Nitrogen Cycling Under Increased N Loads in Two Forested Ecosystems

Stephani Michelsen-Correa

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Reading Committee:
Robert Harrison, chair
Eric Turnblom
Drew Gorman-Lewis

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Abstract

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Stephani Michelsen-Correa

Chair of Supervisory Committee:
Professor Robert Harrison
School of Environmental and Forest Sciences

The coupled role of the terrestrial nitrogen (N) and carbon (C) cycles is of increasing importance, particularly in light of global climate change. Forested ecosystems contain over half of the terrestrial C pool and currently absorb as much as 30% of anthropogenic CO_2 emissions. Many forests, particularly Douglas-fir forests in the Pacific Northwest, are growth limited with respect to N. Fertilizers are therefore applied in commercially managed forests to increase productivity and timber yields. However, high N inputs can saturate the biological demand for N and lead to increased rates of N loss through leaching and gas loss via denitrification and volatilization. To improve N use efficiency one approach is the application of Enhanced Efficiency Fertilizers (EEFs) designed to limit N losses to volatilization. In this dissertation, the recovery rates of three different EEFs including Environmentally Smart Nitrogen (ESN), N-(n-butyl) (NBPT), and Arborite coated urea fertilizer (CUF), are compared to the recovery rates of an unformulated urea fertilizer. Using ^{15}N enriched fertilizer I determined both short term N losses (0-4 weeks after fertilization) and how the applied N is partitioned between ecosystem pools one year after fertilization. After four weeks, a mean of 60% of the applied N was recovered in the soil and forest floor. Retention rates were not significantly improved with the use of EEFs. After 1-year, N recovery was low across all

sites and fertilizer types with a mean of 39.0%. The remaining N could not be accounted for in the pools we sampled. The largest fertilizer pool was the top 20cm of mineral soil. Again, after 1-year, no differences were found between EEFs and the standard urea fertilizer.

Monitoring of nitrogen (N) inputs to forested ecosystems in China has historically been limited despite known negative effects that high N additions can have on environmental and human health. To better understand terrestrial patterns of foliar, soil, and wood $\delta^{15}N$ relative to soil N concentrations (%N) in a heavily polluted region, two Chinese parasol tree (*Firmiana simplex*) groves adjacent to an industrialized basin were examined on the Loess Plateau, approximately 80km east of the city of Xi'an, Shaanxi Province, China. The $\delta^{15}N$ values of ecosystem pools sampled in this study are substantially lower than what is typically observed in temperate forests around the world. Results suggest greater foliar uptake of N in the tree canopies bordering the industrialized basin compared to trees located at the forest interior. N concentrations and $\delta^{15}N$ of soil and plant tissue were inversely correlated along this transect and indicate higher inputs of N from $\delta^{15}N$ depleted sources in the basin. Tree ring $\delta^{15}N$ values declined between 2003 and 2014 during a time of rapid development and expansion of industry in the basin.

The projects in the Pacific Northwest and on the Loess Plateau in China are two separate studies. The differences in experimental design and objectives of the two studies does not facilitate comparisons between them.

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Chapter 1
INTRODUCTION

Over the last 150 years, human activities have altered the global nitrogen (N) cycle to the extent that emissions of reactive N (Nr) now exceed global rates of natural N fixation (Galloway et al. 2008). Despite increases in Nr emissions, the productivity of many forests is still limited by the availability of N, an essential plant nutrient (LeBauer and Treseder, 2008). Nutrient limitations occur when the potential uptake by plants exceeds the available soil nutrient supply (Allen et al. 1990). Theoretically, N fertilization in these forests as a deliberate land management practice or because of atmospheric deposition of N_r should ameliorate these deficiencies and increase tree growth. However, previous research has produced mixed results (Talbert and Marshall, 2005; Peterson et al. 1984). One of the major uncertainties in our understanding of ecosystem response to N fertilization is the fate of approximately 30-40% of N inputs (Morse et al. 2015). Forest productivity and the environmental impacts of Nr inputs depend on where N is retained in the ecosystem, particularly how it is partitioned between ecosystem components (i.e. soil and tree biomass pools).

The purpose of this research is to quantify nitrogen retention efficiency and fate of four commonly used urea based fertilizers and to assess ecosystem uptake of atmospheric N deposition in a heavily polluted forest. This dissertation is divided into four chapters with the following objectives:

[1] Determine nitrogen retention efficiency of different urea-based fertilizers,

[2] Assess nitrogen redistribution in the ecosystem one year after fertilization, and

[3] Evaluate foliar uptake and tree-ring record of atmospheric nitrogen in a polluted forest in China.

Results from this proposed research will improve our understanding of N retention in managed Douglas-fir ecosystems as well as the application of natural abundance isotopes of nitrogen (hereafter referred to as $\delta^{15}N$) in evaluating N cycling in polluted forests in China.

This research is conducted in managed Douglas-fir forests (*Pseudotsuga menziesii*) in the western Douglas-fir region of Oregon and Washington and groves of Chinese Parasol trees (*Firmiana simplex*) on the Loess Plateau in Shaanxi Province, China. The Douglas-fir forests have received a single high N input through deliberate fertilization intended to increase productivity of otherwise N-limited sites. In contrast, the forests in China are not N-limited and are continuously exposed to high levels of atmospheric N deposition from industrial and transportation sources. Despite geographic differences, N retention and partitioning of added N between soil and plant pools is important for the health of both forested sites. The projects in the Pacific Northwest and on the Loess Plateau in China are two separate studies. The differences in experimental design and objectives of the two studies does not facilitate comparisons between them.

1.1 Background

This dissertation research was conducted in managed Douglas-fir forests (*Pseudotsuga menziesii*) in the western Douglas-fir region of Oregon and Washington and groves of Chinese Parasol trees (*Firmiana simplex*) on the Loess Plateau in Shaanxi Province, China. The Douglas-fir forests have received a single high N input through deliberate fertilization intended to increase productivity of otherwise N-limited sites. The forests in China are not N-limited and are continuously exposed to high levels of atmospheric N deposition from industrial and transportation sources.

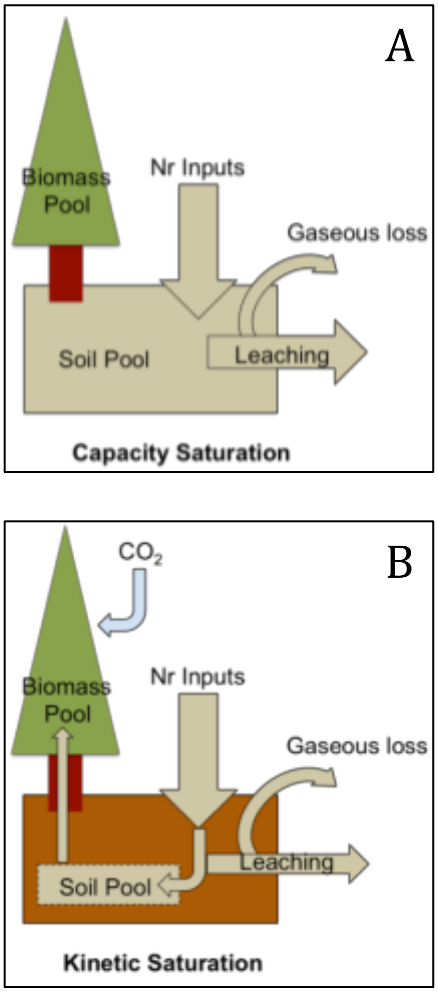

Figure 1.1 Current models of N retention and loss. A: Aber et al. 1998 B: Lovett and Goodale, 2011.

Nitrogen saturation, defined as the accumulation of N in excess of biological demand, develops as a result of N accumulation within an ecosystem (Agren and Bosatta, 1988). One of the earliest models of N saturation describes a sequence in which increased N deposition initially leads to higher productivity and increased N concentrations in vegetation and soil pools (Aber et al. 1998). As N inputs continue, productivity response begins to decline and uptake by biota is saturated, while volatilization, denitrification, and leaching losses from the ecosystem increase. Based on this capacity saturation model, a commonly used indicator of N saturation is increased nitrate (NO_3^-) leaching below the rooting zone and into stream water (Aber et al. 1998). Capacity saturation occurs when the N sinks cannot accumulate additional N, so deposited N is lost from the system. When a site is at capacity saturation the accumulation of new biomass and net carbon sequestration is zero or less, while the inputs of N to the system would be expected to equal the outputs (Lovett and Goodale, 2011). An alternative model recognizes that N can simultaneously enter multiple pools, including aboveground biomass, soil and forest floor, and loss pathways such as leaching, volatilization, and denitrification (Lovett and Goodale, 2011). This kinetic saturation occurs when N is added at a rate exceeding the rate of uptake by N pools in the soil and vegetation (Lovett and Goodale, 2011). During kinetic saturation, N accumulation and losses occur simultaneously (Figure 1.1). Kinetic saturation is likely to occur when rates of fertilization or N deposition exceed the rate of uptake into plant biomass or soil N pools. In natural systems, capacity saturation is rare but can develop because of disturbances such as logging that alter the relative strength of the biomass N pool. The strength of N pools and response of forests to increased N additions vary geographically due to differences in soil base cation saturation, climate, atmospheric deposition of pollution, and forest stand structure (Fenn et al. 2003).

1.1.1 Response of forested ecosystems to N additions varies across sites

Increased growth rates with simultaneous leaching losses in response to N deposition and fertilization across sites suggests that the model proposed by Aber et al. (1998) does not always apply. The N saturation theory developed by Aber et al. (1998) predicts that low level additions of N in N limited sites should increase NPP with negligible N losses. Essentially, N limited sites should have high N retention rates with fertilization. In the western Douglas-fir region of Oregon and Washington, repeated fertilization of N-limited sites yields highly variable results with some sites experiencing increasing NPP and others decreasing NPP (Talbert and Marshall, 2005). If the Abers et al. (1998) theory holds true, fertilization of N limited sites should consistently increase productivity in the absence of other growth limitations.

Previous studies have found that N fertilization can increase tree growth when applied to low productivity sites. Talbert and Marshall (2005) suggest that intensive management with repeated N fertilization can increase the average site productivity from 13 $m^3\ ha^{-1}\ yr^{-1}$ to between 17 and 20 $m^3\ ha^{-1}\ yr^{-1}$. However, other studies have produced less optimistic results. Peterson and Gessel (1984) found a change in the volume of Douglas-fir stands ranging from -5.5 to +13.1 $m^3\ ha^{-1}\ yr^{-1}$ when compared to control sites six years after fertilization. Theoretically, N fertilization should increase tree growth, but the variability in these results suggests a need for further research to determine why some sites respond favorably to fertilizer applications while others do not.

An improved understanding of the temporal and spatial controls on the fate of N is needed to better understand the variable response of N limited temperate forests to N fertilization, deposition, and disturbance. As the Lovett and Goodale (2011) model of N saturation suggests, the assumption that fertilization of N limited sites does not result in high N losses may not be accurate because N can be lost from the system even while NPP is increasing (Swank and Vose, 1997). Quantifying N retention in the western Douglas-fir region of Oregon and Washington can reveal how these N losses vary geographically.

1.1.2 Isotope methods for N cycle reconstructions

Nitrogen stable isotope methods are increasingly employed as a means for understanding the fluxes, transformations, and fate of N in ecosystems (Rothstein et al. 1996; Dawson et al. 2002). The δ^{15}N of soil and plant pools reflects an integration of the dominant fluxes of N into or out of the system and vary across environmental gradients in precipitation and temperature (Pardo et al. 2006; Craine et al. 2009). Current research suggests that soil and plant tissue δ^{15}N are positively correlated with higher leaching losses of N (Pardo et al. 2002). Increasing δ^{15}N of foliage and soil is frequently interpreted as increased N loads and leaching losses of N. Interpretation of the δ^{15}N of tree rings relies on an assumption that the δ^{15}N of a given tree ring reflects the local N cycle for that year (Gerhart and McLauchlan, 2014). Declines in tree-ring δ^{15}N over time have been attributed to declines in N availability (i.e. low leaching rates; McLauchlan and Craine, 2012; Kranabetter et al. 2013) or changes in N source (i.e. new pollution source; Stewart et al. 2002; Guerrieri et al. 2009; Fang et al. 2011). Other studies suggest that increases in tree ring $\delta^{15}N$ also reflect changes in N source, leaching, or increased N availability due to changes in disturbance regimes over time (Caceres et al. 2011). For example, declines in NPP resulting from logging increase N losses due to a decreased capacity for uptake of soil nutrients (Marks and Bormann, 1972) and could consequently increase the $\delta^{15}N$ of the tree rings due to higher N losses.

1.1.3 Enhanced Efficiency Fertilizers

Urea based EEFs are designed to decrease N losses by coordinating the release of fertilizer N with plant demand. Several mechanisms are used in theses fertilizers to theoretically decrease losses of N. Controlled release EEFs work by delaying the plant availability of N through the use of coatings (Trenkel, 2010). Urease inhibitors slow hydrolysis by blocking the active site on urease, the enzyme catalyst that converts urea to CO_2 and ammonia which is readily available in forest soils (AAFPCO, 2015; Bremner and Douglas, 1971; Sanz-Cobena et al. 2008. The benefits in terms of N use efficiency are however unclear. Research comparing N losses with the application of EEFs to standard unformulated urea fertilizers in agricultural plots have produced mixed results with some studies showing decreased losses of N with EEF use (Akiyama et al. 2010; Halvorson et al. 2014) while other studies show an increase or no difference in losses with EEF use (Sistani et al. 2011; Watts et al. 2015). Most of the research comparing EEFs to unformulated urea fertilizers are from studies in food agroecosystems as opposed to agroforestry systems where there is a paucity of research on the topic. A similar study in Loblolly Pine plantations of the southeastern United States shows improved N use efficiency with the application of EEFs (ESN, NBPT, CUF) compared to the unformulated urea (Raymond et al. 2016). Using the same treatment and experimental design, fertilizer-N recovery was higher at the loblolly pine sites than when the same treatments are applied to plantation forests in the western Douglas-fir region of Oregon and Washington (Raymond et al. 2016). The particular EEFs used in both studies were formulated specifically for the southeastern United States. This is especially relevant for the polymer coated EEF (ESN) as the polymer coating breaks down when exposed to water, higher temperatures, and solar radiation (AAFPCO, 2013). Douglas-fir forests have dense canopies with very little sunlight penetrating the canopies and hitting the forest floor relative to loblolly pine stands which are more open and their forest floors receiving more sunlight at the ground level, where the fertilizer rests after application.

Chapter 2

No difference in nitrogen retention with enhanced efficiency fertilizers (EEFS) 4-weeks post-fertilization

Abstract

Enhanced efficiency fertilizers (EEFs) are used to decrease losses of N to volatilization and leaching. Nitrogen lost through leaching ends up in groundwater and streams where it is harmful to both ecosystems and human health. Higher N retention and increased uptake are intended to offset the additional cost of these EEFs. Pacific Northwest Douglas-fir forests have been shown to be N limited and consequently have high N retention rates. We tested this hypothesis by tracing four urea based fertilizers from zero to four weeks after application in five managed Douglas-fir forests in Oregon and Washington. Three fertilizers were urea based EEFs and the fourth an industry standard urea fertilizer. Fertilizers were enriched with ^{15}N which facilitates the tracing of fertilizer as it moves between pools. Retention rates were calculated for the forest floor and mineral soil to a depth of 20cm. After four weeks, a mean of 60% of the applied N was accounted for. Retention rates were not significantly improved with the use of EEFs. No improvement in N retention with EEFs suggests that volatilization may not be a substantial loss pathway at these sites.

2.1 Introduction

Human activities have substantially altered the global nitrogen (N) cycle, to the extent that anthropogenic production of reactive N (Nr) now exceeds global rates of natural N fixation (Galloway et al. 2008). Nitrogen is an essential plant macronutrient needed for the production amino acids and proteins. Consequently, when N is limiting, photosynthetic capacity decreases because of reduced leaf area resulting in decreased growth rates and declines in forest productivity. As a stand grows older the demand for N increases however nutrient availability has decreased since the conditions required for the accumulation of N in the soil are less favorable. Fertilizers are applied to agricultural systems, including commercially managed timber sites, to ameliorate such N deficiencies and maintain growth rates (Fenn et al. 1998; Chapin et al. 2002; Shryock et al. 2014). Unfortunately, the negative consequences of excess N inputs are substantial, having been linked to soil acidification, decreases in biodiversity, eutrophication, and declines in human health (Vitousek, 1997; Fenn et al. 2003). Increased N inputs to ecosystems can stimulate growth and carbon (C) sequestration of some tree species (Thomas et al. 2010), but high rates of fertilizer inputs can saturate the biological demand for N and lead to increased rates of N loss through leaching and gas loss via denitrification and volatilization (Aber et al. 1998). A forest's capacity to retain N when faced with increased N additions varies geographically due to differences in soil base cation saturation, climate, atmospheric N deposition, and forest stand structure (Fenn et al. 2003). The efficient use of N fertilizers is of critical importance to land mangers seeking to balance the demands for high agricultural yields (i.e. timber products), economic costs of fertilizers, and ecosystem health. Improving N management practices and our understanding of the environmental impacts of ecosystem N additions requires a more accurate accounting of the N cycle and the efficient use of N fertilizers (Amponsah et al. 2004; Fang et al. 2011; Fenn et al. 2003).

Enhanced Efficiency Fertilizers (EEFs) are a common management tool employed to limit N losses following fertilization of agroecosystems. Fertilizers are commonly applied in excess of plant demand to maximize gains in productivity (Watts et al. 2015). N applied in excess of plant demand can result in short-term saturation of an otherwise N-limited site (Lovett and Goodale, 2011). This *kinetic saturation* results in the loss of N through increased volatilization, denitrification, or leaching (Lovett and Goodale, 2011). Urea based EEFs are designed to decrease N losses by coordinating the release of fertilizer N with plant demand. Several mechanisms are used in theses fertilizers to theoretically increase their efficiency. Controlled release EEFs work by delaying the plant availability of N through the use of coatings (Trenkel, 2010). Urease inhibitors slow hydrolysis by blocking the active site on urease, the enzyme catalyst that converts urea to CO_2 and ammonia. The benefits in terms of N use efficiency are however unclear. Research comparing N losses with the application of EEFs to standard unformulated urea fertilizers in agricultural plots have produced mixed results with some studies showing decreased losses of N with EEF use (Akiyama et al. 2010; Halvorson et al. 2014) while other studies show an increase or no difference in losses with EEF use (Sistani et al. 2011; Watts et al. 2015). Most of the research comparing EEFs to unformulated urea fertilizers are from studies in food agroecosystems as opposed to agroforestry systems where there is a paucity of research on the topic.

Most forest soils contain large amounts of N, with ranges reported between 2 and 7 Mg ha^{-1} (Fisher and Binkley, 2012). The variability of soil N within forests is high relative to the amount of N that is added through fertilization. An example of this is the soil N content measured at two different Long-term Soil Productivity (LTSP) sites in the Pacific Northwest. For the low productivity Matlock site, the soil N content in the upper 15cm was 1,330 ($\pm$80)kg ha^{-1}. In contrast, the soil N content was 2,810 ($\pm$250) kg N ha^{-1} for the high productivity site at Fall River (Meehan, 2006; Ares et al. 2007). The amount of fertilizer N that is applied to plantation forests is usually equal to or less than 224kg ha^{-1}, and in the case of atmospheric deposition in the region it is estimated between 1-9 kg ha^{-1} yr^{-1} (Geiser et al. 2010). These additions are small relative to the variability and overall quantity of N in forested ecosystems. Consequently, it is difficult to account for changes in the N pools of forested ecosystems by using a simple N mass balance approach or by using changes in tree growth rates as a proxy for N retention. The use of N fertilizer enriched with the stable isotope ^{15}N is a method that has been used for tracing N as it is cycled in the environment (Naddelhoffer et al. 1995; Templer et al. 2004). Because natural abundance $\delta^{15}N$ is low, using ^{15}N enriched fertilizer as a tracer enables the direct measure of the partitioning of applied N between ecosystem components (i.e. vegetation, forest floor, soil) rather than using growth response as a proxy. This technique has been employed in agricultural research for tracing fertilizers in farmed plots (Hauck and Bremner, 1976). While the use of ^{15}N tracers in agricultural research at the single plot scale is relatively common, its use in forest research has been limited because of the high cost and large quantities needed to fertilize trees with extensive root systems and large amounts of above ground biomass. The application of ^{15}N-enriched fertilizers has been used to study N retention in a range of temperate forests (Emmit and Quarmby, 1991; Buchman et al. 1996; Fitzhugh et al. 2003; Nadelhoffer et al. 1995; Tietema et al. 1998; Templer et al. 2004). Results from tracer studies can reveal how N is partitioned between ecosystem components (Nadelhoffer et al. 1995; Templer 2004).

The objective of this study is to determine the differences in short-term (0 – 4 weeks after fertilizer application) N losses between four different commercially available urea based fertilizers. Three of the fertilizers are EEFs and the fourth is an unformulated urea, the industry standard fertilizer. We hypothesize that N losses would increase over the four-week period and the smallest losses would occur in the EEF-fertilized plots. We also expect there to be differences in N retention between sites as the conditions that lead to N loss vary across the five study areas.

2.2 Study Area

Five study sites were selected across the western Douglas-fir region of Oregon and Washington (Figure 2.1). Douglas-fir is the most wide-ranging tree species on the west side of the Cascade mountains and an important crop species which makes the region a leading supplier of high quality wood products (Brubaker et al. 1992; DNR 2012). The study sites all contain approximately 25-years old (since planting), previously unfertilized Douglas-fir stands, located within plantation forests. These sites were selected to include a representative range of parent materials and latitudes of Douglas-fir plantation forests in the region. This study is part of a larger study exploring the varied response of Douglas-fir stands to N fertilization. Each of the five sites are described in further detail below.

Figure 2.1: Locations of study sites in the Western Douglas-fir Region of Oregon and Washington.

2.2.1 Mill Creek

The Mill Creek soils are loams (0-5cm depth) and sandy loams (50cm depth) whose parent material is of glacial outwash origin. Of the five sites Mill Creek has the lower soil N content of the five sites with 7243 Mg ha^{-1}. Mean annual soil moisture at Mill

Creek is 0.34 cm/cm^3 in the surface horizon (5cm depth) and 0.16 cm/cm^3 at 50cm depth. The site is situated at 46m above sea level and has a 0% slope. Plotting density is 809 tree ha^{-1}. C/N of the forest floor is 20 and the soil A horizon has a C/N of 25.

2.2.2 Oppelt Rd

Located to the southwest of Mill Creek, the Oppelt Rd site has a similar glacial outwash parent material. Oppelt Rd has the highest soil N content of the five sites at 10796Mg ha^{-1}. Soil textures are clay loam at 5cm and transitions to a sandy clay loam at 50cm depth. Mean annual soil moisture at this site is higher than Mill Creek with a mean of 0.33 cm/cm^3 at 5cm depth. At 50cm the soil moisture is 0.29 cm/cm^3. The site has a 10% slope and is situated at 91m above sea level. Planting density is 820 trees ha^{-1}. C/N of the forest floor is 20 and the soil A horizon is 18.

2.2.3 *Adna 1*

The Adna 1 site has a sedimentary parent material. The soil texture at both 5cm and 50cm depth is clay. Total soil N content is 9583Mg ha^{-1}. Forest floor C/N is 23 while soil C/N is 17. Mean annual soil moisture increases slightly with depth at this site. At 5cm depth soil moisture is 0.26 cm/cm^3 and at 50cm soil moisture is 0.28 cm/cm^3. The site is located at 213m elevation with a 0% slope. Planting density at Adna 1 is higher than the two northern most sites at 981 trees ha^{-1}.

2.2.4 Les Smith

The Les Smith site is located in Oregon and has an igneous parent material. Soil texture is a silty clay loam at 5cm depth and clay loam at 50cm depth. Total soil N content is 9039Mg ha^{-1}. Forest floor C/N is highest here relative to the other four study sites with a forest floor C/N of 40 while soil C/N is 24. Mean annual soil moisture at 5cms depth is 0.30 cm/cm^{3} and increases to 0.36 cm/cm^{3} at 50cm depth. The site, located at 451m elevation has a 14% slope. Planting density is 805trees ha^{-1}.

2.2.5 Clark Creek

Clark Creek, the southernmost site, is located in Oregon. The parent material here is of igneous origin. The soil at 5cms depth has a loam texture while at 50cm depth the texture is a clay loam. Total soil N content at Clark Creek is 8646 Mg ha^{-1}. Forest floor C/N of 60 is the highest of the five sites in our study. Soil C/N is 28. Mean annual soil moisture is 0.26 cm/cm^{3} at 5cm depth and increases slightly to 0.28 cm/cm^{3} at 50cm. The elevation is at 793m and the site has a 15% slope. A planting density of 769 trees ha^{-1} is the lowest of the five sites.

2.3 Methods

To trace the fate of applied N in the ecosystem and compare the relative N retention between sites and urea fertilizer type, four different urea based ^{15}N-labelled fertilizers were used. Each of the four urea based fertilizers were enriched to 0.5atom% ^{15}N (~370$^{0}/_{00}$$^{15}N$). Using a fertilizer enriched with ^{15}N, the applied fertilizer may be traced as it moves between N pools within the study area. The four fertilizer treatments used were 1) urea, 2) Agrium ESN, 3) urea + NBPT, and 4) Urea + CUF (Table 2.1).

Unformulated urea fertilizer is considered the industry standard fertilizer treatment in production forestry. Environmentally Smart Nitrogen (ESN), N-(n-butyl) (NBPT), and Arborite coated urea fertilizer (CUF) type EEFs each contain a different additive designed to decrease volatile losses and improve uptake efficiency. The application of EEFs assumes that volatilization is a substantial loss pathway and thus additional N retained in the soil is available to plants.

