NSRC Theme 2 Final Report

Project Title: Biological Nitrogen Immobilization as a Control on Soil Nitrogen Retention in Northern Forests

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Most important project outcomes: We show that long-term simulated nitrogen deposition reduces the concentration of manganese available to support fungal decomposition of leaf litter, and that the addition of bioavailable manganese significantly increases fungal activity. As Northern forests respond to altered climate and nitrogen availability, our work provides understanding of the underlying mechanisms affecting long-term soil carbon storage in these systems.

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Project Summary

Long-term atmospheric nitrogen (N) deposition has been shown to reduce leaf litter and lignin decomposition in Northern forest soils, leading to an accumulation of soil carbon (C). Reduced decomposition has been accompanied by altered structure and function of soil fungal communities, the primary decomposers in forest ecosystems; however, a mechanistic understanding of fungal responses to chronic N enrichment is lacking. A reduction in soil and litter manganese (Mn) concentrations under N enrichment (i.e., Mn limitation) may explain these observations, because Mn is a cofactor and regulator of lignin-decay enzymes produced by fungi. We conducted a six-month incubation study to evaluate the effect of Mn availability on decomposition dynamics in chronically N-enriched soils. We measured ligninolytic enzyme activities, mass loss and lignin (% change) in litter, and characterized the whole litter fungal community by internal transcribed spacer (ITS) region metabarcoding. We found a significant positive correlation between Mn availability and ligninolytic enzyme activities in litter. In addition, we observed an increase in the relative abundance of 'weak' decomposers (e.g., yeasts) under long-term N enrichment, and a reversal of this response with Mn amendment. Our results suggest that higher Mn availability may promote fungal communities better adapted to decompose lignin. We conclude that Mn limitation plays an important role in decomposition dynamics under long-term atmospheric N deposition and may represent a mechanism that explains reduced decomposition and soil C accumulation under this global change factor. As Northern forests respond to altered climate and N availability, our work provides understanding of the underlying mechanisms affecting long-term soil C storage in these systems.

Background and Justification

- Human activities have greatly increased the release of nitrogen compounds to Earth's atmosphere.
- Atmospheric nitrogen (N) deposition has risen by 200% since the start of the industrial revolution, and current rates of deposition are projected to double by 2050 in many parts of the world.
- Long-term N deposition has been shown to slow leaf litter and lignin decomposition in forest soils, resulting in an accumulation of soil carbon
- A long history of research has attempted to pinpoint the underlying cause of this C accumulation and has focused much attention on the microbes that regulate decomposition processes in soils, namely fungi.
- Simulated N deposition has been shown to reduce fungal biomass, alter fungal community composition, suppress lignin-decay enzyme activity and down-regulate the expression of genes encoding these enzymes.
- Despite extensive study, a mechanistic understanding of this repression of the soil fungal community is still lacking.
- Studies have demonstrated a strong positive relationship between litter manganese (Mn) concentrations and the rate and extent of fungal-mediated decomposition.
- The importance of Mn in decomposition is thought to derive from its role in lignin-decay enzyme production.
- Our objectives were to (1) examine if Mn limitation contributes to reduced lignin and leaf litter decomposition under long-term N deposition; and (2) to determine if Mn limitation is a factor underlying shifts in fungal community composition that have been observed in long-term simulated N deposition experiments.

Methods

- We quantified nutrient (Ca, Mn, Mg, P, K, Al, B, Cu, Fe and Zn) concentrations in leaf litter collected from the Chronic Nitrogen Amendment Study at the Harvard Forest Long-Term Ecological Research (LTER) site in Petersham, MA (**Table 1**).
- Litter organic matter chemistry was characterized using pyrolysis gas chromatography and mass spectrometry to determine the relative abundance of lignin, phenols and other aromatic compounds.
- Soil pH was quantified in distilled water using a digital pH meter. Soil moisture was determined by oven drying organic horizon material at 60^{o}C for 48 hours and mineral soils at 105^{o}C for 24 hours. Exchangeable soil acidity was evaluated by soil extraction with 1M KCl and subsequent titration with dilute NaOH. Cation exchange capacity (CEC) was calculated thereafter using the equation, CEC = exchangeable acidity (meq) + exchangeable base cations (**Table 2**).
- Leaf litter was assessed for the activities of two ligninolytic enzymes using standard techniques.
- We also conducted an incubation experiment in which we applied Mn amendments to soils from this site.
- Intact soil cores, which included both organic and mineral soil, were incubated at 25^{o}C for ~6 months (167 days) to evaluate the role of Mn in mid- to late-stage litter decomposition. Before incubation, soil moisture was standardized to 60% water-holding capacity and 60% field capacity across the organic horizon and mineral soil components of each core, respectively.
- One of three Mn amendments was applied: ambient (no additional Mn, only native litter Mn); low Mn; or high Mn. The 'low' rate of Mn amendment was based on initial Mn concentrations in control N litter (~3 mg g^{-1} litter; **Table 1**).
- After ~6 months of incubation, we evaluated litter mass loss, the percent change in litter lignin, and the potential activities of ligninolytic enzymes (peroxidase and phenol oxidase; **Figure 1**). We also characterized fungal community composition by ITS2 metabarcoding of the whole litter fungal community (**Figure 2**).

