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Species richness and nitrogen supply regulate the productivity and respiration of ectomycorrhizal fungi in pure culture

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ARTICLE INFO

Article history:
Received 7 April 2011
Revision received 9 August 2011
Accepted 11 August 2011
Available online 20 October 2011
Corresponding editor:
Peter Kennedy

Keywords:
Biodiversity
Carbon:nitrogen ratio
Community ecology
Ecosystem functioning
Ectomycorrhizal fungi
Mycology
Nitrogen availability

ABSTRACT

The effects of biodiversity of aboveground organisms have been widely investigated in a range of ecosystems, yet whether similar responses are also seen in belowground microbial communities, such as ectomycorrhizal (EM) fungi, are little understood. We investigated, *in vitro*, the effects of a gradient of 1–8 species of EM fungi interacting with substratum carbon:nitrogen (C:N) ratio on biomass production and CO_2 efflux. The model experimental systems enabled us to recover and measure biomass of individuals within communities and calculate net selection and complementarity effects. Both biomass and CO_2 efflux increased with species richness particularly under high N concentrations. Moreover, net biodiversity effects were largely positive, driven by both selection and complementarity effects. Our results reveal, in pure culture, the implications of EM species richness on community productivity and C cycling, particularly under high N conditions, and constitute the basis for future experiments under natural conditions.

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Introduction

There is growing concern that reductions in biodiversity will be detrimental to ecosystem functioning (Ehrlich & Wilson 1991; Chapin III *et al.* 1997; Costanza *et al.* 1997; Vitousek *et al.* 1997), and so the effects of diversity have been investigated in a wide range of terrestrial and marine ecosystems worldwide. In many studies it has been proposed that more species-diverse ecosystems are more productive than those that support fewer species (Tilman *et al.* 1996; Engelhardt & Ritchie 2001; Hooper *et al.* 2005). Whether such biodiversity effects can also be seen in belowground microbial systems is less well understood, despite the key roles that soil microorganisms play both in belowground nutrient cycling (Finlay & Söderström 1992) and aboveground productivity and diversity (Setala & Huhta 1991; Moore *et al.* 2003; Smith & Read 2008; van der Heijden *et al.* 2008). Moreover, because the phylogenetic and physiological diversity, abundance, biomass and distribution of microorganisms are

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doi:10.1016/j.funeco.2011.08.007

considerably greater than in plants and animals, current ecological theory is likely to be of limited value if it does not apply to microbes (Prosser et al. 2007). A key challenge in ecology, therefore, is to determine if the effects of biodiversity on communities and ecosystems seen in plants and animals are also seen in soil microbial systems (Fitter 2005).

One of the most important groups of soil microbes is ectomycorrhizal (EM) fungi, which form mutualistic associations with many species of woody trees and shrubs (Smith & Read 2008). In the field EM fungi use organic carbon (C) supplied by their host plants and in turn provide the plants with mineral nutrients (Smith & Read 2008). Host plants support diverse communities of EM fungi (e.g. 15–19 species were found on individual Scots pine roots in ancient woodland in Scotland; Saari et al. 2005), and so there is considerable potential for fungi to interact in most habitats. EM fungi vary both morphologically (for example the extent of hyphal development; Agerer 2001) and functionally (Burgess et al. 1993), and so it is likely that species of EM fungi may exploit distinct niches; this is borne-out by spatial structuring of EM fungal communities (Dickie & Reich 2005; Anderson et al. 2007; Pickles et al. 2010). Whether EM fungal diversity matters for ecosystem functioning has largely been ignored. Baxter & Dighton (2001) discovered that increasing the EM diversity on *Betula populifolia* seedlings led to increased mycorrhizal root biomass as well as increased phosphorus (P) uptake by the birch seedlings. However, this experiment was confounded by limitations in experimental design (Leake 2001). Using a more sophisticated design, Jonsson et al. (2001) found that species richness of fungi colonising *Pinus sylvestris* and *Betula pendula* increased productivity, but this was apparent only under certain nutrient availabilities.

One of the key roles played by EM fungi is in regulating efflux of CO_2 from soils. EM fungi affect C fluxes directly, and it has been estimated that 20–25 % of C transferred belowground is allocated to the growth and maintenance of associated EM symbionts (Smith & Read 2008), and 25 % of the CO_2 efflux from forest soil can be attributed to EM hyphae (Heinemeyer et al. 2007). Whether CO_2 production by EM communities is dependent on their diversity is currently untested, but ecological theory would predict this to be the case because of selection effects (the presence/absence of key species driving ecosystem processes) and complementarity effects, including resource partitioning and interactions (facilitative and/or negative) which lead to increased resource use (Loreau & Hector 2001). EM fungi also have indirect effects on C cycling because the turnover of the extensive mycelial networks produced by many fungi is thought to be relatively fast (Godbold et al. 2006).

A key determinant of EM community structure and function is thought to be the availability of inorganic N. Boreal and temperate forests which are typically dominated by EM plants are characterised by low N availability and the productivity of these systems is highly dependent on N availability (Smith & Read 2008). The main input of N to such ecosystems mainly comes in the form of detrital plant matter (Read & Perez-Moreno 2003), although more recently N inputs from anthropogenic activity into the atmosphere have increased, causing declines in the sporocarp communities of certain EM species (Lilleskov et al. 2001). However, there is a great deal of interspecific variation between EM fungi in their tolerance to N availability, with some species such as *Paxillus involutus* and *Lactarius theiogalus* thriving in high N conditions and others such as species of *Cortinarius* and *Tomentella* preferring lower concentrations (Lilleskov et al. 2002, 2011). Therefore substratum C:N ratio may interact with the diversity of EM in a community to affect productivity.

Through measuring the biomass of all component species in a mixed community it is possible to calculate the net biodiversity effect (i.e. the difference between the observed yield of a mixture and its expected yield based on the performance of the component species in monoculture) and partition it into selection and complementarity effects. Positive selection effects occur when species with higher than average yields in monoculture dominate a mixed community, whereas positive complementarity effects occur when species yields in mixture are on average higher than expected based on their yield in monoculture, possibly as a result of niche differentiation and/or facilitative interactions between species (Loreau & Hector 2001). Under the "insurance hypothesis" (Yachi & Loreau 1999), having more species in a community faced with environmental pressures provides a greater guarantee that some tolerant species will maintain functioning even if others fail, which suggests that selection effects play a role in diverse communities. However, the results of some studies contradict this theory and demonstrate that complementarity effects, and in particular facilitative interaction, are what drive increases in productivity in more diverse communities facing both normal (Cardinale et al. 2002) and variable (Mulder et al. 2001) conditions.

