Ecological Physiology of Nitrogen Use in Trees Following Forest Disturbances

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ABSTRACT

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Nitrogen (N) is considered a key limiting element in northeastern US forests. Recent modifications to N cycling in these ecosystems due to large-scale anthropogenic disturbances have the potential to change forest species composition and productivity by altering soil N availability and ratio of ammonium (NH_4^+) to nitrate (NO_3^-). This thesis investigates the effects of two types of disturbance on tree growth and nitrogen use in forests: oak loss and urbanization. The first study assesses the response of black birch (*Betula lenta* L.) to oak girdling at the Black Rock Forest in southeastern New York, USA. Data were collected from experimental plots composed of three treatments: 100% oaks girdled, 50% oaks girdled, and control. The second study compares the response of serviceberry (*Amelanchier canadensis* (L.) Medik.), blackgum (*Nyssa sylvatica* Marsh.), black cherry (*Prunus serotina* Ehrh.), and red oak (*Quercus rubra* L.) to urbanization. In this study, data were collected at Black Rock Forest and in several urban New York City parks. Both studies found an increase in foliar N content and ^{15}N enrichment in the modified systems compared to the control. This result indicates that the disturbed forests have increased soil nitrogen availability and that nitrogen is cycling at a faster rate. It was more difficult to detect a trend in foliar nitrate reductase activity (NRA) in either study. Foliar NRA actually appeared to decrease in urban sites compared to rural sites, possibly due to drought stress. Changes in N availability and consumption can influence tree growth rates, which was observed following oak loss at the Black Rock Forest. An increase in black birch growth rates in

oak-girdled plots suggests that this species is able to respond to additional N availability and/or increased light availability. Tree species were also found to respond differently to urbanization in terms of their nitrogen use patterns. Together, these findings suggest that physiological differences between tree species could lead to changes in forest canopy composition in ecosystems with altered N cycles.

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GENERAL INTRODUCTION

Nitrogen (N) is an essential macronutrient required for plant growth and is the fourth most abundant element in plants after oxygen, carbon, and hydrogen. It is an element of many plant cell components, including nucleic acids and the amino acids that make up proteins. Assimilation of nitrogen requires a complex series of biochemical reactions that are among the most energy-requiring reactions in living organisms (Taiz and Zeiger 2006). In nitrate (NO_3^-) assimilation, the nitrogen in nitrate is converted to a higher-energy form in nitrite (NO_2^-), then to an even higher-energy form in ammonium (NH_4^+), and finally into the amide nitrogen of glutamine.

Plant roots actively take up nitrate from the soil solution and incorporate it into organic nitrogen compounds. The first reaction in this process is the reduction of nitrate to nitrite in the cytosol (Oaks 1994), which is catalyzed by the enzyme nitrate reductase. The availability of nitrate, light, and carbohydrates influences nitrate reductase at the transcription and translation levels (Sivasankar and Oaks 1996). Nitrite, a highly reactive and potentially toxic ion, is then immediately transported from the cytosol into chloroplasts in leaves and plastids in roots, where the enzyme nitrite reductase reduces nitrite to ammonium. Plants assimilate nitrate in both roots and shoots, and the balance between root and shoot nitrate metabolism varies from species to species. Many plants, including some deciduous tree species, utilize nitrogen from the soil in both ammonium and nitrate form (Templer and Dawson 2004). However, some species appear to prefer ammonium (Lovett and Tobiessen 1993) or appear to prefer a specific ammonium:nitrate ratio (Crabtree and Bazzaz 1992).

Ammonium is the preferred nitrogen source for many plants because the assimilation of ammonium consumes less energy than that of nitrate. Plants are able to directly incorporate ammonium into organic nitrogen, whereas nitrate must be reduced to ammonium before amino acids can be synthesized (Taiz and Zeiger 2006). Ammonium generated from nitrate assimilation or directly absorbed from the soil is rapidly converted into amino acids by the plant. Under extremely N-limited conditions (e.g. tundra and peatlands) microbial activity is strongly N-limited and hardly any N mineralization occurs. In these conditions, dissolved organic N (DON) is the primary form of N absorbed by all soil organisms, including plants (Schimel and Bennett 2004).

Although the atmosphere contains immense quantities (about 78% by volume) of molecular nitrogen (N_2), this large reservoir is not directly available to living organisms. In order to produce ammonia or nitrate, the extremely stable triple covalent bond between two nitrogen atoms must be broken. These reactions, known as nitrogen fixation, can be accomplished by both natural and industrial processes. Atmospheric nitrogen fixation occurs through lightning and photochemical reactions, and the remaining natural nitrogen fixation occurs biologically in bacteria or blue-green algae. Industrial nitrogen fixation employs the Haber-Bosch process in order to produce agricultural fertilizers.

The ammonium and nitrate ions that are generated through fixation or released through decomposition of soil organic matter become the object of intense competition among plants and microorganisms. Many of the forest ecosystems of the northeastern United States are nitrogen limited and exhibit a net retention of N (Aber et al. 1989). However, increased atmospheric nitrogen deposition caused by human agricultural and industrial activity may lead to nitrogen saturation of forest ecosystems, where the availability of nitrogen exceeds the capacity of plants

and soil microbes to accumulate it (Gunderson et al. 1998). The shift of forest ecosystems from nitrogen limitation to nitrogen saturation involves a complex interaction of processes in the nitrogen cycle with implications for aboveground and belowground ecosystem functioning.

N deposition varies on global and regional scales. Currently, wet and dry N deposition levels range from 10 to 12 kg N ha^{-1} yr^{-1} in southern New York and Pennsylvania to less than 4 kg N per ha^{-1} yr^{-1} in eastern Maine (Aber et al. 2003). In the northeastern United States, atmospheric N has increased 5- to 10-fold over preindustrial levels (Galloway et al. 1984) and largely occurs as nitrate (Crabtree 1992). This nitrate-rich N deposition may directly increase the amount of nitrate available to plants and temporarily increase net primary productivity. Historically, northeastern forest soils have had low rates of net nitrification, as plants and the microbial community out-compete nitrifying bacteria for ammonium (Melillo 1981, Melillo et al. 1983, Aber et al. 1983). In the long term, chronic N deposition may lead to nitrogen saturation, removing the substrate limitation on nitrification, and shifting the relative availability of nitrate and ammonium, until most or nearly all mineralized N is nitrified (Aber et al. 1989 and 1998). At this stage, biological retention of N becomes ineffective, net primary productivity decreases, and nitrate leaching increases substantially. The consequences of excess nitrogen and higher levels of nitrate in stream waters could have a lasting effect on water quality and human health. Therefore, it is critical to understand and detect changes in nitrogen levels (Galloway et al. 2003).

Nitrogen cycle flux responses are complex and may depend on a cascade of changes in N pools. For example, microbial immobilization of nitrogen may suppress root uptake and delay response in tree tissue composition, which in turn will affect litterfall N flux and subsequent decomposition (Tietema et al. 1995). When N deposition is experimentally removed, nitrate

leaching has been found to dramatically decrease. If nitrogen is not lost by leaching, the N status of forests will only change through dilution of the N pools by forest growth and accumulation of carbon in the soil (Tietema et al. 1998). This suggests that it will take a long time to change the N status of saturated forests.

In addition to the ongoing changes in nutrient cycling caused by N deposition, land use change and large-scale disturbances, like forest harvests and fires, can impact the pathways of nutrient recycling and alter their levels of retention (Aber et al. 2003, Galloway et al. 2003). By altering the amount of available nutrients, these disturbances disrupt the tight balance between carbon and nitrogen that many forests maintain, with implications for forest structure and function. For example, increased nitrogen availability may alter the tree species composition of a forest. Due to differences in nitrogen uptake preferences, some species may be better able to take advantage of an increase in nitrogen than others (Crabtee and Bazzaz 1993, Jenkins et al. 1999). In order to investigate this process, I chose to study two different types of disturbance that may have increased nitrogen availability in forests: tree mortality and urbanization.

Forests of the northeastern United States have experienced large-scale tree mortality in the past due to a number of pathogens and pests. Currently, the oak-dominated forests (genus *Quercus*) that cover much of the region are threatened by several factors, including sudden oak death (caused by the pathogen *Phytophthora ramorum)* (Brasier et al. 2002). By comparing the growth rates and foliar nitrogen characteristics of black birch trees (*Betula lenta* L.) in control plots and oak-girdled plots, established to mimic pathogen-induced mortality, I examined the nitrogen-mediated response of this common early- to mid-successional tree to the loss of a foundation taxon (*Quercus*). Urbanization is another large-scale disturbance known to have significant impacts on forest nitrogen cycling (Pouyat et al. 1997, Zhu and Carreiro 1999, 2004).

The percentage of the global human population living in cities is projected to reach 60% by 2030. This trend is even more dramatic for North America, where a projected 87% of the population will be living in cities by 2030 (United Nations 2006). As urban areas expand, the scope of their environmental impacts increases, and modified urban nutrient cycles may induce changes in forest structure. In order to examine these impacts, I compared foliar nitrogen characteristics of several native tree species in urban and rural forests.

