

**Hyperspectral Reflectance and Stable Isotopic Nitrogen:
Tools to Assess Forest Ecosystem Nitrogen Cycling**

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Abstract

The use of nitrogenous fertilizers in agricultural and forestry practices coupled with increased fossil fuel combustion and resulting nitrogen (N) deposition across the landscape have contributed to a near doubling of N inputs to terrestrial ecosystems. With such dramatic changes have come adverse environmental consequences including the acidification of soil and water resources and an increased rate of biodiversity loss in both flora and fauna. A method of rapidly predicting ecosystem susceptibility to N loss across large spatial scales would facilitate the identification of those systems most likely to contribute to potentially adverse environmental impacts. To begin the development of such a framework, this research utilizes study sites located throughout the geographic ranges of Douglas-fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*) to explore relationships between hyperspectral remote sensing, N stable isotope ratios ($\delta^{15}\text{N}$) and growth response to nitrogenous fertilizer. In both species multiple linear regression models relating leaf-level reflectance to $\delta^{15}\text{N}$ showed strong predictive capabilities, with some models explaining more than 65% of the variance in $\delta^{15}\text{N}$. Significant correlations between $\delta^{15}\text{N}$ metrics and growth response to N fertilization were also observed in both species. Additional exploratory analysis of the inclusion of $\delta^{15}\text{N}$ metrics with other environmental and edaphic variables to predict fertilizer growth response showed an increase in model performance with the addition of the enrichment factor ($\text{EF} = \delta^{15}\text{N}_{\text{Fol}} - \delta^{15}\text{N}_{\text{Soil}}$). This research demonstrates the ability of hyperspectral reflectance to predict $\delta^{15}\text{N}$ and reveals the potential of $\delta^{15}\text{N}$ to be included in future models to predict fertilizer growth response.

Dedication

For my parents and my fiancé. Thank you for your unconditional love, encouragement and support.

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Chapter 1 - Introduction and Objectives

1. Introduction

Nitrogen (N) is vital to the growth and survival of vegetation as it is utilized in biochemical processes, such as photosynthesis, and is a major component of amino acids and proteins, thereby essential to enzymatic reactions. The largest global pool of N is atmospheric N₂ which is largely unavailable to terrestrial vegetation, with the exception of N-fixing species, and there is little input of N from the weathering of parent material. Productivity in temperate forest ecosystems is most often limited by N (Vitousek and Howarth, 1991).

Human alterations to the N cycle have nearly doubled the rate of N input into terrestrial ecosystems resulting in negative environmental consequences at local and global scales (Vitousek et al., 1997; Gruber and Galloway, 2008). To meet demands for food, fuel and fiber, the addition of nitrogenous fertilizer is a common practice in agriculture and forestry, as N often limits productivity in these systems (Vitousek and Howarth, 1991). Additionally, increased production of reactive N has resulted in enhanced deposition of N across the landscape (Vitousek et al., 1997; Galloway, 1998). With these anthropogenic N inputs to terrestrial ecosystems, there is an increased risk for adverse environmental impacts such as the acidification of aquatic ecosystems and soils, loss of soil nutrients and accelerated biodiversity losses of both flora and fauna (Vitousek et al., 1997). These anthropogenic inputs of N coupled with the resulting environmental degradation are cause for investigation into ecosystem-level N dynamics, particularly with respect to the capacity of terrestrial forest ecosystems to retain this added N.

1.1 Stable Isotopic N

Isotopes are atoms with the same number of protons and electrons but differing numbers of neutrons. Stable isotopes are energetically stable and do not decay, hence they are not

radioactive. The rare, but naturally occurring stable isotope of N is ^{15}N making up only 0.365% of atmospheric N_2 with ^{14}N comprising the remaining 99.635% (Sulzman, 2007).

Patterns in N stable isotope ratios ($\delta^{15}\text{N}$) have the potential to integrate terrestrial N cycling and can be used to identify ecosystems that are susceptible to or have experienced significant N losses (Pardo et al., 2002; Högberg, 1997; Cheng et al. 2010). It is the discrimination against ^{15}N , or fractionation, that occurs during chemical and physical reactions that allows $\delta^{15}\text{N}$ to be used as an ecosystem-level tool of evaluation. In physical reactions, such as diffusion, heavier isotopes usually move or react more slowly, due to the added weight of the extra neutron(s) (Fry, 2006). In chemical reactions, less energy is required to break the bonds formed by the lighter isotopes, thereby causing them to react more rapidly and frequently than the heavier isotopes (Fry, 2006). As N cycles through the system and undergoes the processes of mineralization, nitrification, denitrification and volatilization, fractionation creates products that are depleted and substrates that are enriched in ^{15}N (Shearer and Kohl, 1986). It has been demonstrated that with high net soil nitrification rates and substantial loss of nitrate from the system, soil and foliar $\delta^{15}\text{N}$ are gradually elevated due to the loss of the depleted products (Högberg, 1997; Cheng et al. 2010). Specifically, Högberg (1990, 1993) found that Scots pine (*Pinus sylvestris* L.) foliage showed an increase in $\delta^{15}\text{N}$ when large amounts of added N were lost from the system. Use of the enrichment factor ($\text{EF} = \delta^{15}\text{N}_{\text{Fol.}} - \delta^{15}\text{N}_{\text{Soil}}$) can provide further insights when assessing foliar $\delta^{15}\text{N}$ as it adjusts for the potential variation in foliar $\delta^{15}\text{N}$ that results from variable isotopic compositions of the soil (Garten et al., 2007). The EF has also been positively correlated to nitrification and mineralization rates (Emmett et al., 1998; Garten and Van Miegroet, 1994; Cheng et al., 2010) and has been used as an indicator of ecosystem N availability, with a higher EF indicating higher N availability (Emmett et al., 1998; Fang et al., 2000).

1.2 Hyperspectral Remote Sensing

Hyperspectral remote sensing data typically involves 70 to 350 near continuous bands that sense energy in the visible, near-, mid- and far- infrared regions of the electromagnetic spectrum. Spectroscopy data has been used for over 30 years to study vegetation spectral signatures at the leaf-level. The narrow bandwidth (i.e. approximately 2 to 10 nanometers) of the data facilitates the study of cell structure, water content and foliar biochemistry by observing slight variations in reflectance at specified wavelengths. Of particular interest in forest ecosystems is the use of reflectance data to quantify foliar N concentrations (%N). In fresh vegetation, the close correlation between N and chlorophyll has led to the use of wavelengths in the visible and red-edge regions of the electromagnetic spectrum to develop regression equations for predicting N concentrations in foliage (Yoder and Pettigrew-Crosby, 1995). Wavelengths in the near-infrared, associated with N absorption features due to the vibration of N-hydrogen bonds in proteins (Curran 1989), have also been utilized in the prediction of N concentration from leaf-level reflectance (Peterson et al., 1988; Johnson and Billow, 1996; Yoder and Pettigrew-Crosby, 1995).

With the relationship between reflectance and %N well-established at the leaf-level, researchers have successfully scaled observations to the canopy, stand and regional levels using image-level reflectance data obtained from airborne and satellite platforms (Martin and Aber, 1997; Martin et al., 2008; Coops et al., 2003; Townsend et al., 2003) and top-of-canopy reflectance simulated from radiative transfer models (Doughty et al., 2011). However, recent research (Knyazikhin et al., 2013; Ollinger, 2011; Sullivan et al., 2013) has demonstrated a decoupling of the relationship between reflectance and %N at the canopy scale due to the influence of canopy structure, which is likely facilitating the successful prediction of canopy %N

from hyperspectral data. These findings demonstrate the need to understand how reflectance is related to physiological processes at both the leaf and canopy scale.

Recent work by Wang et al. (2007, 2010) demonstrates the ability to use remotely sensed data, specifically foliar spectral reflectance, to predict foliar $\delta^{15}\text{N}$ of fresh vegetation in specific wavelength ranges (i.e., 481-523 nm, 617-703nm, 1098-1319 nm, and 1,480-1522 nm), at leaf and canopy scales in open savannas and successional fields, respectively. Elmore and Craine (2011) also investigated the feasibility of predicting $\delta^{15}\text{N}$ with hyperspectral data in managed pastures and hay lands, focusing on the use of known absorption features of N and lignin in the near-infrared. Kleinebecker et al. (2009) successfully demonstrated the prediction of foliar $\delta^{15}\text{N}$ in dried and ground plant tissues of a variety of species found in bog complexes of the Chilean Patagonia using near-infrared spectrometry. Though the results of these studies have demonstrated the use of reflectance to predict $\delta^{15}\text{N}$, this research is still in the exploratory phase and the underlying mechanisms driving this relationship are yet to be fully understood. It has been suggested that the ability to quantify foliar $\delta^{15}\text{N}$ from reflectance data results from the well-established relationships between reflectance and %N and the correlation between %N and $\delta^{15}\text{N}$ (Hobbie et al., 2000; Wang et al., 2010; Elmore and Craine, 2011). Wang et al. (2010) also suggest that the elevated $\delta^{15}\text{N}$ of chlorins, relative to whole leaf components (Sachs 1997), and a demonstrated positive linear relationship between chlorophyll $\delta^{15}\text{N}$ and bulk organic matter $\delta^{15}\text{N}$ (Kennicutt et al, 1992) may be influencing the observed relationship between foliar $\delta^{15}\text{N}$ and reflectance due to the strong absorption of visible light by chlorophyll. Additionally, Elmore and Craine (2011) suggest the following three mechanisms: 1) the increased atomic mass of ^{15}N , relative to ^{14}N , may influence the frequency of wave motion in N-containing molecules, such as proteins, thereby influencing the shape of known N absorption features; 2) the covariance of N

availability and $\delta^{15}\text{N}$ with lignin and cellulose may allow for the modeling of foliar $\delta^{15}\text{N}$ by quantifying the concentrations of those biochemicals; 3) environmental variables, soil properties and management regimes that influence $\delta^{15}\text{N}$ may also result in changes to vegetation structure, which are observable with remote sensing at the canopy or stand scale.

If foliar %N and $\delta^{15}\text{N}$ are strongly correlated, as suggested by Wang et al. (2010) and Hobbie et al. (2000) then the previous work done to predict %N at the canopy and stand level could be built upon by simply utilizing the relationship between %N and $\delta^{15}\text{N}$. However, if there is evidence of a decoupling between %N and $\delta^{15}\text{N}$, the relationship between reflectance and $\delta^{15}\text{N}$ must first be established at the leaf-level before it can be successfully scaled to coarser spatial resolutions.

1.3 Nitrogen Fertilization

As the global population continues to rise, the need for wood products is also increasing, thereby necessitating the intensive management of forestlands to meet demands (Fox, 2000). As temperate forest ecosystems are often N limited (Vitousek and Howarth, 1991), the addition of N fertilizer is a common silvicultural practice to increase productivity on a limited land base. Douglas-fir and loblolly pine are two of the most commercially important species, representing nearly half of the standing softwood volume, over 80% of total softwood production and nearly 20% of the forest cover in the United States (Smith et al., 2007). Throughout the Pacific Northwest and southeastern US, estimates suggest that over 65,000 and 485,000 ha, respectively, of forests are fertilized annually (Briggs and Trobaugh, 2001; Fox et al., 2007), typically at a rate of 224 kg N ha⁻¹. Though these species are often N limited, stand response to fertilization is highly variable across the geographic range of each species. Volume growth following fertilization has been shown to vary from -6 to 12 m³ ha⁻¹ year⁻¹ in Douglas-fir stands (Peterson et

al., 1984), with only 70% of stands showing a positive response (Miller et al., 1986). Similar findings have been published in loblolly pine, with average volume response ranging from 1 to 8 m³ ha⁻¹ year⁻¹ (Fox et al., 2007). Previous research has shown that only a small amount, generally 10-15%, of the applied N fertilizer is actually utilized by crop trees (Mead and Pritchett, 1975; Blazier et al., 2006; Mead et al., 2008) with the remaining N allocated to various ecosystem components, such as the soil and competing vegetation, or lost from the system through volatilization and leaching. In order to increase the efficiency and precision of fertilization efforts, forest managers would benefit from a method of assessing which stands will show a positive response to N applications.

