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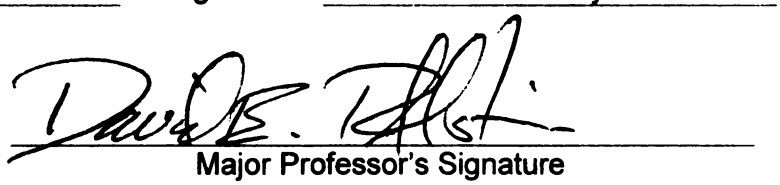
Soil nitrogen cycling and ectomycorrhizal community composition following disturbance in Michigan jack pine forests

presented by

Stephen Daniel LeDuc

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Forestry



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April 30, 2009

Date

SOIL NITROGEN CYCLING AND ECTOMYCORRHIZAL COMMUNITY COMPOSITION FOLLOWING DISTURBANCE IN MICHIGAN JACK PINE FORESTS

By

Stephen Daniel LeDuc

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Forestry

Ecology, Evolutionary Biology, and Behavior

2009

ABSTRACT

NITROGEN CYCLING AND ECTOMYCORRHIZAL COMMUNITY COMPOSITION FOLLOWING DISTURBANCE IN MICHIGAN JACK PINE FORESTS

By

Stephen Daniel LeDuc

Disturbance events, such as wildfire or clearcutting, exert profound influence over the structure, composition and functioning of forest ecosystems. Since nitrogen (N) is generally considered the nutrient most limiting for plant growth in boreal and cold temperate forests, it is critical to understand how disturbances may alter soil N availability and how plants subsequently access that N. In this dissertation, I utilized the jack pine (*Pinus banksiana*) ecosystem of northern Michigan to ask three fundamental questions regarding the effects of disturbance: 1) do the initial recoveries of soil carbon (C) and N pools and dynamics differ following wildfire vs. clearcutting harvesting?; 2) how do available forms of organic and mineral soil N change over time following wildfire?; and, 3) how does community composition of ectomycorrhizal fungi on jack pine roots change following disturbance?

Clearcutting is replacing wildfire as the major disturbance in many forest types, but the implications for soil C and N cycling are little understood. In chapter 3, I compared the initial recovery of C and N pools and dynamics in clearcut, wildfire-burned stands, and intact jack pine stands. Amongst other findings, I observed higher potential nitrification in the clearcut vs. wildfire-burned stands, due to differences in microbial gross nitrate consumption rates. Since the production of nitrate can lead to leaching

losses of N and base cations, this finding has potential implications for the recovery of these forests following clearcutting.

Secondly, studies of successional changes in soil N availability have almost exclusively focused on mineral N; yet, plants can take up forms of organic N, such as amino acids, at biologically important rates. In Chapter 4, I investigated changes in amino acid- and mineral N availability along a ten-site chronosequence of jack pine stands, varying in age from 4 to 60 y post-wildfire. Overall, my results suggest that heterotrophic consumption, not production via proteolysis, controls soil amino acid availability. Moreover, since I found that amino acid N exceeds mineral N in a time period where jack pine growth rates and N demand are highest, I speculate that amino acid N may be important to the N economy of these forests.

Lastly, symbioses between host plants and mycorrhizal fungi can enhance rates of amino acid uptake and allow plants to access complex organic N forms. In Chapter 5, I investigated changes in the belowground community composition of ectomycorrhizal fungi (EMF) using molecular methods along a six-site chronosequence of jack-pine dominated stands (5, 11, 19, 23, 47 and 56 y post-wildfire). Overall, the results of this chapter show that EMF community composition can shift dramatically within the first decades of forest stand development. This compositional shift was primarily driven by higher relative abundances of *Rhizopogon* and *Thelephora* taxa in sites age 5 and 11, and increases in *Cortinarius* in older stands. Since EMF taxa can vary in ability to access N for their plant host, this shift in community may have implications for jack pine nutrition over stand development.

DEDICATION

This work is dedicated to my grandmothers, Ruth LeDuc and Audrey Payne, my parents and my family for their love and support. I also dedicate this work to my fiancée, Rosemary, whose love and companionship has always supported me throughout this process.

ACKNOWLEDGEMENTS

I wish to acknowledge the many people who assisted me throughout my doctoral program. First and foremost, I thank my faculty advisor, David Rothstein, for his encouragement, patience and expert counsel. I not only consider him a mentor, but also a good friend. I extend a warm thanks to my committee members: Tom Horton, Terry Marsh, Mike Walters and Merritt Turetsky. I particularly thank Tom for hosting me at SUNY-ESF in Syracuse, NY for a semester. He and Annette generously welcomed me into their labs, home and family, and I am forever grateful. I also want to acknowledge the late Tom Whittam and members of his Microbial Evolution Lab, most notably Cody Springman. Tom Whittam graciously allowed a forest ecologist to have the run of his lab, constantly peppering his students for advice and assistance. I thank fellow lab members Lynn Holcomb, Melissa McDermott, Gerald Mileski, Emily Scott and Sue Spaulding for their help and forbearance. Though not official lab members, Justin Kunkle and Seth Walk were of great help and even better friends during this process, especially over a cold beverage following a long day in the field. Last but not least, I thank the myriad of undergraduate students who provided many of hours of mundane assistance, everything from washing roots to grinding soil to measuring trees. I cannot thank them enough.

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CHAPTER 1

INTRODUCTION

Disturbance events exert profound influence over the structure, composition and functioning of forest ecosystems. Indeed, current thinking suggests that equilibrium conditions are rare or non-existent in nature, and that all ecosystems are on a trajectory initiated by the last disturbance event (Wu and Loucks 1995). Since nitrogen (N) is generally considered the nutrient most necessary for plant growth in boreal and cold temperate forests (Vitousek et al. 1997), it is critical to understand how disturbances and subsequent stand development may alter soil N availability, the dominant chemical forms of soil N and the access of plants to that N. However, disturbance effects and stand recovery dynamics can be difficult to study because time periods are long and wildfires or clearcuts often occur on sites with substantial confounding differences in ecosystem state factors. The jack pine (*Pinus banksiana*) forests of northern Lower Michigan located on broad, sandy outwash plains with poorly developed soils offer a unique setting that combines frequent disturbance with minimal between-stand variation in topography, soil, vegetation or climate. Thus, by utilizing multiple jack pine stands of differing ages post-disturbance in a chronosequence approach, differences between stands can be inferred as changes over time. In Chapter 2 of this dissertation, I examine the disturbance ecology of these jack pine forests, and in subsequent dissertation chapters, I utilize this particular ecosystem to ask three fundamental questions regarding the effects of disturbance:

1) Do the initial recoveries of soil carbon (C) and N pools and dynamics differ following wildfire vs. clearcutting harvesting?

2) How do available forms of organic and mineral soil N change over stand development following wildfire disturbance?

3) And, how does community composition of ectomycorrhizal fungi on jack pine roots change over time post-wildfire?

The rationale for my first question stemmed from the observation that clearcutting is replacing wildfire as the major disturbance in many forest types (McRae et al. 2001; Bergeron 2004), but the implications for soil C and N cycling are little understood. In Chapter 3, I compare the initial recovery of C and N pools and dynamics in three "treatments": 3-6 y-old clearcuts, 3-6 y-old wildfire-burned stands, and intact, mature jack pine forests (n = 4 stands per treatment). I measure a suite of soil characteristics known to affect C and N cycling; potentially mineralizable C and N fractions; and gross N mineralization and nitrification rates.

For Chapter 4 of this dissertation, I focus on the second question listed above, namely: how do available forms of organic and mineral soil N change over stand development following wildfire disturbance? Studies of changes in soil N availability over stand development have exclusively focused on mineral N (see Vitousek et al. 1989); yet, we now realize that plants can take up certain forms of organic N as free amino acids at biologically important rates. Thus, for this chapter, I investigate the dynamics of free amino acids, over a 10-site chronosequence of jack pine stands, varying in age from 4 to 60 y post-disturbance. Using this chronosequence, I compare free amino acid N and mineral N standing pools, and assess the potential for sources vs. sinks of free amino acids to change over stand development. I measure gross proteolytic rates, a source for amino acids, across the chronosequence, and conduct a ^{15}N leucine tracer

assay on soils from six of the chronosequence sites to evaluate the potential for heterotrophic consumption to act as a sink for free amino-acid N.

While forms of available N may change over stand development, so too might the ability of plants to access that N shift. Ectomycorrhizal fungi (EMF) dominate most boreal and cold-temperate mycorrhizal associations, and these associations are critical for plant nutrient acquisition. Yet, studies have shown variation between EMF taxa in the ability to access nutrients for their plant hosts (Abuzinadah and Read 1986; Finlay et al. 1992; Lilleskov et al. 2002). Thus, a compositional shift in the ectomycorrhizal community over forest development may have important implications for plant nutrition. Therefore, in Chapter 5, I address my last question: how does community composition of ectomycorrhizal fungi on jack pine roots change over time post-wildfire? To answer this question, I characterize the EMF community over six jack pine sites, ranging in age from 5 to 56 y post-wildfire. I identify ca. 60 EMF isolates per site by extracting, amplifying and sequencing the internal transcribed spacer (ITS) regions of the ribosomal DNA. Finally, in Chapter 6, I summarize the main conclusions from the preceding three data chapters, and speculate on potential unifying factors between the chapters.

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CHAPTER 2

DISTURBANCE ECOLOGY OF THE JACK PINE FORESTS OF NORTHERN LOWER MICHIGAN

The jack pine (*Pinus banksiana*) ecosystems within the Highplains district of northern Lower Michigan, USA (44°30´N, 84°30´W) (Fig. 2.1) are maintained by periodic stand-destroying disturbances. These jack pine forests generally grow on extremely dry, nutrient-poor, sandy outwash plains. The flat topography, dry conditions and flammable jack pine vegetation promote frequent stand-destroying wildfires (Cleland et al. 2004). The fires generally kill the overstory jack pine, yet produce conditions necessary for subsequent stand regeneration. Besides jack pine, this ecosystem contains a unique assemblage of plant species, specifically adapted to harsh, fire-prone conditions (Abrams and Dickmann 1982, Houseman and Anderson 2002), and provide habitat for the Kirtland's warbler (*Dendroica kirtlandii*), a federally-endangered songbird. Today, these forests are managed specifically for warbler habitat, and clearcutting and planting have largely replaced wildfire as the dominant disturbance regime.

Species description and native range

A quintessential boreal species, jack pine is a medium sized tree, generally achieving 17 to 20 m in height, and 20 to 25 cm in diameter-at-breast-height (d.b.h.) (Burns and Honkala 1990). It has two short (2-4 cm in length), diverging needles per fascicle, and 3-7 cm long seed cones, with scales tightly closed by a resin bond (Farrar 1995). The native range of jack pine extends across a wide swath of Canada and the

upper latitudes of the United States. Within the boreal zone, jack pine is found as far east as Nova Scotia and as far west as the Northwest Territories. Its southern range reaches into the temperate zone, and includes parts of Maine, northern New York, Michigan, Minnesota and Wisconsin (Burns and Honkala 1990). Hence, the jack pine forests of northern Lower Michigan represent the southern-most extension of its native range.

Climatic and edaphic distribution

Climatic differences are large over this range, in both mean temperatures and precipitation. The Highplains district in northern Lower Michigan experiences a growing season of ca. 80 days and a mean annual temperature of 6.3°C (Albert et al. 1986). The United States Forest Service station in Mio, Michigan, centrally located in this region, recorded an average of 627 mm of precipitation annually between 1995 and 2005, with 331 mm, ca. 53%, occurring during the growing season of May to September (National Climatic Data Center 1995-2005). In contrast, jack pine forests in central Quebec experience much colder temperatures (mean annual temperatures = 0.8-1.2°C), but more precipitation, ca. 840-950 mm—most of this falling as rain (Brais et al. 2000). Moving east to west across boreal Canada, precipitation declines, with jack pine forests in Saskatchewan receiving only approximately 405 mm of precipitation annually (Howard et al. 2004).

Though jack pine usually inhabits very nutrient-poor, coarsely-textured soils, it is generally more restricted to these edaphic conditions in northern Lower Michigan compared to the rest of its range. Jack pine grows best on well-drained loamy sands, but its ability to persist on extremely well-drained, coarse-textured soils allows it to survive

where other species cannot (Burns and Honkala 1990). The jack pine of northern Lower Michigan, for the most part, is relegated to acidic, coarse ice-contact or outwash, sandy soils of the Entisol or Spodosol order (Walker et al. 2003). In its more northerly range, in places like Quebec and Saskatchewan, jack pine is also commonly found on coarse, glacial-deposited material (Howard et al. 2004; Brais et al. 2005); however, it can also inhabit much richer sites. For example in Alberta and Quebec, jack pine can be found on calcareous, lacustrine deposited soils with very fine silts and clays (Visser 1995; Bergeron et al. 2001). In New Brunswick, jack pine can occupy clayey soils and in Minnesota can be found on deep till (Burns and Honkala 1990).

Presettlement fire history to present day management

Historically in Michigan, jack pine inhabited the course outwash sands, as either pure stands or interspersed with the occasional red pine (*Pinus resinosa*) (Whitney 1986). The dominance of jack pine on the outwash sands was maintained by frequent fires, with fire return intervals estimated at ca. 60 y (Cleland et al. 2004). These fires likely burned over large areas, since natural fire breaks are rare across the flat plains. Fires commonly occurred in the spring of the year, especially in the first two weeks of May (Simard and Blank 1982). The reduced snowpack in these forests relative to other areas of northern Lower Michigan (Henne et al. 2007), potentially contributes to low springtime soil moisture and subsequent fires. Perhaps more importantly, dead and dormant vegetation, such as sedge and bracken fern, that readily carry fire, are typically abundant in early May. The establishment of new understory vegetation, combined with jack pine shoot growth, both occurring in mid-to-late May, generally reduce the potential for summer

fires in this region (James Bielecki, Michigan Department of Natural Resources (MDNR), personal communication).

Today, while jack pine forests still inhabit the outwash sites, they are no longer maintained by springtime wildfires, since these disturbances pose a danger to life and property. Instead, forest managers currently employ whole-tree clearcutting and planting on a ca. 50 y rotation as a means to artificially create young stands (ca. 5-23 y-old) for Kirtland's warbler nesting and breeding habitat (Probst and Weinrich 1993). Occasionally, wildfires burn portions of the forest before being suppressed, and as a result, the current landscape consists of a patchwork of mono-dominant jack pine stands of mostly clearcut but also of wildfire origin.

Wildfire disturbance: Jack pine reproduction and establishment

Absent human interference, the life cycle of jack pine is intertwined with wildfire disturbance. Pickett and White (1985) define a disturbance as: "any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability, or the physical environment" (p. 7). Wildfire profoundly alters structure, resource availability and the physical environment of a jack pine forest. Though jack pine is a fire adapted species, it is not tolerant of fire. They may occasionally survive fires (Bergeron 1991), but generally jack pine are girdled and killed (Cayford and McRae 1983). Rather, wildfire promotes the re-establishment of jack pine via the opening of serotinous cones, preparation of the seedbed, and allowing seedlings to establish under an open canopy.

Like most short-lived pioneer species, jack pine reaches maturity relatively quickly, often producing seed cones within the first five years following establishment (Cayford and McRae 1983). The reproductive cycle of jack pine is similar to the three-year cycle that characterizes most pines (Kozlowski and Pallardy 1997). Pollen and seed cone initiation begins in mid-to-late summer of year 1, with pollen cone primordia forming first (Burns and Honkala 1990; Kozlowski and Pallardy 1997). In the middle of May to early June of year 2, the pollen cones elongate and pollination occurs (Burns and Honkala 1990; Kozlowski and Pallardy 1997). Though the development of the pollen tube and ovule begin in year 2, fertilization does not occur until approximately 13 months after pollination, namely June to July of year 3 (Kozlowski and Pallardy 1997). Cones and seeds then develop, ripening by the fall of that year (Burns and Honkala 1990).

Cone serotiny is one of the most important adaptations of jack pine, allowing cones to only open after a fire has created conducive conditions for germination and seedling growth. Jack pine individuals may bear serotinous, non-serotinous or a mix of both types of cones (Schoenike 1976). The resinous bonds of serotinous cone scales melt at ca. 50°C (Cameron 1953), but this temperature is likely lower for non-serotinous cones (Burns and Honkala 1990). Jack pine stands generally exhibit a high degree of cone serotiny, with the notable exception of the southern-most portion of its range (Schoenike 1976; Hyun 1977). In a study that included locations in northern Lower Michigan, Hyun (1977) found a significantly negative trend in cone serotiny moving east to west and a generally negative trend moving north to south. Indeed, non-serotinous cones are relatively common in northern Lower Michigan, whereas virtually all cones are

serotinous in stands located farther north and west in the Upper Peninsula of Michigan (James Bielecki, MDNR, personal communication).

The observation of reduced cone serotiny for Highplains jack pine ecosystems is somewhat surprising, given the historic frequency of wildfire in this region. Several potential explanations for this finding are: present-day fire suppression and clearcutting may favor non-serotinous cones; the historical fire regime may have featured more frequent, non-lethal ground fires; or jack pine in the southern part of its range may represent a distinct genetic population. The characteristic of cone serotiny has been shown to be significantly correlated over a small geographical area with the severity of wildfire disturbance (Gauthier et al. 1996). This suggests that cone serotiny may be a highly plastic trait, potentially affected by the most recent disturbance event. This would further suggest the potential for current fire suppression practices in the Highplains district to alter the frequency of cone serotiny. However, the observation of higher frequencies of cone serotiny in the Upper Peninsula, where fire suppression and jack pine plantings are also practiced, would argue against this as a potential mechanism.

Alternatively, Radeloff et al. (2004) suggest that reduced serotiny in jack pine's southern range in Wisconsin may reflect the historic prevalence of non-lethal ground fires. They argue that the open nature of these forests may not have carried stand-destroying crown fires (Radeloff et al. 2004). Early surveyors in Michigan did note that while jack pine forests grew in "thickets", they also were found in more open areas of drifting sand, often termed "barrens" (Whitney 1987). These barrens may have experienced frequent yet non-lethal fires, perhaps explaining the prevalence of non-serotinous cones in this region. Finally, in perhaps the most parsimonious explanation,

reduced serotiny may simply arise from distinct populations within the Great Lakes region. In a common garden experiment, Hyun (1977) found that Great Lakes jack pine separated phenotypically from boreal populations. Moreover, even within the Great Lakes region, jack pine can vary tremendously in such phenotypic responses as growth rates and branching angles (Burns and Honkala 1990; James Bielecki, MDNR, personal communication). Differences in glacial refugia may explain the presence of multiple phenotypes within the Great Lakes area (Critchfield 1985) and thus differences in cone serotiny.

In addition to opening serotinous cones, wildfire prepares the seedbed for jack pine re-establishment. In a well-stocked stand, over four million seeds per hectare can be released following a wildfire event (Burns and Honkala 1990). The small, winged seeds are dispersed by wind and gravity, usually within two tree heights from the parental source (Burns and Honkala 1990). Germination generally occurs promptly (15-60 d) after seed dispersal—though, slower germination of older seeds can lead to new germinants up to several years post-fire (Beaufait 1962; Burns and Honkala 1990). Jack pine germination rates are highest on mineral soil, intermediate on thin, burned organic (O) horizons, and very low on thick, unburned O horizons (LeBarron 1944; Alhgren and Ahlgren 1960; Cayford and McRae 1983; Burns and Honkala 1990). Undisturbed O horizons serve as poor seedbeds because these layers are prone to desiccation during drought (Eyre and LeBarron 1944). Additionally, the lack of a severe, O-horizon consuming fire may permit the survival of seeds from other species, increasing competition for jack pine seedlings (Benzie 1977).

Wildfire is also critical for stand establishment given that jack pine is highly shade intolerant and wildfire removes the canopy layer. Despite being shade intolerant, in the first few years following establishment, jack pine seedlings actually grow faster with some shade (Logan 1966). Additionally, seedling mortality is often high due to drought and heat (LeBarron 1944; Sims 1975), and so by leaving snags that provide a modicum of shade, wildfire may promote seedling growth and survival. In contrast, clearcutting removes most of the aboveground biomass, leaving no shade for planted seedlings. Reflecting this difference, daily summertime soil temperatures (at 5 cm depth) are substantially higher in clearcut vs. wildfire sites (LeDuc, unpublished data), potentially increasing drought and heat stress to jack pine seedling planted in clearcut stands.

Stand development and successional dynamics

Except for the first few years following disturbance, jack pine generally grows quite rapidly early in stand development, but stands begin to disintegrate after 60 to 80 years post-establishment (Burns and Honkala 1990). Initially, jack pine seedlings grow slowly while establishing a tap root, but, at approximately year four, full sunlight becomes optimal and jack pine growth rates increase sharply (Logan 1966). Over the first twenty years, jack pine grows faster than any other conifer within its native range, with the exception of the tamarack (*Larix laricina*) (Burns and Honkala 1990). In northern Lower Michigan, stands will remain in an exponential growth phase from approximately 10 to 40 years following disturbance, yet by stand age 60 y and beyond, aboveground growth rates are generally quite low to near zero (Rothstein et al. 2004). Older jack pine stands (120+ y) have been noted on finer textured soils (Visser 1995) and

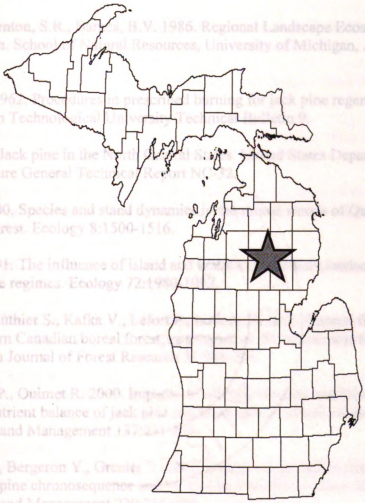
in lake dissected regions (Bergeron 1991), such as the Boundary Waters Canoe Area (BWCA) in Minnesota (Frelich and Reich 1995). Bodies of water often will provide a fire break from severe, large-scale fires, permitting older jack pine stands to survive. The combination of coarse-textured soils and high fire frequency likely made older stands relatively rare historically in northern Lower Michigan. Additionally, the jack pine of this area may be short-lived compared to elsewhere in its range, due to phenotypic differences between populations.

As a result of the historical fire regime and now management practices, jack pine in northern Lower Michigan is maintained as the dominant overstory species, restricting successional dynamics. Given the xeric conditions, the number of species able to survive on these soils is limited (Kilgore and Telewski 2004); nevertheless succession would take place in many sites in the absence of disturbance. For example, advanced regeneration of balsam fir (*Abies balsamea*) will occur in older jack pine stands, undisturbed by harvesting or wildfire (LeDuc personal observation). The lack of succession due to disturbance is similar to the dynamics exhibited by jack pine forests in Western and Central Canada. There, low precipitation leads to drier conditions and greater fire frequency, maintaining jack pine on the landscape (described in Bergeron 2000). In contrast, in the BWCA, the rotation for stand-destroying fire was historically ca. 50-100 years, however, with current fire suppression practices, many even-aged jack pine stands are currently being invaded by balsam fir and white cedar (*Thuja occidentalis*) (Frelich and Reich 1995). Likewise, in southern Quebec, jack pine can be replaced by more shade-tolerant black spruce (*Picea mariana*), as a result of the extended fire cycle due to greater moisture in Eastern Canada (Gauthier et al. 1993).

Summary

Overall, jack pine can persist on highly nutrient-poor, dry soils where other species simply cannot, and its presence on the landscape is predicated upon relatively frequent, stand-destroying disturbance events. These characteristics are exhibited throughout its entire range, but are strikingly apparent in the forests of northern Lower Michigan. These forests are restricted to dry, sandy outwash sites, and do not extend onto finer textured soils, as elsewhere in its range. Despite poor edaphic conditions in this region, jack pine would eventually be replaced in many sites over stand development without frequent disturbance. Historically, this disturbance was wildfire, allowing seedling establishment by opening serotinous cones, preparing the seedbed, and removing the overstory canopy. Before fire suppression, the jack pine forests of northern Lower Michigan probably resembled—in disturbance regime and lack of succession—those of the interior boreal zone, rather than the forests of Eastern Canada. Today, the jack pine ecosystems of northern Lower Michigan still require disturbance, but now clearcutting and planting has replaced wildfire as the major disturbance in these forests.

Figure 2.1: Study location within the Highplains district of northern Lower Michigan, USA (44°30´N, 84°30´W) (Albert et al. 1986).



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CHAPTER 3

INITIAL RECOVERY OF SOIL CARBON AND NITROGEN POOLS AND DYNAMICS FOLLOWING DISTURBANCE IN JACK PINE FORESTS: A COMPARISON OF WILDFIRE AND CLEARCUTTING

ABSTRACT

Forests naturally maintained by stand-replacing wildfires are often managed with clearcut harvesting, yet we know little about how replacing wildfire with clearcutting affects soil processes and properties. I compared the initial recovery of carbon (C) and nitrogen (N) pools and dynamics following disturbance in jack pine (*Pinus banksiana*) stands in northern Lower Michigan, USA, by sampling soils (Oa + A horizons) from three "treatments": 3-6 y-old harvest-regenerated stands, 3-6 y-old wildfire-regenerated stands and 40-55 y-old intact, mature stands (n = 4 stands per treatment). I measured total C and N; microbial biomass and potentially mineralizable C and N; net nitrification; and gross rates of N mineralization and nitrification. Burned stands exhibited reduced soil N but not C, whereas clearcut and mature stands had similar quantities of soil organic matter. Both disturbance types reduced microbial biomass C compared to mature stands; however, microbial biomass N was reduced in burned stands but not in clearcut stands. The experimental C and N mineralization values were fit to a first-order rate equation to estimate potentially mineralizable pool size (C_0 and N_0) and rate parameters. Values for C_0 in burned and clearcut stands were approximately half that of the mature treatment, with no difference between disturbance types. In contrast, N_0 was lowest in the wildfire stands (170.2 μg N/g), intermediate in the clearcuts (215.4 μg N/g) and highest in the

mature stands (244.6 μg N/g). The most pronounced difference between disturbance types was for net nitrification. These data were fit to a sigmoidal growth equation to estimate potential NO_3^- accumulation (Nit_{max}) and kinetic parameters. Values of Nit_{max} in clearcut soils exceeded that of wildfire and mature soils (149.2 vs. 83.5 vs. 96.5 μg NO_3^--N/g, respectively). Moreover, the clearcut treatment exhibited no lag period for net NO_3^- production, whereas the burned and mature treatments exhibited an approximate 8-week lag period before producing appreciable quantities of NO_3^-. There were no differences between disturbances in gross rates of mineralization or nitrification; rather, lower NO_3^- immobilization rates in the clearcut soils, 0.20 μg NO_3^- /g /d compared to 0.65 in the burned soils, explained the difference in net nitrification. Because the mobility of NO_3^- and NH_4^+ differs markedly in soil, my results suggest that differences in nitrification between wildfire and clearcutting could have important consequences for plant nutrition and leaching losses following disturbance.

INTRODUCTION

Disturbance events exert profound influence over the structure, composition and functioning of forest ecosystems (Pickett and White 1985; Attiwill 1994; Oliver and Larson 1996). Indeed, current thinking suggests that equilibrium conditions are rare or non-existent in nature and that all ecosystems are on a trajectory initiated by the last disturbance event (Wu and Loucks 1995). With this understanding, management of forest ecosystems by humans essentially becomes the management of disturbance, and, in theory, the more closely management practices can mimic the intensity, timing and effects of natural disturbance regimes, the more likely our management is to be sustainable over the long-term (Attiwill 1994). Today, many forests once naturally maintained by stand-replacing wildfires are now managed with clearcut harvesting and planting (McRae et al. 2001; Bergeron 2004). By opening the canopy and reinitiating an even-aged stand, clearcutting partially mimics the aboveground effects of stand-destroying wildfire, yet it remains unclear how soil processes are affected and subsequently recover from these different disturbance types. Though many studies have examined either the impacts of clearcutting or wildfire separately, surprisingly few have directly compared the effects of these disturbances on soil processes and properties (e.g. Giardina and Rhoades 2001; Simard et al. 2001).

