PLANT AND MICROBIAL MECHANISMS OF NITROGEN RETENTION IN NORTHERN HARDWOOD FORESTS RECEIVING ATMOSPHERIC NO_3^- DEPOSITION

William C. Eddy

A thesis submitted
in partial fulfillment of the requirements
for the degree of
Master of Science
(Natural Resources and Environment)
at the University of Michigan
April 2006

Thesis Committee:
Professor Donald R. Zak, Chair
Assistant Research Scientist William E. Holmes

TABLE OF CONTENTS

ABSTRACT

Anthropogenic N deposition in industrialized areas in Europe and North America can exceed rates of 30-55 kg N ha^{-1} y^{-1} (Bredemeier et al. 1998, Fenn et al. 1998). The ability of an ecosystem to retain N deposition is dependant upon plant and soil sinks for N, the strengths of which may be altered by the added N. This study examines plant and soil sinks for N deposition in 4 northern hardwood forest tracts in Michigan in which nitrate (NO_3^-) has been added at the rate of 30 kg N ha^{-1} y^{-1} for 10 years.

Sugar maple (*Acer saccharum* Marsh.), the dominant overstory tree species in northern hardwood forests of the Lake States, has been shown to have a limited capacity to take up and assimilate NO_3^- (Rothstein et al. 1996). The first objective of this study is to determine if 10 years of experimental NO_3^- addition has induced sugar maple to uptake and assimilate NO_3^-. The rates of N uptake and nitrate reduction were measured in control and NO_3^- deposition treatments within 4 northern hardwood forest tracts in Michigan. Rates of N uptake were determined by measuring the uptake of isotopically labeled N solutions into excised sugar maple roots. Nitrate reductase activity was determined using an *in vivo* NO_2^- production assay in excised sugar maple roots and leaves.

The rates of NO_3^- and NH_4^+ uptake and the rate of NO_3^- reduction did not differ between the ambient N deposition and experimental N deposition treatments. Furthermore, the rate of NO_3^- uptake was much lower than the rates of NH_4^+ uptake, at the same time that the rates of NO_3^- reduction remained physiologically insignificant. Together, these results indicate that sugar maple is not inducible as a direct sink for NO_3^- deposition by chronic NO_3^- additions. The incapacity of sugar maple to utilize NO_3^-,

means that this dominant tree species cannot be a direct sink for NO_3^- deposition. This finding may partially explain the high rates of NO_3^- leaching in these forests in response to experimental NO_3^- deposition.

Soil microbial community function can alter the capacity of an ecosystem to retain N deposition by controlling the cycling of N into stable and accumulating N pools, such as soil organic matter. The second objective of this study is to examine the short-term flow and fate of N in northern hardwood forest soils receiving experimental NO_3^- deposition. In one forest stand in northern Michigan, $^{15}NO_3^-$ (1927 μmol ^{15}N m^{-2}) was added *in situ* to forest soil within the ambient N deposition and chronic NO_3^- deposition treatments in Aug 2004. The percent recovery of ^{15}N was determined in bulk forest floor, and in inorganic N, dissolved organic N (DON), microbial biomass, soil organic matter, and root biomass N in the upper 10 cm of mineral soil at 12 sampling times from 2 h to 12 wk after tracer addition.

Microbial immobilization of added $^{15}NO_3^-$ appeared to be greater in the treatment receiving only ambient N deposition, thus sequestering NO_3^- more rapidly under low N availability. Furthermore, net DON production was greater, and NH_4^+ production less, under experimental NO_3^- deposition. Although the results of the tracer experience indicated no difference in N retention in soil between N deposition treatments, the recovery of the tracer in more readily leached soil pools (i.e. NO_3^- and DON) in the chronic N deposition treatment is consistent with observations of high rates of NO_3^- and DON loss in this treatment (Pregitzer et al. 2004).

FIGURES

TABLES

ACKNOWLEDGEMENTS

My research was supported by grants from the National Science Foundation and the U.S.D.A.McIntire-Stennis program. I would like to thank my advisor, Donald Zak, for his invaluable guidance, and William Holmes, for without his assistance with the laboratory analyses, this thesis would have not been possible. I am grateful to Kurt Pregitzer and Andrew Burton at Michigan Technological University and the USDA Forest Service North Central Research Station for providing laboratory space and resources. In addition, Jana Gastellum, Rebecca Mau, Seth McGinnis, and Kurt Smemo all assisted greatly in the completion of this thesis.

CHAPTER ONE: INTRODUCTION

Anthropogenic activities have severely altered the global nitrogen (N) cycle (Vitousek et al. 1997). The burning of fossil fuel, the production of commercial N fertilizer, and the widespread cultivation of legumes have doubled the availability of reactive N in the biosphere (Galloway 1995). All of these anthropogenic forces have increased atmospheric N deposition in terrestrial and aquatic ecosystems downwind of sources (Aber et al. 1989, Galloway 1995). This is especially true for temperate forests which are often located close to industrial sources of emission (Galloway and Cowling 2002). Higher rates of atmospheric N deposition in temperate forests are important because their net productivity is usually limited by soil N availability (Pastor et al. 1984, Vitousek and Howarth 1991). For example, in a study of upland forest ecosystems in Michigan, soil N availability limited overstory productivity in all forest ecosystems examined, except northern hardwood forests dominated by sugar maple (*Acer saccharum* Marsh.) and American basswood (*Fraxinus americana* L.; Zak et al. 1989).

Chronic atmospheric N deposition is thought to cause N saturation, a condition in which ecosystem availability of nitrate (NO_3^-) and ammonium (NH_4^+) is greater than plant and microbial nutritional demand, alleviating N limitation of growth and causing an ecosystem to be less retentive of the excess N inputs (Aber et al. 1989, Aber 1992). Nitrogen saturation is thought to have many detrimental effects on terrestrial and aquatic ecosystems. Nitrogen deposition, both as NO_3^- and NH_4^+, can acidify soils (Galloway 1995). When an ecosystem becomes N saturated, NO_3^- and nutrient cations can be leached from the soil, causing surface water acidification and eutrophication (Aber 1992, Galloway 1995). In addition, excess availability of N can increase the emissions of

nitrous oxide (N_2O), a greenhouse gas (Aber et al. 1989). Furthermore, in historically N limited ecosystems, N saturation may reduce fine root allocation and mycorrhizal infection, which may exacerbate limitations of other nutrients (Aber et al. 1989).

Because N is thought to limit plant productivity in most temperate ecosystems, plants are generally considered to be the primary sink for anthropogenic inputs of N (Aber et al. 1989), although studies have both confirmed (Nadelhoffer et al. 1995) and refuted this hypothesis (Zogg et al.2000, Nadelhoffer et al.1999). The ability of plants to act as a direct sink for anthropogenic N deposition depends on their physiological capacity to uptake and assimilate the deposited chemical forms of N (Rothstein et al. 1996). Moreover, the tree species must be able to compete with the soil microbial community for inorganic N (Zak et al. 1990). Experimental evidence indicates that variation exists among tree species in their physiological capacity to uptake NO_3^- (Chapin et al. 1986, Rothstein et al. 1996) and assimilate it into biologically active compounds (Al Gharbi and Hipkin 1984). Thus, trees species likely differ in their capacity to directly take up anthropogenic N deposition, based on their physiological requirements for N and the form of N deposited from the atmosphere.

Sugar maple (*Acer saccharum* Marsh.) is the dominant tree species of the northern hardwood forests in the Upper Lake States (Curtis 1959). Sugar maple in this region has been shown to have an extremely low capacity to take up and assimilate NO_3^- under ambient and short-term NO_3^- fertilization (Rothstein et al. 1996). If sugar maple sustains low NO_3^- uptake and assimilation rates under chronic NO_3^- deposition, then the dominant vegetation is likely not a direct sink for anthropogenic NO_3^- in these forests.

Nitrate uptake and assimilation have been demonstrated to be inducible by NO_3^- additions in other species (Hoff et al. 1992, Oaks 1994). Thus, the first goal of this thesis, was to determine if NO_3^- uptake and assimilation by sugar maple has been induced by 10 years of chronic NO_3^- deposition. Determining whether this dominant tree species is a direct sink for atmospheric N deposition is crucial in understanding how sugar maple influences the patterns of N cycling and retention in northern hardwood forests exposed to atmospheric N deposition. The lack of a sizeable direct plant sink for NO_3^- deposition may increase the rate of NO_3 leaching prior to microbial transformation of NO_3^- to NH_4^+, which is available for sugar maple uptake.

Northern hardwood forests in the Upper Lake States region receive ambient levels of atmospheric N deposition on the order of 7-12 kg N ha^{-1} y^{-1}, of which slightly over half of the deposition is in the form of NO_3^- (MacDonald et al. 1992). Beginning in 1994, four northern hardwood stands lying along a 500 km north-south gradient in Michigan have received experimental NO_3^- additions at the rate of 30 kg ha^{-1} y^{-1} (MacDonald et al. 1992). This rate of addition is comparable to atmospheric N deposition in eastern North America and Europe (Bredemeier et al. 1998, Fenn et al. 1998).

Chronic NO_3^- deposition has rapidly altered the biogeochemical cycling of N and carbon in these northern hardwood forests. Experimental NO_3^- deposition has resulted in higher extractable soil NO_3^- (Zak et al. 2004), and has increased the leaching of NO_3^-, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) from the surface soil (Pregitzer et al. 2004). After 7 years, 72% of the net annual additions of N were being lost as DON and NO_3^- via leaching (Pregitzer et al. 2004). Experimental NO_3^- deposition has also been shown to reduce soil microbial biomass, the activity of cellulose

and lignin degrading enzymes (DeForest et al. 2004a, DeForest et al. 2004b), as well as soil respiration rates (Burton et al. 2004). Fundamental perturbations in the cycling of N through the soil, in turn, have likely altered the ability of the soil to retain NO_3^- deposition.