We created a diversity gradient of 1–8 species of EM fungi in pure culture using an established design (Jonsson et al. 2001) in which all of the fungi were represented in monoculture, as well as in combinations of 2, 4 and 8 species. Our overarching hypothesis was that increased interspecific richness of EM communities will lead to increased productivity in the form of biomass production and CO_2 efflux. However, because the EM species used in this study demonstrate a range of tolerances for N availability we further predicted that the importance of interspecific diversity in regulating productivity will vary depending on the C:N ratios of the substratum, as will the effects (selection and complementarity) driving any diversity effects. With increasing N concentration, biomass production and respiration are likely to decline in species that are N intolerant, such as *Cortinarius glaucopus* (Lilleskov et al. 2001, 2011), yet in more species-rich treatments high diversity may act to maintain production due to the increased likelihood that nitrophilic species will be present in the community.

Materials and methods

A gradient of species richness was created using 8 different species of EM fungi (Table 1). Fifteen unique treatments were created of which 8 were single species monocultures (treatments A–H), 4 were mixtures of 2 species (treatments FH–BG), 2 were mixtures of 4 species (treatments ADFH and BCEG), and 1 comprised all species (treatment ALL). The 2 and 4 species mixtures were drawn at random without replacement. The experiment used individual, gas-tight 500 ml glass Kilner jars

Table 1 – Combinations of the 8 ectomycorrhizal fungal species (isolated from sporocarps) used in the experiment

Treatment identity	Species richness	Species combinations	Isolate identification code
A	1	*Cenococcum geophilum*	Ve-95-12
B	1	*Amanita muscaria*	UP3
C	1	*Lactarius rufus*	GU 98.110
D	1	*Hebeloma crustuliniforme*	UP181
E	1	*Laccaria bicolor*	Lb12
F	1	*Cortinarius glaucopus*	UP21
G	1	*Paxillus involutus*	Pax8
H	1	*Suillus bovinus*	UP63
FH	2	*C. glaucopus* + *S. bovinus*	
AD	2	*C. geophilum* + *H. crustuliniforme*	
CE	2	*L. rufus* + *L. bicolor*	
BG	2	*A. muscaria* + *P. involutus*	
ADFH	4	*C. geophilum* + *H. crustuliniforme* + *C. glaucopus* + *S. bovinus*	
BCEG	4	*A. muscaria* + *L. rufus* + *L. bicolor* + *P. involutus*	
ALL	8	All species	

containing 50 ml pH 5.5 sterile modified Melin Norkrans (MMN; Marx 1969) solid growth media covered with sterile cellophane discs. Three levels of N availability were established in the media (C:N ratios of 10:1, 20:1 and 40:1) by holding C content constant and varying N content. The MMN media therefore contained 15 g l^{-1} agar, 5 g glucose l^{-1} as the C source and 0.900 g l^{-1}, 0.450 g l^{-1} and 0.225 g l^{-1} $(NH_4)_2HPO_4$ as the N source for the 10:1, 20:1 and 40:1 C:N ratio treatments, respectively. Inoculum plugs (3 mm diameter removed from the growing margins of colonies from identical MMN media) were transferred to the cellophane-covered agar in the Kilner jars. The cellophane prevents mycelium from penetrating into the medium below, but also permits exchange of nutrients through it. Eight fungal plugs placed at random in a uniform grid comprising two outer lines of three and an inner line of 2 were used in each treatment. Each microcosm jar had approximately equal amounts of inoculum at the start of the experiment, although it is possible that a small amount of variation in hyphal density could add to variation seen in the data. There were six replicates for each treatment (total number of microcosms = 15 diversity treatments × 3 N treatments × 6 replicates = 270). Each microcosm contained a vial of 10 ml 1 M NaOH to trap evolved CO_2 (i.e. fungal respiration), and an additional series of uninoculated controls accounted for C accumulation through abiotic pathways. The microcosms were kept in the dark at 27 °C. The NaOH samples were removed approximately every 5 d for 25 d and the total amount of CO_2 produced during the experiments was determined by back-titration using a digital burette. After 25 d, the cellophane was removed from the Kilner jars and the total fungal tissue in each microcosm was scraped from it, dried, weighed and corrected for the weight of the initial inoculum.

Individual species in mixed treatment communities were easily distinguishable from one another at the end of the study period due to differences in their appearance and morphology (Fig S1); therefore despite some intermingling in some mixed treatments between hyphae of different species at the growing edges of inoculum patches, we physically separated species to the best of our ability using a scalpel and weighed them individually.

Statistical analysis

A generalized least squares (GLS) statistical mixed modelling approach was used (Bulling *et al.* 2008; Godbold *et al.* 2009; Langenheder *et al.* 2010) to account for the unequal variance imposed by the experimental design using suitable variance-covariate functions. The fixed structure of the model was established by applying backward selection using the likelihood ratio test obtained by Maximum Likelihood (ML). The numerical output of the minimal adequate model was obtained using REML estimation (West *et al.* 2007). These analyses were all performed using the 'nlme' package (ver. 3.1) in the 'R' statistical and programming environment (Pinheiro *et al.* 2006). The statistical tests used cannot be applied directly to mean values with standard errors but instead relate to model predictions; these are therefore what we present in the main paper alongside boxplots showing the spread of the raw data. However, the treatment means (±SEM) are also presented in supplementary material (Figs S1–S4). To determine if species combinations had positive effects on biomass and CO_2 efflux, we compared biomass and respiration in the species combinations relative to the best performing monocultures (transgressive overyielding (D_{max}); Trenbath 1974; Loreau 1998b). $D_{max} > 0$ if a combination mixture produces more biomass or more CO_2 than the corresponding monocultures. The experimental design enabled us to separate and weigh individual species in combination treatments at the end of the study period. We were therefore able to carry out additive partitioning of biodiversity effects as described by Loreau & Hector (2001). In brief, for each mixed treatment the net biodiversity effect is defined as the difference between the observed yield of a mixture and its expected yield based on the weighted average of the component species in monoculture. The selection effect is calculated by the covariance between species yield in monoculture and the change in relative yield when in mixture. Complementarity effects are calculated by comparing expected mixture yields of species based on monoculture yields to their observed yields; these are positive if the observed yields in mixture are on average higher than expected.

