Soil Biology & Biochemistry 66 (2013) 130–138

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Towards a molecular understanding of N cycling in northern hardwood forests under future rates of N deposition

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ARTICLE INFO

Article history:
Received 3 April 2013
Received in revised form
3 June 2013
Accepted 18 July 2013
Available online 31 July 2013

Keywords:
N deposition
Bacteria
Archaea
Climate change
Dispersion
N cycle

ABSTRACT

The combustion of fossil fuels and fertilizer use has increased the amount of biologically available N over the last 150 years. Future rates of atmospheric N deposition may slow organic matter decay and alter microbial community composition and function. However, our understanding of how anthropogenic N enrichment may alter the physiological mechanisms by which soil microorganisms assimilate and cycle N in soil are largely unknown. Since 1994, we have experimentally increased NO_3 deposition to replicate ($n = 4$) northern hardwood forest stands across a 500-km climatic gradient in the Great Lakes region of North America. Our goal was to examine how functional genes mediating N-cycle processes in soil microbial communities have responded to experimental N deposition using the functional gene microarray, GeoChip 4.0. Experimental N deposition decreased the abundance and richness of key protein-coding genes in Archaea and Bacteria responsible for N fixation, ammonification, denitrification and assimilatory nitrate reduction; the same was true for bacterial genes mediating nitrification and dissimilatory nitrate reduction. However, the extent to which experimental N deposition decreased abundance and richness was site-specific, which was revealed by a significant site by treatment interaction. Experimental N deposition also caused a community composition shift via dispersion (increased β-diversity) in archaeal and bacterial gene assemblages. In combination, our observations suggest future rates of atmospheric N deposition could fundamentally alter the physiological potential of soil microbial communities.

1. Introduction

Atmospheric deposition of biologically available nitrogen (N) has increased ten-fold across much of the Northern Hemisphere over the last 150 years (e.g., from 0.5 to 1 to 15–20 kg N ha^{-1} y^{-1}) and is projected to more than double in the next century (Galloway et al., 2004). At present, anthropogenic creation of reactive N surpasses that of all natural terrestrial sources combined (Vitousek et al., 1997). Increased N availability in terrestrial ecosystems, such as temperate forests in which plant growth is often N limited (Vitousek et al., 2002), may lead to a phenomenon called N saturation (Aber et al., 1998, 1989) which models a series of plant and soil responses as N limitation is alleviated. Sugar maple (*Acer saccharum* Marsh.) dominated forests in the upper Great Lakes region, U. S. A., are especially prone to N saturation (Zak et al., 2006) due to their high rates of net N mineralization (80–120 kg N ha^{-1} y^{-1}; Zak and Pregitzer, 1990), and moderate rates of atmospheric deposition (7–12 kg N ha^{-1} y^{-1}; MacDonald et al., 1991).

Since 1994, replicate forest stands have experimentally received experimental NO_3^- deposition across a 500-km climatic gradient spanning the north-south geographic range of the sugar maple dominated northern hardwood forest in the Great Lakes region of North America (Braun, 1950). We have demonstrated that experimental N deposition at a rate expected by 2050 across North America and other regions (Galloway et al., 2004) has increased woody biomass production, as well as organic matter stored in the forest floor and surface mineral soil (Pregitzer et al., 2008). Surprisingly, the observed increase in soil C sequestration has occurred despite no change in above- or belowground litter production (Burton et al., 2004), indicating organic matter has accumulated due to a reduction in plant litter decay (Zak et al., 2008).

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http://dx.doi.org/10.1016/j.soilbio.2013.07.010

It is well established that litter decay rates are correlated with the initial ratios of C:N, lignin:N (Aerts, 1997; Hobbie, 2008; Prescott, 2010), and interactions among N, lignin, and cellulose (Talbot and Treseder, 2012). Thus, our understanding of how microbial communities directly involved in N cycling respond to anthropogenic N enrichment is key to understanding the fate of C and N under environmental change. Nitrogen enrichment can decrease microbial respiration (Burton et al., 2004; Janssens et al., 2010) and biomass (Wallenstein et al., 2006) and alter archaeal (Nemergut et al., 2008), bacterial (Eisenlord and Zak, 2010; Fierer et al., 2011; Nemergut et al., 2008), and fungal (Allison et al., 2007; Edwards et al., 2011) community composition. Our knowledge regarding the function of archaeal communities in the microbial N-cycle is both emerging and essential, as Archaea exhibit a common presence in soils (Bates et al., 2011) and the ability to mediate both assimilatory and dissimilatory processes of the N cycle (Cabello et al., 2004). For example, Archaea are dominant among ammonia-oxidizing prokaryotes across a variety of soil types (Leininger et al., 2006). Although most studies focus on how anthropogenic N enrichment may affect microbial populations, impacts on specific microbial communities responsible for soil N cycling processes is poorly understood.

