ECTOMYCORRHIZAL FUNGAL COMMUNITIES IN WIND-DISTURBED FORESTS

by

CHARLES C. COWDEN

(Under the Direction of Chris J. Peterson)

ABSTRACT

In this dissertation, I describe the effects of wind disturbance in woody plant communities on the composition and structure of ectomycorrhizal fungal (EMF) communities. To evaluate these effects, EMF communities in windthrow gaps were compared to those in nearby undisturbed forests. A combination of morphotype analysis and molecular identification techniques were utilized to identify fungal species from EMF root tips.

I first examined the effects of a catastrophic wind disturbance occurring in the fall of 2002 on EMF communities in a mixed oak and pine forest in northern Georgia. I was able to characterize both seasonal and annual differences between the gap and undisturbed forest EMF communities. The EMF community in the undisturbed forest was consistently more diverse that the EMF community in the windthrow gap, and composition of EMF differed substantially. The EMF assemblages did not differ significantly among seasons in the windthrow gap, but the EMF community during fall season in the undisturbed forest was significantly more diverse than in the spring or summer.

The second study expands the temporal scale of the first study, and investigates the soil inoculum potential of EMF in different stages of forest recovery following windthrow. Soil was collected from disturbance gaps aged 2-, 11-, and 20- years since windthrow and from old-growth (undisturbed) forest stands in the Tionesta Scenic Area of western Pennsylvania, and soil

inoculum potential was evaluated using a seedling bioassay. The results of this study suggest that compositional changes in the plant community strongly influence EMF community composition. Furthermore, there is a severity threshold for wind disturbance that must be crossed in order to affect EMF community composition.

Lastly, I developed a computer simulation based on biological market theory that generates community composition hypotheses of fungal functional types under different environments that affect nutrient availability. The trade of carbon for nutrients based on the demands of both the host plant and the fungal functional types result in different fungal functional type assemblages when mineralized nutrients are readily available or when nutrients are bound in humified organic matter.

INDEX WORDS: mycorrhizal symbiosis, ectomycorrhizal fungi, windthrow, succession, seasonality, diversity, disturbance threshold, soil inoculum potential, biological market simulation, functional diversity

ECTOMYCORRHIZAL FUNGAL COMMUNITIES IN WIND-DISTURBED FORESTS

by

CHARLES C. COWDEN

B. S., The College of William and Mary, 2000

M. S., University of Central Florida, 2002

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2008

ECTOMYCORRHIZAL FUNGAL COMMUNITIES IN WIND-DISTURBED FORESTS

by

CHARLES C. COWDEN

Major Professor:	Chris J. Peterson
Committee:	Ronald L. Hendrick, Jr. Charles W. Mims William J. Otrosina David Porter

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2008

DEDICATION

This dissertation is dedicated to my wife Susan Hastings. Without her support and encouragement, I could never have completed this work.

ACKNOWLEDGEMENTS

I have received help from many during my time at the University of Georgia. Foremost, my wife Susan Hastings provided emotional support and invaluable advice on the molecular techniques central to this dissertation. I am grateful to my friends who have offered their assistance and feedback throughout the development of the research presented in this dissertation. Special thanks must be given to Dr. Eric Fuchs, William Duval, Steve Hovick, Dr. F. Andrew Jones, and Dr. James Kellner for their help.

My committee members - Ron Hendrick, Charles Mims, William Otrosina, and David Porter offered advice and helpful comments on my chapters. I thank my advisor, Chris Peterson for granting me the freedom to pursue a new area of research and learn new techniques. His guidance has helped improve my scientific research and writing. I need to especially thank David Porter and Michelle Momany for allowing me to use their labs and supplies. Also deserving thanks are Lisa Krueger, Leigh Griggs-Nedlo, and Jodi Cohen for assisting me in the field and Melanie Smith for maintaining my plants in the greenhouse. This research was funded in part from the University of Georgia Department of Plant Biology Palfrey Grant.

My extended family, the Hastings, Peterson and Cowden families all offered support and opportunities for rest and relaxation. Finally, I can not express my thanks deeply enough to my wife who has consistently provided the encouragement and perspective necessary to keep moving forward, even during desperate times.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Mycorrhizal symbioses between plants and fungi have been widely recognized by biologists for over a century (Frank 1894) and are found in nearly all terrestrial ecosystems and play critical roles in nutrient and water uptake, protection from root pathogens, and tolerance of heavy metal toxicity. The fungus receives carbohydrates from the host plant in exchange for the supply of water and nutrients to the plant. There are two major categories of mycorrhizae based on the nature of the integration of plant and fungal tissues. Most common are the arbuscular mycorrhizae (AM) in which fungal hyphae penetrate the plant cell walls and the exchange of nutrients and carbohydrate occur across the plant cell plasma membrane and the fungal cell wall. Most terrestrial plants form AM symbioses. In contrast to the high host diversity, only one order of fungi, the Glomales (Zygomycota), form AM. The second category of mycorrhizae is the ectomycorrhizae (EM) which are characterized by a network of fungal hyphae surrounding (not penetrating) root cortical cells and a sheath of densely packed hyphae surrounding the fine root. This sheath is referred to as a mantle and stunts the growth of the fine root resulting in a swollen, coralloid appearance indicative of the EM symbiosis. Unlike the monophyletic group of fungi forming AM symbioses, the fungi forming EM symbioses are from many distantly related

taxonomic groups. The EM symbiosis is thought to have evolved multiple times and is an example of convergent evolution resulting in a polyphyletic group of organisms in the same ecological guild. Only 3% of terrestrial plants are capable of forming EM, mainly boreal and temperate species, but also a few tropical trees. Many of the plant hosts are commercially important timber species, especially those from the Pinaceae, Fagaceae, and Betulaceae families.

Fossil evidence suggests the AM symbiosis arose at least 400 million years ago (Remy *et al.* 1994). The earliest fossil evidence of EM are from 50 million years ago (Le Page et al. 1997); however, molecular clock evidences estimates the EM relationship evolved 130 million years ago (Berbee and Taylor, 1993), approximately the same time as the Pinaceae appear in the fossil record (Axelrod 1986).

The diversity of EM fungi in ecosystems is often in contrast to the host plant diversity - it is common for dozens of EM fungal species to be found in 0.1 ha of a monospecific forest (Bruns 1995). The maintenance of locally high diversity of EM fungi is a major focus of current EM research. Furthermore, the need for integrating the field of plant ecology with that of EM fungi has been recognized as a crucial step forward for our understanding of mycorrhizal community ecology and its role in ecosystem functioning (Dahlberg, 2001, Johnson et al. 2006). Major areas to address include how EM fungal diversity affects ecosystem productivity, the potential role of EM fungi in the translocation of carbon and soil nutrients (Simard et al. 1997), and the influence of EM fungi on plant community diversity (Molina et al. 1992), among many others. Understandably, advancements in EM community ecology have lagged far behind those in plant ecology. Plant ecology, at least the aboveground components, benefits from the high visibility of individuals and readily discernable physical and physiological differences between species. Methods to quantify abundance, diversity, spatial distributions, and succession are

relatively straightforward for plants. For EM fungi, the only aboveground component is the sporocarp, but unlike plants the abundance and spatial location of aboveground components of fungal communities are poor representations of their belowground composition (Gardes and Bruns, 1996).

Molecular identification and quantification techniques and an emphasis on the belowground composition of the fungal community have revitalized the study of EM ecology (Horton and Bruns, 2001). Accurate species identification of EM root tips derived through molecular methods has enhanced our understanding of belowground distribution of fungal species, and these techniques may be applied to assess spatial structure of populations (Guidot *et al*., 2003). Although molecular techniques have simplified taxonomic identification of EM root tips, the sampling efforts remain tedious. Compounding this dilemma, EM fungal communities are often characterized by a high number of rare species (Horton and Bruns 2001). Soil coring and seedling bioassays are the most common methods to sample the EM community, but given the high diversity of EM species, especially in low tree diversity forests (Molina *et al*., 1992), reliable characterizations of the community may require large sample sizes (Horton and Bruns, 2001; Taylor, 2002).

1.2 Disturbance and EM Communities

Disturbances in forest stands, especially fire and logging, have stimulated many EM community level studies (reviewed by Dahlberg 2002, and Jones et al. 2003). High intensity disturbances may greatly alter the physical environment and plant community composition (Pickett and White, 1985) and thereby provide natural experiments (in the case of fire or wind damage; alternatively, logging practices are decidedly anthropogenic treatments) which may

affect EM community structure and succession. Recent disturbances provide an opportunity for establishing long-term EM succession studies, (e.g., Grogan *et al*. 2002); meanwhile, longitudinal studies, such as those of Visser (1995) and Jonsson *et al*. (1999), are surrogates for long term succession following disturbance.

Incorporating temporal dynamics into EM community ecology increases the complexity of sampling efforts. Fine root turnover in trees and seasonal dynamics of EM fungi require multiple samples annually. Multiple-year studies in undisturbed, old growth forests are rare (but see Dahlberg *et al*., 1997; Douglas *et al*., 2005; Izzo *et al*., 2005; and de Román and de Miguel, 2005). Studies of ectomycorrhizal community responses to both natural and anthropogenic disturbances in forests are common (e.g. reviews by Dahlberg, 2002 and Jones *et al*. 2003), but many of these are snapshot studies, relying on only one sampling effort (Taylor 2002). Forest succession, as far as plant components are concerned, may take centuries before achieving old-growth status. Within the first few years to decades following a disturbance, the relatively active dynamics of seedling and sapling establishment accompanied by self-thinning give way to slower, longer term dynamics which may show little change in forest composition or structure over long periods of time. However, the long-term dynamics of EM fungal succession do not necessarily follow the same temporal pattern as forest re-establishment, with the highest species richness and potential for interspecific interactions occurring upon canopy closure (Dighton and Mason, 1985). Indeed, Visser (1995) reported a sharp increase in EM species diversity between 6 and 41 years following wildfires.

1.3 The Effects of Large-Scale Forest Disturbance on EM Communities

Large-scale forest disturbance effects on EM fungal communities provide distinct opportunities to enhance our understanding EM community succession. Literature reviews for fire (Dahlberg, 2002) and clear-cut logging (Jones *et al*., 2003) are available. Here we briefly recap the information presented in these reviews and complement them with more recent studies. Also, we will discuss wind as a source of disturbance in EM forests.

We consider large-scale disturbances as those ranging from a few hectares to thousands of hectares and with intensity great enough to be considered stand replacing. Such disturbances leave few surviving trees, thus the potential for researching EM fungal succession as it coincides with forest establishment is greater. The effects of forest disturbance on the ectomycorrhizal community most often noted are changes in fungal species composition, fungal inoculum potential, relative abundances of different inoculum sources, and the spatial distribution of EM root tips.

1.3.1 Fire

The disturbance of choice for most mycorrhizal research is fire. In coniferous forests of western North America, pine savannahs, eucalypt forest, and boreal forests, fire is the most common and apparent natural disturbance (Oliver 1981, Visser 1995, Dahlberg 2002). Because fire is common and disturbance by fire has occurred regularly over evolutionary time scales, many plant species (and possibly their associate EM species) have been able to adapt to fire as a selective pressure. What is more, proposed management strategies of some temperate and boreal forests worldwide include regular prescribed burning. Facilitating the study of ectomycorrhizal communities, these forests tend to have low tree species diversity, if not monospecific stands,

and regeneration following the fire is often by the same tree species as before the disturbance. Furthermore, these forests are composed almost entirely of ectomycorrhizal tree species (e.g. Fagaceae and Pinaceae). High fungal species richness in these forests contrasts markedly with low woody plant species richness (Molina *et al*., 1992, Dahlberg, 2002).

The effect of fire on the mycorrhizal community depends greatly on intensity. If the fire is of low-intensity with few trees dying the mycorrhizal community may change little because fire damage will likely destroy only a small proportion of EM fungal inocula in the uppermost soil layer (Dahlberg *et al*., 2001). In contrast, severe fires may have drastic impacts on ectomycorrhizal communities since intense heat can destroy fungal inocula deep in the soil (Taylor and Bruns 1999). For example, low-intensity fires alter fungal species frequencies rather than eliminating species from the area (Jonsson *et al*., 1999). High-intensity fires regularly result in dramatic shifts of EM fungal community species composition, often with the most common species in the disturbed area present only at low levels in the undisturbed forest (Visser, 1995; Taylor and Bruns, 1999).

Intense fires in coniferous forests can have a dramatic impact on the tree species composition (Turner *et al*., 1997). Severely disturbed mixed species stands may be replaced by monospecific stands which persist for over a century (Douglas *et al*., 2005). Despite this, Douglas *et al*. (2005) reports dramatically higher fungal species richness in 135-year-old monospecific *Pinus contorta* stands when compared to nearby 300-year-old mixed coniferous forests (81 and 35 EM fungal species, respectively). Furthermore, there was limited EM fungal species overlap between the forest types. These forests represent two seral stages in forest development. The decrease in fungal diversity as the early-stage monospecific forest is invaded by later successional species coincides with the model of Dighton and Mason (1985), albeit at a

longer time series and perhaps not based on nutrient quality as predicted by the model (Douglas *et al*., 2005). However, several studies show EM fungal diversity continues to increase following disturbance, for at least 40 years following disturbance (Visser 1995), and high EM diversity levels are maintained as forests shift towards old-growth tree species (Visser 1995, Kranabetter et al. 2005, Tweig et al 2007).

Major shifts in soil physical and chemical properties occur following even low- intensity fires. The organic soil layers are consumed and the resulting ash deposition alters soil pH and thus nutrient availability to both plant and fungus. Much of the EM fungal biomass is concentrated in the organic soil layers, but various EM fungal species are also present in the lower mineral layers (Stendell et al., 1999; Taylor and Bruns, 1999). The location of fungal species in differing soil strata causes differential responses to fire – those fungi in the organic layers are destroyed and those in the mineral layers are relatively undisturbed, at least by low-intensity fires (Stendell *et al*., 1999, Jonsson *et al*., 1999; Dahlberg 2002).

1.3.2 LOGGING

Second in number to studies of fire disturbance are those concerning clear-cut and partial cut logging practices. Unlike fire, there has been insufficient time for forest species to adapt to logging as a selective pressure. Many logged forests are also of low tree species diversity, similar to those forests in which fire studies are often conducted. Forest regeneration in logged sites is often through seedling planting, seed rain from nearby forests or retained seed trees, or residual undesirable (for timber products) woody species. Occasionally, the regenerating tree communities have similar composition to the original community; if not, the regenerating tree species are mainly EM plant species. In some cases, non-EM plants (shrubby or herbaceous)

may first colonize the clearcut area and suppress EM formation on tree seedlings (Amaranthus and Perry, 1994).

Jones *et al*. (2003) reviewed the literature on ectomycorrhizal fungal communities in forests regenerating after clear-cut logging. Importantly, in the reviewed studies most of the woody plant communities regenerating following logging were similar to the communities prior to logging. They concluded that the component of the EM community that changes most is the species composition, not the percent colonization of root tips. Furthermore, the fungal community composition in the regenerating stands can be attributed to the altered soil conditions as much as from alterations to the fungal inocula following disturbance. Ectomycorrhizal fungi in regenerating stands are better adapted to the soil environment in disturbed areas than in closed canopy forests (Jones *et al.* 2003).

Hyphae of EM fungi will begin to die when carbon transfer from the host plant ceases (Harvey *et al*. 1980). Thus, after only a few years, spores will become the most important inoculum source in clear-cut forests (Deacon and Fleming, 1992). Sclerotia may also be an important inoculum source if the site is replanted or seedlings emerge relatively soon following the disturbance (Fox, 1986). Logging practices and post-logging treatment of the site has considerable effect on the regenerating forest and EM communities (Jones *et al*., 2003). Heavy machinery and log skidding will often compact soil structure (Perry *et al*., 1987). In sites where the top soil horizons have been removed or eroded as well as sites that are burned after logging, the fungal inoculum is greatly reduced (Harvey *et al.*, 1980, Jones *et al.*, 2003). Seed tree retention or other forms of 'advanced regeneration' (Helms, 1998) will affect EM communities by acting as refugia for EM fungi that occurred prior to the logging (Horton *et al*., 1998). The

trees left after logging will influence the spatial structure of both the plant and EM communities (Kranabetter 1999).

1.3.3 WIND

Windthrow caused by hurricanes, tornados, and other windstorms is another source of catastrophic disturbance in temperate forests (Everham and Brokaw 1996, Peterson 2000). However, at this point there exists only one published study in a poorly-known journal addressing the effects of large scale wind disturbance (Egli *et al*., 2002). Egli *et al.* (2002) studied the effects of a windstorm in a Swiss boreal forest and found both reduced fungal species richness and inoculum potential in the disturbance gap when compared to a nearby, undisturbed forest. Only early-stage EM fungi were found on seedlings collected from disturbance plots, and late-stage fungi with some early-stage fungi were found in the undisturbed forest samples. Parsons et al. (1994) created artificial windthrow gaps of various sizes (up to 30 trees), but were unable to show a threshold for gap size effect on EM communities in their simulated gap experiment.

