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Differential responses of total and active soil microbial communities to long-term experimental N deposition

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ABSTRACT

The relationship between total and metabolically active soil microbial communities can provide insight into how these communities are impacted by environmental change, which may impact the flow of energy and cycling of nutrients in the future. For example, the anthropogenic release of biologically available N has dramatically increased over the last 150 years, which can alter the processes controlling C storage in terrestrial ecosystems. In a northern hardwood forest ecosystem located in Michigan, USA, nearly 20 years of experimentally increased atmospheric N deposition has reduced forest floor decay and increased soil C storage. A microbial mechanism underlies this response, as compositional changes in the soil microbial community have been concomitantly documented with these biogeochemical changes. Here, we co-extracted DNA and RNA from decaying leaf litter to determine if experimental atmospheric N deposition has lowered the diversity and altered the composition of the whole communities of bacteria and fungi (*i.e.*, DNA-based) and well as its active members (*i.e.*, RNA-based). In our experiment, experimental N deposition did not affect the composition, diversity, or richness of the total forest floor fungal community, but did lower the diversity (−8%), as well as altered the composition of the active fungal community. In contrast, neither the total nor active forest floor bacterial community was significantly affected by experimental N deposition. Our results suggest that future rates of atmospheric N deposition can fundamentally alter the organization of the saprotrophic soil fungal community, key mediators of C cycling in terrestrial environments.

1. Introduction

Our understanding of microbial communities is largely derived from environmental DNA; however, ~80% of cells, and ~50% of operational taxonomic units (OTUs) in soil may be inactive (Lennon and Jones, 2011). This "microbial seed bank" represents a reservoir of genetic diversity that could shape community composition in response to a changing environment. RNA has a much shorter turnover than DNA and can therefore serve as a tool to gain insight into active microbial community (Moeseneder et al., 2005). In soil, the subset of the microbial community that is putatively active at the time of sampling can be compositionally different than the DNA "seed bank", and it may respond differently to environmental stress (McMahon et al., 2011; Baldrian et al., 2012; Barnard et al., 2013). Furthermore, fluctuations in active microbial community composition can disproportionally contribute to microbial community dynamics, as well as mediate essential ecosystem functions (Shade et al., 2014; Aanderud et al., 2015). The importance of the relationship between the composition of the total microbial community, and the subset that is putatively active, and how this balance can be affected by environmental change is providing novel insight into the dynamics of microbial communities (Lennon and Jones, 2011; Baldrian et al., 2012; Zhang et al., 2014), and is the focus of this study.

Anthropogenic modification of global biogeochemical cycles may be exacerbated by changes in the composition and structure of microbial communities (Falkowski et al., 2008; Zhao et al., 2014). For example, across much of the Northern Hemisphere, atmospheric deposition of biologically available nitrogen (N) has increased by an order of magnitude over the past 150 years (*e.g.*, from 0.5–1 to 15–20 kg N ha^{-1} y^{-1}), with this trend expected to continue through the next century (Galloway et al., 2004; Torseth et al., 2012). For

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twenty years, we have applied chronic experimental N deposition (30 kg N ha^{-1} y^{-1}) to replicate northern hardwood forest stands at a rate expected in some locations by mid-century (Table 1 and Figure S1; Galloway et al., 2004). To date, experimental N deposition has increased net primary productivity (NPP) of wood, reduced litter decay, and has fostered soil organic matter (SOM) accumulation and dissolved organic carbon (DOC) leaching (Pregitzer et al., 2008; Zak et al., 2008). Experimental N deposition has also altered the composition of fungi (Edwards et al., 2011; Entwistle et al., 2013) and bacteria (Eisenlord and Zak, 2010; Freedman and Zak, 2014, 2015b) in the forest floor. Furthermore, experimental N deposition has decreased the abundance and richness of functional genes mediating soil C and N cycling processes (Eisenlord et al., 2013; Freedman et al., 2013), decreased fungal lignocellulolytic gene expression, and increased the abundance of saprophytic bacteria that mediate the decay of polyphenolic compounds (Edwards et al., 2011; Freedman and Zak, 2014). These molecular-level responses appear to be linked to dramatic biogeochemical changes in soil that have fostered greater soil C storage (+10%), which we argue will lead to a potential mechanism reducing the accumulation of anthropogenic CO_2 in the Earth's atmosphere. However, we do not know whether they arise from a change in overall community composition or a change in the organisms actively metabolizing plant detritus into soil organic matter.

