ELEVATED ATMOSPHERIC NITRATE DEPOSITION IN NORTHERN HARDWOOD FORESTS: IMPACTS ON THE MICROBIAL MECHANISMS OF PLANT LITTER DECOMPOSITION

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Natural Resources and Environment) in The University of Michigan 2004

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TABLE OF CONTENTS

ACKNOWLEDGMENTS ................................................................. ii
LIST OF TABLES ........................................................................ v
LIST OF FIGURES................................................................. vii

CHAPTER

I. INTRODUCTION ................................................................. 1
   Literature Cited .............................................................. 5

II. ATMOSPHERIC NITRATE DEPOSITION, MICROBIAL
    COMMUNITY COMPOSITION, AND ENZYME ACTIVITY
    IN NORTHERN HARDWOOD FORESTS ................. 7
   Abstract ................................................................. 7
   Introduction ............................................................. 8
   Methods ................................................................. 9
   Results ................................................................. 16
   Discussion ........................................................... 26
   Literature Cited ....................................................... 30

III. LIGNIN AND CELLULOSE DEGRADATION UNDER
    ATMOSPHERIC NITRATE DEPOSITION: POTENTIAL
    MECHANISMS OF β-GLUCOSIDASE SUPPRESSION ........ 33
   Abstract ................................................................. 33
   Introduction .......................................................... 34
   Methods ............................................................... 35
   Results ................................................................. 38
   Discussion .......................................................... 42
   Literature Cited ....................................................... 44

IV. ATMOSPHERIC NITRATE DEPOSITION AND THE
    MICROBIAL DEGRADATION OF CELLOBIOSE AND
    VANILLIN IN A NORTHERN HARDWOOD FOREST .... 46
   Abstract ................................................................. 46
   Introduction .......................................................... 48
   Methods ............................................................... 50
   Results ................................................................. 55
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Site, climatic, overstory, and soil characteristics of four sugar maple stands receiving experimental NO$_3^-$ additions</td>
<td>12</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Extracellular enzymes involved in plant litter degradation that were measured in northern hardwood ecosystems receiving experimental NO$_3^-$ additions</td>
<td>13</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Repeated measures analysis of variance for enzyme activity in the forest floor</td>
<td>17</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>The influence of N additions on mean enzyme activity. Values in parentheses are standard error of the mean (n = 36)</td>
<td>20</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Repeated measures analysis of variance for enzyme activity in mineral soil</td>
<td>22</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Repeated measures analysis of covariance for the response β-glucosidase, peroxidase, and phenol oxidase activity to chronic NO$_3^-$ additions where soil moisture is the covariate. Repeated measures for the response of phenolic concentrations to chronic NO$_3^-$ additions</td>
<td>40</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>The influence of NO$_3^-$ additions on least square means of enzyme activity and mean soluble soil phenolic concentrations. Values are standard means, averaged across N deposition treatments and time. Values in parentheses are stand error of the mean (n = 36)</td>
<td>41</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Analysis of covariance for the response β-glucosidase, peroxidase and phenol oxidase activities to chronic NO$_3^-$ additions and soil moisture content (covariate)</td>
<td>57</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>The influence of N additions on the mean recovery of $^{13}$C vanillin and $^{13}$C cellobiose in soil organic matter, microbial biomass and respiration, and dissolved organic matter. Means among various C forms with different letters indicate significant difference by Tukey's test $\alpha = 0.05$. Values in parentheses are standard error of the mean (n = 3)</td>
<td>60</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>The influence of Na, Cl, and N on mean enzyme activity and total PLFA. Within a measurement, different letters are significantly different from each other. Values in parentheses are a standard error of the mean (n=3)</td>
<td>76</td>
</tr>
</tbody>
</table>
Table 5.2. The influence of Na, Cl, and N on mean enzyme activity and total PLFA. Within a measurement, different letters are significantly different from each other. Values in parentheses are a standard error of the mean (n=3).
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Distribution of the four northern hardwood sites along a 500-km climatic gradient in Michigan, USA. These stands span the geographic distribution of sugar maple dominated northern hardwood forests in the upper Lake States region.</td>
</tr>
<tr>
<td>2.2</td>
<td>The influence of site and N addition on phenol oxidase activity in the forest floor. Means within a site with the same letter are not significantly different ($\alpha = 0.05$). Error bars indicate standard error of the mean (n = 9).</td>
</tr>
<tr>
<td>2.3</td>
<td>The influence of time and N addition on peroxidase activity in mineral soil. Means within a sampling time with the same letter are not significantly different ($\alpha = 0.05$). Error bars indicate standard error of the mean (n = 18).</td>
</tr>
<tr>
<td>2.4</td>
<td>The influence of N addition on microbial community composition in mineral soil. Error bars represent standard error of the mean (n = 36).</td>
</tr>
<tr>
<td>3.1</td>
<td>The influence of N additions on phenolic concentrations and β-glucosidase, phenol oxidase, and peroxidase activities. Enzyme activities are least square means, where soil moisture is the covariate, across time and sites. Error bars represent standard error of the mean (n = 72).</td>
</tr>
<tr>
<td>4.1</td>
<td>The influence of N additions on enzyme activity in mineral soil. Means with an * are significantly different ($\alpha = 0.05$). Error bars are one standard error of the mean (n = 3).</td>
</tr>
<tr>
<td>4.2</td>
<td>The response of bacterial and fungal PLFAs to chronic NO$_3^-$ additions in surface soils. Error bars are one standard error of the mean (n = 3). No significant difference in PLFA mole fraction existed between control and NO$_3^-$-amended soils.</td>
</tr>
<tr>
<td>4.3</td>
<td>The effect of chronic NO$_3^-$ additions on the assimilation of A) $^{13}$C-labeled vanillin and B) $^{13}$C-labeled cellobiose into PLFA microbial groups. Means with an asterisks are significantly different ($\alpha = 0.05$). Error bars are one standard error of the mean (n = 3).</td>
</tr>
<tr>
<td>6.1</td>
<td>The relative differences in plant litter decay over time as affected by lignin/N ratios and N deposition.</td>
</tr>
</tbody>
</table>
Figure 6.2. The relationship between annual decomposition rate and lignin concentration of leaf litter based on different values of actual evapotranspiration. This figure was adapted from Meentemeyer (1978).
CHAPTER I
INTRODUCTION

On a global scale, human activity has increased the atmospheric input of nitrate (NO$_3^-$) to many terrestrial ecosystems (Galloway, 1998). The impact of anthropogenic NO$_3^-$ deposition on temperate forests is a primary concern, because nitrogen (N) often limits plant growth and therefore has the potential to alter ecosystem function (Vitousek et al., 1997; Aber et al., 1998; Boxman et al., 1998; Gundersen et al., 1998). Nonetheless, the impact of chronic N deposition on terrestrial ecosystem function is still poorly understood. For example, forest ecosystem function is closely linked to microbial function, because microbial degradation of plant litter recycles nutrients that eventually become available for plant uptake. However, we do not understand how anthropogenic NO$_3^-$ deposition might alter microbial community composition and function. Because the functioning of the microbial community may be related to the types of microorganisms present (i.e. composition), a change in microbial composition could alter N and carbon (C) cycling in forested ecosystems, and hence forest net ecosystem productivity. Without this understanding, we cannot predict how the cycling of C by these forested ecosystems will respond to increases in NO$_3^-$ deposition, and in turn how such changes will modify global C cycles.

The goal of my dissertation research is to understand the consequences of anthropogenic NO$_3^-$ deposition on the metabolism of plant litter by the soil microbial community. The focus of my research is on sugar maple-dominated northern hardwood forests in northern Michigan. Since 1994, these stands have received experimental NO$_3^-$
deposition treatments. The nitrogen treatment represents three ambient NO$_3^-$ deposition levels, which is similar to the N inputs of forests near industrial regions in the northeast and Midwest of the United States. These study sites provide the experimental framework for my dissertation.

In Chapter 2, *Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests*, I examine whether microbial community composition is altered by increases in N availability in four northern hardwood forests. I hypothesize that the rapid microbial assimilation of anthropogenic NO$_3^-$ and its subsequent release as NH$_4^+$ (Zogg et al., 2000) suppress the abundance and activity of lignin-degrading fungi (Berg, 1986; Fog, 1988; Dix and Webster, 1995).

Because high levels of NH$_4^+$ can suppress soil lignin oxidation (Keyser et al., 1978), I measured the activity of extracellular enzymes responsible for the degradation of common components in plant litter. I expect that the activity of enzymes responsible for degrading lignin or lignin protected compounds will decrease as NO$_3^-$ deposition increases.

Within Chapter 2, I found that N-amended soils had significantly lower $\beta$-glucosidase and peroxidase activity. I propose that increases in N soil availability, resulting from atmospheric deposition, have reduced the depolymerization of lignocellulose such that lower amounts of cellulose would be available for microbial metabolism. Therefore, the observed reduction in $\beta$-glucosidase activity could be due to a decrease in the available cellulose. Alternatively, an excess of inorganic N could limit the ability of soil microorganism to degrade lignin, which would promote incomplete lignin degradation and potentially increase phenolics in the soil. Soluble soil phenolics
are known to inhibit the activity of β-glucosidase. In Chapter 3, *Lignin and cellulose degradation under atmospheric nitrate deposition: Potential mechanisms of β-glucosidase suppression*, I tested the idea that observed reductions in β-glucosidase activity by N additions may be due to the inhibitory effect that soluble phenolics have on this enzyme (Chapter II).

In Chapters 2 & 3, I found that the activity of β-glucosidase, peroxidase, and phenol oxidase have been reduced due to N additions. In addition, there are significant reductions in soluble soil phenolics for the study site that is the focus of this experiment (Chapter III). This would likely reduce the availability of C from lignocellulose for metabolism by the microbial community. In Chapter 4, *Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest*, I investigate the hypothesis that chronic NO$_3^-$ additions would decrease the flow of C through the heterotrophic soil food web by inhibiting the depolymerization of lignocellulose. To determine changes in the flow of C due to chronic NO$_3^-$ deposition, I conducted an incubation experiment whereby soil from one of my study sites was labeled with either $^{13}$C-cellobiose or $^{13}$C-vanillin. These labeled compounds were used because they are products of cellulose and lignin degradation, respectively. I reason that the observed reduction in β-glucosidase activity in N-amended soils (Chapter II) would limit the amount of energy obtained by the microbial community through the degradation of cellulose. Additionally, I expected that the metabolism and assimilation of vanillin would be lower, and thus, might indicate a change in microbial composition. Therefore, I expect that less $^{13}$C from cellobiose and vanillin will be recovered in microbial biomass or respired from soils experiencing N deposition.
My study sites receive ambient N deposition plus the experimentally added 30 kg N ha\(^{-1}\) y\(^{-1}\) as NaNO\(_3\) and it is possible Na\(^+\) can promote decreases in extracellular enzyme activities and microbial biomass (see Chapter II & III) apart from N additions. The ability of soil microorganisms to degrade plant litter can be suppressed by increases in Na\(^+\) and can also significantly decrease microbial biomass and the relative abundance of soil fungi (Zahran, 1997; Rietz and Haynes, 2003; Sardinha et al., 2003). Therefore, in Chapter 5, *The potentially confounding effect of increased salinity in N deposition experiments*, I address the effects of Na\(^+\), from NaNO\(_3\), on microbial community composition and function. I found that Na\(^+\) has no effect on measured microbial properties and processes and this result is similar to N deposition experiments that add N without adding Na\(^+\) (Carreiro et al., 2000).

In Chapter 6, *Global implications of increases in nitrogen deposition on plant litter decomposition*, I discuss how world-wide increases in N deposition can potential alter global patterns of plant decomposition. Increases in N deposition have the potential to decrease the decomposition of lignin and C protected by lignin (Chapters II and IV). Because lignin decomposition is a control on decay rates within a climatic region, I reason that increases in N deposition have the potential to decrease leaf litter decomposition in terrestrial ecosystems where the majority of leaf litter is high in lignin. Lastly, in Chapter 7, *Conclusions*, I summarize my major research findings from Chapters 2, 3, 4, and 5.
LITERATURE CITED


CHAPTER II

ATMOSPHERIC NITRATE DEPOSITION, MICROBIAL COMMUNITY COMPOSITION, AND ENZYME ACTIVITY IN NORTHERN HARDWOOD FORESTS

ABSTRACT

On a global scale, human activity has increased the atmospheric input of nitrate (NO$_3^-$) to many terrestrial ecosystems. Anthropogenic NO$_3^-$ may be a potent modifier of ecosystem function, especially in temperate forests that are sometimes N limited. However, the impact of chronic N deposition on soil microorganisms is still poorly understood. Nitrate entering Lake States forests is rapidly assimilated by the microbial community and it is subsequently released as NH$_4^+$. Because high levels of NH$_4^+$ inhibit the activity of lignin-degrading soil fungi, I reasoned that chronic N additions could alter the composition and function of heterotrophic microbial communities in soil, and hence the ecosystem-level processes they mediate. The hypothesis was tested in four northern hardwood ecosystems in northern Michigan, which received experimental N additions (30 kg NO$_3^-$-N ha$^{-1}$ y$^{-1}$) during the past 8 years. I quantified microbial community function by measuring the activity of extracellular enzymes involved in plant litter degradation and described microbial community composition using phospholipid fatty acid (PLFA) analysis. Chronic N additions significantly suppressed β-glucosidase activity by 24% in mineral soil and suppressed phenol oxidase activity by 35% in surface litter. I found no evidence that chronic N additions altered microbial community composition; NO$_3^-$ addition did not alter the relative abundance of bacterial, actinomycetal, fungal, or protozoan PLFAs. However, NO$_3^-$ additions significantly reduced microbial biomass by 18% relative to the control treatment. Results indicate that
N additions broadly suppressed all microbial groups, not just the activity and abundance of lignin-degrading microorganisms.