Clearcutting and wildfire are likely to differentially affect the recovery of soil carbon (C) and nitrogen (N) pools and dynamics. Total C and N pools consist of a recalcitrant fraction, turning over relatively slowly, and more labile, bioavailable fractions, which turn over more rapidly. Intense wildfires can consume much of the surface organic matter (O horizons), volatilizing substantial amounts of C and N (Simard

et al. 2001; Wan et al. 2001; Rothstein et al. 2004; Smithwick et al. 2005), reducing the amount of total soil organic matter (SOM). In contrast, O horizon substrate is mostly retained on-site following harvesting, likely resulting in greater amounts of SOM. Disturbance events may directly alter the more labile C and N fractions as well. Heating from wildfire can reduce microbial populations, particularly in the forest floor and upper soil horizons (Neary et al. 1999), whereas clearcutting may leave the microbial pools intact. Wildfire has been shown to reduce the bioavailable fraction of SOM, probably by reducing fresh litter inputs (Hernández et al. 1997). However, clearcutting also substantially reduces fresh litter inputs, and thus, it remains unclear how the disturbances compare in their effect on organic matter quality.

Indirectly, wildfire and clearcutting may differentially affect the recovery of C and N pools and dynamics by altering soil properties. For example, sorption of phenolic compounds, which otherwise can inhibit microbial activity (Hättenschwiler and Vitousek 2000), by fire-produced charcoal (Zackrisson et al. 1996) may result in increased rates of mineralization and N availability to plants (Wardle et al. 1998). Charcoal-induced reductions in phenolic compounds have been particularly implicated in the increase in net nitrification post-wildfire (Berglund et al. 2004; DeLuca et al. 2006). Likewise, the deposition of ash following wildfire and its associated increases in pH, base cation and phosphorus (P) availability (Raison 1979; Kauffman et al. 1993; Certini 2005) may stimulate microbial activity, increasing the turnover of the remaining organic matter. However, wildfire-induced pulses in nutrient availability are likely to be relatively short-lived (Certini 2005). Hence, disturbance effects on SOM quantity and bioavailability may influence the subsequent recovery of C and N dynamics in a more lasting manner.

In this paper, I compare soils from harvested, wildfire-burned and intact, mature jack pine (*Pinus banksiana*) stands in northern Lower Michigan, USA. I ask the following question: Do the initial recoveries of soil C and N pools and dynamics differ following wildfire vs. clearcutting? To answer this question, I assess total organic matter, microbial biomass, potentially mineralizable C and N, net nitrification, and gross rates of N mineralization and nitrification. To interpret my results, I also investigate a suite of soil properties known to affect C and N dynamics.

METHODS

Study sites

Because wildfire is an inherently stochastic phenomenon, in most areas, replicate wildfires will occur on sites with significant differences in topography, soil, vegetation or climate. Hence, many studies of the effects of stand-replacing wildfire on soils often lack true replication (e.g. Fernández et al. 1997; Choromanska and DeLuca 2001; Giardina and Rhoades 2001). In contrast, jack pine forests of northern Lower Michigan provide a unique setting that combines frequent disturbance with minimal confounding variation in climate, topography, soils or vegetation. The landscape of this region is dominated by broad, outwash plains with uniformly sandy and poorly-developed soils (Albert 1986; Werlein 1998). Jack pine has been the dominant vegetation of these outwash plains since prior to European settlement (Comer et al. 1995). The combination of exceedingly dry conditions, flat topography, and highly flammable vegetation has resulted in a return interval of stand-destroying wildfires of ca. 30 y that has remained relatively constant from pre-settlement times to the present day (Simard and Blank 1982). In addition to wildfire disturbance, jack pine forests in this area are aggressively managed using whole-tree harvesting to provide large areas of early-successional jack pine for the endangered Kirtland's warbler (*Dendroica kirtlandii*). The patchwork of mono-dominant jack pine stands of wildfire- or harvest-origin, occurring on nearly uniform soils with little or no variation in topography, allows comparison of wildfire and clearcutting with true replication.

In May and June 2004, I located twelve study sites: four 3-6 y-old wildfire-regenerated stands, four 3-6 y-old clearcut-regenerated stands and four 40-55 y-old

mature stands, which served as undisturbed references (Table 3.1). Consecutive years of substantial wildfires, 1998 to 2001, provided a unique opportunity to study wildfire-burned stands close in age. Using stand inventory databases compiled by the Michigan Department of Natural Resources (MDNR) and the United States Department of Agriculture Forest Service (USDA-FS), I selected candidate sites, each originating from a unique fire or timber sale, based on stand-age and disturbance-history. Despite the general uniformity of this region, variation in topography, relative landscape position and soil texture can influence ecosystem productivity (Kashian et al. 2003; Walker et al. 2003); therefore, I used field-scouting to further restrict our candidate list to jack-pine dominated, uniformly flat, sandy outwash sites, lacking any clay or gravel banding to a depth of 2 m.

I selected wildfire-regenerated sites in which a mature jack pine stand was destroyed by a fire of at least 80 ha in size, and selected clearcut sites that had been whole-tree harvested, a method in which the entire aboveground portion of the tree is harvested and removed off-site. Two of the mature stands lacked stand-age information in the MDNR database, so these sites were aged by tree-coring, following the methods of Stokes and Smiley (1968). Relatively little stand history data existed for the sites beyond the most-recent disturbance. Since I observed no stumps, planting rows or furrows in the mature sites, these were assumed to be of wildfire-origin. Three of four wildfire sites lacked any evidence of prior clearcutting; however, Wildfire 2, did have old furrows, indicating that the preceding stand had originated from harvesting. The disturbance origins of the preceding stands at the clearcut sites were unknown.

Overall, my study sites spanned a 420-km^2 area with a 12-km average distance

between sites within the Highplains district of northern Lower Michigan, USA (44°30′N, 84°30′W). This region is characterized by a harsh, continental climate with a short growing season (82 days) and cold temperatures (mean annual temperature = 6.3°C) (Albert et al. 1986). The closest weather station to our sites at Mio, Michigan recorded a mean annual precipitation of 627 mm (1995- 2005), with 331 mm (53%) occurring during the growing season (May-September) (National Climatic Data Center 1995-2005). All stands were located on poorly developed sands of the Grayling series, classified as mixed, frigid Typic Udipsamments (Werlein 1998). Both types of disturbed sites exhibited abundant growth of groundflora species including: *Carex pensylvanica*, *Pteridium aquilinum*, *Prunus pumila*, *Vaccinium* spp, and *Comptonia peregrina*.

Sampling

Within each stand, I identified a 1 ha sampling area in which we established 8 sampling points in a stratified-random manner (two points per 2500-m^2 quadrant). In the clearcuts, we established the points between planted seedling-rows, in order to sample from un-furrowed soil—in this way, measuring the effects of the clearcutting only and not the added effects of furrowing and planting. On 7 July 2004, I collected soil cores (5.2 cm diameter x 10 cm deep; starting at the top of the Oa horizon) from each point and transported these samples to the laboratory on ice, storing them for 24 h at 4°C prior to processing. I included Oa horizons in our sampling because this horizon has been shown to make a major contribution to N cycling in these systems (Yermakov and Rothstein 2006) and because impacts of disturbance should be greatest in surface organic horizons. Mineralization data were later expressed as a function of total C and N to determine the

relative effect of SOM quantity (methods described below). The soil samples were passed through a 4-mm sieve with any remaining organic matter discarded. The samples were weighed for bulk density and two samples per quadrant were composited to yield a total of four within-site replicates. On 11 September 2004, I collected an additional set of nine soil cores per site in the same manner, randomly extracting an extra soil core from one of the sampling points, and we composited in triplicate for a total of three within-site replicates. Total soil C and N, potentially mineralizable C and N fractions and soluble phenolics were determined on soil samples collected in July. September samples were measured for soil pH, microbial biomass C and N, gross N mineralization and nitrification, available base cations and P.

Soil properties and total C and N

To evaluate potential differences in the initial recovery of C and N pools and dynamics between treatments, I measured a suite of soil properties known to alter C and N cycling, and total C and N pools. Soil pH was determined with a glass electrode in a 1:2 slurry of air-dried soil to 10 mM $CaCl_2$. Base cations and available P were determined by extracting 10 g of air-dried soil with 60 ml of Mehlich-3 extractant (200 mM CH_3COOH, 250 mM NH_4NO_3, 15 mM NH_4F, 13 mM HNO_3, and 1 mM EDTA) (Frank et al. 1998). Base cations, calcium (Ca++), potassium (K+) and magnesium (Mg++), were measured using inductively-coupled plasma atomic emission spectrometry (Optima 2100 DV, Perkin-Elmer, Bridgeport, CT), whereas orthophosphate-P was determined colorimetrically by the Murphy and Riley (1962) method. I measured water-soluble phenolics by extracting 25 g fresh-weight soil in 60 ml deionized water for 2 h on

a rotary shaker. Phenolic compounds were quantified colorimetrically by reaction with sodium carbonate and Folin-Ciocalteau reagent using tannic acid as a standard (Lowe 1993), and all values were expressed as tannic-acid equivalents. Lastly, subsamples for total C and N were pulverized in a ball mill and analyzed in triplicate via dry combustion-gas chromatography (NA1500 elemental analyzer, Carlo-Erba, Milan, Italy).

Microbial biomass and potentially mineralizable C and N

To investigate C and N pools and dynamics in the wildfire-burned, clearcut and mature stands, I measured microbial biomass C and N, potentially mineralizable C and N fractions, net nitrification, and gross N mineralization and nitrification. I utilized a chloroform-fumigation extraction method to determine microbial biomass C and N (Brookes et al. 1985). Briefly, two 20 g fresh-weight subsamples were taken from each within-plot replicate. One set of subsamples was extracted immediately with 50 ml of 0.5 M K_2SO_4 while the other was extracted following a 5-d chloroform fumigation in a sealed desiccator. The C and N in the extracts were determined using a total organic carbon/nitrogen analyzer (TOC-V_{CPN}/TNM-1, Shimadzu, Columbia, MD). Microbial biomass C and N were estimated by the difference in C and N extracted from fumigated and unfumigated samples; correction factors of 2.64 and 2.22 were applied for C and N, respectively (Vance et al. 1987; Jenkinson 1988).

To measure potentially mineralizable pools of C and N, I conducted two long-term incubations. In the first incubation, I placed 100 g fresh-weight subsamples in sealed Mason jars for 259 days at 25°C and 20% gravimetric soil moisture (equivalent to field capacity (0.01 MPa) determined via a pressure plate in a preliminary experiment).

Headspace gas was periodically sampled through an air-tight septum with a gas syringe. I measured CO_2 content using an infrared gas analyzer (S151 CO_2 Analyzer, Qubit Systems, Kingston, Ontario) for the first four sampling points covering 48 days of the incubation, and a gas chromatograph equipped with a thermal conductivity detector (Tracor 540, Tracor Instruments, Austin, TX) for every sampling point afterwards. Following each sampling, I opened the jars, allowing the headspace to equilibrate with the surrounding air, added deionized water as needed to maintain 20% soil moisture content, and then recapped. To measure mineralizable N, in a second incubation, I placed 100 g fresh-weight subsamples into 150-ml membrane filter units fitted with a glass fiber pre-filter, and periodically leached each sample with 150 ml of 10 mM $CaCl_2$. I analyzed the leachate for mineral fractions of N—ammonium (NH_4^+) and nitrate (NO_3^-)—via automated colorimetry (Flow Solution IV, OI Analytical, College Station, TX). To replace nutrients lost during leaching, I added 25 ml of a minus-N nutrient solution to each sample following every leaching (2 mM $CaSO_4$, 2 mM $MgSO_4$, 5 mM $Ca(H_2PO_4)_2$ and 2.5 mM K_2SO_4) (Campbell et al. 1993). I measured potentially mineralizable N in this manner for 257 days. Both the experimental C and N mineralization values were expressed as a function of soil dry-weight and as a percentage of total soil C and N. Lastly, I calculated a ratio of C mineralized to N mineralized over the course of the incubation for each soil.

The C and N mineralization and nitrification values were fit to models in order to estimate potentially mineralizable pool size and rate parameters. The C mineralization values for each soil replicate were fit to the following first-order rate equation using least-

squares nonlinear regression in SYSTAT 10 software (Systat Software Inc., San Jose CA):

$$C_t = C_0[(1-\exp(-k_c t)] \quad (1)$$

where C_t represents C mineralized at time t, C_0 represents the maximum potentially mineralizable pool of C and k_c is the rate constant for C mineralization (Stanford and Smith 1972). Values for N mineralization were fit to the same first-order rate equation (1), estimating the maximum potentially mineralizable pool of N (N_0) and the mineralization rate constant (k_n). As in the case of the experimental values, C_0 and N_0 were also expressed as percentage of total soil C and N mineralized. As in prior studies (Hadas et al. 1986; Badía 2000), my potential nitrification data were more closely described by a sigmoidal function due to the substantial lag time in the wildfire and mature treatments. Values for each soil replicate were fit to the following sigmoidal equation, the Richard's function:

$$Nit_t = Nit_{max}[(1-\exp(-k_{nit} t)]\exp(a_{nit}) \quad (2)$$

where Nit_t represents NO_3^- accumulation at time t, Nit_{max} represents the potential maximum NO_3^- accumulation, k is the rate constant for NO_3^- production, and a_{nit} is a parameter controlling the inflection point of the curve, representing lag time. To my knowledge, the Richard's function has not been previously used to describe net nitrification, yet it has been widely applied to an array of sigmoid-shaped datasets and allows the ready calculation of an inflection point parameter (Causton et al. 1978; Cooper 1983).

Gross N mineralization and nitrification

To further evaluate net nitrification values, I performed a laboratory ^{15}N pool dilution assay to measure gross fluxes of mineral N (Hart et al. 1994). For each composited soil sample, I weighed out four 30 g fresh-weight subsamples into 120 ml plastic specimen cups. Each subsample received 3 ml deionized water and 50 μg ^{15}N as either 1.7 ml of $^{15}NH_4Cl$ or $^{15}KNO_3$ solution, bringing the soils to field capacity. Following solution addition, I stirred each soil with a glass rod to evenly distribute labeled N; then I extracted half the NH_4^+ and NO_3^- subsamples at 30 min in 2 M KCl, while incubating the remaining samples for 24 h before extracting. I measured total NH_4^+ and NO_3^- using an Alpkem Flow Solution IV (OI Analytical, College Station, TX) auto-analyzer, and I diffused an aliquot of each extract containing 40-80 μg N onto acid traps following the methods of Brooks et al. (1989). Acid traps were then rolled in tins and analyzed for atom % ^{15}N on an isotope ratio mass spectrometer (Europa Model 20-20, Crewe, Cheshire, U.K) at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley. Samples extracted at 30 min were measured for atom % ^{15}N to establish a time zero value, and gross rates of mineralization, nitrification and immobilization were calculated using the ^{15}N pool dilution equations in Hart et al. (1994). Given that the soils were highly acidic and were incubated in specimen cups under well-mixed, aerobic conditions, it is highly unlikely the immobilization values were confounded by other processes, such as volatilization, leaching or denitrification.

Statistical Analyses

Stand was the level of replication for comparing among treatments, and all analyses were done on stand level means ($n = 4$). All model estimated parameters were derived for each individual soil sample, and then stand-level means of these parameters were compared statistically. Values for gross NO_3^- immobilization were log-transformed to meet the assumptions of normality. I initially assessed the potential effect of stand-age (i.e. the 3-6 y age range) on all parameters in the harvested and burned treatments using an analysis of covariance (ANCOVA). Because there was no significant effect of stand age on any of my parameters, I evaluated treatment effects using a simple one-way analysis of variance (ANOVA). I used Fisher's Least Significant Difference Test to make pairwise comparisons of individual treatment means. Significance for the overall treatment effects and pairwise comparisons was accepted at $\alpha = 0.05$.

RESULTS

Soil properties and total C and N

I found surprisingly few differences in soil physical and chemical properties among wildfire, clearcut and mature stands. There were no significant effects of treatment on bulk density, soil pH, extractable base cations, extractable P or soluble phenolics (Table 3.2). In contrast, total C and N were affected by treatment; both followed a similar pattern, with comparable values in the clearcut and mature stands and lower values in the wildfire stands (Table 3.3). However, differences were only statistically significant for N ($P = 0.016$), and not for C ($P = 0.158$).

Microbial biomass and potentially mineralizable C and N

Relative to total organic matter, the disturbances had a more pronounced affect on labile pools of C and N. Both disturbances exhibited reduced microbial biomass C relative to mature stands, but only wildfire stands contained significantly reduced microbial biomass N (Table 3.3). The microbial biomass C:N ratio was significantly lower in clearcut soils than in the wildfire or mature treatments. Both disturbance types displayed reduced potentially mineralizable C and N pools, both in absolute and percentage terms, below that of the mature stands (Fig. 3.1A and B; Fig. 3.2; Table 3.3). While percentages of total N mineralized were comparable between disturbance types, percentage of total C mineralized trended lower in the clearcut vs. burned soils ($P = 0.055$). Mean values for the ratio of mineralized C to mineralized N were lower in the clearcuts, intermediate in the wildfire-burned stands and highest in the mature stands;

though the difference between disturbances was not statistically significant ($P = 0.119$; Table 3.3).

Most notably, I observed significantly higher net nitrification in the clearcut soils relative to both the mature and burned treatments (Fig. 3.3; Table 3.3). Moreover, net nitrification in the clearcut treatment exhibited a temporal pattern distinct from that of the wildfire and mature stands, whereby rapid rates of net nitrification (0.61 μg NO_3-N /g / d) began immediately and remained linear for approximately 20 weeks. In contrast, there was a lag period of nearly 8 weeks in the wildfire and mature soils where rates of net nitrification were extremely low (0.14 and 0.02 μg NO_3-N /g /d, respectively).

When fit to the models, the C and N mineralization and nitrification accumulation curves evidenced treatment differences in estimated asymptotic values (C_0, N_0 and Nit_{max}), and, in the case of nitrification, the inflection parameter (a_{nit}); yet showed no significant differences in rate constants (k_c, k_n and k_{nit}) (Table 3.4). The pattern of C mineralization conformed well to the first-order rate equation (1) with an average R^2 of 0.994 and a range of 0.960-1.000. Both disturbances exhibited reduced C_0 compared to the mature stands. When expressed as a function of total C, both disturbances had lower percentages of C mineralized relative to the mature treatment; the trend towards a lower percentage of C mineralized in the clearcut vs. wildfire stands was not evident (Table 3.4). The values for N mineralization were also well described by the first-order rate equation (1) with an average R^2 of 0.995 and a range of 0.979-0.999. In contrast to the clearcuts, only burned stands contained significantly lower potentially mineralizable N pools (N_0) relative to the mature stands (Table 3.4). Compared to the

mature stands, the percentage of N mineralized was significantly lower in the wildfire stands, while trending lower in the clearcuts ($P = 0.058$), with no significant difference between disturbances ($P = 0.311$) (Table 4). Across all treatments, C_0 and N_0 were higher than the experimentally measured cumulative mineralization values. There were no treatment effects on the C and N mineralization rate constants, k_c and k_n.

Values for NO_3^- accumulation conformed well to the Richard's function (2) with a mean-corrected R^2 of 0.996 and a range of 0.987-1.000. Estimated asymptotic NO_3^- accumulation, Nit_{max}, was highest in the clearcut soils and similar in the wildfire and mature stands (Table 3.4). The shorter lag time in the clearcut soils was statistically significant, as indicated by the significantly lower inflection point parameter, a_{nit}, compared to the other treatments (Table 3.4). I did not observe a treatment difference in the nitrification rate constant (k_{nit}).

Gross N mineralization and nitrification

Gross N mineralization and nitrification rates did not differ among treatments, and I observed no difference between disturbances in gross NH_4^+ immobilization rates (Table 3.3). Rather, clearcut soils exhibited markedly lower NO_3^- immobilization rates relative to the burned and mature soils.

DISCUSSION

Though clearcutting partially mimics the aboveground effects of stand-destroying wildfire, my results demonstrate that the initial recovery of SOM pools and dynamics can vary between these disturbance types. Mean values of soil organic C declined from mature to clearcut to wildfire-burned stands, but these differences were not statistically significant ($P = 0.158$). In contrast, soil organic N was significantly lower in the wildfire-burned stands, whereas the clearcut and mature treatments contained similar quantities of soil N. The finding of no significant difference between disturbances in total C is somewhat surprising, given that clearcutting retains the forest floor, while large amounts of SOM can be lost via combustion in a wildfire. These particular wildfires were severe, stand-destroying fires, which combusted substantial portions of the forest floor (LeDuc personal observation), yet its direct effect on the Oa horizon, the only O horizon material included in the sampling, is less clear from my results. Because organic horizon C may volatilize at lower temperatures than N (Saito et al. 2007), it is unlikely that the statistically significant response for N but not for C reflects differential losses of these elements from combustion. My results could reflect spatial variation in the C:N ratio of SOM, in which case greater within-stand replication would show consistent responses between total C and N. Alternatively, my results may reflect differential patterns of accumulation of C and N in the 3-6 years following disturbance.

Whereas disturbance effects on total C were not significant, both disturbance types exhibited substantially smaller labile C pools relative to the intact, mature stands (Fig. 3.1A; Tables 3.3 and 3.4). Notably, even three-to-six years following disturbance, microbial biomass C failed to recover to pre-disturbance levels. Wildfire-induced

microbial mortality alone cannot explain the lower microbial biomass C since similar results were observed for both disturbance types. Rather, the reduced microbial biomass is undoubtedly the effect of reduced available substrate. This is reflected in the smaller potentially mineralizable pool of C in the disturbed stands, where the asymptotic mineralization estimates (C_0), in both absolute values and percentage terms, were approximately half that of the mature stands. The lack of a substantial disturbance effect on total pool size, yet a decline in potentially mineralizable C, is not surprising, given that the total C pool is much larger and more recalcitrant than the active pool, and hence less likely to be affected by disturbance. The opening of the canopy layer following both disturbances sharply reduced fresh organic matter inputs, and, combined with several years of decomposition, likely resulted in the smaller mineralizable pool and reduced percentage of total C available in both the clearcuts and wildfire sites.

In contrast to C, I found significant differences between the disturbance types in the initial recovery of N pools and dynamics (Fig. 3.1B; Tables 3.3 and 3.4). Wildfire soils exhibited significantly lower total N, which may, in part, reflect losses from volatilization (Wan et al. 2001; Smithwick et al. 2005)—though, as mentioned previously, this may also reflect differential patterns of SOM accumulation post-disturbance. Labile N pools followed a similar pattern, with both microbial biomass N and potentially mineralizable N (N_0) significantly lower in the wildfire-burned vs. clearcut soils. The similarity in the percentage of total N mineralized (both experimental and estimated) between disturbance types suggests that differences in N_0 were driven by differences in substrate quantity rather than substrate quality (Table 3.4).

This finding of higher standing pools of mineral N and/or greater mineralization rates post-clearcutting largely fits with the few studies which have directly compared the effects of clearcutting and wildfire on soil properties and processes (Giardina and Rhoades 2001; Simard et al. 2001). However, when making this comparison, it should be noted that I examined these pools and processes three-to-six years post-disturbance and included the Oa horizon in my sampling scheme. My finding that C_0 and N_0 were substantially higher than the cumulative experimental values, despite the long incubation times, likely reflects the inclusion of organic material from the Oa horizon. The dynamics of Oa and mineral horizon material are likely to differ, making it important to compare my findings with similar studies. I am aware of only one such comparable study: Simard et al. (2001) observed significantly higher N mineralization rates post-clearcutting vs. post-wildfire in the O horizons of a 2 y-old site, while a similar pattern in the mineral soil was not significant.

In contrast to field-based studies that generally report higher net N mineralization in recently-disturbed sites relative to that of intact forests (Vitousek and Matson 1985; Vitousek et al. 1989; DeLuca et al. 2002), I observed higher potential mineralization in the intact, mature stands. I believe this finding can be attributed to two factors: 1) the pulse of N mineralization post-disturbance is often ephemeral and 2) measuring mineralizable N in a laboratory setting does not account for the potential stimulatory effect of canopy opening. Generally, the concentration and production of mineral N increases rapidly post-disturbance but is relatively short-lived. As reviewed by Wan et al. (2001), the pulse of NH_4^+ and NO_3^- following fire may last only a year, and then quickly decline to pre-fire levels. In the case of these same jack pine forests, Yermakov

and Rothstein (2006) found in a chronosequence study that N mineralization spiked immediately following stand-destroying wildfire, declined to pre-fire levels by year 4 and continued to decline until year 12. With my study sites ranging from three-to-six years in age, it is likely that the initial pulse of mineral N had already passed prior to sampling; particularly in the wildfire stands, which contained less organic N and hence less substrate for mineralization. One important mechanism driving increases in post-disturbance N mineralization *in situ* is the stimulatory effect of opening the canopy on microbial activity through increased soil temperatures. By examining mineralizable N in a laboratory setting and keeping temperature constant, I examined only the effects of soil chemistry and SOM quantity and quality. In these conditions, as noted above, potentially mineralizable N reflected total N available. Hence, with its greater total N, the mature treatment exhibited a greater mineralization rate than the disturbance treatments in a laboratory setting.

For both C and N mineralization, patterns among treatments appeared to be driven primarily by the quantity and quality of organic C and N pools, in that I observed almost no differences among treatments in the soil environment that may have affected microbial activity. I found virtually no differences in bulk density, pH, base cation or P availability, or soluble phenolics between treatments. Other studies have found that the deposition of ash following wildfire can increase pH, extractable base cations and P (Raison 1979; Kauffman et al. 1993; Certini 2005). However, my results suggest that these increases may be highly transient, and, thus are unlikely to explain treatment differences in the recovery of organic matter cycling several years following disturbance.

Surprisingly, the most marked difference between treatments that I observed was in net nitrification (Fig. 3.3; Tables 3.3 and 3.4). Not only was there greater total NO_3^- production in the clearcut soils, but clearcut soils began accumulating NO_3^- immediately, compared to wildfire soils which exhibited an 8-week lag period. A number of studies have reported an increase in net nitrification following disturbance (Vitousek and Matson 1985; Brais et al. 1995; DeLuca et al. 2002), often associated with decreased competition for NH_4^+ substrate and/or removal of factors inhibiting nitrifiers (e.g. low pH, phenolics, P limitation). Differences in net nitrification between clearcut and wildfire treatments could result from a disparity in the abundance or activity of nitrifying microorganisms, or alternatively, a disturbance-induced difference in the balance between gross NO_3^- production and immobilization. The results from my ^{15}N pool dilution assay clearly demonstrate the latter: that lower rates of immobilization, not production, resulted in the greater NO_3^- accumulation in the clearcut soils. While it should be noted that my pool-dilution assay was performed on disturbed soils in a laboratory setting and that I do not have direct data on nitrifying organisms, my gross nitrification data strongly suggest that differences in the abundance or activity of nitrifiers cannot explain observed differences in net nitrification.

My results are consistent with previous findings that post-disturbance NO_3^- availability is controlled more by the sink of microbial immobilization than by the source of gross production (Vitousek and Matson 1985; Kaye and Hart 1998). I speculate that lower NO_3^- immobilization in clearcut soils may be a result of lower microbial N demand

driven by relatively greater C limitation in clearcut soils. Several lines of evidence suggest a shift in relative C to N limitation between wildfire and clearcut soils. These include significantly greater potentially mineralizable N (N_0) and a significantly lower microbial C:N ratio in clearcut vs. wildfire soils. In addition, I observed a non-significant trend toward a lower ratio of C mineralization to N mineralization ($P = 0.119$) in the clearcuts relative to the wildfire-burned stands, suggesting a lower microbial demand for N in this treatment. Additionally, the experimentally measured percentage of total C mineralized trended lower in the clearcuts vs. the wildfire sites ($P = 0.055$), though this difference was not reflected in the model values, in which C_0 was expressed as a percentage of total C. Notably, I did not find a difference in NH_4^+ immobilization among disturbance types. Microorganisms preferentially assimilate NH_4^+ rather than NO_3^- (Jackson et al. 1989; Puri and Ashman 1999) since the incorporation of NO_3^- requires an energetically costly reduction step (Brown et al. 1974). If the soil microbial community is relatively less N limited in the clearcut soils, this lower demand for N may manifest in reduced uptake of NO_3^-. An important caveat regarding microbial C:N ratio is that it may reflect a change in microbial community composition as well as a change in N status (Paul and Clark 1996).