To investigate the study questions, I chose sites in the rural Black Rock Forest in Cornwall, NY and in several urban forests within New York City public parks. As part of each study, I measured foliar N content (%N), isotopic signature ($\delta^{15}N$), and nitrate reductase activity (NRA). These variables were collected in order to quantify nitrogen consumption in the study trees and to estimate overall nitrogen availability in the forests. As a result, I was able to gain insight into the changes in nitrogen cycling and availability caused by different types of disturbance and the resulting impacts on nitrogen use in forest trees.

CHAPTER ONE: Oak Loss Increases Foliar Nitrogen, $\delta^{15}N$ and Growth Rates of *Betula lenta* in the Black Rock Forest, Hudson Highlands, Southeastern New York State, USA

Summary

Oak forests dominate much of the eastern United States, but their future is uncertain due to a number of threats and widespread failure of oak regeneration. The loss of oaks (genus *Quercus*) may be accompanied by major changes in forest nitrogen cycles with important implications for plant nutrient uptake and tree species composition. In this study we measured the changes in nitrogen use and growth rates of black birch trees (*Betula lenta* L.) following oak girdling at the Black Rock Forest in southeastern New York, USA. Data were collected from nine experimental plots composed of three treatments: 100% oaks girdled (O), 50% oaks girdled (O50), and control. Foliar nitrogen concentration and foliar ^{15}N abundance increased significantly in the oak-girdled plots relative to the control, indicating that the loss of oaks significantly altered N cycling dynamics. Foliar nitrogen concentration increased by 15.5% in the O50 and 30.6% in the O plots relative to the control, while O50 and O plots were enriched in ^{15}N by 33.3% and 102.4% respectively ($p < 0.0001$). A 641% increase in black birch growth rates in O plots suggests that this species is able to respond to additional N availability and/or increased light availability. The loss of oaks and subsequent increase in black birch productivity may have a lasting impact on ecosystem form and function.

Introduction

Over the past century, forests of the United States have experienced the loss of dominant tree species such as the American chestnut (*Castanea dentata* (Marsh.) Borkh.), American beech (*Fagus grandifolia* Ehrh.), and eastern hemlock (*Tsuga canadensis* (L.) Carrière). Oak trees (genus *Quercus*) are currently foundation organisms in eastern US forests, playing critical ecosystem roles such as regulating water yield and quality, carbon storage, nitrogen retention, fire regimes, and biological diversity (Ellison et al. 2005, Lovett et al. 2002 and 2004, Templer et al. 2005, Foster et al. 2002, Jones et al. 1998). The future of oak forests is now uncertain, due to a host of threats including aging canopy trees, climate change, soil acidification, insect herbivory, pathogens, and the potential for increased logging (Abrams 1992, Cha et al. 2010, Lovett et al. 2006). One of the potentially most serious threats is sudden oak death, caused by the pathogen *Phytophthora ramorum*, as red oak (*Quercus rubra* L.) has been found to be susceptible to the disease (Brasier et al. 2002). Oaks are also failing to regenerate through much of their range, primarily due to high browsing pressure and seed predation by white-tailed deer, thus shifting forest composition and potentially compromising many of the benefits provided by oak forests (Côté et al. 2004, Rooney and Waller 2003).

In order to explore the potential ecosystem-level consequences of the loss of oaks an oak-girdling experiment was implemented in 2008 in the Black Rock Forest (BRF), located in the Hudson Highlands region of southeastern New York State. The experimental design includes four treatments which are replicated in three blocks along the North Slope of Black Rock Mountain: 100% oaks girdled, 50% oaks girdled, 100% non-oaks girdled, and control (Figure 1). The focus of the project is primarily on quantifying environmental drivers and identifying

impacts on key forest taxa and higher-level phenomena including energy flow, chemical cycling, and biological diversity. The girdling treatment mimics pathogen-induced mortality by blocking the flow of carbon from the leaves to the roots. This blockage forces the roots to utilize stored reserves of carbon to survive and within about three years girdling starves the roots to the point of mortality (Edwards and Ross-Todd 1979). The mycorrhizal fungi that depend upon these carbon pools experience declines in their productivity, growth and survival as well (Johnson and Edwards 1979).

By altering the amount of available carbon and nitrogen, widespread tree mortality disrupts the tight balance between carbon and nitrogen that many forests maintain. Johnson and Edwards (1979) found that an increase in available ammonium following stem girdling caused an increase in nitrification rates, possibly as a result of decreased belowground competition for ammonium between nitrifiers and other heterotrophs. Similarly, Jenkins et al. (1999) found that net N mineralization, nitrification, and N turnover increased at sites experiencing hemlock mortality.

These findings indicate that oak loss may be accompanied by major changes in forest nitrogen cycles with potentially important implications for plant nutrient uptake and tree species composition leading to a successional change away from oak-dominated forests. Tree species vary in their preferred ratio of ammonium:nitrate uptake and therefore may differ in their response to a change in nitrogen availability. Black birch (*Betula lenta* L.) seedlings in particular have been found to grow larger when nitrate is more readily available (Crabtree 1992, Crabtree and Bazzaz 1992). In addition, an increase in black birch abundance has been found following hemlock decline (Orwig and Foster 1998, Jenkins et al. 1999, Catovsky and Bazzaz 2000, Kizlinski et al. 2002, Stadler 2006, Cobb 2010) and tree harvesting (Smith and Ashton 1993,

Kizlinski et al. 2002), making it a likely species to respond to the loss of the oaks in this experiment.

We predicted that changes in N availability caused by the loss of oaks would have significant impacts on nitrogen use and growth rates of black birch trees at BRF. In order to test this prediction, we measured concentration and isotopic signature of foliar N (%N and $\delta^{15}N$), nitrate reductase activity (NRA), and growth of young black birch trees in oak-girdled and control plots.

Experimental Methods

Study Site

This study was conducted at the Black Rock Forest (BRF), a 1,550 ha preserve located in Cornwall, NY. The study plots are located on the north slope of Black Rock Mountain (41.45° N, 74.01° W). BRF is a mixed hardwood forest with a canopy comprised of about 67% oak and 33% non-oaks (Schuster et al. 2008). Red oak and chestnut oak (*Quercus prinus* L.) dominate the canopy, while the understory is dominated by red maple (*Acer rubrum* L.). Sugar maple (*Acer saccharum* Marsh.), black birch (*Betula lenta* L.), and yellow birch (*Betula alleghaniensis* Britt.) make up a significant composition of the forest as well. Air temperatures are strongly seasonal ranging from a mean of −2.7 °C in January to 23.4 °C in July and the average annual precipitation is 1,200 mm (NOAA 2002). The soils are acidic and low in nutrients, with granite gneiss bedrock or glacial till parent material at 0.25-1 m depth (Olsson 1981).

The forest was colonized by English settlers in 1700 and has been repeatedly logged with some portions converted to agriculture and pastureland until it was abandoned around 1900. In 1928 the land became a research forest, and in 1989 BRF was bought from Harvard and established as part of a consortium of local educational and research institutions (Schuster et al. 2008).

Experimental Design

This study utilized nine out of twelve experimental plots (75m x 75m) in a randomized block design grouped by slope position (upper, middle, and lower) (Figure 1). Each block contained each of four treatments: 100% oaks girdled (O), 50% oaks girdled (O50), girdling of all non-oaks (NO) and the control (C). Our study included all plots except for the three NO plots. The girdling was performed during the summer of 2008, when a chain saw was used to make a 5 cm deep incision at breast height around the circumference of the tree. The cut only penetrated from the bark into the phloem preserving most of the xylem. Trees with a DBH < 2.54 cm were left undisturbed.

Sampling

Five black birch trees with diameters at breast height approximately 2-6 cm were haphazardly selected within each of the nine study plots, as close to the center of the plot as possible to minimize edge effects. Leaf samples were collected from each tree at three times throughout the growing season on May 23-24, July 4-5, and August 26-27, 2010.

Nitrate Reductase Assay

Nitrate reductase activity was measured in black birch leaves in early July and late August. Leaves were collected on two consecutive sunny days between 11 AM and 1 PM in order to ensure the presence of the light-activated nitrate reductase enzyme. All leaves were cut from the middle of the canopy using a pole pruner. Four leaves from each tree were then hole-punched into 6 mm discs and weighed to produce approximately 0.2 mg of fresh leaf tissue per sample. The samples were then analyzed for nitrate reductase activity based on the method of Stewart et al. (1986). Five ml of assay solution (100mM sodium phosphate buffer, pH 7.5; 200mM KNO_3^-; 3% (v/v) n-propanol) were added to the leaf tissue, and the samples were then allowed to incubate for one hour in darkness and were shaken every 15 minutes. Samples were vacuum infiltrated three times to ensure that the solution penetrated into the leaf tissue.