INTRODUCTION

Human activity has globally increased the amount of N entering terrestrial ecosystems from the atmosphere (Galloway, 1998). The northeastern and eastcentral United States receive the greatest amounts of N deposition in North America (Fenn et al., 1998), ranging from 2 to 16 times background levels (Galloway et al., 1984). The impact of anthropogenic N deposition on temperate forests is a primary concern, because increases in soil N availability have the potential to alter species diversity, plant community composition, and ecosystem function (Vitousek et al., 1997; Aber et al., 1998; Boxman et al., 1998; Gundersen et al., 1998).

There are reasons to expect that anthropogenic N deposition also could directly or indirectly alter the composition and function of soil microbial communities. Plant litter provides the primary energy source for heterotrophic microbial growth in soil, and changes in the amount and type of organic substrates entering soil induced by N deposition could indirectly influence the composition and function of microbial communities. Alternatively, anthropogenic N deposition could directly modify soil microbial communities by suppressing the activity of lignin-degrading fungi (Berg, 1986; Fog 1988; Dix and Webster, 1995), thus potentially lowering their abundance and diminishing the overall capacity of microbial communities to degrade lignin and other polyphenols (Carreiro et al., 2000). Such a response could alter rates of soil organic matter formation and the release of plant nutrients from litter. Relatively high levels of
NH$_4^+$ in soil suppress lignin oxidation (Keyser et al., 1978), and the rapid microbial assimilation of anthropogenic NO$_3^-$ and its subsequent release as NH$_4^+$ (Zogg et al., 2000) indicates that anthropogenic NO$_3^-$ could potentially suppress the abundance and activity of lignin-degrading microorganisms.

Nitrate deposition was experimentally manipulated in four northern hardwood stands in Michigan’s Lower and Upper Peninsula. During the past 8 years, the addition of 30 kg NO$_3^-$-N ha$^{-1}$ y$^{-1}$ has significantly increased the export of DOC and DON from these forest stands (Pregitzer et al., 2003), suggesting a change in litter chemistry, a change in microbial community function, or both. Here, I investigate the alternative that long-term NO$_3^-$ deposition has altered microbial community function and composition by suppressing fungal activity and abundance. During the course of one growing season, I measured extracellular enzyme activity to gain insight into microbial community function, and I assessed community composition using PLFA analysis. My primary objective was to determine if chronic N additions have altered the composition and function of the heterotrophic microbial community.

**METHODS**

**Study Area**

Four study sites were established along a 500-km climatic and N deposition gradient extending from northwest Upper Michigan to central Lower Michigan (Figure 2.1). The study sites encompass the geographic extent of *Acer saccharum* Marsh.-dominated, northern hardwood forest in Michigan, and they are similar in composition, history, structure, and soil development (Table 2.1; Burton et al., 1993). The soils are classified
as sandy, mixed, frigid Typic and Alfic Haplorthods (MacDonald et al., 1995). At each of the four study sites, six 30 m x 30 m experimental plots were established. Three plots served as controls receiving ambient levels of N deposition, whereas the remaining three plots received ambient N deposition plus chronic atmospheric N deposition (30 kg NO₃⁻-N ha⁻¹ yr⁻¹). The NO₃⁻ was applied six times during the growing season as small NaNO₃ pellets. I used NO₃⁻ because it is the most common form of N that is atmospherically deposited in this region (Galloway, 1998; MacDonald et al., 1995).

**Field Sampling**

In each site, I collected eight forest floor/mineral soil cores (2-cm diameter and 10-cm deep) in all six plots. I separated forest floor (Oe and Oa) from mineral horizons (A and E) in each core, and then composite the 8 forest floor samples and 8 mineral samples in each plot. This provided me with one composite forest floor sample and one composite mineral horizon sample in each control and NO₃⁻ amended plot. Collection of forest floor and mineral soil occurred in early June, mid-July, and late October 2001.

**Microbial Community Function and Composition**

**Extracellular Enzyme Analysis**

In forest floor and mineral soil, I measured the activity of enzymes responsible for the degradation of four common plant litter compounds, plus chitin and organic phosphate (Table 2.2). Enzyme activity was measured on field-fresh samples within 2 h of collection. I prepared enzyme assays by blending 1 g of forest floor or mineral soil in 150 mL of 50 mM acetate buffer at pH 5.0, which was similar to field soil pH (Saiya-
Figure 2.1. Distribution of the four northern hardwood sites along a 500-km climatic gradient in Michigan, USA. These stands span the geographic distribution of sugar-maple dominated northern hardwood forests in the upper Lake States region.
Table 2.1. Site, climatic, overstory, and soil characteristics of four sugar maple stands receiving experimental NO$_3^-$ additions.

<table>
<thead>
<tr>
<th>Forest Characteristics</th>
<th>Site A</th>
<th>Site B</th>
<th>Site C</th>
<th>Site D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Climate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latitude (N)</td>
<td>46°52'</td>
<td>45°33'</td>
<td>44°23'</td>
<td>43°40'</td>
</tr>
<tr>
<td>Longitude (W)</td>
<td>88°53'</td>
<td>84°51'</td>
<td>85°50'</td>
<td>86°09'</td>
</tr>
<tr>
<td>Mean annual precipitation, 1994-2001 (mm)</td>
<td>821</td>
<td>828</td>
<td>856</td>
<td>793</td>
</tr>
<tr>
<td>Mean annual temperature, 1994-2001 (°C)</td>
<td>4.8</td>
<td>6.1</td>
<td>6.9</td>
<td>7.6</td>
</tr>
<tr>
<td>Wet plus dry NO$_3^-$ -N deposition (kg ha$^{-1}$yr$^{-1}$)</td>
<td>3.8</td>
<td>5.8</td>
<td>7.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Wet plus dry total N deposition (kg ha$^{-1}$yr$^{-1}$)</td>
<td>6.8</td>
<td>9.1</td>
<td>11.7</td>
<td>11.8</td>
</tr>
<tr>
<td>Total N deposited (1994-2001; kg ha$^{-1}$)†</td>
<td>290</td>
<td>310</td>
<td>334</td>
<td>335</td>
</tr>
<tr>
<td><strong>Vegetation and Soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overstory age, 2001</td>
<td>94</td>
<td>88</td>
<td>89</td>
<td>93</td>
</tr>
<tr>
<td>Total basal area, 2001 (m$^2$ ha$^{-1}$)</td>
<td>35</td>
<td>33</td>
<td>33</td>
<td>36</td>
</tr>
<tr>
<td>Overstory biomass (Mg ha$^{-1}$)</td>
<td>261</td>
<td>261</td>
<td>274</td>
<td>234</td>
</tr>
<tr>
<td>Sugar maple biomass, 2001 (%)</td>
<td>91</td>
<td>86</td>
<td>79</td>
<td>71</td>
</tr>
<tr>
<td>Net N mineralization (µg N g$^{-1}$)‡</td>
<td>52</td>
<td>76</td>
<td>81</td>
<td>55</td>
</tr>
</tbody>
</table>

†The sum of naturally deposited N and added N.
‡Zogg et al., 1996;
Table 2.2. Extracellular enzymes involved in plant litter degradation that were measured in northern hardwood ecosystems receiving experimental NO$_3^-$ additions.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Phosphatase</td>
<td>Phosphate Esters</td>
</tr>
<tr>
<td>N-acetyl-glucosaminidase</td>
<td>Chitin</td>
</tr>
<tr>
<td>$\alpha$-glucosidase</td>
<td>Starch</td>
</tr>
<tr>
<td>$\beta$-glucosidase</td>
<td>Cellulose</td>
</tr>
<tr>
<td>Cellobiohydrolase</td>
<td>Cellulose</td>
</tr>
<tr>
<td>$\beta$-xylosidase</td>
<td>Hemicellulose</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>Lignin</td>
</tr>
<tr>
<td>Phenol Oxidase</td>
<td>Lignin</td>
</tr>
</tbody>
</table>
Cork et al., 2002). Activities of non-ligninolytic enzymes were fluorometrically measured in 96-well plates using methylumbelliferone-linked model substrates (Saiya-Cork et al., 2002). Forest floor and mineral soil assayed for N-acetylglucosaminidase and phosphatase activity were incubated for 0.5 h at 25°C, whereas the remaining non-ligninolytic enzymes (α-glucosidase, β-glucosidase, cellobiohydrolase, β-xylosidase) were incubated for 2 h at 25°C (Saiya-Cork et al., 2002). At the termination of the assay, I added 25-μL of NaOH (0.2 M) to each well to enhance fluorescence, which was measured using F-max fluorimeter (Molecular Devices Corp., Sunnyvale, CA). Excitation energy was 355 nm and emission was measured at 460 nm (Saiya-Cork et al., 2002).

I measured peroxidase and phenol oxidase activity using L-dihydroxyphenylalanine (Saiya-Cork et al., 2002). After incubating the samples for 18 h at 25°C in 96-well plates, the optical density (460 nm) of the oxidized reaction product was measured on a spectrophotometer (Bio-Tek Instruments, Winooski, Vt).

**PLFA Analysis**

Phospholipid fatty acid analysis was used to measure the biomass and abundance of bacteria, actinomycetes, fungi, and protozoa in control and NO₃⁻ amended plots (Vestal and White, 1989; Tunlid and White, 1992). If chronic NO₃⁻ deposition lowered the abundance of lignin degrading soil fungi, then I expected to observe a significance change in the relative abundance of fungal biomarkers. From each of the field-fresh homogenized mineral soil samples, I removed and freeze dried a 40-g subsample for PLFA analysis. I added 500 μL of a 21:0 standard (50 nmol/mL) to each sample prior to
analysis to determine recovery. I used a solution containing 10 mL of CH$_3$OH, 5 mL of CH$_3$Cl, and 4 ml of PO$_4^{3-}$ buffer to extract total lipids from 5 g of freeze-dried soil (White et al., 1979). The polar and non-polar lipids were separated by silicic acid chromatography. The separated polar lipids were subjected to an alkaline CH$_3$Cl - CH$_3$OH solution to form fatty acid methyl esters (Guckert et al., 1985). FAMEs were separated using gas chromatography and quantified using a Finnigan Delta plus mass spectrometer with a GC/C III interface (Thermofinnigan, Bremen, Germany). A 19:0 internal standard was added to each sample to determine analytical precision. The concentration of sample FAMEs was determined by a regression equation based from a standard solution containing five common FAMEs (10Me16:0, 12:0, cy19:0a, i15:0, 15:0) of known concentrations analyzed after every fifth sample. I quantified 21 PLFA biomarkers, indicative of bacteria, actinomycetes, fungi, and protozoa, and I used total PLFA as a measure of viable microbial biomass.

**Statistical Analysis**

I used a two-way, repeated-measures analysis of variance to determine the effect of NO$_3^-$ deposition on microbial community function and composition. NO$_3^-$ treatment and site were fixed effects in my model. I used Tukey’s post-hoc test to determine significant differences among means. Significance for all statistical analysis was accepted at $\alpha = 0.05$. 
RESULTS

Forest Floor Enzyme Activity

NO$_3^-$ addition had a significant main effect on phenol oxidase in the forest floor; the mean rate in NO$_3^-$ amended plots (781 ± 137 nmol g$^{-1}$h$^{-1}$; Mean ± SE) was significantly lower than that in the control treatment (1198 ± 152 nmol g$^{-1}$h$^{-1}$; Table 2.3). Time also had a significant effect on phenol oxidase activity (Table 2.3), wherein activity doubled for the October sampling date (~1600 nmol g$^{-1}$h$^{-1}$), as compared to June and July (~750 nmol g$^{-1}$h$^{-1}$). However, closer inspection of interactions between site and treatment shows that phenol oxidase was only suppressed in the two northern-most sites (sites A & B); whereas I observed little suppression of phenol oxidase in the two southern most sites (Figure 2.2). Peroxidase activity also was reduced by NO$_3^-$ addition (32% decrease), but this response was not significant (Table 2.4).

Nitrate addition had no effect on α-glucosidase, cellobiohydrolase, β-xylosidase, or acid phosphatase (Table 2.4). Except for forest floor phenol oxidase, none of the other enzymes measured in forest floor exhibited a change in activity due to NO$_3^-$ addition (Table 2.3). The activity of all enzymes significantly increased with time, making time a significant effect in my analysis (Table 2.3).

Mineral Soil Enzyme Activity

Chronic NO$_3^-$ additions also reduced microbial activity in mineral soil. There was a significant interaction between time and treatment on peroxidase activity in mineral soil, wherein NO$_3^-$ additions reduced peroxidase activity by 50% in June (Figure 2.3). However, this effect dissipated in July and October, resulting in a non-significant treatment main effect (Table 2.5). Nitrate deposition had a significant main effect on β-
Table 2.3. Repeated measures analyses of variance for enzyme activity in the forest floor.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>df</th>
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* *P < 0.05; **P < 0.01; ***P < 0.001
Table 2.3. Continued. Repeated-measures analyses of variance for enzyme activity in the forest floor.

