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

Anna WILKINSON\textsuperscript{a,c,*}, Martin SOLAN\textsuperscript{b}, Ian ALEXANDER\textsuperscript{a}, David JOHNSON\textsuperscript{a}

\textsuperscript{a}Institute of Biological and Environmental Sciences, Cruickshank Building, University of Aberdeen, Aberdeen AB24 3UU, UK
\textsuperscript{b}Oceanlab, University of Aberdeen, Main Street, Newburgh, Aberdeenshire AB41 6AA, UK
\textsuperscript{c}Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK

ASS A B R A C T

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\textsubscript{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\textsubscript{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.

© 2011 Elsevier Ltd and The British Mycological Society. Open access under CC BY license.

doi:10.1016/j.funeco.2011.08.007

Introduction

There is growing concern that reductions in biodiversity will be detrimental to ecosystem functioning (Ehrlich & Wilson 1991; Chapin III \textit{et al.} 1997; Costanza \textit{et al.} 1997; Vitousek \textit{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 \textit{et al.} 1996; Engelhardt & Ritchie 2001; Hooper \textit{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 \textit{et al.} 2003; Smith & Read 2008; van der Heijden \textit{et al.} 2008). Moreover, because the phylogenetic and physiological diversity, abundance, biomass and distribution of microorganisms are...
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 CO₂ 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 CO₂ efflux from forest soil can be attributed to EM hyphae (Heinemeyer et al. 2007). Whether CO₂ 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 CO₂ 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                       |                             |

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 CO2 efflux, we compared biomass and respiration in the species combinations relative to the best performing monocultures (transgressive overyielding (Dmax); Trenbath 1974; Loreau 1998b). Dmax > 0 if a combination mixture produces more biomass or more CO2 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 CO2 efflux

CO2 efflux was measured every 5 d over a 25 d period, but average rates of CO2 efflux peaked across all species and

![Fig 1](image-url)  
**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.
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 CO2 efflux (Model 3 in Table 2, Fig 3; L-ratio = 65.26, \( p < 0.001 \)) with higher levels of CO2 produced in more species-rich systems, although higher N availability also led to significant increases in CO2 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 CO2 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 = 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 CO2 efflux did not necessarily mirror those of biomass production in all treatments. For example, treatment C (L. rufus) produced more CO2 at 20:1 (10:1: 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 CO2 efflux did not necessarily mirror those of biomass production in all treatments. For example, treatment C (L. rufus) produced more CO2 at 20:1 (10:1:

The strong effects of species richness on CO2 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 CO2 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 CO2 efflux did not necessarily mirror those of biomass production in all treatments. For example, treatment C (L. rufus) produced more CO2 at 20:1 (10:1:

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

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                 | 25.9 | 12.2    | SR * C:N ratio    | 24      | <0.001             |      |         |                       |
|          |         | 12                   | 16   | 18      |                      |         |                     |      |         |                       |
|          |         | <0.001               | 0.0011| 0.058   |                      |         |                     |      |         |                       |
| Model 2  | Biomass | 319.8                | 100.4| 98.2    | TID * C:N ratio     | 90      | <0.001             |      |         |                       |
|          |         | 48                   | 60   | 62      |                      |         |                     |      |         |                       |
|          |         | <0.001               | <0.001| <0.001  |                      |         |                     |      |         |                       |
| Model 3  | CO2 efflux | 65.3               | 28.0 | 12.3    | SR * C:N ratio      | 24      | <0.001             |      |         |                       |
|          |         | 15                   | 16   | 18      |                      |         |                     |      |         |                       |
|          |         | <0.001               | <0.001| 0.055   |                      |         |                     |      |         |                       |
| Model 4  | CO2 efflux | 407.4              | 139.0| 114.7   | TID * C:N ratio     | 90      | <0.001             |      |         |                       |
|          |         | 48                   | 60   | 62      |                      |         |                     |      |         |                       |
|          |         | <0.001               | <0.001| <0.001  |                      |         |                     |      |         |                       |
| Model 5  | Dmax Biomass | 5.8               | 5.8  | 11.4    | TID * C:N ratio      | 12      | <0.001             |      |         |                       |
|          |         | 10                   | 10   | 14      |                      |         |                     |      |         |                       |
|          |         | 0.055                | 0.055| 0.023   |                      |         |                     |      |         |                       |
| Model 6  | Dmax CO2 efflux | 30.6             | 11.9 | 11.4    | TID * C:N ratio      | 18      | <0.001             |      |         |                       |
|          |         | 12                   | 12   | 14      |                      |         |                     |      |         |                       |
|          |         | <0.001               | 0.06  | 0.23    |                      |         |                     |      |         |                       |

For each of the four factors, L-ratio, d.f., and p-value are presented sequentially in each cell. ‘e’' 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.