The amount of nitrite in the solutions was calculated using colorimetric analysis. One ml of the reaction mixture was pipetted out and one ml sulphanillic acid plus one ml α-naphtyl ethylenediamine dihydrochloride (NED) were added and new mixture was vortexed. Tubes were incubated for an additional 20 minutes at room temperature and were observed to turn pink. Absorbance was measured on a spectrophotometer (Unico 1100 Series, United Products and Instruments Inc., Dayton, NJ, USA) at 540 nm and zeroed against a blank (water + sulphanillic acid + NED). A standard curve was generated using known concentrations of nitrite. The amount of NRA was calculated based on the amount of nitrite in the solution and scaled to the mass of the sample analyzed using the following equation:

$$\text{NRA} = \frac{\text{nmol}}{\text{ml}} \text{x} \frac{\text{5 ml}}{\text{fresh weight (mg)}}$$

$\delta^{13}C$, %C, $\delta^{15}N$ and %N

In late May, early July, and late August, four leaves from each tree were collected as described above and oven-dried at 60°C, and subsequently ground to a fine powder (Cianflone model 2601, Cianflone Scientific Instruments Corporation, Pittsburgh, PA, USA). Samples were sent to Washington State University for tissue nutrient and stable isotope analysis. Values were obtained for $\delta^{13}C$, %C, $\delta^{15}N$ and %N.

Specific Leaf Area and Light Availability

Specific leaf area (SLA) was calculated for four leaves from every tree at every sampling period. Leaf area was measured with a LiCor Leaf Area Meter (Li – 3000, LiCor, Lincoln, NE, USA), and leaves were then oven-dried at 60°C and their dry weight was used to calculate SLA. Light availability was measured once at each tree on a cloudy day using hemispheric photographs and Gap Light Index (GLI) was calculated using Gap Light Analyzer v2.0.

Birch Growth

Within a center 25m x 25m subplot of each experimental plot, DBH of all black birch trees larger than 2.54 cm was measured annually from July 2007 to July 2010. Allometric equations were used to compute aboveground biomass (Brenneman et al. 1978).

Statistical Analysis

Data were analyzed using an analysis of covariance for each of the three response variables: NRA, %N, and $\delta^{15}N$. Stepwise model selection was used to add and remove variables in the models to solicit only those variables that are statistically significant for explaining

variation. SLA and light availability were found to be highly correlated, so only SLA was used as a covariate with the predictor variables oak-girdling treatment, slope position, and sampling month. No significant differences were found in NRA or $\delta^{15}N$ measurements between the different sampling periods, so these data were pooled. However, sampling month was a significant factor in foliar %N and was therefore included in the model. To meet the assumptions of normality required for ANOVA, %N values were raised to the -0.5 power, $\delta^{15}N$ values were raised to the 0.75 power, and $\delta^{13}C$ values were raised to the 0.5 power after adding a constant to make all isotope values positive. Tukey's HSD values were used to determine significance among the categorical values in each ANOVA. Results with $p < 0.05$ were accepted as statistically significant. All analyses were conducted using statistical software programs R version 2.9.2 (The R Foundation for Statistical Computing 2009) and JMP version 8.0.2 (SAS Institute Inc. 2009).

Results

Birch Growth

Average relative increase in aboveground biomass of black birch in the understory between July 2007 and July 2010 was 641% higher in the girdled plots compared to the control plots (Figure 2). During these first three years of the experiment there was an 11.1±1.3% increase in above ground black birch biomass in the control plots compared to an 82.4±20.0% increase in the girdled plots (Figure 2.) When only half of the oaks were girdled the relative increase in above ground biomass was only 121% compared to the growth on the control plots

(from 11.12±1.29% to 24.61±1.83%). While the three treatments differed significantly ($p < 0.00001$) there was an additional effect of slope position with reduced growth of saplings at the bottom of the slope ($p < 0.05$, data not shown).

Black birch trees at the bottom of the slope had significantly lower SLA ($p < 0.0001$) and experienced a higher light environment ($p < 0.0001$) than trees at the middle or upper slope positions (Table 1). Oak girdling treatment did not significantly affect the GLI at the individual trees ($p = 0.45$). Leaf C:N decreased by 13.5% in the O50 and 20.9% in the O plots relative to the control ($p < 0.0001$, Table 1). Foliar $\delta^{13}C$ increased significantly ($p < 0.0001$) in the O plots relative to the control and O50 plots (by 4.1% and 4.0% respectively) (Table 1). Foliar $\delta^{13}C$ also varied significantly between black birch trees at different slope positions ($p < 0.0001$), with the bottom of the slope being significantly higher in foliar ^{13}C abundance than the middle or top of the slope.

% Nitrogen

Leaf nitrogen increased by 15.5% in the O50 and 30.6% in the O plots relative to the control ($p < 0.0001$, Figure 3). Mean foliar nitrogen concentration was 2.02±0.03%, 2.34±0.03% and 2.64±0.07% in the C, O50, and O plots respectively. Within our nine plots, the best predictive model for foliar %N included girdling treatment, slope position, month, and SLA (Table 2). Sampling period was also a significant factor, with black birch foliar %N significantly lower in July than in May or August ($p < 0.01$). Leaf %N was positively correlated to SLA ($p < 0.0001$) and light ($p < 0.005$).

Slope position was not a significant factor in predicting foliar %N but there was a significant interaction effect between slope position and SLA ($p < 0.01$). This result highlighted

the fact that the six lowest values of SLA were all found in the O plot located at the bottom of the slope. There was also a significant interaction effect between month and girdling treatment ($p < 0.001$). Black birch foliage in the O plots shows a stronger decrease in %N from May to July than the other girdling treatments but a less significant increase from July to August than the other girdling treatments (Figure 3).

$\delta^{15}N$

In addition to changes in the bulk nitrogen concentration, there were significant changes in the isotopic signature of the leaf nitrogen in the treatment plots. The best predictive model for foliar $\delta^{15}N$ included only girdling treatment and slope position (Table 2). Foliar $\delta^{15}N$ was significantly different between different oak girdling treatments, with O50 and O plots increasingly enriched in ^{15}N, by 33.3% and 102.4% respectively ($p < 0.0001$, Figure 4). Mean foliar $\delta^{15}N$ was -3.25±0.07‰, -2.17±0.08‰ and 0.08±0.12‰ in the C, O50, and O plots respectively. Foliar $\delta^{15}N$ also varied significantly between black birch trees at different slope positions ($p < 0.05$), with the bottom of the slope being significantly lower in foliar ^{15}N abundance than the middle of the slope (Figure 4).

Nitrate Reductase Activity

The best predictive model for foliar NRA included girdling treatment, slope position, and SLA (Table 2). Slope position had a significant effect on black birch NRA ($p < 0.01$) with trees at the bottom of the slope having lower NRA than those at the middle or top of the slope, regardless of treatment. Girdling treatment and SLA were not significant independent factors in predicting foliar NRA, but there was significant interaction between girdling treatment and SLA

($p = 0.05$), and marginally significant interactions among girdling treatment and slope ($p = 0.07$), or slope and SLA ($p = 0.08$). Black birch trees at the bottom of the slope show a clear trend of increasing foliar NRA with an increasing level of oak girdling (Figure 5).

Discussion

One of the most apparent biological responses to oak girdling at BRF has been an increase in the growth rate of black birch trees on the oak-girdled plots compared to the control plots. Once a minor component of northern hardwood forests, black birch is now increasing in density over most of its range as a result of partial cutting of the overstory (Ward and Stephens 1996) and hemlock decline (Kizlinski et al. 2002), and has become a common component of forest regeneration in Southern New England (Kittredge and Ashton 1990). In this study, we found that the loss of oaks from this oak-dominated forest has profound effects on nitrogen uptake and the growth of black birch trees that could lead to important changes in ecosystem form and function.

Nitrogen Availability

Girdling alters the flow of carbon and nitrogen in a forest, and with fewer trees taking up nitrogen and increased decaying biomass belowground (roots and mycorrhizal fungi), girdling increases belowground N availability. We hypothesized that the cascade of events resulting from these responses increased available N and led to the increased growth rates of black birch trees in oak-girdled forest stands (Figure 6).

Declines in soil respiration have been detected as a result of oak girdling on the North Slope of the Black Rock Forest (Levy et al. 2011), providing strong evidence of mycorrhizal and root mortality in the girdled plots. Furthermore, soil samples taken before and after oak girdling in a pilot study at BRF show an increase in nitrate and ammonium levels in the girdled stands indicating potentially higher rates of nitrification and more available nitrogen for plant uptake (Melillo and Schuster, unpublished data). Our results further support this hypothesis as we found that, despite seasonal variation, black birch foliage on oak-girdled plots had a significantly higher %N content than foliage on control plots. Correspondingly, black birch leaf C:N decreased significantly on oak-girdled plots. Although foliar ^{13}C was significantly enriched in the 100% oak-girdled plots and at the bottom of the slope, the values were low enough to indicate that none of the trees were water-stressed.