<table>
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<tr>
<th>Source of variance</th>
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<th>peroxidase</th>
<th>phenol oxidase</th>
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Figure 2.2. The influence of site and N addition on phenol oxidase activity in the forest floor. Means within a site with the same letter are not significantly different (α = 0.05). Error bars indicate standard error of the mean (n = 9).
Table 2.4. The influence of N additions on mean enzyme activity. Values in parentheses are standard error of the mean (n = 36)

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<th>Mean Forest Floor Enzyme Activity</th>
<th>Mean Mineral Soil Enzyme Activity</th>
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<td>Fertilized (nmol g(^{-1}) hr(^{-1}))</td>
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<td><strong>α-glucosidase</strong></td>
<td>34.2 (4.6)</td>
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<tr>
<td><strong>β-glucosidase</strong></td>
<td>719.0 (53.8)</td>
<td>759.7 (76.5)</td>
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<tr>
<td><strong>Cellobiohydrolase</strong></td>
<td>218.6 (26.0)</td>
<td>233.4 (32.3)</td>
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<tr>
<td><strong>β-xylosidase</strong></td>
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<td>2094.0 (189.8)</td>
<td>2283.8 (243.9)</td>
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<tr>
<td><strong>Peroxidase</strong></td>
<td>1684.9 (279.8)</td>
<td>1147.5 (207.6)</td>
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<tr>
<td><strong>Phenol oxidase</strong></td>
<td>1197.7 (152.4)</td>
<td>780.9 (136.7)</td>
</tr>
</tbody>
</table>

* *P < 0.05
Figure 2.3. The influence of time and N addition on peroxidase activity in mineral soil. Means within a sampling time with the same letter are not significantly different ($\alpha = 0.05$). Error bars indicate standard error of the mean ($n = 18$).
Table 2.5. Repeated measures analyses of variance for enzyme activity in mineral soil.

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* P < 0.05; ** P < 0.01; *** P < 0.001
Table 2.5. Continued. Repeated-measures analyses of variance for enzyme activity in mineral soil.

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<tr>
<th>Source of variance</th>
<th>df</th>
<th>NAGase</th>
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glucosidase activity, and rates were reduced from $70 \pm 9 \text{ nmol g}^{-1}\text{h}^{-1}$ in control plots to $53 \pm 7 \text{ nmol g}^{-1}\text{h}^{-1}$ in plots receiving chronic NO$_3^-$ additions. Most of the enzymes measured in soil, except $\alpha$-glucosidase and phenol oxidase, significantly increased their activity throughout the growing season, making time a significant main effect (Table 2.5). $\beta$-glucosidase was the only enzyme in mineral soil that was significantly suppressed by chronic NO$_3^-$ additions across all times and sites (Tables 2.5). Peroxidase and phenol oxidase in soil were reduced by NO$_3^-$ additions (Table 2.4), but not significantly.

**PLFA analysis**

Chronic NO$_3^-$ additions significantly lowered total extracted PLFA, indicating a general decline in microbial biomass. Treatment was a significant main effect for total PLFA ($P = 0.039$) in that microbial biomass was suppressed in NO$_3^-$ amended plots from $233.0 \pm 20.8 \text{ nmol g}^{-1}$ in the control treatment to $190.0 \pm 15.4 \text{ nmol g}^{-1}$ in NO$_3^-$ amended plots. Time was a significant main effect ($P < 0.001$), and mean microbial biomass increased from $117 \pm 9 \text{ nmol g}^{-1}$ in June to $265 \pm 17 \text{ nmol g}^{-1}$ in October. However, I found no significant differences in total PLFA among sites ($P > 0.10$). Interactions between time and treatment, and between site and treatment, had no influence on total PLFA ($P > 0.32$). While total PLFA changed due to time, microbial community composition remained consistent. NO$_3^-$ additions did not alter the proportion of bacterial, actinomycetal, fungal, or protozoan PLFA (Figure 2.4).
Figure 2.4. The influence of N addition on microbial community composition in mineral soil. Error bars represent standard error of the mean (n = 36).
DISCUSSION

Chronic NO$_3^-$ additions have the potential to directly modify microbial community composition and function by suppressing the abundance and activity of fungi, which degrade lignin. Alternatively, chronic nitrate deposition could alter the production and biochemical composition of plant litter, and, in turn, substrate availability for heterotrophic microbial communities. My results indicate that chronic NO$_3^-$ deposition significantly lowered microbial biomass, but it did not alter the proportion of bacterial, actinomycetal, fungal, and protozoan PLFAs in soil. There was a significant suppression of phenol oxidase in litter and β-glucosidase in mineral soil, suggesting the chronic NO$_3^-$ deposition may reduce the complete degradation of lignin and cellulose by soil microbial communities. Such a response has the potential to diminish the physiological capacity of microbial communities to degrade plant litter.

Microbial biomass was significantly lower in plots receiving NO$_3^-$ additions (Figure 2.4), and this response may result from an indirect impact NO$_3^-$ additions have on microbial C acquisition, and thus energy available for heterotrophic metabolism. Support for this idea comes from the reduction in β-glucosidase we consistently observed, an important enzyme in both early and later stages of cellulose degradation (Eriksson et al., 1990). Because cellulose is the most common organic substrate produced by plants (Eriksson et al., 1990), a reduction in the metabolism of this substrate could eventually impact the rest of the soil foodweb by reducing energy enzymatically derived from cellulose degradation. For example, a reduction in β-glucosidase activity will lower the physiological capacity of the microbial community to metabolize cellobiose (Deshpande et al., 1978). Moreover, lower rates of peroxidase in forest floor suggest a decline in
ligninolytic activity, which also could reduce access to cellulose contained in lignified cell walls. Although I cannot draw causation for my results, declines in the activity of key degradative enzymes correspond with a reduction in microbial biomass.

The reduction in β-glucosidase activity may result from elevated polyphenol concentrations in soil, because high phenolic concentrations can inhibit β-glucosidase activity (Freeman et al., 2001). My results suggest that the significant 54% decrease in litter phenol oxidase and 18% decrease in soil peroxidase activity could result in incomplete lignin degradation, which, in turn, could elevate soluble phenols in soil solution. Because phenol oxidase oxidizes the benzene ring in a phenolic compound (Hammel, 1997), and peroxidase has the ability to oxidize lignin macromolecules into simple phenols (Tien and Kirk, 1983), a reduction in the activities of these enzymes has the potential to increase the concentration of soluble phenolics. Results show that peroxidase activity was reduced more than phenol oxidase activity in NO₃⁻ amended soils. Because peroxidase breaks lignin into soluble phenolics and phenol oxidase degrades soluble phenolics, then it stands to reason soluble phenolics would increase in concentrations under these circumstances. In my experiment, NO₃⁻ additions significantly increased (300%) the production of DOC from these same sites (Pregitzer et al., 2004). The increase in the leaching of DOC in our plots receiving NO₃⁻ additions (Pregitzer et al., 2004), is consistent with the idea that higher soluble phenolics result from N additions. Nonetheless, it will be necessary to quantify soluble phenolic content in our experiment to determine if our contention is correct.

These observations give rise to a potential chain of events which may cause a reduction in soil microbial biomass. An excess of inorganic N limits the ability of soil
microorganisms to degrade lignin (Burg, 1986; Fog 1988), which would promote incomplete lignin degradation and potentially the accumulation of soil organic matter (Berg and Tamm, 1991; Berg et al., 2001). A potential increase in the concentration of phenols, in turn, might inhibit β-glucosidase activity (Freeman et al., 2001). However, mean β-glucosidase activity during 1999 and 2000 at Site B, showed no response to our treatments (Saiya-Cork et al., 2002). Because DOC leaching also was higher in N amended plots at that time (Pregitzer et al., 2004), it is unlikely that elevated soluble phenolics, alone, were responsible for the reduction in β-glucosidase I observed. Alternatively, the reduction in β-glucosidase activity could be due to a decrease in available cellulose due to lower rates of lignin metabolism (Eriksson et al., 1990). However, if this scenario were important, then cellobiohydrolase and β-xylosidase activities would have likely exhibited larger declines in activity than I observed (Table 2.4). My data does not allow me to discern which alternative idea was the primary mechanism responsible for the reduction of β-glucosidase activity or whether a decline in β-glucosidase was responsible for the reduction in microbial biomass. Answering these questions will be an important part of understanding the indirect impact anthropogenic NO₃⁻-N has on northern hardwood ecosystems (See Chapter III).

One might argue that the decrease in microbial biomass was caused by an increase in the ionic strength of soil due to the application of NaNO₃. Increases in ionic strength can raise soil osmotic potential, which could reduce the availability of water to microbial communities and lower their biomass. Since the start of the fertilization, nearly 400 kg/ha of Na along with 270 kg/ha of N have been added. Increases in ionic strength > 2 dS/m can decrease acid phosphatase and α-glucosidase enzyme activities.
(Frankenberger and Bingham, 1982; Garcia and Hernandez, 1995). However, I did not find a significant decrease in acid phosphatase or $\alpha$-glucosidase in the fertilized plots (Table 4). Moreover, mean electrical conductivity was 0.14 dS/m ± 0.06 in both control and in NO$_3^-$ amended plots (10 cm) for all sites and times (unpublished data). In summary, it does not seem likely that the application of Na in the NaNO$_3$ was a determining factor in the change of microbial biomass or microbial community function (See Chapter V).

In conclusion, a substantial increase in the atmospheric deposition of NO$_3^-$ had a noticeable influence on ecosystem processes that are mediated by microbial communities. My results suggest that excess NO$_3^-$ alters microbial community function by suppressing the activity of enzymes responsible for cellulose and lignin degradation. Although I found no evidence that microbial community composition was altered by NO$_3^-$ addition, microbial biomass decreased significantly. My results suggest that anthropogenic NO$_3^-$ may alter decomposition processes in forest ecosystems by diminishing the physiological capacity of soil microbial communities to degrade plant litter.


CHAPTER III
LIGNIN AND CELLULOSE DEGRADATION UNDER ATMOSPHERIC NITRATE DEPOSITION: POTENTIAL MECHANISMS OF β-GLUCOSIDASE SUPPRESSION

ABSTRACT

The deposition of atmospheric NO₃⁻ has the potential to disrupt the cycling of C in temperate forests by suppressing enzymes responsible for plant litter degradation. Such a response has been observed for phenol oxidase activity, a lignin degrading enzyme and for β-glucosidase activity, a cellulose degrading enzyme. It is possible that a reduction in phenol oxidase activity can trigger an increase in the concentration of phenolic compounds in soil solution, which in turn are known to decrease the activity of β-glucosidase. The objective of our study was to investigate whether the inhibition of lignin degradation by atmospheric NO₃⁻ deposition would increase soluble phenolics in soil and suppress β-glucosidase activity. Our study sites consisted of four *Acer saccharum*-dominated ecosystems in northern Michigan which have received experimental N additions (30 kg NO₃⁻·N ha⁻¹·yr⁻¹) since 1994. The activity of extracellular enzymes involved in cellulose and lignin degradation were quantified 6 times throughout the growing season. Simultaneously, we measured the concentration of soluble soil phenolics. Chronic NO₃⁻ additions suppressed the activity of phenol oxidase, but did not alter the concentration of soil phenolics. I found no evidence to support the hypothesis that the suppression of phenol oxidase increased soluble phenolics, nor did it alter β-glucosidase activity and it is unlikely that β-glucosidase activity in N amended soils is influenced by soluble phenolics.
INTRODUCTION

On a global scale, anthropogenic NO$_3^-$ deposition has the potential to alter the functioning of terrestrial ecosystems (Aber et al., 1989; Fenn et al., 1998; Lovett, 1994). In historically N-limited temperate forests, chronic NO$_3^-$ deposition can increase soil N availability which has the potential to alter ecosystem-scale C cycling by decreasing the decomposition of plant litter (Berg, 1986; Fog, 1988; Vitousek and Howarth, 1991; Berg and Matzner, 1997). Recent evidence suggests that atmospheric N deposition may suppress the activity of phenol oxidase and $\beta$-glucosidase, which can decrease the decomposition of lignin and cellulose (Carreiro et al., 2000; DeForest et al., 2004). Unlike phenol oxidase, whose transcription is inhibited by high inorganic N concentrations (Eriksson and Wood, 1985), there are no direct mechanisms linking increases in N availability to a reduction in $\beta$-glucosidase activity.

Increases in N availability, by suppressing the decay of lignin, lead to increase incomplete lignin degradation such that soluble phenolics produced from lignin depolymerization might accumulate. It is plausible that increases in soluble soil phenolics could inhibit $\beta$-glucosidase activity (Sarkar and Burns, 1983). For example, wetlands with low levels of oxygen have low phenol oxidase activity because oxygen is required for this enzyme to function (McLatchy and Reddy, 1998). These soils often have high concentrations of soluble phenolic compounds because the lack of oxygen prevents phenol oxidase from degrading lignin and other polyphenolics. In addition, decomposition in anaerobic soils is limited, in part, because phenolic compounds can lower the activity of hydrolytic enzymes (Vuorinen and Sacharinen, 1996), especially $\beta$-glucosidase (Freeman et al., 2001). $\beta$-glucosidase is one of an important group of
hydrolytic enzymes responsible for cellulose degradation. The mechanism for inhibition of β-glucosidase by phenols may be copolymerization of β-glucosidase resulting in immobilization of the enzyme (Sarkar and Burns, 1983). Freeman et al. (2001) observed that a decrease in phenol oxidase activity corresponded with an increase in phenolic concentrations, as well as decreased β-glucosidase activity (Freeman et al., 2001). I hypothesize that an increase inorganic N availability, due to atmospheric deposition, will elicit a similar response in well drained upland soils. It is known that experimental NO$_3^-$ deposition can increase the production of dissolved organic carbon (Yano et al., 2000; Pregitzer et al., 2004), which is consistent with higher phenolic concentrations in the soil. Therefore, N additions, could suppress phenol oxidase activity, which could increase phenolic concentrations and cause a reduction in β-glucosidase activity.

