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LETTER

Priority effects are interactively regulated by top-down and bottom-up forces: evidence from wood decomposer communities

Devin R. Leopold,[1]* J. Paula Wilkie,[2] Ian A. Dickie,[3,4] Robert B. Allen,[5] Peter K. Buchanan[6] and Tadashi Fukami[1]*

Abstract

Both top-down (grazing) and bottom-up (resource availability) forces can determine the strength of priority effects, or the effects of species arrival history on the structure and function of ecological communities, but their combined influences remain unresolved. To test for such influences, we assembled experimental communities of wood-decomposing fungi using a factorial manipulation of fungivore (*Folsomia candida*) presence, nitrogen availability, and fungal assembly history. We found interactive effects of all three factors on fungal species composition and wood decomposition 1 year after the fungi were introduced. The strength of priority effects on community structure was affected primarily by nitrogen availability, whereas the strength of priority effects on decomposition rate was interactively regulated by nitrogen and fungivores. These results demonstrate that top-down and bottom-up forces jointly determine how strongly assembly history affects community structure and function.

Keywords

Assembly history, fungivore grazing, historical contingency, priority effects, resource availability, saprotrophic fungi, wood decomposition.

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INTRODUCTION

The order and timing of species arrival during community assembly can affect species composition (Palmgren 1926; Sutherland 1974; Drake 1991) and ecosystem functioning (Körner *et al*. 2008; Tan *et al*. 2012), the phenomenon known as priority effects. Because species arrival order is often highly stochastic and unknown, priority effects introduce historical contingency in community assembly, which complicates the search for general patterns in communities (Diamond 1975; Lawton 1999). However, the strength of priority effects is known to vary with ecological context (e.g. community size, Orrock & Fletcher 2005; phylogenetic diversity, Tan *et al*. 2012; disturbance, Tucker & Fukami 2014). Progress towards a better understanding of community composition and ecosystem function can therefore be made by identifying conditions that make assembly history important (Chase 2003; Fukami 2015).

Factors affecting the strength of priority effects include both top-down forces (e.g. grazing) and bottom-up forces (e.g. resource availability). Grazing by consumers can weaken priority effects by reducing population growth of early-arriving species (Morin 1984) or by altering the competitive hierarchy among species (Louette & De Meester 2007; Chase *et al*. 2009). It is also possible for consumers to strengthen priority effects if they limit the effective size of a local community (Chase *et al*. 2009) or equalise fitness differences among species by preferentially consuming a competitively dominant species (Fukami *et al*. 2016). Greater resource availability generally strengthens priority effects by increasing the growth of early-arriving species (Chase 2003; Fukami 2015; but see Fukami *et al*. 2010; Dickie *et al*. 2012), as shown in plants (Kardol *et al*. 2013), animals (Chase 2010), and microbes (Vannette & Fukami 2014).

Although both top-down and bottom-up forces determine assembly history effects, their potential interactive effects remain unclear. In a pioneering study of grassland plant communities, Ejrnæs *et al*. (2006) found that interactions between species introduction order, nutrient availability, and simulated herbivory (manual defoliation) determined species richness and composition. In that study, however, introduction order was limited to two treatments that were confounded with plant type (specialist vs. generalist) and the simulated herbivory manipulation was applied equally to all species. So far, no studies, to our knowledge, have addressed the possibility that the relationship between assembly history and emergent functional properties of communities depend on interactions between bottom-up and top-down forces.

In this paper, we experimentally test for the joint effects of top-down and bottom-up forces on priority effects. To this end, we assembled communities of wood-decomposing fungi in laboratory microcosms, manipulating species arrival order, initial nitrogen availability, and the presence of fungivorous springtails (Collembola). In wood-decomposing fungi, the history of species arrival can vary among communities (e.g.

[1]*Department of Biology, Stanford University, Stanford, CA 94305, USA*
[2]*Independent Researcher, Auckland 1025, New Zealand*
[3]*BioProtection Research Centre, Lincoln University, Lincoln 7647, New Zealand*
[4]*University of Canterbury, School of Biological Sciences, Christchurch 8140, New Zealand*
[5]*Independent Researcher, Lincoln 7608, New Zealand*
[6]*Landcare Research, Auckland 1142, New Zealand*
**Correspondence: devin.leopold@gmail.com, fukamit@stanford.edu*

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among fallen logs) owing to variation in sporulation timing, mycelial growth, the phenology of insect vectors, and the timing of tree death or log fall (Boddy *et al*. 2017). This variation, coupled with strong local interactions (Boddy 2000), can make priority effects a major driver of community assembly (Fukami *et al*. 2010). In addition, both increased nitrogen availability and fungivore grazing are known to influence the composition and function of fungal communities (Wardle & Yeates 1993; Crowther *et al*. 2012; Morrison *et al*. 2016), yet their potential to interactively regulate priority effects is unexplored.

As resource availability may affect fungal communities more strongly than grazing (Zimmerman *et al*. 1995; Wardle 2002; Moore *et al*. 2003), we predicted that nitrogen addition would influence the strength of priority effects more than fungivore presence. However, because Collembola may also be resource-limited initially (Booth & Anderson 1979; Larsen *et al*. 2011), we also predicted that the influence of fungivores on the strength of priority effects will be greater with, rather than without, nitrogen addition due to more rapid population growth in response to increased fungal resource availability. This study builds on earlier experimental work linking priority effects to functional properties of wood-decomposing fungal communities (i.e. decomposition rate and carbon and nutrient dynamics; Fukami *et al*. 2010; Dickie *et al*. 2012). The novelty of the present work is that, unlike any previous research we are aware of, manipulations of both top-down and bottom-up factors were combined with manipulations of species arrival order.

