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SOIL MICROBIAL COMMUNITY PATTERN AND PROCESS:
IMPACTS ON VASCULAR PLANT COMMUNITIES IN THREE
ECOSYSTEMS OF HIGH CONSERVATION VALUE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

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* * * * *

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ABSTRACT

A growing body of evidence demonstrates that the composition, distribution, and activity of soil organisms may play an important role in determining the outcome of plant dynamics and in maintaining plant diversity in many ecosystems. Nonetheless, plant ecology is still focused aboveground and the effects of soil organisms on community and ecosystem level processes are still poorly understood. This work was conducted to improve our understanding of the impact of the soil microbial community on vascular plant dynamics at the plant community and ecosystem scale at three different ecosystems of high conservation value.

Widescale invasion of grasslands and shrublands by Pinus is a serious threat to the persistence of these biologically diverse ecosystems worldwide. Studies of plant species invasions rarely consider belowground mechanisms facilitating invasions. Much recent work has shown that mycorrhizal community composition and activity can determine plant community structure and influence plant diversity. However, most work on mycorrhizas continues to focus on nutrient dynamics of the root-mycorrhiza symbiosis. Thus, I conducted this work to improve our understanding of the role of the soil microbial community in ecosystem invasions and to enhance our understanding of the importance of mycorrhizas at the ecosystem scale. Using an alkaline limestone
barren complex in Ohio and a serpentine barren complex in Maryland as model systems, I tested the hypothesis that the invasiveness of Virginia pine (P. virgirtiana L.) into grass- and shrublands is regulated by the spatial pattern of ectomycorrhizal (ECM) fungi in the surface soil. Both sites harbor highly unique prairie and endemic plant communities whose persistence is threatened by heavy Pinus invasion. The pattern of pine invasion in open barrens at these sites reflects that observed in other systems worldwide: encroachment by an ECM-dependent woody species threatens plant communities previously dominated by arbuscular mycorrhizal plants.

ECM inoculum disperses into areas devoid of ECM in two ways. Hyphal growth from the root mass of mature ECM pines colonizes seedlings germinating under them when seeds land in their shadow (dispersal by “contagion”). Alternatively, pine seedlings are infected after seeds land in open areas where spores are concentrated in the feces of animals that have consumed sporocarps (dispersal by “centers of infection”). I used geostatistical semivariance analysis, kriging, and spatial mapping of ECM inoculum and soil chemical properties to test these two models of dispersal of ECM fungal inoculum into open barrens in two model systems: The Edge of Appalachia Alkaline Prairie Preserve in Adams County, OH, and Soldier’s Delight Natural Serpentine Environment Area in Owings Mills, MD. Spatial mapping and results from non-parametric ANOVA and correlation analyses strongly suggest that ECM fungal inoculum disperses into open barrens by contagion, thereby facilitating rapid pine colonization in an advancing front from mature pine forests bordering the barrens. Thus, current labor-intensive management techniques that use cutting and fire to curtail pine invasion may be
ineffective because they do not kill ECM or disrupt ECM mycelial mats on mature root systems that infect recolonizing pine seedlings.

My third study was conducted at the Indiana Dunes National Lakeshore, an active dune system where vascular plant dynamics have been extensively studied but where the role of soils in community and ecosystem dynamics has been virtually ignored. Colonization by American beachgrass (*Ammophila breviligulata* Fernald) is the principal mechanism by which coastal dunes are stabilized, and a decline in beachgrass vigor has been associated with invasion by non-dune plant species. Biological soil crusts composed of cyanobacteria and bryophytes often colonize spaces between beachgrass culms. To determine whether this crust affects beachgrass vigor, I conducted a greenhouse experiment utilizing intact soil cores from the Indiana Dunes National Lakeshore. I subjected soil cores to artificial rainfall over a full growing season with rainfall patterns typical for the site and quantified the volume and N content of leachate in relationship to the degree of crust development, crust taxonomic composition, rainfall volume, rainfall intensity, light intensity, and the presence of plant litter.

At rainfall volumes of <2.5 cm, little or no rainfall leached through the cores, regardless of crust cover or composition. Net N throughput significantly exceeded N inputs to cores in rainwater, demonstrating that biological soil crusts and soil organisms associated with crusts contribute substantial inorganic N to this system. Crust composition alone did not affect the amount of rainwater or N that leached through cores; however, rainfall intensity, light intensity, and litter addition interacted with crust composition to affect water and N leaching. High rainfall intensity resulted in greater leachate volume, but not leachate N, than low rainfall intensity, but very heavy (high
volume) rainfall events resulted in substantial increases in N leaching. Less leachate moved through cores exposed to full sun, and crust composition interacted with light intensity to influence NH$_4^+$ leaching through cores. The addition of beachgrass litter to the surface of soil cores significantly increased the amount of N – particularly NO$_3^-$ – in leachate. Biological soil crusts at this site do not appear to compete with American beachgrass for water and N. Instead, these crusts, combined with different properties of the soil surface, substantially increase N inputs to this highly water- and nutrient-limited sand dune ecosystem. Implications for American beachgrass persistence at the Indiana Dunes are discussed.
For women
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CHAPTER 1

INTRODUCTION

The structures and functions of native and rare ecosystems have been severely altered by anthropogenic stressors such as habitat destruction and fragmentation, alien and aggressive species invasion, increased atmospheric CO₂, and nutrient deposition. These stressors have resulted in the fastest reduction in above- and belowground biological diversity since recorded history began (Wilson 1992). For example, habitat loss has resulted in the extinction or threat of extinction of 1,000 plant species in the United States alone, the number of species of soil fungi in Europe has declined by 50% in the past six decades (Wilson 1992), and the decline in ectomycorrhizal fungal species diversity in Europe and Scandinavia due to nutrient deposition threatens to alter forest composition and functioning irreversibly (Arnolds 1991). Thus, a strong mechanistic understanding of plant and soil community dynamics in a wide range of ecosystems is more important than ever if we are to successfully secure the persistence of these systems for the future.

The discipline of plant ecology began in the late 1800’s when European naturalists began to develop ways of conceptualizing and organizing natural species groupings. In the early 20th century, phytosociologist Braun-Blanquet (1932) developed
the relevé system using plant species as the central grouping unit and describing static taxonomic associations based upon their “sociability” in undisturbed systems. In contrast, the Raunkiaer (1934) life form system suggested that individual plants, rather than species, were the primary grouping unit and that different plant life forms were affected differently by environmental conditions.

Plant ecologists in the United States were simultaneously developing and debating plant community concepts as well. Lauded as the father of modern American ecology, Henry Chandler Cowles (1899, 1901) used the Indiana Dunes National Lakeshore as a model system to describe plant community succession. Later Clements (1916, 1936) coined the concept of the climax community as a superorganism with a distinctive structure formed by specific combinations of plant life forms in distinct successional relationships. In contrast, Gleason (1917, 1926) proposed a more individualistic perspective of plant community dynamics, emphasizing individual plant responses to variation in spatial and temporal environmental conditions. Later work by Whittaker (1956, 1961) pointed out that the perspectives of Clements and Gleason directly resulted from the respective biomes in which they worked (western and eastern U.S., respectively), and in turn shed light on the importance of environmental gradients in shaping plant associations and distributions.

Decades of attempts to explain organismal species distributions along environmental gradients followed these early seminal studies. New techniques such as ordination and principal components analysis (e.g., Bray and Curtis 1957) were employed to describe species patterns. Quantitative methods for describing species distributions under competitive and resource limited conditions were developed, giving rise to the
prevailing $R_{\text{max}}$ and $R^*$ theories of resource competition and species coexistence advocated by Grime (1977) and Tilman (1985), respectively. When conservation became a national priority after WWII, the theory of island biogeography set the stage for landscape scale questions about species dispersal, establishment, and coexistence (MacArthur and Wilson 1963; Simberloff and Wilson 1969; Diamond 1976; Forman et al. 1976).

Successional theory has provided a valuable conceptual framework for understanding plant community dynamics (Connell and Slatyer 1977), but even seminal studies of plant succession have focused almost exclusively on aboveground processes to the exclusion of belowground soil communities. In the past two decades, the aboveground bias in plant ecology has shifted as more work is done on the relationship between plant and soil systems. Nonetheless, most plant ecology continues to focus on aboveground processes. For example, a central question in ecology today involves the relationship between biodiversity and ecosystem functioning. Major players in this debate who contend that biodiversity improves and maintains ecosystem functioning still measure ecosystem functioning almost exclusively by plant productivity and plant biomass (Tilman and Downing 1994; Tilman 1999; Naeem et al. 1994; Naeem et al. 2000; but see Naeem and Li 1997; Wardle 1998; Mikola and Setala 1998; Wardle et al. 2000). The exclusion of belowground systems and soil functioning from these assessments risks misrepresenting ecosystem functioning and failing to secure protection for ecosystems in which soil biodiversity is considered an integral component of ecosystem integrity and sustainability.
Soil functions include plant productivity, carbon and nitrogen cycling, carbon sequestration, production and sequestration of trace gases (e.g., CH₄, N₂O, NOₓ, SO₂), degradation of pollutants, development and maintenance of soil structure, and suppression of plant pathogens. Until recent decades, ecologists conceptualized soil as a "black box" whose inputs and outputs could be quantified but whose constituents could not be elucidated. For example, although scientists knew that soil organisms were responsible for carbon and nutrient cycling, we had a poor understanding of particular community constituents, their relationships, and the optimal environmental conditions for their functioning. In the past two decades, improvements in technology have allowed soil ecologists to characterize the composition and activity of soil organisms at progressively finer resolution. Thus, a growing body of work has greatly enhanced our understanding of the important role of soil organisms in influencing and even determining plant productivity and diversity in ecosystems around the world.

Early attempts to characterize the impact of soil organisms on plant communities relied heavily on inference. For example, Coleman et al. (1978) and Elliot et al. (1984) found that an increase in the abundance of bacterial-feeding amoebae and/or nematodes stimulated nitrogen mineralization by reducing bacterial biomass (thereby reducing substrate competition) and/or by making direct contributions to the mineral nitrogen pool. These early studies did not demonstrate whether increased nutrient mineralization was significant for plant growth, but they set in motion a host of experiments that did.

We now know more about how the activity of soil organisms directly and indirectly shapes plant community structure and diversity (e.g., Coleman et al. 1978; Perry et al. 1989; Griffiths 1994; Jentschke et al. 1995; Streitwolf-Engel et al. 1997; van
der Heijden et al. 1998a,b; Laakso and Setala 1999; Packer and Clay 2000; Westover and Bever 2001). For example, Bever et al. (1997) and Mills and Bever (2001) found that plant-induced changes in the composition of the soil microbial community contributed to changes in plant productivity and the maintenance of plant diversity. Van der Heijden et al. (1998a,b) found that altering arbuscular mycorrhizal fungal community composition changed the associated grass and forb species composition in experimental mesocosms. Addition of bacterial-feeding protozoa to soils by Jentschke et al. (1995) reduced bacterial abundance in the rhizosphere, which stimulated plant growth by promoting plant growth hormone production or increasing nutrient mineralization by bacteria. Laakso and Setala (1999) found that the addition of bacterial and fungal grazers to soil food webs in experimental microcosms stimulated plant growth by up to 16% and increased plant biomass allocation to shoots. Packer and Clay (2000) found that host-specific *Pythium* fungal pathogens increased host mortality and subsequently influenced host species distribution. Using a feedback approach, Westover and Bever (2001) demonstrated that host-specific differences between the rhizosphere bacteria populations of *Bacillus mycoides* of two grass species allowed the plant species to coexist and thus may contribute to the maintenance of plant diversity in old-field communities.

A central component of the debate about the relationship between biodiversity and ecosystem functioning centers around whether ecosystem functioning is optimized through the preservation of individual species or functional groups. Some scientists argue that the species-centered view of biodiversity should be abandoned for a focus on preserving functional diversity (Walker 1992; Wardle et al. 1999). Others counter that soil biodiversity must be maintained because much is still unknown about soil systems (it
is hypothesized that < 10% of soil organisms have been identified), and because functionally similar soil organisms often have different optimal environmental tolerances, physiological requirements, and microhabitat preferences (Beare et al. 1995). Many studies show that specific soil organisms perform very specific functions (Bever et al. 1997; Packer and Clay 2000; Mills and Bever 2001; Westover and Bever 2001), suggesting that preservation of soil biodiversity – rather than of ostensibly functionally-redundant groupings – is best for sustaining soil functioning. This is an important area for future research – a recent review of the literature found that only 130 out of 5880 papers about biodiversity addressed soil biodiversity in particular (Smith 2000).

More work is needed to assess the role of soil community composition and functioning in whole communities and ecosystems (Allen and Allen 1990; Francis and Read 1994; Rillig and Allen 1999). The overarching goal of my dissertation was to contribute to our understanding of the role of the soil microbial community in shaping plant community structure, and to thereby enhance our understanding of the influence of soil organisms on plant community and ecosystem level processes. The three substantive chapters in this dissertation are presented as three separate manuscripts, each standing alone.

I conducted research in three different systems, each of particular conservation value: The Edge of Appalachia alkaline barrens in southwestern Ohio, Soldier’s Delight Natural Serpentine Environment Area in Owings Mills, Maryland, and the Indiana Dunes National Lakeshore in Miller, Indiana. The persistence of the cedar barren, prairie, and serpentine-associated plant communities at the Edge of Appalachia and Soldier’s Delight is seriously threatened by widescale aggressive Virginia pine (Pinus virginiana L.)
invasion. The invasion of relict grassland and shrubland communities by *Pinus* species is a serious threat to these systems worldwide, and current attempts to mitigate pine invasion require on-going labor- and resource-intensive management. Anthropogenic suppression of fire and release from grazing contribute to the high invasiveness of pine and to the invasibility of these systems.

*Pinus* invasion into grasslands and savannas is a global problem with a consistent pattern: an obligately ectomycorrhizal pine is colonizing and significantly reducing the area of plant communities previously dominated by arbuscular mycorrhizal plant species. Nonetheless, no work has addressed and characterized the belowground mechanisms that facilitate pine invasion into these systems. Thus, by characterizing and quantifying the distribution and dispersal of ectomycorrhizal fungi at the Edge of Appalachia and Soldier’s Delight, I hope to contribute valuable information to ecologists and managers working to preserve the last remnants of these unique and lovely sites. Results from these studies are highly applicable to other grassland, shrubland, and serpentine systems worldwide that experience pine invasion, where current management strategies involving resource intensive techniques such as cutting and burning may not effectively secure the long term sustainability of these ecosystems.

The objective of my study at the Indiana Dunes National Lakeshore was to characterize the effect of cyanobacterial and bryophytic biological soil crusts on water and nutrient infiltration to the rooting zone of American beachgrass (*Ammophila breviligulata* Fernald), the principal vascular plant responsible for primary sand dune formation and stabilization. Most work in dunes systems has focused on aboveground processes, perhaps because dune soils are nutrient poor and typically considered
biologically bereft. However, biological soil crusts like those I studied from the Indiana Dunes may be the primary source of organismal diversity in some dunes soil systems. Biological soil crusts strongly influence soil nutrient cycling, soil particle stabilization, and food web dynamics in semiarid and arid systems around the world (Belnap and Gillette 1998; West 1990; Belnap and Lange 2001), yet relatively little work has been done on soil crusts inhabiting sand dunes (Belnap and Lange 2001). Through my study of the biological soil crusts at the Indiana Dunes I hope to shed light on the influence of biological soil crusts on soil water and nutrient dynamics in dune systems where crusts comprise a considerable component of the soil biota.

The three studies making up this dissertation will contribute to the growing body of knowledge about the influence of the soil community on vascular plant communities. If ecologists and managers are to preempt and mitigate species losses from the last remaining natural areas on Earth and make decisions that enhance ecosystem functioning and sustainability, we must have a sound understanding of both above- and belowground mechanisms that drive ecosystem functioning. An increased appreciation of belowground processes challenges us to define ecosystem functioning more broadly in a way that incorporates the unique role of soils in preventing soil erosion, sequestering increased atmospheric carbon and nitrogen, and influencing the structures and distributions of plant communities. Focusing principally on aboveground processes hinders our assessment of the effect of biodiversity on ecosystem functioning. Soil biodiversity, activity, and sustainability must be incorporated into this assessment if we are to secure long-term sustainability in a wide range of ecosystems worldwide.
REFERENCES


CHAPTER 2

PREMISE AND BACKGROUND FOR STUDIES AT THE EDGE OF APPALACHIA ALKALINE BARRENS AND SOLDIER'S DELIGHT SERPENTINE BARRENS (CHAPTERS 3 AND 4)

INTRODUCTION

Widescale invasion of native alkaline and serpentine grasslands by *Pinus* is a serious threat to the persistence of these diverse and unique ecosystems worldwide (Richardson and Bond 1991; Richardson et al. 1994). Characterizing the mechanisms of invasion is more relevant than ever as biological invaders threaten the persistence of native ecosystems. Considerable effort has been made to understand why some plant species are such successful invaders (Richardson et al. 1994) and why some plant community types are more vulnerable to invasion than others (e.g., grasslands; Richardson and Bond 1991; Richardson et al. 1994; Williamson 1996; Lonsdale 1999). Thorough assessments of invasions focus on properties of both the invaded system and the invasive species (Richardson et al. 1994; Rejmanek and Richardson 1996; Lonsdale 1999). For example, highly invasible systems are frequently disturbed, low in species richness (but see Lonsdale 1999), and dominated by plants with small life forms (e.g., grasses and shrubs; Richardson and Bond 1991; Richardson et al. 1994). Highly invasive species often have small, wind-dispersed seeds, short juvenile periods, and short intervals...
between large seed crops (Richardson et al. 1994; Rejmanek and Richardson 1995). But while invasion ecology has focused almost exclusively on aboveground processes, very little is understood about the role of the soil microbial community in invasions. In this study I examine the role of the soil microbial community, and of mycorrhizal fungi in particular, on the invasiveness of Virginia pine (*Pinus virginiana* L.) in rare cedar barrens in the eastern United States.

As key players in nutrient cycling, plant uptake of nutrients, and the formation of soil structure, the composition and activity of the soil community directly and indirectly shapes plant community structure and diversity (Coleman et al. 1978; Perry et al. 1989; Griffiths 1994; Jentschke et al. 1995; Laakso and Setala 1999; Packer and Clay 2000; Westover and Bever 2001). Recently soil microbial ecologists have shown that soil microbial activity can influence, and in fact may determine, plant community composition (Wardle and Giller 1996). For example, Bever et al. (1997) and Mills and Bever (1998) found that plant-induced changes in the composition of the soil microbial community contribute to changes in plant productivity and the maintenance of plant diversity. Van der Heijden et al. (1998a,b) found that altering arbuscular-mycorrhizal (AM) fungal community composition changes the grass and forb species composition in experimental microcosms. Addition of bacterial-feeding protozoa to soils by Jentschke et al. (1995) stimulated plant growth by stimulating plant growth hormone production or increasing bacterial nutrient mineralization. Laakso and Setala (1999) found that the addition of bacterial and fungal grazers to soil food webs stimulated plant growth by up to 16% and increased plant biomass allocation to shoots in experimental microcosms.
Most work on mycorrhizas has focused on nutrient dynamics of the root-mycorrhiza association. Surprisingly little work has been done to understand the function of mycorrhizas at the community level or to characterize the importance of the plant-mycorrhiza symbiosis in whole ecosystems (Francis and Read 1994). I conducted this study to enhance our understanding of the importance of ectomycorrhizas at the plant community level and to contribute to our understanding of the role of the soil microbial community in ecosystem invasions. Specifically, my goal was to characterize the mechanism by which the ectomycorrhizal ( ECM )-dependent Virginia pine colonizes rare alkaline cedar barren and serpentine barren ecosystems that were formerly dominated by arbuscular-mycorrhizal plant species.

BACKGROUND

Arbuscular mycorrhizal ( AM ) plants dominate many communities (Walker et al. 1982; Miller 1987). Germinating seedlings of AM plants are generally ensured of being infected by AM fungi in these communities because the copious spore production and dispersal of spores by water and soil fauna results in AM inoculum being ubiquitous and dense. Only when the AM plant community is removed from the site and the soil matrix disturbed, such as by surface mining or conventional agriculture, can the AM inoculum be reduced. Even then the rapid dispersal of spores of AM fungi and extension of hyphal fragments from surrounding vegetation quickly re-establishes a dense inoculum for newly establishing AM plants (Miller 1987; Zakha et al. 1995).

In contrast, ectomycorrhizal ( ECM ) fungi rely on two very different methods for the spread of inoculum. The first is hyphal growth from the root mass of existing ECM
plants; hyphal networks may extend 1-2 m away from the base of ECM trees (Read et al. 1985; Read 1998), and in some cases several meters from mature ECM-infected roots (Schramm 1966). In this way, seedlings of ECM-dependent species successfully establish near the base of existing ECM trees, producing a pattern of colonization resembling a slowly advancing front (Francis and Read 1994). Epidemiologists and landscape ecologists have referred to this model for species spread as the "diffusion" or "contagion" model (Alexander et al. 1992; Forman 1995; Read 1998).

ECM fungi also disperse by means of hypogeous (belowground) and epigeous (aboveground) sporocarps. Consumption of sporocarps by small mammals and subsequent deposition of densely packed fungal spores in feces is an effective means of dispersing ECM inoculum over great distances (up to 100 ha or more) and of infecting incoming and germinating seeds of ECM plant species (Maser et al. 1978, Kotter and Farentinos 1984, Alexander et al. 1992, Claridge et al. 1992; Janos et al. 1995; Johnson 1996). For example, in Australia as much as 80% of the diet of small mammals is composed of sporocarps during some seasons, and survival of ECM-dependent eucalypt seedlings is greater in areas with fecal deposits than away from such areas (Baczocha et al. 1992). Cazares and Trappe (1994) and Johnson (1996) found that small mycophagous mammals move ECM spores between distinct plant community types, specifically from mature established ECM-associated plant communities to early successional patches several meters away. This pattern of ECM inoculum dispersal follows the "centers of infection" model of epidemiology and landscape ecology, in which individual and distinct foci for colonization appear far from the existing infection or species concentration, and spread occurs outward from those centers of colonization (Forman...
1995, Janos et al. 1995). This model depends on relatively simultaneous dispersal of ECM spores and deposition of seeds, as the ECM spores in the deposited sporocarp will be consumed by soil fauna or die if they do not encounter a suitable plant host within one growing season.

Communities dominated by arbuscular mycorrhizal plant species, such as grasses, forbs, and some trees (e.g., tuliptree, maples) resist the invasion of ectomycorrhizal trees (e.g., pines, oaks) because of a lack of ECM inoculum. When invasion by ECM trees does occur, it either follows a “contagion” pattern or a “centers of infection” pattern, or both. Thus, in cases where the conservation or restoration goal is to prevent invasion by ECM trees such as *Pinus*, the spatial pattern of ECM inoculum spread must be documented and appropriate management strategies to counter that pattern of spread implemented. If ECM inoculum is permitted to spread throughout the AM community, intensive, resource consumptive, and long-term management will be necessary to meet conservation or restoration goals to curtail the spread of invasive ECM-dependent plant species. Thus, my study objectives were: (1) to determine if ECM inoculum is present in alkaline and serpentine barren openings whose long-term persistence is threatened by pine invasion; (2) to quantify the spatial distribution and abundance of ECM inoculum in various community types at two model study sites; (3) to determine whether the abundance of ECM inoculum is more closely linked to soil chemical properties or to host distribution and abundance, and; (4) to suggest management strategies for curtailing the spread of pine that incorporate an understanding of the role of ECM distribution and dispersal in the invasion ecology of these ecosystems.
I used two model systems in the eastern United States to characterize the spatial distribution and dispersal of ECM inoculum and to address the above objectives: The Edge of Appalachia alkaline cedar barrens in Adams County, Ohio, and Soldier’s Delight Natural Serpentine Environment Area in Owings Mills, Maryland. Studies at each of these model systems are presented separately in the following two chapters.

REFERENCES


CHAPTER 3

THE EFFECT OF THE SPATIAL DISTRIBUTION AND DISPERsal OF ECTOMYCorrhizAL FUNGI ON THE INVASIVENESS OF VIRGINIA PINE (PINUS VIRGINIANA L.) IN EASTERN U.S. CEDAR BARRENS

ABSTRACT

Widespread invasion of native grasslands and shrublands by Pinus is a serious threat to the persistence of these biologically diverse ecosystems worldwide. Studies of plant species invasions rarely consider belowground mechanisms facilitating invasions despite that soil microbial community composition and activity can determine plant community structure and diversity. This is particularly true for mycorrhizas; however, most work on mycorrhizas continues to focus on nutrient dynamics of the root-mycorrhiza symbiosis. I conducted the current study to improve our understanding of the role of soil microbes in ecosystem invasions and to enhance our understanding of the importance of mycorrhizas at the ecosystem scale. Using The Edge of Appalachia (EOA), a large alkaline cedar barren complex in Ohio, as a model system, I tested the hypothesis that the invasiveness of Virginia pine (P. virginiana L.) into grasslands is regulated by the spatial pattern of ectomycorrhizal (ECM) fungi in the surface soil. The pattern of pine invasion in these barrens is also observed in other systems worldwide: encroachment by an ECM-dependent woody species is threatening plant communities.
previously dominated by arbuscular mycorrhizal plants. The invasiveness of ECM-dependent woody species is facilitated by colonization of AM-dominated soils by ECM inoculum, either in the form of fungal spores or hyphae. ECM inoculum disperses into areas devoid of ECM in two ways. Hyphal growth from the root mass of mature ECM pines colonizes seedlings germinating under them when seeds land in their shadow (dispersal by “contagion”). Alternatively, pine seedlings are infected after seeds land in open areas where spores are concentrated in the feces of animals that have consumed sporocarps (dispersal by “centers of infection”). At four barren openings at EOA, I used a spatially explicit sampling design to collect soils from open barren areas devoid of pine invasion as well as across gradients from pine-dominated forest into open barren areas. After growing Virginia pine seedlings in field-collected soils in a greenhouse for 10 weeks, the proportion of root tips infected by ECM on each seedling was quantified and used to determine the degree of spatial autocorrelation of ECM inoculum potential among samples using semivariance analyses. Spatial mapping (kriging) was used to develop interpolation maps of ECM inoculum density across sampled transects. Results from semivariance analyses and kriging allowed me to quantify patterns of ECM fungal distribution and to illustrate mechanisms of ECM dispersal into open barren areas. Results from semivariance analyses showed strong spatial dependence of ECM inoculum potential among sampled points at all four sites sampled (range of structural variance, C/C+C0: 0.545-1.000) and spatial structuring occurred on scales from 0.5-329.8 m. In general, spatial maps produced by kriging showed that ECM inoculum was abundant in pine forests bordering open areas and in transitional zones between pine forests and open barrens, but was negligible at best in open prairie areas. Combined with the lack of
consistent and strong correlations between ECM inoculum potential and soil chemical properties (particularly organic C), these findings strongly suggest that ECM fungal inoculum disperses into open barrens by contagion, thereby facilitating rapid pine colonization in an advancing front from mature pine forests bordering the barrens. Thus, current labor-intensive management techniques that use cutting and fire to curtail pine invasion may be ineffective because they do not kill ECM or disrupt ECM mycelial mats on mature root systems that infect recolonizing pine seedlings. Mitigation of pine invasion may be more effective if managers trench soil along the perimeter of open barrens to excise ECM mycelial mats from their associated pine hosts, thereby rendering mycelial mats extending into open barrens unviable for incoming pine propagules. This technique could be effectively combined with progressive, direct removal of concentric circles of mature pines bordering the perimeters of prairie barren openings.

