Ecosystem N Cycling through Secondary Succession following Agricultural Abandonment

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Abstract

Secondary succession is occurring worldwide as ecosystems regenerate following human and natural disturbances such as logging, agriculture, fires, and hurricanes. Most of today's forests are in a state of secondary succession (as opposed to old growth), and agricultural abandonment is a significant driver of this trend. Secondary succession is currently taking place throughout the East Coast of the United States due to agricultural clearing and subsequent abandonment as agriculture has moved westward in the 19th and 20th centuries. The consequences of this shift in terms of carbon (C) cycling are relatively well understood, as the C cycle consists predominantly of a simple balance between photosynthesis and ecosystem respiration. However, the effects of large swaths of secondary successional land on nitrogen (N) cycling are less well understood, and most current empirical studies focus simply on N stocks rather than on the numerous N transformations and fluxes that occur within ecosystems. In this dissertation, I assess the current state of knowledge regarding N cycling following agricultural abandonment worldwide in a systematic literature review (Chapter 2), and I examine various changes in N stocks, transformations, and fluxes in a secondary successional chronosequence in Virginia (Chapters 3 and 4). I also explore the implications of the various stages of successional land on estimating foliar N traits using remote sensing (Chapter 4).

In Chapter 2, I find that soil N is generally depleted following agricultural land management, and increases or stays constant as succession proceeds. I also determine that soil N transformations often accelerate through succession, and that NH_4^+ and NO_3^- stocks are often inversely related. While there are numerous studies examining N stocks, and some examining N transformations, very few measure N fluxes through succession, so trends are difficult to discern. Due to the absence of studies measuring multiple aspects of N cycling at once, I conducted my

own study at Blandy Experimental Farm (Chapter 3) looking at changes in several N cycling variables through successional time using two replicate chronosequences. I find evidence of increased N availability and accelerated N cycling later in succession at Blandy, with upward-trending soil N, increased N mineralization and nitrification rates, increased foliar N concentration (among and within species), and high $\delta^{15}N$ late in succession, indicating a relatively open N cycle. As foliar N is a key integrator of ecosystem N cycling, I focus on foliar N in Chapter 4, and find that foliar N concentration and leaf mass per area (LMA), an important indicator of plant strategy, change significantly through succession. Due to growing interest in the estimation of ecosystem N cycling parameters with the use of remote sensing as opposed to typical on-the-ground field methods, I also explore the potential of using leaf reflectance on the electromagnetic spectrum to estimate foliar N and LMA across successional stages. I find that models estimating LMA perform well both within and across succession, but that models predicting foliar N perform less well when making estimations across successional stages.

While N cycling through secondary succession is less well studied than C cycling, I concludex that there are many distinct changes in N cycling as succession proceeds, with potentially vast implications. I also find that common remote sensing techniques may not accurately depict foliar N traits across successional stages. Secondary successional land comprises a significant amount of the land mass worldwide, and changes in N cycling can have cascading effects across systems including changes in primary productivity, health of water bodies, and air quality. Thus, I argue that the consideration of land use history and successional dynamics should be an integral part of forest ecosystem studies, and that these factors merit further research.

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Chapter 1: Introduction

Background and Motivation

Nearly all of Earth's surface has been manipulated by humans in some way, whether through land clearing for agriculture or development, harvesting of natural resources, altered fire regimes, climate change, or other means. The majority of today's forests have been disturbed, with an estimated 64% of forests estimated to be in some stage of secondary succession (FAO, 2010). Worldwide, agricultural abandonment is a leading cause of secondary succession, as large areas of previously cultivated land have been abandoned over the past centuries for various social, economic, political, and technological purposes (Delcourt & Harris, 1980; J. F. Hart, 1968; Kalisz, 1986; McLauchlan, 2006; Ramankutty & Foley, 1999; Yu & Lu, 2018). In the United States in particular, land on the East Coast has been left to undergo secondary succession since the 19th century as agriculture has moved westward toward cheaper and more fertile land (Ramankutty & Foley, 1999).

Throughout secondary succession, vegetation community structure and composition change in several ways (Figure 1). In eastern U.S. temperate forests, traditional early succession is characterized mostly by communities of grasses, forbs, and small shrubs, transitioning to larger shrubs and fast-growing trees such as black locust (*Robinia pseudoacacia)* and tulip poplar (*Lirodendron tulipifera)* (Clebsch & Busing, 1989). These communities are gradually overtaken by slow-growing and long-lived late successional species such as hickory and beech (Thompson et al., 2013). Early successional species tend to be fast growing with a low biomass C:N ratio, whereas later successional species have higher C:N ratios and woodier biomass.

Figure 1: A typical temperate forest secondary successional sequence. Grasses and forbs colonize first, followed by shrubs. Larger trees then colonize as the system progresses toward a climax forest. Image by Davis Coffey.

The impacts of this mass land-use shift in terms of carbon (C) cycling are relatively well understood, as C cycling is primarily a simple equation of photosynthesis versus respiration. Conceptual models and empirical evidence suggest that net primary productivity (NPP) starts low immediately following land abandonment, increases rapidly in early and mid-succession as fast-growing plants colonize the area, and levels out to a steady point in late succession as slower-growing and longer-lived plants take over (Chapin et al., 2012; Gorham et al., 1979; Odum, 1969).

Less well understood, however, are the changes in N cycling that may occur as land proceeds through secondary succession following agricultural abandonment. N cycling is a comparatively complex cycle with many interconnected inputs, outputs, and internal transformations (Figure 2). N may enter a terrestrial ecological system through two primary pathways: through deposition of reactive N, or through biological fixation of inert N_2, which makes up the majority of Earth's atmosphere (Galloway et al., 2003; Gruber & Galloway, 2008). This N is then assimilated into the tissues of living organisms such as microbes and plants as organic N, where it forms proteins and plays an essential role in processes such as photosynthesis (Kokaly, 2001). When these organisms die or are dropped in the form of plant litter, the organic N within them either remains in the soil as organic matter, or is converted to ammonium (NH_4^+) in a process called N mineralization. This ammonium may be directly taken up by plants, or may be further converted to nitrate (NO_3^-) by different nitrifying microbes. NO_3^- is also usable by plants but is also more vulnerable to loss due to its negative charge and the negative charge of soil particles (Chapin et al., 2012; Wen et al., 2016). Some of this N may also be denitrified, or converted into gaseous forms of N (primarily N_2 and N_2O) (Galloway et al., 2003).

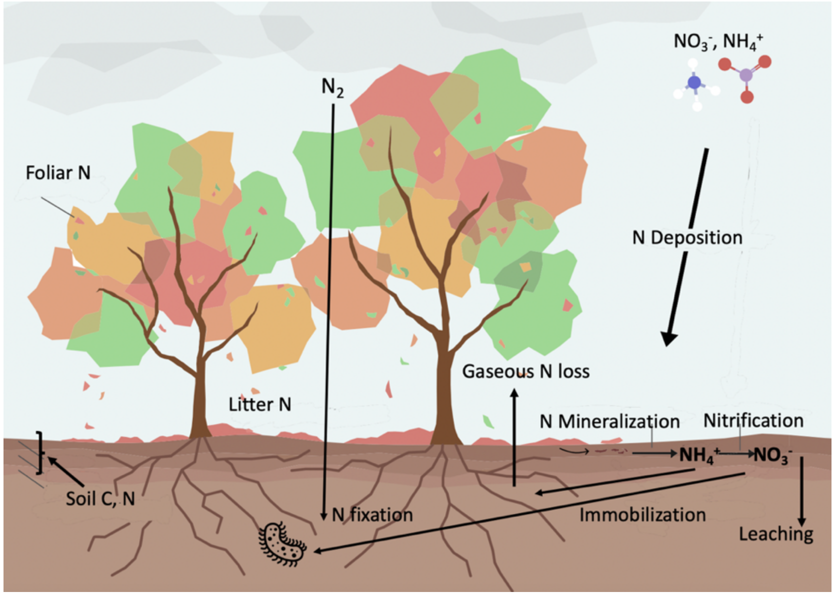

Figure 2: Stocks, transformations, and fluxes in the terrestrial N cycle. Image by Davis Coffey.

Each of these N transformations and fluxes has various controls that may be expected to change over the course of secondary succession. For example, N-fixation rates may be altered as species composition changes through succession; in a typical successional sequence, N-fixing plants are more prevalent in early-mid succession than later in succession (Gorham et al., 1979). N mineralization is strongly controlled by soil C:N and soil N availability (which is itself controlled in part by species composition), with higher mineralization rates at lower C:N and higher N availability (Booth et al., 2005; Yan et al., 2009). Nitrification, in turn, is directly controlled by N mineralization rates, and both are affected by soil temperature, moisture, and pH. Increased temperature and moisture accelerate microbial activity to some extent so increasing temperature and moisture generally increase the rates of soil N transformations (Booth et al., 2005; Hyodo et al., 2016). However, nitrification cannot occur in anoxic environments, so beyond a certain moisture threshold, nitrification may be inhibited. Nitrification also has an inverse relationship with soil pH, with higher nitrification rates at lower pH (Booth et al., 2005). Nitrification rates then serve as a direct control over denitrification and gaseous N losses. Soil temperature, moisture, and pH may fluctuate through succession as forest structure changes, which changes the microenvironment surrounding the soil and the litter inputs into the soil. Changes in these N transformation and fluxes then influence N stocks (for example, higher mineralization rates lead to higher N availability to plants, which may lead to increased N stocks in biomass), which then affect further transformations and fluxes, and so forth.

Despite the complexity of the N cycle, most studies examining changes in N cycling through secondary succession measure only one or two isolated processes, making it difficult to establish a holistic understanding of the cycle as secondary succession proceeds. A comprehensive understanding of how N cycling is changing throughout the process of secondary succession is essential, especially given the large amount of land currently undergoing the secondary succession process. Agricultural land management prior to abandonment may affect ecosystem processes through a variety of practices, including alterations of biomass and hydrology, tillage, and fertilization, which can impact the ecosystem for decades to millennia post-abandonment. (Ellert & Gregorich, 1996; Franzluebbers et al., 2000; Guo & Gifford, 2002; Hernandez-Ramirez et al., 2011; McLauchlan, 2006; Vesterdal et al., 2002). Changes in N cycling in these successional areas can have cascading implications within the system and across other systems, such as runoff or leaching of reactive N into streams and groundwater, outputs of greenhouse gases into the atmosphere, and altered terrestrial NPP (Galloway et al., 2003).

Chapter Summaries

In this dissertation, I address three questions:

1. *What is the current state of knowledge regarding N cycling through secondary succession following agricultural abandonment?*
2. *How do soil and plant components of the N cycle change through succession in two post-agricultural chronosequences in Virginia?*
3. *How do foliar traits change through secondary succession, and how does secondary succession affect our ability to estimate foliar N and other traits using remote sensing?*

In Chapter 2, I address question 1 through a systematic review of the current literature examining N cycling following agricultural abandonment in temperate forested ecosystems; to my knowledge, this is the first review paper of its kind. I evaluate past studies measuring or modeling changes in N stocks, soil N transformations, and N fluxes through the course of secondary succession, while considering differences in successional stages examined, prior agricultural practices, and methodology of each study. Through this review of the literature, I identify subject areas of broad scientific consensus, areas with uncertainty, and areas necessitating more research.

In Chapter 3, I answer question 2 through an observational study of soil and plant N cycling characteristics following agricultural abandonment in two secondary successional sequences at Blandy Experimental Farm in Virginia, each comprised of early, mid, and late successional fields. I examine several aspects of the N cycle at Blandy, including total and inorganic soil N stocks, foliar N, litter N, net N mineralization, net nitrification, inorganic N leaching potential, and soil and foliar natural $\delta^{15}N$ to provide information regarding N fluxes. Through these many measurements, I am able to develop a comprehensive view of how N cycling is changing through succession at Blandy Experimental Farm, and how it may be changing across the East Coast.

In Chapter 4, I focus in specifically on changes in foliar N, a key integrator of ecosystem N cycling, through secondary succession. I answer question 3 by collecting and examining leaves from dominant species across the three stages of succession at Blandy, from various locations in the canopy and throughout the growing season. I measure leaf traits relevant to N cycling, such as foliar N and leaf mass per area (LMA), in addition to collecting hyperspectral reflectance readings of each leaf to explore the relationship between leaf spectra and traits. With these data, I build partial least-square regression (PLSR) models using spectral reflectance to predict foliar traits. I build models across the dataset as a whole, within each successional stage, and using spectra from one successional stage to predict the foliar traits of others. This approach will help establish how secondary succession affects the functionality of these models, and what implications this might have for predicting leaf traits using aerial or satellite hyperspectral imagery, given that most land is in some stage of secondary succession. I also identify wavelengths or sections of the electromagnetic spectrum that are consistently important in predicting these traits.

Chapter 5 summarizes the main findings of the dissertation and explores avenues for future research. In Chapter 2, I find that there is largely a scientific consensus that soil C and N stocks increase or stay the same through secondary succession, but that there are fewer studies measuring – and uncertainty surrounding – N transformations, with many studies reporting conflicting results. I also determine that there is a dearth of research measuring changes in N fluxes through secondary succession, and that few studies measure enough aspects of the N cycle to provide a comprehensive view of its changes as a whole. In Chapter 3, I find that N availability increases as secondary succession proceeds at Blandy Experimental Farm, as evidenced by increasing soil N stocks, increasing rates of net N mineralization and nitrification, increasing foliar N concentration, and high soil $\delta^{15}N$ in late succession, suggesting a relatively open N cycle. In Chapter 4, I find that foliar traits like N concentration and LMA change significantly through succession, both among and within species. PLSR models perform relatively well in predicting foliar N and LMA when trained and validated across multiple successional stages, or when trained and validated within a single successional stage. However, when trained on one successional stage and validated on the others, PLSR models are still able to accurately predict LMA, but are largely unable to predict foliar N.

As a whole, this dissertation sheds light on the effects of agricultural abandonment and subsequent secondary succession – an exceedingly widespread phenomenon – on ecosystem N cycling, which has been understudied to date. Through these reviews, analyses, and findings, I emphasize the need for more comprehensive studies on the topic, and the necessity of consideration of secondary successional processes in predicting and modeling ecosystem dynamics.

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Chapter 2: Soil Nitrogen Cycling through Secondary Succession in Temperate and Subtropical Ecosystems following Agricultural Abandonment: A Review

In review at Ecosystems

Abstract

Secondary succession is a widespread worldwide occurrence, with agricultural abandonment a leading cause. Large areas of formerly cultivated land have been left to regenerate in recent centuries due to movement of agriculture for various social, economic, political, and technological purposes, and the effects of this widespread agricultural abandonment on nitrogen (N) cycling are relatively poorly understood. Here I compile studies in temperate and subtropical ecosystems examining N cycling processes throughout secondary succession following agricultural abandonment, to elucidate general trends and areas bearing the need for more research. Soil C and N both generally increase through successional time, and C generally increases more quickly, resulting in an increasing C:N. Trends regarding soil N transformations are more difficult to discern, but net N mineralization and nitrification often increase throughout succession, and soil NO_3^- and NH_4^+ concentrations are often inversely related. Data on trends in N fluxes through succession are lacking, with some studies finding an increase in fluxes into and out of the ecosystem as succession progresses, but other studies showing a more closed N cycle later in succession. More long-term studies of successional systems, as well as an increase in the comprehensiveness of variables examined at each site and an establishment of consistent sampling procedures, are necessary for a full understanding of N cycling following agricultural abandonment. A better understanding is crucial in understanding and predicting numerous ecosystem processes such as fluxes of nutrients into bodies of water, fluxes of greenhouse gases into the atmosphere, and ecosystem productivity.

Introduction

Nearly all land on Earth bears the marks of human disturbance – from land use and land cover changes, human-mediated fire regimes, climate change, and more. However, the consequences of these disturbances on nitrogen (N) cycling, an essential component of ecosystem functioning, remain poorly understood. Worldwide, secondary succession is occurring where large swaths of previously cultivated land have been abandoned over the past centuries for various social, economic, political, and technological purposes (Delcourt & Harris, 1980; J. F. Hart, 1968; Kalisz, 1986; McLauchlan, 2006; Ramankutty & Foley, 1999; Yu & Lu, 2018).

The United States witnessed a mass shift of croplands from the eastern states to the West and Midwest over the past two centuries; between 1850 and 2016, an estimated 65 Mha of cropland was abandoned, focused in the central and southeastern United States, with expansion elsewhere (Yu & Lu, 2018). Contributing factors to this shift were the presence of more fertile land further west, competition for land on the East Coast, and the Homestead Act of 1862, which encouraged settlement of the Great Plains (Ramankutty & Foley, 1999). Canada experienced a similar westward shift of agriculture between 1850 and 1992, largely driven by immigration to the Canadian West from the American West and the growth of the railway system (Ramankutty & Foley, 1999). This phenomenon is not isolated to North America; croplands are being abandoned across South America, Europe, and Asia as well (McLauchlan, 2006; Smith et al., 2005) (Figure 1). It is estimated that as of 2010, 64% of the world's forests were in a state of secondary succession following some sort of human disturbance (FAO, 2010; Parolari et al., 2017).

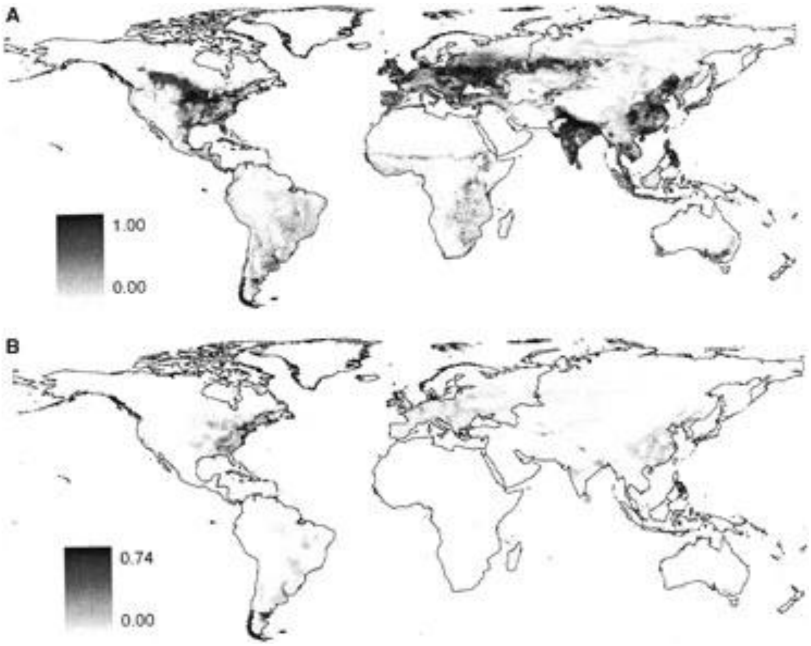

Figure 1: A shows largest extent of farmland as of 1992, and B shows proportion of cropland abandoned between 1850-1992 (McLauchlan, 2006).

Terrestrial N cycling is predominantly a closed cycle mediated by microbial N transformations and subsequent N uptake by plants and microbes, which can be influenced by a range of factors including vegetation structure, soil organic matter, and soil moisture and temperature, all of which can vary over the course of agricultural disturbance and subsequent secondary succession (Compton & Boone, 2000; McLauchlan, 2006). In addition, agricultural management may affect ecosystem processes through a variety of practices, including alterations of biomass and hydrology, tillage, and fertilization, which can impact the ecosystem for decades to millennia post-abandonment (Ellert & Gregorich, 1996; Franzluebbers et al., 2000; Guo & Gifford, 2002; Hernandez-Ramirez et al., 2011; McLauchlan, 2006; Vesterdal et al., 2002).

Despite the widespread phenomenon of agricultural abandonment and the potential for long-term changes to ecosystem function, there currently exists a lack of scientific consensus about how N cycling might change over the course of secondary succession. Conceptual models (Lovett et al., 2018; Vitousek & Reiners, 1975) hypothesize about ecosystem accumulation, import, and export of N through successional time, but these remain largely untested empirically and do not address the complexities in N transformations that may occur. Studies examining changes to N cycling through secondary succession vary widely in geographical location, ecosystem type, disturbance type, and N cycling components examined.

This review seeks to assess the current state of knowledge regarding soil N cycling changes through secondary succession following agricultural abandonment; I exclude studies conducted following non-agricultural disturbance such as fire (eg. Goodale & Aber, 2001; Howard & McLauchlan, 2015; Perakis et al., 2015; Yermakov & Rothstein, 2006) or logging (eg. Davidson et al., 1992; Goodale & Aber, 2001; Nave et al., 2014; J. Wang et al., 2016; Yan et al., 2009), as other disturbance types may have fundamentally different effects on soil and vegetation. To minimize variability, I focus here on temperate and subtropical ecosystems (~20-50° latitude N and S) where the expected climax communities are forests. I include several approaches to studying secondary succession, including long-term monitoring, chronosequences using a space-for-time substitution, and modeling approaches, and I include studies from all stages of successional time.

Conceptual Models

There are two broad conceptual models regarding the inputs and outputs of nutrients, including N, over the course of secondary succession, though neither is specific to post-agricultural succession. Vitousek and Reiners (1975) hypothesized that through early and mid-succession, when plant growth rates are at or near their maximum, inputs of elements such as N from atmospheric, mineral, or biological fixation sources would exceed system outputs, as plants would use most or all of the nutrients available to them. They further hypothesized that in late succession, as net biomass increment approaches zero and plant nutrient needs are reduced, nutrient outputs would become equal to nutrient inputs (Figure 2).

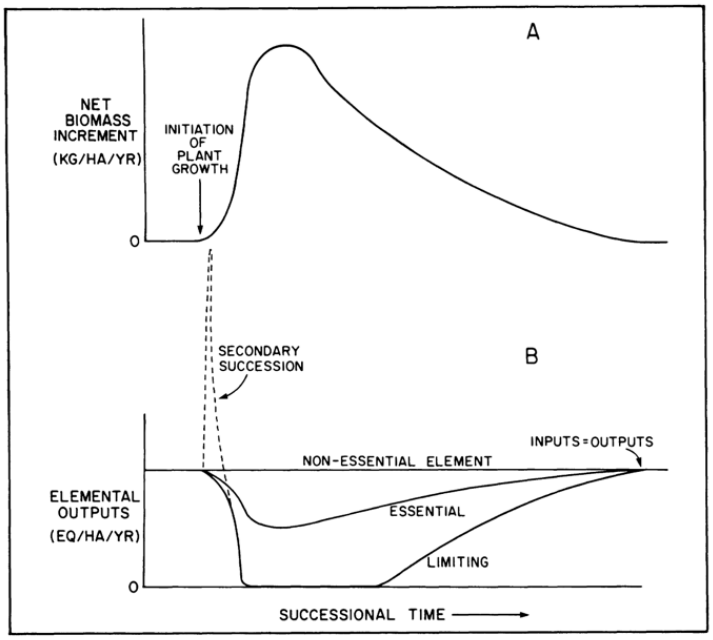

Figure 2: Vitousek and Reiners (1975) hypothesized model of nutrient dynamics through secondary succession. “A” shows net biomass increment, and “B” shows elemental outputs of non-essential, essential, and limiting (e.g. N) nutrients. The dotted line shows a spike in elemental outputs immediately following disturbance in secondary succession.

Recently, Lovett et al. (2018) modified the Vitousek and Reiners (1975) hypotheses regarding nitrogen inputs and outputs through secondary succession. This new conceptual model acknowledges that mineral soil horizons can serve as sources of nutrients to plants early in succession and sinks of nutrients later in succession as plant growth slows. Thus, they argue, nutrient inputs and outputs are not as tightly coupled with biomass accumulation as Vitousek and

Reiners (1975) proposed. In the Lovett et al. (2018) conceptual model, there is an added component of soil N dynamics, where soil N reaches a minimum at maximum net biomass accumulation and plant N accumulation, during what they refer to as the "soil mining phase." Then, as plant N accumulation approaches near-zero levels in late succession, the soil re-accumulates N. N export becomes positive only when both the combined plant biomass and soil N sinks are less than N deposition (Figure 3).

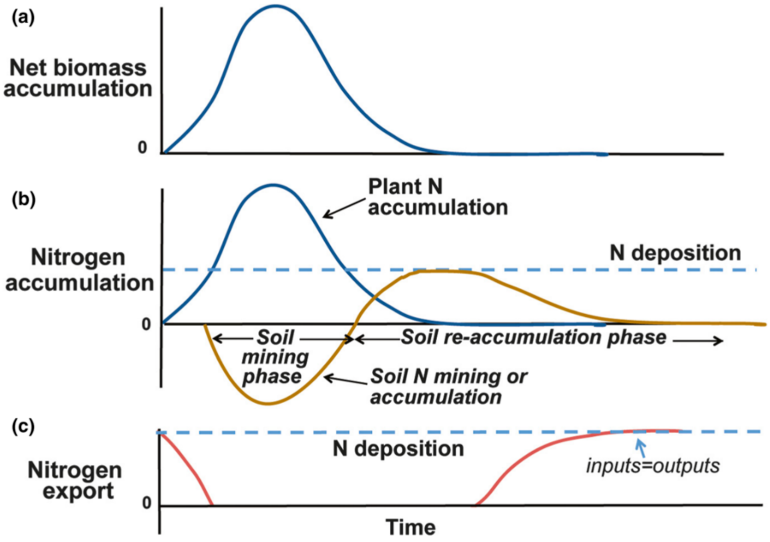

Figure 3: Lovett et al. (2018) revised hypotheses of nutrient retention during succession. (a) shows net biomass accumulation, (b) shows plant N accumulation (blue line) and soil N dynamics (brown line), and (c) shows nitrogen export. The dashed blue line is constant N deposition.