Results/Project outcomes

- We conclude that Mn limitation is a notable mechanism reducing ligninolytic enzyme activity and altering fungal community composition under long-term atmospheric N deposition.
- Our results suggest that Mn limitation may be an important control on decomposition and soil C storage under soil N enrichment in Northern Forests.
- We applied Mn amendments to chronically N-fertilized soils to demonstrate the relationship between Mn availability and ligninolytic enzyme activities.
- We show the first evidence of a strong positive correlation between these two parameters for natural microbial communities in leaf litter and soils.
- We also demonstrate a shift in fungal community composition with Mn addition that helps to explain the enzyme response we observed. Specifically, we show that elevated Mn reduces the relative abundance of fungi thought to be 'weak' decomposers, referring to their poor to intermediate ability to decompose lignin (relative to white-rot fungi, which are considered 'strong' decomposers).
- We conclude that Mn plays a strong role in shaping fungal communities and that Mn amendments reduce the relative abundance of 'weak' decomposers, promoting fungal communities that have greater capacity for litter decay.
- Our results suggest that Mn limitation plays a critical role in decomposition dynamics under long-term atmospheric N deposition and represents a mechanism that may help explain reduced decomposition and soil C accumulation under this global change factor.

Table 1. Initial litter chemistry, representing the starting quality of litter inputs to the incubations. Mean concentrations (n=6) of total aromatics (sum of the %lignin, %phenols and %aromatics), C and N and litter macro and micronutrients are presented with standard errors in parentheses. Means that do not share a letter are significantly different ($P < 0.05$). The percent change from control levels was calculated for each parameter and significant increases/decreases are denoted with asterisks (*$P <$ 0.05; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; one-way ANOVA). Baseline oxidative enzyme (PER, POX) activities were also evaluated. We present these data here to demonstrate common reductions in oxidative enzymes induced by chronic N enrichment.

	Litter origin			Percent change from N0	
Component	N0	N50	N150	N50	N150
Aromatics (%)	28.6 (1.51)^{a}	26.5 (2.96)^{a}	16.1 (2.70)^{b}	-7.57	-43.68**
N (%)	1.50 (0.07)^{a}	1.73 (0.05)^{ab}	1.88 (0.07)^{b}	14.75	24.83**
C:N	33.0 (2.19)^{a}	29.6 (0.85)^{a}	27.9 (1.03)^{a}	-10.32	-15.27
Mn (mg/g)	2.96 (0.06)^{a}	1.28 (0.08)^{b}	0.83 (0.06)^{c}	-56.57***	-71.87***
Ca (mg/g)	5.74 (0.11)^{a}	4.16 (0.12)^{b}	3.08 (0.11)^{c}	-27.53***	-46.35***
K (mg/g)	1.32 (0.09)^{a}	1.19 (0.05)^{a}	1.00 (0.04)^{b}	-9.71	-24.09**
Mg (mg/g)	1.04 (0.04)^{a}	0.93 (0.03)^{ab}	0.83 (0.03)^{b}	-10.91	-20.55**
P (mg/g)	1.06 (0.04)^{ab}	1.13 (0.03)^{a}	0.93 (0.04)^{b}	6.78	-11.83
Al (mg/kg)	515 (180)^{a}	197 (19.6)^{a}	111 (11.0)^{b}	-61.73	-78.48***
B (mg/kg)	15.3 (0.58)^{a}	12.1 (0.42)^{b}	11.9 (0.22)^{b}	-20.87***	-22.50***
Cu (mg/kg)	6.29 (0.34)^{a}	6.13 (0.60)^{ab}	5.08 (0.27)^{b}	-2.62	-19.36**
Fe (mg/kg)	579 (223)^{a}	222 (24.7)^{a}	131 (12.7)^{b}	-61.64	-77.33**
Zn (mg/kg)	55.7 (2.21)^{a}	47.2 (3.10)^{b}	30.1 (0.88)^{c}	-15.18*	-45.93***
PER	5.12 (1.07)^{a}	3.66 (0.77)^{a}	3.41 (1.45)^{a}	-28.52	-33.40
POX	284 (40.2)^{a}	112 (10.1)^{b}	69.6 (32.5)^{bc}	-60.56**	-75.49***