Light availability is a possible contributing factor in the effect of increased N abundance on birch growth, as it generally increases after tree mortality as well. Not only is light directly involved in photosynthetic carbon fixation and therefore growth, but is also necessary to activate the nitrate reductase enzyme and create the large amount of reductant used. However, while oak girdling has been found to increase light availability on a plot level in the BRF experiment (Schuster, unpublished data), we found that the gap light index (GLI) at the individual birch trees we sampled was highly variable and not significantly higher on oak-girdled plots than on control plots. This was likely because the sampled trees were in the understory and may have been shaded by mature non-oak trees that remained standing. Similarly, SLA values did not decrease consistently with oak girdling (Table 1).

The root and mycorrhizal mortality that results from tree mortality provides an increase in belowground organic N, which increases the N mineralization rate and the inorganic N

availability (Johnson and Edwards 1979, Figure 6). The increase in available inorganic N ultimately leads to an increase in nitrification, making more soil nitrate available for plant uptake, but eventually leading to nitrate leaching out of the system (Jenkins et al. 1999). As the rate of nitrification increases, the discrimination against ^{15}N in the system also increases (Figure 6).

Because nitrifying bacteria discriminate against the heavier nitrogen isotope, the products of nitrification (NO_3^- and NO gas) are depleted in ^{15}N. These ^{14}N-enriched compounds are more mobile than ammonium and easily removed from the system *via* leaching or gaseous efflux, leaving behind an enriched ^{15}N soil pool. Collectively, this opens the N cycle, allowing N to leak out of the system while leaving a soil nitrogen pool that is enriched in ^{15}N and, ultimately subject to plant uptake thereby producing foliage which is also enriched in ^{15}N. Many studies have demonstrated the relationship between changes in the N cycle and small changes in ^{15}N values. For example, enrichment of foliar ^{15}N was observed as a result of increased nitrification and loss of nitrate in response to clear-cutting (Pardo et al. 2002), N deposition (Emmet et al. 1998) and N additions (Hogberg et al. 1993). Our foliar ^{15}N data indicate that the girdled plots are enriched relative to the control plots by a difference of 3‰, which is consistent in magnitude with similar studies (Pardo et al. 2002).

In the context of the above mechanism, the isotopic data indicate an increase in nitrification, an increase in the loss of nitrogen from the system and a general opening of nitrogen cycle after oak girdling. Nitrate is easily dissolved in water and can be lost via surface water or groundwater export following rain events. Previous girdling studies that have also collected water samples from the soil have demonstrated significant nitrate losses after girdling (Edwards and Ross-Todd 1979). In addition, Jenkins et al. (1999) found that net N

mineralization, nitrification, and N turnover increased at sites experiencing hemlock mortality, and concluded that nitrate leaching was likely in those areas. Accelerations of N cycling rates have been noted in response to other disturbances as well, including root rot, defoliation, gap formation, and clear-cutting (Matson and Boone 1984, Swank et al. 1981, Mladenoff 1987, Waide et al. 1988, Kim et al. 1995). In this study, it appears that oak loss results in an increase in nitrogen availability that is both altering the foliar isotopic signature through sustained losses of nitrate and simultaneously retaining enough N to increase the foliar %N in black birch trees.

Nitrate Reductase Activity

We predicted that the increased growth of black birch was due to an increase in nitrogen availability within oak-girdled plots and that black birch was specifically able to take advantage of the increased amount of nitrate available in the forest soils following oak girdling. Since nitrate reductase is a substrate inducible enzyme, the observed changes in foliar ^{15}N abundance and likely increase in nitrate availability would logically indicate an increase in NRA. Other studies have demonstrated correlations between soil nitrate availability and leaf NRA (Taylor et al. 1982, Adams and Attiwill 1982). We hypothesized that this mechanism would contribute to the increased foliar N content in black birch. Overall, we did not find the oak girdling treatment to have a significant effect on mean NRA. However, several confounding interactions among ecological factors (e.g. hill-slope position, hydrology, and changes in the density and species composition of the understory vegetation) may have resulted in significant variability within and between plots making the relationship difficult to identify. However, light availability and concomitant changes in SLA were found to have no significant impact on foliar NRA in our study, despite the fact that nitrate reductase is a light activated enzyme (Table 1).

Black birch trees at the bottom of the slope, which had the lowest overall NRA and foliar %N, did show a trend of increasing NRA with oak girdling. If these trees were most nitrogen limited to begin with, the increase in available nitrate after girdling may have had the most impact on trees in those plots, as they also experienced the largest average increase in growth rate. It is also possible that we failed to detect a significant trend because we obtained an incomplete picture of whole plant NRA in *Betula lenta*. The enzyme activity fluctuates extensively diurnally (Lillo 1983, Aslam et al. 2001), seasonally (Koyama et al. 2008), and throughout the plant (Black et al. 2002). Among species, nitrate reductase levels vary between leaves, stems and roots (Black et al. 2002). Many plants divide the processing of nitrate between the roots and shoots, and full evaluation of nitrate reductase activity relies upon the sampling of both, something we urge future research to consider.

Research on nitrogen use in birch species has shown that black birch seedlings grew larger with a nitrate supplement to forest soils supplying largely ammonium and that they may have a preferred balance of ammonium:nitrate (Crabtree 1993, Crabtree and Bazzaz 1992). It is possible that black birch trees must dedicate large quantities of root biomass in pursuit of nitrate to maintain their preferred ratio (Crabtree and Bazazz 1992). This mechanism may help explain the greater growth rates of black birch trees in oak-girdled stands, where nitrate may be more readily available. Although NRA is not necessarily higher, the trees may be benefiting from investing less energy into foraging for nitrate while achieving the desired balance of nitrogen species.

Conclusion

Evidence in the form of an enriched ^{15}N foliar signal and increased foliar %N indicate that the loss of oaks from this forest has significantly altered N cycling dynamics. As mineralization and nitrification rates increase, black birch trees increased N absorption as indicated by higher foliar N content and increased growth rates. A disturbance like large-scale tree mortality can therefore favor the success of certain species, such as black birch, which are able to respond to additional nitrogen by increasing their mechanisms of uptake and use. The incorporation of N into black birch leaves ensures the later return of this N to the soil and its continued cycling within the system. Importantly, through increases in N uptake, black birch will prevent some nitrate leaching from the system, which could otherwise result in water pollution and further exacerbate N limitation. The additional N provides a window of opportunity for rapid growth in the girdled plot, which black birch is utilizing to increase productivity. The forests that emerge from these disturbances and the resulting changes in N cycling are likely to bear the imprint of black birch productivity during this time. Oak mortality is a dynamic change that will have significant but hard to predict ecosystem consequences potentially confounded by anthropogenic alterations of N cycling such as atmospheric N deposition.

The unique foliar and litter properties of some oak species affect carbon and nitrogen cycling by producing litter with low decomposition rates (Lovett et al. 2006). Oak leaf litter also leads to soils with low nitrification rates (Finzi et al. 1998, Lovett et al. 2004), low nitrate leaching into streams (Lewis and Likens 2000, Lovett et al. 2002), and high retention of atmospherically deposited nitrogen (Templer et al. 2005). If oaks disappear from eastern forests and are replaced by black birch trees with nitrogen-rich foliage, these nutrient cycles will be

dramatically changed. Pests and diseases are likely to be the primary cause of species change in eastern forests in the next few decades (Lovett et al. 2006), and have the potential to completely alter species composition and ecosystem functioning in eastern US oak forests.

Table 1. Experimental plot and leaf characteristics from the oak removal experiment of Black Rock Forest. Treatments include: 100% oaks girdled (O), 50% oaks girdled (O50), and control (C) and distributed among the plots as in Figure 1. Values shown are means ± SE. Gap light index (GLI) was measured once at each of experimental tree and may not be representative of the entire plot (n = 5 for each plot). SLA was calculated for four leaves from every tree at every sampling period (n = 60 for each plot). An average value of C:N and $\delta^{13}C$ was calculated for each tree at each sampling period (n = 15 for each plot). Within a column, values not connected by the same letter are significantly different (ANOVA with the Tukey HSD test ($p < 0.05$)).