Results

Species richness, treatment identity and C:N ratio effects on biomass production

Despite a small degree of intermingling of species in certain mixed communities it was still possible to see, from the visible hyphal growth of all species throughout the study period, and confirm that all of the species in mixed treatments grew and

survived until the end of the experiment. Species richness and N concentration both had significant positive effects (L-ratio = 45.50, $p < 0.001$ and L-ratio = 25.90, $p = 0.0011$) on biomass production (Fig 1; Model 1 in Table 2). At the monoculture level, there was a marginally significant decrease in mean biomass production from 59 mg dwt in the higher N concentration treatment (i.e. the 10:1 ratio) to 45–46 mg dwt in the 20:1 and 40:1 ratios (L-ratio = −13.07, $p = 0.062$ and L-ratio = −14.44, $p = 0.041$ respectively). Moreover, the 10:1 ratio produced increasingly higher amounts of biomass as species richness increased in comparison with the other C:N ratio treatments. For example, in mixed communities containing all 8 species biomass production in the 10:1 ratio was double that in the 20:1 and 40:1 ratios (L-ratio = −34.48, $p < 0.001$, and L-ratio = −28.38, $p < 0.001$ respectively). However, the difference in biomass production between the 20:1 and 40:1 treatments was not significant (e.g. at SR = 8, L-ratio = −7.47, $p = 0.39$).

The effects of species richness on biomass production were strongly underpinned by individual treatment identity (Fig 2; Model 2 in Table 2), and the production of the species in monoculture varied greatly, with certain species producing consistently low amounts of biomass (treatments A (*Cenococcum geophilum*), F (*C. glaucopus*) and H (*Suillus bovinus*); Fig 2) and others, such as treatments C (*Lactarius rufus*), D (*Hebeloma crustuliniforme*), E (*Laccaria bicolor*) and G (*P. involutus*) producing high amounts (up to 100 mg dwt in the case of *P. involutus*). However, the performance of individual treatments was very much dependent on C:N ratio (L-ratio = 100.40, $p < 0.001$, Tables 2 and 3). For example, *P. involutus* produced more biomass than all the other fungi in monoculture when N was abundant ($p < 0.010$ for all treatments except *H. crustuliniforme* ($p = 0.020$), *L. rufus* ($p = 0.062$) and *L. bicolor* ($p = 0.193$)), but this was not the case in the 20:1 and 40:1 ratios. Here *H. crustuliniforme* (L-ratio = 40.91, $p = 0.007$, and L-ratio = 43.15, $p = 0.011$) and, marginally, *L. bicolor* (L-ratio = 19.50, $p = 0.182$, and L-ratio = 22.55, $p = 0.163$) were more productive.

Species richness, treatment identity and C:N ratio effects of CO_2 efflux

CO_2 efflux was measured every 5 d over a 25 d period, but average rates of CO_2 efflux peaked across all species and

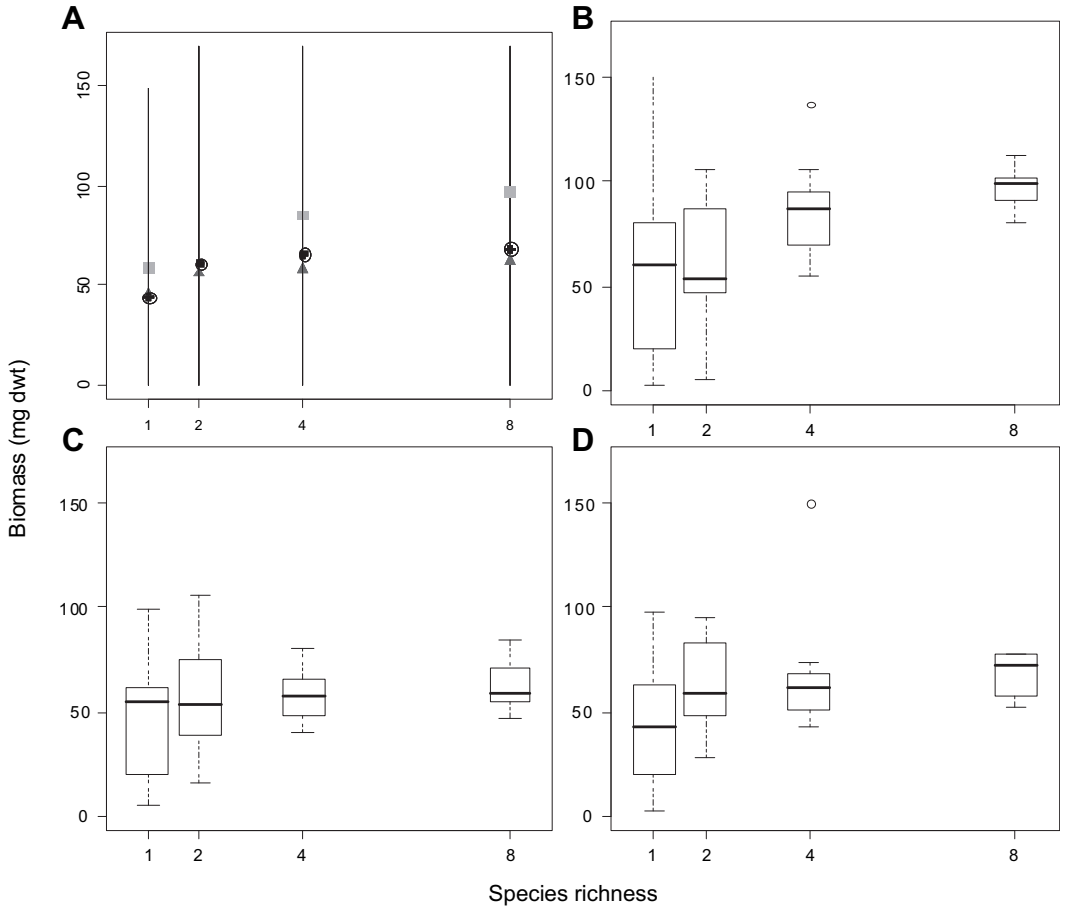

Fig 1 – The effect of species richness and its interaction with substratum C:N ratio on the biomass of fungi. In (A), symbols represent predicted values from the optimal regression model for each C:N ratio (10:1 = light grey square; 20:1 = dark grey triangle; 40:1 = black circle). Species richness was the most important factor influencing fungal biomass (L-ratio = 45.50, d.f. = 12, $p < 0.001$), followed by C:N ratio (L-ratio = 25.90, d.f. = 16, $p = 0.0011$). The spread of the raw data are shown for C:N ratios of (B) 10:1, (C) 20:1 and (D) 40:1. In the latter three plots, the horizontal bars represent predicted median values from the optimal regression model, vertical dashed lines represent the spread of the data, the upper and lower parts of the box indicate the 75 % and 25 % quartile, and circles are outlying values.