The capacity of soil to serve as a long-term sink for N is largely controlled by the cycling of N through the soil microbial community and abiotic incorporation of N into soil organic matter. In mature forest ecosystems, soil microbial biomass is likely to be in steady state, and thus cannot be a large sink for anthropogenic N. In addition, free-living soil microbes are generally considered carbon-limited, not nitrogen limited, and therefore microbial N immobilization should not respond strongly to increased nitrogen availability (Aber et al. 1998). Still, assimilation into microbial biomass has been found to be a large, but short-term sink, for NO_3^- (Jackson et al. 1989, Zak et al. 1990, Davidson et al. 1992, Stark and Hart 1997, Zogg et al. 2000) and could promote N retention if N released from microbial biomass was incorporated into soil organic matter, released as dissolved organic N (DON), or mineralized as NH_4^+ (Stark and Hart 1997). Both NH_4^+ and DON are both less mobile than NO_3^-, as NH_4^+can be absorbed on the cation exchange complex, while DON can be stabilized in soil through adsorption on mineral soils particles (Qualls and Haines 1992).

Thus, the second goal of this thesis was to quantify how chronic anthropogenic NO_3^- deposition has altered the short-term flow and fate of N in these northern hardwood forests. Following the flow of N through the microbial community over short time periods is important for understanding the factors contributing to long-term N retention and loss in response to chronic NO_3^- deposition.

CHAPTER TWO: UPTAKE AND ASSIMILATION OF N BY *ACER SACCHARUM* RECEIVING CHRONIC ATMOPHERIC NO_3^- DEPOSITION

INTRODUCTION:

Anthropogenic activities, especially the combustion of fossil fuels, the production of nitrogenous fertilizer, and the cultivation of legumes, have doubled the availability of reactive nitrogen (N) in the biosphere (Galloway 1995). Fossil fuel emissions enter the atmosphere as NO_x, whereas ammonium (NH_4^+) fertilizer volatizes to gaseous ammonia (NH_3), each of which travel downwind and are deposited onto terrestrial and aquatic ecosystems (Aber et al. 1989, Galloway 1995). Globally, atmospheric N deposition has increased from 31.6 to 103 Tg N yr^{-1} between 1860 and the early 1990's, and this rate is projected to increase to 195 Tg N yr^{-1} by 2050 (Galloway et al. 2004).

Chronic atmospheric N deposition is thought to cause N saturation, a condition in which ecosystem availability of inorganic NO_3^- and NH_4^+ is greater than the plant and microbial demand, alleviating N limitation of growth and causing an ecosystem to be less retentive of the excess N (Aber et al. 1989, Aber 1992). The ability of plants to act as a direct sink for anthropogenic N depends on their N deficit and on their physiological capacity to take up and assimilate NH_4^+ and NO_3^- (Rothstein et al. 1996). Whereas NH_4^+ can be immediately incorporated into biological tissues, NO_3^- must first be enzymatically reduced into NH_4^+ before assimilation can occur (Gutschick 1981). This takes place through a two-step reaction: the reduction of NO_3^- to nitrite (NO_2^-) catalyzed by the enzyme nitrate reductase, and the further reduction of NO_2^- to NH_4^+ by nitrite reductase (Gutschick 1981, Andrews 1986). Experimental evidence indicates that variation exists among tree species in their physiological capacity to take up NO_3^- (Chapin et al. 1986, Rothstein et al. 1996) and assimilate it into biologically active compounds (Al Gharbi and

Hipkin 1984). Furthermore, both NO_3^- uptake and assimilation have been demonstrated to be inducible in other species (Hoff et al. 1992, Oaks 1994). For example, red maple, (*Acer rubrum* L.) had 50% great rates of foliar nitrate reduction in high relative to low nitrate incubations (Downs et al. 1993). Therefore, trees species likely differ in their capacity to directly take up anthropogenic N deposition, based on their physiological requirements for N and the form and amount of N being deposited.

Northern hardwood forests in the Upper Lake States region receive ambient levels of atmospheric N deposition on the order of 7-12 kg ha^{-1} $year^{-1}$ with slightly more N being deposited as NO_3^- than as NH_4^+ (MacDonald et al. 1992). Sugar maple (*Acer saccharum* Marsh.) dominates the overstory of these ecosystems (Curtis 1959), and has been shown to have a limited capacity to take up and assimilate NO_3^- (Rothstein et al. 1996). If sugar maple exposed to chronic NO_3^- fertilization sustains low uptake and assimilation rates of NO_3^-, then the dominant vegetation in northern hardwood forests cannot be a direct sink for atmospheric NO_3^- deposition. No study has yet addressed the ability of chronic NO_3^- deposition to induce NO_3^- uptake by sugar maple, a physiological response that has important implications for the retention of anthropogenic NO_3^- in northern hardwood forests.

Determining the physiological response of sugar maple N uptake and assimilation to NO_3^- addition is crucial in understanding how N deposition influences the patterns of N cycling and loss in northern hardwood forests. For example, longer retention of deposited N in the exchangeable NO_3^- pool, due to a lack of direct plant sinks for NO_3^-, would contribute to greater leaching of NO_3^- from the surface soil. Therefore, the objective of my study was to determine if chronic NO_3^- deposition could induce NO_3^-

uptake and assimilation in *Acer saccharum.* To test this, I examined the rates of NO_3^- and NH_4^+ uptake and rates of NO_3^- reduction by sugar maple in northern hardwood forests receiving ambient N deposition and chronic experimental NO_3^- deposition.

MATERIALS AND METHODS

Study site

My study was conducted in four northern hardwood forest stands along a 500-km north-south gradient from northwestern Upper Peninsula to west central Lower Michigan (Fig. 2.1). All study sites have similar vegetation, stand age, basal area, land-use history, soil type, and soil texture (Burton et al. 1991, MacDonald et al. 1991), but differ in temperature, rate of ambient atmospheric N deposition, and available soil N (Table 2.1). Both the N deposition rate and mean annual temperature decrease from south to north along the gradient sites, whereas soil N availability is lower in the most northern and southern sites than in the middle two sites (Zogg et al. 1996). The stands are approximately 90-year-old second-growth northern hardwood forests dominated by greater than 70% sugar maple basal area (MacDonald et al. 1991). The dominant soil across the gradient sites is Kalkaska sand (Typic Haplorthod).

In each forest stand, there are six 30-m x 30-m plots with a 10-m buffer zone along each side. Three plots within each stand received ambient levels of N deposition, whereas the other three plots received ambient plus 30 kg N ha^{-1} y^{-1} applied as $NaNO_3$. This approximates rates of NO_3^- deposition experienced presently in the northeast U.S (MacDonald et al. 1992, Bredemeier et al. 1998, Fenn et al. 1998). Nitrate is added in dry form during the growing season (late April/early May to September) in six equal

increments. Experimental N deposition treatment began in spring 1994 and has continued through the present year.

Sample Collection

I measured sugar maple NO_3^- and NH_4^+ uptake in fine roots and NO_3^- reductase activity (NRA) in leaves and fine roots using methods similar to those of Rothstein et al. (1996). Fine roots were collected using 5 cm diameter PVC corer extending 10 cm deep into the mineral soil. In each plot, 12 cores were collected within 2 m of randomly chosen *A. saccharum* trees that were greater than 10 cm at DBH. Sun-lit leaves, those likely to have the greatest NRA (Smirnoff and Stewart 1985), were obtained with use of a 12-gauge shotgun loaded with steel shot. Leaves were sampled from accessible branches on randomly selected overstory trees (dbh $\geq$ 10 cm) within each plot. Soil and leaf samples were placed on ice until laboratory analyses, which were completed within 24 h. Root NO_3^- and NH_4^- uptake, and leaf and root NRA, were measured twice during the growing season, in early June 2004 and then again in early September 2004.

Nitrogen Uptake in Roots

The kinetics parameters for NO_3^- and NH_4^+ uptake in fine roots were determined by measuring the rates of $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake (*sensu* Rothstein et al. 1996). Non-woody, very fine roots were collected (<1-mm dia.), gently rinsed free of soil with deionized H_20, and cut to approximately 1-cm lengths. On each sampling date, root segments from each plot were composited. Thirteen samples of fine roots (ea.100-mg) from each plot were rinsed three times with approximately 25 mL of 0.5 m*M* $CaSO_4$.

Each sample was assayed for either $^{15}NO_3^-$ or $^{15}NH_4^+$ uptake by suspending the tissue in 50 mL of a nutrient solution consisting of 0.5 m*M* $CaSO_4$, and 1% sucrose, and a 0, 1, 10, 100, 250, 500 or 1000 µmol of either $K^{15}NO_3$ or $^{15}NH_4Cl$ (both 98 atom% excess) at 25 °C (BassiriRad et al. 1993). The root subsamples were incubated in the ^{15}N solutions for 30 minutes, rinsed with 0.5 m*M* $CaSO_4$ (three 50-ml aliquots), oven dried at 75 ^oC for at least 24 h, and ground with a mortar and pestle. The total N concentration (mg N g^{-1}) and δ ^{15}N was determined for each root sample using a Finnigan Delta Plus isotope ratio mass spectrometer with a Conflo II interface (Thermo Finnagin, San Jose, CA, USA).

Uptake rates of $^{15}NO_3^-$ and $^{15}NH_4^+$ were reported as µmol $^{15}NO_3^-$ or $^{15}NH_4^+$ per gram root dry weight per hour (umol $^{15}NO_3^-$ or $^{15}NH_4^+$ g^{-1} h^{-1}). The atom percent ^{15}N of the root suspended in the blank (i.e., no ^{15}N) uptake solution was subtracted from the atom percent ^{15}N of each root sample suspended in a $^{15}NO_3^-$ or $^{15}NH_4^+$ uptake solution. The $^{15}NO_3^-$ or $^{15}NH_4^+$ uptake rates were used to calculate the maximum velocity (V_{max}) of uptake and the half-saturation constant (K_m) using the Michaelis-Menton equation. Non-linear regression was used to estimate V_{max} and K_m for the population of composite roots collected in each plot.