I tested the hypothesis that previously observed reductions in β-glucosidase activity in soil receiving atmospheric NO$_3^-$ deposition corresponds with high phenolic concentrations (DeForest et al., 2004). I expected to observe an inverse relationship between β-glucosidase activity and phenolic concentrations in plots receiving ambient and experimental NO$_3^-$ deposition treatment. To test this hypothesis, soil enzyme activity and phenolic concentrations were measured in a northern hardwood forest receiving ambient and long-term experimental additions of NO$_3^-$ deposition.

**METHODS**

**Study Area and Field Sampling**

To determine the extent of soil phenolic inhibition of β-glucosidase activity, we used soil from four *Acer saccharum* Marsh.-dominated northern hardwood forests in
Michigan with similar stand composition, history, structure, and soil development. These study sites are located along a 500-km climatic and N deposition gradient and represent a common forest ecosystem found throughout the Great Lake States. Within each of the study sites, six 30 m x 30 m experimental plots were established. Three plots receive ambient levels of N deposition and serve as controls. Since 1994, the remaining plots receive 30 kg NO$_3^-$-N ha$^{-1}$ yr$^{-1}$ in addition to ambient deposition. The amount and type of N added to N amended plots is similar to N inputs of forests near industrial regions of the northeastern United States (Fenn et al., 1998).

I sampled the soil six times during the 2002 growing season (May to September). On each sampling date, I collected eight soils cores (2-cm diameter and 10-cm deep) in all six plots from each of the four study sites. Soil cores contained the Oe and Oa forest floor horizons (1-3 cm) and surface mineral soil (7-9 cm) of A and E horizon. The eight soil samples from each plot were composited and all subsequent analysis were performed on the composited samples. Samples were kept on ice and processed within 48 h of field collection.

**Enzyme Activity Analysis**

I measured the activities of β-glucosidase, a cellulytic enzyme, and the lignin-degrading enzymes peroxidase and phenol oxidase. For each composited soil sample, I used three sub-samples of each composite sample for each enzyme assay. I prepared enzyme assays by mixing 2 g of soil in 150 mL of acetate buffer (50 mM, pH 5). β-glucosidase activity was fluorometrically measured in 96-well plates using 4-methylumbelliferone-β-D-glucoside as a substrate (Saiya-Cork et al., 2002). At the
termination of the assay, I added 25 µL of NaOH (0.2 M) to each well to enhance fluorescence, which was measured using an F-max fluorometer (Molecular Devices Corp., Sunnyvale, CA); excitation energy was 355 nm and emission was measured at 460 nm. I measured peroxidase and phenol oxidase activity using L-dihydroxyphenylalanine (Saiya-Cork et al., 2002). After incubating the samples at 25°C for 18 h in 96-well plates, the optical density (460 nm) of the oxidized reaction product was measured on a spectrophotometer (Bio-Tek Instruments, Winooski, Vt). Enzyme activities are expresses as nmol g⁻¹ soil h⁻¹.

**Phenolic Concentrations**

I determined soluble soil phenolic concentrations by comparing soil solution with a standard phenolic mixture. This standard mixture contained 50 µmol L⁻¹ each of ferulic, p-coumaric, p-hydroxybenoic, vanillic, and syringic acid. I adjusted the standard mixture to pH 6 and then adjusted the mixture to have a graduated range of phenolic concentrations from 3 µmol L⁻¹ to 250 µmol L⁻¹, which encompasses a common range of phenolic concentrations in soil (Sposito, 1989). To extract the soil phenolics, I agitated 5 g of soil and 25 mL of water on a orbital-action shaker for 18 h. The samples were centrifuged and the supernatant was filtered through a 0.45 µm nylon filter (Ohno and First, 1998). I combined 5 mL of filtered supernatant, or standard mixture, with 0.75 mL of Na₂CO₃ (1.9 M) and 0.25 mL of Folin-Ciocalteu reagent (Ohno and First, 1998). Deionized water was used as a negative control. After 1 h incubation at 25 °C in the dark, the absorbance of mixture was measured at 750 nm using a Spectronic 20 Genesis spectrophotometer (Spectronic Instruments, Rochester, NY). A regression of absorbance
and standard phenolic concentration was determined \((r^2 = 0.99)\), and the absorbance of samples were adjusted to represent phenolic concentrations \(\mu\text{mol C g}^{-1}\) of soil C.

**Statistical Analysis**

I used a two way, repeated-measures analysis of covariance, in which soil water was the covariate. In a previous study, I found that soil water had a significant influence on enzyme activity (Chapter IV). Therefore, all values presented for enzyme activities are least square means adjusted for differences in soil water content. I explored the relationship between enzyme activity and phenolic concentrations using regression analysis. Significance for all statistical analysis was accepted at \(\alpha = 0.05\).

**RESULTS**

Nitrate additions significantly decreased the activity of phenol oxidase, when averaged across sampling dates and sites (Figure 3.2). However, \(\beta\)-glucosidase and peroxidase activities in N-amended plots were similar to the control; which is in contrast to previous work (Table 3.1; Chapter II). Sampling date had a significant \((p < 0.001)\) influence on \(\beta\)-glucosidase and peroxidase activity, with enzyme activity peaking in late June. Study sites also were a significant \((p < 0.01)\) influence on enzyme activity, but there was not a clear pattern among sites (Table 3.2). Mean phenolic concentrations across time, site and treatment were \(2240 \pm 80 \ C \mu\text{mol g}^{-1}\) \(\text{C (Mean } \pm \text{ SE)}\). Phenolic concentrations were similar between \(\text{NO}_3^-\) amended and control soils (Table 3.1; Figure 3.1), whereas
Figure 3.1. The influence of N additions on phenolic concentrations and $\beta$-glucosidase, phenol oxidase, and peroxidase activities. Enzyme activities are least square means, where soil moisture is the covariate, across time and sites. Error bars represent standard error of the mean ($n = 72$).
Table 3.1. Repeated measures analysis of covariance for the response β-glucosidase, peroxidase, and phenol oxidase activity to chronic NO$_3^-$ additions where soil moisture is the covariate. Repeated measures for the response of phenolic concentrations to chronic NO$_3^-$ additions.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>β-glucosidase p-value</th>
<th>Peroxidase p-value</th>
<th>Phenol Oxidase p-value</th>
<th>Phenolic Conc. p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within Subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.441</td>
<td>0.089</td>
<td>0.007</td>
<td>0.536</td>
</tr>
<tr>
<td>Site</td>
<td>3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.006</td>
<td>0.026</td>
</tr>
<tr>
<td>Treatment x Site</td>
<td>3</td>
<td>0.074</td>
<td>0.450</td>
<td>0.112</td>
<td>0.700</td>
</tr>
<tr>
<td>Between Subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.059</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>5</td>
<td>0.120</td>
<td>0.494</td>
<td>0.036</td>
<td>0.168</td>
</tr>
<tr>
<td>Time x Site</td>
<td>15</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time x Treatment x Site</td>
<td>15</td>
<td>0.058</td>
<td>0.960</td>
<td>0.412</td>
<td>0.843</td>
</tr>
</tbody>
</table>
Table 3.2. The influence of NO$_3^-$ additions on least square means of enzyme activity and mean soluble soil phenolic concentrations.† Values are standard means, averaged across N deposition treatments and time.

<table>
<thead>
<tr>
<th>Site</th>
<th>β-glucosidase (nmol g$^{-1}$ h$^{-1}$)</th>
<th>Peroxidase (nmol g$^{-1}$ h$^{-1}$)</th>
<th>Phenol Oxidase (nmol g$^{-1}$ h$^{-1}$)</th>
<th>Phenolic Conc. ($\mu$mol C g$^{-1}$ soil C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>85.1 c (5.7)</td>
<td>1649.2 a (145.2)</td>
<td>154.7 a (19.3)</td>
<td>3066.0 a (174.8)</td>
</tr>
<tr>
<td>B</td>
<td>126.1 a (8.5)</td>
<td>471.3 b (184.4)</td>
<td>33.5 b (27.3)</td>
<td>2256.4 b (146.2)</td>
</tr>
<tr>
<td>C</td>
<td>92.5 c (6.8)</td>
<td>1622.0 a (161.2)</td>
<td>161.9 a (21.7)</td>
<td>1939.4 c (109.7)</td>
</tr>
<tr>
<td>D</td>
<td>115.8 b (6.7)</td>
<td>1651.7 a (158.8)</td>
<td>175.7 a (21.2)</td>
<td>1691.2 d (93.9)</td>
</tr>
</tbody>
</table>

† Values in parentheses are stand error of the mean (n = 36)
sampling date and study sites had significant influences on phenolic concentrations (Table 3.1). Phenolic concentrations were highest at Site A and concentrations significantly decreased with latitude (Table 3.2). The amount of phenolics in the soil were not negatively related with $\beta$-glucosidase activity ($p = 0.94$).

**DISCUSSION**

Significant decreases in phenol oxidase activity from NO$_3^-$ additions did not elevate soluble soil phenolics and I found no change in $\beta$-glucosidase activity. Moreover, when phenolic concentrations were the greatest (e.g. 3500 C$\mu$mol g$^{-1}$ C), there were no significant reductions in $\beta$-glucosidase activity. Although, previous research demonstrates that soil phenolics can significantly suppress $\beta$-glucosidase activity (Freeman et al., 2001), it is possible that the modest levels of phenolics present in these soils were insufficient to inhibit $\beta$-glucosidase activity. Therefore, decreases in $\beta$-glucosidase activity due to NO$_3^-$ additions observed previous work (Chapter II) are unlikely caused by increases in soil phenolic concentrations.

The impact of phenolic concentrations on the inhibition of $\beta$-glucosidase activity in upland soils appears to be weak and the mechanism regulating $\beta$-glucosidase activity in these soils is poorly understood. In wetland soils, reductions in $\beta$-glucosidase activity can be attributed to high levels of phenolics (Freeman et al., 2001), but such a mechanisms cannot be applied to upland soils. Alternatively decreases in $\beta$-glucosidase activity observed in Chapter II due to NO$_3^-$ additions could result from a reduction in available cellulose (DeForest et al., 2004). This is supported by a reduction in lignolytic activity and it is hypothesized that a reduction in lignin degradation would also reduce the
decay of lignified cellulose, thus lower amounts of cellulose available for microbial metabolism. However, significant reductions in phenol oxidase did not elicit a reduction in β-glucosidase activity (Figure 3.1). In the present study, it appears that reductions in phenol oxidase were not sufficient to reduce the availability of cellulose, whereas reductions in peroxidase activity were sufficient to reduce the availability of lignified cellulose (DeForest et al., 2004). While these enzymes work in concert to fully degrade lignin (Hatakka, 2001), it is possible that reductions in peroxidase effects cellulose availability more than reductions in phenol oxidase.

Results indicate that chronic NO$_3^-$ additions suppressed the activity of phenol oxidase, but this decrease did not correspond with a reduction in phenolic concentrations (Figure 3.2). It is apparent that phenol oxidase activity is not a major influence on concentrations of soluble soil phenolics in these soils. Therefore, I must reject the hypothesis that chronic NO$_3^-$ additions can increase phenolic concentrations. However, if N additions did increase the production of soil phenolics, then the source of increased production of DOC observed in N amended plots (Pregitzer et al., 2004) remains unclear.

In conclusion, decreases in phenol oxidase activity did not result in higher concentrations of soluble soil phenolics in soils receiving experimental atmospheric NO$_3^-$ deposition. Even when phenolic concentrations were high, β-glucosidase activity was unaffected. This suggests that high concentrations of soluble phenolic may not be a significant inhibitor of β-glucosidase activity in upland forest soils. A decrease in celllobiose availability due to lower lignin degradation may be the primary mechanism responsible for observed decreases in β-glucosidase activity in previous work (Chapter II).
LITERATURE CITED


CHAPTER IV
ATMOSPHERIC NITRATE DEPOSITION AND THE MICROBIAL 
DEGRADATION OF CELLOBIOSE AND VANILLIN IN A NORTHERN 
HARDWOOD FOREST

ABSTRACT

Human activity has increased the amount of N entering terrestrial ecosystems 
from atmospheric NO₃⁻ deposition. High levels of inorganic N are known to suppress the 
expression of phenol oxidase, an important lignin-degrading enzyme produced by white-
rot fungi. I hypothesized that chronic NO₃⁻ addition would decrease the flow of C 
through the heterotrophic soil food web by inhibiting phenol oxidase and the 
depolymerization of lignocellulose. This would likely reduce the availability of C from 
lignocellulose for metabolism by the microbial community. I tested this hypothesis in a 
mature northern hardwood forest in northern Michigan, which has received experimental 
atmospheric N deposition (30 kg NO₃⁻-N ha⁻¹ y⁻¹) for nine years. In a laboratory study, I 
amended soils with ¹³C-labeled vanillin, a monophenolic product of lignin 
depolymerization, and ¹³C-labeled cellobiose, a disaccharide product of cellulose 
degradation. I then traced the flow of ¹³C through the microbial community and into soil 
organic carbon (SOC), dissolved organic carbon (DOC), and microbial respiration. I 
simultaneously measured the activity of enzymes responsible for lignin (phenol oxidase 
and peroxidase) and cellobiose (β-glucosidase) degradation. Nitrogen deposition reduced 
phenol oxidase activity by 83% and peroxidase activity by 74% when compared to 
control soils. In addition, soil C increased by 76%, whereas microbial biomass decreased 
by 68% in NO₃⁻ amended soils. ¹³C cellobiose in bacterial or fungal PLFAs was 
unaffected by NO₃⁻ deposition; however, the incorporation of ¹³C vanillin in fungal PLFA
extracted from NO$_3^-$ amended soil was 82% higher than in the control treatment. The recovery of $^{13}$C vanillin and $^{13}$C cellobiose in SOC, DOC, microbial biomass, and respiration was not different between control and NO$_3^-$ amended treatments. Chronic NO$_3^-$ deposition has stemmed the flow of C through the heterotrophic soil food web by inhibiting the activity of ligninolytic enzymes, but it increased the assimilation of vanillin into fungal PLFAs.
INTRODUCTION

Anthropogenic NO$_3^-$ deposition is a global process that has increased N inputs to terrestrial ecosystems (Aber et al., 1989; 1998; Lovett, 1994; Fenn et al., 1998). Chronic deposition of NO$_3^-$ will likely augment N availability in temperate forests, where N typically limits plant growth (Vitousek and Howarth, 1991). For example, northern hardwood forests in northeastern United States are receiving the greatest amounts of N deposition in North America (Fenn et al., 1998), ranging from 2 to 16 times background levels (Galloway et al., 1994). Increases in N availability in temperate forests is a primary concern, because it has the potential to alter ecosystem-scale C cycling by decreasing the decomposition of plant litter (Berg, 1986; Fog, 1988; Berg and Matzner, 1997).

