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LETTER

Do assembly history effects attenuate from species to ecosystem properties? A field test with wood-inhabiting fungi

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Abstract

Assembly history, or the order of species arrival, can have wide-ranging effects on species, communities and ecosystems. However, it remains unclear whether assembly history primarily affects individual species, with effects attenuating at the level of communities and ecosystems or, alternatively, has consistent effect sizes across increasing levels of ecological organisation. We address this question using a field-based manipulation of assembly history of wood-inhabiting fungi. The largest effect sizes were observed for the frequency of some individual species, and mean effect sizes were lower for community metrics of fungi immigrating from the regional species pool. There was little evidence, however, of attenuation in effect sizes at the ecosystem level (carbon, nitrogen, decomposition) in comparison to the species or community level. These results indicate that assembly history can have strong effects on ecosystem properties even under natural levels of environmental variability.

Keywords

Alternative community states, community assembly, dispersal, effect size, functional redundancy, New Zealand *Nothofagus* forest, priority effects, scaling, wood decomposition, wood-decay fungi.

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INTRODUCTION

Assembly history, or the order of species arrival during community assembly, can affect species establishment and community structure (Lewontin 1969; MacArthur 1972; Gilpin & Case 1976; Drake 1991; Chase 2003; Schröder *et al.* 2005; Fukami *et al.* 2010). These effects, termed priority effects, can in turn result in historically contingent ecosystem properties, including productivity (Zhang & Zhang 2007; Körner *et al.* 2008), decomposition and nutrient cycling (Fukami *et al.* 2010). Such historical effects limit our ability to explain ecological patterns. It is therefore important to understand what species, community and ecosystem properties are affected by assembly history and whether or not some properties are more strongly affected than others.

As one general hypothesis, we propose that the effects of assembly history may be greater at lower levels of ecological organisation (e.g. individual species abundances) and progressively attenuate at higher levels (e.g. emergent community level properties such as species richness; or ecosystem-level properties such as productivity or decomposition rate). Historical differences in species immigration are expected to have direct effects on the establishment and persistence of species, but at higher levels of ecological organisation, spatial and temporal variation in environmental factors (e.g. temperature, nutrients) may overwhelm effects of assembly history. Further, redundancy in the functional traits of potential colonists in the regional species pool may limit assembly history effects on ecosystem properties even when history effects on community composition are large (Fukami *et al.* 2005; Ejrnæs *et al.* 2006). An alternative hypothesis is that assembly history may be consistently important across different levels of ecological organisation. This hypothesis may be correct if differential habitat modification by species strongly affects local environmental conditions. If abiotic factors are changed significantly by the activity of species as they arrive, early-arriving species may be likely to affect not just the establishment and growth of late-arriving species, but also higher-level ecosystem properties.

Few data exist to test these hypotheses. Many studies of assembly history effects have taken place in laboratory experiments or artificial mesocosms, with limited environmental variability, species diversity, and connectivity to species pools. Although these studies show the potential for assembly history effects, the relative importance of assembly history compared with other variables can only be evaluated by field experiments. Moreover, only in the field is it possible to permit immigration from a realistically diverse species pool. Given a diverse species pool, it may be more likely for species with redundant functional traits to establish, reducing assembly history effects at higher levels of ecological organisation (Robinson & Edgemon 1988; Chase 2003). Furthermore, the majority of previous assembly history studies have not compared effect sizes across multiple levels of ecological organisation.

In this article, we test the hypotheses that the effects of assembly history are greater at lower levels of ecological organisation and progressively attenuate at higher levels; or, alternatively, that the effects of assembly history are consistently important across levels of ecological organisation. We use wood-inhabiting fungi as a model system, quantifying effect sizes at the species, community and ecosystem levels. Fungal communities in wood are likely to have a high degree of variation in assembly history, as they are often dominated by uncommon species with, for example, 81% of 151 taxa occurring on <5% of logs (Allen *et al.* 2000). Wood-decay fungi establishing on new substrates, or as latent fungi in living trees, may initially occur in isolation, but eventually encounter a high diversity of other species and undergo strong interspecific interactions (Allen *et al.* 2000; Boddy 2000, 2001; Kennedy & Bruns 2005; Fukami *et al.* 2010). The identity of early-arriving fungi is highly stochastic, as small- and large-scale disturbances provide new wood substrates at varying

timing relative to the phenology of spore-based colonisation (Boddy 2001).

METHODS

Overview and study site

To manipulate initial assembly history, we isolated fungi from a forest, inoculated these species onto wooden discs in different histories, and placed the discs in the forest stand in close contact with the soil. We then measured responses at 6 and 13 months, quantifying species-level (frequency), community-level (species composition and richness) and ecosystem-level (nitrogen and carbon concentration and mass loss of the decomposing wood) properties. The study site was an old-growth *Nothofagus solandri* forest (Allen *et al.* 2000; Clinton *et al.* 2002) at Craigieburn Forest Park, South Island, New Zealand (43°8.556 S, 171°42.825 E, 1090-m elevation, aspect 152°). The only other vascular plant present in the 15 × 23 m study area was the epiphytic mistletoe *Peraxilla tetrapetala*. The ground surface was around 40% covered in moss (dominated by *Dicranoloma billardierei*). Soils comprised a 13-cm-thick F-layer of decaying leaves and wood with dense tree roots and fungal hyphae over a mixed loess–colluvium, yellow-brown acidic (pH 4.1), low-nutrient-availability mineral soil (Allen *et al.* 1997; Clinton *et al.* 2002).

Experimental design

We used 320 wood discs as replicates, as 2 initial nitrogen levels × 8 assembly histories (including a no-inoculum treatment) × 2 harvests × 10 replicates per treatment (see below). To create experimental units, we manufactured 320 discs from *N. solandri*, 80 mm in diameter and 10 mm thick, with 10 flat-bottomed holes 8 mm deep and 9.5 mm in diameter placed equidistant in a circle 30 mm from the disc centre (see Figure S1 of Supporting Information). One additional 6-mm diameter hole was drilled through the centre of each disc, for later use in bolting on a 'cap' disc.

Manipulation of initial nitrogen level

We manipulated initial nitrogen concentration as a factor that frequently influences fungal growth (van der Wal *et al.* 2007; Treseder 2008; Janssens *et al.* 2010) and that limits primary productivity in these forests (Davis *et al.* 2004; Smaill *et al.* 2011). Including initial nitrogen concentration as a reference factor allowed us to evaluate effect sizes of assembly history relative to a known environmental driver. Further, assembly history is expected to have greater effects in nutrient-rich and productive ecosystems by permitting a larger pool of species to exist (Chase 2003, 2010), although we subsequently found that adding nitrogen did not increase decomposition (a surrogate for productivity), consistent with some other studies (Knorr *et al.* 2005; Fukami *et al.* 2010; but see Allison *et al.* 2009). Discs were soaked for 24 h in a 7.04 g L^{-1} solution of L-arginine, for the high-N, or water for the low-N, treatment then autoclaved (121 °C, 30 min), resulting in initial nitrogen concentrations of 0.14 and 0.042%, respectively. These levels span the naturally occurring nitrogen in wood at this site (Clinton *et al.* 2002). Arginine is common in plant tissue, abundant as a source of soil nitrogen in similar mature forests (e.g. Warren & Adams 2007), and readily utilised by many fungi (e.g. McGuire *et al.* 2010). Preliminary data also showed that arginine-N was retained in wood, whereas inorganic-N (NH_4NO_3) was rapidly leached under field conditions (I. A. Dickie, unpublished data).

Manipulation of assembly history

Fungi were obtained from isolation of 96 species from 143 collections taken near the study site in May 2006 as in Fukami *et al.* (2010). This level of diversity is similar to the 151 fungal taxa collected by Allen *et al.* (2000) from 75 logs near the study site in May 1996, but relatively few species were common to both studies, suggesting a higher total diversity. Ten species were selected on the basis of methodological ease of handling and identification and phylogenetic spread, with 7 species randomly selected from the 10 for use as initial species (Fukami *et al.* 2010).