Root and leaf NRA

Fine root and foliar NRA was determined using an *in vivo* NO_2^- production assay (Jaworski 1971, Al Gharbi and Hipkin 1984, Downs et al. 1993, Rothstein et al. 1996). Disks of intervein leaf tissue were cut using a standard hole punch (6-mm diameter). Non-woody very fine roots were collected (<1 mm in diameter), gently rinsed free of soil with deionized H_20, and cut to approximately 1-cm lengths. On each sampling date, all

leaf disks and root segments from each plot were composited and NR activity was determined for 5 replicate samples. For each sample, 250 mg of fresh leaf or root tissue was suspended in 7.5 mL of the optimized assay medium, comprised of 0.016 *M* NaH_2PO_4, 0.084 *M* Na_2HPO_4, 0.04 *M* KNO_3, 5 % $CH_3(CH_2)_2OH$ (n-propanol), and 0.5 mg/mL chloramphenicol (pH 7.5). The plant tissue placed in this medium was vacuum infiltrated and incubated at room temperature (23 ^oC) for 1 h in the dark. Nitrite production was measured colormetrically at 20-min intervals by removing 1.0 mL aliquots and adding 1% sulfanilamide and 0.3 ml 0.02% N-(1-naphthyl)ethylenediamine DI HCl. This solution was incubated for 20 min, diluted with 2.4 mL deonized H_20 and absorbance was measured at 520 nm.

Fine root and foliar NO_3^- NRA was calculated as the slope of the linear regression of NO_2^- production over time, expressed as nmol NO_2^- per g fresh tissue per hour (nmol NO_2^- g^{-1} h^{-1}). Only replicates for which the goodness of fit was greater than 0.80 were used for further statistical analyses.

Statistical Analyses

I used an ANOVA for a randomized complete block design replicated in time to test the effect of chronic N deposition on N uptake and NRA. In this design, stands are blocks ($n = 4$), N deposition is the treatment ($n = 2$), and there were 2 sampling dates. This model included an interaction term between N deposition and time. Tukey's HSD multiple comparison procedure was conducted when there was a significant main or interaction effect. Significance was accepted for all statistical tests at $\alpha = 0.05$.

RESULTS

Nitrogen Uptake

Experimental NO_3^- deposition did not have a significant effect on the uptake of either NH_4^+ or NO_3^-. For example, there was no significant main effect of experimental NO_3^- deposition on the maximum velocity (V_{max}) or the half-saturation constant (K_m) for either NH_4^+ or NO_3^- uptake (Table 2.2). The uptake curves, based on the mean kinetic values for the two N deposition treatments are illustrated in Fig. 2.2. Averaged across N deposition treatment, the mean V_{max} for NH_4^+ uptake was 8 times greater than the mean V_{max} for NO_3^- uptake. The half-saturation constant (K_m) for NH_4^+ uptake was 16 times greater than the mean K_m for NO_3^- uptake. These results indicate that sugar maple has a much greater capacity to uptake NH_4^+ than NO_3^-.

Season had a significant effect on the N uptake kinetics, wherein the mean V_{max} for NH_4^+ uptake in September was significantly greater than that measured in June (6.8 vs. 3.5 μmol $^{15}NH_4^+$ g^{-1} h^{-1}, p = 0.001, Table 2.2). This contrasts with a significantly greater K_m for NH_4^+ uptake in June than in September (1093 vs. 537 μmol $^{15}NH_4^+$ L^{-1}, respectively; p = 0.008, Table 2.2). The mean K_m for NO_3^- uptake in June 2004 was significantly greater than in September 2004 (71.3 vs. 31.4 μmol ^{15} NO_3^- L^{-1}, p=007), although there as no difference between the V_{max} for NO_3^- uptake in June and September (Table 2.2).

There was not a significant interaction between season and chronic NO_3^- deposition for any aspect of uptake N kinetics in sugar maple (Table 2.2). However, there was a marginally significant interaction between NO_3^- deposition and site for V_{max} (P=0.076) and K_m (P=0.066) of NH_4^+ uptake. At Site B, the V_{max} of NH_4^+ uptake

increased with chronic NO_3^- deposition (4.8 vs. 9.0 μmol $^{15}NH_4^+$ g^{-1} h^{-1}), whereas in Site C, the V_{max} of NH_4^+ uptake decreased with chronic N deposition (5.6 vs. 3.2 μmol $^{15}NH_4^+$ g^{-1} h^{-1}). Similarly, at Site B, the K_m of of NH_4^+ uptake increased with N deposition (from 583 to 1507 μmol $^{15}NH_4^+$ L^{-1}), while it decreased with N deposition at Site C (from 1192 to 586 μmol $^{15}NH_4^+$ L^{-1}

Significant differences in the kinetic parameters for both NH_4^+ and NO_3^- uptake occurred among stands, but these differences were relatively small in magnitude and did not appear to be related to patterns of soil N availability or any other characteristics of soil physical or chemical properties.

Nitrate Reductase Activity

Experimental NO_3^- deposition did not have a significant effect on the assimilation of NO_3^- into amino acids by sugar maple, as there was no significant effect of chronic N deposition on NO_3^- reductase activity, the rate limiting enzyme in the assimilation of NO_3^- into amino acids, in sugar maple roots or leaves (Table 2.2). There was no effect of site on sugar maple NRA in roots or leaves (data not shown). Leaf NRA differ significantly between the June and September sampling dates (103.6 vs. 81.5 nmol $N0_2^-$ g^{-1} h^{-1}, respectively; P = 0.006). A significant site by season interaction (p = 0.011) occurred due to higher leaf NRA in Sites A and D in June, relative to the September sampling. There were no main or interactive effects of N treatment, site, or season sampled on sugar maple root nitrate reductase activity. In short, chronic NO_3^- deposition has not induced sugar maple to utilize NO_3^- as N source.

DISCUSSION

Atmospheric N deposition has the potential to alter the retention and loss of N in terrestrial ecosystems. *Acer saccharum* has a limited capacity to take up and reduce NO_3^-, even when seedlings of this plant were fertilized with NO_3^- (Rothstein et al. 1996). Instead, sugar maple was shown to primarily rely on NH_4^+ as its main source of N from the soil. Nonetheless, it is possible that chronic NO_3^- deposition could induce sugar maple NO_3^- uptake and assimilation (Hoff et al. 1992, Oaks 1994). After 10 years of experimental NO_3^- deposition, however, the uptake and assimilation of NO_3^- by sugar maple remained low and did not differ between ambient and experimental NO_3^- deposition treatments. Thus, low rates of NO_3^- use by sugar maple confirm that this species is not a direct sink for anthropogenic NO_3^- deposition in northern hardwood forests and will likely remain as such as N deposition continues to increase over the next several decades (Galloway et al. 2004).

The physiological capacity of the dominant tree species in Lake States northern hardwood forests (i.e., sugar maple) to uptake NO_3^- after 10 years of chronic experimental NO_3^- deposition is low relative to ability to uptake NH_4^+. In the sugar maple roots, V_{max} of NO_3^- was found to be only 12% of the V_{max} of NH_4^+, clearly indicating that sugar maple has a greater capacity to take up NH_4^+. However, sugar maple roots have a higher affinity for NO_3^-, which is suggested by a K_m for NO_3^- uptake that was much lower than that for NH_4^+. As a result, at very low concentrations of N, sugar maple might uptake more NO_3^- than NH_4^+ (See Fig. 2.2). The N uptake kinetics determined in this study differed considerably from the results of a previous experiment in the same stands (Rothstein et al. 1996). For both NH_4^+ and NO_3^-, V_{max} was lower and

K_m was higher in this study, relative to the observations made by Rothstein et al. (1996) Potential explanations for this distinction are that Rothstein et al. (1996) used sugar maple seedlings transplanted to the greenhouse where N uptake was measured late in the fall. Nonetheless, evidence from both studies indicate that sugar maple has a greater capacity for NH_4^+ than NO_3^- utilization and that NO_3^- fertilization did not induce NO_3^- uptake in this species.

Chronic NO_3^- deposition also did not induce NRA in sugar maple leaves and roots, further suggesting that this plant will not become an additional sink for atmospheric NO_3^- deposition in the future. The mean NRA was 26 nmol NO_2^- g^{-1} h^{-1} in roots and 93 nmol NO_2^- g^{-1} h^{-1} in leaves, and did not differ between atmospheric N deposition treatments. Thus, it appears that NO_3^- reduction in sugar maple is not inducible by chronic atmospheric NO_3^- deposition. These rates I report here were an order of magnitude higher than those observed by Rothstein et al. (1996) in the same forest stands. The reason for this discrepancy is not known. Nevertheless, rates of NO_3^- reduction I measured are still very low relative to other plants. For example, a study of over 100 species of ruderal, woodland-edge, and woody plants found that leaf nitrate reductase activity ranged from 1400 to 8750 nmol NO_2^- g^{-1} h^{-1} in the ruderal species, from 100 to 3210 nmol NO_2^- g^{-1} h^{-1} in the woodland-edge species, and from less than 100 to 4800 nmol NO_2^- g^{-1} h^{-1} in the woody species (Al Gharbi and Hipkin 1984). Relative to these observations, the extremely low rates measured in *A. saccharum* suggest that this species does not use NO_3^- as a major N source.

The limited capacity of sugar maple to directly use NO_3^-, even under long-term NO_3^- fertilization, limits the potential these forests to retain anthropogenic NO_3^-

deposition. Sugar maple can only utilize NO_3^- deposition that has first been reduced to NH_4^+ by soil microbes. While studies have demonstrated that microbial immobilization is a rapid and large sink for NO_3^- in Lake States northern hardwood forests (Zogg et al. 2000), the inability of sugar maple to directly uptake NO_3^- likely facilitates longer retention of N within the exchangeable NO_3^- pool and consequently the leaching of this mobile form of inorganic N. Related studies have demonstrated that chronic NO_3^- deposition elicited substantial increases in NO_3^- leaching, which is consistent with the aforementioned contention. (Pregitzer et al. 2004, Zak et al. 2004)

In conclusion, after a decade of chronic NO_3^- deposition, NO_3^- uptake and reduction in sugar maple remained comparable to trees exposed to low rates of ambient atmospheric NO_3^- deposition. Together, this evidence strongly suggest that chronic NO_3^- deposition will not induce a greater capacity to take up and assimilate this form of N, rendering sugar maple unable to function as an additional sink as atmospheric NO_3^- deposition increases over the next several decades.

