota	
			0.57	181.75	0	0.4858	uncultured <i>Atractiellales</i>	D	Basidiomycota	
	0.49	0	85.6	0.5229	<i>Scopulooides hydnooides</i>	D	Basidiomycota			

Pairwise comparisons were performed using Adonis/PERMANOVA, following which a *P*-adjustment was performed to reduce the effect of false discovery rates (Benjamini and Holden, 1995); adjusted *P*-values are shown. *P* < 0.05 indicates a significant difference in community composition between pre-colonizers on the same site. Adonis–Simper tests were conducted to determine which OTUs were responsible for 50% of the variability between pairwise community profiles. See Table 1 for site name abbreviations.

In 12 month samples (Wy1B), each pre-colonizer led to a significantly distinct community (Fig. 3; Table 2). However, in 24 month samples, significant differences were found in community composition between disks pre-colonized by *V. comedens* and *T. versicolor*, but neither was significantly different to the community developing in controls (Fig. 3; Table 2). Differences in community profiles after 12 months were partly driven by *Phanerochaete* sp.; this was only found at very low abundance in 24 month samples, where differences in profiles were instead driven by *Xenasmatella vaga* and *Coprinellus impatiens* (Table 2).

Correlations between community profiles and geographical location or pH

No relationship was found between community composition and position of disks on sites (*P* > 0.05). Community

composition significantly correlated with wood pH (*P* < 0.001) and soil pH (*P* < 0.001). No relationships were found between geographical locations of sites and community composition, or between wood and soil pH.

Discussion

This study showed a clear effect of pre-colonizer on the development of decay communities within dead wood, confirming the findings of Hiscox and colleagues (2015). Moreover, invading species varied between sites, causing different assembly histories between woodlands with similar characteristics. As different assemblages affect the rate of resource decay (Worrall *et al.*, 1997), assembly history could result in different wood decay rates in different woodlands. The profile of invading species was no more similar on sites that were closer together (within the same woodland) than sites further apart. This concurs

with fruit body surveys, where fungal community composition varied highly between forest areas, and also between plots within areas (Ferris *et al.*, 2000; Nordén and Paltto, 2001; Iršénaitė and Kutorga, 2007). Variation in local pools of invading species is driven by biotic and abiotic factors such as habitat variability, forest history and dynamics (including management type), and environmental constraints. These factors impact upon community development history at the woodland level, neutral mechanisms (e.g. the inherent randomness in dispersal and establishment), and biological interactions (Gonthier *et al.*, 2001; Berglund *et al.*, 2011; Bässler *et al.*, 2012; Abrego *et al.*, 2014).

Fungal colonization of wood is influenced both by the dispersal ability of local invading species, as spores or through dispersal organs such as mycelial cords, and by the combative ability of these species, i.e. the ability to displace the resident mycelium (Boddy, 2000; Edman *et al.*, 2004). On all sites, the dominant invading basidiomycete (decomposer) was a cord-forming species. Cord systems grow from colonized resources and forage for new ones, forming extensive (many m²), dynamic networks at the soil–litter interface (Boddy 1993; 1999; Fricker *et al.*, 2008; Boddy *et al.*, 2009). Cord-forming fungi are usually highly combative secondary colonizers; producing cord networks is energetically expensive, and it has been suggested that to be an evolutionarily successful strategy, the cord former must be able to compete against different communities and capture resources at various stages of decay (Stenlid *et al.*, 2008). Single species dominance was most evident at the Usk site, where *M. platyphylla* comprised nearly 63% of fungal OTU counts, and searching for the disks at harvest revealed an extensive mycelial network.

The patchy nature of cord systems might suggest that replacement by cord formers would be concentrated in, or localized to, specific areas within a site, resulting in dispersal limitation (i.e. not all disks would have the opportunity to be invaded by the same set of species, see Fukami *et al.*, 2005). Non-cord-forming invaders may have arrived at disks as spores, and may also be subject to dispersal limitation, although there is limited effect of this over the small size of the experimental grids used (Norros *et al.*, 2012). Our random assortment of treatments and replication should act to control for these effects. Spatial autocorrelation was not detected on any site, suggesting dispersal limitation was not an issue in this experiment. However, any spatial autocorrelation may have been masked by differences in pre-colonizer susceptibility to different invading species.