MATERIALS AND METHODS

Overview and experimental design

Fungal communities, consisting of 10 species, were assembled on sterile wood disks in individual microcosms at one of two initial nitrogen levels and in the presence or absence of Collembola. Assembly history was manipulated by introducing one of four randomly selected initial fungal species 4 weeks prior to introducing the remaining nine species. A control treatment, where no fungi were introduced was also included. The experimental design was fully factorial, with 5 replicates of each treatment destructively harvested at 6 and 12 months, resulting in 200 samples (i.e. 2 nitrogen levels × 2 fungivore treatments × 5 assembly histories (including control) × 5 replicates × 2 harvests, as detailed below). To aid interpretation of the fungal community-level responses to the experimental treatments, a second set of microcosms were inoculated with only one fungal species, resulting in 400 additional, single-species replicates (i.e., 10 species × 2 nitrogen levels × 2 fungivore treatments × 5 replicates × 2 harvests).

Microcosms

Microcosms were prepared as in Fukami *et al*. (2010). Briefly, mineral soil was collected from a monospecific stand of old-growth *Nothofagus solandri* (Allen *et al*. 2000; Clinton *et al*. 2002) at Craigieburn Forest Park, South Island, New Zealand (43°8.556 S, 171°42.825 E, 1000 m elevation) and added to 2 L glass jars. Each jar received 800 g of dry, well-mixed soil and 400 mL water or water with NH_4NO_3 (see below). Wood disks (*c*. 8 cm diameter by 1 cm thick) were prepared from freshly felled *N. solandri* trees and dried to constant weight to determine their initial dry mass. The wood disks were then soaked in water for 48 h and placed on top of the soil in the microcosms. Before inoculation with fungi, the assembled microcosms were capped with loosely fitting lids and autoclaved twice at 121 °C with a 24 h interval. Experimental treatments were randomly assigned to jars, which were then randomly arranged on laboratory shelves and maintained in the dark at *c*. 20 °C and 60% humidity throughout the course of the experiment.

Fungal species

The fungi used in this study were isolated from the same site as soils (above). The 10 species used in this study (Fig. S1) were largely identical to those used by Fukami *et al*. (2010) and Dickie *et al*. (2012), which were selected from the initial collection of 96 species to maximise ease of handling, phylogenetic diversity, and adequate molecular discrimination using nrDNA internal transcribed spacer (ITS) length heterogeneity. In preliminary experiments, we found that one species used in the previous studies, *Phlebia nothofagi*, caused rapid and complete mortality of Collembola *in vitro*. Because *P. nothofagi* was a final dominant species in previous assembly experiments (Fukami *et al*. 2010), we excluded it from the current study to ensure that we could effectively manipulate fungivore presence. Instead of this species, we used *Helicogloea alba* (New Zealand Fungarium, PDD 91620; ICMP 17042; GenBank accession GQ411522.1) in the current study.

Selecting species that are amenable to manipulation in pure culture may have reduced functional diversity relative to the broader species pool in the field. It is also possible, however, that efforts to select a phylogenetically diverse group of species increased the functional diversity of the microcosm communities relative to the diversity of initial colonists under natural conditions. The ten species represent a range of wood-decomposition strategies, including white-rot, brown-rot, and soft-rot fungi (Worrall *et al*. 1997; see Fig. S1).

Assembly history

Assembly history treatments were established by first inoculating sterilised wood disks with one of four randomly selected species, either *Ascocoryne sarcoides*, *Bisporella citrina*, *Daldinia novae-zelandiae,* or *Trametes versicolor*. The feasible number of assembly history treatments was limited due to practical constraints on the number of samples needed for the factorial design. Inoculation consisted of aseptically transferring a 5-mm plug from a colonised agar plate to a predetermined location on the upper surface of each wood disk. The remaining nine species were aseptically transferred to the wood disk 4 weeks later. The inoculation positions for each species, relative to the other species, was invariant across all treatments to avoid potential confounding effects of spatial arrangement (Fig. S1). We used the 4-week interval to allow the initial species to become established without completely colonising the disk. It is uncertain how common this interval

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may be under natural conditions. However, initial colonisation by a single species, followed by simultaneous colonisation by numerous other species after some time interval, is plausible. For example, a dead branch that is colonised by one fungus while still attached to a tree may then fall to the ground and simultaneously come in contact with multiple additional species (Boddy *et al*. 2017).

Nitrogen availability

We chose to manipulate nitrogen availability as a bottom-up factor because fungal decomposition of highly lignified substrates with a high carbon-to-nitrogen ratio, such as wood, is often nitrogen-limited (Allison *et al*. 2009; Boberg *et al*. 2014), although fungal species may vary in their response to nitrogen. The mineral soil collected from the field site and used in the microcosms was nutrient-poor and primary productivity at the site was nitrogen-limited (Clinton *et al*. 2002; Davis *et al*. 2004; Smaill *et al*. 2011). Microcosms assigned to the low-nitrogen treatment received no supplemental nitrogen and reflected the natural nutrient availability in the wood disks and mineral soil. The high-nitrogen treatment received 1 g NH_4NO_3 in the 400 mL water added to each microcosm before autoclaving. The resulting range of soil nitrogen availability in the experimental treatments reflected the range of values naturally present in soils where the fungi were collected, including the relatively nutrient rich organic soil layers (Clinton *et al*. 2002), and coincides with the high- and low-nitrogen treatments used by Fukami *et al*. (2010).