Table 3 – C:N ranked in order of importance in terms of biomass production and CO2 efflux of the 15 unique fungal species combinations

| C:N ratio ranking | Treatment |
|-------------------|-----------|
| Biomass           | A | B | C | D | E | F | G | H | FH | AD | CE | BG | ADFH | BCEG | ALL |
| 1                 | 20:1 | 20:1 | 10:1 | 10:1 | 40:1 | 10:1 | 40:1 | 40:1 | 40:1 | 10:1 | 20:1 | 40:1 | 10:1 | 10:1 | 10:1 |
| 2                 | 40:1 | 10:1 | 20:1 | 40:1 | 40:1 | 20:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 |
| 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 | 20:1 | 20:1 | 20:1 |
| CO2 efflux        | A | B | C | D | E | F | G | H | FH | AD | CE | BG | ADFH | BCEG | ALL |
| 1                 | 20:1 | 10:1 | 10:1 | 10:1 | 40:1 | 10:1 | 40:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 | 10:1 |
| 2                 | 10:1 | 20:1 | 10:1 | 40:1 | 20:1 | 20:1 | 10:1 | 40:1 | 40:1 | 20:1 | 20:1 | 20:1 | 40:1 | 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 | 40:1 |
Transgressive overyielding ($D_{\text{max}}$) and the net biodiversity effect

Transgressive overyielding ($D_{\text{max}}$) 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 $D_{\text{max}}$ 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...
concentrations. However, in the 2-species mixtures under-
yielding 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 perfor-
mance 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 CO2 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
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 CO₂ 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 CO₂ 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 NH₄⁺), thus causing a reduction in biomass and higher

---

**Fig 4** – The effect of (A) treatment identity (i.e. 15 unique fungal assemblages) and its interaction with substratum C:N ratio on fungal CO₂ 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 CO₂ 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.
energy expenditure that lead to increases in CO\textsubscript{2} efflux (Wallenda & Kottke 1998). We also found that other species such as \textit{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 \textit{L. rufus}, are better adapted to utilising labile N forms under high N conditions due to their lower C requirements (Lilleskov et al. 2011).

\textbf{Transgressive overyielding (D\textsubscript{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\textsubscript{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 & Schu (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 5 – Transgressive overyielding of CO\textsubscript{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.
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 damped 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.

References

Aeger R, 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11: 107–114.

Anderson IC, Bastias BA, Genney DR, Parkin PI, Cairney JWG, 2007. Basidiomycete fungal communities in Australian sclerophyll forest soil are altered by repeated prescribed burning. Mycological Research 111: 482–486.

Baxter JW, Dighton J, 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (Betula populifolia) seedlings in host-symbiont culture conditions. New Phytologist 152: 139–149.

Bulling MT, Solan M, Dyson KE, Hernandez-Milian G, Luque P, Pierce GJ, Raffaelli D, Paterson DM, White PCI, 2008. Species effects on ecosystem processes are modified by faunal responses to habitat composition. Oecologia 158: 511–520.

Burgess TJ, Malajczuk N, Grove TS, 1993. The ability of 16 ectomycorrhizal fungi to increase growth and phosphorus uptake of Eucalyptus globulus Labill. and E. diversicolor F. Muell. Plant and Soil 153: 155–164.

Cardinale BJ, Palmer MA, Collins SL, 2002. Species diversity enhances ecosystem functioning through interspecífic facilitation. Nature 415: 426–429.

Chapin III FS, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D, 1997. Biotic control over the functioning of ecosystems. Science 270: 500–504.

Costanza R, D’Arge R, De Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O’Neill RV, Paruelo J, Raskin RG, Sutton P, Van Den Belt M, 1997. The value of the world’s ecosystem services and natural capital. Nature 387: 253–260.

Dickie IA, Reich PB, 2005. Ectomycorrhizal fungal communities at forest edges. Journal of Ecology 93: 244–255.

Ehrlich PR, Wilson EO, 1991. Biodiversity studies: science and policy. Science 253: 758–762.

Engelhardt KAM, Ritchie ME, 2001. Effects of macrophyte species richness on wetland ecosystem functioning and services. Nature 411: 687–689.

Erland S, Taylor AFS, 2002. Diversity of ectomycorrhizal communities in relation to the abiotic environment. In: van der Heijden MGA, Sanders IR (eds), Mycorrhizal Ecology. Ecological Studies, vol. 157. Springer, Berlin, Heidelberg, New York, pp. 470–485.

Finlay R, Soderstrom B, 1992. In: Allen MF (ed), Mycorrhizal Functioning: an Integrative Plant-fungal Process. Chapman and Hall, London, pp. 134–160.

Fitter AH, 2005. Darkness visible: reflections on underground ecology. Journal of Ecology 93: 231–243.

Godbold DL, Hoosbeek MR, Lakuc M, Cotrufo MF, Janssens IA, Ceulemans R, Polle A, Velthorst EJ, Scarascia-Mugnozza G, De Angelis P, Miglietta F, Peressotti A, 2006. Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. Plant and Soil 281: 15–24.

Godbold JA, Solan M, Killham KS, 2009. Consumer and resource diversity effects on marine macroalgal decomposition. Oikos 118: 77–86.

Hediund K, Ohrn MS, 2000. Tritrophic interactions in a soil community enhance decomposition rates. Oikos 88: 585–591.

Heinemeyer A, Hartley JP, Evans SP, Carreira De La Fuente JA, Ineson P, 2007. Forest soil CO2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology 13: 1786–1797.