Table 2. *In situ* soil characteristics showing the effect of chronic N on pH, exchangeable acidity and cation exchange capacity (CEC) of the O (organic) and A (mineral) soil horizons. Average values (n=6) are presented with standard errors in parentheses. Means that do not share a letter are significantly different ($P < 0.05$). The percent change from control levels was calculated for each parameter and significant increases/decreases are denoted with asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; one-way ANOVA).

	Soil origin			Percent Change from N0	
Component	N0	N50	N150	N50	N150
pH (O)	4.03 (0.10)^{a}	3.82 (0.18)^{ab}	3.38 (0.07)^{b}	-5.21	-16.13**
pH (A)	4.66 (0.07)^{a}	4.45 (0.06)^{ab}	3.93 (0.16)^{b}	-4.44	-15.59**
Acidity (O)	10.5 (0.67)^{a}	13.5 (0.73)^{b}	13.5 (0.45)^{b}	28.57*	28.57*
Acidity (A)	5.70 (0.51)^{a}	6.24 (0.58)^{a}	9.42 (0.72)^{b}	9.47	65.26**
CEC (O)	15.2 (1.15)^{a}	18.0 (0.97)^{a}	18.9 (1.30)^{a}	18.18	24.23
CEC (A)	6.22 (0.54)^{a}	6.79 (0.61)^{a}	9.94 (0.75)^{b}	9.20	59.77**

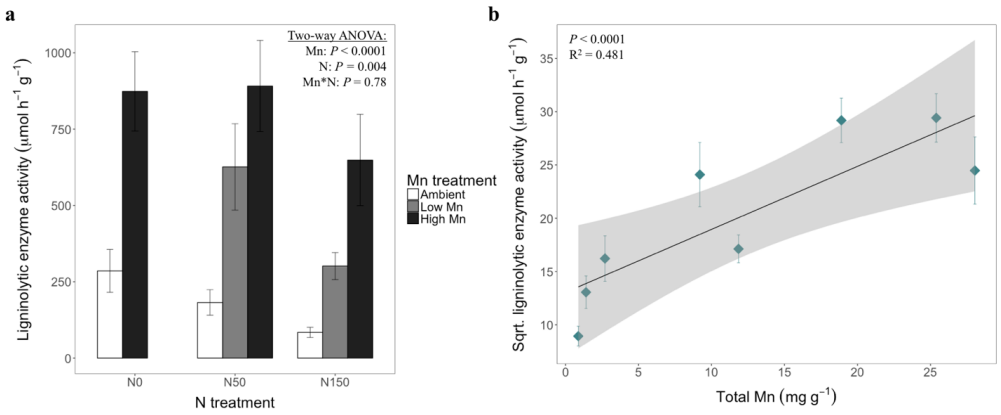

Figure 1. (**a**) Ligninolyitc enzyme activity (umol h^{-1} g^{-1}) across N and Mn treatments. The activities of peroxidase and phenol oxidase have been summed (*sensu* Ng et al. 2014) because responses to Mn amendment (% change from ambient) were highly similar. Bar color is representative of Mn treatments, where white is ambient, gray is low Mn, and black is high Mn. Two-way ANOVA results are presented for the square-root transformed enzyme data. (**b)** Linear regression showing the effect of total Mn (mg g^{-1}) on ligninolytic enzyme activity (square-root transformed), where total Mn represents the initial litter Mn concentration plus the cumulative amount of Mn added over the course of the incubation. The p-value and R^2 presented are for a linear model which includes all observations (n = 48), not average values.