Fungivore presence

We manipulated the presence of fungivorous mesofauna in the experimental microcosms by adding a species of Collembola, *Folsomia candida*. This species was chosen because it is primarily mycophagous and could be easily reared under laboratory conditions, which helped to avoid unintended introduction of contaminant fungi to the microcosms (Fountain & Hopkin 2005). In addition, *F. candida* has been previously used in studies of the ecological impacts of grazing by soil fungivores (e.g. Lussenhop 1996; Cragg & Bardgett 2001). To ensure that the initial fungal species had an opportunity to establish on the wood disk and that there would be sufficient resources for the *F. candida* population, *c*. 30 individuals of the fungivores were aseptically transferred into each of half of the microcosms belonging to each assembly and nutrient treatment 4 weeks after the final fungal inoculation. It should be noted, however, that fungivores can be present throughout fungal community assembly under natural conditions.

Harvest and data collection

Microcosms were destructively harvested at one of two time points, 6 or 12 months after the introduction of the initial fungal species, to determine if priority effects were persisting or attenuating over time. Specifically, each wood disk was removed from the jar within a laminar flow hood and split along eight radial lines at predetermined locations relative to the initial inoculation points (Fig. S1). A custom, sterile wood-splitting device was used to avoid contamination of the interior wood, as detailed in Fukami *et al*. (2010) and Dickie *et al*. (2012). Approximately 5.5 mg of sawdust was removed from the interior wood at nine predetermined locations (Fig. S1) using a flame-sterilised 1.5-mm drill bit, and collected in individual sterile 0.2 mL tubes.

To characterise fungal species composition, each of the nine sawdust samples from each disk were analysed separately using a modified length-heterogeneity polymerase chain reaction (LH-PCR) assay. First, total fungal DNA was extracted and PCR amplified using the Sigma REDExtract-N-amp Plant PCR kit and the labelled primers ITS1F-6FAM and ITS4-VIC (Dickie *et al*. 2009). Fungi present in each PCR product were then identified by analyzing ITS fragment lengths with 50-cm capillary electrophoresis in a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Peak profiles were compared against a database of the known ITS sequence lengths and converted to species presence data for each sawdust sample using TRAMPR (FitzJohn & Dickie 2007).

Functional outcomes of fungal community assembly were characterised by measuring the wood mass lost through decomposition and the final carbon and nitrogen concentrations in the wood disks. Following sampling for molecular analysis, the wood disks were dried at 40 °C to a constant weight and the mass was recorded. The change in dry mass for each disk was used as a proxy for decomposition, but also includes fungal tissue. In a laboratory experiment, Jones & Worrall (1995) found that 3–30% of the dry biomass remaining after 12 weeks was likely fungal. Although this study is not directly comparable to ours, it indicates that fungal biomass might be relatively minor compared to wood mass. The mass of sawdust removed for molecular analysis was not accounted for in the final dry weights, but was negligible relative to mass lost to decomposition and was consistent across treatments. Carbon and nitrogen were analysed after determining the final dry mass of each disk by collecting a composite sawdust sample from new locations along each of the eight split edges using a 2-mm drill bit.

In addition, immediately before the harvest, we visually inspected the microcosms and recorded the abundance of *F. candida* ordinally. We classified each microcosm into one of four categories (i.e. 0 = no *F. candida* individual in the microcosm; 1 = 1–10 individuals; 2 = 10–100 individuals; and 3 = more than 100 individuals). The *F. candida* population persisted until the end of the experiment in most microcosms (Figs S2 and S3).

Statistical analysis

To determine whether the effects of assembly history were regulated interactively by nitrogen availability and fungivore grazing, we tested for significant three-way interactions of these experimental treatments as predictors of fungal community composition and function. To quantify fungal species composition, we used, as a measure of species prevalence in the wood, the numbers of subsamples from each wood disk in which each species was observed to construct a species by sample matrix of relative abundance. The relationships between species composition and experimental treatments were visualised using non-metric multidimensional scaling

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(NMDS) and the significance of these relationships were tested with a permutational multivariate analysis of variance (permanova) using the adonis function in the R-package vegan (Oksanen *et al*. 2017). For both the NMDS and permanova analyses, community dissimilarity was calculated using the modified Gower dissimilarity index proposed by Anderson *et al*. (2006). In addition, to examine how experimental treatments affected individual species, the proportion of subsamples per microcosm where a species was detected was modelled as a binomial response variable using generalised linear models. The full model for each species was simplified using reverse model selection and likelihood-ratio chi square tests.

To test whether the strength of priority effects on species composition was influenced by our experimental treatments, we compared the compositional dissimilarity among assembly history treatments (i.e. a measure of the variation in species composition that can be attributed to species arrival order) for each factorial combination of nitrogen availability and fungivore presence. To quantify compositional dissimilarity, we first calculated the beta-dispersion among samples with the same nitrogen and fungivore treatment using the modified Gower dissimilarity index and the function betadisper in the R-package vegan. We then extracted the distance of each sample to its group centroid and tested whether they were correlated with the nitrogen and fungivore treatments and their interaction, including the assembly history treatment as a random effect, using the function lme in the R-package nlme (Pinheiro *et al*. 2016).

The effect of the experimental treatments on wood decomposition were tested using analyses of variance (anovas), with the proportion of dry wood mass lost, the percent nitrogen, and the carbon-to-nitrogen ratio as response variables. The carbon-to-nitrogen ratio was log-transformed to better meet the assumption of normality. The full models, including all experimental treatments and interactions, were simplified for each response variable using F tests. In order to link the properties of the wood substrate to fungal community composition, the mass loss, percent nitrogen, and carbon-to-nitrogen ratio for each sample were correlated with the NMDS ordination by vector fitting, using the envfit function in the R-package vegan.