Because my study of wildfire and clearcutting is comparative not experimental, it is important to evaluate the potential for confounding variation in site factors or disturbance history that may affect my interpretation of treatment differences. Studies on these forests have demonstrated that soil texture has a major influence on soil and ecosystem processes (Walker et al. 2003; Rothstein et al. 2004). I had no systematic

variation in soil texture among my treatments (Table 3.1) so soil texture is unlikely to confound my observed treatment effects. Another potential confounding factor could be the disturbance history of the preceding stand. For example, DeLuca and Sala (2006) showed that fire-effects on soil N cycling could persist for 12-17 years following disturbance. To evaluate the potential for legacy effects to confound my study, I estimated preceding stand age using a relationship between diameter at breast height and known age developed from 17, intact jack-pine stands (age 12-69 y), also located on mixed, frigid Typic Udipsamments in the Highplains District (data not shown). Diameter at breast height values were measured for wildfire snags and estimated for clearcuts from stump diameters (Raile 1977). These data produced estimates of preceding stand age in our disturbed treatments ranging from 41 to 66 y. I did observe that estimated age of the preceding stand was lower on average in the wildfire sites (46 y; range 41-60 y) relative to the clearcut treatment (61 y; range 57-66 y). However, differences in preceding stand age between treatments are unlikely to be a confounding factor given that preceding stand age estimates far exceed the demonstrated time period of a legacy effect on N cycling (DeLuca and Sala 2006). Furthermore, soil C and N dynamics following disturbance in these jack pine forest change little over this entire age range (Yermakov and Rothstein 2006), and I observed no correlation between stand age in my mature treatment and any of my C and N cycling parameters (data not shown).

CONCLUSIONS

Overall, my results demonstrate the potential for wildfire and clearcutting to alter the recovery of organic matter pools and dynamics in an opposing manner. I observed no difference between disturbance types in total or labile pools of C, but significantly lower total N, labile N and nitrification in the wildfire soils. The N mineralization data are consistent with the findings of Simard et al. (2001), yet the nitrification results were surprising. These findings need to be tested in situ in order to draw further conclusions; however, a shift in the predominant form of mineral N from NH_4^+ to NO_3^- is likely to have important community and ecosystem-level consequences. Production of NO_3^- in soils is a key control for many biogeochemical processes in that it promotes soil acidification (Brady and Weil 2002), leaching losses of N (Vitousek et al. 1982) and leaching of nutrient cations (Ca++, K+and Mg++) (Harrison et al. 1996, Jussy et al. 2000). Furthermore, many plant species show strong adaptations for utilizing different forms of mineral N, and jack pine in particular appears to grow poorly on NO_3^--N (Lavoie et al. 1992). My results highlight the need for a better understanding of the effects of replacing natural disturbance regimes with management treatments on ecosystem processes.

ACKNOWLEDGEMENTS

I thank the USDA-Forest Service and the Michigan Department of Natural Resources, particularly Phil Huber and Don Torchia, for access to field sites and logistical help. I thank M.A. LeDuc, J. Kunkle, R. Fanelli and S. Spaulding for field assistance and M. Cook, A. Holcomb, M.E. LeDuc, L. Marx, M. McDermott, G. Mileski, A. Mueller, J. Randall, M. Selvaraj, G. Smith, E. White and A. Zemke for technical and laboratory assistance. This project was supported by funding from the Hanes Fund, Michigan State University's Plant Science Initiative and the Michigan Agricultural Experiment Station.

Table 3.1: Treatment, location, vegetation and soil properties of study sites.

Treatment/ Site #	Age (years)[a]	Lat. (N)	Long (W)	% jack pine[b]	Mean jack pine seedling height (cm)	Mean jack pine DBH (cm)[c]	Silt + clay (%)[d]
Clearcut 1	3	44°29′15″	84°19′47″	ND	37.5	ND	8
Clearcut 2	5	44°32′56″	84°22′16″	ND	39.6	ND	5
Clearcut 3	5	44°28′42″	84°20′58″	ND	93.0	ND	4
Clearcut 4	6	44°32′47″	84°24′38″	ND	78.7	ND	5
Wildfire 1	3	44°30′25″	84°14′51″	ND	23.9	9.2	10
Wildfire 2	4	44°35′31″	84°00′31″	ND	37.3	17.1	5
Wildfire 3	5	44°35′57″	84°03′00″	ND	33.1	9.5	8
Wildfire 4	6	44°28′05″	84°19′48″	ND	82.5	9.3	4
Mature 1	43	44°29′19″	84°19′51″	91	ND	11.5	11
Mature 2	45	44°29′35″	84°21′01″	95	ND	10.5	7
Mature 3	49	44°32′19″	84°26′57″	94	ND	13.6	5
Mature 4	54	44°32′58″	84°15′15″	86	ND	15.4	12

Note: ND=No Data

[a] years since disturbance event

[b] (jack pine basal area/total stand basal area) x 100

[c] Diameter at breast height (1.3m) for live overstory in mature stands and standing dead trees in wildfire-burned stands

[d] in upper B horizon

Table 3.2: Soil properties in burned, clearcut and mature jack pine stands.

Variable	Burned Stands	Clearcut Stands	Mature Stands
Bulk Density (Mg m ^ 3)	0.89 (0.07)a	1.08 (0.03)a	0.98 (0.07)a
pH	3.77 (0.05)a	3.54 (0.07)a	3.53 (0.14)a
Extractable Base Cations (μg/g)			
Ca(++)	188.7 (36.6)a	226.7 (38.2)a	194.4 (41.3)a
K(+)	17.9 (4.5)a	22.0 (1.6)a	25.1 (5.0)a
Mg(++)	11.8 (2.4)a	12.3 (1.9)a	12.9 (3.8)a
Extractable P (μg/g)	8.9 (0.9)a	5.5 (1.2)a	5.7 (1.9)a
Soluble Phenolics (μg/g)	28.0 (4.9)a	33.2 (1.8)a	40.2 (3.1)a

Note: All values are means (n = 4 for each treatment) with ± 1 SE given in parentheses. Asterisks represent a significant main effect (*P<0.05 and **P<0.01). For pairwise comparisons, means followed by a different letter within each row are significantly different at P < 0.05 according to Fisher's Least Significant Difference Test.

Table 3.3: Soil carbon and nitrogen pools and fluxes in burned, clearcut and mature jack pine stands.

Variable	Burned Stands	Clearcut Stands	Mature Stands
Total C (mg/g)	23.49 (1.84)a	26.54 (0.79)a	27.50 (1.33)a
Total N (mg/g)*	**0.98 (0.04)a**	**1.13 (0.03)b**	**1.09 (0.02)b**
C:N	24.00 (1.07)a	23.59 (0.32)a	25.37 (0.94)a
Microbial Biomass C and N (μg/g)			
C*	**76.0 (8.0)a**	**86.0 (9.5)a**	**124.3 (9.8)b**
N*	**8.8 (0.8)a**	**11.5 (1.1)b**	**13.7 (1.3)b**
C:N*	**8.6 (0.3)a**	**7.4 (0.2)b**	**9.0 (0.4)a**
Long-term Incubations			
Mineralized C (mg CO_2-C/g)**	**1.9 (0.2)a**	**1.8 (0.2)a**	**3.4 (0.2)b**
Percentage of C mineralized (g C_t 100/g C)**	**8.5 (0.6)a**	**6.9 (0.3)a**	**12.7 (0.6)b**
Net mineralized N (μg NH_4^++NO_3^--N/g)**	**129.1 (6.0)a**	**149.4 (5.2)a**	**182.8 (14.0)b**
Percentage of N mineralized (g N_t 100/g N)*	**13.5 (0.7)a**	**13.4 (0.2)a**	**16.9 (1.1)b**
Mineralized C/mineralized N*	**15.1 (1.8)a,b**	**11.7 (0.7)a**	**18.9 (1.7)b**
Net nitrified N (μg NO_3-N/g)*	**73.5 (10.6)a**	**110.0 (3.4)b**	**72.9 (12.1)a**
Gross N Transformations			
Mineralization (μg NH_4^+-N/g /d)	1.80 (0.15)a	2.21 (0.08)a	2.02 (0.26)a
Nitrification (μg NO_3^--N/g /d)	0.19 (0.07)a	0.25 (0.09)a	0.18 (0.05)a
NH_4^+ immobilization (μg NH_4^+-N/g /d)*	**2.33 (0.12)a,b**	**2.23 (0.04)a**	**2.73 (0.15)b**
NO_3^- immobilization (μg NO_3^--N/g /d)**	**0.65 (0.20)a**	**0.20 (0.03)b**	**0.60 (0.08)a**

Note: All values are means (n = 4 for each treatment) with ± 1 SE given in parentheses. Asterisks represent a significant main effect (*P<0.05 and **P<0.01). For pair-wise comparisons, means followed by a different letter within each row are significantly different at P < 0.05 according to Fisher's Least Significant Difference Test.

Table 3.4: Kinetic parameters for C and N mineralization and nitrification models in burned, clearcut and mature jack pine stands.

Variable	Burned Stands	Clearcut Stands	Mature Stands
C_0 (mg CO_2-C/g)**	**2.2 (0.2)a**	**2.0 (0.1)a**	**4.5 (0.7)b**
Percentage of C mineralized (g C_0 100/g C)*	**9.6 (0.8)a**	**7.9 (0.3)a**	**17.4 (3.6)b**
k_c (year ^ -1)	3.4 (0.1)a	3.7 (0.2)a	2.8 (0.3)a
N_0 (µg NH_4^++NO_3^--N/g)**	**170.2 (7.8)a**	**215.4 (10.3)b**	**244.6 (17.6)b**
Percentage of N mineralized (g N_0 100/g N)*	**17.8 (0.7)a**	**19.5 (1.1)a,b**	**22.7 (1.4)b**
k_n (year ^ -1)	2.2 (0.1)a	1.9 (0.2)a	2.3 (0.3)a
Nit_{max} (µg NO_3-N/g)**	**83.5 (11.9)a**	**149.2 (10.1)b**	**96.5 (6.8)a**
k_{nit} (year ^ -1)	5.2 (0.5)a	3.6 (0.6)a	6.2 (0.8)a
a_{nit}**	**6.3 (1.4)a**	**2.0 (0.6)b**	**10.0 (1.6)a**

Note: All values are means (n = 4 for each treatment) with ± 1 SE given in parentheses. Asterisks represent a significant main effect (*P<0.05 and **P<0.01). For pair-wise comparisons, means followed by a different letter within each row are significantly different at P < 0.05 according to Fisher's Least Significant Difference Test.

Figure 3.1: Cumulative carbon (A) and net nitrogen mineralized (B) per g soil in burned, clearcut and mature jack pine stands.

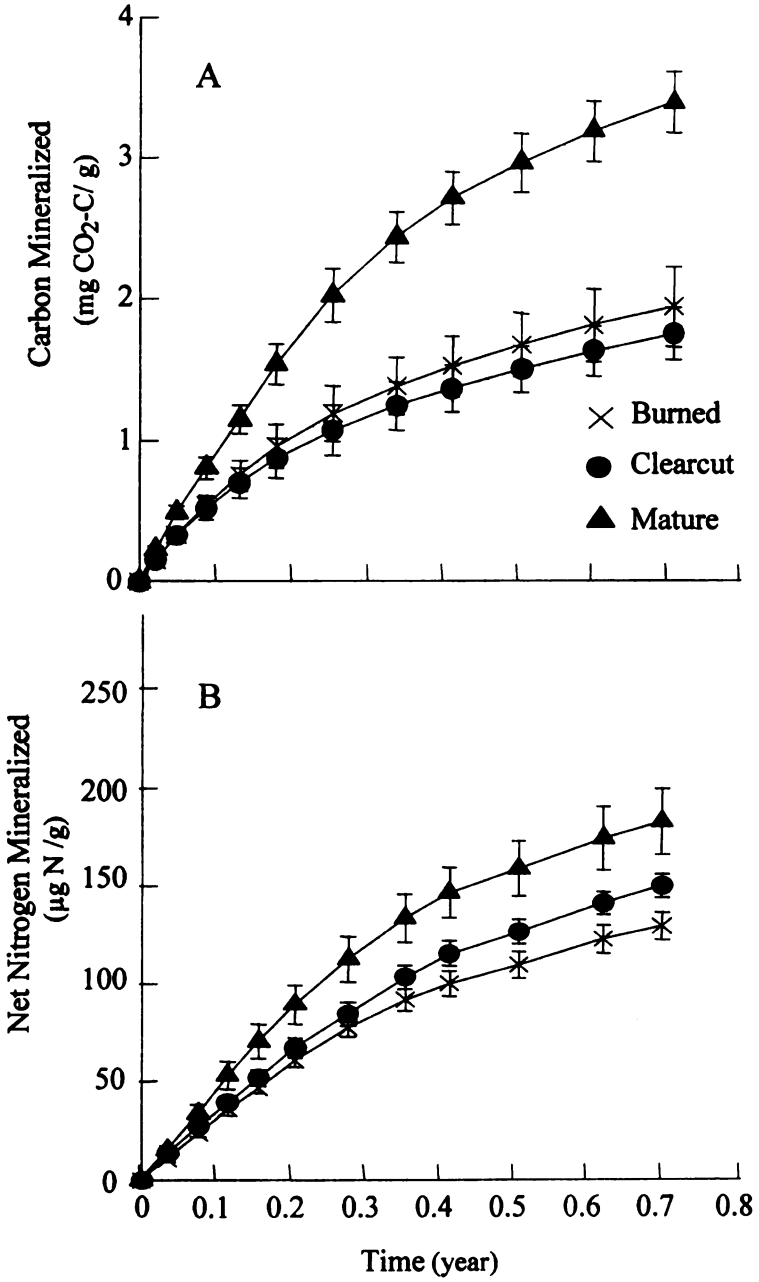


Figure 3.2: Cumulative carbon and net nitrogen mineralized expressed as percentage of total soil C and N in burned, clearcut and mature jack pine stands.

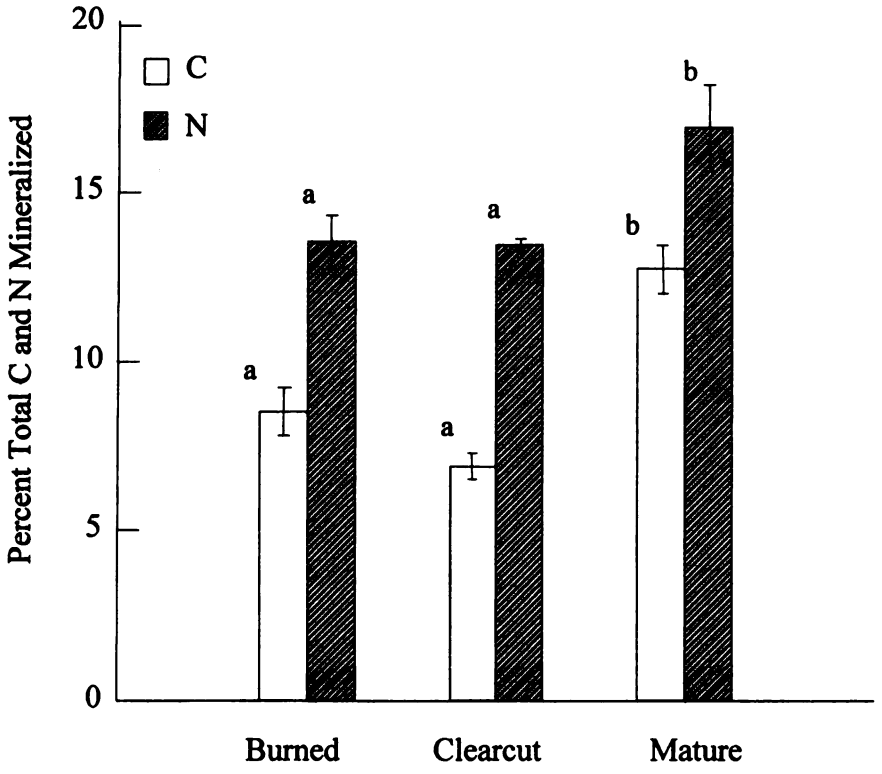
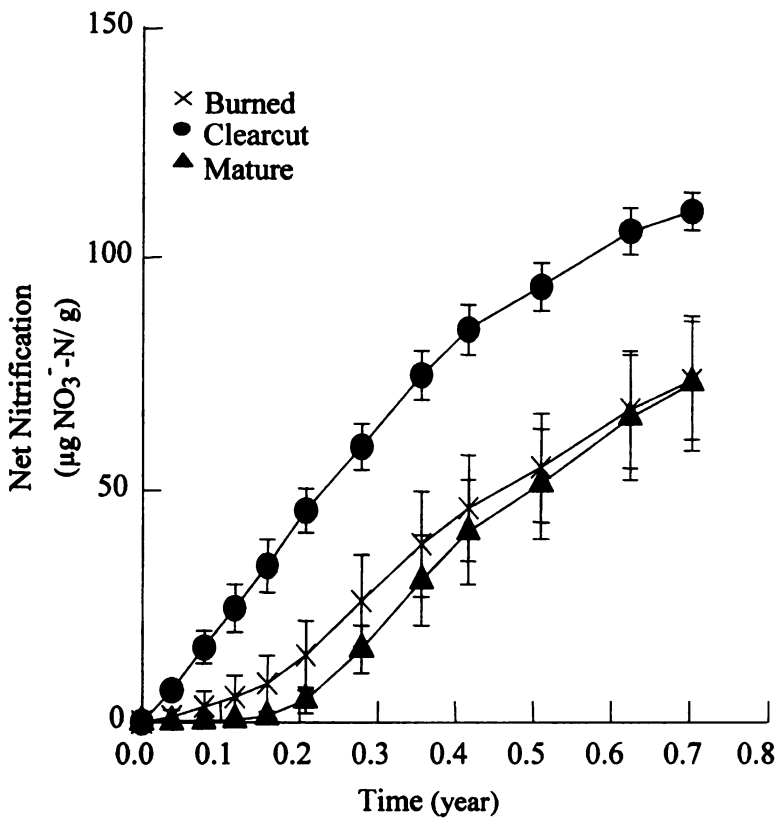


Figure 3.3: Cumulative net nitrification for soils in burned, clearcut and mature jack pine stands.



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CHAPTER 4

DOES PLANT-AVAILABLE ORGANIC NITROGEN INCREASE OVER FOREST STAND DEVELOPMENT? CONTRASTING PATTERNS OF FREE AMINO ACID VS. MINERAL NITROGEN AVAILABILITY WITH STAND AGE

ABSTRACT

Studies of soil nitrogen (N) availability over stand development have almost exclusively focused on mineral N; yet, we increasingly recognize that plants can take up organic N in the form of amino acids at biologically important rates. I investigated amino acid and mineral N availability along a ten-site chronosequence of jack pine stands, varying in age from 4 to 60 y post-wildfire. I measured free amino acid N and mineral N in soil extracts; native proteolytic rates; net N mineralization; and microbial amino acid consumption via a ^{15}N leucine tracer assay in six of the ten sites (4, 10, 18, 22, 46, and 55-y old). Amino acid N was consistently low in the youngest sites (4-10 y), increased rapidly in mid-aged sites (15-22 y), and was highest in stand age 46. In contrast, mineral N exhibited a parabolic shape ($R^2 = 0.499$; $P < 0.0001$), with the youngest site and the four oldest sites containing the highest amounts of mineral N. As a result, amino acid N as a percentage of labile N (amino acid N + mineral N) was greatest in mid-aged stands (e.g. 67% in the 22-y old stand). I observed no trend in proteolytic rates across the chronosequence ($P = 0.632$). Percent ^{15}N tracer recovery was lowest in the soluble organic N (SON) pool for the 4, 10 and 18 y-old sites—though only site age 10 was significantly different from the older sites. Percent recovery in the SON pool was significantly, positively related ($R^2 = 0.798$; $P < 0.05$) to standing pools of amino acid N.

Overall, my results suggest that heterotrophic consumption, not production via proteolysis, controls soil amino acid availability. Higher microbial demand for free amino acids in younger vs. older sites likely results from greater microbial C and N limitation early in stand development due to the lack of fresh litter inputs. Since amino acid N exceeds mineral N in a time period of stand development where jack pine growth rates and N demand are highest, I speculate that amino acid N may be important to the N economy of these forests.

INTRODUCTION

Since nitrogen (N) typically limits plant growth in boreal and cold temperate forests (Vitousek et al. 1997), shifts in N availability over stand development can profoundly impact ecosystem dynamics and functioning. For example, changes in the amount and form of mineral N are thought to influence plant species composition across successional gradients (Kronzucker et al 1997). Many studies have explored changes in mineral N (ammonium (NH_4^+) and nitrate (NO_3^-)) over stand development (see Vitousek et al. 1989; DeLuca et al. 2002; Yermakov and Rothstein 2006); however, over the last decade, a new paradigm of N cycling has emerged which recognizes the potential for plants to "short-circuit" the microbial mineralization of soil organic matter (SOM) and acquire N directly from organic sources (Chapin et al. 1993; Kielland 1994). In particular, a series of studies have shown that mycorrhizal and some non-mycorrhizal plants can take up free amino acid N at rates that equal or exceed those for mineral N (Chapin et al. 1993; Kielland 1994; Raab et al. 1996, 1999; Näsholm et al. 1998). Indeed, the ability to take up intact free amino acids has been demonstrated in a wide-variety of plants, from non-mycorrhizal sedges (Chapin et al. 1993; Raab et al. 1996) to ericaceous shrubs (Näsholm et al. 1998) to arbuscular- and ectomycorrhizal trees (Näsholm et al. 1998; Bennett and Prescott 2004; Hofmockel et al. 2007). Despite this realization, it remains unclear how potentially available forms of organic N, such as free amino acids, might shift over time post-disturbance.

In contrast to amino acid N, changes in mineral N availability through forest development or forest succession have been well studied. Since until recently, plants were widely assumed to be poor competitors for mineral N relative to soil

microorganisms, the net production of mineral N in the absence of live roots (net N mineralization) was considered an adequate metric of plant available N (reviewed in Schimel and Bennett 2004). Rates of net N mineralization are generally highest in the first few years following a disturbance event, with this pulse of mineral N availability persisting for several years to a decade (Wan et al. 2001). Nitrate is typically the dominant form of mineral N immediately following disturbance but often gives way to predominantly NH_4^+ later in secondary succession (Vitousek et al. 1989; Brais et al. 1995; DeLuca et al. 2002; MacKenzie et al. 2004). Following this initial pulse of mineral N, net N mineralization rates typically decline sharply as the ecosystem recovers (Vitousek et al. 1989; DeLuca et al. 2002; Yermakov and Rothstein 2006). Some studies have found that net N mineralization rates continue to decline over the entire successional sequence (see Vitousek et al. 1989; DeLuca et al. 2002), whereas others have found that rates slowly recover from this "trough" to levels comparable to those within the first few years following disturbance (Brais et al. 1995; MacKenzie et al. 2004; Yermakov and Rothstein 2006).

Compared to mineral N cycling, we lack even a basic understanding of how the availability of free amino acids may vary with stand age, either in absolute terms or relative to mineral N. Research to date has primarily focused on spatial patterns, demonstrating that standing pools of amino acids are high in arctic and alpine ecosystems where mineralization is strongly limited by cold temperatures (Chapin et al. 1993; Keiland 1994; Näsholm et al. 1998; Lipson et al. 2001; Jones and Kielland 2002), or in extremely low fertility sites where poor litter quality retards decomposition (Yu et al. 2002; Rothstein *In Press*). I am aware of only one study to have examined changes in

plant-available organic N over a successional sequence. Using a buried core method, Kielland et al. (2006) measured amino acid accumulations across a five stage (three sites per stage), 350-y primary floodplain successional sequence, representing a substantial shift in dominant vegetation (willow (*Salix*) to alder (*Alnus*) to balsam poplar (*Populus balsamifera*) to white and black spruce (*Picea glauca* and *mariana*)). They observed negative to very low net amino acid accumulations in early successional stages, while considerable variation in net amino acids—with both high and low accumulations—later in succession. However, because this represents a primary succession from un-vegetated substrate, these findings may not be relevant to secondary succession following wildfire or harvesting.

Conventional wisdom holds that organic forms of N should increase through stand development as the forest floor and SOM accumulates (Read 1991); however, whether availability of free amino acids coincides with this increase in total organic matter is unclear. How standing pools of free amino acids change over forest development will ultimately depend upon potential shifts in the balance between sources vs. sinks for these molecules. The two major sources for amino acids in the soil are: 1) the depolymerization of proteins and peptides (Schimel and Bennett 2004); and 2) direct inputs of amino acids via leaching of fresh plant litter, root exudates, root turnover and microbial turnover (Hicks et al. 1991; Jones and Darrah 1994; Lipson and Näsholm 2001). Conversely, the sinks for free amino acids are: 1) microbial consumption via uptake or mineralization; 2) direct plant uptake; and 3) abiotic soil sorption. These sources and sinks are likely to shift with stand development. For example, Schimel and Bennett (2004) argue that depolymerization of peptides (i.e. proteolysis) ultimately

controls the availability of free amino acids for uptake by plants and microorganisms. If soil peptide content increases with SOM over stand development, older stands may exhibit higher amounts of free amino acids than younger stands. However, microbial consumption of amino acids is also likely to change over stand development. Young stands exhibit reduced labile soil C and N pools (Chapter 3), likely because fresh litter inputs to the soil are small compared to intact, mature stands. Under these conditions, microorganisms are potentially more C limited and may exhibit comparatively greater demand for amino acids, a source of labile C as well as N. As a result, greater microbial consumption in younger stands may limit amino acid standing pools early in stand development.

In this study, I examine temporal patterns in standing pools of free amino acid N and potential underlying mechanisms (i.e. sources vs. sinks) over a chronosequence of post-wildfire jack pine (*Pinus banksiana*) stands in northern Lower Michigan, USA. I hypothesized that pools of amino acid N would increase with stand age, as a result of low depolymerization rates and high microbial demand for amino acids in the youngest sites giving way to higher rates of depolymerization and lower demand in the older sites. Because previous research in these systems has shown that mineral N availability follows a "U-shaped" pattern with stand age (Yermakov and Rothstein 2006), I further hypothesized that, relative to mineral N, amino acid availability should be greatest at intermediate-aged stands.

METHODS

Study sites and sampling

The jack pine forests of northern Lower Michigan, USA, provide an ideal landscape for studying changes in ecosystem processes over stand development because climatic, edaphic and floristic variation, that would otherwise confound temporal dynamics, are held to a minimum. This area is characterized by a short growing season (82 days) and cold temperatures (mean annual temperature = 6.3°C) (Albert et al. 1986). The landscape predominantly consists of broad outwash plains, with soils generally classified as mixed, frigid Typic Udipsamments (Albert et al. 1986; Werlein 1998). Jack pine is the dominant overstory species on the outwash plains, and its highly flammable vegetation has resulted in a short return interval for stand-destroying fires (59 y) (Cleland et al. 2004). Due to frequent fires and extensive harvesting in this region, the vast majority of jack pine acreage is composed of stands 70 y of age or younger (Yermakov and Rothstein 2006).

In May 2005, I developed a chronosequence of ten fire-origin, jack pine stands, ranging in age from 4 to 60 y (Table 4.1; Fig. 4.1). I used stand inventory databases compiled by the Michigan Department of Natural Resources (MDNR) and the United States Department of Agriculture Forest Service (USDA-FS) to select candidate sites based on age, fire-disturbance and site characteristics. Despite the general uniformity of this region, variation in site topography, relative landscape position and soil texture can influence jack pine productivity and species composition (Kashian et al. 2003); therefore, to minimize confounding between-site variation, we selected jack pine dominated, uniformly flat, sandy outwash sites, lacking any clay or gravel banding to a depth of 2 m.

In all sites, the overstory was destroyed by fires of at least 80 ha in size. Two of my sites (age 22 and 46 y) lacked fire history information and were aged by tree-coring. Since these sites were even-aged, lacked the presence of cut tree stumps and contained substantial amounts of charcoal, charred snags and coarse woody debris, I assumed these sites were of wildfire origin.

At each site, I identified a 1-ha sampling area of uniform terrain, and established 8 sampling points in a stratified-random manner (two points per 2500-m^2 quadrant). Three times over the growing season of 2005 (7 June, 20 July, and 10 September), I collected soil cores (4 cm diameter x 10 cm deep; starting at the top of the Oe horizon) from each point and transported these samples to the laboratory on ice, storing them for less than 24 h at 4°C prior to processing. All samples were passed through a 4-mm sieve, weighed and two samples per quadrant were composited to yield a total of four within-site replicates.