Table 2 – Summary of the statistical analyses for the 6 GLS models (Models 1–6)

	Response	Factors				Variance covariates	d.f. *p*-Value
		SR	TID	C:N ratio	2 Way interaction		
Model 1	Biomass	45.5 12 <0.001	–	25.9 16 0.0011	12.2 18 0.058	SR * C:N ratio	24 <0.001
Model 2	Biomass	319.8 48 <0.001	100.4 60 <0.001	–	98.2 62 <0.001	TID * C:N ratio	90 <0.001
Model 3	CO_2 efflux	65.3 15 <0.001	–	28.0 16 <0.001	12.3 18 0.055	SR * C:N ratio	24 <0.001
Model 4	CO_2 efflux	407.4 48 <0.001	–	139.0 60 <0.001	114.7 62 <0.001	TID * C:N ratio	90 <0.001
Model 5	D_{max} Biomass	–	–	5.8 10 0.055	–	SR * C:N ratio	12 <0.001
Model 6	D_{max} CO_2 efflux	30.6 12 <0.001	–	11.9 12 0.06	11.4 14 0.023	TID * C:N ratio	18 <0.001

For each of the four factors, L-ratio, d.f., and *p*-value are presented sequentially in each cell. '–' Indicates that a factor had no significant effect. SR = species richness; TID = treatment identity (i.e. the 15 unique populations within a given C:N ratio). See Methods and Supporting information for details of models.

species combinations (with the exception of treatment H (*S. bovinus*) in the 10:1 ratio) at 20 d (Fig S2) so data were analysed statistically from this time point. Species richness was the main factor driving CO_2 efflux (Model 3 in Table 2, Fig 3; L-ratio = 65.26, $p < 0.001$) with higher levels of CO_2 produced in more species-rich systems, although higher N availability also led to significant increases in CO_2 efflux (L-ratio = 28.04, $p = 0.0005$) between the 10:1 ratio and the 20:1 and 40:1 ratios. As seen previously with biomass production, the difference in CO_2 efflux between the 10:1 ratio and the other two treatments expands with increasing species richness level (for example, the 40:1 ratio at SR = 1: L-ratio = −1.71, $p = 0.037$, and at SR = 8: L-ratio = −3.25, $p = 0.017$).

The strong effects of species richness on CO_2 efflux were also underpinned by the effects of the individual communities (L-ratio = 407.38, $p < 0.001$) interacting with C:N ratio (Fig 4, Model 4 in Table 2; L-ratio = 139.01, $p < 0.001$). There was a preference of species in monoculture towards certain C:N ratios (Table 3). Treatment G (*P. involutus*) produced higher levels of CO_2 in the 10:1 ratio (20:1: L-ratio = −4.49, $p < 0.001$, 40:1: L-ratio = −5.03, $p < 0.001$), and treatment H (*S. bovinus*) at 40:1 (10:1: L-ratio = −2.72, $p = 0.018$, 20:1: L-ratio = −0.72, $p = 0.502$), reflecting patterns in biomass production. However, the optimum C:N ratios for CO_2 efflux did not necessarily mirror those of biomass production in all treatments. For example, treatment C (*L. rufus*) produced more CO_2 at 20:1 (10:1:

Table 3 – C:N ranked in order of importance in terms of biomass production and CO_2 efflux of the 15 unique fungal species combinations

C:N ratio ranking	Treatment														
	A	B	C	D	E	F	G	H	FH	AD	CE	BG	ADFH	BCEG	ALL
Biomass															
1	20:1	20:1	10:1	10:1	10:1	40:1	10:1	40:1	40:1	40:1	10:1	20:1	40:1	10:1	10:1
2	40:1	10:1	20:1	40:1	40:1	20:1	40:1	10:1	10:1	10:1	20:1	10:1	10:1	20:1	40:1
3	10:1	40:1	40:1	20:1	20:1	10:1	20:1	20:1	20:1	20:1	40:1	40:1	20:1	40:1	20:1
CO_2 efflux															
1	20:1	10:1	20:1	10:1	10:1	40:1	10:1	40:1	10:1	10:1	10:1	20:1	10:1	10:1	10:1
2	10:1	20:1	10:1	40:1	20:1	20:1	20:1	10:1	40:1	40:1	20:1	10:1	20:1	20:1	20:1
3	40:1	40:1	40:1	20:1	40:1	10:1	40:1	20:1	20:1	20:1	40:1	40:1	40:1	40:1	40:1