Analyses of species relative abundance and functional outcomes were repeated for the single-species microcosms using the same statistical methods, with the exception that assembly history was not included as a factor. All statistical analyses were conducted in R v3.3.1 (R Development Core Team 2016).

RESULTS

Patterns of fungal community composition and wood decomposition were qualitatively similar at both harvest time points and statistical support for treatment effects after 12 months were as strong as, or stronger than, those observed at 6 months. We will focus here on the 12-month results and report the 6-month results as supplemental materials (Figs S4–S7). When inoculated alone, all fungal species colonised wood by 12 months, at least in the absence of fungivores and nitrogen addition (Figs 1, S5 and S7).

Fungal community composition

The composition of the fungal community after 12 months varied with assembly history, initial nitrogen availability, fungivore presence, and their interactions (permanova, $F_{15,64} = 9.32$, $P < 0.001$; Fig. 2a), including a significant effect of the three-way interaction ($F_{3,64} = 2.246$, $P = 0.019$). The greatest amount of variation in community composition was explained by assembly history ($R^2 = 0.36$), followed by nitrogen availability ($R^2 = 0.21$) and fungivore presence ($R^2 = 0.11$). Beta-dispersion among assembly history treatments (i.e. the strength of priority effects; Fig. 2b) was reduced with nitrogen addition ($F_{1,73} = 16.59$, $P < 0.001$), but was unaffected by fungivore presence ($F_{1,73} = 0.153$, $P = 0.69$) or the interaction between nitrogen and fungivores ($F_{1,73} = 0.038$, $P = 0.85$).

Species varied in complex ways in their response to the experimental treatments, both in the mixed communities (Fig. 3) and in the single-species microcosms (Fig. 1). For example, the proportion of wood disk subsamples where *T. versicolor* was observed was strongly influenced by assembly history, averaging 86.1% when arriving ahead of other species and only 3.9% otherwise, but was not significantly influenced by fungivore presence or nitrogen availability. The abundance of *Sistotrema brinkmannii* also responded to assembly history, but only under low-nitrogen conditions and did not appear to be influenced strongly by fungivore grazing. For *A. sarcoides*, *B. citrina*, *Calocera sinensis,* and *D. novaezelandiae,* the effect of assembly history on abundance varied with both nitrogen availability and fungivore presence. For example, *B. citrina* was ubiquitous in the wood disk when arriving first under low-nitrogen conditions, irrespective of grazing. However, under high-nitrogen conditions, *B. citrina* colonised only 40 and 4.4% of the wood disk in the absence and presence of fungivores, respectively. When arriving after another species, the prevalence of *B. citrina* depended on the initial species identity, nitrogen availability and fungivores. For example, the prevalence of *B. citrina* when preceded by *T. versicolor,* relative to its prevalence when arriving after *T. versicolor*, was dependent on the nitrogen and fungivore treatments. Under low-nitrogen conditions, the prevalence of *B. citrina* was reduced when *T. versicolor* arrived first, irrespective of fungivore presence. Under high-nitrogen conditions, initial colonisation by *T. versicolor* had no effect on the prevalence of *B. citrina* when fungivores were absent, but, facilitated growth when fungivores were present. Three species, *Armillaria sp*., *H. alba* and *Pleurotus purpureoolivaceus*, were not detected at all, and one species, *Artomyces* (≡ *Clavicorona*) *candelabrum*, was only detected twice at 12 months and once at 6 months.

Wood decomposition

The physical and chemical properties of the decomposing wood disk were correlated with assembly history, initial nitrogen availability, fungivore presence and their interactions (Fig. 4). Significant three-way interactions were observed for wood mass loss ($F_{3,64} = 8.99$, $P < 0.001$), final nitrogen concentration ($F_{3,64} = 11.36$, $P < 0.001$), and the carbon-to-nitrogen ratio ($F_{3,64} = 4.96$, $P = 0.004$). Fungal species