Methods

Studies were compiled from several databases, including the University of Virginia's VIRGO catalog, Web of Science, and Google Scholar. Multiple searches were conducted on each database using various combinations of search terms for nutrient cycling ("nutrient cycl[ing]," "N/nitrogen cycl[ing]," "C/carbon cycl[ing]"), secondary succession ("secondary succession," "forest regeneration," "forest regrowth," "afforestation"), and land use ("agriculture," "agricultural abandonment," "land use change"). Of the studies matching search terms in two or more of the above categories, the abstracts were examined to determine their relevance to this review.

The papers compiled through this search process were evaluated, and papers were eliminated if they were not within the scope of this review in terms of location (~20-50° latitude N and S), climax ecosystem type (forest), disturbance type (agriculture), or stages of post-agricultural succession examined (at least two), or if they did not contain N cycling variables. Variables recorded for each paper, in addition to the above variables were: study type (model, experimental, observational, theoretical), specific location, specific agricultural disturbance type, status of fertilization during agricultural use (if known), length of time land used for agriculture (if known), soil type (if specified), successional stages evaluated, total C and N in soil of each successional stage, inorganic and organic N in soil of each successional stage, changes in rates of N transformations in soil, changes in isotopic enrichment in soil and plants, changes in N fixation, and changes in gaseous N fluxes through secondary succession.

Results

Within the aforementioned criteria, 17 studies evaluated total and/or organic soil carbon (C) and N. Of these, 15 were in secondary succession following abandonment of cropland, and two were in secondary succession following abandonment of pastureland/animal agriculture. Seven of the studies took place in previously fertilized sites, four took place on previously unfertilized land, and in six of the studies, the prior fertilization status was unknown or unspecified.

Ten studies evaluated inorganic N and soil N transformations, and all took place on land previously used as cropland. In six of the studies, the land was previously fertilized, and in four, the prior fertilization status was unknown or unspecified. Additionally, eight studies examined N fluxes, either within or into and out of the successional system; five were on previously fertilized land (three from the same site), and three were on land where prior fertilization status was not known (Figure 4).

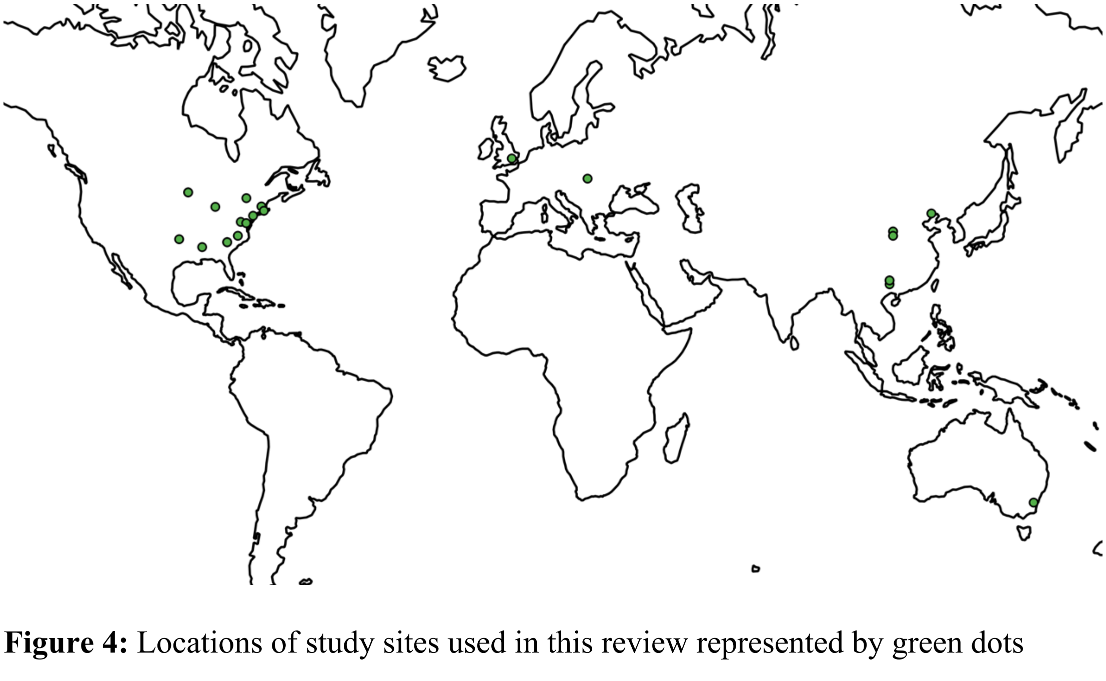

Figure 4: Locations of study sites used in this review represented by green dots

Soil C and N

Though C cycling is not the focus of this review, the soil N cycle is tightly coupled with the carbon cycle; therefore, I assess both elements with respect to soil properties for the identified studies. Many studies show an increase in soil C and N contents during secondary succession following agricultural abandonment, though the specific forms of C and N examined vary by study. For example, Foote & Grogan (2010) found that surface soil (0-5 and 5-10 cm depth) total C and N contents were significantly greater in mature forests than in adjacent agricultural fields, and significantly increased with successional age up to ~100 years, in southeastern Ontario, Canada. On the Loess Plateau of China, similar trends were observed, with soil organic carbon (SOC) and total nitrogen (TN) increasing consistently with successional age up to climax forested ecosystems in the top 10 cm of soil (Liu et al., 2020). Total organic carbon (TOC) and TN accumulated following vineyard abandonment in the top 10 cm of soil in a study in Hungary, yet the accumulation of TOC leveled off after the first 50 years of secondary succession (Spohn et al., 2016).

While C and N generally increase through secondary succession, the relative rates of C and N accumulation, and the resulting specific C:N dynamics, vary by study. Hu et al. (2018) found accumulation of soil C and N down to 50 cm depth, with SOC stocks returning to primary forest levels in ~74 years, and soil N stocks recovering in ~100 years in a karst region in China. Poulton et al. (2003) similarly found N accumulation rates to be less than those of C accumulation at 0-69 cm depth (including the litter layer) in two successional fields in southeast England, with C:N increasing in both fields over time. Foote and Grogan (2010) noted similar patterns, with significant C:N increases in the top 10 cm through the first 80-100 years of secondary succession, and C:N trending upward in the 10-20 cm fraction. Spohn et al. (2016)

observed that in early succession soil, TOC in the top 10 cm increased more rapidly than soil N, potentially due to agricultural fertilizer N still present in the soil, but that after approximately 40 years of succession, the TOC:TN ratio stabilized. In these cases, C accumulation outpacing N accumulation seems to suggest a lack of N limitation in these successional systems.

However, a study in a karst region in China revealed soil N stocks recovering more rapidly than C, in ~67 years post-agricultural abandonment (Wen et al., 2016), with a follow-up study incorporating additional sampling sites in the same region producing similar results (Xiao et al., 2018). In Minnesota, Knops and Tilman (2000) estimate that the required recovery time for C and N to approach pre-agricultural levels is 230 years and 180 years, respectively, with N recovering more quickly than C, suggesting N as a limiting resource to biomass accumulation. In several studies, N is initially a strongly limiting resource, but as N stocks recover, the N cycle becomes progressively more open (Knops & Tilman, 2000; Poulton et al., 2003; Wen et al., 2016), consistent with the proposed conceptual models discussed above (Vitousek and Reiners 1975, Lovett et al. 2018).

While many studies have shown soil C and N to be depleted during cultivation and to gradually increase throughout succession, this finding is not uniform across all studies. Soil amendments applied specifically for agricultural use of land may yield high soil N levels that are gradually depleted over time. For example, a New England study found greater N in the top 15 cm of soil in formerly cultivated soils ~90 years post-abandonment when compared with a permanent woodlot ~110 years following logging management (Compton & Boone, 2000). Another study on the Loess Plateau in China found varying soil N levels throughout 25 years of secondary succession, with the lowest levels 15 years post agricultural abandonment and the highest levels 20 years following agricultural abandonment in the top 20 cm of soil (Jiao et al., 2013). In the Piedmont of South Carolina, mineral soil N decreased in the first ~25 years following agricultural abandonment, representing the “soil mining phase” in the Lovett et al. (2018) model, but ecosystem N accumulation was positive due to biomass and litter accumulation (Richter et al., 2000). Similarly, in a formerly cropped northeastern China chronosequence, forest floor (litter) C and N consistently increased until the oldest aged field at 20 years, but mineral soil C and N in the top 100 cm decreased over the first 10 years, after which it began to increase (Mao et al., 2010). Near Canberra, Australia, a combined sampling and modeling approach demonstrated a decrease in mineral soil C and N 18 years following pasture land use (which may have fundamentally different impacts from cropping (Franzluebbers et al., 2000)) and subsequent afforestation with *Pinus radiata,* but an increase in forest floor and total ecosystem C and N (Kirschbaum et al., 2008).

N Transformations

There is a general lack of synthesis and consensus regarding soil N transformations throughout secondary succession. Some research has found a general acceleration of net N mineralization over time; two studies in the subtropical karst region of China observed an increase in net N mineralization from a ~10-year-old grassland to a ~59-year-old secondary forest (Wen et al., 2016; Xiao, Li, Wen, Yang, Luo, & Chen, 2018). Similarly, potential N mineralization was found to increase from one to 60 years following agricultural abandonment near Minneapolis, Minnesota (Zak et al., 1990). This may be due to accumulation of N in the soil following agricultural abandonment, as well as potential changes in microbial communities and soil microenvironments.

However, Robertson and Vitousek (1981) found no significant change in net N mineralization through succession on the New Jersey Piedmont, except in the oldest site (an old-growth forest), where the rates were the highest. One study in the southeastern United States found a U-shaped relationship between mineralizable N in the 0-7.5 cm soil fraction, measured as net N mineralization rates of incubated soil samples, and successional stage between 5-45 years (Richter et al., 2000), with the rates starting relatively high shortly following abandonment, then quickly dropping off, and then steadily recovering. A chronosequence in northeast China had a similar pattern following afforestation of agricultural land; potential net N mineralization decreased for 5 years following cessation of cultivation, remained relatively stable for 5 more years, and then increased for the following 10 years (Mao & Zeng, 2010).

Net nitrification has been hypothesized to be low in older forested ecosystems, potentially due to inhibition by tannins in mature forests (E. A. Davidson et al., 1992; Rice & Pancholy, 1972a). While they did not measure nitrification rates directly, Rice and Pancholy (1972) found steadily decreasing numbers of nitrifying bacteria *Nitrosonomas* and *Nitrobacter* from early succession to a climax ecosystem in Oklahoma. Another study in North Carolina identified decreasing rates of nitrate production (ΔNO_3-N) from 4-7-year-old fields to mature hardwood forests, but with high variability among sites in the same successional stage (Christensen & MacAller, 1985). However, several studies have turned up conflicting patterns. For example, Robertson and Vitousek (1981) found net nitrification to be closely correlated with net N mineralization, and to be greatest in old-growth systems. Xiao et al. (2018) discovered increasing net nitrification from ~5-10 to ~30-50 years post-agricultural abandonment in southwest China, and Zak et al. (1990) found increasing potential net nitrification through the first 60 years of secondary succession in Minnesota. Wen et al. (2016) saw increasing net nitrification rates over successional time (~10 to ~60 years), but determined that primary forests had lower net nitrification rates than secondary forests that were previously cultivated. Similarly, a study in Harvard Forest found higher net nitrification rates in formerly cultivated sites than in permanent woodlots, even ~100 years after cultivation (Compton and Boone, 2000).

Total ammonium and nitrate levels have been used by many studies as metrics to assess N cycling. Wen et al. (2016) found ammonium concentrations to peak in the early grassland stage of secondary succession, and to reach a minimum in secondary forest ~50 years following agricultural abandonment, which had lower ammonium concentrations than the primary forest. They found an opposite pattern with nitrate concentrations, which reached a maximum in the secondary forest. Xiao et al. (2018) similarly found nitrate to peak in the secondary forest stage of succession, but found no significant differences among successional stages for ammonium concentrations. In contrast, Liu et al. (2020) found ammonium to generally peak in the "pioneer forest" stage of succession before declining in the "climax forest" stage, with no generalizable differences in nitrate, and Yesilonis et al. found lower nitrate levels in older (120-150 yr) successional plots than in younger ones (50-70 yr) (2016). Rice and Pancholy (1972) saw an inverse relationship between ammonium and nitrate concentrations, with ammonium increasing and nitrate decreasing through succession, in accordance with their hypothesis of nitrification inhibition in late succession.

N Fluxes

In addition to variation of soil chemical transformations of N-containing molecules, a subset of studies also examined changes in land-atmosphere-water fluxes of N through successional time. For example, some measured the isotopic composition of N in various components of the ecosystem, which can provide insight into the openness of the N cycle, including N fixation and gaseous losses (Wang et al., 2007). Wen et al. (2016) found similar $\delta^{15}N$ in the top 15 cm of soil of a cropland and early successional field, and elevated $\delta^{15}N$ in a later successional shrubland and secondary forest. Because microbial processes leading to gaseous soil N losses favor ^{14}N over ^{15}N, an enrichment in ^{15}N in the later successional fields indicates an increase in soil N losses in these successional stages. However, a study in northwestern Virginia found an opposite pattern; there, $\delta^{15}N$ decreased between 5 and 85 years of secondary succession, correlating with an increase in soil C:N. Here, it was hypothesized that the N cycle was more open in early succession, where C:N was low, and became more closed over time (Wang et al., 2007).

Billings and Richter (2006) found ^{15}N enrichment in soil layers from 7.5 cm depth down to 60 cm attributed to increasing plant associations with ectomycorrhizal (ECM) fungi through 40 years of succession. However, in this study, $\delta^{15}N$ was relatively stable in the soil organic matter of the top 7.5 cm of soil, owing to inputs of $\delta^{15}N$-depleted plant litter. Similar trends were observed in Rhode Island, USA; mineral soil experienced an increase in $\delta^{15}N$ through successional time while foliar and forest floor $\delta^{15}N$ decreased. These trends are hypothesized by the authors to be largely driven by internal cycling of N rather than by N fluxes into and out of the system, and the increase in $\delta^{15}N$ through time may be due to preferential uptake of ^{15}N depleted molecules by plants. They hypothesize that the decrease in foliar and forest floor $\delta^{15}N$ is partially driven by a tightening of the N cycle and decrease in N availability through succession, as mycorrhizal fractionation increases with decreasing N availability, thus providing growing biomass with increasingly isotopically depleted N as the element becomes more limited (Compton et al., 2007).

Some studies also report on processes directly providing external N to the study system, such as the presence of N-fixing organisms and factors affecting N deposition, and processes removing N from the system, such as leaching. Gorham et al. (1979) predict that N-fixation should be high in early-mid secondary succession due to the presence of N-fixing plants, low in mid-late succession, and then increase again late in succession due to fixation in epiphytes, decaying logs, and other factors specific to late successional systems. In the United Kingdom, Poulton et al. (2003) qualitatively attribute differing rates of N accumulation between two successional fields to more N-fixing legumes, higher edge-to-area ratio leading to more effective capturing of dry N deposition, and closer proximity to farm buildings with roosting birds with N-rich excrement in the field with greater N accumulation rates. In both fields, N-fixing legumes were present early on and disappeared in late successional woodlots. In Minnesota, a study found N accumulation across successional fields from 0-60 years old to be consistent with N deposition rates, with a very small amount of N fixation throughout succession (Zak et al., 1990). Richter et al. (2000) observed similar patterns in a ~40 year old successional pine forest in South Carolina, with ecosystem N accretion through succession closely matching N deposition rates and low leaching of N, indicating negligible rates of N fixation at any successional stage. Following their findings of decreased nitrification and nitrate concentrations through succession in Oklahoma discussed above, Rice and Pancholy (1972) theorized that leaching would decrease through succession and reach a minimum in a climax ecosystem. N_2O production, rarely measured in temperate forests, increased with age in a Michigan chronosequence (Robertson & Tiedje, 1984).

Also of interest is the movement of N within an ecosystem through succession. While Richter et al. (2000) found an overall accumulation of total ecosystem N through 40 years of succession, mineral soil N was depleted and transferred to the biomass and forest floor. A follow-up study revealed that in the subsequent decade, as forest growth slowed and self-thinning occurred, deeper mineral soil N stabilized and began to replenish (Mobley et al., 2015), consistent with the "soil mining phase" followed by a "soil re-accumulation phase" as theorized by Lovett et al. (2018).

Discussion

General trends

Though the studies above vary in conclusions regarding the changes in N cycling over the course of secondary succession following agricultural abandonment, some general trends can be observed. Most studies that examined either total or organic C and N found an increase in both through successional time, and all found either an increase or no significant change in C:N over time, corroborating reviews on this topic (Yang et al., 2011; Zou et al., 2021). Some found a decrease in N in the mineral soil, but an increase in N in the forest floor layer, with an overall ecosystem increase in N (Hooker & Compton, 2003; Kirschbaum et al., 2008; Richter et al., 2000). While the decrease in mineral soil N persisted through the 114-year chronosequence in the Hooker and Compton study (2003), Kirschbaum et al. (2008) and Richter et al. (2000) had their oldest successional stages at the relatively young ages of 18 and 40 years, respectively. Two studies, both on land known to have been previously amended with fertilizer, observed an initial decrease of N in the mineral soil, followed by an increasing trajectory beginning between 10-15 (Mao et al., 2010) and ~20 (Parolari et al., 2017) years into succession. These two cases of decreasing soil N demonstrate the "soil mining phase" of the Lovett et al. (2018) conceptual model, with a subsequent increase in mineral soil N indicating a switch to the "soil re-accumulation phase." However, this pattern is not seen in the other studies discussed here, either because not all systems experience this phase (perhaps due to differences in starting soil N status, species composition/N-fixer presence or absence, or ecosystem type), or because sampling frequency was not high enough to observe it.

Agriculture may deplete surface soil C and N stocks in several ways, including biomass removal and tilling, which mixes the soil layers, increases erosion, and accelerates decomposition rates (Foote and Grogan, 2010; McLauchlan, 2006). Forest regeneration following agricultural abandonment increases soil C stocks through increased biomass litter inputs, especially from woody biomass with a high C:N ratio (Liu et al., 2020; Poulton et al., 2003; Wen et al., 2016). The C accumulation rate is tightly linked to the N accumulation rate in many ecosystems, as N is frequently a limiting resource to biomass growth (Hu et al., 2018; Poulton et al., 2003; Wen et al., 2016). N accumulation during secondary succession can be attributed to atmospheric deposition, symbiotic fixation, asymbiotic fixation, and recycling and retention of N within the plant-soil system (Knops & Tilman, 2000; Wen et al., 2016); the latter three all have the potential to increase during ecosystem regeneration, though to varying degrees in different systems.

These general trends are in partial agreement with metanalyses conducted evaluating C and N stocks over the course of secondary succession worldwide following any disturbance type (not limited to agricultural disturbance). These found generally insignificant increases in C and N in mineral soil during succession, but significant forest floor and total ecosystem increases in both (Yang et al., 2011; Zou et al., 2021). Also observed was that, in cases where mineral soil C and N did increase, they increased at roughly the same rate, or C increased slightly more quickly, maintaining a stable or slowly increasing C:N. The mineral soil degradation that results from agricultural disturbance can substantially deplete soil N stocks through processes such as tilling and biomass removal, and therefore a pattern of significant increases in N in the mineral soil through successional time may be more likely following agricultural practices than other disturbance types (Foote and Grogan, 2010; McLauchlan, 2006). A countering viewpoint may suggest that soil amendments could cause elevated soil N stocks at the time of abandonment in previously fertilized fields, leading to a potential decrease in soil N through successional time. However, this phenomenon is less frequently observed, and the magnitude of, and time scale over which these increases in N stocks may persist have been poorly quantified (McLauchlan, 2006).

Trends in inorganic N and N transformations over secondary succession have been studied much less frequently than total stocks, and thus patterns are more difficult to discern due to the relative scarcity of measurements and variability in study characteristics. In many studies, NO_3^- and NH_4^+ were inversely related, but the trends over time were not consistent (Christensen & MacAller, 1985; Rice & Pancholy, 1972a; Wen et al., 2016). Of the studies that measured net N mineralization or potential net N mineralization, either an increase through successional time (Wen et al., 2016; Xiao et al., 2018; Zak et al., 1990) or an initial decrease followed by an increase (Mao & Zeng, 2010; Richter et al., 2000) was observed, reflecting patterns noted in a review by Vitousek et al. (1989). Where an initial decrease followed by an increase in net mineralization was observed, this corresponded to a similar pattern in total mineral soil N stocks in one study (Mao & Zeng, 2010), and with a decrease in mineral soil N but an increase in forest floor N in the other (Richter et al., 2000). Studies measuring net nitrification tended to see an increase throughout the course of secondary succession (Wen et al., 2016; Xiao et al., 2018; Zak et al., 1990), though one study found a decrease in nitrifying bacteria through successional time, and thus surmised a decline in nitrification (Rice & Pancholy, 1972a).

N mineralization and nitrification are of particular importance to vegetation, as these processes generate the dominant forms of N for plant uptake (Gorham et al., 1979; Lovett et al., 2002). Net N mineralization is generally accelerated by the quantity of dissolved organic nitrogen (DON) in the soil, soil temperature, and soil moisture to a point, and is slowed by increasing C:N ratio (Chen et al., 2019). An important factor controlling nitrification is ammonium concentration (Robertson & Vitousek, 1981). Oxygen concentration is also a direct control over nitrification, as nitrifying bacteria are largely aerobic. Therefore, varying patterns in these N transformations and resulting inorganic N concentrations across studies may arise from a number of factors, including specific agricultural practices (e.g. differences in soil amendments), the state of the system at the start of secondary succession, and climate dynamics over time.

Due to the small number of studies and broad diversity in N flux variables measured, trends in N fluxes among soil, air, and water through successional time are more difficult to discern.

Complicating Factors

The variability in nutrient cycling patterns from study to study indicates the importance of several considerations when examining successional dynamics. Though this paper focuses specifically on post-agricultural terrestrial forest succession, other site-specific factors, such as prior agricultural type and practices, climate/ecosystem type, soil type, and soil depths, are wide-ranging. For example, as discussed above, in cropped systems frequent tilling may greatly deplete soil organic matter prior to secondary succession, and frequent fertilizer application may lead to higher soil N prior to secondary succession. In addition, agricultural practices may have divergent effects in different systems; for example, studies have shown soils with higher clay content to be less susceptible to degradation by tilling than soils with higher sand content (McLauchlan, 2006), and Paul et al. (2002) found widely variable responses of soil C to fertilizer application. Additionally, different soil textures and types may respond differently to successional processes (Pastor & Post, 1986).

Although I focus only on temperate and subtropical systems here, several sub-climates exist within these climate zones, which may impact conclusions drawn from secondary successional studies. For example, faster-growing systems may pass through successional stages more quickly than slower-growing systems, and thus comparing systems by number of years following abandonment may yield misleading conclusions; Yang et al. (2011) found a strong climatic influence on rates of change of C and N stocks in successional forests worldwide. Also, even following the abandonment of agricultural disturbance, other human-mediated ecological disturbances, such as removal of seed banks, introduction of highly competitive invasive species, and changes in temperature and water regimes, may lead otherwise "undisturbed" systems to undergo secondary succession in divergent ways, and may even lead to cases of "arrested succession," where the original pre-disturbance ecosystem is unable to return (Dey et al., 2019; Gill & Marks, 1991).

Future Research Directions

Many knowledge gaps remain regarding N cycling dynamics over secondary successional time in temperate forest ecosystems following agricultural abandonment. It is clear that a number of system-specific factors play a role in altering the successional dynamics at any given location, and thus caution should be used when attempting to generalize trends over large areas. A meta-analysis of secondary successional studies to date in wide-ranging ecosystem and disturbance types that includes N transformations and fluxes, in addition to C and N stocks, may help to quantify the effects of various site-specific characteristics, but is beyond the scope of this paper. More long-term or repeated studies in single locations will help to better elucidate the processes at play at those sites. Future research may also work toward establishing consistency in methods (for example, soil depths examined) in order to allow for comparisons among studies.

Current studies also examine widely variable aspects of the N cycle, leaving some N processes and transformations largely unexplored. Frequently discussed in the current literature are soil and plant nutrient stocks and soil N transformations (e.g. net [and less frequently gross] mineralization and nitrification), whereas N fluxes into and out of ecosystems are less commonly assessed. For example, while many studies of secondary succession in tropical forests measure gaseous N fluxes to and from the soil (Erickson et al., 2001; Keller & Reiners, 1994; Winbourne et al., 2018), very few measure this aspect of the N cycle and its relative importance in different successional stages in higher latitude (e.g. temperate) forests. There are also very few studies quantifying N fixation or N leaching rates throughout secondary succession in temperate forests.

N cycling dynamics have far-ranging implications worldwide. As N is frequently the limiting element for plant growth, the N cycle and levels of plant-available N are directly linked to C cycling and the productivity of ecosystems (Vitousek & Howarth, 1991). N cycling dynamics must also be understood in order to quantify and constrain processes of concern, such as leaching, nitrate runoff and subsequent eutrophication in downstream bodies of water, or the contribution of gases produced during nitrification and denitrification to atmospheric processes and the greenhouse effect. Given the widespread agricultural abandonment over the past few centuries in all corners of the globe (Delcourt & Harris, 1980; Hart, 1968; Kalisz, 1986; McLauchlan, 2006; Ramankutty & Foley, 1999; Yu & Lu, 2018), as well as the importance of N cycling dynamics to many environmental processes, a comprehensive understanding of how N cycling varies over the course of secondary succession is essential.