We inoculated species onto discs, manipulating assembly history by inoculating the initial species 3 weeks before the remaining nine species, while holding constant the set of species introduced. We placed autoclaved cylindrical (8 mm diameter × 8 mm long) *Nothofagus menziesii* wooden plugs (Leech Wood Turning, Doyleston, New Zealand) around or onto growing fungal cultures on agar, and incubated these for 2–6 months, depending on the intrinsic growth rate of isolates. *Nothofagus menziesii* wooden plugs were used because *N. solandri* is difficult to machine. We inoculated discs by inserting a single colonised plug into one of the pre-drilled holes, incubated the disc in a Petri dish for 21 days at 18 °C in a clean incubation room, and then added colonised plugs of all nine other species (Figure S1). After 21 days superficial growth of the initial species onto wood varied from only a few mm (*Calocera*) to over a third (*Ascocoryne*, *Pleurotus*) or more of the disc surface (*Daldinia*, *Trametes*). We placed colonised plugs in the same relative positions in all treatments, so that the only difference between treatments was which species was inoculated earliest. No-inoculum discs received three sterile wooden plugs (rather than 10 due to limited supplies of plugs). Although the use of inoculum plugs is artificial, it may mimic the colonisation of wood by fungal hyphae from adjacent substrates, or the period following spore colonisation of new substrate where a fungus may grow in isolation from other species (Boddy 2000).

On 29 April 2008, 7 days following the second round of inoculation, all discs were emplaced at 25 mm depth into the soil F-layer at 1 m × 1 m spacing in a 15 m × 23 m grid on the forest floor. To retain plugs, we 'capped' each disc with a second wooden disc (10 mm thick × 80 mm diameter) with a 6-mm stainless steel bolt inserted through a hole in the centre of both discs (caps were soaked in the same nutrient solution as the disc and autoclaved). After bolting two discs together, we inserted disc pairs into nylon mesh bags (150 mm × 150 mm; 1.6-mm mesh size), which were closed with two staples and attached with stainless steel wire to an aluminium peg and identity tag; the bag prevented material loss due to fragmentation during decomposition while allowing most fungivorous soil fauna access, and the buried metal peg aided in re-locating discs with a metal detector (Figure S1). All non-sterile handling of discs occurred within 5 m of the experimental area; hence any fungi inadvertently introduced to discs were likely local in origin. We did not use inoculated wooden discs visibly contaminated with non-target fungi; this resulted in the pre-experimental loss of eight samples.

Harvest and measurement

Discs were destructively harvested on 20 October 2008 and 28 May 2009. At harvest, discs were removed from the F-layer, placed into

plastic bags, transported to the lab and stored at 4 °C. Discs were split along eight radial lines, exposing internal wood without contamination from the surface, using custom-made ethanol-flame-sterilised samplers (Figure S2). Ethanol-flamed 1.5-mm drill bits were used to obtain 5.5 mg (mean; $n = 20$, range 2.0–9.0 mg) of sawdust from the centre of the eight exposed interior surfaces of discs, directly into sterile 0.2-mL tubes. Fungal DNA was extracted and PCR of the ITS region performed using the Sigma REDExtract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, MO, USA), and primers ITS1F-6FAM and ITS4-VIC following standard protocols (Dickie *et al.* 2009). The eight PCR products per disc were combined and cleaned using the MinElute 96 UF PCR purification kit (Qiagen, Valencia, CA, USA), digested with *HpyCH4*IV (NEB, New England Biolab, Ipswich, MA, USA) and *Hae*III (Roche Diagnostics, Berlin, Germany) restriction enzymes, denatured with highly de-ionised formamide and 0.1× diluted samples were run through capillary electrophoresis on a 3130xl genetic analyser (Applied Biosystems, Carlsbad, CA, USA) with MapMaker 1000 standard (Bioventures Inc., Murfreesboro, TN, USA).

The frequency of the 10 inoculated species was determined using TRAMPR software (Fitzjohn & Dickie 2007) to detect the presence of known terminal-restriction fragment length polymorphism (T-RFLP) profiles across all four channels (2 primers × 2 digests) of data. To detect immigrant species we used peak-profile T-RFLP (Dickie & Fitzjohn 2007) to indicate total taxa richness and shifts in fungal community composition. Peaks were binned, using cluster analysis in R (R Development Core Team 2010), based on complete-linkage clustering with a cut height of 2.5, resulting in median bin-width of 1.34 base pairs. Peaks from inoculated species were eliminated from data before this analysis, so that the second analysis was based on immigrant (non-inoculated) taxa only. Peak-profile T-RFLP may not separate all species, hence taxa richness of immigrants provides a relative metric within the study rather than an absolute metric of diversity (Dickie & Fitzjohn 2007). The small size of our wood discs relative to fungal individuals (Figure S3) and relatively short duration of our experiment led us to conclude that diversity of fungi was likely to be within the resolution limits of T-RFLP. Most other molecular methods are not cost-effective for highly replicated experimental designs (Dickie & Fitzjohn 2007).

At both harvests, percentage wood mass remaining (including fungal biomass in wood) was measured for each disc as the proportion of dry weight at the harvest to initial dry weight (after drying at 40 °C). After weighing, nitrogen and carbon concentrations of each wooden disc were measured from a combined sawdust sample comprising eight sub-samples each from a different split-edge interior disc surface obtained using 2-mm drill bits.

Statistical analysis

The test of assembly history is whether a response variable differed significantly across histories, not whether the seven histories differed from the no-inoculum treatment. We therefore excluded the no-inoculum treatment from statistical analyses, and report no-inoculum results only to indicate which inoculated species may also have been present as immigrant taxa and to provide some context for ecosystem-level responses. All response variables were analysed following the model response ~ history × nitrogen separately for each harvest, using type II sums of squares. Frequencies of species were analysed using general linear models with a binomial response variable, and species richness using function aov in R, which permits unbalanced data. Community composition was analysed using permutational multivariate analysis of variance, with beta diversity (dispersion) tested following Anderson (2001) as implemented in the R-package vegan (Oksanen *et al.* 2010), except that Raup–Crick distances were calculated following Chase *et al.* (2011). We visualised community composition with non-metric multidimensional scaling (NMDS, as metaMDS in R-package vegan) combining both harvests for visualisation purposes, but analysing each harvest separately for statistical tests. Ecosystem response variables (nitrogen, carbon, mass loss) were analysed using function aov; mass loss was strongly non-normal in distribution, and was therefore square root-transformed.

Effect sizes were calculated as the proportion of variance explained by a factor, after accounting for other factors, which is equivalent to a partial R^2 and includes variance explained by a significant interaction term as well as main effects. Although there are limitations to using R^2 as a metric of effect size (Nakagawa & Cuthill 2007), R^2 values have the inherent advantage of immediate interpretability by indicating the proportion of variation explained by a factor and integrating across multiple assembly history treatments. Alternative methods for quantifying effect sizes were inappropriate for comparing response variables with different underlying variances (e.g. log response ratios) or were not designed for multiple treatment contrasts (e.g. Cohen's *d*, log response ratios). To test for attenuation, we compared the effect size of each response variable at community and ecosystem levels with the mean effect size for individual species-level responses. Specifically, we tested the null hypothesis $\rho = \rho_0$ where ρ is the square root of effect size and ρ_0 is the square root of the mean effect size for the individual species-level responses (Zar 1999, pp. 383) based on Fisher's z transformed ρ and ρ_0 as z and ζ, respectively, in the test:

$$Z = \frac{z - \zeta}{\sqrt{1/n - 3}}; Z_{0.05(2)} = t_{0.05(2),\infty}$$

RESULTS

Effects on individual species

The effect of history on the frequency of individual inoculated species was significant for six species at 6 months and five species at 13 months and explained, respectively, 12–80 and 8–76% of the variance at the two harvests (Figs 1 and 2). Excluding *Armillaria*, which was never detected, and *Calocera*, which was only detected twice, history had a mean effect size (partial R^2) of 0.45 and 0.31 at 6 and 13 months for individual species. The frequency of most species at 6 months was highest when that species was inoculated first (e.g. *Pleurotus*, *Phlebia*, *Daldinia*, *Trametes*, *Calocera*), whereas some species established well regardless of history (*Bisporella*, *Ascocoryne*, *Sistotrema*). *Phlebia* was only present when inoculated first and never present in other histories (Fig. 1). Few inoculated species were detected in the no-inoculum treatment (*Ascocoryne* twice at 6 month, and *Ascocoryne* and *Bisporella* three times and *Sistotrema* once at 13 months).