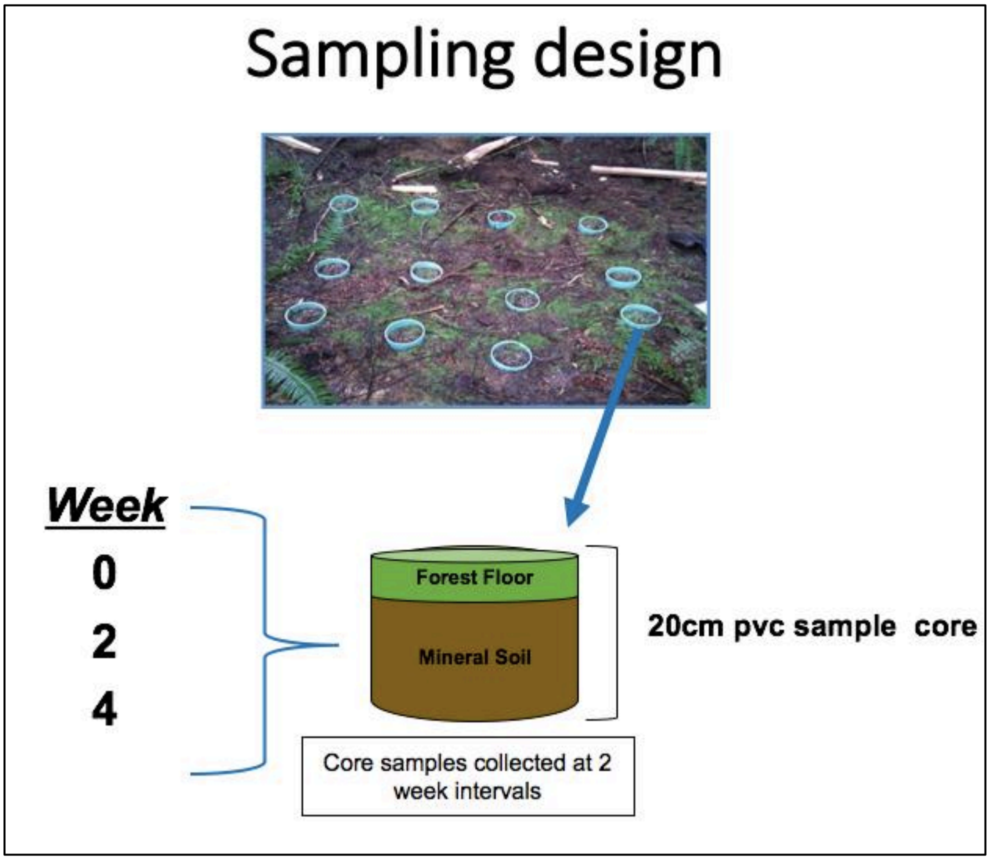

Figure 2.2: Study plot showing PVC cores inserted in forest floor. Cross section of a single PVC core. One PVC core was removed and analyzed at times 0, 2, and 4 for each treatment type (i.e. ESN, Cuf, NBPT, and Urea).

2.3.1 Experimental Design

Short-term (0-4 week) N losses have been measured at each of the 5 installations using a randomized block design. Thirteen PVC loss tubes were installed to a depth of 20cm at each of the five sites. Each tube received one of the four fertilizer treatments for a total of three tubes for each treatment and a single loss tube for the control per site (Figure 2.2). The PVC cores cut off the roots from the soil and provided an estimate of N losses irrespective of plant uptake. Forest floor and mineral soil samples were collected from within the tubes immediately after fertilization (week 0) and every two weeks thereafter for a total of 4 weeks (at weeks 2 and 4). The soil samples were all dried and sieved to

<2mm. Organic matter >2mm was removed, weighed, and ground in a ball mill. The ground organic component was then added back to the <2mm mineral soil. The soil and ground organic matter was then mixed to ensure uniform subsampling. Four ~20g samples were scooped from the mixture and ground to a fine powder for isotope analysis. The forest floor component was air dried and weighed. The dry sample was then passed through the Wiley mill and ground to <2mm. A subset of the forest floor was sampled and ground to a fine powder to be used in the isotope analysis. Enriched samples were analyzed by the UC Davis Isotope Lab using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope mass spectrometer. For each mineral soil and forest floor samples, the $\delta^{15}N$ signature obtained was converted to a total a value for N attributed to the fertilizer application. The $\delta^{15}N$ value obtained from the analyses is converted to atom% ^{15}N using Equation 2.1. A simple mass balance equation was used to calculate N loss over the four week period (Equation 2.1). The environmental and site conditions which best account for the short-term N losses across the study sites and between fertilizer types will be determined.

[Equation 2.1]

$$\%^{15}N_{rec} = 100 * m_{pool} * \frac{(atom\%^{15}N_{pool} - atom\%^{15}N_{ref})/100}{^{15}N_{tracer}}$$

$\%^{15}N_{rec}$ = percent of applied ^{15}N tracer recovered in labeled N pool
m_{pool} = N mass of labeled pool
atom % $^{15}N_{ref}$ = atom percent ^{15}N of the reference pools
atom % $^{15}N_{pool}$ = atom percent ^{15}N of the ecosystem pools
$^{15}N_{tracer}$ = amount of ^{15}N added to each pools

Equations based on calculations describe by Buchmann et al. 1996; Templer et al. 2005

Table 2.1: Summary of the four fertilizer treatments applied at each of the 5 study sites.

Fertilizer	Description	Mechanism
Urea	Industry standard	Volatilization loss controlled via timing of application
ESN (Agrium)	Polymer coated urea	Delays hydrolysis of urea for 60-90 days; release of urea is temp. and moisture dependent
Urea+ NBPT (Agrotain)	Coated with N-(n-butyl) thiophosphoric triamide	Slows hydrolysis by blocking active site on urease for 7-21 days
Urea+CuF (Arborite)	Coated with phosphate	Granular urea pellets coated with boron solution- acts as both an inhibitor and a binder for additional nutrient layer (PO_4)
Unfertilized Control	No fertilizer added within the $100m^2$ boundary	N/A

2.3.2 Statistical Analyses

Differences in N recovery among sites and fertilizer treatments were assessed with an ANOVA and all statistical significance is assessed based on $\alpha < 0.05$. A Tukey's HSD test was then applied to determine which means were significantly different from each other ($\alpha = 0.05$). A multiple linear regression analysis was used to determine which environmental site conditions, if any, best predict N retention across the study sites. The subset of predictor variables included in the final model were selected using a forward step-wise approach.

2.4 Results

Over the 4-week sample period, a mean of 30.2% of the applied fertilizer was lost across all study sites and treatments (Figure 2.3). During this time, the forest floor was the largest reservoir for the applied fertilizer. A mean of 66.7% of the applied fertilizer was recovered in the forest floor after 4-weeks, and a mean of only 2.5% of the applied fertilizer N was recovered from the soil at 4-weeks. During this 4-week study, the pool of N recovered in the forest floor declined by 26.7%, with only a negligible increase in the soil N pool (Figure 2.3). During this time the portion of unaccounted for N increased. At 2-weeks, 16.5% of the applied N fertilizer was not recovered and then at 4-weeks 30.2%.

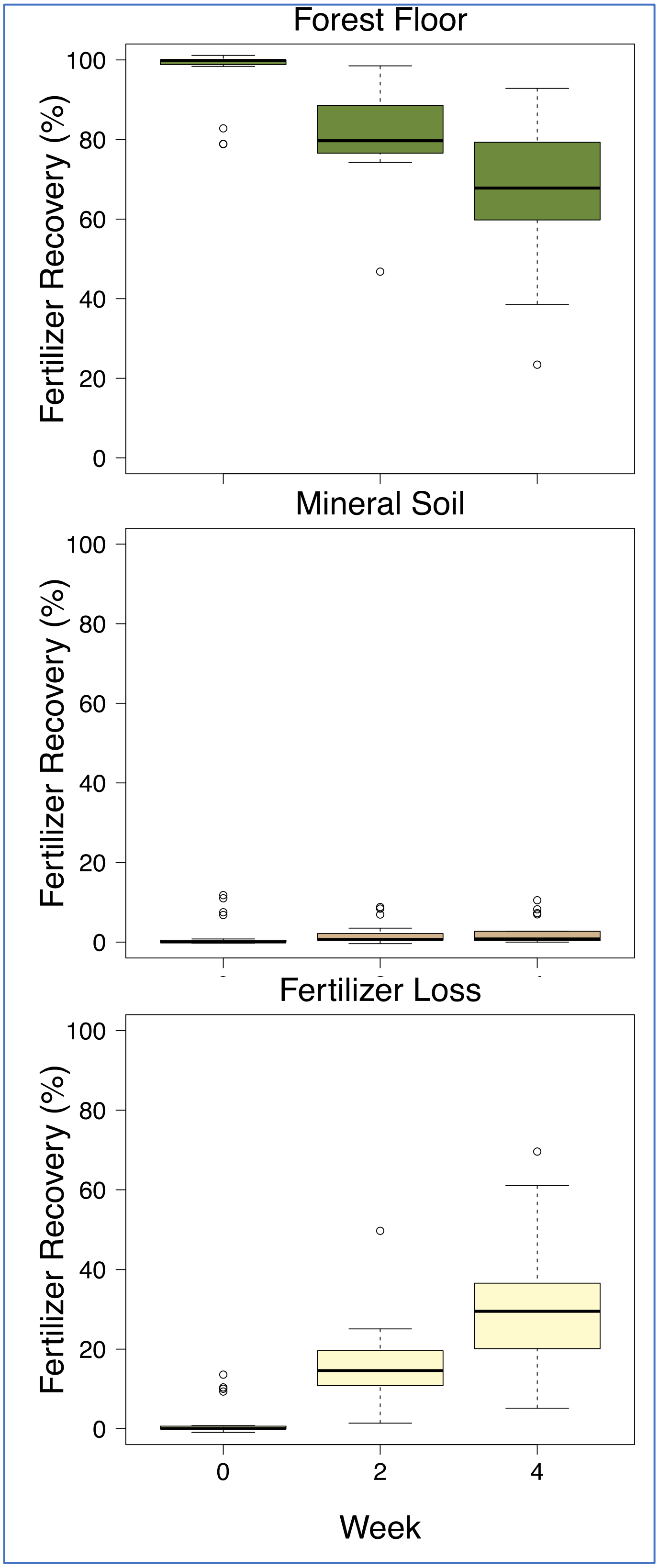

Figure 2.3: N recovery in forest floor and mineral soil (0-20cm) at 0, 2, and 4 weeks. Fertilizer loss represents the unaccounted-for fertilizer at 0, 2, and 4 weeks.

Table 2.2: Mean fertilizer recovered (% of fertilizer application) in the forest floor and soil (0-20cm) for each of the four fertilizer treatments. The total loss represents the fractions of fertilizer that was unaccounted for in the loss tubes containing the forest floor and 0 – 20cm of soil.

Forest Floor				
	ESN	CUF	NBPT	UREA
Week 2	76.9	86.9	81.2	77.2
Week 4	53.0	71.3	71.9	80.6
Soil				
	ESN	CUF	NBPT	UREA
Week 2	0.80	2.5	2.2	2.7
Week 4	2.0	2.6	2.7	2.6
Total Loss				
	ESN	CUF	NBPT	UREA
Week 2	21.9	10.6	16.1	19.4
Week 4	42.1	26.1	27.7	25.9

Table 2.3: Mean fertilizer recovered (% of fertilizer application) in the forest floor and soil (0-20cm) across the five study sites. The total loss represents the fractions of fertilizer that was unaccounted for in the loss tubes containing the forest floor and 0 – 20cm of soil.

Forest Floor					
	Adna 1	Clark Creek	Les Smith	Mill Creek	Oppelt Rd.
Week 2	96.6^{a}	NA	86.0	69.0^{a}	81.7
Week 4	80.6^{x}	83.1^{y}	46.5^{wy}	55.0	71.8
Soil					
	Adna 1	Clark Creek	Les Smith	Mill Creek	Oppelt Rd.
Week 2	0.50^{a}	NA	0.32^{b}	6.9^{abc}	0.47^{c}
Week 4	0.78^{w}	1.2^{x}	0.26^{y}	8.3^{wxyz}	0.44^{z}
Total Loss					
	Adna 1	Clark Creek	Les Smith	Mill Creek	Oppelt Rd.
Week 2	3.1	NA	13.5	24.2	17.8
Week 4	18.9	15.7^{x}	51.5^{x}	36.7	27.8

2.4.1 Differences between treatments

At both the 2-week and 4-week sample points, there no significant differences in N recovery between treatment types (i.e. ESN, CUF, NBPT, UREA) for either the forest floor or the soil samples (Table 2.2, Figure 2.4). None of the Enhanced Efficiency Fertilizer (EEF) treatments improved N recovery over the standard unformulated urea treatment. At the end of the 4-weeks, the plots fertilizer with unformulated urea had the lowest N loss of the four treatments with 25.9% of the applied fertilizer unaccounted for compared to 42.1%, 26.1%, and 27.7% for ESN, CUF, and NBPT respectively. However, these differences were not statistically significant. The forest floor was the largest source of recovered N fertilizer at both the two and four-week sample points. As the forest floor pool declined with time, the mineral soil showed a small increase in the proportion of fertilizer N recovered (Figure 2.4). The increase in the soil fertilizer pools however does not offset the losses in the forest floor pool as the amount of unaccounted for N increased significantly at both the two and four week sample points (Figure 2.5). No significant differences in N recovery were observed between treatment types.

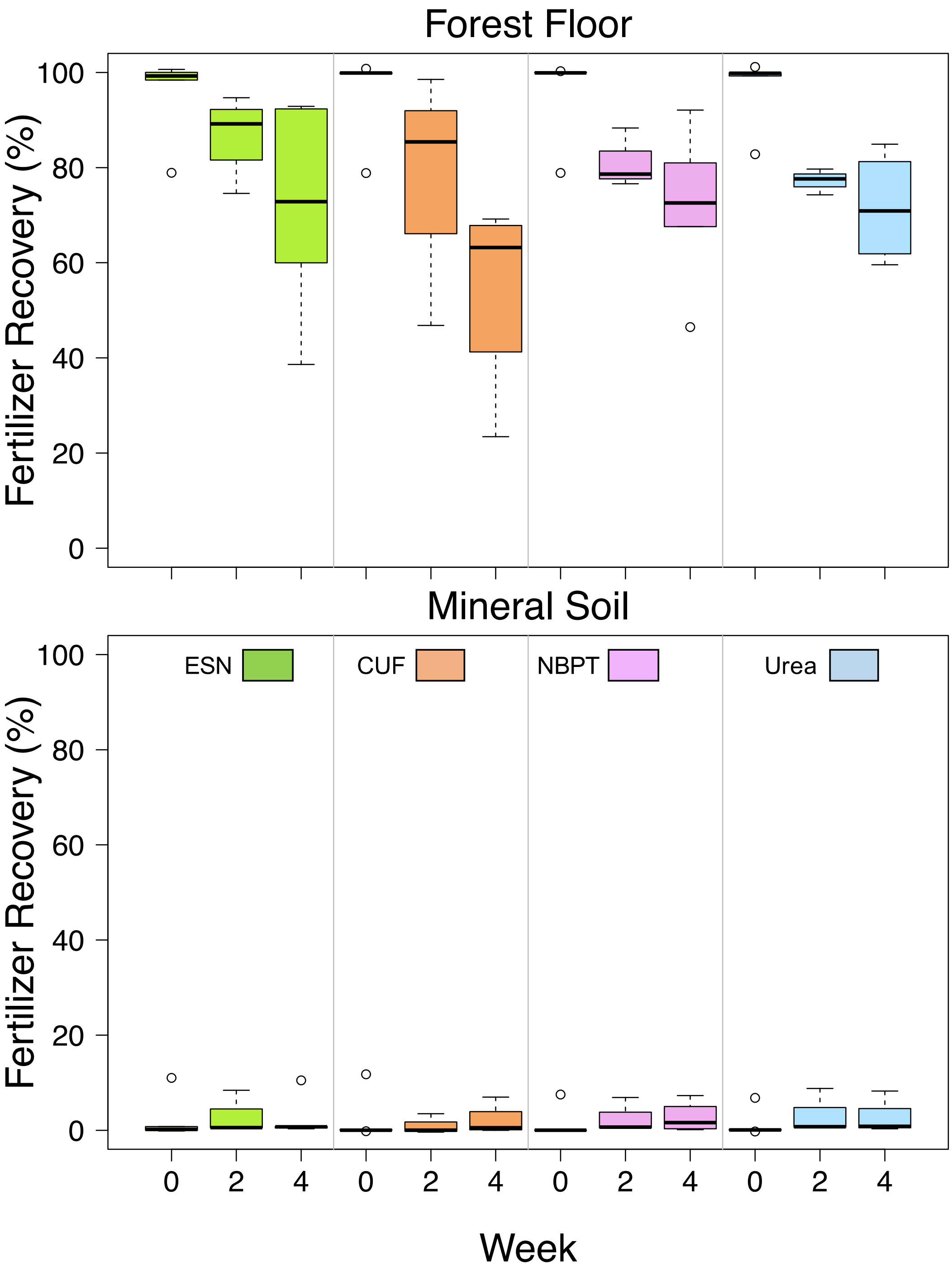

Figure 2.4: Forest floor and mineral soil fertilizer recovery at 0, 2, and 4 weeks subdivided by treatment types.

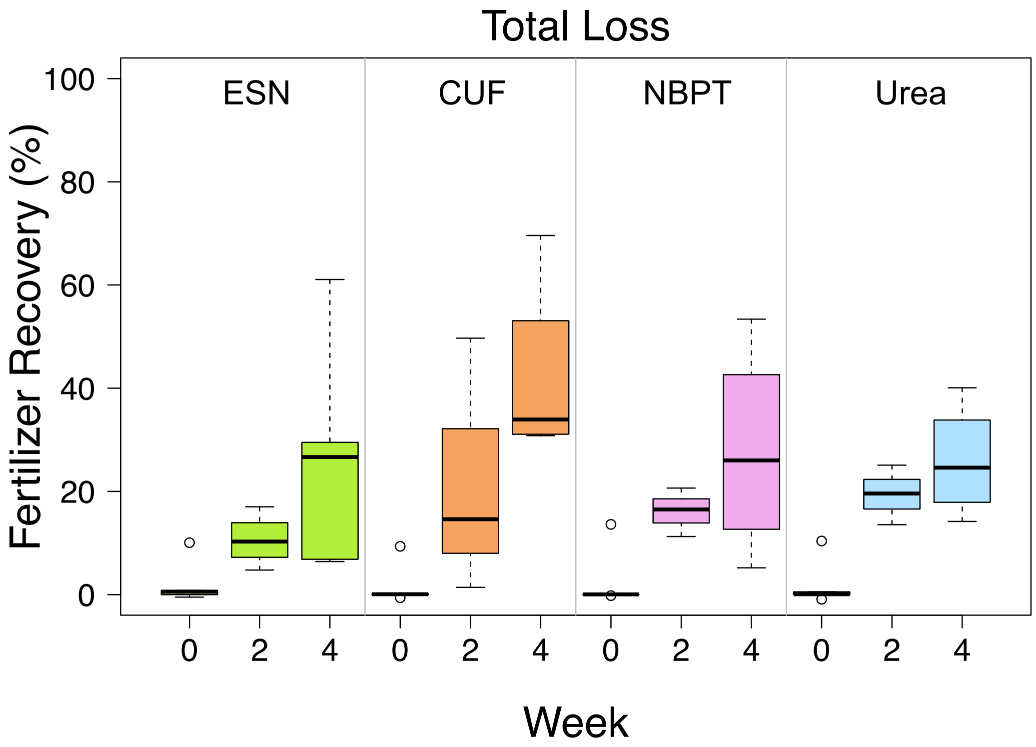

Figure 2.5: Total unaccounted-for fertilizer (i.e. losses) for weeks 0, 2, and 4 subdivided by treatment type.

2.4.2 Differences between sites

Significant differences in N recovery emerge when the data are partitioned by site as opposed to treatment type (Table 2.3, Figure 2.6). At week-2 Adna 1 had a mean fertilizer recovery rate of 96.6% in the forest floor which was significantly higher than the recovery at Mill Creek which only had 69.0% recovery in the forest floor. Both Les Smith and Oppelt Rd. with mean forest floor recovery rates of 86.0% and 81.7% respectively, were not significantly different from either Adna 1 or Mill Creek. Soil N recovery at week two was significantly higher at Mill Creek which had the lowest forest floor N recovery. (Table 2.3, Figure 2.6). However, with regards to total N losses, Mill Creek did not have significantly higher losses compared to any of the other sites at either the 2-week or 4-week sample points. The highest losses with a mean of 51.5% of the applied N unaccounted for at the end of 4-weeks were recorded at Les Smith (Table 2.4, Figure 2.7). Les Smith had the lowest recovery rates in both the soil and forest floor pools with only 46.5% and 0.26% of the fertilizer recovered in the forest floor and soil pools at 4-weeks. Losses at Les Smith were only significantly higher than the losses at Clark Creek which had the lowest loss rate with only 15.7% of the applied N unaccounted for at the end of the 4-week sample period. Soil moisture was recorded during the four week study period. Mill Creek had the highest rainfall with 358mm, Les Smith 52mm, Clark Creek 42mm, Adna 1 10mm, and no rainfall was recorded at Oppelt Rd during this time. Mean soil temperatures at 5cm depth were 6.3, 4.2, -0.2, 4.9, and 4.2^{o}C at Mill Creek, Les Smith, Clark Creek, Adna 1, and Oppelt Rd respectively.

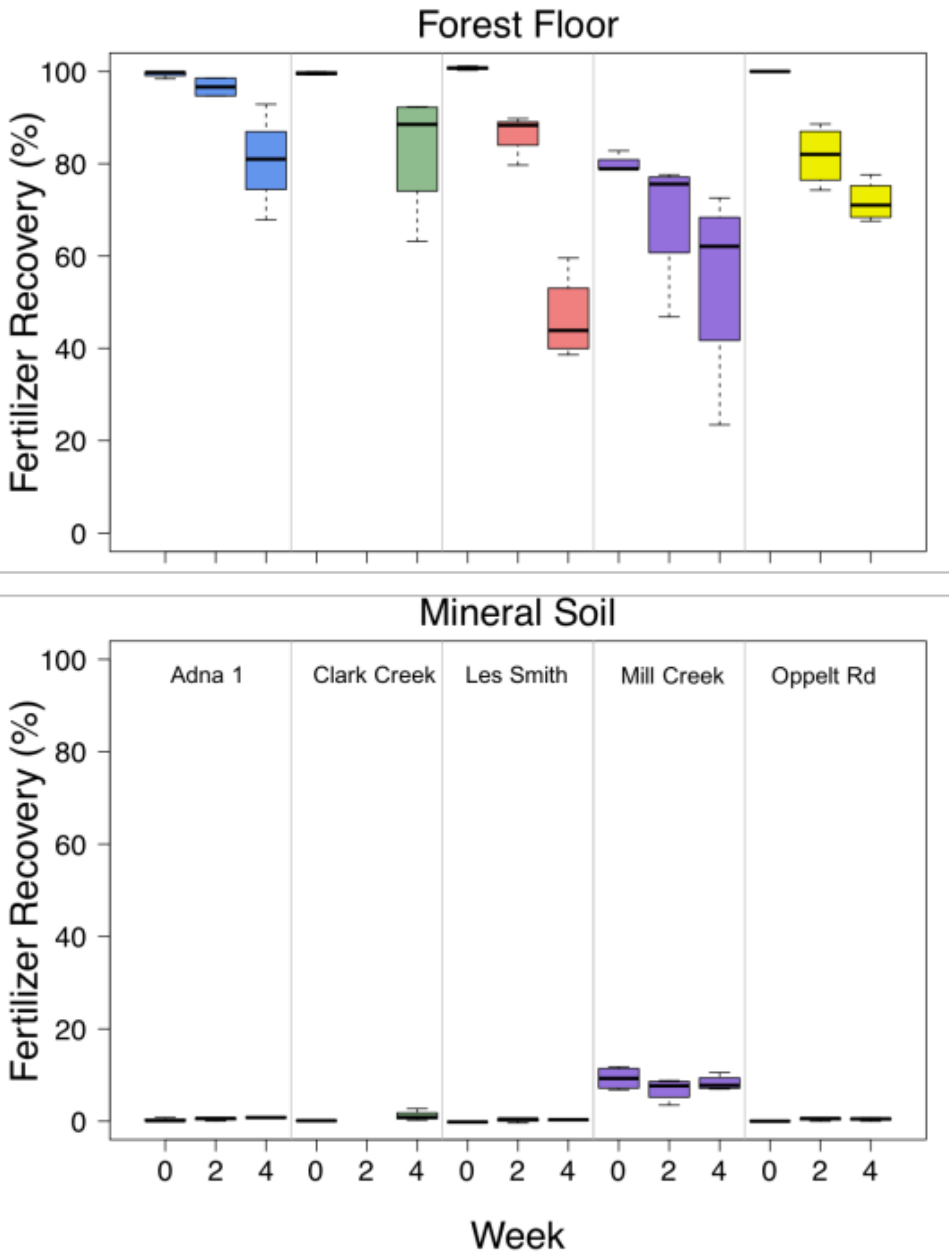

Figure 2.6: Fertilizer recovered in forest floor and mineral soil (0 - 20cm) at weeks 0, 2, and 4 across the five study sites.