Mounting evidence indicates that decreases in plant litter decomposition due to chronic N deposition can be partially explained by the inhibitory effect excess N has on extracellular enzymes that mediate microbial decomposition of plant litter (Berg, 1986; Carreiro et al., 2000; DeForest et al., 2004). Specifically, the rapid microbial assimilation of anthropogenic NO$_3^-$ and its subsequent release as NH$_4^+$ (Zogg et al., 2000) has the potential to suppress the activity of white rot fungi, due to the inhibition of their ligninolytic enzymes by high levels of NH$_4^+$ (Berg, 1986; Fog, 1988). Because lignin protects plant tissue from decomposition, a reduction in lignin degradation could reduce the quantities C available for metabolism by other heterotrophic microorganisms. For example, cellulose is a major substrate for heterotrophic metabolism; however, the decomposition of plant litter containing cellulose associated with lignin is suppressed in soils with high N availability (Berg, 1986; Fog, 1988). Therefore, anthropogenic N
deposition could potentially reduce the decomposition of lignin and other plant cell wall components.

The extent to which anthropogenic NO$_3^-$ deposition has altered the flow of C through the microbial foodweb is poorly understood. We are unsure how the suppression of lignin-degrading fungi will alter soil microbial communities and, thus, the flow of C in soil. I have observed that chronic NO$_3^-$ addition can reduce microbial biomass, indicating a reduction in substrate availability (DeForest et al., 2004). Furthermore, NO$_3^-$ additions also can suppress enzymes responsible for cellobiose ($\beta$-glucosidase) and lignin (peroxidase and phenol oxidase) degradation, which suggests the microbial community has a reduced capacity to degrade recalcitrant plant litter (DeForest et al., 2004)

Based upon this knowledge, I investigated the hypothesis that NO$_3^-$ additions, by reducing the depolymerization of lignocellulose, will also reduce the ability of soil microorganisms to metabolize and assimilate cellobiose and vanillin. Cellobiose is a disaccharide produced during the enzymatic hydrolysis of cellulose, which can be further metabolized to glucose by $\beta$-glucosidase (Eriksson and Wood, 1985). I reason that the observed reduction in $\beta$-glucosidase activity in N amended soils would limit the amount of energy obtained by the microbial community through cellulose degradation. Vanillin is a monophenolic compound that is a common product of lignin depolymerization, a process mediated by phenol oxidase and peroxidase (Pearl, 1967). The reduction in these ligninolytic enzymes would reduce the amount of monophenolic compounds available in the soil. Thus, NO$_3^-$ amended soils have the potential to limit the amount of C and energy obtained by white-rot fungi that use lignin as a C and energy source. My objective is to determine if NO$_3^-$ additions is reducing the flow of C from cellulose and
lignin through the soil food web. To determine changes in the flow of C due to NO₃⁻ deposition, I used ¹³C-cellobiose and ¹³C-vanillin and traced the flow of ¹³C through the microbial community and into common pools of C in the soil.

**METHODS**

**Study site and soil sampling**

My study site was located (N 45°33’ by W 84°51’) in a mature northern hardwood forest in northern, Lower Michigan, USA. This stand is dominated by sugar maple (*Acer saccharum* Marsh.) and is representative of northern hardwood forest in the upper Great Lake States. Total basal area is 33 m² ha⁻¹ and sugar maple represents 86% of the biomass. The mean annual temperature is 6.1°C and receives 828 mm of precipitation annually. The soil is classified as sandy, mixed, frigid Typic Haplorthods (MacDonald et al., 1995). Within this study site, six 30 m x 30 m experimental plots were established. Three plots serve as controls, whereas the remaining three plots receive ambient N deposition plus 30 kg NO₃⁻-N ha⁻¹ yr⁻¹. Since 1994, NaNO₃ pellets have been applied six times during the growing season. Over the 2002 growing season, extractable total inorganic N in control soils is 10.1 ± 1.3 µg N g⁻¹ (Mean ± SE), whereas NO₃⁻ amended soil contain 17.3 ± 1.2 µg N g⁻¹ (Chapter III). Ammonium represents 76% of extractable inorganic N in control soils and 61% in NO₃⁻ amended soils. In late June, 2002, we collected twelve mineral soil cores (2-cm diameter and 10-cm deep; A and E horizons) in all six plots. In each plot, soil samples were composited and placed in plastic bags, kept at 4°C, and processed within 24 h of field collection.
Soil enzyme analysis

I measured the activities of β-glucosidase, an enzyme responsible for the degradation of celllobiose, and the lignin degrading enzymes peroxidase and phenol oxidase. I used three analytical replicates for each enzyme for each plot. I prepared enzyme assays by mixing 2 g of soil in 150 ml of acetate buffer (50 mM, pH 5). β-glucosidase activity was fluorometrically measured in 96-well plates using 4-methylumbelliferone-β-D-glucoside as a substrate (Saiya-Cork et al., 2002). At the termination of the assay, we added 25 µl of NaOH (0.2 M) to each well to enhance fluorescence, which was measured using a F-max fluorometer (Molecular Devices Corp., Sunnyvale, CA); excitation energy was 355 nm and emission was measured at 460 nm. I measured peroxidase and phenol oxidase activity using L-dihydroxyphenylalanine (Saiya-Cork et al., 2002). After incubating the samples at 25°C for 18 h in 96-well plates, the optical density (460 nm) of the oxidized reaction product was measured on a spectrophotometer (Bio-Tek Instruments, Winooski, Vt).

13C Labeling and Incubation

I labeled field-fresh soil (12 g) from each control and N-amended plot with 13C celllobiose (70 nmol 13C g⁻¹) or 13C vanillin (580 nmol 13C g⁻¹). The celllobiose (atom % 27.5) was labeled with one 13C with 99% purity (Omicron Biochemicals, Inc., South Bend, IN), whereas the vanillin (atom % 42.6) was 13C ring-labeled with a 99% purity (Cambridge Isotope, Andover, MA). The 13C substrate was delivered in 1 ml of deionized water, which did not cause saturation. I also amended soil subsamples from each plot with 1 ml of deionized water without any 13C substrate to quantify the initial 13C abundance in each
soil pool. For each control and N-amended plot, two analytical replicates of each of the
three treatments (\(^{13}\)C cellobiose, \(^{13}\)C vanillin, deionized water) were used to measure
microbial respiration and subsequently were analyzed for phospholipid fatty acids. In
addition, for each control and N amended plot, another two analytical replicates of each
plot were used for sequential extractions to follow \(^{13}\)C into dissolved organic carbon,
微生物 biomass, and soil organic C pools. The purpose for using two different sets of
soils was to ensure an even distribution of labeled substrate while providing enough soil
for all the analysis. In all, the 72 \(^{13}\)C labeled and 36 non-labeled soil samples were
incubated for 48 h at 25\(^{\circ}\)C.

\(^{13}\)C Microbial respiration

On one half of the replicates, I determined microbial respiration by placing the samples
(12 g field-fresh) in 50 ml vials within 1 qt mason jars. These jars had air-tight lids
containing rubber septa for gas sampling. Using an air-tight syringe, I withdrew a 30 ml
aliquot of headspace (940 ml) from each sample. Headspace CO\(_2\) concentrations and
\(\delta^{13}\)C were determined by introducing headspace air into a Finnigan Delta Plus isotope
ratio mass spectrometer (IRMS) with a Conflo II interface (Thermofinnigan, Bremen,
Germany). I determine the initial headspace concentration of CO\(_2\) and \(\delta^{13}\)C from a sealed
jar that contained no soil. The headspace concentration of CO\(_2\) and \(\delta^{13}\)C was measured
once after the 48 h incubation period. The rate of respiration was calculated by using the
time-linear change in headspace CO\(_2\) (\(\mu\)mol CO\(_2\) h\(^{-1}\)) during the 48 h incubation. I
determined the amount of substrate \(^{13}\)C respired by multiplying the moles of C respired
by the atom percent excess (APE) \(^{13}\)C. APE was calculated by subtracting natural
abundance atom % $^{13}$C from atom % $^{13}$C. The natural abundance of $^{13}$C respired was
determined from the samples amended with deionized water.

$^{13}$C Phospholipid fatty acid analysis

After determining microbial respiration, I freeze dried this soil (~10 g) for PLFA
analysis. To determine analytical recovery, I added 500 µl of an 11:0 and 21:0
phospholipid standards (50 pmol ml$^{-1}$; 99% purity) to each soil sample (Sigma Co., St.
Louis, MO). I used a solution containing 10 ml of CH$_3$OH, 5 ml of CH$_3$Cl, and 4 ml of
PO$_4^{3-}$ buffer to extract total lipids from 5 g of freeze-dried soil (White et al., 1979). The
polar and non-polar lipids were separated by silicic acid chromatography. The separated
polar lipids were subjected to an alkaline CH$_3$Cl-CH$_3$OH solution to form fatty acid
methyl esters (FAMEs; Guckert et al., 1985). FAMEs were separated using gas
chromatography and quantified using a Finnigan Delta plus mass spectrometer with a
GC/C III interface (Thermofinnigan, Bremen, Germany). The GC column was an
Agilent HP-1 column (50 m x 0.200 mm) and samples passed through the column with
constant pressure and splitless. The initial temperature was 60 °C and held for 2 min,
which increased 10 °C min$^{-1}$ until 150 °C then increased 3 °C min$^{-1}$ to 312 °C. The output
of this process was a chromatogram where PLFA identity was establish from peak
retention time; the area below the peak was used to determine C mass. My analysis
allowed me to quantify the $\delta^{13}$C of each peak. The mass and $\delta^{13}$C of each of the FAMEs
was determined by a regression equation based on a standard solution that contained five
common FAMEs (10Me16:0, 12:0, cy19:0a, i15:0, 15:0) of known mass of C and $\delta^{13}$C.
This FAME mix was analyzed after every fifth sample. The excess $^{13}$C recovered in each
PLFA was determined as the product of the mass of PLFA (nmol C g\(^{-1}\)), the mass of soil (g), and APE.

I organized specific PLFAs into three microbial groups: bacterial PLFAs, fungal PLFAs, and PLFAs occurring in both bacteria and fungi. Bacteria specific PLFAs are i15:0, a15:0, i16:0, 10Me16:0, a17:0, i17:0, cy17:0, cy19:0a, 16:1\(\omega9c\), 18:1\(\omega7t\), and 18:1\(\omega5c\) (Asselineau, 1962; Gitaitis & Beaver, 1990; Annous et al., 1999; Grayston et al., 2001). Fungal specific PLFAs are 18:1\(\omega9c\) and 18:2\(\omega6\) (Federle et al., 1986; Feofilova et al., 1998). PLFAs that occur in bacteria and fungi include 14:0, 16:0, 18:0, 16:1\(\omega7c\), 18:1\(\omega7c\), and 16:1\(\omega5c\) (Stahl & Klug, 1996; Funtikova et al., 1998; Feofilova et al., 1998; Schie & Young, 1998; Annous et al., 1999; Olsson, 1999). I used the sum of all these PLFAs to calculate total PLFA, a measure of living microbial biomass.

\textbf{\(^{13}\text{C} \text{Sequential Extractions}\)}

On the remaining group of replicates, I extracted DOC with 40 ml of K\(_2\)SO\(_4\) (0.5 M) and passed the extract through a 1.2 µm glass fiber filter from ~10 g oven dried soil. The remaining soil and filter were fumigated with CH\(_3\)Cl for 5 days, after which microbial C was extracted with 40 ml of K\(_2\)SO\(_4\) (0.5 M). A 1 ml subsample of DOC and microbial C extracts were then evaporated, and the C content and \(\delta^{13}\text{C}\) were determined by mass-spectrometry using a CE Elantech NC2500 interfaced to a Finnigan Delta Plus IRMS. The remaining soil was freeze-dried and pulverized with a ball mill prior to determining soil organic C content and \(\delta^{13}\text{C}\). Carbon content and \(\delta^{13}\text{C}\) was determined by mass-spectrometry as described above. The excess of \(^{13}\text{C}\) recovered from DOC, microbial C,
and SOM was determined by multiplying the product of C (µmol C g\(^{-1}\)) by the oven-dry mass of soil (g) and its APE.