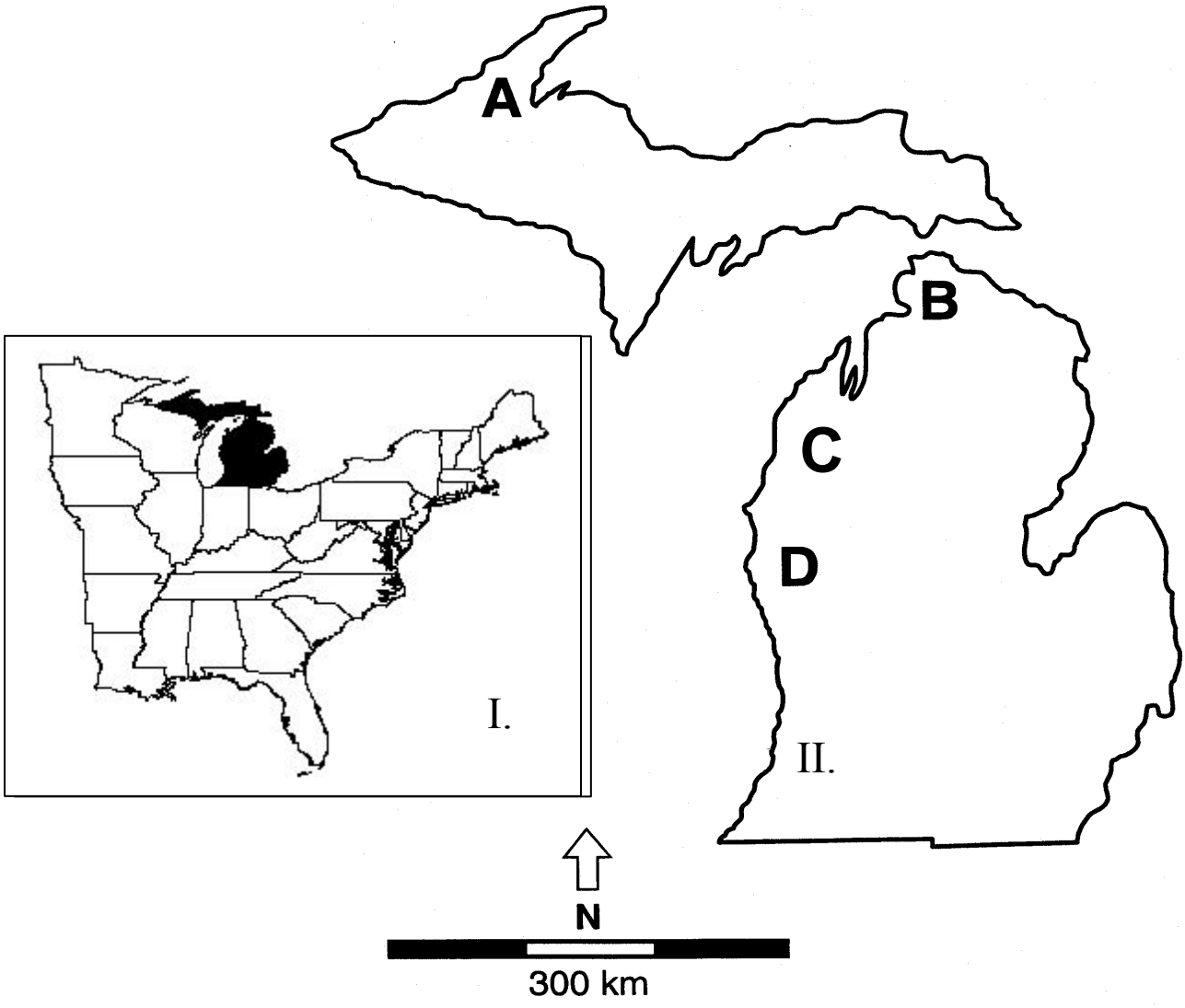

Figure 2.1. The geographic distribution of the study sites, showing the location of Michigan in the eastern United States (I) and the location of the four study sites within Michigan (II). Adapted from (Pregitzer et al. 2004)

Table 2.1. Climatic, edaphic and floristic characteristics of four northern hardwood stands located along a latitudinal gradient in Michigan, USA.

Characteristic	**Site A**	**Site B**	**Site C**	**Site D**
Latitude (N)	46° 52'	45° 33'	44° 23'	43° 40'
Longitude (W)	88° 53'	84° 51'	85° 50'	86° 09'
Net N mineralization (mg N g soil^{-1})[†]	0.29b	0.46a	0.48a	0.32b
pH of A+E soil horizons[‡]	4.6-5.1	4.6-5.3	4.4-4.5	4.3-5.2
pH of upper B soil horizons[‡]	4.7-5.7	4.8-6.3	5.2-6.9	5.0-5.7
Forest floor (Oe+Oa) C:N ratio[±]	16.7	18.4	20.6	17.9
Mean annual precipitation, 1994-2001 (mm)[≠]	821	828	856	793
Mean annual temperature, 1994-2001 (°C)[√]	4.8	6.1	6.9	7.6
Wet plus dry NO_3^--N (g m^{-2} yr^{-1})[◊]	0.38	0.58	0.78	0.76
Wet plus dry total N (g m^{-2} yr^{-1})[◊]	0.68	0.91	1.17	1.18
Total basal area, 2001(m^2 ha^{-1})	35	33	33	36
Sugar maple basal area, 2001 (% of total)	91	86	79	71
Overstory age, 2005	98	92	93	97

[†] N mineralization data from (Zogg et al. 1996) for the top 10 cm of soil and organic matter below the litter (O_i) layer measured using the buried bag technique. Sites indicated by different letters had significantly different rates ($p<0.05$).

[‡] Soil pH values from N.W. MacDonald, unpublished data, from samples collected as described in MacDonald et al. (1991) for the control plots.

[±] Forest floor C:N data are calculated from MacDonald et al. (1991).

[≠] Precipitation amounts collected in open areas within 5 km of each site.

[√] Air temperature was measured at 2 m within all plots at all sites, using thermistors that were read every 30 minutes throughout the year.

[◊] Atmospheric deposition data from MacDonald et al. (1992).

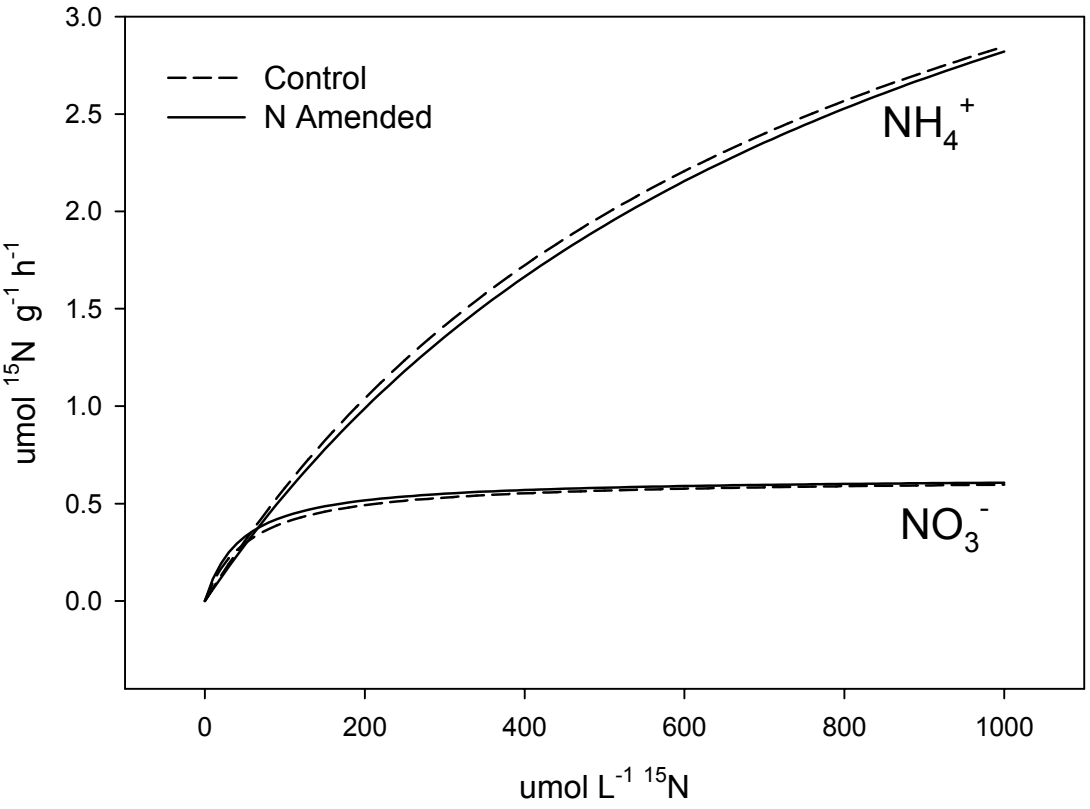

Figure 2.2. The velocity of N uptake by *Acer saccharum* roots as a function of substrate N concentration. Dashed lines indicated the ambient N deposition, whereas the solid line indicates the experimental NO_3^- deposition treatment. The N substrate (NO_3^- or NH_4^+) is indicated on the figure.