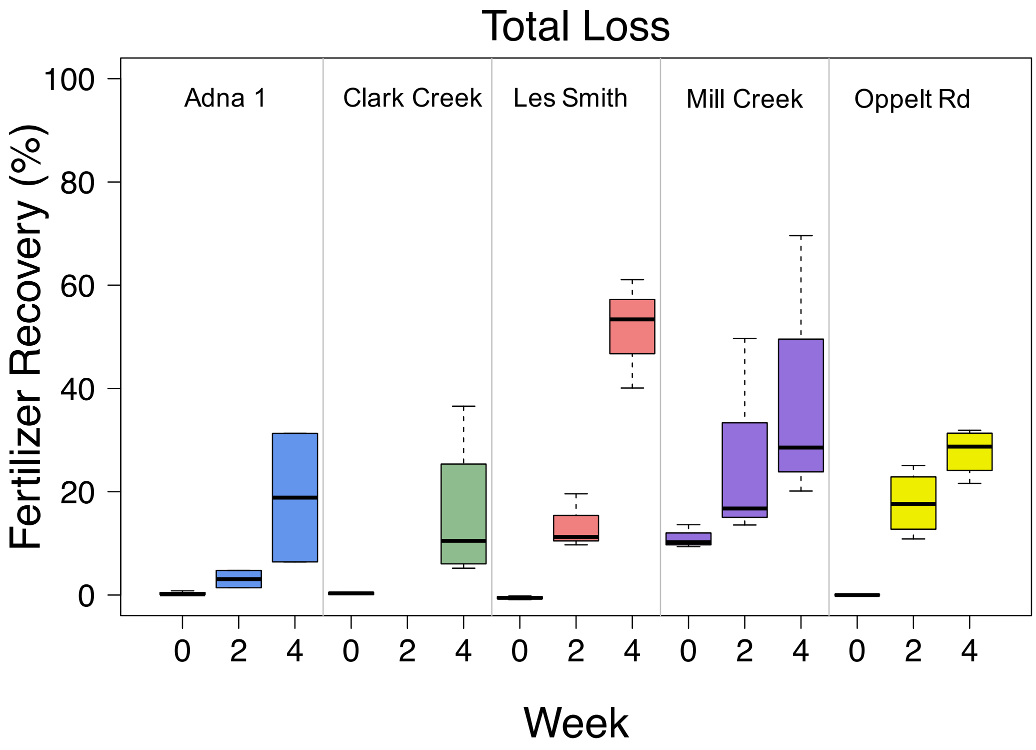

Figure 2.7: Total unaccounted-for fertilizer (i.e. N loss) at weeks 0, 2, and 4 at each of the five study sites.

2.5 Discussion

When urea based fertilizers are applied to the soil surface, urea can undergo hydrolysis resulting in the formation of $NH_{3(gas)}$ which is then lost from the system. To limit losses of the applied fertilizers to volatilization, several methods are employed. One approach is to apply the urea prior to rainfall because the water will react with NH_3 to produce NH_4^+ which is a plant available N source (Black et al. 1987; Kissel et al. 2004) that can be retain on the soil cation exchange capacity. Alternatively, a wet soil surface may increase the rate that urea is hydrolyzed thus increasing the rate of ammonia loss to volatilization (Freney et al. 1992). A second approach is the use of EEFs designed to release the urea when conditions are optimal for rapid incorporation into the soil and maximum plant uptake, or urea granules formulated with urease inhibitors which slows the hydrolysis reaction thus increasing the time for plants to consume the available N (AAPFCO, 2013). Given that fertilizer application rates that are half of what our study used (224kg/ha) can result in substantial N losses (Salazar et al. 2012), N losses were expected to occur following fertilization at our sites. With the purported benefits in N use efficiency with the application of EEFs, we also expected the plots treated with ESN, NBPT, or CUF type fertilizers to have lower N losses compared to the unformulated urea treated plots.

Result from this 4-week study however, revealed no difference in N retention in the forest floor or upper 20cm of soil with the application of EEFs compared to the unformulated urea fertilizer. The 30.2% of the applied fertilizer lost after 4-weeks is consistent with results from other studies with losses of up to 40% recorded after a 4-week time span (Cabrera et al. 2010). Across our range of field sites in Oregon and Washington, N recovery at both 2-weeks and 4-weeks is driven by site properties as opposed to fertilizer treatment type. Finally, substantial N losses occurred in just the first 4-weeks after the fertilizer was applied which raises questions about the fate of the missing N, the mechanisms of N loss, and the efficiency of fertilizer applications at these sites.

2.5.1 Use of Enhanced Efficiency Fertilizers

With no significant differences in N recovery between plots fertilized with the EEFs compared to the unformulated urea fertilizer, our results suggest that the benefits of EEFs cannot be generalized to the region as a whole, at least when fertilizer is applied under the conditions of this study. The particular EEFs used in our study are designed to decrease volatile losses of NH_3 through two different mechanisms; ESN via polymer coatings around the urea and urease inhibitors combined added to the urea granules in both NBPT and CUF. The use of EEFs as a management approach to improving nitrogen use efficiency assumes that volatile losses at a site are a substantial loss pathway for the applied N fertilizer. The failure of the EEFs in our study to increase N recovery rates relative to plots fertilized with the unformulated urea could be the result of either low volatile losses at these sites, in which case the added urease inhibitors or polymer coatings would yield little to no increase in final recovery rates. However, if substantial losses of N at both week-2 and week-4 are actually due to volatilization then it is possible that the EEFs do not function as intended under the particular environmental conditions found at these forested sites. Other studies that have examined the effectiveness of EEFs have produced mixed results across a wide range of agricultural plots (Akiyama et al. 2010; Halvorson et al. 2010; Halvorson et al. 2014; Kibet et al. 2016). The benefits of EEF use appears to be site specific based on environmental properties such as soil type and climatic conditions (Akiyama et al. 2010; Kissel et al. 2004; Sommer et al. 2004; Cabrera et al. 2010).

2.5.2 Influences of site properties on N retention

Differences in N recovery rates emerged when the data were partitioned by site as opposed to fertilizer treatment across the region. Clark Creek had the lowest rates of N loss after 4-weeks with just 15.7% of the applied N unaccounted for. In contrast, the Les Smith site had the highest rates of N loss with nearly half (51.5%) of the applied N lost by week 4. During the study period the Clark Creek soil had a mean temperature just below freezing (-0.2 ºC). Soil temperature and volatilization rates are positively correlated with volatile losses of N (Cabrera et al. 2010). Additionally, the frozen ground may have inhibited the hydrolysis of urea and also slowed the rates of microbial activity responsible for assimilating N into the plant available soil pool. Enzymatic nutrient uptake processes are also slowed with low soil temperatures (Thomas and Strain, 1991). The two sites with the highest precipitation rates during the study period were also the two sites with the highest N losses. Mill Creek had the highest rainfall at 358mm which may explain why this site was the only one that had substantial N recovery in the soil pool after 4-week. With a mean of 8.3% of N recovered in the soil pool it is possible that some N was lost through leaching. Les Smith which had the highest N losses (although only significantly different from the lowest N loss site, Clark Creek) had the second highest rainfall of the five sites, after Mill Creek. Rainfall after fertilization with urea can increase the hydrolysis of urea to NH_3 (Kissel et al. 2004). If the rainfall is not sufficiently high enough to carry the products of the hydrolysis reaction into the soil where it is available to plants as NH_4^+ it will be lost to the atmosphere as $NH_{3(gas)}$.

2.5.3 Fertilizer losses and the fate of missing N

The productivity of forests in western Douglas-fir region of Oregon and Washington is generally limited with respect to N. Our results suggest that N losses can still occur despite the application of N fertilizer to otherwise N limited sites. Nitrogen losses from N-limited sites suggests that saturation and losses can occur when fertilizer is applied at a rate exceeding the rate at which plants and microbes can immobilize the nutrients in their respective biomass (Lovett and Goodale, 2011). Since the loss chambers in our study essentially cut off root access from the sides of the chambers, the losses we measured, particularly from Mill Creek which had 8.3% N recovery in the soil, may have been at the high end for these particular sites. With N retention at a site correlated with the strength of the N uptake pathways into roots and thus plants pools (Lovett and Goodale, 2011), higher losses to leaching, volatilization, and denitrification could be expected. Recovery in the soil pool across sites and treatments was low after 4-weeks with a mean of only 2.5% of the N recovered from the soil. This would suggest that leaching losses were low during this time period and that the unaccounted-for N can be attributed to gaseous losses of N. Two loss pathways that could therefore produce these losses are either denitrification or volatilization and a combination of the two. The challenge is that without direct measurements we are unable to quantify the partitioning of N to these two loss pathways.

2.5.4 Conclusions

Regardless of whether the N losses from the forest floor are attributed to denitrification or volatilization, we were able to conclude that the use of EEFs did not improve N recovery rates across our study sites in the western Douglas-fir Region of Oregon and Washington. N recovery was attributed to environmental conditions that make up each site in our study as the only significant N recovery differences were observed when the data were analyzed by site. The fertilizers were all applied in early spring when conditions are optimal for limiting volatile losses. While temperatures are still cool and rainfall is high enough to wash the urea hydrolysis products into the soil where it becomes accessible to plants, the benefits of EEFs are not pronounced. Because these EEFs delay the hydrolysis of urea to times when conditions are optimal for plant uptake, we would expect there to be a greater advantage to their application in the warm dry months when volatile losses of applied fertilizers would be high (warm, dry weather). Further research is however needed to determine if EEF use maybe warranted for a summer fertilizer application at these sites. In a similar study in loblolly pine forests of the southeastern United States, which used the same treatments and experimental design, fertilizer-N recovery was higher (Raymond et al. 2016) than when the same treatments are applied to the plantation forests in the western Douglas-fir region of Oregon and Washington. The particular EEFs used in both studies were formulated specifically for the southeastern United States. This is especially relevant for the polymer coated EEF (ESN) as the polymer coating breaks down when exposed to water, higher temperatures, and solar radiation (AAFPCO, 2013). Douglas-fir forests have dense canopies with very little sunlight penetrating the canopies and hitting the forest floor relative to loblolly pine stands which are more open and their forest floors sunlit. With site conditions the greatest driver of short term N recovery, management decisions regarding the efficient application and use of fertilizers need to be geographically customized at the site scale as opposed to region wide.

2.5.6 Limitations and assumptions

With only one of each treatment type applied per study area we lack the statistical power to determine if any particular treatment improves N use efficiency at given site. The variable response in terms of N recovery with EEFs compared to the unformulated urea across sites suggests that environmental conditions dictate how much N is retained in the ecosystem. Unfortunately, given the limited sample size I was only able to determine that the aggregate of environmental conditions that comprise each *site* best predict N recovery as opposed to being able to determine which fertilizers are best suited for improved N use efficiency at each site. Additionally, given the study design, I was not able to partition the N loss pathways that account for the missing N. With only a small quantity of N recovered in the soil after 4-weeks it is possible to infer that leaching is unlikely a substantial loss pathway during this short time frame. This leaves gaseous losses of N as the likely cause of the N loss but we cannot quantify the relative size of the flux pathways for volatilization or denitrification without measuring N_2O and NH_3 fluxes directly.

Chapter 3

Less than 50% nitrogen retention under high N additions after 1-year in Douglas-fir forests

Abstract

In Pacific Northwest forests, N is known to be a limiting nutrient particularly in Douglas-fir (*Pseudotsuga menziesii*) ecosystems. Fertilizers are applied in order to increase timber yields in managed commercial forests in Oregon and Washington. Despite these N limitations, Douglas-fir uptake of the applied fertilizers is typically low and highly variable depending on the location of the forest. The highest rates of uptake have been found to occur in the first year after fertilization. We measured N retention within this time frame at five sites using a fertilizer enriched in ^{15}N as a tracer. Retention was determined as the proportion of applied fertilizer accounted for in ecosystem pools after one year. The pools include mineral soil (0-60cm), forest floor, bole wood, bark, and foliage. Comparisons were also made between three urea based enhanced efficiency fertilizers (EEFs) and the industry standard urea fertilizer. Retention was low across all sites and fertilizer types with a mean of 39.0% after 1-year. The remaining N could not be accounted for in the pools we sampled. The largest fertilizer pool was the top 20cm of mineral soil. No differences were found between EEFs and the standard urea fertilizer.

3.1 Introduction

In Pacific Northwest forests, N is known to be a limiting nutrient, particularly in Douglas-fir (*Pseudotsuga menziesii*) ecosystems (Gessel et al. 1973). Nutrient limitations occur when the potential uptake by plants exceeds the available soil nutrient supply (Allen et al. 1990). Theoretically, N fertilization in these forests as a deliberate land management practice should ameliorate these deficiencies and increase tree growth (Fenn et al. 1998; Fisher and Binkley, 2012; Chapin et al. 2002). However, previous research has produced mixed results (Peterson and Gessel, 1984; Talbert and Marshall, 2005; Jandl et al. 2007). Despite increases in Nr emissions either from deliberate fertilization or Nr deposition, the productivity of many forests is still limited by the availability of N, an essential plant nutrient (LeBauer and Treseder, 2008). Of the total N applied, only between 12-43% is ever accounted for in the above ground biomass (Amponsah et al. 2004; Salifu and Timmer, 2003). This contrasts with more controlled greenhouse pot studies where 88-95% of the fertilizer is accounted for in the above ground biomass (Amponsah et al. 2004). The fate of the remaining N is largely unknown. Quantifying the fate of this missing N is essential to determine the environmental site conditions that best predict N retention and uptake in the western Douglas-fir region of Oregon and Washington.

With the demand for forest products exceeding the current and projected supply, intensively managed bioenergy plantations with rapid turnover may be a sustainable practice to meet the needs for additional raw material. In Washington, the efficient and rapid growth rates of high quality Douglas-fir make the state one of the country's leading suppliers of timber products (DNR, 2012). In the Pacific Northwest, fertilization is a common practice meant to increase tree growth and productivity in plantation forests. Since 1990, an estimated 250,000 acres of Pacific Northwest Coast Douglas-fir plantation forests have been fertilized (Sucre et al. 2008). Previous research highlights the benefits of N fertilization, showing increased tree growth when applied to low productivity Douglas-fir sites (Miller et al. 1989; Chappell et al. 1991). Talbert and Marshall (2005) suggest that intensive management with repeated N fertilization increases the average site productivity from 13 m^3 ha^{-1} yr^{-1} to between 17 and 20 m^3 ha^{-1} yr^{-1}. However, other studies have produced less optimistic results. Peterson and Gessel (1984) found a change in the volume of Douglas-fir stands ranging from -5.5 to +13.1 m^3 ha^{-1} yr^{-1} when compared to control sites six years after fertilization. Theoretically, N fertilization should increase tree growth, but the variability in past results suggests a need for further research to determine why some sites respond with increased productivity to fertilizer applications while others do not.

Variable response to N fertilization is attributed to differences in environmental site conditions. Environmental site conditions that are positively correlated with growth response to fertilization include forest floor carbon (C) to N (C/N) ratios (Edmonds and Hsiang, 1987; Littke et al. 2014), pre-fertilization soil N concentrations (Hopmans and Chappell, 1994), and foliar N concentrations (Turner et al. 1988). One of the major uncertainties in our understanding of ecosystem response to N deposition is the fate of approximately 30-40% of N inputs (Morse et al. 2015). However, direct field measurements of these losses are complicated by large background levels of N_2 in the atmosphere and the high temporal and spatial variability in the processes that dictate gaseous losses of N (Bai et al. 2012). Quantification of the pools in which fertilizer N accumulates across a range of site conditions is an important part of understanding this variability in growth response to fertilizer treatments.

Enhanced Efficiency Fertilizers (EEFs) are a common management tool employed to limit N losses following fertilization of agroecosystems. Fertilizers are commonly applied in excess of plant demand to maximize gains in productivity (Watts et al. 2015). N applied in excess of plant demand can result in short-term saturation of an otherwise N-limited site (Lovett and Goodale, 2011). This *kinetic saturation* results in the loss of N through increased volatilization, denitrification, or leaching (Lovett and Goodale, 2011). Urea based EFFs are designed to decrease N losses by coordinating the release of fertilizer N with plant demand. Several mechanisms are used in theses fertilizers to theoretically decrease losses of N. Controlled release EEFs work by delaying the plant availability of N through the use of coatings (Trenkel, 2010). Urease inhibitors slow hydrolysis by blocking the active site on urease, the enzyme catalyst that converts urea to CO_2 and ammonia which is readily available in forest soils (AAFPCO, 2015; Bremner and Douglas, 1971; Sanz-Cobena et al. 2008. The benefits in terms of N use efficiency are however unclear. Research comparing N losses with the application of EEFs to standard unformulated urea fertilizers in agricultural plots have produced mixed results with some studies showing decreased losses of N with EEF use (Akiyama et al. 2010; Halvorson et al. 2014) while other studies show an increase or no difference in losses with EEF use (Sistani et al. 2011; Watts et al. 2015). Most of the research comparing EEFs to unformulated urea fertilizers are from studies in food agroecosystems as opposed to agroforestry systems where there is a paucity of research on the topic.

Forest soils contain large amounts of N relative to the size of typical fertilizer applications, with ranges in the Pacific Northwest typically reported between 2 and 11 Mg ha^{-1} (Fisher and Binkley, 2012). Additionally, the variability of soil N concentrations within forested sites is high relative to the amount of N that is added through fertilization. Across the Western Douglas-fir region, soil N contents vary between low and high productivity plots. At the low end in productivity soil N concentrations have been measured at 1,330 ($\underline{+}$80) kg ha^{-1}. In contrast, the soil N content is 2,810 ($\underline{+}$250) kg N ha^{-1} for high productivity sites near Fall River, WA (Ares et al. 2007). The amount of fertilizer N that is applied to plantation forests is usually equal to or less than 224kg ha^{-1}, and in the case of atmospheric deposition is estimated between 1-9 kg ha^{-1}yr^{-1} (Geiser et al. 2010). These additions are small relative to the variability and overall quantity of N in forested ecosystems. Consequently, it is difficult to account for changes in the N pools of forested ecosystems by using a simple mass balance approach or by using changes in tree growth rates as a proxy for N retention.

The objective of this study is to quantify N retention and redistribution within the mineral soil, forest floor, bole wood, and foliage of commercially managed Douglas-fir forests in Oregon and Washington, one year after fertilization. We compare the distribution of retained N for three commonly used urea based enhanced efficiency fertilizers (EEFs) and the industry standard urea fertilizer to determine if N retention and distribution is altered with the addition of fertilizers containing adjuvants designed to decrease volatile losses following fertilizer application. A N-fertilizer enriched in the stable isotope ^{15}N is used to trace the movement of N between ecosystem pools (Buchmann et al. 1996; Templer et al. 2005). Management for both timber productivity and ecosystem health requires a greater accounting of applied N, particularly its partitioning between ecosystem components (i.e. soil and tree biomass pools) and determination of the site conditions that best predict retention in the various N pools.

3.2 Study Area

This project is comprised of 5 study sites in the Western Douglas-fir region of Oregon and Washington (Figure 3.1). The study sites are all 25-years old, previously unfertilized stands, within privately managed plantation forests. The sites were selected to include a representative range of parent materials and latitudes of Douglas-fir plantation forests in the region. The three primary parent materials are of glacial, volcanic, and sedimentary origin. Douglas-fir is the widest ranging tree species on the west side of the Cascade mountains (Brubaker et al. 1992). It is an important crop species as the production of the high-quality Douglas-fir tree that make Washington state one of the country's leading suppliers of timber products (DNR, 2012).

This research benefits from the infrastructure already in place from a much larger fertilization project with 73 field sites extending from southern Oregon, north through

British Columbia. The purpose of the larger study is to determine the environmental conditions and geographic variables that best predict a stand's growth response to fertilizer applications (Littke et al. 2014). The set up and installation of this larger project was initiated through the Center for Advanced Forestry Systems (CAFS), which is a National Science Foundation Industry and University Cooperative Research Center. With support from CAFS, a subset of the 73 sites were selected for inclusion in this ^{15}N fertilizer tracer study. From north to south the five sites included in our study are Mill Creek, Oppelt Rd, Adna 1, Les smith, and Clarks Creek respectively (Figure 3.1). The region has a maritime Mediterranean climate with mild wet winters and warm dry summers, an ideal growing condition for highly productive conifer forests.

Figure 3.1: Locations of the five study sites in the Western Douglas-fir Region of Oregon and Washington.

3.2.1 Mill Creek

The Mill Creek soils are loams (0-5cm depth) and sandy loams (50cm depth) whose parent material is of glacial outwash origin. Of the five sites, Mill Creek has the lowest soil N concentration with 7243 Mg ha^{-1}. Mean annual soil moisture at Mill Creek is 0.34 cm/cm^3 in the surface horizon (5cm depth) and 0.16 cm/cm^3 at 50cm depth. The site is situated at 46m above sea level and has a 0% slope. Plotting density is 809 tree ha^{-1}. C/N of the forest floor is 20 and the soil A horizon has a C/N of 25.

3.2.2 Oppelt Rd

Located to the southwest of Mill Creek, the Oppelt Rd site has a similar glacial outwash parent material. Oppelt Rd has the highest soil N concentration of the five sites at 10796Mg ha^{-1}. Soil textures are clay loam at 5cm and transitions to a sandy clay loam at 50cm depth. Mean annual soil moisture at this site is higher than Mill Creek with a mean of 0.33 cm/cm^{3} at 5cm depth. At 50cm the soil moisture is 0.29 cm/cm^{3}. The site has a 10% slope and is situated at 91m above sea level. Planting density is 820 trees ha^{-1}. C/N of the forest floor is 20 and the soil A horizon is 18.

3.2.3 *Adna 1*

The Adna 1 site has a sedimentary parent material. The soil texture at both 5cm and 50cm depth is clay. The total soil N concentration is 9583Mg ha^{-1}. Forest floor C/N is 23 while soil C/N is 17. Mean annual soil moisture increases slightly with depth at this site. At 5cm depth soil moisture is 0.26 cm/cm^{3} and at 50cm soil moisture is 0.28 cm/cm^{3}. The site is located at 213m elevation with a 0% slope. Planting density at Adna 1 is higher than the other four sites at 981 trees ha^{-1}.

3.2.4 Les Smith

The Les Smith site is located in Oregon. This site has an igneous parent material. Soil texture is a silty clay loam at 5cm depth and clay loam at 50cm depth. Total soil N content is 9039Mg ha^{-1}. Forest floor C/N is highest here relative to the other four study sites with a forest floor C/N of 40 while soil C/N is 24. Mean annual soil moisture at 5cms depth is 0.30 cm/cm^{3} and increases to 0.36 cm/cm^{3} at 50cm depth. The site, located at 451m elevation has a 14% slope. Planting density is 805trees ha^{-1}.

3.2.5 Clark Creek

Clark Creek, the southernmost site, is located in Oregon. The parent material here is of igneous origin. The soil at 5cms depth has a loam texture while at 50cm depth the texture is a clay loam. Total soil N content at Clark Creek is 8646 Mg ha^{-1}. Forest floor C/N of 60 is the highest of the five sites in our study. Soil C/N is 28. Mean annual soil moisture is 0.26 cm/cm^{3} at 5cm depth and increases slightly to 0.28 cm/cm^{3} at 50cm. The elevation is at 793m and the site has a 15% slope. A planting density of 769 trees ha^{-1} is the lowest of the five sites.

3.3 Methods

3.3.1 Fertilizer treatments

To trace the fate of applied N in the ecosystem, four different ^{15}N-labelled urea based fertilizers were used. The fertilizers were enriched to 0.5atom% ^{15}N (~$370^{0}/_{00}{}^{15}$N). Enrichment to 0.5atom% ^{15}N is expected to be sufficiently high to register a signal in the various N pools even if fractionation occurs as the fertilizer-N moves through the soil and into the trees. By using a fertilizer enriched with the stable isotope ^{15}N, the applied fertilizer may be traced as it moves between N pools within the study plots. The four fertilizers used were 1) urea, 2) Agrium ESN, 3) urea + NBPT, and 4) Urea+CuF (Table 3.1). Urea without any enhancements is the industry standard fertilizer while ESN, NBPT, and CUF each contain a different adjuvant designed to decrease volatile losses and improve nitrogen use efficiency (Table 3.1). The use of EEFs in agroforestry management assumes that volatilization is a substantial loss pathway and that additional N retained in the soil as a result of their use is available to plants.

Table 3.2: Summary of the four fertilizer treatments applied at each of the 10 study sites.

Fertilizer	Description	Mechanism
Urea	Industry standard	Volatilization loss controlled via timing of application
ESN (Agrium)	Polymer coated urea	Delays hydrolysis of urea for 60-90 days; release of urea is temp. and moisture dependent
Urea+ NBPT (Agrotain)	Coated with N-(n-butyl) thiophosphoric triamide	Slows hydrolysis by blocking active site on urease for 7-21 days
Urea+CuF (Arborite)	Coated with phosphate	Granular urea pellets coated with boron solution- acts as both an inhibitor and a binder for additional nutrient layer (PO_4)
Unfertilized Control	No fertilizer added within the $100m^2$ boundary	N/A

3.3.2 Experimental design

A complete randomized block design was used where each site (Figure 3.1) is a replicate that includes five treatment plots. Each plot consists of a target tree at the center of a $100m^2$ fertilized treatment area (Figure 3.2). The five plots were treated with ^{15}N-enriched Urea, Agrium ESN, Urea + NBPT, or Urea + CUF type fertilizer at a rate of 224kg N ha^{-1}. The remaining plot was an unfertilized control.

The five study sites were fertilized in March of 2012. The ecosystem components sampled within each plot boundary included the forest floor, mineral soil (0-60cm in 15 cm increments), bark, bole, foliage, and understory vegetation (Figure 3.2). Samples of all these ecosystem components were collected prior to fertilization to form a baseline for comparison. They were again sampled one year after the fertilizer application. All samples were collected from within the $100m^2$ fertilized boundary around each target tree. All the trees within the plot boundaries had their diameter at breast height (DBH) measured before and after fertilization. The air-dried mineral soil was then sieved to <2mm. Large organic pieces were removed from the sample during sieving and weighed. This organic component was then ground in a Wiley mill and added back to the <2mm component. The mixture was blended so that uniform subsamples could be collected. Each subsample contained approximately 20g of soil and was ground to a fine powder with a mortar and pestle. A small subset of the powdered sample was then weighed and tinned for isotope analysis. Sub samples of individual forest floor, foliage, bark, and bole wood samples were each ground to a fine powder using a ball mill and then weighed and tinned for isotope analysis. The individual samples were analyzed for their %N and $\delta^{15}N$. Samples were analyzed by UC Davis Isotope Lab using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20-20 isotope mass spectrometer.