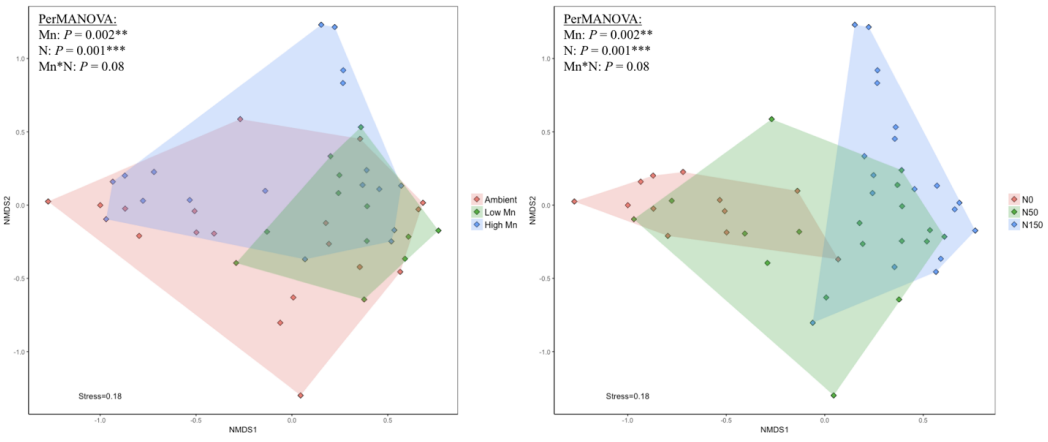

Figure 2. NMDS ordinations of fungal ITS2 data (stress = 0.18). Panel **(a)** represents the effects of Mn amendments on litter fungal community composition evaluated after 6 months of decomposition and panel **(b)** shows the effects of long-term N enrichment on fungal communities (evaluated in litter after 6 mo. of decomposition). Polygons outline the bounds of samples within each Mn **(a)** or N **(b)** treatment group; they are not representative of any statistical parameter. Polygon color represents the level of Mn or N application: control/ambient (red), intermediate (green), or high (blue) levels of each nutrient. PerMANOVA with a two-way interaction was used to test for significant differences in community composition across treatments; the same PerMANOVA results are shown in the Mn and N panels.

Future directions

- We have established an international collaboration with Dr. Marie Spohn at the University of Bayreuth in Germany and she evaluated our samples for phosphorus dynamics.
- Dr. Spohn observed that total P concentrations in the organic layer decreased on average by 15% due to N fertilization, while C:P ratios increased by 60%.
- Phosphatase activity was elevated in the N fertilized soils in all experiments by a factor of 2 to 5, and the ratio and chitinase:phosphatase activity was on average decreased by 30%, indicating that specifically phosphatase production was upregulated.
- The results imply that trees and/or microorganisms invested more N in the production of phosphatases in the N fertilized soils than in the non-fertilized controls.
- A future direction is to now compare these data with experiments in Europe.

List of Products

Peer-reviewed publications

Whalen, E. and S.D. Frey. Manganese limitation as a mechanism for reduced decomposition in soils under long-term atmospheric nitrogen deposition. In prep for Soil Biology & Biochemistry (expected submission by March 1, 2018).

Other publications

Whalen, E.D. 2017. Manganese limitation as a mechanism for reduced decomposition in soils under long-term atmospheric nitrogen deposition. M.S. Thesis. University of New Hampshire, Durham, NH.

Conference presentations

Spohn, M., C. Heuck, S.D. Frey, P. Gundersen, F. Moldan, G. Smolka, E.D. Whalen. 2017. Phosphorus and nitrogen cycling in temperate forest soils depending on long-term nitrogen inputs. Annual meeting of the German Soil Science Society, Göttingen, Germany.

Whalen, E.D. and S.D. Frey. 2017. Effect of manganese availability on decomposition under simulated nitrogen deposition. Ecological Society of America Annual Meeting, Portland, OR.

Whalen, E.D. and S.D. Frey. 2017. Effect of manganese availability on decomposition under simulated nitrogen deposition. University of New Hampshire Graduate Research Conference, Durham, NH.

Whalen, E.D. and S.D. Frey. 2017. Effect of manganese availability on decomposition under simulated nitrogen deposition. Northeastern Ecosystems Research Cooperative Meeting, Saratoga Springs, NY. Awarded Best Poster Presentation

Whalen, E.D. and S.D. Frey. 2016. Effect of manganese limitation on the decay capacity of saprotrophic fungi. MassMyco, Amherst, MA.

Whalen, E.D. and S.D. Frey. 2016. Effect of manganese limitation on decay capacity of saprotrophic fungi. Mycological Society of America Meeting, Berkeley, CA.

Whalen, E.D. and S.D. Frey. 2016. Effect of manganese limitation on the decay capacity of saprotrophic fungi. 2016. International Symposium on Microbial Ecology, Montreal, QC, Canada.