Soil properties

Using the 20 July samples, I measured a suite of soil properties known to influence N cycling. Soil pH was determined with a glass electrode in a 1:2 slurry of air-dried soil to 10 mM $CaCl_2$. Base cations (calcium (Ca++), potassium (K+) and magnesium (Mg++)) and available phosphorus (P) were determined by extracting 6 g of air-dried soil with 60 mL of Mehlich-3 extractant (Frank et al. 1998). Base cation concentrations were measured using inductively-coupled plasma atomic emission spectrometry (Optima 2100 DV, Perkin-Elmer, Bridgeport, CT), summed and expressed as cmol(+)/ kg. Extracted orthophosphate-P was determined colorimetrically by the

Murphy and Riley (1962) method. Subsamples for total C and N were pulverized in a ball mill and analyzed in triplicate via dry combustion-gas chromatography (NA1500 elemental analyzer, Carlo-Erba, Milan, Italy). I measured total soil proteins by extracting 7 g air-dried soil in 35 mL 1 M NaOH for 1 h on a rotisserie shaker; centrifuging for 10 min; and diluting 1/100 in deionized water (Raab et al. 1999). Samples were analyzed by the Bradford (1976) method using a Coomassie Protein Assay Kit (Pierce Product #23200; Pierce, Rockford, IL) and expressed in μg bovine serum albumin (BSA) equivalence per m^2.

Standing pools of soluble mineral and amino acid N

For all three sampling times throughout the growing season, I measured standing pools of soluble free amino acids and mineral N by extracting an 18-g fresh-weight subsample of each composited soil in 27 mL of 4 mM $CaCl_2$ contained in 50 ml centrifuge tubes. The tubes were placed on a rotisserie shaker for 10 min and then spun on a centrifuge for another 10 min. I then filtered the supernatant via a syringe through a 0.4 μm polycarbonate filter, and the samples were frozen at -20°C for later analysis. I determined total soluble N in each extract using a total organic carbon/nitrogen analyzer (TOC-V_{CPN}/TNM-1, Shimadzu, Columbia, MD). To measure mineral N standing pools, I analyzed each extract for NH_4^+ and NO_3^- via automated colorimetry (Flow Solution IV, OI Analytical, College Station, TX).

I used a fluorometric, microplate procedure (modified from Jones et al. 2002) to measure free amino acid content for each $CaCl_2$ extract. Briefly, in each well of the

microplate, I mixed 100 μL of sample or standard with 100 μL of a derivation solution containing 0.02 mM *o*-phthaldialdehyde (OPA) and 1 mM 3-mercaptopriopionic acid (MPA) in 0.2 M potassium tetraborate buffer (pH adjusted to 9.30). Following addition of the OPA-MPA solution, fluorescence was read at 60 min using a microplate reader (FLUOstar Optima, BMG Labtech Inc., Chicago, IL), with the excitation and emission wavelengths set to 370 and 440 nm, respectively. To measure any background fluorescence, I analyzed a negative control for each sample by replacing the derivation solution with 100 μL of the potassium tetraborate buffer; in all cases, the background was negligible and was ignored. Likewise, the derivation solution failed to react with an NH_4^+ standard at concentrations comparable to those in our extracts; hence, it was unnecessary to account for fluorescence due to NH_4^+ in my samples. Glycine solutions were used to generate linear standard curves (0 - 5 μM) in triplicate for each individual plate. Samples were run in duplicate and averaged. In a separate study of free amino acids across a soil fertility gradient in northern Lower Michigan, Rothstein (*In Press)* found the average N content of $CaCl_2$-extractable amino acids was 1.4 μmol N per μmol amino acid. Therefore, to estimate N content of the amino acids measured in this study, I multiplied each result by a factor of 1.4.

Net N mineralization and proteolytic activity

To quantify mineral N and amino acid N production, I measured both net N mineralization in the field and proteolytic activity in the laboratory. I used a buried core method (Raison et al. 1987) to measure in situ net N mineralization twice over the 3-month sampling period (7 June to 10 September). I collected initial samples on 7 June

and 20 July, and time final samples on 20 July and 10 September. Extracts were analyzed for NH_4^+ and NO_3^- via automated colorimetry, and net N mineralization was calculated as the difference in mineral N between the initial and final samples. The two measurements were summed to yield a total mineralization value and expressed as $g\ N\ m^{-2}$.

Using the methods of Watanabe and Hayano 1995, I measured proteolytic activity in each of the four within-site replicates collected on 10 September 2005. Four subsamples of each replicate (2 g fresh weight) were weighed into 15 mL centrifuge tubes, each receiving 10 mL of 50-mM sodium acetate buffer (pH adjusted to 4.25), and 0.5 mL toluene to prevent microbial amino acid uptake. The samples were placed on a rotisserie shaker and subsamples were extracted across sites at 2, 4, 8 and 12 h. Reactions were stopped by adding 2 ml trichloroacetic acid (0.846 M acetic acid; 0.566 M sodium acetate; 0.282 M trichloroacetic acid), centrifuged, and passed through a 0.4 μm polycarbonate filter. The samples were frozen at -20°C and later analyzed for amino acid N content by the above described fluorometric procedure. For each replicate, proteolytic rates (amino acid N /g dry soil / h) were calculated based on the linear slope of the four extraction time values.

Microbial consumption of amino acid N

Finally, I conducted a laboratory-based, ^{15}N-tracer assay to measure microbial amino acid consumption. At the end of the following growing season (24 September 2006), I collected 8 soil cores per site in the stands that in 2005 were 4, 10, 18, 22, 46 and 55 y-old. The cores (4 cm diameter x 10 cm deep; starting at the top of the Oe horizon)

were collected at 8 stratified-random points along 2 plot-length transects (two points per 50 m of transect). The soil cores were transported to the laboratory on ice, stored for less than 24 h at 4°C, passed through a 4-mm sieve and weighed. The samples were then combined by 50 m transect section to yield 4 within-site replicates. Subsamples were taken for an additional proteolytic assay, conducted in the manner described above, except subsamples were extracted at 0 and 5 h.

For the tracer study, I weighed two subsamples (12 g fresh weight each) for every replicate into 50 mL centrifuge tubes. One subsample received a 1 mL solution of 98 atom% ^{15}N leucine (30 mg N / L), while the other received a 1 mL deionized water blank. I used leucine as a tracer since it is readily metabolized by soil microorganisms (Lipson et al. 1999; Gonod et al. 2006) and should have minimal sorption potential due to its net neutral charge at acidic soil pH. Labeling solutions and water blanks were injected into the soil in five 0.2-mL aliquots, with solution dispensed evenly as the needle was removed from the soil. The samples were incubated for exactly 4 h at 25°C, and extracted using a modified version of the serial extraction method of Holmes et al. (2003). In this extraction procedure, each sample received 24 mL 0.5 M K_2SO_4, and was placed on a rotisserie shaker for 20 min. Following shaking, the sample was centrifuged for 10 min, decanted into a 60 mL syringe and passed through a 0.4 μm filter. This procedure was repeated with an additional 24 mL aliquot, and the filtrate was stored at -20°C for later diffusion. The filter was carefully removed and placed with its respective soil sample back in the centrifuge tube, and chloroform fumigated following the procedure of Brookes et al. (1985). After 5 d, I again extracted each sample using two separate 24-mL K_2SO_4 aliquots, as described above. This filtrate was also stored at -

20°C, while each filter and soil sample were dried at 65°C for 48 h prior to analysis.

I determined the percent recovery of the ^{15}N tracer in the extractable mineral, organic, microbial biomass and residual soil N pools. First, I measured total NH_4^+ and NO_3^- in the non-fumigated filtrate using the Alpkem Flow Solution IV, and then diffused an aliquot of each extract containing 25-50 μg N onto acid traps following a modified method of Brooks et al. (1989). Both NH_4^+ and NO_3^- were diffused together in a capped specimen cup for 12 d by adding MgO and Devarda's alloy simultaneously to each aliquot. Acid traps were then dried, rolled in tins, and analyzed for atom % ^{15}N on an isotope ratio mass spectrometer (Europa Model 20-20, Crewe, Cheshire, U.K) at the Center for Stable Isotope Biogeochemistry, University of California, Berkeley. Second, to measure percent recovery in the extractable organic and microbial biomass N pools, aliquots of the non-fumigated and fumigated filtrates were digested in $K_2S_2O_8$ (Cabrera and Beare 1993), and the NO_3^--N was diffused onto acid traps and analyzed for atom % ^{15}N as described above. Digest blanks were diffused to account for background ^{15}N. Finally, for the residual N pool, the dried filter and soil were pulverized together in a ball mill and analyzed for atom % ^{15}N.

Data analysis

I used a combination of linear and polynomial regressions in order to evaluate potential changes in measured variables with stand age. When necessary, data were log transformed to meet the assumptions of normal distribution and homogeneity of variance.

I used conceptual strength and a lack-of-fit test of the mean square error values to select between significant reduced and full models. The results of the ^{15}N tracer and proteolytic assays, conducted on the subset of chronosequence stands, were analyzed by a one-way analysis of variance (ANOVA). In the case of a significant overall effect, I used Tukey's Honestly Significant Difference (HSD) test to make multiple pairwise comparisons between sites. Significance for all regression analyses, overall treatment effects and pairwise comparisons was accepted at $\alpha = 0.05$.

Though most measurements in this study were conducted in 2005, as noted above, the ^{15}N tracer and the proteolytic assay on the reduced number of chronosequence sites were conducted in 2006. However, for the sake of continuity, sites are referred to by age in 2005 throughout all text and figures.

RESULTS

Soil properties

The chronosequence approach, substituting space for time, requires holding all factors, besides age and age-related variables, as constant as possible. Jack pine was the dominate overstory vegetation (≥90% basal area) across the chronosequence, and soil properties generally exhibited a narrow range in characteristics (Table 4.1). Percent silt and clay did not vary systemically with age, rather it ranged from 7 to 16% in the 46 and 10 y-old sites, respectively. Similarly, soil pH ranged from 3.46 to 3.83 with no apparent pattern associated with stand age. Site age 10 did have notably higher exchangeable bases; whereas extractable P was highest in the youngest stand and highly variable in the other sites. There were no significant trends in total C and N with stand age ($P = 0.864$ and $P = 0.380$, respectively). Soil NaOH-extractable proteins across the sites were weakly described by a quadratic model (Total proteins = $4.52 - 0.07x + 0.001x^2$, $R^2 = 0.216$, $P < 0.01$), with the highest levels in the youngest and oldest sites.

Standing pools of soluble mineral and amino acid N

I observed consistent patterns in standing pools of soluble N, mineral N and amino acid N across the chronosequence throughout the growing season. In contrast to the lack of a clear trend in total soil N, soluble N generally declined from an initial intermediate value in the youngest site (4 y) to low values in the 6, 10 and 15 y-old sites, and subsequently increased and remained high in the 18-60 y-old sites (Fig. 4.2A). In the September sampling, I observed the same general pattern, but an overall decline in standing pools of soluble N, particularly in the two oldest sites. I selected a cubic

function ($R^2 = 0.524$; $P < 0.0001$) to describe soluble N averaged over the growing season (Fig. 4.2B) since it trended towards a significantly better fit ($P = 0.053$) compared to a linear model and best-represented the data conceptually. Average soluble N values initially declined from the youngest site to site age 10, subsequently increased to levels exhibited by site age 46, and then declined moderately again in the two oldest sites (55-60 y). Overall, site age 10 had the smallest pool of total soluble N (328 mg N m^2), whereas site age 46 contained the greatest (763 mg N m^2).

I found substantial within-site variation in the mineral and amino acid fractions of the total soluble N pool; yet, there were clear patterns across both the chronosequence and the three sampling dates. With the notable exception again of the two oldest sites in the September sampling date, standing pools of mineral N exhibited a rather consistent pattern, with the youngest site and the four oldest sites—in particular sites age 55 and 60—containing higher amounts of mineral N compared to sites age 6 through 22 (Fig. 2C). When averaged across the growing season (Fig. 4.2D), this parabolic shape was significant ($R^2 = 0.499$; $P < 0.0001$), driven primarily by high values in the youngest and oldest sites. In marked contrast to mineral N, standing pools of amino acid N were consistently low in all three of the youngest sites, increased rapidly in sites age 15-22 y, and exhibited high variability among the four oldest sites (Fig. 4.2E). Seasonal average values followed an inverted parabolic pattern (Fig. 2F) ($R^2 = 0.517$; $P < 0.0001$): increasing from lows of 24 and 19 mg N m^2 in the 4 and 10 y-old sites, respectively, to a high of 127 mg N m^2 in the 46 y-old. Values then declined to intermediate levels in the 55 and 60 y-old sites.

Comparatively, standing pools of mineral N exceeded amino acid N in the three youngest sites (age 4, 6 and 10 y) and three of the four oldest sites (age 39, 55 and 60) (Fig. 4.3A). This was particularly the case for site age 4 with 131 mg mineral N m^2 compared to 24 mg amino acid N m^2, and also site age 55 with 271 and 67 mg N m^2 of mineral and amino acid N, respectively. In contrast, in the mid-aged sites, most notably site age 22, amino acid N exceeded that of mineral N. Expressing amino acid N as a percentage of labile N (amino acid N + mineral N) yielded an inverted parabolic curve ($R^2 = 0.408$; $P < 0.0001$), with the highest percentages generally in the mid-aged sites (Fig. 4.3B). Amino acid N made up 67% of the labile N pool in the 22 y-old site, and over 50% in three other mid-aged-to-older sites (15, 18 and 46 y).

Net N mineralization and proteolytic activity

Across the chronosequence, net N mineralization mirrored the parabolic pattern of standing mineral N pools (Fig. 4.4A) ($R^2 = 0.486$; $P < 0.0001$), whereas proteolytic rates lacked any significant trend (Fig. 4.4B). Total mineralization values were intermediate in the youngest site (1.8 g N m^2), lowest in the remaining young-to-mid-aged sites (e.g. 0.5 g N m^2 for site age 15), and highest in the oldest sites, particularly sites age 55 and 60 (3.0 and 2.5 g N m^2, respectively). In marked contrast to standing pools of amino acid N, there was no distinct pattern in proteolysis across the chronosequence. In the 2006 assay, I did observe an overall site effect ($P = 0.046$) in proteolytic rates, which ranged from a high of 0.382 to a low of 0.202 μg N / g soil / h in the 10 and 18 y-old sites, respectively

(Fig 4.5). There was a non-significant trend towards higher production rates in site age 10 relative to the 4, 18 and 22 y-old sites (P = 0.068, 0.062, 0.079, respectively).

Microbial consumption of amino acid N

The results of the ^{15}N leucine assay demonstrated variation among sites in the partitioning of the added label. Total recovery of tracer in all pools combined (mineral N, microbial biomass N, soluble organic N, and residual soil N) averaged 97% with no significant differences among sites (P = 0.588). Percent of the tracer recovered in the mineral N pool was highest in the 4 y-old site (19%), but there were no significant differences among sites (Fig 4.6A). The 10 y-old site exhibited significantly higher percent tracer incorporated into microbial biomass (58%) relative to sites age 22 and 46 (24 and 27%, respectively), while sites age 4, 18 and 55 (33, 34 and 36%, respectively) were intermediate. Combining the mineral and microbial biomass N pools to yield percent microbial consumption, site age 10 exhibited significantly higher proportions of the tracer consumed relative to the four oldest sites, with site age 4 again intermediate between site groupings. In the residual soil N pool, the pattern in percent tracer recovery was similar to that for microbial biomass N. Site age 10 exhibited significantly higher proportion of the tracer recovered in the residual soil compared to sites age 22 and 46 (Fig. 4.6B). As a result of mineralization, microbial uptake and residual soil N, the percentages of the tracer remaining in the soluble organic N (SON) pool were lowest in site age 10, highest in the 22, 46 and 55 y-old sites, and intermediate in sites age 4 and 18.

To further evaluate the influence of sinks (microbial consumption) vs. sources

(proteolysis) on free amino acids in the field, I regressed stand-level averages of laboratory proteolytic rates and percent ^{15}N recovery in the SON pool against growing season average amino acid standing pools. There was a significant, positive, linear relationship ($R^2 = 0.798$; $P < 0.05$) between free amino acid N pools and recovery in the SON pool (Fig. 4.7). In contrast, there was no relationship between free amino acid N pools and protease activity across either the entire chronosequence ($R^2 = 0.000$; $P = 0.461$) or the subset of sites ($R^2 = 0.000$; $P = 0.391$).

DISCUSSION

Patterns in amino acid and mineral N with stand age

Though we have a fairly well-informed understanding of mineral N dynamics over forest stand development, we know almost nothing about how plant-available organic forms of N may change with stand age. Here, I observed a clear pattern of one such form of available organic N, free amino acids. Consistent with my initial hypothesis, standing pools of amino acid N generally increased with stand age. Values of free amino acids were very low in the three youngest stands, increased rapidly after stand age 10 and reached a peak at stand age 46. Free amino acids then moderately declined to intermediate levels in the two oldest stands. In contrast, both standing pools of mineral N and net N mineralization followed a parabolic pattern, with the highest values in the youngest (4 y) and oldest sites (55-60 y) and the lowest values in the intermediate-aged sites (10 – 22 y). Because free amino acid N recovery preceded the recovery of mineral N availability, I found support for my hypothesis that the relative availability of free amino acid N would be greatest at intermediate-aged sites.

My finding of a parabolic pattern of mineral N availability is consistent with other studies (Brais et al. 1995; MacKenzie et al. 2004) and mirrors the results of Rothstein and Yermakov (2006), who used the chronosequence approach to measure mineral N cycling in these same jack pine forests (three of my study sites—the 4, 15 and 39 y-old sites—overlapped with this previous study). Net mineral N originates from organic matter with relatively low C-to-N ratios, residing primarily in the more well-decomposed layers of the O horizon (Wagener and Schimel 1998). Conversely, it is fresh litter inputs, with high C:N ratios, that provide a sink for mineral N through microbial immobilization

(Vitousek and Matson 1985; Kaye and Hart 1998). Following disturbance, the lack of microbial immobilization due to the loss of fresh litter inputs (Vitousek and Matson 1985; Kaye and Hart 1998), and the stimulation of microbial decomposition of the remaining organic matter—likely through a combination of opening the canopy, increasing soil temperatures, and often an increase in base cations and phosphorus (P) from ash deposition—result in an initial pulse in net mineralization (Raison 1979; Vitousek et al. 1989; Certini 2005). Following this initial increase, net N mineralization rates generally decline, potentially due to decreases in P, soil temperature, and/or labile N pools (Vitousek et al. 1989; Yermakov and Rothstein 2006). Eventually, within 20 y post-wildfire in these jack pine systems, mineral N begins a slow trajectory back towards pre-disturbance levels, driven by the re-accumulation of Oa horizon (Yermakov and Rothstein 2006).

This pattern in mineral N contrasts sharply with the pattern of free amino acid N availability observed here, resulting in the two classes of available N alternating in dominance over stand development. As I hypothesized, mineral N dominated the labile N pool (mineral + amino acid N), in both absolute and relative terms, in the three youngest sites (Fig. 4.3B). This was particularly the case for the site age 4, where mineral N was ca. 83% of total labile N. In contrast, the sharp rise in amino acid N after stand age 10 and the concomitant decline in mineral N resulted in over 50% of the labile N pool consisting of amino acid N in the 15, 18, 22 and 46 y-old stands. Though absolute values of amino acids only moderately declined in the two oldest sites, mineral N dominated in percentage terms due to a marked increase of mineral N in these stands.

Underlying mechanisms: Sources for amino acid N

The finding of a consistent pattern in free amino acid N over these chronosequence sites raises the fundamental question: What are the underlying mechanisms controlling free amino acid N availability over forest stand development? Logically, any change in free amino acids must result from shifts in the balance between sources and sinks for these molecules. In contrast to my hypothesis of increased proteolytic production of free amino acids with stand age, I found no pattern in proteolytic rates across the entire chronosequence (Fig. 4.4B). In the second assay based on a smaller number of sites (Fig. 4.5), I did observe a trend towards higher production of free amino acids in site age 10, and, although not statistically significant, this site also had the highest site mean in the first assay (Fig. 4.4B). However, despite this trend towards higher proteolytic rates, site age 10 contained the smallest standing pools of free amino acids (Fig. 4.2F). Overall, I observed no relationship between proteolytic rates and free amino acid pools across the entire chronosequence ($P = 0.461$).

Proteolytic rates reflect both protease enzyme activity and the availability of substrate, namely combined amino acids. I found no evidence that substrate availability was driving the observed pattern in free amino acids. There were no significant trends in total C and N over the chronosequence and no relationship between total N and standing pools of amino acids ($P = 0.965$). Likewise, I found no relationship between total proteins and free amino acids ($P = 0.857$). It should be pointed out that I did not measure easily soluble proteins and peptides. Moreover, the proteolytic rate measurements are laboratory-based assays on disturbed soils and may not necessarily reflect field conditions. Nonetheless, I could find no evidence to suggest that gross production rates

of free amino acids via proteolysis of SOM are controlling the pattern in amino acid N standing pools observed here.

While it has been posited that depolymerization ultimately regulates plant available N—both mineral and organic forms (Schimel and Bennett 2004)—in the case of free amino acids, there exists a distinct alternative possibility: *direct* inputs of free amino acids to the soil via fresh litter, root exudates, root turnover and microbial turnover (Hicks et al. 1991; Jones and Darrah 1994; Lipson and Näsholm 2001). Thus, rather than resulting from increased depolymerization of soil peptides, the pattern in free amino acid pools I observed may alternatively be explained by changes in *direct* inputs to the soil. Though I did not measure fresh litter, root exudates and root biomass directly, these inputs should theoretically follow changes in aboveground plant biomass. I estimated aboveground jack pine biomass across our chronosequence using biomass equations for Great Lakes area jack pine (Perala and Alban 1993; Rothstein et al. 2004), and found a classic sigmodial pattern with maximum biomass accumulation between sites age 10 and 22 y (Fig. 4.8). Early in stand development, inputs of above- and belowground fresh litter and root exudates are likely relatively small. As vegetation recovers, fresh litter inputs and root biomass increase rapidly. It is during this growth phase that amino acid N pools also sharply increase (Fig. 4.2F). This raises the possibility that direct amino acid inputs from leaching of the litter layer, root turnover, root exudates, mycorrhizal hyphae or microbial turnover may be contributing to amino acid standing pools.

Underlying mechanisms: Sinks for amino acid N

In addition to sources, sinks for free amino acid N are also likely to affect

standing pools of these molecules. As listed above, the sinks for free amino acids are: 1) microbial consumption via uptake or mineralization; 2) direct plant uptake; or 3) soil sorption. It is unlikely that direct plant uptake explains the pattern observed in free amino acids found in this study because, despite low plant N demand, I observed low amino acid concentrations early in the chronosequence. Similarly, sorption of amino acids to soil colloids is an unlikely mechanism because I did not observe systematic differences in soil texture or total soil organic matter across the chronosequence (Table 4.1), suggesting limited between-site variation in soil sorption capacities. Furthermore, the pattern of recovery of ^{15}N tracer in residual soil (Fig. 4.6B) was not consistent with sorption as a driver of free amino acid pools.

Instead, in support of one of my original hypotheses, the results of the ^{15}N tracer study suggest microbial consumption of free amino acids may be a key factor in holding free amino acid pools low early in stand development. Supporting this conclusion, I recovered smaller percentages of tracer in the SON pool in the 4 and 10 y-old sites compared to the three oldest sites—though, due to high intra-site variability, this difference was significant only for site age 10. The tracer remaining in the SON pool is a function of the label added minus the consumptive processes of mineralization, microbial biomass uptake and sorption by soil solids. Though not significant, percent tracer recovery in the mineral N pool was highest in site age 4; whereas, recovery in the microbial biomass and the residual soil N pools in site age 10 were significantly higher than two of the three older stands (22 and 46 y). Given the slightly finer textures in site age 10 (Table 4.1), it is possible that higher recovery of ^{15}N in the residual soil pool in this site reflects greater soil sorption. However, since only about half of the N contained

in microbial biomass is rendered extractable by $CHCl_3$ fumigation (Brookes et al. 1985; Jenkinson 1988), some of this ^{15}N undoubtedly reflects microbial uptake. Regardless of the particular mechanism, the tracer remaining in the SON pool should reflect the total consumptive potential for free amino acids in these soils. The results suggesting higher consumptive potential in the younger sites fits with the pattern observed in standing pools of free amino acids. Overall, I observed a strong linear relationship between stand-level averages of free amino acids and percent tracer recovery in the SON pool in the six sites measured ($R^2 = 0.798$, $P < 0.05$) (Fig. 4.7). Though not conclusive, this finding suggests that microbial consumption, potentially in conjunction with a lack of direct amino acid inputs, may result in the low standing pools of free amino acids observed in the youngest sites.

The dynamics of site age 10 may be particularly informative regarding the effects of microbial consumption vs. proteolytic production on free amino acid availability. As mentioned previously, this site trended higher in rates of free amino acid production via proteolysis (Figs. 4.4B & 4.5). Despite this, site age 10 maintained the smallest amino acid pools of any site along the chronosequence, at levels lower than predicted by the fitted model (Fig. 4.2F). Higher microbial uptake of amino acid N in this site, as evidenced by the tracer assay, likely accounts for this finding. This site demonstrates that microbial consumptive processes can control amino acid availability in the soil. This finding fits with a number of studies, which have shown high microbial demand for amino acids in a wide-variety of soils (Jones 1999; Jones and Kielland 2002; Berthrong and Finzi 2006).

Given that wildfires reduce labile C and N pools (Chapter 3), it is perhaps not surprising that microbial demand for free amino acids, a readily available source for both C and N, may be higher in the youngest sites. Disturbance-induced reductions in fresh litter inputs often decrease microbial N demand, as evidenced by declines in microbial N immobilization (Vitousek and Matson 1985; Kaye and Hart 1998). Instead, in the earliest stages post-disturbance, soil microorganisms should be relatively more C limited, and it is likely they would sequester C from available amino acids while mineralizing the N. Potentially reflecting this dynamic, mineralization of ^{15}N from the labeled amino acid was highest in site age 4, but this was not statistically significant. Further investigation, employing multiple replicates per site age class, would be required to evaluate whether mineralization of amino acid N is indeed higher in the first few years post-disturbance. Following an initial pulse in mineral N post-disturbance, net N mineralization rates generally decline for a period with stand age, suggesting increasing microbial N limitation. Since N mineralization across the chronosequence was lowest in site age 10 and 15 (Fig. 4.4A), it is not unexpected that microbial ^{15}N uptake was greatest at site age 10. Possibly due to the finer textured soils, this site did exhibit proportionally higher microbial biomass N, as recovered in the tracer experiment (data not shown); however, even accounting for microbial biomass, microorganisms in this site incorporated higher amounts of amino acid tracer relative to the other sites. As labile C and N pools increase with stand age (Chapter 3), microbial demand for amino acids as a source of C and N may comparatively decline in older sites.

Potential Importance of Amino Acid N for Jack Pine N Demand

As noted previously, the marked increase in free amino acids coincides with the rapid growth of overstory vegetation in these jack pine forests (Fig. 4.8). As a result, pools of free amino acid N exceed mineral N at a period of time in stand development when plant N demand should be relatively high. This raises the prospect that amino acid N uptake may make an important contribution to the N economy in actively growing, intermediate-aged jack pine stands. I did not measure net N mineralization for an entire growing season in this study, but, based on data from Yermakov and Rothstein (2006), net N mineralization rates should range from 6 – 8 kg N / ha / y between stand ages 10 and 22 y. Coupling rates of tree growth in these sites with literature data on jack pine N concentrations (Alban 1988) yields an estimate of 12.5 kg N / ha / y accumulating in aboveground jack pine biomass between these stand ages. This suggests that mineral N may not account for all plant N demand in these rapidly growing forests and that amino acid N may contribute to plant nutrition during this stage of stand development. Further research is required to evaluate plant uptake of free amino acid N in these forests as a function of stand age.

CONCLUSIONS

We increasingly recognize that the definition of plant-available N has shifted to include simple N monomers, such as free amino acids, in addition to mineral forms of N. Despite this realization, we do not understand how the cycling of these plant-available organic forms of N may shift over stand development, which may have important implications for ecosystem structure and function (e.g. plant-growth, mycorrhizal communities, plant species composition, etc.) In this study, I observed a clear pattern in free amino acid N as a function of age since stand-destroying wildfire: amino acid N was very low in the three youngest sites, increased rapidly between 10 to 46 y post-fire, and then declined to intermediate levels in the two oldest stands. The observed pattern in amino acid N was in marked contrast to that of mineral N, which exceeded amino acid N early and late in stand development but not in mid-aged sites. The results of this study illustrate that the dynamics of plant-available forms of organic N, such as free amino acids, are not likely to simply track total organic N or total peptides over stand development. In an evaluation of the underlying mechanisms behind the pattern in free amino acids, I observed no difference in proteolytic rates across the chronosequence. Rather, the pattern in free amino acid N closely tracked microbial consumption of amino acids, with the lowest standing pools at sites with the greatest potential for microbial consumption. Finally, my finding that amino acid N availability can exceed mineral N at a period coinciding with rapid stand growth, raises the prospect that free amino acid N may be important for plant nutrition in these systems.