Determining a reliable, efficient and economical method of predicting fertilizer growth response has been a challenge. Previous research efforts have investigated the use of foliar mass and nutrient concentrations (Valentine and Allen, 1989; McNeil et al., 1988; Brockley, 2000), soil nutrient indices (Lea and Ballard, 1982; Hart et al., 1986), the growth of the unfertilized trees (Hart et al, 1986) and various measures of site productivity such as site index (Carter et al., 1998). Varying degrees of success have been achieved using these predictive variables, with the combination of one or more variables typically yielding the best models (Ballard, 1984).

As an integrated metric of ecosystem N cycling, $\delta^{15}\text{N}$ may aid in identifying forest stands that would be expected to respond to N fertilization as well as identifying those more susceptible to N losses.

2. Objectives

The primary objectives of this research are as follows:

1. Develop models to predict foliar $\delta^{15}\text{N}$ utilizing leaf-level hyperspectral reflectance.
2. Explore the relationships between $\delta^{15}\text{N}$ metrics and fertilizer growth response.

Each objective is covered in a separate research chapter.

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Chapter 2 - Hyperspectral Prediction of Foliar Isotopic Nitrogen

Abstract

Anthropogenic influences on the nitrogen (N) cycle have nearly doubled the rate of N inputs to terrestrial ecosystems resulting in environmental degradation of adjacent water resources through the leaching of nitrate from terrestrial to aquatic systems. Patterns in foliar nitrogen stable isotope ratios ($\delta^{15}\text{N}$) have been shown to reveal trends in terrestrial N cycles including the identification of ecosystems susceptible to N losses. This research investigates the relationship between leaf-level hyperspectral data and $\delta^{15}\text{N}$ collected throughout the geographic ranges of Douglas-fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*). Foliage was measured for reflectance using a handheld spectroradiometer (FieldSpec 3), and further analyzed for $\delta^{15}\text{N}$ and N concentration. Reflectance and various transformations of the data, including derivatives and logarithms, were correlated to $\delta^{15}\text{N}$. Resulting multiple linear regression models suggest a strong predictive ability of hyperspectral data to quantify $\delta^{15}\text{N}$, with some models explaining more than 65% of the variance in the $\delta^{15}\text{N}$. Additionally, the relationships between $\delta^{15}\text{N}$, site productivity and reflectance were explored. Moderate to strong correlations were found between site index and $\delta^{15}\text{N}$ ($R^2=0.49$) and reflectance and site index ($R^2=0.84$) in the Douglas-fir data set. The development of relationships between foliar spectral reflectance, $\delta^{15}\text{N}$ and measures of ecosystem N retention/loss will provide a framework for the rapid prediction of ecosystems susceptible to N leaching losses across large spatial scales.

1. Introduction

Human alterations to the nitrogen (N) cycle have nearly doubled the rate of N input into terrestrial ecosystems resulting in negative environmental consequences at local and global scales (Vitousek et al., 1997; Gruber and Galloway, 2008). To meet demands for food, fuel and fiber, the addition of nitrogenous fertilizer is a common practice in agriculture and forestry, as N often limits productivity in these systems (Vitousek and Howarth, 1991). Additionally, increased production of reactive N has resulted in enhanced deposition of N across the landscape (Vitousek et al., 1997; Galloway, 1998). With these anthropogenic N inputs to terrestrial ecosystems, there is an increased risk for adverse environmental impacts such as the acidification of aquatic ecosystems and soils, loss of soil nutrients and accelerated biodiversity losses of both flora and fauna (Vitousek et al., 1997). These anthropogenic inputs of N coupled with the resulting environmental degradation are cause for investigation into ecosystem-level N dynamics, particularly with respect to the capacity of terrestrial forest ecosystems to retain this added N.

Patterns in natural abundance N stable isotope ratios ($\delta^{15}\text{N}$) have been shown to integrate terrestrial N cycling dynamics and can be used to identify ecosystems that are susceptible to N losses. This is due to the isotopic discrimination, or fractionation, that occurs throughout the N cycle as various processes, such as mineralization and nitrification, which creates products that are depleted and substrates that are enriched in ^{15}N (Shearer and Kohl, 1986). With high net soil nitrification rates and substantial loss of nitrate from the system, soil and foliar $\delta^{15}\text{N}$ are gradually elevated due to the loss of the depleted products from the system (Högberg, 1997; Martinelli et al., 1999; Cheng et al. 2010; Pardo et al., 2002). For example, Högberg (1990, 1993) found that Scots pine (*Pinus sylvestris* L.) foliage showed an increase in $\delta^{15}\text{N}$ when large

amounts of added N were lost from the system. The ability to predict foliar $\delta^{15}\text{N}$ across large spatial and temporal scales would provide a framework to rapidly assess ecosystem susceptibility to N losses. Establishing a relationship between leaf-level reflectance and foliar $\delta^{15}\text{N}$ is the first step to developing a framework that would provide an efficient and non-destructive method of mapping foliar $\delta^{15}\text{N}$.

Spectroscopy data has been used for over 30 years to study vegetation spectral signatures at the leaf-level. The narrow bandwidth of the data facilitates the study of cell structure, water content and foliar biochemistry by observing slight variations in reflectance at specified wavelengths. Of particular interest in forest ecosystems is the use of reflectance data to quantify foliar N concentrations (%N). In fresh vegetation, the close correlation between N and chlorophyll has led to the use of wavelengths in the visible and red-edge regions of the electromagnetic spectrum to develop regression equations for predicting N concentrations in foliage (Yoder and Pettigrew-Crosby, 1995). Wavelengths in the near-infrared, associated with N absorption features due to the vibration of N-hydrogen bonds in proteins (Curran 1989), have also been utilized in the prediction of N concentration from leaf-level reflectance (Peterson et al., 1988; Johnson and Billow, 1996; Yoder and Pettigrew-Crosby, 1995).

With the relationship between reflectance and %N well-established at the leaf-level, researchers have successfully scaled observations to the canopy, stand and regional levels using image-level reflectance data obtained from airborne and satellite platforms (Martin and Aber, 1997; Martin et al., 2008; Coops et al., 2003; Townsend et al., 2003) and top-of-canopy reflectance simulated from radiative transfer models (Doughty et al., 2011). However, recent research (Knyazikhin et al., 2013; Ollinger, 2011; Sullivan et al., 2013) has demonstrated a decoupling of the relationship between reflectance and %N at the canopy scale due to the

influence of canopy structure which is likely facilitating the successful prediction of canopy %N from hyperspectral data.

Recent work by Wang et al. (2007, 2010) demonstrates the ability of foliar spectral reflectance to predict $\delta^{15}\text{N}$ of fresh vegetation in specific wavelength ranges (i.e., 481-523 nm, 617-703nm, 1098-1319 nm, and 1,480-1522 nm), at leaf and canopy scales in grasslands and successional fields. Elmore and Craine (2011) also investigated the feasibility of predicting $\delta^{15}\text{N}$ with hyperspectral data in managed pastures and hay lands, focusing on the use of known absorption features of N and lignin in near-infrared wavelengths. Kleinebecker et al. (2009) successfully demonstrated the prediction of foliar $\delta^{15}\text{N}$ using dried and ground plant tissues of a variety of species found in bog complexes of the Chilean Patagonia using near-infrared spectrometry. If foliar %N and $\delta^{15}\text{N}$ are strongly correlated, as suggested by Wang et al. (2010) and Hobbie et al. (2000) then the previous work done to predict %N at the canopy and stand level could be built upon by simply utilizing the relationship between %N and $\delta^{15}\text{N}$. However, if there is evidence of a decoupling between %N and $\delta^{15}\text{N}$, the relationship between reflectance and $\delta^{15}\text{N}$ must first be established at the leaf-level before it can be successfully scaled to coarser spatial resolutions.

Instead of studying the relationship between $\delta^{15}\text{N}$ and leaf-level reflectance in multiple species at a small spatial scale, as previous studies have, this research aims to develop the relationship in Douglas-fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*) throughout their geographic ranges in the Pacific Northwest and southeastern United States, respectively. Douglas-fir and loblolly pine have a dominant presence on the landscape, representing nearly half of the standing softwood volume and nearly 20% of the forest cover in the United States (Smith, 2007). With the establishment of the relationship between reflectance and $\delta^{15}\text{N}$, these

relatively homogeneous systems should facilitate future efforts to scale from leaf to canopy level observations, as the impact of species on reflectance and $\delta^{15}\text{N}$ should be minimal. In addition to developing models to predict $\delta^{15}\text{N}$ from leaf-level reflectance, this research explores the relationship between $\delta^{15}\text{N}$ and site productivity as well as the relationship between reflectance and site productivity.

2. Materials and Methods

2.1 Study Sites

This research leveraged existing regional trials of the National Science Foundation Center for Advanced Forestry Systems (NSF CAFS) with study sites located throughout the species' ranges of Douglas-fir and loblolly pine in the Pacific Northwest and southeastern United States, respectively (Figures 1 and 2). Ten sites were located in Washington and Oregon for Douglas-fir, and 18 sites located in loblolly pine plantations across eleven different states, ranging north to south from Virginia to Florida and east to west from Oklahoma to the coast of North Carolina (Figure 2). The intent of the NSF CAFS research trials is to examine the ecosystem fate and uptake efficiency of added N in mid-rotation plantations. At each site 100 m² plots were established. Plantation age varied from 6 to 21 years and density ranged from 2 to 25 trees per plot in loblolly pine. Throughout the Douglas-fir study sites, plantation age ranged from 13 to 19 years with trees per plot ranging from 7 to 13.

2.2 Field Data Collection

Fieldwork was conducted throughout the growing season of 2012. Douglas-fir study sites were visited in March and loblolly pine study sites were visited in July through early September. Foliage was collected from one tree of the control plot at each study site to measure natural abundance $\delta^{15}\text{N}$ and reflectance. Tree branches were collected from the upper one-third of the

canopy using a shotgun or pole pruner. Following the approach of Wang et al. (2007) foliage was sampled for reflectance using an ASD FieldSpec3 handheld spectroradiometer (ASD, Inc., Boulder, CO) equipped with a plant probe containing its own halogen light source and a leaf clip attachment. The FieldSpec3 has a spectral range of 350 to 2500 nm with sampling intervals of 1.4 nm from 350 to 1000 nm and 2 nm from 1000 to 2500 nm. Reflectance was measured as the ratio of the energy reflected from the sample to the energy reflected off a white reference standard of known reflectance (i.e., a Spectralon panel; Labsphere, Inc., North Sutton, NH). Dark current and white reference readings were taken after each sample, or as needed, to recalibrate the FieldSpec3 and ensure quality reflectance data collection. To minimize noise, an average of 10 spectral signatures was generated per foliage sample.

Each foliage sample of Douglas-fir consisted of 12 needles aligned to minimize overlap and maximize field of view coverage. At each Douglas-fir site the spectral signature of 10 separate foliage samples was measured. Loblolly pine foliage samples consisted of 6 to 10 fascicles of needles and varied due to the number needed to cover the entire field of view of the plant probe. Needles were sampled near their center to avoid inclusion of the fascicle sheath. Loblolly pine was sampled during the height of the growing season when foliage from the previous year (2011) and current year (2012) was present. Samples were separated in the field by flush and only foliage from 2011 or, when unavailable, the first flush from 2012 was used in subsequent analysis. The number of foliage samples collected at each loblolly pine site varied due to the amount of foliage available from the desired year and flush.