Table 2.2. The Michaelis-Menten kinetics of *Acer saccharum* root N uptake and nitrate reductase activity in control and N-amended plots grouped by N amendment treatment and season sampled. P values are shown for the ANOVA model used test significant main effects and interaction of N amendment and season sampled Significance is denoted as *P < 0.10, ** P < 0.05, ***P < 0.01, ****P < 0.001. One standard error is shown in parentheses

	Control			N-amended			Significant effects		
	June	Sept	Mean	June	Sept	Mean	N addition P	Season P	Interaction P
V_{max} (umol ^{15}N g^{-1} h^{-1})									
NH_4^+	3.5 (0.49)	6.6 (0.76)	5.0 (0.54)	3.5 (0.53)	7.1 (1.8)	5.3 (0.97)	0.799	****	0.208
NO_3^-	0.64 (0.08)	0.62 (0.05)	0.63 (0.05)	0.59 (0.08)	0.68 (0.09)	0.63 (0.06)	0.908	0.404	0.106
K_m (umol L^{-1})									
NH_4^+	497 (106)	1039 (189)	768 (120)	576 (130)	1147 (339)	861 (187)	0.639	***	0.308
NO_3^-	79.8 (25)	33.3 (4.1)	56.6 (13)	62.9 (19)	29.5 (3.9)	46.2 (10)	0.459	***	0.639
NRA (nmol NO_2 g^{-1} h^{-1})									
Root	24.0 (15)	31.2 (4.3)	27.7 (7.5)	22.8 (22)	26.2 (2.3)	24.6 (10)	0.850	0.521	0.866
Leaf	105 (9.4)	79.5 (5.0)	92.2 (5.9)	102 (12)	83.5 (7.3)	92.9 (7.5)	0.922	***	0.668

CHAPTER 3: BELOWGROUND NITROGEN CYCLING IN NORTHERN HARDWOOD FORESTS RECEIVING CHRONIC ATOMPSHEREIC NO_3^- DEPOSITION

INTRODUCTION:

The burning of fossil fuels, the production of commercial N fertilizer, and the widespread cultivation of legumes have doubled the availability of reactive N in the biosphere (Galloway 1995). Emissions of N to the atmosphere have increased N deposition onto terrestrial and aquatic ecosystems downwind of sources (Aber et al. 1989, Galloway 1995). The rate of N deposition is particularly high in temperate forests which are often located close to industrialized sources of N emission (Galloway and Cowling 2002). Chronic atmospheric N deposition is thought to cause N saturation, a condition in which ecosystem availability of nitrate, (NO_3^-) and ammonium, (NH_4^+) is greater than the plant and microbial nutritional demand, alleviating N limitation of growth and causing an ecosystem to be less retentive of the excess N (Aber et al. 1989, Aber 1992).

Atmospheric NO_3^- deposition can be retained within ecosystems by plant (Aber et al. 1989, Nadelhoffer et al. 1995, Zak et al. 2004), microbial, (Nadelhoffer et al. 1999, Zogg et al. 2000) and physical processes (Johnson et al. 2000, Fitzhugh et al. 2003). The ability of plants to act as a sink for anthropogenic N depends on the degree to which their growth is limited by N, their physiological capacity to take up and assimilate the available forms of N, and their competitiveness with soil microorganisms for this resource (Rothstein et al. 1996). Although microbial communities in soil are more effective competitors for N in soil solution, rapidly taking up available NO_3^- (Jackson et al. 1989, Zak et al. 1990, Davidson et al. 1992), they function only as a short term sink for N. Nonetheless, the flow of N through microbial biomass over short time scales, might be very important in controlling long-term N retention. For

example, the microbial immobilization of N can promote N retention if N released from microbial biomass is incorporated into soil organic matter (SOM), released as dissolved organic N (DON), or mineralized as NH_4^+ (Stark and Hart 1997). The microbial conversion of highly mobile NO_3^- into less mobile NH_4^+ that can be retained by cation exchange, and into DON that can be stabilized in soil through adsorption by mineral soils particles (Qualls and Haines 1992), can prevent N from leaching from the soil. Absorption of DON by the mineral soil, however, does not appear limitless as DON leaching can increase significantly in response to experimental N deposition (Magill et al. 2000, Pregitzer et al. 2004). Physical processes contributing to ecosystem N retention are important mechanisms of rapid N retention in many ecosystems, but the exact mechanisms responsible for this are not well understood (Johnson et al. 2000, Fitzhugh et al. 2003).

Northern hardwood forests in the Upper Lake States region receive moderate rates of atmospheric N deposition, and may be particularly prone to N saturation due to high soil N availability (Zak and Pregitzer 1990, Zogg et al. 1996) and coarsely textured soils. In these ecosystems, ambient levels of atmospheric NO_3^- deposition are initially assimilated by the microbial community in soil and released into soil solution as NH_4^+ where it is taken up by plant roots (Zogg et al. 2000). However, recent evidence indicates there have been fundamental changes in processes regulating soil C and N cycling in response to chronic N deposition. Experimental NO_3^- deposition has resulted in increased leaching of NO_3^-, dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) from the surface soil (Pregitzer et al. 2004), as well as reduced soil microbial biomass, activity of cellulose and lignin degrading enzymes (DeForest et al. 2004a, DeForest et al. 2004b), and soil respiration (Burton et al. 2004). These observations suggest that both plant and soil sinks for atmospheric NO_3^- have been

saturated by less than a decade of atmospheric NO_3^- deposition at rates comparable to those presently being input into the northeastern U.S. and Europe.

The overall objective of my study was to quantify how chronic anthropogenic NO_3^- deposition has altered the short-term flow and fate of N in northern hardwood forests. The flow of N over shorter time frames are important in controlling the initial cycling of N deposition through the biotic and physical processes that in part determine the long-term N retention in the soil. My specific objectives were to understand i) how chronic NO_3^- deposition has altered the flow of N through soil microbial communities, and ii) how documented declines in microbial activity in response to chronic NO_3^- deposition (DeForest et al. 2004a) have influenced the fate of anthropogenic NO_3^- in soil. Previous studies have suggested that alteration of soil C and N cycling in response to chronic NO_3^- deposition has been primarily biologically driven (Burton et al. 2004, DeForest et al. 2004a, DeForest et al. 2004b, Pregitzer et al. 2004) Therefore, we focus our attention on the of the flow of N through the microbial community. To address these objectives, a tracer experiment was conducted in which $^{15}NO_3^-$ was added *in situ* to the soil surface of ambient and experimental N deposition treatments in a northern hardwood forest. I examined the fate of ^{15}N tracer in plant, microbial and soil pools at 12 samples times from 2 h to 12 wks after tracer addition.

MATERIALS AND METHODS

Study Site

I conducted this study in one northern hardwood forest stand (Site B, 45° 33' N lat., 88° 53' W long.) near Pellston, Michigan. This stand was chosen, because previous work has established the pattern and rate at which NO_3^- flowed from soil solution into microbial, plant,

and soil pools (Zogg et al. 2000, Zak et al. 2004). The stand is a 92-year-old second-growth northern hardwood forest with 85% of the basal area consisting of sugar maple (*Acer saccharum* Marsh). American beech (*Fagus grandifolia* Ehrh.), white ash (*Fraxinus Americana* L.), American basswood (*Tilia americana* L.), and yellow birch (*Betula alleghaniensis* Britton) each comprise the remainder of basal area in this stand. The mean annual temperature is 6.1°C and the mean annual precipitation rate is 828 mm. The soil is a sandy Typic Haplorthod of the Kalkaska series.

In this forest stand, there are six 30-m x 30-m plots with a 10-m buffer zone along each side. Three plots within the stand receive ambient levels of N deposition (5.8 kg N ha^{-1} yr^{-1} wet + dry deposition as NO_3^-, 9.1 kg N ha^{-1} yr^{-1} wet + dry total N deposition), and three plots and buffers received ambient plus 30 kg N ha^{-1} y^{-1} applied as dry $NaNO_3$. This treatment is comparable to rates of NO_3^- deposition experienced presently in the northeast U.S and Europe (MacDonald et al. 1992, Bredemeier et al. 1998, Fenn et al. 1998). Nitrate additions occur during the growing season (late April/early May to September) in six equal increments. Treatment began in spring 1994 and has continued through the present year.

$^{15}NO_3^-$ Tracer Experiment

In order to follow the flow of NO_3^- in soil, I conducted a $^{15}NO_3^-$ tracer experiment in plots receiving ambient and experimental N deposition. This study closely replicates the experimental design of Zogg et al. (2000), but with an additional goal: to determine the effect of 10 years of chronic N amendment on the short-term flow and fate of N. One 9-m^2 subplot was established in the buffer zone of each ambient and N-amended plot (n = 3 for each treatment). On 15 August 2004, isotopic tracer was applied using a backpack sprayer at a rate of 1927 μmol ^{15}N m^{-2} to each

subplot ; the isotope was delivered as $K^{15}NO_3$ (98%) dissolved in deionized H_20 (4 L/m^2). Forest floor sampling consisted of collecting the loose litter (O_i and O_e) from three randomly 10-cm x 20-cm quadrants within the 9-m^2 plot. Within the quadrant with forest floor removed, two soil samples (5.1-cm diameter) were taken in random locations to a depth of 10 cm. The samples were transported on ice to the laboratory for analysis. Each plot was sampled prior to $^{15}NO_3^-$ application, and at 5 h, 10 h, 1 d, 2 d, 1 wk, 2 wk, 4 wk, 6 wk, 9 wk, and 12 wk after ^{15}N addition

Within 24 hours of field sampling, the roots were sorted by hand, removing as much of the adhered soil as possible. The soil was passed through a 2-mm sieve. Soil samples were immediately processed to determine ^{15}N in soil solution, microbial biomass, and soil organic matter, whereas the roots and forest floor were frozen immediately at -20ºC for subsequent isotopic analyses. The N content (g m^{-2}) and the δ ^{15}N were determined in the forest floor, fine root (0.5-2 mm), very fine root (<0.5 mm), inorganic N (NO_3^- and NH_4^+), dissolved organic matter, microbial biomass, and soil organic matter pools.

The N concentration and δ ^{15}N in NO_3^-, NH_4^+, microbial biomass N, and soil organic matter N pools were determined by using a sequential extraction procedure (Holmes et al. 2003). Field moisture soil (12-g) was extracted twice with 20 mL of 0.5*M* K_2SO_4, thereby removing NH_4^+, NO_3^-, and dissolved organic N (DON). The extracted soil was then fumigated with chloroform for 5 d and extracted twice again with 20 mL of 0.5*M* K_2SO_4. This extract contained microbial biomass N. The N remaining in the soil after both extractions and $CHCl_3$ fumigation was considered to be the soil organic matter (SOM) N.