The proportion of ^{15}N tracer (i.e. fertilizer) recovered within each pool is calculated using Equation 3.1. An allometric equation was used to scale the fertilizer pools from the individual foliage, bark, and bole samples up to the entire tree. The allometric equation (Equation 3.2) used in this study was developed by Gholz et al. (1979). The equation describes the relationship between diameter at breast height (DBH) of Pacific Northwest Douglas-fir trees and their foliage, stem wood, stem bark, live branches, and dead branches in units of kg. Using %N, atom% ^{15}N of the sample, and total N in each ecosystem component relative to the control plot provides a best estimate of the total amount of fertilizer retained in each ecosystem pool. The %N and $\delta^{15}N$ of each sample was converted to fertilizer recovered (i.e. fertilizer recovery) using Equation 3.1. The R code used for these calculations is available in Appendix 1.

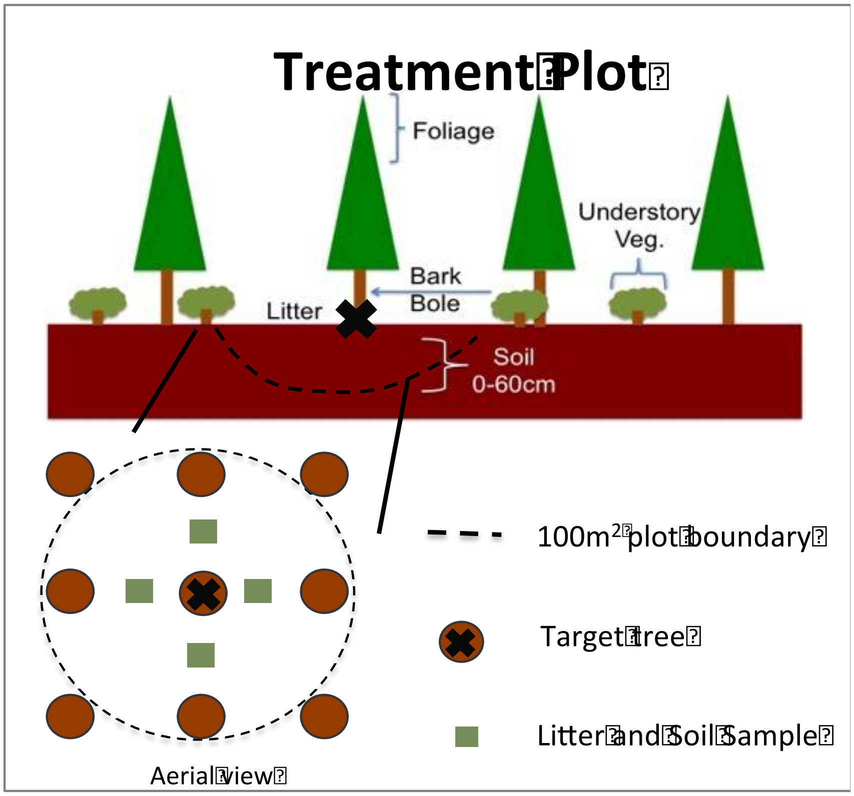

Figure 3.2: Layout of a single treatment plot.

[Equation 3.1]

$$\%^{15}N_{rec} = 100 * m_{pool} * \frac{(\text{atom}\%^{15}N_{pool} - \text{atom}\%^{15}N_{ref})/100}{^{15}N_{tracer}}$$

$\%^{15}N_{rec}$ = percent of applied ^{15}N tracer recovered in labeled N pool
m_{pool} = N mass of labeled pool
atom % $^{15}N_{ref}$ = atom percent ^{15}N of the reference pools
atom % $^{15}N_{pool}$ = atom percent ^{15}N of the ecosystem pools
$^{15}N_{tracer}$ = amount of ^{15}N added to each pools

Equations based on calculations described by Buchmann et al. 1996; Templer et al. 2005

[Equation 3.2] $Y=e^{(a + b*\ln X)}$
a and b are constants
X = DBH (cm)
Y = biomass
From (Gholz et al. 1979)

3.3.3 Statistical Analyses

Differences in N recovery among sites and fertilizer treatments were assessed with an ANOVA and all statistical significance is assessed based on $\alpha = 0.05$. A Tukey's HSD test was then applied to determine which means were significantly different from each other ($\alpha = 0.05$). A multiple linear regression analysis was used to determine which environmental site conditions, if any, best predict N retention across the study sites. The subset of predictor variables included in the final model were selected using a forward step-wise approach. Expected relationships between environmental site conditions and total ecosystem fertilizer N recovery are summarized in Table 3.2. All analyses were conducted in R (R Statistical Program Version 3.0.1). The R functions used for converting $\delta^{15}N$ to atom % ^{15}N, the Gholz (1979) equation, and N retention calculator are included in the appendix.

Table 3.2: Ecosystem properties used as parameters in multivariate linear model selection.

Parameter	Hypothesized Correlation
Foliage	
δ^{15}N	-
%N	+ / -
Forest Floor	
δ^{15}N	-
C/N	+
%N	+ / -
Soil	
δ^{15}N	-
Total N%	+ / -
Total C%	+
A-Horizon C/N	-
Mineral Soil C/N	-
Ca^{+2}	+
K^{+}	+
Mg^{+2}	+
5cm Sand%	-
5cm Clay%	+
50cm Sand%	-
50cm Clay%	+

3.4 Results

3.4.1 Total N recovery

Total N recovery, one year after fertilization, averaged 39.0% across sites and fertilizer treatment types. Total N recovery (sum of foliage, bark, bole, forest floor, and mineral soil for a given plot) as a function of fertilizer treatment was not significant (p = 0.84). Therefore, N recovery was not improved with EEFs relative to the standard urea fertilizer (Table 3.3, Figure 3.3). As expected, N recovery was highly variable across sites and treatments. Total N recovery ranges from a low of 29.6% to a high of 47.2% between study sites, while N recovery for the four fertilizer treatments ranged from 34.3% for CUF to 39.3% for NBPT (Table 3.3). Total N recovery was significantly different between sites (p = 0.01). The Oppelt Road site had the highest 1-year N recovery rate of 47.2% while Les Smith and Clark Creek had the lowest N recovery rates of 29.6% and 30.0% respectively (Table 3.4, Figure 3.4). The N recovery at Oppelt Road was significantly higher than both Les Smith (p =0.01) and Clark Creek (p = 0.02). Mill Creek and Adna 1 had mean N recovery rates of 37.4% and 40.6% respectively but were not significantly different from any of the other sites (i.e. Oppelt Road, Les Smith, or Clark Creek).

Table 3.3: Mean N retention for each of the four fertilizers. Letters in superscript denote significant difference (α = 0.05).

Fertilizer	Total	Above Ground	Below Ground	Foliage	Bole	Forest Floor	Mineral Soil
CUF	34.3	6.9	27.4	4.4	2.5	1.2	26.2
ESN	39.9	5.7	34.2	2.3	3.4	3.7	30.5
NBPT	39.3	14.7	24.6	9.4	5.3	1.2	23.4
UREA	38.4	12.2	26.2	7.0	5.2	1.6	24.6

Table 3.4: Mean N retention for the five study sites. Letters in superscript denote significant difference (α = 0.05).

Fertilizer	Total	Above Ground	Below Ground	Foliage	Bole	Forest Floor	Mineral Soil
Les Smith	29.6	12.3	17.3	7.9	4.4	2.0	15.3
Adna 1	40.5	8.7	31.8	5.4	3.3	1.8	30.0
Clark Creek	30.0	5.8	24.2	3.1	2.7	2.3	21.9
Mill Creek	40.4	15.6	24.8	9.2	6.4	2.0	22.8
Oppelt Road	47.3	6.8	40.5	3.3	3.5	1.5	39.0

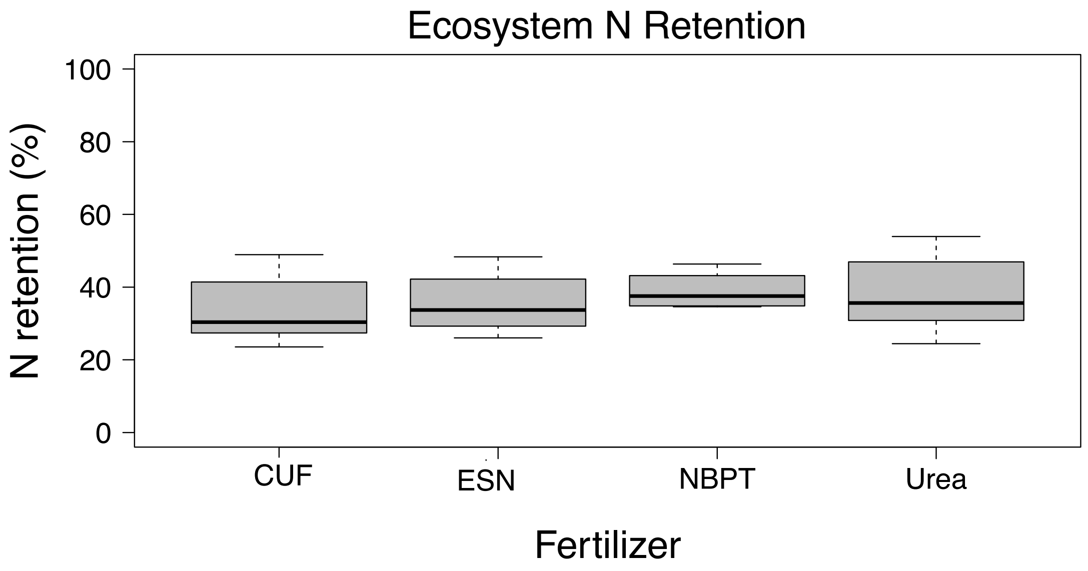

Figure 3.3: Total fertilizer retained in ecosystem under the four different treatment types

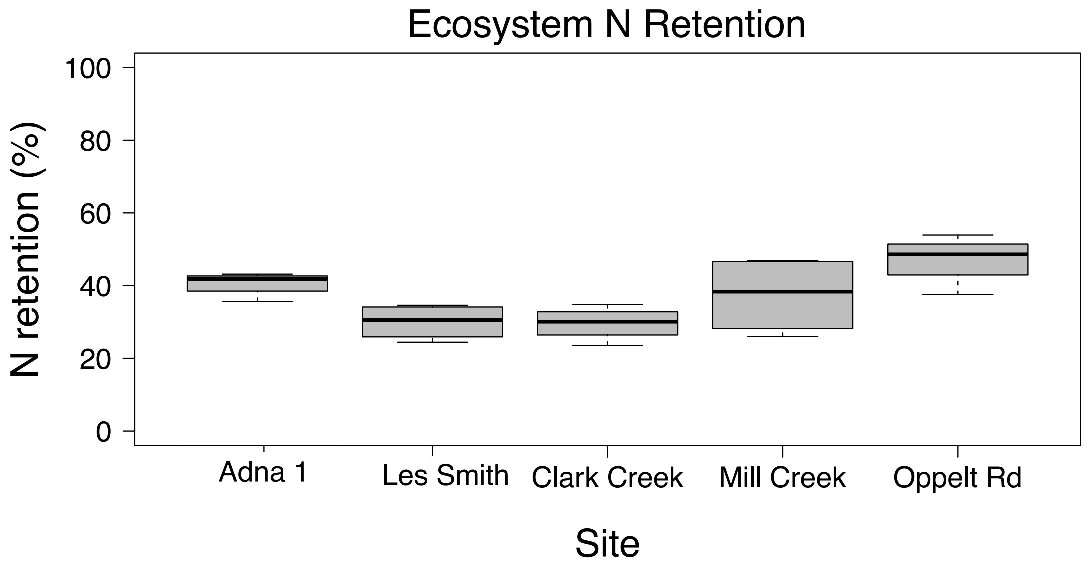

Figure 3.4: Total fertilizer recovered 1-year after fertilization at the five study sites

3.5.2 Above and below ground N recovery

Significantly more N was recovered in the below ground pools (combined forest floor and mineral soil) relative to the above ground biomass pools (foliage and bole wood) ($p < 0.000$). A mean of 27.1% of the applied fertilizer was accounted for in the below ground pools while only 9.9% was accounted for in the above ground biomass pools one year after fertilization. Below ground N recovery was not significantly different between fertilizer treatment types (p = 0.07), so again EEFs did not significantly improve N recovery relative to the unformulated urea fertilizer (Table 3.3). Just as with overall N recovery, differences in below ground N recovery were driven largely by site conditions rather than treatment type (p = 0.001). Below ground recovery was highest at Oppelt Road (relative to Les Smith (p = 0.001), Mill Creek (p = 0.001), and Clark Creek (p = 0.04)). Les Smith had significantly lower N recovery compared to Adna 1 (p = 0.01) (Table 3.4). Recovery in the above ground pools was not improved with the use of EEFs (p = 0.08), and unlike the below ground pools, above ground N recovery did not differ between sites (p = 0.16) (Table 3.1, Figure 3.5).

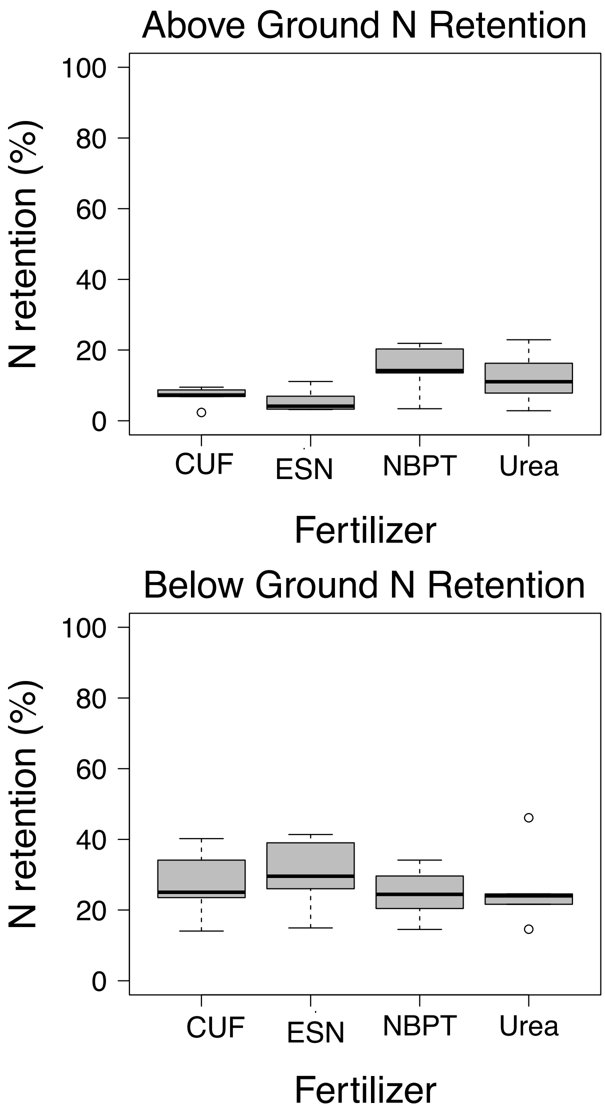

Figure 3.5: Above and below ground N retention 1-year after fertilization. The data are grouped by fertilizer treatment.

3.4.3 N retention by ecosystem component

Of the individual ecosystem pools sampled in this study (i.e. foliage, bole wood, forest floor, and mineral soil), mineral soil contained the greatest proportion of retained N one-year after fertilization ($p < 0.000$) (Figure 3.6, Figure 3.7, Figure 3.8, and Figure 3.9). Across sites, 24.9% of the applied N was recovered in the mineral soil (0-60cm depth).

The highest proportion of recovered mineral soil N was retained in the upper 15cm of the profile and recovery declines with depth (Figure 3.7). The upper 15cm has significantly higher N recovery than each of the lower sampling depths. The 15 – 30cm sampling depth has significantly higher N recovery than 45 – 60cm depth but not the 30 – 45 cm (Figure 3.7).

N recovery in the foliage, bole wood, and forest floor components were not significantly different from each other, accounting for 5.8%, 4.1%, and 1.9% of the total N applied respectively (Figure 3.6). Foliar N recovery did not differ significantly across study sites (Table 3.4, Figure 3.9). However, significantly higher N recovery was recorded in foliage of plots fertilized with NBPT (9.2%) compared to plots fertilized with ESN (2.3%) (Table 3.3, Figure 3.10). Bole wood N recovery did not differ across study areas or between treatment plots (Figure 3.11 and 3.12). Fertilizer recovery in the forest floor pool yielded the greatest variability between treatment plots. Plots fertilized with ESN had the highest N recovery in the forest floor at 3.7% relative to the three other fertilizer treatments where recovery was 1.2%, 1.2%, and 1.6% for CUF, NBPT, and Urea, respectively (Table 3.3, Figure 3.13 and 3.14).

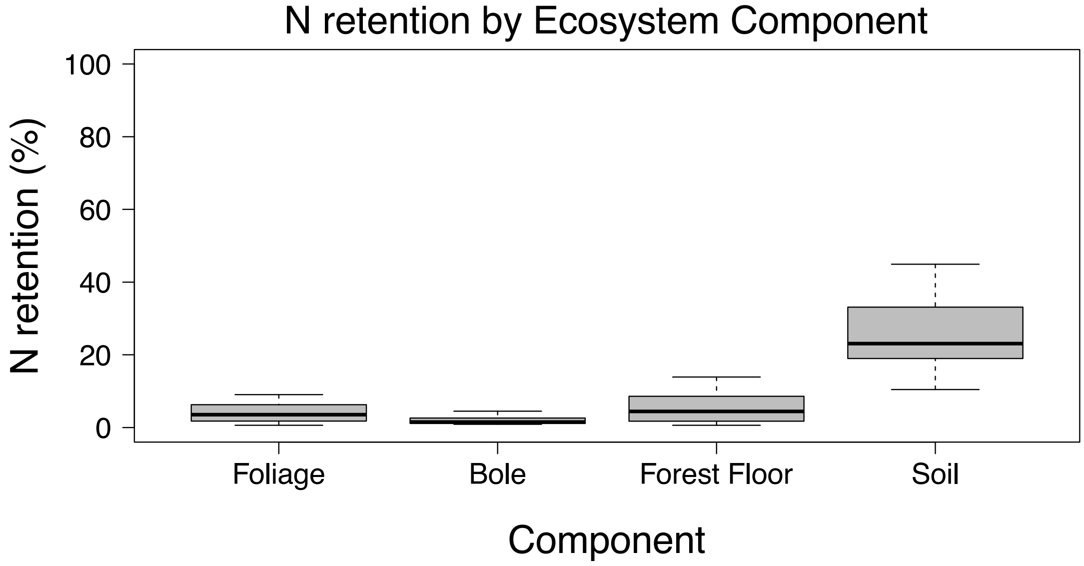

Figure 3.6: N recovery by ecosystem type. The greatest portion of the applied fertilizer was recovered in the mineral soil 1-year after fertilization.

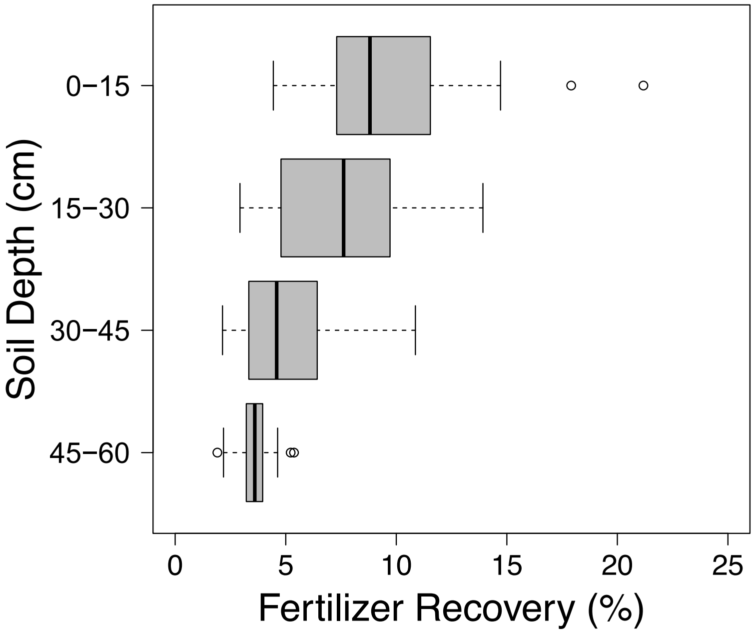

Figure 3.7: N recovery in mineral soil with depth. The highest proportion of fertilizer recovery occurred in the top 0-15cm depth.

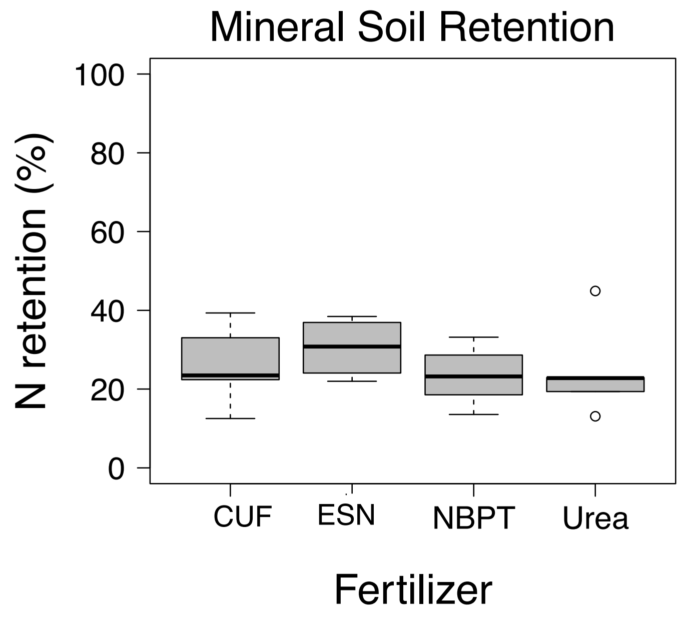

Figure 3.8: Mineral soil N recovery by fertilizer type.

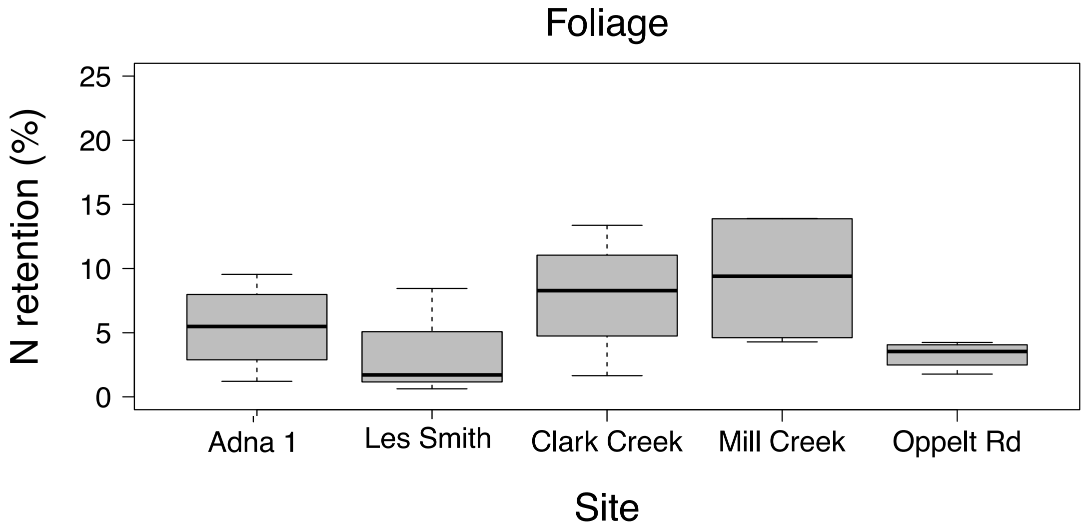

Figure 3.9: Foliar N recovery 1-year after fertilization. Data are divided by site.

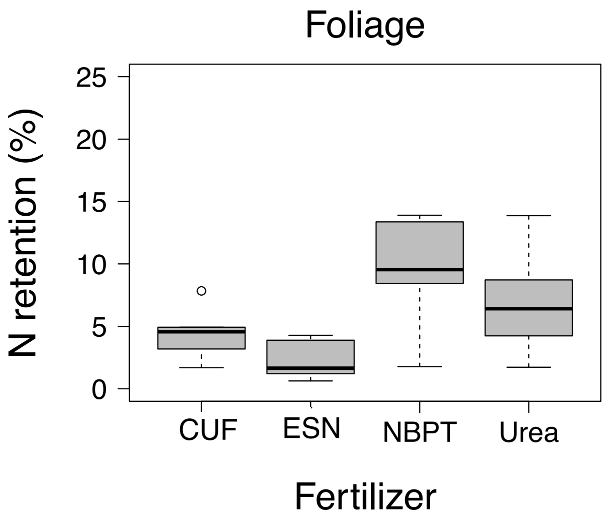

Figure 3.10: Foliar N recovery 1-year after fertilization. Data are divided by fertilizer treatment.

Figure 3.11: Fertilizer recovery in bole wood 1-year after fertilization. Data are divided by site.

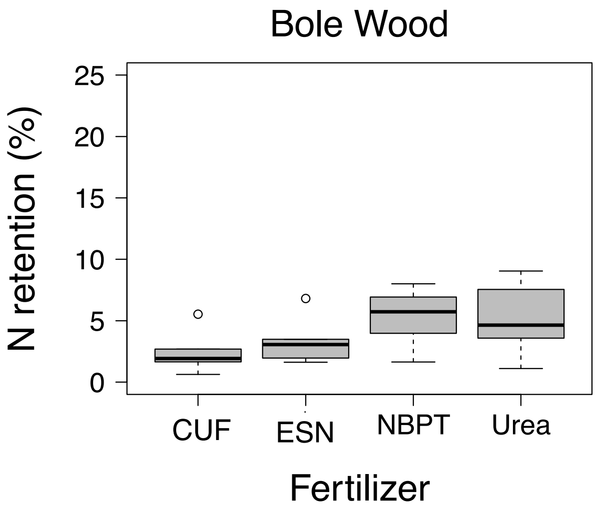

Figure 3.12: Fertilizer N recovery 1-year after fertilization. Comparing recovery in bole wood by treatment type.

