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

Anna WILKINSON[a,c,*], *Martin SOLAN*[b], *Ian ALEXANDER*[a], *David JOHNSON*[a]

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

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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.

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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* *Corresponding author*. Institute of Biological and Environmental Sciences, Cruickshank Building, University of Aberdeen, Aberdeen AB24 3UU, UK. Tel.: +44 01524 592931.

E-mail addresses: a.wilkinson4@lancs.ac.uk (A. Wilkinson), m.solan@abdn.ac.uk (M. Solan), i.alexander@abdn.ac.uk (I. Alexander), d.johnson@abdn.ac.uk (D. Johnson).

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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