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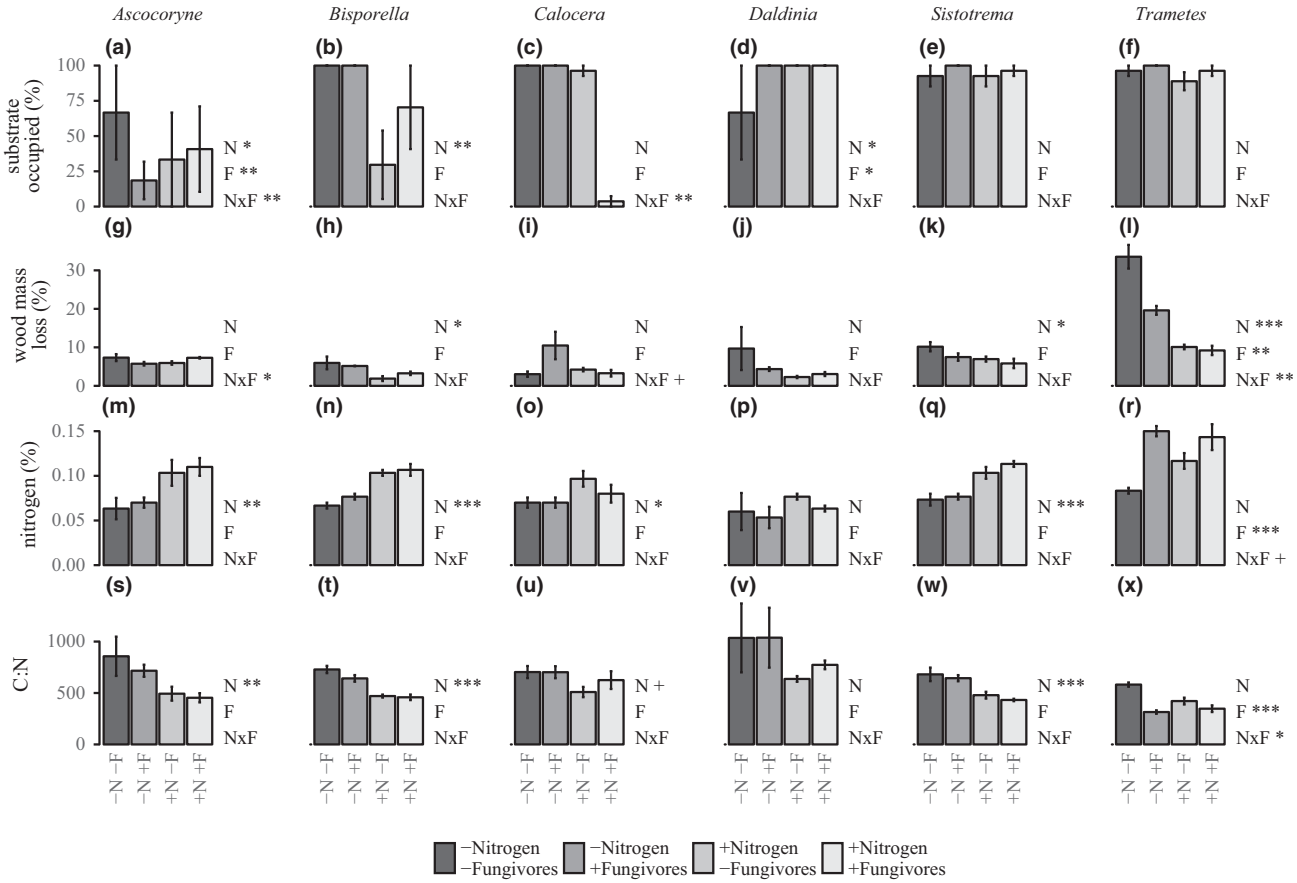

Figure 1 Effect of nitrogen addition (N) and fungivore presence (F) on wood volume occupied (a–f), % wood mass loss (g–l), % nitrogen (m–r), and carbon-to-nitrogen ratio (s–x) for single-species inoculations. Significant predictors are indicated for each response, where $+P < 0.1$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. See also Figs S5 and S7 for additional results for singe-species inoculations.

composition in NMDS was correlated with these functional outcomes, based on *envfit* (mass lost, $R^2 = 0.44$, $P < 0.001$; % N, $R^2 = 0.26$, $P < 0.001$; C:N, $R^2 = 0.46$, $P < 0.001$; Fig. S8).

The effect of assembly history on decomposition rate depended on nitrogen availability and fungivore presence (Fig. 4a–d). Under low-nitrogen conditions, decomposition rate varied among assembly history treatments to a greater degree when fungivores were not present (Fig. 4a and b). However, under high-nitrogen conditions decomposition rate was only minimally influenced by assembly history or fungivores. This pattern can be largely explained by the response of *T. versicolor* to the experimental treatments. In the single-species microcosms, decomposition by *T. versicolor* was about three times as quick as by any other species under low-nitrogen conditions without fungivores, twice as quick with the addition of fungivores, but only slightly quicker than the across-species average under high-nitrogen conditions, with or without fungivores (Fig. 1g–l). Because the prevalence of *T. versicolor* was strongly influenced by introduction order (Fig. 3u–x) and *T. versicolor* function was strongly influenced by the nitrogen and fungivore treatments (Fig. 1l, r and x), decomposition rate was much higher when *T. versicolor* arrived first to colonise a large portion of the wood, but this effect was reduced by nitrogen addition and fungivore presence. The introduction order of *T. versicolor* was also largely responsible for the effect of assembly history on nitrogen concentration and carbon-to-nitrogen ratio during decomposition (Fig. 4e–l). However, in contrast to decomposition rate, the nitrogen and carbon patterns in the single-species microcosms (Fig. 1r and x) did not correspond clearly to the mixed species treatments (Fig. 4e–l).

DISCUSSION

Our results provide experimental evidence that nutrient availability and fungivore presence interact with fungal inoculation history to determine species composition (Fig. 2) and wood decomposition (Fig. 4). Prior research has shown that fungivore grazing can alter competitive interactions among fungi by suppressing or stimulating hyphal growth, depending on the species involved (Crowther *et al.* 2011a). Our results suggest that these effects of fungivores may depend on resource availability. For species composition, the joint effects of nitrogen availability and fungivore presence can be illustrated by considering the prevalence of *B. citrina*. Under low-nitrogen conditions, this species occupied the smallest volume of wood when arriving after *T. versicolor*, but at high nitrogen and in the presence of fungivores, had its highest occupancy when arriving after the same species (Fig. 3e–h). The exact mechanisms remain uncertain, but increased nitrogen availability