INTRODUCTION AND DESCRIPTION OF STUDY SITES

The Edge of Appalachia

The Richard and Lucile Durrell Edge of Appalachia Preserve System, or Edge of Appalachia (EOA), is part of a network of several bedrock outcrops supporting tallgrass prairie vegetation at the western edge of the Allegheny Plateau in Adams County, OH (Figure 3.1). The scattered prairie relics of EOA — also referred to as alkaline cedar barrens or cedar glades — punctuate the north-south oriented border between the Bluegrass Physiographic Region and the Unglaciated Allegheny Plateau (Braun 1928a), and are likely the remnants of a once much more extensive prairie community spanning from the true “prairie states” into Ohio (Braun 1928b). Prairie plant communities are
very unusual in this geographic location; the only other prairie outcrops in the state of Ohio are in the more northern glaciated Lake Plains or Till Plains (Braun 1928a), and prairie communities were not typically believed to exist south of the glacial boundary in the deciduous forest region of the state (Gleason 1923; Sears 1926). The conditions and vegetation of the prairies of Adams County are strikingly similar to those in the more western tallgrass prairie; both are underlain by calcium carbonate (though of different origins) and both are dominated by the *Andropogon-Bouteloua* plant community association (Braun 1928a).

The prairie plant associations in Adams County became established and persist because of edaphic factors above all else (Braun 1928a). The calcareous soils at EOA are primarily Alfisols and Ultisols of the Opequon-Bratton series formed of Silurian Cedarville dolomitic limestone parent material. The residual limestone soils are shallow to moderately deep, well to moderately well drained, and lack the glacial till that covers limestone bedrock in all other areas of Ohio (Braun 1928a). Where forested vegetation occurs, Mississippian and Devonian sandstones and Crab Orchard shales overlay the dolomite, providing the acidity preferred by eastern red cedar and other woody species making up the deciduous forest in this region (Braun 1928a).

Over 100 rare species of plants and animals populate the EOA Preserve. In 1967 the National Park Service designated Lynx Prairie a National Landmark, and currently the Ohio Nature Conservancy (TNC) manages the preserve with the goal of maintaining the barrens as suitable habitat for rare and endangered prairie plants. The most common plant association at EOA is the big- and little bluestem (*Andropogon scoparius* Michx. and *A. gerardii* Vitman) and side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.)
association. Indian grass (*Sorghastrum nutans* (L.) Nash) and plains muhlenbergia (*Muhlenbergia cuspidata* (Torr.) Rydb.) are also common, and more than a dozen rare plant species grow in the barrens (Braun 1928a; Rankin and O’Bryan 1982). The alkaline barren outcrops of EOA exist within a forest matrix of Virginia pine, eastern red cedar (*Juniperus virginiana* L.), and a mixture of oaks including post oak (*Quercus stellata* Wangenh.), white oak (*Q. alba* L.), shingle oak (*Q. imbricaria* Michx.), black oak (*Q. veluntina* Lam.), and blackjack oak (*Q. marilandica* Muenchh.). Pre-settlement Native American fires and post-settlement cattle grazing likely prevented the encroachment of the alkaline barrens by woody species until release from grazing in the early 1900’s. Virginia pine was not recorded in early vegetation surveys of the area (Braun 1928a), but some woody encroachment was apparent in aerial photographs from 1938 (Annala and Kapustka 1983; Annala et al. 1983). In the absence of these limits to woody spread at EOA, invasion by woody species and Virginia pine in particular has threatened the persistence and integrity of the unique ecosystem of EOA, reducing prairie coverage from 47% to 16% between 1938-1971 (Kapustka and Annala 1982; Annala and Kapustka 1983).

Prior to pine invasion, the oak barrens of EOA were stable and did not succeed to closed-canopy oak forests. The punctuated distribution of oak trees in the barrens suggests that a “centers of infection” model might apply to their colonization; i.e., oaks appear to have entered the prairie community individually, rather than in an “advancing front” from established oak forests. The apparent stability of this pattern suggests that successful establishment of oak is sporadic and may be limited by interactions between ECM inoculum spread, seed production, dispersal and predation, and environmental...
factors (e.g., fire and dryness of the soil). In contrast, the greater seed production of pine than oak, smaller pine seed size (thus reducing predation risk), and lower dispersal distance of pine seeds (leaving seeds within the hyphal "shadow" of the adults) seems to result in a very different pattern of colonization of open barrens by pine. This invasion appears to follow the contagion model and may result in the development of closed forest if management is unsuccessful.

To curtail the encroachment of pine into open barren areas, the Ohio Nature Conservancy uses a labor-intensive management program of cutting, burning, and selective herbicide application (Peter Whan, The Ohio Nature Conservancy, pers. comm.). The Nature Conservancy targets primarily Virginia pine for removal, as eastern red cedar was historically a natural component of these plant communities (Braun 1928a), and oak is not an aggressive invader. Although cutting and burning kills young and intermediate-aged pines, they resprout, prompting managers to selectively apply herbicides to stumps. However, low intensity fires like those used at EOA are unlikely to kill the network of ECM fungal hyphae on living pine roots and in the soil, because soil microbes living 5-10cm deep are killed only by high intensity or very hot fires (Jorgensen and Hodges 1970), and some ECM inocula potential can persist even after severe fires in some areas (Amaranthus and Perry 1994). Thus, surviving ECM hyphae can colonize incoming pine seeds and can support the growth of resprouts from cut or burned trunks.

I sampled two EOA alkaline openings for this study, Lynx 3 and Lynx 5, because these openings are currently experiencing heavy pine encroachment. Both openings are of high priority for active management, and thus they are both managed to mitigate pine invasion and to restore habitat for native prairie plants.
Helen's Prairie and the adjacent perennial pasture

Helen's Prairie is a 0.025 ha opening with a diverse assemblage of tall grass prairie species that borders the Lynx Prairies at EOA (Figure 3.1). Like the Lynx Prairies, Helen's Prairie also occurs in a forest matrix of Virginia pine, eastern red cedar, and oaks, but unlike the Lynx barrens, its soils are not calcareous. Adjacent to Helen's Prairie is a 1.273 ha privately-owned perennial pasture, which is periodically mowed and actively grazed by cattle; I included the pasture in the study for comparison with the two Lynx prairies and Helen's Prairie, and to determine how vulnerable the pasture would be to Virginia pine invasion if it were converted to prairie. Both Helen's Prairie and the adjacent pasture were forested until they were cleared in 1965 for grazing. In 1983, Helen's prairie was released from grazing and sold to the Ohio Nature Conservancy. In the early 1990's, Ms. Helen Black, a philanthropist and conservationist from Cincinnati, OH, planted the area with an Adams County prairie seed mix. The prairie plants at Helen's Prairie have established very well; Helen's Prairie is one of the most diverse plant communities in the EOA complex (Kendra Cipollini, The Ohio Nature Conservancy, pers. comm.). Pine encroachment into Helen's prairie from a bordering mature pine forest is severe but the opening is not managed.

Before severe Pinus invasion into the EOA prairie openings became a problem, the plant communities at EOA were dominated by arbuscular mycorrhizal (AM) plant species. Although post oaks (traditionally believed to by obligately ectomycorrhizal [but see Egerton-Warburton and Allen 2001]) are present, they do not aggressively invade prairie openings and their colonization of outcrops appears sporadic and isolated. The successful colonization and establishment of Pinus in alkaline openings depends upon the
presence of sufficient ECM inoculum to infect incoming *Pinus* seedlings.

To characterize the distribution and dispersal of ECM inoculum in the prairie openings at EOA, I quantified the density of ECM inoculum in open barren areas and along gradients of pine or oak forest transitioning to open barrens using geostatistical semivariance analyses and interpolation mapping (described in detail below). I hypothesized that semivariance analyses and spatial maps depicting high spatial heterogeneity, or having small patches of ECM inoculum scattered throughout barren openings devoid of ECM, would support a "centers of infection" model of ECM dispersal. In contrast, spatial maps and semivariance analyses supporting the "contagion" model of ECM dispersal would depict lower spatial heterogeneity, heterogeneity on a larger spatial scale, or a concentration of ECM inoculum in areas of heavy pine density and in transitional zones between pine-dominated and open barren areas, with little ECM inoculum in open barren areas.

**METHODS**

I tested which model of ECM inoculum dispersal (contagion or centers of infection) is operating at the Edge of Appalachia (EOA) by quantifying the spatial pattern of ECM fungal inoculum in two prairie openings at EOA, Lynx Prairie openings 3 and 5, and in Helen's Prairie and the adjacent perennial pasture (Figure 3.1). In each opening I established two or three belt transects 3 m wide and 6-10 m long moving from forested areas through the transitional ecotone into open barrens or pasture (Figure 3.2). "Forested areas" refers to two types of forest: (1) oak-dominated mixed deciduous forest comprised of various oak species, tulip tree, and maple (includes some pine in borders
along the perennial pasture) and (2) pine-dominated evergreen forest with some cedar. At Lynx 3, one of three belt transects was established in open barren only (no forested samples), and both transects in Helen’s Prairie were along pine-barren gradients (Figure 2.2). Hereafter, transects along gradients from pine-dominated evergreen forests to open barrens will be referred to as PB (for “pine-barren”), transects along gradients from oak-dominated forests to open barren areas will be referred to as OB (for “oak-barren”), and the transect in open barren as O (“open”).

In each belt transect, 2.5cm-diameter soil cores were collected to 7.5cm deep at 0.5-1m intervals in a rectangular grid pattern. Soil samples were also taken from below three isolated (i.e., growing in open barren away from other woody plants) post oak, redbud, and pine trees to determine whether these trees were using ECM fungi in open barren areas. At each sample point, I removed the litter layer and sampled the A horizon (in open barrens) and the O_h/A horizons (in pine-dominated and transitional areas) to ensure sampling of soil horizons where fine mycorrhizal roots are most abundant. Soil samples were returned to the laboratory under refrigeration, where each sample was transferred within 48 hours to its own 2.5cm diameter x 5cm long Conetainer® pot (Stuewe and Sons, Corvallis, OR). Soil in each Conetainer® pot was planted with three seeds of Virginia pine (Lawyer nursery, Plains, MT), and in the second week germinated seedlings were thinned to one seedling per pot. On each day of planting, pots containing only sterilized Sunshine® soil mix were seeded to serve as controls, i.e., to ensure that no contamination by ECM fungi occurred during the experiment. Pots were randomized and watered with deionized water as needed; care was taken to prevent splashing of water and soil among pots.
Seedlings could not be grown in the greenhouse because insects infesting the greenhouse bays ate several seedlings upon initial sowing of the seeds. Instead, seedlings were kept next to a large window at room temperature (not air conditioned) under typical summer day/night length in the laboratory, where they proved to be safe from herbivory. Pine seedlings were harvested after 8-9 weeks, rinsed carefully in a tap water bath, and refrigerated in a formalin:acetic acid:ethyl alcohol solution until analysis.

Quantification of ECM infection was done using a dissecting microscope (x 10.5-60) by counting the number of root tips infected with ECM fungi on the basis of altered root morphology and the presence of a conspicuous mantle and/or fungal hyphae (Natarajan et al. 1992; Visser 1995; Gehring et al. 1998) based on the criteria of Ingleby et al. (1990). The number of root tips uninfected by ECM fungi was also counted. The total number of root tips per seedling encountered on pine seedlings grown in soils from Lynx 3 ranged from 1 to 201 (mean ± s.e.: 59.91 ± 3.14); from 1 to 144 in soils from Lynx 5 (mean ± s.e.: 52.96 ± 3.35); from 1 to 224 in soils from Helen’s Prairie (mean ± s.e.: 87.42 ± 4.99); and from 1 to 103 in soils from the active pasture (mean ± s.e.: 35.93 ± 2.62). Throughout this paper I use the term infection instead of colonization to refer to the presence of a mycorrhiza on a root system to differentiate between individual root mycorrhizae and the migration of mycorrhizal fungi into a site (Allen 2001). The number of different ECM morphotypes on each seedling root system, at each study site, and at each location (forested, ecotonal, or open barren) was noted.

The abundance of ECM fungi is very closely linked to soil organic carbon (Harvey et al. 1979), and can also vary according to soil pH, moisture, and bulk density (Francis and Read 1994). I expected that soil pH and organic C would be most
influential on ECM abundance and patterning at EOA, due, respectively, to the distinctive high alkalinity and low productivity of the site. Thus, to determine if fungal inoculum potential was correlated with soil chemical properties, each soil sample was analyzed for pH in 0.01M CaCl$_2$ in a 1:2 soil:solution ratio (Hendershot et al. 1993) and for organic C by Walkley-Black wet chemical oxidation (Allison 1965).

Statistical analyses

In any system structured by non-random processes, samples taken closer together are more likely to be positively correlated than samples taken farther apart. The degree to which ECM inoculum density and soil chemical properties exhibited non-random patterning was determined at the plot level (i.e., for PB, OB, and O transects) using semivariance analysis (GS* Version 2.0, Gamma Design Software, Plainwell, MI). Semivariance analysis is premised on the idea that if sample points show spatial patterning at the scale measured, then samples will be autocorrelated (Issaks and Srivastava 1989; Morris 1999). Semivariance analysis allowed me to quantify the degree of spatial autocorrelation in ECM root infection and soil chemical properties that existed among my samples, and to produce semivariograms, which are used to convert the ECM infection and soil chemical data to spatially explicit contour maps using kriging. Kriging is an interpolation technique that uses the semivariograms to estimate levels of a measured parameter in areas between sample points. This technique was used successfully by Boerner et al. (1996) to quantify the spatial distribution of ECM inoculum in plant communities along a successional chronosequence, by Morris (1999) to evaluate spatial autocorrelation in soil microbial communities, and by Choesin and

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Boerner (2000) to evaluate spatial patterning of soil and ground water chemical properties in an Ohio fen.

Because of the inherent lack of independence among my soil samples, non-parametric statistics were used for all statistical analyses. To make stand-level comparisons in ECM infection intensity, soil pH, and organic C among open barren, pine and oak forests, and transitional zones, I used a non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute, 1996). Ryan-Einot-Gabriel-Welsch multiple F tests were used for mean separation. To assess whether ECM infection intensity was correlated with soil chemical properties at the plot and stand levels, I performed non-parametric Spearman’s rank-order correlation coefficient analyses (PROC CORR OUTS, SAS Institute, 1996). Significance for all statistical analyses was determined at p<0.05 except where otherwise noted.

RESULTS

Mycorrhizal infection

Pine seeds germinated in Conetainer® pots within 10 days of planting in soils from all four barren openings. Two of 18 control pine seedlings were contaminated with ECM morphotype 5 (Table 3.1), but very few root tips on control pines were infected. I observed no significant differences when statistical tests were performed on root infection data with and without morphotype 5, thus I included it in my analyses.

Nine ECM morphotypes were identified at the four sites sampled, and ECM species composition at all four sites was strongly dominated by three morphotypes (Table 3.1). Five ECM morphotypes were found in Lynx 3, and despite the low infection
potential of soils from Lynx 5, the site contained six ECM morphotypes. I identified seven ECM morphotypes at Helen’s Prairie, while only four ECM morphotypes were found in the active perennial pasture (Table 3.1).

At Lynx 3, ECM fungi infected over half (53.7%) of pine seedlings grown in samples from the pine-to-barren (PB) transect. Infection intensity was relatively high and varied widely; of the root tips infected with ECM, the range in the proportion of tips infected was 0.01-0.85 (Figure 3.3). In contrast, only three pine seedlings growing in soils from the oak-to-barren (OB) transect were infected by ECM fungi. Of the root systems grown in soils from this transect infected, the range in the proportion of tips infected was only 0.01-0.11 (Figure 3.3). No pine seedlings grown in soils from the O transect were infected with ECM (Figure 3.3). None of the pine seedlings grown in soils from below three separate redbud (Cercis canadensis L.) trees were infected, but pines growing in soil taken from below one of three separate post oak trees was highly infected (the third seedling was killed by a pathogenic fungus) (Table 3.1).

In contrast to Lynx 3, PB at Lynx 5 had very low ECM inoculum potential. ECM fungi infected only 16.6% of pine seedlings growing in soils from this transect, and of infected tips, the range in the proportion of root tips infected was 0.04–0.36 (Figure 3.3). Similarly, ECM fungi infected only 18.2% of pine seedlings grown in soils from the OB transect at Lynx 5, and infection intensity was low. Of infected tips, the range of the proportion of infected root tips was 0.02 – 0.14 (Figure 3.3).

Almost all soil samples from Helen’s Prairie had heavy ECM inoculum potential. ECM infected >67% of pine seedlings grown in soils from PB. Of infected tips, the proportion of root tips infected ranged from 0.02-0.39 (Figure 3.3). ECM fungi infected
78.4% of pines grown in soils from PB, and of infected tips, the proportion of roots tips infected by ECM ranged from 0.03-0.58 (Figure 3.3). Pine seedlings grown in soils from below two of three isolated post oaks (i.e., growing in open barren) in Helen's Prairie were infected with ECM. I also collected samples from below three isolated pines, and two pine seedlings grown in soils from below these pines were infected with ECM. A seedling grown in one of the three samples collected below separate redbuds was also infected.

The ECM infection intensity in the active perennial pasture was intermediate, with 38.2% of pine seedlings grown in soils from the PB transect infected. Of ECM-infected seedlings, the proportion of root tips infected ranged from 0.02-0.43 (Figure 3.3). The OB transect in the pasture had the same ECM inoculum potential; 38.2% of pines grown in soils from OB were infected. Of infected seedlings, the proportion of root tips infected ranged from 0.03-0.42 (Figure 3.3).

Spatial structure

Root infection and site colonization by ECM

GS+ produced significant linear-to-sill models with moderately good fit for root ECM infection in all transects at Lynx 3 and Lynx 5 (Tables 3.2, 3.3). Moderate spatial structuring occurred at an intermediate spatial scale for the proportion of roots infected by ECM in the PB transect at Lynx 3 (Table 3.2). Both PB and OB transects at Lynx 5 showed intermediate to strong spatial structuring of root ECM infection, with spatial structuring occurring on large spatial scales in both transects (Table 3.3). The interpolation maps of ECM inoculum density for Lynx 3 and Lynx 5 reflect the
surprisingly low ECM infection potential of soils from OB transects at Lynx 3 and Lynx 5, and of soils from the O transect at Lynx 3 (Figures 3.4, 3.5). The interpolation map produced for the Lynx 3 PB transect reflects good ECM inoculum potential throughout the forested end of the transect with hot spots of heavy inoculum potential in both forested and transitional zones (Figure 3.4).

GS+ produced significant linear-to-sill models of root ECM infection with moderately good strength for Helen's Prairie and with very good strength for the adjacent perennial pasture (Tables 3.4, 3.5). Spatial autocorrelation between samples was significant and occurred at a small spatial scale in all transects at both sites, although model fit was weak for PB$_2$ at Helen's Prairie and for OB in the perennial pasture (Tables 3.4, 3.5). Interpolation maps of ECM inoculum density produced by kriging for Helen's Prairie showed good inoculum potential in pine forests and in transitional zones; inoculum potential was good for all of transect PB$_1$ (Figure 3.6). Hot spots of inoculum density occurred in the transitional zones of both transects at Helen's where juvenile pines are abundant, and inoculum potential decreased abruptly in PB$_2$ moving from the transitional zone into open barren (Figure 3.6).

Interpolation maps of ECM density produced for the active perennial pasture revealed a very different pattern of ECM inoculum potential. The pattern of ECM distribution varied strongly between PB and OB transects and from the pattern at Helen's Prairie (Figure 3.7). In the PB transect in the pasture, ECM inoculum was distributed in high-density clumps across the entire transect. In contrast, very little ECM inoculum occurred in the OB transect except for five low-density spots of inoculum, including one hot spot in the transition zone that was located at the base of an oak tree (Figure 3.7).
Interpolation maps of ECM fungal inoculum for both Helen's Prairie and the adjacent perennial pasture show some areas of inoculum that appear as "donuts" on the maps (see Figures 3.6, 3.7). These "donuts" are an artifact of the kriging interpolation procedure that, when estimating values between measured sampled points, weights sample points closer to a measured point more heavily than sample points further away (Issaks and Srivastava 1989). This weighted search radius can produce the "donut" effect observed on some of my interpolation maps. Such artifacts are typical in geostatistical mapping procedures that use estimation techniques (Issaks and Srivastava 1989). Attempts to correct for these artifacts would be arbitrary and would make this study impossible to reproduce by other researchers; thus, no attempt was made to correct them. Nonetheless, interpolated values are based solidly on data measured at sample points.

Soil chemical properties

GS+ produced significant linear-to-sill models for soil pH in all three transects at Lynx 3, for organic C in OB and O at Lynx 3 (Table 3.2), and for pH and organic C in both PB and OB at Lynx 5 (Table 3.3). Both pH and OC showed strong spatial structuring in all three transects at Lynx 3, and moderate to strong spatial structuring at Lynx 5. With the exception of OB at Lynx 3, pH in the Lynx barrens was autocorrelated across a larger spatial range than OC (Tables 3.2, 3.3). Notably, pH was consistently lower in pine-dominated areas of the PB transect of Lynx 3 than in transitional and open barren areas (Figure 3.8). Organic C in Lynx 3 was highest in transitional areas of PB but lowest in transitional areas of OB. Interpolation maps of soil chemical properties in OB at Lynx 5 showed lower pH and higher organic C in mixed oak forest than in open
barren areas (Figures 3.10, 3.11). Despite a hot spot of high pH in the pine forest at Lynx 5, generally the pattern of soil pH was similar to Lynx 3, where pH was lower in pine forests and transitional areas than in open barrens (Figure 3.10). Organic C showed no apparent pattern in PB at Lynx 5, i.e., some hot spots of organic C are present, but generally organic C was structured on a very small spatial scale (Figure 3.11).

GS+ produced significant linear-to-sill models of soil chemical properties with moderate to good spatial structuring for both Helen's Prairie and the adjacent perennial pasture (Tables 3.4, 3.5). In general, transects in the adjacent perennial pasture showed very strong spatial patterning at a smaller scale than at Helen's Prairie (Tables 3.4, 3.5). Interpolation maps of soil chemical properties at Helen's Prairie showed high variation in pH in forested and open barren areas with areas of consistent pH in transitional areas (Figure 3.12). Soil pH and organic C were generally lower at Helen's Prairie than in the Lynx outcrops, and areas of high organic C occurred in forested areas of the active pasture (Figure 3.15).

Stand level effects of sampling location on root infection and soil chemical properties

Root infection by ECM

For this analysis, soil samples were divided into five stand types: open barren (O); pine forest (P); transitional between pine and barren (TP); oak forest (K); and transitional between oak and barren (TK). Pine seedlings grown in soils taken from P and TP areas at Lynx 3 had significantly higher ECM infection potential than soils from O, K, and TK at this site (Table 3.6). This was not true at Lynx 5, Helen's Prairie, or the perennial
pasture, where there was no difference in ECM infection potential across stand types (at p<0.05; Table 3.8). However, at the p<0.10 level, soils from P and TP at both Helen’s Prairie and the perennial pasture had significantly higher ECM infection potential than O (Table 3.8).

Soil chemical properties

At Lynx 3, soil pH was significantly lower in P and TP soils than O and K soils (Table 3.6). While P and K soils at Lynx 5 were more similar to each other, soils from both types were lower in pH than O soils (Table 3.7). Organic C in Lynx 3 was significantly lower in O soils than in other community types, and pine and oak forests at this site there did not differ in organic C (Table 3.6). In contrast, at Lynx 5, organic C was significantly higher in K soils than in other types (Table 3.7). At Helen’s Prairie, pH was significantly lower in P and TP soils than in open barrens, but organic C did not differ among community types (Table 3.8). K and TK soils in the perennial pasture were significantly lower in pH and higher in organic C than O soils (Table 3.9).

Effects of soil properties on ECM infection potential

ECM fungal inoculum potential in both Lynx outcrops was dependent upon soil chemical properties, particularly pH, and this relationship was especially strong at Lynx 3. ECM inoculum potential was negatively correlated with soil pH in both Lynx 3 and 5, and pH accounted for almost 65% of the variation in ECM inoculum potential at Lynx 3 in the PB transect and when all three transects were pooled (Table 3.10). When transects at Lynx 3 were pooled, organic C was positively correlated with ECM infection and
accounted for almost 50% of the variation in ECM inoculum potential at this site. ECM inoculum potential at Lynx 5 was also positively correlated with organic C (at p<0.10) in the OB transect and when both transects were pooled, but the relationship was not strong. Trends were similar when all data from all five transects at both Lynx 3 and Lynx 5 were pooled; ECM inoculum potential was negatively and significantly correlated with pH and positively and significantly correlated with organic C (Table 3.10).

Correlations between soil chemical properties and ECM infection potential were weaker at Helen’s Prairie and the active perennial pasture. When both transects at Helen’s were pooled, ECM inoculum potential was negatively correlated with pH (at p<0.10), but pH accounted for only a small proportion of the variation in ECM infection (Table 3.10). In transect OB in the perennial pasture, organic C was negatively correlated with ECM inoculum potential, accounting for 43% of the variation in ECM infection in this transect (Table 3.10).

DISCUSSION

The pattern of pine invasion at the Edge of Appalachia is very similar to that observed in other systems worldwide: encroachment by an ECM-dependent woody species threatens plant communities dominated by primarily arbuscular mycorrhizal plants. My goal in this study was to understand the influence of the soil microbial community, and of ectomycorrhizae in particular, on the invasiveness of Virginia pine in the alkaline cedar barrens of Ohio. I also hoped to contribute to our understanding of the impact of ECM fungi on community-level processes, and to make suggestions to
managers to address this impact for more effective long-term mitigation of pine
recruitment into ecosystems of conservation value.