ACKNOWLEDGEMENTS

I thank the USDA-Forest Service and the Michigan Department of Natural Resources for access to field sites and logistical support. I particularly acknowledge A. Holcomb, R. Fanelli, J. Kunkle, M. McDermott, G. Mileski, A. Mueller, S. Spaulding, E. White and many others for field, technical and laboratory assistance. This project was supported by funding from a National Science Foundation grant 0448058 to D. E. Rothstein, a Michigan Botanical Society's Hanes Fund grant to S.D. LeDuc, the Michigan Agricultural Experiment Station, and a Michigan State University's Plant Science fellowship to S.D. LeDuc.

Table 4.1: Age, vegetation and soil properties of chronosequence sites.

Age (y)[a]	% jack pine[b,c]	Median jack pine DBH (cm)[c,d]	%Silt + clay[e]	pH[f,g]	Base cations (cmol+ /kg)[f,h]	Soil P ($g\ m^{-2}$)[f]	Total C ($kg\ m^{-2}$)[f]	Total N ($g\ m^{-2}$)[f]	Total proteins ($\mu g\ BSA\ m^{-2}$)[f]
4	ND	ND	10	3.83	1.70 (0.31)	3.19 (0.61)	6.8 (0.5)	285 (22)	4.11 (0.27)
6	ND	ND	8	3.56	1.58 (0.17)	1.66 (0.19)	7.1 (0.6)	265 (20)	4.35 (0.26)
10	ND	ND	16	3.74	2.85 (0.36)	1.41 (0.13)	7.0 (0.8)	298 (10)	4.41 (0.31)
15	100	5.0	13	3.80	1.43 (0.33)	1.09 (0.15)	6.2 (1.2)	243 (53)	3.37 (0.15)
18	100	4.5	10	3.70	0.96 (0.09)	2.59 (0.32)	5.9 (0.5)	221 (09)	3.55 (0.19)
22	98	7.1	12	3.81	1.52 (0.54)	2.04 (0.51)	5.9 (0.5)	209 (20)	3.50 (0.22)
39	95	10.2	13	3.71	1.66 (0.38)	2.19 (0.68)	5.2 (0.8)	233 (27)	3.84 (0.24)
46	95	10.5	7	3.47	1.11 (0.19)	0.99 (0.22)	7.0 (1.6)	258 (49)	4.00 (0.48)
55	90	14.3	12	3.46	1.62 (0.18)	2.35 (0.64)	7.7 (0.8)	268 (22)	4.07 (0.16)
60	98	12.9	12	3.55	1.12 (0.18)	1.09 (0.09)	5.9 (0.5)	229 (23)	4.55 (0.23)

Note: ND=No Data; Values in parentheses are SEs based on $n = 4$.

[a] Years since stand-destroying wildfire.

[b] (Jack pine basal area/total stand basal area) x 100.

[c] Data for %jack pine basal area and DBH for stands age 18, 39, 55 and 60 y taken from Spaulding (2008).

[d] Diameter (≥ 2 cm) at breast height (1.3 m).

[e] In upper B horizon

[f] For combined O horizon and upper 10 cm of mineral horizon.

[g] Measured in a 1:2 soil-0.01 M $CaCl_2$ slurry.

[h] Calcium (Ca++), potassium (K+) and magnesium (Mg++).

Figure 4.1: Chronosequence site locations, noted by year since stand destroying wildfire.

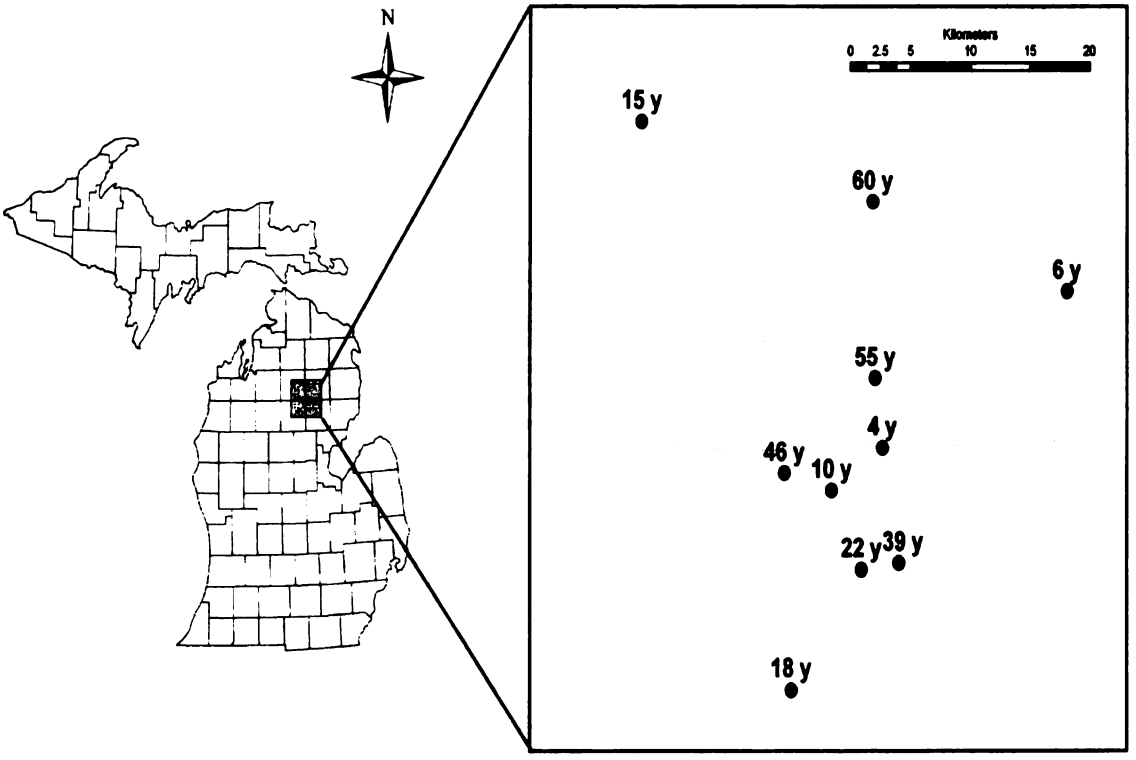


Figure 4.2: Standing pools of soil N as a function of stand age. Individual panels represent total soluble N (**A**) and growing season average (**B**); mineral N (**C**) and growing season average (**D**); and amino acid N (**E**) and growing season average (**F**); data are stand means ± 1 SE. Lines represent the following best fit equations: Total soluble N = 382.80 - 3.52 * age + 0.56 * age ^ 2 – 0.007 * age ^ 3, R ^ 2 = 0.524, $P < 0.0001$ (**B**); Mineral N = 104.33 - 4.82 * age + 0.12 *age ^ 2, R ^ 2 = 0.499, $P < 0.0001$ (**D**); Amino acid N = -7.11 + 6.00 * age - 0.08 * age ^ 2, R ^ 2 = 0.517, $P < 0.0001$ (**F**).

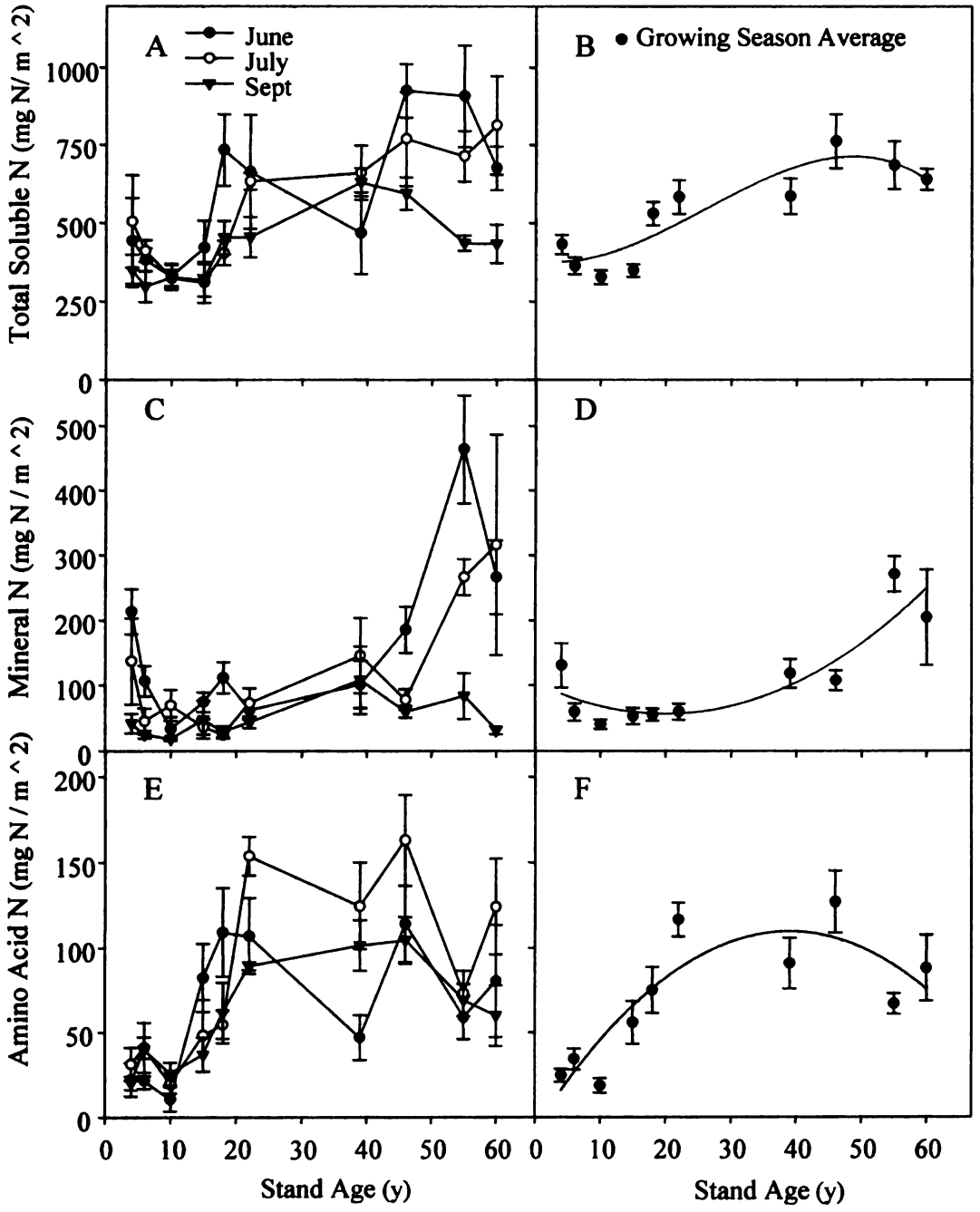


Figure 4.3: Standing pools of amino acid N and mineral N as a function of stand age **(A)**; and amino acid N as percent labile N (amino acid N + mineral N) **(B)**; data are stand means ±1 SE. See Figure 3.4 for amino acid and mineral N best fit equations. The line in B represents the following best fit equation: Amino acid N as percent labile N = -1.30 + 5.86 * age - 0.17 * age ^ 2 + 0.001 * age ^ 3, R ^ 2 = 0.408, $P < 0.0001$ **(B)**.

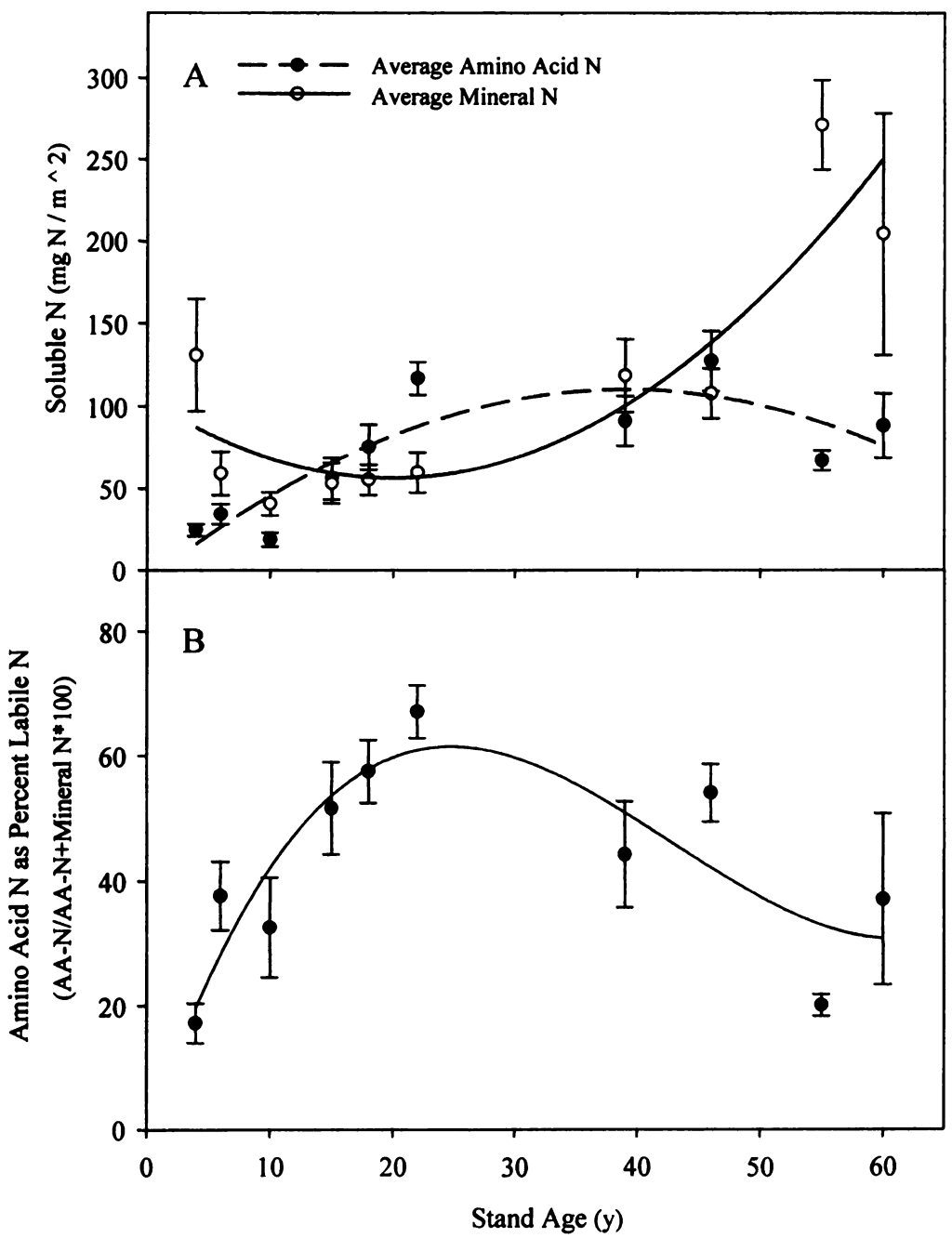


Figure 4.4: Net N mineralization for 7 June to 10 September 2005 **(A)**; and proteolytic activity across the chronosequence sites, as measured in September 2005 **(B)**; data are stand means ± 1 SE. Line represents the following best fit equation: Total N mineralization = 1.57 - 0.069 * age + 0.002 * age ^ 2, R ^ 2 = 0.486, $P < 0.0001$. There was no significant relationship of proteolytic activity with stand age ($P = 0.632$).

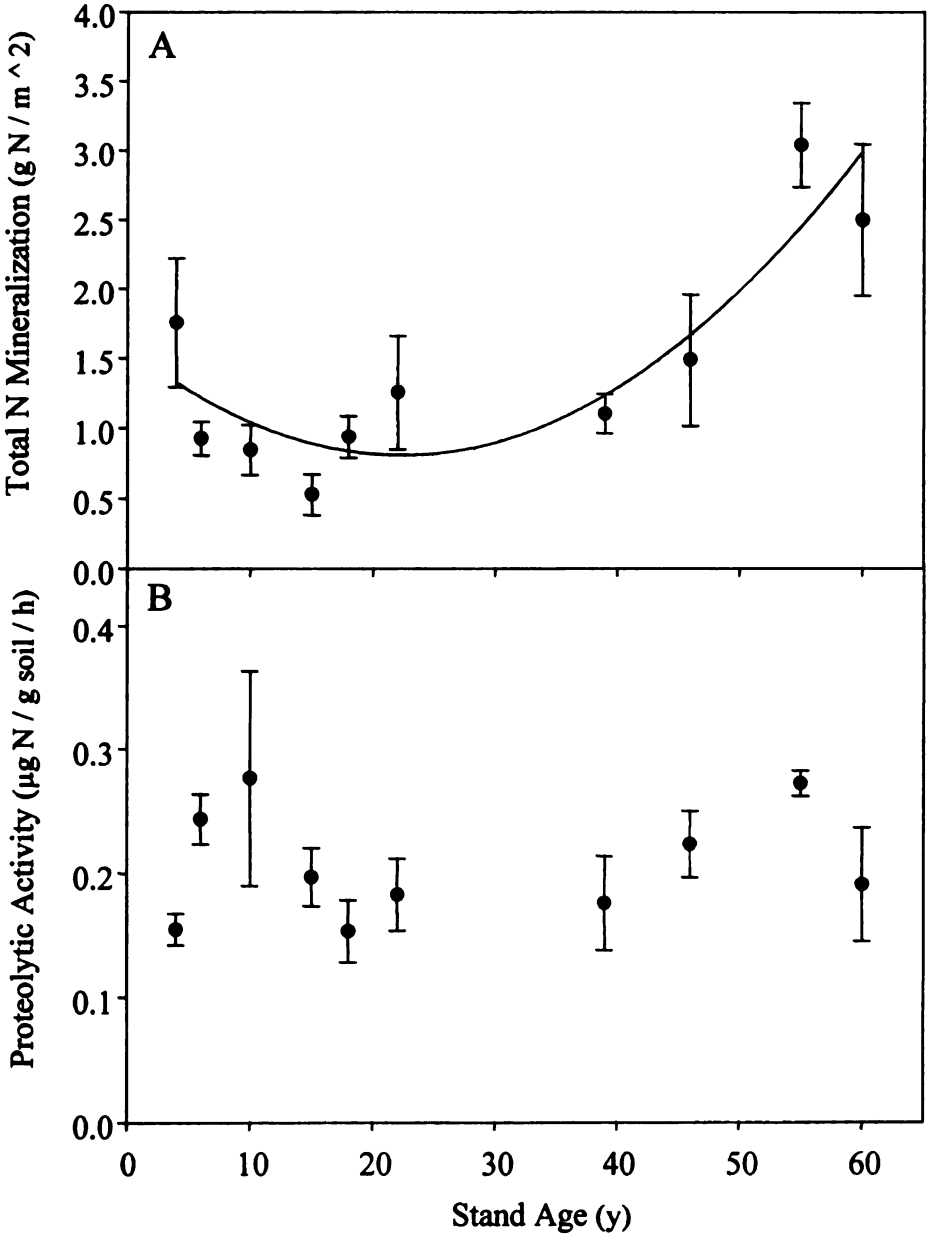


Figure 4.5: Proteolytic activity in six of the chronosequence sites, as measured in September 2006; data are stand means for ± 1 SE. There was an overall site effect (P = 0.046), but no significant pair-wise comparison.

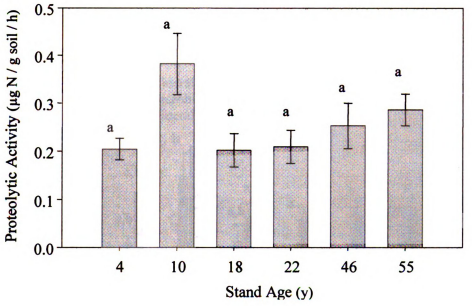


Figure 4.6: Percent ^{15}N tracer recovery in the mineral N, microbial biomass N, and mineral N + microbial biomass N pools **(A)**; and in the soluble organic N, and residual soil N pools **(B)**. For pair-wise comparisons, means followed by a different letter within each N pool type are significantly different at $P \leq 0.05$ according to Tukey's Honestly Significant Difference test.

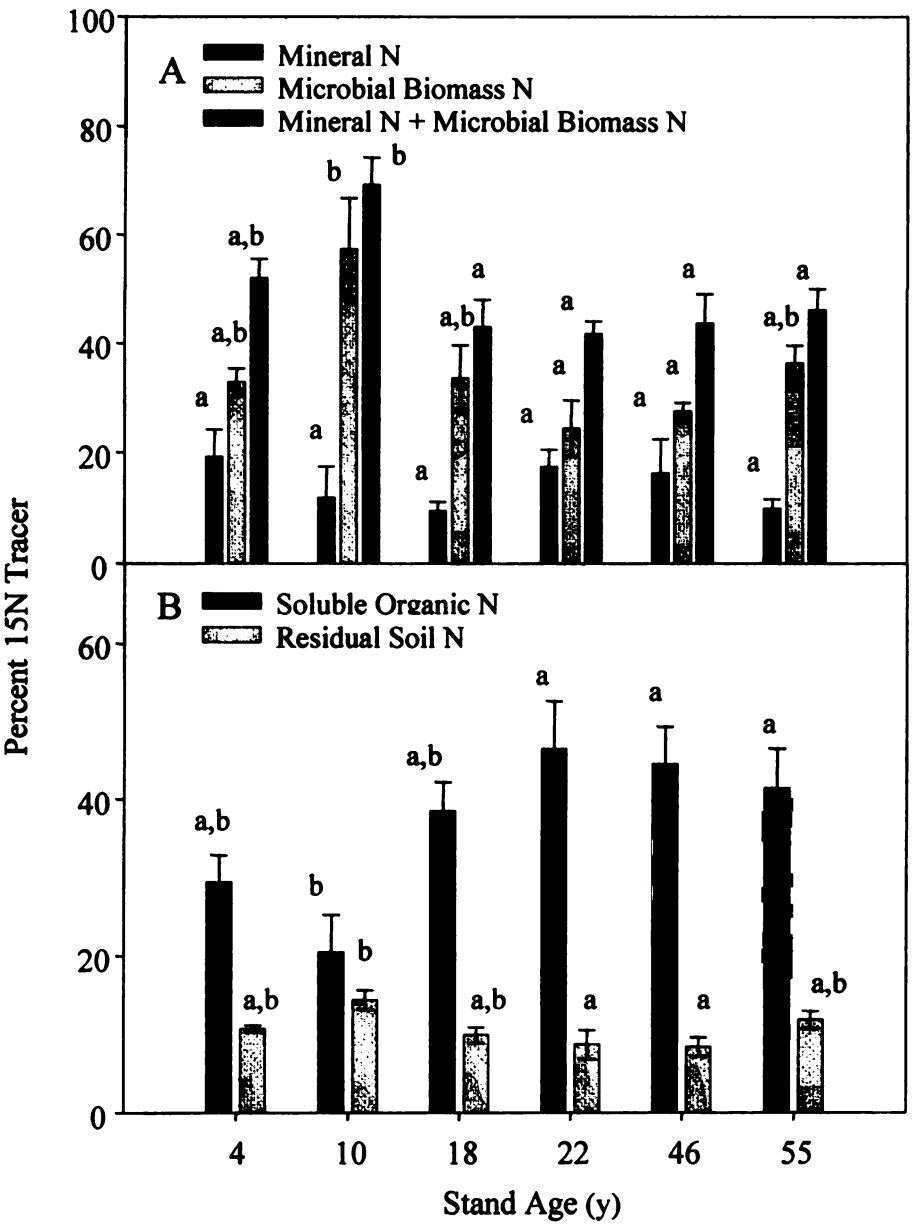


Figure 4.7: Average standing amino acid N pools in six of the chronosequence sites regressed against percent recovery of 15N tracer in the soluble organic N pool; sites noted by year since wildfire and data are stand means ± 1 SE. Line represents the following linear equation: Amino acid N = -80.87 + 4.15 * Percent 15N tracer, R ^ 2 = 0.798, $P < 0.05$.

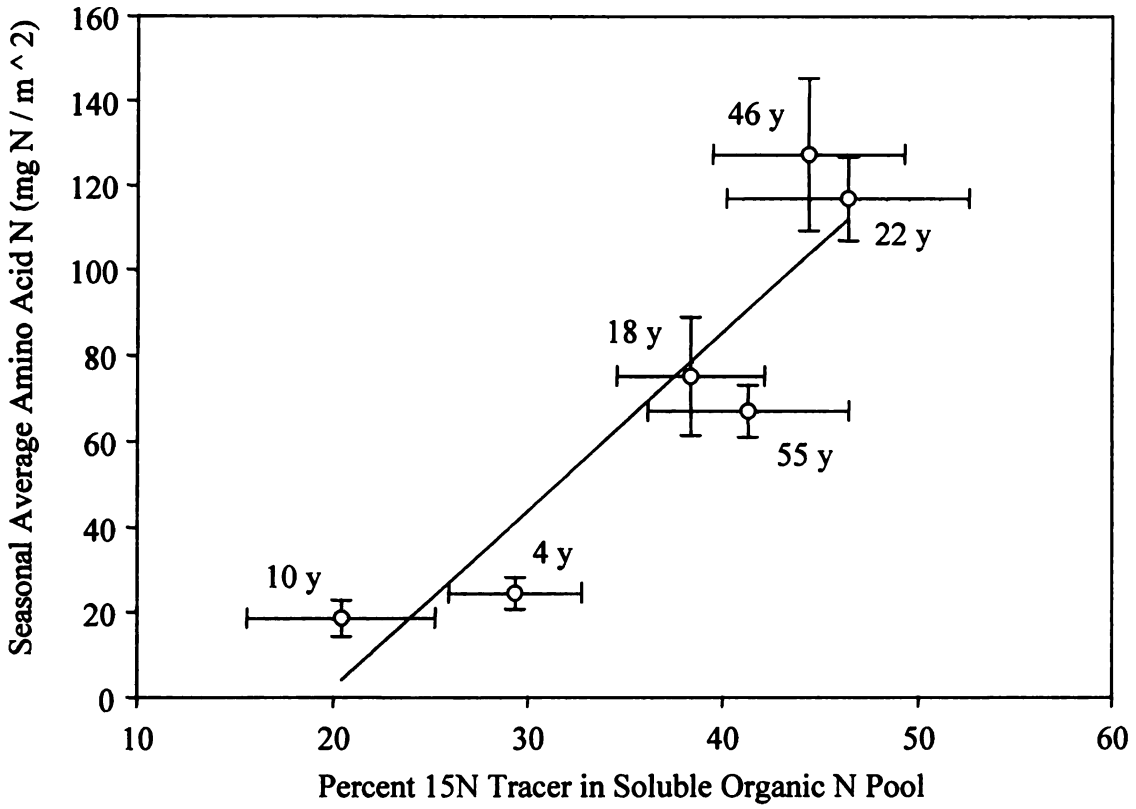
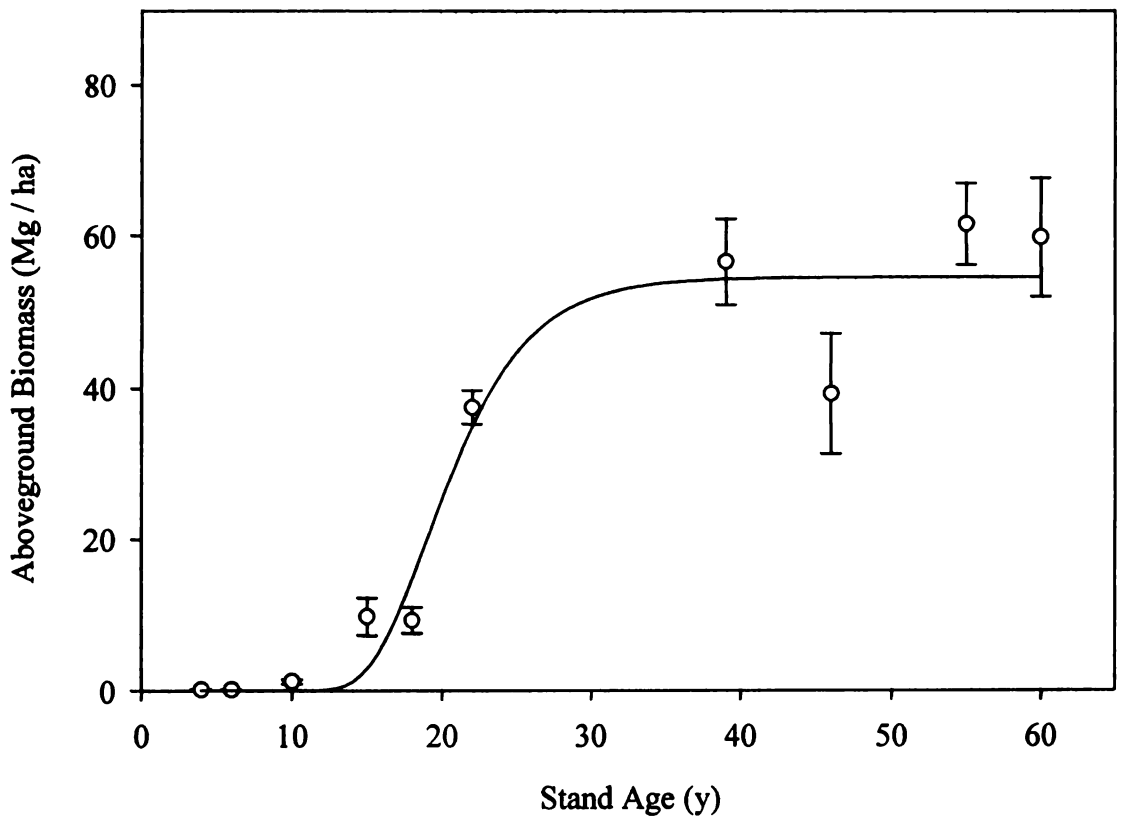


Figure 4.8: Estimated recovery of live jack pine aboveground biomass following wildfire as a function of stand age; data are stand means ± 1 SE. Line represents the following best fit function: Biomass = 54.57 * (1 – exp(-0.27 * age)) ^ 163.38 (R ^ 2 = 0.936).