When we excluded the treatment where a particular species was inoculated first, we found that assembly history remained a significant predictor for frequency of five species at 6 months and two species at 13 months, explaining up to 53 and 35% of the variance in species frequency, respectively (not shown), largely reflecting suppression of particular species when another species was introduced first. For example, *Trametes* was suppressed when *Daldinia* was inoculated first;

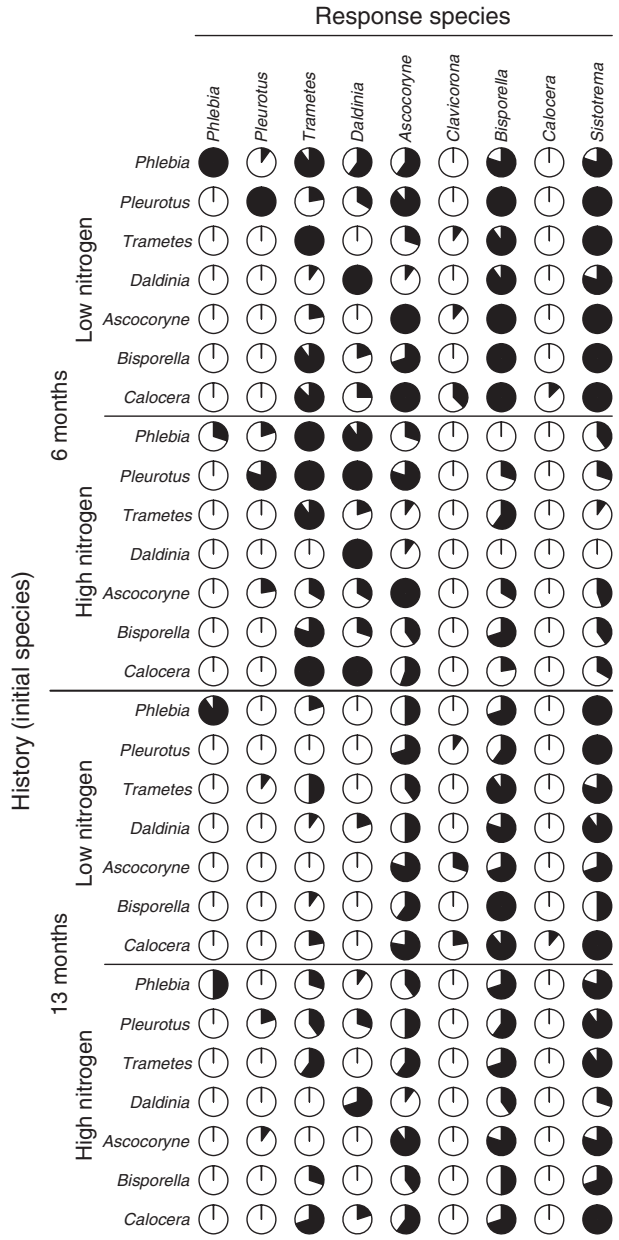

Figure 1 Frequency, or the proportion of wood discs (out of 8–10 replicates) where a species was present (columns), as a function of initial species history, nitrogen treatment and harvest (rows). Order of species indicates largest to smallest assembly history effect (see Fig. 2). Circle with line indicates frequency of zero; *Armillaria* was inoculated but never detected, and hence is not shown.

Daldinia was suppressed by *Trametes* or *Ascocoryne*; and *Ascocoryne* was suppressed by both *Daldinia* and *Trametes* (Fig. 1).

The N-addition treatment had a significant effect on the frequency of seven species at 6 months and six species at 13 months and explained a mean 22 and 11% of the variance at 6 and 13 months, respectively (maximum 46 and 19%; Fig. 2). For most species, N-addition resulted in a decrease in species frequency (Fig. 1). There were only two significant interactions between history and nitrogen: for the frequency of *Trametes* at 6 months, partly reflecting a higher abundance in the *Pleurotus*-first history at high-N rather than low-N; and for the frequency of *Sistotrema* at 13 months, which had lower abundance in the *Daldinia*-first history at high-N.

Effects on community composition: inoculated species

An average of 3.3 ± 0.1 (mean and SE) of the 10 inoculated species were detected at 6 months, varying from 2.0 ± 0.23 to 4.3 ± 0.20 species across different assembly histories (Fig. 3a), with history explaining 34% of variance in species richness (Fig. 2). By 13 months, mean richness had lowered to 2.6 ± 0.09 species, varying from 2.0 ± 0.23 to 3.2 ± 0.18 species across histories, with history explaining 22% of variance. The richness of inoculated species was higher in low-N than high-N discs at 6 months (3.9 ± 12 vs. 2.7 ± 11 species) with nitrogen addition explaining 22% of the variance; the effect of nitrogen addition on the richness of inoculated species at 13 months was only 5% and not significant.

The community composition of inoculated species was influenced by both assembly history and nitrogen addition treatments, with assembly history explaining more of the variance than nitrogen addition (39 vs. 23% and 23 vs. 9% at 6 and 13 months, respectively). Beta diversity of inoculated species was affected by assembly history, which explained 18% of the variance, at 6 months, but not by nitrogen treatment. No significant treatment effects on beta diversity were found at 13 months. NMDS visualisation supported both assembly history effects (e.g. *Daldinia*-first tended to cluster opposite *Ascocoryne*-first treatments) and nitrogen treatment effects at 6 months, as well as the general convergence of the inoculated species community by 13 months (Fig. 3b). The mean effect size of assembly history on inoculated species community metrics was 0.30 and 0.20 at 6 and 13 months, respectively. Only beta diversity showed a significantly lower effect size of assembly history than the mean effect size for individual species ($P = 0.0015$ at 6 months, $P = 0.060$ at 13 months), with all other effect sizes not significantly different ($P > 0.2$).

Effects on community composition: immigrant species

From peak-profile T-RFLP analysis, a richness of 25.7 ± 0.6 and 33.2 ± 0.6 operational taxonomic units (OTUs; hereafter 'taxa') were detected at 6 and 13 months, respectively. Excluding possible matches to inoculated species, 21.2 ± 0.6 and 30.1 ± 0.6 taxa were considered immigrant (T-RFLP peaks that could not be unequivocally assigned to either inoculated species or immigrant taxa were excluded, this represented 1.2 and 0.5 taxa at 6 and 13 months, respectively). Assembly history explained 22% of the variance in immigrant taxa richness at 6 months (significant interaction with nitrogen; Fig. 2), with more than twofold differences in immigrant richness depending on history in the high-N treatment (Fig. 4a). By 13 months, all treatment effects on taxa richness had disappeared.