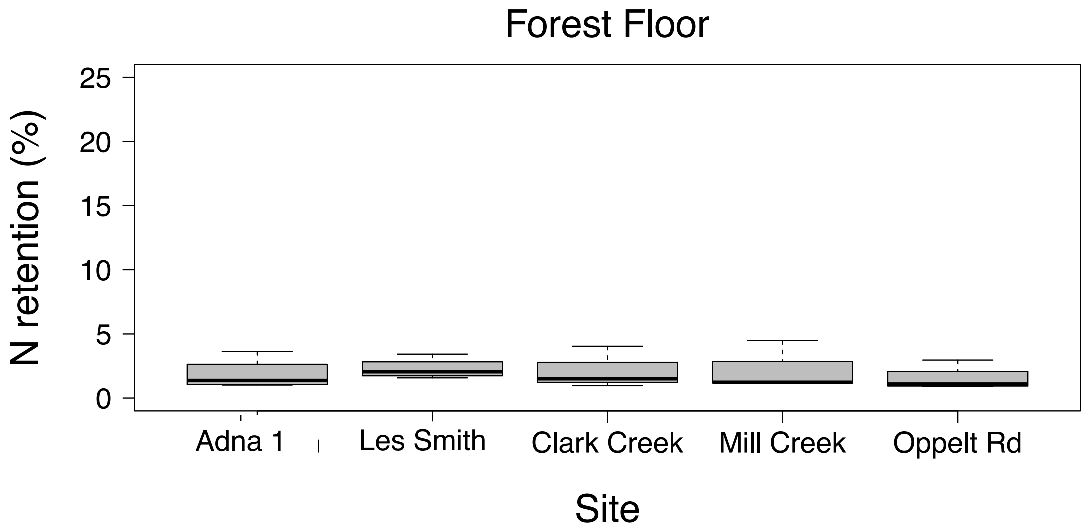

Figure 3.13: Fertilizer recovery in the forest floor across the five study sites, 1-year after fertilization.

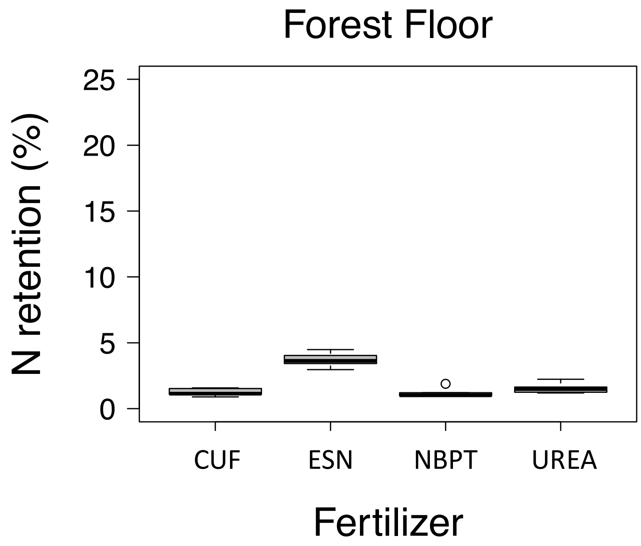

Figure 3.14: Fertilizer recovery in the forest floor across the treatment types, 1-year after fertilization.

Parameters from the multiple linear regression that were significantly correlated ($p<0.05$) with total N retention include forest floor $\delta^{15}N$, soil C concentration, forest floor C/N, A horizon C/N, and latitude. A forward stepwise approach to model selection using the parameters summarized in Table 3.2 revealed that total 1-year N recovery across the five sites in this study was best predicted by *Site*. The *Site* parameter is an amalgamation of the different environmental properties that make up each individual study area. The individual environmental properties of each site (Table 3.2) that were significant predictive variables in the linear regression analysis included forest floor C/N, soil C/N soil C, latitude, soil %Mg, A-horizon C/N, and forest floor $\delta^{15}N$. The inclusion of these additional parameters to the linear model did not improve the model's ability to predict total ecosystem N recovery over *Site*.

3.5 Discussion

The important results of this study are as follows. First, there was no significant improvement in total N retention with the use of enhanced efficiency fertilizers compared to the unformulated urea fertilizer. This suggests that volatilization is not a substantial loss pathway at these sites, otherwise urea fertilized plots should have the lowest N recovery rates of all the treatment types. While the differences between treatments were not significant, it is possible that a difference in N recovery of 34.3% for CUF (the lowest) up to 39.3% for NBPT (the highest) is ecologically significant in terms of forest productivity, environmental health, and longer-term N budgets. Second, significant differences in N recovery between sites as opposed to treatment type indicate that, as expected, ecosystem properties are driving N retention. Third, only 39% of the applied N was recovered with the largest proportion retained in the mineral soil as opposed to the above ground vegetation pools.

3.5.1 Effects Enhanced Efficiency Fertilizers

The primary mechanisms of N losses from forested ecosystems include leaching of nitrate, denitrification, and ammonia volatilization (Brady and Weil, 2008). Management approaches to decreasing N losses include timing of fertilizer applications and the use of EEFs. EEFs are used in place of conventional fertilizers to minimize volatile losses of ammonia and nitrous oxide (AAPFCO, 2013). CUF and NBPT contain urease inhibitors which slow the conversion of urea to NH_4^+ which in turn reduces the concentrations of

NH_3 in the soil, thus decreasing volatile losses of N (Watson et al. 2008; AAPFCO, 2013). Selecting EEFs such as NBPT, CUF, or ESN as a management approach to increase N use efficiency assumes that volatile losses of N are a substantial loss pathway. If volatilization of NH_3 is a substantial loss pathway then the controlled release of N or inhibition of urease enzymes should result in increased retention and ecosystem uptake of the fertilizer (Motavalli et al. 2013). We expected to see higher one year N recovery for the plots treated with EEFs because of their indicated ability to decrease volatile losses of N. However, our results did not support the hypothesis of increased N recovery with EEFs, which suggests that volatile losses of N may not be a substantial loss pathway at these sites and therefore there is no benefit to the use of EEFs for improving N use efficiency at our study sites. Because volatilization was not directly measured in this study, we cannot confirm the percentage of unaccounted for N that is attributed to volatile losses. It is also possible that volatilization does occur at these sites but the EEFs are not sufficiently effective at inhibiting ammonia volatilization. Other studies have produced mixed results with regards to N use efficiency and EEF use. The benefits of EEFs in agricultural settings is shown to vary based on environmental properties (i.e. soil and climate) and management practices (Akiyama et al. 2010). While the use of urease inhibitors (i.e. the mechanism in CUF and NBPT treatments) to reduce gaseous N losses has produced mixed results in other studies (Akiyama et al. 2010; Halvorson et al. 2010; Halvorson et al. 2014), focused on N losses in the form of N_2O (a potent greenhouse gas) rather than N losses in the form of NH_3. If EEFs were reducing N_2O emissions at the sites in our study we should see that as an increase in N recovery after one year, which was not the case.

The plots treated with unformulated urea (i.e. the conventional fertilizer treatment) have the second highest total N recovery after one year. When retention is partitioned by ecosystem pool, urea fertilized plots had the second highest retention in their above ground, foliar, bole wood, and forest floor pools. However, the only significant differences in N recovery by treatment were in the foliar and forest floor pools (Table 3.1). Foliar uptake was highest in the NBPT treated plots but only compared to ESN which had the lowest foliar uptake. Above ground (i.e. bole wood and foliage) uptake of ESN is lowest compared to all the treatments, but this difference was not significant. When the above ground pools are further partitioned into bole wood and foliar pools, ESN treated plots reveal low total N recovery. The difference in foliar uptake was significantly lower for ESN treatments compared to foliage at plots treated with NBPT. We attribute the low above ground recovery of ESN to the significantly higher N recovery of ESN in the forest floor pools relative to all three other treatments. Of the applied ESN fertilizer, 3.7% was recovered in the forest floor which is more than double the amount recovered in the NBPT, CUF, and urea treated forest floors. When the sites were sampled one year after fertilization, polymer coated beads of the ESN fertilizer were still present in the forest floor. When the beads were opened, some still contained fertilizer. The polymer coating is designed to dissolve and release the fertilizer when conditions are optimal for the translocation of the N into the soil where it becomes available for plant uptake. The undissolved polymer beads with fertilizer still inside are likely a contributing factor in the additional N recovery in the forest floor at the ESN treated sites.

3.5.2 Ecosystem Controls on N Recovery

A forest's capacity to retain N following N-fertilizer application varies geographically due to differences in soil base cation saturation, climate, atmospheric N deposition, and forest stand structure (Fenn et al. 2003). Nitrogen recovery after 1-year was highly variable when the data are partitioned by study site, but no significant differences were observed when partitioned by fertilizer treatment. Individual site characteristics are therefore likely driving N recovery as opposed to the type of urea based fertilizer used. Of the five sites studied, Oppelt Rd had the highest N recovery after 1 year with a mean of 47.2%. The Oppelt Rd site also has the highest pre-fertilization total soil N content of any of the five sites at 10796 Mg ha^{-1} and also the lowest forest floor C/N ratio at 20. Soil total N and forest floor C/N are two common indicators of a site's capacity to retain N (Edmonds and Hsiang, 1987; Littke et al. 2014; Hopmans and Chappell, 1994). Oppelt Rd, with the highest fertilizer recovery rate, was the only site to yield significantly higher 1-year total N recovery. Results of the linear regression analysis were not particularly revealing in that *Site* was the strongest predictor of N recovery after 1-year. When the individual environmental properties of each site (Table 3.1) were partitioned out and used as predictive variables in the linear regression analysis, forest floor C/N, soil C/N, soil C, latitude, soil %Mg, A-horizon C/N, and forest floor $\delta^{15}N$ were also significant predictors of total 1-year N recovery. These trends are consistent with previous research on the environmental site conditions that correlate with N fertilizer response and retention

(Edmonds and Hsiang, 1987; Littke et al. 2014; Hopmans and Chappell, 1994, Templer et al. 2005).

3.5.3 N Recovery in Different Ecosystem Pools

Ecosystem N recovery in this study was comparable to the recovery rates obtained in similar ^{15}N tracer studies (Salifu and Timmer, 2003; Amponsah et al. 2004; Templer et al. 2005). Mean fertilizer N recovery across sites and treatments is 36.9%. Consistent with other studies, the largest pool of retained fertilizer is the mineral soil (Templer et al. 2005). Les Smith has the lowest proportion of fertilizer N recovered in the mineral soil with only 15.3% of the fertilizer accounted for in this pool after 1year. Mineral soil N recovery at Les Smith is significantly lower than the site with the highest proportion of fertilizer N recovery in the mineral soil pool, Adna 1 (30.0% recovered) and Oppelt Rd (39.0% recovered). The Les Smith site is one of the two wettest sites which may have contributed to the high N losses if urea hydrolysis proceeded without the fertilizer being incorporated into the soil for plant uptake. Oppelt Rd has significantly higher N recovery in its mineral soil pool relative to Mill Creek (22.8%), Clark Creek (21.9%), and Les Smith (15.3%). Forest floor C/N ratios are used as predictors of an ecosystem's capacity to retain fertilizer applications (Edmonds and Hsiang, 1987; Littke et al. 2014).

3.5.4 Conclusions

Future EEF use to improve N use efficiency at the five sites in our study is not warranted as no significant differences in total 1-year N recovery or tree uptake of N were observed. This suggests that volatile losses of N are not a substantial loss pathway at these sites. The fertilizers were all applied in early spring when conditions are optimal for limiting volatile losses. While temperatures are still cool and rainfall is high enough to wash the urea hydrolysis products into the soil where it becomes accessible to plants, the benefits of EEFs are not pronounced. Because these EEFs delay the hydrolysis of urea to times when conditions are optimal for plant uptake, we would expect there to be a greater advantage to their application in the warm dry months when volatile losses of applied fertilizers would be high (warm, dry weather). Further research is however needed to determine if EEF use maybe warranted for a summer fertilizer application at these sites. Further research is however needed to determine if EEF use maybe warranted for a summer fertilizer application at these sites. In a similar study in loblolly pine forests of the southeastern United States, which used the same treatments and experimental design, fertilizer-N recovery was higher (Raymond et al. 2016) than when the same treatments are applied to the plantation forests in the western Douglas-fir region of Oregon and Washington. The particular EEFs used in both studies were formulated specifically for the southeastern United States. This is especially relevant for the polymer coated EEF (ESN) as the polymer coating breaks down when exposed to water, higher temperatures, and solar radiation (AAFPCO, 2013). Douglas-fir forests have dense canopies with very little sunlight penetrating the canopies and hitting the forest floor relative to loblolly pine stands which are more open and their forest floors sunlit. The low N recovery after 1-year but simultaneous increases in above ground biomass support a kinetic saturation model of N loss where the ecosystem can continue to accumulate biomass with simultaneous leaching and gaseous losses of N (Lovett and Goodale, 2011). This conclusion contrasts with the commonly held assumption that fertilization of N limited sites, such as the ones included on this study, should yield negligible losses of N and high recovery of the applied fertilizer (Aber et al. 1998). Additionally, we conclude that management decisions regarding fertilizer use efficiency and the benefits of fertilization with regards to productivity need to be site specific due to the variable N recovery rates based on site factors as opposed to fertilizer treatment type. Finally, despite differences in the size of the mineral soil fertilizer pools the amount of N recovered in the above ground pools (i.e. bole wood and foliage) were not significantly different despite differences in the size of the soil N pools. This suggests that N uptake by the plants pools may have been at its maximum, thus additional N in the soil pools would not make a difference in terms of productivity over just one year. It remains to be seen what the longer-term impacts of the fertilizer treatments are, as the sites with a larger reservoir of plant available N are expected to maintain their growth rates for longer than the more limited site.

3.5.6 Limitations and Assumptions

An assumption and therefore possible limitation of this study is that total N recovery for each plot represents the true N retained in the system after one year and that unaccounted-for N is in fact lost from the system through the three dominant loss mechanisms (i.e. leaching, denitrification, or volatilization). Nitrogen missing from our final N recovery calculations may have been taken up in understory vegetation or held in the roots, both of which were not sampled and we are therefore unable to account for in our estimate of N retention. However, since each site was sampled in the same way the relative differences in N recovery between sites should still hold true. Additionally, we assume that the small subsamples used for the isotopic analyses are indeed a representative sample of each ecosystem component. This was accounted for by running repeat samples to test for variability between samples of the same ecosystem component for each site and treatment. Repeated sample runs did not yield significantly different $\delta^{15}N$ or N% between the same samples. With regards to the conclusion that volatilization may not be a substantial loss pathway at these sites we are assuming that the EEFs function as expected and therefore no difference in N recovery between treatment types can be attributed to low volatile losses. If volatilization were high across the plots and the EEFs substantially decrease volatile losses following fertilizer application, we would expect to have measured substantially higher N recoveries in the EEF fertilized plots. Finally, the Gholz (1979) allometric equation may under estimate the fertilizer pool recovered in the foliar biomass. Some studies have shown that substantial growth response in the stem wood of a fertilized tree is delayed beyond the first year after fertilization (Blackmon, 1973). The allometric equation uses change in diameter at breast height to estimate foliar biomass. Initial increases in foliar biomass without a corresponding change in bole wood have been observed in other studies (Garrison et al. 2000; Amponsah et al. 2005). Therefore the Gholz (1979) allometric equation may underestimate the foliar pool because it does not account for an increase in foliar growth rates in the year proceeding fertilization.

Chapter 4

Local alterations to the nitrogen cycle as indicated by soil, foliage, and tree-ring total N and δ^{15}N: A case study in Shaanxi Province, China

Abstract

Monitoring of nitrogen (N) inputs to forested ecosystems in China has historically been limited despite known negative effects that high N additions can have on environmental and human health. To better understand terrestrial patterns of foliar, soil, and wood δ^{15}N relative to soil N concentrations (%N) in a heavily polluted region, two Chinese parasol tree (*Firmiana simplex*) groves adjacent to a industrialized basin were examined on the Loess Plateau, approximately 80km east of the city of Xi'an, Shaanxi Province, China. The δ^{15}N values of ecosystem pools sampled in this study are substantially lower than what is typically observed in temperate forests around the world. Results suggest greater foliar uptake of N in the tree canopies bordering the industrialized basin compared to trees located at the forest interior. N concentrations and δ^{15}N of soil and plant tissue were inversely correlated along this transect and indicate higher inputs of N from δ^{15}N depleted sources in the basin. Tree ring δ^{15}N values decline between 2003 and 2014 during a time of rapid development and expansion of the industry in the basin.

4.1 Introduction

China's economic development over the past 30 years has led to rapid expansion of their industrial and agricultural sectors, two of the major sources of reactive nitrogen (Nr) emissions (Richter et al. 2005; Liu et al. 2013a). The negative consequences of increased Nr deposition are substantial, having been linked to soil acidification (Yang et al. 2012; Duan et al. 2013), eutrophication, decreases in biodiversity, and declines in human health (Vitousek, 1997; Fenn et al. 2003). Understanding the spatial and temporal scale of Nr pollution is necessary for monitoring its impacts as well as the outcomes of mitigation efforts (Zhang et al. 2015). Nr deposition monitoring across China has however been limited and large gaps in the geographic extent of monitoring programs still exist (Liu et al. 2013b). Proxies for direct pollution monitoring, particularly in forested ecosystems, are therefore necessary when historical records do not exist (Leonelli et al. 2012).

Forests near pollution sources can improve local air quality by serving as a sink for atmospheric pollutants (Speak et al. 2012; Sæbø et al. 2012; Nowak et al. 2013; Zhao et al. 2017). A forest's capacity to dictate the fate of pollutants is dependent on several forest properties (i.e. stand structure, age, and species; Nizzetto et al. 2006; Sæbø et al. 2012; Janhäll, 2015). When plants retain pollutants in their tissue they may serve as effective bio-monitors for changing environmental conditions and for pollution monitoring purposes. For example, plant tissue including tree rings and foliage, have been used to monitor trace element inputs to ecosystems (Gratani et al. 2008). Tree rings are especially well suited for historical reconstructions when annual growth rings retain compounds absorbed during a given year (Padilla and Anderson, 2002). Due to the mobile nature of N within plants, the N concentration (% N) of tree ring tissue is not a sufficiently sensitive bioindicator of ecosystem N status (Poulson et al. 1995; Sheppard and Thompson 2000; Gerhart and McLauchlan, 2014). Alternatively, the natural abundance stable isotopes of N (hereafter referred to as $\delta^{15}N$) are increasingly used as indicators of source, retention, and transformations of N compounds within ecosystems (Rothstein et al. 1996; Dawson et al. 2002). As a record of N cycling status, $\delta^{15}N$ of ecosystem components can serve as an important proxy for N cycling in regions with limited historical Nr monitoring programs (i.e East Asia). In addition to serving as a proxy for ecosystem N inputs and cycling processes, measuring $\delta^{15}N$ is advantageous because it is both simple and relatively inexpensive to measure.

The $\delta^{15}N$ of plant tissue (i.e. foliage and tree rings) reflect an integration of the dominant fluxes of N into or out of the ecosystem (Pardo et al. 2006; Craine et al. 2009). Stable N isotopes have previously been used to evaluate changes in N source and its incorporation into plant tissue (Fang et al. 2011; Guerrieri et al. 2009; Savard et al. 2009). Current research suggests that globally, soil and plant tissue $\delta^{15}N$ are positively correlated with N inputs and subsequent leaching losses of N while varying across gradients in temperature and precipitation (Pardo et al. 2002; Craine et al. 2015). Increases in $\delta^{15}N$ of tree rings have also been observed following changes in N source from one relatively depleted in $\delta^{15}N$ to one with higher $\delta^{15}N$ and vice versa (Stewart et al. 2002; Guerrieri et al. 2009; Fang et al. 2011).

Interpretations of ecosystem $\delta^{15}N$, particularly for tree rings are however based almost entirely on research in forests across North America, Europe, and South America where

N is frequently the limiting nutrient with regards to net primary productivity and atmospheric deposition is relatively low compared to China (Pardo et al. 2006; Craine et al. 2009, Guerrieri et al. 2009; Templer et al. 2012). A more complete global synthesis of tree ring $\delta^{15}N$ trends requires inclusion of data from understudied regions and sites exposed to biogeochemical extremes in N deposition (Gerhart and McLauchlan, 2014). Studies using the natural abundance $\delta^{15}N$ of foliage and soil in forested ecosystems across China are currently not well represented in published literature (Fang et al. 2011; Fang et al. 2013). Studies that include measurements of tree-ring $\delta^{15}N$ as a means for reconstructing historical N cycles in China are few (Sun et al. 2010; Zeng et al. 2014). Forested ecosystems in China are unique in that they exist on the extreme high end for Nr deposition. The limited available data from Chinese forests exposed to high pollution levels highlights the need for additional research because results from the limited number of existing studies suggest that the local trends observed in heavily polluted Chinese forests do not follow global patterns of foliar and soil $\delta^{15}N$ (Fang et al. 2011; Fang et al. 2013). For example, in East Asian forests exposed to high atmospheric deposition of N, $\delta^{15}N$ of foliage decreases with increasing soil and foliar N concentrations (Fang et al. 2011; Fang et al. 2013). Negative $\delta^{15}N$ values have been recorded in foliar samples collected in both broadleaf and coniferous forests in southeastern China (Fang et al. 2011). The $\delta^{15}N$ signatures of plant tissue in China are negatively correlated with common indicators of N saturation status including N concentrations, carbon (C) to N ratios (C/N) of soil and forest floor pools, as well as atmospheric N deposition (Fang et al. 2013).

The objective of our study is to determine if the natural abundance $\delta^{15}N$ signatures of soil and plant pools (i.e foliage and tree rings) are a useful indicator of local differences in ecosystem N status between two forested sites in a region exposed to high levels of atmospheric N deposition. Additionally, we integrate tree-ring $\delta^{15}N$ records spanning a 12-year period to determine if the current relationships between N concentrations and $\delta^{15}N$ hold true for the previous decade. We analyze differences in N concentrations and trends in $\delta^{15}N$ of tree-rings, foliage, and soil of a forest exposed to the N emissions of a recently industrialized basin to test the following hypotheses: [1] Foliage and soil sampled closest to an industrialized basin will have higher N concentrations and thus higher $\delta^{15}N$ than samples collected from the forest interior 4km away, [2] Positive correlations exist between soil N concentrations (%N) and $\delta^{15}N$ of foliage, soil, and tree rings, [3] $\delta^{15}N$ of tree rings increases over time with the likely increase in N emissions as the basin's land use transitioned from predominantly farmland to factories and higher density residential dwellings over the period of record for the sampled tree rings (1996 – 2014). Emission sources in the basin are predominantly from a N fertilizer factory, vehicle exhaust, and coal burning.

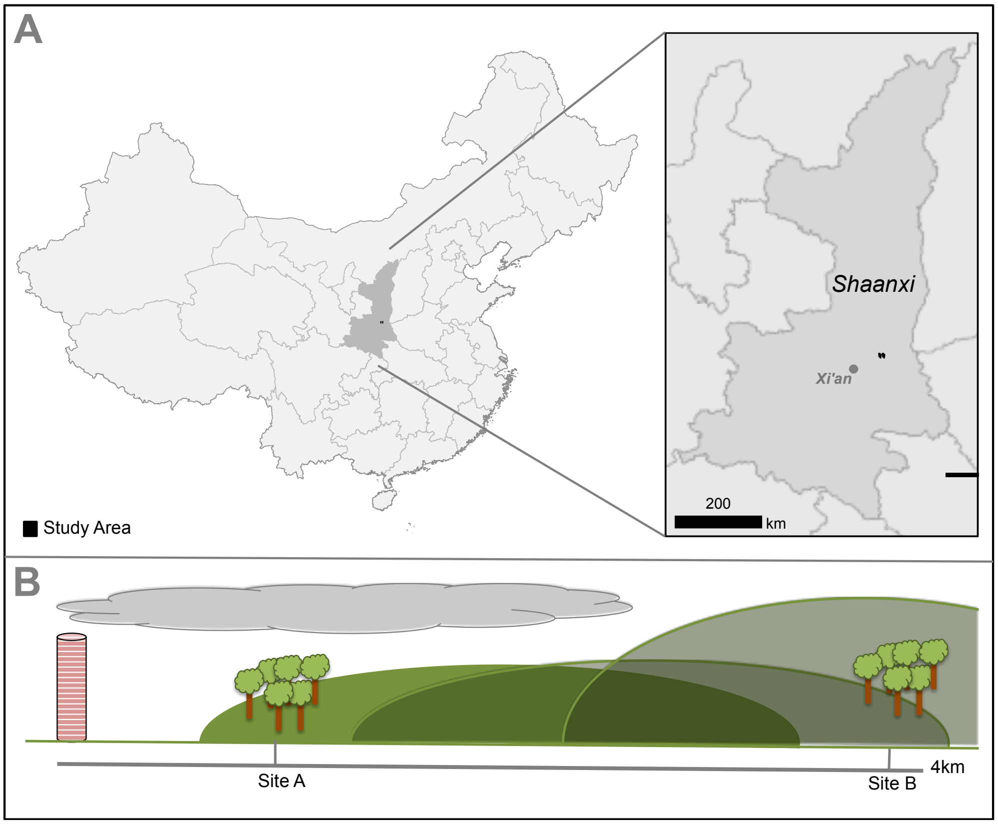

Figure 4.1: (A) Study area located on the Loess Plateau, 80km East of Xi'an and bordering. (B) Location of study plots relative to the heavily industrialized basin.

4.2. Methods

4.2.1 Study Area

This study was conducted within a patchwork of forest and farmland on the Loess Plateau approximately 80km east of the city of Xi'an, Shaanxi Province, China (Figure 4.1). The region has a semi-arid climate with rains from the East Asian Monsoon falling mostly during the hot summer months. Winters are typically cold and dry. Soil on the plateau is deep, with loess deposits ranging from 30 to 250m (Xianmo et al. 1983). The fertile soils of the Loess Plateau facilitate the region's rich agricultural history. However, intensive agricultural practices have led to severe erosion issues (Chen et al. 2007; Jiao et al. 2012). As a means for reducing soil loss, aforestation efforts on the plateau started in the late

1990’s and resulted in the expansion of forests across the region (Wang et al. 2009; Bullock and King, 2011). The forested plots we sampled border an industrialized basin surrounded by smaller villages. The basin has multiple sources of Nr including vehicle exhaust, a fertilizer factory, coal burning, and agriculture.