We hypothesized that the experimental N deposition has altered the functional potential of forest floor microbial assemblages. From this hypothesis, we predict a decline in abundance and diversity of archaeal and bacterial functional genes related to N-cycling, as well a shift in community composition of functional gene assemblages mediating N cycling processes in response to chronic N deposition. To test our hypothesis, we used GeoChip 4.0, a PCR-independent microarray encompassing 73 archaeal and 2803 bacterial genes mediating N cycling processes in soil.

2. Materials and methods

2.1. Site description

The influence of simulated N deposition on soil microbial communities was investigated in four sugar maple (*A. saccharum* Marsh.) dominated northern hardwood forest stands in lower and upper Michigan, USA (Fig. 1). These locations span the north-south geographic range of the northern hardwood forests in the Great Lakes region of North America (Braun, 1950). The sites are floristically and edaphically similar, but span a climatic and atmospheric N deposition gradient (Table 1; MacDonald et al., 1991; Zak et al., 2008). The forest floor is composed of a thin Oi horizon dominated by sugar maple leaf litter, and a thicker Oe horizon interpenetrated by a dense root mat. At each study site, six 30-m by 30-m plots were established in 1994. Three plots in each stand receive ambient N deposition, and three receive experimental N deposition which consists of six equal applications of $NaNO_3$ pellets delivered to the forest floor over the growing season (30 kg N ha^{-1} y^{-1}). In our study sites, NO_3^- comprises ~60% of wet plus dry atmospheric N deposition (MacDonald et al., 1992).

Fig. 1. The geographic distribution of the study sites A–D in Lower and Upper Michigan. In each stand beginning in 1994, three plots received ambient atmospheric N deposition and three plots received ambient plus 30 kg NO_3–N ha^{-1} yr^{-1}.

2.2. Forest floor collection and DNA extraction

Forest floor sampling was performed in early October 2009, a time in which ample moisture favors high rates of microbial activity. In each 30-m by 30-m plot, 10 random 0.1-m by 0.1-m forest floor samples (Oe/Oa horizons) were collected after manually removing the freshly fallen Oi horizon. Following manual removal of large organic debris, samples were composited, homogenized by hand, and were immediately frozen in the field using liquid N_2.

Genomic DNA was extracted from 2.5 g of forest floor samples using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) following manufacturer's instructions. Extracted DNA was quantified by the Quant-iT PicoGreen (Invitrogen, Carlsbad, CA, USA) assay according to manufacturer's instructions and stored at −80 °C until further analysis.

2.3. Target preparation, GeoChip hybridization, and data preprocessing

GeoChip 4.0 was applied in this study as described previously (Lu et al., 2011). A full list of functional genes and the number of associated probes (gene variants) mediating N cycling processes are included in the supplementary information (Table S1). Briefly, 1 μg of genomic DNA from each replicate sample was purified by the Genomic DNA Clean & Concentrator kit (Zymo Research, Irvine, CA, USA) and labeled with fluorescent dye Cy-3 using random primers (Wu et al., 2006). The labeled gDNA was dried and rehydrated with 2.7 μl of sample tracking control to confirm sample identity, followed by incubation at 50 °C for 5 min. This DNA solution was mixed with 7.3 μL of hybridization buffer containing the universal

Table 1
Site, climactic, overstory, and ambient nitrogen deposition rates of four study sites receiving experimental NO_3^- 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	4.7	6.0	6.9	7.6
Mean annual precipitation	873	871	888	812
Wet + dry NO_3–N deposition, g N m^{-2} yr^{-1}	0.38	0.58	0.78	0.76
Wet + dry total N deposition, g N m^{-2} yr^{-1}	0.68	0.91	1.17	1.18
Vegetation				
Overstory biomass, Mg ha^{-1}	261	261	274	234
Acer saccharum biomass, Mg ha^{-1}	237	224	216	201
Soil (0–10 cm)				
Sand, %	85	89	89	87
pH (1:1 soil/H_2O)	4.8	5.0	4.5	4.7
Cation exchange capacity, $mmol_c$ kg^{-1}	3.4	3.8	2.6	3.0
Base saturation, %	71	96	73	80

Table adapted from Eisenlord and Zak (2010).