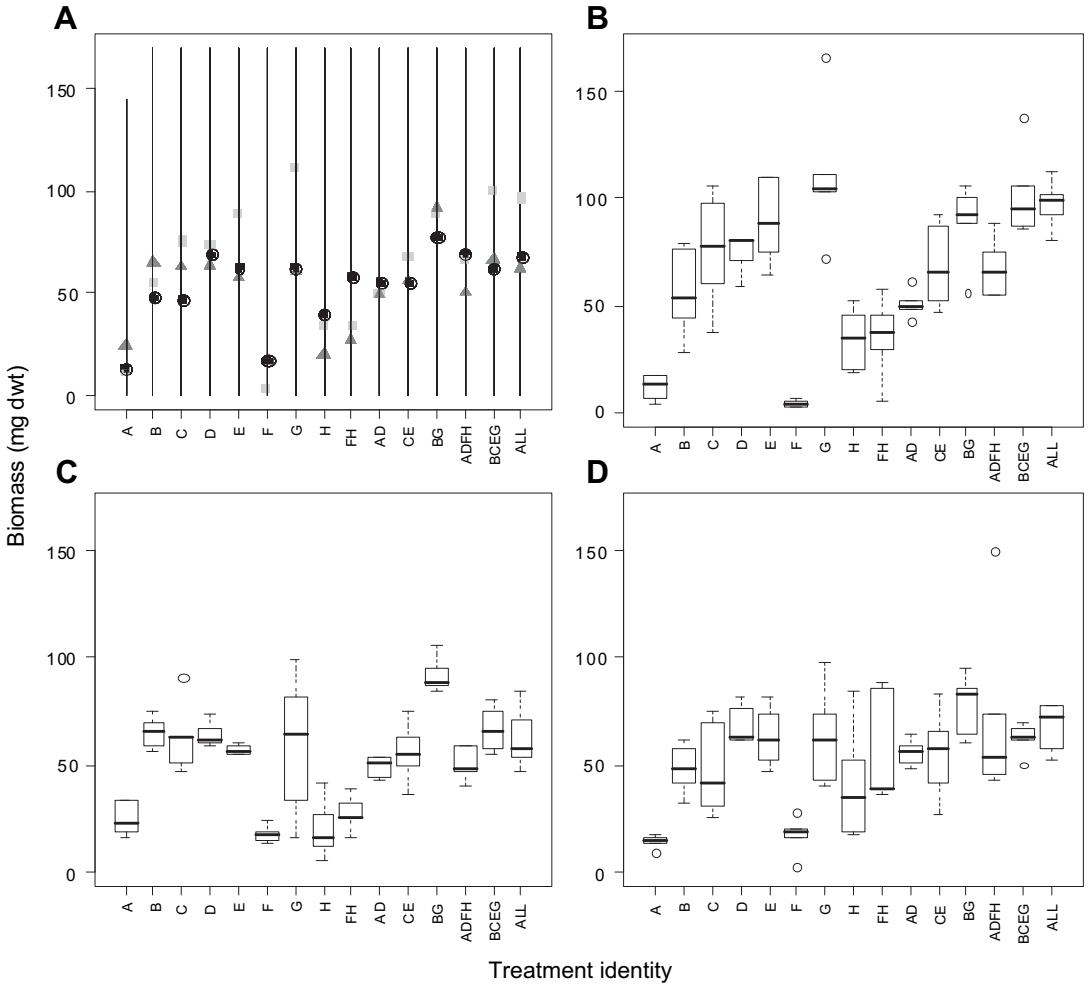

Fig 2 – The effect of treatment identity (i.e. 15 unique fungal assemblages) and its interaction with substratum C:N ratio on the biomass of fungal assemblages. In (A), the symbols are the predicted values from the optimal regression model for each of the 15 unique fungal populations for each C:N ratio (10:1 = light grey square; 20:1 = dark grey triangle; 40:1 = black circle). Each letter (A–H) represents an individual species and combinations describe the particular diversity treatments (Table 1). Treatment identity was the most important factor influencing fungal biomass (L-ratio = 319.80, d.f. = 48, $p < 0.001$) followed by C:N ratio (L-ratio = 100.40, d.f. = 60, $p < 0.001$). The spread of the raw data (lines interpreted as for Fig 1) are shown for C:N ratios of (B) 10:1, (C) 20:1 and (D) 40:1.

L-ratio = −2.35, $p = 0.061$, 40:1: L-ratio = −4.45, $p < 0.001$) but had greater biomass at 10:1 (20:1: L-ratio = −25.93, $p = 0.064$, 40:1: L-ratio = −31.09, $p = 0.028$).

Transgressive overyielding (D_{max}) and the net biodiversity effect

Transgressive overyielding (D_{max}) was calculated in order to compare the yields (biomass and CO_2 efflux) of mixed EM communities to those of the highest yielding replicate component species in monoculture. This gives an indication of whether a mixed community is likely to produce more biomass or CO_2 than a monoculture of its most productive species. Both biomass production (Model 5 in Table 2) and CO_2 efflux (Model 6 in Table 2; Fig 5) in mixed community treatments were lower than the highest performing component species at all species richness levels. Transgressive overyielding of biomass production in mixed communities was not affected by species richness (L-ratio = 2.80, $p = 0.246$), but D_{max} values were significantly lower in the 10:1 ratio compared to the 20:1 ($t = 0.073$, $p = 0.027$) and 40:1 ratios ($t = 0.096$, $p = 0.014$). CO_2 production of mixed communities relative to their highest performing monocultures was significantly affected by the species richness of the community (L-ratio = 30.57, $p < 0.001$), and although communities underyielded compared to their highest performing component species, this was less pronounced in more diverse communities (up to 4 species). Transgressive overyielding was also significantly affected by the C:N ratio of the treatment substratum (L-ratio = 11.94, $p = 0.006$); whether this was positive or negative depended on the species richness of the treatment. In mixed communities consisting of 4 or 8 species, underyielding was less pronounced at higher N

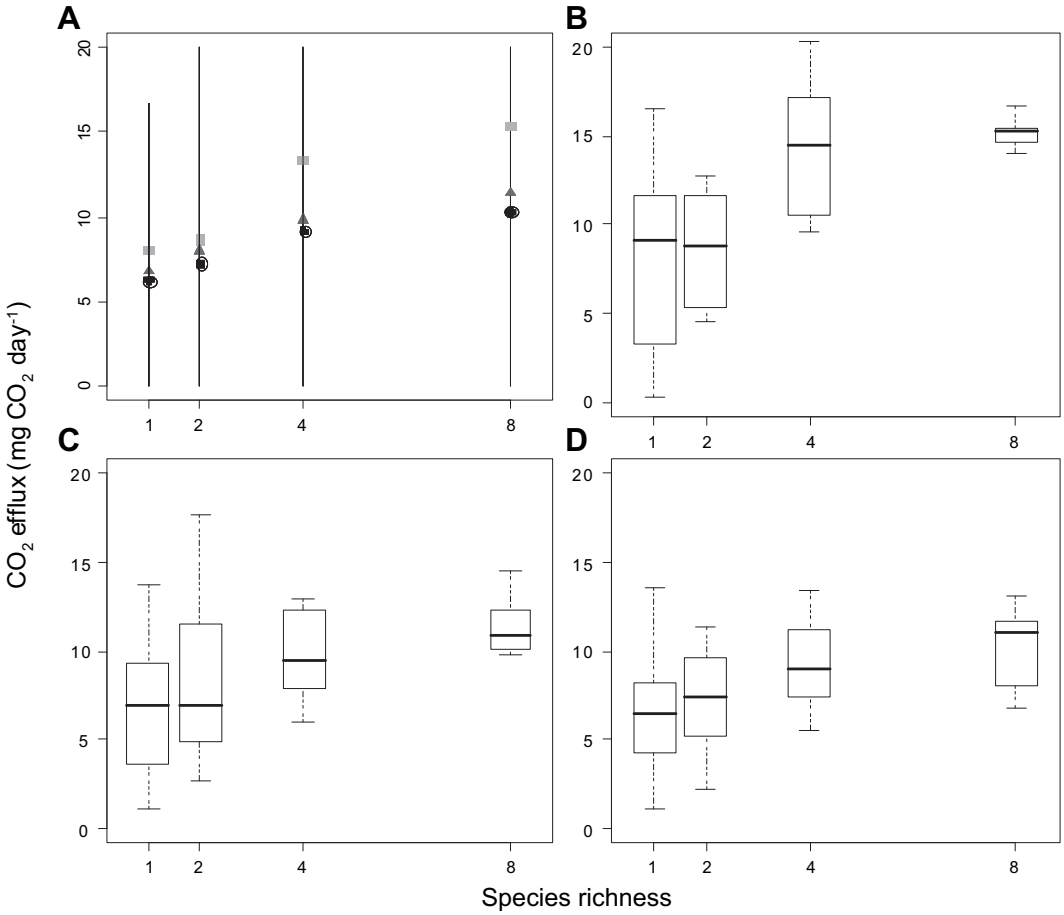

Fig 3 – The effect of fungal species richness on CO_2 efflux (mg CO_2 d^{-1}). In (A), horizontal bars represent predicted values from the optimal regression model for each C:N ratio (10:1 = light grey square; 20:1 = dark grey triangle; 40:1 = black circle). Species richness was the most important factor influencing CO_2 efflux (L-ratio = 65.26, d.f. = 15, $p < 0.001$), followed by C:N ratio (L-ratio = 28.04, d.f. = 16, $p = 0.0005$). The spread of the raw data are shown for C:N ratios of (B) 10:1, (C) 20:1 and (D) 40:1.

concentrations. However, in the 2-species mixtures underyielding was lower at low N concentrations.