On the majority of sites, there were clear differences in communities formed as a result of primary resource capture (following initially uncolonized disks, where

species with r-selected characteristics have an advantage) versus secondary resource capture (following *V. comedens* or *T. versicolor*, where combative ability is an advantage). However, clear priority effects occurred on two of the seven sites, with distinct successor communities following *V. comedens* and *T. versicolor*. Priority effects within woody resources, therefore, vary between woodlands. Variation in abiotic factors between sites, such as microclimate or soil pH, influences local species pools (Fukami *et al.*, 2005; 2010; Dickie *et al.*, 2012). Variation in abiotic factors may further affect community development by altering the progress and outcomes of species interactions, which can be affected by temperature, moisture and gaseous regime (Boddy, 2000). Previous field studies on priority effects, species associations and succession in wood decay fungal communities have either used single sites (e.g. Dickie *et al.*, 2012), or merged data from multiple sites with similar characteristics (e.g. Lindner *et al.*, 2011; Ottosson *et al.*, 2014). Similarly, use of multiple sites has been overlooked in studies of priority effects and assembly history in other ecosystems. Our results suggest that to draw meaningful conclusions about priority effects and assembly history, it is essential that location and/or the effect of abiotic factors on local species assemblages and species interactions be considered.

Resource modification may explain differences in community development following different pre-colonizers (Woodward and Boddy, 2008; Allison, 2012): *T. versicolor* decays beech wood faster than *V. comedens* (Hiscox *et al.*, 2015a), but *V. comedens* decreases the pH of colonized wood more than *T. versicolor* (Hiscox *et al.*, 2015). Fungi alter their resource pH, with different species (and hence assemblages) generating different pH (Tudor *et al.*, 2013). The initial pH of wood disks was suspected partly to explain differences in successor communities in beech disks in a previous experiment (Hiscox *et al.*, 2015). We found a significant correlation between successor community composition within disks and pH of the underlying soil, despite an absence of correlation between soil and wood disk pH. This might indicate that soil pH is important in determining local pools of successor species. Future studies should profile the abiotic conditions on each site much more thoroughly, including precise temperature and precipitation measurements, canopy coverage and distance to forest edges. Similarly, attempts to profile the pools of potential successor species could be undertaken, although this is an enormous undertaking as it would need to incorporate profiling air and soil spora at regular intervals, mapping large cord systems and determining species composition in adjacent dead woody resources.

Differences in subsequent community development may also be determined by combative ability, as

T. versicolor (a secondary colonizer) is more combative than *V. comedens* (a primary colonizer; Hiscox *et al.*, 2015). Differences in susceptibility to replacement, especially by cord formers, may explain the variation in priority effects at different sites. An intransitive species hierarchy was implied (Boddy, 2000); *V. comedens* was frequently replaced by *Phanerochaete* sp. on Wytham site 1 and by *M. platyphylla* at Usk, while *T. versicolor* was not. However, on the Garth site, *V. comedens* was much better able to resist replacement by *Mutinus caninus* than *T. versicolor*. The third pre-colonizer species, *H. fasciculare*, a later secondary colonizer, is more combative than *V. comedens* and *T. versicolor* (Hiscox *et al.*, 2015a), and was hardly replaced over the experiment. This differs from a previous study, where after 12 months there was significant replacement of the same strain of *H. fasciculare* (Hiscox *et al.*, 2015), probably reflecting poor pre-colonization in the lab in the earlier experiment. *Hypholoma fasciculare*'s persistence within the resource means that priority effects driven by this species would only be revealed after longer in the field.

Length of time in the field affected the successor communities. After 12 months, distinct successor communities followed both the pre-colonizers *V. comedens* and *T. versicolor* and control disks, driven mostly by susceptibility to replacement by *Phanerochaete* sp., *X. vaga* and *C. impatiens*. However, after 24 months, neither pre-colonizer led to a significantly different successor community compared with control disks, supporting the suggestion that priority effects become weaker as decay progresses (Rajala *et al.*, 2011; Dickie *et al.*, 2012). This may indicate that while priority effects drive differences in decay rate during the early stages of succession, due to the different species assemblages present (Worrall *et al.*, 1997), these effects on decay rate attenuate during later successional stages. Despite a high prevalence of *Phanerochaete* sp. in the 12 month samples (2012–2013), this species hardly colonized the 24 month samples (2011–2013). This implies that *Phanerochaete* sp. cord systems developed more extensively on Wytham site 1 in the latter 12 months. The 24 month disk communities were dominated instead by *X. vaga* and *C. impatiens*, and if these developed a foothold within the disks in the first 12 months of the study, this could have prevented subsequent colonization by *Phanerochaete* sp.