Though all practical measures were taken to ensure maximum field of view coverage this was not always achieved due to needle length and shape (i.e., the short needle length of Douglas-fir foliage). Therefore, the black background of the leaf clip attachment was used when

measuring reflectance of all foliage samples. After the spectral signatures were recorded, each sample was placed in a marked coin envelope and transported back to Virginia Tech for $\delta^{15}\text{N}$ and %N analysis.

2.3 Laboratory Analysis

Foliar samples were dried at 65°C for a minimum of three days then ground in a ball mill to ensure sample homogeneity. A subsample of foliar material was analyzed for $\delta^{15}\text{N}$ and %N using an IsoPrime 100 continuous-flow isotope ratio mass spectrometer coupled with an elemental analyzer (EA-IRMS; IsoPrime Ltd., Cheadle Hulme, UK). $\delta^{15}\text{N}$ is reported relative to the accepted standard of atmospheric N in parts per thousand (‰) deviation from the standard using the following equation:

$$\delta^{15}\text{N}(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ where } R = \frac{^{15}\text{N}}{^{14}\text{N}} \text{ (Sulzman, 2007).}$$

Foliar $\delta^{15}\text{N}$ and %N values were later averaged by site.

2.4 Hyperspectral Data

A second order Savitzky-Golay smoothing filter with a window of 5 bands was applied to further reduce noise in the signature (Cho and Skidmore, 2006). Various transformations including first and second derivatives, log 1/reflectance and the first derivative of log 1/reflectance were also calculated. These various transformations highlight features of the vegetation spectral signature that may not be apparent in the original reflectance curve. In particular the log transformation of reflectance (log 1/R), also referred to as pseudoabsorbance, simulates an absorption curve, creating peaks at the absorbing wavelengths (Serrano et al., 2002). The first derivative has also been utilized to identify features of the reflectance curve such as the location of the red-edge inflection point and the magnitude of reflectance at that

wavelength (Horler et al., 1983; Filella and Peñuelas, 1994). Signatures were averaged by study site resulting in 10 signatures for Douglas-fir and 18 signatures for loblolly pine.

Correlation curves were created by calculating the Pearson correlation coefficient for $\delta^{15}\text{N}$ and each wavelength of reflectance and its various transformations. The correlation curves were used to assist with feature selection for subsequent multiple linear regression models. Given that our understanding of the relationship between reflectance and foliar $\delta^{15}\text{N}$ in natural ecosystems is in its infancy, we identified wavelength regions with significant correlations with $\delta^{15}\text{N}$ (i.e., 3 or more channels), as well as regions known to be well correlated with N. Areas of the electromagnetic spectrum that may be affected by atmospheric water vapor (i.e. ~940, ~1140, 1350-1470, 1800-2000 nm) were excluded from selection as they may compromise the use of resulting models in future remote sensing applications. More than thirty vegetation indices related to various properties of vegetation, such as biochemicals, structure, and physiology, were calculated and included in the best subsets regression (Table 1). Also included were wavelengths previously identified for the prediction of foliar $\delta^{15}\text{N}$ (Wang et al. 2007) and %N (Gökkaya, 2012), as were the lignin and N absorption features explored by Elmore and Craine (2011) (Table 1).

2.5 Statistical Analysis

Multiple regression analysis was performed using JMP Statistical Discovery Software (JMP, Version 10). Multiple linear regression models to predict natural abundance foliar $\delta^{15}\text{N}$ from hyperspectral data were built using a best subsets approach. In this approach all possible models are compared with a user-defined number of independent variables. A maximum of three variables was chosen, however, the number of independent variables in the final models for each data set varies from one to three based on sample size and strength of the model output. The

coefficient of determination (R^2) and adjusted R^2 were used to initially assess the overall adequacy of the models. To avoid multicollinearity the variable inflation factor (VIF) was limited to 6 (Montgomery et al., 2006). Studentized residuals from each model were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Model validation was performed using a leave-one-out cross validation technique reported as the Press RMSE. The Press statistic can be used to assess how well a model will perform when predicting new data, with smaller Press values being more desirable (Montgomery et al., 2006). If the model with the highest R^2 and adjusted R^2 did not meet all of the above requirements, the next best model was chosen and the process was repeated until a satisfactory model was selected. The relationship between foliar %N and $\delta^{15}\text{N}$ was also explored by performing a simple linear regression model with %N as the independent variable and $\delta^{15}\text{N}$ as the dependent variable. These analyses were performed separately on the Douglas-fir and loblolly pine data sets as well as a combined data set of both species.

In addition, an exploratory analysis of the relationships between reflectance, $\delta^{15}\text{N}$ and site index was performed using data provided by the Stand Management Cooperative (SMC) and the Forest Productivity Cooperative (FPC). Site index (King, 1966) was provided for the Douglas-fir sites and calculated for the loblolly pine data utilizing the equation provided by Amateis and Burkhart (1985). Multiple linear regression, utilizing a best subsets approach, was performed on each data set to ascertain which, if any, of the previously identified wavelengths useful in predicting $\delta^{15}\text{N}$ may also predict site index. Furthermore, a simple linear regression was performed to explore the relationship between $\delta^{15}\text{N}$ and site index.

3. Results

3.1 Foliar $\delta^{15}\text{N}$ and %N

The distributions of $\delta^{15}\text{N}$ and %N for Douglas-fir and loblolly pine are described in Table 2. All samples across both species had negative $\delta^{15}\text{N}$ and fell within the range of natural abundance levels for foliage observed in other studies conducted in temperate forest ecosystems (Martnelli et al., 1999; Emmet et al., 1998; Pardo et al., 2006). The loblolly pine data set had a larger range of $\delta^{15}\text{N}$ (6.4‰ compared to 2.3‰) and foliar %N (0.3% compared to 0.2%) than Douglas-fir (Table 2).

Results demonstrate a decoupling of foliar %N and $\delta^{15}\text{N}$. There is no significant relationship for Douglas-fir, and only 25% of the variance in $\delta^{15}\text{N}$ is explained by %N for loblolly pine (Table 3, Figure 3). Some explanatory power is evident when the datasets are combined, but still less than half of the variance in $\delta^{15}\text{N}$ is explained by %N. This may suggest an underlying environmental gradient that is partially driving response of both %N and $\delta^{15}\text{N}$ across the large geographic extent of the study.

3.2 Reflectance and Foliar $\delta^{15}\text{N}$

The strong predictive ability of leaf-level hyperspectral data to quantify natural abundance foliar $\delta^{15}\text{N}$ is demonstrated in the multiple linear regression models for each of the data sets (Table 4, Figure 4). Wavelengths selected for inclusion in the final models were located in the visible and near-infrared regions of the spectrum. One ($R^2=0.65$, RMSE=0.43) and two ($R^2=0.81$, RMSE=0.35) variable models were selected for Douglas-fir, two ($R^2=0.54$, RMSE=1.11) and three ($R^2=0.68$, RMSE=0.96) variable models were selected for loblolly pine, and a two variable model was selected for the combined data set of both species ($R^2=0.63$, RMSE=1.03).

Selected wavelengths and vegetation indices varied across each data set. Variables selected for the Douglas-fir models included a wavelength located in the green visible region of the spectrum, specifically the second derivative of reflectance at 543 nm, and the Anthocyanin Reflectance Index (ARI) (Gitelson et al., 2001). The two-variable loblolly pine model included wavelengths located in the green and red-edge regions of the spectrum, specifically the first derivative of reflectance at 587 nm and the log of 1/reflectance at 748 nm. The Pigment Specific Simple Ratio (PSSR) (Blackburn, 1998) and reflectance at 619 nm, a wavelength previously identified by Wang et al (2007) as being useful in predicting foliar $\delta^{15}\text{N}$, were selected for inclusion in the three-variable loblolly pine model. The combined data set model included the log of 1/reflectance at 1167 nm and the second derivative of reflectance at 570 nm located in the near-infrared and green visible regions of the spectrum, respectively.

3.3 Reflectance, Site Index and $\delta^{15}\text{N}$

Site index ranged from 37 to 55m (base age 50) and 16 to 36m (base age 25) in Douglas-fir and loblolly pine, respectively. In the Douglas-fir data set, a strong positive linear relationship was found between the second derivative of reflectance at 543 nm, the same variable used in the one and two variable models for predicting $\delta^{15}\text{N}$, and site index ($R^2=0.84$). The use of site index to predict $\delta^{15}\text{N}$ also yielded interesting results with a correlation of $R^2=0.49$.

In the loblolly pine data set, the first derivative of reflectance at 677 nm explained 25% of the variation in site index. This specific wavelength was not chosen for any of the models, but it falls within the visible light region of the spectrum from which many of the selected wavelengths in the models were chosen. The relationship between site index and $\delta^{15}\text{N}$ was statistically insignificant in this data set ($p=0.43$).

4. Discussion

4.1 Hyperspectral Prediction of $\delta^{15}\text{N}$

The simplest mechanism facilitating the prediction of $\delta^{15}\text{N}$ from hyperspectral reflectance data would be the relationship between $\delta^{15}\text{N}$ and %N and the demonstrated ability of hyperspectral data to predict %N. Our results show a decoupling of the relationship between %N and $\delta^{15}\text{N}$, most notably in the Douglas-fir data set from which the strongest models were developed (Table 3, Figure 3). There is a moderate correlation between %N and $\delta^{15}\text{N}$ in the combined data set which may result from the increased range of both variables when the data are pooled (Tables 2 and 4, Figure 4), however, none of the vegetation indices specific to foliar %N nor any of the wavelengths associated with known N absorption features were selected for use in the regression models. Instead, many of the selected wavelengths and vegetation indices were located in the visible region of the electromagnetic spectrum (400-700 nm), similar to the results of Wang et al. (2007), and have been correlated to various vegetation physiological properties and biochemicals. Wavelengths at 543, 570 and 587 nm are located within the green peak of the vegetation reflectance curve which has been correlated with photosynthetic efficiency and seasonal changes in N and chlorophyll content (Peñuelas et al., 1994). The wavelength at 619 nm, previously identified by Wang et al. (2007, 2010) as being highly correlated to foliar $\delta^{15}\text{N}$, is associated with red visible light absorption by chlorophyll. The wavelength at 748 nm is located near the shoulder of the red-edge. The red-edge is a feature of the vegetation spectral signature, specific to hyperspectral data, that is caused by the shift from visible light absorption by pigments to the scattering of near infrared light by plant cellular structure (Horler et al., 1983). It is a feature that is highly correlated to total chlorophyll content and has been used to estimate various measures of productivity, such as leaf area index (LAI) and biomass, and vegetation

nutritional status such as N concentration and content (Horler et al., 1983; Filella and Penuelas, 1994; Cho and Skidmore, 2009; Lamb et al., 2002). The wavelength selected in the near-infrared at 1167 nm may be associated with the vibration of a carbon-hydrogen bond related to lignin located at 1120 nm or the vibration of an oxygen-hydrogen bond associated with water, cellulose, starch and lignin located at 1200 nm (Curran, 1989). The ARI uses wavelengths in the visible light, specifically in the green and red regions of the spectrum, and was developed to assess foliar anthocyanin content (Gitelson et al., 2001). The PSSR index was developed for the prediction of the accessory pigment chlorophyll b, utilizing wavelengths located in the red and near-infrared regions (Blackburn, 1998).

Although our experiment was not designed to investigate the relationships between $\delta^{15}\text{N}$, chlorophyll and/or other indicators of greenness, our results suggests that these mechanisms, as opposed to %N, are driving the relationships between reflectance and $\delta^{15}\text{N}$ across our experiment. Additional research investigating the relationships to $\delta^{15}\text{N}$, chlorophyll and pigments across large environmental gradients is required.