We investigated the impact of windthrow in forests on EM fungal communities at the Dawsonville Wildlife Management Area in northern Georgia in Chapter 2. We found that the EM fungal community in the undisturbed forest was consistently more diverse that the EM fungal community in the windthrow gap, and composition of EM fungal communities differed substantially. Our research suggests that reduction in carbon availability from the host plant community has a greater influence on the EM fungal communities in windthrow gaps than does the reduction of fungal inoculum. Moreover, catastrophic wind disturbance in forests enhances landscape scale diversity among EMF communities.

1.4 EM Community Succession

EM fungal communities undergo shifts in species composition as forest stands age and the physiognomy shifts from young seedlings and saplings to closely-packed young adults and eventually to multi aged old-growth forests. Early work on EM fungal communities was based on fruiting bodies formed near isolated seedlings and saplings (Mason et al. 1983), and this work suggested that there are 'early-stage' and 'late-stage' fungi, based on the age at which they produce ectomycorrhizae and fruiting bodies. These categories have not withstood scrutiny (Arnolds 1991), and the occurrence of so called 'multi-stage' fungi (Danielson 1984) further confound attempts to produce simple models of EM fungal succession. The conceptual model presented by Last et al. (1987) utilized the broad categories of ruderal and stress-tolerant species (following Grime 1977), and others have suggested that describing EM fungal species as r- and K-selected (following Gadgil and Solbrig 1972) may be more promising (Smith and Read 1997). The greatest challenge is placing EM fungal species into such broadly defined categories, especially when high intraspecific variability is present in many species (Cline et al. 1987, Wong and Fortin 1990, Bonfante et al. 1997).

The model proposed by Last et al. (1987) predicted that young forests would be dominated by non-host specific, ruderal mycorrhizal fungi and have low mycorrhizal diversity. Mycorrhizal diversity would peak when the forest developed a closed canopy (25 to 30 year old forest), and mycorrhizal diversity would then decline as forests matured to old growth. The model further predicts that mycorrhizal communities in old growth forests would tend to be host-specific; and that these late-seral fungi would be best described as stress-tolerant, able to persist when litter is plentiful, but of extremely low nutrient quality. The temporal trend in species richness presented by Last et al. (1987) is broadly similar to that of Connell's (1978)

Intermediate Disturbance Hypothesis - a high diversity peak is expected at intermediate ages in community development. The Last et al. (1987) model seemed to adequately describe mycorrhizal communities in forests growing on abandoned agriculture fields (Mason et al. 1983, Dighton et al. 1986), yet, the results from research in forested landscapes contradict the model predictions, especially concerning host-specificity and fungal diversity in old-growth forests (Molina et al. 1992, Visser et al. 1995, Kranabetter et al. 2005, Tweig et al. 2007). Mycorrhizal species with high host specificity have been found in young forests, and these species persist as forests mature to old-growth status. Also, fungal diversity remains high even in late-succession and old-growth forests, suggesting that fungal diversity may plateau, rather than decrease as forests mature.

A recent model proposed by Jumponnen and Egerton-Warburton (2005) considers the host plant community, environmental factors, and biotic factors as successive filters to organize the mycorrhizal fungal community. Although this model does not directly address succession in EM communities, changes in the relative importance of each filter and the effects of the filters over time are easily conceivable.

In Chapter 3, we examine the soil inoculum potential of EMF in different stages of forest recovery following windthrow (2-, 11-, and 20-years since windthrow and in old growth). We found no differences in levels of colonization (percentage of seedlings colonized) from soils in the gaps and undisturbed forests; however, the species composition of the colonizing EMF differed in the different age categories. The results suggest that compositional changes in the plant community strongly influence EMF community composition. Furthermore, there may be a severity threshold for wind disturbance that must be crossed in order to affect EMF community composition. Thus, full understanding of EMF response to disturbance and forest recovery

clearly requires knowledge on not only time elapsed (since disturbance), but disturbance severity as well.

Myriad factors influence plant community succession (Pickett et al. 1987), and development of general models for EM communities face challenges similar to those of plant communities - propagule dispersal, suitability of edaphic conditions, and competition for resources, among many others. The symbiotic nature of EM fungi complicates attempts to predict patterns of EM community diversity since succession in the host plant community is central to EM fungal succession. Interactions between EM fungal species are poorly understood, and existing studies include only a few of the many species potentially found in EM communities (Kennedy et al. 2007). Thus, there are many questions yet to be answered to develop new models of EM fungal succession.

1.5 Functional Diversity of EM

Life history strategies have been integral in understanding the response to disturbance in plant and animal communities (Grime 1977, Petraitis et al 1989, Didham et al 1996, and many others). In fungal community research, life histories have played a major part in our understanding dynamics of coprophilous fungi on animal dung (Richardson 2002) and the succession of wood rotting fungi (Boddy and Rayner 1983, Boddy 1993). For ectomycorrhizal fungi, life histories have been harder to describe. Advances in molecular techniques allow for more accurate estimations of ectomycorrhizal fungal community composition and diversity (Gardes and Bruns 1993, Horton and Bruns 2001, Lilleskov and Bruns 2004). Still, sampling and measuring ectomycorrhizal communities involves destructive or at least invasive methods. Indeed, both the nature of fungal growth and the soil matrix handicap the in situ investigation of

ectomycorrhizal communities. It is difficult to imagine practical methods to study the recruitment, development, growth and reproduction of an individual ectomycorrhizal fungus and it is currently impossible to do so for an entire community of ectomycorrhizal fungi.

Since the EM fungal community is obligatorily associated with its plant host community, there is a tendency to model EM community dynamics after existing models of plant community dynamics and to fit EM fungi into ecological roles originally for plants (e.g., ruderal, stress tolerant, r-selected, K-selected, etc.). But, efforts to categorize fungi have not proven applicable in many circumstances; there are too many fungi that seem to fit multiple categories or those that fit no categories. The only successful categorizations up to this point have dealt with the ability of particular fungi to obtain nitrogen or phosphorus from organic compounds (e.g., Abuzinadah and Read 1986, Finlay et al. 1992); however, these categories appear not to predict successional status of the fungi. The most promising advances in succession models for mycorrhizal fungi focus on the functional diversity of the mycorrhizal relationship. These models define functional species, hypothetical species with certain suites of physiological attributes, in lieu of using true EM species found in nature. The benefit of utilizing functional species is that basic hypotheses regarding mycorrhizal succession can be generated based on simple functional attributes, rather than complex suites of interactions that are presently not well understood. The hypotheses generated provide direction for both field experimentation and future model development.

Models of the maintenance and stability of mutualisms are heavily rooted in an economic framework (Boucher et al. 1982, Noë and Hammerstein 1994, Bronstein 2001). Most recently, biological market models have illustrated the mutual benefit between partners in the mycorrhizal symbiosis(Schwartz and Hoeksema 1998, Hoeksema and Kummel 2003, Hoeksema and Schwartz 2003) and how multiple fungal functional types may be simultaneously associated

with a single plant host (Kummel and Salant 2006). The fourth chapter of this dissertation presents a step beyond these biological market models in the development of a computer simulation that generates community composition hypotheses of fungal functional types under different environments that affect nutrient availability. The trade of carbon for nutrients based on the demands of both the host plant and the fungal functional types result in different fungal functional type assemblages when mineralized nutrients are readily available or when nutrients are bound in humified organic matter.

1.6 Concluding Remarks

Disturbance is critical in maintaining plant species diversity (Connell 1978) and is thought to be equally important in maintaining ectomycorrhizal species diversity (Bruns 1995). The study of mycorrhizal communities following disturbance has necessarily lagged behind that of plant communities – to a large extent, the types of plants in an area determine the potential for particular mycorrhizal associations (Molina et al 1992, Johnson *et al*. 2005). Although plant succession is affected by myriad factors at multiple levels (Pickett et al. 1987), conceptual models supported by field observations of forest regeneration following disturbance provide generalized predictions of forest composition and succession. Furthermore, spatially explicit forest models predict plant community composition and the spatial distribution of individual tree species (SORTIE model, Pacala et al. 1993, Pacala et al 1996). Highly sophisticated simulation models, particularly those with spatial distributions, may allow for spatially explicit models of ectomycorrhizal communities following disturbance.

CHAPTER 2

ECTOMYCORRHIZAL FUNGAL COMMUNITIES IN FORESTS RECOVERING FROM CATASTROPHIC WINDTHROW IN NORTHERN GEORGIA, U.S.A[1]

[1] Cowden, C.C. and Peterson, C.J. To be submitted to *New Phytologist*

2.1 INTRODUCTION

The obligatory relationship of ectomycorrhizal fungi (EMF) to their host plants fundamentally drives EMF community dynamics; however, the processes that maintain high local EMF diversity (α-diversity) are poorly understood. Forest disturbance has been proposed as a factor influencing EMF diversity (Bruns 1995). The most common natural disturbances arise from windstorms and fire; furthermore, due to the high timber value of the host trees, commercial logging impacts many forests that support EMF. Although wind disturbance is the dominant natural disturbance in many forests (Everham and Brokaw 1996), the effects of wind disturbance in forests on the EMF community remain largely uninvestigated. The literature on wind disturbance and EMF community is scant; we know of only one study (Egli et al. 2002); conversely, studies of EMF communities in fire disturbed forests and in logged forests are plentiful. This study focuses on the EMF fungal community in a catastrophically wind disturbed forest and emphasizes important differences between the effects of wind disturbance compared to both fire and logging.

EMF communities in naturally fire disturbed forests show low α-diversity (Visser 1995, Grogan et al. 2000); however, diversity at the landscape scale (β-diversity) often is enhanced - the EMF community in the disturbed areas are compositionally different from EMF communities in adjacent undisturbed forests (Taylor and Bruns, 1999). The many studies of EMF communities in harvested forests indicate that the shifts in the EMF community during forest regeneration are due to both the loss of inocula and changes in the soil environment (Jones et al. 2003). While the damage to the forest canopy in clearcut logging may be similar to that of catastrophic wind damage, the activities associated with logging result in distinctly different

disturbance effects on the soil environment (McIver and Star 2001, Jones et al. 2003, Bennett and Adams 2004).

Under regular exposure to disturbance, organisms may evolve life history strategies to cope with the typical disturbance regime. Indeed, many plant communities are maintained only when a disturbance regime is maintained, and if the disturbance regime has been suspended, the composition of the plant community changes. This is especially true when dominant plants require disturbance to reproduce, such as the many coniferous species with serotinous cones, or for plants that require mineral soil for seed germination. Likewise, EMF communities adapt to disturbance regimes, and well adapted species are capable of forming resistant propagules following disturbance (Taylor and Bruns 1999).

Fire, logging, and wind disturbance have very different effects on plant community composition (Turner et al. 1997). Low intensity fires can remove understory vegetation and organic soil horizons without damaging canopy species, but shift the EMF community to favor species forming resistant propagules (Taylor and Bruns 1999). Importantly, fire selects against species that are not capable of forming resistant propagules, and regular fires reinforce the specialized EMF community composition. However, wind disturbance does not impose the same selection pressures on the EMF propagule bank. The most frequent wind disturbance in forests results in small gaps, damaging a few canopy trees, but leaving the understory relatively intact and imposing little selection pressure on the EMF community.

Catastrophic wind disturbance is far less frequent than the small gap disturbances (Turner et al. 1998); therefore, organisms are unlikely to have evolved coping strategies specific to large, severe wind disturbances although some traits and responses that are beneficial after other types of disturbance may be beneficial after wind disturbance. Furthermore, the physical

and chemical changes in soils in wind disturbed forests are minimal since there is no loss of organic material nor is the soil exposed to intense heat (Foster et al. 1997). This allows us to frame two possible null hypotheses, which provide the primary point of departure for this study. First, there would likely be no shift towards an EMF community of "wind-disturbance specialists", but towards a community of general colonizers - ruderal EMF species (species that are effective as colonizers from spores) - and EMF species that may be common are those that remain viable inocula as hyphae while receiving reduced carbohydrates from a host (as would occur when mycelia are attached to the root system of a recently snapped tree). Second, as an alternative null hypothesis, the EMF community following catastrophic windthrow may simply be random subset of the pre-disturbance community, the disturbance serving to bottleneck the EMF community.

A second component of this study investigates seasonal variability in α-diversity. The forests of northern Georgia are a mix of deciduous broadleaf (*Quercus*) and evergreen (*Pinus*) trees, and temperate forests have different photosynthetic rates during different times of the year with the highest rates occurring when broadleaf trees are fully leafed out in the summer and fall. Since EMF rely on carbohydrates from their host plant for energy, we predicted that the EMF community would have greater diversity in the summer and fall. Furthermore, we expect greater seasonal variation in the undisturbed forest than in the disturbance gap because the plant community is well established and has greater seasonal variability in carbohydrate production (the disturbance gap has few EM hosts, and those present are predominantly *Pinus* seedlings).

We utilize a combination of a greenhouse bioassay (similar to Brundrett and Abbott 1994) and seedling collection in order to characterize EMF community composition in the windthrow gap and the adjacent undisturbed forest. We sampled naturally regenerating seedlings

from the gap and undisturbed forest several times throughout the year over a three year time span in order to assess annual and seasonal differences in EMF community composition.

2.2 METHODS

2.2.1 STUDY SITE

The Dawsonville Wildlife Management Area (DWMA) is located in northern Georgia, U.S.A (42° 25' N, 82° 9' W). The forest is dominated by *Quercus sp.* (Fagaceae) and *Pinus strobus* (Pinaceae) with some *Pinus virginiana* (Pinaceae), *Acer rubrum* (Aceraceae), *Liriodendron tulipifera* (Magnoliaceae), and *Oxydendrum arboretum* (Ericaceae). Soils in the DWMA are ultisols, and have a sandy clay texture. The disturbance gap is the result of a tornado that occurred in fall 2002, and is >500 m in length and up to 120 m wide. Complete canopy removal occurred over much of the gap, although a few areas had only partial canopy removal.

Five transects were established across the width of the disturbance gap in a stratified random fashion along the length of the gap. Three transects were established in the adjacent undisturbed forest. These transects were utilized for the duration of the study for both soil and seedling collection.

2.2.2 GREENHOUSE BIOASSAY

Soil collection for the greenhouse bioassay occurred in August 2005. Ninety soil samples, 15 cm diameter, 10 cm deep, were collected at random locations near the established transects in both the disturbance gap and the undisturbed forest for a total of 180 soil samples. To avoid edge effects, sampling locations were at least 40 m from the edge of the gap. Upon collection, each soil sample was placed in a plastic bag in a cooler and transported to a

greenhouse in Athens, Georgia (33° 57' N, 83° 19' W). The soil cores were handled carefully to keep the structure of the soil as intact as possible.

Soil cores were placed into appropriately sized pots at the greenhouse within 24 hours of collection. In order to have control pots, 30 pots each from the gap and the undisturbed forest soil pots were selected at random and were steam pasteurized for 1 hour to destroy any EMF inocula present in the soil. The 60 remaining gap soil pots and 60 undisturbed forest soil pots were left untreated. All of the pots were then planted with sterile (EMF free), 1 month-old *Pinus strobus* seedlings to 'trap' the EMF present in the pots. The *P. strobus* seedlings were grown in sterilized vermiculite, and the root systems were washed and visually inspected for EMF prior to the planting in the experimental pots. After the seedlings were planted in the experimental pots, the pots were arranged randomly on a greenhouse bench, and the seedlings were allowed to grow for 5 months under normal light conditions. The seedlings were watered 3 to 5 times per week and received pesticide treatments once per month, but they were never fertilized. The seedlings were harvested after 5 months; the roots were washed and inspected for EMF. The roots were stored in distilled deionized water (ddH_20) at 4°C

2.2.3 Field Bioassay

In addition to the greenhouse bioassay, naturally regenerating, approximately 1 to 2 year old (based on counting of whorls) *P. strobus* seedlings were collected for a field bioassay. Using the established transects, 30 seedlings were collected from each of the gap and the undisturbed forest, three times per year (in May, July and October during the first week of the month), for three years (2004 - 2006). The timing of the May collection was just before deciduous trees leaf out, and deciduous trees in northern Georgia lose their leaves in late October to early November.