Ectomycorrhizal inoculum was ubiquitous at all of my study sites except Lynx 5. Based on the patterns of the distribution and abundance of ECM inoculum at these four sites, Lynx 3 and Helen's Prairie should be of highest priority for active management, because pine forests and transitional areas between pines and open barrens at these two sites contain ubiquitous ECM inoculum, often in high density. Spatial maps of transects in Lynx 3 and Helen's Prairie depict ubiquitous ECM inoculum in pine forests and transition areas where inoculum potential drops off abruptly past the transition zone (~2m into open barrens). Taken in conjunction with negligible to no ECM inoculum in open barren and oak-to-barren (OB) transects at Lynx 3, these maps suggest that ECM is dispersed into open areas by contagion, and that this process has the potential to facilitate rapid pine recruitment in the future.

Documented field patterns of Virginia pine colonization in other cedar barren communities also support a contagion model of dispersal. For example, invading pine saplings in serpentine outcrops in southeastern PA and western MD grow clustered under established mature pine trees (Wallenstein 1996; personal observation). Natural vegetative patterns of pine colonization at EOA are difficult to discern because managers periodically remove them. However, at Lynx 3 and Helen's Prairie, pine saplings are regenerating most successfully at the pine forest border. Other studies have consistently documented that contagion of ECM fungal infections commonly occurs by extensive ECM hyphal networks linking plant root systems (Molina and Trappe 1982; Read et al. 1985; Read 1988; Molina et al. 1992; Perry et al. 1989a; Amaranthus et al. 1990).
Hyphae on mature root systems aid in the establishment of neighboring linked seedlings by providing them with nutrients before their photosynthetic output can support the energy cost of an ECM association (Amaranthus and Perry 1994). However, direct linkage of hyphal tissue is not necessary for germinating seedlings to benefit from the mycelium on infected mature root systems, due to the ease and rapidity with which new ECM plants are infected when hyphal propagules are abundant (Amaranthus and Perry 1994; Francis and Read 1994).

The clumped and isolated distribution pattern of ECM fungal inoculum in the perennial pasture OB transect ostensibly suggests that dispersal of ECM at this site may occur in part by centers of infection. This is possible, given the intense and consistent disturbance this system encounters. Mowing and grazing of the pasture physically suppresses mushroom emergence and can favor the formation of hypogeous sporocarps (Thiers 1983; Trappe 1988), which are preferred over epigeous sporocarps by small mycophagous mammals (Maser et al. 1978; Claridge and May 1994). Hypogeous fungi make up a major component of ECM communities in ecosystems prone to seasonal drought (Trappe 1988; Luoma et al. 1991; Johnson 1994) and/or with low soil nutrient status (Taylor 1993) like those at the EOA barrens. Mammals using the network of openings making up EOA may deposit basidiospores in their feces in the perennial pasture. Nonetheless, this does not explain why a similar pattern of ECM inoculum potential does not occur in open areas at Lynx 3 and 5 and Helen's Prairie, which small mammals may be even more likely to traverse then the more disturbed perennial pasture.

A more likely interpretation of the OB map of the perennial pasture is that my sampling of this transect captured sporadic and mild ECM infection of the roots of the
large mature oak located at the top of the transect. ECM infection intensity was consistently low in soils collected from oak forests and below isolated oaks at all of my study sites. It appears that oaks at these sites may not be heavily ectomycorrhizal. Although oaks are traditionally considered to be obligately ECM species, recent work has shown that *Quercus* can harbor both AM and ECM (Molina et al. 1992). For host taxa that can use both types of mycorrhizae, which type is used depends upon ecological conditions and upon the presence and abundance of ECM vs. AM fungal propagules.

Lapeyrie and Chilvers (1985) suggest that predominantly ECM tree species may use AM for a short time in the seedling stage until they encounter ECM or until they can support the energy demands of an ECM association, and that this early use of AM might be important to the initial establishment of plants in low nutrient or calcareous soils.

Chilvers et al. (1987) found that in *Eucalyptus* stands, initially high inoculum potential of AM fungal propagules yields early root colonization by AM over ECM; however once ECM fungi enters the stand, ECM dominates AM in secondary root infections, especially as soil humus builds up (Molina et al. 1992).

The persistence of oak in the EOA barrens may be primarily dependent upon AM infection, and the low grade ECM infection we observed on some oaks in this study may be secondary. Although I have no way of proving this, pines in and surrounding the study sites – which are strongly ectomycorrhizal – may have provided oaks with the ECM inoculum necessary for this secondary ECM infection. In this case, increasing ECM use by oaks at these sites may provide ECM fungal propagules for incoming pines in the future. Egerton-Warburton and Allen (2001) found the *Quercus agrifolia* Nee. seedlings inoculated with AM had higher productivity and higher foliar N than control
seedlings but that AM were replaced with ECM as seedlings aged. This suggests that *Quercus* may benefit from AM infection early in establishment, but it is not clear whether *Quercus* can retain functional AM associations as trees mature. More work is needed to test the level of functionality of AM fungi infecting species typically considered obligately ECM like *Quercus* spp.

I am surprised by the very different abundance of ECM inoculum at the adjacent outcrops Lynx 3 and 5. It is not clear what accounts for the relative lack of ECM inoculum in pine forests and transitional areas in Lynx 5. My field observations support my experimental results; unlike in Lynx 3 and Helen’s Prairie, Lynx 5 has no observable pine regeneration below mature pines, along pine forest edges, or in open barren areas. Managers at EOA confirm this observation, claiming that Lynx 3 has always been more prone to pine invasion than Lynx 5 (David Minney, Ohio Nature Conservancy, pers. comm.). I cannot discern any differences in soil chemical parameters between the two sites that might explain this discrepancy.

One possible suggestion for the discrepancy in ECM inoculum potential between Lynx 3 and 5 is the difference between their dominant plant communities. Lynx 3 is dominated by little bluestem and three-awn grass (*Aristida* spp. L.), while big bluestem and Indian grass dominate Lynx 5. In addition, heavy pine encroachment into Lynx 3 has reduced prairie-plant fuel loads (David Minney, Ohio Nature Conservancy, pers. comm.). These differences between the two outcrops may cause fires in Lynx 5 to burn hotter and longer. Further, over the past 15 years Lynx 5 has been burned twice as frequently as Lynx 3; Lynx 5 was burned in 1986, 1993, 1995, and 1998, while Lynx 3 was burned only in 1993 and 1998 (David Minney, Ohio Nature Conservancy, pers. comm.). Thus,
hotter and more frequent fires at Lynx 5 may have reduced ECM inoculum density and
delayed recolonization of transitional and open areas of this outcrop by ECM fungi.
Further, while generally ECM fungal hyphae are thought to proliferate in the substantial
litter and humus layers that develop under host plants (Persson 1979), some studies
suggest that removal of the litter layer by low-intensity burning in *Pinus* stands increases
ECM fungal diversity, sporocarp production, and the reappearance of critical ECM
specialist species (Kuyper 1988; Jansen and vanDobben 1987). Thus, low-intensity
burns in Lynx 3 that temporarily curtail pine encroachment may inadvertently improve
pine recruitment in the long run by improving ECM fungal inoculum potential, especially
since burns at Lynx 3 have been infrequent. Studies are needed that characterize the
effects of different fire intensities and frequencies on the distribution and abundance of
ECM fungi at similar sites where fire is used to control pine (e.g., PA and MD serpentine
sites).

One of my objectives was to determine whether ECM inoculum potential was
correlated with soil properties. My correlation analyses showed that ECM infection was
consistently negatively correlated with pH at the Lynx outcrops, and that this dependence
was particularly strong in PB transects. This is not surprising given the high alkalinity of
the Lynx outcrops, and it suggests that as pine encroachment acidifies soils in pine forests
and transitional areas at the Edge of Appalachia, ECM fungi will be even more successful
at colonizing open barren areas.

The relatively low and inconsistent correlation between ECM inoculum potential
and organic C supports my suggestion that contagion accounts for ECM colonization of
these sites rather than centers of infection. If isolated clumps of germinated ECM spores
were important for large-scale infection of seedlings, I would expect a stronger relationship between ECM fungal distribution and the soil chemical properties that strongly influence nutritional growth requirements (e.g., soil organic matter). Infection of seedlings by isolated clumps of ECM inoculum requires either that pine seeds land on areas of very high ECM basidiospore density within one growing season of their deposition in feces, or that ECM fungi at EOA subsist saprophytically for some time without a host plant. The synchrony required for simultaneous deposition of seeds and spores may occur, but it is unlikely to account for heavy pine recruitment in the field. The number of spores required to infect one seedling in the field may be greater than $10^6$ (Castellano et al. 1985), even for ECM species like *Rhizopogon* that germinate readily from basidiospores. However, Kotter (1981) estimated that tassel-eared squirrels disseminated 200 million to 1 billion fungal spores per gram of fecal material, although in another similar study only 33% of pine seedlings inoculated with squirrel feces developed an ECM infection (Kotter and Farentinos 1984). In general, spores alone don’t appear to have strong inoculation potential in the field, and their infection potential declines rapidly in the absence of host plants (Perry et al. 1989b).

Further, it is unlikely that any ECM fungal spores germinating in the droughty, nutrient poor, alkaline soils of EOA are very successful saprophytically; this is supported by my finding that pH was significantly higher in Lynx prairie openings than in pine forests or transitional zones, and that ECM inoculum density was negatively and significantly correlated with pH. Further, while some ECM fungi may have limited saprophytic capability through extraction of nutrients from soil organic matter (Read 1988), most ECM fungi probably cannot compete well with saprophytic fungi for carbon.
Soderstrom and Read (1987) measured the respiratory output of the mycelium of a number of ECM fungal species before and after severing their connection to the plant and found that ECM fungal activity was almost completely dependent upon the supply of current carbon assimilate from the host plant.

The small scale at which spatial structuring of soil chemical properties occurs at these sites suggests that pH and organic C are controlled by small-scale microsite differences, e.g., the presence of limestone bedrock, different microbial communities associated with plant species rhizospheres, or other small-scale microsite differences. Gehring et al. (1998), found that ECM species richness was not dependent upon soil or other environmental conditions, although species composition was dependent upon soil type. Villeneuve et al. (1989), using sporocarp surveys, found that ECM species richness was less sensitive to environmental stressors that reduce ecosystem productivity than saprophytic basidiomycete species (the applicability of this study to my results should be considered cautiously, however, since ECM basidiomycete sporocarps do not always accurately reflect ECM species composition or infection belowground (Gardes and Bruns 1996)). Although ECM species richness should not be confused with ECM infection potential, these studies suggest that while ECM fungal proliferation does depend somewhat on soil conditions, ECM inoculum density is controlled principally by an association with a host plant.

The low number of ectomycorrhizal morphotypes at the four study sites is not unusual. Many community- and ecosystem-level studies have found low ECM species diversity with domination by a few abundant species. Based on both morphological and molecular analyses, Gehring et al. (1998) found only 5.0 (± 1.73) ECM types in a pinyon
pine forest on limestone-derived soils, with one to three dominant ECM types accounting for 33-53% of ectomycorrhizas across six different sites and two different soil types. Natarajan et al. (1992) found that one ECM fungus, *Amanita muscaria*, infected 45% of the roots tips of 17-year-old *Pinus patula* in plantations in India. Together with the current study, these results suggest that dominance by a few species of ECM at a site is common, regardless of whether ECM is typed using molecular or morphological techniques (Gehring et al. 1998). The dominance of my sites by morphotype 1, which I believe is *Cenococcum geophilum*, is not surprising, as Piggott (1982) found C. *geophilum* to be characteristically tolerant of soil droughtiness.

**Suggestions for management of the EOA cedar barrens**

My evidence strongly suggests that colonization of open cedar barrens at the Edge of Appalachia by Virginia pine occurs primary by contagion, and would not be effective without the mycelial mat that extends from mature root systems of established pines. Thus, management strategies that address the spread of ECM fungal inoculum into areas of conservation value may be more effective and less labor intensive than current management techniques. I suggest that trenching around established pine forests would more effectively curtail pine recruitment into open barrens by breaking up the mycelial network that supports and infects encroaching seedlings. Trenching a pit 15-20 cm deep around the perimeter of open barrens would render ECM fungal hyphae inviable for incoming pine seeds, and pits could be trenched every few years to ensure removal of accumulated soil and organic matter that could support ECM fungal growth into open barrens.
Prior laboratory and field experiments suggest that this technique may work. Fleming (1983) found that mycelial strands severed from the roots of their hosts plants were not capable of infecting seedlings planted around established hosts, and Soderstrom and Read (1987) showed that ECM fungal respiration depends almost entirely upon photosynthetic input from host plants. Excised ECM hyphae remain viable for only 8 months after being severed from their hosts (Harvey et al. 1980; Ferrier and Alexander 1985). Clearly seedlings germinating below mature parent plants show strong association with the mycelial mats on parent plant root systems (Griffiths et al. 1991). Thus, in addressing the invasion ecology of Pinus into these systems, the movement of Pinus propagules into open prairie outcrops is less important than colonization of openings by ECM mycelial mats associated with the roots of mature pines bordering the outcrops. Thus, managers may more effectively mitigate pine invasion by targeting ECM fungal hyphal colonization of open barrens rather than by targeting mature pine trees themselves.

Mitigating ECM fungal hyphal movement into open barrens may be successful using a management strategy in which saplings and mature pine trees are progressively removed in concentric circles from the perimeters of prairie outcrops (R. Wayne Tyndall, Maryland Natural Heritage Program, personal communication). Combined with trenching, progressive removal of pines in concentric circles bordering openings would successfully reduce the expanse and infectivity of ECM mycelial mats associated with mature pines bordering prairie openings. In contrast to the current removal method in which managers cut and burn entire pine stands, this concentric management method would save considerable money, time, and labor. It would also permit managers to target
more openings more frequently than limited resources currently allow. By increasing the number of outcrops that are actively and effectively managed, managers will be able to incrementally expand and improve the continuity of the areas occupied by native prairie plant communities before Virginia pine invasion eradicates them entirely.

CONCLUSIONS

The potential for managers and scientists to effectively contain and preempt invasions requires a sound and comprehensive understanding of both above- and belowground mechanisms facilitating the invasions. Examining the role of mycorrhizal fungi on community- and ecosystem-level processes is relevant when the pattern of invasion is characterized by the movement of a strongly ECM-dependent woody species like pine into previously AM-dominated ecosystems. The colonization of rare alkaline barren communities by pine may be regulated in large part by movement into these systems of ECM propagules in the form of hyphal inoculum networks resembling an advancing front. Thus, unless management efforts at these sites address the underlying issue of ECM fungal dispersal, current management efforts to eliminate pine and to conserve habitat for the tallgrass prairie plant associations unique in this region of the country may be in vain.

ACKNOWLEDGMENTS

I thank the Ohio Nature Conservancy and the Cincinnati Museum of Natural History for granting me access to the Edge of Appalachia cedar barrens. Peter Whan and David Minney at the Ohio Nature Conservancy provided me with valuable information
about the natural history of the region and about recent management regimes. I thank Kelly Decker, William Dress, and Joy Dress for field assistance and Jennifer Brinkman for lab assistance. This research was funded in part by a Janice Carson Beatley Travel Grant.

REFERENCES


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SAS Institute, Inc. 1996. Cary, NC, USA.


Figure 3.1. Location of the Edge of Appalachia Prairie Preserve System in Adams County, OH. Shaded areas are outcrops within a matrix of mixed deciduous-evergreen forest. The current study was conducted at Lynx 3 and Lynx 5 (circled), as well as in nearby Helen's Prairie and an adjacent active perennial pasture, approximately 1 km southeast of the Lynx Prairies (not pictured). Figure adapted from Annala and Kapustka (1983).
Figure 3.2. Location of sample transects in the four sites sampled at and near the Edge of Appalachia Prairie Preserve, Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open barren; O = open barren (no forested samples) and; PB = pine-dominated forest transitioning to open barren.
Figure 3.3. Mean (+SE) proportion of root tips infected with ECM fungi in my Conetainer® pot experiment. Transect type abbreviations are: PB = Pine-dominated forest to open barren gradient; OB = oak-dominated forest to open cedar barren gradient, and; O = open cedar barren (no forested sample points). Helen’s (1) and (2) signify that both transects at Helen’s Prairie were PB. Numbers above error bars are N.
Figure 3.4. Spatial maps of the proportion of root tips infected by ectomycorrhizal fungi in Lynx 3 cedar barren at the Edge of Appalachia Prairie Preserve, Adams Co., OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open barren; O = open barren (no forested sample points); PB = pine-dominated forest transitioning to open barren. The legend applies to all three transects at Lynx 3. Circles in transects represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.
Figure 3.5. Spatial maps of the proportion of root tips infected by ectomycorrhizal fungi in Lynx 5 cedar barren at the Edge of Appalachia Prairie Preserve in Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open barren; and PB = pine-dominated forest transitioning to open barren. The legend applies to both transects. Circles represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.

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Figure 3.6. Spatial maps of the distribution of ectomycorrhizal fungal inoculum at Helen’s Prairie near the Edge of Appalachia Prairie Preserve, Adams County, OH. Both transects are PB = pine-dominated forest transitioning to open barren. The legend applies to both transects. Circles represent trees; all circles in these transects represent pines. Units on transect grids are meters.
Figure 3.7. Spatial maps of ectomycorrhizal fungal inoculum density in the active perennial pasture near the Edge of Appalachia Prairie Preserve, Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open pasture; and PB = pine-dominated forest transitioning to open pasture. The legend applies to both transects. Circles represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.
Figure 3.8. Spatial maps of pH in transects in Lynx 3 cedar barren at the Edge of Appalachia Prairie Preserve, Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open barren; O = open barren (no forested sample points); PB = pine-dominated forest transitioning to open barren; The legend applies to all three transects at Lynx 3. Circles in transects represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.
Figure 3.9. Spatial maps of pH in Lynx 5 cedar barren at the Edge of Appalachia Prairie Preserve, Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open barren; and PB = pine-dominated forest transitioning to open barren. The legend applies to both transects. Circles represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.
Figure 3.10. Spatial maps of pH at Helen's Prairie near the Edge of Appalachia Prairie Preserve, Adams County, OH. Both transects are PB = pine-dominated forest transitioning to open barren. The legend applies to both transects. Circles represent trees; all circles in these transects represent pines. Units on transect grids are meters.
Figure 3.11. Spatial maps of pH in the active perennial pasture adjacent to Helen’s Prairie, near the Edge of Appalachia Prairie Preserve, Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open pasture; and PB = pine-dominated forest transitioning to open pasture. The legend applies to both transects. Circles represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.
Figure 3.12. Spatial maps of organic C content (%) in Lynx 3 cedar barren at the Edge of Appalachia Prairie Preserve, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open barren; O = open barren (no forested sample points); PB = pine-dominated forest transitioning to open barren. The legend applies to all three transects at Lynx 3. Circles in oak-dominated forest transect represent oaks; circles in pine-dominated forest transect represent pines. Units on transect grids are meters.
Figure 3.13. Spatial maps of organic C (%) in Lynx 5 Prairie at the Edge of Appalachia Prairie Preserve, Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open barren; and PB = pine-dominated forest transitioning to open barren. The legend applies to both transects. Circles represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.
Figure 3.14. Spatial maps of organic C (%) at Helen's Prairie near the Edge of Appalachia Prairie Preserve in Adams County, OH. Both transects are PB = pine-dominated forest transitioning to open barren. The legend applies to both transects. Circles represent trees; all circles in these transects represent pines. Units on transect grids are meters.
Figure 3.15. Spatial maps of organic C (%) in the active pasture adjacent to Helen’s Prairie, near the Edge of Appalachia Prairie Preserve, Adams County, OH. Transect abbreviations are: OB = oak-dominated forest transitioning to open pasture; and PB = pine-dominated forest transitioning to open pasture. The legend applies to both transects. Circles represent trees; black circles represent oaks, grey circles represent pines. Units on transect grids are meters.
<table>
<thead>
<tr>
<th>Morphotype</th>
<th>Distinctive features</th>
<th>Abundance</th>
<th>Locations found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Black mantle; thick, long black hyphae; occur primarily on tips of lateral roots; root morphology branched or unbranched (probably <em>Cenococcum geophilum</em> Far.)</td>
<td>Abundant</td>
<td>Lynx 3: PB; Lynx 5: OB and PB; Helen’s: PB₁ and PB₂; Pasture: OB and PB</td>
</tr>
<tr>
<td>2</td>
<td>Dark brown/black, long hyphae; hyphae envelop base of lateral roots; root morphology unbranched</td>
<td>Common</td>
<td>Lynx 3: PB; Helen’s: PB₂</td>
</tr>
<tr>
<td>3</td>
<td>Shiny, club-like white mantle; no apparent hyphal development; root morphology bulbous</td>
<td>Common</td>
<td>Lynx 3: PB and individual oaks; Lynx 5: OB</td>
</tr>
<tr>
<td>4</td>
<td>Shiny, translucent/white mantle; no apparent hyphal development; root morphology altered, often branched</td>
<td>Rare</td>
<td>Lynx 5: OB and PB</td>
</tr>
<tr>
<td>5</td>
<td>Rusty brown mantle; no apparent hyphal development; root characteristically bifurcated</td>
<td>Abundant</td>
<td>Lynx 3: OB, PB, and individual oaks; Lynx 5: OB and PB; Helen’s: PB₁, PB₂, and individual pines; Pasture: OB and PB</td>
</tr>
<tr>
<td>6</td>
<td>Black mantle; cream colored, opaque, dense hyphae; occurs characteristically on root tip</td>
<td>Rare</td>
<td>Lynx 3: PB</td>
</tr>
<tr>
<td>7</td>
<td>Thick white mantle; dense, powdery golden mustard-colored hyphae; forming thick clumps of bifurcated and branched roots</td>
<td>Abundant</td>
<td>Lynx 5: PB; Helen’s: PB₁, PB₂, and individual oaks, pines and one redbud; Pasture: OB</td>
</tr>
<tr>
<td>8</td>
<td>Dark brown, smooth bulbous mantle; occurs characteristically on root tips; bulbous</td>
<td>Rare</td>
<td>Helen’s: PB₁</td>
</tr>
<tr>
<td>9</td>
<td>White mantle; obvious fuzzy hyphal development around mantle; occurs on root tips; bulbous</td>
<td>Common</td>
<td>Lynx 5: PB; Helen’s: PB₁ and PB₂; Pasture: PB</td>
</tr>
</tbody>
</table>

Table 3.1. Ectomycorrhizal morphotypes observed in the cedar barrens of the Edge of Appalachia (EOA) and in nearby Helen’s Prairie and the adjacent pasture in Adams County, OH. Location abbreviations are: OB = oak-dominated forest transitioning to open pasture; PB = pine-dominated forest transitioning to open pasture and; O = open barren (no forested samples).
Table 3.2. Semivariance analysis of spatial structure in proportion of Virginia pine roots infected by ectomycorrhizal (ECM) fungi, pH, and organic carbon (%) in our greenhouse study using soils collected at spatially-explicit intervals in transects at Lynx 3 at the Edge of Appalachia Preserve in Adams County, OH. Structural variance is the proportion of structural + nugget variance accounted for by spatial structuring. All models are linear-to-sill models where range (m) represents the distance at which the semivariogram asymptotes to the sill. Ranges given as intervals are for anisotropic models of best fit. Transect abbreviations are: PB = pine-dominated forest to open barren gradient; OB = oak-dominated forest to open barren gradient; O = open barren (no forested sample points).
Table 3.3. Semivariance analysis of spatial structure in proportion of Virginia pine roots infected by ectomycorrhizal (ECM) fungi, pH, and organic carbon (%) in our greenhouse study using soils collected at spatially-explicit intervals in transects at Lynx 5 at the Edge of Appalachia Preserve in Adams County, OH. Structural variance is the proportion of structural + nugget variance accounted for by spatial structuring. All models are linear-to-sill models where range (m) represents the distance at which the semivariogram asymptotes to the sill. Ranges given as intervals are for anisotropic models of best fit. Transect abbreviations are: PB = pine-dominated forest to open barren gradient; OB = oak-dominated forest to open barren gradient.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Transect</th>
<th>Nugget variance (C₀)</th>
<th>Total model variance (C + C₀)</th>
<th>Structural variance (C/(C + C₀))</th>
<th>r²</th>
<th>Range (m)</th>
<th>RSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop. roots</td>
<td>PB</td>
<td>0.003</td>
<td>0.025</td>
<td>0.880</td>
<td>0.524</td>
<td>14.10-34.65</td>
<td>4.32*E⁻⁰⁴</td>
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<tr>
<td></td>
<td>OB</td>
<td>0.001</td>
<td>0.003</td>
<td>0.667</td>
<td>0.666</td>
<td>329.8-329.8</td>
<td>1.09*E⁻⁰³</td>
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<tr>
<td>pH</td>
<td>PB</td>
<td>0.362</td>
<td>1.103</td>
<td>0.672</td>
<td>0.880</td>
<td>4.08</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>OB</td>
<td>0.018</td>
<td>0.560</td>
<td>0.968</td>
<td>0.931</td>
<td>5.15</td>
<td>0.015</td>
</tr>
<tr>
<td>Organic C (%)</td>
<td>PB</td>
<td>0.001</td>
<td>2.304</td>
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<td></td>
<td>OB</td>
<td>0.461</td>
<td>1.774</td>
<td>0.740</td>
<td>0.942</td>
<td>1.53</td>
<td>0.038</td>
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<td>Variable</td>
<td>Transect</td>
<td>Nugget variance (C₀)</td>
<td>Total model variance (C + C₀)</td>
<td>Structural variance (C/(C + C₀))</td>
<td>r²</td>
<td>Range (m)</td>
<td>RSS</td>
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<tr>
<td>Prop. roots PB₁</td>
<td>0.003</td>
<td>0.011</td>
<td>0.727</td>
<td>0.843</td>
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<td>8.18E⁻⁰⁷</td>
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<td>infected</td>
<td>PB₂</td>
<td>0.004</td>
<td>0.020</td>
<td>0.800</td>
<td>0.0</td>
<td>0.49</td>
<td>5.69E⁻⁰⁵</td>
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<tr>
<td>pH PB₁</td>
<td>0.035</td>
<td>0.130</td>
<td>0.731</td>
<td>0.559</td>
<td>8.41-19.67</td>
<td>1.72E⁻⁰³</td>
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<tr>
<td></td>
<td>PB₂</td>
<td>0.002</td>
<td>0.272</td>
<td>0.993</td>
<td>0.953</td>
<td>8.08</td>
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<td>Organic C (%) PB₁</td>
<td>0.066</td>
<td>0.302</td>
<td>0.781</td>
<td>0.927</td>
<td>1.04</td>
<td>9.42E⁻⁰⁴</td>
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<td></td>
<td>PB₂</td>
<td>0.085</td>
<td>0.396</td>
<td>0.785</td>
<td>0.896</td>
<td>4.45</td>
<td>8.33E⁻⁰³</td>
</tr>
</tbody>
</table>