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Chapter 3: Nitrogen Cycling through Secondary Succession in Northwestern Virginia

Prepared for submission to JGR Biogeosciences

Abstract

Forest clearing for agricultural use followed by cropland or pasture abandonment is a leading cause of forest disturbance. While theoretical models broadly predict the biogeochemical and structural dynamics of secondary forest succession following disturbances, much remains unknown regarding how specific components of biogeochemical cycling vary through secondary succession. Here I investigate two post-agricultural disturbance chronosequences at Blandy Experimental Farm in Boyce, VA, each consisting of an early, a mid, and a late successional field (~20, ~35, and ~100 years old, respectively). I collected data observing a wide range of ecosystem N pools, transformations, and fluxes, including soil, litter, and live foliar N; net N mineralization and nitrification; soil N leaching potential; and soil and foliar ^{15}N natural abundance. I found that total soil N trended upward through secondary succession. While litter N concentration decreased in late succession, total litter mass increased, so total litter N increased as well. Foliar N concentration increased from early to late succession, both among and within species. While soil ammonium concentration decreased through succession, soil nitrate concentration increased. Net N mineralization and nitrification both significantly increased through succession, and a greater proportion of mineralized N was nitrified later in succession, corresponding to the decreasing ammonium and increasing nitrate. Isotopic analysis indicates high N-fixation in mid-succession, and high N availability and a relatively open N cycle late in succession. Comprehensive field observations such as mine are essential in understanding and making predictions about the biogeochemical cycling and ecosystem function of current and future successional forests in a changing world.

Introduction

Secondary succession in the United States

Nearly all land in eastern North America has undergone some degree of human disturbance, particularly from agricultural activity, yet the consequences of these disturbances on components of nitrogen (N) cycling remain poorly understood. In the eastern United States, secondary succession is occurring where large swaths of previously cultivated land have been abandoned over the past century due to advances in farming efficiency and a move westward to more fertile soils (Compton & Boone, 2000). In New England, 45-65% of the land was cleared for agriculture by the mid-1800s; many of these agricultural fields were abandoned, and as of 2000, 70-90% of these lands were once again forested (Compton & Boone, 2000). In the southeastern United States between 1750 and 1960, there was a 42.1 gigaton decrease in carbon stored in virgin forest – largely due to agricultural clearing in the 1800s – and an 11.2 gigaton increase in secondary forest carbon storage (Delcourt & Harris, 1980). These trends were also observed in the Appalachian region of eastern Kentucky, where intensive cultivation of corn (*Zea mays* L.) between 1800 and 1930 produced decreasing yields as the soil A horizon eroded and the land was subsequently abandoned. Successional vegetative communities established rapidly in these areas, dominated by early successional tree species such as chestnut (*Castanea dentata*) and tulip poplar (*Liriodendron tulipifera*) (Kalisz, 1986). Similar trends of agricultural abandonment have occurred worldwide, with about 64% of present-day forests estimated to be in some stage of secondary succession (FAO, 2010).

Throughout secondary succession, vegetation community structure and composition change considerably. In eastern U.S. temperate forests, traditional early succession is characterized mostly by grasses, forbs, and small shrubs, transitioning to larger shrubs and fast-growing trees such as black locust (*Robinia pseudoacacia)* and tulip poplar (*Lirodendron tulipifera)* (Clebsch & Busing, 1989). These communities are gradually overtaken by slow-growing and long-lived late successional species such as hickory and beech (Thompson et al., 2013). Early successional species tend to be fast growing with a low biomass C:N ratio, whereas later successional species have higher C:N ratios and more woody biomass. In a study in the Great Smoky Mountains of Tennessee, species richness was found to peak around mid-succession, whereas other indices of diversity, such as Shannon's Diversity Index and species evenness, peaked later (Clebsch & Busing, 1989).

However, this standard route of secondary succession is increasingly altered by other human-mediated ecological disturbances, even following agricultural abandonment and in the absence of direct human management. Actions such as deforestation and removal of seed banks, introduction of highly competitive invasive species, and climate change-induced alterations to temperature and water regimes may fundamentally change the course and outcome of secondary succession today compared to those decades or centuries ago (Dey et al., 2019; Gill & Marks, 1991).

Carbon cycling through secondary succession

The dynamics of carbon (C) cycling over the course of secondary succession have been widely studied, and change in land use is considered the leading driver of changes in the carbon budget (Delcourt & Harris, 1980). The general model of ecosystem carbon balance during secondary succession shows net primary production (NPP) starting low and increasing to a maximum in mid-succession when plant growth rates are high, and then decreasing and leveling out in late-succession as growth rates slow and the respiratory costs of woody biomass increase.

Heterotrophic respiration, on the other hand, theoretically starts high immediately following disturbance and declines to a somewhat lower level by late succession. Together, these two processes compose net ecosystem production (NEP), which starts below zero immediately following disturbance when heterotrophic respiration dominates, increases to a peak during mid-succession when plant growth is greatest, and then declines to near-zero levels in mature forest stands as woody biomass levels increase relative to photosynthesizing foliar biomass (Chapin et al., 2012; Gorham et al., 1979; Odum, 1969) (Figure 1).

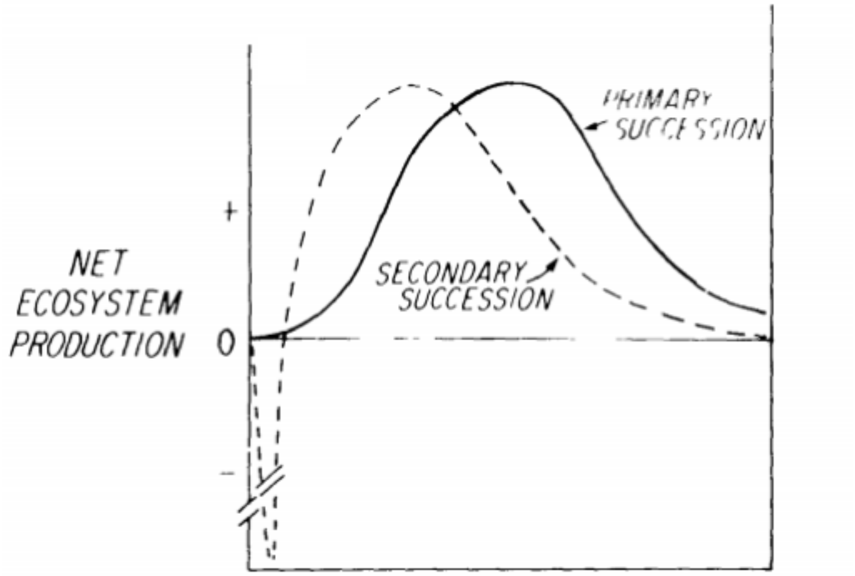

Figure 1: Conceptual diagram of NEP through secondary forest succession (adapted from Gorham et al., 1979)

Nitrogen cycling through secondary succession

Two broad conceptual models depict the inputs and outputs of nutrients, including N, over the course of secondary succession. In 1975, Vitousek and Reiners hypothesized an initial pulse of elemental (e.g. N) outputs immediately following disturbance caused by erosion and accelerated decomposition with minimal plant acquisition. They further hypothesized that following this initial loss of nutrients, in early and mid-succession when plant growth rates are at their maxima, inputs of elements such as N would exceed outputs, as plants would use more or all of the nutrients available to them. However, in late succession, as NPP and therefore plant nutrient needs level out, nutrient outputs would become equal to nutrient inputs (Vitousek & Reiners, 1975) (Figure 2).

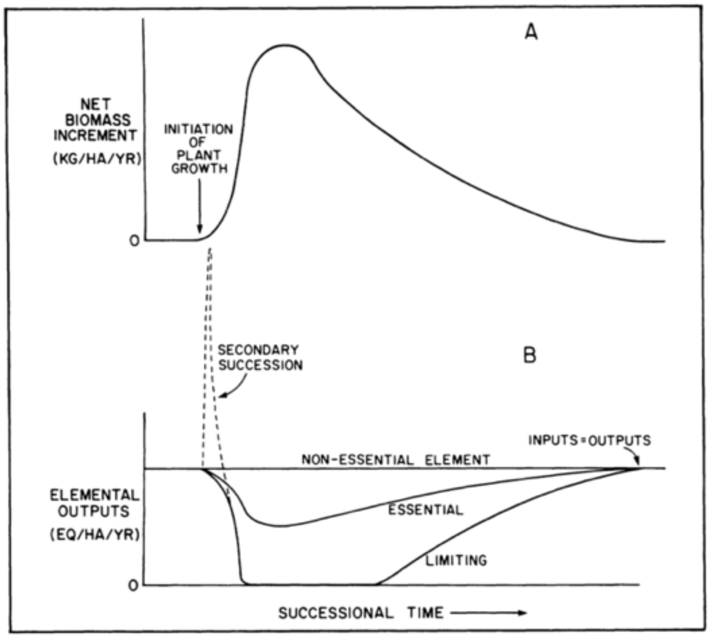

Figure 2: Vitousek and Reiners conceptual model of nutrient dynamics through secondary succession. “A” shows net biomass increment, synonymous with annual NPP, and “B” shows elemental outputs of non-essential, essential, and limiting (e.g. N) nutrients. The dotted line shows a steep increase in elemental outputs immediately following disturbance in secondary succession (Vitousek & Reiners, 1975).

More recently, Lovett et al. (2018) revised the conceptual model regarding nutrient inputs and outputs through succession. These new hypotheses suggest that mineral soil horizons can serve as sources of nutrients to plants early in succession and sinks of nutrients later in succession as plant growth slows. Thus, they argue, nutrient inputs and outputs are not as clearly coupled with biomass accumulation as Vitousek and Reiners (1975) theorized. In the Lovett et al. (2018) conceptual model, there is an added component of soil N dynamics, where soil N reaches a minimum during maximum NPP and plant N accumulation, in what they refer to as the "soil mining phase." Subsequently, as plant N accumulation levels off to near-zero levels in late succession, the soil reaccumulates N. N export becomes positive only when both the combined plant biomass and soil N sinks are less than N deposition (Figure 3).

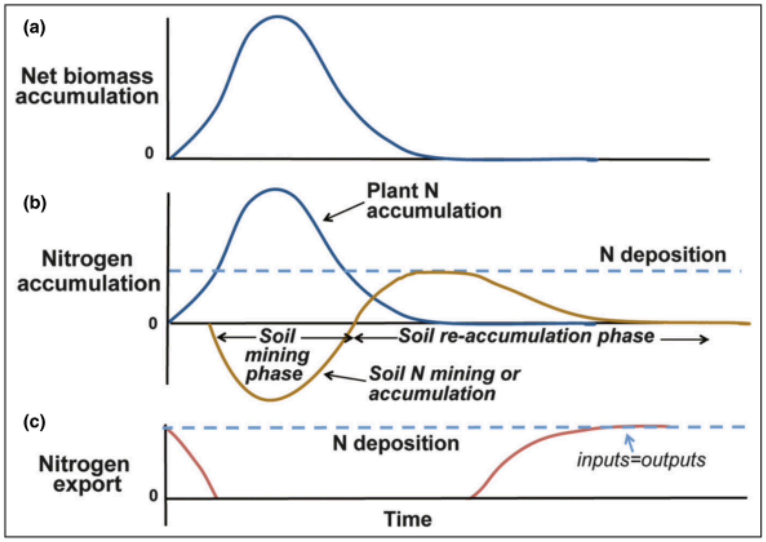

Figure 3: Lovett et al. (2018) revised conceptual model of nutrient retention during succession (a) shows net biomass accumulation, (b) shows plant N accumulation (blue line) and soil N dynamics (brown line), and (c) shows nitrogen export.

While these conceptual models have been developed to describe import and export of N through succession, few studies have focused on characterizing specific N transformations over secondary succession, and the studies that have examined these processes have yielded inconsistent results. These conflicting findings may stem from the narrowness of the studies –

many of which have measured one or two isolated components of the N cycle – and the fact that current studies examine varying climates, ecosystems, and disturbance types with minimal integration. Whereas the majority of the carbon budget in forests is essentially a balance between photosynthesis and ecosystem respiration, the N cycle is more complex, and thus a simple conceptual model is more difficult to create. Terrestrial N cycling is largely a closed cycle mediated by microbial N transformations and subsequent N uptake by plants, which can be influenced by a range of factors including vegetation structure, soil organic matter, and soil moisture and temperature, all of which can vary over the course of secondary succession (Compton & Boone, 2000).

Soil N stocks (and C stocks, which are tightly coupled with N) are the best understood aspects of N cycling over the course of secondary succession. There is general consensus that total soil C and N are depleted during cultivation and increase throughout succession (Knops & Tilman, 2000; Lamb, 1980; Pastor & Post, 1986; J. Wang et al., 2016; Xiao, Li, Wen, Yang, Luo, Chen, et al., 2018; Y. Yang et al., 2011; Zou et al., 2021). However, a study of succession following cropland abandonment in the Piedmont region of North Carolina found no clear differences in C and N throughout succession (Christensen & MacAller, 1985), and a New England study found higher N in cultivated soils when compared with a secondary woodlot (Compton & Boone, 2000).

Most studies also observe either an increase (Foote & Grogan, 2010; Liu et al., 2020; Parolari et al., 2017; Poulton, Pye, Hargreaves, & Jenkinson, 2003; Richter et al., 2000; Spohn, Novák, Incze, & Giani, 2016; Xiao, Li, Wen, Yang, Luo, & Chen, 2018) or no significant change (Hu et al., 2018; Wen et al., 2016) in the soil C:N ratio over the course of secondary succession following agricultural abandonment. Some studies note a decrease in mineral soil N early in succession, coinciding with an increase in biomass N, followed by an increase in mineral soil N (Kirschbaum et al., 2008; Mao et al., 2010; Richter et al., 2000), corroborating the Lovett et al. (2018) theory of a "soil mining phase" and subsequent "soil re-accumulation phase."

There is a general lack of consensus regarding soil N transformations through secondary succession. Some studies have found an acceleration of net N mineralization (Wen et al., 2016; Xiao et al., 2018) or potential net N mineralization (Zak et al., 1990), or higher NH_4^+ concentrations (Christensen & MacAller, 1985; Liu et al., 2020; Rice & Pancholy, 1972) in late successional forests compared to early successional fields. However, one study in North Carolina found a U-shaped relationship between net N mineralization and successional age (Richter et al., 2000), and another found high variability and no significant differences in net N mineralization over successional time (G P Robertson & Vitousek, 1981). Net nitrification has been hypothesized to decrease from early to late succession due to inhibition by tannins in mature forests (Christensen & MacAller, 1985; Rice & Pancholy, 1972), but several studies have found the opposite pattern (Wen et al., 2016; Xiao et al., 2018; Zak et al., 1990).

There are also conflicting findings regarding vertical N fluxes through secondary succession following agricultural abandonment. Some studies have examined the ^{15}N natural abundance in plants and soils, which can provide insight into the movements of N into, out of, and within the system. Whereas one study found elevated $\delta^{15}N$ in late succession, suggesting an increase in gaseous loss and a more open N cycle (Wen et al., 2016), another found a decrease in $\delta^{15}N$ in soil and leaves from 5 to 85 years of secondary succession, suggesting a more closed cycle later in succession (Wang et al., 2007). N fixation can serve as a substantive input of N into a system, and its rate has been hypothesized to peak in early-mid succession, due to the common occurrence of N-fixing legumes and shrubs in these stages (Gorham et al., 1979; Poulton et al., 2003). However, some studies observe that N accumulation rates follow N deposition rates, with apparently low rates of N fixation at any successional stage (Richter et al., 2000; Zak et al., 1990). Leaching represents a flux of N out of the system, and Rice and Pancholy (1972) theorize that it decreases through succession due to decreased nitrification and NO_3^- concentrations late in succession.

Clearly, more comprehensive datasets are needed to elucidate patterns in N cycling throughout succession. Here, I investigate a wide range of N stocks, transformations, and fluxes in northwestern Virginia chronosequences, with the aim of understanding how these processes vary through secondary succession following agricultural abandonment.

Methods

Site Description

Blandy Experimental Farm (BEF), located in Boyce, Clarke County, Virginia (39.06, -78.07), is a 300-hectare research, education, and outreach field station operated by the University of Virginia. Situated in the Shenandoah Valley within the Blue Ridge Mountains, it has a mean annual precipitation of 975 mm and a mean annual temperature of 12°C. The soils at BEF are classified as ultisols, largely due to the long history of agriculture.

BEF has two replicate chronosequences consisting of adjacent fields in three successional stages – early, mid, and late. All fields were formerly used for agriculture and were abandoned in 2001 and 2003 (early), 1986 and 1987 (mid), and prior to 1910 and 1920 (late) (Wang et al., 2016) (Figure 4). These chronosequences offer a unique opportunity to examine nutrient cycling through time in a series of plots experiencing the same environmental conditions but with different times elapsed since agricultural abandonment.

Figure 4: Two replicate chronosequences at BEF (outlined in red), each consisting of an early, mid, and late successional field.

Within each field, five 10x10 m plots were randomly selected, for a total of 30 plots (Figure 5).

Figure 5: Plots within each field (yellow stars) a) in the western chronosequence and b) the eastern chronosequence

Baseline Ecosystem Properties

Soil temperature, moisture, and CO_2 efflux were measured biweekly during the 2018 and 2019 growing seasons, at three randomly selected points per plot. Soil temperature was measured using a digital soil thermometer probe to 10 cm depth. Soil moisture was measured using a HydroSense II soil moisture probe (Campbell Scientific, Inc., Logan, UT, USA) to a depth of 12 cm. Soil CO_2 efflux was measured using an EGM-5 gas analyzer with attached soil respiration chamber (PP Systems, Amesbury, MA, USA).

N Pools

Soil C and N

At each plot, three sampling locations were randomly selected, and soil C and N, along with bulk density, were determined at three depths: 0-10 cm, 10-20 cm, and 20-30 cm. Samples were collected with a soil corer of known volume in 10 cm increments. The soil was oven dried at 60°C for 48 hours or until mass remained constant, and then sieved with a 2 mm sieve. The >2 mm fraction was separated into organic and mineral matter, and the total volume of non-soil was calculated using the theoretical densities for organic matter (0.2 g/cm^3) and mineral matter (1.65 g/cm^3). The <2 mm fraction was weighed, and bulk density calculated using Equation 1:

$$Eq.\ 1{:}\ Bulk\ density = \frac{Dry\ weight\ of < 2\ mm\ fraction}{Total\ volume\ of\ soil\ core - calculated\ volume\ of\ > 2\ mm\ fraction}$$

The sieved soil was homogenized and ground to a fine powder using a ball mill, and soil percent C and N was determined using a Flash 2000 NC Analyzer (Thermo Scientific, Waltham, MA, USA). From the C and N percent by mass, total soil C and N per unit area was calculated by multiplying the fraction of C and N by the bulk density.

Soil Inorganic N

Soil ammonium (NH_4^+) and nitrate (NO_3^-) pools were determined at three soil depths (0-10 cm, 10-20 cm, and 20-30 cm) twice during the growing season of 2019: peak growing season (mid-July) and during senescence (mid-October). During each collection period, at one randomly chosen location per plot, a soil corer was used to collect a sample at each depth. Following collection, soil samples were immediately frozen for later analysis.

At the time of analysis, soil samples were thawed and passed through a 2 mm sieve to separate out non-soil components. One 10 g subsample of each sample of field-moist soil was set aside for drying to determine soil moisture content. This subsample was dried at 60° C for 48 hours and then weighed. Another 10 g subsample was used for KCl extraction with 40 mL of 2 M KCl. The KCl and soil mixture was shaken at 200 rpm for one hour, and then allowed to rest for an hour. The liquid supernatant was then filtered, and the KCl solution containing the extracted NH_4^+ and NO_3^- was analyzed colorimetrically using a Lachat QuikChem 8500 Flow Injection Analyzer (Hach Company, Loveland, CO, USA) to determine their concentrations.

The calculated percent mass loss of the dried subsample [(field moist weight-dry weight)/field moist weight] was used to calculate the mass of soil used for the field moist extraction. From this, concentrations of NH_4^+-N and NO_3^--N per unit mass of soil were calculated, and using bulk density, inorganic N concentrations per unit area were calculated.

Litter C and N

Litter was collected following the 2017 and 2019 growing seasons. In late August, prior to senescence, one litter basket of known area was anchored to the ground in a randomly selected location in each plot. In plots with minimal overstory and a large area of herbaceous plants, a 0.5x0.5 m quadrat was established and marked. Existing litter within the quadrat was removed prior to senescence.

In early November, following senescence, the contents of each litter basket were collected. In plots with quadrats, new litter within the quadrat was also collected. Within the quadrats, “litter” was defined as any senesced biomass that was no longer attached to the ground or to a live plant (i.e., not standing dead biomass). The contents of the litter baskets and/or quadrats from each plot were dried at 40° C until they maintained a constant mass. Contents were weighed, and then a representative sample of each basket/quadrat was ground to a fine powder for C and N analysis with a FLASH 2000 NC Analyzer (ThermoFisher Scientific, Waltham, MA, USA). The C and N content and mass of the litter within the known areas of the litter basket or quadrat were used to calculate the mass of C and N in senesced biomass produced per unit area per year.

Foliar C and N

Foliar C and N were measured during the 2021 growing season in three plots per field (18 plots total, six per successional stage). Three to four dominant species in each field were selected as focal species (Table 1). If a species was present within a plot, an individual of that species was selected and marked, and two leaf samples collected (one shade leaf and one sun leaf). Within each species, plants of similar sizes and ages were selected as the representative individuals.

Leaves were collected from each marked individual at three stages throughout the growing season; mid-June (late spring), mid-July (summer), and mid-September (fall). Shade leaves were characterized as leaves low in the canopy receiving minimal direct sunlight. These were collected either by hand, or for taller trees and shrubs, with a tree pruner. Sun leaves were characterized as leaves either high in the canopy or growing in gaps of the canopy, and receiving direct sunlight for a substantial portion of the day. For smaller plants, sun leaves were collected by hand, and for taller plants, they were collected using a tree pruner or by firing a shotgun at the desired branch and retrieving the fallen leaves.

Table 1: Focal species in each field

Field Age	Chronosequence 1	Chronosequence 2
~20 years (Early successional)	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • autumn olive (*Elaeagnus umbellate)*	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • Amur honeysuckle (*Lonicera mackii)*
~35 years (Mid-successional)	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • autumn olive (*Elaeagnus umbellate)* • Amur honeysuckle (*Lonicera mackii)*	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • autumn olive (*Elaeagnus umbellate)* • Amur honeysuckle (*Lonicera mackii)*
100 + years (Late successional)	• Dahurian buckthorn (*Rhamnus davurica)* • tree of heaven (*Ailanthus altissima)* • mockernut hickory (*Carya tomentosa)*	• Dahurian buckthorn (*Rhamnus davurica)* • white oak (*Quercus alba)* • mockernut hickory (*Carya tomentosa)* • paw paw *(Asimina triloba)*

Following collection, the surface area of each fresh leaf was calculated using an LI-3000C leaf area meter with attached transparent belt conveyor (LI-COR Biosciences, Lincoln, NE, USA). Leaves were then dried at 40° C for 48 hours, and their mass was recorded. Leaf mass per area (LMA) was calculated as the dry weight of the leaf divided by its surface area.

Each leaf was then ground into a fine powder individually to be homogenized and then prepared for C and N content analysis with a FLASH 2000 NC Analyzer (ThermoFisher Scientific, Waltham, MA, USA). C and N per unit area were determined by multiplying the percent C and N by the LMA of each leaf. C:N was calculated on a mass basis.

N transformations

Net N mineralization and nitrification

At three locations per plot (90 total), net N mineralization and net nitrification were estimated using a paired soil core-resin bag approach, as described by Raison et al. (1987). These soil N transformations were estimated twice during the growing seasons of 2017 and 2019; once during the time period of mid-June to mid-July, and once during the time period of mid-July to mid-August. A soil core to 10 cm depth was taken at each location and immediately frozen to serve as the "initial" sample. An adjacent soil core was then taken and placed back in the ground in a PVC tube with an anion-cation exchange resin bag secured beneath it to capture any leaching NH_4^+ and NO_3^-. These cores were allowed to incubate *in situ* for 28 days during the time periods specified above, after which time the soil and resin bags were frozen separately for later analysis.

Soil samples and resin bags were thawed in the lab, and the field moist soil samples sieved through a 2 mm sieve to separate out non-soil components. NH_4^+ and NO_3^- were extracted from each soil and resin bag sample using 2M KCl and the same methods described above for determining inorganic N concentrations. An 80% extraction efficiency was assumed for resin bags (Hart & Binkley, 1984). Net N mineralization per incubation period was calculated using Equation 2, and net nitrification was calculated using Equation 3.

Equation 2:

$$\frac{\left(NH_4^+ - N_{final\ core} + NH_4^+ - N_{resin\ bag} + NO_3^- - N_{final\ core} + NO_3^- - N_{resin\ bag}\right) - \left(NH_4^+ - N_{initial\ core} + NO_3^- - N_{initial\ core}\right)}{1\ month}$$

Equation 3:

$$\frac{\left(NO_3^- - N_{final\ core} + NO_3^- - N_{resin\ bag}\right) - \left(NO_3^- - N_{initial\ core}\right)}{1\ month}$$

N Fluxes

Inorganic N Leaching Potential

Potential leaching of NH_4^+ and NO_3^- was estimated using the soil inorganic N values from 0-10, 10-20, and 20-30 cm depth (measured in July and October of 2019 as described above) and the mass of roots in each 10 cm soil layer. Root mass in each soil layer was measured during determination of bulk density as described above. An index estimating the potential of inorganic N leaching was calculated using the NO_3^- and NH_4^+ concentrations in each 10 cm soil layer, divided by the mass of roots within and below that soil layer. The greater the root mass within and below the soil layer, or the lower the inorganic N concentrations in the soil layer, the lower the leaching potential index.

Soil ^{15}N natural abundance

Soil δ^{15}N (‰) was determined in the top 10 cm of soil at three locations per plot, from samples collected in July 2019. Following collection, the soil was dried at 60°C for 48 hours or until it maintained constant mass, after which it was passed through a 2 mm sieve to remove non-soil components (i.e., rocks and roots). The samples were then ground to a fine powder, tinned, and sent to the UC Davis Stable Isotope Facility for isotopic analysis. An Elementar Vario EL Cube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK) was used for sample analysis.