**Statistical Analyses**

I used an analysis of variance to determine the effect of chronic NO\(_3^-\) additions on enzyme activity, PLFA, and % recovery of \(^{13}\)C substrates into C pools. Because moisture content has a major influence on enzyme activity, I used moisture content as a covariate in my analysis of enzyme activity. I used Tukey’s post-hoc test to determine significant differences among means. Significance for all statistical analyses was accepted at \(\alpha = 0.05\).

**RESULTS**

**Soil Enzyme Analysis and Organic C Content**

Nitrate additions consistently suppressed the activity of enzymes responsible for degrading cellobiose and lignin (Figure 4.1). I found that \(\beta\)-glucosidase activity was suppressed by 21% in NO\(_3^-\) amended soils, but not to a significant degree. NO\(_3^-\) additions significantly suppressed peroxidase activity by 74% and phenol oxidase activity by 83% (Figure 4.1). Moisture content was a significant covariate for \(\beta\)-glucosidase, peroxidase, and phenol oxidase (Table 4.1). NO\(_3^-\) amended total soil C was significantly (\(p < 0.001\)) higher than control soils. Control soils contained 2.2 ± 0.1 µmol C g\(^{-1}\) (mean ± SE) and NO\(_3^-\) amended soil has 3.9 ± 0.4 µmol C g\(^{-1}\).
Figure 4.1. The influence of N additions on enzyme activity in mineral soil. Means with an * are significantly different ($\alpha = 0.05$). Error bars are one standard error of the mean ($n = 3$).
Table 4.1. Analysis of covariance for the response $\beta$-glucosidase, peroxidase, and phenol oxidase activities to chronic NO$_3^-$ additions and soil moisture content (covariate).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>$\beta$-glucosidase</th>
<th>Peroxidase</th>
<th>Phenol Oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$ addition</td>
<td>1</td>
<td>0.802</td>
<td>0.082</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO$_3^-$ addition x soil moisture</td>
<td>1</td>
<td>0.567</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Microbial Biomass and Community Composition

Microbial biomass, as indicated by total PLFA, was significantly (p = 0.012) lower in N amended soils (4.6 ± 0.6 nmol PLFA µg soil C⁻¹) than control soils (7.8 ± 1.0 nmol PLFA µg soil C⁻¹). Mean bacteria PLFAs were significantly reduced by 75%, whereas fungal PLFAs were significantly reduced by 48%. Nitrate addition did not significantly change the bacteria:fungal PLFA ratio. Directly extracted microbial C in control soils was 832 ± 58 (mmol C µg soil C⁻¹), compared to 521 ± 13 (mmol C µg soil C⁻¹) in N amended soils. The 68% reduction of total PLFA coincides with a 37% reduction in microbial C in N amended soils. NO₃⁻ addition did not have any observed effect on the microbial community composition as determined by the mole fractions of specific bacterial and fungal PLFAs (Figure 4.2).

¹³C Substrate Assimilation and Processing

I found no treatment effect on the mean percent recovery of ¹³C vanillin or ¹³C cellobiose in SOC, microbial biomass, respiration, or DOC (Table 4.2). The majority of ¹³C vanillin (~60%) was recovered in SOC, whereas the majority of recovered ¹³C cellobiose was distributed evenly between microbial biomass (~22%) and SOC (~18%; Table 2). Almost three times more ¹³C from cellobiose was recovered in microbial biomass than from vanillin, but ¹³C recovered in microbial respiration was similar between cellobiose and vanillin (Table 4.2). The mean total recovery of ¹³C vanillin was ~73%, whereas ¹³C cellobiose was ~53%.

N additions significantly increased the incorporation of ¹³C vanillin into 10:Me16:0, cy17:0, and 18:2ω6. I recovered significantly more ¹³C vanillin in fungal PLFA in NO₃⁻ amended soils, as compared to soil from the control treatment (Figure 4.3).
Figure 4.2. The response of bacterial and fungal PLFAs to chronic NO₃⁻ additions in surface soils. Error bars are one standard error of the mean (n = 3). No significant difference in PLFA mole fraction existed between control and NO₃⁻-amended soils.
Table 4.2.
The influence of N additions on the mean recovery of $^{13}$C vanillin and $^{13}$C cellobiose in soil organic matter, microbial biomass and respiration, and dissolved organic matter. Means among various C forms with different letters indicate significant difference by Tukey's test $\alpha = 0.05$. Values in parentheses are standard error of the mean ($n = 3$).

<table>
<thead>
<tr>
<th></th>
<th>$^{13}$C Vanillin</th>
<th></th>
<th>$^{13}$C Cellobiose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Mean)</td>
<td>N Amended (Mean)</td>
<td>Control (Mean)</td>
</tr>
<tr>
<td>Soil organic matter</td>
<td>64.4a (9.9)</td>
<td>53.6a (5.0)</td>
<td>20.3a (6.8)</td>
</tr>
<tr>
<td>Microbial biomass</td>
<td>6.6a (1.9)</td>
<td>6.9a (0.7)</td>
<td>21.8a (1.3)</td>
</tr>
<tr>
<td>Microbial respiration</td>
<td>6.8a (2.1)</td>
<td>7.4a (2.1)</td>
<td>7.8a (0.7)</td>
</tr>
<tr>
<td>Dissolved organic matter</td>
<td>0.7a (0.4)</td>
<td>0.3a (0.2)</td>
<td>3.7a (2.7)</td>
</tr>
<tr>
<td>Total recovery</td>
<td>78.4a (7.1)</td>
<td>67.8a (7.9)</td>
<td>55.2a (7.0)</td>
</tr>
</tbody>
</table>
Figure 4.3. The effect of chronic NO\textsubscript{3} additions on the assimilation of A) \textsuperscript{13}C-labeled vanillin and B) \textsuperscript{13}C-labeled cellobiose into PLFA microbial groups. Means with an asterisks are significantly different (\(\alpha = 0.05\)). Error bars are one standard error of the mean (\(n = 3\)).
Specific microbial respiration for $^{13}$C vanillin decreased from $1.01 \pm 0.52$ (nmol $^{13}$CO$_2$ nmol total PLFA$^{-1}$) in N control soils compared to $0.37 \pm 0.14$ (nmol $^{13}$CO$_2$ nmol PLFA$^{-1}$) in N amended soils. I found no difference in the incorporation of $^{13}$C cellobiose into any specific PLFA. In addition, there was no treatment effect in $^{13}$C excess of any PLFA microbial group amended with $^{13}$C cellobiose (Figure 4.3). There was no observed difference in $^{13}$C cellobiose specific respiration. Bacteria and fungal PLFAs and total PLFA also exhibited more incorporation of $^{13}$C vanillin, when compared to $^{13}$C cellobiose.

**DISCUSSION**

My study provides evidence that NO$_3^-$ additions can inhibit the ability of soil microorganisms to metabolize cellobiose or vanillin by suppressed the activity of lignin-degrading enzymes. However, this decrease does not appear to significantly decrease the ability of the microbial community to assimilate cellobiose or vanillin. The assimilation of $^{13}$C cellobiose into fungal PLFAs was unaffected by NO$_3^-$ deposition, whereas, more $^{13}$C vanillin was incorporated into fungal PLFAs in NO$_3^-$ amended soil. Although fungal PLFA was more enriched in $^{13}$C from vanillin, I did not observe an increase in $^{13}$C vanillin recovered in microbial C, estimated by direct extraction. A possible explanation is that the lignin-degrading organisms represent only a small fraction of total microbial biomass. The increase in $^{13}$C vanillin incorporated into fungal PLFAs in NO$_3^-$ amended soils cannot be explained by enzyme activities. Peroxidase and phenol oxidase activity can reflect the depolymerization of lignin and the production of vanillin, but not assimilation of vanillin (Kirk and Shimada, 1985). Therefore, it appears that NO$_3^-$
additions can simultaneous inhibit lignin depolymerization and stimulate the assimilation of vanillin evident by the accumulation of $^{13}$C in PLFAs.

My results indirectly suggest that NO$_3^-$ additions have altered the biochemistry of soil organic C by increasing the lignin content. Previous research demonstrates that NO$_3^-$ additions increased the decomposition of labile plant litter, whereas it decreased the decomposition of lignocellulose (Pinck et al., 1950; Berg, 1986; Fog, 1988; Carreiro et al., 2000). The suppression of lignolytic enzymes is likely to reduce the degradation of lignin, along with plant tissue protected by lignin. The 68% decrease in microbial biomass is consistent with the idea that less substrate is available for microbial growth in NO$_3^-$ amended soils. It stands to reason that chronic NO$_3^-$ deposition, by suppressing ligninolytic activity, has decreased the decomposition of lignin, thus increasing SOC. I have found that SOC is 76% higher in NO$_3^-$ amended soil, than in control soils. NO$_3^-$ amended soils from the same study site as this experiment exhibit a significant decrease in soluble phenolics (Chapter III). The decreases in ligninolytic activity coupled with reductions in soluble phenolic monomers indicates that more C from lignin is remaining as polyphenolic compounds in NO$_3^-$ amended soils rather than undergoing humification (Orlvo, 1986). However, I have no direct evidence of increases in the lignin content of SOC. Determining if NO$_3^-$ deposition has changed the biochemistry of SOC warrants future study.

Increases in soil organic carbon, decreases in microbial biomass, and potential increases in soil lignin content indicate that chronic NO$_3^-$ deposition has reduced the flow of C through the heterotrophic soil food web. The increase in SOC cannot be explained by greater leaf or root litter biomass, which has not changed in NO$_3^-$ amended plots (Zak
et al., 2003). Increases in SOC may be explained by decreases in peroxidase and phenol oxidase activity, which are responsible for degrading lignin in plant litter. The suppression of ligninolytic enzymes is a typical response to NO$_3^-$ additions and this is a likely mechanism explaining decreases in decomposition of recalcitrant plant litter (Carreiro et al., 2000; Sinsabaugh et al., 2002). Other experiments found that the response of these ligninolytic enzymes to chronic NO$_3^-$ addition show similar decreases in enzyme activity (Carreiro et al., 2000; Saiya-Cork et al., 2002; Sinsabaugh et al., 2002; DeForest et al., 2004). It stands to reason that if lignolytic enzymes are inhibited, then SOC would accumulate. Therefore, it appears that NO$_3^-$ deposition may have the capacity to increase soil C sequestration in northern hardwood forest.

In conclusion, the depolymerization of lignin by lignolytic enzymes has been reduced by NO$_3^-$ additions. This, in turn, has probably altered SOC biochemistry by increasing the lignin content of SOC. This is consistent with the 76% increase in SOC in NO$_3^-$ amended soils. The increases in SOC are not consistent with reductions in fungal biomass, which suggests a reduction in the availability of C from lignocellulose. Nevertheless, I have no evidence the NO$_3^-$ additions have reduced cellobiose or vanillin metabolism or assimilation. Increases in incorporation of vanillin by the fungal community in NO$_3^-$ amended soils indicated that the assimilation of lignin monomers are not directly coupled to lignin decomposition. Nevertheless, it is clear that NO$_3^-$ additions have reduced the flow of C through the microbial community by increasing the amount of soil organic carbon.
LITERATURE CITED


Anderson, T.H. and K.H. Domsch. 1993. The metabolic quotient for CO₂ ($q_{CO₂}$) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biology & Biochemistry 25, 393-395.


CHAPTER V

THE POTENTIALLY CONFOUNDING EFFECT OF INCREASED SALINITY IN NITRATE DEPOSITION EXPERIMENTS

ABSTRACT

The burning of fossil fuels and the subsequent atmospheric deposition of NO$_3^-$ can be a potent modifier of ecosystem function, especially in N-limited temperate forests. In order to determine how atmospheric N deposition alters ecosystem function, it is a common practice to apply N fertilizer to simulate anthropogenic atmospheric deposition. However, the application of sodium nitrate (NaNO$_3$) in some experiments may create an experimental artifact by adding Na$^+$, which could potentially affect a variety of microbial-mediated processes in the soil. It is possible that changes in microbial community composition, reductions in microbial biomass, and lower extracellular enzyme activities attributed to atmospheric NO$_3^-$ deposition may result from Na$^+$, and not the addition of NO$_3^-$. This experiment was designed to determine if Na$^+$ contained in NaNO$_3$ contributes to decreases in microbial biomass and extracellular enzyme activities, previously observed in our field experiment. To determine if there is a “salt effect”, I applied NaCl, NaNO$_3$, NH$_4$NO$_3$, NH$_4$Cl with the same equivalents of charge to a mature sugar maple-dominated northern hardwood forest. By measuring phospholipid fatty acids (PLFA) and the activities of extracellular enzymes, I was able to gauge the influence of Na$^+$ on microbial community composition, biomass, and function. I found that Na$^+$ did not significantly alter microbial community composition or total PLFA. When compared with the control, total PLFA values were reduced virtually the same for NaNO$_3$ and NH$_4$NO$_3$ treatments; therefore, any potential Na$^+$ effect appears to be trivial.
N-acetylglucosaminidase (NAGase) activity was significantly suppressed by the application of NaCl. However, because the N-containing fertilizers elicited a similar response, we consider a Na$^+$ effect to be of minor importance. My results suggest that microorganisms responding to NaNO$_3$ or NH$_4$NO$_3$ additions do so in a comparable manner. After two years of fertilization, I have no strong evidence to support the idea that Na$^+$ alters microbial community composition and processes apart from the effects of NO$_3^-$ additions.
INTRODUCTION

The burning of fossil fuels has altered the biogeochemical cycling of N by increasing the deposition of atmospheric nitrate (NO$_3^-$; Galloway 1998). This is a primary concern because N cycling rates in N-limited temperate forests are likely to change by increases atmospheric N deposition (Fenn et al., 1998). For example, high rates of N deposition can decrease net primary productivity and N mineralization, while stimulating N leaching loss (Boxman et al., 1998; Gundersen et al., 1998; Magill et al., 2000). In addition, the rapid microbial assimilation of anthropogenic NO$_3^-$ and its subsequent release as NH$_4^+$ (Zogg et al., 2000) has the potential to suppress the ability of white-rot fungi to depolymerize lignin (Keyser et al., 1978; Berg 1986; Berg and Tamm, 1991). In addition, a reduction in lignin degradation could alter soil C cycling such that soil C storage could increase (Waldrop et al., 2003). For example, nitrogen deposition experiments have found that lignolytic activity is suppressed in N amended soils, but cellulase activity has a variable response (Carreiro et al., 2000; Sinsabaugh et al., 2002; DeForest et al., 2004). It is plausible that the aforementioned responses could result from the addition of Na$^+$ in experiments using NaNO$_3$ to simulate atmospheric NO$_3^-$ deposition (DeForest et al., 2004). Therefore, the type of N fertilizer used might confound results on microbial properties.