Table 3.4. Semivariance analysis of spatial structure in proportion of Virginia pine roots colonized by ectomycorrhizal (ECM) fungi, pH, and organic carbon (%) in our greenhouse study using soils collected at spatially-explicit intervals in transects at Helen's Prairie in Adams County, OH. Structural variance is the proportion of structural + nugget variance accounted for by spatial structuring. All models are linear-to-sill models where range (m) represents the distance at which the semivariogram asymptotes to the sill. Ranges given as intervals are for anisotropic models of best fit. Transect abbreviations are: PB = pine-dominated forest to open barren gradient.
Table 3.5. Semivariance analysis of spatial structure in proportion of Virginia pine roots infected by ectomycorrhizal (ECM) fungi, pH, and organic carbon (%) in our greenhouse study using soils collected at spatially-explicit intervals in transects at the Active Pasture in Adams County, OH. Structural variance is the proportion of structural + nugget variance accounted for by spatial structuring. All models are linear-to-sill models where range (m) represents the distance at which the semivariogram asymptotes to the sill. Ranges given as intervals are for anisotropic models of best fit. Transect abbreviations are: PB = pine-dominated forest to open barren gradient; OB = oak-dominated forest to open barren gradient.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Transect</th>
<th>Nugget variance (C₀)</th>
<th>Total model variance (C + C₀)</th>
<th>Structural variance (C/(C + C₀))</th>
<th>r²</th>
<th>Range (m)</th>
<th>RSS</th>
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</thead>
<tbody>
<tr>
<td>Prop. roots infected</td>
<td>PB</td>
<td>0.001</td>
<td>0.015</td>
<td>0.933</td>
<td>0.559</td>
<td>1.15</td>
<td>5.09*E-05</td>
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<tr>
<td></td>
<td>OB</td>
<td>0.000</td>
<td>0.008</td>
<td>1.0</td>
<td>0.000</td>
<td>0.49</td>
<td>5.37*E-05</td>
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<td>pH</td>
<td>PB</td>
<td>0.049</td>
<td>0.247</td>
<td>0.802</td>
<td>0.977</td>
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<td>0.217</td>
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<td>1.14</td>
<td>8.87*E-04</td>
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<td>Organic C (%)</td>
<td>PB</td>
<td>0.023</td>
<td>0.884</td>
<td>0.974</td>
<td>0.920</td>
<td>3.00</td>
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<td></td>
<td>OB</td>
<td>0.538</td>
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<td>0.779</td>
<td>0.930</td>
<td>0.79</td>
<td>0.028</td>
</tr>
<tr>
<td>Location</td>
<td>Prop. roots infected</td>
<td>pH</td>
<td>Organic C (%)</td>
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<td></td>
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<tr>
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<tr>
<td>O</td>
<td>0.003 (0.00) a</td>
<td>7.058 (0.03) a</td>
<td>2.966 (0.13) a</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>P</td>
<td>0.233 (0.06) c</td>
<td>4.977 (0.15) d</td>
<td>4.018 (0.17) bc</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TP</td>
<td>0.056 (0.04) b</td>
<td>6.558 (0.14) c</td>
<td>4.793 (0.34) c</td>
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<td></td>
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<tr>
<td>TK</td>
<td>0.0 (0.0) a</td>
<td>7.045 (0.02) ab</td>
<td>3.724 (0.12) b</td>
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<td>K</td>
<td>0.009 (0.01) a</td>
<td>6.940 (0.03) b</td>
<td>4.125 (0.26) bc</td>
<td></td>
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Table 3.6. Results from non-parametric ANOVA on ranked root infection, pH, and organic C data (PROC RANK GLM, SAS Institute, 1996) for Lynx 3 cedar barren at the Edge of Appalachia, OH. Numbers are mean (± SE) for all samples from that particular location; different letters indicate significant differences at p<0.05. Location abbreviations are: O = open barren samples; P = samples from pine-dominated forests; TP = transition areas between pine forests and open barrens; TK = transition areas between oak-dominated forests and open barrens; K = oak-dominated mixed deciduous forest.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Location</th>
<th>O</th>
<th>P</th>
<th>TP</th>
<th>TK</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop. roots infected</td>
<td>0.0 (0.0) a</td>
<td>0.054 (0.03) a</td>
<td>0.005 (0.01) a</td>
<td>0.017 (0.01) a</td>
<td>0.025 (0.01) a</td>
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<tr>
<td>pH</td>
<td>7.006 (0.04) a</td>
<td>5.501 (0.23) d</td>
<td>6.476 (0.21) bc</td>
<td>6.75 (0.12) b</td>
<td>6.073 (0.14) cd</td>
<td></td>
</tr>
<tr>
<td>Organic C (%)</td>
<td>4.72 (0.18) a</td>
<td>4.83 (0.44) a</td>
<td>4.729 (0.53) a</td>
<td>5.77 (0.36) ab</td>
<td>6.588 (0.40) b</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.7. Results from non-parametric ANOVA on ranked root infection, pH, and organic C data (PROC RANK GLM, SAS Institute, 1996) for Lynx 5 cedar barren at the Edge of Appalachia, OH. Numbers are mean (± SE) for all samples from that particular location; different letters indicate significant differences at p<0.05. Location abbreviations are: O = open barren samples; P = samples from pine-dominated forests; TP = transition areas between pine forests and open barrens; TK = transition areas between oak-dominated forests and open barrens; K = oak-dominated mixed deciduous forest.
<table>
<thead>
<tr>
<th>Location</th>
<th>Prop. roots infected</th>
<th>P</th>
<th>TP</th>
<th>TK</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>0.076 (0.02) a</td>
<td>0.126 (0.02) a</td>
<td>0.140 (0.02) a</td>
<td>not sampled</td>
<td>not sampled</td>
</tr>
<tr>
<td>P</td>
<td>0.126 (0.02) a</td>
<td>0.126 (0.02) a</td>
<td>0.140 (0.02) a</td>
<td>not sampled</td>
<td>not sampled</td>
</tr>
<tr>
<td>TP</td>
<td>0.140 (0.02) a</td>
<td>0.140 (0.02) a</td>
<td>0.140 (0.02) a</td>
<td>not sampled</td>
<td>not sampled</td>
</tr>
<tr>
<td>TK</td>
<td>not sampled</td>
<td>not sampled</td>
<td>not sampled</td>
<td>not sampled</td>
<td>not sampled</td>
</tr>
<tr>
<td>K</td>
<td>not sampled</td>
<td>not sampled</td>
<td>not sampled</td>
<td>not sampled</td>
<td>not sampled</td>
</tr>
<tr>
<td>pH</td>
<td>4.741 (0.06) a</td>
<td>4.484 (0.05) b</td>
<td>4.576 (0.04) b</td>
<td>not sampled</td>
<td>not sampled</td>
</tr>
<tr>
<td>Organic C (%)</td>
<td>2.173 (0.11) a</td>
<td>2.164 (0.13) a</td>
<td>1.993 (0.07) a</td>
<td>not sampled</td>
<td>not sampled</td>
</tr>
</tbody>
</table>

Table 3.8. Results from non-parametric ANOVA on ranked root infection, pH, and organic C data (PROC RANK GLM, SAS Institute, 1996) for Helen's Prairie near the Edge of Appalachia, OH. Numbers are mean (± SE) for all samples from that particular location; different letters indicate significant differences at p<0.05. Asterisk denotes statistical significance at p<0.10. Location abbreviations are: O = open barren samples; P = samples from pine-dominated forests; TP = transition areas between pine forests and open barrens; TK = transition areas between oak-dominated forests and open barrens; K = oak-dominated mixed deciduous forest.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Location</th>
<th>O</th>
<th>P</th>
<th>TP</th>
<th>TK</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop. roots infected</td>
<td></td>
<td>0.043 (0.02) a</td>
<td>0.090 (0.04) a</td>
<td>0.080 (0.05) a</td>
<td>0.096 (0.05) a</td>
<td>0.014 (0.01) a</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>5.177 (0.06) a</td>
<td>4.753 (0.09) ab</td>
<td>4.97 (0.13) ab</td>
<td>4.378 (0.24) b</td>
<td>4.755 (0.08) b</td>
</tr>
<tr>
<td>Organic C (%)</td>
<td></td>
<td>5.471 (0.25) a</td>
<td>5.693 (0.23) a</td>
<td>4.179 (0.11) c</td>
<td>6.561 (0.71) ab</td>
<td>7.819 (0.31) b</td>
</tr>
</tbody>
</table>

Table 3.9. Results from non-parametric ANOVA on ranked root infection, pH, and organic C data (PROC RANK GLM, SAS Institute, 1996) for the perennial pasture adjacent to Helen’s Prairie, near the Edge of Appalachia, OH. Numbers are mean (± SE) for all samples from that particular location; different letters indicate significant differences at p<0.05. Location abbreviations are: O = open barren samples; P = samples from pine-dominated forests; TP = transition areas between pine forests and open barrens; TK = transition areas between oak-dominated forests and open barrens; K = oak-dominated mixed deciduous forest.
<table>
<thead>
<tr>
<th></th>
<th>Lynx 3 pH</th>
<th>OC</th>
<th>Lynx 5 pH</th>
<th>OC</th>
<th>Helen’s pH</th>
<th>OC</th>
<th>Pasture pH</th>
<th>OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transects pooled</td>
<td>-0.648 (0.000)</td>
<td>0.456 (0.000)</td>
<td>-0.360 (0.002)</td>
<td>0.198 (0.098)</td>
<td>-0.171 (0.085)</td>
<td>-0.058 (0.563)</td>
<td>-0.044 (0.725)</td>
<td>-0.175 (0.153)</td>
</tr>
<tr>
<td>PB</td>
<td>-0.648 (0.000)</td>
<td>0.047 (0.771)</td>
<td>-0.468 (0.006)</td>
<td>0.215 (0.231)</td>
<td>-0.104 (-0.172)</td>
<td>-0.108 (-0.159)</td>
<td>-0.044 (0.806)</td>
<td>0.092 (0.605)</td>
</tr>
<tr>
<td>OB</td>
<td>-0.234 (0.102)</td>
<td>-0.094 (0.518)</td>
<td>-0.298 (0.070)</td>
<td>0.299 (0.068)</td>
<td>N/A</td>
<td>N/A</td>
<td>-0.029 (0.871)</td>
<td>-0.429 (0.011)</td>
</tr>
<tr>
<td>O</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

- Lynx 3 and Lynx 5 data pooled
- Data from all four sites pooled

Table 3.10. Results from a rank-order Spearman’s correlation coefficient analysis (PROC CORR OUTS, SAS Institute, 1996) for the effects of pH and organic carbon (%) on the proportion of pine root tips colonized by ECM fungi. First value is the Spearman correlation coefficient (r), and the value in parentheses denotes statistical significance (i.e., p-value). Double numbers for Helen’s demonstrate that both transects sampled were PB. Ectomycorrhizal infection in transect O at Lynx 3 was zero, thus correlation coefficients could not be calculated. Unsampled transects are designated as N/A for “not applicable.” Transect abbreviations are: PB = pine-dominated forest to open barren gradient; OB = oak-dominated forest to barren gradient, and; O = open barren (no forested sample points). “Lynx 3 and Lynx 5 pooled” denotes that only data from all five transects were pooled for the correlation analysis; “Data from all four sites pooled” denotes that all data from all transects at all four sample sites (Lynx 3, Lynx 5, Helen’s Prairie, and the active pasture) were pooled for the correlation analysis.
CHAPTER 4

THE EFFECT OF THE SPATIAL DISTRIBUTION AND DISPERsal OF ECTOMYCORRHIZAL FUNGI ON THE INVASIVENESS OF VIRGINIA PINE (*PINUS VIRGINIANA* L.) IN RARE SERPENTINE BARRENS

ABSTRACT

Plant communities associated with serpentine soils include rare, endemic, and endangered plant species that have been the subject of important work on speciation, microevolution, and biogeography. Serpentine systems have been highly degraded and fragmented by human activity, and widespread invasion of serpentine grasslands by *Pinus* is a serious threat to the persistence of these biologically diverse ecosystems worldwide. Studies of plant species invasions rarely consider belowground mechanisms facilitating invasions despite that soil microbial community composition and activity can determine plant community structure and diversity. This is particularly true for mycorrhizas; however, most work on mycorrhizas continues to focus on nutrient dynamics of the root-mycorrhiza symbiosis. The current study was conducted to improve our understanding of the role of soil microbes in ecosystem invasions and to enhance our understanding of the importance of mycorrhizas at the ecosystem scale. Using Soldier’s Delight Natural Environment Area, a 770 ha serpentine barren complex in eastern Maryland, as a model system, I tested the hypothesis that the invasiveness of Virginia pine (*P. virginiana* L.)
into serpentine and other grasslands is regulated by the spatial pattern of ectomycorrhizal (ECM) fungi in the surface soil. The pattern of pine invasion in these barrens is also observed in other systems worldwide: encroachment by an ECM-dependent woody species is threatening plant communities previously dominated by arbuscular mycorrhizal plants. The invasiveness of ECM-dependent woody species is facilitated by colonization of AM-dominated soils by ECM inoculum, either in the form of fungal spores or hyphae. ECM inoculum disperses into areas devoid of ECM in two ways. Hyphal growth from the root mass of mature ECM pines colonizes seedlings germinating under them when seeds land in their shadow (dispersal by "contagion"). Alternatively, pine seedlings are infected after seeds land in open areas where spores are concentrated in the feces of animals that have consumed sporocarps (dispersal by "centers of infection"). In two serpentine outcrops at Soldier's Delight, I used a spatially explicit sampling design to collect soils from open barren areas devoid of pine invasion as well as across gradients from pine-dominated forest into open barren areas. After growing Virginia pine seedlings in field-collected soils in a greenhouse for 10 weeks, the proportion of root tips infected by ECM on each seedling was quantified and used to determine the degree of spatial autocorrelation of ECM inoculum potential among samples using semivariance analyses. Spatial mapping (kriging) was used to develop interpolation maps of ECM inoculum density across sampled transects. Results from semivariance and kriging analyses allowed me to quantify patterns of ECM fungal distribution and to illustrate mechanisms of ECM dispersal into open barren areas. Results from semivariance analyses showed strong spatial dependence of ECM inoculum potential among sampled points (range of structural variance, C/C+C₀: 0.75-1.0) and spatial structuring occurred on
scales from 0.66-16.49 m. In general, ECM inoculum was abundant in pine forests bordering open areas and in transitional zones between pine forests and open barrens, but was negligible at best in open serpentine areas. Combined with the lack of correlation between ECM inoculum potential and soil chemical properties, these findings strongly suggest that ECM fungal inoculum disperses into open barrens by contagion, thereby facilitating rapid pine colonization in an advancing front from mature pine forests bordering the barrens. Thus, current labor-intensive management techniques that use cutting and fire to curtail pine invasion may be ineffective because they do not kill ECM fungi or disrupt ECM mycelial mats on mature root systems that infect recolonizing pine seedlings. Mitigation of pine invasion may be more effective if managers trench soil along the perimeter of open barrens to excise ECM mycelial mats from their associated pine hosts, thereby rendering mycelial mats extending into open barrens unviable for incoming pine propagules. This technique could be effectively combined with progressive, direct removal of concentric circles of mature pines bordering the perimeters of prairie barren openings.

INTRODUCTION

Serpentine soils and the unique plant communities associated with them have been widely studied by ecologists and evolutionary biologists (Brooks 1987; Baker et al. 1992; Roberts and Proctor 1992). Serpentine soils have high heavy metal content, very low Ca:Mg ratios, low nutrient status, and high droughtiness and alkalinity (Brooks 1987). Studies of the dynamics of serpentine plant communities have enhanced our understanding of speciation (Stebbins 1942; Kruckeberg 1954; Wild and Bradshaw
Despite the harshness of serpentine soils, serpentine outcrops exclude common local species and support endemic and globally and regionally rare plant species (Harshberger 1903, Brooks 1987; Roberts and Proctor 1992). These plants are poor competitors under more benign soil conditions, but are superior competitors under the stressful edaphic conditions unique to serpentine because they have adapted drought and heavy metal (e.g., Ni, Mg, and Cr) tolerance and high nutrient-use efficiency (Harshberger 1903; Brooks and Yang 1984; Brooks 1987; Roberts and Proctor 1992). Further, recent work with a common mycorrhiza, *Cenococcum geophilum* Far. associated with Virginia pine on serpentine and non-serpentine soils shows that common ECM fungal symbionts have also adapted strategies for establishing and persisting in serpentine soils (Panaccione et al. 2001).

Habitat alteration, development, and mining have severely degraded and fragmented serpentine areas around the world, and many outcrops remain ignored or insufficiently protected (Wolf 2001). Small, fragmented serpentine outcrops have lower local species richness, more alien grasses, and more non-serpentine plant species than large outcrops (Harrison 1997, 1999). This problem is exacerbated by the widescale invasion of serpentine grasslands and savannas by *Pinus* (Richardson and Bond 1991; Richardson et al. 1994), which seriously threatens the persistence of these ecosystems worldwide by further fragmenting and reducing their expanse. For example, in some serpentine areas in the piedmont region of Maryland, more than 80% of serpentine grasslands and savannas have succeeded to closed-canopy stands of Virginia pine (*Pinus*...

Congruent with generalized concepts of woody invasion into grassland and savanna systems worldwide, invasion of serpentine barrens in the eastern United States by Virginia pine has been attributed to fire suppression and release from grazing (Tyndall 1992). But while invasion ecologists continue to focus almost exclusively on aboveground processes (e.g., DeSimone and Zedler 2001), very little is understood about the role of the soil microbial community in invasions, despite a growing body of evidence that the soil community influences plant interactions and may in fact determine plant community composition (Coleman et al. 1978; Crowell and Boerner 1988; Perry et al. 1989; Griffiths 1994; Jentschke et al. 1995; Laakso and Setala 1999; Wardle and Giller 1996; Packer and Clay 2000; Westover and Bever 2001). In this study I examine the role of the soil microbial community, and of mycorrhizal fungi in particular, on the invasiveness of Virginia pine in rare serpentine barrens in the piedmont region of the eastern United States.

Serpentine openings and the woody species that surround them are dominated by arbuscular mycorrhizal plant species. The successful colonization and establishment of *Pinus* in serpentine openings depends upon the presence of sufficient ECM inoculum to infect incoming *Pinus* seedlings. To characterize the distribution and dispersal of ECM inoculum in the serpentine openings at Soldier’s Delight, I quantified the density of ECM inoculum in open barren areas and along gradients of pine forest transitioning to open barrens using geostatistical semivariance analyses and interpolation mapping (described in detail below). I hypothesized that semivariance analyses and spatial maps depicting
high spatial heterogeneity, or having small patches of ECM inoculum scattered throughout barren openings devoid of ECM, would support a “centers of infection” model of ECM dispersal. In contrast, spatial maps and semivariance analyses supporting the “contagion” model of ECM dispersal would depict lower spatial heterogeneity, heterogeneity on a larger spatial scale, or a concentration of ECM inoculum in areas of heavy pine density and in transitional zones between pine-dominated and open barren areas, with little ECM inoculum in open barren areas.

METHODS

Description of the study site

The Soldier's Delight Natural Environment Area is a 770 ha serpentine grassland/savanna located 15 km west of Baltimore in Owings Mills, MD (Figure 4.1). Soldier’s Delight is one of the largest contiguous tracts of serpentine oak barren spanning the Piedmont Upland, which extends from Alabama to New Jersey. The site contains several plant communities, including barren openings dominated by prairie grasses (particularly little bluestem \( \textit{Schizachyrium scoparius} \) Michx. Nash), dense pine-\textit{Smilax} stands, mixed oak (especially \textit{Quercus marilandica} Muench.) and Virginia pine forests, and some black-oak \( \textit{Q. velutina} \) Lam.) dominated deciduous stands (Knox 1984; Panaccione et al. 2001).

Pine and eastern red cedar were not present in early land surveys or published aerial photographs of Soldier’s Delight (Shreve 1910), and oak was scattered or absent (Tyndall 1992). During Native American inhabitation, regular fires maintained the open oak barrens of Soldier’s Delight (Marye 1955a). After European colonization circa 1750
Porter, 1975, 1979), livestock grazing prevented woody species from entering openings and foresting the area (Marye 1955b). In 1910, over 80% of Virginia pine populations were located east of Baltimore, but by 1937 conifers were scattered in upland areas of Soldier’s Delight (Tydall 1992). Since then, the open serpentine barren areas of Soldier’s Delight have steadily declined due to aggressive invasion by Virginia pine (eastern red cedar is not a problem at this particular serpentine site, Tyndall 1992). In some places more than 80% of serpentine savannas have been replaced by closed-canopy pine or pine-cedar forests (Knox 1984; Tydall and Farr 1989; Tyndall 1992), and pine invasion has reduced the area of open savanna at Soldier’s Delight by 50% since 1938 (Tyndall 1992). The speed with which Virginia pine has formed closed canopy forests at Soldier’s Delight is remarkable; since 1970, even-aged stands of pine have become dominant in many openings, despite that most pines are about 55 years younger than their Quercus marilandica Muench. codominants (Knox 1984). Thus, rare serpentine plant populations are threatened with permanent loss from Soldier’s Delight due to consequences resulting from pine invasion such as shading, pine-induced changes in soil properties, and loss of serpentine habitat.

Management of Soldier’s Delight is overseen by the Maryland Natural Heritage Program of the MD Department of Natural Resources. To maintain the habitat for the prairie plants and several serpentine endemics and state and regionally very rare species that grow in these openings (Tyndall and Farr 1989), managers implement a large-scale cutting and burning program to curtail pine encroachment into open barrens under the assumption that pine cannot recover from burning. However, removing adult pines may not be sufficient to curtail their spread; the low-intensity fires used for management at
Soldier’s Delight are unlikely to kill the network of ECM fungal hyphae on living pine roots and in the soil, because soil microbes living 5-10 cm deep are killed only by high intensity or very hot fires (Jorgensen and Hodges 1970; Boerner et al. 2000), and in some areas ECM inoculum can persist even after severe fires (Amaranthus and Perry 1994). Thus, surviving ECM hyphae can colonize incoming pine seeds and can support the growth of resprouts from cut or burned trunks.

**Experimental design**

I tested which model of ECM inoculum dispersal (contagion or centers of infection) is operating at Soldier’s Delight by quantifying the spatial pattern of ECM fungal inoculum in two serpentine outcrops there, Areas F and G (Figure 4.2). In each opening two to three belt transects 3 m wide and 6-9 m long were randomly established moving from forested areas through the transitional ecotone into open serpentine outcrops (Figure 4.2). “Forested areas” refers to pine-dominated forest with some eastern red cedar and greenbriar. In both areas, one belt transect was established in open serpentine barren only (no forested samples). Hereafter, transects along gradients from pine-dominated forests to open barrens will be referred to as PB (for “pine-barren”), and transects in open barren areas as O (“open barren”).

In each belt transect, 2.5 cm-diameter soil cores were collected to 7.5 cm depth at 0.5-1 m intervals in a rectangular grid pattern; sampling included the A horizon in open barrens and the O<sub>h</sub>/A horizons in pine-dominated and transitional areas because fine mycorrhizal roots are most abundant in these horizons. Soil samples were returned to the laboratory at Ohio State University under refrigeration, where each sample was
transferred within 48 hours to its own 2.5 cm diameter x 5 cm long Conetainer® pot (Stuewe and Sons, Corvallis, OR). Soil in each Conetainer® pot was planted with three seeds of Virginia pine (Lawyer nursery, Plains, MT), and in the second week germinated seedlings were thinned to one seedling per pot. On each day of planting, pots containing only sterilized Sunshine® soil mix were seeded to serve as controls. Pots were randomized and watered with deionized water as needed and care was taken to prevent splashing of water and soil among pots. Pine seeds germinated within 10 days of planting in soils from both Areas F and G at Soldier’s Delight and control pine seedlings were not infected with ECM fungi.

Seedlings could not be grown in the greenhouse because insects infesting the greenhouse bays ate several seedlings in a prior similar experiment. Instead, seedlings were kept next to a large window at room temperature (not air conditioned) under typical summer day/night length in the laboratory, where they proved to be safe from herbivory. Pine seedlings were harvested after 8-9 weeks, rinsed carefully in a tap water bath, and refrigerated in a formalin:acetic acid:ethyl alcohol solution until analysis. Quantification of ECM infection was done using a dissecting microscope (x 10.5-60) by counting the number of root tips infected with ECM fungi on the basis of altered root morphology and the presence of a conspicuous mantle and/or fungal hyphae (Natarajan et al. 1992; Visser 1995; Gehring et al. 1998) based on the criteria of Ingleby et al. (1990). The number of root tips uninfected by ECM fungi was also recorded, as was the number of different ECM morphotypes on each seedling root system and at each study site. The range in the total number of root tips encountered on pine seedlings grown in soils from Area F was 1 to 124 (mean ± s.e.: 44.94 ± 4.15) and in soils from Area G the range was 3 to 143.
Throughout this paper I use the term infection instead of colonization to refer to the presence of a mycorrhiza on a root system to differentiate between individual root mycorrhizae and the migration of mycorrhizal fungi into a site (Allen 2001).

The abundance of ECM fungi is very closely linked to soil organic carbon (Harvey et al. 1979), and can also vary according to soil pH, moisture, and bulk density (Francis and Read 1994). Thus, to determine if fungal inoculum potential was correlated with soil chemical properties, each soil sample was analyzed for pH in 0.01M CaCl$_2$ in a 1:2 soil:solution ratio (Hendershot et al. 1993) and for total C and total N using Dumas combustion in a Carlo Erba 2100 NCS analyzer.

**Statistical analyses**

In any system structured by non-random processes, samples taken closer together are more likely to be positively correlated than samples taken farther apart. The degree to which ECM inoculum density and soil chemical properties exhibited non-random patterning was determined at the plot level (i.e., for PB and O transects) using semivariance analysis (GS* Version 2.0, Gamma Design Software, Plainwell, MI). Semivariance analysis is premised on the idea that if sample points show spatial patterning at the scale measured, then samples will be autocorrelated (Issaks and Srivastava 1989; Morris 1999). Semivariance analysis allowed me to quantify the degree of spatial autocorrelation in ECM root infection and soil chemical properties that existed among my samples, and to produce semivariograms that are used to convert the ECM infection and soil chemical data to spatially explicit contour maps using kriging. Kriging...
is an interpolation technique that uses the semivariograms to estimate levels of a measured parameter in areas between sample points. This technique was used successfully by Boerner et al. (1996) to quantify the spatial distribution of ECM inoculum in plant communities along a successional chronosequence, by Morris (1999) to evaluate spatial autocorrelation in soil microbial communities under trees in an Ohio deciduous forest, and by Choesin and Boerner (2000) to evaluate spatial patterning of soil and ground water chemical properties in an Ohio fen.