Foliar ^{15}N natural abundance

Foliar δ^{15}N (‰) was determined in live-collected leaves for four commonly found species; Dahurian buckthorn (*Rhamnus davurica)*, wineberry (*Rubus sp.)*, autumn olive (*Elaeagnus umbellate)*, and white oak (*Quercus alba)*. Shade leaves were collected three times during the 2018 growing season (early June, mid-July, and mid-September), and sun leaves were collected three times during the 2019 growing season. Shade and sun leaves were defined and collected as described above.

Following collection, leaves were dried at 40°C for 48 hours, after which they were ground to a fine powder, tinned, and sent to the UC Davis Stable Isotope Facility for isotopic analysis. A PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) was used to determine foliar δ^{15}N.

Statistical analysis

Linear mixed effects models were used to determine differences in ecosystem properties among the early, mid, and late successional age classes. Variables were log-transformed as needed to correct for normality of residuals (assessed visually using histograms and Q-Q plots), and variance structures were added as needed to correct heterogeneity of variance (assessed visually and quantitatively using Levene's test). To ensure a lack of pseudoreplication, when plots were subsampled or sampled repeatedly, "plot" was added as a random effect in the model. When multiple years of data were collected, "plot" was nested within "year" as a random effect, unless "year" was included as a fixed effect in the model to assess year-to-year variability. Models were fitted using the restricted maximum likelihood (REML) procedure. Significance of fixed effects was evaluated using marginal F-tests, and post-hoc pairwise comparisons were conducted using Tukey's HSD test.

All statistical analyses were conducted using R (R Core Team, 2020). Models were fitted and hypothesis tests were conducted using the nlme package (Pinheiro et al., 2022). Post-hoc pairwise comparisons and estimated marginal means were calculated using the emmeans package (Lenth et al., 2022).

Results

Baseline ecosystem properties

Soil temperature significantly decreased through successional stage, whereas soil moisture significantly increased ($p=0.03$, $p<0.001$, respectively; Figure 6a and 6b). Soil temperature increased through the growing season (late May-August), whereas the trend in soil moisture differed by year (increasing trend in 2018, decreasing trend in 2019). Soil CO_2 efflux decreased from a mean of 1.05 g CO_2/m^2/hr in early succession to a mean of 0.76 g CO_2/m^2/hr in late succession (p=0.004, Figure 6c), and did not vary significantly through the growing season.

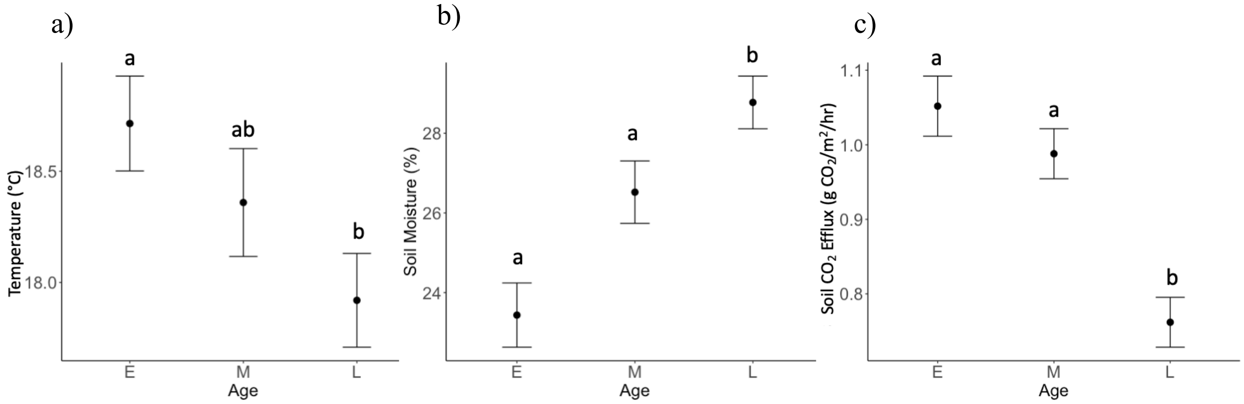

Figure 6: Soil temperature (a), moisture (b), and CO_2 efflux (c) in early, mid, and late succession, averaged over both growing seasons. Different letters indicate significant differences (p<0.05). Error bars indicate standard error.

N pools

Soil C and N

Soil C increased significantly from early to late succession (p=0.01), with the increases primarily occurring in the 0-10 and 10-20 cm soil fractions (Figure 7a). Soil N trends upward insignificantly through succession, with the trend visible in the 0-10 and 10-20 cm soil fractions, but not in the 20-30 cm soil fraction (Figure 7b). Soil C:N varied insignificantly by soil depth, but increased through successional time, with the C:N in late succession being significantly higher than in early or mid- succession (p=0.005) (Table 2, Figure 8).

Table 2: Mean soil C, N, and C:N

Age	Depth (cm)	C (g/m^2) (SE)	N (g/m^2) (SE)	C:N (SE)
E	0-10	2003.1 (105.6)	216.4 (8.4)	9.23 (0.30)
	10-20	1379.6 (111.4)	136.9 (7.0)	10.11 (0.68)
	20-30	802.9 (60.4)	87.2 (4.2)	9.12 (0.39)
M	0-10	2150.1 (87.6)	230.0 (11.0)	9.51 (0.21)
	10-20	1512.2 (67.9)	175.3 (9.8)	8.95 (0.32)
	20-30	836.6 (51.9)	111.8 (12.8)	8.90 (0.71)
L	0-10	2722.6 (118.8)	263.0 (12.1)	10.58 (0.39)
	10-20	1956.2 (133.9)	183.5 (14.6)	11.46 (0.63)
	20-30	837.0 (52.7)	89.3 (9.5)	10.55 (0.57)

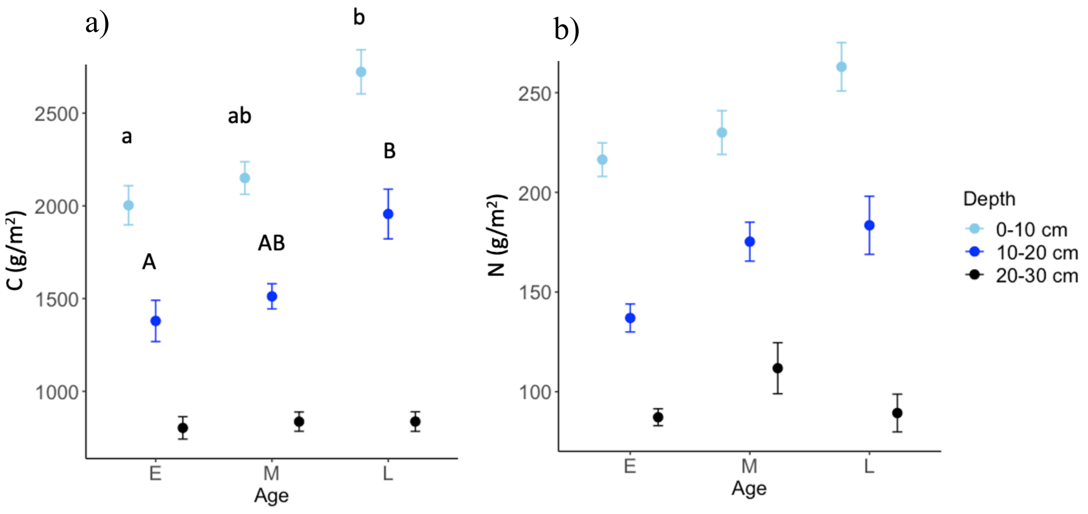

Figure 7: Mean soil C (a) and N (b) through secondary succession at each soil depth measured. Different letters indicate significant differences through succession in that specific depth category. Error bars indicate standard error.

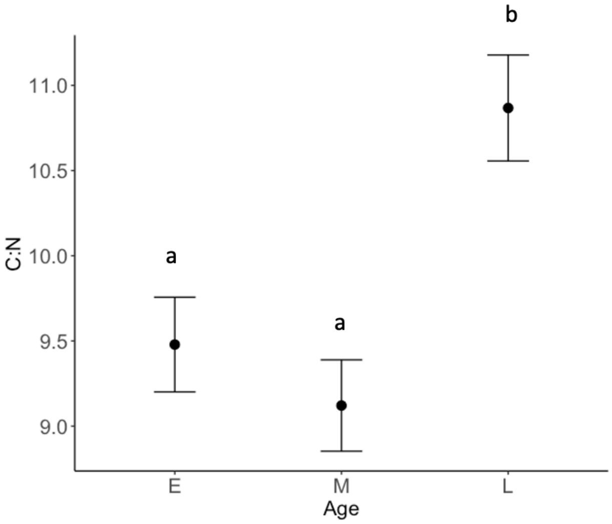

Figure 8: Mean soil C:N through secondary succession. Different letters indicate a significant difference. Error bars indicate standard error.

Soil inorganic N

Soil ammonium significantly decreased through successional time (p=0.04) and by soil depth (p<0.001), but did not vary by sampling month (Figure 9a). Conversely, soil nitrate concentration significantly increased through successional time (p=0.003), and was higher in October than in July (p<0.001, Figure 9b). Soil nitrate concentration showed increasing variability from early to mid to late succession. Total inorganic N decreased with depth (p<0.001), but did not vary significantly by age.

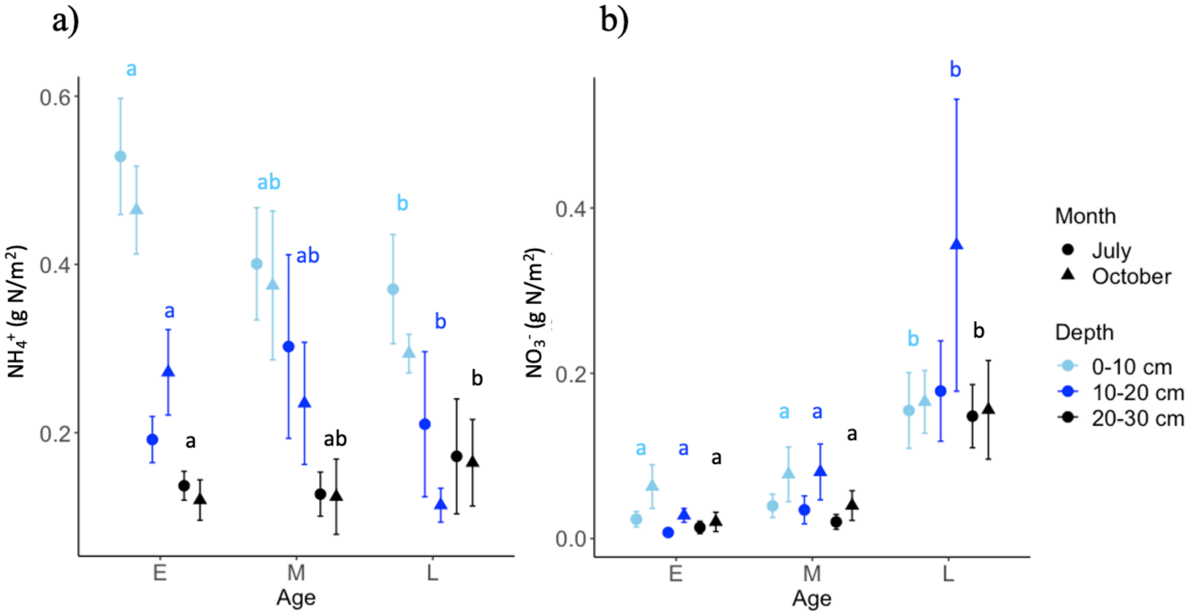

Figure 9: Soil NH_4^+ (a) and NO_3^- (b) through secondary succession. Different letters indicate significant differences between the successional stages in that particular depth category. Error bars indicate standard error.

Litter C and N

Across all successional stages, litter N concentration ranged from 0.91-3.21%, with an overall mean of 1.77%. While the N concentration of litter sharply decreased in late succession (p=0.03, Figure 10a), average litter quantity increased, and thus the N per unit area significantly increased through succession (p=0.005, Figure 10b). Carbon concentration ranged from 38.86-79.97% and varied insignificantly by field age, but C per unit area was highest in late succession (p<0.001), along with litter C:N (p=0.02, Figure 10c).

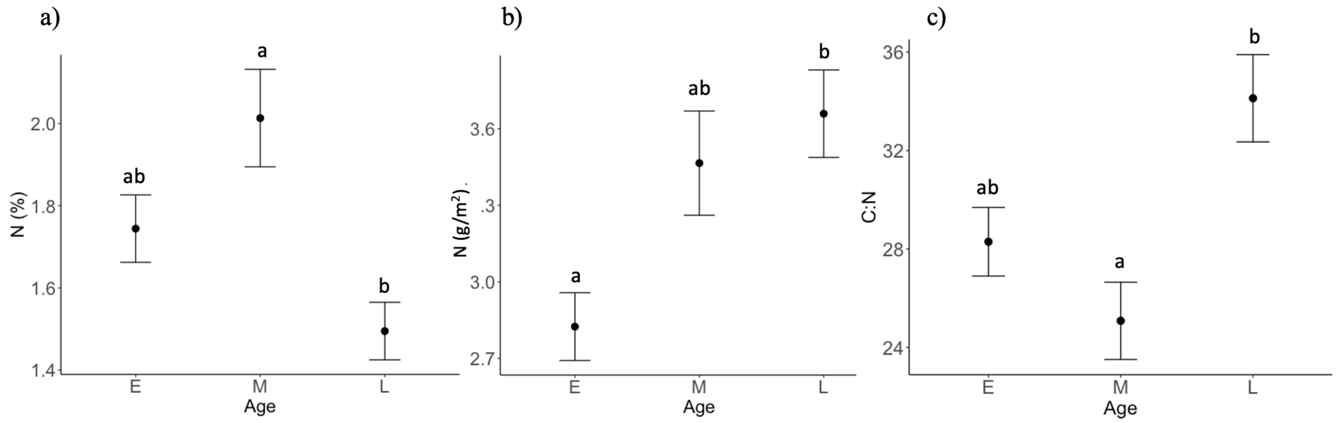

Figure 10: Litter N concentration (a), N per unit area (b) and C:N (c) through secondary succession. Different letters indicate significant differences between the successional stages. Error bars indicate standard error.

Foliar C and N

Foliar N concentration across all focal species increased through successional time (p=0.05, Figure 11a), decreased through the growing season (p=0.05), and varied insignificantly by leaf type (sun vs. shade). Foliar C:N increased through successional time (p=0.04), and varied insignificantly by season and leaf type. Despite an increase in foliar percent N through succession, foliar LMA trended downward (Figure 11b), and thus foliar N per unit area significantly decreased through succession (p=0.03). LMA, and relatedly, foliar N per unit area, was significantly lower in shade leaves than sun leaves (p<0.001, Figure 11b), and increased through the growing season (p<0.001).

Species-specific linear mixed effects models revealed similar patterns. For example, *Rhamnus davurica*, which was a focal species in all three successional stages, trended upward in foliar N concentration through succession (though not significantly, Figure 12), decreased in foliar N per unit area from early to late succession (p=0.03), increased in foliar N per unit area through the growing season (p=0.02), and had higher area-based foliar N in sun leaves than shade leaves (p<0.001).

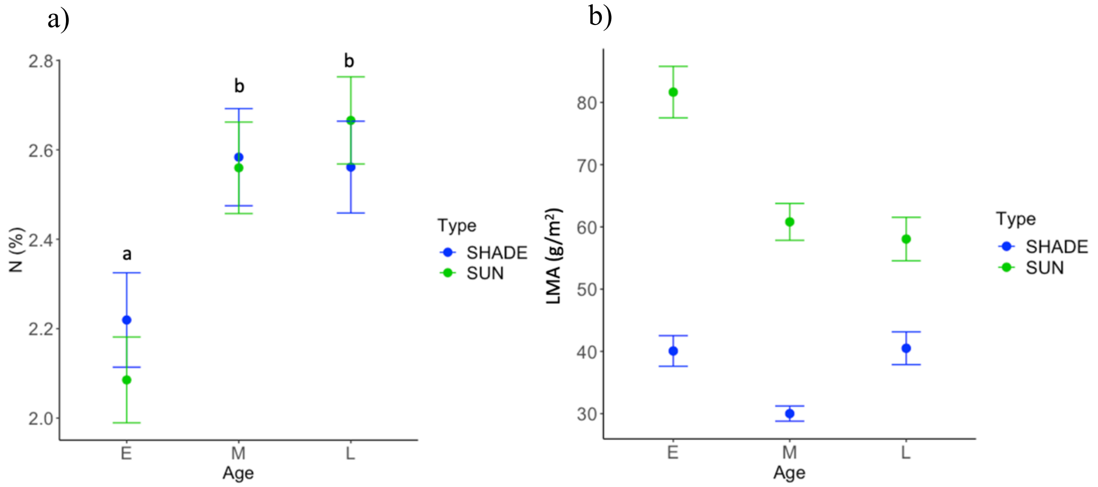

Figure 11: Foliar N concentration (a) and LMA (b) in shade and sun leaves through secondary succession. Different letters represent significant differences through succession in a linear mixed effects model with both leaf types. Error bars represent standard error.

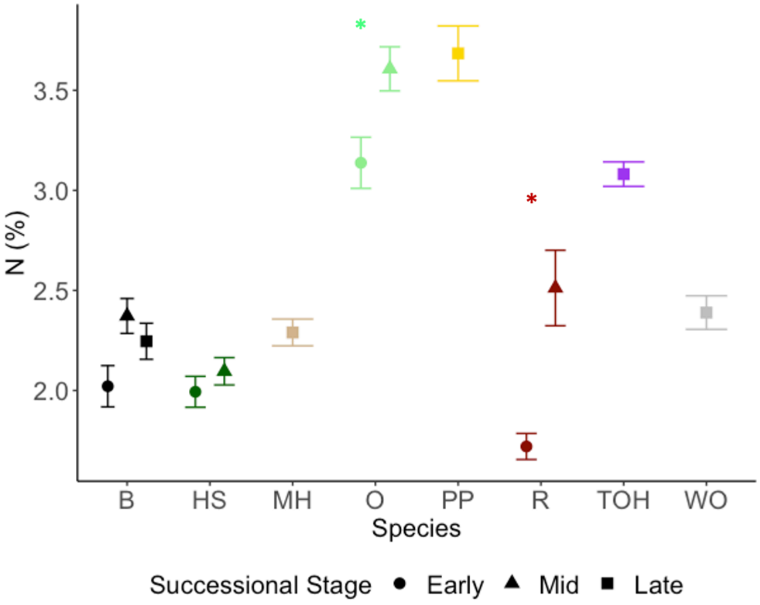

Figure 12: Foliar N concentration through secondary succession in each focal species. Stars indicate significant differences through the successional stages in a separate linear mixed effects

model for that particular species. B=Dahurian buckthorn, HS=Amur honeysuckle, O=autumn olive, R=wineberry, MH=mockernut hickory, PP=pawpaw, TOH=tree of heaven, WO=white oak. Error bars represent standard error.

N transformations

Net N mineralization and nitrification

Net N mineralization and nitrification both increased through successional time (p=0.001, p=0.009, respectively; Figure 13a and 13b). Net N mineralization and nitrification both varied insignificantly by incubation (June-July vs. July-August).

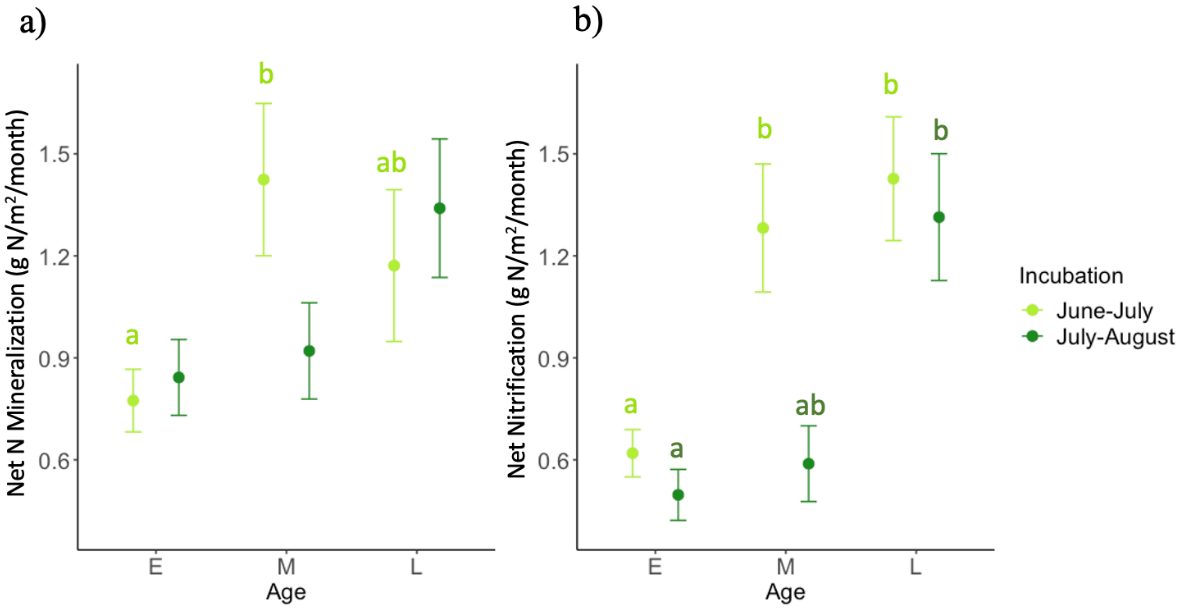

Figure 13: Net N mineralization (a) and net nitrification (b) averaged over both years in the June-July and July-August incubations. Different letters represent significant differences in the N transformations through succession in that particular incubation. Error bars represent standard error.

N fluxes

Inorganic N leaching potential

Despite a significant increase in inorganic N from early to late succession, the leaching potential index varied insignificantly through successional time (Figure 14). Leaching potential index also did not significantly differ between the July and October sampling periods. The index did differ between soil depths, with the 10-20 cm soil fraction having the lowest mean value (p=0.04).

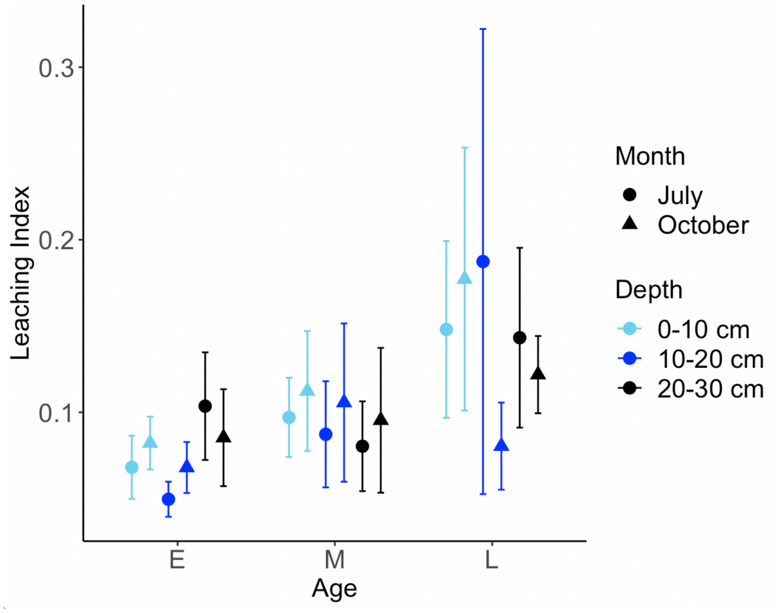

Figure 14: Leaching potential index through succession, grouped by month and soil depth. Error bars represent standard error.

Soil ^{15}N natural abundance

Soil $\delta^{15}N_{Air}$ (‰) in the top 10 cm was significantly lower in mid succession than in early or late succession (p=0.01, Figure 15a); mean $\delta^{15}N$ was 3.68‰, 2.48‰, and 3.72‰ in early, mid and late succession, respectively. Soil in all successional stages was enriched in ^{15}N relative to air. Soil $\delta^{13}C_{VPDB}$ (‰) was also significantly lower in mid succession than in early or late succession ($p=0.05$, Figure 15b).

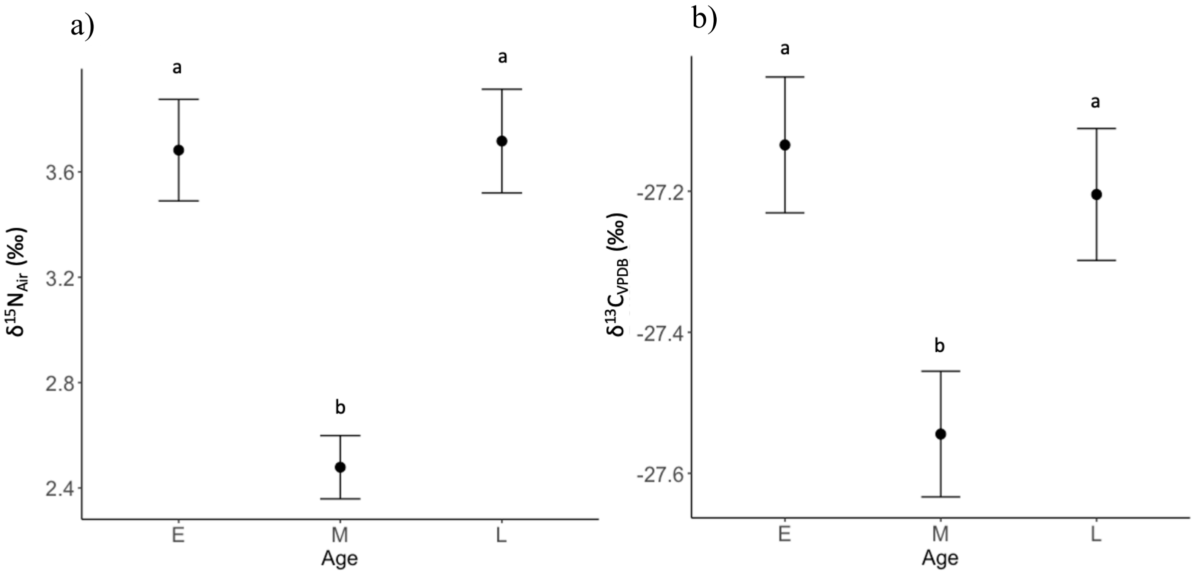

Figure 15: Soil $\delta^{15}N$ (a) and $\delta^{13}C$ (b) in each successional stage. Different letters represent significant differences between the successional stages. Error bars represent standard error.