Nitrate and NH_4^+ concentrations in the first (i.e. unfumigated) soil extract were determined colormetrically on an FS3000 Automated Flow Analyzer (O.I. Analytical Corp., College Station, Texas). Diffusion was used to collect NH_4^+ and NO_3^-, isolate each N pool from

the liquid phase, and prepare them for stable isotope analysis (Brooks et al. 1989). In this procedure, aqueous NH_4^+ was converted to gaseous NH_3 by addition of magnesium oxide (MgO). The NH_3 is trapped onto an acidified paper disk, as the gaseous NH_3 is protonated back into aqueous NH_4^+. The acidified disk was dried and used to determine $\delta\ ^{15}N$ by isotope ratio mass spectrometry. Ammonium was diffused from the unfumigated extract onto an acidified disk by addition of MgO. Next, NO_3^- was diffused from the unfumigated extract onto acidified disks by adding Devarda's reagent and MgO to this extract. Devarda's reagent is used to catalyze the conversion of NO_3^- and NH_4^+. After, NO_3^- and NH_4^+ were diffused from the unfumigated extract, the N remaining in this extract was DON, which was converted to NO_3^- by means of persulfate digestion. To accomplish this, 15 mL of extract , 5 mL of 0.148 M $K_2S_2O_8$, and 1 mL of 3*N* NaOH were combined and autoclaved at 150 ºC for 55 minutes. A sample was removed to determine DON concentration (as NO_3^-) as described above. DON (as NO_3^-) was diffused onto acidified disks by the approach described above. Microbial biomass N was collected by persulfate digestion, then diffusion of the second (i.e. $CHCl_3$ fumigated) soil extract. The $\delta\ ^{15}N$ was determined for the NO_3^-, NH_4^-, DON, and microbial N using a Finnigan Delta Plus isotope ratio mass spectrometer with a Conflo II interface (Thermo Finnagin, San Jose, CA, USA). Nitrogen concentration and $\delta\ ^{15}N$ in soil organic N was determined by mass spectrometry after the extracted soil was air-dried and ground

Forest floor samples were oven dried at 70 ^oC for at least 48 h and were then ground with a Wiley mill. Fine roots (<2-mm) were thawed, and sorted into two size classes (<0.5-mm and 0.5-2.0-mm), dried at 70 ^oC for at least 48 h, and ground with a Wiley mill. The total N concentration (mg N g^{-1}) and $\delta\ ^{15}N$ the in forest floor, fine roots, and very fine roots was determined as described above.

The excess ^{15}N content of each ecosystem pool was calculated as the product of the atom % excess ^{15}N, the N concentration (μmol N m^{-2}), and the mass of the pool (g m^{-2}). The areal mass of bulk soil and roots were determined as the mean measurement for each plot over all sampling times, thus assuming no change in the bulk density of soil or roots over the course of the experiment. The areal mass of forest floor was determined for every plot and sampling date. For each sampling date, the percent ^{15}N recovered in each pool was calculated by dividing the excess ^{15}N content in a given pool (μmol ^{15}N m^{-2}) by the total ^{15}N added per unit area (1927 umol ^{15}N m^{-2}).

Statistical Analyses

The effect of chronic NO_3^- deposition on the flow and fate of ^{15}N among soil pools was determined using a repeated-measures one-way ANOVA. Plot means of percent recovery in each pool was used in this analysis, and these data were log transformed to meet the assumptions of normality. Due to a limited sample size (n = 3), significance was accepted for all statistical tests at $\alpha = 0.10$.

RESULTS

The N content of ecosystem pools differed between ambient and N amended treatments. While, no statistical tests were performed on these data, it appears that the NO_3^- and soil organic matter (SOM) N pools are larger in the N-amended soils (Table 3.1). In contrast, the NH_4^+, dissolved organic N (DON), microbial N, fine root N, very fine root N, and forest floor N pool were larger in the soils only receiving ambient N deposition (Table 3.1)

The ^{15}N recovered in the forest floor, extractable NO_3^-, and DON pools, as well as the total tracer recovery decreased significantly with time, while the ^{15}N recovered in SOM and very fine roots increased significantly with time. The isotope first appeared, as expected, in the extractable NO_3^- pool, where the amount of the ^{15}N tracer that was recovered was high immediately after tracer addition. Likewise, ^{15}N entered the DON pool immediately after tracer addition, peaking at the mean recovery of 2.4% 10 hours after tracer addition, and then decreased in ^{15}N until the percent ^{15}N recovery was steady at less than 0.5% beginning 2 weeks after tracer addition. In contrast, the recovery of ^{15}N in both N deposition treatments in SOM increased steadily from 4.4% recovery at 5 hours after tracer application to plateau at roughly 21% ^{15}N recovery from 2 weeks until the last sampling time, 12 weeks after tracer addition. The amount of ^{15}N recovered in forest floor was 82% 5 hours after ^{15}N addition and declined to 28% at the last sampling time, 12 weeks after tracer addition. The total amount of tracer recovered in all N soil pools measured decreased from 111% of the added ^{15}N 5 hours after tracer addition to 61% 12 weeks after tracer addition.

There were no significant main effects of experimental N deposition on the total recovery of ^{15}N or the recovery of ^{15}N in any soil pool. Significant time by treatment interactions were observed in the recovery of $^{15}NO_3^-$ ($p = 0.099$) and DON ($p=0.039$; Fig. 3.1). Following isotope addition (5h to 48h), more ^{15}N was recovered as extractable NO_3^- in the experimental NO_3^- deposition treatment, after which the amount of tracer recovered in this pool diminished greatly in both treatments and did not appear to differ. The recovery of the tracer in DON appeared to be greater in N-amended soils at 10 and 24 h after tracer addition, but there were no differences at 48h and later sampling times. Although not significant, there was a trend of greater ^{15}N recovery in the NH_4^+ and microbial N pools in the control treatment than the experimental NO_3^-

treatment from 5 hours to 1 week after tracer addition. There were no consistent differences in tracer recovery between treatments for any of the other measured soil pools (forest floor, SOM, fine root, and very fine root percent tracer recovery).

DISCUSSION

Atmospheric N deposition has the potential to alter the retention and loss of N by terrestrial ecosystems. In mature forest ecosystems, microbial biomass is unlikely to accrue and is a relatively small soil N pool, and thus is not likely to be a large sink of anthropogenic N deposition. Still, microbial assimilation could promote N retention, if N released from microbial biomass was incorporated into stable and accumulating N pools, such as soil organic matter (Stark and Hart 1997). This study examined whether the short-term flow and fate of NO_3^- has been altered by 10 years of experimental NO_3^- deposition. Forest floor N and extractable NO_3^- pools initially (i.e., after 5 h) contained the majority of ^{15}N applied. As ^{15}N moved out of these pools, it entered microbial N, then DON, NH_4^+, and soil organic matter, and eventually fine roots. Short-term N cycling differed significantly between ambient and N amended treatments, with a significantly larger recovery of ^{15}N in NO_3^- and DON in the experimental NO_3^- deposition treatment, relative to the ambient treatment. Moreover, non-significant trends suggest that more ^{15}N was present in the microbial biomass and NH_4^+ in the control treatment. There was no difference in SOM and total N retention between the two treatments, but higher recovery of the tracer in more readily leached N pools (NO_3^- and DON) suggest that chronic N deposition may reduce N retention in soil. The apparent alteration of soil N cycling in the chronic NO_3^- deposition treatment is consistent with the results of past studies that demonstrate that a large proportion of the experimental NO_3^- additions are being lost to leaching (Pregitzer et al. 2004).

Forest floor was the largest initial sink for the tracer, after which tracer in this pool decreased over time as the tracer entered other soil pools. Five hours after adding the $^{15}NO_3^-$ tracer to control and N-amended soils, the majority of the tracer (83%, mean for both N treatments) was recovered in the forest floor, with no differences in recoveries between the N deposition treatments (Fig. 3.1). This result is not unexpected, as the tracer was added in solution to the forest floor, and forest floor ^{15}N was measured in bulk, not separated by N fraction. The high initial recovery of ^{15}N tracer in the forest floor decreased steadily over the experiment, from 83% of the 5 h after application to 28% of the tracer 12 weeks as the tracer moved from forest floor and exchangeable NO_3^- into microbial N, then DON, NH_4^+, and SOM, and eventually plant roots. Nonetheless, the recovery of more than 25% of the applied tracer in the forest floor after 12 weeks, suggests that there may be important microbially-mediated or physical mechanisms of N retention within this soil horizon.

In the first two days after $^{15}NO_3^-$ tracer addition, ^{15}N recovery in the extractable NO_3^- pool remained high, with more tracer recovered in this pool in the experimental NO_3^- deposition treatment than the control treatment (Fig. 3.1). At the same time, there was greater ^{15}N recovery in soil microbial biomass in the control treatment than under chronic NO_3^- deposition, although this difference was not significant. Together these results suggest that experimental NO_3^- deposition reduced the microbial sink for N.

By 1 week, however, the recovery of the tracer in extractable NO_3^- did not appear to differ between treatments and had decreased to 3% (mean for both N treatments). From 2 weeks after tracer addition until the end of the experiment at 12 weeks, the recovery of ^{15}N as extractable NO_3^- remained less than 0.20%, demonstrating that N cycled quickly out of the soil

NO_3^- pool, and there was no significant return of N to this pool via re-mineralization and nitrification.

The flow of ^{15}N from the microbial biomass pool appeared to differ between the two treatments, with more NH_4^+ being produced in the control treatment, and more DON being produced in the N-amended treatment. The $^{15}NH_4^+$ pool was greater (although not significantly) in the N-amended treatment from 5 h to 1 wk after tracer addition. Moreover, DON was significantly greater in the chronic NO_3^- deposition treatment 24 to 48 h after tracer addition. The tracer accumulated in SOM over the course of this experiment, but no difference in the recovery of ^{15}N was found between NO_3^- deposition treatments. Taken together, these results suggest that there is differential flow of ^{15}N through soil pools in the control and chronic NO_3^- deposition treatment. In the ambient treatment, a greater amount of the tracer ^{15}N entered the microbial biomass pool. Presumably, microbial biomass ^{15}N can either be mineralized to $^{15}NH_4^+$, or can form DON or SOM. A greater fraction of microbial biomass ^{15}N, however, appeared to be mineralized to$^{15}NH_4^+$ in the control treatment, and DON in the experimental NO_3^- deposition treatment. However, movement of isotope into SOM did not differ between the two treatments.