Because the assumption of independence between sample points was not met for use of parametric statistics, a non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute, 1996) was used to make stand-level comparisons in the proportion of root tips infected by ECM fungi, soil pH, total C, and total inorganic N among soil samples taken in open serpentine barren, pine forest, and transition areas from pine forest to open barren. Ryan-Einot-Gabriel-Welsch multiple F tests were used for means separation. To assess whether ECM infection intensity was correlated at the plot level with soil chemical properties, a Spearman’s non-parametric correlation analysis (PROC CORR OUTS, SAS Institute, 1996) was performed. Significance for all statistical analyses was determined at p<0.05.

RESULTS

Mycorrhizal infection

Five ectomycorrhizal morphotypes were found in both serpentine openings at Soldier’s Delight, and ECM species composition at both sites was strongly dominated by
two morphotypes (Table 4.1). The typically common ECM fungus *Cenococcum geophilum* Far. was found rarely and only in Area G.

In Area F, only 18% of pine seedlings grown in samples from the first pine-barren transect (PB1) were infected by ECM, and of infected seedlings, the range in the proportion of root tips infected was 0.1-0.41 (Figure 4.3). In the second pine-barren transect (PB2) in Area F, 39% of pine seedlings were infected by ECM, and of infected seedlings, the proportion of infected root tips ranged from 0.02-0.23 (Figure 4.3).

Twenty-three percent of pine seedlings grown in soil samples from the open serpentine transect (O) in Area F were infected with ECM and the proportion of infected roots tips ranged from 0.01-0.23 (Figure 4.3). However, when one highly-infected root system (proportion of root system infected = 0.23) was not included in analyses, the range for infected tips in the O transect of Area F was substantially lower (0.01-0.07).

In Area G, 27% of pine seedlings grown in the PB transect were infected with ECM, and of infected seedlings, the proportion of root tips infected by ECM ranged from 0.01-0.47. ECM fungi infected only 12.5% of pine seedlings grown in the O transect of Area G, and infection intensity was low, ranging from 0.02-0.08 (Figure 4.3).

**Spatial structure**

Semivariance analysis by GS+ produced significant linear-to-sill models for all variables in all five transects sampled in Areas F and G (Tables 4.2, 4.3). Root infection was strongly spatially structured in both serpentine openings, and model fit was moderate to strong. Spatial structuring of root infection in PB transects at both openings occurred on a small spatial scale (0.66 – 1.60 m) (Tables 4.2, 4.3). Spatial structuring of root
infection in O transects in both Areas F and G occurred on a larger spatial scale (16.49 m and 78.57 m) (Tables 4.2, 4.3), reflecting the sparse distribution of ECM inoculum in the O transects.

Interpolation maps of ECM inoculum density in PB1 in Area F show areas of high ECM density in the pine forest, with areas of low to intermediate ECM density in the transition area (Figure 4.4). In PB2, the pine forest contained spots of low inoculum density, but hot spots of inoculum were present in the transition zone. Although a very small amount of ECM infection occurred in the O transect in Area F, semivariance analysis did not interpret the amount to be significant; thus, the interpolation map of inoculum density produced for this transect showed no inoculum (Figure 4.4). ECM inoculum density was heavy and patchily distributed throughout the entire PB transect in Area G (Figure 4.5). As in transect O in Area F, semivariance analysis did not consider ECM inoculum density in the O transect in Area G significant enough to be reflected in the interpolation maps for this transect (Figure 4.5).

The interpolation map of ECM fungal inoculum in the open barren of Area F shows some areas of inoculum that appear as “donuts” on the map (see Figure 4.4). These “donuts” are an artifact of the kriging interpolation procedure that, when estimating values between measured sampled points, weights sample points closer to a measured point more heavily than sample points further away (Issaks and Srivastava 1989). This weighted search radius can produce the “donut” effect observed on some of my interpolation maps. Such artifacts are typical in geostatistical mapping procedures that use estimation techniques (Issaks and Srivastava 1989). Attempts to correct for these artifacts would be arbitrary and would make this study impossible to reproduce by other
researchers; thus, no attempt was made to correct them. Nonetheless, interpolated values are based solidly on data measured at sample points.

Spatial structuring of soil pH at both serpentine openings was strong and occurred a small to intermediate spatial scale (1.74 – 4.13 m) in all five transects in both openings, and model fit was generally good (Tables 4.2, 4.3). Soil pH was very patchy in the O transect in Area F and in both transects in Area G, and there was some evidence of reduced soil pH in pine-forested areas in both Areas F and G (Figures 4.6, 4.7).

Spatial structuring of total carbon and nitrogen was strong in all transects in both serpentine openings and occurred on a small to intermediate spatial scale (0.50 – 3.93 m) for both soil chemical properties (Tables 4.2, 4.3). Interpolation maps of total carbon show strong patchiness (Figures 4.8, 4.9). Total carbon in both areas was generally lower in O transects than in PB transects, but some areas of very high carbon occurred in the O transect in Area G (Figures 4.8, 4.9). Maps of total nitrogen also show strong patchiness, with the exception of the O transect in Area F (Figures 4.10, 4.11). Total nitrogen was generally lower in O transects in both serpentine openings, but some areas of very high nitrogen occurred in the O transect in Area G (Figures 4.10, 4.11).

Stand-level effects of sampling location on root infection and soil chemical properties

Root infection by ECM

For this analysis, soil samples were divided into three stand-level community types: open barren, transitional between pine and barren, and pine forest. Pine seedlings grown in soils taken from pine forest areas at both sites had higher rates of ECM
infection than those grown in soil taken from open and transitional areas, and open and transitional areas had similar levels of ECM infection (Tables 4.4, 4.5). The difference in ECM infection between open and transitional areas was substantially increased, however, when one datum reflecting unusually high ECM infection (proportion of roots infected = 0.23) was removed from the open barren data.

Soil chemical properties

In both serpentine openings, soil pH was consistently higher in O soils than in TP and P soils, while total carbon was consistently higher in TP and P soils than in O soils (Tables 4.4, 4.5). Total inorganic nitrogen in Area F was highest in TP areas, while in Area G total nitrogen was higher in TP and P soils than in O soils (Tables 4.4, 4.5).

Effects of soil chemical properties on ECM fungal infection potential

ECM fungal inoculum potential was not dependent upon any soil chemical properties measured in transects in either serpentine Area F or G, or when data from all five transects at both openings were combined. Correlations were very low and none were statistically significant (Table 4.6).

DISCUSSION

The pattern of pine invasion into serpentine barrens at Soldier’s Delight Natural Environment Area reflects that observed in other systems worldwide: encroachment by an ECM-dependent woody species is threatening plant communities that were previously dominated by arbuscular mycorrhizal plants. My goal in this study was to understand the
influence of the soil microbial community, and of ectomycorrhizal fungi in particular, on the invasiveness of Virginia pine in rare serpentine barrens. I also hoped to contribute to our understanding of the impact of ECM fungi on community-level processes, and to make recommendations to managers to address this impact for more effective long-term mitigation of pine recruitment into ecosystems of conservation value.

Ectomycorrhizal fungal inoculum was ubiquitous in pine-dominated and transitional areas in both serpentine openings sampled and ECM were notably absent from open barren (non-woody) areas. Both Areas F and G should be of high priority for active management, because pine forests and transitional areas between pines and open barrens at both sites contain ubiquitous ECM inoculum, often in high density. Spatial maps of ECM inoculum density in Area F depict very good ECM inoculum potential in pine forests and transition areas where inoculum potential drops off abruptly past the transition zone (~2 m into open barrens). The map of the PB transect in Area G shows several areas of very high ECM inoculum potential extending into open barren areas, because while the open barren end of this transect did not contain any pine trees, the relatively small size of the Area G opening necessitated locating the transect in a matrix of relatively heavy pine colonization. Taken in conjunction with the lack of ECM inoculum in open barren transects, these maps suggest that ECM are dispersed into open areas by contagion, and that this process has the potential to facilitate rapid and continued pine recruitment into open serpentine areas in the future. Support for the contagion model of ECM inoculum dispersal is especially strong in light of the lack of ECM inoculum potential in open barren transects in both Areas F and G. If pine colonization of open areas were facilitated by the dispersal of ECM spores into open areas via centers
of infection, I would expect ECM inoculum potential to be much higher in open areas, especially in areas of high organic matter content (Francis and Read 1994).

The results of my comparison of ECM inoculum potential between open, transitional, and forested sample points further supports the suggestion that ECM inoculum dispersal occurs by contagion, because pine forests had consistently higher ECM inoculum potential than open barren areas. The similarity in ECM inoculum potential between open and transitional areas in Area F was likely caused by one pine root system from an open barren that was highly infected (proportion of root system infected = 0.23). When this datum was removed from the analysis, transitional areas had higher mean root infection than open areas, but the difference was still not statistically significant. Similarly, in Area G, ECM inoculum potential was high even on the “open barren” end of the PB transect because the transect had to be located within a matrix of heavy pine colonization. Thus, because the soils sampled from the open barren end of this transect had heavy ECM inoculum potential and because they were treated as open barren sample points in the ranked ANOVA, no significant differences were detected between open and transitional areas in this outcrop.

Another of my objectives was to determine whether ECM distribution and abundance were dependent upon soil chemical parameters that are important for fungal proliferation. For example, ectomycorrhizal hyphae tend to proliferate in patches where organic residues are abundant (Francis and Read 1994) due to their unique ability to access nutrients bound in the soil organic matter fraction (Harvey et al. 1979; Carleton and Read 1991; Francis and Read 1994). The small scale at which spatial structuring of soil chemical properties occurs at these sites suggests that pH, total C, and total N are
controlled by small-scale microsite differences, e.g., the presence of ultramafic bedrock, different microbial communities associated with plant species rhizospheres, or other small-scale microsite differences. No correlation was observed between ECM inoculum potential and any of three soil chemical properties tested at these sites. This lack of correlation supports the suggestion that contagion accounts for ECM colonization of these sites rather than centers of infection. If isolated clumps of germinated ECM spores were important for large-scale infection of seedlings, I would expect a stronger relationship between ECM fungal distribution and the soil chemical properties that strongly influence nutritional growth requirements (e.g., soil organic matter).

The very heavy ECM inoculum potential in soil from one sample point in the open barren (O) transect of Area F suggests that occasionally ECM inoculum is present in high density in open barrens. The ECM inoculum in this spot may have been ECM basidiospores deposited in the feces of a fungivorous mammal or bird, or may have been hyphal outgrowth from an extensive root system of a nearby mature pine tree. Infection of seedlings by isolated clumps of ECM inoculum requires either that pine seeds land on areas of very high ECM basidiospore density within one growing season of their deposition in feces, or that ECM fungi at Soldier's Delight subsist saprophytically for some time without a host plant. The synchrony required for simultaneous deposition of seeds and spores may occur, but it is unlikely to account for heavy pine recruitment in the field. The number of spores required to infect one seedling in the field may be greater than $10^6$ (Castellano et al. 1985), even for ECM species like *Rhizopogon* that germinate readily from basidiospores. However, Kotter (1981) estimated that tassel-eared squirrels disseminated $2 \times 10^8$ - $1 \times 10^9$ fungal spores per gram of fecal material, although in
another similar study only 33% of pine seedlings inoculated with squirrel feces developed an ECM infection (Kotter and Farentinos 1984). In general, spores alone do not appear to have strong inoculation potential in the field, and their infection potential declines rapidly in the absence of host plants (Perry et al. 1989).

Further, it is unlikely that any ECM fungal spores germinating in the droughty, nutrient poor, alkaline soils of Soldier’s Delight are very successful saprophytically. Some ECM fungi may have limited saprophytic capability through extraction of nutrients from soil organic matter (Read 1988), but most ECM fungi probably cannot compete well with saprophytic fungi for carbon (Amaranthus and Perry 1994). Gehring et al. (1998) found that ECM species richness was not dependent upon soil or other environmental conditions, although species composition was dependent upon soil type. Villeneuve et al. (1989), using sporocarp surveys, found that ECM species richness was less sensitive to environmental stressors that reduce ecosystem productivity than saprophytic basidiomycete species (the applicability of this study to my results should be considered cautiously, however, since ECM basidiomycete sporocarps do not always accurately reflect ECM species composition or infection belowground (Gardes and Bruns 1996)).

Soderstrom and Read (1987) measured the respiratory output of the mycelium of a number of ECM fungal species before and after severing their connection to the plant and found that ECM fungal activity was almost completely dependent upon the supply of current carbon assimilate from the host plant. Although ECM species richness should not be confused with ECM infection potential, these studies suggest that while ECM fungal proliferation does depend somewhat on soil conditions, ECM inoculum density is controlled principally by an association with a host plant. More work is needed to
determine whether ECM fungal infection rates are decreased in harsh serpentine soils, although some research suggests that ECM fungi also have adapted to the unique stressors of serpentine (Panaccione et al. 2001).

Field observations of pine recruitment at Soldier’s Delight also suggest that ECM disperse by contagion (Tyndall 1992; personal observation). Colonization of open barrens is pronounced at pine forest edges, and in many small openings at Soldier’s Delight clumps of pines punctuate open areas. These clumps always appear even-aged, with the exception of one or two older pines in each clump (personal observation). Knox (1984) documented even-aged stands of pine at Soldier’s Delight, and invading pine saplings in serpentine outcrops in southeastern PA also grow clustered under established mature pine trees (Wallenstein 1996). Field studies have shown that germinating seedlings can be infected very rapidly by ECM fungi (Fleming 1985; Newton and Piggott 1991), and confirm laboratory observations that the fungi on already-established plants are largely responsible for the infection of new seedlings (Francis and Read 1994). While isolated clumps of pine in open barrens ostensibly suggest ECM disperse by centers of infection, it is more likely that the clumps result from the successful ECM fungal infection and establishment of an isolated pine seedling that later becomes the source of ECM hyphal propagules for incoming seeds (Finlay and Read 1986; Francis and Read 1994). As outcrops are reduced in size by pine encroachment from established pine forests, the probability of having ECM inoculum present even in areas devoid of pine trees increases due to the convergence of root systems and their associated fungal mycelia growing inward from the outcrop periphery.
Several tiny pine seedlings (~10-15 weeks old) were observed in the O transect of Area G—a transect that was conspicuously devoid of ECM inoculum. These seedlings were evaluated and found to be uninfected, thus their presence in open areas of Area G suggests that pines are capable of germinating in open areas without ECM inoculum. Most of these seedlings likely die in their second or third growing season if they do not encounter ECM propagules. If one or two isolated pine seedlings survive this initial recruitment period by encountering sufficient ECM propagules in open areas (e.g., from expansive root systems of mature pines), hyphal nets extending from these nascent foci may set in motion a contagion pattern of dispersal from one or two successfully established trees. It is well accepted that extensive ECM hyphal networks link plant root systems (Molina and Trappe 1982; Read et al. 1985; Read 1988; Molina et al. 1992; Perry et al. 1989; Amaranthus et al. 1990), and that hyphae on mature root systems aid in the establishment of neighboring linked seedlings by providing them with nutrients before their photosynthetic output can support the energy cost of an ECM association (Finlay and Read 1986; Amaranthus and Perry 1994). However, direct linkage is not necessary for germinating seedlings to benefit from the hyphal network on mature infected root systems, due to the ease and rapidity with which new ECM plants are infected when propagules are abundant (Amaranthus and Perry 1994; Francis and Read 1994). Spatial maps from Area G suggest that this is especially likely as serpentine outcrops become smaller, again because the area of serpentine openings devoid of ECM hyphae decreases as invading pines colonize from the outcrop periphery.

ECM fungi are often concentrated on surface roots just below the litter layer of host plants (Persson 1979). This affinity of ECM for soil conditions with heavier litter...
layers created by hosts with highly recalcitrant leaf litter such as oaks and pines suggests that pine recruitment will only improve as ECM-associated pine increases in abundance. Although it cannot be concluded with certainty that the pattern of lower pH and higher total carbon and nitrogen observed in pine-dominated and transitional areas of my study sites is caused by pine, it is likely that soil conditions in serpentine outcrops are indeed changing in response to pine colonization. If, as these and other data suggest (Barton and Wallenstein 1997), pine invasion is causing soil nutrient enrichment and acidification of serpentine openings, widespread pine colonization could reduce the competitive advantage of many serpentine endemic plant species. Increased competition with non-serpentine plants could threaten the persistence of serpentine plants and alter the structure and function of these unique systems irreversibly.

The low diversity of ECM fungal morphotypes at Soldier's Delight is not unusual. Many community- and ecosystem-level studies have found low ECM species diversity with domination by a few abundant species. Based on both morphological and molecular analyses, Gehring et al. (1998) found only 5.0 (± 1.73) ECM types in a pinyon pine forest on limestone-derived soils, with one to three dominant ECM types accounting for 33-53% of ectomycorrhizas across six different sites and two different soil types. Natarajan et al. (1992) found that one ECM fungus, Amanita muscaria, infected 45% of the roots tips of 17-year-old Pinus patula in plantations in India. Together with the current study, these results suggest that dominance by a few species of ECM at a site is common, regardless of whether ECM is typed using molecular or morphological techniques (Gehring et al. 1998).
Stand-level ECM diversity was lower at Soldier’s Delight than in a dolomitic oak-
cedar barren complex in southwestern Ohio also experiencing Virginia pine invasion,
where similar unfavorable soil conditions such as alkalinity and droughtiness occur
(Thiet and Boerner, manuscript in review; also see Chapter 3). The range of ECM
species responses to heavy metals varies widely and is poorly understood. In some
systems, ectomycorrhizas may form under heavy metal stress and confer metal tolerance
onto their host plants, but in other systems ECM may not form under heavy metal stress
(Harris and Jurgensen 1977; Jones and Hutchinson 1986). It is possible that the high
metal content of the Soldier’s Delight soils (Tyndall and Farr 1989; Panaccione et al.
2001) restricts ECM formation and exacerbates the common community dominance by a
few ECM species, in this case by the most metal tolerant ECM species. Thus, pines
growing at Soldier’s Delight may resist heavy ECM infection or may selectively promote
infection by ECM species with the lowest photosynthetic cost for conferring heavy metal
tolerance. This suggestion begs the question of host versus mycobiont control of the
symbiosis and more work is needed to characterize the nature of this relationship in a
variety of ecosystems and soil types.

Panaccione et al. (2001) documented genetic divergence of the ectomycorrhiza
Cenococcum geophilum Far. isolated from roots of Virginia pine growing on serpentine
soils at Soldier’s Delight in contrast to C. geophilum Far. from pines in non-serpentine
soils. The divergence may be an adaptive response to serpentine soils, which led the
authors to underscore appeals by other soil ecologists for the conservation of soil
microbial diversity across a range of ecosystem types (Beare et al. 1994; Gehring et al.
1998; Panaccione et al. 2001). I agree that ecosystem resistance and resilience is
promoted by conserving soil microbial diversity in addition to plant species assemblages. However, the pattern of invasion by ECM-dependent Virginia pine into unique AM-dominated ecosystems challenges us to consider that in some cases, preservation of diverse ectomycorrhizal assemblages may counteract management efforts to preserve diverse habitats for rare serpentine and other native or endemic prairie plants.

**Suggestions for management of Soldier’s Delight serpentine barrens**

Evidence from this work strongly suggests that colonization of open serpentine barrens at Soldier’s Delight by Virginia pine occurs primary by contagion, and would not be effective without the mycelial mat that extends from mature root systems of established pines. Thus, management strategies that address the spread of ECM fungal inoculum into areas of conservation value may be more effective and less labor intensive than current management techniques. I suggest that trenching around established pine forests would more effectively curtail pine recruitment into open barrens by breaking up the mycelial network that supports and infects encroaching seedlings.

Prior laboratory and field experiments suggest that this technique may work. Fleming (1983) found that mycelial strands severed from the roots of their hosts plants were not capable of infecting seedlings planted around established hosts, and Soderstrom and Read (1987) showed that ECM fungal respiration depends almost entirely upon photosynthetic input from host plants. Excised ECM hyphae remain viable for only 8 months after being severed from their hosts (Harvey et al. 1980; Ferrier and Alexander 1985). Clearly seedlings germinating below mature parent plants show strong association with the mycelial mats on parent plant root systems (Griffiths et al. 1991). Thus, in
addressing the invasion ecology of Pinus into these systems, the movement of Pinus propagules into open serpentine outcrops is less important than colonization of openings by ECM mycelial mats associated with the roots of mature pines bordering the outcrops. Thus, managers may more effectively mitigate pine invasion by targeting ECM fungal hyphal colonization of open barrens rather than by targeting mature pine trees themselves.

Mitigating ECM fungal hyphal movement into open serpentine barrens may be successful using a management strategy in which saplings and mature pine trees are progressively removed in concentric circles from the perimeters of prairie outcrops (R. Wayne Tyndall, Maryland Natural Heritage Program, personal communication). Combined with trenching, progressive removal of pines in concentric circles bordering openings would successfully reduce the expanse and infectivity of ECM mycelial mats associated with mature pines bordering serpentine openings. In contrast to the current removal method in which managers cut and burn entire pine stands, this concentric management method would save considerable money, time, and labor. It would also permit managers to target more openings more frequently than limited resources currently allow. By increasing the number of outcrops that are actively and effectively managed, managers will be able to incrementally expand and improve the continuity of the areas occupied by native serpentine plant communities before Virginia pine invasion eradicates them entirely.
CONCLUSIONS

The potential for managers and scientists to effectively contain and preempt invasions requires a sound and comprehensive understanding of both above- and belowground mechanisms facilitating the invasions. Examining the role of mycorrhizal fungi on community- and ecosystem-level processes is relevant when the pattern of invasion is characterized by the movement of a strongly ECM-dependent woody species like pine into previously AM-dominated ecosystems. The colonization of rare serpentine barren communities by pine may be regulated in large part by movement into these systems of ECM propagules in the form of hyphal inoculum networks resembling an advancing front. Thus, unless management efforts at these sites address the underlying issue of ECM fungal dispersal, current management efforts to eliminate pine and to conserve habitat for globally rare serpentine plant communities may be in vain.

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Figure 4.1. Serpentine sites of the Piedmont Upland Region of the Eastern United States. Arrows denote the general area where the current study took place at Soldier’s Delight Natural Environmental Area. Maps are adapted from Pearre and Heyl (1960).
Figure 4.2. Location of Areas F and G at Soldier's Delight Natural Environmental Area in Owings Mill, MD. Arrows denote serpentine openings sampled. Transect abbreviations are PB$_1$ and PB$_2$ = pine-dominated forests transitioning to open barren area and O = open serpentine barren (no forested samples).
Figure 4.3. Mean (+SE) proportion of root tips infected with ECM fungi in my Conecontainer® pots experiment. Transect type abbreviations are PB₁ and PB₂ = pine-dominated forest to open barren gradient and O = open barren (no forested sample points). No PB₂ was sampled in Area G due to its smaller size relative to Area F. Numbers above error bars are N.
Figure 4.4. Spatial maps of ectomycorrhizal inoculum density in Area F at Soldier’s Delight Natural Environmental Area, as interpolated from ECM fungal infectivity in my greenhouse Conetainer® pot experiment. Location abbreviations for transects are: PB₁ and PB₂ = pine-dominated forest transitioning to open barren; O = open barren (no forested samples). Circles inside transects represent pine trees. The legend applies to all three transects. Units on transect grids are meters.
Figure 4.5. Spatial maps of the ectomycorrhizal fungal inoculum density in Area G at Soldier's Delight Natural Environmental Area, as measured by the proportion of root tips infected by ECM in my Conetainer® pot greenhouse experiment. Location abbreviations are: PB = pine-dominated forest transitioning to open cedar barren and O = open cedar barren (no forested samples). Circles represent trees; black circles represent post oaks, grey circles represent pines. The legend applies to both transects. Units on transect grids are meters.
Figure 4.6. Spatial maps of pH in Area F at Soldier's Delight Natural Environmental Area. Location abbreviations for transects are: PB₁ and PB₂ = pine-dominated forest transitioning to open barren; O = open barren (no forested samples). Circles inside transects represent pine trees. The legend applies to all three transects. Units on transect grids are meters.
Figure 4.7. Spatial maps of pH in Area G at Soldier’s Delight Natural Environmental Area. Location abbreviations are: PB = pine-dominated forest transitioning to open cedar barren; O = open cedar barren (no forested samples). Circles represent trees; black circles represent post oaks, grey circles represent pines. The legend applies to both transects. Units on transect grids are meters.
Figure 4.8. Spatial maps of total carbon (%) in Area F at Soldier's Delight Natural Environmental Area. Location abbreviations for transects are: PB₁ and PB₂ = pine-dominated forest transitioning to open barren; O = open barren (no forested samples). Circles inside transects represent pine trees. The legend applies to all three transects. Units on transect grids are meters.
Figure 4.9. Spatial maps of total carbon (%) in Area G at Soldier's Delight Natural Environmental Area. Location abbreviations are: PB = pine-dominated forest transitioning to open barren and O = open barren (no forested samples). Circles inside and outside transects represent pine trees. The legend applies to both transects. Units on transect grids are meters.