Because we were able to calculate the biomass of the component species of mixed treatments, it was possible to partition the net biodiversity effect (i.e. the difference between the observed yield of a mixture compared with its expected yield based on the performance of all of the component species in monoculture) into selection and complementarity effects (Fig 6). All but two of the twenty-one mixed treatments exceeded their expected biomass yields (based on the performance of the component species in monoculture), leading to high net biodiversity effects in the case of the 4 species (ADFH) and 8 species mixture treatments in the 10:1 ratio. Observed responses in the mixed treatments were driven by positive complementarity effects and some smaller effects of both positive and negative selection. There was a noticeable effect of species richness on the net effect in the 10:1 ratio, but less so in the other ratios. In fact, the greatest net effect seen in the entire study was in the 8 species community in the 10:1 C:N substratum ratio, and this was driven by complementarity effects (facilitative interactions/niche differentiations). The size of the net effect and its cause also varied in treatments between the different C:N ratios. For example, in the 10:1 ratio, treatment BG (*Amanita muscaria* and *P. involutus*) had a small net effect caused by a prevailing selection effect, but this was counteracted by a negative complementarity effect. However in the 20:1 ratio, the net effect trebled in size, this time driven largely by complementarity effects with a very small selection effect caused by an underperforming species. In the 40:1 C:N treatment the large positive net effect was mostly due to positive complementarity effects, but also by a small positive effect of species dominance.

Discussion

The effects of species richness and treatment identity on productivity

Our data demonstrate that species richness positively affects both biomass production and CO_2 efflux of EM fungi and supports our overarching hypothesis. Underpinning the effects of species richness substrate C:N ratio interactions were strong effects of individual communities, which also

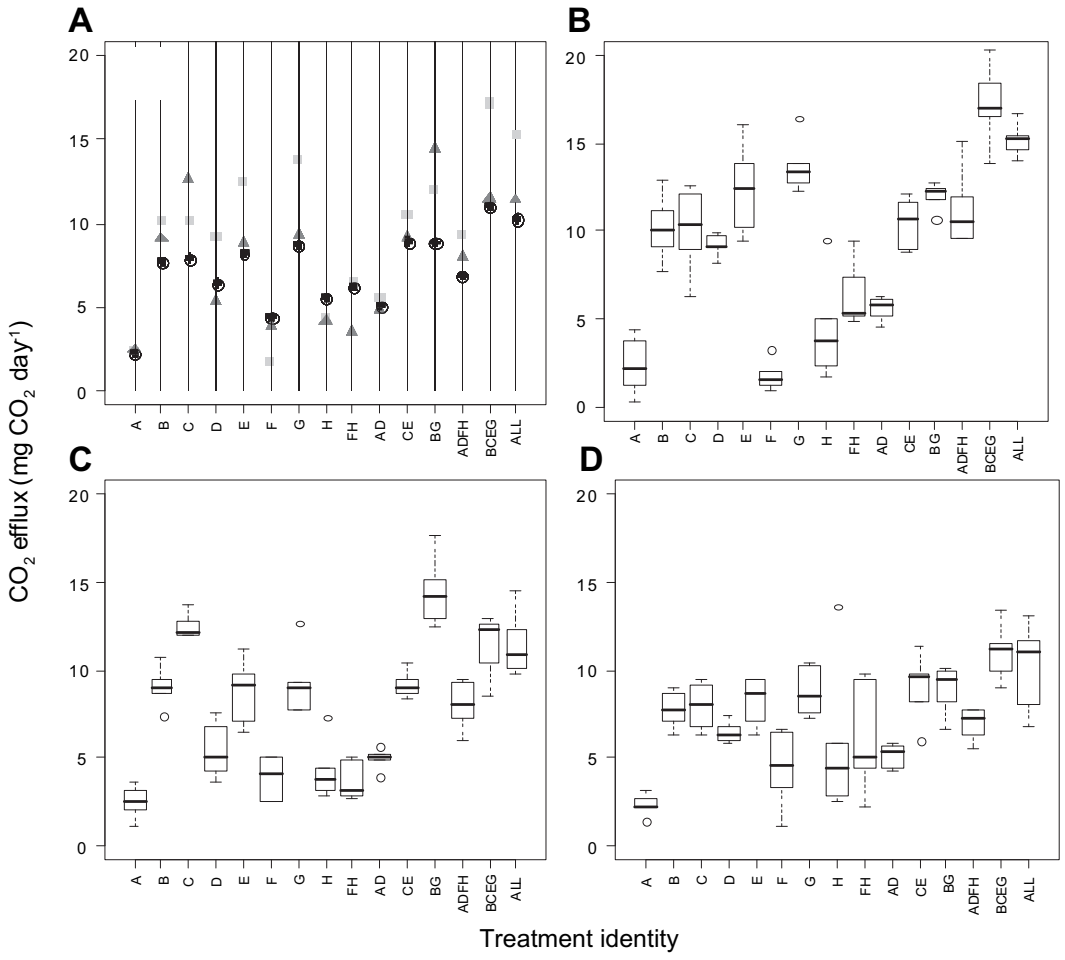

Fig 4 – The effect of (A) treatment identity (i.e. 15 unique fungal assemblages) and its interaction with substratum C:N ratio on fungal CO_2 efflux. In (A), the symbols are the predicted values from the optimal regression model for each of the 15 unique fungal populations for each C:N ratio (10:1 = light grey square; 20:1 = dark grey triangle; 40:1 = black circle). Each letter (A–H) represents an individual species and combinations describe the particular diversity treatments (Table 1). Treatment identity was the most important factor influencing CO_2 efflux (L-ratio = 407.38, d.f. = 48, $p < 0.001$) followed by C:N ratio (L-ratio = 139.01, d.f. = 60, $p < 0.001$). The spread of the raw data (lines interpreted as for Fig 1) are shown for C:N ratios of (B) 10:1, (C) 20:1 and (D) 40:1.

interacted with substratum N concentration. Species varied in their ability to produce biomass at different C:N ratios, and this finding supports previous field observations (Lilleskov *et al.* 2001) where species were classified as 'nitrophobic' (e.g. *Cortinarius* and *Piloderma* genera) or 'nitrophilic' (such as *P. involutus*) depending on their change in abundance over a N deposition gradient. Many nitrophilic fungi tend to be adapted to high inorganic nutrient and pH conditions and in the field they often rapidly colonise roots in conditions where inorganic nutrients are plentiful, such as former agricultural sites (Visser 1995). Nitrophobic species such as *Cortinarius* spp. are more typical of later-stage forests (Visser 1995) where inorganic N concentrations are low (Van Cleve & Viereck 1981). However, species that favour different nutrient conditions may coexist, although the fungi that are poorly adapted to the prevailing environmental conditions often form part of the larger number of 'rare' species typically found in communities (Erland & Taylor 2002).