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CHAPTER 5

COMPOSITIONAL SHIFTS OF ECTOMYCORRHIZAL COMMUNITIES WITH STAND AGE IN POST-WILDFIRE JACK PINE FORESTS

ABSTRACT

Studies of successional changes in forest community composition have almost exclusively focused on aboveground biota; yet, we increasingly realize that above- and belowground communities are intimately linked. I investigated changes in the belowground community composition of ectomycorrhizal fungi (EMF) along a six-site chronosequence of jack-pine dominated stands (5, 11, 19, 23, 47 and 56 y post-wildfire). In each site, I collected 20 soil cores, one every 4 m along four 80-100 m transects, for a total of 80 samples per site. In each sample, a mycorrhizal root-tip was randomly selected. Fungal isolates were identified by extracting, amplifying and sequencing the internal transcribed spacer (ITS) regions of the rDNA. I grouped sequences (≥400 base pairs) by family or genus according to the 10 top-scoring BLAST search matches in GenBank (≥95% base-pair similarity). Sequences were edited in Seqman 7.0, aligned using ClustalW in Mega 4.0, and further categorized into individual ITS type groups (≥95% base-pair similarity). Employing the ITS groupings, I compared community diversity between sites via the Simpson's diversity indices. At the more conservative family/genus level, community composition was evaluated using principal components analysis (PCA), correspondence analysis (CA) and non-metric multi-dimensional scaling (NMDS). Overall, taxonomic richness and evenness were lowest in the 5 y-old stand, and EMF community composition clearly shifted in all orientations between the youngest (5 and 11 y) and older sites (19-56 y). This compositional shift was primarily driven by

higher relative abundances of *Rhizopogon* and *Thelephora* taxa in sites age 5 and 11, and increases in *Cortinarius* later in stand development. It has been previously hypothesized that declines in mineral nitrogen (N) and increases in soluble organic N may drive changes in EMF taxa over stand development. Therefore, the taxonomic shift observed here was compared to standing pools of mineral and organic N. I found no correlation between the change in taxa and mineral N availability, yet a strong negative correlation for organic N and this taxonomic shift ($R^2 = 0.661$, $P < 0.05$). Overall, the results of this study show that EMF community composition can shift dramatically within the first decades of forest stand development. Furthermore, my results indicate that mineral N dynamics are not a primary driver of EMF taxa change across stand ages, but I cannot rule out the potential of organic N contributing to the shift in EMF taxa observed here.

INTRODUCTION

Studies of changes in community composition over forest development have almost exclusively focused on aboveground biota; yet, ecologists increasingly realize that above- and belowground communities are intimately linked (Wardle et al. 2004). Despite this realization, we have a poor understanding of how soil microbial communities may change with forest stand age and the driving factors behind any potential shifts in belowground composition. Given their intimate symbiosis with plant roots, compositional shifts in the soil mycorrhizal community may have important implications for ecosystem structure and functioning (van der Heijden et al. 1998). Many boreal and cold-temperate mycorrhizal associations involve ectomycorrhizal fungi (EMF) that form a characteristic sheath or mantle on colonized plant root-tips (Peterson et al. 2004). These EMF communities are critical for plant nutrient acquisition, yet studies have shown variation between EMF taxa in the ability to access nutrients for their plant hosts (Abuzinadah and Read 1986; Finlay et al. 1992; Lilleskov et al. 2002a). Hence, shifts in the ectomycorrhizal community over forest development may have important implications for plant nutrition.

Prior studies have generally observed changes in EMF community composition over forest development, yet they have been typically restricted to visual-based assays on a limited number of samples. In a sporocarp survey in lodgepole pine and Sitka spruce chronosequences, Dighton et al. (1986) observed a shift in relative species abundance between younger and older stands. Likewise, Visser (1995), employing both sporocarp surveys and visual inspection of colonized root-tips, found an overall shift in EMF species abundance with stand age in Canadian jack pine forests. Generally, these visual-

based studies have observed certain fungal genera, such as *Laccaria, Rhizopogon, Thelephora*, to be most prevalent in young stands, while finding increases in such genera as *Cortinarius*, *Piloderma* and *Russula* later in stand development (Dighton et al. 1986; Visser 1995; reviewed in Deacon and Fleming 1992). Despite these findings, sporocarp surveys often poorly reflect the compositional makeup of the underground EMF community as a whole (Gardes and Bruns 1996; Dahlberg et al. 1997; Kårén and Nylund 1997; Lilleskov et al. 2002b). Considerable effort has been made to identify mycorrhizal taxa on colonized root-tips based on visual morphology. However, relatively few species are described in this manner and sample sizes are typically small, given the time and effort imposed by this procedure. The advent of molecular methods has provided a powerful means by which the underground EMF community can be characterized in finer detail using many more samples than previously possible (Horton and Bruns 2001).

In addition to the uncertainty surrounding visual-based methods, the underlying mechanisms behind any potential shift in fungal taxa over stand development remain unclear. Deacon and Fleming (1992) suggested that differences in life-history strategies and photosynthate requirements between fungal taxa likely explain changes in EMF community composition with stand age. For example, "ruderal" taxa, such as *Rhizopogon* and *Thelephora* species, are generally prolific fruiters and able to quickly colonize root-tips from spores (Colpaert 1999; Molina et al. 1999), characteristics that undoubtedly confer an advantage in young, disturbed stands. However, this fails to explain why these fungi may be subsequently replaced by other EMF species in older stands. It has been argued that this model ignores the potential influence of changes in

soil characteristics accompanying stand development (Jumpponen and Egerton-Warburton 2005).

One such characteristic that changes profoundly with stand age, soil nitrogen (N) availability, has been shown to be a critical modifier of the EMF community (Taylor et al. 2000; Lilleskov et al. 2002). Mineral N, ammonium (NH_4^+) and nitrate (NO_3^-), availability is generally elevated in young, recently disturbed stands (reviewed in Wan et al. 2001), while organic forms of N, such as amino acids, proteins and chitin, are thought to predominate later in stand development with the accrual of organic horizons (Read 1991). Many EMF species can access organic forms of N, such as protein N, whereas others cannot—or have a limited ability to do so—relying instead on mineral forms of N (Abuzinadah and Read 1986; Finlay et al. 1992; Smith and Read 1997; Lilleskov et al. 2002a). It has been hypothesized that declines in mineral N and concomitant increases in soluble peptides and proteins may drive changes in EMF taxa over stand development (Abuzinadah and Read 1986; Finlay et al. 1992). Regulatory mechanisms within host plants, such as higher mortality rates of root-tips colonized by poor vs. good mutualists, could result in a shift from fungal taxa with a high affinity for mineral N early in stand development to those able to access more complex, organic forms of N in older stands (Hoeksema and Kummel 2003).

The jack pine (*Pinus banksiana*) forests of northern Lower Michigan provide an ideal setting to examine potential changes in EMF community composition with stand age and possible underlying factors. These forests exhibit frequent disturbance with minimal confounding variation in climate, topography, soils or vegetation, facilitating comparison of multiple stand ages within a small geographical area. Most importantly

for the following study, this ecosystem allows plant-host type, in this case jack pine, to be held constant. Additionally, this ecosystem exhibits a "U-shaped" pattern in soil mineral N availability with stand development: high mineral N availability in the first few years following a disturbance event, followed by a sharp decline, and, finally, a slow upwards trajectory from this "trough" to levels comparable, or exceeding, those within the first few years following the disturbance (Yermakov and Rothstein 2006; Chapter 4). This experimental system allows the comparison of N availability vs. other mechanisms as drivers of EMF community change.

I hypothesized that overall EMF community composition in these jack pine stands would shift dramatically with stand age; yet, I also hypothesized that the abundance of "young-site" taxa would increase again in the oldest stands in response to higher mineral N availability. To test these hypotheses, I compared EMF community composition, using molecular methods, along a six-site chronosequence of Michigan jack pine stands of wildfire-origin (ages 5, 11, 19, 23, 47 and 56 y post-fire). As part of a larger chronosequence study conducted a year earlier, these sites were measured for standing pools of mineral N, net N mineralization, total soluble N, soluble organic N, and total N (Chapter 4). Using these pre-existing data, I compared mineral N and organic N availability with EMF community composition.

METHODS

Study sites and sampling

My chronosequence study sites were located within the Highplains district of northern Lower Michigan, USA (44°30′N, 84°30′W), a region characterized by a harsh, continental climate with a short growing season (82 days) and cold temperatures (mean annual temperature = 6.3°C) (Albert et al. 1986). The landscape of this area is dominated by broad, outwash plains generally consisting of acidic, excessively drained, poorly developed sands of the Grayling series (mixed, frigid Typic Udipsamments) (Albert et al. 1986; Werlein 1998). The outwash plains are inhabited by jack pine, and the combination of dry conditions, flat topography and highly flammable vegetation has resulted in a frequent fire return interval of approximately 59 y (Cleland et al. 2004). Due to frequent disturbance (a short fire-return interval and extensive harvesting), the vast majority of jack pine acreage is composed of stands 70 y of age or less (Yermakov and Rothstein 2006).

From a larger chronosequence study, I selected 6 jack-pine dominated, flat, sandy outwash sites, in which the overstory was destroyed by wildfire of at least 80 ha in size (for complete site description and location see Chapter 4). At the time of this study, the six chronosequence sites were 5, 11, 19, 23, 47 and 56 y-old (Table 5.1). These sites had been characterized a year prior (2005) to the present study for standing pools of mineral N, net N mineralization, soluble organic N, and total N (Table 5.1), and the mineral N dynamics of these 6 sites followed the "U-shaped" pattern in soil mineral N availability evidenced by the chronosequence as a whole (Fig. 5.1; Chapter 4). At each site, I identified an 80 x 100 m sampling area of uniform terrain, surrounded by at least a 20 m

buffer from any site boundary. In September 2006, I collected 80 soil cores (4 cm diameter x 10 cm below the O horizon; starting at the Oe horizon) per site, along 4 transects (80-100 m in length). The cores were collected at the drip-line of jack-pine seedlings/trees, spaced at least 4 m apart. The 4 m spacing was selected in order to better sample the diversity of each stand, since many EMF taxa exhibit patchiness at 3 m or less (Lilleskov et al. 2004). In the youngest sites, sampling near any few remaining live trees from the preceding stand was specifically avoided. All samples were transported to the laboratory on ice, storing them for less than 7 d at 4°C prior to processing. Using tap water, the roots in each core were washed over a 1-mm sieve, placed in Whirlpaks® and frozen at -20°C for later analysis.

Molecular identification of EMF taxa

To identify EMF isolates, each frozen root sample was slowly thawed by first placing the sample in 50% ethanol and water for 1 h, transferring to 75% ethanol for 1 h, and then finally transferring to 100% ethanol prior to sampling (Lilleskov et al. 2002b). A random subsample was spread out on a 1 cm grided Petri dish, and starting in the upper right-hand corner, each grid was systematically viewed under a stereomicroscope, with the ectomycorrhiza (EM) closest to each grid center selected. In this manner, a primary EM and 5-to-6 backup tips were chosen, while clearly desiccated root tips were purposefully avoided. The backup tips were stored in 100% ethanol at -20°C.

Deoxyribonucleic acid (DNA) was extracted from each primary tip, using a method modified from Avis et al. (2003). I used a plant DNA extraction kit (REDExtract-N-Amp Plant PCR Kit, Sigma-Aldrich, Inc., St. Louis, Missouri, USA),

following the manufacturer's instructions, except for the following: the EM was removed from the 100% ethanol and blotted dry on a clean Kimwipe® prior to placing in extraction solution; and 20 µl each of extraction and dilution solutions were used. The internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) were amplified by polymerase chain reaction (PCR) using the PCR mix supplied with the kit, and the primer pair, ITS1-F and ITS4 (White et al. 1990). Amplified DNA was purified using a Qiagen QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA, USA), and submitted to Michigan State University's Research Technology Support Facility (E. Lansing, MI, USA) with the primer ITS5 for a forward sequencing reaction.

Sequences (≥400 base pairs) were grouped into genera or family according to the consensus of the 10 top-scoring BLAST search matches in GenBank (≥ 95% base-pair similarity), and each sequence was edited with SeqMan 7.0 (DNAStar, Inc., Madison, WI, USA). I aligned sequences and compared base-pair similarities using ClustalW in MEGA 4.0 (Tamura et al. 2007). If isolates displayed less than 95% base-pair similarity within a given genera or family, the sequences were further categorized into individual ITS type groups. Exceptions were made for *Cortinarius*, *Lactarius*, *Russula*, *Thelephoraceae* and *Tricholoma* isolates, which were grouped at ≥ 90% base-pair similarity, given the greater variation in the ITS region exhibited by these isolates. One representative isolate per family, genus or ITS type was submitted to Genbank (Table 5.2). In some cases, sequences failed to match with any identified taxa. These "unknown" sequences were then aligned and compared by base-pair similarities. The unknown sequences exhibiting ≥ 95% base-pair similarity were grouped into the same ITS group type.

Colonization rate counts

To assess the prevalence of ectomycorrhizal fungi at each stand age, colonization rate counts were conducted across all six sites. The sites were re-sampled in October 2007, and five samples per transect were randomly chosen for colonization rate assays, yielding a total of 20 samples per site. From each core, a random subsample of fine roots ($\leq$ 2 mm in diameter) were spread out over the 1 cm grided Petri dish, and mycorrhizas were counted by the line intersection method under a stereomicroscope (Reich and Barnard 1984). A minimum of 100 counts were made, and percentage mycorrhizal colonization was expressed as lines intersected by EM divided by the total number of intersections.

Data analysis

To examine how much of the EMF community was captured by my sampling intensity, I used the software EstimateS 7.52 to compute taxa (at the family/genus level) accumulation curves and first-order Jackknife richness estimators (100 randomizations without sample replacement) for each site. Numbers of taxa observed were divided by Jackknife richness estimators and multiplied by 100 to yield a percent estimate of the community captured.

Family or genus level categories, rather than ITS groupings, were used to evaluate potential compositional shifts in the EMF community to reduce the potential for un-sampled percentages of the community undermining the comparison. I compared EMF communities between sites by first conducting a principal components analysis (PCA) of the unstandardized count data. Since PCA uses Euclidean distances which can inflate site

similarities based on taxa absence (Legendre and Legendre 1998), I also conducted a correspondence analysis (CA) on the unstandardized data, and a non-metric multi-dimensional scaling (NMDS) using Bray-Curtis distances of the original data. All ordinations were conducted in R 2.6.1. Finally, I used the less conservative ITS groupings to calculate Simpson's diversity and evenness indices (Magurran 2004).

I used a combination of linear and polynomial regressions to evaluate potential changes in colonization rates with stand age. I used conceptual strength and a lack-of-fit test of the mean square error values to select between significant reduced and full models. For the sake of continuity throughout the text and figures, all sites are referred to by age when sampled for EMF community composition, even though the sites were one year older when colonization rate counts were conducted.

RESULTS

Chronosequence characteristics

The validity of the chronosequence approach requires that all factors, outside of those influenced by stand age, be held as constant as possible. Overall, the sites exhibited very similar vegetation and soil characteristics (Table 5.1). Jack pine was the dominant overstory vegetation across all stands, ranging from 94% basal area in the 47 y-old stand to 100% in both the 11 and 19 y-old stands. Similarly, jack pine accounted for 93% of the total number of stems in the 5 y-old stand. Soils in all sites were acidic, extremely well-drained, loamy-sand to sand, with site age 11 containing the finest-textured soils amongst the sites.

EMF community composition

I successfully identified EMF isolates in approximately 71% of my samples, with a range of 69% in the 23, 47 and 56 y-old sites to 74% in the 5 y-old site. Taxa accumulation curves (Fig. 5.2) in all sites deviated from a linear slope, and, in the case of the 19 y-old site, approached an asymptote. Numbers of observed taxa expressed as a percentage of jackknife values (data not shown) suggested that my sampling effort captured 77% of the family and genera groups present across all sites, with a range of 74% to 82% in the 5 and 11 y-old sites, respectively.

In the PCA, the 5 and 11 y-old sites clearly separated from the cluster of 19 to 56 y-old sites along the first PC axis, which explained 55.4% of the data (Fig. 5.3). The genera *Rhizopogon*, *Laccaria* and *Thelephora* received the highest negative loadings along axis 1 (|>0.8|), indicating their importance in the EMF community of the 5 and 11

y-old sites. However, despite the heavy loading, it should be noted that *Laccaria*, with only 5 isolates across all sites, was a relatively small component of the EMF community in the youngest sites compared to *Rhizopogon* or *Thelephora* (Fig. 5.4). In contrast, *Cortinarius*, with 47 isolates across the chronosequence and a high positive loading on axis 1, was a particularly important component of the EMF community in the four oldest sites. The 5 and 11 y-old sites separated along the second PC axis, explaining 24.6% of the data, while the four oldest sites remained clustered. According to the PC 2 loadings, the difference between the 5 and 11 y-old sites was primarily driven by *Pseudotomentella, Phialocephala* and *Tuber* (Fig. 5.3), which had 2, 2 and 3 isolates in the 11 y-old site, respectively, with no identified isolates in the 5 y-old site.

In addition to the taxonomic groups receiving high loadings in the PCA, shifts occurred in the relative abundance of many other taxa with stand age (Fig. 5.5). For example, *Suillus* represented ca. 19% of the isolates in the 5 y-old site, while only 1.8 and 0% of the isolates in the 47 and 56 y-old sites, respectively. Conversely, *Russula* increased in relative abundance from ca. 3% in the 5 y-old site to almost 15% in site aged 56 y. In the CA, I again observed a separation of the 5 and 11 y-old sites from the cluster of the four oldest sites, with many accompanying taxonomic changes (Fig. 5.6). Since a substantial percentage of the data (19%) remained unexplained by PCA axes 1 and 2, and, as mentioned above, PCA can inflate similarities based on taxa absence, the NMDS was used to reduce all count data into a 2-dimension plot (Fig. 5.7; Stress = 3.20 x 10 ^ -14). The NMDS yielded a similar orientation to the PCA, with a few notable exceptions: 1) the 19 and 23 y-old sites separated slightly from the 47 and 56 y-old sites

along the second axis in the NMDS; and 2), along the same axis, the 5 and 11 y-sites clustered closer than in the PCA orientation (Fig. 5.7).

To assess the potential of mineral vs. soluble organic N availability accounting for this observed shift in EMF taxa over stand development, I regressed the relative abundance of negative PC 1 loadings (hence, "young" site taxa) for each site against standing pools of mineral N (Fig. 5.8A), soluble organic N (Fig. 5.8B), and mineral vs. soluble organic N (Fig. 5.8C). There was no relationship between either mineral N or mineral vs. soluble organic N and the relative abundance of taxa receiving negative PC 1 loadings. However, there was a strong, negative linear relationship between soluble organic N and the taxa with negative PC 1 loadings ($R^2 = 0.661$, $P < 0.05$).

EMF community diversity and colonization rates

According to the Simpson's indices, taxonomic richness and evenness were lowest in the 5 y-old stand, intermediate in the 19, 23 and 47 y-old sites, and highest in the 11 and 56 y-old sites (Fig. 5.9). I selected a quadratic function (R^2 = 0.200, $P <$ 0.0001) to describe colonization rates across the chronosequence (Fig. 5.10). A cubic model provided a significantly better fit; yet, given the number of sites, I ultimately selected the quadratic model because it provided a more conservative representation of the data. Fungal colonization rates generally increased from sites age 5 to 23, and then moderately declined to an intermediate level in the 56 y-old site.

DISCUSSION

EMF community composition

The results of this present study clearly demonstrate that the EMF community can change in composition with forest stand age. Notably, these changes occurred in the absence of any variation in the species of plant-host. The most striking difference was between sites age 5 and 11 and sites age 19 through 56 y-old. In all ordinations, these site groupings separated consistently. As noted by the PC 1 loadings, this change in composition was a shift in relative abundance away from certain genera, namely *Rhizopogon*, *Laccaria* and *Thelephora*, and towards *Cortinarius* (Figs. 5.3 & 5.4). These changes in taxa were accompanied by many others (Figs. 5.5 & 5.6). For example, *Suillus* and a group of specific *Atheliaceae* isolates tended to be found in the two youngest sites, while *Amanita*, *Russula* and *Piloderma* were most prevalent in the remaining older sites.

This overall shift in EMF taxa with stand age observed here largely confirms the results of previous studies, based on visual techniques. Within the first 6 years following the establishment of birch plantations, Mason et al. (1982) found that sporocarps were dominated by *Laccaria* spp., *Lactarius pubescens*, *Inocybe lanuginella* and *Hebeloma crustuliniforme*, while also noting the presence of *Thelephora terrestris*. By year 6 in the same plantations, they reported the first presence of *Cortinarius*, and by year 10, had found species of *Russula* (Mason et al. 1987). Similarly, in two studies conducted in Alberta jack pine forests, Danielson (1984) and Visser (1995) both observed a shift in EMF taxa over stand development. Visser (1995) described higher relative abundances of *Rhizopogon rubescens*, *Suillus brevipes* and *Thelephora terrestris* sporocarps in her 4

y-old site, while finding more *Cortinarius* spp. and *Russula* spp. in the three older sites (41, 65 and 122 y-old). Likewise, she found that jack pine root-tips in the youngest site were dominated by *Suillus brevipes*, while *Russula* spp. was particularly abundant in the older stands. Besides my study, I am aware of only one other study to employ molecular methods to examine changes in EMF communities across multiple forest stand ages. Over four age classes (5-100 y), Twieg et al. (2007) observed a shift in relative abundance of EMF genera in mixed Douglas-fir and paper birch stands in the interior of British Columbia. They found a *Rhizopogon* vinicolor-type to be dominant on 5 y-old Douglas-fir seedlings, while *Russula*, *Piloderma* and *Cortinarius* increased in abundance on Douglas-fir roots in older stands. The results of Tweig et al. (2007) and my study confirm prior visual-based assays in concluding that a shift in EMF taxa can occur with forest stand age. Certain taxa, such as species of *Rhizopogon* and *Thelephora*, are particularly associated with young, disturbed stands, while species of *Amanita*, *Cortinarius*, *Russula* and others are more prevalent in older stands (Fig. 5.6).

Strikingly, the overall shift in taxa observed here occurred quite rapidly in forest stand development, within the first 20 years following disturbance. This relatively rapid change in EMF community may be a function of these short-lived jack pine forests, where net primary productivity is very low to near zero by 50 years post-disturbance (Rothstein et al. 2004). However, Visser (1995) found most changes in community composition between her 4 and 41 y-old sites, with little difference between the 41, 65 and 122 y-old sites. Likewise, Twieg et al. (2007) observed most change in EMF composition prior to stand age 26 and a stabilization of the EMF community by age 65 in their Douglas-fir/paper-birch forests. These results, combined with this present study,

suggest that EMF community composition is likely to shift quite rapidly, perhaps within the first few decades of forest development, while remaining relatively stable afterwards. The first 20 years of stand development in these Michigan jack pine forests coincide with a movement from stand initiation to a stem-exclusion phase, either at the stand or patch-level (Oliver and Larson 1996; Spaulding 2008). Further study is needed to specifically assess whether this shift in taxa does correlate with forest dynamics, namely the end of stand initiation and beginning of canopy closure.

It should be noted that these changes in EMF composition reported here occurred over forest development following severe, stand-destroying wildfire. A lighter, less severe fire that does not consume substantial portions of the forest floor and remove the overstory may, in contrast, leave the pre-fire EMF community relatively intact (Jonsson et al. 1999; Dahlberg et al. 2001). Dahlberg et al. (2001) found a severe-burn treatment—achieved through drying the forest floor layer and supplementing fuel loads—killed all mycorrhizae in the organic and upper mineral soil horizons, whereas a low-severity treatment allowed some mycorrhizal fungi to persist in the mineral soil. In low severity fires, the survival of the overstory trees ensures the allocation of substantial photosynthate belowground and likely continuation of mycelial networks. In such a case, the pre- and post-disturbance EMF communities are more likely to be similar, and substantial shifts in the fungal community may not occur.

Potential underlying mechanisms

The findings here of a shift in EMF community composition over stand development raise the question: what are the underlying mechanisms behind these

changes in EMF community composition? Logically, the mechanisms are likely either one, or a combination, of the following factors: 1) changes in the soil environment; 2) the variation in life history strategies between EMF taxa; and/or 3) shifts in the amount carbon (C) allocated belowground.

Changes in the soil environment with stand age may favor certain fungi over others, ultimately structuring the EMF community. Ectomycorrhizal taxa appear to vary in the ability to access mineral vs. organic N (Abuzinadah and Read 1986; Finlay et al. 1992: Lilleskov et al. 2002a), and it has been hypothesized that shifts in EMF composition may be driven by declines in mineral N and increases in organic N over stand development (Abuzinadah and Read 1986; Finlay et al. 1992). Finlay et al. (1992) explicitly tested the N uptake capabilities of early vs. late successional EMF species. In general, they found that EMF species at late successional stages could better acquire amino acid and protein N, while fungi from younger stands were more reliant on mineral N. However, they reported notable exceptions, such as protein N acquisition by *Hebelome crustuliniforme* and *Thelephora terrestris*, two classic "early-stage" fungi.

Contrary to my original hypothesis, I found no relationship between the shift in EMF taxa observed and mineral N availability, either in absolute terms or in relationship to organic N (Fig. 5.8A & C). In these jack pine systems, mineral N availability increases sharply late in stand development (Fig. 5.1), and so I anticipated a "re-emergence" of some young site taxa in the oldest stands. In such a case, site age 56, in particular, with its high mineral N availability, would have been oriented between the 5 and 11 y-old and the other older sites along the PC 1, CA 1 or NMDS 1 axes. Instead, in all ordinations, the four oldest sites remained clustered. Similarly, site age 11 did not

group with the older stands, despite the lowest mineral N standing pools and net N mineralization rates across the entire chronosequence (Fig. 5.1). This site also exhibited a low soluble mineral N-to-organic N ratio (Table 5.1), indicating the predominance of organic N in this site. If mineral vs. organic N availability was the sole driver of EMF composition, the shift to older site EMF taxa would have likely occurred between the 5 and 11 y-old sites. These results suggest that mineral N dynamics alone or in relation to organic N do not drive changes in EMF composition.