The sampling scheme allowed both seasonal and annual differences to be investigated. All collected seedlings were located at least 40 m from the edge of the gap. A small hand trowel was used for collection to minimize damage to the seedling root system. Sixty seedlings were harvested at each collection, and a total of 540 seedlings were collected over the three year span. Fungal fruiting bodies were also collected from the site. Each seedling was placed in an individual plastic bag for transport to the laboratory for identification. The roots were washed and stored in the same manner as the greenhouse bioassay roots.

2.2.4 EMF IDENTIFICATION

Both morphological and molecular techniques were utilized to identify EMF species. Initially, EMF root tips were placed into morphotype groups based on size, color, texture and EMF root tip architecture using a dissecting microscope. Based on published descriptions (Agerer 1988), several EMF could be identified based on the morphotype (e.g., *Cenococcum geophilum*, *Rhizopogon sp.*). From each morphotype group, 10 randomly selected root tips were used for identification using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). We chose to include fungi from the *Mycelium radicis atrovirens* group (MRA, also referred to as dark septate endophytes in the literature) in the EMF community although their functional status is not well defined (Menkis et al. 2004). Danielson and Visser (1989) and Visser (1995) provide precedence for including MRA fungi in EMF communities.

A CTAB-chloroform extraction was used to extract DNA from the root tips and fruiting bodies. A single root tip (or a small portion of a fruiting body) was placed in a 1.5 ml microcentrifuge tube containing 600 µl of a 2% CTAB and ground using a micropestle. The sample was then incubated at 65°C for at least one hour. The sample was then centrifuged using

a benchtop microcentrifuge at 13 000 rpm for 5 minutes, after which the supernatant was transferred into a new 1.5 ml tube. Next, 600 µl of chloroform was added to the tube, and then the tube was shaken to mix the solution. The samples were centrifuged at 13 000 rpm for 5 minutes, and again the supernatant was transferred to a new 1.5 ml tube. Then, 750 µl of -20°C isopropanol was added to the tube to precipitate the DNA. The sample was shaken to mix the solution, and the sample was chilled to -20°C for at least 30 min to fully precipitate the DNA. After precipitation, the sample was centrifuged at 13 000 rpm for 30 minutes. The isopropanol solution was carefully poured off as to not disturb the DNA pellet, which was washed with -20°C ethanol. After centrifuging at 7000 rpm for 5 minutes, the ethanol was carefully poured off and the DNA sample was dried in a 65°C heating block. Once the sample was completely dry, the pellet was resuspended in at least 200 µl ddH_2O (more ddH_2O for larger pellets).

The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using the primer pairs with at least one fungal specific primer (ITS1F and ITS4, or ITS1F and ITS4B depending on success of the PCR reaction) developed by Gardes and Bruns (1993). Each 50 µl PCR reaction contained 50 - 100 ng of template DNA (5 to15 µl of the extraction mixture), 5 µl of 10X PCR buffer (New England Biolabs), 0.5 µl 50mM dNTP solution, 0.4 µl each of the 10mM forward and reverse primers, 2.5 µl DMSO, 0.5 µl BSA, 0.3 µl $MgCl_2$, 0.4 µl taq polymerase (New England Biolabs), and adding the appropriate amount of ddH_20 to bring the volume of the reaction mixture to 50 µl. The typical PCR cycle was an initial 2 minute hold at 95°C for initial denaturing, followed by 25 to 35 cycles of 30 s at 95°C, 30 s at 50°C and 75 s at 68°C to amplify the number of DNA copies, and finally a 5 minute hold at 68°C for final extension. Success of the PCR reactions was confirmed using electrophoresis of 5 µl aliquots of the PCR reactions (0.9% agarose gel for 1 hour at 100 V).

Three separate restriction digests were performed on aliquots from each PCR product tube using Mbo I, Taq α I, and Hinf III enzymes (New England Biolabs). One-hour digestions were performed under conditions specified for each enzyme. Gel electrophoresis was utilized to view RFLP patterns resulting from the enzyme digestions (2% agarose gel, 100 V for at least 2 hours). RFLP patterns from root tip samples were compared to those from fruiting bodies in order to identify species. When root tip patterns could not be matched to fruiting bodies, predicted digestion patterns from published sequences were used to match species.

EMF root tips were separated into 22 morphotypes and a total of 39 RFLP species were identified. These species were grouped into 15 genera (Table 2.1) in order to characterize the EMF community. EMF community diversity was calculated using Shannon's Diversity Index ($H' = \Sigma\, p_i\, (\ln p_i)$; in which p_i is the proportion of the ith species in the total sample, with proportion calculated as the number samples of the ith species divided by the total number of individuals). Similarity between EMF communities was calculated using Sørenson's similarity coefficient ($S_S = 2a / (2a + b + c)$; in which a is the species common to both the gap and the undisturbed forest, b is the species found only in the gap, and c is the species found only in the undisturbed forest). The Sørenson similarity coefficient ranges from 0 to 1, with 0 representing no similarity and 1 representing communities of identical floristic composition.

2.3 Results

2.3.1 Greenhouse Bioassay

All seedlings survived in the forest, gap, and control samples. There was no EMF colonization in the control samples, and the number of seedlings colonized in the forest (57 out of 60) and gap (55 out of 60) samples were not significantly different (chi-square, P = 0.714).

The mean number of EMF species colonizing a single seedling differed significantly (Mann-Whitney Rank Sum Test, $P < 0.001$) between the forest samples (1.51 ± 0.62, mean ± standard deviation) and the disturbance gap samples (1.07 ± 0.48; Figures 2.1 and 2.2). EMF from 6 genera colonized the gap samples, and 9 EMF genera colonized the undisturbed forest samples (Table 2.1). There was high overlap in the EMF communities in the forest and gap samples, but several genera (*Amanita*, *Cortinarius*, and *Suillus*) were found only in the forest samples (Figure 2.3). The EMF diversity was significantly greater in the forest samples ($H' = 1.97$) than in the disturbance gap samples ($H' = 1.57$) ($t = 3.821$, $df = 6$, $P < 0.01$), and the similarity between the forest and gap communities was $S_S = 0.52$.

2.3.2 Field Bioassay

All of the naturally regenerating seedlings collected from the disturbance gap and the undisturbed forest over the three years of this study had EMF colonization. As in the greenhouse bioassay, seedlings collected from the undisturbed forest had a greater mean number of EMF genera colonizing each seedling (1.94 ± 0.93) than the seedlings from the disturbance gap (1.55 ± 0.83; Mann Whitney Rank Sum Test, $P = 0.009$; Figure 2.1). Many of the EMF genera colonized seedlings both in the disturbance gap and the undisturbed forest (Table 2.1); however, there were two genera that were found only on seedlings from the disturbance gap (*Hebeloma* and *Scleroderma*) and five genera found only on seedlings from the undisturbed forest (*Cantharellus*, *Cortinarius*, *Laccaria*, *Piloderma*, and *Tricholoma*).

To monitor annual changes in the EMF communities, the spring, summer, and fall samples for each year were pooled for both the disturbance gap and the undisturbed forest, and pooled samples compared among years. Diversity was consistently greater in the forest than in

the gap (Figure 2.4a). Diversity was highest in both the disturbance gap and undisturbed forest in 2004; however, differences in diversity between years were not significant for either the disturbance gap or the undisturbed forest. The relative abundances of EMF genera found in the field bioassay for the gap and undisturbed forest are shown in Figure 2.5. The three most abundant EMF genera are the same for the forest and the gap: *Russula, Cenococcum*, and *Rhizopogon*, albeit not the same rank. *Hebeloma* and *Scleroderma* EMF were found only in the gap.

Seasonal EMF data were compared across all three years to examine seasonal changes in the EMF community in the disturbance gap and the undisturbed forest. Diversity for both the gap and the forest were lowest in spring, and diversity increased as the year progressed (Figure 2.4b). The tendency to increase in diversity for the disturbance gap was not significant (t-test, P = 0.391). In the gap samples, the fall 2004 diversity (H' = 1.84) was much greater than in fall 2005 (H' = 1.59) and fall 2006 (H' = 1.68). In the undisturbed forest, the increase from the average spring diversity (H' = 2.09 ± 0.02) to the average summer diversity (H' = 2.14 ± 0.03) was nearly significant (t-test, P = 0.057), and the increase from the average summer diversity to the average fall diversity (H' = 2.3 ± 0.03) was significant (t-test, P = 0.002). The EMF communities in the gap and the forest had low similarity in spring (S_S = 0.46), summer (S_S =0.47), and fall (S_S = 0.41). Many EMF genera were abundant throughout the year in both the disturbance gap and the undisturbed forest (e.g. *Cenococcum*, MRA, *Rhizopogon*, and *Russula*), and the majority of EMF genera present in the spring were also found throughout the year (*Suillus* and *Tricholoma* were not found in the summer in the undisturbed forest; Figure 2.6a). In the disturbance gap, several species were found in very low abundance (in only 1 sample each) in the fall of 2004 (*Amanita*, *Boletus*, and *Suillus*); *Lactarius* was also found in the gap only in

TABLE 2.1. Ectomycorrhizal fungi (EMF) found in samples from the greenhouse bioassay (GH) and the field bioassay (FB). The presence of a particular EMF genus in the bioassay is indicated with a (+); if the EMF genus was not found in the samples, the column is left blank.

	Gap		*Undisturbed Forest*	
EMF genera	*GH*	*FB*	*GH*	*FB*
Amanita.		+	+	+
Boletus.		+		+
Cantharellus				+
Cenococcum	+	+	+	+
Cortinarius			+	+
Hebeloma	+	+	+	
Laccaria				+
Lactarius	+	+	+	+
MRA	+	+	+	+
Piloderma				+
Rhizopogon	+	+	+	+
Russula	+	+	+	+
Scleroderma		+		
Suillus		+	+	+
Tricholoma				+

the summer and fall of 2004. In the undisturbed forest, *Amanita*, *Cortinarius,* and *Laccaria* EMF were found only in the summer and fall, but were found in all three years.

2.4 Discussion

Analysis of both the greenhouse bioassay results and the field samples indicate that EMF community composition and diversity within a 2 - 4 year old windthrow gap differs from that of adjacent undisturbed forest. The EMF community in the undisturbed forest is more diverse (Figures 2.5 and 2.6) and individual seedlings are on average colonized by more EMF species than in the disturbance gap (Figure 2.1). There are many EMF species common to both the gap and the undisturbed forest; however, there are several species found predominantly in either the gap or the undisturbed forest. The prevalence of *Hebeloma* and *Scleroderma* in the windthrow gap rejects the proposed null hypothesis that the disturbance would simply create a bottleneck on the EMF community since neither genus is found in the undisturbed forest (Figure 2.5). There is a clear shift in the EMF community following disturbance. Wind disturbance does not destroy EMF inocula, and in the gap studied here did not greatly alter the soil structure. However, the tornado removed the host plants, thus removing the carbon source for the EMF. In this gap, the loss of a carbon source appears to have a greater effect on the EMF community than the destruction of inoculum or the alteration of soils.

In comparing the results of the greenhouse bioassay with the field bioassay there are several important differences in community composition. The EMF community composition is similar in the greenhouse and field bioassays for the windthrow gap. However, *Scleroderma* EMF were present in the field bioassay and absent from the greenhouse bioassay, but this may be due to the smaller sample size for the greenhouse bioassay. The larger sample sizes for the field

bioassay enabled detection the presence of EMF genera that are rare, but present in the gap (Figure 2.5). Likewise, comparing Figures 2.3 and 2.5, the greenhouse bioassay underestimated EMF community richness in the undisturbed forest. Greenhouse conditions do not mimic field conditions, and the conditions of the greenhouse may favor certain EMF. *Hebeloma* species were common in the undisturbed forest samples in the greenhouse bioassay (Figure 2.3), but were never found in any of the seedlings in the undisturbed forest from the field bioassay (Figure 2.5). The removal of the soil from the field, even if the soil sample remains intact, disturbs the soil enough to favor disturbance specialist (ruderal) fungi such as *Hebeloma*. Removal of the soil from the site disrupts the plant-fungus association, thus EMF species that colonize mainly through hyphal contact would be less common in greenhouse studies (Taylor and Bruns 1999). Species that colonize predominantly via spores or sclerotia are therefore probably favored. Importantly, when species are found in the greenhouse bioassay, but not in field samples, there is an indication that the EMF species are present in the inoculum bank either as long term persistent spores and sclerotia or are recent arrivals via spore rain. The absence of *Hebeloma* from the field sampled seedlings in the undisturbed plot can not be attributed to a lack of inoculum, but some other factor reduces the potential of *Hebeloma* inocula in older forests.

MRA fungi colonized seedlings in the windthrow gap and the undisturbed forest in both the greenhouse and the field bioassay. MRA appear to be common in forests; Visser (1995) found MRA to be common in forests ranging from 6 to 122 years since wildfire; likewise, Twieg et al. (2007) reported MRA fungi at many stages of forest succession. Menkis et al. (2004) were able to isolate MRA fungi from live roots, decayed roots, stumps, and fine woody debris in forests, indicating that these fungi remain capable of colonizing roots from dead tissues. Thus, wind disturbance in forests would likely not reduce the capability of MRA to colonize roots.

Russula EMF were commonly found in both the windthrow gap and the undisturbed forest in both the greenhouse bioassay (Figure 2.3) and the seedlings from the field bioassay (Figure 2.5). We expected *Russula* EMF would be common in the undisturbed forest as they are considered late-stage fungi (Deacon and Fleming 1992); however, the high relative abundance of *Russula* EMF in the disturbance gap was surprising. In the undisturbed forest, fruiting bodies of *Russula spp.* were extremely common, but were not found in the windthrow gap (C. Cowden personal observation). Like *Russula spp., Amanita spp.* are generally considered late-stage fungi. The occurrence of *Amanita spp.* in this study follows the predicted pattern of being more common in the undisturbed forest and nearly absent in the windthrow gap. In contrast to *Russula spp.*, fruiting bodies of *Amanita* were rare throughout the three year span of this study.

We propose two possible explanations for the abundance of *Russula* EMF in the windthrow gaps. First, high input of spores from the nearby undisturbed forest coupled with the relatively favorable soil conditions in wind disturbed gaps (as compared to areas damaged by fire, logging, or prolonged agriculture) may provide a high inoculum potential from spores. Second, *Russula* mycelia may remain viable for extended periods following disturbance if they remain attached to the root system of snapped trees; the fungi may survive on the residual carbohydrates stored in the roots (Bâ et al. 1991, Hagerman et al. 1999). There are likely other possible explanations, but further experimentation is needed in order to address these hypotheses.

Annual differences in both the windthrow gap and the undisturbed forest were minimal. Regeneration of the plant community in the windthrow gap has been dominated by *Rubus sp.* (Rosaceae), with some *Kalmia latifolia* (Ericaceae) and many herbaceous Asteraceae. Notably, the regeneration of *Pinus* and *Quercus* seedlings has been minimal even 5 years post disturbance (C. Cowden, personal observation, 2007). With little change in the ectomycorrhizal host plant

community during our sample period, we expect similarly little change in the EMF community. The 2004 field bioassay samples from the windthrow gap contained several genera that were not found in either the 2005 or 2006 samples, and this may be an indication that inocula of these genera (*Amanita*, *Lactarius,* and *Suillus*) were able to persist only up to two years post disturbance without plant community regeneration including suitable EM hosts. The plant community in the undisturbed forest was stable during the duration of this study, and we found the EMF community to be likewise stable when comparing seedling EMF colonization across years.

Seasonal differences in EMF community diversity and composition are evident in the undisturbed forest (Figures 2.4 and 2.6). Diversity increased throughout the growing season, and many EMF genera were more abundant during the summer and fall. The forests in the DWMA are mixed coniferous and deciduous broadleaf forests, and during the spring samplings in early May, the deciduous trees are at the early stages of leafing out. Carbon allocation to EMF during the leafing out period of deciduous trees is considerably less than during the growing season, thus EMF mycelia associated with these trees may be either dormant or at least constrained in their ability to grow and colonize nearby root tips. As the trees leaf out, photosynthesis increases and so presumably does the carbohydrate allocation to EMF. Therefore, higher levels of EMF activity should be seen during the summer and fall when carbon fixation is at its highest in temperate forests (Lopez et al. 2001, Newman et al. 2006) . The diversity increase in the undisturbed forest in the summer and fall support this hypothesis. The seasonal increase in diversity in the windthrow gap (Figure 2.4) was found only in fall 2004, when *Amanita, Boletus, Lactarius,* and *Suillus* EMF colonized seedlings. In all three years, the EMF community in the windthrow gap in all seasons was consistently composed of *Cenococcum, Hebeloma,* MRA,

Rhizopogon, Russula, and *Scleroderma* EMF. In the gap, carbohydrate availability to EMF is consistent (and low) throughout the year, making seasonal differences in EMF activity less likely.

