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Simulated nitrogen deposition favors stress-tolerant fungi with low potential for decomposition

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ABSTRACT

Global changes such as atmospheric nitrogen (N) deposition can alter the structure of microbial communities, but a mechanistic understanding of the linkages between community structure and ecosystem function is lacking. Here we apply a trait-based framework to an analysis of litter fungal communities in a temperate forest exposed to > 20 years simulated N addition in order to develop hypotheses regarding the mechanisms underlying community responses and resulting changes in ecosystem function. We performed metabarcoding of the total and active fungal communities and measured extracellular enzyme activity in leaf litter after it decomposed for approximately two years in a long-term simulated N deposition experiment. We found that N fertilization decreased the relative abundance of six species in the active community that were highly correlated with lignin decomposing enzyme activity. Four of these species have currently unknown taxonomic identity and should be targets for isolation and further characterization. Nitrogen fertilization also increased species richness and relative abundance of yeasts in the total community and decreased their relative activity levels, suggesting these species may be dormant or otherwise inactive. Together these responses may contribute to accumulation of organic matter in soils by favoring yeasts that are not strong enzyme producers and by disfavoring the fungal species that are most active in litter decomposition.

1. Introduction

Fungi, as the primary agents of plant litter decomposition in temperate forest soils (Schneider et al., 2010, 2012), are likely to mediate changes in ecosystem carbon (C) and nutrient cycling that occur with global change. The various species of fungi involved in decomposition of plant litter have a wide array of potential functions – ranging from lignin-decomposers (e.g., white-rot fungi) that can produce a variety of cellulose- and lignin-decomposing extracellular enzymes (Floudas et al., 2012) to yeast species that are better-adapted for rapid consumption of sugar monomers and other simple compounds (Botha, 2011; Treseder and Lennon, 2015). A change in the distribution of species in these different guilds is therefore likely to alter ecosystem functioning in important ways, but evidence for causal relationships between taxonomic composition of communities and ecosystem function has remained elusive (Nemergut et al., 2013; Crowther et al., 2014). This is partly due to the lack of a cohesive framework to describe the relationship between microbial community structure and ecosystem functioning – taxonomic composition is often assessed independently of microbial functional traits that control ecosystem processes like litter decomposition (Crowther et al., 2014). However, traits are not independent of species, and fungi in particular can be defined in terms of functional groups for which a suite of traits is consistently present (Treseder and Lennon, 2015), providing a means to link taxonomic identity to functioning in the environment. To this end, a response-effect trait framework (*sensu* Lavorel and Garnier, 2002) has been proposed to understand how environmental perturbations cause changes in fungal community composition that then feed back to ecosystem functioning (Crowther et al., 2014; Koide et al., 2014; Treseder and Lennon, 2015). In this framework, a response trait determines how an organism *responds* to its environment (e.g., change in fitness) while an effect trait determines how an organism *affects* ecosystem processes. When response and effect traits are linked (e.g., the traits consistently occur in the same groups of organisms), then an environmental

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perturbation that affects species abundance will also alter ecosystem processes.

Long-term simulated N deposition experiments provide a particularly cogent study system within which to apply a response-effect trait framework, because of the breadth of research on fungal community and ecosystem responses to chronic N addition. For example, N addition to temperate forest ecosystems consistently causes an accumulation of C in soils and reduces organic matter decomposition rates (Pregitzer et al., 2008; Liu and Greaver, 2010; Zak et al., 2011; Lovett et al., 2013; Weber et al., 2013; Frey et al., 2014), depresses activity of lignin decomposing enzymes (Carreiro et al., 2000; Sinsabaugh et al., 2002; DeForest et al., 2004; Frey et al., 2004), and causes accumulation of lignin compounds in soil and litter (Magill and Aber, 1998; Frey et al., 2014). Despite that fungal community responses are relatively well studied (e.g., Allison et al., 2007; Osono, 2007; Entwistle et al., 2013; Eisenlord et al., 2013; Weber et al., 2013; Mueller et al., 2014; Freedman et al., 2015; Hesse et al., 2015; Morrison et al., 2016; Entwistle et al., 2017) it is still not clear whether there is a general response of the fungal community that underlies the observed ecosystem responses.

There are several examples of fungal groups or specific fungal traits that fit a response-effect framework in the context of long-term N deposition and that could contribute to the ecosystem responses observed (e.g., soil organic matter accumulation). We focus on traits such as potential for stress resistance and production of extracellular decomposition enzymes that are broadly correlated with different fungal body forms, specifically single-celled yeasts versus filamentous body forms (Treseder and Lennon, 2015), and use these groupings of traits to circumscribe fungal functional groups. For example, yeasts, defined here as fungi within Ascomycota and Basidiomycota with a single-cell body form (*sensu* Kurtzman et al., 2011), can be an important component of the forest litter and soil fungal community in terms of both relative abundance in the community and the number of species present (i.e. species richness; Masinova et al., 2017). Yeasts have greater genetic capacity for inorganic N uptake than filamentous fungi suggesting that they may be favored in N-rich environments, but have less capacity to produce cellulose and lignin-degrading enzymes (Treseder and Lennon, 2015). Yeasts are also enriched in traits conferring stress resistance (Singaravelen et al., 2008; Kurtzman et al., 2011; Treseder and Lennon, 2015) suggesting they may be favored in high N environments in which total fungal biomass is often suppressed (Frey et al., 2004; Wallenstein et al., 2006; Treseder, 2008). Potential mechanisms explaining suppression of fungal biomass in high-N soil environments include formation of reactive nitrogen compounds in soils (e.g., nitric oxide, nitric acid) and subsequent nitrosative or oxidative stress (Brown et al., 2009; Cánovas et al., 2016), osmotic stress induced by added N salts (Averill and Waring, 2018), lower availability of lignin-complexed cellulose and hemicellulose resulting from transcriptional down regulation of genes encoding lignin-degrading enzymes (Edwards et al., 2011; Zak et al., 2011), and/or reduced exudation by plant roots resulting in lower availability of labile soil C (Carrara et al., 2018), though the relative contributions of these or other mechanisms is unknown. Yeasts therefore represent a functional group that may exhibit a positive response to chronic N deposition and have the potential to effect an increase in soil organic matter accumulation by competing with other fungi that are stronger decomposers of plant litter.

