Atmospheric Nitrogen Deposition and Microbial Mechanisms Enhancing Soil Carbon Storage

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http://www.webpages.uidaho.edu/nitrogen-gradient/Default.htm

Project Goals: Since the industrial revolution, the amount of reactive nitrogen (N) deposited from the atmosphere has increased across large areas of the Northern Hemisphere (e.g., from 50-100 to 1500-2000 mg N m^{-2} y^{-1}). Agricultural and industrial activities are largely responsible for this increase, which has the potential to disrupt many of the fundamental ways in which ecosystems function—ranging from the growth and vigor of trees to the microbial mechanisms mediating the cycling and storage of carbon (C) in soil. Our long-term experiment was established to understand the mechanisms by which atmospheric N deposition could alter the ecosystem processes in sugar maple dominated forests of the Great Lakes region. Exposure to 18 years of experimental N deposition, at a rate expected mid-century, has slowed the decay of plant litter, which has increased soil C storage as well as export of phenolic DOC; this biogeochemical response has occurred in the absence of a change in litter biochemistry or production. Presently, we are testing the hypothesis that the slowing of litter decay and the accumulation of soil organic matter in response to anthropogenic N deposition is governed by the environmental regulation of microbial gene expression. We predict that chronic experimental N deposition has down regulated the transcription of fungal genes encoding lignocellulolytic enzymes, thereby slowing litter decay and opening a niche for other, less effective lignocellulolytic soil microorganisms to occupy. To understand whether these hypothesized mechanisms are at work in our experiment, we have employed molecular genetic approaches to examine the community and physiological responses of saprotrophic fungi and *Actinobacteria* residing in forest floor and surface soil.

Experimental Design: Since 1994, we have experimentally simulated increased atmospheric N deposition in replicate stands of a northern hardwood forest ecosystem stretching across a 500-km climatic gradient (Fig. 1). Our study sites deliberately encompass the north-south geographic range of the of the sugar maple-dominated (*Acer saccharum* Marsh.) northern hardwood forest in the Great Lakes region of North America, enabling us to generalize our experimental results across this geographic region. These sites are floristically and edaphically matched (> 80% sugar maple on Typic Haplorthods), but they differ in climate along the north-south latitudinal gradient (Fig. 1). The study sites also span an atmospheric N deposition gradient, over which NO_3^--N composes ~ 60% of wet-plus-dry deposition. There are six 30-m x 30-m plots at each study site; every plot is surrounded on all sides by a 10-m wide treated buffer. Three plots at each site receive ambient atmospheric N deposition. The other three plots at each site receive ambient N deposition plus 3 g NO_3^--N m^{-2} y^{-1}, a rate approaching that expected by 2050 across large portions of North America and other regions of the Earth. The additional N is

Fig. 1. Distribution of replicate sites spanning the north-south range of northern hardwood forests in the Upper Great Lakes region.

delivered over the growing season in six equal applications (0.5 g N m^{-2} $month^{-1}$) of solid $NaNO_3$ pellets, which are broadcast over the forest floor.

Hypothesis Testing: Basidiomycete and ascomycete fungi are the primary agents of plant cell wall decay, and a change in their composition or decline in activity could slow litter decay under experimental N deposition. To characterize the actively metabolizing community of these organisms, we used cDNA clone libraries constructed from 28S fungal rRNA. The active basidiomycete communities under ambient and experimental atmospheric N deposition differed significantly in terms of membership as well as their dispersion across a phylogenetic tree. Furthermore, suggestive, albeit nonsignificant, differences in the fraction of unique phylogenetic branch length (i.e., the UniFrac metric) between ambient and experimental atmospheric N deposition were observed for forest floor basidiomycetes. In contrast, the active ascomycete communities under ambient and experimental atmospheric N deposition did not exhibit significant differences in these same metrics. Collectively, our observations indicate that experimental N deposition has altered the composition of litter decaying fungi and that these changes have ecosystem-level implications for the cycling and storage of C in forest ecosystems.

Actinobacteria also are one of the few groups of saprotrophic microorganisms which oxidatively depolymerize lignin, producing substantial soluble polyphenolics during the process. These organisms could plausibly become more important agents of lignin decay, if atmospheric N deposition suppresses the activity or alters the composition of lignolytic basidiomycete and ascomycete fungi. To test this idea, we quantified actinobacterial abundance and community composition under ambient and experimental N deposition. Actinobacterial abundance was assessed using quantitative PCR of 16S rRNA and community composition was evaluated using clone libraries and phylogenetic community analyses (i.e., Libshuff and Unifrac). Contrary to our expectation, experimental atmospheric N deposition had no effect on actinobacterial abundance in the forest floor (~10^{10} gene copies/g); however, it significantly decreased actinobacterial abundance by 47% in surface mineral soil. Our analyses revealed experimental N deposition further elicited a significant membership change in forest floor and surface soil communities, as well as significant differences in the phylogenetic diversity of forest floor *Actinobacteria*. This shift in community composition occurred in concert with the slowing of plant litter decay, the accumulation of soil organic matter, and the greater production of phenolic DOC.

To determine whether slower decomposition rates resulted from down-regulation of the transcription of key lignocellulolytic genes, we quantified the expression of fungal genes encoding key cellulolytic (cellobiohydrolase, *cbhI*) and lignolytic (laccase, *lcc*) enymes. Our results indicate that the community-scale expression of *cbhI* under experimental N deposition did not differ significantly from that under ambient N deposition. In contrast, expression of *lcc* was significantly down-regulated by a factor of 2-4 fold relative to its expression under ambient N deposition. Our results suggest that chronic atmospheric N deposition may lower decomposition rates through a combination of reduced expression of ligninolytic genes such as *lcc*, as well as compositional changes in the fungal community. More importantly they indicate that ecosystem response to atmospheric N deposition, a wide-spread agent of global change, is controlled by the environmental regulation of fungal gene expression.

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