Competitive networks have been proposed as a method for maintaining high EMF diversity at small scales (Bruns 1995), but little progress has been made to elucidate them (Kennedy et al. 2007). Seasonal variation in carbon availability may help explain the maintenance of locally high diversity of EMF communities in temperate mixed evergreen and deciduous forests in a similar manner as proposed by Hutchison (1961) for the maintenance of plankton diversity. Winters in the DWMA are mild with average daily highs > 9°C. Evergreen trees are capable of photosynthesis year round and in the proposed scenario EMF associated with evergreens may remain active all year. The deciduous trees are active only in late spring through the end of the fall season, and EMF associated solely with deciduous trees are likely to be dormant or have low levels of activity while the host trees are leafless. During winter and early spring, the EMF associated with evergreen trees have a competitive advantage over the EMF associated with deciduous trees in colonizing vacant fine root tips. However, broadleaf trees are generally considered to have a greater photosynthetic rate than coniferous evergreens (Givnish 2002), and during the active growing season, EMF associated with broadleaf deciduous trees would have an advantage over those on coniferous evergreens. Central to this hypothesis is that the seasonal advantage of one group over the other is insufficient for competitive exclusion. Different EMF would be competitively superior at different times of the year depending on their host plant; moreover, the competitive ability of fungi that associate simultaneously with multiple hosts (potentially with evergreen and deciduous hosts) may be spatially correlated with the locations of their associations - the fungus would be more competitive closer to the host with the

greater current carbohydrate allocation. Tests of this hypothesis would require fine scale spatial and temporal measurements in forests using rhizotrons to minimize sampling disruption and to allow measurement of hyphal growth rate. Greenhouse experiments with large pots (allowing for separate rooting zones with seedling "bait" positioned in the different zones) containing 2 or more host species would also be appropriate, and in such experiments it would be easier to monitor EMF colonization than in field studies.

Large catastrophic wind disturbances that destroy aboveground components of the plant community create spatial heterogeneity in soil carbohydrate input at a landscape scale, and potentially enhance β-diversity of EMF assemblages in forests. If the host plant community regenerates quickly, or if there is sufficient advanced regeneration or residual surviving plants, alterations to the EMF community composition will likely be minimal. However, the plant community in the windthrow gap studied here had no residual EM host plants, and even 4 years following the disturbance, EM host plant regeneration was minimal. Thus, the EMF community in the windthrow gap was compositionally different than in adjacent undisturbed forest. Although there are several EMF genera common to both the windthrow gap and the undisturbed forest, there are genera that are specific to either the gap or the forest, creating unique EMF communities in each location.

FIGURE 2.1. The average number of different ectomycorrhizal fungi (EMF) colonizing individual seedlings in the windthrow gap and undisturbed forest in both the greenhouse bioassay (GH) and the field bioassay (FB). The effects of both the location of the sample (gap vs. undisturbed forest) and sampling scheme (GH vs. FB) are significant ($P < 0.001$ for both, Holm-Sidak t-test).

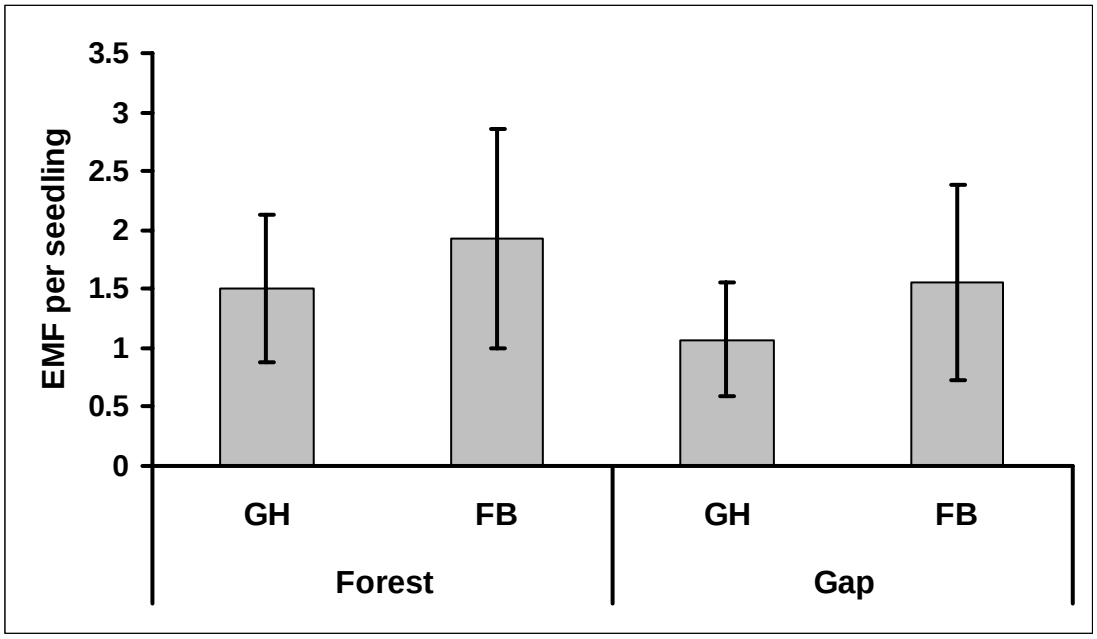


FIGURE 2.2. The number of seedlings with multiple ectomycorrhizal fungi (EMF) in the undisturbed forest and the windthrow gap samples from the greenhouse bioassay (GH) and the field bioassay (FB).

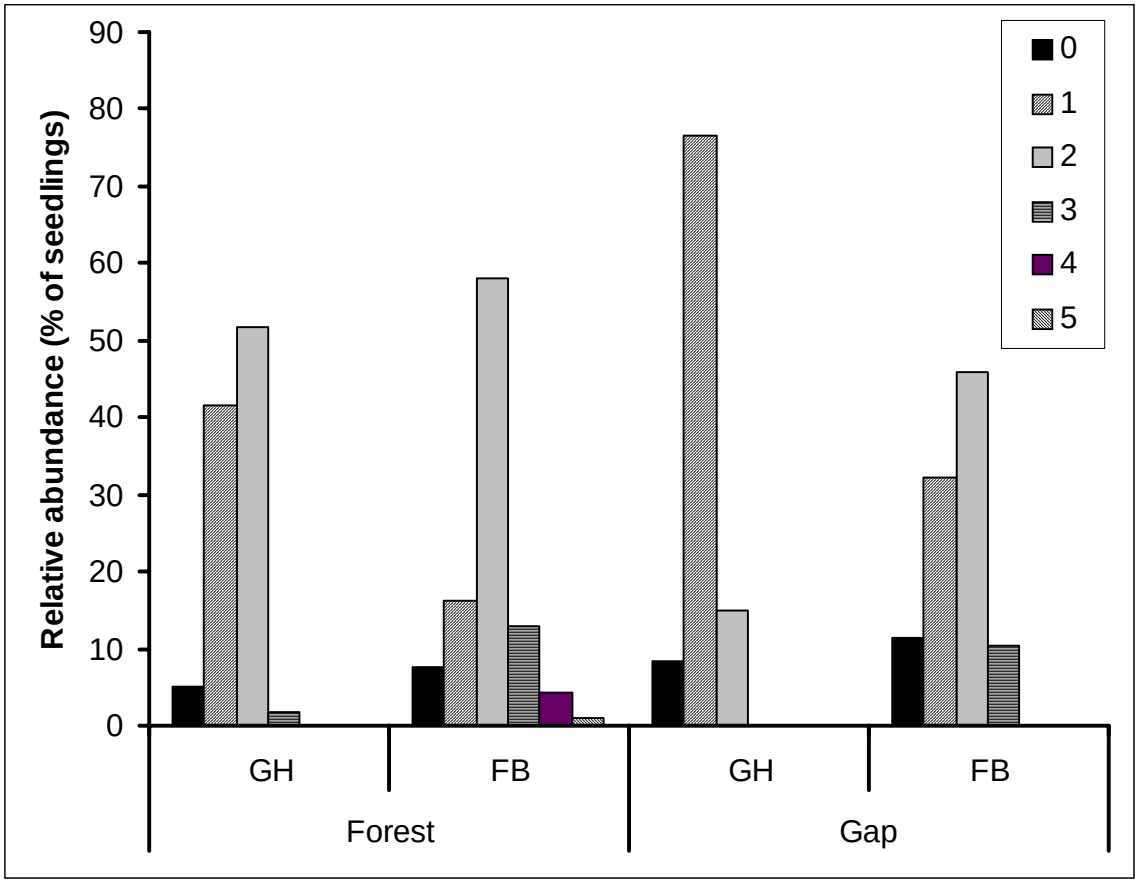


FIGURE 2.3. Relative abundances of ectomycorrhizal fungi (EMF) found in the greenhouse bioassay samples. EMF genera are ordered based on their relative abundance in the undisturbed forest samples. This figure emphasizes the differences in the EMF communities found in the windthrow gap (gray bars) and the undisturbed forest (black bars). There is high overlap in the EMF communities.

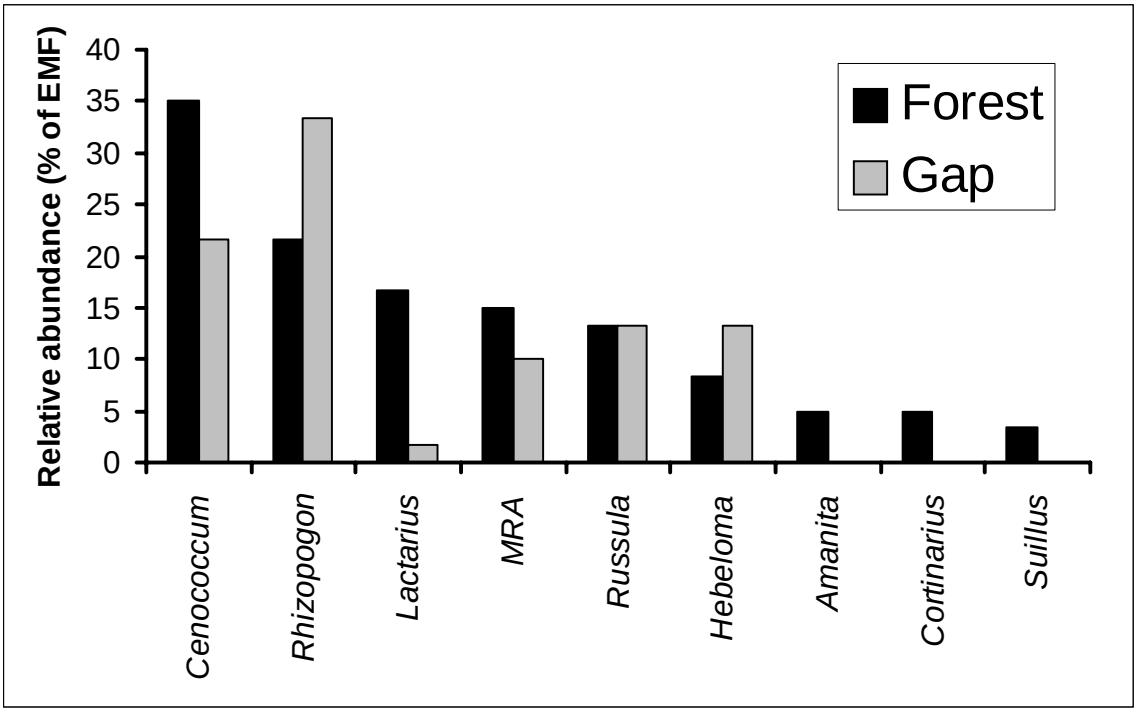


FIGURE 2.4. Shannon's Diversity Index (H') for the undisturbed forest (solid triangles, solid line) and windthrow gap (empty diamonds, dashed line). (a) Annual diversity values for the three years of the study. The EMF community diversity of the undisturbed forest is consistently greater than in the windthrow gap, and the differences between years are not significant for either the undisturbed forest or the windthrow gap. (b) Seasonal diversity values. The diversity for the undisturbed forest is significantly greater in the fall than other times of the year, and there are no significant seasonal differences in diversity in the windthrow gap.

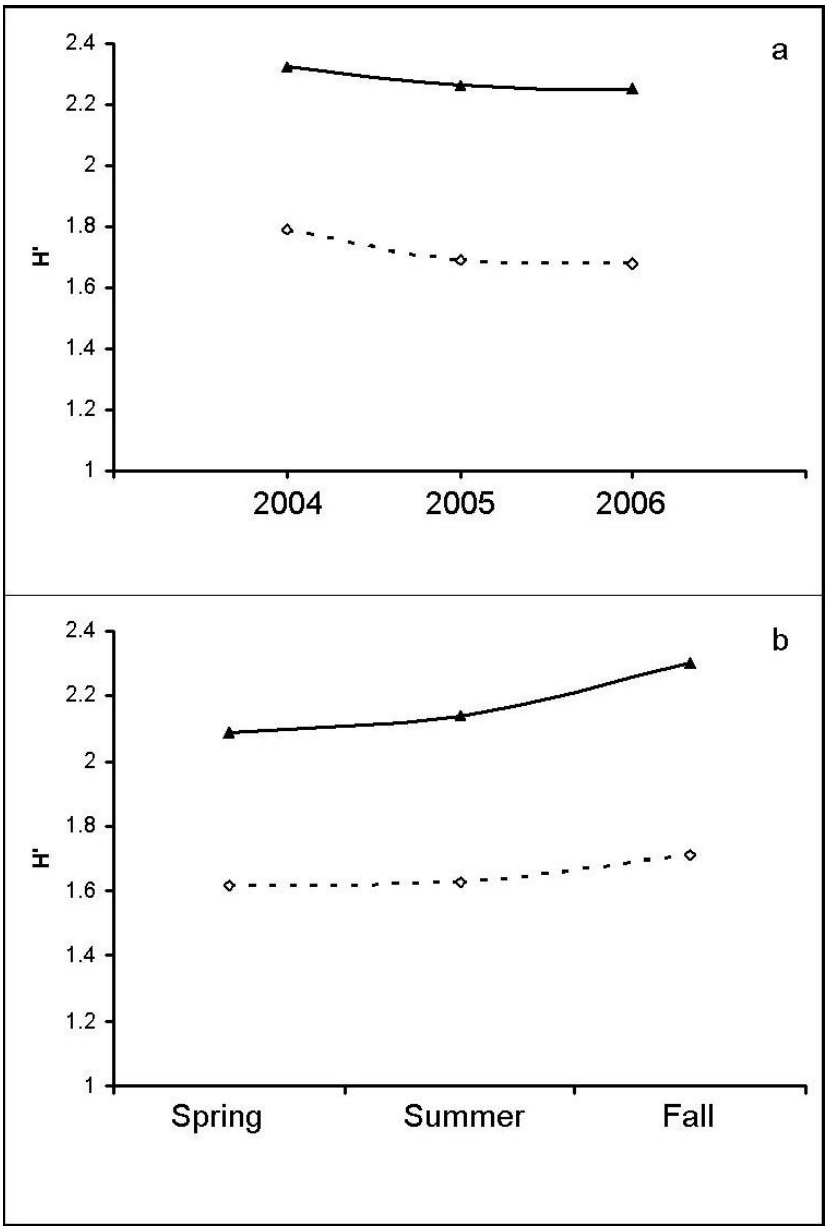


FIGURE 2.5. Relative abundances of ectomycorrhizal fungi (EMF) found in the field bioassay samples. EMF genera are ordered based on their relative abundance in the undisturbed forest samples. This figure emphasizes the differences in the EMF communities found in the windthrow gap (gray bars) and the undisturbed forest (black bars). It is noteworthy that many species are found in both areas, but several species are unique either to the windthrow gap or the undisturbed forest.