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Figure 4.10. Spatial maps of total nitrogen (%) in Area F at Soldier's Delight Natural Environmental Area. Location abbreviations for transects are: PB1 and PB2 = pine-dominated forest transitioning to open barren; O = open barren (no forested samples). Circles inside transects represent pine trees. The legend applies to all three transects. Units on transect grids are meters.
Figure 4.11. Spatial maps of total nitrogen (%) in Area G at Soldier’s Delight Natural Environmental Area. Location abbreviations for transects are: PB = pine-dominated forest transitioning to open barren and O = open barren (no forested samples). Circles inside and outside transects represent pine trees. The legend applies to both transects. Units on transect grids are meters.
<table>
<thead>
<tr>
<th>Morphotype</th>
<th>Distinctive features</th>
<th>Abundance</th>
<th>Locations found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Black mantle; thick, long black hyphae; occur primarily on tips of lateral roots; root morphology branched or unbranched (probably <em>Cenococcum geophilum</em> Far.)</td>
<td>Rare</td>
<td>Area G: PB</td>
</tr>
<tr>
<td>2</td>
<td>Dark brown/black, long hyphae; hyphae envelop base of lateral roots; root morphology unbranched</td>
<td>Rare</td>
<td>Area F: O</td>
</tr>
<tr>
<td>3</td>
<td>Shiny, club-like white mantle; no apparent hyphal development; root morphology bulbous</td>
<td>Abundant</td>
<td>Area F: <strong>PB₁, PB₂, and O</strong>; Area G: PB and O</td>
</tr>
<tr>
<td>4</td>
<td>Shiny, translucent/white mantle; no apparent hyphal development; root morphology altered, often branched</td>
<td>Abundant</td>
<td>Area F: <strong>PB₁, PB₂, O, and pines</strong>; Area G: PB and O</td>
</tr>
<tr>
<td>5</td>
<td>White mantle; obvious fuzzy hyphal development around mantle; occurs characteristically on root tips; bulbous</td>
<td>Rare</td>
<td>Area F: <strong>PB₁</strong></td>
</tr>
</tbody>
</table>

Table 4.1. Ectomycorrhizal morphotypes observed in the serpentine barrens of Areas F and G at Soldier’s Delight Natural Environmental Area near Baltimore, MD. Transect abbreviations are: PB = pine-dominated forest transitioning to open barren; O = open serpentine barren.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Transect</th>
<th>Nugget variance ((C_0))</th>
<th>Total model variance ((C + C_0))</th>
<th>Structural variance ((C/(C + C_0)))</th>
<th>(r^2)</th>
<th>Range (m)</th>
<th>RSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop. roots infected</td>
<td>PB(_1)</td>
<td>0.0</td>
<td>0.007</td>
<td>1.000</td>
<td>0.886</td>
<td>1.6</td>
<td>7.31*E^-06</td>
</tr>
<tr>
<td></td>
<td>PB(_2)</td>
<td>0.001</td>
<td>0.004</td>
<td>0.750</td>
<td>0.554</td>
<td>0.66</td>
<td>4.63*E^-07</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.001</td>
<td>0.005</td>
<td>0.800</td>
<td>0.467</td>
<td>16.49(16.49)</td>
<td>2.43*E^-05</td>
</tr>
<tr>
<td>pH</td>
<td>PB(_1)</td>
<td>0.028</td>
<td>0.119</td>
<td>0.765</td>
<td>0.976</td>
<td>3.63</td>
<td>1.53*E^-04</td>
</tr>
<tr>
<td></td>
<td>PB(_2)</td>
<td>0.001</td>
<td>0.343</td>
<td>0.997</td>
<td>0.977</td>
<td>4.13</td>
<td>3.04*E^-03</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.003</td>
<td>0.019</td>
<td>0.842</td>
<td>0.761</td>
<td>1.91</td>
<td>8.10*E^-06</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>PB(_1)</td>
<td>0.207</td>
<td>0.758</td>
<td>0.727</td>
<td>0.915</td>
<td>1.33</td>
<td>8.70*E^-02</td>
</tr>
<tr>
<td></td>
<td>PB(_2)</td>
<td>0.122</td>
<td>0.864</td>
<td>0.859</td>
<td>0.829</td>
<td>1.53</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.099</td>
<td>0.414</td>
<td>0.762</td>
<td>0.770</td>
<td>1.27</td>
<td>9.069*E^-03</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>PB(_1)</td>
<td>0.001</td>
<td>0.005</td>
<td>0.816</td>
<td>0.568</td>
<td>0.78</td>
<td>0.178*E^-06</td>
</tr>
<tr>
<td></td>
<td>PB(_2)</td>
<td>0.001</td>
<td>0.008</td>
<td>0.900</td>
<td>0.908</td>
<td>2.76</td>
<td>4.535*E^-06</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.000</td>
<td>0.006</td>
<td>0.998</td>
<td>0.616</td>
<td>0(3.93)</td>
<td>2.180*E^-03</td>
</tr>
</tbody>
</table>

Table 4.2. Semivariance analysis of spatial structure in the proportion of Virginia pine roots infected by ectomycorrhizal (ECM) fungi, pH, total carbon (%), and total nitrogen (%) in my greenhouse study using soils collected at spatially-explicit intervals in transects in Area F at Soldier’s Delight Natural Environmental Area. Structural variance is the proportion of structural + nugget variance accounted for by spatial structuring. All models are linear-to-sill models where range (m) represents the distance at which the semivariogram asymptotes to the sill. Ranges with parentheses are for anisotropic models of best fit. Transect abbreviations are: PB\(_1\) and PB\(_2\) = pine-dominated forest transitioning to open barren; O = open barren.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Transect</th>
<th>Nugget variance $(C_o)$</th>
<th>Total model variance $(C + C_o)$</th>
<th>Structural variance $\frac{C}{C + C_o}$</th>
<th>$r^2$</th>
<th>Range (m)</th>
<th>RSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prop. roots infected</td>
<td>PB</td>
<td>0.0</td>
<td>0.011</td>
<td>1.000</td>
<td>0.917</td>
<td>1.60</td>
<td>8.45E-06</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.0</td>
<td>0.001</td>
<td>1.000</td>
<td>0.573</td>
<td>78.57</td>
<td>8.48E-07</td>
</tr>
<tr>
<td>pH</td>
<td>PB</td>
<td>0.023</td>
<td>0.145</td>
<td>0.841</td>
<td>0.780</td>
<td>1.74</td>
<td>1.92E-03</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.003</td>
<td>0.016</td>
<td>0.813</td>
<td>0.851</td>
<td>1.99</td>
<td>3.06E-06</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>PB</td>
<td>0.214</td>
<td>1.253</td>
<td>0.829</td>
<td>0.000</td>
<td>0.50</td>
<td>0.099</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.121</td>
<td>0.690</td>
<td>0.825</td>
<td>0.682</td>
<td>1.64</td>
<td>7.71E-03</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>PB</td>
<td>0.001</td>
<td>0.007</td>
<td>0.891</td>
<td>0.100</td>
<td>0.59</td>
<td>6.18E-06</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>0.001</td>
<td>0.005</td>
<td>0.816</td>
<td>0.778</td>
<td>1.95</td>
<td>6.48E-07</td>
</tr>
</tbody>
</table>

Table 4.3. Semivariance analysis of spatial structure in the proportion of Virginia pine roots infected by ectomycorrhizal (ECM) fungi, pH, total carbon (%), and total nitrogen (%) in my greenhouse study using soils collected at spatially-explicit intervals in transects in Area G at Soldier's Delight Natural Environmental Area. Structural variance is the proportion of structural + nugget variance accounted for by spatial structuring. All models are linear-to-sill models where range (m) represents the distance at which the semivariogram asymptotes to the sill. Ranges with parentheses are for anisotropic models of best fit. Transect abbreviations are: PB = pine-dominated forest transitioning to open barren; O = open barren.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Location</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>TP</td>
<td>P</td>
</tr>
<tr>
<td>Prop. roots infected</td>
<td>0.02 (0.01) a</td>
<td>0.02 (0.01) a</td>
<td>0.06 (0.02) a</td>
</tr>
<tr>
<td>pH</td>
<td>5.90 (0.02) a</td>
<td>5.42 (0.07) b</td>
<td>5.40 (0.10) b</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>4.57 (0.13) a</td>
<td>5.24 (0.16) b</td>
<td>4.96 (0.17) ab</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.43 (0.01) ab</td>
<td>0.46 (0.01) a</td>
<td>0.42 (0.01) b</td>
</tr>
</tbody>
</table>

Table 4.4. Results from non-parametric ANOVA on ranked root infection, pH, total C, and total N data (PROC RANK GLM, SAS Institute, 1996) for Area F at Soldier's Delight Natural Environmental Area, Owings Mill, MD. Numbers are mean (± SE) for all samples from that particular location; different letters indicate significant differences at p<0.05. Location abbreviations are: O = open barren samples; TP = transition areas between pine forests and open barrens; P = samples from pine-dominated forests.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Location</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O</td>
<td>TP</td>
<td>P</td>
</tr>
<tr>
<td>Prop. roots infected</td>
<td>0.02 (0.01) a</td>
<td>0.02 (0.01) a</td>
<td>0.05 (0.03) a</td>
</tr>
<tr>
<td>pH</td>
<td>6.15 (0.03) a</td>
<td>5.56 (0.13) b</td>
<td>5.78 (0.06) b</td>
</tr>
<tr>
<td>Total C (%)</td>
<td>4.40 (0.19) a</td>
<td>5.87 (0.40) b</td>
<td>5.45 (0.24) b</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.37 (0.02) a</td>
<td>0.48 (0.02) b</td>
<td>0.48 (0.02) b</td>
</tr>
</tbody>
</table>

Table 4.5. Results from non-parametric ANOVA on ranked root infection, pH, total C, and total N data (PROC RANK GLM, SAS Institute, 1996) for Area G at Soldier's Delight Natural Environmental Area, Owings Mill, MD. Numbers are mean (± SE) for all samples from that particular location; different letters indicate significant differences at p<0.05. Location abbreviations are: O = open barren samples; TP = transition areas between pine forests and open barrens; P = samples from pine-dominated forests.
Table 4.6. Results from a rank-order Spearman’s correlation coefficient analysis (PROC CORR OUTS, SAS Institute, 1996) for the effects of pH, total carbon (%) (TC), and total nitrogen (TN) (%) on the proportion of pine root tips colonized by ECM fungi. First value is the Spearman correlation coefficient (r), and the value in parentheses denotes statistical significance (i.e., p-value). Transect abbreviations are: PB1 and PB2 = pine-dominated forest to open barren gradient and O = open barren (no forested sample points). Unsampled transects are designated as N/A for “not applicable.”

<table>
<thead>
<tr>
<th>Transects</th>
<th>TC</th>
<th>TN</th>
<th>pH</th>
<th>OC</th>
<th>TN</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>pooled</td>
<td>0.086</td>
<td>0.059</td>
<td>-0.069</td>
<td>0.166</td>
<td>0.124</td>
<td>-0.146</td>
</tr>
<tr>
<td></td>
<td>(0.361)</td>
<td>(0.533)</td>
<td>(0.467)</td>
<td>(0.201)</td>
<td>(0.341)</td>
<td>(0.263)</td>
</tr>
<tr>
<td>PB1</td>
<td>0.104</td>
<td>0.087</td>
<td>0.151</td>
<td>0.046</td>
<td>-0.056</td>
<td>-0.009</td>
</tr>
<tr>
<td></td>
<td>(0.495)</td>
<td>(0.569)</td>
<td>(0.322)</td>
<td>(0.785)</td>
<td>(0.743)</td>
<td>(0.959)</td>
</tr>
<tr>
<td>PB2</td>
<td>-0.028</td>
<td>-0.065</td>
<td>-0.044</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>(0.858)</td>
<td>(0.676)</td>
<td>(0.778)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>0.065</td>
<td>0.066</td>
<td>-0.112</td>
<td>0.024</td>
<td>0.027</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>(0.752)</td>
<td>(0.748)</td>
<td>(0.587)</td>
<td>(0.911)</td>
<td>(0.900)</td>
<td>(0.425)</td>
</tr>
<tr>
<td>Areas F, G</td>
<td>0.112</td>
<td>0.094</td>
<td>-0.109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>combined</td>
<td>(0.139)</td>
<td>(0.217)</td>
<td>(0.151)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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CHAPTER 5

THE EFFECT OF BIOLOGICAL SOIL CRUSTS ON RAINWATER AND NITROGEN INFILTRATION IN AMERICAN BEACHGRASS (AMMOPHILA BREVILIGULATA FERNALD) STANDS ON LAKE MICHIGAN SAND DUNES

ABSTRACT

Colonization by American beachgrass (Ammophila breviligulata Fernald) is the principal mechanism by which coastal dunes are stabilized. Biological soil crusts composed of cyanobacteria and bryophytes often colonize spaces between beachgrass culms. To determine whether this crust affects beachgrass vigor, I conducted a greenhouse experiment utilizing intact soil cores from the Indiana Dunes National Lakeshore. I subjected crusted soil cores to artificial rainfall over a full growing season with rainfall patterns typical for the site and quantified the volume and N content of leachate in relationship to the degree of crust development, crust taxonomic composition, rainfall volume, rainfall intensity, light intensity, and the presence of plant litter. At rainfall volumes of <2.5 cm, little or no rainfall leached through the cores, regardless of crust cover or composition. Net N throughput significantly exceeded N inputs to cores in rainwater, clearly demonstrating that crust organisms and soil organisms associated with crusts contribute substantial inorganic N to this system. Ammonium-N inputs by crusts ranged from 0.063 kg NH$_4^+$-N ha$^{-1}$ yr$^{-1}$ (0.0063 g NH$_4^+$-N m$^{-2}$ yr$^{-1}$) to 1.91 kg NH$_4^+$-N ha$^{-1}$ yr$^{-1}$.
Nitrate-N inputs ranged from 0.091 kg NO$_3$-N (0.00914 g NO$_3$-N m$^{-2}$ yr$^{-1}$) to 6.101 kg NO$_3$-N (0.6101 g NO$_3$-N m$^{-2}$ yr$^{-1}$). Thus, total inorganic N inputs by biological soil crusts at the Miller Dunes ranged from 0.154 kg N ha$^{-1}$ yr$^{-1}$ to 8.011 kg N ha$^{-1}$ yr$^{-1}$. Crust composition alone did not affect the volume of rainwater or N that leached through cores; however, rainfall intensity, light intensity, and litter addition interacted with crust composition to affect rainwater and N leaching. High rainfall intensity resulted in greater leachate volume, but not leachate N, than low rainfall intensity, but very heavy (high volume) rainfall events resulted in substantial increases in N leaching. Less leachate moved through cores exposed to full sun, and crust composition interacted with light intensity to influence NH$_4^+$ leaching through cores. The addition of beachgrass litter to the surface of soil cores significantly increased the amount of N (particularly NO$_3$) in leachate. Biological soil crusts do not appear to compete with American beachgrass for water and N. Instead, these crusts, combined with different properties of the soil surface, substantially increase N inputs to this strongly water- and nutrient-limited sand dune ecosystem. Implications for American beachgrass persistence at the Indiana Dunes are discussed.

**INTRODUCTION**

Biological soil crusts significantly influence the physical and chemical properties of soils in arid and semiarid ecosystems by influencing soil water retention following rainfall, reducing wind and water erosion through soil particle stabilization (Belnap and Gillette 1998), producing chelating compounds that bind essential plant nutrients (Belnap 1994), and increasing carbon and nutrient inputs to vascular plants, soil fungi and
bacteria, and soil fauna (Millbank 1978, 1982; Rogers and Burns 1994; Dodds et al. 1995; Belnap 2001a). Biological soil crusts play a central role in the carbon and nitrogen dynamics of these systems due to the capacity for crust cover to approach 100% in plant interspaces, to photosynthesize, and to fix nitrogen (West 1990; Evans and Johansen 1999; Evans and Lange 2001). Many studies report increased nitrogen and mineral uptake and enhanced growth of vascular plants growing among N-fixing soil crusts (Mayland and MacIntosh 1966, Harper and Pendleton 1993, Belnap and Harper 1995). However, the high spatial and temporal variability of crust cover and crust species composition and functioning within and among ecosystems makes generalizations about the contributions of biological soil crusts to carbon and nitrogen dynamics difficult (Evans and Johansen 1999).

Most soil crust organisms are photosynthetic (e.g., phycobionts, cyanobacteria, bryophytes), thus their growth and activity are moisture, carbon, and nutrient limited (Lange 2001; Belnap 2001a). The functional responses of soil crusts to hydration and other abiotic conditions affecting crust functioning such as light intensity and temperature vary widely depending upon the composition and relative abundances of crust constituents and varying spatial and temporal environmental conditions (West 1990; Belnap 2001a; Lange 2001). For example, rainwater infiltration to the vascular plant rooting zone may be increased or decreased in crust-covered areas depending upon the specific biotic components of particular crusts (e.g. cyanobacteria vs. lichens and bryophytes; West 1990). In particular, lichen and bryophyte-dominated crusts absorb high volumes of surface water in their thalli upon rehydration after dessication (Lange 2001). Cyanobacterial-dominated crusts can reduce water infiltration to the rooting zone.
of vascular plants when their polysaccharide sheaths absorb water and swell during rainfall events (Kidron and Yair 1997; Edlridge et al. 2000).

A growing body of evidence demonstrates that soil community structure and functioning can influence and even determine the outcome of plant competition and coexistence (Bever et al. 1997; van der Heijden et al. 1998a,b; Westover and Bever 2001). Most work on biological soil crusts has focused on their influence on soil physical and chemical properties, particularly in desert ecosystems (West 1990; Evans and Johansen 1999). While it is generally believed that biological soil crust cover is positively correlated with vascular plant cover (Kleiner and Harper 1977, Graetz and Tongway 1986, Mucher et al. 1988; but see Eldridge 1993), a clear understanding of the influence of biological soil crusts on vascular plant communities in various ecosystem types is still lacking.

Biological soil crusts on sand dunes are dominated by large filamentous cyanobacteria (e.g., Lyngbya, Microcoleus) that can survive and disperse after sand covering by gliding to the soil surface. Although work on soil crusts of sand dune systems is limited, existing studies have focused exclusively on the impact of crust organisms on water infiltration. Typically soil crusts in very sandy soils (>80% sand) decrease water infiltration through the soil profile by absorbing large quantities of water (Yair 1990; Mazor et al. 1996; Kidron and Yair 1997; Edlridge et al. 2000). Despite sand dune systems being strongly nutrient limited, I am aware of no research in sand dune systems that has evaluated the influence of biological soil crusts on nutrient movement through the soil profile.
Active dune ecosystems are ideal for studies of plant succession because they are continually retrograding to early successional stages, and thus sand dune systems exist as a 'landscape mosaic' of various successional stages occurring simultaneously. Early studies at the Indiana Dunes National Lakeshore have contributed substantially to the development of theoretical models of plant community dynamics and succession (e.g. Hill 1896; Cowles 1899, 1901; Lyon 1927; Olson 1958), and work on plant succession continues there currently (Poulson 1999; Poulson and McClung 1999).

I conducted this study to expand our understanding of the potential influence of soil crust organisms on plant community dynamics in this well-studied sand dune system where soils in general are understudied and where biological soil crusts in particular have been observed but whose role in ecosystem structure and functioning has been ignored. By evaluating the influence of biological soil crusts on rainwater and nutrient infiltration through the soil profile, this study has the potential to shed light on the role of soil crusts in shaping the outcome of vascular plant interactions on leeward dunes and in pannes where crusts are prevalent. I hope the current study will inspire more work on the relationship between the soil community and vascular plant dynamics at the Indiana Dunes and in other dune ecosystems.

BACKGROUND

Located in northwestern Indiana, the Indiana Dunes National Lakeshore (IDNL) is a system of active sand dunes that has been the subject of botanical and ecological studies since the late 1800's (Figure 4.1; Hill 1896, Cowles 1899, 1901, Lyon 1927). Plant community composition at IDNL is characterized by heavy American beachgrass...
(Ammophila breviligulata Fernald) colonization of fore- and mid-dunes, which coexists with even-aged cohorts of cottonwood (Populus deltoides Marshall); these two perennial pioneer species play an important role in dune formation. On middunes beachgrass and cottonwood give way to perennial grasses and forbs (e.g., Solidago sp., Andropogon gerardii Vitman, Panicum sp.), jack pine (Pinus banksiana Lam.), black oak (Quercus velutina Lam.), and beech-oak-maple (Fagus-Quercus-Acer) mixed deciduous forests further inland (Olson 1958; Poulson 1999; Poulson and McClung 1999).

Studies of plant-soil relationships at IDNL have been cursory and biased in favor of aboveground processes. While work at IDNL has contributed substantially to our understanding of plant community succession, most attention to soils in dune systems has focused exclusively on the movement of sand across the dune system, or on the accumulation of organic matter and nutrients below isolated clumps of plants (Cowles 1899, Olson 1958, Poulson 1999). For example, Cowles (1899) suggested that humus accumulation and decomposition of litter of early successional species (e.g., American beachgrass, perennial grasses) improved soil conditions for incoming later successional species (e.g., pine, black oak, maple, and beech). Olson (1958) also suggested that organic matter deposition and decomposition drove successional relationships among later successional species. He attributed soil development to organic matter deposition by American beachgrass, believing this facilitated cottonwood establishment and other woody species establishment. In successional studies of sand dunes at Wilderness State Park, Michigan, Lichter (1999) suggested that environmental constraints during early succession include physical stresses related to soil properties, especially sand movement and nutrient competition. He also noted the presence of soil lichens and mosses on
northern Michigan dune soils, but his work did not assess their functional significance in the system.

In a recent study at the Miller Dunes at IDNL, Poulson and McClung (1999) observed a severe decline in American beachgrass vigor on the backs of foredunes, which they attributed to lack of covering by windblown sand. Several other authors have documented beachgrass decline soon after sand accumulation ceases (Marshall 1965; Hope-Simpson and Jefferies 1966; Huiskes 1979). Poulson and McClung (1999) also documented the presence of algae and mosses on the soil surface in this area, as well as elevated nutrient status (K and P) and CEC, which they attributed to nutrient leaching from a nearby slag heap (Poulson and McClung 1999). They suggested that where the lack of covering by windblown sand leads to American beachgrass decline and nutrient status is higher, non-dune ruderals invade the backs of foredunes and preclude further succession to dune perennials (e.g., *Andropogon gerardii* Vitman), jack pine and black oak (Poulson and McClung 1999). Further, regularly occurring fires have the potential to increase colonization of the dunes by later successional species where American beachgrass decline increases litter fuel loads (Poulson 1999; Poulson and McClung 1999).

American beachgrass decline changes successional dynamics at the Miller Dunes by intensifying the effects of fire and speeding succession toward invasion by non-dune species, and I agree that lack of sand accumulation on the backs of foredunes may in part account for beachgrass decline. Because cyanobacterial and bryophytic biological soil crusts have been observed among declining beachgrass ramets, I was interested in testing whether these soil crusts may contribute to American beachgrass decline. Cyanobacterial
crusts may compete with vascular plants for water and nutrients (e.g., P and Fe; Belnap et al. 2001), particularly in sandy soils where nutrients and minerals are highly limiting (Black 1968; Warren 2001). In sandy soils, cyanobacteria-dominated crusts typically decrease rainfall infiltration because their polysaccharide sheaths can absorb water up to 12 times their dry weight and increase their volume up to 10 times (Wang et al. 1981; Brotherson and Rushforth 1983). In contrast, moss-dominated crusts tend to increase rainfall infiltration in sandy soils (Brotherson and Rushforth 1983; Warren 2001), perhaps because their leaf architecture channels water toward the center of the plant (Eldridge 2001).

The goal of this study was to evaluate the impact of biological soil crusts and surface soil characteristics at IDNL on water and inorganic nitrogen infiltration through the soil profile in American beachgrass stands. I hypothesized that the presence of biological soil crusts would decrease rainfall and nutrient leaching through the soil profile, particularly in crusts dominated by cyanobacteria, by intercepting water and nutrients in this highly water and nutrient limited environment. My objectives were: (1) to characterize the species composition of the biological soil crusts at the Indiana Dunes National Lakeshore; (2) to determine whether crust species composition affects leachate and nitrogen infiltration through the soil profile; (3) to determine whether light intensity, rainfall intensity, and litter addition affect leachate and nitrogen infiltration and; (4) to determine whether crust composition and surface soil characteristics interact to affect leachate and nitrogen infiltration.
METHODS

Description of the study site

I conducted this experiment with biological soil crusts and soils collected from the Miller Dunes, a dune complex in northwestern Indiana on the southern shore of Lake Michigan, approximately 1 km north of Miller, IN (Figure 5.1). The Miller Dunes occur within a mosaic of non-contiguous land owned by the National Park Service making up the Indiana Dunes National Lakeshore (Figure 5.1). The area sampled is owned by the city of Miller, is not developed or managed, and is subject to severe disturbances such as dumping, ATV use, foot traffic, arson, and lightning fires.

Sampling method and experimental design

In June, 2000, 90 soil cores were collected randomly from a population of mixed cyanobacterial and bryophytic soil crusts in an area of the Miller Dunes dominated by American beachgrass and free from human disturbance. Six of the 90 cores collected had no apparent cyanobacterial or bryophytic growth on them and served as controls. Samples were collected to a depth of 7 cm using 5 cm-diameter transparent plastic soil sleeves and returned intact to a greenhouse at the Ohio State University. A pilot study conducted in 1999 revealed that 7 cm was the optimal depth for obtaining leachate under various rainfall and crust cover regimes.

In the greenhouse, intact crusted soil cores were kept in the cylindrical soil sleeves and suspended 8 cm above a greenhouse bench by affixing the cores into holes cut in wax cardboard boxes (Figure 5.2). To prevent loss of the soil core through the bottom of the soil sleeve, layers of sterilized bridal veil and window screen were affixed
to the bottom of the sleeve with rubber bands. This layering method prevented loss of sand grains but allowed leachate to pass through the sleeves. The 90 cores were distributed randomly on the bench along no apparent temperature or moisture gradients and rotated weekly (Figure 5.2). Mean temperature in the greenhouse over the course of the experiment was 25.2 °C (range: 15.3 – 48.7 °C) and mean humidity was 79.18% (range: 53 - 97%).

The experiment was set up as a full factorial design with cores randomly assigned to binary light intensity, rainfall intensity, and litter addition treatments. At Miller Dunes, crusts establish between beachgrass ramets where they are totally exposed to full sunlight as well as below beachgrass clumps where they are shaded. I simulated these two light intensities in the greenhouse by placing 45 of the 90 cores under a shade cloth under which incident photon flux densities (PPFD) were approximately 200 μM s⁻¹ m⁻², equal to the average incident photon flux density measured under beachgrass ramets in the field by a Li-Cor quantum sensor. PPFD under full sun in the greenhouse were approximately 1630 μM s⁻¹ m⁻² (Figure 5.2).

Biological soil crusts function differently under different levels of hydration, light intensities, and temperatures, but hydration is the primary limiting factor for crust activity (Lange 2001). Many studies have assessed the impact of biological soil crusts on water infiltration. However, only one study in North America has evaluated the effect of rainfall intensity on the relationship between biological soil crusts and water infiltration (Faust 1971; Warren 2001) and I am aware of no work that has evaluated the effect of rainfall intensity on the relationship between soil crusts and nutrient infiltration.
Thus, to simulate different rainfall intensities that occur in Lake Michigan dune systems, 45 of the crusted soil cores were randomly assigned to receive rainfall simulating frontal storm events while the other 45 received rainfall simulating convective storms. 'Raining' was simulated to reflect the precise rain amount recorded at the Miller Dunes during the months of April and May, 1995; periods of dessication occurred between rainfall events on days that no precipitation was recorded (National Atmospheric Deposition Program 1995). During each rainfall event, crusts subjected to convective and frontal 'storm' events received the same amount of rain but at different intensities: convective 'storms' consisted of raining the full amount on crusts at one time (e.g., 1.5 cm s\(^{-1}\)), and crusts subjected to frontal 'storms' received rain intermittently over the period of 8-15 hours (e.g., 0.188 cm s\(^{-1}\)-0.1 cm s\(^{-1}\)). Rainfall was simulated with a pipettor using a Hubbard Brook rainfall recipe that delivers 0.17 mg l\(^{-1}\) NH\(_4\)-N and NO\(_3\)-N (Likens et al. 1996).