In the previous experiment, also on Wytham site 1 (Hiscox *et al.*, 2015), disks were set out at the same time as the 24 month samples here, but collected after 12 months. The dominant replacing decomposer species were *X. vaga*, *C. impatiens* and *Coprinopsis lagopus*, consistent with the dominant successor species in the current 24 month samples. However, the pattern of priority effects in the previous study was not the same as those in the 12 or 24 month disks used here, but was

instead consistent with the majority of the other sites in the current study: initially uncolonized controls led to a different successor community compared with disks pre-colonized with *V. comedens* and *T. versicolor*. This difference between experiments in subsequent years may have been driven by the increased colonization of the site by *Phanerochaete* sp., or other uncontrollable factors, demonstrating the dynamic nature of fungal interactions and underlining the importance of stochastic rather than deterministic factors in fungal community succession.

Another difference between the present and previous study (Hiscox *et al.*, 2015) is the relationship between decomposer and co-colonizer taxa. Previously, differences in community composition following different pre-colonizers were driven by changes in co-colonizer but not decomposer taxa. Here, significant relationships with site and pre-colonizer species were found for both decomposers and co-colonizers, in part perhaps due to greater sequencing depth in the current study. The dominant co-colonizer taxa may be more consistent between sites than the decomposers, with *Phialocephala dimorphospora*, *Lasio-sphaeris hispida*, *Meliniomyces* sp. and *Chalara* sp. occurring on multiple sites. Although these co-colonizers are thought to be opportunistic scavengers rather than directly contributing to wood decay, they clearly play a large role in wood decay communities. Exactly what this role is, and how they affect the decomposition process, remains an important avenue for future research.

Conclusions

Priority effects following different pre-colonizers varied depending on geographical location: at two sites all pre-colonizer treatments led to distinct successor communities. At the other five sites, priority effects followed a different pattern, with different communities found in initially uncolonized disks (primary resource capture) compared with disks pre-colonized with *V. comedens* or *T. versicolor* (secondary resource capture). Further, length of time in the field altered the pattern of priority effects within a site. Abiotic factors are presumed to be major drivers of these differences in assembly history, determining the pools of invading species, which differed on all sites, and influencing the progress and outcomes of species interactions. The dominance of cord-forming fungi in developing communities at all sites highlights the importance of these fungi in wood decomposition on the forest floor. While it was not possible in this study, future research should employ experimental designs that can more rigorously evaluate priority effects in different locations, as opposed to dispersal limitation and other forces that affect community assembly.

Experimental procedures

Colonization of wood disks

Beech (*Fagus sylvatica*) wood disks (diameter 10 cm, thickness 2 cm) were colonized by *V. comedens*, *T. versicolor* and *H. fasciculare*, respectively, representing fungi from primary, secondary and late secondary/tertiary stages of succession as in Hiscox and colleagues (2015; Table S8). Briefly, freshly felled branches were cut, sterilized by autoclaving three times, placed onto mycelia growing on 0.5% MA (malt agar) in plastic 400 ml lidded pots and incubated in the dark at 20°C for 12 weeks.

Field site characteristics and experimental layouts

Seven woodland sites predominantly comprised of beech (*Fagus sylvatica*), 0.8–137 km apart, were selected in the southern UK (Tables 1 and S8). On six sites, a 15 × 15 m grid was divided into 6 × 6 sections and experimental units allocated to grid squares. Colonized disks (scraped free of adhering mycelium; nine replicates/species) and nine sterile control disks were placed in the litter layer, randomly distributed across the grids (Fig. S2). On the remaining site (Wytham site 1), colonized and control disks (10 replicates) were distributed in a larger grid (25 × 25 m; 10 × 10 sections) as part of a larger experiment (Hiscox *et al.*, 2015). Disks were placed on the forest floor for 12 months (September 2012–2013). An additional set of disks (termed Wy1A) were placed on the Wytham site 1 in the previous year, harvested after 24 months (September 2011–2013), and compared only with those on the same site for 12 months (Wy1B).