4.2 $\delta^{15}\text{N}$, Reflectance and Site Index

The use of remote sensing to directly assess aboveground forest productivity has been demonstrated at the canopy scale by developing relationships between reflectance, canopy %N and various measures of productivity such as aboveground net primary production and aboveground wood production (Smith et al., 2002). Recent work by Knyazikhin et al. (2013) and Sullivan et al. (2013) has demonstrated the strong influence of canopy structure (i.e. LAI and the number of leaves per canopy volume) on reflectance. Their findings suggest that it is canopy structure driving the relationship between reflectance and %N at the canopy-level as opposed to the leaf-level relationships between reflectance and %N. The significant correlations observed

between leaf-level reflectance and site index in Douglas-fir and loblolly pine indicate that various physiological properties known to be correlated to reflectance in the green and red visible regions of the spectrum, as previously mentioned, may also be related to measures of site productivity. Additionally, the significant correlation between $\delta^{15}\text{N}$ and site index observed in Douglas-fir demonstrates a relationship between leaf-level processes and canopy scale physiology, measured here as site index. This observed relationship may facilitate future efforts for scaling from the leaf to the canopy level as it is increasingly important to identify the driving mechanisms controlling reflectance at both leaf and canopy scales.

As site index is a measure of productivity, sites with higher site index values would be expected to be less resource deficient and specifically, less N deficient. Garten and Van Miegroet (1994) observed a strong positive correlation between foliar $\delta^{15}\text{N}$ and net N mineralization rates and several regional-scale studies have shown strong positive relationships between N mineralization and productivity (Joshi et al., 2003; Nadelhoffer et al., 1985; Pastor et al., 1984). With higher rates of N cycling and N availability, those sites would be expected to be more susceptible to N loss. As N is lost from the system, foliar $\delta^{15}\text{N}$ has been shown to become more positive over time (Högberg, 1997; Cheng et al. 2010). Therefore, a positive relationship between $\delta^{15}\text{N}$ and site index would be expected, as observed in Douglas-fir. The lack of a significant relationship between $\delta^{15}\text{N}$ and site index in loblolly pine may be due to how this species utilizes N in foliage. Loblolly pine produce 2 to 4 flushes each growing season, and nutrient concentration and weight have been shown to decrease with each subsequent flush (Schultz, 1997). Therefore, a measure of $\delta^{15}\text{N}$ scaled to the canopy, as opposed to leaf-level observations, may be more appropriate when assessing the relationship between $\delta^{15}\text{N}$ and measures of site productivity in loblolly pine.

5. Summary and Conclusions

With increasing terrestrial N additions from anthropogenic sources and atmospheric deposition there is a need to assess ecosystem N dynamics across large spatial and temporal scales. Developing the relationships between foliar spectral reflectance, $\delta^{15}\text{N}$, and ecosystem N loss would provide a method for the rapid prediction of systems susceptible to N losses at the regional-level. These results demonstrate the ability of leaf-level hyperspectral data to predict foliar $\delta^{15}\text{N}$ in Douglas-fir and loblolly pine. The exploratory analyses of the relationships between reflectance, $\delta^{15}\text{N}$ and site index provide intriguing results but require further investigation to identify the underlying interactions driving these correlations.

Future research efforts should focus on scaling from leaf to canopy level, investigating the underlying relationships between foliar $\delta^{15}\text{N}$ and reflectance and exploring the relationship between natural abundance $\delta^{15}\text{N}$ and measures of ecosystem N retention or loss of added N. A better understanding of the relationship between reflectance and foliar $\delta^{15}\text{N}$ may improve predictive capabilities and facilitate the use of reflectance to map foliar $\delta^{15}\text{N}$ at larger spatial scales and in ecosystems that are more heterogeneous in species composition and land use history.

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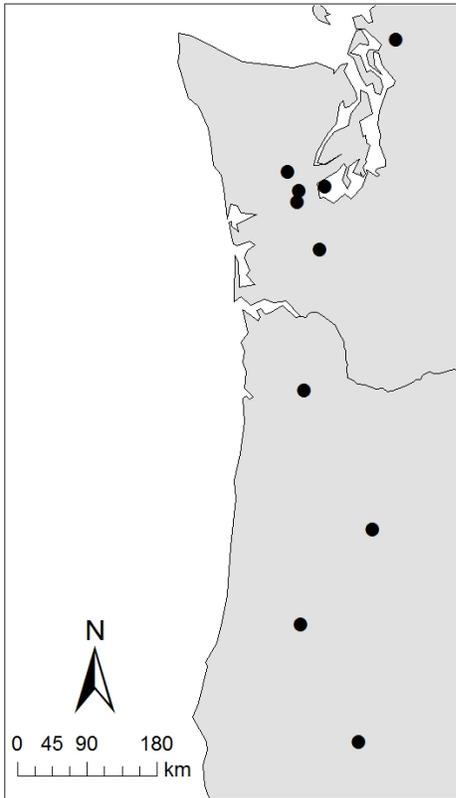


Figure 1. Map of the NSF CAFS ^{15}N fertilization study sites located in Douglas-fir plantations in Pacific Northwest region of the United States.

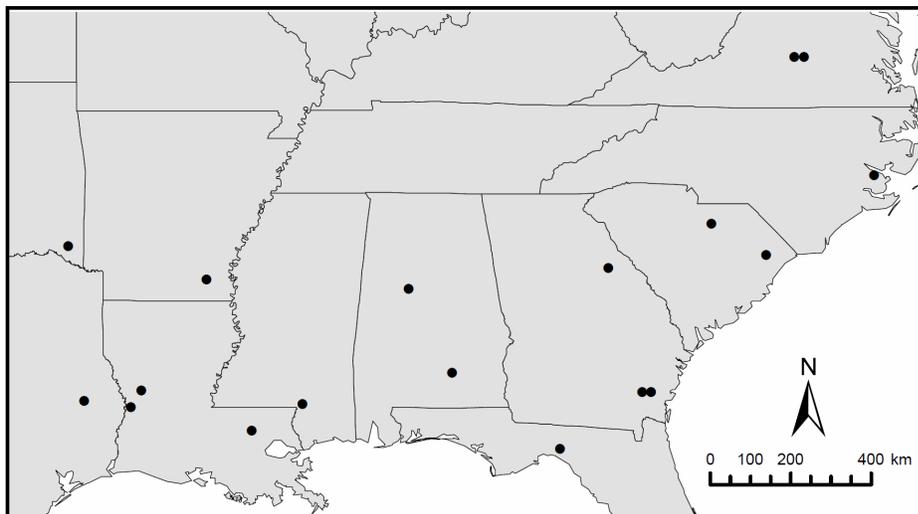


Figure 2. Map of the NSF CAFS ^{15}N fertilization study sites located in loblolly pine plantations of the southeastern United States.

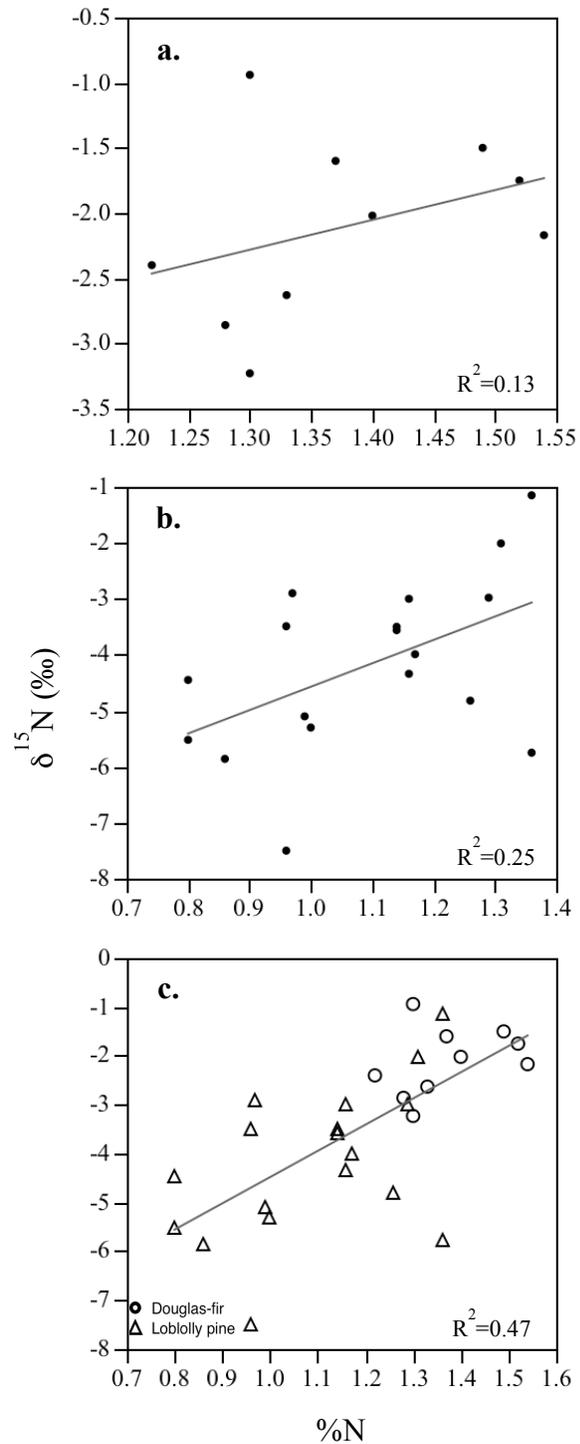


Figure 3. Relationship between foliar %N and $\delta^{15}\text{N}$ (‰) for a. Douglas-fir b. loblolly pine and c. combined data set of both species (Table 3).

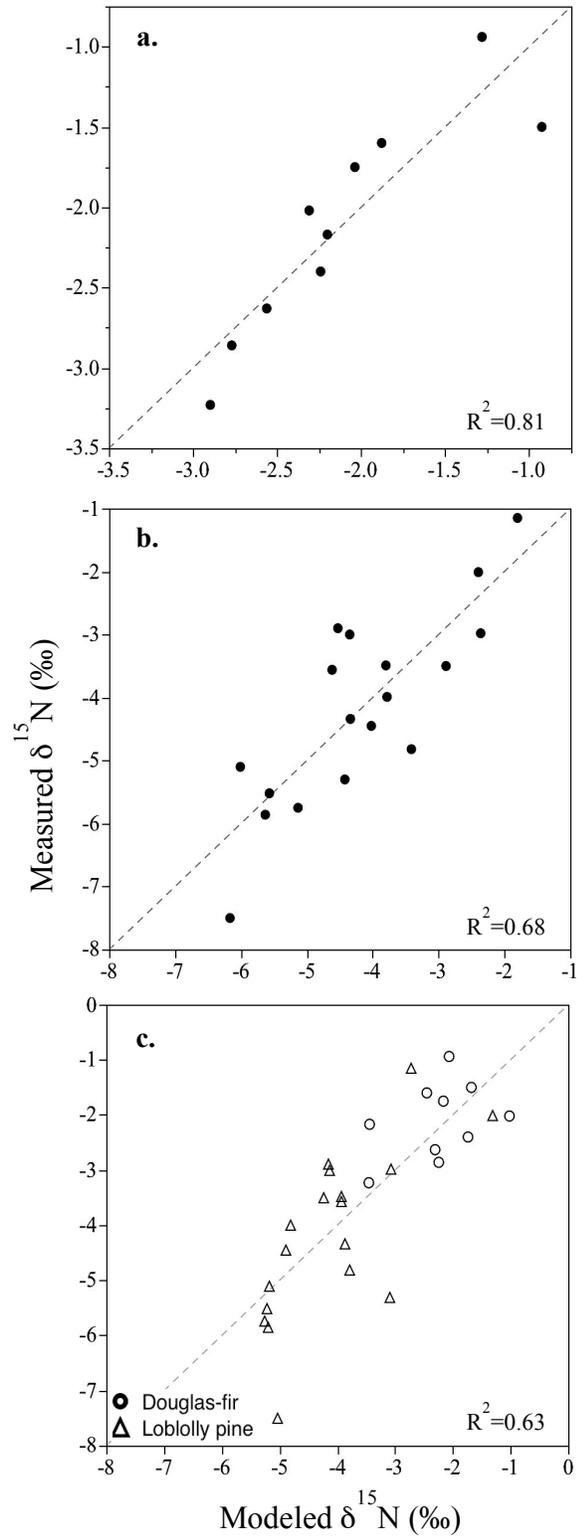


Figure 4. Multiple linear regression models predicting foliar $\delta^{15}\text{N}$ from leaf-level hyperspectral reflectance data for a. Douglas-fir (two-variable model) b. loblolly pine (three-variable model) and c. combined data set of both species (two-variable model) (Table 4).