In the field, I observed that some crusted areas were overlain with beachgrass litter while others were not. To simulate the potential effects of this litter on water and nutrient leaching, I added 3 g of field-collected beachgrass litter on the soil crust surface to 28 randomly chosen crusted soil cores.

After each simulated rainfall event, leachate that infiltrated through each crusted soil core was collected in a sterilized beaker placed below each suspended soil sleeve (Figure 5.2). The volume of leachate from each core was measured and the leachate was stored in an airtight plastic bottle, dated, and frozen until nitrogen analyses were performed. NH\(_4\)-N and NO\(_3\)-N in each leachate sample were analyzed on an ELx800 microplate reader (Bio-Tek Instruments 2000). Net NH\(_4\)-N and NO\(_3\)-N leached through
each core during each rainfall event were calculated by subtracting the amount of nitrogen introduced in rainwater to the cores during that particular rainfall event.

After the greenhouse experiment was concluded, each crust was analyzed to quantitatively determine the relative percent cover of cyanobacteria and moss using a 5 cm-diameter circle drawn on a petri dish and divided into a grid of 3 x 3 mm squares. The grid was placed over each 5 cm-diameter crust, each 3 x 3 mm square was recorded as either cyanobacteria, moss, or open sand, and the percentage of each of the three crust constituents was calculated for each crust. This allowed me to obtain continuously distributed estimates of the percent cover of cyanobacteria and moss in each crust. I am confident that little bias was introduced by my two-dimensional quantification of crust constituents because preliminary dissections revealed little or no layering of organism types, i.e., areas of crusts with high moss density were not underlain by cyanobacteria and vice versa. After quantifying the relative percent cover of cyanobacteria and moss in each crust, a representative sample of crusts was dissected in a Calgon®-water surfactant solution to microscopically identify and taxonomically characterize the cyanobacterial and bryophytic crust organisms.

Data analysis

Leachate amount, NH$_4^+$, and NO$_3^-$ were summed for each crust to achieve a cumulative measurement of each response variable over the growing season and these cumulative data were used for statistical analyses. The activity of biological soil crusts is highly sensitive to temporal and spatial changes in dessication and hydration and the responses of crusts to these forces vary by crust composition and cover. Thus, using
cumulative numbers allowed us to evaluate how crusts were functioning through the series of dessication and rehydration events I subjected them to over the course of the entire growing season. Further, summing raw data allowed me to effectively use conventional statistical analyses despite my experimental units being subjected to repeated measures.

Data were non-normally distributed due to high NH$_4^+$ and NO$_3^-$ leaching during heavy rainfall events and data transformation did not improve normality. Thus, non-parametric methods were used to assess the impact of crust composition, light intensity, litter addition, and rainfall intensity on leachate volume and nitrogen infiltration. Non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute, 1996) was used to determine the effects of crust composition, the binary variables, and their interactions on leachate amount and nitrogen infiltration. Non-parametric Spearman’s correlations (PROC CORR OUTS, SAS Institute, 1996) were used to determine whether crust cover composition and the binary treatment variables were correlated with the response variables leachate volume, NH$_4^+$ and NO$_3^-$. Although I was able to prove that putative outlier data (n=4 for NH$_4^+$ and n=7 for NO$_3^-$ out of 540 data) were indeed statistical outliers (Dixon 1953), I analyzed data and report results both with outlier data included and excluded because I believe these 11 outlier points are biologically significant. Statistical significance was determined at p<0.05 unless otherwise noted.
RESULTS

Crust species composition

Biological soil crusts at the Miller Dunes were composed of cyanobacteria, bryophytes, and mixed cyanobacteria-bryophytes. Cyanobacterial crusts were strongly dominated by the nitrogen-fixing filamentous cyanobacterium *Lyngbya* spp. (Table 5.1). *Lyngbya* spp. were highly abundant in all crusts with cyanobacteria and I observed strong physical binding of sand particles by the polysaccharide sheaths of these species. Other common cyanobacteria observed were heterocystous *Nostoc* and *Microcoleus* spp. Consistently present but less common were the non-heterocystous nitrogen-fixing cyanobacterium *Chroococcus* sp. and the green alga *Klebsormidium* sp. (formerly *Hormidium* sp.) (Table 5.1). Moss spores and protonemata were present among the cyanobacteria but were not abundant.

Bryophytic crusts were dominated strongly by the common weedy moss *Ceratodon purpureus* Snow, and small patches of *Sphagnum* were interspersed throughout some *C. purpureus* crusts. Crusts made up of only dense mats of mosses had no cyanobacteria present (e.g., on top of or beneath moss gametophytes), however most of the crusts had a mixture of both cyanobacteria and mosses on the soil surface. I observed some moss gametophyte growth atop cyanobacterial crusts over the course of my 10-week experiment. No moss gametophytes germinated over the course of my experiment in control cores or on small areas of open sand in some crusted cores.

Dissection of crusts revealed that three of the six control (i.e., uncrusted) soil cores had sparse communities of cyanobacteria approximately 3 mm below the sand surface, but it is impossible to discern whether this growth occurred prior to or during my
experiment. Cyanobacterial species composition in these three cores was the same as in crusts with abundant cyanobacteria on the soil surface, but the density of the cyanobacteria in control cores was very low relative to crusts without windblown sand coverage.

**Rainwater infiltration**

Over the course of the experiment 27.39 cm (495.68 ml) of rainwater were added to crusts and the cumulative infiltration through crusts over the growing season ranged from 3.06 – 7.81 cm (55.30-141.40 ml). Cumulative infiltration was higher during high volume (not to be confused with high rainfall intensity) storm events than low volume storm events at all crust composition levels (Figure 5.3), and percent cover of cyanobacteria or mosses alone did not affect cumulative leachate amount over the growing season (Figure 5.4).

Light intensity significantly affected leachate amount regardless of crust composition or percent cover. Crusts exposed to full sunlight leached significantly less volume over the growing season than those in shade (Figure 5.5, Table 5.2). Rainfall intensity also had a significant impact on leachate volume, with high intensity ‘frontal’ storms resulting in significantly higher cumulative leachate over the growing season (Figure 5.6, Table 5.2). In addition, leachate amount was affected by the interaction between rainfall intensity and percent cover of both cyanobacteria and mosses. Specifically, as percent cover of cyanobacteria decreased and percent cover of mosses increased, high rainfall intensity increased infiltration while low intensity rainfall
decreased infiltration (Figure 5.6, Table 5.2). Litter addition had no significant effect on infiltration amount regardless of crust composition or percent cover (Figure 5.7, Table 5.2).

**Inorganic nitrogen infiltration**

Overall, net N infiltration exceeded N inputs to crusts in rainwater. Total NH$_4^+$ and NO$_3^-$ inputs to crusts over the growing season were 0.084 µg each, while NH$_4^+$ and NO$_3^-$ throughput in leachate ranged from 2.83 - 92.70 µg and 15.27 - 1019.73 µg, respectively. Cumulative N infiltration through control crusts also exceeded N inputs, but NH$_4^+$ and NO$_3^-$ infiltration through control crusts only ranged from 3.67 - 5.96 µg and 82.13 - 244.45 µg, respectively.

Cumulative N infiltration was higher during high volume storm events (range: 2.83 - 92.70 µg for NH$_4^+$ and 15.27 - 1019.73 µg for NO$_3^-$) than low volume storm events (range: 0.00 - 4.55 µg for NH$_4^+$ and 0.00 - 141.17 µg for NO$_3^-$) at all crust composition levels, especially for NO$_3^-$ (Figures 5.8, 5.9). The effect of different rainfall volumes on nitrogen infiltration was decreased when outlier data reflecting heavy nitrogen leaching on two days with very high rainfall volume were removed from the analysis, but the trend of higher inorganic N infiltration during high volume rain events remained the same.

When considered alone, percent cover of cyanobacteria had a significant impact on NO$_3^-$ infiltration but not on NH$_4^+$ infiltration, and percent cover of mosses had no significant effect on either NH$_4^+$ or NO$_3^-$ infiltration (Figures 5.10, 5.11, Table 5.2). However, when outlier data points were removed, percent cover of cyanobacteria no longer had a significant impact on NO$_3^-$ infiltration (Table 5.2).
Light intensity had a significant effect on \( \text{NH}_4^+ \) infiltration (at \( p < 0.10 \)), but not on \( \text{NO}_3^- \) infiltration in all crust types (Figures 5.12, 5.13, Table 5.2) both when outlier data were included and omitted (Table 5.2). As the percent cover of cyanobacteria increased and percent cover of mosses decreased, shaded crusts had increased \( \text{NH}_4^+ \) leaching while crusts exposed to full sun showed decreased \( \text{NH}_4^+ \) leaching; however, interactions between light intensity and crust composition and percent cover were not significant (Table 5.2).

In general, rainfall intensity had no impact on inorganic N infiltration, and interactions between rainfall intensity and crust composition and percent cover were not significant (Figures 5.14, 5.15, Table 5.2). When outlier data were included in the analysis, crusts subjected to low intensity rainfall leached more \( \text{NH}_4^+ \) than crusts subjected to high intensity rainfall (at \( p < 0.10 \)) as the percent cover of mosses increased, but this difference was not significant when 11 outliers were omitted (Table 5.2). Rainfall intensity had no significant effect on \( \text{NO}_3^- \) leaching, with or without the outliers (Table 5.2). Interactions between rainfall intensity and crust composition and percent cover were not significant.

The addition of litter to the surface of crusts significantly increased \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) leaching at all levels of crust composition and percent cover (Figures 5.16, 5.17, Table 5.2). When 11 outlier data were removed from the analysis the effect of litter addition on \( \text{NH}_4^+ \) leaching was not significant but the effect of litter on \( \text{NO}_3^- \) leaching was unchanged (Table 5.2). A significant interactive effect on \( \text{NO}_3^- \) occurred between litter addition and the percent cover of cyanobacteria in crusts, both when outlier data were included and omitted (Figures 5.16, 5.17; Table 5.2).
Correlation analyses

Results from non-parametric Spearman's correlations (PROC CORR OUTS, SAS Institute 1996) with outliers included showed that leachate amount was positively but very weakly correlated with percent cover of cyanobacteria (Table 5.3). NO$_3^-$ infiltration was negatively but weakly correlated with percent cover of cyanobacteria, and NH$_4^+$ was positively correlated with leachate amount both when outliers were included and omitted from the analysis. When outliers were removed from the correlation analysis, NO$_3^-$ was positively correlated with leachate amount (Table 5.3).

DISCUSSION

Crust contribution to ecosystem N budget

This study clearly demonstrates that biological soil crusts on surface soils at the Indiana Dunes National Lakeshore contribute substantial N inputs to this highly nutrient-limited ecosystem. On an ecosystem scale, NH$_4^+$-N inputs by crusts ranged from 0.063 kg NH$_4^+$-N ha$^{-1}$ yr$^{-1}$ (0.0063 g NH$_4^+$-N m$^{-2}$ yr$^{-1}$) to 1.91 kg NH$_4^+$-N ha$^{-1}$ yr$^{-1}$ (0.191 g NH$_4^+$-N m$^{-2}$ yr$^{-1}$). Nitrate-N inputs ranged from 0.091 kg NO$_3^-$-N (0.00914 g NO$_3^-$-N m$^{-2}$ yr$^{-1}$) to 6.101 kg NO$_3^-$-N (0.6101 g NO$_3^-$-N m$^{-2}$ yr$^{-1}$). Thus, total inorganic N inputs by biological soil crusts at the Miller Dunes ranged from 0.154 kg N ha$^{-1}$ yr$^{-1}$ to 8.011 kg N ha$^{-1}$ yr$^{-1}$.

Estimates of rates of N input by biological soil crusts at IDNL approximate and in some cases exceed rates of N input recorded in other ecosystems. Rychert and Skujins (1974), Rychert et al. (1978), and West and Skujins (1977) estimated nitrogen fixation rates of 10 to 100 kg N ha$^{-1}$ yr$^{-1}$ in cold deserts. Jeffries et al. (1992) estimated that crusts
contribute 0.7 to 3.6 kg N ha\(^{-1}\) yr\(^{-1}\) for various areas of the Colorado Plateau. Estimates of N inputs by crusts in the Sonoran desert and Australia range from 7 to 18 kg N ha\(^{-1}\) yr\(^{-1}\) and 1.3 kg N ha\(^{-1}\) yr\(^{-1}\), respectively (Rychert et al. 1978; Evans and Johansen 1999). Rates of crust-mediated N fixation and ecosystem inputs do not necessarily indicate that all fixed nitrogen is available to plants and microorganisms, i.e., all fixed N is not necessarily retained in the nitrogen pool of the system (West 1990; Evans and Johansen 1999). Considerable N is lost via denitrification and volatilization (see discussion below); however, in the current study N was measured after it had leached through the soil profile to a depth of at least 7 cm. This suggests that my estimates of crust-mediated N inputs to soils at IDNL reflect available N for potential plant and microbial immobilization. Further studies are needed to quantify the relative amounts of fixed N contributed by soil crusts in this ecosystem that are assimilated by vascular plants, bryophytes, and soil organisms.

Evans and Ehleringer (1993) used stable isotopes (\(\delta^{15}N\)) to quantify the relative contributions of atmospheric and soil crust-mediated N to a pinyon-juniper ecosystem in southeastern Utah. They determined that although a substantial amount of crust-fixed inorganic N was lost by denitrification and volatilization, crust organisms still contributed the majority of total N input to this system (Evans and Ehleringer 1993). Rates of N addition to eastern forests by dry and wet atmospheric deposition are estimated at < 2 kg N ha\(^{-1}\) yr\(^{-1}\) in areas unaffected by high industrial inputs, and up to 40 kg N ha\(^{-1}\) yr\(^{-1}\) in areas with heavy atmospheric N pollution (Aber et al. 1989). Wet N deposition for the state of Indiana is estimated at approximately 3 to 3.5 kg NH\(_4^+\)-N ha\(^{-1}\) yr\(^{-1}\) and 12 to 15 kg NO\(_3^-\)-N ha\(^{-1}\) yr\(^{-1}\), and dry deposition N concentrations (e.g., particulates, gases, and
nitric acid) in Indiana are estimated as approximately 2.0 μg m$^{-3}$ NH$_4^+$-N and 4 to 5 μg m$^{-3}$ NO$_3^-$-N (United States Environmental Protection Agency 1999).

Ecosystems in eastern states experience substantially higher N deposition rates than western states (U.S. EPA 1999). Thus, when considering ecosystem N budgets, the relative contribution of N fixed by biological soil crusts relative to dry and wet N deposition is probably lower in the eastern U.S. than in western states where N deposition is much lower. The rates of N deposition given above are for the state of Indiana, not for the Indiana Dunes in particular; I am aware of no studies documenting N deposition rates at IDNL or the Miller Dunes in particular. Used in conjunction with research characterizing N deposition rates at the Miller Dunes, stable isotope analyses that quantify the relative contributions of atmospheric and crust-mediated N inputs to this dune system would shed light on the relative importance of crust-mediated fixed N to the overall N budget of this ecosystem.

**Crust species composition**

Species diversity of the biological soil crusts at the Indiana Dunes National Lakeshore was relatively low compared to diversity of crusts in more fine-texture soils, but this is typical of crusts that develop on sand dunes (Kleiner and Harper 1977; Verrecchia et al. 1995). Most of the cyanobacterial species I observed are nitrogen fixers; I observed heterocysts on the *Nostoc* colonies in these crusts, and high rates of nitrogen fixation occur even in non-heterocystous cyanobacteria such as *Lyngbya*, *Oscillatoria*, and *Phormidium* (Stewart 1973; Rogers and Gallon 1988; Belnap 1996). High rates of N-fixation by *Lyngbya* spp. are well documented (Prufert-Bebout and
Garcia-Pinchel 1994; Zehr et al. 1995; Steppe et al. 1996). The positive and sometimes very high net inorganic N infiltration through my soil cores strongly suggests that the non-heterocystous *Lyngbya* in these crusts function in N-fixation. Although *Microcoleus* alone are not capable of N-fixation, epiphytic diazotrophic bacteria associated with this cyanobacterial genus are capable of fixing atmospheric N and account for N contributions where *Microcoleus* is present (Steppe et al. 1996). The low density of heterocystic *Nostoc* alone is unlikely to account for the high inorganic N production observed.

The large, filamentous cyanobacterium *Lyngbya* sp. strongly dominated the cyanobacterial crust organisms at the Indiana Dunes. Soil crusts on coarse-textured, unstable soils like sand dunes are usually characterized by large filamentous cyanobacteria such as *Lyngbya* and *Microcoleus* because these species are highly motile and effective dispersers that can survive sand burial and active disturbance by gliding longitudinally and latitudinally through the soil profile (Kleiner and Harper 1977; Verrecchia et al. 1995). Other smaller cyanobacteria die when covered by windblown sand particles (Campbell 1979; Belnap and Eldridge 2001), but some cyanobacteria can remain photosynthetically active in low light below the soil surface. Although cyanobacterial density was much lower in control crusts, cyanobacteria in controls may have survived sand burial to actively photosynthesize and fix nitrogen, which may account for the positive – albeit lower – net N infiltration through control crusts in my experiment. Active bacterial and fungal communities associated with buried cyanobacteria may also have contributed to N flushing through control crusts.

Cyanobacterial soil crusts are early successional crusts that often become more diverse later in crust succession by moss and lichen development if suitable soil
conditions exist (Harper and Marble 1988; Eldridge 2001). This trajectory of crust succession appears to occur in crusts from the Indiana Dunes as well, because I observed spore germination and moss gametophyte development on top of cyanobacterial crusts over the course of my 10-week experiment. In water- and nutrient-limited systems, cyanobacteria and their associated microbial communities provide moisture, C, and N necessary for bryophytic and lichen spore germination and gametophyte establishment. High sand content and the absence of suitable soil conditions for lichen establishment such as high alkalinity and long-term stability may account for the absence of lichens in crusts at the Indiana Dunes (Eldridge 2001). I cannot be certain of this because these crusts have never before been characterized; thus, they could be at a relatively early successional stage. Lichens may establish in the future if the area where crusts are prevalent remains relatively undisturbed; however, dune movement and active sand burial will probably preclude lichen establishment altogether as it does on most sand dunes.

**Crust and treatment influence on infiltration of rainwater through the soil profile**

One of my primary objectives was to determine how crust composition and percent cover, light intensity, rainfall intensity, and litter addition influence the amount of rainwater that infiltrates through the soil profile to the vascular plant rooting zone. Light and rainfall intensity had the strongest influence on the amount of rainfall that infiltrated through the soil profile, and rainfall intensity interacted with percent cover of both cyanobacteria and mosses to affect leachate amount. Not surprisingly, less rainwater leached through crusts exposed to full sunlight. Full sunlight likely caused higher
evapotranspiration rates and greater crust dessication between rehydration events, increasing the amount of rainwater absorbed by crusts upon rehydration during rainfall events.

Rainwater leaching through the soil profile significantly increased during high intensity rainfall events, due to higher crust surface evaporation over the many hours it often took to simulate low intensity rainfall events. High intensity rainfall had a stronger effect on increasing infiltration as the percent cover of mosses increased. This is consistent with other work that has documented higher rates of rainwater infiltration through moss-dominated crusts than cyanobacterial crusts (Rushforth and Brotherson 1982; Brotherson and Rushforth 1983; Ladyman and Muldavin 1996). The differential effect of cyanobacteria and mosses on rainwater infiltration may be especially pronounced in very sandy soils where typical high porosity is blocked by the swelling of cyanobacterial sheaths during rehydration (Verrecchia et al. 1995; Warren 2001).

The differential effect of cyanobacterial- and moss-dominated crusts on rainwater infiltration through the soil profile in this and other studies suggests that the influence of crusts on infiltration may vary temporally depending upon the phase of crust development (Warren 2001). Different phases of crust development are represented by different crust constituents (e.g., cyanobacteria vs. bryophytes and lichens) and early and late successional crust stages usually exist simultaneously as a landscape mosaic. This suggests that the influence of crusts on water (and nutrient) infiltration may vary on the spatial scale at which crust composition varies, especially during heavy storm events. At the Indiana Dunes, if rainwater infiltration translates into water availability for American beachgrass roots growing in crusted areas, crust-mediated spatial patterning of infiltration

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may contribute to spatial patterning of beachgrass vigor and decline. More work is needed in situ in this and other systems to determine how temporal and spatial patterning of crust compositions and successional stages affect rainwater infiltration to the vascular plant rooting zone at the stand scale.

**Crust and treatment influence on nutrient infiltration through the soil profile**

My other primary objective was to determine how crust composition and percent cover, light intensity, rainfall intensity, and litter addition influence nutrient infiltration through the soil profile. I observed positive net N infiltration through both cyanobacterial and moss crusts over the duration of my experiment, clearly showing that the organisms comprising these crusts are fixing substantial amounts of N and that crust-associated microbial and faunal communities are actively mineralizing N that is flushed through the soil profile upon rehydration.

Cumulative net NO$_3^-$ throughput was notably much higher than NH$_4^+$ infiltration, which may be explained in two ways. Nitrifying bacteria associated with crust organisms and crust organic byproducts may have oxidized NH$_4^+$ immediately upon its release into the soil profile following N fixation and/or mineralization. Nitrifying bacteria obtain carbon from the gelatinous sheaths and extracellular carbon secretions of cyanobacteria (Evans and Lange 2001); these cyanobacterial carbon inputs can increase soil polysaccharides and total carbon by as much as 300% (Rao and Burns 1990; Rogers and Burns 1994; Belnap et al. 2001). Carbon leaching by mosses and rhizoid decomposition may also be a reliable source of carbon for bacterial communities. Soil crusts dominated by lichens and mosses contribute substantially more carbon to systems than
cyanobacterial crusts (estimates are 12-37 g C m$^{-2}$ yr$^{-1}$ and 0.4-2.3 g C m$^{-2}$ yr$^{-1}$, respectively; Evans and Lange 2001), due in part to the higher photosynthetic capacity and 'leakiness' of mosses than cyanobacteria (Lange 2001).

Alternatively or in addition, higher NO$_3^-$ than NH$_4^+$ throughput may have resulted from NH$_4^+$ volatilization and loss from the system as NH$_3$. Ammonium volatilization is favored over other processes where N-fixation rates or extracellular nitrogen leaking by cyanobacteria are high (Haynes and Sherlock 1986; Schlesinger and Peterjohn 1991), and where surface soil temperatures are high. West and Skujins (1977) estimate that 70% of N fixed by biological soil crusts is lost through volatilization and denitrification.

While there was generally no strong effect of rainfall intensity (frontal vs. convective) on cumulative nitrogen infiltration, rainfall volume did affect N throughput and this effect varied across cyanobacterial cover. Cumulative N infiltration was consistently higher during high volume (> 2 cm) storm events than low volume (< 2 cm) events. NO$_3^-$ infiltration was significantly reduced under high percent cover of cyanobacteria, but only when outlier data were included in data analyses; cyanobacterial percent cover had no significant effect on NO$_3^-$ leaching when these data were omitted. In other words, crusts dominated by cyanobacteria leached less NO$_3^-$ when rainfall was very heavy. This suggests that different types of rainfall events (e.g., high vs. low volume rain storms) differentially affect the functioning of cyanobacterial crusts at this site.

Reduced rates of cyanobacterial N fixation may have resulted from high soil saturation during my simulations of high volume rainstorms. Cyanobacterial metabolism and N fixation is maximized at 40-60% soil saturation; respiration and photosynthesis of
most cyanobacteria decrease rapidly above 50% soil saturation (Jeffries et al. 1993a). However, this does not explain why NO$_3^-$ leaching decreased as cyanobacterial cover increased, because presumably high saturation would impede N fixation by crusts with lower cyanobacterial cover as well. An alternative explanation for reduced NO$_3^-$ leaching by crusts with high cyanobacterial cover is that temporary soil anaerobiosis produced by high soil saturation activated a denitrifier bacterial community associated with heavy cyanobacterial colonization, resulting in loss of NO$_3^-$ as N$_2$ and N$_2$O (Peterjohn and Schlesinger 1991). Denitrification can be especially high where cyanobacterial cover is abundant, because denitrifying bacteria rely heavily on the carbon provided by cyanobacterial crusts (West and Skujins 1977). Evans and Belnap (1999) found higher $\delta^{15}$N in crusts dominated by cyanobacteria in contrast to lichen-dominated crusts, which they attributed to greater gaseous N loss in cyanobacterial-dominated crusts. Work that characterizes the composition and functions of bacterial communities associated with various crust communities would enhance our understanding of the role of these organisms in soil crust functioning under different rainfall regimes.

In general, I observed very heavy N flushing during high volume rain events; various explanations may account for this. Because biological soil crusts require sufficient liquid water for metabolic activity, photosynthetic rates and subsequent N-fixation increase only when hydration has reached a high enough threshold for activity (Lange 2001). Once rehydration has occurred, actual photosynthetic rates are controlled by the degree of hydration (Lange et al. 1993). Thus, insufficient hydration levels during low volume rain events may have limited photosynthetic and subsequent nitrogenase
activity, reducing the amount of fixed N flushed through the soil profile during light rainfall events.

In addition, a nitrifying bacterial community associated with the polysacharride sheaths, organic matter, carbon excretions, and rhizoids of biological soil crust organisms may have become activated upon rehydration. Weakly bound NO$_3^-$ oxidized by nitrifier bacteria may be flushed through the soil profile during heavy rainfall events, especially in the absence of vascular plant uptake of NH$_4^+$. Bacterial numbers and biomass below biological soil crusts can be several orders of magnitude higher than in uncrusted soils (Rogers and Burns 1994; Steppe et al. 1996; see States et al. 2001; Belnap 2001b), although I am aware of no study that distinguished among the bacteria functionally.

Alternatively or in addition, spikes in N infiltration during heavy rainfall events may have been caused by the cyanobacterial release of extracellular nitrogenous compounds (Millbank 1982). N-fixing cyanobacteria can release up to 70% of their fixed N and most nitrogenous compounds released to the surrounding soil are released as NH$_4^+$ and NO$_3^-$ (Belnap 2001a). N release to the surrounding soil substrate has been shown to be higher during rehydration after dessication, perhaps resulting from membrane deformation with dessication and rehydration (Millbank 1982; Potts 1984). This extracellular N loss may be especially pronounced in low N environments, where N loss may occur in part by diffusion (Dodds et al. 1995).