The community composition of immigrant taxa was strongly affected by both history and nitrogen treatments, not only with strong separation of community composition by nitrogen addition, but also notable history effects within the high-N treatment (Fig. 4b). Immigrant community composition was notably more dispersed (i.e. had higher beta diversity) in the high-N treatment than low-N treatment at 6 months, but there was no significant effect of either assembly history or nitrogen treatment on beta diversity at 13 months.

The mean effect size of assembly history on immigrant species community metrics was 0.18 and 0.09 at 6 and 13 months, respectively. All immigrant community metrics at both harvests showed smaller assembly history effect sizes than the mean effect size for individual species ($P < 0.05$ for all; Fig. 2).

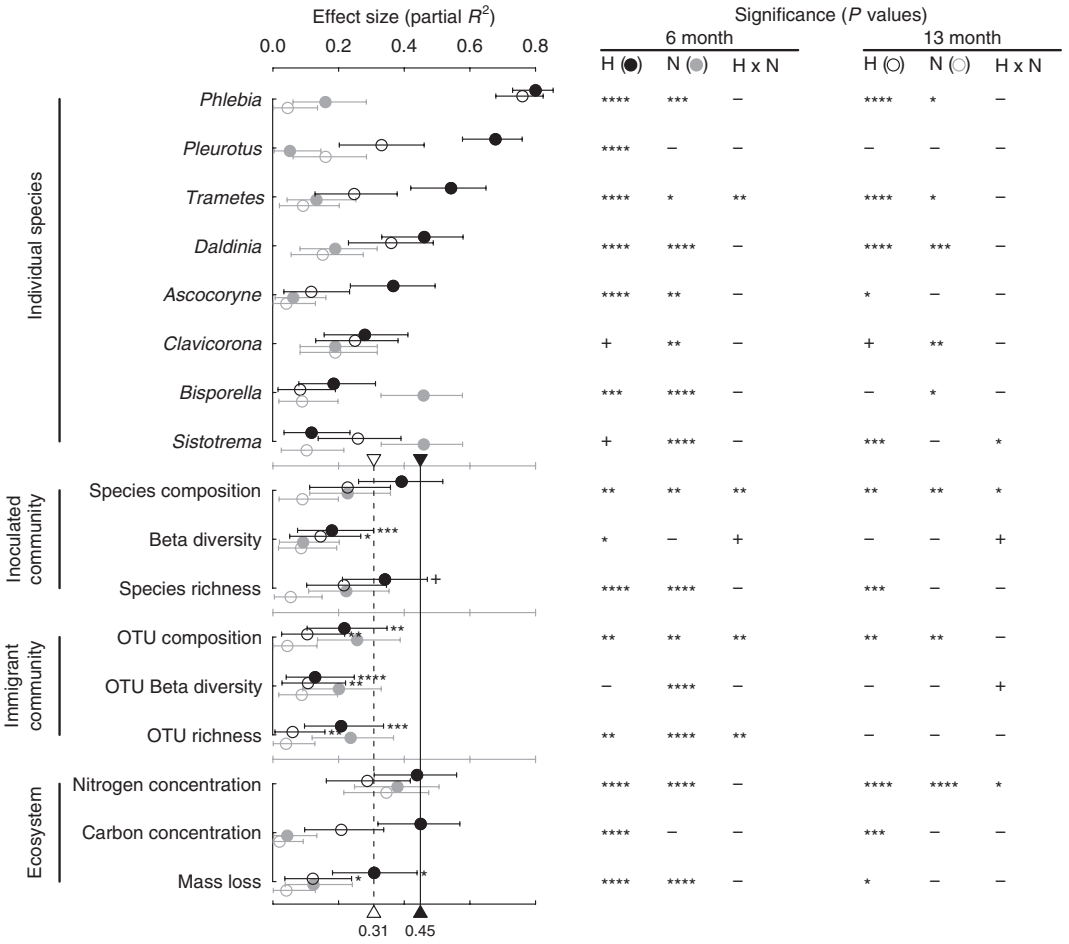

Figure 2 Effect sizes (partial R^2) with 95% confidence intervals of assembly history (black) and nitrogen addition (grey) at 6 (solid symbols) and 13 months (open symbols) on species, community, and ecosystem properties (rows). Significance of each term is given by *< 0.05, **< 0.01, ***< 0.001, ****< 0.0001; P values < 0.1 indicated by +, and P values > 0.1 by a dash. Species ordered by assembly history effect size. Triangles and vertical lines in the lower portion of figure show the mean effect of assembly history at the level of species in comparison to effect sizes at higher levels of ecological organisation for 6 (black triangles, solid line) and 13 months (open triangles, dashed line). Assembly history effects at community and ecosystem levels that are significantly lower than the mean effect size of assembly history at the individual species level are indicated by * or + (as above) adjacent to confidence interval bars.

Effects on ecosystem-level properties

At 6 months, nitrogen concentrations varied between 0.032 and 0.134% in the low-N treatment and 0.092 and 0.18% in the high-N treatment depending on assembly history (Fig. 5a). The effect of assembly history on nitrogen concentration (explaining 44 and 29% of variance at 6 and 13 months) was similar in effect size to the effect of adding nitrogen (Fig. 2; Table S1). The effects of both assembly history and nitrogen addition on nitrogen concentration remained highly significant at 13 months, but there were reversals in the rank order of species. For example, in the low-N treatment the *Daldinia*-first history had the highest nitrogen concentration at 6 months, but the lowest at 13 months, whereas the two histories with the lowest nitrogen concentrations at 6 months (*Pleurotus*- and *Phlebia*-first) had the highest at 13 months. Carbon concentrations showed quantitatively small (< 1%) but highly significant responses to assembly history at both 6 and 13 months, with history explaining 45 and 21% of variance, respectively. Mass loss had lower effect sizes than other ecosystem-level properties (31 and 12%) and only mass loss at 13 months showed a lower effect size of assembly history than the mean effect size for individual species ($P = 0.0015$), with all other effect sizes not significantly lower ($P > 0.2$ for all). The mean effect size of assembly history on ecosystem-level properties was 0.40 and 0.20 at 6 and 13 months, respectively.

Across all treatments there was an average mass loss of 3.5 ± 0.09% (mean and SE) at 6 months and 7.4 ± 0.4% at 13 months (Fig. 5c). There was also a significant ($P = 0.013$) but weak ($R^2 = 0.03$) negative correlation of mass loss with species richness at 13 months. Despite the loss of total mass, total nitrogen (not just nitrogen concentration) increased over time in most of the low-N treatments either at 6 months (e.g. *Daldinia*, *Trametes*, *Calocera*) or at 13 months (*Pleurotus*, *Phlebia*, *Bisporella*). The high-N treatment showed more variable responses, but no strong gains or losses of total nitrogen by 13 months.

DISCUSSION

Assembly history had stronger effects on some individual species than on community- or ecosystem-level properties, but the mean effect size showed little attenuation at the ecosystem level compared with the species or community level (Fig. 6). The lowest mean effect sizes of assembly history were on the immigrant community (Fig. 6), with the