Table 1. Vegetation indices and wavelengths of interest included in a best subsets approach to building multiple linear regression models for the prediction of natural abundance foliar $\delta^{15}\text{N}$ from hyperspectral data in Douglas-fir and loblolly pine. R=reflectance, FDR=1st derivative of R, L=log of 1/R, FDL=1st derivative of the log of 1/R.

Index or λ	Equation (nm)	Reference
ACI	$R_{\text{green}}/R_{\text{NIR}}$	Van den Berg and Perkins, 2005
ARI	$(1/R_{550}) - (1/R_{700})$	Gitelson et al., 2001
CARI	$[(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})]$	Kim, 1994
CI _{red edge}	$R_{\text{NIR}}/R_{\text{red edge}} - 1$	Gitelson et al., 2006
CRI 1	$(1/R_{510}) - (1/R_{550})$	Gitelson et al., 2002
CRI 2	$(1/R_{510}) - (1/R_{700})$	Gitelson et al., 2002
DCI	FDR_{705}/FDR_{722}	Zarco-Tejada et al., 2002
D _{max} RE	$FDR_{\text{max}(680-750)}$	Zarco-Tejada et al., 1999
D _{max} RE/D ₇₀₃	$FDR_{\text{max}(680-750)}/D_{(703)}$	Zarco-Tejada et al., 1999
EVI	$2.5 * (R_{\text{NIR}} - R_{\text{red}})/(R_{\text{NIR}} + 6 * R_{\text{red}} - 7.5 * R_{\text{blue}} + 1)$	Huete et al., 1997
Gökkaya 590	R_{590}	Gökkaya, 2012
Gökkaya 1023	R_{1023}	Gökkaya, 2012
Gökkaya 1507	R_{1507}	Gökkaya, 2012
Gökkaya 2173	R_{2173}	Gökkaya, 2012
G&M 2	R_{750}/R_{700}	Gitelson and Merzylak, 1997
HNDVI	$(R_{827} - R_{668}) / (R_{827} + R_{668})$	Opelt and Mausser, 2004
Lic 1	$(R_{800} - R_{680}) / (R_{800} + R_{680})$	Lichtenthaler et al., 1996
Lignin 1730	R_{1730}	Elmore and Craine, 2011
Lignin 2300	R_{2300}	Elmore and Craine, 2011
mARI	$[(1/R_{550}) - (1/R_{700})] * R_{800}$	Gitelson et al., 2006
MCARI	$[(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})] * (R_{700} / R_{670})$	Daughtry et al., 2000
MNDVI	$(R_{750} - R_{705}) / (R_{750} + R_{705})$	Gitelson and Merzylak, 1996
NDNI	$(L_{1510} - L_{1680}) / (L_{1510} + L_{1680})$	Serrano et al., 2002
NDVI	$(R_{\text{NIR}} - R_{\text{red}}) / (R_{\text{NIR}} + R_{\text{red}})$	Rouse et al., 1973
Nitrogen 2100	R_{2100}	Elmore and Craine, 2011
PRI	$(R_{531} - R_{570}) / (R_{531} + R_{570})$	Gamon et al., 1997
PSND	$(R_{800} - R_{650}) / (R_{800} + R_{650}); (R_{800} - R_{675}) / (R_{800} + R_{675})$	Blackburn, 1998
PSRI	$(R_{680} - R_{500}) / R_{750}$	Merzylak et al., 1999
PSSR	$(R_{800} / R_{650}); (R_{800} / R_{675})$	Blackburn, 1998
REIP	λ of $FDR_{\text{max}(650-750)}$	Horler et al., 1983
RGRI	$R_{\text{red}}/R_{\text{green}}$	Gamon and Surfus, 1999
SIPI	$(R_{800} - R_{445}) / (R_{800} - R_{680})$	Peñuelas et al., 1995
SR	$R_{\text{NIR}}/R_{\text{red}}$	Jordan, 1969
VIgreen	$(R_{\text{green}} - R_{\text{red}}) / (R_{\text{green}} + R_{\text{red}})$	Gitelson et al., 2002
Vog 1	R_{740}/R_{720}	Vogelmann et al., 1993
Vog 2	$(R_{734} - R_{747}) / (R_{715} + R_{726})$	Vogelmann et al., 1993
Wang 619	R_{619}	Wang et al., 2007
Wang 695	R_{695}	Wang et al., 2007
Wang 1135	R_{1135}	Wang et al., 2007
Wang 603	FDL_{603}	Wang et al., 2007
Wang 639	FDL_{639}	Wang et al., 2007
Wang 702	FDL_{702}	Wang et al., 2007
Wang 704	FDL_{704}	Wang et al., 2007

Table 2. Summary statistics of $\delta^{15}\text{N}$ (‰) and %N in Douglas-fir and loblolly pine foliage collected during the growing season of 2012.

	Douglas-fir		Loblolly pine	
	$\delta^{15}\text{N}$	%N	$\delta^{15}\text{N}$	%N
Min	-3.2	1.2	-7.5	0.8
Max	-0.9	1.5	-1.2	1.4
Mean	-2.1	1.4	-4.2	1.1
Range	2.3	0.2	6.4	0.3

Table 3. Linear regression models using foliar nitrogen concentration (%N) to predict natural abundance foliar $\delta^{15}\text{N}$ (‰) in Douglas-fir, loblolly pine and a combined data set of both species. An asterisk (*) indicates model significance at the $\alpha=0.05$ level.

Data Set	p-value	Coefficient	R ²	Adj. R ²	RMSE (‰)	Press RMSE (‰)
Douglas-fir (n=10)	0.30	2.29	0.13	0.02	0.68	0.74
Loblolly pine (n=18)	0.04*	4.18	0.25	0.20	1.34	1.50
Combined (n=28)	<0.01*	5.37	0.47	0.45	1.21	1.25

Table 4. Multiple linear regression models selected by a best subsets approach using leaf-level hyperspectral reflectance data to predict foliar $\delta^{15}\text{N}$ (‰) in Douglas-fir, loblolly pine and a combined data set of both species. R=reflectance, FDR=1st derivative of R, SDR=2nd derivative of R, L=log of 1/R, FDL=1st derivative of the log of 1/R.

Data Set	Wavelength (nm) or Vegetation Index	Coefficient	R ²	Adj. R ²	RMSE (‰)	Press RMSE (‰)	Shapiro-Wilk W	p-value
Douglas-fir (n=10)	SDR 543	85291.43	0.65	0.61	0.43	0.57	0.96	0.77
	SDR 543 ARI	109936.02 -1.10	0.81	0.75	0.35	0.53	0.91	0.29
Loblolly pine (n=18)	FDR 587 L 748	10909.27 -28.37	0.54	0.48	1.11	1.19	0.94	0.26
	FDR 587 PSSR	18171.06 1.71	0.68	0.61	0.96	1.07	0.97	0.78
	Wang 619	166.64						
Combined (n=28)	FDL 1167 SDR 570	9118.40 54698.60	0.63	0.61	1.03	1.07	0.95	0.21

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Chapter 3 – Predicting Forest Growth Response to Fertilization Using Natural Abundance Stable Isotopic Nitrogen

Abstract

To meet the demands of a growing global population, intensively managed forests regularly include the addition of nitrogen (N) to the system to maintain or increase productivity. Although N is most often the limiting nutrient in temperate forest ecosystems, not all stands show a positive response to N applications. Identifying a reliable method to predict fertilizer growth response would be beneficial to forest managers, but has been difficult historically. One method that has not previously been investigated is the use of natural abundance stable isotopic N signatures ($\delta^{15}\text{N}$) of the foliage and soil. Patterns in $\delta^{15}\text{N}$ can provide insights into N cycling across large geographic scales, including the identification of stands that are susceptible to N losses. This research focuses on the use of $\delta^{15}\text{N}$ as a predictor of N fertilizer growth response with study sites located throughout the geographic ranges of Douglas-fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*). Foliar $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and the enrichment factor ($\text{EF} = \delta^{15}\text{N}_{\text{Fol}} - \delta^{15}\text{N}_{\text{Soil}}$) of control trees were correlated to fertilizer response metrics including basal area, height and volume growth. Significant correlations were found between both soil $\delta^{15}\text{N}$ and the EF and growth response. When the $\delta^{15}\text{N}$ metrics were incorporated with environmental and edaphic data, results from a multiple linear regression analysis revealed increased model strength when the EF was included as an independent variable. These results demonstrate that the addition of $\delta^{15}\text{N}$ metrics in multivariate models may enhance the ability to predict fertilizer growth response across large spatial scales.

1. Introduction

As the global population continues to rise, the need for wood products is also increasing, thereby necessitating the intensive management of forestlands to meet demands (Fox, 2000). As temperate forest ecosystems are often nitrogen (N) limited (Vitousek and Howarth, 1991), the addition of N fertilizer is a common silvicultural practice to increase productivity on a limited land base. Douglas-fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*) are two of the most commercially important species, representing nearly half of the standing softwood volume, over 80% of total softwood production and nearly 20% of the forest cover in the United States (Smith et al., 2007). Throughout the Pacific Northwest and southeastern US, estimates suggest that over 65,000 and 485,000 ha, respectively, of forests are fertilized annually (Briggs and Trobaugh, 2001; Fox et al., 2007), typically at a rate of over 200 kg N ha⁻¹. Though these species are often N limited, stand response to fertilization is highly variable across the geographic range of each species. Volume growth following fertilization has been shown to vary from -6 to 12 m³ ha⁻¹ year⁻¹ in Douglas-fir stands (Peterson et al., 1984), with only 70% of stands showing a positive response (Miller et al., 1986). Similar findings have been published in loblolly pine, with average volume response ranging from 1 to 8 m³ ha⁻¹ year⁻¹ (Fox et al., 2007). Previous research has shown that only a small amount, generally 10-15%, of the applied N fertilizer is actually utilized by crop trees (Mead and Pritchett, 1975; Blazier et al., 2006; Mead et al., 2008) with the remaining N allocated to various ecosystem components, such as the soil and competing vegetation, or lost from the system through volatilization and leaching. In order to increase the efficiency and precision of fertilization efforts, forest managers would benefit from a method of assessing which stands will show a positive response to N applications.

Determining a reliable, efficient and economical method of predicting fertilizer growth response has been a challenge. Previous research efforts have investigated the use of foliar mass and nutrient concentrations (Valentine and Allen, 1989; McNeil et al., 1988; Brockley, 2000), soil nutrient indices (Lea and Ballard, 1982; Hart et al., 1986), the growth of the unfertilized trees (Hart et al, 1986) and various measures of site productivity such as site index (Carter et al., 1998). Varying degrees of success have been achieved using these predictive variables, with the combination of one or more variables typically yielding the best models (Ballard, 1984).