Isolation, DNA sample generation and direct incubation

After harvest, disks were scraped clean and attached cords sampled by placing sections onto 2% MA following surface sterilization (30 s immersion in 5–10% domestic bleach). The disk surface was also sterilized, and then skimmed off using a sterile chisel, and six 3–4 mm chips excised aseptically from each face and placed onto 2% MA. Plates were incubated in the dark at 20°C until mycelia emerged. Pre-colonizer fungi were identified based on mycelial morphology. Mycelia that grew and sporulated very rapidly were not considered to be true colonizers, but rather 'contaminants' that grew opportunistically on artificial media. Disks were drilled at 20 + points through their width, using a sterile 4 mm drill bit, the sawdust flash frozen and stored at –80°C. Disks were sprayed with distilled water and incubated at 20°C in the dark for 1–2 months, and outgrowth of mycelia recorded. pH was measured in disks, by shaking 0.5 g sawdust in 5 ml distilled water for 1 h and of soil taken immediately beneath the disks at harvest.

Molecular identification of unknown fungi

Deoxyribonucleic acid (DNA) was extracted from unknown mycelia (in pure culture) and from outgrowing mycelia and cords attached to disks, and polymerase chain reaction (PCR) amplifications performed using ITS1F/ITS4 (Gardes and

Bruns, 1993) following Parfitt and colleagues (2010). Polymerase chain reaction products were purified (PCR purification kit; Qiagen, USA), and sequenced using the 3710xl DNA analyser with BIG DYE TERMINATOR v3.1 (Life Technologies, UK) by Eurofins Genomics (Ebersberg, Germany). Sequences were identified by BLASTN comparison with the UNITE and INSD databases (PLUTOF; Abarenkov *et al.*, 2010). Where fungi could not be identified through sequencing, they were grouped based on morphology and assigned an identification number.

Preparation of samples for 454 pyrosequencing

454 amplicon sequencing was performed on 144 samples four to five replicates of each treatment per site. Deoxyribonucleic acid was extracted from sawdust using the PowerSoil DNA extraction kit (MoBio, USA) modified to include a bead-beating step [$3 \times (4 \text{ m s}^{-1} \text{ for } 20 \text{ s})$; FastPrep-24, MP Biomedicals, UK]; the ITS2 region was PCR amplified using the primer ITS4 (extended with 8 bp sample identification tags; Integrated DNA Technologies Belgium; Table S9) in combination with gITS7 (Ihrmark *et al.*, 2012). Polymerase chain reaction was performed using a Dyad-Peltier thermal cycler (Bio Rad, Herts, UK) in 50 µl reactions [0.25 ng template, 300 nM tagged-ITS4, 500 nM gITS7, 0.025 U µl⁻¹ Taq polymerase (PCR Biosystems, London, UK) in supplied buffer; 5 min at 94°C; 22–30 × (30 s at 94°C; 30 s at 56°C; 30 s at 72°C); 7 min at 72°C]. Cycle numbers were optimized to ensure reactions stopped in log phase due to two degenerate positions in the gITS7 primer. Triplicate PCRs were combined prior to electrophoresis through ultrapure agarose (Life Technologies, UK), excision and purification of bands using the Qiaquick gel extraction kit (Qiagen, USA). Purified products were quantified using the Quant-iT PicoGreen dsDNA assay kit (Life Technologies, UK). Samples were merged (equimolar mixing) into four amplicon libraries (36 samples library⁻¹). Each library was sequenced on 1/4 plate using the Roche GS FLX + 454 pyrosequencing platform (Hoffman La-Roche, Germany) by the NERC-Biomolecular Analysis Facility (Centre for Genomic Research, Liverpool, UK).

Sequence analysis

Sequences were analysed using the SCATA pipeline (scata.mykopat.slu.se; Ihrmark *et al.*, 2012). Sequences were filtered by screening for primer sites, and removing sequences with an average quality score below 20, or a score below 10 at any position. This resulted in 701 338 high-quality sequences, which were compared for similarity using BLAST, with a pairwise alignment scoring function with 1 as penalty for mismatch, 0 for gap opening and 1 for gap extension. Homopolymers were collapsed to 3 bp before clustering. Sequences were clustered by single linkage clustering with 1% maximum distance to nearest neighbour demanded for sequences to enter clusters. Sequences occurring only once in the entire data set were excluded. Representative sequences of all clusters (OTUs) were compared with all fungal sequences in the UNITE and INSD databases by BLASTN using the MASSBLASTER function in PLUTOF (Abarenkov *et al.*, 2010), and taxonomic information for each OTU obtained using the Galaxy project toolkit (<http://>

usegalaxy.org/). Sequence data are archived at NCBI SRA under accession no. SRP056529.