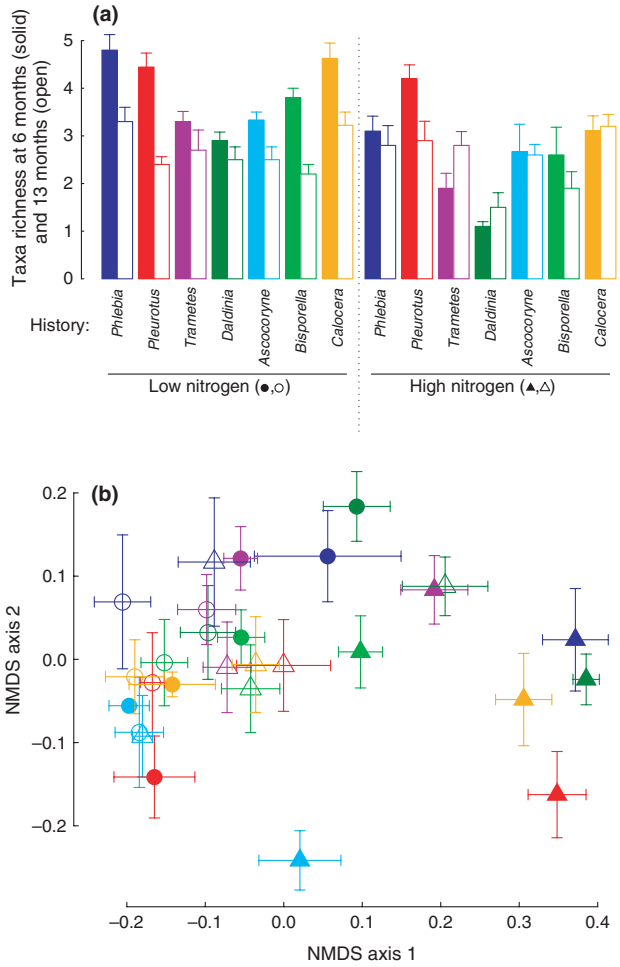

Figure 3 Effect of assembly history on richness (a) and composition (b) of the 10 inoculated species at 6 (solid bars and symbols) and 13 months (open bars and symbols) at low (circles) and high-N (triangles). Colours in (a) indicate species in (b); order of species follows Fig. 2 (greatest to lowest assembly history effect size). Composition is shown as mean axis scores from non-metric multidimensional scaling (as metaMDS in R-package vegan) based on Raup–Crick distances with error bars showing one standard error.

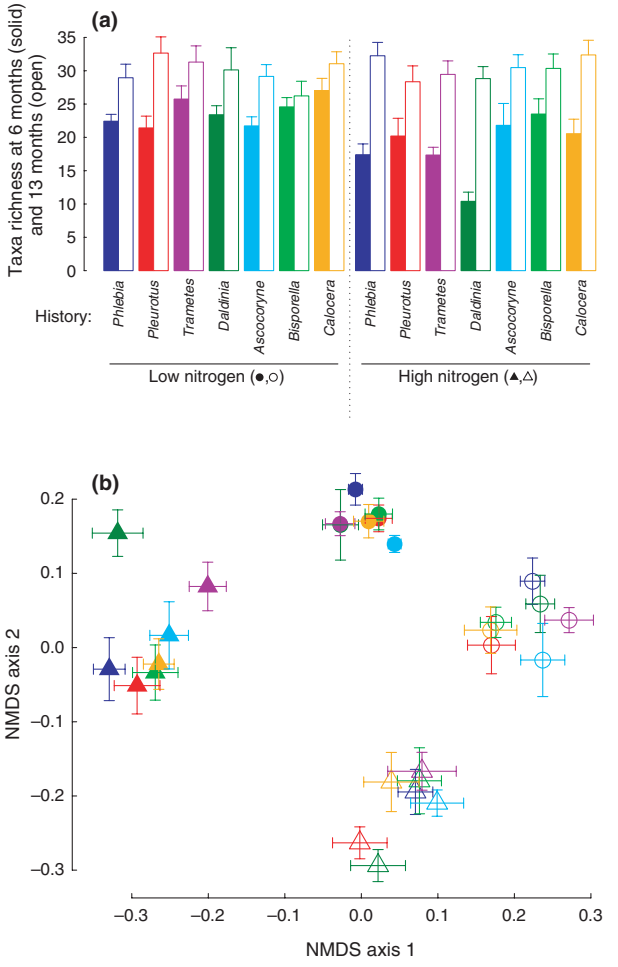

Figure 4 Effect of assembly history on richness (a) and composition (b) of immigrant taxa as indicated by terminal-restriction fragment length polymorphism (T-RFLP) taxa at 6 (solid bars and symbols) and 13 months (open bars and symbols) at low (circles) and high-N (triangles). Colours in (a) indicate history treatments in (b); order of species follows Fig. 2. Composition is shown as mean axis scores from non-metric multidimensional scaling (as metaMDS in R-package vegan) based on Raup–Crick distances with error bars showing one standard error.

effect sizes of all three immigrant community metrics significantly lower than the mean effect size of individual species. In contrast, at the ecosystem level, only mass loss showed significantly lower effect sizes compared to the mean effect size on individual species (Fig. 2). Our results provide evidence that strong effects of assembly history on ecosystem properties, comparable or greater in strength to those on communities, can occur even with natural environmental variability and despite potential functional redundancy in a diverse regional pool of immigrants (Ejrnæs *et al.* 2006; Körner *et al.* 2008; Fukami *et al.* 2010).

Comparison of species, community and ecosystem effects

The strongest effects of assembly history were on the establishment of some individual species, with most species having the greatest establishment when that species was introduced first. The phenomena of early-arriving species having a greater ability to establish may be common in fungi (Boddy 2000; Kennedy & Bruns 2005; Fukami *et al.* 2010). Fungi compete strongly through the production of secondary metabolites and direct hyphal interactions, including high investment in non-nutrient assimilating, combative hyphae (Boddy 2000; Heilmann-Clausen & Boddy 2005). Given a period of initial establishment without competition, a fungus may have a greater resource pool with which to support metabolically expensive interactions (Holmer & Stenlid 1997), which may also explain the reduction in decomposition in many treatments compared to discs with no inoculum.

There were also individual species for which assembly history had negligible effects, resulting in the mean effect size of assembly history being the same as for the inoculated community and ecosystem level. Small effect sizes were found for species that were rare across all treatments (e.g. *Clavicorona*) or, alternatively, uniformly common across all treatments (e.g. *Sistotrema*, *Bisporella*). Whether this is a statistical artefact driven by the lack of presences and absences, respectively, limiting power of binomial tests or whether common species, due to the very traits that make them common, are less affected by assembly history than rare species is worth further investigation.

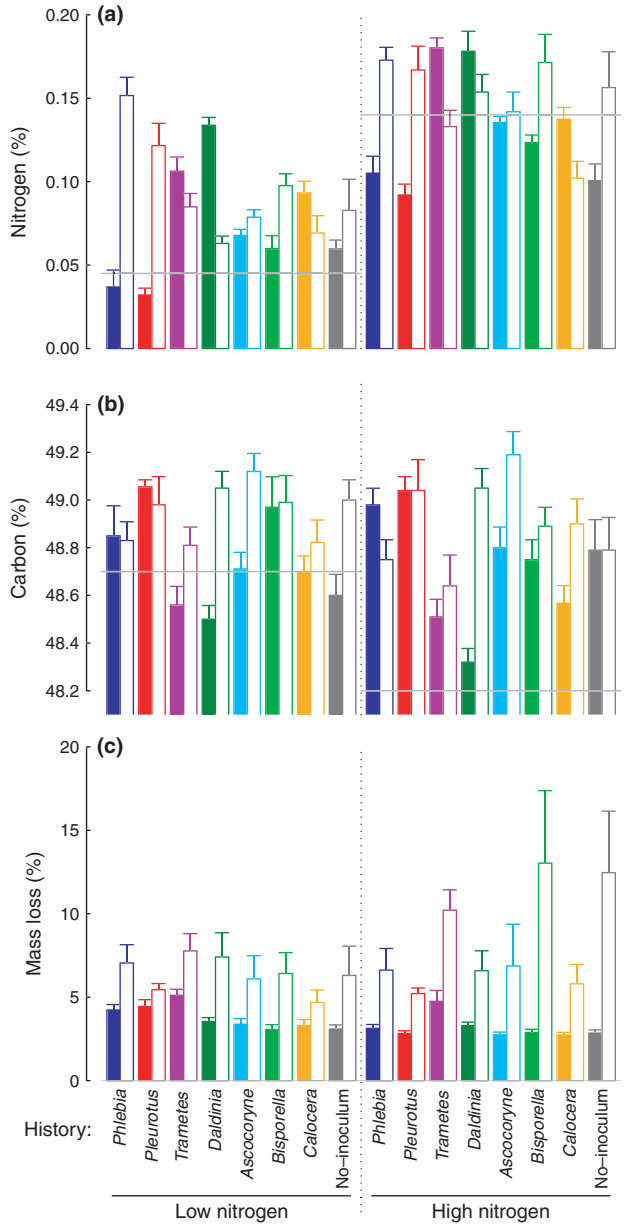

Figure 5 Ecosystem-level responses to assembly history for nitrogen (a) and carbon (b) concentrations and mass loss (c) at 6 (solid bars) and 13 months (open bars). Error bars indicate one standard error; horizontal grey lines indicate initial conditions for low-N and high-N treatments; order of species follows Fig. 2. The no-inoculum results are shown for context, but not included in any statistical analyses.