Soil fungi and bacteria exhibit different community dynamics (Prewitt et al., 2014), thus, to understand soil ecosystem processes it is essential to address the fungal and bacterial community simultaneously (Baldrian et al., 2012). Here, we sought to determine if experimental N deposition altered the composition of the total (DNA-based) and active (RNA-based) fungal and bacterial communities on decaying forest floor. We refer to the microbial community as "saprotrophic" because we expect the majority of the organisms living in the Oe/Oa horizon are sustained by the metabolism of decaying organic matter. It is plausible that chronic N deposition can alter the composition of the total microbial community, as well as the subset of the community that are metabolically active, which may elicit a functional response consistent with our biogeochemical observations. To address our objectives, we i) determined the ligonocellulolytic decay potential of the forest floor microbial community by quantifying the activity of two enzymes, cellobiohydrolase and peroxidase, that mediate the decay of common plant cell wall compounds, cellulose and lignin, respectively and ii) co-extracted genomic DNA and ribosomal RNA from forest floor, from which, we amplified and sequenced fungal 28S and bacterial 16S rRNA genes.

2. Materials and methods

2.1. Site description and sample collection

We investigated the influence of experimental N deposition on the total (DNA-based) and active (RNA-based) saprotrophic microbial community in four northern hardwood forest stands in Lower and Upper Michigan, USA (Table 1; Figure S1). The stands span the north-south geographic range of the northern hardwood forests in the Great Lakes region (Braun, 1950) and lie along a 500-km climatic and atmospheric N deposition gradient. All sites are floristically and edaphically similar and are dominated by sugar maple (*Acer saccarum* Marsh.). The thin Oi horizon is comprised of sugar maple leaf litter and the Oe/Oa horizons are interpenetrated by a dense root mat. The soils are sandy (85–90%), well-drained, isotic, frigid Typic Haplorthods of the Kalkaska series. Six 30-m by 30-m plots were established at each stand in 1994; three receive ambient N deposition and three receive experimental N deposition. Experimental N deposition consists of $NaNO_3$ pellets broadcast over the forest floor in six equal applications during the growing season (30 kg N ha^{-1} y^{-1}); NO_3^- comprises ~60% of atmospheric N deposition (wet plus dry) in our study sites (Zak et al., 2008; Barnard et al., 2013).

Forest floor sampling occurred in late May to early June 2013. In this way, samples from all four sites occurred during a phenologically-similar period, a time at which ample moisture supports high rates of microbial activity. Within each 30-m-by-30-m plot, 10 random 0.1-m-by-0.1-m forest floor samples (Oa/Oe horizons) were collected by hand after removing the freshly fallen Oi horizon. All samples were composited within each plot and homogenized by hand in the field. A portion of the homogenized sample was immediately flash frozen on liquid N_2 for nucleic acid extraction and the remainder was kept on ice for enzyme analyses. Samples were transported to the University of Michigan within 48 h of sampling, where they were stored at −4 or −80 °C for enzyme analysis and nucleic acid extraction, respectively. Enzyme analysis and nucleic acid extractions were initiated 72 h after sampling.

2.2. Enzyme analysis

To determine if experimental N deposition reduced the lignocellulytic potential of the forest floor microbial community, we measured the activity potential of cellobiohydrolase (EC 3.2.1.91) and peroxidase (EC 1.11.1.7), extracellular enzymes that catalyze the

Table 1
Site, climatic, overstory, and ambient nitrogen deposition rates of four study sites receiving experimental N additions.

Characteristic	Site A	Site B	Site C	Site D
Location				
Latitude (N)	46°52″	45°33″	44°23″	43°40″
Longitude (W)	88°53″	84°52″	85°50″	86°9″
Climate				
Mean annual temperature (°C)	4.8	6.1	6.5	7.7
Mean annual precipitation (cm)	91.9	93.3	92.8	86.6
Ambient N Deposition (Kg N ha^{-1} yr^{-1})	5.9	6.1	7.4	7.4
Vegetation				
Overstory biomass (Mg ha^{-1})	261	261	274	234
Acer saccharum biomass (Mg ha^{-1})	237	224	216	201
Environment				
Leaf Litter (Oe/Oa horizons)				
Litter C:N	63.7	57.1	52.9	43.4
Litter mass (g)	412	396	591	550
Soil (0–10 cm)				
Sand (%)	85	89	89	87
pH (1:1 soil/H_2O)	4.8	5.0	4.5	4.7
Base saturation, %	71	96	73	80