Foliar ^{15}N natural abundance

Foliar $\delta^{15}N_{Air}$ (‰) was highest in early succession, and was not significantly different in mid and late succession ($p<0.001$, Figure 16a). Foliar $\delta^{13}C_{VPDB}$ (‰) followed a similar pattern; it was highest in early succession, and lowest in mid succession, but was intermediate in late succession ($p<0.001$, Figure 16b). Within individual focal species, foliar $\delta^{15}N$ showed no consistent patterns (Figure 17a). However, within individual focal species, foliar $\delta^{13}C$ was inversely related to field age, significantly decreasing from early to mid to late succession (Figure 17b).

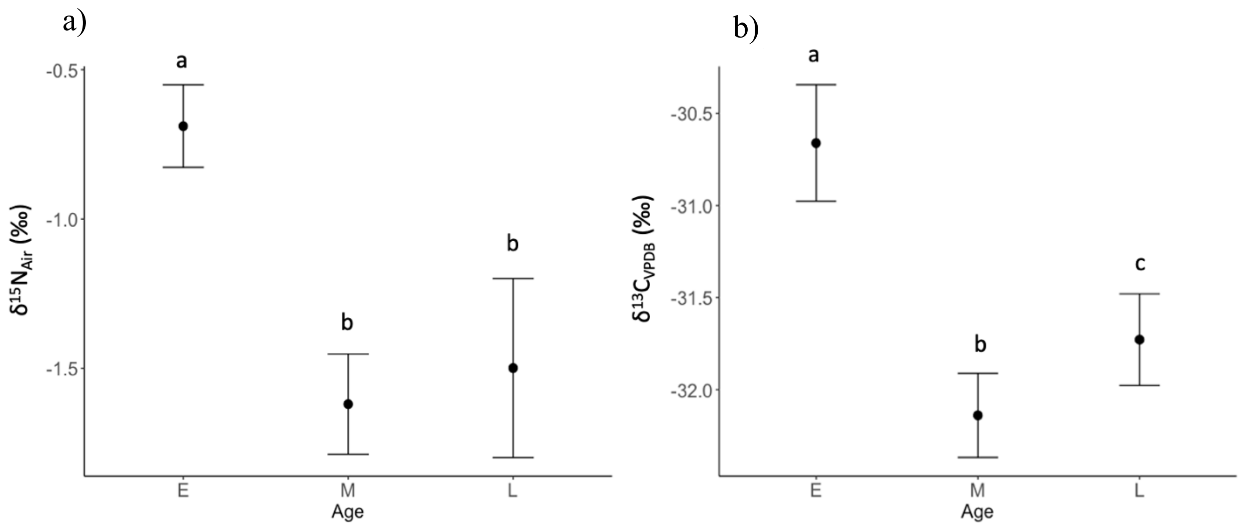

Figure 16: Foliar δ^{15}N (a) and δ^{13}C (b), averaged over seasonal time and all four focal species. Different letters represent significant differences between the successional stages. Error bars represent standard error.

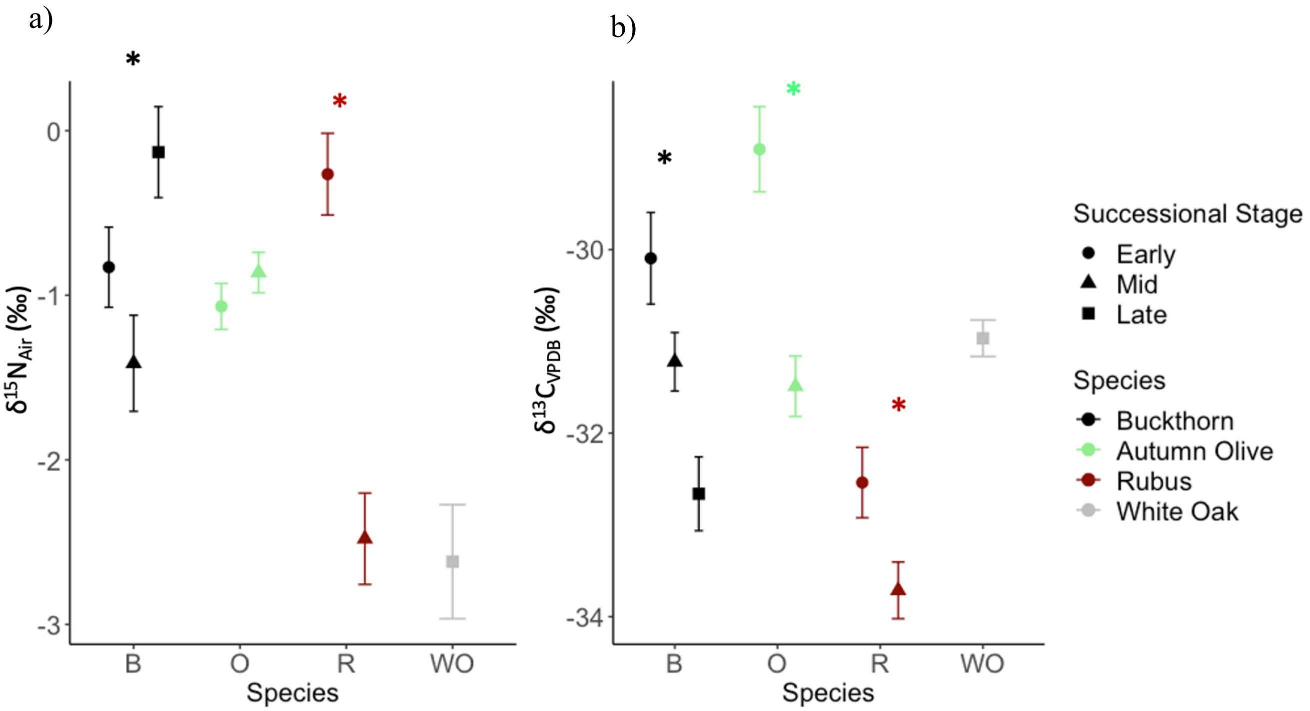

Figure 17: Foliar δ^{15}N (a) and δ^{13}C (b) through succession by focal species. Stars represent significant differences between the successional stages in a separate linear mixed effects model for that particular species. B=Dahurian buckthorn, O=autumn olive, R=wineberry, WO=white oak. Error bars represent standard error.

Discussion

N Pools

I found significant increases in soil C and N through successional time, consistent with other studies examining soil C and N pools over the course of secondary succession (Foote & Grogan, 2010; Hu et al., 2018; Knops & Tilman, 2000; Poulton et al., 2003; Spohn et al., 2016; Wen et al., 2016; Xiao et al., 2018; Zhang et al., 2015). This indicates a depletion of soil C and N during cultivation followed by a reaccumulation during vegetation regrowth. Agriculture may deplete soil C and N in multiple ways, including biomass removal and tilling, which mixes the soil layers, increases erosion, and accelerates decomposition rates (Foote and Grogan, 2010; McLauchlan, 2006). Forest regeneration may increase soil C stocks in this system through increased biomass litter inputs (Liu et al., 2020; Poulton et al., 2003; Wen et al., 2016). Woody biomass (with a high C:N) and litter mass increased through the successional stages in this system and reached a maximum in the late successional plots, feeding carbon-rich biomass back into the soil.

N accumulation during secondary succession, which is closely linked to C accumulation, can be attributed to atmospheric deposition, symbiotic fixation, asymbiotic fixation, and recycling and retention of N within the plant-soil system (Knops & Tilman, 2000; Wen et al., 2016); the latter three all have the potential to increase during ecosystem regeneration. I did not observe an initial decrease in soil N, or a “soil mining phase” in this study as predicted by Lovett et al. (2018). However, my fields of ~20, ~35, and ~100 years old may have undergone this phase earlier in the successional process, or I may lack the temporal resolution necessary to observe this phenomenon.

I also found C:N to be highest in late succession, implying a more rapid recovery of C compared to N in this system, and in accordance with other post-agricultural studies of secondary succession (Foote & Grogan, 2010; Hooker & Compton, 2003; Knops & Tilman, 2000; Liu et al., 2020; Mao et al., 2010; Parolari et al., 2017; Poulton et al., 2003; Richter et al., 2000; Spohn et al., 2016; Switzer, GL; Shelton, MG; Nelson, 1979; Xiao et al., 2018). This is caused in part by the observed increase in C:N in the litter from early to late succession; while foliar N concentration increases through the successional stages in this system, likely due to the increase in N availability, litter N concentrations sharply decrease in late succession, suggesting that the late successional plants have a more conservative N-use strategy and are reabsorbing more foliar N prior to senescence. Though the C:N in this system increases through successional time, it remains relatively low compared to other forested systems in the southern and Mid-Atlantic United States (e.g. Richter et al., 2000; Yesilonis et al., 2016). This suggests legacy effects of agriculture on the soil even after 100 years of succession, as agricultural practices tend to decrease C:N (McLauchlan, 2006).

Although I observed no significant change in total inorganic soil N through the successional chronosequence, I observed divergent trends in different forms of inorganic N. Whereas NH_4^+ significantly decreased with age, NO_3^- significantly increased. While the literature describes no consistent trends regarding NH_4^+ and NO_3^- through successional time, my results are consistent with those of other studies finding an inverse relationship between the two

(Christensen & MacAller, 1985; Rice & Pancholy, 1972; Wen et al., 2016). This increase in NO_3^- corresponding to a decrease in NH_4^+ suggests either a shift in microbial composition and an increased number of nitrifiers later in succession (Liu et al., 2020) or N saturation late in succession, reducing the proportion of NH_4^+ immobilized and leaving more in the soil to be nitrified (Eric A. Davidson et al., 2007; Wen et al., 2016).

N Transformations

The relative pools of NH_4^+ and NO_3^- are consistent with N transformation trends I observed; while net N mineralization and nitrification both significantly increased through succession, the ratio of mineralized N to subsequently nitrified N changed. N mineralization and nitrification are of particular importance to plants, as NH_4^+ and NO_3^- are the primary forms of plant-available N (Rice & Pancholy, 1972). Early in succession, a relatively low proportion of mineralized N was nitrified (0.80 g N/m^2/month mineralized vs. 0.57 g N/m^2/month nitrified), but in late succession, net nitrification exceeded net N mineralization due to NH_4^+ immobilization during the incubation (1.26 g N/m^2/month mineralized vs. 1.37 g N/m^2/month nitrified).

The increase in both net N mineralization and nitrification is consistent with other studies conducted in the karst region of China (Wen et al., 2016; Xiao et al., 2018) and in Minnesota, (Zak et al., 1990), and suggest an increase in N availability and a more open N cycle later in succession. A shift to increasing nitrification relative to N mineralization and increasing NO_3^- later in succession may have implications for ecosystem N losses due to the additional chemical reactions that must be carried out to convert mineralized NH_4^+ to NO_2^- and then NO_3^-, and then back to NH_4^+ to be assimilated into plants in the form of amino acids. These additional reactions consume energy, and each carries the risk for loss of N through various gaseous pathways (Rice & Pancholy, 1972; Firestone & Davidson, 1989). This shift may also have implications for other ecosystem N losses, as the negative charge of NO_3^- makes it more mobile in the soil than NH_4^+, and thus more vulnerable to loss through leaching or runoff (Wen et al., 2016). Indeed, my measurements of inorganic N in different soil layers indicate depth-dependence of both NH_4^+ and NO_3^-, but stronger depth dependence in NH_4^+, as microbial processes are generally most active in the upper soil layers and NO_3^- is more mobile and able to move to deeper soil layers (Rice & Pancholy, 1972).

N fluxes

Despite an increase in NO_3^- relative to NH_4^+ later in succession, I observed no significant change in leaching potential index from early to late succession. Though total inorganic N trended upward (though insignificantly), root mass also increased with field age, balancing out the increase in more mobile NO_3^-. However, it is important to note that this index does not examine inorganic N values below 30 cm, and thus does not explicitly describe how much inorganic N may pass below the rooting zone.

There were, however, significant trends in natural abundance of ^{15}N in soil and leaves, suggesting differing N fluxes through successional time. Soil $\delta^{15}N$ in the top 10 cm was lowest in mid succession and highest in early and late succession. Values ranging from ~1-5 ‰ are in alignment with other soil $\delta^{15}N$ values in temperate forest successional ecosystems (e.g. Billings & Richter, 2006; Compton et al., 2007; Wen et al., 2016), but the pattern is in contrast to another study conducted at BEF in 2005, which showed increasing $\delta^{15}N$ from early to late succession (L. Wang et al., 2007). Generally, biological nitrogen fixation introduces ^{15}N-depleted N into the system (-2 to 0‰), so low ^{15}N soil signatures are often associated with higher rates of N fixation (Wen et al., 2016). This suggests that N fixation is highest in mid succession in the BEF system, where known N-fixing shrubs such as autumn olive (*Elaeagnus umbellata*) are at their most dense.

In contrast, high $\delta^{15}N$ values are often associated with a more open N cycle, as higher N availability leads to higher rates of microbial processing, and microbes preferentially utilize ^{14}N. Because ^{14}N is preferentially used by soil microbes, ^{14}N is lost at a higher rate than ^{15}N, as each soil reaction (mineralization, nitrification, denitrification) carries the risk of N loss through gaseous or leaching pathways (L. Wang et al., 2007; Wen et al., 2016). This suggests that ecosystem openness, including ecosystem N losses, may be highest in early and late succession at BEF. This is consistent with my findings of peak NH_4^+ concentrations in early succession and peak NO_3^- concentrations in late succession. In addition, soil temperature and CO_2 efflux were at their peak in early succession, indicating high microbial activity, and total N availability and transformation rates were at their maximum in late succession, also indicating high microbial activity.

In contrast with soil ^{15}N, foliar ^{15}N pooled across all four species examined [Dahurian buckthorn (*Rhamnus davurica)*, wineberry (*Rubus sp.)*, autumn olive (*Elaeagnus umbellata)*, and white oak (*Quercus alba)*] was highest in early succession, and lowest in mid and late succession. I hypothesize that foliar ^{15}N decreased from early to mid succession due to the observed decrease in soil ^{15}N. However, whereas soil ^{15}N was relatively high in late succession, foliar ^{15}N was relatively low. This could be due to an increase in N availability, and thus increased fractionation due to preferential transformation by microbes of ^{14}N into plant-available forms such as NH_4^+ and NO_3^-. In addition, with increasing N availability, fractionation during plant uptake increases due to preferential uptake of the lighter isotope (Högberg et al., 1999).

Conclusions and Future Work

Several lines of evidence (increasing total soil N, increasing NO_3^- to NH_4^+ ratio, increasing net N mineralization and nitrification, high soil $\delta^{15}N$ in late succession) point to increasing N availability through the course of secondary succession at BEF, and potentially even N saturation in the latest stage of succession. Despite the high N availability in late succession, these plants still display higher N-use efficiency than early or mid-successional plants, with increased resorption of foliar N prior to senescence. In addition, $\delta^{15}N$ analysis points to the highest rates of N-fixation in mid-succession, and more open N cycling in early and late succession, potentially indicating higher N losses.

While current N-cycling conceptual models (Lovett et al., 2018; Vitousek & Reiners, 1975) provide a useful framework with which to describe general N cycling through succession, they do not address the complexities of various pools, transformations, and fluxes within the N cycle. Moreover, while many studies examine some aspects of N cycling through succession, few examine and integrate multiple components to provide a comprehensive understanding of the intricacies of N cycling. This makes it difficult to generalize about N cycling during succession following various disturbance types, in different climate zones, or in different ecosystem types. Given the vastness of successional land worldwide (FAO, 2010; McLauchlan, 2006), future work should integrate as many aspects of N cycling as possible from individual sites in order to better estimate and quantify widespread implications of N cycling changes, including nutrient fluxes into bodies of water, land-atmosphere gas exchanges, and primary productivity rates in successional ecosystems.

Finally, it is important to acknowledge that succession today may follow a different trajectory from succession centuries or even decades ago, due to ecosystem alterations such as human removal of seed banks, introduction of invasive species, and climate change (Dey et al., 2019; Gill & Marks, 1991). Thus, chronosequence-based studies such as this one may come with the caveat that the latest stage of succession might not represent what the earlier stages will look like when they reach the same age. Therefore, care should be taken when considering studies from the past versus studies today, and successional sequences affected by these widespread disturbances should be considered and examined.

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Chapter 4: Changes in Foliar Traits through Secondary Succession and Detection using Remote Sensing

Abstract

Most forests today exist in some stage of secondary succession following land disturbance, and significant questions surround the effects of this land disturbance and subsequent regrowth on nitrogen (N) cycling and foliar traits. While there is general scientific consensus regarding how N stocks on a community or ecosystem level change through secondary succession, little work has specifically examined changes in foliar traits over successional time, especially within species. As an alternative to labor-intensive on-the-ground foliar sampling, remote sensing with hyperspectral data has proven to be a promising way to assess foliar characteristics. Partial least squares regression (PLSR) models have demonstrated accuracy in estimating foliar traits across latitudes, species, and seasonal time utilizing hyperspectral datasets. However, it remains unknown how secondary successional dynamics and associated changes in foliar traits following land disturbance might affect the predictive capabilities of these models. Here, I investigate foliar traits in three different stages of succession following agricultural abandonment at the Blandy Experimental Farm in Boyce, VA: early (~20 years old), mid (~40 years old), and late succession (~100 years old). Three to four dominant species in each successional stage were selected, and leaves were collected and analyzed throughout the growing season and at two different heights in the canopy (shade and sun leaves). For each leaf, foliar N concentration (N_{mass}), leaf mass per area (LMA), and reflectance from 400-2400 nm were examined. N_{mass} and LMA changed significantly through succession, among species and/or within single species; while N_{mass} increased, LMA decreased over successional time. PLSR models were able to predict N_{mass} and LMA within the full dataset and within a single successional stage relatively well (R^2>0.2 and R^2>0.8, respectively). Across successional

stages, PLSR models predicted LMA successfully (R^2>0.8), but were unsuccessful in predicting N_{mass}, except when calibrating on early succession and validating on mid succession or vice versa (R^2=0.17 and 0.3, respectively). Due to increased availability of and interest in hyperspectral imaging to predict foliar traits, investigating the breadth to which these models can be applied is important. Given widespread land disturbance and subsequent secondary succession occurring worldwide, and because foliar traits and spectra vary by time since disturbance, successional stage will be an important factor to consider in future applications of PLSR models of hyperspectral data.

Introduction

Globally, about 64% of forests today are in a state of secondary succession following some sort of land disturbance such as logging, agriculture, or fire (FAO, 2010). However, it is unclear how foliar traits change through the process of secondary succession. Also in question is how land in various successional stages might affect our ability to predict plant physiological or morphological traits such as foliar nitrogen (N) concentration and leaf mass per area (LMA) via remote sensing.

In a typical successional chronosequence, species composition changes from faster-growing species with lower C:N early in succession to slower-growing and longer-lived species with higher C:N later in succession (Clebsch & Busing, 1989; Thompson et al., 2013). Accordingly, a global meta-analysis found that the majority of studies examining foliar C:N on a community level found either no significant change or a significant increase through secondary succession (Y. Yang et al., 2011). In addition, one study examining temperate forest post-agricultural successional dynamics found a decrease in foliar N concentration (%) over time, indicating a tightening of the N cycle as succession proceeded in that Rhode Island, USA system (Compton et al., 2007).

While there is a general consensus that ecosystem carbon (C) and N accumulate over the course of secondary succession (Lovett et al., 2018; Vitousek & Reiners, 1975; Y. Yang et al., 2011), with C and N largely accumulating in biomass, there is no consensus regarding how the distribution and concentration of N might change within plants through succession, or how other plant traits might vary. It is also unclear how N concentrations and other foliar traits might change through succession within the same species or within a group of similar species. Successional sequences are increasingly taking divergent paths due to human-induced ecological disturbances such as deforestation and removal of seed banks, climate change, and introduction of invasive species, and therefore the traditional shift from lower C:N to higher C:N species might not occur (Dey et al., 2019).

Foliar traits such as nutrient concentrations and LMA can be important indicators of ecosystem biogeochemical cycling, photosynthetic rates, and net primary productivity (NPP) (Asner & Martin, 2016; Kokaly et al., 2009). Despite the relatively low concentration of N in leaves compared to other elements such as C, it is a crucial component in many physiological processes. For example, N is a main component of the protein Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO), which acts to fix CO_2 in photosynthesis and accounts for 30-50% of the N in green leaves (Kokaly et al., 2009). N is also present in chlorophyll, the green pigment in leaves that harvests sunlight for photosynthesis (Kokaly et al., 2009; Serbin et al., 2014; X. Yang et al., 2016). LMA can vary both among and within species, and even within a single plant, based on the local environmental conditions. It is an indicator of plant strategy, as there is a tradeoff between leaf construction costs to the plant, increased by a higher leaf thickness, and surface area for intercepting and capturing light (Poorter et al., 2009; Serbin et al., 2014; X. Yang et al., 2016).

Traditional on-the-ground methods for measuring foliar traits have been employed for decades but are relatively time and labor intensive and require destructive sampling. Thus, there is increasing interest in the use of remote sensing to predict foliar traits more easily and for larger extents (Asner & Martin, 2016). Partial least-squares regression (PLSR) is a commonly used chemometric technique for predicting foliar traits using spectral reflectance; it is optimized for spectral data in that it accounts for collinearity between neighboring wavelengths and allows for datasets with more predictors than observations (Serbin et al., 2014). The approach has shown promise in predicting a wide range of foliar traits from spectral reflectance, including nutrient, pigment, and C concentrations; LMA; and δ^{15}N (Asner et al., 2009; Serbin et al., 2014; X. Yang et al., 2016). PLSR has also been effective in predicting foliar traits from spectra across canopy locations, various sites, and seasonal time (X. Yang et al., 2016). However, the accuracy of PLSR modeling across successional stages has not been directly investigated.

In this study, I examine foliar trait dynamics through secondary succession, and include leaves from upper and lower canopy locations and from three points in the growing season to capture spatial and temporal variability. I aim to answer the following questions: 1) How do foliar N (N_{mass}) and LMA vary by canopy location, seasonal time, and successional time? 2) How well do PLSR models perform within a single successional stage and across successional time?

Methods

Site description

Blandy Experimental Farm is a 300-hectare research, education, and outreach field station operated by the University of Virginia. Located in the Shenandoah Valley within the Blue Ridge Mountains, it has a mean annual precipitation of 975 mm and a mean annual temperature of 12°C. The soil at Blandy is classified as an ultisol, largely due to its long history of agriculture.

Blandy has two replicate chronosequences consisting of adjacent fields in three successional stages – early, mid, and late. All fields were formerly used for agriculture and were abandoned in 2001 and 2003 (early), 1986 and 1987 (mid), and prior to 1910 and 1920 (late) (Wang et al., 2016) (Figure 1). Within each field, five 10x10 m plots were randomly selected, for a total of 30 plots (Figure 2).

Figure 1: Two replicate chronosequences at Blandy Experimental Farm (outlined in red), each consisting of an early, mid, and late successional field.

Sample collection

Three to four dominant species in each field were selected as focal species (Table 1). If a species was present within a plot, an individual of that species was selected and marked, and shade and sun leaf samples were collected. Shade leaves were characterized as leaves low in the canopy receiving minimal direct sunlight, and sun leaves were characterized as leaves either high in the canopy or growing in gaps of the canopy and receiving direct sunlight for a significant portion of the day. Samples were collected at three points in the growing season in two-month intervals; late spring (mid-May), summer (mid-July), and early fall (mid-September).

Shade leaves were collected by hand or with an arborist pruner, and sun leaves were collected with an arborist pruner or by firing a shotgun at the desired branch and retrieving the fallen leaves. Within each species, plants of similar sizes and ages were selected as the representative individuals. Immediately following collection, leaves were placed in a sealed bag with a moistened paper towel and into a cooler until analysis.

Proportion of plots in which each species was present is shown in Figure 2.