The recovery of ^{15}N in roots did not differ between NO_3^- deposition treatments, suggesting that this is no difference in plant uptake of N between the two N treatments. Moreover, uptake of ^{15}N by fine roots likely occurred after $^{15}NO_3^-$ was reduced to $^{15}NH_4^+$. Recovery of tracer in fine roots was low in the first 24 h after isotope addition, when the concentration of $^{15}NO_3^-$ was the greatest. The percent recovery of ^{15}N in roots did not peak until *ca.* 2 wks after tracer addition, when the percent recovery of ^{15}N in NO_3^- was less than 0.2%. Furthermore, a rise tracer recovery in the $^{15}NH_4^+$ pool closely mirror the rise in the recovery of

the tracer in fine and very fine roots. These results indicate that the roots were not a large initial sink for added NO_3^-, but instead utilized NH_4^+ mineralized from microbial biomass. This supports findings that *Acer saccharum* had a limited capacity to uptake and reduce nitrate, but instead relies on NH_4^+ as its main source of N from the soil (Rothstein et al. 1996, Eddy 2005, previous chapter).

There was no difference in the sum of ^{15}N recovery in all pools, between the two treatments, although, several potential mechanisms suggest that the chronic NO_3^- deposition treatment might be less retentive of added N than the control treatment. The total amount of the tracer recovered after 12 wks did not differ between the control and experimental NO_3^- deposition treatments. Five hours after the tracer was applied, total recovery was 101% in the control treatment, whereas 122% of the ^{15}N was recovered in the experimental NO_3^- deposition treatment. By the end of the experiment, total recovery was 61% in the ambient N deposition treatment and 62% in the experimental NO_3^- deposition treatment In addition, there was no difference in the recovery of ^{15}N within soil organic matter. These results suggest that there is no difference in NO_3^- retention between N deposition treatments. Nonetheless, a greater amount of isotope recovered in stable soil N pools in the control treatment might allow for a greater N retention over longer time periods. For example, the residence time of the N within the NO_3^- pool was greater in the NO_3^- deposition treatment than in the control treatment. During the first week of this experiment, no precipitation fell. If there were a precipitation event while the pool of NO_3^- was greater in the chronic NO_3^-deposition treatment, it is likely that much more of the NO_3^- would have been leached from the upper soil. In addition, more ^{15}N entered the DON pool in the experimental NO_3^- treatment, whereas more ^{15}N from the control treatment entered the

NH_4^+ pool. Dissolved organic N is likely to be more mobile than NH_4^+, further suggesting that the N-amendment treatment might be less retentive of added N.

In conclusion, this study demonstrates that 10 years of experimental NO_3^- deposition has altered the short-term flow of N through the soil, which has important implications for soil N retention and is consistent with documented patterns of N leaching (Pregitzer et al. 2002). Forest floor N and extractable NO_3^-pools were the initial sinks for $^{15}NO_3^-$, regardless of N deposition treatment. Microbial immobilization of the added $^{15}NO_3^-$ appeared to be greater in the treatment receiving ambient N deposition, thus depleting the added NO_3^- faster in this control than under experimental NO_3^- deposition. As ^{15}N flowed out of the microbial biomass pool, it entered into DON, NH_4^+, and SOM, and eventually into plant roots. More tracer was recovered in DON in the N-amended treatment, while non-significant trends suggest that more ^{15}N was present in the NH_4^+ in the control treatment. Furthermore, the results of this study are consistent with recent observations that chronic NO_3^- deposition has caused N-saturation (Pregitzer et al. 2004), greater NO_3^- and DON leaching from the surface soil (Pregitzer et al. 2004), and reduced soil microbial biomass (DeForest et al. 2004a) in these northern hardwood forests.

Table 3.1. The N content of soil N pools in a northern hardwood forest receiving experimental NO_3^- deposition. For each ecosystem N pool, control and NO_3^- amended treatments are the mean of 10 measurements spread over 12 weeks late in the growing season in 2004. Standard errors are in parentheses.

	g N m^{-2}							
	NO_3^-	**NH_4^+**	**Dissolved Organic N**	**Microbial N**	**Soil Organic N**	**Fine Root N**	**Very Fine Root N**	**Forest Floor N**
Control	1.3 (0.18)	4.9 (0.25)	4.6 (0.33)	54.9 (2.0)	2034 (76)	5.4 (0.18)	35.5 (1.1)	625 (25)
N Amended	2.0 (0.19)	4.8 (0.34)	4.5 (0.27)	48.1 (1.8)	2056 (101)	5.0 (0.13)	30.7 (1.2)	539 (25)

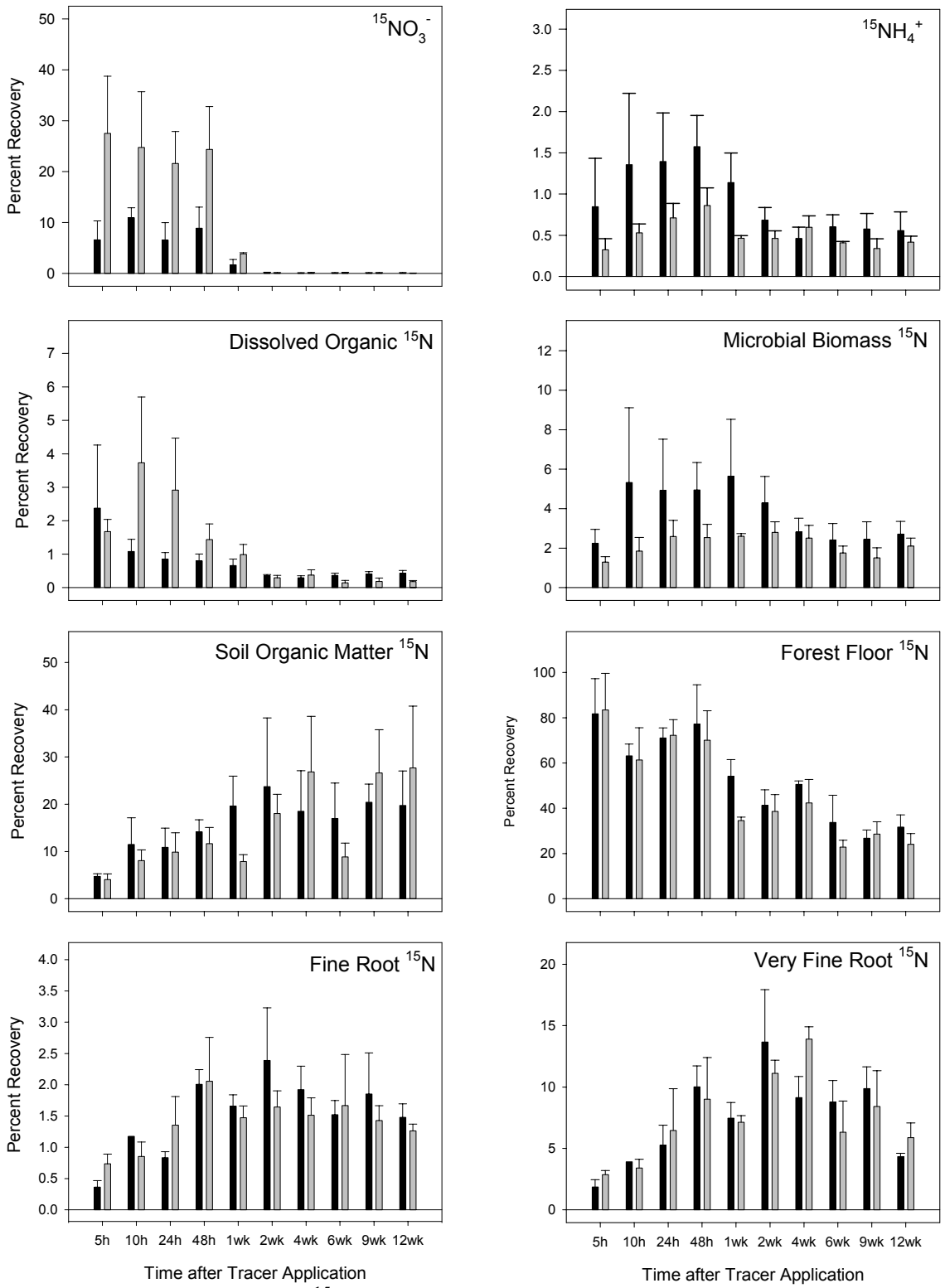

Figure 3.1. Percent recovery of ^{15}N in soil N pools over time. The control treatment (ambient N deposition) is shown in black. The chronic N03- deposition treatment (ambient + 30 kg N ha^{-1} $yr^{-1)}$ is shown in grey. Error bars are +/- 1 SE.

CHAPTER FOUR: CONCLUSIONS

In this thesis, I examined plant and microbial mechanisms for N retention in northern hardwood forests receiving chronic experimental NO_3^- deposition. Plants are typically considered to be the primary sink for N inputs to an ecosystem. Sugar maple (*Acer saccharum* Marsh.), the dominant tree species in northern hardwood forests in Michigan has been found to have a limited ability to uptake and assimilate NO_3^- (Rothstein et al. 1996), the dominant chemical form of N deposition in much of the industrialized world. The first goal of this thesis was to determine if NO_3^- uptake and assimilation by sugar maple has been included by 10 years of experiment NO_3^- deposition at the rate of 30 kg N ha^{-1} y^{-1}. In short, I found that the ability of sugar maple to utilize NO_3^- did not differ between trees in the control and experimental NO_3^- deposition treatments. There was no difference in NO_3^- or NH_4^+ uptake and NO_3^- reduction between the two treatments. Moreover, the rates of NO_3^- uptake in both treatments were low relative to the rates of NH_4^+ uptake. These results demonstrate that sugar maple is not a direct sink for NO_3^- deposition and suggest that these northern hardwood forests have a limited ability to retain excess N.

Soil microbial processes that occur over short time-spans can be important mechanisms for N retention, if the N released from microbial biomass is incorporated into stable and accumulating N pools. Thus, the second goal of this thesis was to determine if the short-term fate and retention of NO_3^- has been altered by 10 years of chronic NO_3^- deposition. In a ^{15}N tracer experiment in these forests, forest floor N and extractable NO_3^-pools were the initial sinks for $^{15}NO_3^-$. The microbial community appeared to assimilate N from these initial pools into their biomass. Nitrogen isotope flow from the microbial biomass pool was into dissolved organic N, NH_4^+, and soil organic N, and eventually into plant roots. Several important differences existed

in the flow of ^{15}N between the control and chronic N deposition treatments. First, the microbial immobilization of ^{15}N appeared to be greater in the control treatment, as this treatment had a greater tracer recovery in the microbial biomass and more rapid depletion of ^{15}N from the NO_3^- pool. Next, microbial release of ^{15}N appeared to differ between the treatments with greater release of microbial biomass N to dissolved organic N in the N-amended treatment, and higher net mineralization of microbial N to NH_4^+ in the control treatment. There was no difference in recovery of the tracer between the treatments after 12 weeks, although higher concentrations of ^{15}N within easily leached soil pools (i.e. NO_3^- and DON) suggest that the capacity of these soils to retain NO_3^- deposition may be diminished by NO_3^- inputs.