Oak-girdling treatment	Plot	Slope position	GLI (%)	SLA (cm^2/g)	C:N	$\delta^{13}C$ (‰)
C	A3	Lower	6.00(±0.61) bc	334.0(±6.92) ab	22.48(±0.45) ab	-31.53(±0.13) bc
C	B4	Middle	7.65(±0.64) b	304.3(±9.40) b	25.05(±0.82) a	-32.06(±0.82) c
C	C2	Upper	7.60(±0.41) b	326.0(±7.66) ab	22.16(±0.41) ab	-31.99(±0.15) bc
O50	A2	Lower	7.67(±0.77) b	320.6(±8.35) ab	20.39(±0.50) bc	-31.55(±0.13) bc
O50	B1	Middle	5.65(±0.43) bc	339.6(±8.87) ab	20.03(±0.59) bc	-31.97(±0.14) bc
O50	C3	Upper	8.16(±1.58) b	344.3(±8.22) ab	19.88(±0.47) bc	-31.92(±0.12) bc
OG	A4	Lower	17.49(±2.83) a	244.9(±16.08) c	20.80(±1.33) bc	-29.22(±0.46) a
OG	B2	Middle	6.69(±1.06) b	321.0(±15.53) ab	17.76(±0.76) cd	-31.24(±0.24) bc
OG	C1	Upper	0.47(±0.00) c	358.3(±14.83) a	16.54(±0.68) d	-31.18(±0.23) b

Table 2. Best fit model summaries and effect tests for predicting leaf N characteristics in the nine plots from the oak removal experiment at the Black Rock Forest.

Model Response Variable	Model R2	Model Degrees of freedom	Model F ratio	Model p-value	Model Parameters	Parameter Degrees of freedom	Parameter F ratio	Parameter *p*-value
%N	0.68	15	16.96	<.0001*	Girdling Treatment	2	80.13	<.0001*
					Slope position	2	2.43	.09
					Month	2	6.31	<.005*
					SLA	1	22.32	<.0001*
					Girdling Treatment*Month	4	5.81	<.0005*
					Girdling Treatment*SLA	2	2.87	.06
					Slope position*SLA	2	7.10	<.005*
$\delta^{15}N$	0.84	4	172.77	<.0001*	Girdling Treatment	2	342.18	<.0001*
					Slope position	2	3.36	<.05*
NRA	0.30	13	2.48	<.01*	Girdling Treatment	2	0.95	0.39
					Slope position	2	5.70	0.005*
					SLA	1	2.93	0.09
					Girdling Treatment*Slope position	4	2.30	0.06
					Girdling Treatment*SLA	2	3.06	0.05
					Slope position*SLA	2	2.68	0.08

Figure 1. Map of the north slope study plots in the Black Rock Forest, Cornwall NY depicting the layout of the experimental plots and treatments.



Figure 2. Percent growth in aboveground biomass from July 2007 to July 2010 of all black birch trees larger than 2.5 cm DBH in the central 25 m x 25 m area of each experimental plot. Mean values ± SE for each experimental treatment: control (*n* = 44), 50% oak-girdled (*n* = 63), and 100% oak-girdled (*n* = 13).

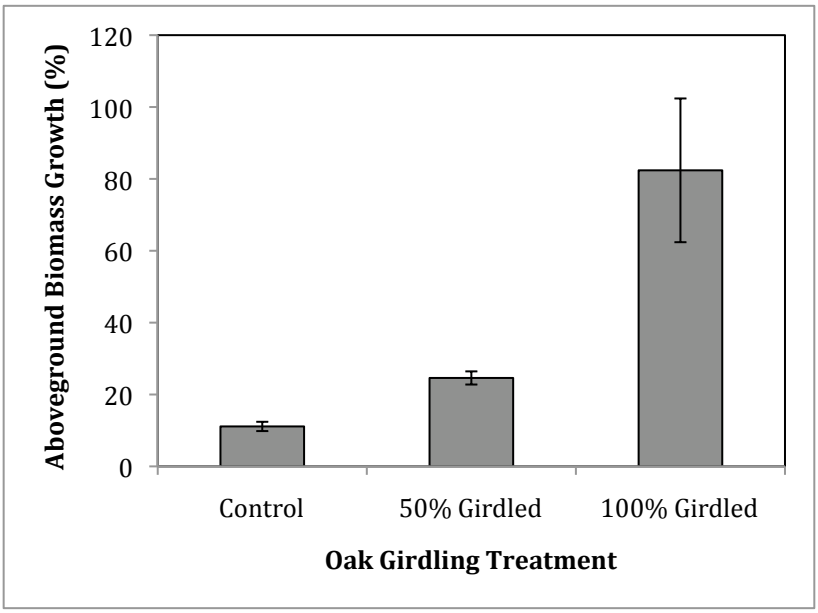


Figure 3. Foliar nitrogen content by mass (mean ± SE) for black birch leaves collected on each experimental treatment at each sampling time. Fifteen trees per treatment type were sampled three times, spaced evenly throughout the growing season in late May, early July, and late August (n = 135). Values not connected by the same letter are significantly different (ANOVA with the Tukey HSD test ($p < 0.05$)).

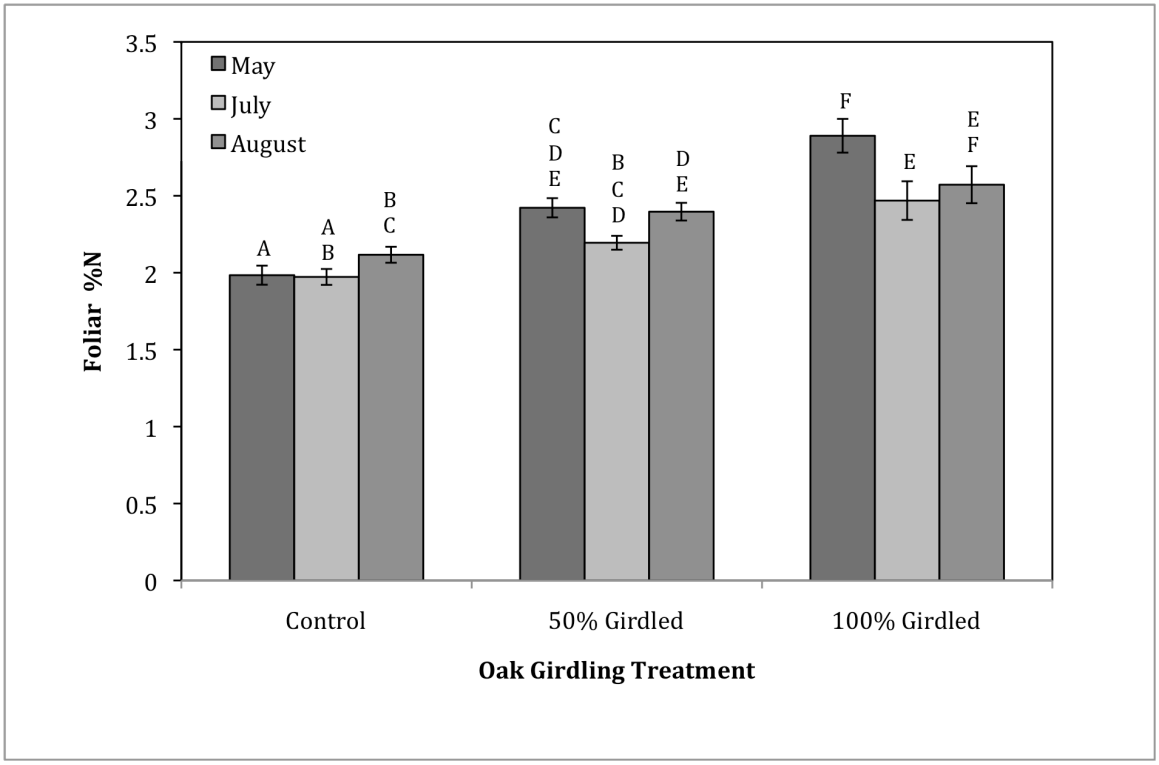


Figure 4. Foliar $\delta^{15}N$ signatures (‰) for black birch leaves collected from each experimental treatment (at each slope position). Mean values with error bars (SE) for 15 trees per treatment sampled three times throughout the growing season (n = 135). Among girdling treatments, values not connected by the same letter are significantly different (ANOVA with the Tukey HSD test ($p < 0.05$)).