Table 1: Focal species in each field

Field Age	Chronosequence 1	Chronosequence 2
~20 years (Early successional)	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • autumn olive (*Elaeagnus umbellate)*	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • Amur honeysuckle (*Lonicera mackii)*
~35 years (Mid-successional)	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • autumn olive (*Elaeagnus umbellate)* • Amur honeysuckle (*Lonicera mackii)*	• Dahurian buckthorn (*Rhamnus davurica)* • wineberry (*Rubus sp.)* • autumn olive (*Elaeagnus umbellate)* • Amur honeysuckle (*Lonicera mackii)*

100 + years (Late successional)	• Dahurian buckthorn (*Rhamnus davurica)* • tree of heaven (*Ailanthus altissima)* • mockernut hickory (*Carya tomentosa)*	• Dahurian buckthorn (*Rhamnus davurica)* • white oak (*Quercus alba)* • mockernut hickory (*Carya tomentosa)* • paw paw *(Asimina triloba)*

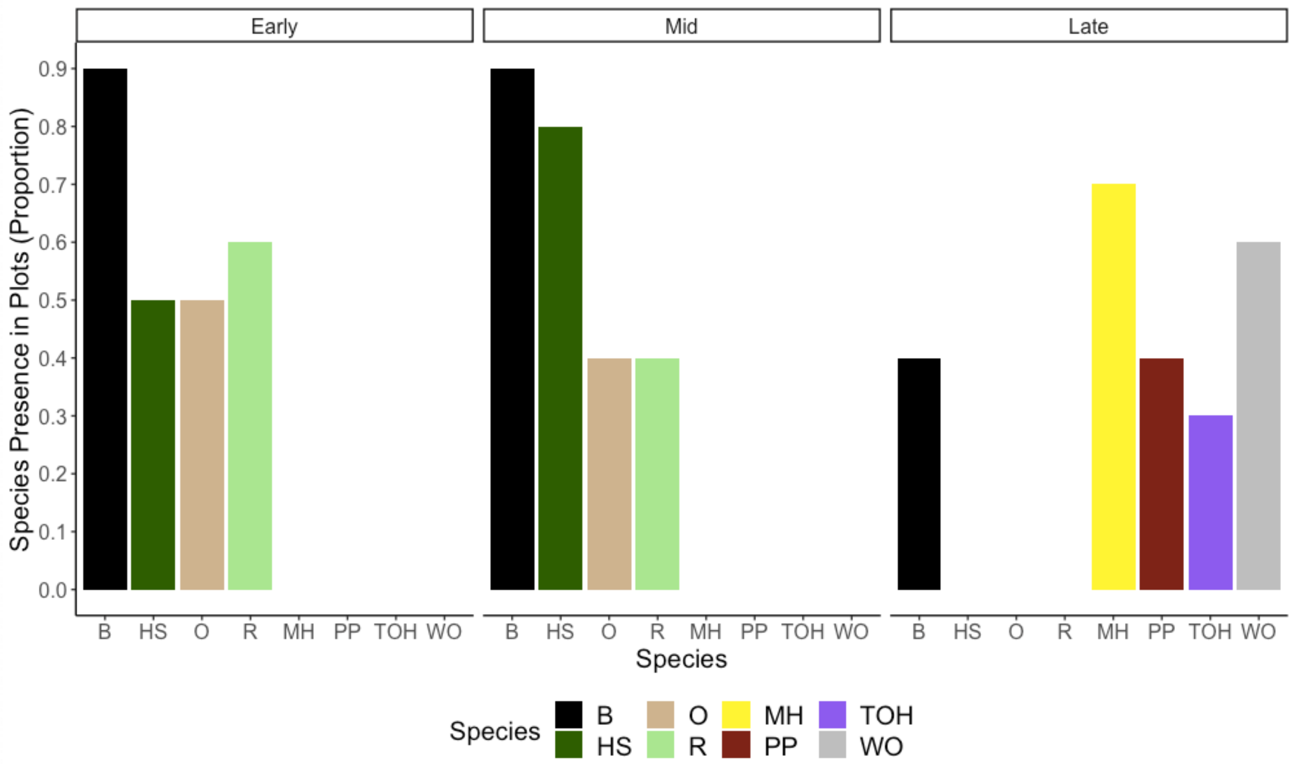

Figure 2: Proportion of plots in which each focal species was present in each successional stage**.** B=Dahurian buckthorn, HS=Amur honeysuckle, O=autumn olive, R=wineberry, MH=mockernut hickory, PP=pawpaw, TOH=tree of heaven, WO=white oak

Spectral data collection

Foliar spectral reflectance was measured in the laboratory within two hours of sample collection using a spectroradiometer with attached leaf clip (SVC HR-1024i, Spectra Vista Corp, Poughkeepsie, NY) over visible, NIR, and SWIR wavelengths (350-2500 nm). Reflectance spectra were recorded for each leaf, avoiding the leaf midrib and edges. White referencing was performed prior to sampling each leaf.

Foliar traits

Following collection, area of fresh leaves was measured using a LiCor LI-3050C Transparent Belt Conveyor (LI-COR, Lincoln, NE, USA.). Leaves were dried at 60° C for 48 hours, or until leaf mass remained constant. Dried leaves were then weighed to calculate LMA. Leaves were then ground into a fine powder and analyzed for C and N content with a FLASH 2000 NC Analyzer (ThermoFisher Scientific, Waltham, MA, USA).

Statistical analysis

Foliar C and N

Foliar nutrient content was compared among successional stages and growing season periods with linear mixed effects models. N_{mass} and LMA were log-transformed as needed to improve normality of residuals, which were assessed visually using residual distribution histograms and Q-Q plots. Variance structures were added to improve heterogeneity of variance, which was assessed visually and quantitatively using Levene’s test. Models were fitted using the restricted maximum likelihood (REML) procedure. Significance of fixed effects was evaluated using marginal F-tests, and post-hoc pairwise comparisons were conducted using Tukey’s HSD test.

All statistical analyses were conducted using R (R Core Team, 2020). Models were fitted and hypothesis tests were conducted using the nlme package (Pinheiro et al., 2022). Post-hoc pairwise comparisons and estimated marginal means were calculated using the emmeans package (Lenth et al., 2022).

PLSR

Reflectance spectra of the leaves were evaluated against N_{mass} and LMA using PLSR in order to determine the relationship between foliar traits and reflectance spectra. I built 20 PLSR models estimating N_{mass} and LMA from foliar reflectance in a range of scenarios. First, I modeled each using the entire dataset of leaves from all successional stages. I next modeled each using only leaves from one successional stage. I then modeled each using foliar traits and reflectance from one successional stage to predict foliar traits from reflectance in another successional stage to test performance of PLSR models across successional time.

To avoid noise at the limits of the spectroradiometer measurement range, wavelengths <400 nm and >2400 nm were excluded from analysis. For the PLSR analyses using the whole dataset or only one successional stage, the dataset was split into a calibration dataset (70%) and a validation dataset (30%). For the analyses using one successional stage to predict foliar traits in another, one successional stage served as the calibration dataset and the other served as the validation dataset. One thousand PLSR models were fitted to the calibration dataset, and the optimum number of components selected to minimize the root-mean-square error of prediction (RMSEP) based on the mean of the 1000 PLSR permutations. Models were cross-validated internally using leave-one-out validation. N_{mass} and LMA were log-transformed to improve normality.

The model parameters were then fit to the validation dataset in order to test model performance on independent data. Variable importance in projection (VIP) values were calculated using the model parameters (Wold, 1994). The relationship of spectral reflectance and foliar chemistry was examined over two time scales: the course of a growing season and successional time using a space-for-time assumption. PLSR models were build using the pls package in R (Liland et al., 2022; R Core Team, 2020).

Results

Foliar N

N_{mass} concentration ranged from 1 to 4% across all samples. Among all species, N_{mass} increased significantly by successional stage ($p=0.03$, Figure 3) and varied significantly by species ($p<0.001$), but not by season (Figure 4) or canopy location. When controlling for the other variables, mean N_{mass} in early succession was significantly lower than mid and late succession, with estimated marginal mean values of 2.42%, 2.80%, and 2.67%, respectively.

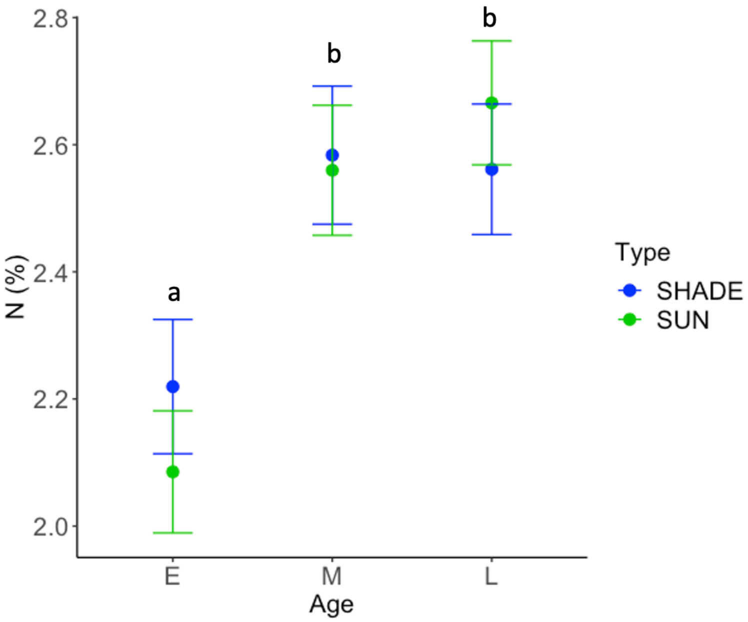

Figure 3: Mean N_{mass} in shade and sun leaves through the successional stages. Different letters represent significant differences ($p<0.05$), and error bars represent standard error.

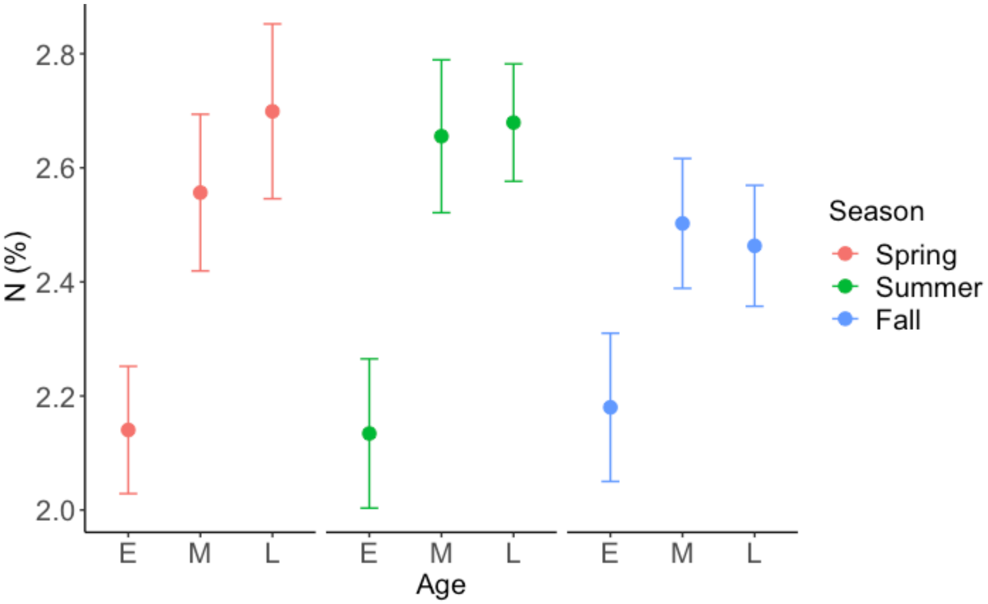

Figure 4: Mean N_{mass} through the successional stages by season. Error bars represent standard error.

Four species were represented in two or more successional stages: Dahurian buckthorn (B), Amur honeysuckle (HS), autumn olive (O), and wineberry (R). Within Dahurian buckthorn, which was present in all three successional stages, N_{mass} trended upward through successional time, though not significantly (p=0.1). Amur honeysuckle, present in early and mid succession, did not change in N_{mass} between the two stages. Autumn olive and wineberry, also present in both early and mid succession, both significantly increased in N_{mass} through successional time (p=0.03 and p=0.02, respectively, Figure 5).

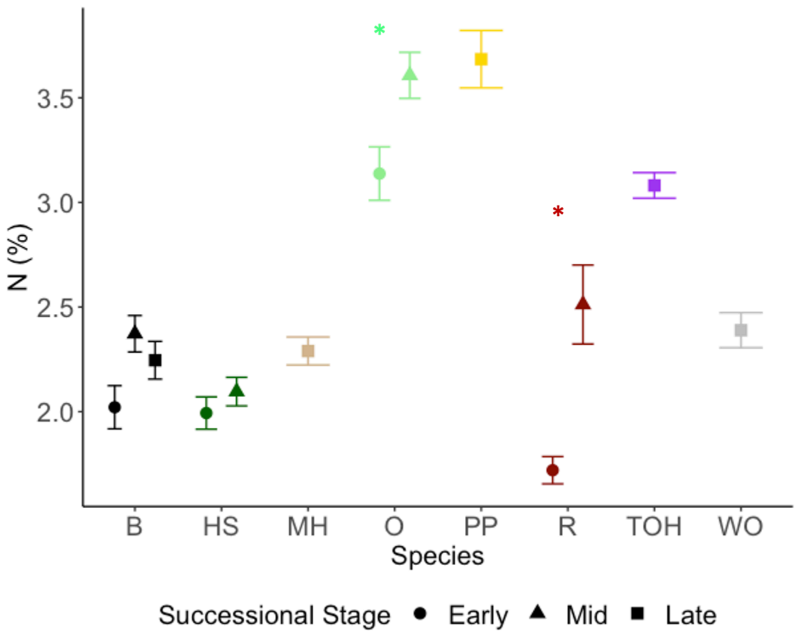

Figure 5: Mean N_{mass} by successional stage and species. Stars indicate significant differences between the successional stages in that individual species ($p<0.05$), and error bars represent standard error. B=Dahurian buckthorn, HS=Amur honeysuckle, O=autumn olive, R=wineberry, MH=mockernut hickory, PP=pawpaw, TOH=tree of heaven, WO=white oak.

LMA

LMA ranged from ~15 to ~145 g/m^2, with a mean of 51.99 g/m^2. LMA trended downward insignificantly with successional stage (Figure 6), varied significantly among species ($p<0.001$), increased through the growing season ($p<0.001$, Figure 7), and was significantly greater in sun leaves than in shade leaves ($p<0.001$).

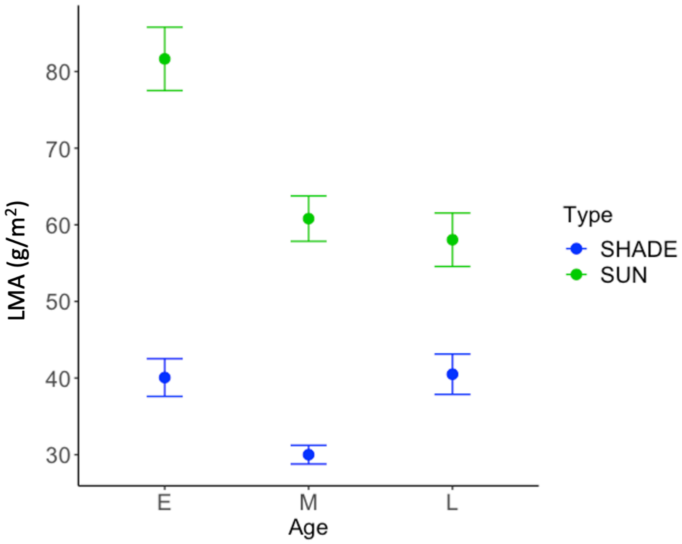

Figure 6: Mean LMA in shade and sun leaves through secondary succession. Error bars represent standard error

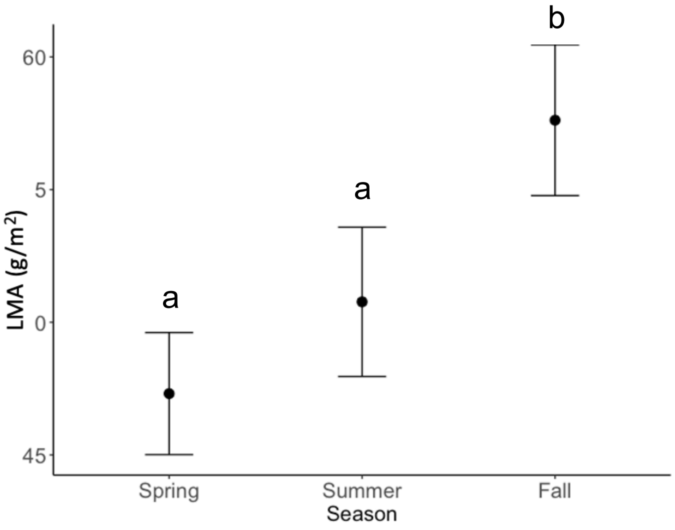

Figure 7: Mean LMA by time in the growing season. Different letters indicate significant differences ($p<0.05$), and error bars represent standard error

Within the species present in two or more successional stages, similar trends were noted (Figure 8). In Dahurian buckthorn, LMA decreased by successional stage (p=0.04), increased through the growing season (p=0.02), and was greater in sun leaves than in shade leaves (p<0.001). In autumn olive, LMA was significantly lower in mid succession than in early succession (p<0.001) and was significantly higher in sun leaves than in shade leaves (p<0.001), but did not change significantly by season. In wineberry, LMA did not vary significantly by successional stage (though it trended downward), but did increase through the growing season (p=0.02) and by canopy height (p<0.001). Amur honeysuckle leaves did not vary in LMA by successional or seasonal time, but LMA was higher in sun leaves than in shade leaves (p<0.001).

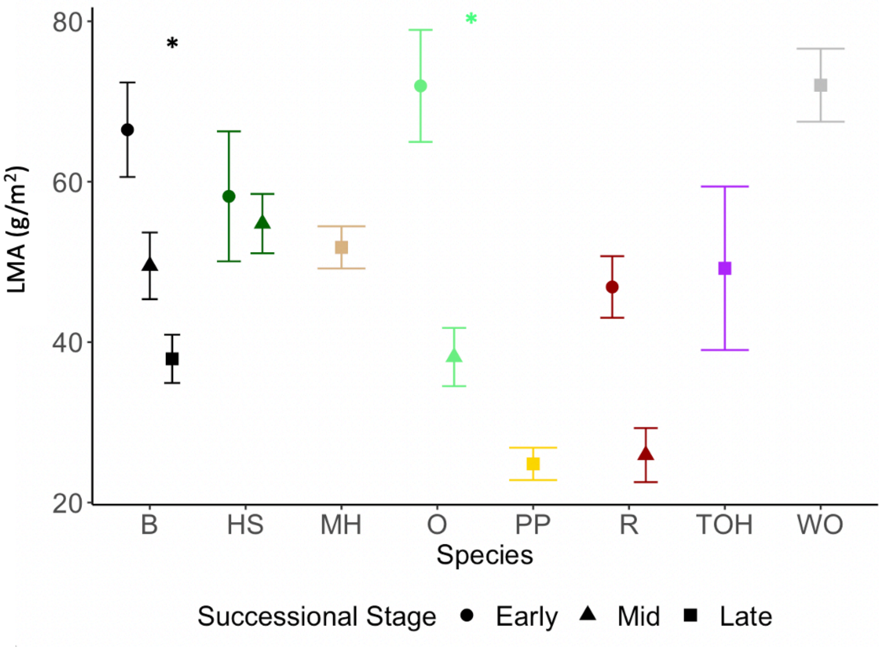

Figure 8: Mean LMA by successional stage and species. Stars indicate significant differences between the successional stages in that individual species (p<0.05), and error bars represent standard error. B=Dahurian buckthorn, HS=Amur honeysuckle, O=autumn olive, R=wineberry, MH=mockernut hickory, PP=pawpaw, TOH=tree of heaven, WO=white oak.

Spectral Reflectance

Average reflectance spectra for each species examined are shown in Figure 9. The general shape remains the same across species, with some species-based variability. For example, wineberry (red) has higher average reflectance than the other species in the visible range of the spectrum (400-700 nm), whereas autumn olive (light green) has higher average reflectance than the other species in the near-infrared (NIR) range of the spectrum (700-1300 nm). Across the spectrum, late successional leaves generally had the lowest coefficient of variation (CV, Figure 10), and early successional leaves generally had the highest. For all successional stages, the CV was highest in the visible range, around 1450 nm (corresponding to a local minimum in reflectance), and around 1950 nm (also corresponding to a local minimum in reflectance).

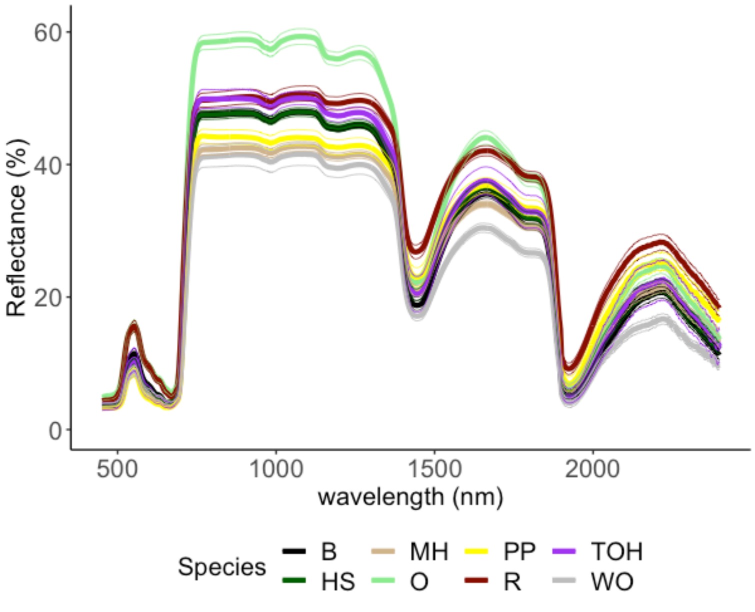

Figure 9: Reflectance spectra by species. Bold lines are mean reflectance spectra across successional stages, time in the growing season, and canopy location, and thin lines are standard error. B=Dahurian buckthorn, HS=Amur honeysuckle, O=autumn olive, R=wineberry, MH=mockernut hickory, PP=pawpaw, TOH=tree of heaven, WO=white oak.

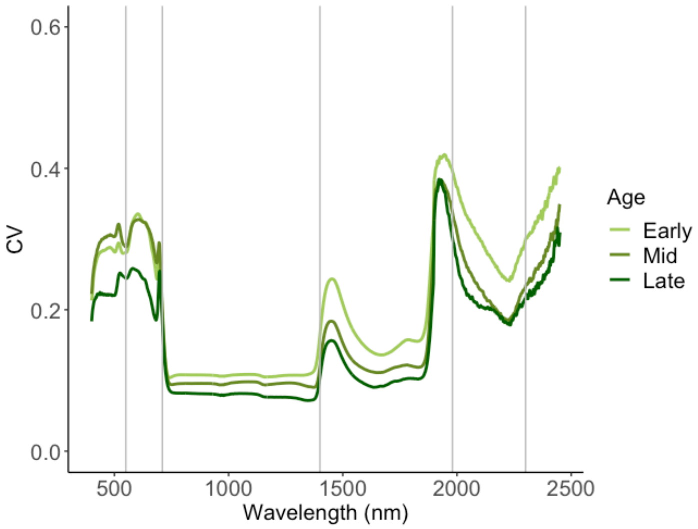

Figure 10: CV across the spectra by successional stage. Vertical grey lines represent theoretically important wavelengths in predicting foliar traits (pigments (including chlorophyll) at 550 and 710 nm, water content at 1400 nm, and proteins at 1980 and 2300 nm) (Curran, 1989)

PLSR

Within the full dataset (using all successional stages), PLSR was able to predict both N concentration and LMA (validation R^2 of 0.49 and 0.91 respectively). Within individual successional stages, PLSR predicted N concentration less accurately and with greater uncertainty; models performed best in the mid-successional stage (validation R^2 of 0.43) and less well in the early and late successional stages (validation R^2 of 0.25 and 0.20, respectively). LMA was predicted with greater accuracy, with validation R^2 of 0.94, 0.94, and 0.90 in early, mid, and late succession respectively. Models trained on early succession and tested on mid succession and vice versa moderately succeeded in predicting N, with R^2 of 0.17 and 0.30 respectively. However, models trained on early and tested on late, trained on mid and tested on late, and vice versa failed to predict N_{mass}. The models using one successional stage to predict LMA of another performed well, with R^2 values ranging from 0.82-0.95 (Table 2).

Table 2: Calibration dataset, validation dataset, validation model R^2, and validation model % RMSEP for each model run in this study. Models for which R^2 and % RMSEP are not included failed to predict foliar traits (R^2~0)

Cal. Data	Val. Data	log(N_{mass}) (%) R^2 (%RMSEP)	log(LMA) (g/m^2) R^2 (%RMSEP)
Full Dataset	Full Dataset	0.49 (17.30)	0.91 (6.61)
Early	Early	0.25 (23.98)	0.94 (6.97)
Mid	Mid	0.43 (21.00)	0.94 (6.45)
Late	Late	0.20 (19.05)	0.90 (8.48)
Early	Mid	0.17 (22.69)	0.95 (5.8)
Early	Late	-	0.86 (7.79)
Mid	Early	0.30 (16.36)	0.93 (6.91)
Mid	Late	-	0.82 (8.77)
Late	Early	-	0.87 (9.73)
Late	Mid	-	0.85 (9.98)

I determined the relative importance of each wavelength in modeling each foliar trait by calculating variable importance in projection (VIP) values. For models predicting LMA, VIP values were generally >1 around 710 nm, and then from ~1300-2400 nm, indicating particular importance of those wavelengths (Mehmood et al., 2012) (Figure 11). For models predicting N_{mass}, VIP values were >1 around 550 nm, 710 nm, and 1400 nm, and were generally ~1 or below beyond 1500 nm (Figure 12).