Filamentous saprotrophic fungi represent a second functional group with key traits that may be important in determining ecosystem responses to chronic soil N enrichment. Filamentous fungi generally have a lower genetic capacity for uptake of inorganic N and show lower stress-tolerance than yeasts (Treseder and Lennon, 2015); they may therefore be disfavored relative to yeasts in high-N environments, though to our knowledge the responses of yeasts relative to filamentous fungi have not been specifically examined in N-enriched soils. Filamentous fungi also have a higher capacity to produce plant cell wall degrading enzymes than yeasts overall (Treseder and Lennon, 2015), and some groups of filamentous fungi, especially those typically classified as “white-rot,” harbor high capacity for production of ligninases (Floudas et al., 2012; Kohler et al., 2015). White-rot fungi have been shown to have reduced relative abundance with N addition in high-lignin substrates like wood, but have increased relative abundance in N-amended soil (Entwistle et al., 2017), and their response to N addition in leaf litter is yet unclear. In general, high soil N availability may disfavor filamentous saprotrophs relative to yeasts, thereby reducing extracellular enzyme production and decomposition rates.

In the present study we applied a response-effect trait lens to an examination of the fungal communities of decomposing leaf litter in a long-term simulated N deposition experiment in a temperate hardwood forest. We used fungal species traits inferred from previously published literature (e.g., morphology and trophic guild categories) to classify fungal species into broad functional groups. Because traits may be variable within groups and may display plasticity in different environments we performed more direct measurements of two key traits – extracellular enzyme production and relative activity levels. First, we compared taxon relative abundance to extracellular enzyme activities measured in the same litter samples as an indication of extracellular enzyme production by individual taxa, and as a means to confirm the distribution of traits inferred from functional group classification. We also performed measurements of relative activity levels as an indication of dormancy by comparing ratios of ribosomal RNA (rRNA) to ribosomal RNA genes (rDNA) (i.e. rRNA:rDNA ratio, *sensu* Jones and Lennon, 2010), expecting that groups with higher levels of dormancy and lower activity would be enriched in general stress-tolerance traits (Lennon and Jones, 2011; Treseder and Lennon, 2015). General stress-tolerance is likely to be an important response trait in high-N conditions where total fungal biomass is reduced as is the case at our study site (Frey et al., 2004; Wallenstein et al., 2006). Specifically, we focused our analyses on filamentous saprotrophic fungi, filamentous saprotrophs identified as white-rot fungi, and yeasts. We expected these functional groups to have strong responses to N addition because of high stress-tolerance and inorganic N uptake potential of yeasts, and the association of filamentous saprotrophs and white-rot fungi with extracellular enzyme production (Treseder and Lennon, 2015), especially ligninases (Floudas et al., 2012) which are often reduced by N addition (Carreiro et al., 2000; Sinsabaugh et al., 2002; DeForest et al., 2004; Frey et al., 2004).

2. Methods

2.1. Site description, experimental design, and sample collection

This research was performed at the Chronic Nitrogen Amendment Study at the Harvard Forest Long-term Ecological Research (LTER) site in Petersham, Massachusetts, USA (42° 30′ N, 72° 10’ W). The experiment, which is described in full elsewhere (Aber et al., 1989; Aber and Magill, 2004; Frey et al., 2014), is situated in a hardwood forest stand dominated by *Quercus rubra* and *Q. velutina* (red and black oak) with lesser contributions of *Fagus grandifolia* (American beech) and *Acer rubrum* (red maple). The soils are Typic Dystrudepts of the Gloucester series (Peterjohn et al., 1994), with no significant variation amongst N treatments in pH or concentrations of the base cations Ca and Mg after 20 years of N addition (Turlapati et al., 2013). The experiment is divided into three 30 × 30 m megaplots that have received one of three N addition treatments since the inception of the experiment in 1988: ambient (control) N deposition (hereafter, N0), which is currently 7–9 $\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$ at this site (Schwede and Lear, 2014); 50 $\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$, representing current (Vet et al., 2014) or future (Galloway et al., 2008; Reay et al., 2008) rates of N deposition in areas of the world that are susceptible to high N deposition; or 150 $\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$, a space-for-time substitution meant to push the ecosystem toward N saturation in order to examine the long-term consequences of high cumulative rates of N deposition (J. Aber, pers. comm.). Nitrogen is applied to soils