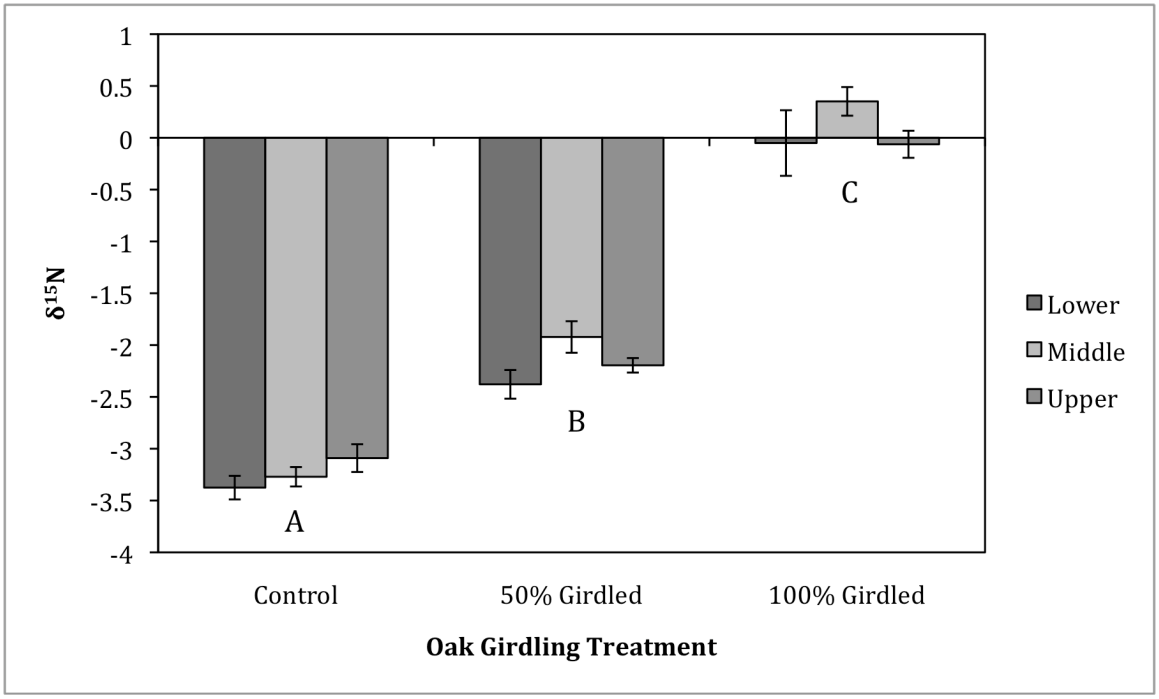


Figure 5. Black birch foliar NRA levels (nmol NO_2 produced per hour per gram fresh leaf weight) for each experimental treatment at each slope position. Mean values with error bars (SE) for 15 trees per treatment sampled three times throughout the growing season (n = 135). Values not connected by the same letter are significantly different (ANOVA with the Tukey HSD test ($p < 0.05$)).

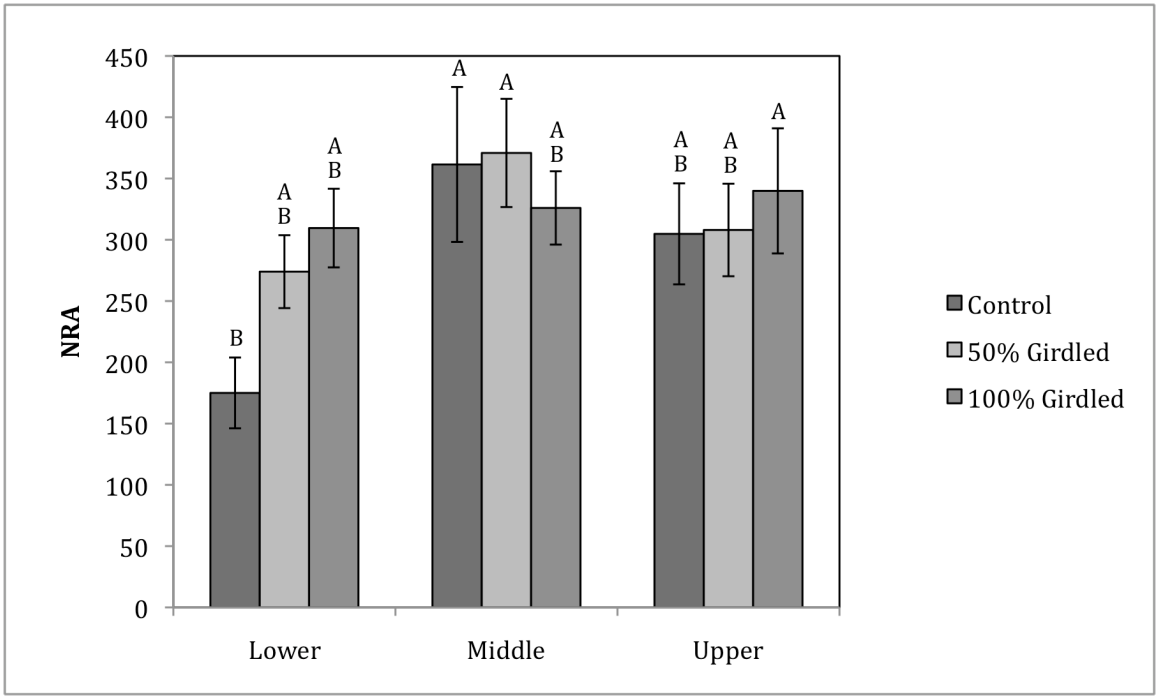
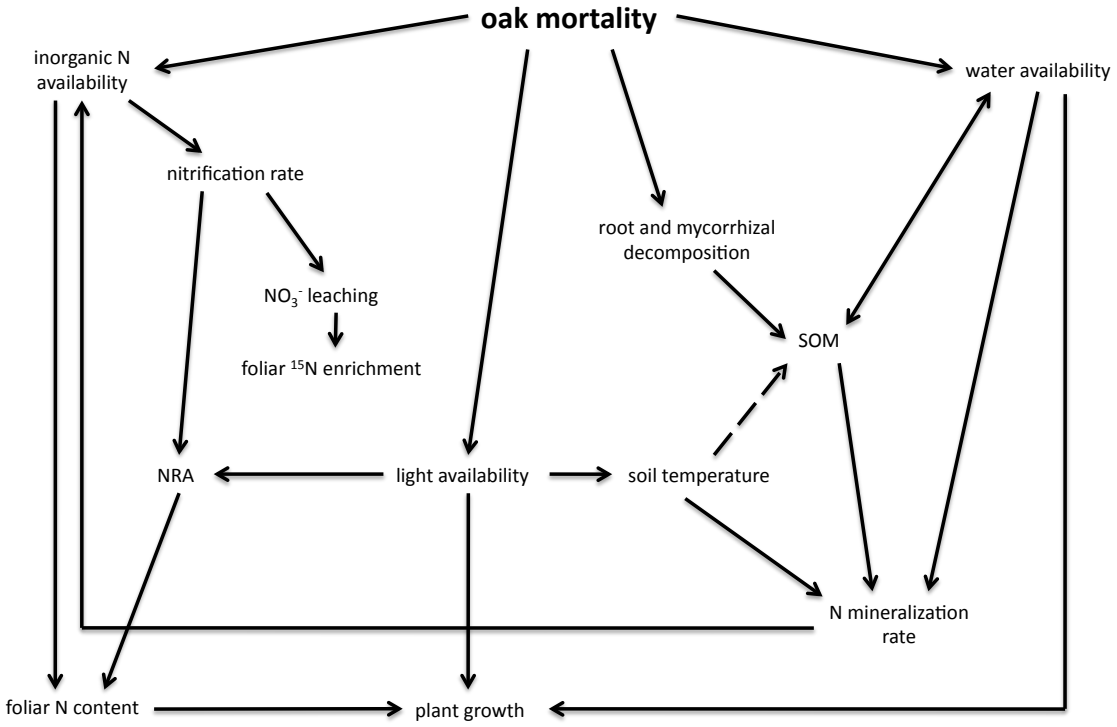


Figure 6. Hypothesized relationships among forest community structure and N cycling rates following oak mortality. All arrows indicate positive relationships (an increase in one variable causes an increase in the next), except for the dashed arrow, which indicates a negative relationship (an increase in one variable causes a decrease in the next).



CHAPTER TWO: An Urban-Rural Comparison of the Foliar Nitrogen Characteristics of Four Tree Species in Southeastern New York State

Summary

Urban forests provide important environmental benefits, leading many municipal governments to initiate citywide tree plantings. However, nutrient cycling in urban ecosystems is not yet well understood, and nitrogen use in urban trees may be quite different from use in rural forests. My objective in this study was to examine the response of several common northeastern deciduous tree species to a modified urban nitrogen cycle. I found significant differences in the foliar nitrogen characteristics of urban trees compared to trees in a rural forest. These differences reveal changes in nitrogen cycling and availability that could lead to important differences in urban ecosystem structure and function. All tree species measured showed similar trends of decreased nitrate reductase activity (NRA), increased %N, and ^{15}N enrichment in the foliage of urban trees compared to trees from a rural forest. However, linear mixed effects models revealed that the tree species varied in the strength of these responses. Understanding nutrient cycling in urban systems and the associated physiological changes in such important taxa is critical to the success of urban forest restoration, and may have implications for carbon sequestration and water quality, two important areas of management concern.

Introduction

Urbanization is an important force of environmental change impacting ecosystem functioning on continental and global scales (Miller and Small 2003). Habitat destruction, invasive species, air and water pollution, and the urban heat island effect are all associated with increased urbanization (Baxter 2002, Nowak 2000, Oke 1982). One outcome of these human impacts is altered nitrogen cycling and availability, which can have profound effects on ecosystem structure and function (Rusek 1993). Forests embedded within an urban matrix are directly affected by environmental impacts associated with urban land use change, and thus provide a useful context for investigating the effects of these anthropogenic factors on forest ecosystems (Groffman et al. 2006).