Statistical analysis

Statistical analyses were performed using R v3.1.0 (R Core Team, 2013), using the VEGAN package (Oksanen *et al.*, 2013) unless otherwise stated, and graphs generated using the package GGPLOT2 (Wickham, 2009).

Operational taxonomic unit counts corresponding to the pre-colonizer species were removed from the data set. Samples were excluded if the pre-colonizer accounted for > 80% of the OTU counts, resulting in the exclusion of two *T. versicolor* samples and 36 *H. fasciculare* samples. This left insufficient replicates of *H. fasciculare* samples, so all were removed.

Samples were rarefied to the lowest OTU count in any one sample (1321 reads), and clusters corresponding to < 1% of reads in any one sample (i.e. < 13 reads) removed. Rarefying data and read deletion did not alter the outcome of significance testing between groups. The data were fourth root transformed, reducing the influence of abundant taxa relative to less dominant taxa and allowing community-wide assessment of changes in taxon composition (Clarke and Warwick, 2001). Differences in community composition between treatments were visualized using Canonical Analysis of Principal Coordinates based on Discriminant Analysis (CAPdiscrim). Distance matrices for sequence data were constructed using the Bray–Curtis dissimilarity index (Clarke and Warwick, 2001). Fungal diversity estimates (Shannon diversity, Fisher's alpha and Pielou's evenness), wood pH and soil pH, were compared using one-way analysis of variance (ANOVA), with Tukey–Kramer *a posteriori* tests.

Permutational ANOVA (999 permutations; Anderson, 2001) assessed whether pre-colonizer species, site and the species–site interaction resulted in significantly different successor communities. Betadisper tests confirmed equal dispersion between treatment groups. Pairwise tests were used to compare differences in successor communities following different pre-colonizer species, and between woodlands, with *P*-adjustment (false discovery rate method; Benjamini and Hochberg, 1995). Operational taxonomic units were divided into species actively contributing to wood decomposition (decomposers: basidiomycetes and xylariaceous ascomycetes), and those purportedly incapable of white or brown rot, instead living opportunistically off other mycelia or their extracellular enzyme products (co-colonizers). Permutational ANOVA was run on both groups separately to detect whether community structure of decomposer versus co-colonizer species changed following different pre-colonizers.

Mantel tests were performed to detect whether community composition was linked to: position on the site, pH of disks at the end of the experiment or pH of underlying soil (tests performed as a correlation between a Euclidean distance matrix and the Bray–Curtis dissimilarity matrix; Legendre and Legendre, 1998).

Conflict of interest

The authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1. Relationship between wood and soil pH on different sites.

- A. pH within disks after 12 months at different sites, comprising data from all samples (regardless of treatment).
- B. pH of soil underneath disks at point of harvest.
- C. Correlation between pH within disks and that of the underlying soil.

For A and B, bars show the mean pH of all samples from a site, \pm 95% confidence intervals. Different letters indicate significant ($P < 0.05$) differences between pH between sites. Data were not collected for Wytham site 1 samples.

Fig. S2. Distribution of samples on the field sites. Disks were placed in the centre of each grid square, in a randomized control block design (Latin square). Numbers indicate sample ID tags. Figures show all of the samples put out in the field, although not all of these were retrieved. Disks were either uncolonized controls (C), or pre-colonized with *V. comedens* (Vc), *T. versicolor* (Tv) or *H. fasciculare* (Hf). On Wytham site 1 (Wy1A and Wy1B), samples were placed in a 10 \times 10 grid (25 \times 25 m) as part of a larger experiment, whereas on all other sites samples were arranged in a 6 \times 6 grid (15 \times 15 m).

Table S1. Re-isolation data arranged by site and pre-colonizer species. Mismatch can occur between fungi identified from isolation points and those that were present from disks (i.e. no invading fungi grew from isolations, but invading fungi were present following disk incubation). See Table 1 for site name abbreviations.