4.2.2 Experimental Setup

In July of 2014, two sites were sampled, 1km (*Site A*: 34.459994°N, 109.714204°E) and 4km (*Site B*: 34.459994°N, 109.714204°E) from the basin edge. Samples collected at Site A are from a patchwork of forest along the southeastern edge of the basin. While samples collected at Site B are from the forest interior, 4km further to the southeast. The soil at these two sites are part of the Lou soil series (Xianmo et al. 1983). Elevation increases from 535m to 750m between the two sites. Chinese parasol trees (*Firmiana simplex*), a deciduous tree that can grow to a height of 16m (Miller et al. 2010), were selected for this study because stands are ubiquitous throughout the area due to their use as an ornamental species. The trees are young, ranging in age from 11 to 18 years. Agricultural terraces are still visible at the forested sites we sampled. With much of the area used for agriculture, the sites were selected to limit possible N inputs from fertilizer and manure runoff upslope of the study plots. Additionally, we attempted to keep site factors such as forest composition, slope, aspect, and soil type similar between the two study sites.

4.2.3 Sample Processing

Within the two sites, 6 Chinese Parasol trees were sampled. Foliage was collected from the sunlit upper third of the canopy. Foliage samples were oven dried at 40^{o}C. Dried samples were ground with a Wiley-mill outfitted with a size 40 mesh. Four soil cores were collected to a depth of 10cm in each cardinal direction, 1m from the base of each tree. The four samples were consolidated to comprise the sample for that tree. Soil samples were air dried and sieved to 2mm. The forest floor samples above each soil core were consolidated to comprise a single forest floor sample per tree. A subset of each foliage, soil, and forest floor sample was ground to a fine powder using a ball mill, weighed, and then tinned for chemical analysis.

Three trees from each site were randomly selected for dendrochronological analysis. Each tree was cored three times at an equal distance (i.e 120^o degree spacing) from each other around the trunk. Annual growth rings were identified under a microscope. The rings were dated and cut at each annual growth interval. The cut tree rings were ground in a ball mill and weighed into tin capsules for analysis. Raw ring wood was analyzed rather than performing any extraction techniques to remove mobile N compounds. The benefits of extraction are unclear and not indicated for natural abundance studies (Gerhart and McLauchlan, 2014). The rings were analyzed in one-year increments. The years covered in our study are 1996 – 2014. All isotope analyses were conducted using a Costech elemental analyzer coupled to a Thermo MAT253 isotope ratio mass spectrometer at the University of Washington Stable Isotope Laboratory (IsoLab), Seattle WA.

4.2.4 Statistical Analyses

We examined the possible relationships between %N, $\delta^{15}N$, %C, C/N, and pH of soil, %N, $\delta^{15}N$, and $\delta^{13}C$ of foliage, and %N, $\delta^{15}N$, and %C of forest floor samples at both forested sites. With the tree rings we evaluated the temporal variability in $\delta^{15}N$. Pearson's product moment was used to test for correlation between the measured variables. An assumption that each group of parameters is normally distributed, a requirement of the Pearson's product movement, was tested using a Shapiro-Wilk test for normality ($p= 0.05$). Determining whether significant differences exist between ecosystem components or sites was assessed using an analysis of variance (ANOVA) with $\alpha = 0.05$. Tukey HSD test was then applied to determine which means were significantly different from each other ($\alpha = 0.05$).

4.3 Results

4.3.1 Soil

Soil sampled along the basin edge (Site A) has a mean N concentration of 0.17%. N concentrations increase at the interior forest site with a mean of 0.27% (Table 4.1). Soil pH is consistently alkaline across both sites (Table 4.2). As expected, soil N concentrations are negatively correlated with soil pH ($\rho = -0.68$, $p < 0.00$) (Figure 4.2). Soil pH is significantly higher at Site A (mean 7.9) where soil N concentrations are lower relative to Site B that has higher soil N concentrations and a lower soil pH (7.6).

Table 4.1: Mean N concentrations and $\delta^{15}N$ for soil, forest floor, and foliage samples at the two study sites (i.e Site A at the basin edge and Site B in the forest interior).

		%N soil	δ^{15}N soil	%N forest floor	δ^{15}N forest floor	%N foliage	δ^{15}N foliage
Site A	mean	0.17	-1.1	1.9	-8.5	3.4	-11.8
	s.d.	0.05	1.4	0.44	1.4	0.37	0.7
Site B	mean	0.28	1.62	1.6	-5.2	3.0	-4.6
	s.d.	0.07	0.08	0.46	0.7	0.38	1.0

Table 4.2: Mean and standard deviation (s.d) for pH, carbon concentration (%), and C:N ratios of the soil and the carbon concentration and $\delta^{13}C$ of the foliage samples at the two study sites (i.e Site A at the basin edge and Site B in the forest interior).

		pH soil	%C soil	C:N soil	%C foliage	δ^{13C} foliage
Site A	mean	7.9	3.3	19.7	49.8	-27.9
	s.d.	0.06	0.60	2.2	2.4	1.1
Site B	mean	7.6	3.0	10.6	49.0	-25.6
	s.d.	0.11	0.84	0.42	3.5	0.56

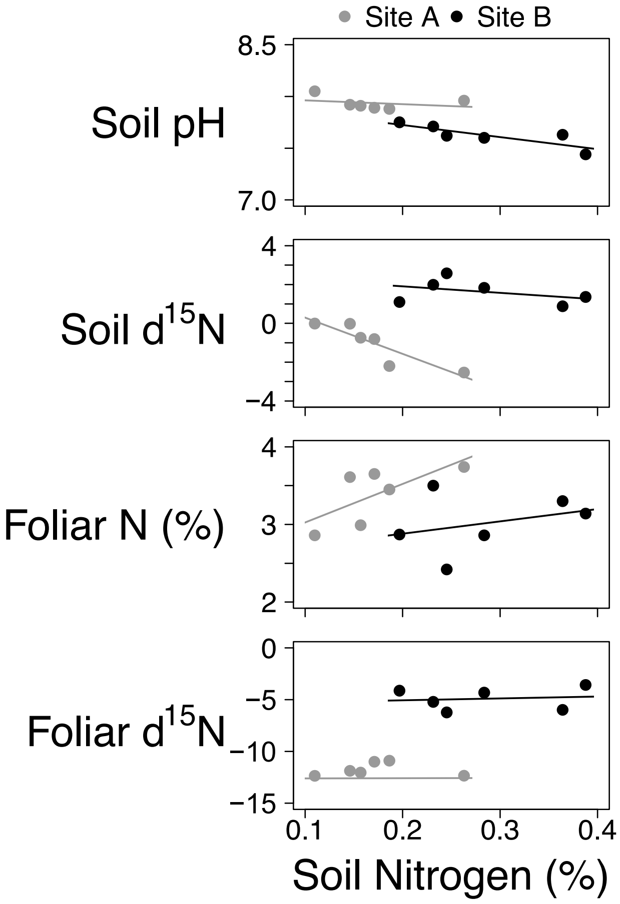

Figure 4.2: Scatter plots with regression lines showing the relationship between soil nitrogen concentration (%N) and soil pH, soil δ^{15}N, foliar N, and foliar δ^{15}N. The data and regression lines are plotted by site (i.e Site A along the basin edge and Site B at the forest interior).

Soil carbon concentrations are not significantly different (p = 0.5) between Site A at the basin edge and Site B in the forest interior (Table 4.2). Site A has a mean soil C concentration of 3.3%, while Site B has a mean concentration of 3.0%. Soil C/N ratios are low across sites (Table 4.2). Site A the soil C/N is significantly higher at 19.7 compared to Site B at the interior of the forest which has a C/N of 10.6 (p <0.00), a reflection of the higher soil N concentrations of the site.

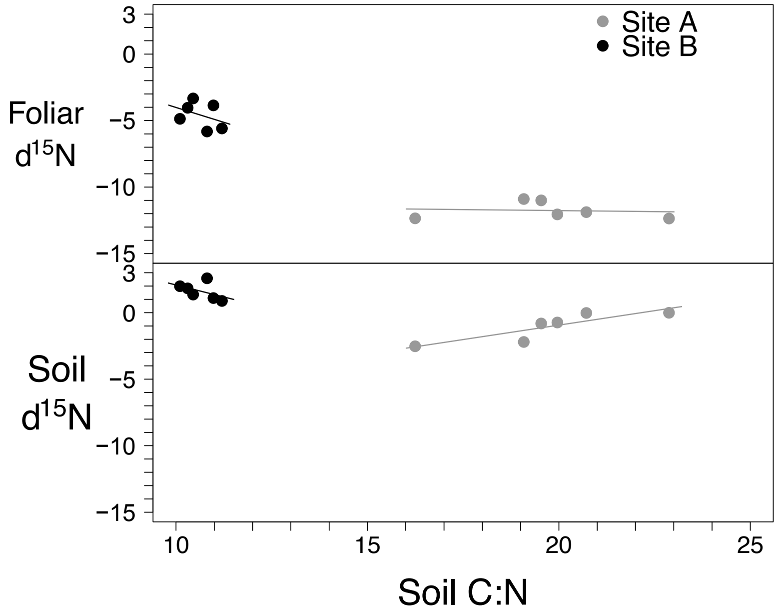

Figure 4.1: Scatter plots of the relationship between soil C:N and foliar δ^{15}N and soil δ^{15}N. The data points and regression lines are plotted by site.

Soil δ^{15}N values at Site A, along the basin edge average -1.1‰. Soil δ^{15}N is significantly higher ($p = 0.01$) at Site B with a mean of 1.62‰. Soil N concentrations at Site A are negatively correlated with soil δ^{15}N ($\rho = -0.89$, $p = 0.01$) (Figure 2.2). Site B, shows a weak negative correlation between soil N and soil δ^{15}N ($\rho = -0.41$, $p = 0.42$). Conversely, soil N and soil δ^{15}N have a weak positive correlation (higher soil δ^{15}N with higher soil N concentrations) when the samples from Site A and Site B are analyzed in aggregate ($\rho = 0.36$, $p = 0.24$). Soil δ^{15}N and soil C/N are positively correlated along the basin edge at Site A ($\rho = 0.87$, $p = 0.02$). In contrast, at Site B soil δ^{15}N and soil C/N have a weak negative correlation ($\rho = -0.48$, $p = 0.33$) (Figure 4.3).

4.3.2 Forest Floor

The forest floor (O_i horizon) is less than 1cm thick at both sites and predominantly composed of Chinese parasol tree leaf litter. The forest floor has a mean N concentration of 1.9% at Site A while forest floor N concentrations are lower (1.6 %N) at Site B. However, this difference is not significant ($p = 0.24$). The $\delta^{15}N$ of the forest floor is negative for all samples across both study sites. Forest floor $\delta^{15}N$ at Site A is significantly lower than at Site B with means of -8.5‰ and -5.2‰ respectively ($p < 0.00$) (Table 4.1). N concentrations of the forest floor are not significantly correlated with forest floor $\delta^{15}N$ at either Site A ($\rho = 0.17$, $p = 0.74$) or Site B ($\rho = -0.41$, $p = 0.42$).

4.3.4 Foliage

Foliage sampled at Site A, the basin edge, has a mean N concentration of 3.4% (Table 2.1). Foliar N concentrations are lower at Site B with a mean of 3.0% ($p = 0.12$). All foliar $\delta^{15}N$ signatures are negative at both sites, however they are significantly lower along the edge of the basin, Site A relative to the forest interior at Site B ($p < 0.000$) (Figure 4.2). Foliage at Site A has a mean $\delta^{15}N$ of -11.8‰ (Table 4.1). Foliar $\delta^{15}N$ is higher with a mean of -4.6‰ at Site B. Foliar $\delta^{15}N$ and foliar N concentrations are not significantly correlated within either Site A ($\rho = 0.37$, $p = 0.47$) or Site B ($\rho = 0.14$, $p = 0.78$).

Foliar $\delta^{13}C$ is lower ($p = 0.001$) at Site A with a mean of -27.90‰ compared to Site B that has a mean of -25.64‰ (Table 4.1). Foliar $\delta^{13}C$ and foliar $\delta^{15}N$ are positively correlated at Site A ($\rho = 0.90$, $p = 0.02$). At Site B, there is a weak but not significant positive correlation between foliar $\delta^{13}C$ and foliar $\delta^{15}N$ ($\rho = 0.31$, $p = 0.56$).

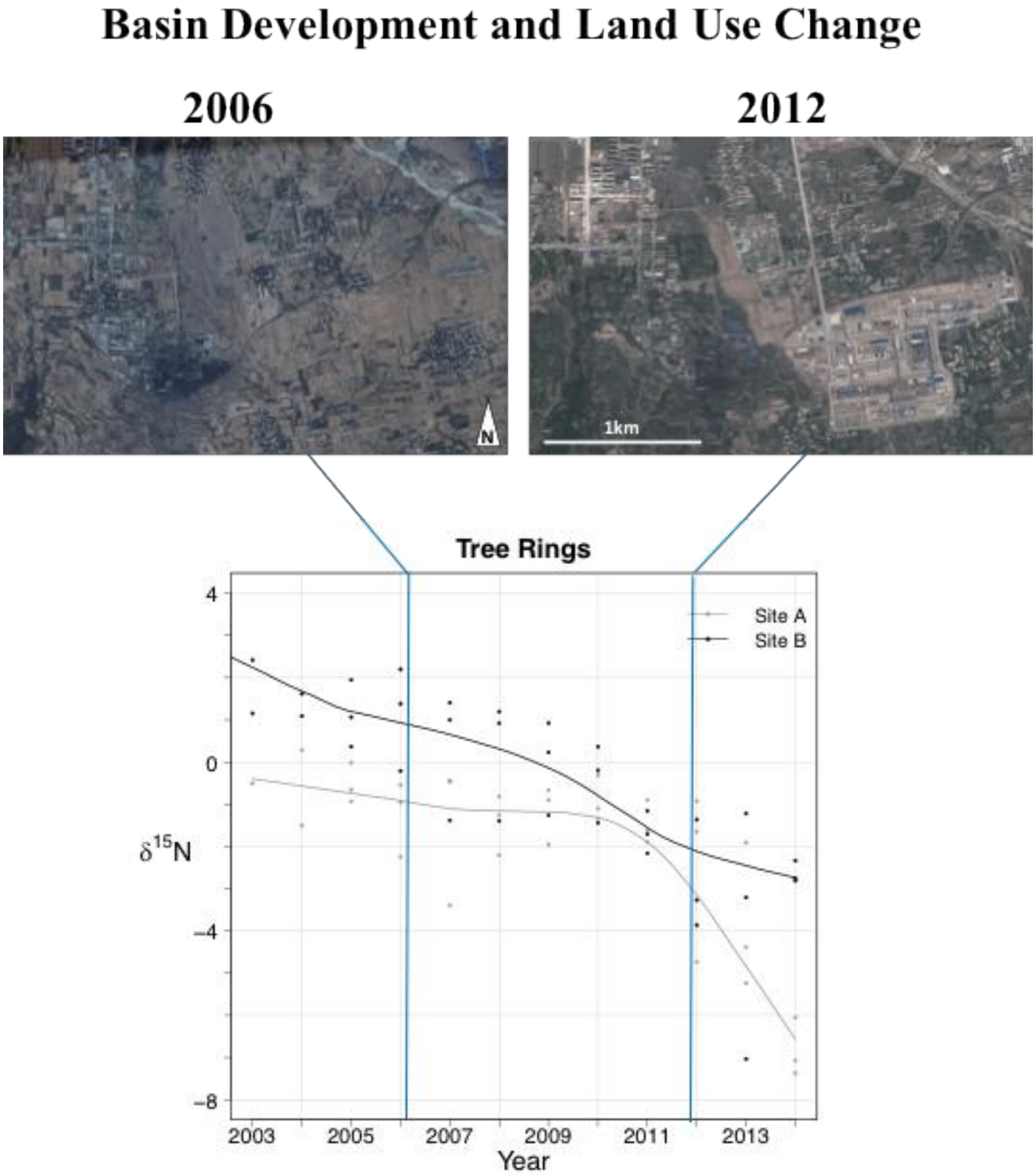

Figure 4.4: Satellite imagery reveals rapid development of the basin with conversion of farm land to industry, roads, and denser housing between 2006 and 2012, particularly in the southeastern corner of the maps. The study sites are located directly south of the area featured in the satellite images. The tree ring record is a scatter plot with loess regression line showing declining wood $\delta^{15}N$ in annual growth rings between 2003 and 2014 at both Site A and Site B. The rapid decline in $\delta^{15}N$ starts in 2010, around the time the major development projects feature in the 2012 image were completed.

4.3.5 Tree Rings

Tree-ring δ^{15}N decreases between 2003 and 2014 at both the basin edge Site A and forest interior Site B (Figure 4.4). Consistent with foliage, soil, and forest floor samples, the δ^{15}N of wood is lower within Site A compared to Site B. This trend holds true for the range of rings sampled (2003 – 2014). The wood at Site A has negative δ^{15}N across all years represented by the annual growth rings and have a greater rate of decline between 2011 and 2014 compared to Site B whose δ^{15}N signatures only first fall below 0‰ starting in 2010 (Figure 4.4).

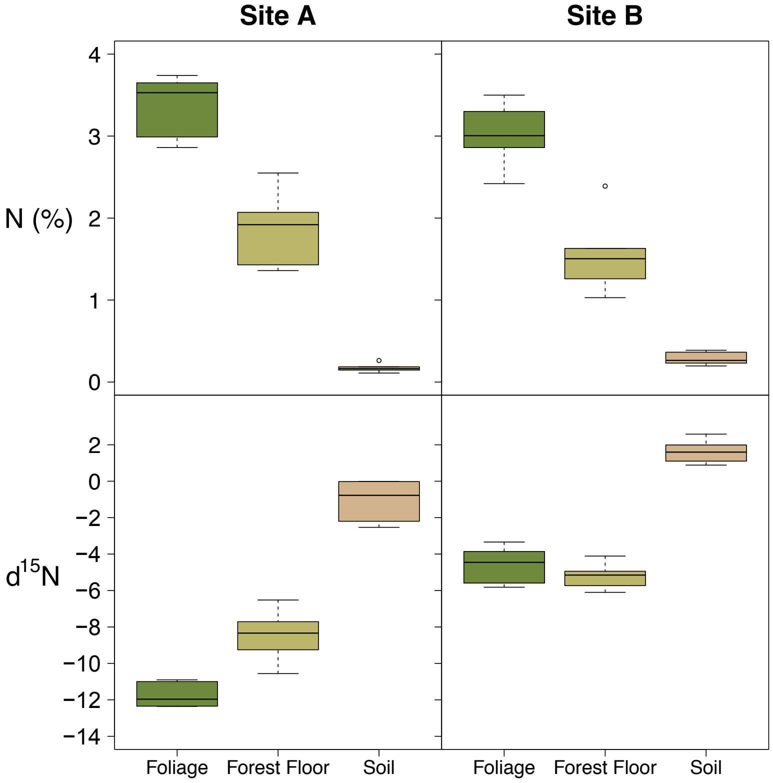

Figure 4.5: Box plots for N concentrations (%N) and δ^{15}N of ecosystem components at each site.

3.6 Comparison of Ecosystem Components

Along the basin edge Site A, foliar $\delta^{15}N$ is significantly lower than both the $\delta^{15}N$ of soil and forest floor samples, and forest floor and soil $\delta^{15}N$ are also significantly different from one another ($p < 0.000$) (Figure 4.5). Forest floor $\delta^{15}N$ fall between foliage and soil $\delta^{15}N$ at Site A. At Site B however, both foliar $\delta^{15}N$ and forest floor $\delta^{15}N$ are significantly different from the soil $\delta^{15}N$ ($p < 0.000$), but not from each other ($p = 0.40$). Soil $\delta^{15}N$ at Site B is however significantly higher than both the foliage and forest floor $\delta^{15}N$. As expected, N concentrations increase from the soil, to forest floor, and are highest in the live foliage sampled at both Sites A and B (Figure 5). Soil N concentrations and foliar $\delta^{15}N$ are not correlated when analyzed within each site (Site A: $\rho = 0.01$, $p = 0.96$; Site B: $\rho = 0.13$, $p = 0.81$). There is a positive correlation (higher foliar $\delta^{15}N$ at the higher soil N site) when the data for both sites are pooled ($\rho = 0.70$, $p = 0.01$). Foliar $\delta^{15}N$ and soil C/N are not significantly correlated within each site (Site A: $\rho = -0.10$, $p = 0.85$; $\rho = -0.38$, $p = 0.46$). When the sites are pooled, there is a negative correlation between soil C/N and foliar $\delta^{15}N$ ($\rho = -0.94$, $p < 0.00$). Site A has both higher soil C/N and lower foliar $\delta^{15}N$ relative to Site B (Figure 4.3).

4.4 Discussion and Conclusion

The $\delta^{15}N$ values of ecosystem pools sampled in this study are substantially lower than what is typically observed in temperate forests around the world, particularly at Site A along the basin edge (Martinelli et al. 1999; Pardo et al. 2002; Templer et al. 2007; Fang et al. 2013). As expected, our results are most similar to the results of studies in temperate East Asian forests with high N deposition where foliar $\delta^{15}N$ ranges from -7.1‰ to 0.5‰ (mean = -4.6‰) (Fang et al. 2013). The low foliar $\delta^{15}N$ across the region has been attributed to the high inputs of ^{15}N depleted N deposition (Fang et al. 2011; Fang et al. 2013; Sun et al. 2010). Foliar $\delta^{15}N$ at Site A was substantially lower than the values obtained in similar forested sites in China. The $\delta^{15}N$ values recorded at Site B in our study fall within the range observed elsewhere in the region (Fang et al. 2011; Fang et al. 2013; Sun et al. 2010). The negative values for foliar $\delta^{15}N$ in our study and similar East Asian forests are all lower than observations from forested sites in North America and Europe. Forests across the East Asian region are exposed to some of the highest N deposition rates in the world (Fang et al. 2011; Liu et al. 2013a). N deposition has been linked to declines in the $\delta^{15}N$ of ecosystem pools when the $\delta^{15}N$ of the atmospheric deposition is relatively low compared to the other sources of plant available N (Savard et al. 2009; Sun et al. 2010). For example, negative $\delta^{15}N$ of tree rings near urban and industrial centers has been observed in variety of species (i.e *Quercus rubra* and *alba* Bukata and Kyser, 2007; *Pinus densiflora* Choi et al. 2005)

Of the ecosystem pools sampled, the lowest $\delta^{15}N$ values were recorded in the foliar tissue followed by the most recent year's tree ring, forest floor, and soil respectively. Several mechanisms may explain the patterns of soil, bolewood, foliar, and tree ring $\delta^{15}N$ observed in our study including [1] direct foliar uptake of ^{15}N depleted N species (Vallano and Sparks, 2008; Sparks 2009), [2] uptake of ^{15}N depleted N species from the soil (Pardo et al. 2002), [3] fractionation as plant available N moves between soil and plant pools due to high N availability as indicated by total soil N concentrations and soil C/N (Evans, 2001).

4.4.1 Tree rings

Tree ring $\delta^{15}N$ declines between 2003 and 2014 at both the basin edge Site A and the interior Site B. Consistent with the $\delta^{15}N$ values of other ecosystem components sampled, the $\delta^{15}N$ of tree rings along the basin edge were lower for comparable years at the interior site. This between site trend persists across all tree ring years. Interpretations of tree ring $\delta^{15}N$ relies on an assumption that the $\delta^{15}N$ of a given tree ring reflects the local N cycle for that year (Gerhart and McLauchlan, 2014). Declines in $\delta^{15}N$ over time have been attributed to declines in N availability (i.e. low leaching rates; McLauchlan and Craine, 2012; Kranabetter et al. 2013) or changes in N source (i.e. new $\delta^{15}N$ depleted pollution source; Stewart et al. 2002; Guerrieri et al. 2009; Fang et al. 2011). Other studies suggest that increases in tree ring $\delta^{15}N$ also reflect changes in N source, leaching, or increased N availability due to changes in disturbance regimes over time (Caceres et al. 2011). The $\delta^{15}N$ of tree rings can also increase with increasing rates of N losses from soils that trees get their N from (Gerhart and McLachlan, 2014). Between 2003 and 2014 the basin was rapidly developing with new factories, housing, and road construction. The difference in land-use in the basin is apparent between 2006 and 2012 when satellite images of the area are available (Figure 4.4). In just six years between 2006 and 2012 there is a dramatic decline in farmland and an increase in factories, power plants, roads, and housing density.

While deposition data for this site is not available, regional trends (Liu et al. 2013b) and the increased industrial development observed in the basin suggest that Nr emissions in the basin have likely been increasing during this time as well. The steep decline in $\delta^{15}N$ beings in the 2010 tree rings which corresponds with the new industrial development and the regional trends of increased N deposition. Additionally, the low C/N ratios of the soil pools at both sample sites indicates that these sites are not N limited. With high soil N concentrations and the increased industrial development in the basin between 2003 and 2014, the decline in tree ring $\delta^{15}N$ is best explained by uptake of increasingly ^{15}N depleted N from the tree's N sources.