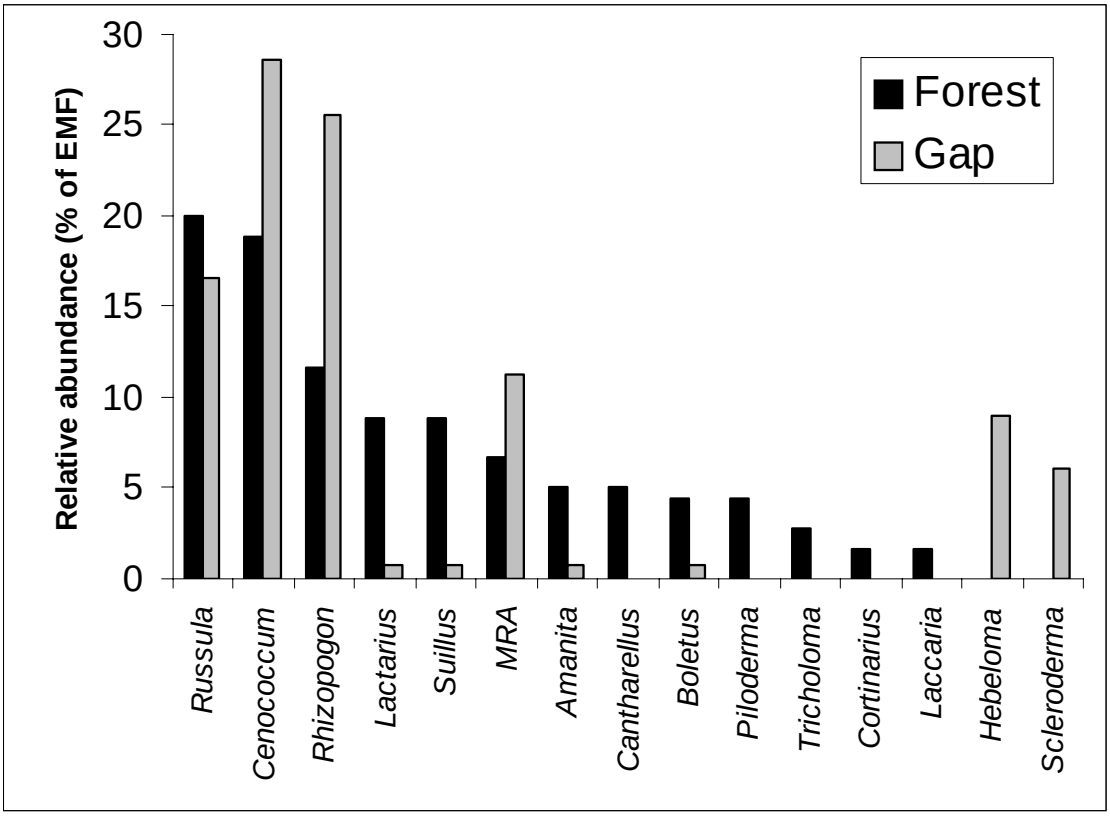
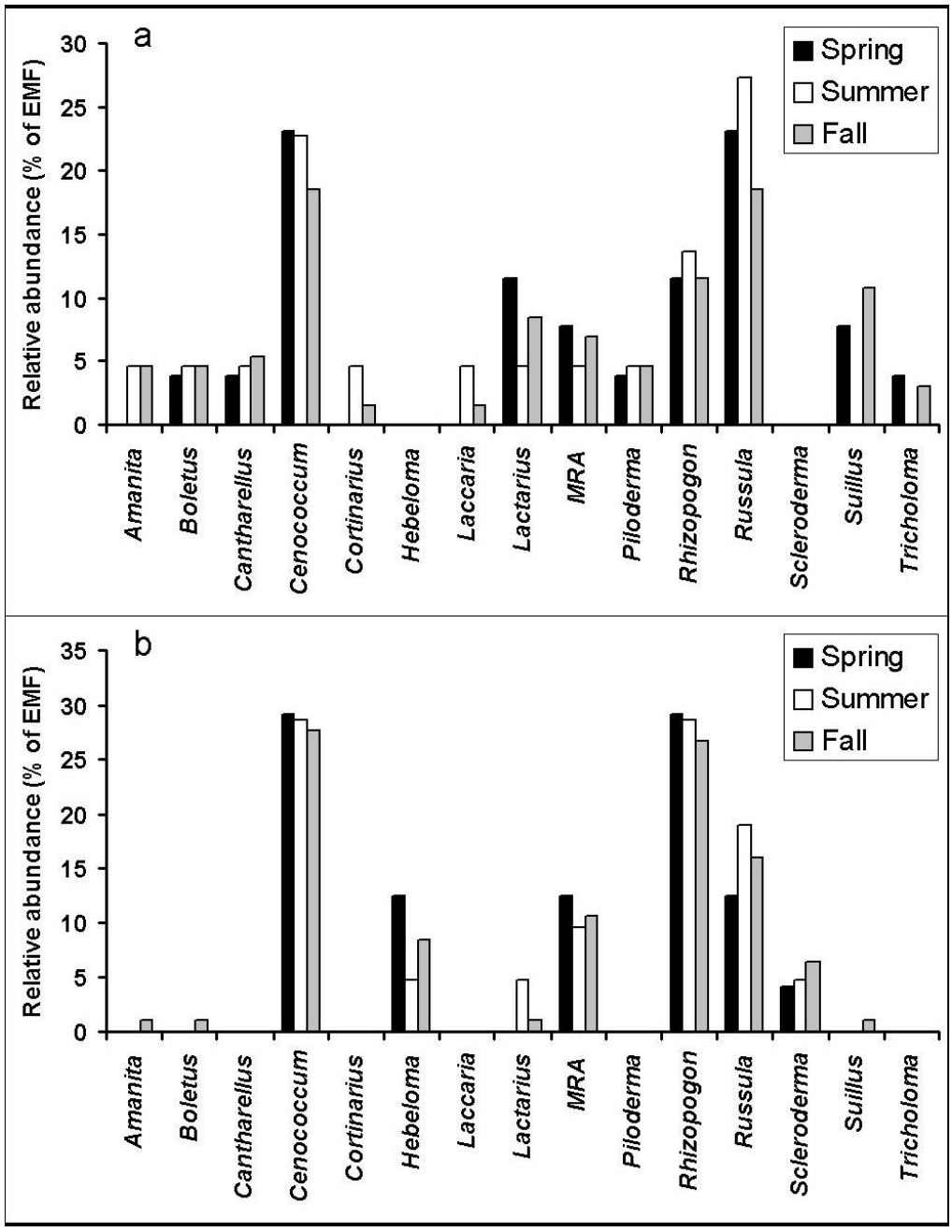


FIGURE 2.6. Seasonal ectomycorrhizal fungal (EMF) community composition in the undisturbed forest (a) and the windthrow gap (b). The composition of the EMF community in the windthrow gap remains consistent throughout the year while the EMF community in the undisturbed forest becomes more diverse as the seasons progress. In the windthrow gap, *Amanita, Boletus, Lactarius* and *Suillus* EMF were found only in samples from 2004.



CHAPTER 3

SOIL ECTOMYCORRHIZAE INOCULUM POTENTIAL IN FOREST GAPS REGENERATING FOLLOWING WINDTHROW: A LONGITUDINAL STUDY FROM WESTERN PENNSYLVANIA, U.S.A.[1]

[1] Cowden, C.C. and Peterson, C.J. Submitted to *Mycorrhiza* 6/12/2008

3.1 INTRODUCTION

The richness and compositional relationships between ectomycorrhizal fungi assemblages and plant communities can be difficult to characterize, having aspects of both tightly-linked and loose, facultative relationships. On one hand, mycorrhizal fungi depend on their host plants for carbon, and are therefore inexorably linked to the host plant communities (DeBellis et al. 2006), leading to an expectation that diversity, composition, and dynamics of plant communities should be closely reflected by fungal assemblages. Indeed, host plant identity can strongly constrain the type of mycorrhizal symbionts; for EMF, the absence of certain plant families may preclude host specific fungi from the EMF assemblage (e.g. the Suilloid fungi specific to plant family Pinaceae).

On the other hand, even simple patterns of diversity diverge between the plant and fungal communities. For example, the temperate and boreal forests that host most ectomycorrhizal fungi are often species-poor in the tree component, yet they can maintain strikingly diverse EMF communities in which dozens of fungal species may be found within 0.1 ha (Trappe 1977, Bruns 1995). Thus it is perhaps most appropriate to view the plant community as a biological filter, which shapes but does not determine in detail the fungal symbiont community (Jumponen and Egerton-Warburton 2005).

The similarities and differences between plant and fungal responses to disturbance are even more poorly understood. Empirically, long-term dynamics of EM fungal succession do not necessarily follow the patterns seen in plant succession; EMF reach highest diversity about the time of canopy closure and this high diversity is maintained after forests mature (Visser 1995). However, detailed characterization of fungal response to disturbances is difficult because understanding fine root turnover in trees and seasonal dynamics of EMF require multiple-year

sampling, which is rare (but see Dahlberg et al. 1997; Douglas et al. 2005; Izzo et al. 2005; and de Romain and de Miguel 2005). In contrast, snapshot studies of ectomycorrhizal community responses to both natural and anthropogenic disturbances, which rely on only a single sampling effort, are more common (e.g. reviews by Dahlberg 2002; Jones et al. 2003). Obviously, generalizing from snapshot studies to patterns of fungal dynamics over time would be problematic, suggesting that different sampling approaches are necessary.

In comparison to concepts of plant community response to disturbance, conceptual frameworks for the response dynamics of mycorrhizal fungi remain elusive. The most recent models predict plant community response as a function of disturbance size, severity, and/or type (e.g., Frelich and Reich 1999, Frelich 2002, Roberts 2004), but we yet have little empirical basis for generalizing how these components of a disturbance regime influence mycorrhizal fungi. Nevertheless, numerous concepts suggest that among plants, disturbances can facilitate coexistence of numerous species, with the greatest diversity occurring at some intermediate time: diversity is low soon after a disturbance, peaks at some intermediate time, and declines again in old-growth assemblages (Connell 1978, Last et al. 1987). This very broad model may provide a conceptual starting point for fungal assemblage response to disturbance. Severe disturbances can provide natural experimental treatments (in the case of fire or wind damage) in which EMF community structure and succession can be monitored to test the model.

This study investigates the change in soil inoculum potential following catastrophic wind disturbance and during subsequent forest succession. Wind is one of the dominant agents of disturbance in many forests of the world (Everham and Brokaw 1996), yet several characteristics that are distinct from other types of disturbance may influence the response of the EM fungal community. Ironically, nearly all existing studies of EM fungal response to disturbance focus on

logging or fire as the agents of disturbance. Fire, for example, physically and chemically alters soil characteristics, and depending on the intensity of the fire, may destroy the host plant community. Even low intensity fires destroy the organic layers of soil, the site of the majority of potential EMF inocula for colonizing seedlings (Dahlberg 2002). Logging practices often include prescribed burning as site preparation before replanting, but the activity of logging itself often disrupts or destroys much of the inocula present in forest soils (Jones et al. 2003). Wind disturbance is the only natural disturbance for which it is possible to isolate the effects of host removal on EMF communities without the compounding factors of widespread chemical or physical alteration of soil or the destruction of the EMF inocula present.

We use a glasshouse bioassay approach; the seedlings act as baited traps for EMF, and the fungal species colonizing the seedlings are assumed to be present as inocula in the forest soil. Our sampling design is longitudinal (e.g. Visser 1995; Jonsson et al. 1999; Twieg et al. 2007), as a surrogate for long-term monitoring that might require decades or more. We sampled from similar forests in close proximity to each other, with matching soils, land use history, and dominant forest vegetation, but differing in age (time elapsed) since recent disturbance: 2-yr, 11-yr, and 20-yr since disturbance sites, as well as undisturbed old-growth forest. The youngest gap chosen for this study was 2 years since disturbance, and given the lack of regeneration of EMF host plants at the time of sampling, the inocula present in the soil are likely mostly spores, since there is no carbon available for EMF fungi. As EMF host plants recruit within a gap, EMF mycelia begin to grow, thus potential inoculum sources include spore, mycelia, and sclerotia. As forests mature, EMF mycelia are ubiquitous throughout forest soils and become the predominant colonizing inoculum (Amaranthus and Perry 1994). Thus EMF species colonizing the bioassay seedlings in soils from 2 year old gap do so exclusively from germinating spores, while EMF

species in the older gaps and undisturbed forest may be colonizing from spores, mycelia, or sclerotia inocula.

3.2 Methods

3.2.1 Study Site

The Tionesta Scenic Area (TSA) is located in the Allegheny National Forest in western Pennsylvania, U. S. A. (41° 40' N, 78° 58' W). The undisturbed, old growth forest is dominated by *Tsuga canadensis* (Pinaceae), *Acer saccharum* (Aceraceae), and *Fagus grandifolia* (Fagaceae) with some *Prunus serotina* (Rosaceae). Within the TSA, wind disturbances in 1985, 1994, and 2003 provide three different post-disturbance "ages". The first disturbance was a large tornado occurring in May 1985. The forest gap left by the tornado was >10 km long and up to 900 m wide, with 84.1% reduction of tree density and 97% basal area reduction (Peterson and Pickett 1991). The second and considerably smaller storm occurred in 1994. The gap left was approximately 2 ha with a 29% reduction in density and 38% reduction in basal area (Peterson 2000). The 2003 disturbance was caused by a severe thunderstorm in July 2003, and it created many small or medium sized gaps scattered across the forest near the 1985 and 1994 damage areas (Evans et al. 2007). For this study, a medium sized gap (approximately 3 Ha) with complete canopy destruction was selected from the many gaps resulting from the 2003 storm. Sampling of the undisturbed forest and all three gaps occurred in September 2005. At the time of sampling, the 1985 gap (20 years since disturbance) was dominated by *Betula alleghaniensis* and *Betula lenta* (Betulaceae). The 1994 gap (11 years since disturbance) was dominated by *Tsuga canadensis* and *Fagus grandifolia*, and the 2003 gap (2 years since disturbance) had undergone little tree community recovery with very few seedlings located within the gap.

Soils in the TSA are spodosols overlaying siltstone and sandstone bedrock. The soil is moist, stony and acidic, with pH 5.1 - 5.5 (Hough and Forbes 1943, Aguilar 1981). The climate is temperate, humid continental with mean winter temperature -5°C and mean summer temperature 16.5°C; mean annual rainfall is 1070 mm, evenly distributed throughout the year (Bjorkbom and Larson 1977).

3.2.2 Soil Collection

Several transects were arranged in a stratified-random fashion in each gap and in an area of undisturbed forest near the disturbance. No samples were taken within 40 m of the gap edge in order to preclude edge-effects from the study. Seventy-five soil samples were collected from each of the undisturbed forest and the three disturbance gaps using a 15 cm diameter soil corer to collect the top 10 cm of soil. An additional smaller sampling was performed in the 20 year old gap to test the influence of canopy species on EMF inoculum potential. The majority of the 20 year old gap is dominated by *Betula spp.*; however, there are many patches within the large gap that are dominated by *Acer pensylvanicum* and *Acer rubrum*. For the *Betula* dominated areas, locations were selected that contained only *Betula* individuals within a radius of 10 m. Four such sites were located, and 5 soil samples were taken at each, for a total of 20 soil samples (these samples are hereafter identified as *Betula* samples). The same method was used for *Acer* dominated patches, and 20 soil samples were collected (*Acer* samples). Each soil sample was placed in a sealed plastic bag and placed in a cooler for transport from TSA to the greenhouses in Athens, Georgia. A total of 340 soil samples were collected for this study. Special efforts were made to minimize disturbance and compaction of the sampled soil. Fruiting bodies of any epigeous fungus were also collected.

3.2.3 GLASSHOUSE BIOASSAY

Within 24 hours of collection, the soil samples were transported to a glasshouse in Athens, GA (33° 57' N, 83° 19' W) and placed in pots 15 cm wide and 10 cm deep. From each of the 75 samples taken from the gaps and undisturbed forest, 15 potted samples were randomly selected to serve as controls for potential greenhouse contamination. Thus, a total of 60 samples were used as control, and 60 samples were left for each of the gaps and the undisturbed forest, along with 20 samples each for the *Betula* and the *Acer* patches. The control samples were steam pasteurized for >1 hour to destroy any potential EMF inocula present in the soil.

A 1 month-old, sterile grown *Pinus strobus* seedling was planted in each plot to act as a colonization opportunity, or 'trap' for EMF in the soil samples. *Pinus strobus* was selected because it can be easily grown in a glasshouse compared to the limited success of other plant species found in TSA (*Tsuga canadensis* and *Fagus grandifolia* seedlings had mortality rates too high to be useful for this study). Seedlings were inspected prior to planting for any EMF, and only those seedlings that had no EMF were used. Following seedling planting, the pots were arranged randomly on benches in the greenhouse and grown for 5 months under ambient light conditions. The seedlings were watered regularly and received pesticide treatments monthly, but were not fertilized. After 5 months, the seedlings were collected; the roots were washed and visually inspected for EMF. All EMF root tips were stored in distilled deionized water at 4°C.

3.2.4 EMF IDENTIFICATION

Ectomycorrhizal fungal species were identified using a combination of morphological and molecular techniques. Root tips were separated into 19 morphotype groups based on size, color, texture, and tip branching patterns using a dissecting microscope. Several fungal

morphotype groups could be identified either to species (e.g., *Cenococcum geophilum*) or to genus (*Rhizopogon sp.* and *Suillus sp.*) based on their morphology alone (Agerer 1991). From each morphotype group, 10 root tips were randomly selected for identification using molecular techniques. Although the functional status of *Mycelium radicis atrovirens* (MRA) - also referred to as a dark septate endophyte - is unclear and possibly weakly pathogenic (Menkis et al 2004), we choose to include MRA as a component of the EMF community as in Danielson and Visser (1989) and Visser (1995).