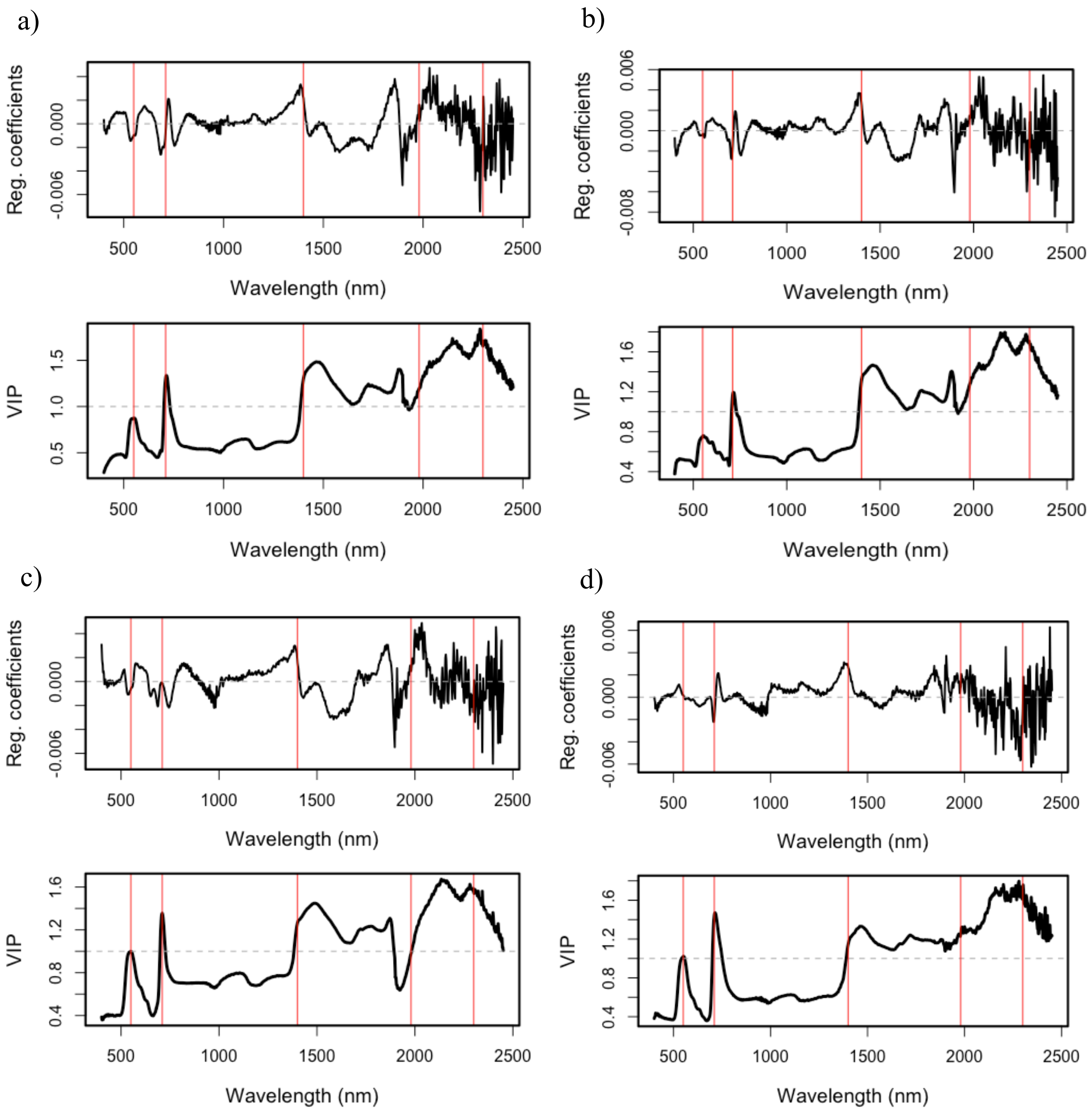

Figure 11: Regression coefficients and VIP values of PLSR models predicting LMA in a) the full dataset, b) early succession, c) mid succession, and d) late succession. Red lines represent theoretically important wavelengths in predicting foliar traits (pigments (including chlorophyll) at 550 and 710 nm, water content at 1400 nm, and proteins at 1980 and 2300 nm) (Curran, 1989)

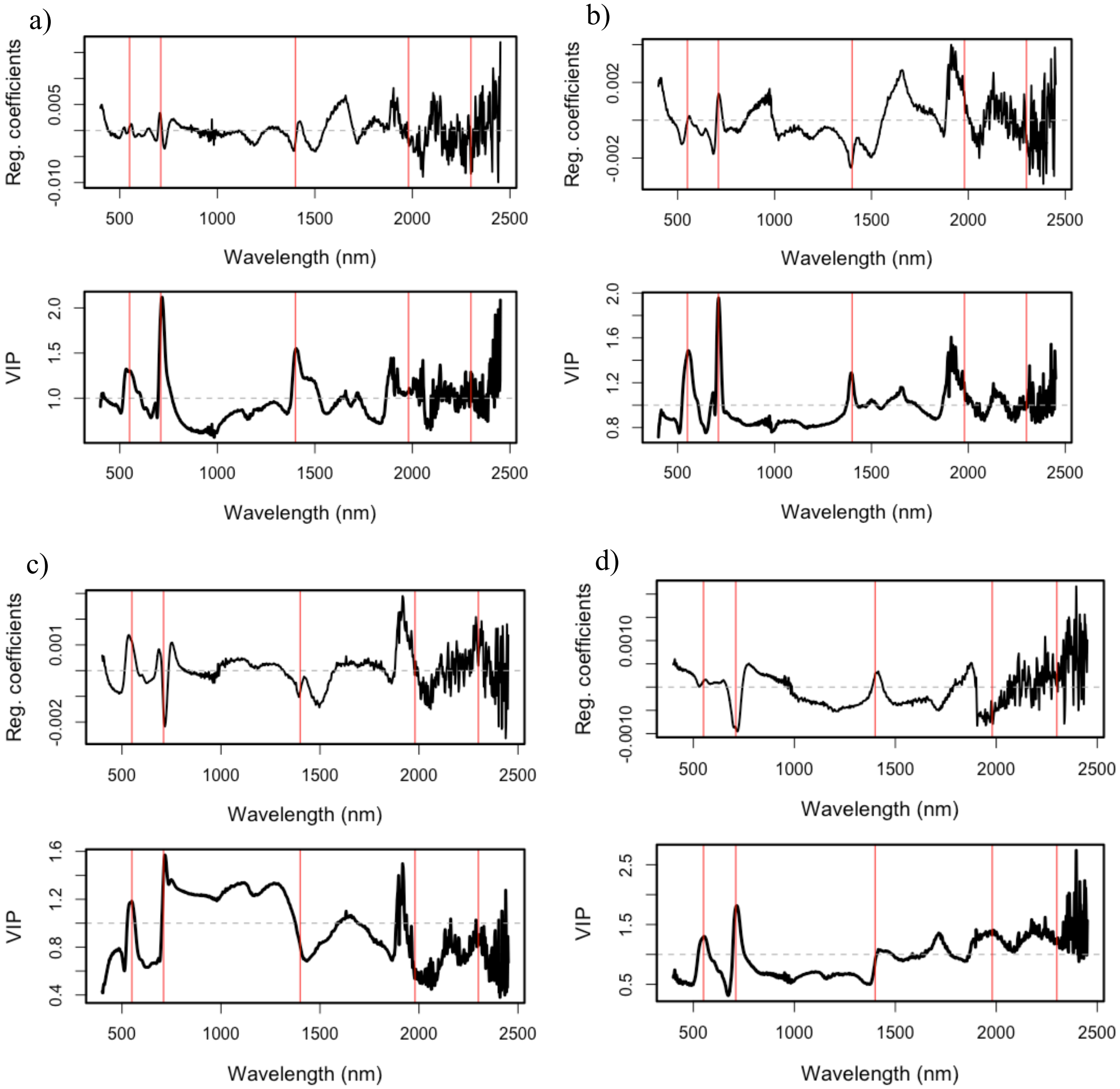

Figure 12: Regression coefficients and VIP values of PLSR models predicting N_{mass} in a) the full dataset, b) early succession, c) mid succession, and d) late succession. Red lines (pigments (including chlorophyll) at 550 and 710 nm, water content at 1400 nm, and proteins at 1980 and 2300 nm) (Curran, 1989)

Discussion

Both plant traits examined in this study (N_{mass} and LMA) varied significantly through secondary succession, within and/or among species. N_{mass} increased through successional time, in contrast to a secondary successional chronosequence in Rhode Island, USA (Compton et al., 2007), in which foliar N concentration decreased over time. While in Rhode Island this signified a tightening of the N cycle, in my study, we can infer an opening of the N cycle as succession proceeds. This corresponds with other data from this site, showing accelerated soil N cycling later in succession as well (Parisien & Epstein, *in prep*.). This may be due to the prevalence of invasive shrubs at Blandy Experimental Farm such as autumn olive, Amur honeysuckle, and Dahurian buckthorn; all of these grow quickly and have high quality, fast-decomposing litter, and autumn olive is a known N-fixer (Malinich et al., 2017). As N is present in RuBisCO and chlorophyll, both of which are essential in the process of photosynthesis (Kokaly, 2001), an increase in foliar N through secondary succession may have a profound impact on ecosystem-scale processes such as NPP.

While N concentration increased, LMA decreased through successional time within species and trended downward among all species. Among species, plants with higher LMA invest more resources into a single leaf and employ a slower and more conservative growing strategy, and thus tend to have an advantage in adverse growing environments. Conversely, plants with lower LMA employ a faster growing strategy and perform better in high-resource environments (Poorter et al., 2009). A shift from higher to lower LMA corresponds with the increase in N concentration and may also reflect higher N availability as succession proceeds in these chronosequences.

Within single successional stages, and using the full dataset with all successional stages, PLSR was able to predict LMA very well and N concentration reasonably well. My models predicting LMA performed comparably to other models used to predict LMA or its inverse, specific leaf area (SLA), but my models predicting N_{mass} across the dataset or within a single successional stage performed less well when compared to studies with a similar modeling methodology (e.g. Asner et al., 2011; Asner & Martin, 2008; Serbin et al., 2014; X. Yang et al., 2016). This discrepancy may be due to the fact that this study consisted of only one location and eight species. While my foliar measurements revealed a large range and high variation of LMA values (15.43-146.57 g/m^2, CV=51.21), I observed a lower variability in N values (0.95-4.99%, CV=30.26), which may have inhibited the predictive capabilities of the model. Regardless, PLSR successfully predicted LMA when trained on one successional stage and tested on the others but were less successful in predicting N_{mass}.

For the models that succeeded in predicting foliar traits from foliar spectral reflectance, the VIP values were relatively consistent across models and were similar among models predicting LMA and models predicting N_{mass}. There was generally a peak in VIP around 550 nm, corresponding to reflectance of chlorophyll and carotenoid pigments (Curran, 1989; Ecarnot et al., 2013). There was also generally a peak at around 710 nm, corresponding to the red edge/NIR plateau (Ecarnot et al., 2013). In my models, higher reflectance values around 550 nm were typically positively correlated with N_{mass} and LMA, whereas higher reflectance values around 710 nm were negatively correlated with N_{mass} and LMA. Further peaks in VIP around 1400, 1980, and 2300 nm correspond to water (1400) and proteins (1980 and 2300) (Curran, 1989). Especially for models predicting LMA, VIP values remain high between 1300-2400 nm, consistent with studies demonstrating these wavelengths to be most important in predicting LMA (Asner et al., 2011).

While previous studies demonstrate that foliar traits can be predicted by PLSR models across seasonal time and through different canopy locations (e.g. X. Yang et al., 2016), this study demonstrates that PLSR can also predict foliar traits when calibrated over multiple successional stages. However, models accurately predicted LMA when calibrated on one successional stage and validated on another, but failed to predict N_{mass} except in the cases of calibrating the model on the early successional dataset and validating on mid succession, and vice versa. This may be due to the fact that the early and mid-successional plots in this study consist of many of the same species, though in different proportions. N_{mass} PLSR models calibrated on late succession and tested on either early or mid-succession, or vice versa, were unable to make accurate predictions, perhaps due to the different species composition of the late successional plots. This indicates that species composition may be a more important factor to consider when generalizing models predicting N_{mass} than mean N_{mass}. Early and mid succession significantly differed in terms of $N_{mass,}$ yet models calibrated on one to predict the other succeeded, whereas mid and late succession do not significantly differ in terms of N_{mass}, but models calibrated on one to predict the other failed.

Due to the predominance of land currently in secondary succession worldwide (Delcourt & Harris, 1980; FAO, 2010; J. F. Hart, 1968; Kalisz, 1986; McLauchlan, 2006; Ramankutty & Foley, 1999; Yu & Lu, 2018), as well as increasing interest in estimating foliar traits from aircraft or satellite hyperspectral products (e.g. AVIRIS, Hyperion), I suggest that the age of the land and the composition of species be strongly considered when building PLSR models to predict foliar characteristics.

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Chapter 5: Conclusion

Already widespread globally, secondary successional land is likely to continue to expand as human use of land shifts and evolves. While research and analysis dating back decades make headway in assessing or predicting the effects of secondary succession on N cycling (e.g. Gorham et al., 1979; Rice & Pancholy, 1972; Robertson & Vitousek, 1981; Switzer et al., 1979; Vitousek & Reiners, 1975), few studies consider the full array of variables needed for a comprehensive view of changes in the system. In addition, past reviews on the process (e.g., Yang et al., 2011; Zou et al., 2021) focus only on total C and N pools and stocks, ignoring changes in various forms of N, as well as the important underlying N transformations and fluxes that provide context regarding the stocks.

A primary goal of this dissertation was to clarify the current state of knowledge of the phenomenon, including of lesser-studied factors like N transformations and fluxes. In Chapter 2, I compile and review all studies examining any aspect of N cycling during secondary succession following agricultural abandonment in temperate systems worldwide. I focus specifically on post-agricultural successional land because land clearing for agriculture and subsequent abandonment is a leading cause of secondary succession, and because agricultural practices may have unique effects on the land that can persist for centuries (McLauchlan, 2006; Ramankutty & Foley, 1999). I find that total N stocks, especially in the soil, are relatively well-studied through secondary succession, with a general consensus that soil C and N increase or stay the same over time (Y. Yang et al., 2011). Sometimes observed is the "soil mining phase" theorized by Lovett et al. (2018), in which soil N initially decreases at the start of succession, followed by a subsequent increase (Mao et al., 2010; Parolari et al., 2017). Although soil N transformations and stocks of inorganic plant-available N are less frequently measured, my review suggests that NH_4^+

and NO_3^- are often inversely related, and that net N mineralization and nitrification often increase through succession following agricultural abandonment. Due to the scarcity of data, trends in N fluxes over time are difficult to discern.

In Chapter 3, my objective was to measure N stocks, transformations, and fluxes in a variety of forms in order to gain a comprehensive understanding of how N cycling is changing following agricultural abandonment in Virginia. I use two secondary successional chronosequences, each consisting of an early, mid, and late successional field, to observe several ecosystem traits and functions. I find that N availability appears to increase as succession proceeds in this system, with total soil N that trends upwards, increasing net N mineralization and nitrification, and increasing foliar N, even within individual species. These soil stock and transformation results are consistent with many of the studies in my review (e.g., Knops & Tilman, 2000; Lamb, 1980; Pastor & Post, 1986; Wang et al., 2016; Wen et al., 2016; Xiao et al., 2018; Zak et al., 1990). Additional evidence of increased N availability in late succession is relatively high $\delta^{15}N$ values, indicating an opening of the N cycle ~100 years following agricultural abandonment. This is in contrast to other studies indicating a tightening of N cycling later in succession (e.g. Compton et al., 2007), suggesting that N cycling trajectories through secondary succession may be highly context-dependent, and that specific traits such as prior agricultural practices, sub-climate zones, and soil types must be considered when making predictions about various areas of land.

Finally, in Chapter 4, I focus on lesser-studied foliar N traits, and find that foliar N concentration increases through succession both among and within species, in accordance with an increase in N availability, and that LMA decreases insignificantly among species but significantly within specific species. A decrease in LMA through succession means fewer structural resources allocated to leaves by plants, which may also be indicative of higher resource (N) availability in late succession (Poorter et al., 2009). Also in this chapter, I explore the implications of successional landscapes on the ability to predict foliar traits through remote sensing, which is an increasingly popular approach to estimating N cycling characteristics on community and ecosystem scales. I find that models built on one successional stage can successfully predict LMA of leaves from other successional stages. Models built on one successional stage to predict foliar N concentration in leaves of another succeed when the differently-aged fields have similar species composition, despite differences between the stages in terms of foliar N, but fail when the differently-aged fields have different species compositions, despite no significant differences in foliar N. This result suggests that care should be taken to identify and appropriately categorize successional land when making broad foliar N predictions using hyperspectral remote sensing information.

Altogether, this dissertation summarizes the state of knowledge of secondary successional N cycling following agricultural abandonment; conducts a thorough investigation of N cycling through succession in Virginia, providing a framework for future studies; and assesses the ability of remote sensing to detect these changes. My findings highlight the need for further research on the topic, especially comprehensive research like mine, given the wide-ranging implications of changes in N cycling on conditions like forest productivity, waterway health, and air quality (Galloway et al., 2003).

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Appendix A: Supplemental Figures for Chapter 2

Table A1: Trends in soil C, N and C:N through secondary succession. Studies included examine two or more successional stages. Successional stages are provided in terms of age in years. If age in years is unspecified in the study, successional stage is defined by vegetational community. Fertilization of prior agricultural land is indicated by a Y (yes), N (no), or ? (unknown/unspecified). Trends in soil C, N and C:N indicated by a + (increase through successional time), - (decrease through successional time), NA (not measured), NSC (no significant change), or NCC (no consistent change).

Authors and Year	Location	Successional Stages	Fertilized?	Soil Depth (cm)	Soil C	Soil N	Soil C:N
Foote and Grogan 2010	Ontario, Canada	0-~100 years	N	0-5	+	+	+
				5-10	NSC	NSC	+
Hooker and Compton 2003	RI, USA	10-114 years	?	0 (forest floor)	+	+	NSC
				0-20	NSC	-	+
				20-70	+	NSC	+
Hu and others 2018	S China	cropland, grassland, shrubland, secondary forest	?	0-20	+	+	NSC
				20-50	+	+	NSC
Kirschbaum and others 2008	SE Australia	0-18 yr	N	0 (forest floor)	+ (organic)	+	NSC
Knops and Tilman 2000	MN, USA	61-yr chronosequence, repeated sampling over 12 years	N	0-10	+	+	+
Liu and others 2020	NE China	0-160 years	?	0-10	+ (organic)	+	+
				10-20	NSC (organic)	NSC	NSC
Mao and others 2010	NE China	5-20 years	Y	0 (forest floor)	+ (organic)	+	+

				0-15	initial -, then + (organic)	initial -, then +	+
				15-30	peak at 5 years	peak at 5 years	+
				30-45	initial -, then + (organic)	initial -, then +	+
				45-60	+ (organic)	+	+
				60-100	initial -, then + (organic)	initial -, then +	+
Parolari and others 2017	SC, USA	100 yr model, 50 yr field study	Y	0-60	+	-, then +	+
Jiao and others 2013	NE China	0-25 years	N	0-20	NA	+, peak @ 20 years	NA
Poulton and others 2003	SE England	0-120 years	?	0-69	+	+	+
Richter and others 2000	SC, USA	5-40 years	Y	0 (forest floor)	+	+	NA
				0-60	+	-	+
Spohn and others 2015	Hungary	~14-~193 years	Y	0-10	+ (organic)	+	+ (levels off at 40 years)
Switzer 1979	MS, USA	~15-~100 years	?	0 (forest floor)	NSC	NSC	NSC
				0-3	+	+	+
Wen and others 2016	S China	10-59 years	Y	0-15	+	+	NSC
Xiao and others 2018	S China	~5-~50 years	Y	0-15	+ (organic)	+	+
Yesilonis and others 2016	MD, USA	50-70 years, 120-150 years	Y	0-20 cm	NSC	NSC	NSC
Zhang and others 2015	S China	~25-~50 years	?	0-15	+ (organic)	+	NA

Table A2: Soil NO_3^- and NH_4^+ concentrations & nitrification and N mineralization rates. Studies included examine two or more successional stages. Successional stages are provided in terms of age in years. If age in years is unspecified in the study, successional stage is defined by vegetational community. Fertilization of prior agricultural land is indicated by a Y (yes), N (no), or ? (unknown/unspecified). Trends in the variables in question are indicated by a + (increase through successional time), - (decrease through successional time), NA (not measured), NSC (no significant change), or NCC (no consistent change).

Source	**Location**	**Successional Stages**	**Fertilized ?**	**Soil Depth (cm)**	**NO_3^-**	**NH_4^+**	**Nitrification**	**N Mineralization**
Christensen 1985	NC, USA	4-7 years, 40-60 years, hardwood forest	?	0-5 cm	-	+	NA	NA
Liu and others 2020	N China	0-160 years	?	0-10	+	NSC	NA	NA
				10-20	NSC, minimum @ ~30 yr	+	NA	NA
Mao and others 2010	NE China	5-20 years	Y	0-15	NA	NA	NA	-, then + (net potential)
Rice and Pancholy 1972	OK, USA	2 years, 8 years	?	0-15	-	+	- (predicted by - in nitrifying bacteria) (net)	NA
				45-60	-	+	NA	NA
		1 years, 25 years		0-15	-	+	- (predicted by - in nitrifying bacteria) (net)	NA
				45-60	-	+	NA	NA
Richter and others 2000	SC, USA	5-40 years	Y	0-7.5	NA	NA	NA	-, then + (net)
				35-60	NA	NA	NA	-, then + (net)
Robertson and Vitousek 1981	NJ, USA	1 year, 4 years, 19 years	Y	0-15	-, then +	NSC	NCC	NCC
Wen and others 2016	S China	10-59 years	Y	0-15	+	-	+ (net)	+ (net)
Xiao and others 2018	S China	~5-~50 years	Y	0-15	+	NSC	+ (net)	+ (net)

Zak and others 1990	MN, USA	1-60 years	?	0-10	peak @ 1 year, - and variable after	NCC	+ (potential net)	+ (potential net)
Yesilonis and others 2016	MD, USA	50-70 years, 120-150 years	Y	0-10	-	NA	NA	NA

Appendix B: Supplemental Figures for Chapter 3

Table B1: Fixed effects and results of linear mixed effects models run in this study. *Significant at α=0.05, **significant at α=0.01, ***significant at α=0.001

Modeled Variable	Fixed Effects	Num. DF	Den. DF	F	p
Soil Respiration (g $CO_2/m^2/hr$)	Intercept	1	566	4.49	0.03*
	Age	2	27	6.69	0.004**
	DOY	1	566	1.37	0.24
	Year	1	566	99.39	<0.001***
Soil Moisture (%)	Intercept	1	566	1412.09	<0.001***
	Age	2	27	9.49	<0.001***
	DOY	1	566	868.30	<0.001***
	Year	1	566	262.99	<0.001***
Soil Temperature (°C)	Intercept	1	566	147.56	<0.001***
	Age	2	27	4.02	0.03*
	DOY	1	566	776.40	<0.001***
	Year	1	566	18.63	<0.001***
Soil C (g/m^2)	Intercept	1	235	10964.33	<0.001***
	Age	2	27	5.16	0.01**
	Depth	2	235	92.16	<0.001***
	Age:Depth	4	235	3.25	0.01**
Soil N (g/m^2)	Intercept	1	235	5485.45	<0.001***
	Age	2	27	1.43	0.24
	Depth	2	235	149.18	<0.001***
	Age:Depth	4	235	3.66	0.007**
Soil C:N	Intercept	1	241	5883.20	<0.001***
	Age	2	27	6.47	0.005**
Soil NH_4^+ (g N/m^2)	Intercept	1	124	53.65	<0.001***
	Age	2	26	3.59	0.04*
	Depth	2	124	63.14	<0.001***
	Month	1	124	0.01	0.94
Soil NO_3^- (g N/m^2)	Intercept	1	123	7.48	0.007**
	Age	2	26	7.20	0.003**
	Depth	2	123	4.55	0.01*
	Month	1	123	15.82	0.001***
Total Inorganic N (g N/m^2)	Intercept	1	123	34.07	<0.001***
	Age	2	26	1.32	0.28
	Depth	2	123	57.78	<0.001***
	Month	1	123	0.88	0.35

Litter N (%)	Intercept	1	38	7.48	0.009**
	Age	2	38	3.96	0.03*
Litter N (g/m^2)	Intercept	1	38	31.66	<0.001***
	Age	2	38	6.21	0.005**
Litter C (%)	Intercept	1	38	67222.14	<0.001***
	Age	2	38	0.52	0.60
Litter C (g/m^2)	Intercept	1	38	85138.74	<0.001***
	Age	2	38	209.31	<0.001***
Litter C:N	Intercept	1	38	432.47	<0.001***
	Age	2	38	4.48	0.02*
Foliar N (%)	Intercept	1	266	289.49	<0.001***
	Age	2	15	3.60	0.05*
	Type	1	266	0.32	0.57
	Species	7	266	49.23	<0.001***
	Season	2	266	3.10	0.05*
Foliar C:N	Intercept	1	266	10577.98	<0.001***
	Age	2	15	4.21	0.04*
	Type	1	266	41.99	0.13
	Species	7	266	2.71	<0.001***
	Season	2	266	1.63	0.20
LMA (g/m^2)	Intercept	1	266	4180.45	<0.001***
	Age	2	15	0.96	0.40
	Type	1	266	384.00	<0.001***
	Species	7	266	52.90	<0.001***
	Season	2	266	20.99	<0.001***
Foliar N (g/m^2)	Intercept	1	266	8.50	0.004**
	Age	2	15	4.72	0.03*
	Type	1	266	294.85	<0.001***
	Species	7	266	41.26	<0.001***
	Season	2	266	14.09	<0.001***
Net N Min (g N/m^2/month)	Intercept	1	153	3.92	0.05*
	Age	2	55	7.49	0.001***
	Incubation	1	153	2.33	0.13
	Age:Incubation	2	153	1.85	0.16
Net Nit (g N/m^2/month)	Intercept	1	153	15.36	<0.001***
	Age	2	55	5.64	0.009**
	Incubation	1	153	1.04	0.31
	Age:Incubation	2	153	2.35	0.10
Leaching Potential	Intercept	1	122	127.30	<0.001***
	Age	2	26	0.23	0.79