Experiments that investigate N deposition and microbial processes apply N fertilizer as either NaNO$_3$ (Saiya-Cork et al., 2002; DeForest et al., 2004; Waldrop et al., 2004) or NH$_4$NO$_3$ (Carreiro et al., 2000; Sinsabaugh et al., 2002) to simulate future anthropogenic N deposition. Experiments using NaNO$_3$ may be inadvertently increasing soil salinity. Because Na$^+$ is not required for plant growth, it could reside in the soil and
increase in concentration over time. It is possible that Na$^+$ could promote decreases in extracellular enzyme activities and microbial biomass apart from N additions (Frankenberger and Bingham, 1982; Rietz and Haynes, 2003).

Soil microorganisms and their ability to degrade plant litter can be influenced by increases in salinity (Zahran, 1997; Sardinha et al., 2003). High concentrations of soluble salts (> 4 EC$_e$) are known to reduce the activity of cellulases (Frankenberger and Bingham, 1982). Moreover, increases in Na$^+$ concentrations can significantly decrease microbial biomass and alter microbial community composition in favor of prokaryotic microorganisms (Rietz and Haynes, 2003; Sardinha et al., 2003). Because high levels of Na$^+$ can alter microbial community composition and decrease microbial biomass and activity, it is possible that modest additions of Na$^+$ in N deposition experiments using NaNO$_3$ could reduce microbial biomass and activity, potentially confounding the effect of added N. Therefore, this experiment was designed to determine if possible changes in microbial community composition, biomass, and enzyme activities could result from adding Na$^+$ rather than NO$_3^-$.

**MATERIALS AND METHODS**

**Study Area and Field Sampling**

My study was conducted in a mature sugar maple (*Acer saccharum* Marsh.)-dominated northern hardwood forest stand. This stand is located in the northwest region (46°39’ x 88°28’) of the upper peninsula of Michigan, USA. Within this stand, fifteen 3 m x 3 m plots that consisted of five treatments were established; each treatment was replicated three times. NaCl, NaNO$_3$, NH$_4$NO$_3$, and NH$_4$Cl were added to provide 0.214
mEq m\(^{-2}\) yr\(^{-1}\) in each treatment. By applying the same equivalents of charge, we were able to add the same amounts of Na\(^+\) and N used in previous work (DeForest et al., 2004). An exception is the NH\(_4\)NO\(_3\) treatment, which received twice as much N as the other N-containing treatments. Since 2001, the compounds were applied six times a year, over the growing season. After two years of treatment, I collected 4 mineral soil cores (2-cm diam. and 10-cm deep) from each plot, which were composited in the field. Composite samples were placed in plastic bags and kept on ice. The influence of Na\(^+\) was determined on microbial community composition using phospholipid fatty acids (PLFA) analysis and extracellular enzyme activities. If there was a Na\(^+\) effect on microbial activity, I expected similar responses in soils receiving NaCl and NaNO\(_3\), but the NaNO\(_3\) treatment would be different from the NH\(_4\)NO\(_3\) and NH\(_4\)Cl treatments. Likewise, a similar response of only N-containing fertilizers would be indicative of a N effect on the microbial community.

Microbial Community Function and Composition

*Extracellular Enzyme Activity*

Using field-fresh soil samples, I measured the activity of eight enzymes responsible for degrading common components in plant litter: N-acetylglucosaminidase (NAGase), phosphatase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, cellobiohydrolase, \(\beta\)-xylosidase, peroxidase, and phenol oxidase. I blended 2 g of mineral soil in 150 mL of 50 mM acetate buffer (Saiya-Cork et al., 2002). Using methylumbelliferone (MUB)-linked model substrates, I measured the activity of NAGase, phosphatase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, cellobiohydrolase, and \(\beta\)-xylosidase (Saiya-Cork et al., 2002). Soils assayed
for NAGase and phosphatase activity were incubated for 0.5 h at 25°C, whereas α-glucosidase, β-glucosidase, cellobiohydrolase, β-xylosidase assays were incubated for 2 h at 25°C. Because the fluorescence of MUB is inhibited below pH 7, we added 25 mL of NaOH (0.2 M) at the termination of the assay (Saiya-Cork et al., 2002). An F-max fluorimeter (Molecular Devices Corp., Sunnyvale, CA) was used to measure the fluorescence; excitation energy was 355 nm and emission was measured at 460 nm (Saiya-Cork et al., 2002). The activity of peroxidase and phenol oxidase was measured using L-dihydroxyphenylalanine (Saiya-Cork et al., 2002). The optical density (460 nm) of the oxidized reaction product was measured on a spectrophotometer (Bio-Tek Instruments, Winooski, Vt) after incubating the samples for 24 h at 25°C.

**Phospholipid Fatty Acid Analysis**

In order to determine if Na⁺ altered microbial community composition and biomass, we analyzed phospholipid fatty acids. From the composite soil samples, a 5 g subsample was freeze-dried for PLFA analysis. Total lipids were extracted from the soil using a solution containing 10 mL of CH₃OH, 5 mL of CH₃Cl, and 4 mL of phosphate buffer (White et al., 1979). In addition, we added 500 µL of an 11:0 and 21:0 PLFA standard (50 pmol mL⁻¹) to each sample to determine analytical recovery. Silicic acid chromatography was used to separate the polar lipids from neutral and nonpolar lipids and samples were subjected to an alkaline CH₃Cl -CH₃OH solution to form fatty acid methyl esters (FAMEs; Guckert et al., 1985). FAMEs were separated using gas chromatography and quantified using a Finnigan Delta plus mass spectrometer with a GC/C III interface (Thermofinnigan, Bremen, Germany). The mass of each FAME was
determined using a standard FAME mix solution (Chapter IV). I categorized 21 PLFA bioindicators, indicative of bacteria, actinomycetes, fungi, and protozoa. Bacteria PLFAs have signatures of i15:0, i16:0, 10Me16:0, cy17:0, 18:1ω7t, cy19:0a, a15:0, 16:1ω9c, i17:0, a17:0, 18:1ω5c (Asselineau, 1962; Gitaitis and Beaver, 1990; Annous et al., 1999; Grayston et al., 2001), whereas the fungal PLFAs are 18:1ω9c and 18:2ω6 (Federle et al., 1986; Feofilova et al., 1998). Additionally, total PLFAs were used as an indicator of living microbial biomass.

Statistical Analysis

My experimental plots were arranged as a complete randomized block design. Using an analysis of variance, I determined the effect of each compound on enzyme activity (nmol g⁻¹ h⁻¹) and total PLFA (nmol g⁻¹). Any measurement with a rejected null hypothesis was furthered investigated using a Fisher’s protected least significant difference procedure.

RESULTS AND DISCUSSION

Sodium apart from N had no noticeable influence on the microbial community composition (Table 5.1). NaCl significantly decreased the abundance of bacterial bioindicator 18:1ω5c, however, this decrease was not apparent in the N containing treatments (Table 5.1). Cy17:0, a minor bacterial bioindicator in these soils, was significantly decreased by N containing treatments, but not for the NaCl treatment (Table 5.1). Total PLFA, a measure of microbial biomass, was unchanged by Na⁺ as indicated by no difference among treatment means (Table 5.2). It is interesting to note
Table 5.1. Analysis of variance for the percent mole fraction of PLFA bioindicators. Values are p-values for null hypothesis that Na\(^+\) alters microbial community composition.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>i15:0</th>
<th>i16:0</th>
<th>10Me16:0</th>
<th>cy17:0</th>
<th>18:1ω7t</th>
<th>cy19:0a</th>
<th>a15:0</th>
<th>16:1ω9c</th>
<th>i17:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO(_3)</td>
<td>1</td>
<td>0.20</td>
<td>0.41</td>
<td>0.83</td>
<td>0.03</td>
<td>0.64</td>
<td>0.56</td>
<td>0.71</td>
<td>0.25</td>
<td>0.65</td>
</tr>
<tr>
<td>NH(_4)NO(_3)</td>
<td>1</td>
<td>0.88</td>
<td>0.74</td>
<td>0.10</td>
<td>0.01</td>
<td>0.99</td>
<td>0.34</td>
<td>0.89</td>
<td>0.64</td>
<td>0.10</td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>1</td>
<td>0.61</td>
<td>0.21</td>
<td>0.23</td>
<td>0.06</td>
<td>0.06</td>
<td>0.92</td>
<td>0.41</td>
<td>0.99</td>
<td>0.67</td>
</tr>
<tr>
<td>NaCl</td>
<td>1</td>
<td>0.25</td>
<td>0.10</td>
<td>0.46</td>
<td>0.14</td>
<td>0.21</td>
<td>0.14</td>
<td>0.21</td>
<td>0.25</td>
<td>0.87</td>
</tr>
</tbody>
</table>
Table 5.1. Continued. Analysis of variance for the percent mole fraction of PLFA bioindicators. Values are p-values for the null hypothesis that Na$^+$ alters microbial community composition

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>a17:0</th>
<th>17:0</th>
<th>18:1ω5c</th>
<th>14:0</th>
<th>15:0</th>
<th>16:0</th>
<th>18:0</th>
<th>19:0</th>
<th>24:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO$_3$</td>
<td>1</td>
<td>0.18</td>
<td>0.25</td>
<td>0.66</td>
<td>0.37</td>
<td>0.19</td>
<td>0.35</td>
<td>0.13</td>
<td>0.99</td>
<td>0.64</td>
</tr>
<tr>
<td>NH$_4$NO$_3$</td>
<td>1</td>
<td>0.07</td>
<td>0.25</td>
<td>0.52</td>
<td>0.35</td>
<td>0.24</td>
<td>0.74</td>
<td>0.83</td>
<td>0.68</td>
<td>0.21</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>1</td>
<td>0.42</td>
<td>0.25</td>
<td>0.99</td>
<td>0.27</td>
<td>0.35</td>
<td>0.11</td>
<td>0.02</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>NaCl</td>
<td>1</td>
<td>0.26</td>
<td>0.52</td>
<td>0.04</td>
<td>0.35</td>
<td>0.19</td>
<td>0.39</td>
<td>0.12</td>
<td>0.37</td>
<td>0.52</td>
</tr>
</tbody>
</table>
Table 5.1. Continued. Analysis of variance for the percent mole fraction of PLFA bioindicators. Values are p-values for the null hypothesis that Na\(^+\) alters microbial community composition.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>df</th>
<th>16:1ω7c</th>
<th>18:1ω7c</th>
<th>16:1ω5c</th>
<th>18:1ω9c</th>
<th>18:2ω6</th>
<th>10Me18:0</th>
<th>20:4ω6</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO(_3)</td>
<td>1</td>
<td>0.75</td>
<td>0.10</td>
<td>0.60</td>
<td>0.24</td>
<td>0.78</td>
<td>0.19</td>
<td>0.99</td>
</tr>
<tr>
<td>NH(_4)NO(_3)</td>
<td>1</td>
<td>0.61</td>
<td>0.36</td>
<td>0.99</td>
<td>0.80</td>
<td>0.00</td>
<td>0.02</td>
<td>0.99</td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>1</td>
<td>0.27</td>
<td>0.04</td>
<td>0.01</td>
<td>0.08</td>
<td>0.38</td>
<td>0.22</td>
<td>0.99</td>
</tr>
<tr>
<td>NaCl</td>
<td>1</td>
<td>0.62</td>
<td>0.02</td>
<td>0.26</td>
<td>0.29</td>
<td>0.67</td>
<td>0.85</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Table 5.2. The influence of Na, Cl, and N on mean enzyme activity and total PLFA. Within a measurement, different letters are significantly different from each other. Values in parentheses are a standard error of the mean (n=3).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NaCl</th>
<th>NaNO₃</th>
<th>NH₄NO₃</th>
<th>NH₄Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total PLFA†</strong></td>
<td>245.0a</td>
<td>177.7a</td>
<td>168.5a</td>
<td>168.0a</td>
<td>169.6a</td>
</tr>
<tr>
<td>α-glucosidase‡</td>
<td>2.2a</td>
<td>1.9a</td>
<td>2.2a</td>
<td>2.5a</td>
<td>1.9a</td>
</tr>
<tr>
<td>β-glucosidase‡</td>
<td>120.0ab</td>
<td>101.0abc</td>
<td>136.9a</td>
<td>83.5c</td>
<td>68.3c</td>
</tr>
<tr>
<td>Cellobiohydrolase‡</td>
<td>17.7a</td>
<td>14.7a</td>
<td>16.7a</td>
<td>15.6a</td>
<td>11.1a</td>
</tr>
<tr>
<td>β-xylosidase‡</td>
<td>31.5a</td>
<td>36.7a</td>
<td>48.3a</td>
<td>30.7a</td>
<td>27.5a</td>
</tr>
<tr>
<td>N-acetylglucosaminidase‡</td>
<td>75.0a</td>
<td>40.7c</td>
<td>44.2bc</td>
<td>42.1c</td>
<td>48.4bc</td>
</tr>
<tr>
<td>Phosphatase‡</td>
<td>563.9a</td>
<td>559.8a</td>
<td>528.9a</td>
<td>537.7a</td>
<td>583.6a</td>
</tr>
<tr>
<td>Peroxidase‡</td>
<td>1714.0a (286.9)</td>
<td>1722.2a (443.9)</td>
<td>1375.2a (530.1)</td>
<td>1041.3a (307.5)</td>
<td>1195.7a (411.4)</td>
</tr>
<tr>
<td>Phenol Oxidase‡</td>
<td>161.5a (61.4)</td>
<td>187.3a (29.0)</td>
<td>180.3a (25.0)</td>
<td>146.2a (29.7)</td>
<td>142.0a (39.2)</td>
</tr>
</tbody>
</table>