In contrast to mineral N, my finding of strong, negative relationship between young site taxa and organic N ($R^2 = 0.661$, $P < 0.05$) may indicate that changes in organic N by itself may favor certain EMF taxa over others (Fig. 5.8B). However, this finding should be viewed cautiously, since, in contrast to mineral forms of N, organic N increases over the entire chronosequence (Table 5.1). Many other factors that may influence EMF community composition (e.g. belowground C allocation) also change along this same stand-age sequence, raising the potential for conflation of these results by a co-variable. If changes in the form and amount of N availability did indeed favor certain fungi over others, it would seem likely that the relative proportion of mineral N vs. organic N would be more important than one or the other by itself. The lack of a relationship between the shift in EMF taxa and the ratio of mineral-to-organic N suggests that N may not structure the EMF community. However, given the results of this present study, I ultimately cannot rule out the potential for available organic N alone, not in relationship to mineral N, to alter EMF community composition.

Variation in the life history strategies of EMF taxa and C allocation belowground offer alternative explanations for the overall shift in EMF taxa observed here. According

to the PC 1 loadings, *Rhizopogon* and *Thelephora* taxa were distinguishing components of the EMF community in the younger (5 and 11 y-old) vs. the older (19-56 y-old) sites. Species of these genera are generally considered "ruderal" taxa, able to quickly respond to increases in resource availability (Dighton and Mason 1985; Taylor et al. 2000). *Rhizopogon* species can be found in younger and older forests, but they are particularly associated with young seedlings on disturbed sites (Molina et al. 1999). They are generally prolific fruiters and quickly colonize root tips via spores, which can remain viable in the soil, surviving extended periods of desiccation (Molina et al. 1999; Ashkannejhad and Horton 2005). The *Thelephora* isolates encountered in this study matched most closely in Genbank with *Thelephora terrestris*. This species is often found in greenhouses, nurseries or reforested sites, due to its ability to disperse and quickly colonize a plant host from spores (Colpaert 1999). These fungi appear better able to exploit the open niche space of a disturbed stand, becoming more abundant for a period of time.

The question then arises: why are these disturbance-related fungi eventually replaced by other taxa? Belowground C allocation via photosynthate undoubtedly increases over stand development, likely making an older stand a more resource rich environment for mycorrhizal fungi. It is generally thought that the importance of competition, as an influence on community composition, increases under high resource availability (Grime 1977). Thus the shift in EMF taxa as shown in this study may result from more competitive fungi replacing ruderal taxa (Grime 1977). Fungi in older stands may be more competitive because they confer an advantage to their plant host. Other soil

characteristics, in addition to N, can change over stand development, possibly favoring taxa prevalent in older stands.

Alternatively, the taxa observed in the older sites, such as *Cortinarius* and *Russula*, generally spread from existing mycelial networks connecting multiple individual trees (Deacon and Fleming 1992). Once established, this mycelial network could yield the resource base for colonizing new root-tips, providing a competitive advantage to these fungal species (Deacon and Fleming 1992). Perhaps, the most compelling piece of evidence comes from a series of trenching experiments, where seedlings planted beneath established canopies were colonized by taxa typical of older stands (Fleming 1983, 1984; reviewed in Deacon and Fleming 1992). But, when they were surrounded by a trench, precluding the spread of mycelial networks, the seedlings were colonized by taxa more prevalent in younger stands. I suggest further research should be directed towards mycelial networks as the mechanism for a shift in EMF taxa with stand age.

Study limitations

The results of this study should be cautiously interpreted as a change in the dominant EMF taxa over different stand ages, since, as the species accumulation curves and jackknife estimators suggest, my sampling failed to capture the entire community. Even with grouping at the family/genus level, this is unsurprising, given other studies have found that EMF communities generally exhibit a left-hand skewed distribution in relative abundance: a community dominated by a few taxa with "long tail" of relatively rare isolates (Taylor 2002). This limits the comparison of relatively rare taxa across

stands, but is unlikely to affect the overall shifts of the most common or dominant taxa observed here.

CONCLUSIONS

My results clearly demonstrate that the dominant EMF community can change in composition through forest development. In particular, I observed higher relative abundances of *Rhizopogon* and *Thelephora* species in the 5 and 11 y-old sites, while finding greater abundance of other taxa, like *Cortinarius*, *Russula* and *Piloderma*, in the mid-aged and older sites. This shift in taxa occurred within the first 20 y of stand development in these jack pine forests. I found no correlation between this overall change in EMF community composition and soluble mineral vs. organic N availability. Rather, I found a strong, negative correlation between EMF community composition and soluble organic N by itself. Thus, though my results suggest that mineral N dynamics are not a driving factor, there is remains a potential for organic N alone to alter EMF community composition with stand age. Overall, given the apparent variation of EMF taxa in their ability to acquire nutrients, such as N, for their plant hosts, the change in community composition shown here may have implications for plant nutrition over stand development.

ACKNOWLEDGEMENTS

Personnel of the USDA-Forest Service and the Michigan Department of Natural Resources provided access to field sites and logistical help. I particularly thank the late Tom Whittam and his lab members, most notably Cody Springman and Seth Walk, for their generosity in assistance and advice. Annette Kretzer and David Burke provided much needed lab support. Finally, I thank M. Cook, E. Grman, R. Fanelli, J. Kunkle, N. Millar, E. Scott, G. Smith, S. Tourtellot, and many others for field and lab assistance. This project was supported by funding from the Michigan Botanical Society's Hanes Fund, Michigan State University's Plant Science Initiative and Michigan State University's Dissertation Completion Fellowship.

Table 5.1: Age, plant community, soil characteristics and nitrogen for chronosequence sites.

Stand Age (y)[a]	**5**	**11**	**19**	**23**	**47**	**56**
Plant community (%)[b]						
Jack pine (*P. banksiana*)	93	100	100	98	94	97
Red pine (*P. resinosa*)	-	-	-	-	6	-
Northern pin oak (*Q. ellipsoidalis*)	7	-	-	2	-	3
Median jack pine DBH (cm)[c]	ND	2.5	5.7	7.5	11.5	15.4
Soil characteristics						
pH[d,e]	3.83	3.74	3.70	3.81	3.47	3.46
Silt + clay (%)[f]	10	16	10	12	7	12
Soil nitrogen[d]						
Total N (g m^{-2})	285 (20)	298 (10)	221 (09)	209 (20)	258 (49)	268 (22)
Total soluble N (mg m^{-2})	432.5 (31.0)	327.5 (22.6)	531.0 (37.7)	584.4 (54.2)	763.1 (86.1)	686.1 (76.4)
Mineral N (mg m^{-2})	130.7 (34.1)	40.5 (7.1)	55.2 (9.3)	59.6 (12.2)	107.6 (15.1)	271.3 (27.5)
NH_4^+	109.2 (28.4)	25.6 (5.7)	40.5 (10.7)	51.2 (11.3)	92.4 (9.5)	255.4 (28.0)

Table 5.1 (cont'd):

NO_3^-	21.6 (8.3)	14.9 (5.8)	14.7 (3.7)	8.3 (2.4)	15.2 (6.5)	15.9 (1.7)
Total N mineralization (g N m^{-2})	1.76 (0.46)	0.84 (0.17)	0.94 (0.15)	1.25 (0.40)	1.49 (0.47)	3.04 (0.30)
Soluble mineral N/organic N ratio	0.43	0.14	0.12	0.11	0.16	0.65

Note: ND=No Data; Values in parentheses are SEs (n = 4).

[a]Years since stand-destroying wildfire.

[b]Values expressed in percent number of stems for the 5 y-old site ((no. jack pine stems/no. total stems) x 100); while values expressed in percent basal area for sites age 11-56 y-old ((jack pine basal area/total stand basal area) x 100).

[c]Diameter at breast height (1.3m).

[d]For combined upper 10 cm of Oe/Oa and mineral horizons; measured in 2005 (Chapter 4).

[e]measured in a 1:2 soil-0.01 M $CaCl_2$ slurry.

[f]Fine earth fraction ($\leq$ 2 mm) in upper B horizon.

Table 5.2: Isolate counts and Genbank accession numbers for fungal taxa identified in each chronosequence site. Bolded genera and families denote taxa and total isolate counts per taxa used in the multivariate analyses (PCA, CA and NMDS).

Stand Age (y)[a]	5	11	19	23	47	56	
Family/Genus/ITS Type[b]							GenbankAccession #[c]
Amanita	**1**	**0**	**3**	**3**	**6**	**5**	-
Type 1	1	0	0	2	4	5	FJ715922
Type 2	0	0	1	0	0	0	FJ715923
Type 3	0	0	0	0	2	0	FJ715924
Type 4	0	0	2	1	0	0	FJ715925
Atheliaceae	**1**	**3**	**0**	**0**	**0**	**0**	FJ807983
Boletus	**1**	**0**	**0**	**0**	**0**	**0**	FJ768688
Cantharellaceae	**0**	**0**	**0**	**0**	**0**	**1**	FJ768692
Cenococcum	**0**	**0**	**7**	**2**	**2**	**1**	-
Type 1	0	0	5	1	2	1	FJ768689
Type 2	0	0	2	1	0	0	FJ768690
Clavulina	**0**	**1**	**7**	**6**	**8**	**0**	FJ768691
Clavulinaceae	**0**	**0**	**0**	**0**	**1**	**0**	FJ768693
Coltricia	**1**	**0**	**0**	**0**	**0**	**1**	FJ769546
Cortinarius	**0**	**1**	**16**	**13**	**8**	**9**	-
Type 1	0	0	0	0	1	2	FJ769527
Type 2	0	1	2	0	0	2	FJ769528
Type 3	0	0	0	2	0	0	FJ769529
Type 4	0	0	14	11	7	5	FJ769530
Cortinariaceae	**2**	**0**	**0**	**2**	**1**	**2**	-
Type 1	2	0	0	2	1	1	FJ769547
Type 2	0	0	0	0	0	1	FJ769548
Elaphomyces	**0**	**0**	**0**	**1**	**5**	**5**	FJ769549
Laccaria	**2**	**3**	**0**	**0**	**0**	**0**	FJ787044

Table 5.2 (cont'd):

Lactarius	**6**	**11**	**5**	**5**	**5**	**7**	-
Type 2	3	0	3	0	0	0	FJ769532
Type 3	0	0	0	1	0	1	FJ769533
Type 4	0	1	0	0	0	0	FJ769534
Type 5	0	1	0	0	0	0	FJ769535
Type 6	0	1	0	0	0	0	FJ769536
Type 7	0	1	0	0	0	0	FJ769537
Type 8	0	1	0	1	0	0	FJ769538
Type 9	0	2	0	0	0	0	FJ769539
Type 10	1	0	0	0	0	0	FJ769540
Type 11	0	1	0	0	0	0	FJ769541
Type 12	0	0	0	0	0	1	FJ769542
Type 13	0	0	1	0	0	0	FJ769543
Type 14	0	0	0	1	0	0	FJ769544
Type 15	0	0	0	0	0	1	FJ769545
Phialocephala	**0**	**2**	**0**	**0**	**0**	**0**	FJ803971
Piloderma	**0**	**0**	**0**	**0**	**4**	**0**	FJ803972
Pseudotomentella	**0**	**2**	**0**	**0**	**0**	**1**	FJ803973
Rhizopogon	**4**	**4**	**0**	**1**	**0**	**0**	FJ803974
Russula	**2**	**0**	**5**	**8**	**3**	**8**	-
Type 1	2	0	3	4	1	2	FJ803975
Type 2	0	0	0	0	0	1	FJ803976
Type 3	0	0	0	2	0	3	FJ803977
Type 4	0	0	1	0	1	0	FJ803978
Type 5	0	0	0	1	1	0	FJ803979
Type 6	0	0	1	0	0	0	FJ803980
Type 7	0	0	0	0	0	1	FJ803981
Type 8	0	0	0	1	0	1	FJ803982
Suillus	**11**	**3**	**3**	**0**	**1**	**0**	FJ803983

Table 5.2 (cont'd):

Thelephora	**21**	**4**	**1**	**0**	**0**	**0**	FJ807974
Thelephoraceae	**4**	**20**	**1**	**4**	**3**	**2**	-
Type 1	0	0	0	1	0	0	FJ807966
Type 2	0	8	0	0	0	0	FJ807967
Type 3	0	0	0	0	1	0	FJ807968
Type 4	1	5	0	1	0	1	FJ807969
Type 5	0	2	0	0	1	0	FJ807970
Type 6	1	0	0	0	0	0	FJ807971
Type 7	0	0	1	1	0	0	FJ807972
Type 8	2	5	0	1	1	1	FJ807973
Tomentella	**0**	**1**	**0**	**0**	**1**	**6**	FJ807975
Tricholoma	**1**	**0**	**7**	**7**	**2**	**0**	-
Type 1	0	0	2	4	1	0	FJ807976
Type 2	1	0	2	1	0	0	FJ807977
Type 3	0	0	3	0	0	0	FJ807978
Type 4	0	0	0	1	1	0	FJ807979
Type 5	0	0	0	1	0	0	FJ807980
Tuber	**0**	**3**	**0**	**0**	**0**	**0**	FJ807981
Unknown EMF 1	**0**	**0**	**0**	**0**	**4**	**5**	FJ807982
Unknown EMF 2	**2**	**0**	**0**	**1**	**0**	**2**	FJ807984
Unknown EMF 3	**0**	**0**	**0**	**1**	**0**	**0**	FJ807987

Table 5.2 (cont'd):

Unknown EMF 4	**0**	**0**	**1**	**0**	**1**	**0**	FJ807988
Unknown Fungus 1	**0**	**0**	**1**	**0**	**0**	**0**	FJ807985
Unknown Fungus 2	**0**	**0**	**0**	**1**	**0**	**0**	FJ807986
Total numbers of isolates	59	58	57	55	55	55	

[a] Years since stand-destroying wildfire.

[b] Taxa were grouped according to the consensus of the 10 top-scoring BLAST search matches in Genbank (≥95% base-pair similarity). Sequences were further categorized into ITS type groups if isolates displayed less than 95% base-pair similarity. All isolates within each ITS type group had ≥95% base-pair similarity, except for *Cortinarius*, *Lactarius*, *Russula*, *Thelephoraceae* and *Tricholoma* isolates which were grouped at ≥ 90% base-pair similarity.

[c] One representative isolate per family, genus or ITS type was submitted to Genbank.

Figure 5.1: Mineral N standing pools **(A)** and total N mineralization **(B)** as functions of stand age; data are stand means ±1 SE. Lines represent the following best fit equations: Mineral N = 170.79 - 11.12 * age + 0.22 *age ^ 2, R ^ 2 = 0.722, $P < 0.0001$ **(A)**; Total N mineralization = 2.11 - 0.11 * age + 0.002 * age ^ 2, R ^ 2 = 0.472, $P < 0.0009$ **(B)**.

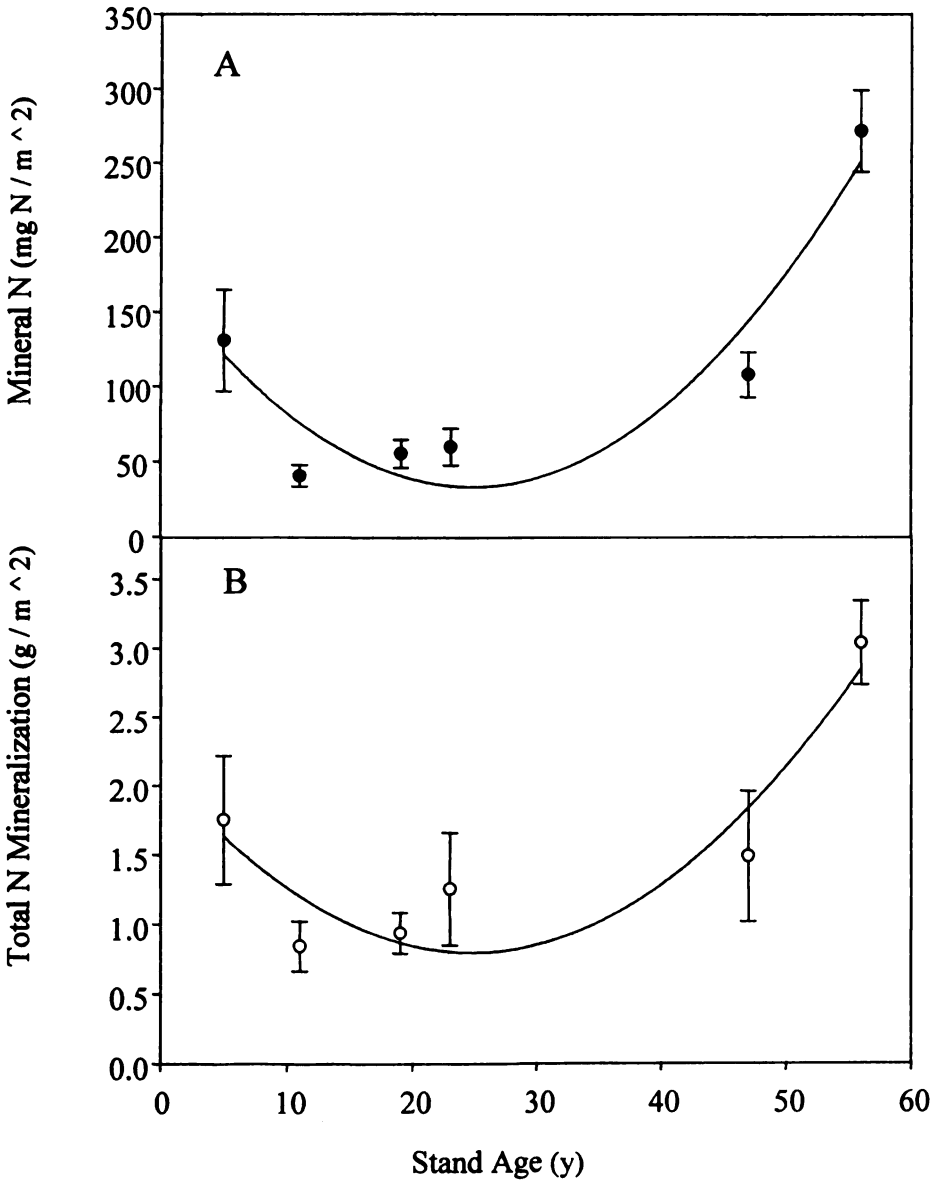


Figure 5.2: Taxa accumulation curves for each site, calculated in EstimateS 7.52 by averaging 100 randomizations without replacement.

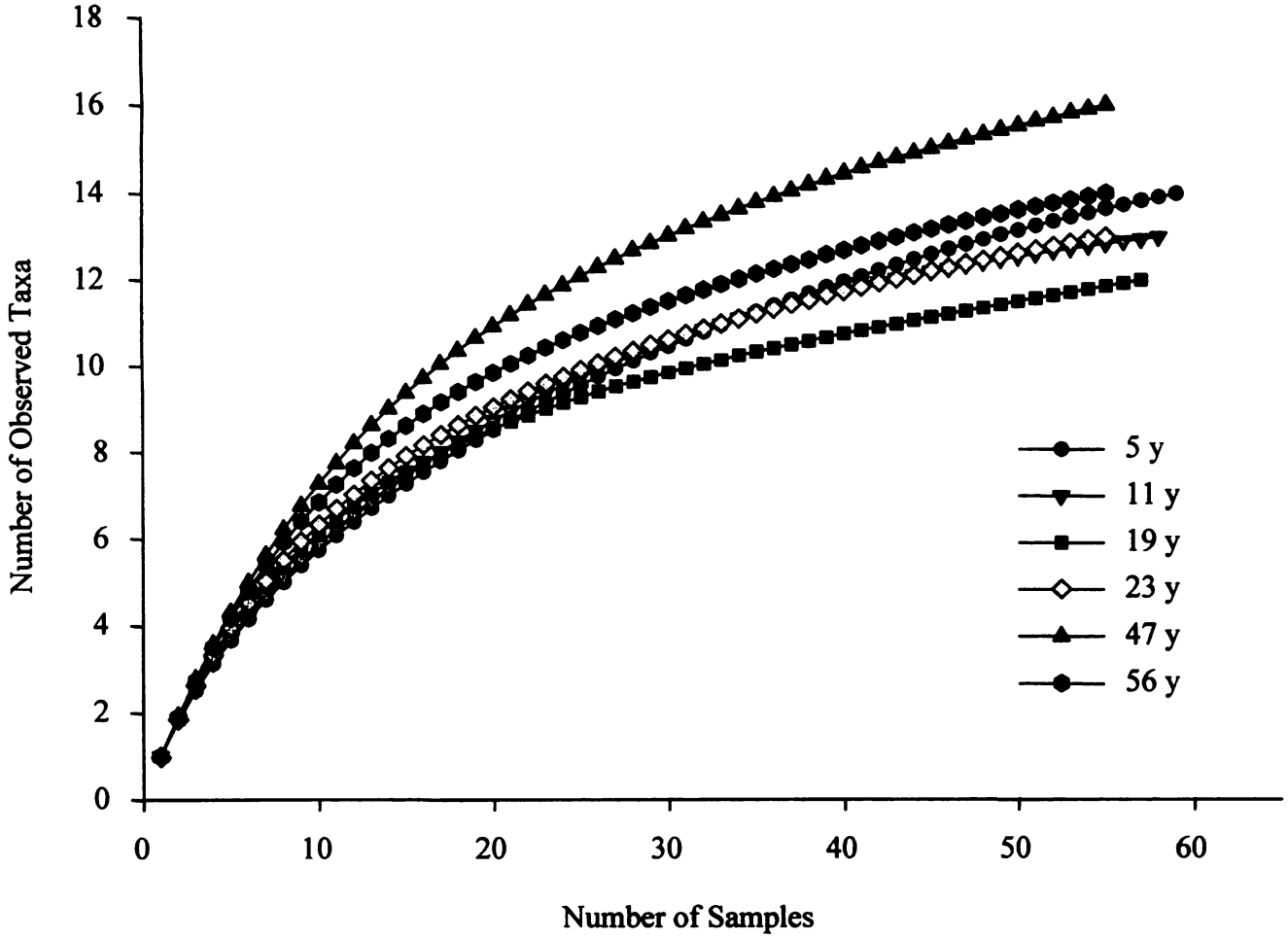
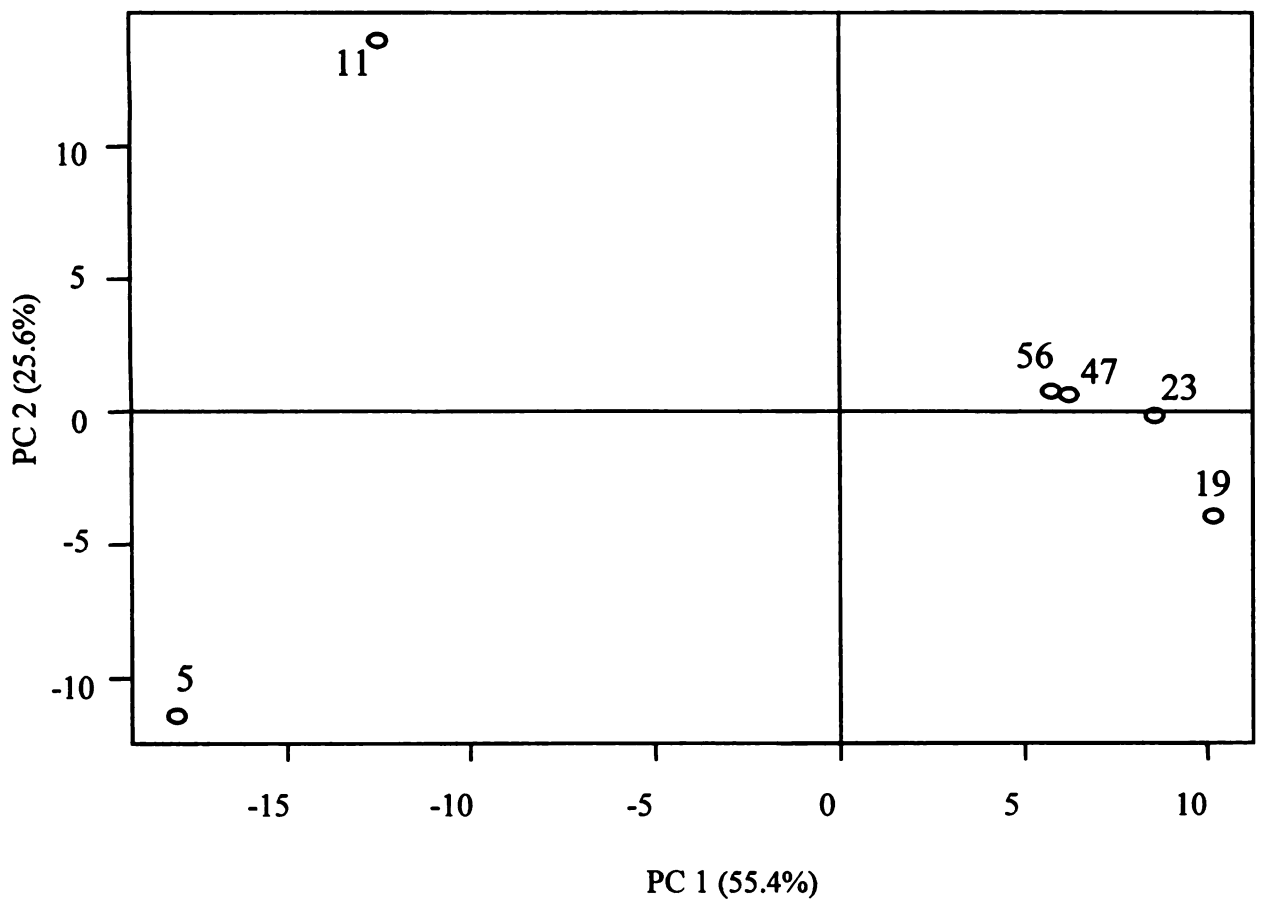


Figure 5.3: PCA biplot and loadings of EMF community composition across the chronosequence sites. Sites are noted by year since fire, and percentage of data explained by each axis is noted in parentheses.



PC 1 Loadings |>0.8|
-*Rhizopogon*: -0.954
-*Cortinarius*: 0.940
-*Laccaria*: -0.918
-*Thelephora*: -0.835

PC 2 Loadings |>0.8|
-*Pseudotomentella*: 0.830
-*Phialocephala*: 0.827
-*Tuber*: 0.827

Figure 5.4: Relative abundance of EMF taxa across the chronosequence sites receiving high loadings along PC axis 1 (|>0.8|).

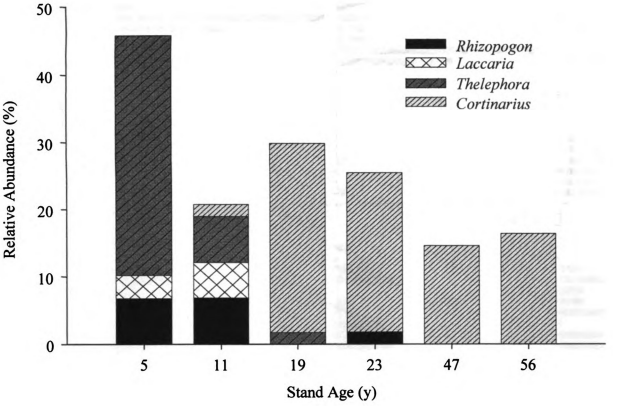


Figure 5.5: Percent relative abundance of EMF taxa in each chronosequence site. Taxa arranged by PC 1 scores (negative to positive) along vertical axis, moving top-to-bottom. Rare taxa (≤ 2 isolates across all sites) were removed for ease of reader interpretation.