	Depth	2	122	3.52	0.03*
	Month	1	122	1.62	0.21
Soil ^{13}C (‰)	Intercept	1	64	48262.90	<0.001***
	Age	2	26	3.35	0.05*
Soil ^{15}N (‰)	Intercept	1	64	162.04	<0.001***
	Age	2	26	5.53	0.01**
Foliar ^{13}C (‰)	Intercept	1	168	6668.07	<0.001***
	Age	2	168	20.70	<0.001***
	Type	1	168	49.82	<0.001***
	Species	3	168	30.84	<0.001***
	Season	2	168	6.82	0.001***
Foliar ^{15}N (‰)	Intercept	1	168	1.30	0.25
	Age	2	168	17.07	<0.001***
	Type	1	168	19.31	<0.001***
	Species	3	168	14.37	<0.001***
	Season	2	168	2.81	0.06

Appendix C: Supplemental Figures for Chapter 4

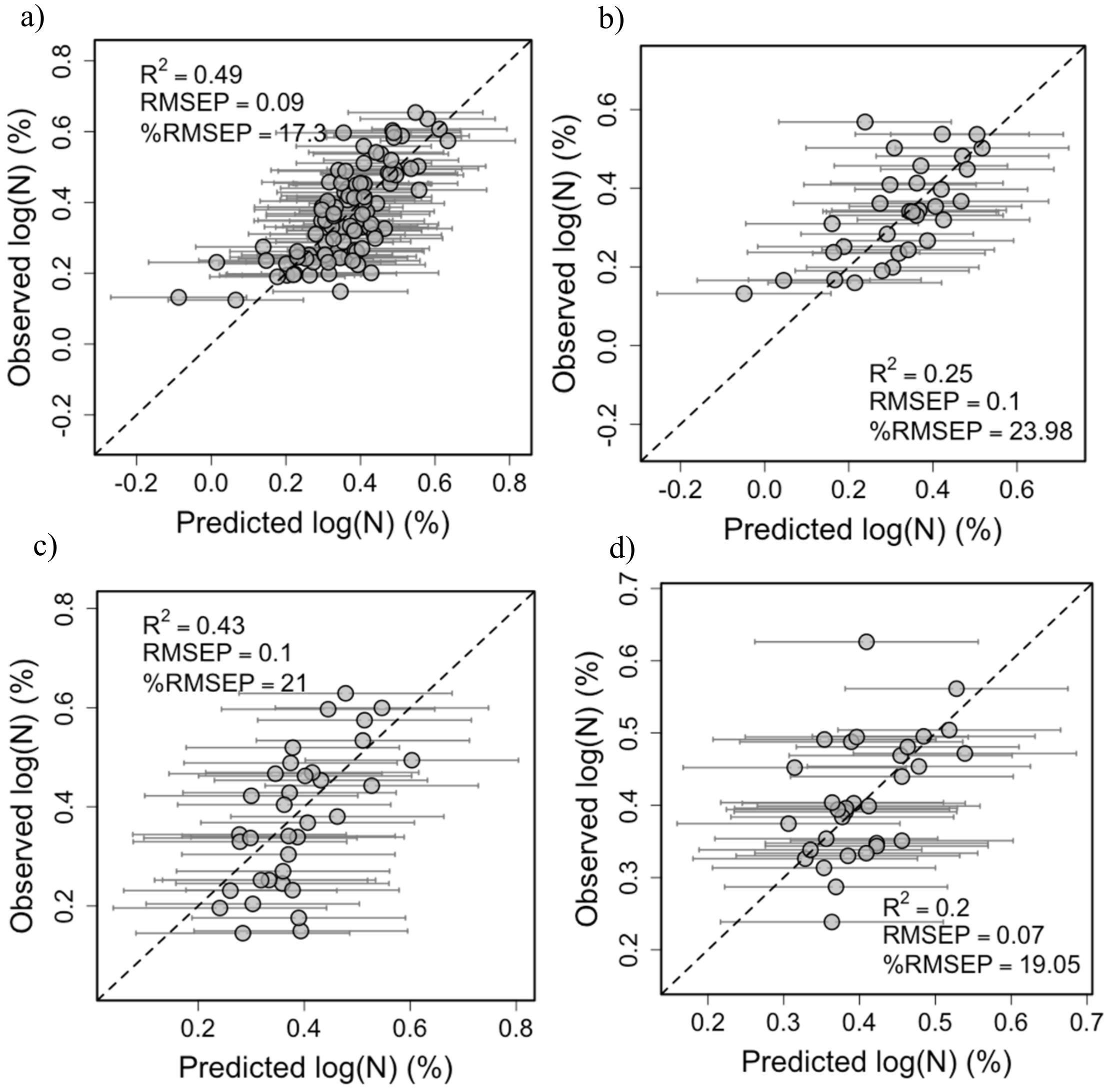


Figure C1: Modeled vs. observed values of log-transformed N_{mass}, modeled within a) the full dataset, b) early succession, c) mid succession, and d) late succession. Dotted line is 1:1 line, error bars represent standard deviation of the 1000 PLSR permutations

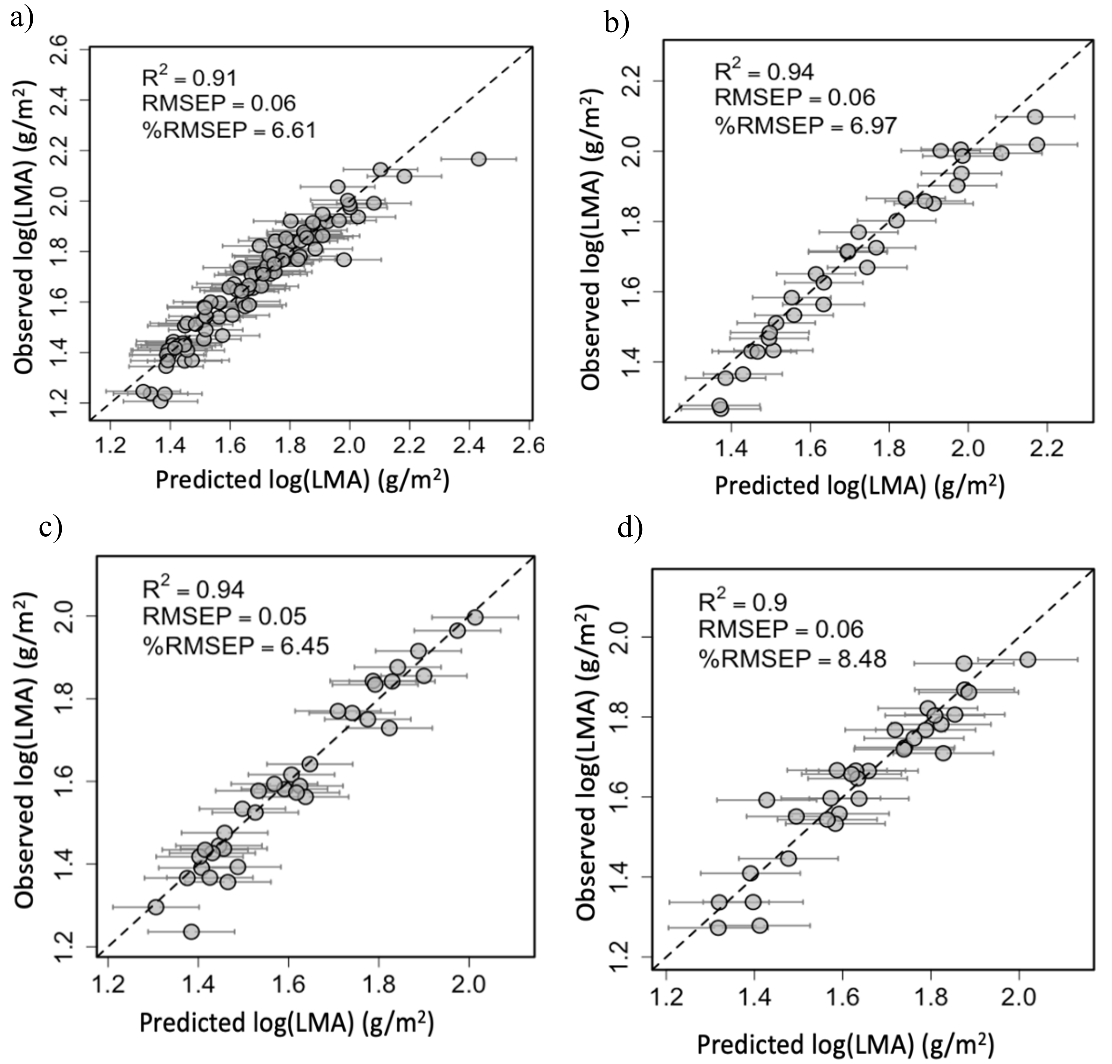


Figure C2: Modeled vs. observed values of log-transformed LMA, modeled within a) the full dataset, b) early succession, c) mid succession, and d) late succession. Dotted line is 1:1 line, error bars represent standard deviation of the 1000 PLSR permutations

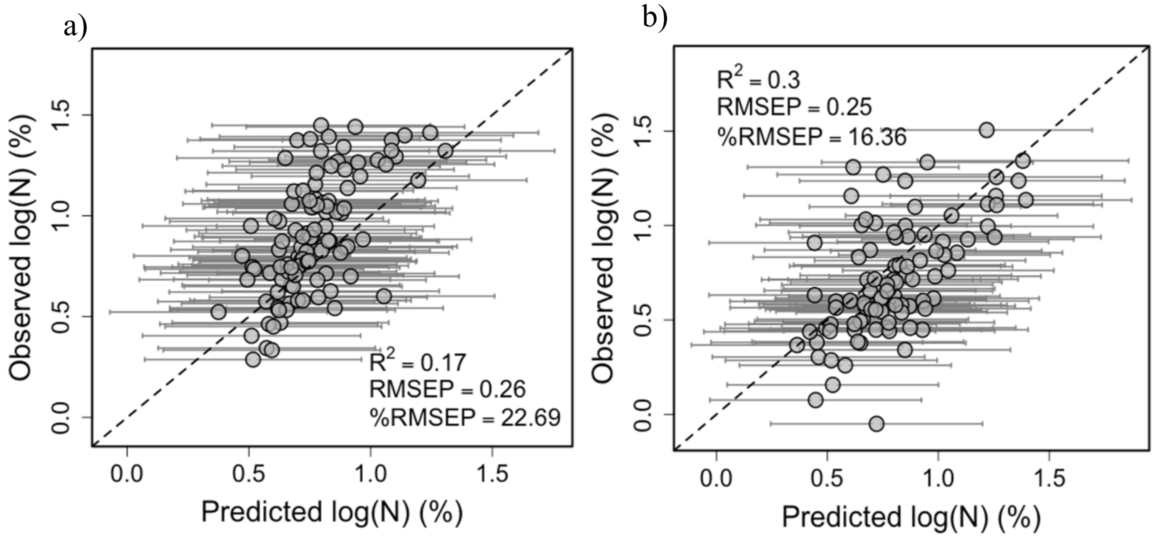


Figure C3: Modeled vs. observed values of log-transformed N_{mass}, modeled by a) calibrating on early succession and validating on mid succession, and b) calibrating on mid-succession and validating on early succession, Dotted line is 1:1 line, error bars represent standard deviation of the 1000 PLSR permutations

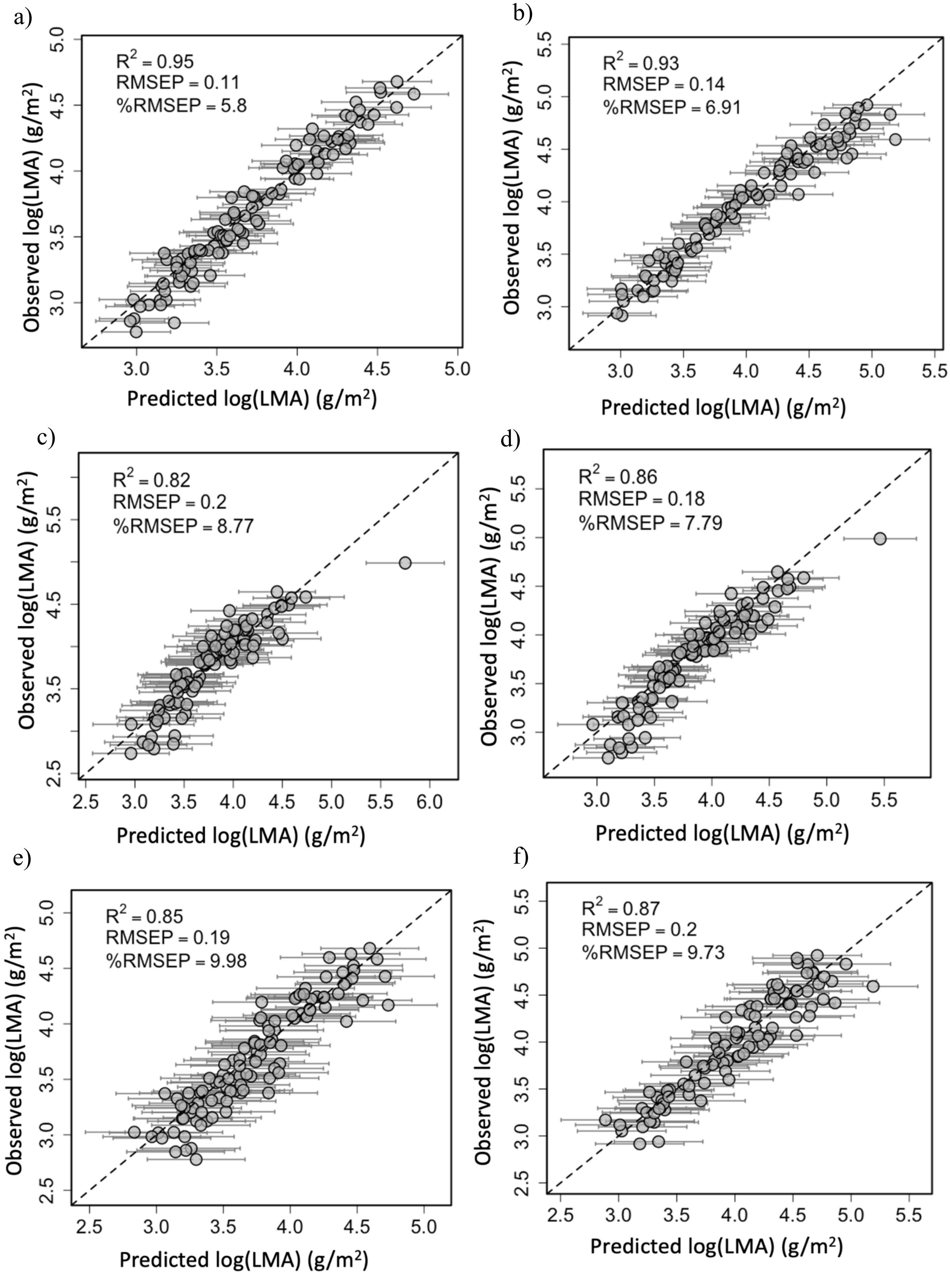

a)
R^2 = 0.95
RMSEP = 0.11
%RMSEP = 5.8
b)
R^2 = 0.93
RMSEP = 0.14
%RMSEP = 6.91
c)
R^2 = 0.82
RMSEP = 0.2
%RMSEP = 8.77
d)
R^2 = 0.86
RMSEP = 0.18
%RMSEP = 7.79
e)
R^2 = 0.85
RMSEP = 0.19
%RMSEP = 9.98
f)
R^2 = 0.87
RMSEP = 0.2
%RMSEP = 9.73
Observed log(LMA) (g/m²)
Predicted log(LMA) (g/m²)

Figure C4: Modeled vs. observed values of log-transformed LMA, modeled by a) calibrating on early succession and validating on mid succession, b) calibrating on mid-succession and validating on early succession, c) calibrating on mid succession and validating on late succession, d) calibrating on early succession and validating on late succession, e) calibrating on late succession and validating on mid succession, and f) calibrating on late succession and validating on early succession. Dotted line is 1:1 line, error bars represent standard deviation of the 1000 PLSR permutations

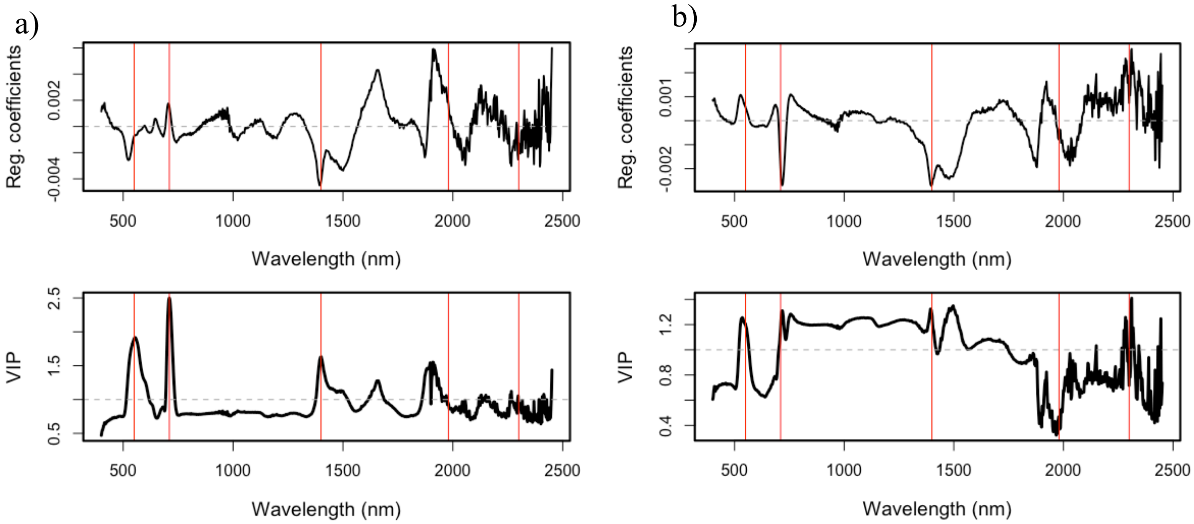
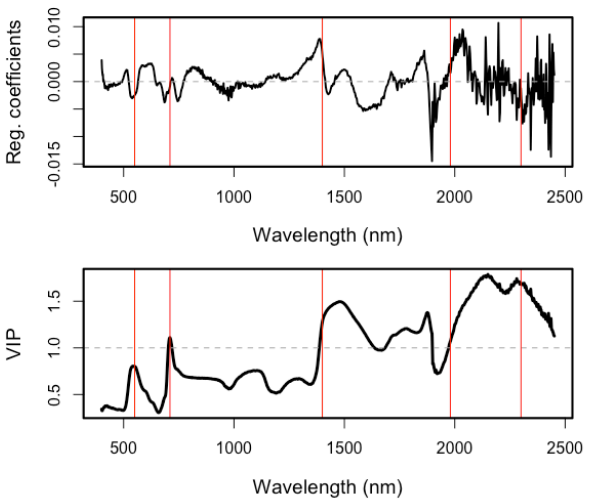
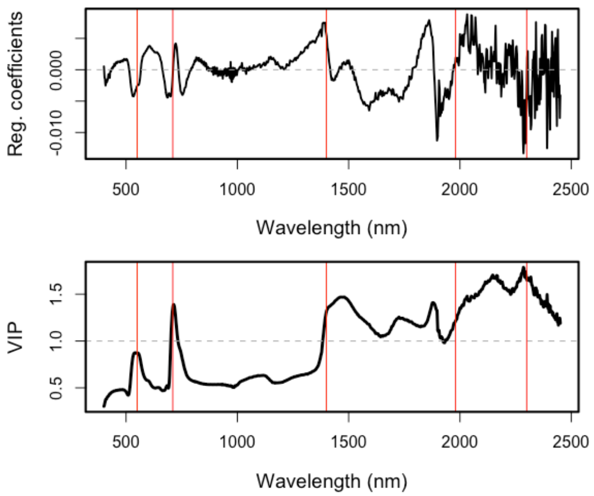


Figure C5: Regression coefficients and VIP values of PLSR models predicting N_{mass}, modeled by a) calibrating on early succession and validating on mid succession, and b) calibrating on mid succession and validating on early succession. Red lines represent theoretically important wavelengths in predicting foliar traits (pigments (including chlorophyll) at 550 and 710 nm, water content at 1400 nm, and proteins at 1980 and 2300 nm).

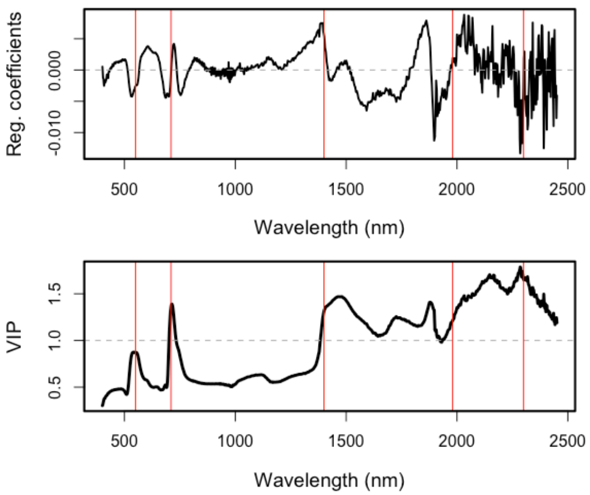
a)


Reg. coefficients
-0.015
0.000
0.010
500
1000
1500
2000
2500
Wavelength (nm)
VIP
0.5
1.0
1.5
500
1000
1500
2000
2500
Wavelength (nm)

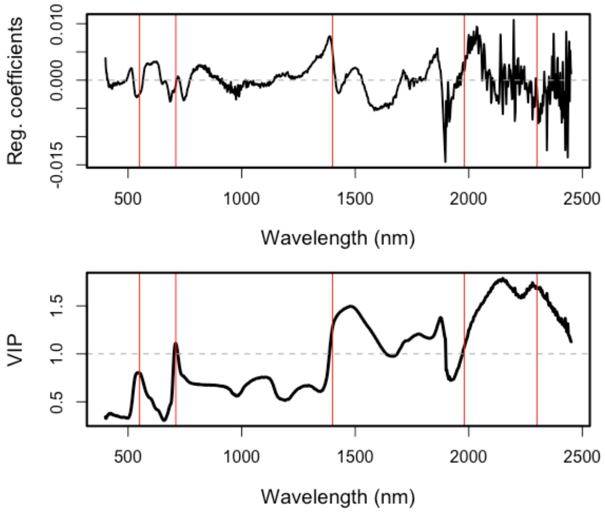
b)


Reg. coefficients
-0.010
0.000
500
1000
1500
2000
2500
Wavelength (nm)
VIP
0.5
1.0
1.5
500
1000
1500
2000
2500
Wavelength (nm)

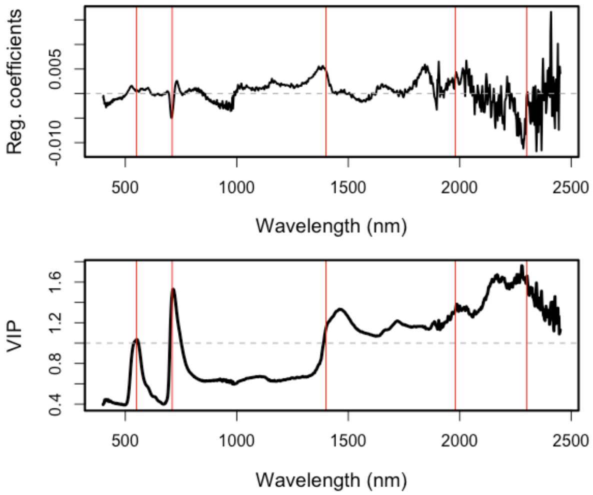
c)


Reg. coefficients
-0.010
0.000
500
1000
1500
2000
2500
Wavelength (nm)
VIP
0.5
1.0
1.5
500
1000
1500
2000
2500
Wavelength (nm)

d)


Reg. coefficients
-0.015
0.000
0.010
500
1000
1500
2000
2500
Wavelength (nm)
VIP
0.5
1.0
1.5
500
1000
1500
2000
2500
Wavelength (nm)

e)


Reg. coefficients
-0.010
0.005
500
1000
1500
2000
2500
Wavelength (nm)
VIP
0.4
0.8
1.2
1.6
500
1000
1500
2000
2500
Wavelength (nm)

f)

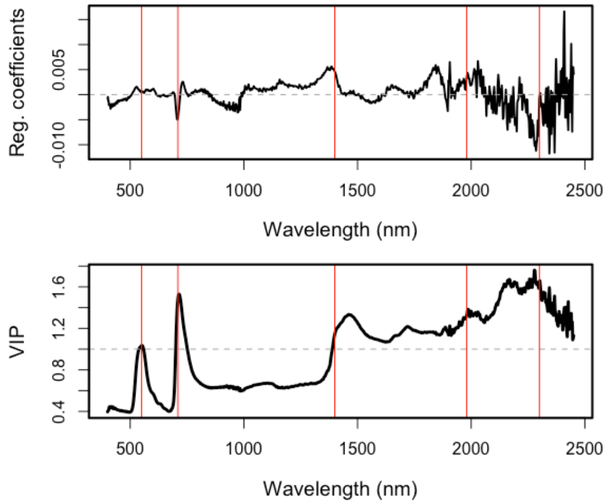

Reg. coefficients
-0.010
0.005
500
1000
1500
2000
2500
Wavelength (nm)
VIP
0.4
0.8
1.2
1.6
500
1000
1500
2000
2500
Wavelength (nm)

Figure C6: Regression coefficients and VIP values predicting LMA, modeled by a) calibrating on early succession and validating on mid succession, b) calibrating on mid-succession and validating on early succession, c) calibrating on mid succession and validating on late succession, d) calibrating on early succession and validating on late succession, e) calibrating on late succession and validating on mid succession, and f) calibrating on late succession and validating on early succession. Red lines represent theoretically important wavelengths in predicting foliar traits (pigments (including chlorophyll) at 550 and 710 nm, water content at 1400 nm, and proteins at 1980 and 2300 nm).