DNA was extracted from fungal fruiting bodies and the root tips using CTAB-chloroform extraction. Individual root tips were ground using a micropestle in 600 µl 2% CTAB in a 1.5 ml microcentrifuge tube and placed in a 65°C heating block for at least one hour (up to 4 hours). The tubes were then centrifuged at 13 000 rpm for 5 minutes and the supernatant was transferred into a new 1.5 ml microcentrifuge tube; 600 µl of chloroform was added to the tube. The tubes were then shaken to mix the solution and centrifuged at 13 000 rpm for 5 minutes. The supernatant was transferred to another new 1.5 ml microcentrifuge tube to which 750 µl of -20°C isopropanol was added and the tubes shaken to mix the solution. The tubes were then placed in a -20°C freezer for at least 1 hour to precipitate the DNA. The samples were then centrifuged for 30 minutes at 13 000 rpm at 4°C. The isopropanol was discarded and the DNA pellet was washed with -20°C ethanol. After centrifuging the tube at 7 000 rpm for 5 minutes, the ethanol was discarded and the DNA sample was dried for 5 minutes on a 65°C heating block. The pellet was resuspended in 100 - 250 µl distilled deionized water (dd H_20) depending on the size of the pellet.

Identification of the root tips was achieved using a combination of polymerase chain reaction (PCR) followed by restriction enzyme digestion to produce restriction fragment length

polymorphism (RFLP) patterns (Henrion et al. 1992). The internal transcribed spacer region (ITS) was amplified using the ITS1F fungal specific primer with either ITS4 or ITS4B depending on the success of the amplification (Gardes and Bruns 1993). For each 50 µL PCR reaction, between 50 and 200 ng of template DNA was added to a PCR tube (5 to 15 µL of the DNA extraction mixture) containing 5.0 µL of 10X reaction buffer (New England Biolabs), 0.5 µL 50 mM dNTP mix, 0.4 µL each of 10 mM primers, 2.5 µL DMSO, 0.5 µL BSA, 0.3 µL $MgCl_2$, 0.4 µL taq polymerase (New England Biolabs), and ddH_20 to bring the volume of the reaction mixture to 50 µL. A typical PCR reaction began with a 2 minute hold at 95°C for initial denaturing followed by 35 cycles of 30 s at 95°C, 30 s at 50°C, and 75 s at 68°C and ending with a 5 minute hold at 68°C for final extension.

Restriction enzyme digestion was performed separately with Mbo I, Taq α I, and Hinf III enzymes for one hour under conditions specific to each enzyme. Using a 2% agarose gel, elecrophoresis was performed at 100 volts for at least 2 hours to ensure good separation of the RFLP bands. Products of the digestion ranged from approximately 800 base pairs (bp) to <100 bp. The RFLP patterns of root tip samples were compared to patterns produced by fruiting bodies and to patterns predicted from published sequences in order to identify the samples to species.

RFLP-species were grouped at the genus level to characterize the EMF inocula in the gaps and the undisturbed forest, and to calculate taxonomic richness and diversity. Note that this grouping is conservative in that it underestimates actual species richness. Shannon's diversity index (H') is calculated as $H' = \Sigma\, p_i\, (\ln p_i)$; in which p_i is the proportion of the ith species in the total sample. Nonmetric multidimensional scaling (NMDS) was used to graphically represent dissimilarity between gaps based on the Sørenson statistic (PC-ORD version 4.01, MjM

Software, Gleneden Beach, OR, USA). Relative abundances were used to calculate dissimilarity due to the smaller number of soil cores taken from the *Acer* and *Betula* dominated areas in the 20 year old gap.

3.3 RESULTS

Seedling survival was high and was similar for all groups. Colonization by EMF was lowest in the 2 year old gap, 43 out of 56 surviving seedlings, which differed significantly from the other groups ($\chi^2 = 20.1$, df = 3, $P < 0.001$). Fifty-two of the 55 surviving seedlings from the 11 year old gap had EMF, all 53 of the surviving seedlings from the 20 year old gap were colonized, and 53 of the 57 surviving seedlings from the undisturbed forest were colonized. Only one seedling from the control group was colonized by EMF (1 out of 58 surviving seedlings, colonized by *Hebeloma sp.*). In the *Betula* samples, 17 of 20 surviving seedling were colonized, and 17 out of 20 surviving seedlings were colonized from the *Acer* samples. Seedlings were most often colonized by a single morphotype per pot, but seedlings colonized by multiple morphotypes per pot were not uncommon. The average numbers of EMF colonizing each surviving seedling for each group are shown in Figure 3.1. A total of 19 morphotypes were identified, and from those a total 26 RFLP-species were identified. Most RFLP species were identified to genus level (Table 3.1).

Diversity (H') and richness of the inoculum communities were calculated based on the genera data. The samples from the 2 year old gap had the fewest RFLP genera, 5, and the lowest diversity, H' = 1.42. In samples from the 11 year old gap, 10 genera colonized with H' = 2.0; samples from the 20 year old gap contained 8 genera with H' = 1.86; and samples from the

undisturbed forest contained 10 genera with H' = 2.08. Many EMF genera were common to all of the areas sampled (*C. geophilum*, MRA, *Rhizopogon spp.* and *Russula spp.*); nevertheless, EMF community composition (Figure 3.2) was strongly influenced by host community composition and by canopy closure. The NMDS ordination (Figure 3.3) shows the strong similarity between the EMF community in the undisturbed forest and the 11 year old gap. Not surprisingly, the EMF community in the *Betula* dominated patches within the 20 year old gap is similar to the EMF community from the 20 year old gap samples. However, the EMF community in the *Acer* dominated patches in the 20 year old gap is most similar to the 2 year old gap.

3.4 DISCUSSION

The diversity of inocula contributing to EMF communities in disturbance gaps increased rapidly following disturbance. The 2 year old gap lacked EMF host plants at the time of sampling, and colonization from existing mycelia is unlikely since there is insufficient carbon input to sustain EMF (Based on results of Hagerman et al. (1999) who reported few active EMF roots 2 years after clearcutting in forests). The EMF inocula colonizing bioassay seedlings for the 2 year old gap are similar to the early colonizing species (*Cenococcum geophilum*, *Rhizopogon sp.*, and MRA fungi) from other surveys (Visser 1995, Grogan et al. 2000, Kranabetter et al. 2005, Twieg et al. 2007). Surprisingly, some typical late-stage fungi also colonized the bioassay seedlings from the 2 year old gap (*Leccinum*), indicating that late-stage species may be surviving at low frequencies during early secondary forest succession. For the forest gaps in this study, EMF community diversity reaches a plateau upon canopy closure, and high diversity was maintained in older forests, supporting results reported by Visser (1995),

TABLE 3.1. Ectomycorrhizal fungi colonizing seedlings in the bioassay. The number of unique RFLP patterns attributed to each group is indicated in the RFLP types column. The presence (+) or absence (-) of each EMF group for the sampling locations is also included.

EMF species	RFLP types	2 year old	11 year old	20 year old	Undisturbed
Amanita spp.	3	-	+	+	+
Boletus spp.	2	-	+	-	+
C. geophilum	2	+	+	+	+
Cortinarius spp.	2	-	+	+	-
Lactarius spp.	4	-	+	+	+
Leccinum sp.	1	+	-	+	+
MRA fungi	1	+	+	+	+
Piloderma sp.	1	-	+	-	+
Rhizopogon spp.	2	+	+	+	+
Russula spp.	6	+	+	+	+
Suillus spp.	2	-	+	-	+

Kranabetter et al. (2005), and Twieg et al. (2007). Thus the temporal trend in EMF communities over time may not match the richness peak at intermediate ages and subsequent decline often seen in plant communities (Connell 1978).

In most cases, the severity of disturbance is neglected when discussing the intermediate disturbance hypothesis. The severity of forest disturbance is often characterized by the amount of canopy destroyed; furthermore, the trajectory of subsequent forest succession is greatly influenced by the individuals remaining following disturbance. These residual individuals contribute to advanced regeneration. For EMF communities following wind disturbance, there is minimal disturbance to the soil (as compared to other disturbances), and thus high levels of residual EMF inocula. If there are numerous residual host plants, as following the disturbance in the 11year old gap, the impact of wind disturbance on EMF diversity will be low, especially if the residual host plant community is similar to the pre-disturbance forest. Disturbances in the gaps selected for this study ranged from complete canopy destruction in the 2 year old and 20 year old gaps, to partial canopy removal in the 11 year old gap. When compared to the undisturbed forest, there was little compositional change in the 11 year old gap, but the plant community in the 20 year old gap differed greatly from the pre-disturbance forest (Peterson 2000). Alteration of the host plant community clearly affects host specific fungi as indicated by the absence of *Suillus spp.* in the 20 year old gap which lacks conifers.

EMF community composition is strongly influenced by the composition of the plant community at an even finer scale within gaps. Disturbances create patchy environments within gaps, and microsite differences in the gap created by the 1985 tornado impacted forest regeneration (Peterson and Pickett 1990). Although the sample sizes were much smaller than for the entire gaps, the results from the *Betula* and *Acer* patches suggest that plant canopy plays a

major role in maintaining EMF inoculum diversity at a very fine scale. A comparison between EMF inoculum potential beneath *Betula* and *Acer* dominated canopies is an extreme example because *Acer spp.* are predominantly arbuscular mycorrhizal. The dissimilarity between the *Acer* patch and *Betula* patch communities (Figure 3.3) emphasizes the importance of the pathway that fixed carbon takes into the soil in order to maintain EMF. The maples and the birches in the 20 year old plot are of similar size, and likely fix similar amounts of carbon, but only the carbon fixed by birches is available to EMF. Moreover, the effect of low carbon availability on EMF diversity is emphasized by the similarity between the *Acer* patch and the 2 year old gap EMF communities (Figure 3.3); there is little carbon for EMF in either location.

The composition of forests with suitable ectomycorrhizal hosts also influences EMF community composition. The plant community in the 11 year old gap is similar to that in the undisturbed forests, thus the similarity of EMF communities in these two sites is not surprising. Compositional differences between the 20 year old gap and both the 11 year old gap and undisturbed forest lead to differences in the EMF community. Several of the *Cortinarius* species are birch specialists; thus, *Cortinarius spp.* were prominent components of the 20 year old birch dominated gap and mostly absent from the other areas sampled. Likewise, suilloid fungi were not found in the 20 year old gap due to the lack of suitable Pinaceae hosts (Figure 3.2). Intense deer browsing is likely the primary reason for absence of hemlocks (*Tsuga canadensis*) from the sapling generation in the 20 year old gap (Krueger and Peterson 2006), suggesting a possible indirect cascade between deer browsing, [lack of] hemlock regeneration, and abundance of suilloid EMF. The 20 year old gap is surrounded by old growth forests with hemlocks; therefore, the lack of suilloid EMF in the gap is not likely due to limitations in spore dispersal.

Estimations of inoculum diversity based on glasshouse bioassays are likely to underestimate the EMF diversity present *in situ* in the forest (Jones et al. 2003). Furthermore, soil inoculum potential does not indicate the activity of EMF, simply the ability of seedlings to be colonized. It is unlikely that there were active mycelia in the 2 year old gap as there were no living EMF hosts at the time of soil collection, thus the colonization of seedlings occurs largely through spore germination. Conversely, in established forests, most colonization is thought to occur via hyphal contact (Amaranthus and Perry 1994), and glasshouse studies may overestimate the contribution of species present only as spores in the soil rather than active EMF symbionts. Seedlings in this experiment were colonized by EMF typical of old growth forests (e.g., *Lactarius spp.* and *Piloderma spp.*) indicating that the mycelia of these species are still viable inocula when separated from their hosts at least for the relatively short duration of the bioassay.

The results of this study indicate that there may be a severity threshold that must be surpassed in order to affect EMF community composition. The 1985 tornado was considerably more devastating than the 1994 windstorm, and the 11 year old disturbance gap had recovered to closed canopy status at the time of sampling. As such, the composition of the plant communities in the undisturbed forest and the 11 year old gap most similar, as were the EMF communities in these areas. Since there was only a 38% reduction in basal area in the 11 year old disturbance gap, it is possible that the 1994 disturbance lacked the severity necessary to affect the EMF community. Thus, the EMF community could persist in the partially disturbed forest gap on the undamaged hosts. Furthermore, *Fagus grandifolia* is common in the area of the forest disturbed in 1994 and this species sprouts readily from roots (Peterson 2000). The recovery of the plant community was rapid, and the EMF community would have been stressed by low carbon availability for only a short period of time. Conversely, the 2003 windstorm completely

removed the canopy in many gaps (Evans et al. 2007), and recruitment of EMF hosts in the studied gap had not occurred at the time of sampling. Thus, the EMF community was diminished due to extremely low carbon availability. In sum, these comparisons emphasize the importance of disturbance severity for EMF community composition via its influence on plant community structure and composition; simple predictions of trends based solely on age (time since disturbance) are inadequate.

FIGURE 3.1. The average number of unique ectomycorrhizal morphotypes found on the roots of seedlings grown in soils from each sampling location. Standard deviation is indicated with error bars. Sites with matching letters do not differ significantly (Mann-Whitney U, $P > 0.05$).

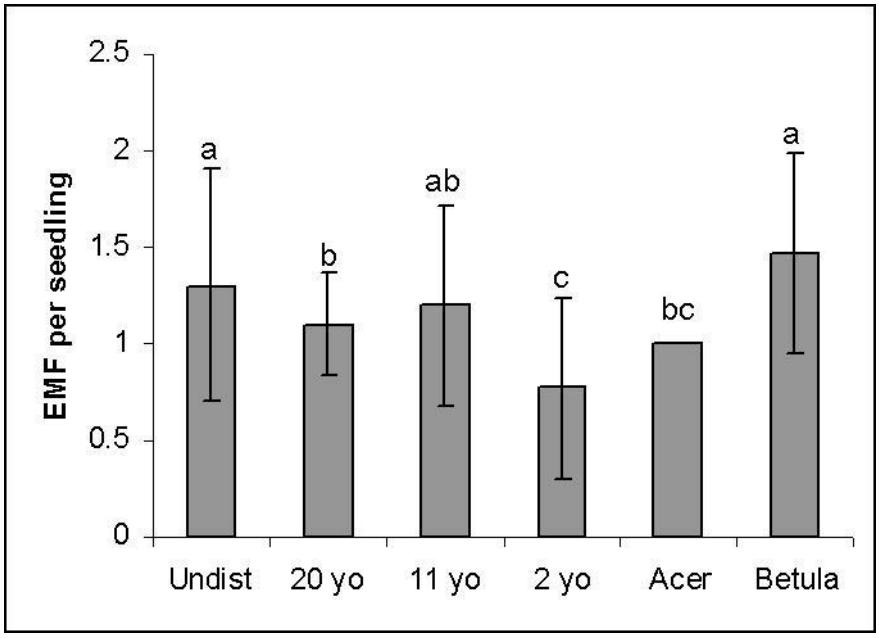


FIGURE 3.2. Distribution of EMF genera in the three disturbance gaps and the undisturbed forests. EMF were identified by RFLP and the bars represent the number of seedlings that were colonized by the ectomycorrhizal genera.