One approach that has not previously been investigated to predict fertilizer growth response is the use of natural abundance stable isotope ratios of N ($\delta^{15}\text{N}$). Patterns in $\delta^{15}\text{N}$ have the potential to integrate N cycling and can be used to identify ecosystems that are susceptible to or have experienced significant N losses (Pardo et al., 2002; Högberg, 1997; Cheng et al. 2010). It is the discrimination against ^{15}N , or fractionation, that occurs during chemical and physical reactions that allows natural abundance isotope ratios to be used as an ecosystem-level tool of evaluation. As N cycles through the system and undergoes the processes of mineralization, nitrification, denitrification and volatilization, fractionation creates products that are depleted and substrates that are enriched in ^{15}N (Shearer and Kohl, 1986). The amount of discrimination that occurs during each of these processes is variable and dependent upon a number of factors including the degree to which the soil is depleted relative to the foliage, N availability and the organisms transforming the N (Pardo and Nadelhoffer, 2010). It has been demonstrated that with high net soil nitrification rates and substantial loss of nitrate from the system, soil and foliar $\delta^{15}\text{N}$ are gradually elevated due to the loss of the depleted products (Högberg, 1997; Cheng et al. 2010). Specifically, Högberg (1990, 1993) found that Scots pine (*Pinus sylvestris* L.) foliage showed an increase in $\delta^{15}\text{N}$ when large amounts of added N were lost from the system. Use of

the enrichment factor ($EF = \delta^{15}N_{Fol.} - \delta^{15}N_{Soil}$) can provide further insights when assessing foliar $\delta^{15}N$ as it adjusts for the potential variation in foliar $\delta^{15}N$ that results from variable isotopic compositions of the soil (Garten et al., 2007). The EF has also been positively correlated to nitrification and mineralization rates (Emmett et al., 1998; Garten and Van Miegroet, 1994; Cheng et al., 2010) and has been used as an indicator of ecosystem N availability, with a higher EF indicating higher N availability (Emmett et al., 1998; Fang et al., 2000).

As an integrated metric of ecosystem N cycling, $\delta^{15}N$ may aid in identifying forest stands that would be expected to respond to N fertilization as well as identifying those more susceptible to N losses. This research focuses on exploring the relationships between $\delta^{15}N$ of the foliage, soil and the EF and the growth response of the two most prominent managed tree species in the United States, Douglas-fir and loblolly pine, to N fertilization. In addition, the influence that $\delta^{15}N$ metrics may have on predictive models when coupled with site, climatic and edaphic data is explored.

2. Materials and Methods

2.1 Study Sites

This research utilized data and samples from previously existing regional fertilization trials of the Stand Management Cooperative (SMC) and the Forest Productivity Cooperative (FPC) with study sites located throughout the geographic ranges of Douglas-fir (Figure 5) and loblolly pine (Figure 6) in the Pacific Northwest and southeastern US, respectively.

Sites located in Douglas-fir stands were part of SMC paired-tree fertilization studies with fifty-six sites established in the winter seasons of 2007 to 2009 in mid-rotation Douglas-fir plantations distributed from northern Washington to southern Oregon (Figure 5). Study sites were dispersed over a range of latitudes, elevations, slopes and slope positions to explore the

influence of site and soil characteristics on Douglas-fir growth response to N fertilization (Littke, 2012). At each study site, 48 dominant and codominant trees were included in the paired-tree study. From those 48, 12 to 20 pairs of trees were chosen based on similarity in diameter at breast (1.4m) height (dbh) and crown length. One tree from each pair was fertilized at a rate of 224 kg N ha⁻¹ in the spring following plot establishment. The treatment area was 79.5m² with the target tree located in the center. Of the 56 study sites, 54 were utilized in subsequent analysis due to data and archived sample availability.

Determining optimal rates and frequencies of fertilizer application to maintain high productivity in juvenile loblolly pine plantation forests are among the objectives of the FPC regional study. Twenty-six study sites were established throughout the southeastern US from 1998 to 2003, ranging north to south from Virginia to Florida and east to west from Texas to North Carolina (Figure 6). Of these 26 study sites, 22 were used in this research due to data and archive sample availability. At each study site, experimental plots received varying rates and frequencies of N fertilizer application resulting in seven treatments (Table 5). Fertilization rates varied from 67 to 269 kg N ha⁻¹ with frequency of application ranging from every-other year to every six years. In addition to N fertilization, phosphorus (P) was applied at a rate of 10% the N application rate. Potassium (K) and boron (B) were also applied at a rate of 40% and 0.5% the N application rate, respectively.

2.2 Foliage and Soil Samples

Douglas-fir foliage samples were collected during the dormant seasons of 2009 to 2011, two years following study plot installation. Six unfertilized trees from each plot were chosen at random and current year foliage was shot or cut down from the upper crown. Foliage was then dried at 65°C and ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Soil samples

were collected from one central location at each plot, avoiding primary roots of trees, and separated by horizon. Surface mineral soil samples were air-dried to a constant weight, sieved to 2mm, and ground.

Loblolly pine foliage samples were collected from December to mid-February in 1998 to 2003, during the year of study site installation. A composite sample of 100 fascicles from five dominant or codominant trees was collected from the first flush of the most recent growing season from the primary lateral branches of the upper one-third of the live crown. A horizon soil samples were collected during the dormant season and were a composite of at least ten randomly located soil cores taken throughout each plot. Subsurface soil samples (E or B horizons) were collected from only a subset of plots at each study site as described for the A horizon. However, if the A horizon was less than 2 inches thick, subsurface soil samples were collected at each plot.

Paired sub-samples of foliar and soil material from each study site were obtained from SMC and FPC sample archives and further processed at Virginia Tech as described below.

2.3 Stable Isotopic Analysis

Following Templer et al. (2007) foliar and soil samples were dried at 65°C and further ground in a ball mill to ensure sample homogeneity. A small amount of foliar and soil material (3.5 – 5.0 mg and 40.0 – 70.0 mg, respectively) from each sample was analyzed for $\delta^{15}\text{N}$ and %N using an IsoPrime 100 continuous-flow isotope ratio mass spectrometer coupled with an elemental analyzer (EA-IRMS; IsoPrime Ltd., Cheadle Hulme, UK) with 10% of the samples replicated in triplicate for quality assurance/quality control. Stable isotopic N is reported relative to the accepted standard of atmospheric N in parts per thousand (‰) deviation from the standard using the following equation:

$$\delta^{15}\text{N}(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ where } R = \frac{^{15}\text{N}}{^{14}\text{N}} \text{ (Sulzman, 2007).}$$

Therefore, $\delta^{15}\text{N}$ can be either positive or negative depending on the relative abundance of ^{15}N in the sample compared to atmospheric N. Utilizing $\delta^{15}\text{N}$ values obtained for the foliage ($\delta^{15}\text{N}_{\text{Fol}}$) and soil ($\delta^{15}\text{N}_{\text{Soil}}$), the enrichment factor ($\text{EF} = \delta^{15}\text{N}_{\text{Fol}} - \delta^{15}\text{N}_{\text{Soil}}$) was calculated for each pair of samples from each study site.

2.4 Fertilizer Growth Response

Growth response metrics of basal area, height and dbh were provided or calculated from the data made available by the SMC and FPC. Two-year N fertilizer growth response data was provided for the Douglas-fir sites and calculated as the average percent difference in growth between paired fertilized and control trees at each plot. The regional study located in loblolly pine is a long-term research study and growth response data was provided for a large range of response duration. An eight-year post initial fertilization response duration was chosen for these sites as it provided the longest post-fertilization response period while maintaining the largest number of study sites. Growth response data for the loblolly pine sites were calculated in the same manner as the Douglas-fir sites using the following equation:

$$\text{Growth response (\%)} = \left[\frac{\text{Growth of Fertilized} - \text{Growth of Control}}{\text{Growth of Control}} \right] * 100$$

Therefore, growth response can be zero or negative if trees showed no response to fertilization or exhibited less growth than the control plots, respectively.

2.5 Ancillary Data

Climatic (i.e., seasonal precipitation and temperature), site (i.e., elevation and stocking density), and edaphic (i.e., parent material and forest floor C:N ratio) data were provided by the SMC for each of the Douglas-fir study sites. The minimum, maximum, mean and standard deviation of a subset of the continuous variables are provided in Table 6. These data were compiled by Littke (2012) and originally obtained from various sources including the

ClimateWNA program, field data collection, United States Geological Survey maps and Natural Resources Conservation Services soil series descriptions.

2.5 Statistical Analysis

Distributions of the $\delta^{15}\text{N}$ and growth response metrics were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Many of the growth response metrics from the loblolly pine data set had non-normal distributions, as did $\delta^{15}\text{N}_{\text{Fol}}$ and the EF from the Douglas-fir data set. Therefore, Spearman's correlation coefficient was utilized to relate $\delta^{15}\text{N}$ metrics to each of the growth response metrics as it is a nonparametric rank statistic that measures the strength of association between two variables but does not require the data to be normally distributed (Darity, 2008).

In order to explore the effect of $\delta^{15}\text{N}$ metrics on improving more traditional fertilizer growth response predictions, a subset of our data will be used in a multiple linear regression approach with and without $\delta^{15}\text{N}$ data. Specifically, we will utilize available ancillary data for the Douglas-fir sites to develop multiple linear regression models that predict fertilizer growth response, utilizing a best subsets approach. Studentized residuals of the models were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965).

3. Results

The distributions of the $\delta^{15}\text{N}$ values are presented in Figure 7. In both data sets, median values of $\delta^{15}\text{N}_{\text{Fol}}$ and the EF were negative and median $\delta^{15}\text{N}_{\text{Soil}}$ was positive. Slight differences between the two systems were apparent. The median $\delta^{15}\text{N}_{\text{Fol}}$ of Douglas-fir was more negative than that of loblolly pine (-2.7 and -1.2‰, respectively) while the median $\delta^{15}\text{N}_{\text{Soil}}$ was more positive (2.8 and 1.2‰, respectively). Thus, the EF was generally more negative in Douglas-fir. The range of $\delta^{15}\text{N}_{\text{Fol}}$ observed in Douglas-fir was larger than that of loblolly pine (6.8 and 2.9‰,

respectively), while the range in $\delta^{15}\text{N}_{\text{Soil}}$ was smaller (5.1 and 7.0‰, respectively). The range of EF values observed in both data sets was approximately 7.0‰.

The minimum, maximum, average, range and standard deviation of growth response for each species across all sites are shown in Table 7. Growth response to N fertilization was highly variable in both species with negative growth responses observed in both data sets. Loblolly pine showed a larger range of response than Douglas-fir. The maximum ranges of growth response were 210% for volume and 74% for height growth of loblolly pine and Douglas-fir, respectively.

Spearman's rank coefficients of correlation relating $\delta^{15}\text{N}$ to growth response are provided in Tables 8 and 9. Three significant correlations were found in Douglas-fir while four were observed in loblolly pine across all treatments. In the Douglas-fir data, significant relationships were found between both $\delta^{15}\text{N}_{\text{Soil}}$ and the EF and basal area growth response. A significant correlation was also observed between the EF and volume growth response (Table 8). Significant relationships in the loblolly pine data set occurred in two of the seven treatments, both fertilized at a frequency of once every four years at rates of 202 and 269 kg N ha⁻¹. $\delta^{15}\text{N}_{\text{Soil}}$ and both height and volume growth response were significantly correlated, as was the EF and height growth response (Table 9). In both species, no significant correlations were found between $\delta^{15}\text{N}_{\text{Fol}}$ and growth response. Across both data sets there were mostly positive correlations between $\delta^{15}\text{N}_{\text{Soil}}$ and each of the growth response metrics. Negative correlations between the EF and growth response were consistent across both species.

Focusing on a subset of data with the most significant results from the Spearman's rank correlations (Table 8), the addition of $\delta^{15}\text{N}$ metrics to the climatic, site and edaphic variables facilitated the prediction of basal area growth response in Douglas-fir using multivariate models

(Table 10). When the regression was performed without the $\delta^{15}\text{N}$ metrics, a satisfactory model was not obtained due to the lack of significance in one or more of the independent variables. With the addition of the $\delta^{15}\text{N}$ metrics, the best two-variable model selected included the EF and the forest floor C:N ratio, explaining 39% of the variance in basal area growth. When effective depth, defined as the depth of the soil profile after removing rocks and gravel, was included to form a 3-variable model R^2 increased to 0.44.