Nitrogen is an essential nutrient required by plants for protein synthesis. Northeast temperate forests are typically nitrogen limited systems with tightly controlled rates of nitrogen cycling (Aber et al. 1983, Melillo et al. 1983). The soils in these systems generally have low rates of net nitrification, as plants and the microbial community out-compete nitrifiers for ammonium (Melillo 1981). However, nitrate-rich N deposition from anthropogenic emissions may be increasing nitrogen availability in the northeastern U.S. (Aber et al. 1998). Furthermore, urban areas such as New York City receive increased nitrogen deposition relative to nearby rural forests (Lovett et al. 2000). Additional environmental factors associated with urbanization that are known to alter nutrient cycling and availability include increased effects of heavy metals (Berg et al. 1991), exotic invasive species (Steinberg et al. 1997), and human disturbance (Pardo et al. 1995).

Previous studies have examined soil nitrogen dynamics on urban to rural gradients, with inconsistent conclusions. Studies in the New York City metropolitan area found significant increases in nitrogen mineralization and nitrification in urban forest stands relative to rural stands, suggesting that urban forests may be approaching nitrogen saturation (Pouyat et al. 1997, Zhu and Carreiro 1999, 2004). Baxter et al. (2002) found higher rates of nitrification and a lower availability of ammonium in urban forest stands, possibly due to exotic earthworm activity and lower litter quality. However, White and McDonnell (1988) found lower N mineralization rates in urban soils and Goldman et al. (1995) found no difference in N mineralization when controlling for the earthworm effect. Groffman et al. (2006) suggest that natural soil factors and changes in species composition are ultimately stronger determinants of soil N dynamics than urban atmospheric conditions.

Despite the attention paid to urban soil N dynamics, the physiological response of plants to these changes in nutrient cycling remains unstudied. Many northeastern temperate tree species are not naturally exposed to large amounts of nitrate, as they tend to grow in nutrient-poor soils with low rates of nitrification (Melillo 1981). However, they may have the ability to take advantage of increased nitrate availability following disturbance (Truax et al. 1994). These biological changes may affect the structure and function of urban forests, with implications for carbon sequestration and water quality.

I predicted that trees in urban forests would have significantly different nitrogen use patterns than trees of the same species in a rural forest, due to the changes in nitrogen availability caused by urbanization. In order to test this prediction, I measured the concentration and isotopic signature of foliar N (%N and $\delta^{15}N$) and foliar nitrate reductase activity (NRA) of saplings in both urban and rural forests in late summer. The tree species studied include *Amelanchier*

canadensis (L.) Medik. (serviceberry), *Nyssa sylvatica* Marsh. (blackgum), *Prunus serotina* Ehrh. (black cherry), and *Quercus rubra* L. (red oak).

Experimental Methods

Study Sites

This study included four urban sites and one rural site (Figure 1). The urban sites were recently reforested urban parks in New York City: Clove Lakes in Staten Island, Marine Park in Brooklyn, and Roy Wilkins and Alley Pond Park in Queens. The trees at these sites were saplings planted in open areas adjacent to intact forest. The rural forest used for comparison was the Black Rock Forest (BRF), a 1,550 ha preserve located in Cornwall, NY. The urban and rural sites both experience strongly seasonal air temperatures, ranging from a mean of 5.6 °C in January to 24.7 °C in July in New York City, and a mean of -2.8 °C in January to 23.6 °C in July at BRF. Average annual precipitation is 1262 mm in New York City and 1285 mm at BRF (NOAA 2002). All sites are composed of a similar mix of northeastern hardwood tree species.

Sampling

At each site, five saplings were selected of each study species: *Amelanchier canadensis*, *Nyssa sylvatica*, *Prunus serotina*, and *Quercus rubra*. At the urban sites, trees were selected at random from recently reforested sites within the parks, while the trees at BRF were chosen haphazardly based on their size and were often found within deer exclosures. There are few saplings in the BRF understory due to heavy deer browsing, so the trees sampled were the

smallest available trees of each species. Saplings were found for all tree species at BRF except for *Nyssa sylvatica*, in which case mature tree leaves were used. All leaf samples were collected on bright sunny days between 11 AM and 1 PM from August 30 to September 2, 2010 to limit variation in climate or light availability.

Nitrate Reductase Assay

To measure nitrate reductase activity, leaves were collected on sunny days between 11 AM and 1 PM in order to ensure the presence of light-activated nitrate reductase. All leaves were cut from the middle of the canopy using a pruning shears. Two to four leaves from each tree were then hole-punched into 6 mm discs and weighed to produce approximately 0.2 mg of fresh leaf tissue per sample. The samples were then analyzed for nitrate reductase activity based on the method of Stewart et al. (1986). Five ml of assay solution (100mM sodium phosphate buffer, pH 7.5; 200mM KNO_3^-; 3% (v/v) n-propanol) were added to the leaf tissue, and the samples were then allowed to incubate for one hour in darkness and were shaken every 15 minutes. Samples were vacuum infiltrated three times to ensure that the solution penetrated into the leaf tissue.

The amount of nitrite in the solutions was calculated using colorimetric analysis. One ml of the reaction mixture was pipetted out and one ml sulphanillic acid plus one ml α-naphtyl ethylenediamine dihydrochloride (NED) were added and new mixture was vortexed. Tubes were incubated for an additional 20 minutes at room temperature and were observed to turn pink. Absorbance was measured on a spectrophotometer (Unico 1100 Series, United Products and Instruments Inc., Dayton, NJ, USA) at 540 nm and zeroed against a blank (water + sulphanillic acid + NED). A standard curve was generated using known concentrations of nitrite. The

amount of NRA was calculated based on the amount of nitrite in the solution and scaled to the mass of the sample analyzed using the following equation:

$$\text{NRA} = \frac{\text{nmol}}{\text{ml}} \text{x} \frac{\text{5 ml}}{\text{fresh weight (mg)}}$$

$\delta^{15}N$ and %N

Four leaves from each tree were collected as described above and oven-dried at 60°C, and subsequently ground to a fine powder (Cianflone model 2601, Cianflone Scientific Instruments Corporation, Pittsburgh, PA, USA). Samples were sent to Washington State University for tissue nutrient and stable isotope analysis. Values were obtained for %N and $\delta^{15}N$.

Statistical Analysis

Data was analyzed using linear mixed effects models to determine the effect of forest type (urban vs. rural) on NRA, %N, and $\delta^{15}N$ for each tree species. Site was included in the model as a random effect to account for variation between the urban sites, which have different land use histories and may differ significantly in biogeochemistry. NRA and %N values were transformed in order to meet the assumption of normally distributed model residuals, except in the case of *Prunus serotina* NRA values, which could not be transformed to a normal distribution. Significant differences between species leaf N characteristics were determined using a one-way ANOVA. Tukey's HSD values were used to determine significance among the categorical values in the ANOVA. Results with $p < 0.05$ were accepted as statistically significant. All analyses were conducted using statistical software programs R version 2.9.2 (The R Foundation for Statistical Computing 2009) and JMP version 8.0.2 (SAS Institute Inc. 2009).

Results

% Nitrogen

Only *Nyssa sylvatica* showed a significant increase in leaf nitrogen of 37.6% from the rural forest to the urban forests ($p < 0.05$). All other tree species showed a similar increase to *Nyssa sylvatica*, but not at a significant level (Table 1). There were significant differences in leaf nitrogen between species ($p < 0.0002$), with *Prunus serotina* and *Quercus rubra* having significantly higher values than *Amelanchier canadensis* (2.44 ± 0.14 and 2.30 ± 0.09 compared to 1.84 ± 0.06 % nitrogen, respectively) (Table 4).

$\delta^{15}N$

Both *Amelanchier canadensis* and *Nyssa sylvatica* foliage were significantly enriched in ^{15}N at the urban sites compared to the rural site ($p < 0.05$), with increases of 119.6% and 125.1% respectively (Table 2). *Prunus serotina* showed a marginally significant increase of 106.0% ($p < 0.10$). There were no significant differences in foliar isotope signature between species ($p = 0.77$) (Table 4).

Nitrate Reductase Activity

Only *Prunus serotina* showed a significant change in foliar NRA between the rural and urban forests ($p = 0.01$), decreasing by 100% (Table 3). Foliar NRA varied significantly between species ($p < .0001$), with *Nyssa sylvatica* having significantly more NRA than *Quercus rubra*, and *Prunus serotina* being significantly lower than all other species (96.42± 7.89, 63.49 ± 8.34, and 0.62 ± 0.34 nmol $NO_2\ hr^{-1}\ g^{-1}$ fresh leaf weight, respectively, Table 4).

Discussion

In this study, I found significant differences in foliar nitrogen characteristics of urban and rural forest trees. These differences reveal changes in nitrogen cycling and availability that could lead to important differences in urban ecosystem structure and function. All tree species showed similar, though not always significant, trends of increased %N, ^{15}N enrichment, and decreased NRA in the urban forests compared to BRF. However, the linear mixed effects models revealed that the tree species varied in the strength of their responses.