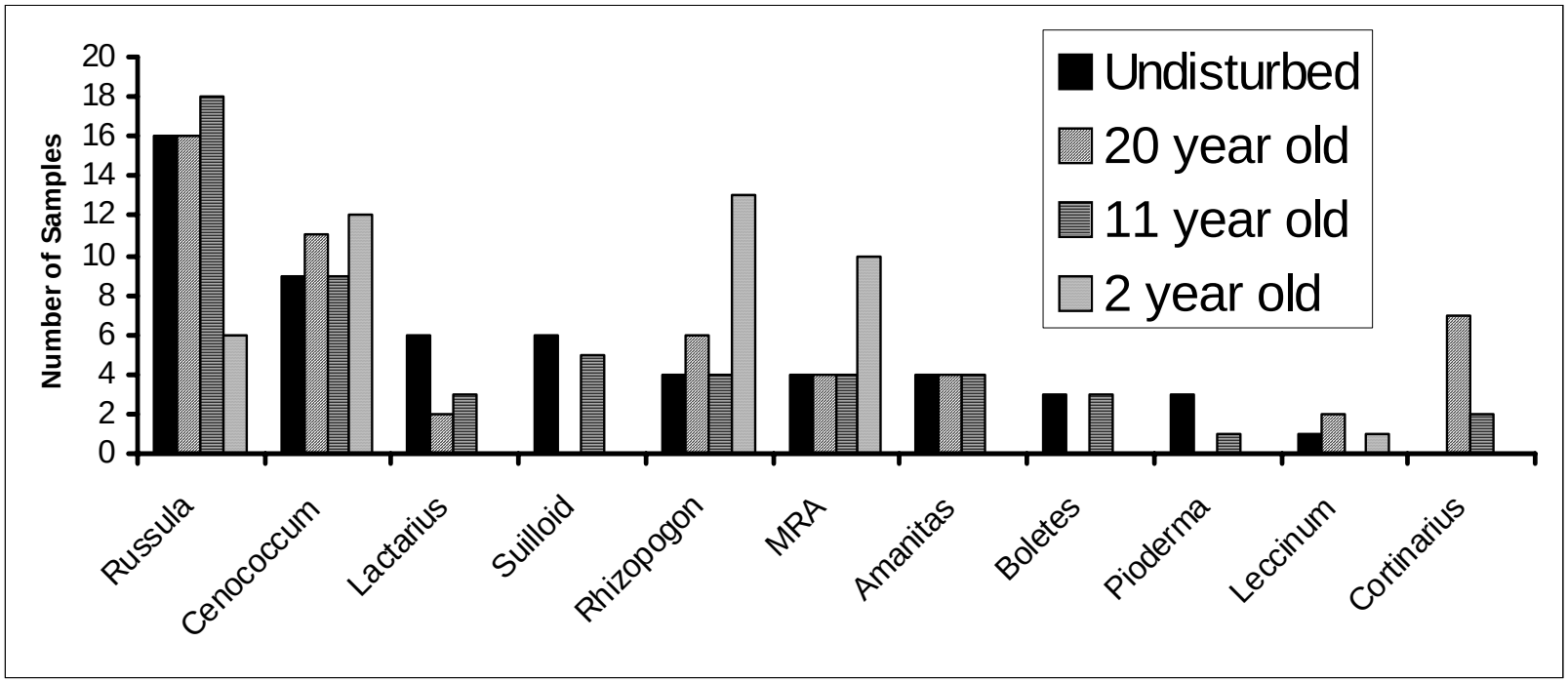
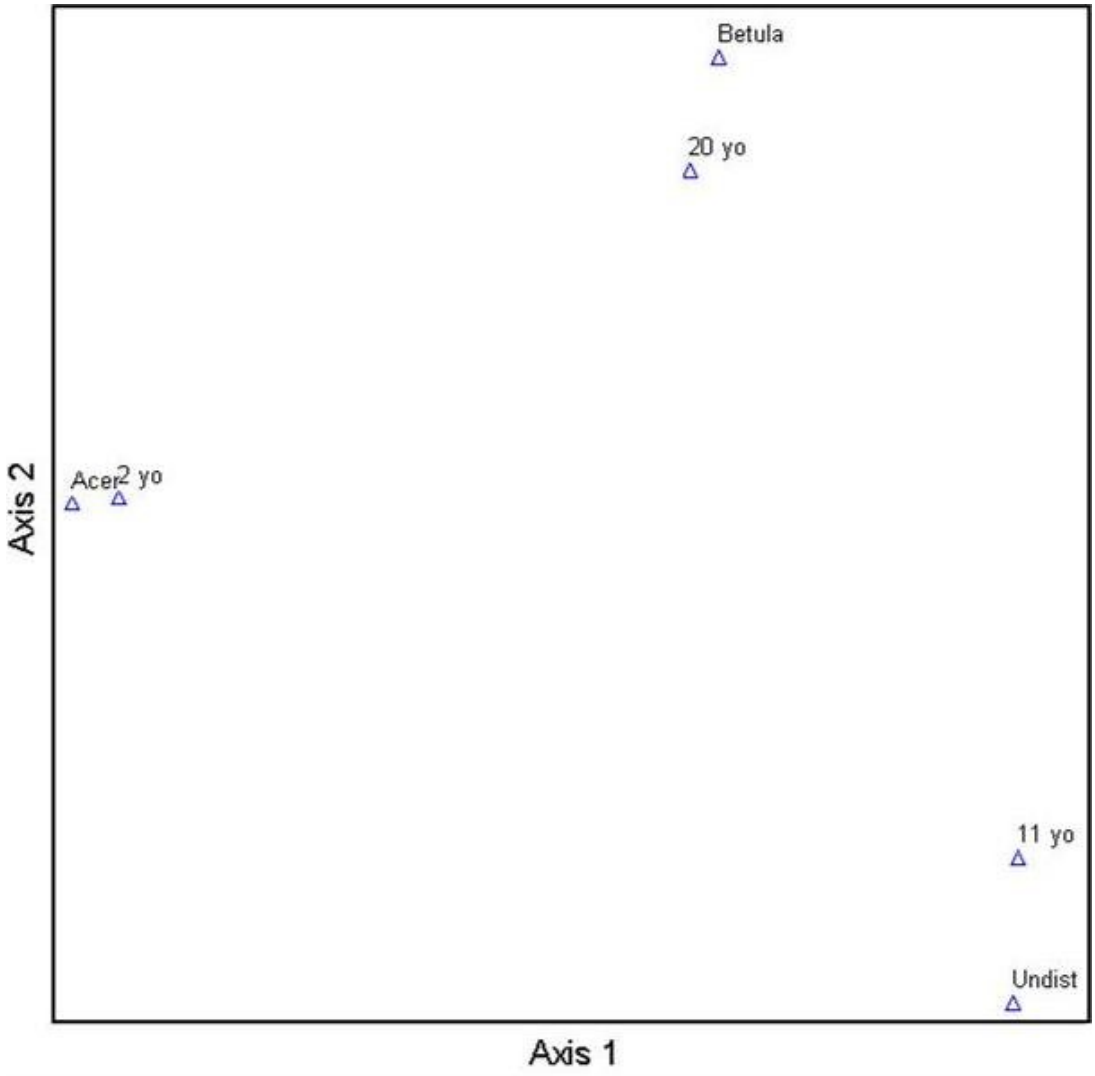


FIGURE 3.3. Nonmetric Multidimensional Scaling ordination based on EMF community composition. The distance between points represents dissimilarity between communities calculated using the Sørenson statistic.



CHAPTER 4

A MULTI-MUTUALIST SIMULATION: APPLYING BIOLOGICAL MARKET MODELS TO DIVERSE MYCORRHIZAL COMMUNITIES[3]

[3] Cowden, C.C and Peterson, C. J. to be submitted to *Ecological Modelling*

4.1 INTRODUCTION

Interspecific mutualisms are often conceptually viewed as the sum of costs and benefits to the involved organisms over the duration of the symbiosis (Roughgarden 1975, Koide and Elliot 1989, Bronstein 1994, Bronstein 2001 and references cited within). This perspective has led to the application of economic theory towards describing and understanding the evolutionary stability of mutualisms in nature (Axelrod and Hamilton 1981, Noë and Hammerstein 1994, Schwartz and Hoeksema 1998). More particularly, some theoretical approaches to understanding mutualist associations have relied on economic relationships between potential partners in which each member of the mutualism specializes in the acquisition of a particular commodity (Noë and Hammerstein 1994, Schwartz and Hoeksema 1998, Hoeksema and Schwartz 2003, Kummel and Salant 2006). In these biological market models, mutualistic relationships are stable and maintained because both partners experience a net gain in resources required for growth. Such a "division of labor" perspective has also provided insight into cooperation and competition among semi-autonomous ramets of clonal plants (Stuefer et al. 1996, Roila et al. 2007, Tannenbaum 2007).

Biological market models postulate how two heterospecific individuals benefit from a mutualistic relationship (e.g. Schwartz and Hoeksema 1998). Early versions of these models were based on pair wise relationships between species, but more recent models have been extended to systems including multiple potential partners (Kummel and Salant 2006). The host is generally assumed to select for or against the symbiont depending on whether the association is perceived as beneficial or detrimental. In cases with multiple potential

partners, the association between the two partners that yields the highest mutual fitness should be the most desirable according to the market models.

The relationship between ectomycorrhizal fungi and their woody plant associates is an ideal context in which to apply biological market models (to simplify, the term host will refer to the plant and the term symbiont to the fungus, as in Wilkinson and Sherratt 2001). Mycorrhizal mutualisms are prevalent in all terrestrial biomes, with ectomycorrhizal fungi especially common in temperate and boreal forests (Smith and Read 1997). Moreover, in temperate and boreal forests, the ectomycorrhizal fungal community is strikingly diverse (Trappe 1977, Molina *et al*. 1992, Bruns 1995, Gardes and Bruns 1996, DeBellis et al. 2006). In mycorrhizal symbioses, the traded commodities are sugar from the host plant and resources bound in the soil matrix, especially nitrogen and phosphorus, from the fungal symbiont. The "goal" of the plant host is to allocate as little carbon to the fungus as possible, while still acquiring sufficient nutrients to maintain plant functions and support new growth and reproduction; this allows greater carbon allocation to growth and reproduction, thereby maximizing fitness. The fungus must provide sufficient nutrients to the plant in order to maintain its association with the plant while obtaining the most carbon possible in order to maximize its own fitness.

A simulation model is the appropriate step from theoretical models towards empirical studies, especially for developing testable hypotheses based on trade relationships between a host and multiple potential symbionts (as suggested in Johnson et al. 2006). A cellular automaton is particularly appropriate for biological market models. Each cell in the simulation may be viewed as an individual root tip in the root system of a single plant available for colonization by mycorrhizal fungi. Occupation probability is determined at the

level of the cell (i.e., root tip), but the overall strategy behind the decisions is based on whole-plant abundances of carbon and nutrients.

The biological market approach to ectomycorrhizal interactions overlain on a cellular automaton framework can make testable predictions concerning the structure of a symbiont assemblage associated with a given host. Since many ectomycorrhizal fungi are obligate mutualists, rather than facultative, association with any available host is preferable to no association. Likewise, many plants are unable to absorb organically bound nutrients and are thus obligate mutualists with so called protein-fungi (Abuzinadah and Read 1986, Read and Perez-Moreno 2003) under certain environmental conditions. However, given multiple potential fungal symbionts, the mutualistic interaction between an individual host plant and any single symbiont species is facultative rather than obligate since the host may interact preferentially with another fungal species given differing environmental conditions and symbiont availability. Environmental conditions, especially those related to the available forms of nutrients, are thus likely to determine the demand for and cost of maintaining associations with mutualistic fungi. The effect of environmental conditions on nutrient availability and economic relationships between host and symbiont is central to the simulations presented here.

During succession, the plant community changes, forests mature and soil conditions are modified. Consequently, the composition of the ectomycorrhizal fungal community changes, and different fungal functional types may be present at various times during forest succession (Last et al. 1987). These functional types are likely to have different interactions with hosts. For example, during forest establishment a particular functional type may be a mutualist, and as resource availability changes, the same functional type may become a

cheater (receiving sugars from the host but offering few or no nutrients in payment). Host plants would perceive a symbiont as a cheater if the price of the nutrients at the symbiont's location is greater than the average price paid at the whole-plant level. If host plants are able to selectively associate with more beneficial fungi, the economic relationship determined by the physiological attributes of fungal functional types and nutrient availability would shape the mycorrhizal fungal community.

For the purposes of the simulations reported herein, the key axes by which fungal functional types differ are: (1) the ability to access nutrients in inorganic molecules or bound in organic compounds and (2) the amount of carbon the fungus requires (from the plant) for a given amount of nutrients. We assume a strict positive correlation between these functional traits; therefore, the greater ability the fungus has to obtain nutrients from organic compounds, the more costly that fungus is to maintain for the plant (based on Gibson and Deacon 1990).

Three major plant strategies for allocating carbon to roots are tested using the simulation: carbon pool adjustment, symbiont selection, and selective carbon allocation. In the carbon pool adjustment strategy, carbon is equally allocated to all roots irrespective of the colonizing fungi, and adjustments in the amount of carbon allocated to the root system are made at the whole-plant level. The fate of the fungal symbiont in each cell is based on the probability of the fungus remaining viable given the amount of carbon made available by the host in that cell. In the symbiont selection strategy, the fungus involved in the mutualism determines the amount of carbon allocated to each cell, and the plant determines the probability of the fungus remaining in the cell. If the fungus is beneficial (from the host's perspective), it will remain in the cell; if it is not beneficial, it will be expelled. In the

selective carbon allocation strategy, the tree increases carbon allocation to the more beneficial symbionts and decreases carbon allocated to less beneficial symbionts.

4.2 METHODS

In the model, a plant in full sun produces a pool of carbon beyond its requirements for maintenance. This excess pool is hereafter referred to as C_{TOTAL}. There are three fates for carbon in C_{TOTAL}: (1) the carbon is allocated to new growth (C_G); (2) the carbon is used in the trade for nutrients via the fungal symbiosis (C_F); or (3) the carbon is mismanaged (C_{MIS}), either allocated to fungal symbionts that do not provide adequate nutrients or allocated to growth when there are insufficient nutrients for growth. The plant maximizes fitness by allocating as much carbon to C_G as possible, and obtaining a sufficient amount of nutrients, all while minimizing C_{MIS}. The relationship between the amount of nutrients acquired (N_{ACQ}) and the amount of nutrients required (N_{REQ}) determines if the plant is acquiring sufficient nutrients ($N_{ACQ} = N_{REQ}$), excess nutrients (($N_{ACQ} > N_{REQ}$), or has nutrient deficiency ($N_{ACQ} < N_{REQ}$). N_{REQ} is calculated as the minimum amount of nutrients to sustain the tree based on its size (N_{MIN}) plus an N value proportional to the amount of carbon allocated to growth calculated as $N_g \times C_G$ (described below underneath tree parameters), similar to the consumption vector of Hoeksema and Schwartz (1998). In general, if there is a nutrient deficiency, the plant must allocate more carbon to C_F at the expense of C_G. If there are excess acquired nutrients (i.e., exceeds the requirement of nutrients to maintain the tree and support the new growth), the plant may allocate more carbon to C_G and less to C_F (Figure 4.1). Importantly, the tree in this model is not able to store either carbon produced or

nutrients acquired in a previous step to be utilized in a later step. For reference, the abbreviations are explained in Table 4.1.

4.2.1 Fungal Functional Type Definitions

Simulations utilize a range of fungal functional types, which span the range of likely traits, with no other attempt to mimic any particular fungal species. The fungal types are defined based on their ability to acquire and transfer inorganic and organic nutrients, the minimum amount of carbon required, the cost of transferring a unit of nutrients to the plant, and the probability of remaining in the cell given an amount of carbon. There are six defined mutualist fungal types, numbered from 1 to 6 (Table 4.2). These mutualist fungi range from those that are unable to acquire nutrients bound in organic substrates to fungi that can acquire almost all of the organically bound nutrients. The more organically bound nutrients the functional type is able to acquire, the more carbon it requires to function. The proportion of inorganic nutrients that can be acquired is the same for all fungal types (IN = 1.0). Likewise, the total amount of inorganic nutrients that can be transferred from the fungus to the host in one time step is also identical for all fungal types (IN_{max} = 2.0).

The probability of a fungus remaining in a cell ($prob_f$) is based on the following equation:

$$prob_f = (c_f - C_{min})/(N_{max} \times C_{cost}) \qquad (1)$$

where c_f is the amount of carbon allocated to each cell, C_{min} is the minimum amount of carbon on which the fungus can survive, N_{max} is the maximum amount of nutrients the fungus can transfer to the tree in one time step, and C_{cost} is the amount of carbon required by the fungus to transfer one unit of nutrients. The greater c_f is relative to C_{min}, the greater the

TABLE 4.1. A key to notations used to define parameters and functions in the cellular automaton. Symbols for fungal functional types are defined in Table 4.2.