In our study *P. involutus* and *Lactarius* sp. showed preferred growth when N was abundant, but *Cortinarius* sp. did not grow very well in the same conditions, and this is in line with observations from the field (Lilleskov *et al.* 2001). Because increases in biomass and CO_2 efflux with increasing diversity were more pronounced in substrata with high N concentrations, it is possible that facilitative interactions between different fungi and dominance by species well adapted to inorganic N utilisation may have acted to maintain productivity in the species-rich communities where the substratum C:N ratio was least.

Certain species differed in their optimum C:N ratio for biomass production and CO_2 efflux. For example, *A. muscaria* produced more biomass at mid-range N concentrations, but respired more at high N concentrations. It has been suggested that increasing levels of N availability place more demand on the fungus to obtain carbohydrate in order to assimilate the N (as NH_4^+), thus causing a reduction in biomass and higher

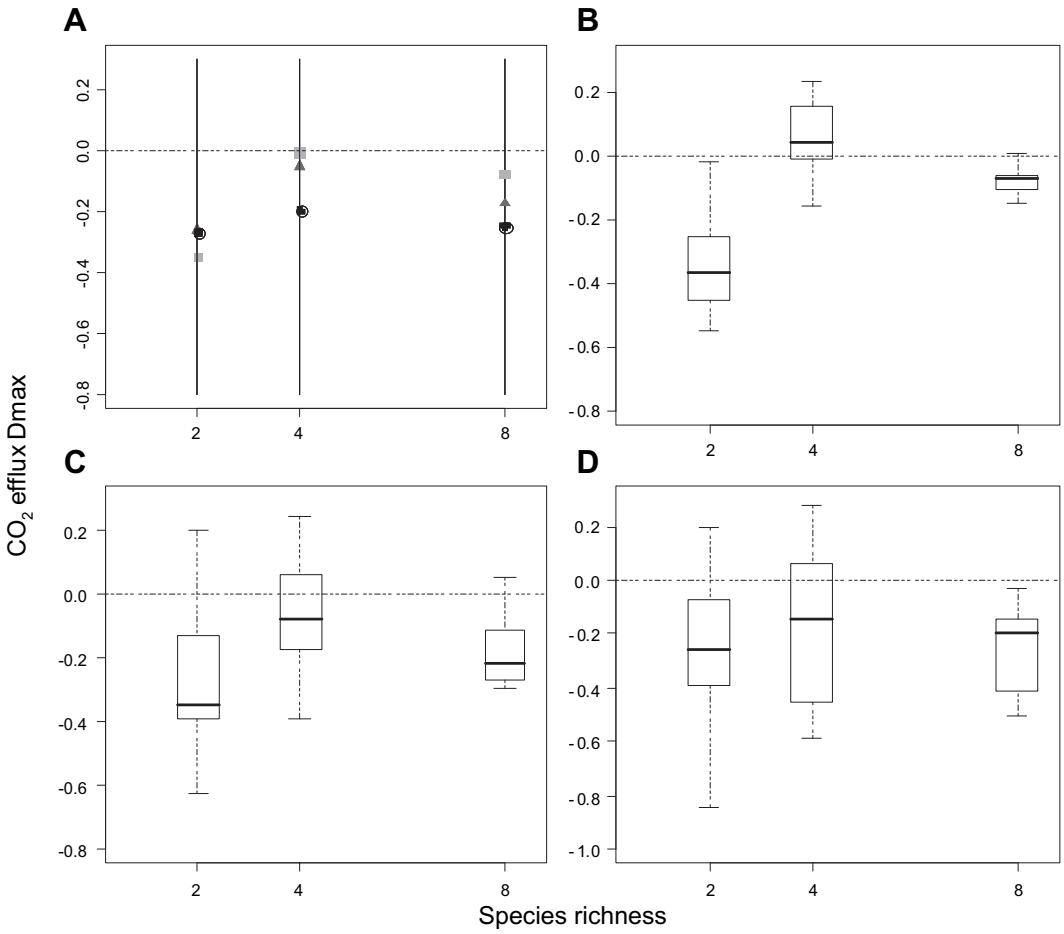

Fig 5 – Transgressive overyielding of CO_2 efflux in response to species richness and substratum C:N ratio (10:1 = light grey square; 20:1 = dark grey triangle; 40:1 = black circle) based on (A) predicted values from the optimal regression model. Species richness was the most important factor (L-ratio = 30.57, d.f. = 12, $p < 0.001$), followed by C:N ratio (L-ratio = 11.94, d.f. = 12, $p = 0.06$). The spread of the raw data for each level of species richness are shown for C:N ratios of (B) 10:1, (C) 20:1 and (D) 40:1. Interpretation of bars and lines follows Fig 1.

energy expenditure that lead to increases in CO_2 efflux (Wallenda & Kottke 1998). We also found that other species such as *L. rufus* (treatment C) showed the opposite trend, producing more biomass at high N concentrations and elevated respiration levels under lower N concentrations, possibly because this species is more adapted to utilising labile N forms and must expend more energy acquiring N when concentrations are lower. These results support recent claims that EM species with contact, short-distance and medium-distance smooth explorative strategies that produce few emanating hyphae, such as *L. rufus*, are better adapted to utilising labile N forms under high N conditions due to their lower C requirements (Lilleskov *et al.* 2011).

Transgressive overyielding (D_{max}) and the net biodiversity effect in biomass production

Overall our mixed treatments produced less biomass compared to the single highest performing counterpart in monoculture. Similar results were obtained by Janzen *et al.* (1995) and Hedlund & Öhrn (2000) with soil basidiomycetes. They found that litter decomposition by 2 or 3 species mixtures did not exceed the best performing monocultures. However, in the case of CO_2 efflux in our study, underyielding did become less pronounced with species richness (up to 4 species), indicating that increasing complementarity effects could be taking place at higher species richness levels.