4. Discussion

In both data sets, median $\delta^{15}\text{N}_{\text{Fol}}$ was depleted relative to $\delta^{15}\text{N}_{\text{Soil}}$, demonstrating the fractionation that occurs in soil microbial processes and plant N uptake (Shearer and Kohl, 1986; Hogberg, 1997; Robinson, 2001). The greater overlap of foliar and soil $\delta^{15}\text{N}$ in loblolly pine, compared to Douglas-fir, may suggest that a greater portion of organic N turns over annually in loblolly pine systems. Additionally, the degree to which these species are associated with mycorrhizal fungi is likely to influence $\delta^{15}\text{N}$. Mycorrhizae have been shown to contribute significantly to fractionation during N uptake (Hogberg, 1990) and host plants are likely to receive depleted N due to the preferential transfer of isotopically depleted N demonstrated by mycorrhizae (Hobbie, 1997). Therefore, vegetation exhibiting a greater dependence on mycorrhizae to obtain N may have $\delta^{15}\text{N}_{\text{Fol}}$ that are more depleted, relative to $\delta^{15}\text{N}_{\text{Soil}}$, than would be observed in species and stands that are less dependent on this symbiotic relationship. Though the degree of mycorrhizal association was not measured for these study sites, both Douglas-fir and loblolly pine are known to commonly form ectomycorrhizal associations. An additional interpretation of the greater separation between $\delta^{15}\text{N}_{\text{Fol}}$ and $\delta^{15}\text{N}_{\text{Soil}}$ observed in Douglas-fir is that these sites obtain more of their N from their fungal associations than the loblolly pine sites. Furthermore, species preference, or utilization, of nitrate over ammonium would influence

$\delta^{15}\text{N}_{\text{Fol}}$ resulting in foliage that is more depleted in ^{15}N compared to species that utilize ammonium (Pardo and Nadelhoffer, 2010). This is due to the higher degree of fractionation observed during nitrification, compared to ammonification, with the resulting product of nitrate have a depleted, or more negative, $\delta^{15}\text{N}$ relative to the remaining ammonium substrate (Pardo and Nadelhoffer, 2010). Therefore, the more negative median $\delta^{15}\text{N}_{\text{Fol}}$ observed in Douglas-fir may suggest a stronger preference, or utilization, of nitrate over ammonium in Douglas-fir when compared to loblolly pine.

A high degree of variability in growth response was observed across all sites of both species, consistent with other studies (Peterson et al., 1984; Miller et al., 1986). Variation in response may be due to a number of pre-fertilization site conditions. Growth response in Douglas-fir has been shown to decrease as site index increases (Heath and Chappell, 1989) as highly productive stands are less likely to be nutrient deficient, therefore, showing less response to N additions. Moisture availability has also been shown to limit productivity in Douglas-fir (Mika et al., 1992). Sites with low available moisture have shown little response to fertilization as it is moisture, not N (post-fertilization), that is limiting productivity (Mika et al., 1992). In loblolly pine, natural soil drainage class has been shown to influence height growth response, with sites classified as being more poorly drained showing greater height growth than those classified as being well drained (Amateis et al., 2000). Deficiencies in K (Mika and Moore, 1990) and sulfur (S) (Brockley and Swift, 1990), determined from foliar ratios of K:N and S:N, have also been shown to hinder Douglas-fir response to N fertilization.

The consistent negative correlation observed between the EF and the growth response metrics indicates that sites with higher EF values are less responsive to fertilization. Previous research has demonstrated significant positive correlations between the EF and a number of N

cycling transformations such as mineralization and nitrification rates (Garten and Van Miegroet, 1994; Cheng et al., 2010; Takebayashi et al., 2010), and has found that sites with greater N availability have increased, or less negative, EF values (Fang et al., 2010). Though rates of mineralization and nitrification were not directly measured for this research, if we utilize the aforementioned relationships we can presume that stands with increased EF values are likely to have increased N availability and cycling rates which may explain why they are less responsive to fertilization as they are less N limited.

The positive correlation between $\delta^{15}\text{N}_{\text{Soil}}$ and growth response was unexpected as positive linear relationships have been observed between $\delta^{15}\text{N}$ of organic soil and net mineralization and nitrification rates (Templer et al., 2007) and higher rates of N cycling have been shown to cause an overall enrichment of $\delta^{15}\text{N}_{\text{Soil}}$ (Mariotti et al., 1981). Using the same rationale as presented above, a negative correlation with growth response would be anticipated.

The lack of significant relationships between $\delta^{15}\text{N}_{\text{Fol}}$ and growth response and the unexpected relationship between $\delta^{15}\text{N}_{\text{Soil}}$ and growth response indicate that use of the EF provides a better method of predicting growth response to fertilization. This is further evidenced by the inclusion of the EF, as opposed to the other $\delta^{15}\text{N}$ metrics, in multiple linear regression models to predict basal area growth response in the Douglas-fir sites (Table 6). Previous studies have identified the forest floor C:N ratio as being correlated to volume growth response following urea fertilization (Edmunds and Hsiang, 1987) and the EF has been shown to be correlated measures of N cycling and availability (Garten and Van Miegroet, 1994; Cheng et al., 2010; Takebayashi et al., 2010; Fang et al., 2010). Both of these variables are related to nutrient cycling rates, with higher C:N ratios and more negative EF values being indicative of sites more likely to respond to N fertilization. In addition, effective depth provides a measure of the amount of soil available to

roots and can be indicative of the capacity of the soil to hold moisture and nutrients (Littke, 2012). While this was an exploratory analysis, our results demonstrate that the inclusion of the EF in regression models may further enhance future research efforts to predict fertilizer growth response.

The ability to predict $\delta^{15}\text{N}_{\text{Fol}}$ utilizing hyperspectral reflectance has been demonstrated at the leaf-level in Douglas-fir (Chapter 2), but our results show no significant correlations between $\delta^{15}\text{N}_{\text{Fol}}$ and growth response metrics. Instead, our results show that the EF would need to be modeled at the landscape-level for inclusion in regression models to assess growth response at large spatial scales. Since the EF is calculated as the difference between foliar and soil $\delta^{15}\text{N}$, remote sensing could be used to obtain $\delta^{15}\text{N}_{\text{Fol}}$ values once observations are successfully scaled to the regional level, however, determining $\delta^{15}\text{N}_{\text{Soil}}$ would require field sampling and isotopic analysis.

5. Summary and Conclusions

Our results show significant correlations between $\delta^{15}\text{N}_{\text{Soil}}$ and the EF and growth response of the two predominant managed softwood timber species in the United States. Though the correlations may have limited predictive power on their own, an increase in R^2 was observed when the EF was included in a multiple linear regression model along with climatic, site and edaphic variables to predict basal area growth response in Douglas-fir. These observations suggest that inclusion of $\delta^{15}\text{N}$ metrics in future efforts to predict fertilizer growth response may provide useful, particularly for certain species and under certain environmental conditions.

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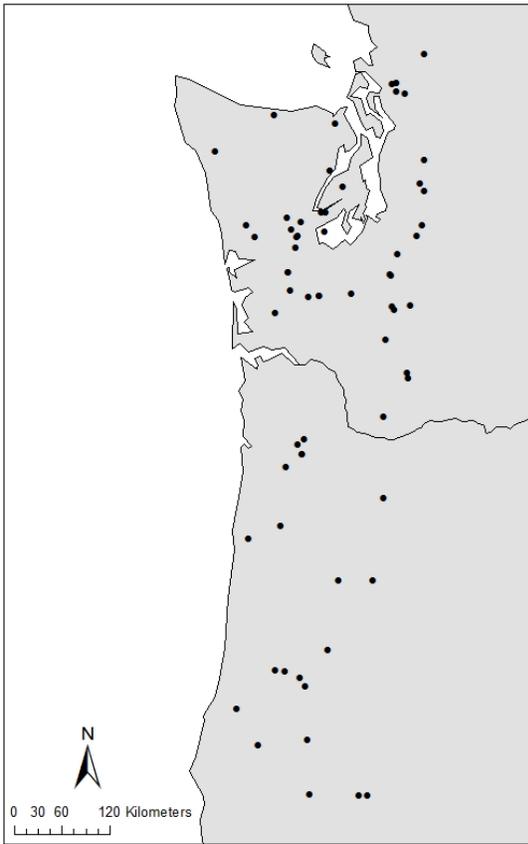


Figure 5. Study site locations of the Stand Management Cooperative paired-tree fertilization trials distributed throughout the geographic range of coastal Douglas-fir in the United States.

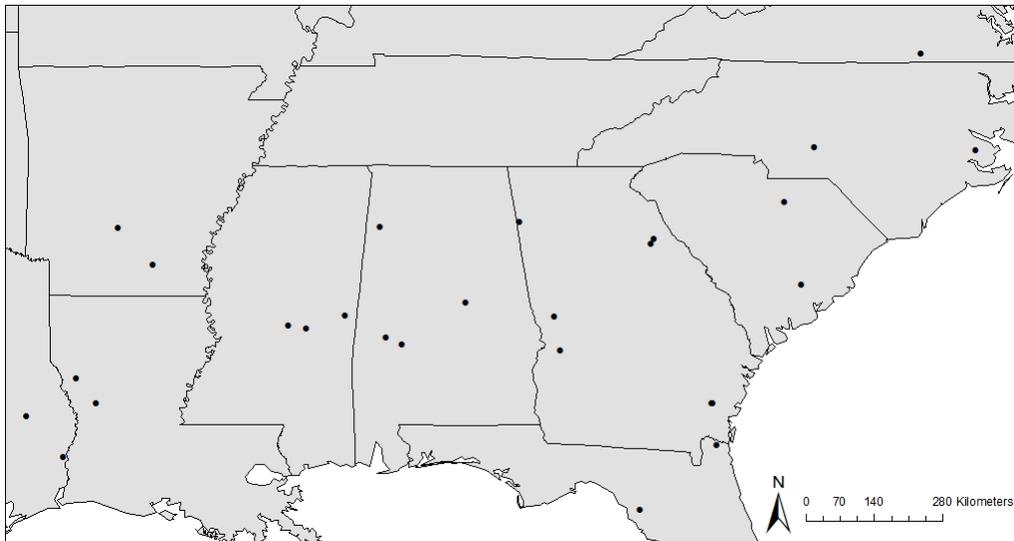


Figure 6. Study site locations of the Forest Productivity Cooperative regional fertilization trials distributed throughout the geographic range of loblolly pine.