Hooper DU, Chapin III FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setala H, Symstad AJ, Vandermeer J, Wardle DA, 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75: 3–35.

Janzen RA, Dormaar JF, McGill WB, 1995. A community-level concept of controls on decomposition processes: decomposition of barley straw by Phanerochaete chrysosporium or Phlebia radiata in pure or mixed culture. Soil Biology and Biochemistry 27: 173–179.

Jonsson LM, Nilsson M-C, Wardle DA, Zackrisson O, 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Oikos 93: 353–364.

Langenheder S, Bulling MT, Solan M, Prosser JI, 2010. Bacterial biodiversity ecosystem functioning relations are modified by environmental complexity. PLoS ONE 5: e10834. doi:10.1371/journal.pone.0010834.

Lawton JH, Brown VK, 1993. Redundancy in ecosystems. In: Schulze E-D, Mooney HA (eds), Biodiversity and Ecosystem Function. Springer, Berlin, pp. 225–270.

Leake JR, 2001. Is diversity of ectomycorrhizal fungi important for ecosystem function? New Phytologist 152: 1–3.

Lilleskov EA, Fahey TJ, Horton TR, Lovett GM, 2002. Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83: 104–115.

Lilleskov EA, Fahey TJ, Lovett GM, 2001. Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. Ecological Applications 11: 397–410.

Lilleskov EA, Hobbie EA, Horton TR, 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. Fungal Ecology 4: 174–183.

Loreau M, 1998a. Biodiversity and ecosystem functioning: a mechanistic model. Proceedings of the National Academy of Sciences of the United States of America 95: 5632–5636.

Loreau M, 1998b. Separating sampling and other effects in biodiversity experiments. Oikos 82: 600–602.

Loreau M, Hector A, 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412: 72–76.

Marx DH, 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pathogenic infections. I. Antagonism of mycorrhizal fungi to pathogenic fungi and soil bacteria. Phytopathology 59: 59–153.
Moore JC, McCann K, Setälä H, De Ruiter PC, 2003. Top-down is bottom-up: does predation in the rhizosphere regulate aboveground dynamics? *Ecology* **84**: 846–857.

Mulder CPH, Uliassi DD, Doak DF, 2001. Physical stress and diversity-productivity relationships: the role of positive interactions. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 6704–6708.

Naem S, 1998. Species redundancy and ecosystem reliability. *Conservation Biology* **12**: 39–45.

Pickles BJ, Genney DR, Potts JM, Lennon JJ, Anderson IC, Alexander IJ, 2010. Spatial and temporal ecology of Scots pine ectomycorrhizas. *New Phytologist* **186**: 755–768.

Prosser JI, Bohannan BJM, Curtis TP, Ellis RJ, Firestone MK, Freckleton RP, Green JL, Green LE, Kilham K, Lennon JJ, Osborn AM, Solan M, van der Gast CJ, Young JPW, 2007. The role of ecological theory in microbial ecology. *Nature Reviews Microbiology* **5**: 384–392.

Read DJ, Perez-Moreno J, 2003. Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? *New Phytologist* **157**: 475–492.

Saatii SK, Campbell CD, Russell J, Alexander IJ, Anderson IC, 2005. Pine microsatellite markers allow roots and ectomycorrhizas to be linked to individual trees. *New Phytologist* **165**: 295–304.

Setala H, Huhta V, 1991. Soil fauna increase *Betula pendula* growth: laboratory experiments with coniferous forest floor. *Ecology* **72**: 665–671.

Smith SE, Read DJ, 2008. *Mycorrhizal Symbiosis*. Academic Press, London.

Tilman D, Lehman CL, Thomson KT, 1997. Plant diversity and ecosystem productivity: theoretical considerations.

*Tilman D, Wedin D, Knops J, 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* **379**: 718–720.

Tiunov AV, Scheu S, 2005. Facilitative interactions rather than resource partitioning drive diversity–functioning relationships in laboratory fungal communities. *Ecology Letters* **8**: 618–625.

Trenbath BR, 1974. Biomass productivity of mixtures. *Advances in Agronomy* **26**: 177–210.

van der Heijden MGA, Bardgett RD, van Straalen NM, 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* **11**: 296–310.

Van Cleve K, Viereck LA, 1981. Forest succession in relation to nutrient cycling in the boreal forest of Alaska. In: West DC, Shugart HH, Botkin DB (eds), *Forest Succession, Concepts and Application*. Springer-Verlag, New York, pp. 185–210.

Visser S, 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytologist* **129**: 389–401.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM, 1997. Human domination of Earth’s ecosystems. *Science* **277**: 494–499.

Walker BH, 1992. Biodiversity and ecological redundancy. *Conservation Biology* **6**: 18–23.

Wallenda T, Kottke I, 1998. Nitrogen deposition and ectomycorrhizas. *New Phytologist* **139**: 169–187.

West BT, Welch KB, Galecki AT, 2007. *Linear Mixed Models: a Practical Guide using Statistical Software*. Chapman & Hall/CRC, Boca Raton.

Yachi S, Loreau M, 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 1463–1468.