Partitioning of net biodiversity effects in biomass production revealed evidence of strong positive complementarity effects occurring in mixed communities, as well as some positive and negative selection effects. It is thought that species complementarity effects are more likely to play important roles in soil microbial community function due to the complexity and heterogeneity of most soils (Tilman *et al.* 1997) and the intricate biochemical pathways operating in this environment (Tilman *et al.* 1997; Loreau 1998a). Tiunov & Scheu (2005) found that both sampling and complementarity effects contributed to higher rates of organic matter decomposition brought about by increasing saprotrophic fungal community diversity. However, they found that complementarity effects were more pronounced in substrata where cellulose availability was homogenous as opposed to complex forest soil, indicating that

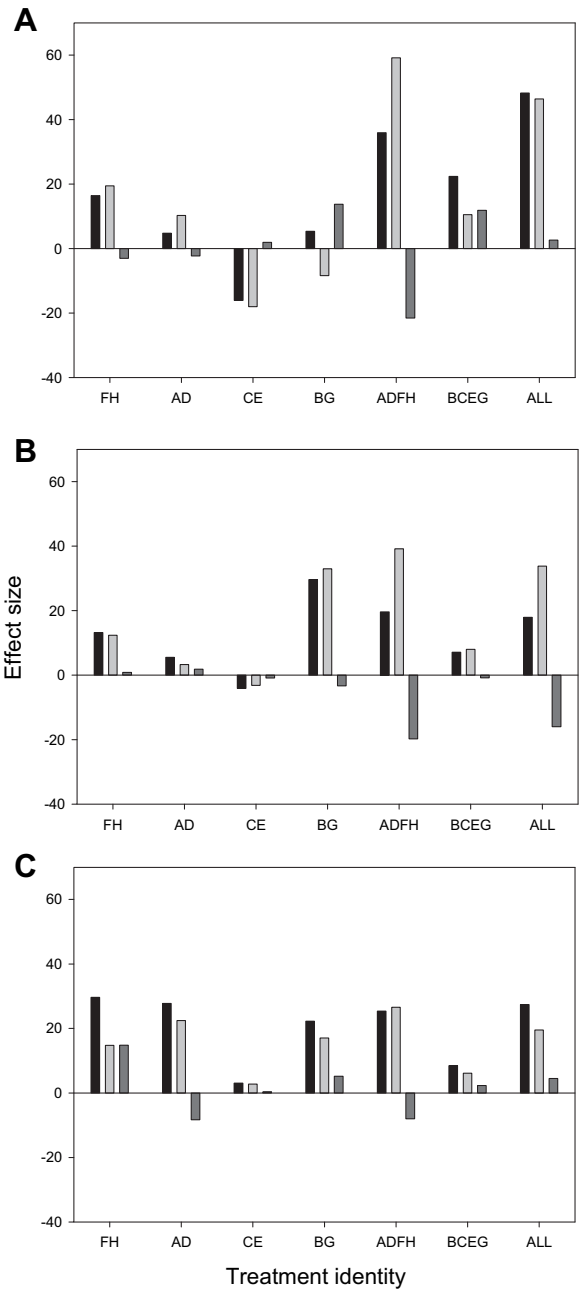

Fig 6 – The net biodiversity effect in C:N ratio treatments (A) 10:1, (B) 20:1 and (C) 40:1 partitioned into complementarity and selection effects. For each treatment, the black vertical bars represent the net effect, the light grey bars represent the complementarity effect and the dark grey bar represents the selection effect.

facilitative interactions, rather than resource partitioning, were driving the community response. Our results provide evidence that, at least with homogenous substrata, species complementarity may be an important driver of biomass production in species-rich fungal communities. Even with the initially simple substrata in our experiment, complementarity effects may occur through time as fungi release more complex secondary compounds like organic acids, which may open up new niches. However, whether these complementarity effects are dampened or even enhanced by substratum complexity in the field remains untested.

Biomass production increased with species richness when N was abundant, but species richness had less of an effect when N was less concentrated. Complementarity effects also increased with species richness under high N conditions, and it is likely that the observed increase in biomass production with community richness was caused in part by increased facilitative interactions between nitrophilic and nitrophobic species in a N regime which is not favourable to a proportion of the community. This contradicts the insurance hypothesis, which predicts biomass increases in a community under stressful conditions as a result of an increase in biomass of the most resistant species (Walker 1992; Lawton & Brown 1993; Naeem 1998; Yachi & Loreau 1999). In our study, there were dominant nitrophilic species, such as *P. involutus*, in the high diversity mixture. However, the yields of the mixtures were on average greater than the expected yield based on the performance of individual species in monoculture, suggesting positive complementarity. In addition *S. bovinus*, a known nitrophobic species, contributed to the very high biomass in mixture ADFH when the substratum C:N was 10:1, which suggests facilitative interactions were taking place. It is possible that nitrophilic species such as *P. involutus* with a high capacity to assimilate ammonium may rapidly reduce the concentration of substratum N, making conditions more tolerable to nitrophobic species. Such complementarity effects may be seen in recently disturbed forest stands or forest developing on old agricultural land; nitrophilic species may rapidly colonise patches where mineral N concentrations will be high, but with little or no influx of new mineral N, concentrations will be lowered, allowing for colonisation of species that are better adapted to utilising organic N forms.

Conclusions

Species richness of fungal communities has already been suggested to regulate aboveground productivity of the host communities (Baxter & Dighton 2001; Jonsson *et al.* 2001). Our data suggest, in pure culture, that the species richness of EM communities also plays important roles in the productivity of the fungi themselves, although this is dependent on the species composition of the communities and the availability of N.

Due to the complexity of EM fungi in their natural environment (i.e. interactions with host plant, homogeneity of substratum, temporal dynamics, interactions with other organisms) it was necessary to simplify our microcosm conditions in order to reduce confounding factors. We have provided a conservative test for the effects of EM biodiversity on productivity, however further examination of factors such as substratum complexity and the presence of a host partner is required to fully understand how EM diversity effects on productivity operate in the field. In contrast to the mineral growth media used in this study, C and N are mostly present in complex organic forms in forests and require enzyme degradation prior to uptake by EM fungi. Thus it is most likely

that facilitative interactions and niche differentiation will play a significant role in driving ecosystem functioning under these conditions. If, as our data suggest, facilitation takes place to increase biomass production in less tolerable conditions (for example, high N availability), more diverse communities should be more productive than less diverse communities or monocultures in the face of the dynamic and heterogeneous conditions found in nature.

Acknowledgements

We thank J Brodie for technical support, the Natural Environment Research Council for providing a studentship to AW, and Dr AFS Taylor for providing some of the fungal isolates.

Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.funeco.2011.08.007.

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