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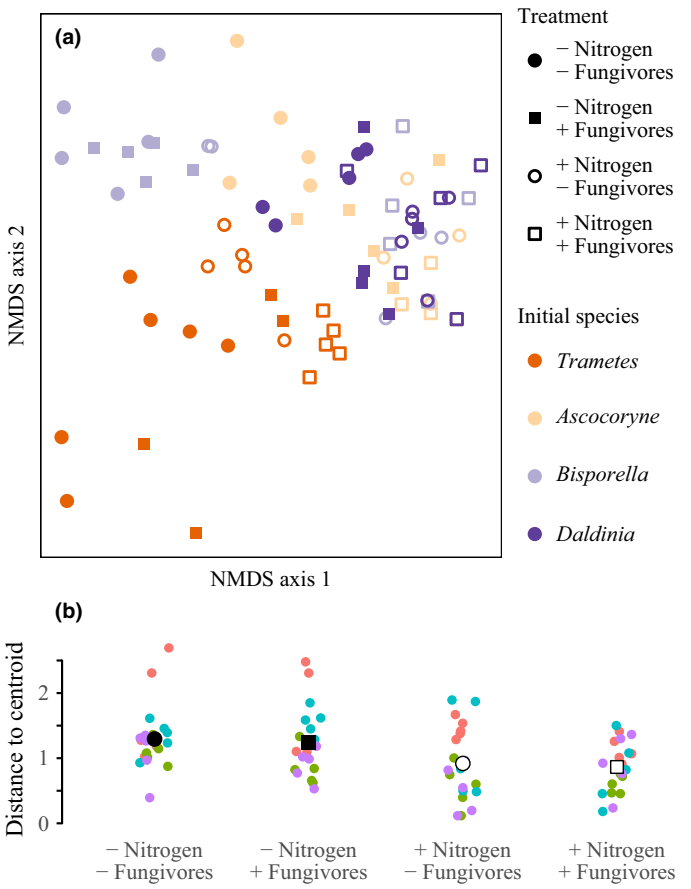

Figure 2 (a) Non-metric multidimensional scaling (NMDS) of fungal community composition (stress = 0.17), using the modified Gower dissimilarity index proposed by Anderson *et al.* (2006). Colours indicate assembly history treatment, fill (empty or solid) indicates the omission or addition of supplemental nitrogen and shape (square vs. circle) indicates the presence or absence of fungivores. (b) Compositional dissimilarity (modified Gower) for each combination of nitrogen and fungivore treatments, calculated using the betadisper function in the R-package vegan. Large points indicate the mean distance to the multivariate group centroid and small points (coloured by initial species treatment) indicate the distance for individual samples.

may have altered the effects of fungivores by altering the composition of the fungal community that the Collembola were interacting with (Kardol *et al.* 2016; Morrison *et al.* 2016); by facilitating defensive responses to fungivore grazing, such as the production of nitrogen-rich secondary metabolites (Crowther *et al.* 2011b); or by altering hyphal growth rate and morphology (Dickie *et al.* 1998).

Although greater resource availability can strengthen priority effects (Chase 2003, 2010; Fukami 2015), we found that nitrogen addition weakened priority effects for species composition. In this system, nitrogen addition appears to have reduced the inhibition of late-arriving species by early-arriving ones by allowing more species to occupy a larger portion of the wood substrate (Fig. 3). There are several possible explanations for this observation. For example, if priority effects are primarily the result of niche preemption, nitrogen addition may have modified hyphal morphology by increasing local hyphal density while reducing the spatial extent of initial resource exploration (Dickie *et al.* 1998). Alternatively, priority effects can be the result of niche modification by early-arriving species. There is some evidence that the strength of priority effects for wood-decomposing fungi is linked to their ability to degrade lignocellulose (Cline & Zak 2015; Hiscox *et al.* 2015a,b), presumably as a result of biochemical modification of the substrate material. In our study, initial colonisation by *T. versicolor*, a ligninolytic, white-rot fungus, led to a distinct species composition, but this effect was less apparent when nitrogen was added (Fig. 2a). Cline & Zak (2015) observed a similar phenomenon, reporting that priority effects exerted by ligninolytic fungi were stronger on low-nutrient, high-lignin leaf litter and weaker on more nutrient-rich litter. However, these observations may reflect either a change in the relative impact of the initial coloniser, due to a change in extracellular enzyme production (Leatham & Kirk 1983), or greater nutrient availability facilitating colonisation by later arriving species.

For wood decomposition, we found that the strength of priority effects was modified by the interactive effects of nitrogen availability and fungivore presence, primarily due to the response of *T. versicolor* to the experimental treatments. This species induced a relatively high decomposition rate compared to the other species, but this distinction was diminished by fungivore presence and nearly eliminated by nitrogen addition (Fig. 1l). For some wood-decomposing fungi, nitrogen supplementation can inhibit or delay ligninolytic enzyme production, resulting in lower decomposition rates (Keyser *et al.* 1978; Leatham & Kirk 1983). Grazing by fungivores has also been shown to affect extracellular enzyme production and decomposition rate (Crowther *et al.* 2011c,d). Both nitrogen and fungivores may have affected decomposition by modifying enzyme production in *T. versicolor*, but the stronger effect of nitrogen addition may have precluded any additional effect of fungivory. Alternatively, if fungivores reduced decomposition rate by requiring that the fungus reallocate resources to defence or to repair damage caused by grazing, nitrogen supplementation may simply satisfy the additional demand imposed by fungivore grazing.