Soil fauna associated with soil crusts may be activated by hydration and may account for substantial amounts of C and N mineralization during and after rainfall events. The soil faunal communities associated with moss-dominated soil crusts are remarkably diverse, and moss cover is highly correlated with nematode abundance.
(Yeates 1979; Belnap 2001b). For example, lichen-moss crusts from Colorado supported
three times more bactivorous nematodes and seven times more fungivorous nematodes
than adjacent cyanobacterial crusts (Anderson et al. 1984). Liverwort-dominated crusts
in the Arctic supported more bacteria and fungi than cyanobacterial crusts (Smith and
Griggs 1932). Given that soil nematodes often account for a disproportionately high
percentage of soil N mineralization (Coleman et al. 1978; Hunt et al. 1987), high
nematode activity, and a rich soil microbial and faunal community associated with both
cyanobacterial- and bryophyte-dominated crusts may account for the high N leaching I
observed through crusts during heavy rainfall events.

Heavy nutrient flushing during high volume storm events may have reflected a
delay in the onset of metabolic activity of crust organisms. The response of
cyanobacterial crusts to rewetting varies highly by species composition, but initial
nitrogenase activity is generally very low (Belnap 2001a). For example, the onset of
photosynthesis by Microcoleus and Nostoc begins within minutes of rewetting (Garcia-
Pichel and Belnap 1996), but there is usually a lag time between rehydration and the
onset of N-fixation, and a further lag time between the onset of N-fixation and maximum
nitrogenase activity (Belnap 2001a). Many mixed cyanobacterial crusts (e.g., Nostoc-
Anabaena, Nostoc-Scytonema-Microcoleus) can take 25-36 hours to reach maximum
nitrogenase activity after rewetting (Englund 1978; Belnap 2001a), and nitrogenase
activity levels can take three hours to two days to attain full activity following several
consecutive days of dessication (Jeffries et al. 1993a,b; Belnap 2001a). The lag before
maximum nitrogenase activity is achieved may in part account for the very heavy N
leaching I observed on high rainfall days because high volume rainfall simulations took several hours to complete.

More work is needed to distinguish among these mechanisms of N flushing in this system. Nonetheless, results from this study suggest that summer and autumn convective storms along southern Lake Michigan may cause substantial N inputs to this system in heavily crusted areas. Field studies that assess these inputs in different spatial and temporal scales would contribute valuable information to our understanding of how these crusts influence water and nutrient dynamics on an ecosystem scale.

Influence of other surface soil characteristics on water and nutrient infiltration through the soil profile

Light intensity treatments affected cyanobacterial- and moss-dominated crusts differently. NH₄⁺ infiltration decreased in cyanobacterial-dominated cores subjected to full sun but increased in moss-dominated crusts subjected to full sun. Maximum cyanobacterial nitrogenase activity and bryophytic photosynthesis can be reached at relatively low light levels (e.g., 100-200 μM m⁻² sec⁻¹; Ohki and Fujita 1988; Jones, 1977a,b; Coxson and Kershaw 1983b; Jeanfils and Tack 1992), and most cyanobacterial nitrogen fixation occurs between -5 and 30 C (optimal: 20-30 C), above which nitrogenase activity drops off rapidly (DuBois and Kapustka 1983; Fritz-Sheridan 1988; Lennihan et al. 1994; Belnap 2001a).

Thus, shading of cores at 200 μM s⁻¹ m⁻² and greenhouse temperatures (mean: 25.2 C) were probably not responsible for the differential NH₄⁺ leaching observed between light intensity treatments or the differential responses of cyanobacteria- and
moss-dominated crusts to light intensity treatments. Instead, high surface temperatures may have exacerbated the dessication of crusts with high cyanobacterial cover that were exposed to full sun, thereby reducing nitrogen fixation rates and increasing response rates for resumption of nitrogenase activity in cyanobacterial-dominated crusts exposed to high light.

The addition of beachgrass litter to the soil surface significantly increased nitrogen leaching through the soil profile, and the effect of litter addition was especially pronounced when the percent cover of mosses increased. An active microbial decomposer community associated with beachgrass litter may account for this, as surface soil characteristics atop mosses may be more optimal for microbial activity than those atop cyanobacteria, as a result of the lower temperatures and higher humidity above mosses. Litter addition may also have decreased surface soil temperatures and dessication, thereby increasing N-fixation rates and prolonging N-fixation duration in crusts with significant cyanobacterial communities (Belnap 2001a).

Conclusions and implications for American beachgrass vigor

Biological soil crusts growing among beachgrass ramets at the Miller Dunes do not appear to compete with vascular plants for water and N. Instead, my results suggest that these crusts have the potential to contribute substantial N inputs to this system, particularly under certain environmental conditions such as heavy rainfall events and beachgrass litter deposition.

Beachgrass decline was observed in an area of the Miller Dunes where soil nutrient status was two orders of magnitude higher than other dune areas (Poulson and
McClung 1999), although no formal correlation analyses between soil nutrient status and beachgrass vigor were performed. It is possible that higher nutrient status in some areas of the dunes – due either to leaching from slag heaps (Poulson and McClung 1999) or to biological soil crust activity – may somehow contribute to beachgrass decline through a mechanism that is not currently apparent to us.

Accumulation of detrimental soil pathogens (pathogenic soil nematodes) has been implicated in beachgrass decline on European sand dunes (van der Putten and Troelstra 1990; van der Putten et al. 1993), although these pathogens were not specifically associated with biological soil crusts. Nonetheless, the presence of biological soil crusts at the Indiana Dunes may create chemically and physically favorable microsites for pathogenic soil organisms to accumulate and compromise beachgrass persistence. Studies that characterize the soil microbial and faunal communities associated with these biological soil crusts would shed light on this possible mechanism for beachgrass decline and would enhance our understanding of the information presented here regarding the role of biological soil crust organisms in water, nutrient, and vascular plant dynamics at the Indiana Dunes and in other dune ecosystems.

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organisms. Gary Floyd, Jeff Johansen, and Moria Nagy contributed their considerable expertise for taxonomic identifications of crust organisms.

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SAS Institute, Inc. 1996. Cary, NC, USA.


Figure 5.1. Location of study site where biological soil crusts and soils were sampled. The star denotes the precise sampling location at the Miller Dunes in Miller, IN.
Figure 5.2. Diagram of the experimental set up for my greenhouse experiment on the effects of crust composition and percent cover, light intensity, rainfall intensity, and litter addition on rainwater and N infiltration through biological soil crusts from the Indiana Dunes National Lakeshore.
Figure 5.3. Comparison of cumulative leachate volume between low (< 2.0 cm) and high (> 2.0 cm) rain volume events over the growing season. Data here are pooled, i.e., not sorted by binary treatment variables of light intensity, rainfall intensity, and litter addition.
Figure 5.4. The effect of crust composition and crust percent cover on cumulative leachate volume (ml). Data here are pooled, i.e., not sorted by binary treatment variables of light intensity, rainfall intensity, and litter addition.
Figure 5.5. The effects of differential light intensity (shade vs. full sun) on leachate volume by the percent cover of cyanobacteria and mosses. Asterisks denote a significant difference between light intensity treatments at p < 0.05, as determined by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996). No significant interactions occurred between crust cover and light intensity.

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Figure 5.6. The effects of differential rainfall intensity (high intensity/convective vs. low intensity/frontal) on leachate volume by the percent cover of cyanobacteria and mosses. Asterisks denote a significant difference between shade and sun and crosses denote a significant interaction between crust cover and rainfall intensity, as determined by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996).
Figure 5.7. The effects of litter addition treatments on leachate volume by the percent cover of cyanobacteria and mosses. No significant effects of litter treatment or interaction between litter and crust composition or percent cover on leachate were detected by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996).
Figure 5.8. Comparison of cumulative NH$_4^+$ amount (µg) between low (< 2.0 cm) and high (> 2.0 cm) rain volume events over the growing season.
Figure 5.9. Comparison of cumulative NO$_3^-$ amount (µg) between low (< 2.0 cm) and high (> 2.0 cm) rain volume events over the growing season.
Figure 5.10. The effect of crust composition and percent cover on cumulative NH$_4$+ (µg) in leachate. Data here are pooled, i.e., not sorted by binary treatment variables light intensity, rainfall intensity, and litter addition.
Figure 5.11. The effect of crust composition and percent cover on cumulative NO$_3^-$ (μg) in leachate. Data here are pooled, i.e., not sorted by binary treatment variables light intensity, rainfall intensity, and litter addition.
Figure 5.12. The effects of light intensity treatments on cumulative $\text{NH}_4^+$ (μg) by the percent cover of cyanobacteria and mosses. Asterisks denote a significant difference between shade and sun (at $p < 0.10$) as determined by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996). There was no significant interaction between crust cover and light intensity.
Figure 5.13. The effects of light intensity treatments on cumulative $\text{NO}_3^-$ (µg) by the percent cover of cyanobacteria and mosses. No significant difference between light intensity treatments was detected by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996).
Figure 5.14. The effects of rainfall intensity treatments on cumulative NH$_4^+$ (µg) by the percent cover of cyanobacteria and mosses. Asterisks denote a significant difference between high and low rainfall intensities (at $p < 0.10$) as determined by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996). No significant interaction between crust cover and rainfall intensity was detected.

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Figure 5.15. The effects of rainfall intensity treatments on cumulative NO$_3^-$ (µg) by the percent cover of cyanobacteria and mosses. No significant effect of rainfall intensity treatments on NO$_3^-$ leaching were detected by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996), and no interaction between crust composition and percent cover was detected.
Figure 5.16. The effects of litter addition treatments on cumulative $\text{NH}_4^+$ (µg) by the percent cover of cyanobacteria and mosses. Asterisks denote a significant difference between litter addition treatments as determined by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996). No significant interactions between litter treatments and crust composition or percent cover were detected.
Figure 5.17. The effects of litter addition treatments on cumulative $\text{NO}_3^-$ ($\mu$g) by the percent cover of cyanobacteria and mosses. Asterisks denote a significant difference between litter addition treatments and crosses denote a significant interaction between litter addition treatment and crust cover, as determined by non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996).
<table>
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<th>Taxon</th>
<th>Abundance</th>
<th>N-fixing?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
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<tr>
<td><em>Leptolyngbya</em> sp.</td>
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<td>Yes</td>
</tr>
<tr>
<td><em>Microcoleus</em> sp.</td>
<td>Less common</td>
<td>No*</td>
</tr>
<tr>
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<td>Common</td>
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</tr>
<tr>
<td><em>Chroococcus</em> sp.</td>
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<tr>
<td><em>Sphagnum</em> sp.</td>
<td>Less common</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 5.1. Taxonomic composition, relative abundances, and nitrogen fixing functions of biological soil crust organisms from the Miller Dunes at the Indiana Dunes National Lakeshore near Miller, IN. Nitrogen fixation determination is based on information in Stewart (1973) and Steppe et al. (1996). Asterisk denotes that *Microcoleus* alone does not fix N, but an epiphytic bacterial community associated with *Microcoleus* does demonstrate considerable nitrogenase activity (Steppe et al. 1996).
<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Response variable</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent cover of cyanobacteria</td>
<td>Leachate (ml)</td>
<td>26.021</td>
<td>0.05</td>
<td>0.829</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>293.629</td>
<td>0.44</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>2182.808</td>
<td>3.22</td>
<td>0.076</td>
</tr>
<tr>
<td>Percent cover of mosses</td>
<td>Leachate (ml)</td>
<td>28.779</td>
<td>0.05</td>
<td>0.820</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>1047.450</td>
<td>1.59</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>514.782</td>
<td>0.74</td>
<td>0.393</td>
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<tr>
<td>Light</td>
<td>Leachate (ml)</td>
<td>13156.086</td>
<td>23.81</td>
<td>0.000</td>
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<tr>
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<td>NH₄⁺ (µg)</td>
<td>1968.110</td>
<td>2.97</td>
<td>0.089</td>
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<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>26.903</td>
<td>0.04</td>
<td>0.843</td>
</tr>
<tr>
<td>Cyanobacteria*light</td>
<td>Leachate (ml)</td>
<td>32.756</td>
<td>0.06</td>
<td>0.808</td>
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<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>1432.948</td>
<td>2.16</td>
<td>0.145</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>252.776</td>
<td>0.37</td>
<td>0.543</td>
</tr>
<tr>
<td>Mosses*light</td>
<td>Leachate (ml)</td>
<td>127.685</td>
<td>0.23</td>
<td>0.633</td>
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<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>1137.486</td>
<td>1.72</td>
<td>0.193</td>
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<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>158.752</td>
<td>0.23</td>
<td>0.635</td>
</tr>
<tr>
<td>Rainfall intensity</td>
<td>Leachate (ml)</td>
<td>15797.386</td>
<td>31.40</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>1682.995</td>
<td>2.47</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>1239.807</td>
<td>1.86</td>
<td>0.176</td>
</tr>
<tr>
<td>Cyanobacteria*rainfall</td>
<td>Leachate (ml)</td>
<td>1651.929</td>
<td>3.28</td>
<td>0.074</td>
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<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>205.662</td>
<td>0.30</td>
<td>0.584</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>139.826</td>
<td>0.21</td>
<td>0.648</td>
</tr>
<tr>
<td>Mosses*rainfall</td>
<td>Leachate (ml)</td>
<td>4041.542</td>
<td>8.53</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>121.077</td>
<td>0.18</td>
<td>0.672</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>399.949</td>
<td>0.59</td>
<td>0.445</td>
</tr>
<tr>
<td>Litter addition</td>
<td>Leachate (ml)</td>
<td>0.741</td>
<td>0.00</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>2410.147</td>
<td>3.59</td>
<td>0.062</td>
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<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>8255.153</td>
<td>15.32</td>
<td>0.000</td>
</tr>
<tr>
<td>Cyanobacteria*litter</td>
<td>Leachate (ml)</td>
<td>7.295</td>
<td>0.01</td>
<td>0.919</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>237.290</td>
<td>0.35</td>
<td>0.554</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>3292.395</td>
<td>6.04</td>
<td>0.016</td>
</tr>
<tr>
<td>Mosses*litter</td>
<td>Leachate (ml)</td>
<td>6.441</td>
<td>0.01</td>
<td>0.924</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ (µg)</td>
<td>1034.488</td>
<td>1.59</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>NO₃⁻ (µg)</td>
<td>567.232</td>
<td>0.95</td>
<td>0.334</td>
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</table>

(continued on next page)
Table 5.2 (continued)

<table>
<thead>
<tr>
<th>Light*rainfall</th>
<th>Leachate (ml)</th>
<th>NH$_4^+$ (µg)</th>
<th>NO$_3^-$ (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>385.217</td>
<td>3856.357</td>
<td>235.532</td>
</tr>
<tr>
<td>Litter*rainfall</td>
<td>Leachate (ml)</td>
<td>NH$_4^+$ (µg)</td>
<td>NO$_3^-$ (µg)</td>
</tr>
<tr>
<td></td>
<td>12.521</td>
<td>90.927</td>
<td>1.542</td>
</tr>
<tr>
<td>Light*litter</td>
<td>Leachate (ml)</td>
<td>NH$_4^+$ (µg)</td>
<td>NO$_3^-$ (µg)</td>
</tr>
<tr>
<td></td>
<td>154.668</td>
<td>1156.252</td>
<td>260.262</td>
</tr>
<tr>
<td>Light<em>litter</em>rainfall</td>
<td>Leachate (ml)</td>
<td>NH$_4^+$ (µg)</td>
<td>NO$_3^-$ (µg)</td>
</tr>
<tr>
<td></td>
<td>2.682</td>
<td>13.315</td>
<td>2.682</td>
</tr>
</tbody>
</table>

Table 5.2. Results from a non-parametric ranked ANOVA (PROC RANK GLM, SAS Institute 1996) on the effects of treatment variables crust percent cover, light intensity, rainfall intensity, and litter addition on response variables cumulative leachate volume (ml), cumulative NH$_4^+$ (µg) in leachate, and cumulative NO$_3^-$ (µg) in leachate. Results are included both with proven outliers (Dixon 1953) included and with NH$_4^+$ (n = 4 outliers) and NO$_3^-$ (n = 7 outliers) outliers omitted. Bolded significant p-values (< 0.10) were non-significant when outlier data were removed from the analysis.
Table S.3. Results from a non-parametric Spearman's Correlation analysis (PROC CORR OUTS, SAS Institute 1996) on the effects of treatment variables crust percent cover, light intensity, rainfall intensity, and litter addition on response variables cumulative leachate volume (ml), cumulative NH$_4^+$ (µg) in leachate, and cumulative NO$_3^-$ (µg) in leachate. Data here are pooled, i.e., not sorted by binary treatment variables light intensity, rainfall intensity, and litter addition treatment. Numbers not in parentheses are Spearman's correlation coefficients (r), and numbers in parentheses are p-values. Bolded significant p-values (at <0.05 or 0.10) were non-significant when outlier data were removed from the analysis, and italicized p-values were significant (at <0.05) when outlier data were removed from the analysis.

<table>
<thead>
<tr>
<th>Outliers included</th>
<th>Leachate volume (ml)</th>
<th>NH$_4^+$ (µg) in leachate</th>
<th>NO$_3^-$ (µg) in leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent cover of cyanobacteria</td>
<td>0.021 (0.005)</td>
<td>-0.070 (0.515)</td>
<td>-0.190 (0.074)</td>
</tr>
<tr>
<td>Percent cover of mosses</td>
<td>0.022 (0.839)</td>
<td>0.131 (0.217)</td>
<td>0.092 (0.388)</td>
</tr>
<tr>
<td>Leachate volume (ml)</td>
<td>1.000 (0.0)</td>
<td>0.300 (0.005)</td>
<td>0.146 (0.170)</td>
</tr>
</tbody>
</table>
CHAPTER 6

CONCLUSIONS AND AREAS FOR FUTURE RESEARCH

AREAS FOR FUTURE RESEARCH RELATED TO THESE DISSERTATION STUDIES

Like any research, the studies making up this dissertation have inspired ideas for future work. My research at the Edge of Appalachia (EOA) and Soldier’s Delight demonstrated that the patterning of an active ectomycorrhizal fungal community associated with ECM-dependent *Pinus virginiana* L. is facilitating pine invasion into these ecosystems of high conservation value. Follow up studies would further enhance our understanding of the influence of the ECM fungal community on the invasiveness of Virginia pine and the invasibility of ecosystems like EOA and Soldier’s Delight. For example, an understanding of the impact of different fire frequencies and intensities on the ectomycorrhizal fungal community across plant community types (e.g., serpentine barrens, alkaline cedar barrens, and grasslands) would allow managers to ascertain what type of fire management – if any – is most effective at mitigating ECM fungal colonization of open barren communities. This question is particularly interesting in light of the notable difference in ECM inoculum potential in open and transitional areas of Lynx 3 and Lynx 5 at EOA, which may be due in part to differences between the two
barrens in vascular plant cover and fire frequency and intensity. A comparison of ECM fungal survival and diversity at different soil depths (e.g., 0-5 cm, 5-10 cm, 10-20 cm) following fire would allow researchers to ascertain ECM recolonization patterns by quantifying ECM infection potential at both the soil surface and in the soil mineral layer. Further, field tests that compare different management strategies, including varying fire regimes and trenching around established pine stands, would help managers determine the best way to curtail pine colonization into these systems in the long run.

Other belowground mechanisms of pine invasion at EOA and Soldier’s Delight may exist, and exploring these mechanisms would help ecologists and managers improve mitigation of pine colonization by deepening their understanding of the invasion ecology of these systems. Research that characterizes the interactions between other soil organisms and the ECM fungal community would enhance our understanding of how other constituents of the soil community influence pine invasion at these sites – both from the perspective of pine invasiveness and native ecosystem invasibility. Of specific interest is the interaction between arbuscular mycorrhizae and ectomycorrhizae. The outcome of competitive interactions between these two different types of mycorrhizae may influence the vulnerability of these previously arbuscular mycorrhizal communities to invasion by ECM-dependent woody species like pine.

The presence and activity of other host-mediated soil organisms have been shown to determine the outcome of vascular plant competition (e.g., pathogenic nematodes, van der Putten et al. 1993; pathogenic fungi, Mills and Bever 1998; soil rhizosphere bacteria, Westover and Bever 2001). Thus, a comprehensive characterization of the soil communities associated with pine and with plant hosts in open barren areas devoid of
pine would improve our understanding of other belowground mechanisms that may be involved in the invasion ecology of these systems. This characterization would be especially insightful if combined with reciprocal transplant experiments that determined whether host-induced changes to the soil community facilitate pine invasion and prohibit the establishment and regeneration of more desirable plant species at these sites (Mills and Bever 1998).

Without previous soil chemical data we cannot be sure whether pine is changing the soil chemistry in these systems; however, results from this and other research (Barton and Wallenstein 1997) strongly suggests that heavy pine colonization is acidifying and enriching soils in these alkaline, nutrient-poor systems. Thus, pine-induced changes to soil chemistry may facilitate further pine invasion. For example, heavy litter layers created by pine may improve conditions for ECM fungal proliferation at the periphery of established mature pine stands and hinder establishment and reproduction of native grassland plant species. Studies that assess these questions may assist managers in targeting sites of highest conservation priority for intensive and experimental management.

Interesting questions have also grown out of my work on the biological soil crusts at the Indiana Dunes. My finding that these crusts contribute substantial N inputs to crust-covered areas suggests that crusts play a significant role in the nutrient dynamics of the Indiana Dunes, and probably other dunes systems where biological soil crusts develop in plant interspaces. Although research on plant community dynamics at the Indiana Dunes has shaped important paradigms in plant ecology, such as plant succession, the contribution of soil organisms to ecosystem dynamics in this and other dune systems has
been largely ignored. Thus, work on the biological soil crusts and other soil organisms at the Indiana Dunes will improve our understanding of the mechanisms of plant succession and the relationship between soils and vascular plant dynamics at this site and in other systems.

Several follow up studies would enhance our understanding of the functioning of the biological soil crusts at the Indiana Dunes. Quantification of chlorophyll \( a \) would permit biomass estimates of crust organisms and thereby facilitate the characterization of the effect of different crust constituents and densities on rainwater and nutrient leaching at various spatial scales and crust successional stages. Quantification of nitrogenase activity in crusts of varying compositions and densities, and under various treatments like those imposed in my study, would improve our understanding of the relative amount of N contributed to this system directly by N-fixing cyanobacteria.

In addition, a comprehensive, quantitative characterization of the microbial and faunal soil communities associated with these crusts would shed light on the relative contributions of crust organisms (e.g., cyanobacteria) and crust-associated organisms (e.g., epiphytic, heterotrophic N-fixing bacteria associated with *Microcoleus* sp.) to N fixation, mineralization, and leaching. Stable isotope analyses could be utilized *in situ* to assess how much of the N produced by crusts and crust-associated organisms is taken up and incorporated by vascular plants (Hawkes 2000). Further, measurements of N volatilization of different crust constituents and densities under various conditions like those imposed in my study would enhance our understanding of N dynamics at this site, as influenced by the interaction of crust organisms and environmental conditions.
Belowground contributions to vascular plant establishment and persistence are complex and result from a combination of interacting factors. Beachgrass decline at the Indiana Dunes may be a function of the lack of windblown sand covering on leeward dunes, as has been suggested for European beachgrass (*Ammophila arenaria* (L.) Link (Hope-Simpson and Jefferies 1966; Huiskes 1979). Results from my study and others (Poulson and McClung 1999) suggest that beachgrass decline at the Indiana Dunes may somehow be related to soil nutrient enrichment, but the mechanism for this is unclear and should be examined mechanistically *in situ*. Some attention should be paid in future research to soil pathogens below American beachgrass in crusted and uncrusted areas. Pathogenic nematodes accumulate below beachgrass and contribute to beachgrass decline on European sand dunes where windblown sand accumulation is minimal (van der Putten and Troelstra 1990; van der Putten et al. 1993). While it is well known that soil biodiversity and organismal activity increase under crusts, the impact of biological soil crusts on the accumulation of pathogenic soil organisms has not been evaluated. The influence of biological soil crusts on the presence, density, and activity of pathogenic soil organisms that influence vascular plant establishment and survival is an important area for continued study.

**AREAS FOR FUTURE RESEARCH IN SOIL ECOLOGY – GENERAL**

Advances in technology and the maturation of the field of ecology have permitted soil ecologists to open the “black box” and identify and characterize the constituents of soil communities and their myriad functions. In doing so, we can evaluate soil community dynamics within the context of traditional ecological principles, and assess
whether these principles apply to soil communities. Traditional paradigms that may be applied to future soils research include evaluation of competition among soil organisms, the impact of invasive microbial species on soil food web diversity and functioning and on vascular plant diversity, the impact of food web structure on soil functioning and vascular plant diversity, and evolutionary lineages and relationships among soil organisms. Research of this nature will allow soil scientists to assess whether traditional ecological paradigms apply to soil systems, to contribute new paradigms suggested by studies in soils that may be applicable to aboveground systems, and to expand and improve our understanding of ecosystem dynamics.

With improvements in our ability to characterize the soil community at increasingly finer resolution, we can better determine the effect of anthropogenically-mediated stress and disturbance on soil community structure and functioning. In light of increasing threats to ecosystem sustainability under elevated atmospheric CO$_2$ and nutrient deposition, understanding the role of soil communities in global carbon and nitrogen cycling and sequestration in various ecosystem types is paramount. Many researchers are evaluating the impact of increased atmospheric CO$_2$ and nitrogen deposition on the biodiversity and functioning of soil organisms (e.g., Arnolds 1991; Rillig et al. 1998; Rillig and Allen 1999). Research is also underway to characterize the contributions of the soil microbial community to carbon pools and carbon sequestration within different soil aggregate sizes in soils from agroecosystems, and to evaluate the effect of different cropping systems (conventional vs. no-tillage) on the capacity for soil organisms to sequester C and N (S.D. Frey and J. Six, in progress; B.F. Tracy, in progress).
It is imperative that soil ecologists be active in one of the most significant and contentious debates currently in ecology: the relationship between biodiversity and ecosystem functioning. Studies by David Tilman and his colleagues have produced evidence that increased plant diversity improves the sustainability and functioning of grassland ecosystems (Tilman 1999; Naeem et al. 2000). However, these researchers have assessed the effect of plant diversity on ecosystem functioning from a primarily aboveground perspective while virtually excluding evaluation of belowground processes (Wardle et al. 2000). For ecosystem functioning to be characterized accurately and meaningfully toward the goal of ensuring ecosystem sustainability, many functions of soils must also be characterized and quantified under different levels of plant species diversity and various anthropogenic stressors and disturbances (Wardle 1998; Mikola and Setala 1998). Soil ecologists must contribute important information to this timely debate by characterizing the structure and functions of different soil communities under varying environmental conditions, quantifying the contribution of soil organisms to various ecosystem functions, and evaluating whether soil organisms exhibit functional redundancy. Our best chance at ensuring long-term ecosystem sustainability will be gained through dialogue among scientists representing all ecosystem components—both above- and belowground.

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