Table S2. Taxa recovered by isolation and disk incubation following uncolonized control (C) disks, or disks pre-colonized

with *V. comedens* (Vc), *T. versicolor* (Tv) or *H. fasciculare* (Hf). Named taxa were identified by Internal Transcribed Spacer (ITS) sequencing (Seq), or macroscopic morphological similarity to other named cultures (similarity). Where pure culture or sequencing was not possible, taxa are described as 'unknown' groups of similar phenotype or individuals, and their mycelial morphology described. The recovery method of each taxon refers to whether it was obtained through re-isolation onto agar from wood (Isol) or cords (Cords), or as outgrowth following disk incubation (Disk).

Table S3. Summary OTU statistics following different pre-colonizer species. Number of OTUs pre- and post-rarefaction are given for data including and excluding OTUs corresponding to the pre-colonizer species; where there was no pre-colonizer (Control), data are the same in both instances. Data are given as total number of occurrences of each OTU (Counts) and number of OTUs (# OTUs) across all sites. All subsequent analyses were performed on data from which pre-colonizer OTUs had been excluded. Operational taxonomic units belonging to the 'Decomposer' or 'Co-colonizer' communities are defined in Table S7. Results from one-way ANOVA comparisons of Shannon diversity (H), Fishers' alpha (α) and Pielou's evenness (E) are given as degrees of freedom (df), the *F*-statistic and *P*-value; no significant differences ($P > 0.05$) were found in any of these measures between different pre-colonizers.

Table S4. Variability in OTU profiles between different species and sites. Betadisper tests were performed to assess variability in OTU profiles separately for individual species on different sites ('Site'), or between different species on the same site ('Species'). All tests were performed on fourth root transformed data, and the degrees of freedom (groups, residuals), *F*-statistic and *P*-value are shown. See Table 1 for site name abbreviations.

Table S5. Summary OTU statistics for different sites. Numbers of OTUs pre- and post-rarefaction are given for data including and excluding OTUs corresponding to the pre-colonizer species; where there was no pre-colonizer (Control) data are the same in both instances. All analyses were performed on data with pre-colonizer OTUs excluded. Data are given as total number of occurrences of each OTU (Counts) and number of OTUs (# OTUs) across all sites. All subsequent analyses were performed on data with pre-colonizer OTUs excluded. Operational taxonomic units belonging to the 'Decomposer' or 'Co-colonizer' communities are defined in Table S7. Results from one-way ANOVA comparisons of Shannon diversity (H), Fishers' alpha (α) and Pielou's evenness (E) are given as degrees of freedom (df), the *F*-statistic and *P*-value; no significant differences ($P > 0.05$) were found in any of these measures between different pre-colonizers. See Table 1 for site name abbreviations.

Table S6. Pairwise comparisons between different sites, split by pre-colonizer species. Comparisons were performed using Adonis/PERMANOVA, following which a *P*-adjustment was performed to reduce the effect of false discovery rates (Benjamini and Holden, 1995); adjusted *P*-values are shown. $P < 0.05$ indicates a significant difference in community composition following the same pre-colonizer on different sites. See Table 1 for site name abbreviations.

Table S7. Occurrence and identification of OTUs by pre-colonizer species across different sites. Each OTU is given

as a % of the total OTUs detected across all replicates of each pre-colonizer species on each site. The total number of OTU counts across all treatments gives a measure of the overall abundance of each OTU. Operational taxonomic unit identity was determined using MASSBLASTER, and the taxon with the best e-value was selected, and the relevant details are given. Taxonomy was determined by Galaxy454. –, not detected or data unavailable. Operational taxonomic units were divided into one of two groups based on their ecological role: those that are known to directly contribute to wood decomposition were assigned to the decomposer group (D), whereas those that are secondary saprotrophs (live off exu-

dates or the mycelium of wood decomposers) assigned to the co-colonizer group (C). Broadly, the Ascomycota were group C and the Basidiomycota plus xylariaceous Ascomycota comprised group D. See Table 1 for site name abbreviations; C, uncolonized controls; Vc, *V. comedens*; Tv, *T. versicolor*; Hf, *H. fasciculare*.

Table S8. A. Details of species used. All cultures were obtained from the Cardiff University Culture Collection. B. Geodesic distances (km) between sites.

Table S9. Details of primers used. Primers were designed by Ihrmark and colleagues (2012).