In summary, the lack of a direct sink for NO_3^- retention in the dominant overstory tree species (i.e. sugar maple) and the higher residence time of N in more mobile soil pools within the chronic NO_3^- deposition treatment are consistent with recent observations of higher extractable soil NO_3^- (Zak et al. 2004), increased NO_3^- DOC, and DON leaching (Pregitzer et al. 2004), and rapid N saturation (Pregitzer et al. 2004) with experimental NO_3^- deposition. Overall, it appears that the lack of plant and stable soil sinks is important in understanding why these forest stands have experienced rapid nitrogen saturation, when studies elsewhere have seen much greater quantities of N added without N saturation (e.g. Magill et al. 2000).

LITERATURE CITED

Aber, J., W. McDowell, K. Nadelhoffer, A. Magill, G. Berntson, M. Kamakea, S. McNulty, W. Currie, L. Rustad, and I. Fernandez. 1998. Nitrogen saturation in temperate forest ecosystems - Hypotheses revisited. Bioscience **48**:921-934.

Aber, J. D. 1992. Nitrogen Cycling and Nitrogen Saturation in Temperate Forest Ecosystems. Trends in Ecology & Evolution **7**:220-224.

Aber, J. D., K. J. Nadelhoffer, P. Steudler, and J. M. Melillo. 1989. Nitrogen Saturation in Northern Forest Ecosystems. Bioscience **39**:378-386.

Al Gharbi, A., and C. R. Hipkin. 1984. Studies on Nitrate Reductase in British Angiosperms .1. A Comparison of Nitrate Reductase-Activity in Ruderal, Woodland-Edge and Woody Species. New Phytologist **97**:629-639.

Andrews, M. 1986. The partitioning of nitrate assimilation between root and shoot of higher plants. Plant, Cell, and Environment. **9**:511-519.

BassiriRad, H., M. M. Caldwell, and C. Bilbrough. 1993. Effects of soil-temperature and nitrogen status on kinetics of $^{15}NO_3^-$ uptake by roots of field-grown *Agropyron-desertorum* (Fisch. ex Link) Schult. New Phytologist **123**:485-489.

Bredemeier, M., K. Blanck, Y. J. Xu, A. Tietema, A. W. Boxman, B. Emmett, F. Moldan, P. Gundersen, P. Schleppi, and R. F. Wright. 1998. Input-output budgets at the NITREX sites. Forest Ecology and Management **101**:57-64.

Brooks, P. D., J. M. Stark, B. B. Mcinteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated N-15 analysis. Soil Science Society of America Journal **53**:1707-1711.

Burton, A. J., K. S. Pregitzer, J. N. Crawford, G. P. Zogg, and D. R. Zak. 2004. Simulation chronic NO_3^- deposition reduces soil respiration in northern hardwood forests. Global Change Biology. **10**:1080-1091.

Burton, A. J., D. D. Ramm, D. D. Reed, and K. S. Pregitzer. 1991. Use of multivariate methods in forest research site selection. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere **21**:1573-1580.

Chapin III, F. S., K. Van Cleve, and P. R. Tryon. 1986. Relationship of ion adsorption to growth rate in tiaga trees. Oecologia **69**:238-242.

Curtis, J. T. 1959. The Vegetation of Wisconsin. University Wisconsin Press, Madison, Wisconsin, USA.

Davidson, E. A., S. C. Hart, and M. K. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology. **73**:1148-1156.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., and Burton, A.J. 2004a. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal. **68**:132-138.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., and Burton, A.J. 2004b. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology and Biochemistry. **36**:965-971.

Downs, M. R., K. J. Nadelhoffer, J. M. Melillo, and J. D. Aber. 1993. Foliar and Fine Root Nitrate Reductase-Activity in Seedlings of 4 Forest Tree Species in Relation to Nitrogen Availability. Trees-Structure and Function **7**:233-236.

Fenn, M. E., M. A. Poth, J. D. Aber, J. S. Baron, B. T. Bormann, D. W. Johnson, A. D. Lemly, S. G. McNulty, D. E. Ryan, and R. Stottlemyer. 1998. Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecological Applications **8**:706-733.

Fitzhugh R. D., G. M. Lovett and R.T. Venterea. 2003. Biotic and abiotic immobilization of ammonium, nitrite, and nitrate in soils developed under different tree species in the Catskill Mountains, New York, USA. Global Change Biology **9**:1591-1601.

Hoff, T., B. M. Stummann, and K. W. Henningsen. 1992. Structure, function, and regulation of nitrate reductase in higher plants. Physiologia Plantarum **84:**616-624.

Holmes, W. E., D. R. Zak, K. S. Pregitzer, and J. S. King. 2003. Soil nitrogen transformations under *Populus tremuloides, Betula papyrifera,* and *Acer saccharum* following 3 years exposure to elevated CO_2 and O_3. Global Change Biology. **9**:1743-1750.

Galloway, J. N. 1995. Acid deposition: Perspectives in time and space. Water Air and Soil Pollution **85**:15-24.

Galloway, J. N., and E. B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. Ambio **31**:64-71.

Galloway, J. N., F. J. Dentener, D. G. Capone, E. W. Boyer, R. W. Howarth, S. P. Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green, E. A. Holland, D. M. Karl, A. F. Michaels, J. H. Porter, A. R. Townsend, and C. J. Vörösmarty. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry **70**:153-226.

Gutschick, V. P. 1981. Evolved strategies in nitrogen acquisition by plants. American Naturalist **118**:607-637.

Jackson, L. E., D. S. Schimel, and M. K. Firestone. 1989. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Biology and Biochemistry **21**:409-415.

Jaworski, E. G. 1971. Nitrate reductase assay in intact plant tissues. Biochemical and Biophysical Research Communications **43**:1274-1279.

Johnson, D.W., W. Cheng, and I. C. Burke. 2000. Biotic and abiotic nitrogen retention in a variety of forest soils. Soil Science Society of America Jounral **64**:1503-1514.

Magill, A. H., J. D. Aber, G. M. Bernston, W. H. McDowell, K. J. Nadelhoffer, J. M. Melillo, and P. Steudler. 2000. Long-term nitrogen additions and nitrogen saturation in two temperate forests. Ecosystems **3**:238-253.

MacDonald, N. W., A. J. Burton, A. J. Jurgensen, J. W. McLaughlin, and G. D. Mroz. 1991. Variation in forest soil properties along a Great Lakes air pollution gradient. Soil Science Society of America Journal **55**:1709-1715.

MacDonald, N. W., A. J. Burton, H. O. Liechty, J. A. Witter, K. S. Pregitzer, G. D. Mroz, and D. D. Richter. 1992. Ion leaching in forest ecosystems along a Great Lakes air pollution gradient. Journal of Environmental Quality **21**:614-623.

Nadelhoffer, K. J., M. R. Downs, and B. R. Fry. 1999. Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. Ecological Applications **9**:72-86.

Nadelhoffer, K. J., M. R. Downs, B. R. Fry, J. D. Aber, A. H. Magill, and J. M. Melillo. 1995. The fate of ^{15}N-labelled nitrate additions to a northern hardwood forest in eastern Maine, USA. Oecologia **103**:292-301.

Oaks, A. 1994. Primary nitrogen assimilation in higher plants and its regulation. Canadian Journal of Botany **72**:739-750.

Pastor, J., J. D. Aber, C. A. McClaugherty, and J. M. Melillo. 1984. Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecology **65**:256-268.

Pregitzer, K. S., D. R. Zak, A. J. Burton, J. A. Ashgy, and N. W. MacDonald. 2004. Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems. Biogeochemistry **68:**179-197.

Qualls, R. G., and B. L. Haines. 1992. Biodegradability of dissolved organic matter in forest throughfall, soil solution, and stream water. Soil Science Society of America Journal **56**:578-586.

Rothstein, D. E., D. R. Zak, and K. S. Pregitzer. 1996. Nitrate deposition in northern hardwood forests and the nitrogen metabolism of Acer saccharum marsh. Oecologia **108**:338-344.

Smirnoff, N., and G. R. Stewart. 1985. Nitrate assimilation and translocaton by higher plants: comparative physiology and ecological consequences. Physiologia Plantarum **64**:133-140.

Stark, J. M., and S. C. Hart. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature **385**:61-64.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications **7**:737-750.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry **13**:87-115.

Zak, D. R., P. M. Groffman, K. S. Pregitzer, S. Christensen, and J. M. Tiedje. 1990. The vernal dam: plant-microbe competition for nitrogen in northern hardwood forests. Ecology **71**:651-656.

Zak, D. R., G. E. Host, and K. S. Pregitzer. 1989. Regional variability in nitrogen mineralization, nitrification, and overstory biomass in northern Lower Michigan. Canadian Journal of Forest Research **19**:1521-1526.

Zak, D. R., K. S. Pregitzer. 1990. Spatial and temporal variability of nitrogen cycling in northern Lower Michigan. Forest Science **36**:367-380.

Zak, D. R., K. S. Pregitzer, W. E. Holmes, A. J. Burton, and G. P. Zogg. 2004. Anthropogenic N deposition and the fate of $^{15}NO_3^-$ in a northern hardwood ecosystem. Biogeochemistry **69**:143-157.

Zogg, G. P., D. R. Zak, A. J. Burton, and K. S. Pregitzer. 1996. Fine root respiration in northern hardwood forests in relation to temperature and nitrogen availability. Tree Physiology **16**:719-725.

Zogg, G. P., D. R. Zak, K. S. Pregitzer, and A. J. Burton. 2000. Microbial immobilization and the retention of anthropogenic nitrate in a northern hardwood forest. Ecology **81**:1858-1866.