† (nmol PLFA g⁻¹)
‡ (nmol g⁻¹ hr⁻¹)
that the addition of each compound reduced total PLFA, but values were not different from the control (Table 5.2). Although total PLFA content was not significantly different between treatments, N-containing fertilizers reduced total PLFA content by 31% and the NaCl treatment reduced it by 27% (Table 5.2). Because the NaNO₃ and NH₄NO₃ treatments contained virtually the same total PLFA (Table 5.2), I consider any possible effect of Na⁺ in NaNO₃ to be unimportant.

Although most enzyme activities were unaffected by Na⁺, NAGase activity was significantly suppressed by the NaCl and N-containing compounds (Table 5.2). This result is not universal because NAGase activity was not significantly altered in long-term experiments using NaNO₃ (Saiya-Cork et al., 2002; DeForest et al., 2004). Nevertheless, I can reject the idea that Na⁺ contained in NaNO₃ has a major influence on NAGase activity, because NaNO₃ and NH₄NO₃ treatments suppressed NAGase in a similar manner when compared to the control (Table 5.2). It is interesting to note that doubling the amount of N (NaNO₃ vs. NH₄NO₃) suppressed NAGase by virtually the same amount. This result suggests that, for the short-term, increasing N deposition from 3 to 6 (g N m⁻² y⁻¹) does not have a cumulative effect on NAGase activity. β-glucosidase activity was significantly (p = 0.01) decreased by the NH₄-containing treatments, when compared to the control (Table 5.2). While this observation excludes a Na⁺ effect, I am unaware of any mechanism that could explain an NH₄⁺ induced suppression of β-glucosidase activity. It is interesting to note that the NaNO₃ treatment increased β-glucosidase activity, which was also observed by Waldrop et al., (2004) in a sugar-maple dominated forest. However, NO₃⁻ additions also have been observed to significantly
decrease β-glucosidase activity (DeForest et al., 2004). I am unable to explain the discrepancy between these experiments.

I found several similar responses in this experiment and in our previous work on atmospheric N deposition and microbial activity (DeForest et al., 2004; Chapter IV). Total PLFA was reduced by 31% due to NaNO$_3$ in this experiment, which can be compared to the significant 19% reduction in total PLFA from our previous work (DeForest et al., 2004). Additionally, peroxidase activity was reduced by 19% by NaNO$_3$ when compared to the control, which is similar to a reduction in peroxidase observed in my previous work (DeForest et al., 2004). However, we did not observe significant reductions in total PLFA or peroxidase activity (DeForest et al., 2004), which differs from our previous observations. This discrepancy may be explained by a much smaller (3 m$^2$ vs. 30 m$^2$) plot size than used in previous work (DeForest et al., 2004). Due to the heterogeneity of soil, samples collected from the smaller plot are likely to have higher variation.

My results provide evidence that changes microbial biomass and extracellular enzyme activities due to N additions are comparable for NaNO$_3$ and NH$_4$NO$_3$. It also indicates that increasing N deposition, from 3 to 6 (g N m$^{-2}$ y$^{-1}$), does not have a cumulative effect on these properties. I found that NaCl and N-containing treatments significantly decreased NAGase activity. However, because NaNO$_3$ and NH$_4$NO$_3$ had similar responses, a Na$^+$ affect appears to be trivial. After two years of fertilization, I have no strong evidence to demonstrate that Na$^+$ altered microbial properties and processes apart from the NO$_3^-$ additions.
LITERATURE CITED


CHAPTER VI
GLOBAL IMPLICATIONS OF INCREASES IN NITROGEN DEPOSITION ON LIGNIN DECOMPOSITION

My dissertation demonstrates that in sugar-maple dominated temperate hardwood forests, atmospheric N deposition can reduce the flow of C by suppressing the activity of enzymes responsible for the depolymerization of lignin (Chapters 2 & 4). Recognizing what factors influence plant litter decomposition on a global basis is important in order to place my research in context with other ecosystems. Moreover, it is important to understand how anthropogenic N deposition will effect other terrestrial ecosystems in different climates, because increases in N deposition are a global phenomenon (Galloway, 1984). The same mechanism that decreases lignin decomposition in my study sites could be used to predict the impact anthropogenic N deposition on broad global patterns of plant litter decomposition.

The decomposition of plant litter is an important component of the global C cycle because plant decay contributes about 70% to the total global annual respired CO₂ flux of ~120 Pg C (Raich and Schlesinger, 1992). The rate of plant litter decomposition is controlled by environmental conditions, the chemical composition of the litter, and by soil organisms (Swift et al., 1979). On a global scale, climate is the best predictor of plant litter decomposition, followed by litter chemistry and soil organisms (Meentemeyer, 1978; Aerts, 1997; Silver and Miya, 2001). Climatic controls of decomposition can be expressed by actual evapotranspiration (AET), which represents temperature and precipitation. Humid tropical regions have the highest AET, whereas polar or desert regions have the lowest AET values (Aerts, 1997). Tropical regions typically have
higher litter decomposition rates than other terrestrial ecosystems. For example, the average time it takes to decompose ~95% of leaf litter is 6 months in a tropical rain forest, whereas in a temperate deciduous forests it is 4 years (Swift et al., 1979).

Leaf litter chemistry, as characterized by lignin/N ratio, is a good chemical predictor of decomposition within a climatic region (Aerts, 1997). Higher lignin concentrations in leaf litter normally decrease decomposition, whereas higher litter N concentrations increase decomposition (Meentemeyer, 1978; Fog, 1988; Berg et al., 1993). The lower the lignin/N ratio, the faster leaf litter can be decomposed.

Increases in N deposition can be a potent modifier of global patterns of decomposition because increases in N availability can change leaf litter decomposition by altering litter chemistry and the activity of soil microorganisms. Plants that experience experimental increases in atmospheric N depositions usually have significantly higher leaf N concentrations (Magill et al., 2000; Zak et al., 2004). However, the lignin content of leaf litter appears to be unaffected by increases in N availability (K.S. Pregitzer, unpublished data). If increases in N deposition raise litter N concentrations, then lignin/N ratios will decrease and this can increase decomposition. Conversely, increases in soil inorganic N availability can reduce the lignin-degrading activity of white-rot fungi (Fog, 1988). White-rot fungi produce lignin-degrading extracellular enzymes, which are induced by low N concentrations that are insufficient for growth (Keyser et al., 1978). Therefore, increases in N deposition can elevate N starvation in white-rot fungi, thus preventing the production of enzymes responsible for lignin depolymerization. A reduction in the depolymerization of lignin also would decrease the decomposition of plant tissue protected by lignin (Berg, 1986; Fog, 1988).
Because lignin can inhibit plant litter decay and increases in N deposition can suppress lignin decomposition (Chapter II), increases in N deposition can reduce plant litter decomposition. However, the overall impact of N deposition on decomposition would depend on the lignin content of litter. Lignin control of decomposition may be absent in leaf litter that has relatively low (< 10%) lignin concentrations (Taylor et al., 1989). Increases in N deposition are likely to increase the decomposition of leaf litter with low lignin content because of the decrease in the lignin/N ratio (Aerts, 1997). Because the amount of plant tissue protected by lignin is relatively small, reductions in lignin decomposition would be unimportant to overall plant decomposition (Figure 6.1; Taylor et al., 1989). I reason that increases in N deposition will have the greatest influence on leaf litter that has the highest lignin/N ratio. Figure 6.1 illustrates this hypothesis. Leaf litter with high lignin content has the slowest decomposition rates (Meentemeyer, 1978; Taylor et al., 1989; Berg et al., 1993), and increases in N depositions are likely to suppress overall leaf litter decomposition (Carreiro, et al., 2000; Chapter 2).

The global effect of N deposition on broad patterns of plant litter decomposition is likely to be ecosystem-specific. Decomposition would be minimally influenced in ecosystems with low lignin plant litter, whereas decomposition would be the most suppressed in ecosystems where most plant litter has high lignin concentrations. For example, a prairie ecosystem containing grasses and forbs with relatively low lignin would likely have less suppression of leaf litter decomposition then a coniferous forest with similar AET. Moreover, lignin appears to have a larger role in leaf litter
Figure 6.1. The relative differences in plant litter decay over time as affected by lignin/N ratios and N deposition.
Figure 6.2. The relationship between annual decomposition rate and lignin concentration of leaf litter based on different values of actual evapotranspiration. This figure was adapted from Meentemeyer (1978).
decomposition in moist-tropical climates (Meentemeyer, 1978). Figure 6.2, adapted from Meentemeyer (1978), shows the relationship between annual decomposition rate and lignin concentrations as effected by AET. Therefore, anthropogenic N deposition is likely to have a higher impact in tropical regions than in temperate regions because lignin content in leaf litter has a greater influence on annual decomposition rates at higher AET (Meentemeyer, 1978).

In summary, actual evapotranspiration is the main control of decomposition on a global scale, followed by leaf litter chemistry (lignin/N ratio). Increases in N deposition are likely to decrease the ability of white-rot fungi to produce lignin-degrading extracellular enzymes. Because lignin is a major control of leaf litter decomposition, the amount of lignin in litter will determine the impact of N deposition on decomposition. Nevertheless, ecosystems where leaf litter has high lignin content would experience the greatest reductions in decomposition. This would be more prevalent in climates with high AET. In conclusion, increases in anthropogenic N deposition, by suppressing lignin decomposition, would have the most pervasive control of litter mineralization in wet tropical environments and the least in polar or arid environments.
LITERATURE CITED


CHAPTER VII

SUMMARY AND CONCLUSIONS

My results demonstrate that increases in the atmospheric deposition of N have the potential to diminish the physiological capacity of the microbial community to depolymerize lignin. This, in turn, has likely caused a reduction in the cycling of C in the soil by these ecosystems. The major results of my dissertation were:

i) increases in nitrate deposition have suppressed the activity of β-glucosidase and lignolytic enzymes (Chapter II).

ii) increases in nitrate deposition have broadly suppressed all microbial groups, not just the activity of lignin-degrading microorganisms (Chapter II).

iii) decreases in β-glucosidase activity are likely due to a reduction in cellulose availability due to the suppression of enzymes that depolymerize lignin which protects cellulose from microbial degradation (Chapter III).

iv) increases in nitrate deposition did not increase the concentration of soil phenolics and it is unlikely that soils are the source of reported increases in DOC production on nitrate treated soils (Chapter III).

v) while nitrate additions can suppress the activity of lignin and cellulose degrading enzymes, this does not appear to alter the ability of the microbial community to assimilate vanillin or cellobiose (Chapter IV).

vi) increases in SOC and decreases in microbial biomass indicate that nitrate deposition has the potential to reduce the flow of C through the microbial soil food web (Chapter IV).

vii) increases in salinity from the nitrate fertilizer, apart from nitrogen additions, have apparently not changed microbial properties or activity in soils (Chapter V).

viii) increases in anthropogenic nitrogen deposition, by suppressing lignin decomposition, would have the most pervasive control of litter decay in wet tropical environments.
My research contributes to our understanding of the patterns and mechanisms of anthropogenic nitrate deposition control on carbon cycles at the ecosystem scale. The results of my research are important because they demonstrate that increases in nitrogen deposition have the potential to significantly decrease the decomposition of lignin and lignified carbohydrates contained with plant cell walls. A decrease in plant litter decomposition would slow the cycling of carbon by sugar maple-dominated northern hardwood forests. The capacity for these ecosystems to accumulate carbon could increase through soil organic matter formation. Therefore, anthropogenic N deposition, by slowing the cycling of C through the microbial community can be a potent modifier of ecosystem-level patterns of C cycling.