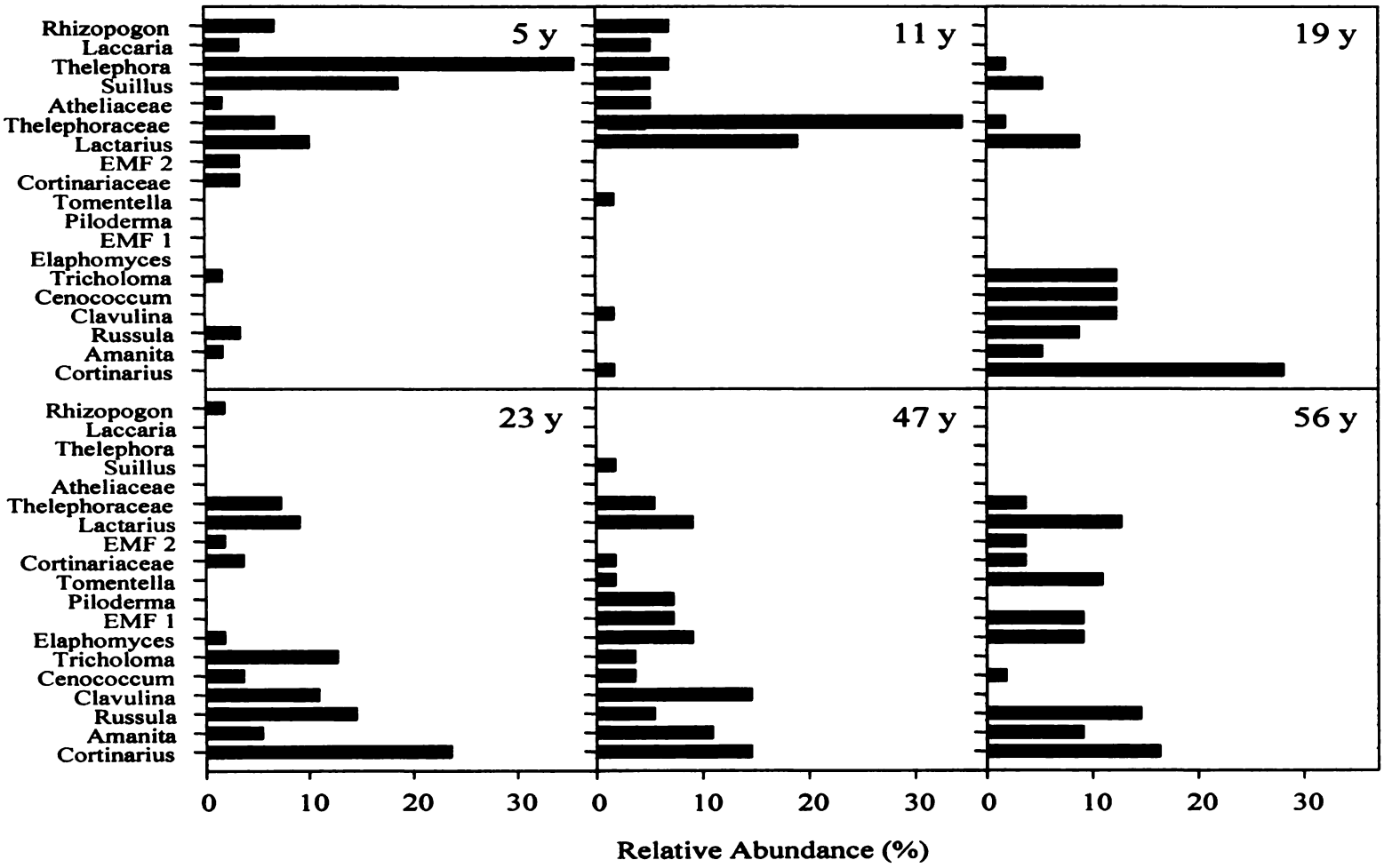


Figure 5.6: Correspondence analysis biplot of EMF community composition across the chronosequence sites, preserving distances between sites. Sites are noted by year since fire, and percentage of data explained by each axis is noted in parentheses. Rare isolates (1 isolate across all sites) were removed following analysis for ease of reader interpretation.

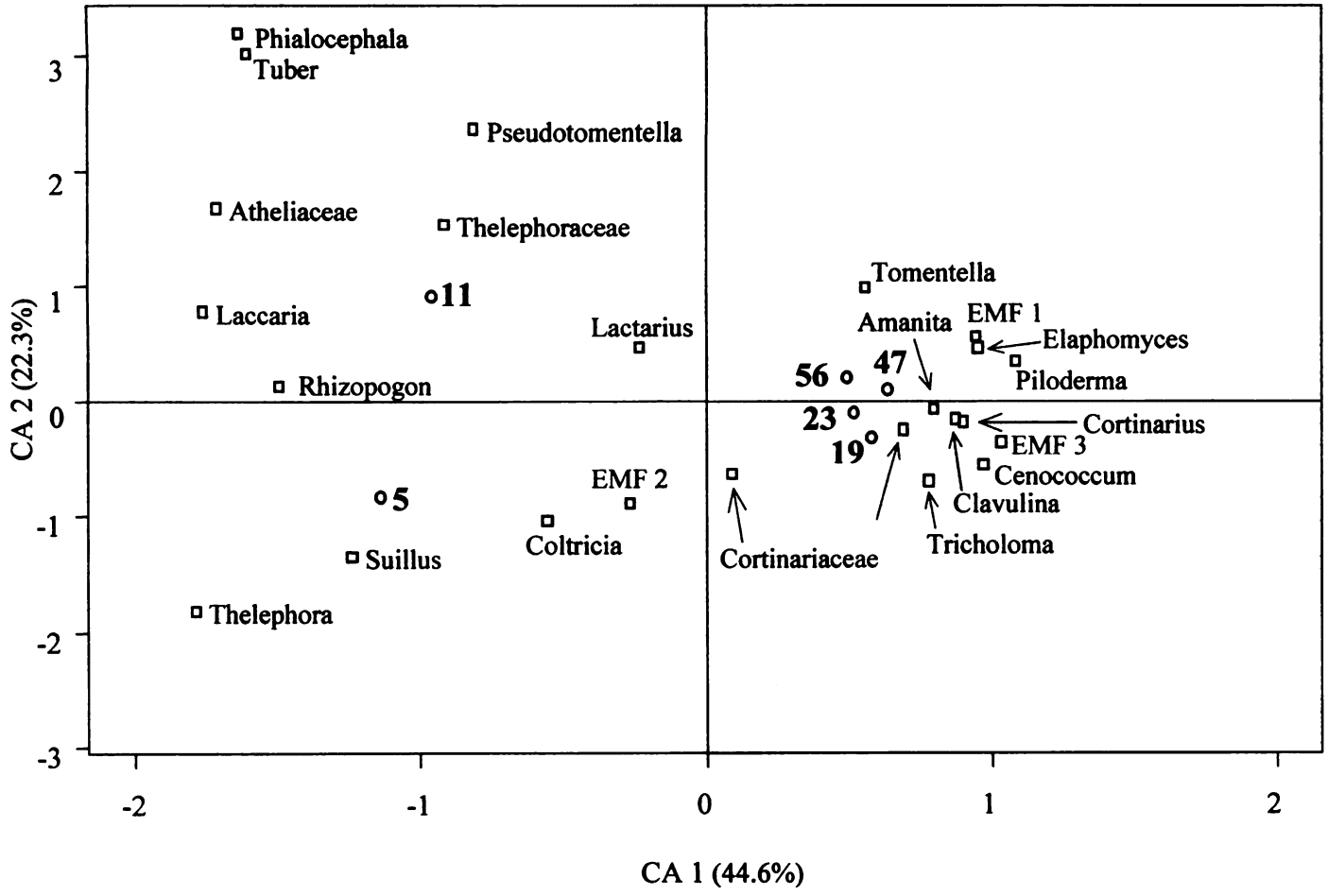


Figure 5.7: NMDS biplot of EMF community composition across the chronosequence sites (Stress = 3.20 x 10 ^ -14). Sites are noted by year since fire.

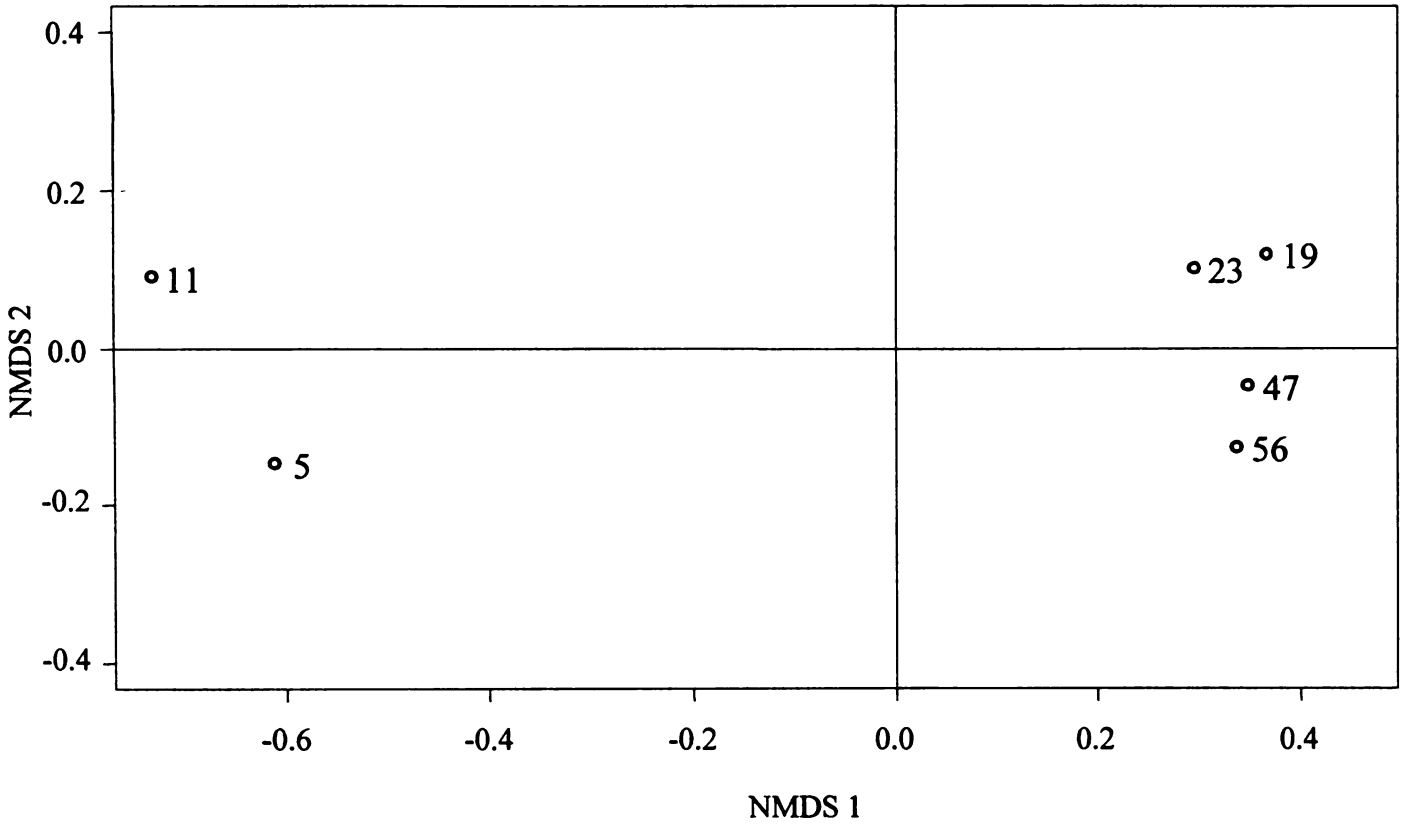


Figure 5.8: Percent relative abundance of taxa receiving negative PC 1 loadings as a function of standing pools of mineral N **(A)**; soluble organic N **(B)**; and soluble mineral vs. organic N ratios **(C)**. There was a significant negative linear relationship for organic N and taxa receiving negative PC 1 loadings (**B**; R ^ 2 = 0.661, $P < 0.05$), while the other relationships were not significant (**A**; R ^ 2 = 0.000, $P = 0.753$) (**C**; R ^ 2 = 0.000, $P = 0.821$).

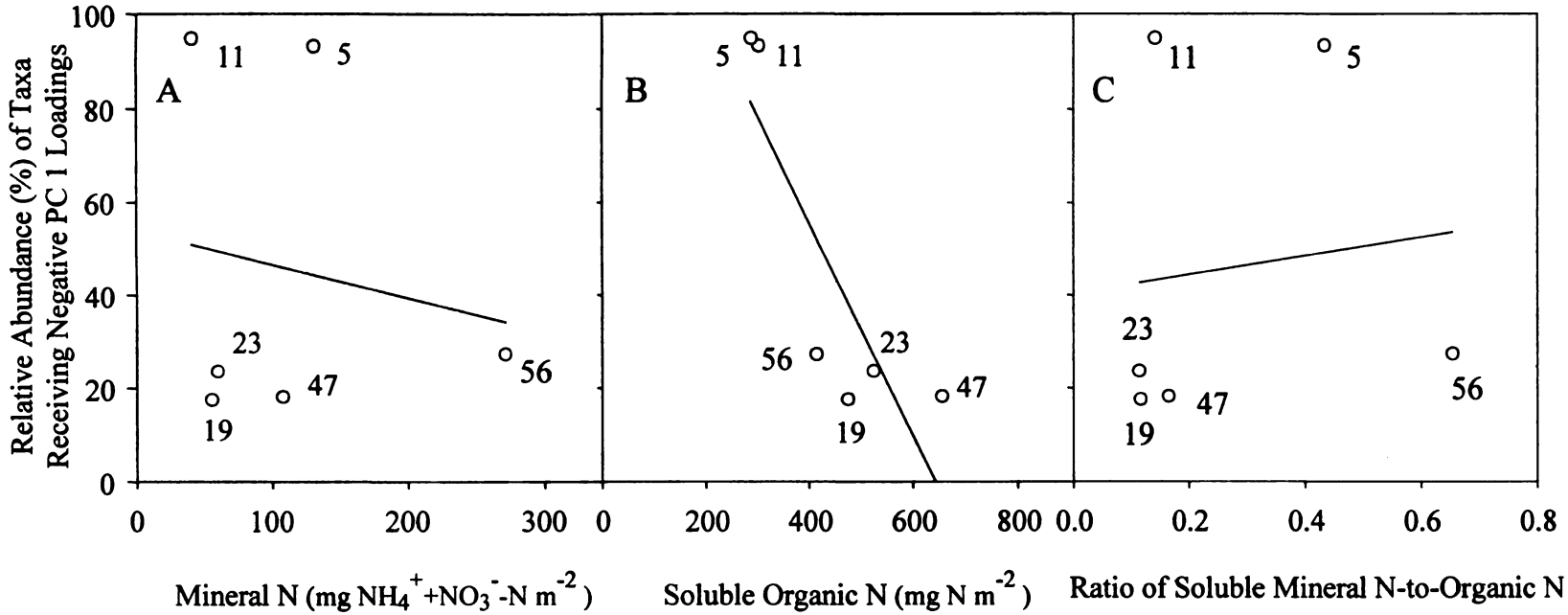


Figure 5.9: Simpson's diversity (A) and evenness (B) indices for the EMF community across the chronosequence sites.

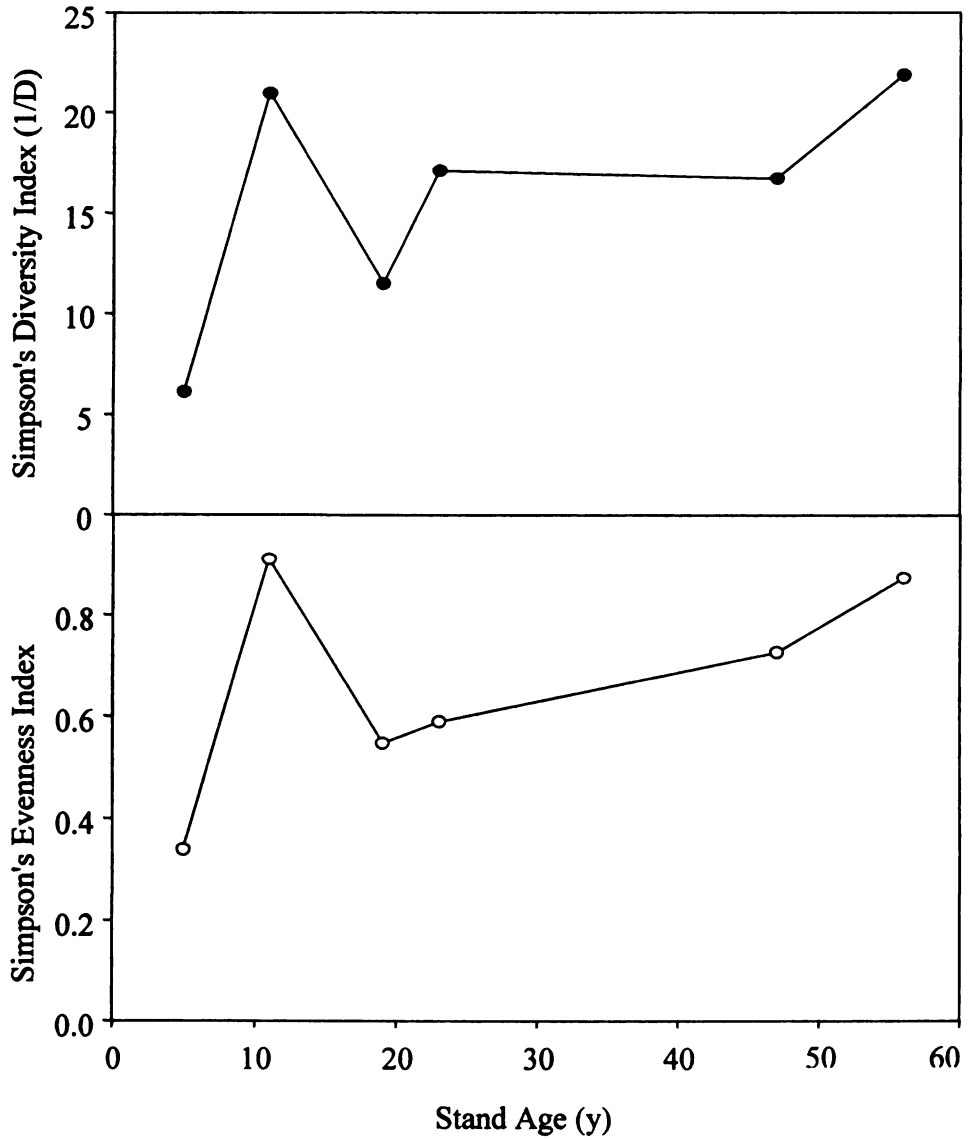
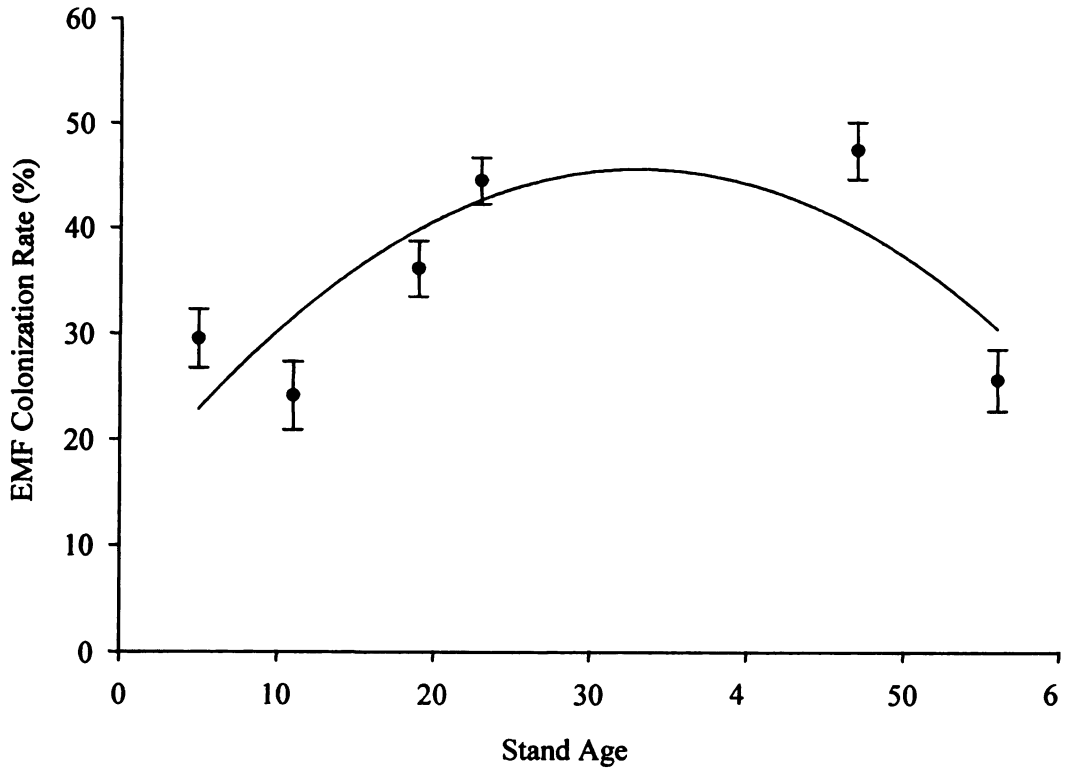


Figure 5.10: Percent EMF colonization rates across the chronosequence sites; data are stand means ±1 SE. Line represents the following best fit equations: Colonization rate = 13.99 + 1.91 * age - 0.03 *age ^ 2, R ^ 2 = 0.200, $P < 0.0001$.



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CHAPTER 6

CONCLUSIONS: THE ROLE OF LABILE CARBON

Throughout my dissertation research, I utilized the jack pine ecosystem of northern Lower Michigan to gain insights into the effects of disturbance and subsequent stand dynamics on soil nitrogen (N) cycling and ectomycorrhizal community composition. Though each research chapter asked separate questions, I believe several unifying themes run throughout the entire dissertation. One such theme is the effect of disturbance on microbial N immobilization and ectomycorrhizal communities mediated through labile carbon (C) inputs.

Organic C enters terrestrial ecosystems through plant fixation of carbon dioxide (CO_2) via photosynthesis. Carbon flux from the plant to soil organic matter (SOM) then occurs through a variety of processes, including, but not limited to: 1) litterfall inputs; 2) woody inputs, including plant mortality; 3) root turnover; and 4) root exudates. Much of the organic C entering the soil is in the form of complex polymers, such as lignin, which are difficult for soil microorganisms to metabolize. In contrast, more microbial accessible forms of C, such as amino acids, proteins, sugars, nucleotides, etc., make up only about 5-10% of plant matter (Horwath 2007). Moreover, organic C may be either chemically or physically shielded from decomposition through incorporation into humic compounds, sorption onto mineral particles or the formation of mineral-humic complexes (reviewed in Allison 2006). As a result, the vast majority of soil organic C turns over quite slowly, while a smaller fraction, often termed labile C, turns over much more rapidly.

In Chapter 3, I used potentially mineralizable C as an index for labile C, and I found that disturbed soils, either following wildfire or clearcutting, contained significantly smaller amounts of labile C relative to soils from intact stands. Additionally, I observed that microbial biomass C, generally included in discussions of labile soil C, was also significantly lower in the disturbed stands. Labile C pools may be directly affected by a disturbance event. For example, given that by definition labile C is readily metabolized by soil microorganisms, fresh plant inputs will contain higher percentages of labile C than older litter. Both disturbances substantially reduced fresh plant litter inputs by killing or removing the overstory. This was particularly the case for the clearcut treatment, since the entire overstory was removed off-site via whole-tree harvesting. The smaller labile C pools observed here are also due to the 3-6 years of decomposition following disturbance but prior to measurement. In the years following disturbance, microorganisms undoubtedly preferentially metabolized labile C vs. recalcitrant C, while the labile C pools were not replenished through fresh litter inputs.

The reduction in labile C pools following disturbance likely drove many of the N dynamics and, possibly, the shift in the ectomycorrhizal community observed throughout these dissertation chapters. The cycling of N in the soil in many ways hinges on the relative availability of C vs. the availability of N for microbial metabolism. When C availability is high relative to N, soil microorganisms will immobilize additional N, whereas under low C to N availability, they will mineralize N as a waste product (Paul and Clark 1996). These processes can occur simultaneously, yet in spatially discrete locations, in the soil. For example, Wagener and Schimel (1998) demonstrated that organic matter in the upper O horizon tends to have high C:N ratios and, as result, induce

N immobilization; whereas, more well-decomposed organic matter deeper in the profile with low C:N ratios is a site for net N mineralization.

A decline in labile C pools following disturbance caused lower C to N availability in the youngest sites, likely resulting in reduced microbial demand for N. As shown in Chapter 3, mean values for the ratio of mineralizeable C to mineralizable N were lowest in the clearcuts, intermediate in the wildfire-burned, and highest in the intact soils. This appears to have particularly reduced microbial N demand in clearcut sites, where I found greater nitrification as a result of lower microbial gross nitrate (NO_3^-) consumption. Likewise, my finding of higher net N mineralization in the youngest site of my Chapter 4 chronosequence suggests a decrease in microbial N demand, again due, at least in part, to decreases in C availability.

The lack of labile C and a shift towards greater C limitation post-disturbance also likely explains both the smaller standing pools of amino acid N and the higher microbial demand for amino acids in the youngest chronosequence site (Chapter 4). Free amino acids represent a readily available source for C as well as N, and in the youngest sites, soil microorganisms may have tended to sequester C from available amino acids while mineralizing the N. Potentially reflecting this dynamic, I found higher mineralization of the labeled amino acid tracer in my youngest site. This was not statistically significant, but, given this finding fits with our knowledge of N cycling in this ecosystem, it merits further investigation.

The results of my chronosequence study also suggest that following the first 4-5 years post-disturbance labile N pools become exhausted as well. Total N mineralization values declined sharply until reaching the lowest values in the 10 y-old site, suggesting

increased microbial N limitation. The exponential growth phase of jack pine biomass does not occur until ca. 7-10 years post-wildfire (Rothstein et al. 2004; Figure 4.8); thus, by stand age 10 fresh litter inputs have remained quite low for an extended period. At this point, the remaining soil organic matter is likely relatively depleted in both mineralizable C and N. Because of this dynamic, it was not too surprising consumption of the amino acid tracer was highest at the 10 y-old site, the majority of the label being directly incorporated into microbial biomass (Chapter 4). Unlike site age 4, mineralization of the label in this site was low, reflecting a shift towards greater N limitation.

Following this first decade of stand recovery, the resumption of litter inputs may have increased labile C pools, comparatively reducing microbial demand for free amino acids. I observed a clear pattern in free amino acid N over stand development. Standing pools of amino acid N were consistently low in the youngest sites (4-10 y), increased rapidly in mid-aged sites (15-22 y), and were highest in stand age 46. As labile C and N pools increase with stand age (Chapter 3), microbial demand for amino acids as a source of C and N may decline relatively in older sites; hence, resulting in the observed pattern. Conversely, the sharp rise in total N mineralization in the oldest stands, particularly in the 55 and 60 y-old sites, is likely due increases in organic matter with relatively low C-to-N ratios, residing primarily in the more well-decomposed layers of the O horizon. My finding of a marked rise in amino acids prior to an increase in mineral N fits with this labile vs. recalcitrant pool hypothesis, given that more labile inputs must precede the formation of more humified material.

Finally, a shift in the relative amounts of labile C, in the form of plant photosynthate allocated belowground, may contribute to the clear pattern in ectomycorrhizal fungal (EMF) community composition observed in Chapter 5. This compositional shift was primarily driven by higher relative abundances of *Rhizopogon* and *Thelephora* taxa in sites age 5 and 11, and increases in *Cortinarius* later in stand development. Shifts in the relative abundance of many other taxa, such as *Suillus* and *Russula*, accompanied these changes. There is some laboratory-based evidence to suggest that ectomycorrhizal taxa prevalent in older stands may exhibit greater C demand than fungi establishing following disturbance. Gibson and Deacon (1990) found that fungi, which they called "late-stage" fungi, such as *Amanita muscaria*, required higher glucose concentrations than "early-stage" fungi for hyphal growth on agar plates or full colonization of aseptic birch seedlings. Belowground C allocation via photosynthate undoubtedly increased over my chronosequence of stands. Taxa observed in the older sites, such as *Cortinarius* and *Russula*, generally spread from existing mycelial networks connecting multiple individual trees (Deacon and Fleming 1992). By establishing a C source on one tree, or a resource base (Deacon and Fleming 1992), these taxa may have a competitive advantage colonizing new root-tips in older stands. Thus, it may be that increases in labile C allocated by plants to their belowground symbionts can explain the shift in EMF taxa shown in Chapter 5. It is generally thought that the importance of competition, as an influence on community composition, increases under high resource availability (Grime 1977). I speculate that competition between rhizosphere microorganisms, in general, and EMF taxa, specifically, increases over stand development.

Since soil microorganisms are responsible for N transformations in the soil, it is perhaps not surprising that available C inputs, which ultimately regulate heterotrophic microbial activity, have a profound impact on soil N dynamics. Depending on the relative availability of C vs. N, soil organic matter will either be a sink for N through microbial immobilization, or a net source through net mineralization. The lack of labile C reduced microbial demand for N, while increasing C demand, in the young, disturbed stands. Subsequent shifts through stand development in the relative proportion of C vs. N availability likely explain the changes in N dynamics over my chronosequence. And, increases in symbiotic C allocation may be partially responsible for the shift observed in EMF taxa. As demonstrated in these dissertation chapters, disturbance events can have a profound influence on belowground resource availability and community composition, changes that are likely mediated through labile C inputs.

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