The lack of attenuation at higher levels of ecological organisation likely reflects two properties of fungi. First, fungi are strong habitat modifiers through chemical antagonism and through decomposition of different compounds at different rates (Coates & Rayner 1985; Boddy 2000; McGuire & Treseder 2010). Second, fungal species may have limited redundancy in ecological traits (Coates & Rayner 1985; Dickie & Moyersoen 2008; Hanson *et al.* 2008; McGuire *et al.* 2010) such that different communities translated into different ecosystem-level properties. Assumptions in major decomposition models that the fungal community can be treated as a 'black box' may need to be re-examined in light of the significant community-driven effects we have observed (McGuire & Treseder 2010).

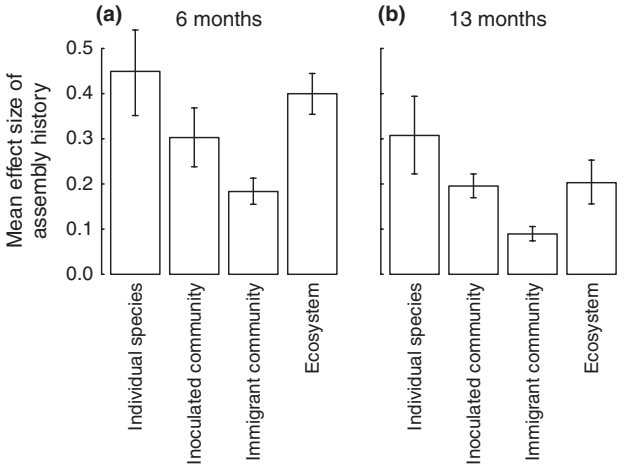

Figure 6 Summary of mean effect sizes at individual species, inoculated and immigrant community, and ecosystem levels at 6 (a) and 13 (b) months. Means and standard errors back-transformed from zeta-transformed R^2 values (Zar 1999).

The three ecosystem-level properties we measured (nitrogen, carbon and mass loss) all potentially include fungal, bacterial and other organismal biomass, which cannot be readily separated from decaying wood. Thus, the increase in nitrogen observed in the low-N treatment may reflect either importation of exogenous nitrogen, via fungal hyphal translocation (Laiho & Prescott 2004) or the immigration of soil animals, or endogenous N-fixation by bacteria (Jurgensen *et al.* 1989). N-immobilisation by wood is well known (Zimmerman *et al.* 1995; Laiho & Prescott 2004). Our results show that both the degree and timing of this immobilisation can be strongly responsive to the assembly history of fungal communities. This likely reflects changes in species composition, with some fungal species driving greater immobilisation than others (Clinton *et al.* 2009). The inclusion of fungal biomass is also likely to reduce the apparent wood mass loss, and reduce carbon concentrations.

Effect sizes of both assembly history and nitrogen addition decreased from 6 to 13 months (Fig. 2). Communities appeared to generally converge in composition. This was evidenced by an overall loss of inoculated species richness (increasing only in the two treatments where richness had been lowest), the loss of treatment effects on OTU richness of immigrant taxa, and the convergence of communities in ordination (NMDS). In contrast, ecosystem properties (mass loss, carbon concentrations) did not converge. Rather, the diminishing effect sizes of assembly history on mass loss and carbon concentrations reflected an increased variability within treatments, rather than convergence in treatment means. Wood substrates can be considered as degrading ecosystems, with progressive loss of substrate to the point of ecosystem extinction. Assembly history may have persistent effects on ecosystem properties due to this degradative process; once a fungus modifies the substrate, there is limited scope for ecosystem properties to recover. Modification of immigrant fungal communities by inoculated fungi may also have long-lasting effects on ecosystem-level properties, as immigrants as well as inoculated species influence ecosystem properties.

Contrasting effect sizes of assembly history and nitrogen addition

The mean effect size of assembly history on ecosystem properties, R^2 of 0.21, is small to moderate by conventional metrics (Nakagawa &

Cuthill 2007). However, the assembly history effects we observed were comparable with, and in many cases larger than, the effects of the experimentally imposed threefold difference in initial nitrogen concentration (Fig. 2), even though nitrogen is frequently considered important to fungi (van der Wal *et al.* 2007; Treseder 2008; Janssens *et al.* 2010) and is a limiting resource at this site (Davis *et al.* 2004; Smaill *et al.* 2011). Interpretation of the difference in effect size is complex, as it is influenced both by the number of levels of each factor and by the degree to which factors span their possible range, both of which are arbitrary. We explored the first of these using adjusted R^2 values (Table S1) and found that the effects of history on all three ecosystem properties after accounting for nutrients were consistently comparable to, or larger than, the effects of nitrogen addition after accounting for history, even after adjusting for the higher number of factor levels of the history treatment (7) compared to the nitrogen treatment (2). In addition, the threefold differences in initial nitrogen represent a major portion of the possible range of wood nitrogen values for this system. In contrast, the seven history treatments represent only a tiny fraction of possible histories. These results support the view that assembly history effects had effect sizes on ecosystem-level properties comparable to nitrogen, a factor generally considered a significant ecosystem driver.

Comparison to laboratory microcosms

The effect sizes we observed were generally smaller than those reported from an earlier laboratory experiment conducted in microcosms with the same fungal isolates, wood substrate type and with soil from the same site (Fukami *et al.* 2010). Individual species responses were quite different between the field and microcosm experiments. *Phlebia*, for example, dominated in the microcosm experiments in most treatments (Fukami *et al.* 2010), but was only detected in this field experiment when it was inoculated first. In contrast, *Bisporella*, *Pleurotus*, and *Daldinia* all established better in the field than in the microcosms. Both abiotic (e.g. more variable moisture, colder temperatures, tannins from surrounding leaf litter) and biotic factors (immigrant species, fungivores, bacteria) may have contributed to these differences. Despite differences in individual species responses, emergent trends at the level of communities and ecosystems were generally similar between the microcosm and field experiments.

Our use of sterile wood and inoculum plugs imperfectly mimics the natural pattern of substrate colonisation by fungi, our selection of species may have resulted in some initial species that are rarely primary colonists, and wood substrates do not naturally occur as small wood discs in nylon mesh bags. The manipulation of fungal communities also forced us to initiate the experiment for 28 days under microcosm conditions. Future studies might consider whether observational or other more 'natural' approaches could be used, although the high diversity of fungal communities might make other study systems more tractable. Finally, our study took place within a single site. It would be valuable to test assembly history across a wider range of environmental conditions, to determine whether or not assembly history effect sizes vary with, for example, moisture, temperature and environmental extremes.