The ligninolytic capabilities of *T. versicolor* may largely explain the differences in decomposition rate among assembly history treatments, but our results suggest that this species was also more adept at translocating available nitrogen than the other species: highest mean nitrogen concentrations at 6 months were observed when *T. versicolor* arrived first (Fig. S6e–h), but at 12 months these treatments had the lowest mean nitrogen concentrations (Fig. 4e–h). These changes in nitrogen concentration are not consistent with mass loss to respiration and suggest that *T. versicolor* transported nitrogen from the soil to the wood substrate during the early stages of decomposition and away during the later stages. However, these patterns varied with nitrogen addition and fungivore presence and were not directly correlated with decomposition rate. In addition, patterns of nitrogen concentration in the mixed-species treatments did not directly reflect the patterns observed in the single-species treatments (Fig. 1m–x). These discrepancies suggest that nitrogen dynamics during decomposition were influenced by interactions between initial nitrogen availability, fungivore presence and interspecific fungal interactions, possibly involving the production of nitrogen-rich secondary defence compounds or modified, combative hyphae

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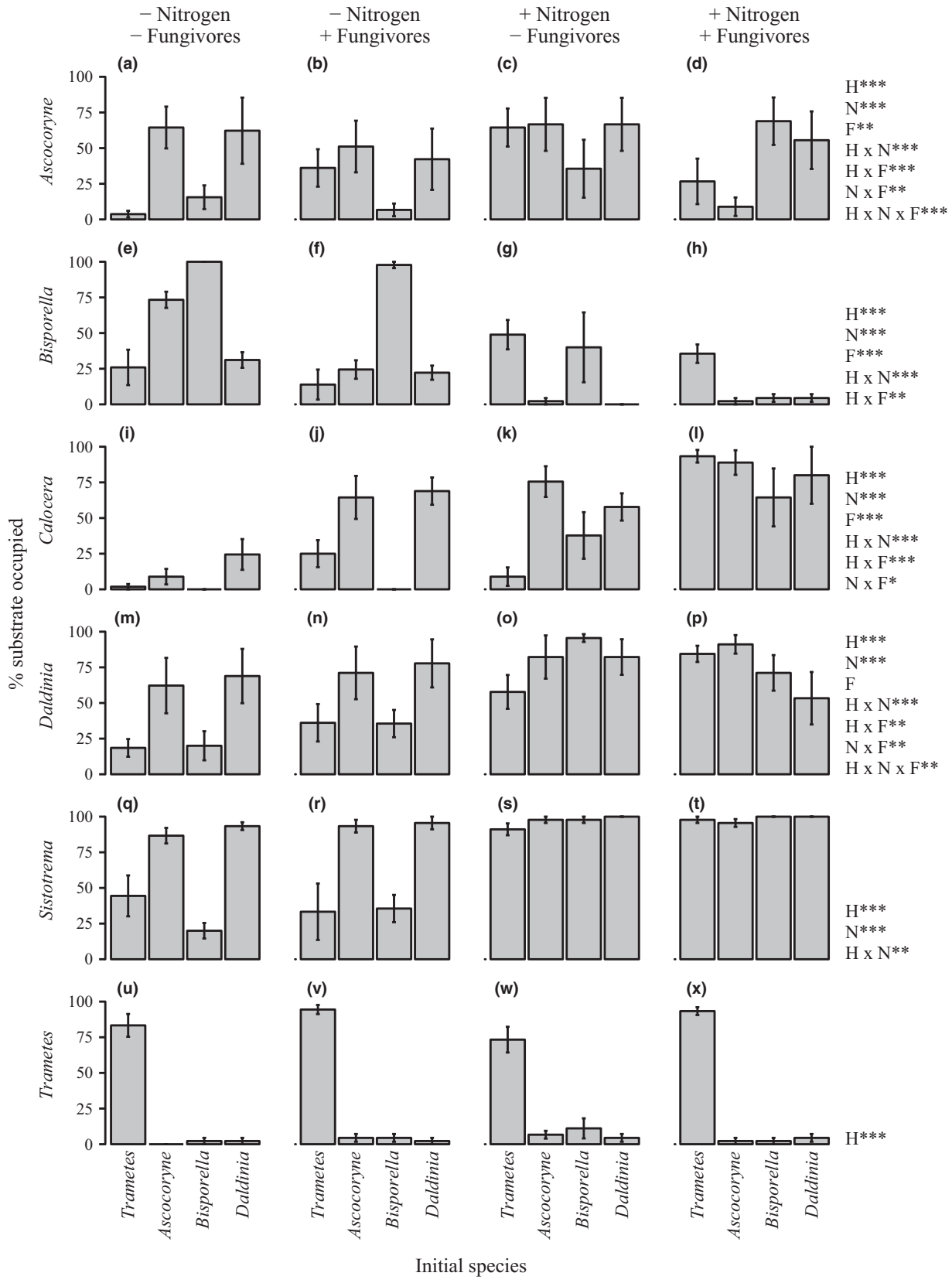

Figure 3 Effect of assembly history (H), nitrogen addition (N) and fungivore presence (F) on the abundance (mean % sub-samples occupied ± SE) of individual fungal species, excluding species that were not observed in more than one sample. Results are show for *Ascocoryne sarcoides* (a-d), *Bisporella citrina* (e-h), *Calocera sinensis* (i-l), *Daldinia novae-zelandiae* (m-p), *Sistotrema brinkmannii* (q-t), and *Trametes versicolor* (u-x). Predictors remaining after model selection and their significance levels are shown for individual binomial generalised linear models, where $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