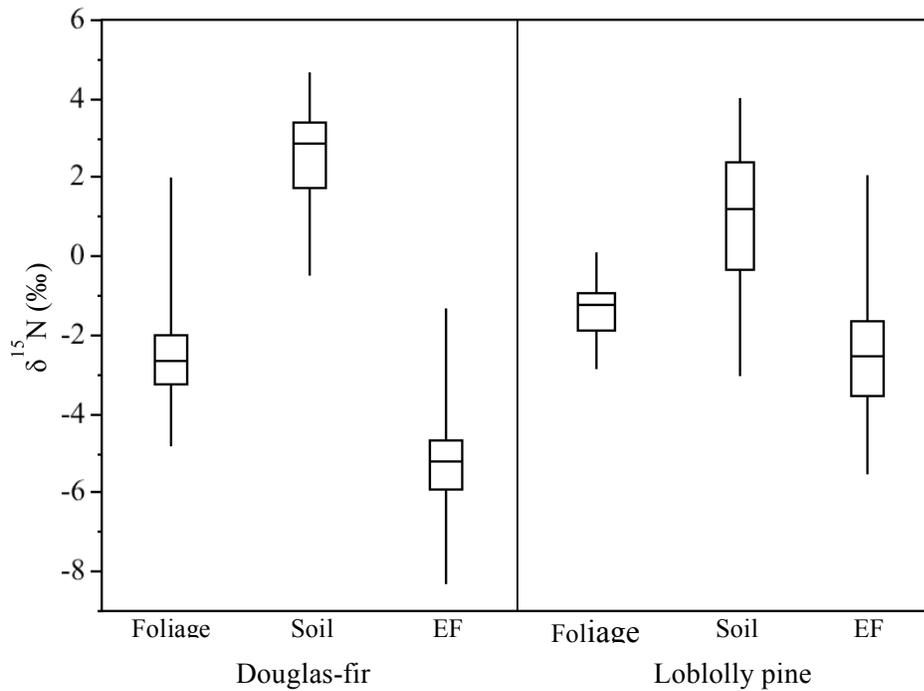


Figure 7. Box-whisker plot of $\delta^{15}\text{N}$ of the foliage, soil and the enrichment factor ($\text{EF} = \delta^{15}\text{N}_{\text{Fol}} - \delta^{15}\text{N}_{\text{Soil}}$) for Douglas-fir and loblolly pine samples collected throughout the geographic ranges of each species. Boxes show the interquartile range, the line in the box is the median and the bars show the range.

Table 5. Rates and frequencies of nitrogen fertilizer application for plot treatments of the Forest Productivity Cooperative regional fertilization trials.

Trt. Code	Freq. (yrs)	Rate (lbs ac ⁻¹)	Rate (kg ha ⁻¹)
206	2	60	67
212	2	120	135
218	4	180	202
412	4	120	135
418	4	180	202
424	4	240	269
624	6	240	269

Table 6. Minimum, maximum, mean and standard deviation for a subset of site, climatic and edaphic variables, provided by the Stand Management Cooperative, for Douglas-fir study sites located throughout the Pacific Northwest. Seasonal temperatures are an average while seasonal precipitation is cumulative.

Variable	Min.	Max.	Mean	Std. Dev.
Slope (%)	0	60	14	13
Elevation (m)	46	1341	335	251
Trees ha ⁻¹	475	1134	799	158
Height:dbh ratio	60	93	74	7
King's site index	32	55	43	4
Stand age	11	25	18	3
Foliar N (%)	0.8	1.7	1.3	0.1
Needle area (cm ²)	24.5	52.7	35.5	5.2
Winter temp. (°C)	0.8	7.0	4.3	1.2
Spring temp. (°C)	5.5	11.4	9.3	1.1
Summer temp. (°C)	14.7	18.8	16.8	0.9
Autumn temp. (°C)	7.8	12.8	10.7	0.9
Winter precip. (mm)	303.2	1325.9	707.0	248.7
Spring precip. (mm)	225.7	677.7	402.1	111.5
Summer precip. (mm)	52.8	223.1	131.0	44.0
Autumn precip. (mm)	229.6	865.0	477.4	144.6
Organic matter (%)	1.0	10.8	3.6	2.4
Clay content (%)	3.0	47.8	23.3	12.8
Sand content (%)	7.6	86.1	34.6	22.3
Effective depth (cm)	12	100	77	22
A horizon depth (cm)	0	63	24	15
Lowest soil moisture (%)	0.0	24.0	9.9	5.6
Plant available water (%)	3.3	22.0	11.8	4.2
Total soil N content (kg N ha ⁻¹)	280	23462	9958	5230
Soil A horizon C:N ratio	11	37	21	4
Forest floor C:N ratio	17	63	33	10

Table 7. Summary statistics of percent growth response, calculated relative to the growth of the control plots, to nitrogen fertilization in study sites located throughout the geographic ranges of Douglas-fir and loblolly pine utilizing growth response durations of two and eight years, respectively.

	Douglas-fir			Loblolly pine		
	BA	Ht.	Vol.	BA	Ht.	Vol.
Min	-23.5	-12.6	-12.7	-34.3	-12.5	-30.4
Max	41.5	61.1	34.1	118.8	49.7	179.7
Mean	9.2	6.2	7.3	30.1	8.4	36.9
Range	65.0	73.7	46.8	153.1	62.2	210.1
Std. Dev.	15.2	12.3	10.1	26.5	12.9	40.4

Table 8. Spearman’s rank coefficient of correlation between two-year growth response metrics, calculated as percent growth of fertilized plots relative to the growth of the control plots, and $\delta^{15}\text{N}$ metrics in Douglas-fir. P-values are shown in parentheses. An asterisk (*) denotes significance at the $\alpha=0.10$ level.

	$\delta^{15}\text{N}_{\text{Fol}}$	$\delta^{15}\text{N}_{\text{Soil}}$	EF
Basal area	-0.14 (0.30)	0.29 (0.03)*	-0.34 (0.01)*
Height	-0.17 (0.20)	-0.04 (0.76)	-0.04 (0.75)
Volume	-0.17 (0.21)	0.19 (0.16)	-0.27 (0.05)*

Table 9. Spearman’s rank coefficient of correlation between eight-year growth response metrics, calculated as percent growth of fertilized plots relative to the growth of the control plots, and $\delta^{15}\text{N}$ metrics in loblolly pine separated by fertilization treatment. P-values are shown in parentheses. An asterisk (*) denotes significance at the $\alpha=0.10$ level.

Trt	Basal Area			Height			Volume		
	$\delta^{15}\text{N}_{\text{Fol}}$	$\delta^{15}\text{N}_{\text{Soil}}$	EF	$\delta^{15}\text{N}_{\text{Fol}}$	$\delta^{15}\text{N}_{\text{Soil}}$	EF	$\delta^{15}\text{N}_{\text{Fol}}$	$\delta^{15}\text{N}_{\text{Soil}}$	EF
206	0.22 (0.33)	0.22 (0.39)	-0.12 (0.64)	0.18 (0.43)	0.20 (0.43)	-0.11 (0.66)	0.25 (0.26)	0.23 (0.35)	-0.13 (0.62)
212	0.25 (0.26)	0.24 (0.33)	-0.21 (0.39)	0.12 (0.58)	0.12 (0.64)	-0.18 (0.47)	0.26 (0.24)	0.30 (0.22)	-0.26 (0.30)
218	0.16 (0.48)	0.00 (1.00)	-0.03 (0.92)	-0.10 (0.67)	0.19 (0.45)	-0.35 (0.15)	0.18 (0.42)	0.11 (0.65)	-0.14 (0.57)
412	0.20 (0.36)	-0.08 (0.75)	0.06 (0.80)	-0.03 (0.91)	0.16 (0.52)	-0.33 (0.18)	0.14 (0.53)	0.04 (0.87)	-0.14 (0.57)
418	0.26 (0.24)	0.24 (0.34)	-0.13 (0.62)	0.04 (0.86)	0.40 (0.10)*	-0.44 (0.07)*	0.22 (0.33)	0.40 (0.10)*	-0.36 (0.14)
424	0.21 (0.35)	0.14 (0.59)	-0.07 (0.77)	-0.02 (0.94)	0.39 (0.11)	-0.47 (0.05)*	0.17 (0.44)	0.27 (0.29)	-0.22 (0.38)
624	0.28 (0.21)	0.21 (0.41)	-0.14 (0.60)	0.36 (0.11)	0.36 (0.15)	-0.25 (0.32)	0.36 (0.11)	0.26 (0.30)	-0.16 (0.53)

Table 10. Multiple linear regression models, selected by a best subsets approach, using $\delta^{15}\text{N}$ metrics and climatic, site and edaphic data to predict the basal area growth of Douglas-fir in response to N fertilization. Basal area growth response was calculated as percent growth of fertilized plots relative to the growth of the control plots.

Variables	R²	Adj. R²	RMSE (%)	Press RMSE (%)	p-value	Shapiro-Wilk W	p-value
Forest Floor C:N Ratio	0.24	0.23	13.4	13.5	<0.01	0.97	0.16
Forest Floor C:N Ratio EF	0.39	0.36	12.4	12.6	<0.01	0.98	0.33
Forest Floor C:N Ratio EF Effective Depth (cm)	0.44	0.41	11.9	12.1	<0.01	0.99	0.76

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Chapter 4 – Summary and Conclusions

Due to the increase of N deposition in terrestrial ecosystems from anthropogenic sources such as fertilization and fossil fuel combustion, there is a need to develop a method of assessing ecosystem N dynamics across large spatial and temporal scales. Developing the relationships between hyperspectral reflectance, $\delta^{15}\text{N}$, and a measure of ecosystem N retention would provide a method for the rapid prediction of systems susceptible to N losses at the regional-level. This research focused on developing the relationship between leaf-level hyperspectral reflectance and $\delta^{15}\text{N}$ and the relationship between $\delta^{15}\text{N}$ and fertilizer growth response utilizing study sites distributed throughout the species' ranges of Douglas-fir (*Pseudotsuga menziesii*) and loblolly pine (*Pinus taeda*).

The results of this research demonstrate a strong predictive ability of leaf-level hyperspectral reflectance to quantify foliar $\delta^{15}\text{N}$ in both species with single and multivariate regression models explaining more than 60% of the variance in the data. The decoupling of $\delta^{15}\text{N}$ and %N observed in this study demonstrates the need to develop models specific to $\delta^{15}\text{N}$. Many of the wavelengths selected for use in the predictive models fell within the visible portion of the electromagnetic spectrum (400-700 nm). Though this research was not designed to identify the underlying mechanism facilitating the prediction of $\delta^{15}\text{N}$ from reflectance, our results suggest that pigments, such as chlorophyll, and other biochemicals that influence vegetation reflectance of visible light may be driving the relationship, as opposed to %N.

Additionally, this research explored the relationships between $\delta^{15}\text{N}$ of the foliage, soil and the enrichment factor ($\text{EF} = \delta^{15}\text{N}_{\text{Fol}} - \delta^{15}\text{N}_{\text{Soil}}$) and N fertilizer growth response. Predicting growth response has been a challenge as it necessitates incorporating many processes occurring at multiple temporal and spatial scales. Patterns in $\delta^{15}\text{N}$ have been previously identified to

integrate N cycling and have been correlated to specific N cycling processes, such as net nitrification and mineralization rates. Additionally, the enrichment factor has been shown to adjust for the spatial heterogeneity of soil $\delta^{15}\text{N}$ and has been positively correlated to N availability. Significant correlations were observed in this research between $\delta^{15}\text{N}_{\text{Soil}}$ and the EF and the growth response metrics in both Douglas-fir and loblolly pine. When the $\delta^{15}\text{N}$ metrics were included with climatic, edaphic and sites variables in a multiple linear regression to predict fertilizer growth response in Douglas-fir, the EF facilitated an increase in model explanatory power.

Future research efforts should focus on scaling from leaf-level to canopy level, investigating the underlying relationships between foliar $\delta^{15}\text{N}$ and reflectance and exploring the relationship between natural abundance $\delta^{15}\text{N}$ and direct measures of ecosystem N retention or loss of added N. As data from the NSF CAFS ^{15}N fertilization mapping study is synthesized into a mass balance of the added N, relationships between $\delta^{15}\text{N}$ and N allocation will be explored. Based on previous studies, we expect the sites with more positive foliar $\delta^{15}\text{N}$ to show increased susceptibility to N loss, however the influence of the climatic and edaphic factors on fractionation and soil N cycling dynamics will need to be addressed. A better understanding of the relationship between reflectance and foliar $\delta^{15}\text{N}$ may improve predictive capabilities and facilitate the use of reflectance to map foliar $\delta^{15}\text{N}$ at larger spatial scales and in ecosystems that are more heterogeneous in species composition and land use history. The inclusion of the EF with other site, climate and edaphic variables in multivariate regression models to predict fertilizer growth response in species other than those included in this research should also be explored.