CONCLUSION

Strong assembly history effects limit our ability to understand and predict ecological properties. A first step towards dealing with this challenge is to understand when assembly history matters and what ecological properties are more or less sensitive to assembly history. Our results show that assembly history effects do not necessarily attenuate at increasing levels of ecological organisation. This finding may reflect non-redundant functional differences among fungal species (Coates & Rayner 1985; Hanson *et al.* 2008; McGuire & Treseder 2010). Earlier work on plant communities provided contrasting results, where functional redundancy among plant traits appeared to limit the effects of compositional differences at higher levels of organisation (Fukami *et al.* 2005). We propose that attenuation (or lack thereof) of assembly history effects across ecological levels of organisation should be investigated in a variety of systems to advance the general understanding of historical contingency in community assembly and its implications for ecosystem properties.

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AUTHORSHIP STATEMENT

TF and IAD contributed equally to this work. TF initially conceived this study and led the proposal for funding with substantial input from RBA, IAD and PKB. IAD led the field-based work with input from TF and RBA, designed and analysed molecular identifications and performed all statistical analyses. JPW led the laboratory-based components (pre- and post-field). PKB led the collection, identification, and selection of fungal isolates. IAD and TF wrote the manuscript with input from all other authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Schematic of disc design.
Figure S2 Customised devices used to sample interior of wood disc without surface contamination.
Figure S3 Photographs showing (a) site, (b) wood discs *in situ*, and (c) wood discs following harvest.
Table S1 R^2 and adjusted R^2 comparisons for ecosystem-level responses for full model (response ~ history × nitrogen) and for history and nitrogen after accounting for the other factor (history residuals ~ nitrogen; nitrogen residuals ~ history).

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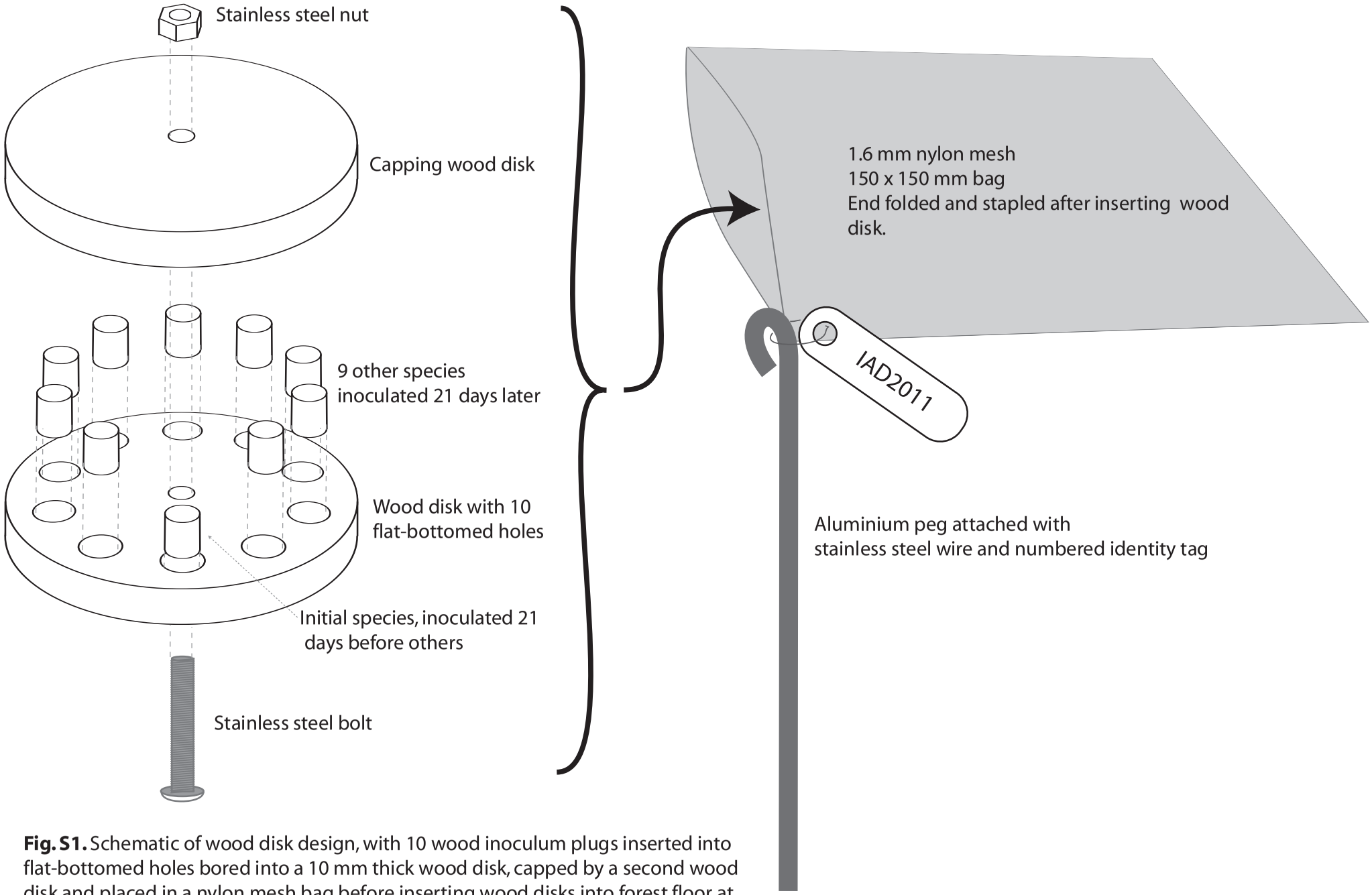

Fig. S1. Schematic of wood disk design, with 10 wood inoculum plugs inserted into flat-bottomed holes bored into a 10 mm thick wood disk, capped by a second wood disk and placed in a nylon mesh bag before inserting wood disks into forest floor at ~25 mm depth.