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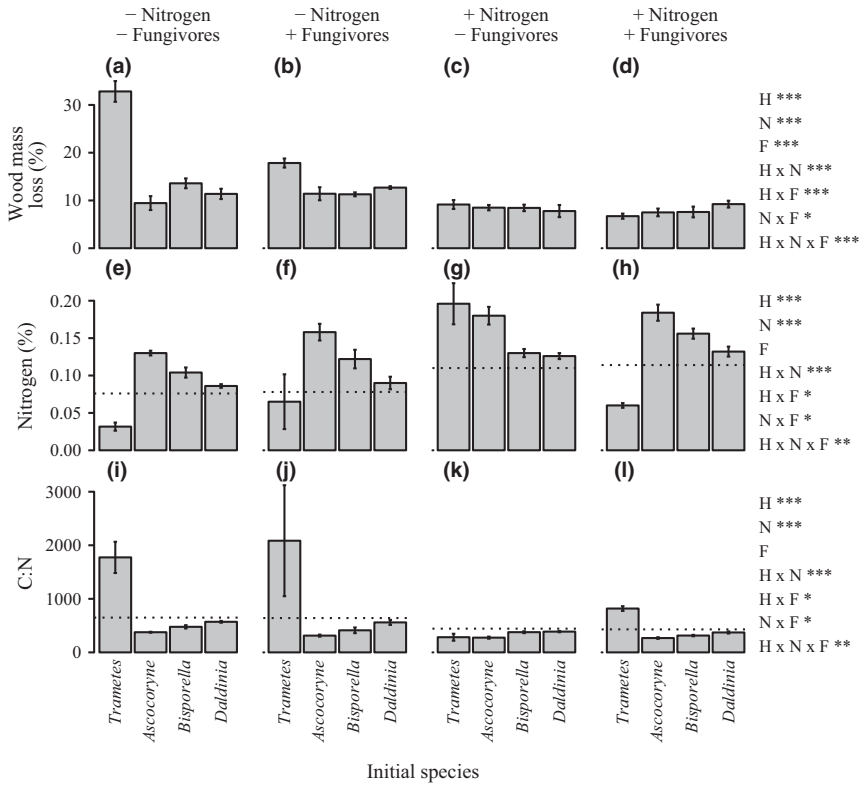

Figure 4 Effect of assembly history (H), nitrogen addition (N) and fungivore presence (F) on wood substrate characteristics 12 months after inoculation. Bar heights for all response variables (dry mass [a–d], % nitrogen [e–h] and carbon-to-nitrogen ratio [i–l]) indicate mean ± SE. Horizontal dotted lines indicate the mean % nitrogen or carbon-to-nitrogen ratio of the non-inoculated controls. Significant predictors are indicated for each response, where $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

(Hedlund *et al.* 1991; Heilmann-Clausen & Boddy 2005; Crowther *et al.* 2012).

Our study used a small number of fungal species and assembly history treatments so that the strength of priority effects was primarily attributed to the influence of the experimental treatments on one species, *T. versicolor*. A larger species pool may include a broader spectrum of functionally diverse species and may also increase the potential for priority effects by increasing the probability that some species will have high niche overlap (Fukami 2015). More data on fungal traits than currently available would facilitate testing of this hypothesis. Similarly, we used only one fungivore species to introduce top-down pressure, but fungi are likely to be consumed by a wide range of organisms in natural systems. Recent evidence suggests that fungivorous macrofauna in soils, such as isopods or millipedes, can have greater effects on fungal community assembly than Collembola and other mesofauna (Crowther *et al.* 2011a, 2013). In this sense, our use of Collembola might be viewed as a conservative test of top-down effects. However, depending on the level of specialisation and functional differences among fungivores, multiple species of fungivores may cancel out one another's effect on community assembly. Future efforts to extend our results could incorporate mesh bags to exclude different size-classes of soil fauna in field-based experimental manipulations (Dickie *et al.* 2012).

It remains uncertain how applicable the results from our experiment are to natural community assembly, given the artificial conditions. For example, we used agar plugs for experimental ease, but inoculation with pre-colonised wood blocks (e.g. Boddy 2000) may have yielded different results and may better represent scenarios in which wood is colonised by mycelia from neighbouring wood substrates. However, recent field studies suggest that the types of priority effects observed in our 1-year experiment may have long-term consequences for fungal and arthropod community assembly and wood decomposition (Weslien *et al.* 2011; Ottosson *et al.* 2014). Long-term priority effects may be important to consider for understanding how climate and land-use change affect wood decomposition as climate variability (Kauserud *et al.* 2012; Diez *et al.* 2013; Boddy *et al.* 2014) and the size and connectivity of forest reserves (Abrego *et al.* 2015) alter fungal phenology. Furthermore, knowledge of long-term priority effects and their joint regulation by top-down and bottom-up forces may help to design effective reintroduction of threatened fungal species for conservation (Abrego *et al.* 2016).

Although we have focused here on a simple community of wood-decomposing fungi, interactive regulation of priority effects by top-down and bottom-up forces may be common in natural systems. For example, Bakker *et al.* (2006) demonstrated that the effects of herbivores on plant diversity in grasslands can vary from negative to positive with increasing resource availability, suggesting that the joint effects of top-down and bottom-up factors may affect priority effects in these communities. In addition, if herbivores preferentially consume more productive species, or certain plant functional groups, they could modify the effects of assembly history and

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local resource availability on ecosystem function (Ejrnæs *et al.* 2006). Similarly, in aquatic invertebrate communities the strength of priority effects on community composition is also known to vary with resources (Chase 2010) and predation (Louette & De Meester 2007; Chase *et al.* 2009). If resource availability also influences the abundance of predators in these systems, interactive control of the strength of priority effects may be likely. Interactive effects could also arise if predator presence limits prey species' access to resources, indirectly modifying resource availability. We suggest that further efforts to understand how multiple factors interact to regulate priority effects will contribute to identifying the conditions that make assembly history important to community structure and function.

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AUTHORSHIP

TF designed the study with input from RBA, IAD, JPW, and PKB. JPW led implementation of the experiment. IAD developed molecular identification. DRL performed data analysis with input from IAD and TF and wrote the manuscript with input from all other authors.

DATA ACCESSIBILITY STATEMENT

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.7p2cv

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