4.4.2 Foliar uptake of N

Forest canopies increase deposition rates of Nr (Sparks 2009). Forests can filter out atmospheric pollutants (i.e. NH_3, NO_x) close to the emission source due to the high surface roughness of tree canopies and their physiological capacity for direct foliar uptake of nutrients (Adriaenssens et al. 2011, Sparks, 2009; Vallano and Sparks, 2008; Nguyen et al. 2015). It has been estimated that forest canopies can retain up to 40% of deposited N (Lovett and Lindberg, 1993). The two pathways by which N is absorbed and incorporated into vegetation include direct foliar uptake of atmospheric deposition through the stomata or from the soil via the roots. Canopy uptake of N from atmospheric deposition, particularly NH_3, is well documented (Bruckner et al. 1993; Gessler et al. 2002; Adriaenssens et al. 2011). The uptake of N through the stomata is largely driven by stomatal conductance (Hanstein et al. 1999; Gessler et al. 2002; Sparks 2009). The

$\delta^{13}C$ value of foliage is related to both stomatal conductance and rates of photosynthesis (Dawson et al. 2002). Trees stressed by exposure to atmospheric pollutants (i.e SO_2, O_3, and NO_x) have lower rates of photosynthesis due degradation of their chlorophyll (Farquhar et al. 1989; Shan, 1998). Decreased photosynthesis rates yield lower foliar $\delta^{13}C$ (Wuytack et al. 2013). The foliar $\delta^{13}C$ and N concentrations recorded in our study support the hypotheses that trees closer to a pollution source will have higher N concentrations in their foliage due to direct foliar uptake of N. Foliar $\delta^{13}C$ measured in trees at Site A along the basin edge was significantly lower than the interior Site B. Higher stomatal conductance as suggested by the significantly lower foliar $\delta^{13}C$ values at Site A can lead to greater foliar uptake of N. Accordingly, Site A has slightly higher (although not significant as $p = 0.12$) foliar N concentrations despite having lower soil N concentrations than Site B. The elevated foliar N concentrations coupled with the significantly lower foliar $\delta^{15}N$ suggest that canopy uptake of atmospheric N sources (with negative $\delta^{15}N$) contributes to the differences observed between the basin edge and the more distant and therefore less exposed interior site.

4.4.3 $\delta^{15}N$ depleted N uptake directly from the soil

In addition to foliar uptake of Nr, atmospheric deposition also drives rates of N uptake from the soil. Negative soil $\delta^{15}N$ in temperate forests is typically associated with a more closed N cycle (i.e. one where added N is retained in the ecosystem as opposed to being leached out) and high N inputs (Pardo et al. 2002; Guerrieri et al. 2009). Conversely, in East Asian forests, the negative $\delta^{15}N$ values of soil and plant biomass have been correlated with increased atmospheric N deposition and low soil C/N ratios (Fang et al. 2013). If the N deposition rates at this study site are consistent with regional trends then the addition of N with negative $\delta^{15}N$ to the soil surface may be driving the overall negative $\delta^{15}N$ at these two forested sites as well. This may in part explain the lower $\delta^{15}N$ values in the soil along the basin edge compared to the forest interior. Additionally, as soil N with low $\delta^{15}N$ is metabolized by the trees, the low $\delta^{15}N$ signature of this N source will drive negative $\delta^{15}N$ values in the plants that consume it. This does not fully explain the negative $\delta^{15}N$ in the above ground biomass sampled because the $\delta^{15}N$ of foliage and bole wood at both sites is more negative than the soil $\delta^{15}N$ would otherwise suggest.

4.4.4 Fractionation

Current research suggests that soil and plant tissue $\delta^{15}N$ are positively correlated with leaching losses of N (Pardo et al. 2002; Craine et al. 2009). Positive $\delta^{15}N$ values in plant tissue (i.e foliage and bole wood) and soil is commonly attributed to high N loads and the subsequent leaching losses of N. The bonds between the lighter N isotopes require less energy to break and therefore more readily enter the soil solution where they are leached out of the system. This effectively leaves behind the heavier N (i.e. ^{15}N) thus enriching the soil and the plants that use this N. In environments with an abundant supply of N relative to the demands of the plants and fungi or when the nutrient pool is rapidly replaced will show greater fractionation than N deficient sites (Evans, 2001; Henn and Chapela, 2004). With low C/N ($C/N < 20$) across both forested sites we would expect to find elevated soil and plant $\delta^{15}N$. This indicates that either our study sites have a more closed N cycle than the C/N ratios and N concentrations would otherwise suggest or the dominant N source to the soil is highly negative $\delta^{15}N$, diluting the impact of N losses on enrichment of the soil N pools. Finally, the isotope fractionation effect of plant uptake can result in plant tissue with lower $\delta^{15}N$. This effect is greater in N limited sites where mycorrhizae play a greater role in plant nutrient uptake (Treseder, 2004).

4.4.5 Conclusions and limitations

Likely some combination of these three factors is driving the trends observed this study. Low $\delta^{15}N$ of atmospheric Nr sources is likely driving the overall low $\delta^{15}N$ of soil and plant tissue while the between site differences could be a combination of both fractionation and greater foliar uptake of N at the basin edge. The results of this study need to be considered in the context of their small sample size and small geographic range. Because the two sites sampled are relatively close to one another local factors as opposed to regional N deposition trends are likely driving the differences between the two sites. More detailed field sampling including measurements of atmospheric deposition, concentrations of specific nitrogen species (i.e. nitrate and ammonia) in the soil, and nitrification rates are needed to more precisely elucidate which mechanisms are driving the $\delta^{15}N$ trends at these sites and the degree to which tree-ring and foliar $\delta^{15}N$ can be used to determine the dominant processes driving the cycling of N in the region. Local trends may exert a greater control on foliar and tree ring $\delta^{15}N$ than global patterns of ecosystem $\delta^{15}N$ would otherwise predict. However, the lack of local N deposition data makes direct correlations between the $\delta^{15}N$ of the ecosystem components at these two sites and N inputs impossible. Further research comparing ecosystem $\delta^{15}N$ to more complete N cycle accounting (i.e. denitrification and nitrification rates, soil and atmospheric deposition N-species and concentrations) would allow for more robust interpretations on the links between $\delta^{15}N$ and ecosystem N status. Despite these limitations, the data provide further support to the observation that $\delta^{15}N$ trends in China are not entirely consistent with trends across North America and Europe. Finally, a more complete understanding of the relationship between ecosystem $\delta^{15}N$ and ecosystem N status would benefit from additional data sampled from East Asian forests because the trends differ from what is typically observed in less polluted, N limited sites.

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Chapter 5
REFERENCES CITED

5.1 Chapter 1: Introduction

AAFPCO. (2013). *Official publication number 65. Association of American Plant Food Control Officials.* Retrieved from http://www.aapfco.org/publications.html

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., . . . Fernandez, I. (1998). Nitrogen Saturation in Temperate Forest Ecosystems. *BioScience, 48*(11), 921-934. doi:10.2307/1313296

Allen, H. L., Dougherty, P. M., & Campbell, R. G. (1990). Manipulation of water and nutrients — Practice and opportunity in Southern U.S. pine forests. *Forest Ecology and Management, 30*(1), 437-453. doi:10.1016/0378-1127(90)90153-3

Craine, J., Elmore, A., Aidar, M., Bustamante, M., Dawson, T., Hobbie, E., . . . Wright, I. (2009). Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. In (Vol. 183, pp. 13). New Phytologist.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002). Stable Isotopes in Plant Ecology. *Annual Review of Ecology and Systematics, 33*, 507-559.

Fang, Y., Koba, K., Yoh, M., Makabe, A., & Liu, X. (2013). Patterns of foliar δ 15 N and their control in Eastern Asian forests. *Ecol Res, 28*(5), 735-748. doi:10.1007/s11284-012-0934-8

Fang, Y., Yoh, M., Koba, K., Zhu, W., Takebayashi, Y., Xiao, Y., . . . Lu, X. (2011). Nitrogen deposition and forest nitrogen cycling along an urban- rural transect in southern China. *Global Change Biology, 17*(2), 872-885. doi:10.1111/j.1365-2486.2010.02283.x

Fenn, M. E., Baron, J. S., Allen, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., . . . Neitlich, P. (2003). Ecological Effects of Nitrogen Deposition in the Western United States. *BioScience, 53*(4), 404-420. doi:10.1641/0006-3568(2003)053[0404:EEONDI]2.0.CO;2

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., . . . Sutton, M. A. (2008). Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science, 320*(5878), 889-892. doi:10.1126/science.1136674

Gerhart, L., & McLauchlan, K. (2014). Reconstructing terrestrial nutrient cycling using stable nitrogen isotopes in wood. *An International Journal, 120*(1), 1-21. doi:10.1007/s10533-014-9988-8

Guerrieri, M. R., Siegwolf, R. T. W., Saurer, M., Jäggi, M., Cherubini, P., Ripullone, F., & Borghetti, M. (2009). Impact of different nitrogen emission sources on tree physiology as assessed by a triple stable isotope approach. *Atmospheric Environment, 43*(2), 410-418. doi:10.1016/j.atmosenv.2008.08.042

Kranabetter, J., Saunders, S., MacKinnon, J., Klassen, H., & Spittlehouse, D. (2013). An Assessment of Contemporary and Historic Nitrogen Availability in Contrasting Coastal Douglas- Fir Forests Through δ 15 N of Tree Rings. *Ecosystems, 16*(1), 111-122. doi:10.1007/s10021-012-9598-z

Larry Lopez Caceres, M., Mizota, C., Yamanaka, T., & Nobori, Y. (2011). Effects of pre- treatment on the nitrogen isotope composition of Japanese black pine (Pinus thunbergii) tree-rings as affected by high N input. *Rapid Communications in Mass Spectrometry, 25*(21), 3298-3302. doi:10.1002/rcm.5227

Lebauer, D. S., & Treseder, K. K. (2008). NITROGEN LIMITATION OF NET PRIMARY PRODUCTIVITY IN TERRESTRIAL ECOSYSTEMS IS GLOBALLY DISTRIBUTED. *Ecology, 89*(2), 371-379. doi:10.1890/06-2057.1

Lovett, G., & Goodale, C. (2011). A New Conceptual Model of Nitrogen Saturation Based on Experimental Nitrogen Addition to an Oak Forest. *Ecosystems, 14*(4), 615-631. doi:10.1007/s10021-011-9432-z

Morse, J., Durán, J., Beall, F., Enanga, E., Creed, I., Fernandez, I., & Groffman, P. (2015). Soil denitrification fluxes from three northeastern North American forests across a range of nitrogen deposition. *Oecologia, 177*(1), 17-27. doi:10.1007/s00442-014-3117-1

Pardo, L. H., Hemond, H. F., Montoya, J. P., Fahey, T. J., & Siccama, T. G. (2002). Response of the natural abundance of 15 N in forest soils and foliage to high nitrate loss following clear- cutting. *Canadian Journal of Forest Research, 32*(7), 1126-1136. doi:10.1139/x02-041

Pardo, L. H., Templer, P. H., Goodale, C. L., Duke, S., Groffman, P. M., Adams, M. B., . . . McGee, G. (2006). Regional Assessment of N Saturation using Foliar and Root d15N. *Biogeochemistry, 80*(2), 143-171. doi:10.1007/s10533-006-9015-9

Peterson, C. E., Ryan, P. J., & Gessel, S. P. (1984). Response of Northwest Douglas- fir Stands to Urea: Correlations with Forest Soil Properties1. *Soil Science Society of America Journal, 48*(1), 162. doi:10.2136/sssaj1984.03615995004800010030x

Raymond, J., Fox, T., Strahm, B., & Zerpa, J. (2016). Differences in the recovery of four different nitrogen containing fertilizers after two application seasons in pine plantations across the southeastern United States. *Forest Ecology and Management* 380:161-171.

Rothstein, D., Zak, D., & Pregitzer, K. (1996). Nitrate deposition in northern hardwood forests and the nitrogen metabolism of Acer saccharum marsh. *Oecologia, 108*(2), 338-344. doi:10.1007/BF00334659

Stewart, G., Aidar, M., Joly, C., & Schmidt, S. (2002). Impact of point source pollution on nitrogen isotope signatures (δ 15 N) of vegetation in SE Brazil. *Oecologia, 131*(3), 468-472. doi:10.1007/s00442-002-0906-8

Sun, F., Kuang, Y., Wen, D., Xu, Z., Li, J., Zuo, W., & Hou, E. (2010). Long-term tree growth rate, water use efficiency, and tree ring nitrogen isotope composition of Pinus massoniana L. in response to global climate change and local nitrogen deposition in Southern China. *J. Soils Sediments, 10*(8), 1453-1465. doi:10.1007/s11368-010-0249-8

Swank, W. T., & Vose, J. M. (1997). Long- term nitrogen dynamics of Coweeta Forested Watersheds in the southeastern United States of America. *Global Biogeochemical Cycles, 11*(4), 657-671. doi:10.1029/97GB01752

Talbert, C., & Marshall, D. (2005). Plantation Productivity in the Douglas- Fir Region Under Intensive Silvicultural Practices: Results from Research and Operations. *Journal of Forestry, 103*(2), 65-70.

Templer, P. H., Mack, M. C., Chapin, F. S., Christenson, L. M., Compton, J. E., Crook, H. D., . . . Zak, D. R. (2012). Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of 15N tracer field studies. *Ecology, 93*(8), 1816.

5.2 Chapter 2: Little difference in nitrogen retention with enhanced efficiency fertilizers (EEFS)

AAFPCO. (2013). *Official publication number 65. Association of American Plant Food Control Officials.* Retrieved from http://www.aapfco.org/publications.html

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., . . . Fernandez, I. (1998). Nitrogen Saturation in Temperate Forest Ecosystems. *BioScience, 48*(11), 921-934. doi:10.2307/1313296

Akiyama, H., Yan, X., & Yagi, K. (2010). Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N2O and NO emissions from agricultural soils: meta-analysis. *Global Change Biology, 16*(6), 1837-1846. doi:10.1111/j.1365-2486.2009.02031.x

Amponsah, I. G., Lieffers, V. J., Comeau, P. G., & Landhäusser, S. M. (2004). Nitrogen-15 Uptake by Pinus contorta Seedlings in Relation to Phenological Stage and Season. *Scandinavian Journal of Forest Research, 19*(4), 329-338. doi:10.1080/02827580310019301

Ares, A., & Pacific Northwest Research, S. (2007). *The Fall River long- term site productivity study in coastal Washington: site characteristics, methods, and biomass and carbon and nitrogen stores before and after harvest*: Portland, OR: U.S. Dept. of Agriculture, Forest Service, Pacific Northwest Research Station.

Bremner, J. & Douglas, L. (1971). Inhibition of urease activity in soils. *Soil Biology and Biochemistry* 3(4): 297-307.

Brubaker, L., Vega-Gonzalez, S., Ford, E., Ribic, C., Earle, C., & Segura, G. (1992). *Old-growth Douglas-fir in western Washington* (R. K. Olson, D. Binkley, M. Böhm, & M. Arbaugh Eds.). New York: New York : Springer-Verlag.

Cabrera, M., Kissel, D., Craig, J., Qafoku, N., Vaio, N., Rema, J., & Morris, L. (2010). Relative Humidity Controls Ammonia Loss from Urea Applied to Loblolly Pine. *Soil Science Society of America Journal, 74*(2), 543-549.

Chapin, F. S. (2002). *Principles of terrestrial ecosystem ecology*. New York: New York : Springer.

(DNR), D. o. N. R. (2012). *Washington Mill Survey 2010*. Retrieved from http://www.dnr.wa.gov/Publications/obe_econ_rprt_millsurv_2010.pdf.

Fang, Y., Yoh, M., Koba, K., Zhu, W., Takebayashi, Y., Xiao, Y., . . . Lu, X. (2011). Nitrogen deposition and forest nitrogen cycling along an urban- rural transect in southern China. *Global Change Biology, 17*(2), 872-885. doi:10.1111/j.1365-2486.2010.02283.x

Fenn, M. E., Baron, J. S., Allen, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., . . . Neitlich, P. (2003). Ecological Effects of Nitrogen Deposition in the Western United States. *BioScience, 53*(4), 404-420. doi:10.1641/0006-3568(2003)053[0404:EEONDI]2.0.CO;2

Fisher, R. F., Binkley, D., & Fisher, R. (2012). *Ecology and management of forest soils* (4th ed. ed.). Oxford: Oxford : Wiley-Blackwell.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., . . . Sutton, M. A. (2008). Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science, 320*(5878), 889-892. doi:10.1126/science.1136674

Geiser, L. H., Jovan, S. E., Glavich, D. A., & Porter, M. K. (2010). Lichen- based critical loads for atmospheric nitrogen deposition in Western Oregon and Washington Forests, USA. *Environmental Pollution, 158*(7), 2412-2421. doi:10.1016/j.envpol.2010.04.001

Halvorson, A., Snyder, C., Blaylock, A., & Del Grosso, S. (2014). Enhanced- Efficiency Nitrogen Fertilizers: Potential Role in Nitrous Oxide Emission Mitigation. *Agronomy Journal, 106*(2), 715-722.

Halvorson, A. D., Del Grosso, S. J., & Francesco, A. (2010). Nitrogen source effects on nitrous oxide emissions from irrigated no- till corn. *Journal of environmental quality, 39*(5), 1554. doi:10.2134/jeq2010.0041

Kibet, L. C., Bryant, R., Buda, A., Kleinman, P., Saporito, L., Allen, A. L., . . . May, E. (2016). Persistence and Surface Transport of Urea-Nitrogen: A Rainfall Simulation Study. *J. Environ. Qual., 45*(3), 1062-1070. doi:10.2134/jeq2015.09.0495

Kissel, D., Cabrera, M., Vaio, N., & Craig, J. (2004). Rainfall Timing and Ammonia Loss from Urea in a Loblolly Pine Plantation. *Soil Science Society of America Journal, 68*(5), 1744-1750. doi:10.2136/sssaj2004.1744

Lovett, G., & Goodale, C. (2011). A New Conceptual Model of Nitrogen Saturation Based on Experimental Nitrogen Addition to an Oak Forest. *Ecosystems, 14*(4), 615-631. doi:10.1007/s10021-011-9432-z

Raymond, J., Fox, T., Strahm, B., & Zerpa, J. (2016). Differences in the recovery of four different nitrogen containing fertilizers after two application seasons in pine plantations across the southeastern United States. *Forest Ecology and Management* 380:161-171.

Salazar, F., Martínez-Lagos, J., Alfaro, M., & Misselbrook, T. (2012). Ammonia emissions from urea application to permanent pasture on a volcanic soil. *Atmospheric Environment, 61*, 395-399. doi:10.1016/j.atmosenv.2012.07.085

Sanz-Cobena, A., Misselbrook, T., Arce, A., Mingot, J., Diez, J., & Vallejo, A. (2008). An inhibitor of urease activity effectively reduces ammonia emissions from soil treated with urea under Mediterranean conditions. *Agriculture, Ecosystems, & Environment* 126:243-255.

Sistani, K., Baptiste, M., Lovanh, N., & Cook, K. (2011). Atmospheric Emissions of Nitrous Oxide, Methane, and Carbon Dioxide from Different Nitrogen Fertilizers. *Journal of Environmental Quality, 40*(6), 1797-1805.

Sucre, E., Harrison, R., Turnblom, E., & Briggs, D. (2008). The use of various soil and site variables for estimating growth response of Douglas-fir to multiple applications of urea and determining potential;l long-term effects on soil properties. *Canadian Journal of Forestry*, 38:1458-1469. Doi: 10.1139/X08-007.

Templer, P., Lovett, G., Weathers, K., Findlay, S., & Dawson, T. E. (2005). Influence of Tree Species on Forest Nitrogen Retention in the Catskill Mountains, New York, USA. *Ecosystems, 8*(1), 1-16. doi:10.1007/s10021-004-0230-8

Thomas, R. B., & Strain, B. R. (1991). Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. [Gossypium hirsutum L]. *Plant Physiology, 96*(2). doi:10.1104/pp.96.2.627

Thomas, R. Q., Charles, D. C., Kathleen, C. W., & Christine, L. G. (2010). Increased tree carbon storage in response to nitrogen deposition in the US. *Nature Geoscience, 3*(1), 13. doi:10.1038/ngeo721

Trenkel, M. E. (2010). Slow- and controlled-release and stabilized fertilizers: an option for enhancing nutrient use efficiency in agriculture. . In: International Fertilizer Industry Association.

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., . . . Tilman, D. G. (1997). Technical Report: Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications, 7*(3), 737-750. doi:10.2307/2269431

Watts, D. B., Runion, G. B., Smith Nannenga, K. W., & Torbert, H. A. (2015). Impacts of Enhanced- Efficiency Nitrogen Fertilizers on Greenhouse Gas Emissions in a Coastal Plain Soil under Cotton. *Journal of environmental quality, 44*(6), 1699. doi:10.2134/jeq2015.01.0036

5.3 Chapter 3: Less than 50% nitrogen retention under high N additions after 1 year Douglas-fir forests

AAFPCO. (2013). *Official publication number 65. Association of American Plant Food Control Officials.* Retrieved from http://www.aapfco.org/publications.html

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., . . . Fernandez, I. (1998). Nitrogen Saturation in Temperate Forest Ecosystems. *BioScience, 48*(11), 921-934. doi:10.2307/1313296

Akiyama, H., Yan, X., & Yagi, K. (2010). Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N2O and NO emissions from agricultural soils: meta-analysis. *Global Change Biology, 16*(6), 1837-1846. doi:10.1111/j.1365-2486.2009.02031.x

Allen, H. L., Dougherty, P. M., & Campbell, R. G. (1990). Manipulation of water and nutrients — Practice and opportunity in Southern U.S. pine forests. *Forest Ecology and Management, 30*(1), 437-453. doi:10.1016/0378-1127(90)90153-3

Amponsah, I. G., Lieffers, V. J., Comeau, P. G., & Landhäusser, S. M. (2004). Nitrogen-15 Uptake by Pinus contorta Seedlings in Relation to Phenological Stage and Season. *Scandinavian Journal of Forest Research, 19*(4), 329-338. doi:10.1080/02827580310019301

Ares, A., & Pacific Northwest Research, S. (2007). *The Fall River long- term site productivity study in coastal Washington: site characteristics, methods, and biomass and carbon and nitrogen stores before and after harvest*: Portland, OR: U.S. Dept. of Agriculture, Forest Service, Pacific Northwest Research Station.

Amponsah, I.G.; Comeau, P.G.; Brockley, R.P.; Lieffers, V.J. (2005). Effects of repeated fertilization on needle longevity, foliar nutrition, effective leaf area index, and growth characteristics of lodgepole pine in interior British Columbia, Canada. *Canadian Journal of Forest Research* 35, 440–451.

Bai, E., Houlton, B. Z., & Wang, Y. P. (2012). Isotopic identification of nitrogen hotspots across natural terrestrial ecosystems. *Biogeosciences, 9*(8), 3287-3304. doi:10.5194/bg-9-3287-2012

Brady, N. C. (2008). *The nature and properties of soils* (14th ed. ed.). Upper Saddle River, N.J.: Upper Saddle River, N.J. : Pearson Prentice Hall.

Bremner, J. & Douglas, L. (1971). Inhibition of urease activity in soils. *Soil Biology and Biochemistry* 3(4): 297-307.

Briggs, D. G. (2008). The use of various soil and site variables for estimating growth response of Douglas- fir to multiple applications of urea and determining potential long-term effects on soil properties. *Canadian Journal of Forest Research, 38*(6), 1458-1469.

Brubaker, L., Vega-Gonzalez, S., Ford, E., Ribic, C., Earle, C., & Segura, G. (1992). *Old-growth Douglas-fir in western Washington* (R. K. Olson, D. Binkley, M. Böhm, & M. Arbaugh Eds.). New York: New York : Springer-Verlag.

Buchmann, N., Gebauer, G., & Schulze, E.-D. (1996). Partitioning of 15 N- labeled ammonium and nitrate among soil, litter, below- and above- ground biomass of trees and understory in a 15- year- old Picea abies plantation. *Biogeochemistry, 33*(1), 1-23. doi:10.1007/BF00000967

Chapin, F. S. (2002). *Principles of terrestrial ecosystem ecology*. New York: New York : Springer.

Chappell, H., Cole, D., Gessel, S., & Walker, R. (1991). Forest fertilization research and practice in the Pacific Northwest. *Fertilizer research, 27*(1), 129-140. doi:10.1007/BF01048615

(DNR), D. o. N. R. (2012). *Washington Mill Survey 2010*. Retrieved from http://www.dnr.wa.gov/Publications/obe_econ_rprt_millsurv_2010.pdf.

Edmonds, R. L., & Hsiang, T. (1987). Forest Floor and Soil Influence on Response of Douglas- fir to Urea1. *Soil Science Society of America Journal, 51*(5), 1332. doi:10.2136/sssaj1987.03615995005100050043x

Fenn, M. E., Baron, J. S., Allen, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., . . . Neitlich, P. (2003). Ecological Effects of Nitrogen Deposition in the Western United States. *BioScience, 53*(4), 404-420. doi:10.1641/0006-3568(2003)053[0404:EEONDI]2.0.CO;2

Fisher, R. F., Binkley, D., & Fisher, R. (2012). *Ecology and management of forest soils* (4th ed. ed.). Oxford: Oxford : Wiley-Blackwell.

Garrison, M.T, Moore, J.A., Shaw, T.M., & Mika, P.G. (2000). Foliar nutrient and tree growth response of mixed-conifer stands to three fertilization treatments in northeast Oregon and north central Washington. *Forest Ecology and Management* 132, 183–198.

Geiser, L. H., Jovan, S. E., Glavich, D. A., & Porter, M. K. (2010). Lichen- based critical loads for atmospheric nitrogen deposition in Western Oregon and

Washington Forests, USA. *Environmental Pollution, 158*(7), 2412-2421. doi:10.1016/j.envpol.2010.04.001

Gessel, S. P., Cole, D. W., & Steinbrenner, E. C. (1973). Nitrogen balances in forest ecosystems of the Pacific Northwest. *Soil Biology and Biochemistry, 5*(1), 19-34. doi:10.1016/0038-0717(73)90090-4

Gholz, H. L., & Oregon State University. Forest Research, L. (1979). *Equations for estimating biomass and leaf area of plants in the Pacific Northwest*. Corvallis: Corvallis : Forest Research Lab., Oregon State University, School of Forestry.

Halvorson, A., Snyder, C., Blaylock, A., & Del Grosso, S. (2014). Enhanced- Efficiency Nitrogen Fertilizers: Potential Role in Nitrous Oxide Emission Mitigation. *Agronomy Journal, 106*(2), 715-722.