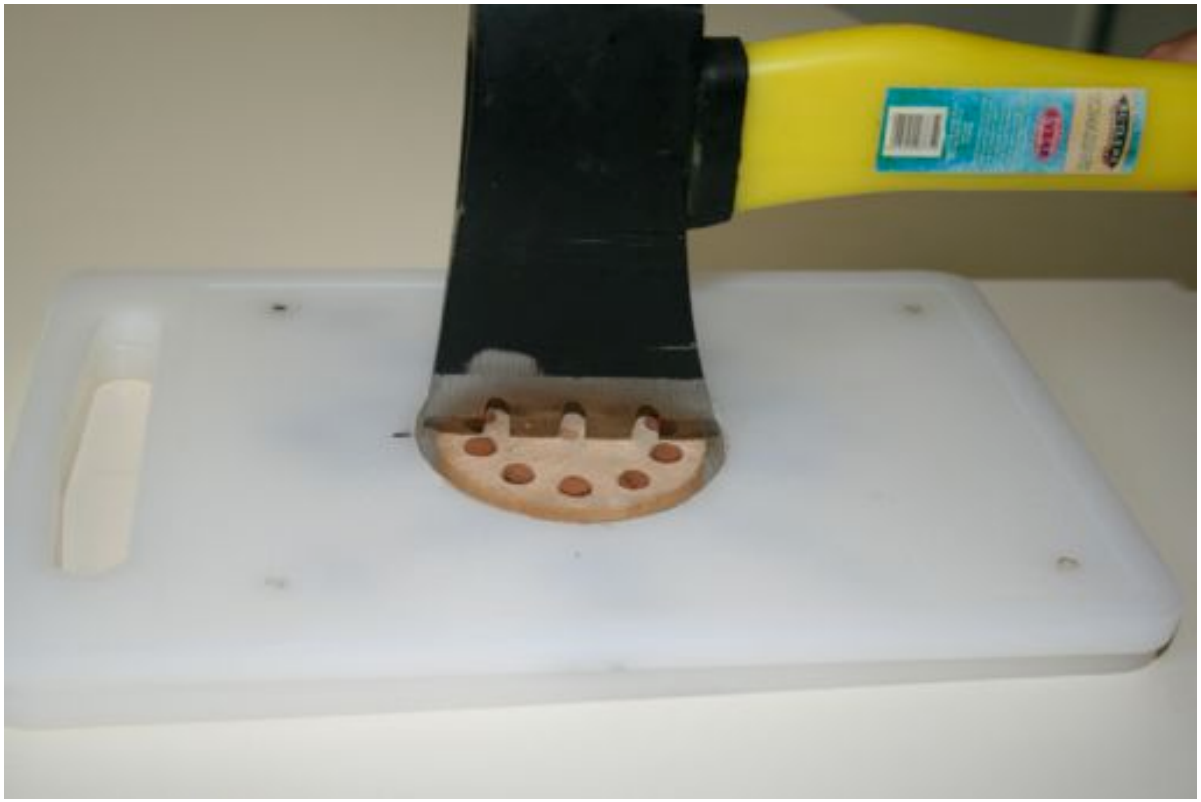

Fig. S2. To avoid carrying surface material into the interior of wood disk, we split the disk first with a triple-notched, straight-ground edge axe (A-Xenic Extractor; AXE) into two parts and then split each half radially three more times with a single-notched chisel. We then drilled eight sawdust samples from the exposed wood surface on the interior of disk underneath the notched portion of the splitting devices. The middle notch under the AXE sampler was not used for this experiment. (Note that here a non-inoculated, dry wood disk used for illustration purposes).

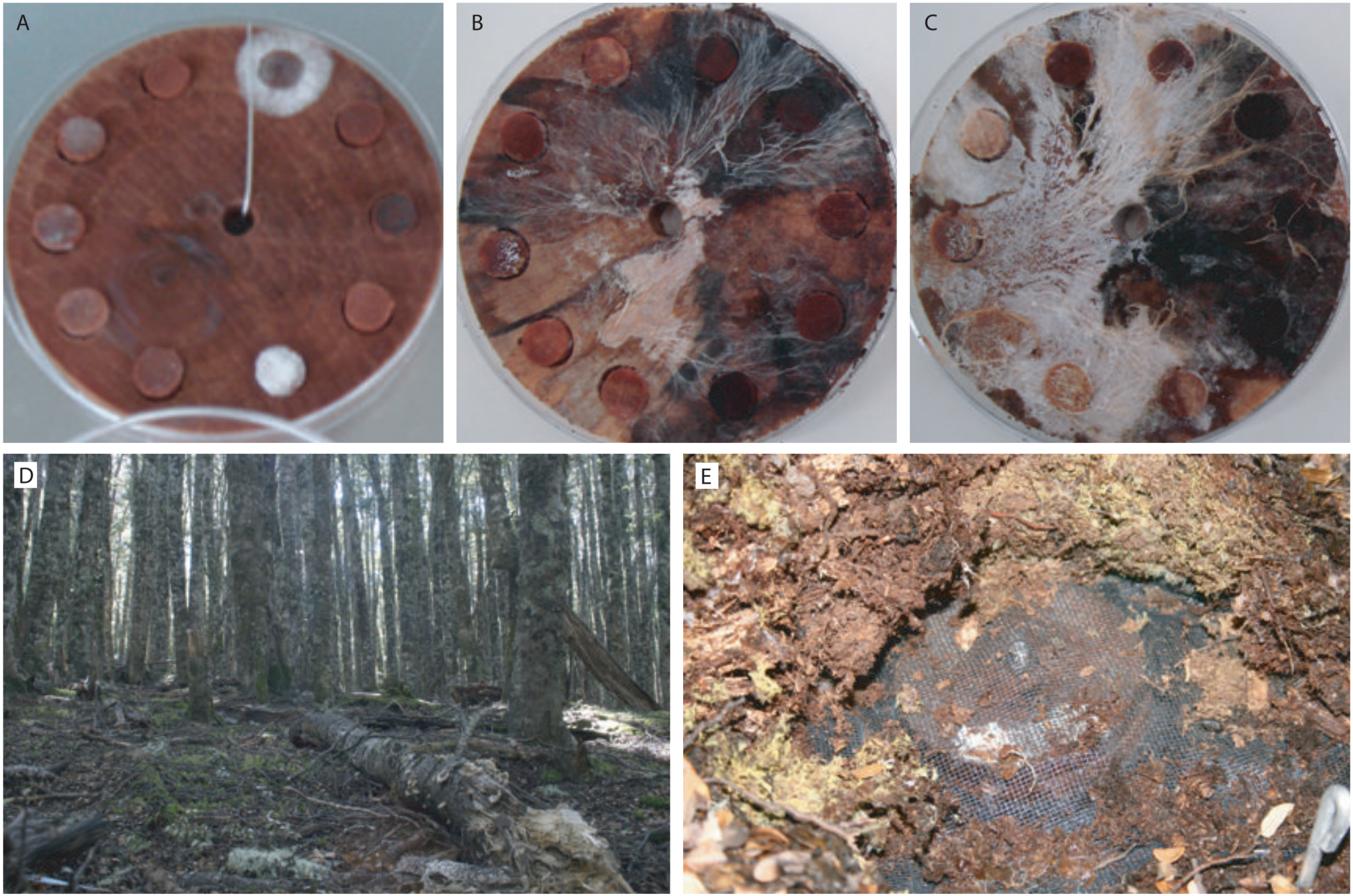

Figure S3. Images of a wood disk immediately after the second inoculation round (A), two examples after 13 months (B, C), the research site (D) and a disk emplaced in soil (E) with mesh bag and metal peg.

Table S1. R^2 and adjusted R^2 (R^2_{adj}) comparisons for ecosystem-level responses for full model (response ~ history x nitrogen) and for history and nitrogen after accounting for the other factor (history residuals ~ nitrogen; nitrogen residuals ~ history). In evaluating the relative effect sizes of history and nitrogen addition it was questioned whether the effect size of history was increased due to having seven levels, while nitrogen had only two. A common approach to comparing models is the calculation of R^2_{adj} as:

$$R^2_{adj} = 1 - \frac{\text{residual MS}}{\text{total MS}} = 1 - \frac{n-1}{n-m-1}(1-R^2)$$

where m is the model degrees of freedom (Zar 1999). Here we present the R^2 and R^2_{adj} values for the full model and four partial models, either without (N given H, H given N) or with an interaction term (N + N:H given H, H + H:N given N). The results show that the total model R^2 values are not excessively inflated (R^2 versus R^2_{adj} for full model) and that the effects of history on all three ecosystem-level response metrics are consistently comparable to (nitrogen) or larger (carbon, mass loss) than the effects of nitrogen addition even after accounting for the greater number of levels for the history treatment.

	Model:	Full model		N given H		H given N		N + N×H given H		H + H×N given N	
	Formula:	response ~ H × N		$H_{residuals}$ ~ N		$N_{residuals}$ ~ H		$H_{residuals}$ ~ N + N:H		$N_{residuals}$ ~ H + H:N	
		(m = 13)		(m = 1)		(m = 6)		(m = 7)		(m = 13)	
Response	Harvest	R^2	R^2_{adj}	R^2	R^2_{adj}	R^2	R^2_{adj}	R^2	R^2_{adj}	R^2	R^2_{adj}
Nitrogen	1	0.81	0.79	0.65	0.64	0.68	0.66	0.65	0.63	0.70	0.66
	2	0.59	0.54	0.39	0.39	0.34	0.31	0.40	0.37	0.42	0.36
Carbon	1	0.47	0.41	0.01	0.00	0.43	0.40	0.02	-0.04	0.46	0.41
	2	0.21	0.12	0.00	-0.01	0.19	0.15	0.00	-0.05	0.21	0.12
Mass loss	1	0.39	0.33	0.11	0.11	0.30	0.26	0.12	0.07	0.34	0.27
	2	0.13	0.04	0.01	0.00	0.09	0.05	0.02	-0.04	0.12	0.03