Urban-Rural Comparison

The higher levels of foliar nitrogen found in the urban trees indicate that nitrogen is more readily available in those environments than at BRF. Furthermore, the higher values of $\delta^{15}N$ found in the urban foliage suggest that nitrogen is cycling at a faster rate in urban soils and that some is being lost from the system. Increased nitrification leads to leaching of mobile nitrate ions and the enrichment of the soil ^{15}N pool. The lighter ^{14}N isotope is preferentially incorporated into nitrate by nitrifying bacteria, and is then leached out into nearby water sources (Aber et al. 1998).

Overall foliar NRA was found to be lower in the urban forests, despite the higher light levels at those sites. Trees at BRF were often found under lower light conditions due to the intact forest canopy, while the urban trees were grown in open, recently reforested areas. The higher nitrogen content of the urban foliage suggests that there is more nitrogen available in urban soils, likely due to increased deposition from anthropogenic sources (Lovett et al. 2000). The unexpectedly lower NRA values may therefore reflect one or more of the following mechanisms.

It is possible that a large amount of nitrate is leaching out of the urban parks, as indicated by the enrichment of ^{15}N in the urban foliage. Urban parks receive heavy stormwater flows and experience a great deal of erosion because they are often surrounded by impermeable surfaces and have relatively little vegetation to hold soil or water in place. Another possible explanation for the low NRA values is that the trees in the urban forests may have experienced drought stress when sampled at the end of a hot, dry summer. An energy intensive process like NRA may decrease when a plant experiences environmental stress and depleted energy stores. The urban trees likely experienced a greater degree of drought stress than the rural trees, as they were planted in exposed areas with no canopy cover.

Species Differences

Some species were significantly more successful than others in incorporating the available urban soil nitrogen into their foliage. This result is probably linked to the differences in NRA between tree species and may reflect a preference for nitrate vs. ammonium nitrogen forms. Little is known about nitrogen use in *Amelanchier canadensis* or *Nyssa sylvatica*, but *Quercus rubra* has been found to prefer ammonium (Bigelow and Canham 2007). *Prunus serotina* foliage showed very little NRA at either site, although a previous study found this species to grow best in a greenhouse experiment when provided with a high ratio of nitrate:ammoium (Horsley 1988). The fact that *Prunus serotina* showed almost no NRA, but experienced the same increase in $\delta^{15}N$ as the other species, suggests that the observed changes in concentration and isotopic signature of foliar N may reflect elevated levels of ammonium in the urban soils compared to rural soils. However, an alternative explanation for this result is that *Prunus serotina* reduces nitrate primarily in its roots rather than its leaves.

Quercus rubra and *Amelanchier canadensis* showed a sharper decline in foliar NRA from the rural to urban forests, and a smaller increase in %N, while *Prunus serotina* and *Nyssa sylvatica* showed a smaller decline in foliar NRA and a larger increase in %N. These patterns may reflect the nitrogen form preference of the species and the availability of ammonium vs. nitrate in urban soils compared to BRF. If there is a significant loss of nitrate from the urban forest soils, trees with an affinity for nitrate over ammonium may not be able to take advantage of the increased available nitrogen in the city. However, without data on the soil nitrogen content in each forest, it is difficult to interpret these results.

It is possible that I obtained an incomplete picture of whole plant NRA in these urban and rural trees. The enzyme activity fluctuates extensively diurnally (Lillo 1983, Aslam et al. 2001), seasonally (Koyama et al. 2008), and throughout the plant (Black et al. 2002). Among species, nitrate reductase levels vary between leaves, stems and roots (Black et al. 2002). Many plants divide the processing of nitrate between the roots and shoots, and full evaluation of nitrate reductase activity relies upon the sampling of both, something I urge future research to consider. It would also be beneficial to repeat this experiment throughout a growing season, as these foliar measurements were taken at the end of a hot, dry summer and some trees may have experienced stress due to drought conditions.

Conclusion

Modified nitrogen use by trees in urban forests may have cascading, ecosystem-level effects on urban watersheds. Increased levels of nitrification found in urban soils may lead to excess nitrate, which is easily dissolved in water and subsequently leaches into nearby water sources. If too much nitrate is leached out of the system, waterways become depleted of oxygen, disrupting the ecological functioning of the watershed. Uptake of nitrate by urban forests may prevent the loss of some nitrogen from the system. If urban trees are able to assimilate more nitrate than their rural counterparts, nitrogen limitation may be alleviated, with implications for growth rates and therefore carbon sequestration. Nitrogen-rich enzymes are required for photosynthesis, and increased leaf %N may therefore indicate increased growth rates of forest trees (Reich et al. 1997).

Understanding modified nutrient cycling in urban systems and the associated physiological changes in important tree species is critical to the success of urban forest restoration. Nitrogen use in urban trees has implications for carbon sequestration and water quality, two areas of great concern for ecosystem management. Urban forests are the focus of much ecological restoration work and will become increasingly important with the rapid urbanization of the world's population.

Table 3. Urban-rural comparison of foliar %N for each study species. Values shown are means ± SE. n = 20 for each species at the urban sites and n = 5 at the rural site.

Species	Mean Urban %N	Mean Rural %N	Degrees of freedom	p-value
Amelanchier canadensis	1.86 (±0.07)	1.77 (±0.19)	3	0.77
Prunus serotina	2.59 (±0.15)	1.86 (±0.15)	3	0.18
Nyssa sylvatica	2.27 (±0.06)	1.65 (±0.07)	3	0.04*
Quercus rubra	2.33 (±0.11)	2.18 (±0.14)	3	0.62

Table 4. Urban-rural comparison of foliar $\delta^{15}N$ for each study species. Values shown are means ± SE. n = 20 for each species at the urban sites and n = 5 at the rural site.

Species	Mean Urban $\delta^{15}N$	Mean Rural $\delta^{15}N$	Degrees of freedom	p-value
Amelanchier canadensis	0.76 (±0.23)	-3.87 (±0.25)	3	0.02*
Prunus serotina	0.18 (±0.29)	-2.92 (±0.57)	3	0.08
Nyssa sylvatica	0.60 (±0.17)	-2.38 (±0.31)	3	0.02*
Quercus rubra	0.39 (±0.29)	-1.59 (±0.12)	3	0.28

Table 5. Urban-rural comparison of foliar NRA for each study species. Values shown are means ± SE. n = 20 for each species at the urban sites and n = 5 at the rural site. NRA is measured in nmol NO_2 produced per hour per gram fresh leaf weight.

Species	Mean Urban NRA	Mean Rural NRA	Degrees of freedom	p-value
Amelanchier canadensis	65.34 (±6.98)	122.70 (±29.66)	3	0.10
Prunus serotina	0.00 (±0.00)	3.12 (±1.20)	3	0.01*
Nyssa sylvatica	95.14 (±9.85)	101.53 (±3.50)	3	0.78
Quercus rubra	56.45 (±9.77)	91.65 (±4.98)	3	0.17

Table 6. Leaf N characteristics by species. $n = 25$ for each species. NRA is measured in nmol NO_2 produced per hour per gram fresh leaf weight. Within a column, values not connected by the same letter are significantly different (ANOVA with the Tukey HSD test ($p < 0.05$)).

Species	%N	$\delta^{15}N$ (‰)	NRA
Amelanchier canadensis	1.84 (±0.06) b	-0.17 (±0.42) a	76.81 (±9.06) ab
Prunus serotina	2.44 (±0.14) a	-0.44 (±0.36) a	0.62 (±0.34) c
Nyssa sylvatica	2.14 (±0.07) ab	0.00 (±0.28) a	96.42 (±7.89) a
Quercus rubra	2.30 (±0.09) a	0.00 (±0.29) a	63.49 (±8.34) b

Figure 7. Study sites included the Black Rock Forest in Cornwall, NY and four urban parks in New York City.



GENERAL CONCLUSIONS

Drivers of global ecosystem change, including N deposition, spread of pathogens, and urbanization are all expected to increase in the coming century (Ayres and Lombardero 2000, Vitousek et al. 1997, United Nations 2006). These processes are likely to alter forest ecosystem dynamics through changes in plant physiology and productivity as carbon and nitrogen cycles shift from baseline rates, altering soil chemistry and soil biochemical processes, which can cascade into large-scale shifts in species composition and ecosystem functions (Rothstein et al. 1996).

In this study, it is clear that both oak mortality and urbanization had significant effects on foliar N characteristics. Both types of disturbance led to an increase in foliar N content and ^{15}N enrichment, indicating that the disturbed systems had more nitrogen available and that it was cycling at a higher rate. It was more difficult to detect a trend in nitrate reductase activity, which actually appeared to decrease in urban sites compared to rural sites, possibly due to drought stress. Changes in nitrogen availability and consumption can influence tree growth rates, as seen in black birch trees following oak loss at the Black Rock Forest. Different species were also found to respond differently to urbanization in terms of their nitrogen use patterns. Together, these findings suggest that such physiological differences between species could lead to changes in forest canopy composition in systems with modified N cycles.

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