Halvorson, A. D., Del Grosso, S. J., & Francesco, A. (2010). Nitrogen source effects on nitrous oxide emissions from irrigated no- till corn. *Journal of environmental quality, 39*(5), 1554. doi:10.2134/jeq2010.0041

Hopmans, P., & Chappell, H. N. (1994). Growth response of young, thinned Douglas- fir stands to nitrogen fertilizer in relation to soil properties and tree nutrition. *Canadian Journal of Forest Research, 24*(8), 1684-1688. doi:10.1139/x94-217

Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., . . . Byrne, K. A. (2007). How strongly can forest management influence soil carbon sequestration? *Geoderma, 137*(3), 253-268. doi:10.1016/j.geoderma.2006.09.003

Lebauer, D. S., & Treseder, K. K. (2008). NITROGEN LIMITATION OF NET PRIMARY PRODUCTIVITY IN TERRESTRIAL ECOSYSTEMS IS GLOBALLY DISTRIBUTED. *Ecology, 89*(2), 371-379. doi:10.1890/06-2057.1

Littke, K. M., Harrison, R. B., Zabowski, D., & Briggs, D. G. (2014). Assessing nitrogen fertilizer response of coastal Douglas- fir in the Pacific Northwest using a paired-tree experimental design. *Forest Ecology and Management, 330*, 137-143. doi:10.1016/j.foreco.2014.07.008

Lovett, G., & Goodale, C. (2011). A New Conceptual Model of Nitrogen Saturation Based on Experimental Nitrogen Addition to an Oak Forest. *Ecosystems, 14*(4), 615-631. doi:10.1007/s10021-011-9432-z

Miller, R. E., McNabb, D. H., & Hazard, J. (1989). Predicting Douglas Fir Growth and Response to Nitrogen Fertilization in Western Oregon. *Soil Science Society of America Journal, 53*(5), 1552. doi:10.2136/sssaj1989.03615995005300050043x

Morse, J., Durán, J., Beall, F., Enanga, E., Creed, I., Fernandez, I., & Groffman, P. (2015). Soil denitrification fluxes from three northeastern North American forests across a range of nitrogen deposition. *Oecologia, 177*(1), 17-27. doi:10.1007/s00442-014-3117-1

Motavalli, P. P., Nelson, K. A., & Bardhan, S. (2013). Development of a Variable-Source N Fertilizer Management Strategy Using Enhanced- Efficiency N Fertilizers. *Soil Science, 178*(12), 693-703. doi:10.1097/SS.0b013e31827dddc1

Peterson, C. E., Ryan, P. J., & Gessel, S. P. (1984). Response of Northwest Douglas- fir Stands to Urea: Correlations with Forest Soil Properties1. *Soil Science Society of America Journal, 48*(1), 162. doi:10.2136/sssaj1984.03615995004800010030x

Raymond, J., Fox, T., Strahm, B., & Zerpa, J. (2016). Differences in the recovery of four different nitrogen containing fertilizers after two application seasons in pine plantations across the southeastern United States. *Forest Ecology and Management* 380:161-171.

Salifu, K., & Timmer, V. (2003). Nitrogen retranslocation response of young Picea marianna to nitrogen- 15 supply. *Soil Science Society of America Journal, 67*(1), 309-317. doi:10.2136/sssaj2003.0309

Sanz-Cobena, A., Misselbrook, T., Arce, A., Mingot, J., Diez, J., & Vallejo, A. (2008). An inhibitor of urease activity effectively reduces ammonia emissions from soil treated with urea under Mediterranean conditions. *Agriculture, Ecosystems, & Environment* 126:243-255.

Sistani, K., Jn-Baptiste, M., Lovanh, N., & Cook, K. (2011). Atmospheric Emissions of Nitrous Oxide, Methane, and Carbon Dioxide from Different Nitrogen Fertilizers. *Journal of Environmental Quality, 40*(6), 1797-1805.

Talbert, C., & Marshall, D. (2005). Plantation Productivity in the Douglas- Fir Region Under Intensive Silvicultural Practices: Results from Research and Operations. *Journal of Forestry, 103*(2), 65-70.

Templer, P., Lovett, G., Weathers, K., Findlay, S., & Dawson, T. E. (2005). Influence of Tree Species on Forest Nitrogen Retention in the Catskill Mountains, New York, USA. *Ecosystems, 8*(1), 1-16. doi:10.1007/s10021-004-0230-8

Trenkel, M. E. (2010). Slow- and controlled-release and stabilized fertilizers: an option for enhancing nutrient use efficiency in agriculture. . In: International Fertilizer Industry Association.

Turner, J., Lambert, M., & Gessel, S. (1988). Nitrogen requirements in young Douglas-fir of the Pacific Northwest. *Fertilizer research, 15*(2), 173-179. doi:10.1007/BF01050679

Watson, C. J., Akhonzada, N. A., Hamilton, J. T. G., & Matthews, D. I. (2008). Rate and mode of application of the urease inhibitor N -(n- butyl) thiophosphoric triamide on ammonia volatilization from surface- applied urea. *Soil Use and Management, 24*(3), 246-253. doi:10.1111/j.1475-2743.2008.00157.x

Watts, D. B., Runion, G. B., Smith Nannenga, K. W., & Torbert, H. A. (2015). Impacts of Enhanced- Efficiency Nitrogen Fertilizers on Greenhouse Gas Emissions in a Coastal Plain Soil under Cotton. *Journal of environmental quality, 44*(6), 1699. doi:10.2134/jeq2015.01.0036

5.4 Chapter 4: Local alterations to the nitrogen cycle as indicated by soil, foliage, and tree-ring total N and δ^{15}N: A case study in Shaanxi Province, China

Adriaenssens, S., Staelens, J., Wuyts, K., Schrijver, A., Wittenberghe, S., Wuytack, T., . . . Boeckx, P. (2011). Foliar Nitrogen Uptake from Wet Deposition and the Relation with Leaf Wettability and Water Storage Capacity. *An International Journal of Environmental Pollution, 219*(1), 43-57. doi:10.1007/s11270-010-0682-8

Andreas, R., John, P. B., Hendrik, N., Claire, G., & Ulrike, N. (2005). Increase in tropospheric nitrogen dioxide over China observed from space. *Nature, 437*(7055), 129. doi:10.1038/nature04092

Bruckner, G., Gebauer, G., & Schulze, E. D. (1993). Uptake of 15 NH 3 by Picea abies in Closed Chamber Experiments. *Isotopenpraxis Isotopes in Environmental and Health Studies, 29*(1-2), 71-76. doi:10.1080/10256019308046137

Bukata, A., & Kyser, T. (2007). Carbon and Nitrogen Isotope Variations in Tree- Rings as Records of Perturbations in Regional Carbon and Nitrogen Cycles. *Environmental Science & Technology, 41*(4), 1331. doi:10.1021/es061414g

Bullock, A., & King, B. (2011). Evaluating China’s Slope Land Conversion Program as sustainable management in Tianquan and Wuqi Counties. *Journal of Environmental Management, 92*(8), 1916-1922. doi:10.1016/j.jenvman.2011.03.002

Chen, L., Wei, W., Fu, B., & Lu, Y. (2007). Soil and water conservation on the Loess Plateau in China: review and perspective. In *Prog. Phys. Geogr.* (Vol. 31, pp. 389-403).

Choi, W.-J., Lee, S.-M., Chang, S., & Ro, H.-M. (2005). Variations of δ 13 C AND δ 15 N in Pinus Densiflora Tree- Rings and their Relationship to Environmental Changes in Eastern Korea. *An International Journal of Environmental Pollution, 164*(1), 173-187. doi:10.1007/s11270-005-2253-y

Craine, J., Brookshire, E., Cramer, M., Hasselquist, N., Koba, K., Marin-Spiotta, E., & Wang, L. (2015). Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *An International Journal on Plant-Soil Relationships, 396*(1), 1-26. doi:10.1007/s11104-015-2542-1

Craine, J., Elmore, A., Aidar, M., Bustamante, M., Dawson, T., Hobbie, E., . . . Wright, I. (2009). Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. In (Vol. 183, pp. 13). New Phytologist.

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002). Stable Isotopes in Plant Ecology. *Annual Review of Ecology and Systematics, 33*, 507-559.

Duan, L., Liu, J., Xin, Y., & Larssen, T. (2013). Air- pollution emission control in China: Impacts on soil acidification recovery and constraints due to drought. *The Science of the Total Environment, 463 464*, 1031.

Evans, R. D. (2001). Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science, 6*(3), 121-126. doi:10.1016/S1360-1385(01)01889-1

Fang, Y., Koba, K., Yoh, M., Makabe, A., & Liu, X. (2013). Patterns of foliar δ 15 N and their control in Eastern Asian forests. *Ecol Res, 28*(5), 735-748. doi:10.1007/s11284-012-0934-8

Fang, Y., Yoh, M., Koba, K., Zhu, W., Takebayashi, Y., Xiao, Y., . . . Lu, X. (2011). Nitrogen deposition and forest nitrogen cycling along an urban- rural transect in southern China. *Global Change Biology, 17*(2), 872-885. doi:10.1111/j.1365-2486.2010.02283.x

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon Isotope Discrimination and Photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol., 40*(1), 503-537. doi:10.1146/annurev.pp.40.060189.002443

Fenn, M. E., Baron, J. S., Allen, E. B., Rueth, H. M., Nydick, K. R., Geiser, L., . . . Neitlich, P. (2003). Ecological Effects of Nitrogen Deposition in the Western United States. *BioScience, 53*(4), 404-420. doi:10.1641/0006-3568(2003)053[0404:EEONDI]2.0.CO;2

Gerhart, L., & McLauchlan, K. (2014). Reconstructing terrestrial nutrient cycling using stable nitrogen isotopes in wood. *An International Journal, 120*(1), 1-21. doi:10.1007/s10533-014-9988-8

Gessler, A., Rienks, M., & Rennenberg, H. (2002). Stomatal uptake and cuticular adsorption contribute to dry deposition of NH 3 and NO 2 to needles of adult spruce (Picea abies) trees. *New Phytologist, 156*(2), 179-194. doi:10.1046/j.1469-8137.2002.00509.x

Gratani, L., Crescente, M. F., & Varone, L. (2008). Long-term monitoring of metal pollution by urban trees. *Atmospheric Environment, 42*(35), 8273.

Guerrieri, M. R., Siegwolf, R. T. W., Saurer, M., Jäggi, M., Cherubini, P., Ripullone, F., & Borghetti, M. (2009). Impact of different nitrogen emission sources on tree physiology as assessed by a triple stable isotope approach. *Atmospheric Environment, 43*(2), 410-418. doi:10.1016/j.atmosenv.2008.08.042

Hairong, Z., Wanqin, Y., Fuzhong, W., & Bo, T. (2017). Mixed forest plantations can efficiently filter rainfall deposits of sulfur and chlorine in Western China. *Scientific Reports, 7*. doi:10.1038/srep41680

Hanstein, S., Mattsson, M., Jaeger, H. J., & Schjoerring, J. K. (1999). Uptake and utilization of atmospheric ammonia in three native Poaceae species: leaf conductances, composition of apoplastic solution and interactions with root nitrogen supply. *New Phytologist, 141*(1), 71-83. doi:10.1046/j.1469-8137.1999.00330.x

Henn, M. R., & Chapela, I. H. (2004). Isotopic fractionation during ammonium assimilation by basidiomycete fungi and its implications for natural nitrogen isotope patterns. *New Phytologist, 162*(3), 771-781. doi:10.1111/j.1469-8137.2004.01068.x

Janhäll, S. (2015). Review on urban vegetation and particle air pollution – Deposition and dispersion. *Atmospheric Environment, 105*, 130-137. doi:10.1016/j.atmosenv.2015.01.052

Jiao, J., Zhang, Z., Bai, W., Jia, Y., & Wang, N. (2012). Assessing the Ecological Success of Restoration by Afforestation on the Chinese Loess Plateau. *Restoration Ecology, 20*(2), 240-249. doi:10.1111/j.1526-100X.2010.00756.x

Kranabetter, J., Saunders, S., MacKinnon, J., Klassen, H., & Spittlehouse, D. (2013). An Assessment of Contemporary and Historic Nitrogen Availability in Contrasting Coastal Douglas- Fir Forests Through δ 15 N of Tree Rings. *Ecosystems, 16*(1), 111-122. doi:10.1007/s10021-012-9598-z

Larry Lopez Caceres, M., Mizota, C., Yamanaka, T., & Nobori, Y. (2011). Effects of pre- treatment on the nitrogen isotope composition of Japanese black pine (Pinus thunbergii) tree-rings as affected by high N input. *Rapid Communications in Mass Spectrometry, 25*(21), 3298-3302. doi:10.1002/rcm.5227

Leonelli, G., Battipaglia, G., Siegwolf, R. T. W., Saurer, M., Morra Di Cella, U., Cherubini, P., & Pelfini, M. (2012). Climatic isotope signals in tree rings masked by air pollution: A case study conducted along the Mont Blanc Tunnel access road (Western Alps, Italy). *Atmospheric Environment, 61*, 169-179. doi:10.1016/j.atmosenv.2012.07.023

Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., . . . Zhang, F. (2013). Enhanced nitrogen deposition over China. *Nature, 494*(7438), 459. doi:10.1038/nature11917

Liu, Z.-P., Shao, M.-A., & Wang, Y.-Q. (2013). Spatial patterns of soil total nitrogen and soil total phosphorus across the entire Loess Plateau region of China. *Geoderma, 197-198*, 67-78. doi:10.1016/j.geoderma.2012.12.011

Lovett, G. M., & Lindberg, S. E. (1993). Atmospheric deposition and canopy interactions of nitrogen in forests. *Canadian Journal of Forest Research, 23*(8), 1603-1616. doi:10.1139/x93-200

Martinelli, L., Piccolo, M., Townsend, A., Vitousek, P., Cuevas, E., McDowell, W., . . . Treseder, K. (1999). Nitrogen stable isotopic composition of leaves and soil: Tropical versus temperate forests. *Biogeochemistry, 46*(1), 45-65. doi:10.1007/BF01007573

Mclauchlan, K. K., & Craine, J. M. (2012). Species- specific trajectories of nitrogen isotopes in Indiana hardwood forests, USA. *Biogeosciences, 9*(2), 867-874. doi:10.5194/bg-9-867-2012

Miller, J. H. (2010). *A field guide for the identification of invasive plants in southern forests*. Asheville, NC: Asheville, NC : U.S. Dept. of Agriculture, Forest Service, Southern Research Station.

Nguyen, T. B., Crounse, J. D., Teng, A. P., St Clair, J. M., Paulot, F., Wolfe, G. M., & Wennberg, P. O. (2015). Rapid deposition of oxidized biogenic compounds to a temperate forest. *Proceedings of the National Academy of Sciences of the United States of America, 112*(5), E392. doi:10.1073/pnas.1418702112

Nizzetto, L., Cassani, C., & Di Guardo, A. (2006). Deposition of PCBs in mountains: The forest filter effect of different forest ecosystem types. *Ecotoxicology and Environmental Safety, 63*(1), 75-83. doi:10.1016/j.ecoenv.2005.05.005

Nowak, D. J., Hirabayashi, S., Bodine, A., & Hoehn, R. (2013). Modeled PM2.5 removal by trees in ten U.S. cities and associated health effects. *Environmental Pollution, 178*, 395-402. doi:10.1016/j.envpol.2013.03.050

Padilla, K. L., & Anderson, K. A. (2002). Trace element concentration in tree- rings biomonitoring centuries of environmental change. *Chemosphere, 49*(6), 575-585. doi:10.1016/S0045-6535(02)00402-2

Pardo, L. H., Hemond, H. F., Montoya, J. P., Fahey, T. J., & Siccama, T. G. (2002). Response of the natural abundance of 15 N in forest soils and foliage to high nitrate loss following clear- cutting. *Canadian Journal of Forest Research, 32*(7), 1126-1136. doi:10.1139/x02-041

Pardo, L. H., Templer, P. H., Goodale, C. L., Duke, S., Groffman, P. M., Adams, M. B., . . . McGee, G. (2006). Regional Assessment of N Saturation using Foliar and Root d15N. *Biogeochemistry, 80*(2), 143-171. doi:10.1007/s10533-006-9015-9

Poulson, S. R., Chamberlain, C. P., & Friedland, A. J. (1995). Nitrogen isotope variation of tree rings as a potential indicator of environmental change. *Chemical Geology, 125*(3), 307-315. doi:10.1016/0009-2541(95)00097-6

Rothstein, D., Zak, D., & Pregitzer, K. (1996). Nitrate deposition in northern hardwood forests and the nitrogen metabolism of Acer saccharum marsh. *Oecologia, 108*(2), 338-344. doi:10.1007/BF00334659

Savard, M. M., Begin, C., Smirnoff, A., Marion, J., & Rioux-Paquette, E. (2009). Tree-Ring Nitrogen Isotopes Reflect Anthropogenic NOx Emissions and Climatic Effects. *Environ. Sci. Technol., 43*(3), 604-609. doi:10.1021/es802437k

Shan, Y. (1998). Effects of Simulated Acid Rain on Pinus densiflora: Inhibition of Net Photosynthesis by the Pheophytization of Chlorophyll. *An International Journal of Environmental Pollution, 103*(1), 121-127. doi:10.1023/A:1004921511269

Sheppard, P. R., & Thompson, T. L. (2000). Effect of Extraction Pretreatment on Radial Variation of Nitrogen Concentration in Tree Rings. *Journal of Environment Quality, 29*(6), 2037. doi:10.2134/jeq2000.00472425002900060042x

Sparks, J. P. (2009). Ecological ramifications of the direct foliar uptake of nitrogen. In *Oecologia* (Vol. 159, pp. 1-13).

Speak, A. F., Rothwell, J. J., Lindley, S. J., & Smith, C. L. (2012). Urban particulate pollution reduction by four species of green roof vegetation in a UK city. *Atmospheric Environment, 61*, 283-293. doi:10.1016/j.atmosenv.2012.07.043

Stewart, G., Aidar, M., Joly, C., & Schmidt, S. (2002). Impact of point source pollution on nitrogen isotope signatures (δ 15 N) of vegetation in SE Brazil. *Oecologia, 131*(3), 468-472. doi:10.1007/s00442-002-0906-8

Sun, F., Kuang, Y., Wen, D., Xu, Z., Li, J., Zuo, W., & Hou, E. (2010). Long-term tree growth rate, water use efficiency, and tree ring nitrogen isotope composition of Pinus massoniana L. in response to global climate change and local nitrogen deposition in Southern China. *J. Soils Sediments, 10*(8), 1453-1465. doi:10.1007/s11368-010-0249-8

Sæbø, A., Popek, R., Nawrot, B., Hanslin, H. M., Gawronska, H., & Gawronski, S. W. (2012a). Plant species differences in particulate matter accumulation on leaf surfaces. *Science of the Total Environment, 427-428*, 347-354. doi:10.1016/j.scitotenv.2012.03.084

Sæbø, A., Popek, R., Nawrot, B., Hanslin, H. M., Gawronska, H., & Gawronski, S. W. (2012b). Plant species differences in particulate matter accumulation on leaf surfaces. *Science of the Total Environment, 427-428*, 347-354. doi:10.1016/j.scitotenv.2012.03.084

Templer, P. H., Mack, M. C., Chapin, F. S., Christenson, L. M., Compton, J. E., Crook, H. D., . . . Zak, D. R. (2012). Sinks for nitrogen inputs in terrestrial ecosystems: a meta-analysis of 15N tracer field studies. *Ecology, 93*(8), 1816.

Treseder, K. K. (2004). A meta- analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO 2 in field studies. *New Phytologist, 164*(2), 347-355. doi:10.1111/j.1469-8137.2004.01159.x

Vallano, D. M., & Sparks, J. P. (2008). Quantifying foliar uptake of gaseous nitrogen dioxide using enriched foliar δ 15 N values. *New Phytologist, 177*(4), 946-955. doi:10.1111/j.1469-8137.2007.02311.x

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., . . . Tilman, D. G. (1997). Technical Report: Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications, 7*(3), 737-750. doi:10.2307/2269431

Wang, B., Yang, Q., & Liu, Z. (2009). Effect of conversion of farmland to forest or grassland on soil erosion intensity changes in Yanhe River Basin, Loess Plateau of China. *Front. For. China, 4*(1), 68-74. doi:10.1007/s11461-009-0015-5

Wuytack, T., Abdelgawad, H., Staelens, J., Asard, H., Boeckx, P., Verheyen, K., & Samson, R. (2013). The response of the foliar antioxidant system and stable isotopes (δ13C and δ15N) of white willow to low-level air pollution. *Plant Physiology and Biochemistry, 67*, 154-161. doi:10.1016/j.plaphy.2013.03.007

Yang, Y., Ji, C., Ma, W., Wang, S., Wang, S., Han, W., . . . Smith, P. (2012). Significant soil acidification across northern China's grasslands during 1980s–2000s. *Global Change Biology, 18*(7), 2292-2300. doi:10.1111/j.1365-2486.2012.02694.x

Zeng, X., Liu, X., Xu, G., Wang, W., & An, W. (2014). Tree- ring growth recovers, but δ 13 C and δ 15 N do not change, after the removal of point-source air pollution: a case study for poplar (Populus cathayana) in northwestern China. *Environ Earth Sci, 72*(6), 2173-2182. doi:10.1007/s12665-014-3127-7

Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P., & Shen, Y. (2015). Managing nitrogen for sustainable development. *Nature, 528*(7580), 51. doi:10.1038/nature15743

Zhu, X., Li, Y., Peng, X., & Zhang, S. (1983). Soils of the loess region in China. *Geoderma, 29*(3), 237-255.

APPENDIX

6.1 $\delta^{15}N$ to atom percent ^{15}N converter

```
#atmPercent function converts d15N notation to atom percent 15N
atm.Percent <- function(x)
{
 r <- 0.366/99.634 #R Standard for nitrogen (mean atmospheric 15N)
 step1 <- (x/1000 + 1)
 step2 <- step1 * r
 step3 <- 1 + step2
 step4 <- step2/step3

 return(step4*100) #atm % 15N
}
```

6.2 Ecosystem N retention calculator

```
# 15N recovery calculation (Buchmann et al. 1996; Templer et al. 2004)
# %15Nrecovery = 100 * (mpool * (atm%15Npool - atm%15Nreference) / 100) /
15Ntracer
# mpool = N mass of the labeled pool
# atm%15N = atom percent 15N of the reference (non-labeled) plots
# 15N tracer = amount of 15N added to each plot prior to sample collection
# Equation used to estimate net N retention in a given pool (i.e. soil, foliage, forest floor)
at a given point in time
#N retention based on the bulk density, 15N (atm %), %N of the sample
retention <-function(N15pool, N15ref, percent.N, BD, N15fert = 0.4975, Soildepth = 15,
type = "soil", dbh = NA, appRate = 0.224){
  if(type == "soil"){
    MgN.ha <- (((percent.N/100) * BD) / 1000 *(10^8) * 0.001) * Soildepth
    Nt <- MgN.ha / appRate
    diff <- (N15pool - N15ref) / (N15fert - N15ref)
    N15rec <- Nt * diff
    return(N15rec)
    }

  else if (type == "foliage"){
    bm <- gholz(dbh, component = type)
    mpool <- percent.N/100 * bm/1000 * (10^8) * 1000  #mpool in MgN/ha, for foliage
BD is kg/ha of foliage
    N15tracer <- appRate * (N15fert/100) / 1000 #amount of 15N added to each plot prior
to sample collection Mg/ha
    N15rec <- 100 * ((mpool * (N15pool - N15ref) / 100) / N15tracer)
    return(N15rec)
  }

  else if (type == "forest floor"){   mpool <- percent.N/100 * BD/1000 * (10^8) * 1000
#mpool in MgN/ha, for foliage BD is kg/ha of foliage
  N15tracer <- appRate * (N15fert/100) / 1000 #amount of 15N added to each plot prior
to sample collection Mg/ha
  N15rec <- 100 * ((mpool * (N15pool - N15ref) / 100) / N15tracer)
  return(N15rec)
  }

  else {
    warning("type not recognized: use 'soil', 'foliage', or 'forest floor'")
  }
}
```

6.3 Allometric equation calculator

```
#Douglas-fir biomass equations from H.L. Gholz, C.C. Grier, A.G. Campbell and A.T.
Brown. 1979.
#Equations for estimating biomass and leaf area of plants in the Pacific Northwest.
#Research Paper 41. Forest Research Lab. Oregon State University, Corvallis, OR
#Data from Table A-1 (Gholz et al. 1979)

gholz <- function(dbh, component){
 # biomass = EXP^(a+b*ln(dbh))
 FoliageTotal <- c( -2.8462,  1.7009, 123, 0.86) #c(a, b, n, r^2)
 BranchLive <- c( -3.6941, 2.1382, 123, 0.92)
 BranchDead <- c( -3.5290, 1.7503, 85,       0.84)
 StemWood <- c( -3.0396,     2.5951, 99, 0.99)
 StemBark <- c( -4.3103, 2.4300, 99, 0.99)
 Roots <- c( -4.6961, 2.6929, 26,       0.96)
 FoliageNew <- c( -5.7265, 2.0390, 29, 0.93)

 if (component == "stemwood"){
  Ystemwood <- exp(StemWood[1] + StemWood[2] * log(dbh))
  return(Ystemwood) #kg of stem wood
 }

 else if (component == "stembark"){
  Ystembark <- exp(StemBark[1] + StemBark[2] * log(dbh))
  return(Ystembark)
 }

 else if (component == "branchlive"){
  YBranchLive <- exp(BranchLive[1] + BranchLive[2] * log(dbh))
  return(YBranchLive) #kg of live branches
 }

 else if (component == "branchdead"){
  YBranchDead <- exp(BranchDead[1] + BranchDead[2] * log(dbh))
  return(YBranchDead) #kg of dead branches
 }

 else if (component == "foliage") {
  YFoliageTotal <- exp(FoliageTotal[1] + FoliageTotal[2] * log(dbh))
  return(YFoliageTotal) #kg of total foliage
 }

 else if (component == "foliagenew"){
  Yfoliagenew<- exp(FoliageNew[1] + FoliageNew[2] * log(dbh))
  return(Yfoliagenew) #kg of new foliage
```

```
  }
  else warning("component not recognized")
}
```