Notation	Description
C_{TOTAL}	the total amount of carbon the tree is able to allocate to the root system
C_G	the portion of C_{TOTAL} that is allocated to plant growth
C_F	the portion of C_{TOTAL} that is allocated to fungal symbionts
C_{MIS}	the portion of C_{TOTAL} that is mismanaged, either the portion C_G for which there is insufficient nutrients, or allocated to C_F but not used optimally to acquire nutrients
C_{REMAIN}	the amount of C left for colonizing fungi (symbiont selection and selective carbon allocation strategies)
N_{ACQ}	the total amount of nutrients acquired by the host in one time cycle
N_{MIN}	the minimum amount of nutrients required to sustain the host
N_{REQ}	the amount of nutrients required to sustain the host and new growth
N_{EXCESS}	N_{ACQ} - N_{REQ} when $N_{ACQ} > N_{REQ}$
PRICE	the average price paid for nutrients by the host
p_{cell}	the price paid for nutrients within a single cell
$prob_f$	the probability of the fungus remaining in the cell for the next cycle
c_f	the amount of carbon allocated to a cell
n_{total}	the maximum amount of nutrients a fungus can acquire in the cell it occupies
$n_{transfer}$	the amount of nutrients the fungus transfers to the plant in the cell
in_{cell}	the amount of inorganic nutrients in a cell
on_{cell}	the amount of organically bound nutrients in a cell
in_{uptake}	the amount of inorganic nutrients taken up by the fungus
on_{uptake}	the amount of organic nutrients taken up by the fungus
N_g	the amount of nutrients require to support one unit of C_G

TABLE 4.2. Fungal functional types defined based on characteristic parameters: C_{min} is the minimum amount of carbon on which the fungus can survive; C_{cost} is the amount of carbon the fungus requires to transfer one unit of nutrients; IN is the proportion of inorganic nutrients the fungus can access; IN_{max} is the maximum amount of inorganic nutrients the fungus can transfer to the host in one step; ON is the proportion of organic nutrients the fungus can access; ON_{max} is the maximum amount of organic nutrients the fungus can transfer to the host in one step; and N_{max} is the maximum amount of nutrients the fungus can transfer to the host in one step.

Functional Type	C_{min}	C_{cost}	IN	IN_{max}	ON	ON_{max}	N_{max}
1	0.25	1.00	1.0	2.0	0.00	0.00	2.00
2	0.40	1.15	1.0	2.0	0.15	0.25	2.25
3	0.55	1.30	1.0	2.0	0.30	0.50	2.50
4	0.70	1.45	1.0	2.0	0.45	0.75	2.75
5	0.85	1.60	1.0	2.0	0.60	1.00	3.00
6	1.00	1.75	1.0	2.0	0.75	1.25	3.25

probability of the fungus remaining in the cell. The denominator calculation scales the carbon provided relative to the maximum capability of the fungus to transfer nutrients to the tree. It is possible that $c_f < C_{min}$, and that $(c_f - C_{min}) > (N_{max} \times C_{cost})$; however, since probability is defined $0 \leq prob_f \leq 1$, if the calculated $prob_f$ is less than zero, it is treated as 0, and if it is greater than 1, it is treated as 1. The range is truncated because these simulations consider each fungus within each cell as a unique individual, unable to increase in size - future simulations may allow functional types to colonize adjacent cells when c_f is high ($prob_f > 1$), or for multi-celled individuals to contract in size when c_f is low ($prob_f < 0$).

4.2.2 Cellular Automaton

The simulations were performed using a two-dimensional cellular automaton format on a 100*100 grid of cells. Each cell contains spores of all fungal functional types, thus dispersal limitation is not considered in these simulations. There are four major occurrences in each cell during each discrete time step: (1) colonization; (2) the trade of nutrients for carbon; (3) the calculation of the trade value – the price of nutrients for each cell; and (4) modification of carbon allocation and/or fungal probability alteration. For the simulations, colonization occurs solely via spore germination; therefore, hyphal growth into neighboring cells to compete for colonization is not considered. Nutrients taken up by the fungus are then transferred to the tree in exchange for the carbon sugars produced by the tree. The amount of nutrients the fungi offer depends on the functional type of the fungus, the amount of available nutrients within the cell, and the amount of carbon supplied to the fungus by the tree (Figure 4.1B). The fungus in each cell attempts to deliver nutrients appropriate to the amount of carbon it receives; however, the environmental conditions may prevent fair trade. For

example, different fungal types have different capabilities for taking up nutrients bound in organic compounds (Table 4.2). Thus, if functional type 1 occupies a cell with a vast quantity of organically bound nutrients and no inorganic nutrients, it will be unable to deliver any nutrients to the plant regardless of the amount of carbon that is supplied.

4.2.3 COLONIZATION

Cell order is randomized for colonization such that the identity and location of the cell has no bearing on the sequence in which the simulation evaluates the cell. This randomization is critical for colonization in the symbiont selection and selective carbon allocation strategies, in which the fungal symbiont determines the initial c_f, as explained below. Likewise, the order for the fungi is randomized for each colonization attempt. For the initial colonization step in the carbon pool adjustment strategy, colonization is attempted in all cells, with successful colonization based on the output of a random number generator and the probability of a spore germinating (equal for all functional types). For each cell, the simulation cycles through a random order of fungal functional types and moves on to test the next cell if there is either a successful colonization event (the cell is occupied) or if all fungal functional types are unsuccessful in germinating (the cell remains vacant).

Initial colonization in the symbiont selection and selective carbon allocation strategies differs from that in the carbon pool adjustment strategy. Since the fungal functional type determines c_f, and there is a finite amount of available carbon (C_{TOTAL}), there is a possibility that a fungal community will draw more carbon than is available to the roots. Since the cellular automaton simulation cycles through the cells one at a time, the cells assessed towards the end of the cycle would have a greater probability of vacancy than those earlier in

the cycle. Thus randomization of cell order is critical in the colonization cycles. For the initial colonization cycle in the symbiont selection and selective carbon allocation strategies, the c_f for the cell is subtracted from the remaining available pool (C_{REMAIN}) until the available pool is exhausted. For the first colonization cycle, the initial C_{REMAIN} is equal to C_{TOTAL}.

In all three strategies, all colonization after the first cycle occurs only in vacant cells. Cells are vacant if an earlier colonization attempt fails and the cell is thus vacant in the previous cycle, or if the fungus fails to remain in the cell based on $prob_f$ (through either the action of the host or the fungus, based on the operating strategy). The order in which colonization attempts occur in vacant cells is randomized as in the initial colonization cycle. In the carbon pool adjustment strategy, colonization attempts occur in all vacant cells. In the symbiont selection and selective carbon allocation strategies, colonization attempts occur in vacant cells until C_{REMAIN} is exhausted.

4.2.4 Calculation of Trade Coefficients

After cells are colonized by fungi, the simulation calculates for each cell the amount of nutrients the fungus in that cell can access, and then the amount of nutrients it can transfer based on c_f. The amount of nutrients transferred is calculated using the following algorithm:

$$on_{cell} \times ON = on_{uptake} \quad (2)$$

$$\text{If: } on_{uptake} > ON_{max}, \text{ then: } on_{uptake} = ON_{max} \quad (3)$$

$$in_{cell} \times IN = in_{uptake} \quad (4)$$

$$\text{If: } in_{uptake} > IN_{max}, \text{ then: } in_{uptake} = IN_{max} \quad (5)$$

$$on_{uptake} + in_{uptake} = n_{total} \quad (6)$$

$$c_f / C_{cost} = n_{transfer} \quad (7)$$

$$\text{If: } n_{transfer} > n_{total}, \text{ then } n_{transfer} = n_{total} \quad (8)$$

In which on_{cell} and in_{cell} are the available amounts of organic nutrients and inorganic nutrients in each cell, respectively. If $n_{transfer} > n_{total}$, the fungus transfers the maximum amount of inorganic nutrients (in_{uptake} as calculated in equations 4 and 5) and the remaining $n_{transfer}$ is of the organic nutrients ($on_{uptake} = n_{transfer} - in_{uptake}$).

After $n_{transfer}$ is calculated, the cell price (p_{cell}) is determined by $c_f / n_{transfer}$. Tree wide pools of nutrients acquired and total carbon transferred to the roots are calculated by summing the corresponding values of c_f and $n_{transfer}$ from all cells. Also, average price paid for nutrients (PRICE) is calculated as the average of all cell prices.

4.2.5 Modification of Cell Carbon and Probability

Methods of cell attribute modification depend on which strategy is being simulated. There are several general attributes calculated tree-wide for each time step that apply to all three strategies. First is the calculation of carbon partitioning of the C_{TOTAL} pool into C_F, C_G and C_{MIS} sub-pools. Tree-wide C_F is calculated by summing c_f of all cells in the grid. Tree-wide C_G is generally a pre-determined amount calculated by the simulation to maximize C_G, and is detailed in the carbon pool adjustment strategy. There are two conditions that create the C_{MIS} pool: (1) when nutrients are acquired in excess to the nutrient requirement of the plant, and (2) when nutrient acquisition is insufficient to support the growth of the plant. Calculation of C_{MIS} from the first condition is through multiplying the amount of excess acquired nutrients ($N_{EXCESS} = N_{ACQ} - N_{REQ}$) by the average price the tree paid for nutrients. In the second condition, the excess amount of carbon allocated to C_G (calculated as C_G -

(N_{ACQ}-N_{MIN})/N_g); the total C_G pool minus the portion of the C_G pool that was utilized because the acquired nutrients could satisfy that portion) is transferred to the C_{MIS} pool.

Second, the calculation of the tree-wide nutrient pools is common to all strategies. Tree-wide N_{ACQ} is calculated as the sum of all $n_{transfer}$ values in the grid. N_{REQ} is calculated by adding the N_{MIN} (minimum amount of nutrients needed to sustain the tree without growth and reproduction) to (C_G x N_g), which is the amount of nutrients required to satisfy the carbon allocated to growth. Based on the relationship of N_{ACQ} to N_{REQ}, the tree may have a nutrient deficiency, have a nutrient surplus, or acquire a sufficient amount of nutrients. Furthermore, each strategy for modifying carbon pools and fungal probabilities is based on the relationship of N_{ACQ} to N_{REQ}. Strategies are considered successful under given environmental conditions if sufficient nutrients are acquired to satisfy the minimum requirements of the tree. Strategies are considered more successful if the carbon pools are managed effectively – maximizing carbon allocated to growth and reproduction while minimizing C_{MIS}.

4.2.6 Carbon Pool Adjustment

The carbon pool adjustment strategy is the most basic strategy as all cells receive the same amount of carbon from the tree regardless of the fungal functional type or even the presence of a fungus. For the first step, the entire C_{TOTAL} pool is distributed to all cells – each cell receiving C_{TOTAL} /10 000 units of carbon (there are 10 000 cells in the plot). The carbon distribution is adjusted after each time step in order to maximize C_G while maintaining sufficient nutrient uptake. The strategy adjusts the C allocation based on nutrient uptake. If the amount of nutrients acquired is exactly sufficient, such that $N_{ACQ} = N_{REQ}$, the pool sizes

are not adjusted. If there is a nutrient surplus (which often occurs after the initial step, when all of C_{TOTAL} is allocated to C_F), the C_{MIS} pool as calculated above for nutrient excess, is partitioned into C_G and C_F as to maximize the amount of C allocated to C_G while taking into account the new nutrient requirement needed to accommodate the new size of the C_G pool based on the nutrient price from the previous step. The new tree-wide C pools are calculated as follows:

$$C_{MIS} = (N_{ACQ} - N_{REQ}) \text{ x PRICE} \quad (9)$$

$$C_{MIS} = (C_{Gj} - C_{Gi}) + (C_{Gj} - C_{Gi}) \text{ x } (N_g \text{ x PRICE}) \quad (10)$$

$$C_{Gj} = C_{Gi} + C_{MIS} / (1 + N_g \text{ x PRICE}) \quad (11)$$

$$C_{Fj} = C_{TOTAL} - C_{Gj} \quad (12)$$

C_{Gi} is the C_G pool from the previous step, C_{Gj} is the C_G pool for the next step. Equation 9 calculates the size of the C_{MIS} pool based on the results of the previous step. Equation 10 redefines C_{MIS} in terms of how the pool will be allocated to C_G in the next step: (C_{Gj}-C_{Gi}) represents the new carbon allocated to C_G, and (C_{Gj} - C_{Gi}) x (N_g x PRICE) reflects the amount of carbon that must be allocated to C_F in order to satisfy the new nutrient requirements based on the C_G pool. Equation 11 is an algebraic transformation of Equation 10 solved for C_{Gj}. The new tree-wide C_F pool (C_{Fj}) is then equally distributed to all cells. Thus, if nutrients are acquired in excess in one time step, less carbon will be allocated to each cell in the next step. The probability of the fungus remaining in the cell is based on the amount of carbon it receives; therefore, if less carbon is allocated to c_f in each cell, fungi with greater carbon demands (i. e., greater C_{min}) will have lower probability of remaining in the cell.

If the tree fails to acquire a sufficient level of nutrients, the tree must allocate more carbon to C_F, while still maximizing C_G. There are two conditions in which the tree may not

acquire enough nutrients. In the first condition, the plant has allocated too much carbon to C_G, and thus the C_{MIS} pool consists of all of the C_G that can not be supported by the nutrients. The second condition occurs when all of C_{TOTAL} is allocated to C_F and the tree still fails to acquire sufficient nutrients (N_{MIN} is the nutrient requirement for the plant to exist at its current state with no growth; thus, even when $C_G = 0$, the plant still requires some nutrients, see Figure 4.1); essentially, the price of the nutrients is too high. To ameliorate the first condition, the tree must convert some of the C_G pool to C_F. The C pool sizes for the next time step are adjusted using the following equations:

$$PRICE_i = C_{Fi} / N_{ACQi} \quad (13)$$

$$\text{Nutrient Deficit} = N_{REQi} - N_{ACQi} \quad (14)$$

$$C_{MIS} = C_{Gi} - (N_{ACQi} - N_{MIN}) / N_g \quad (15)$$

$$N_{REQi} = N_{MIN} + C_{Gi} \times N_g \quad (16)$$

$$C_{Fj} = N_{REQi} \times PRICE - (C_{Gf} \times N_g) \times PRICE \quad (17)$$

$$C_{G\,f} = C_{G\,i} - C_{G\,j} \quad (18)$$

$$C_{Fj} = C_{TOTAL} - C_{Gj} \quad (19)$$

$$C_{TOTAL} - C_{Gj} = (N_{REQi}) \times PRICE - [(C_{G\,i} - C_{G\,j}) \times N_g] \times PRICE \quad (20)$$

$$CGj = [C_{TOTAL} - PRICE\ (N_{REQi} - C_{Gi} \times N_g)] / (1 + PRICE \times N_g) \quad (21)$$

Variables with the i subscript represent values from the previous step, those with the j subscript represent values for the next step, and C_{Gf} is the amount of C_{Gi} transferred to C_F. Equation 13 is used to calculate PRICE, Equation 14 is used to calculate the nutrient deficit, and Equation 15 is used to calculate C_{MIS} when adequate nutrients are not acquired. Equation 16 is used to calculate the amount of nutrients required. Equation 17 is used to calculate the

optimum size of the C_F pool for the next step based on the values of the previous step. The N_{REQi} x PRICE portion of the equation is the total amount of carbon required to satisfy the nutrient needs from the previous step, and the (C_{Gf} x N_g) x PRICE portion accounts for the reduction of N_{REQ} based on the reduced size of the C_G pool due to C_{Gf}. Equations 18 and 19 are listed to explain substitutions for values in Equation 17, which are represented in Equation 20. Finally, Equation 20 is algebraically solved for C_{Gj}, resulting in Equation 21. These C pool adjustments allow the tree to convert a sufficient amount of carbon to C_F while still maintaining as large a C_G pool as possible. The second condition requires the plant to adjust the probabilities of the fungi involved in the mutualism, which in the carbon pool adjustment strategy is beyond the tree's capabilities.

4.2.7 Symbiont Selection

In the symbiont selection strategy, the fungal symbiont determines the amount of carbon allocated to each cell. The fungus is the carbon sink and determines the amount it requires from the source (i.e., the plant). For the symbiont selection simulations, $c_f = 1.5$ x C_{cost}, the amount of carbon required to transfer 1.5 units of nutrients. The level of 1.5 units of nutrients per cell was selected because it maximizes the host carbon utilization when the host is associated with the cheapest symbiont, functional type 1, simply due to the definitions of the model parameters. Also, $prob_f = 1$ for all functional types when $c_f = 1.5$ x C_{cost}. The plant host determines the probability of a fungus remaining in the cell based on the N_{ACQ} and N_{REQ} relationship, the p_{cell} value relative to PRICE, and the $n_{transfer}$ for each cell relative to the tree-wide average $n_{transfer}$ value. The host's goal is to maximize C_G, and the tree does so by selectively adjusting the symbiont community. If nutrients are acquired at sufficient or

excess levels ($N_{ACQ} \geq N_{REQ}$) the tree will select against symbionts with p_{cell} > PRICE, by reducing $prob_f$ to 0; the simulation will attempt colonization in the newly vacant cell in the next step. For beneficial symbioses in this situation, $prob_f$ is considered equal to 1, since the fungi determine c_f. The PRICE decreases when supply exceeds demand, resulting from selection against costlier symbionts.

If nutrient levels are insufficient ($N_{ACQ} < N_{REQ}$), and if $n_{transfer}$ for the cell is less than the average $n_{transfer}$ value for the plot, fungal symbionts will be selected against by reducing $prob_f$ to 0. The PRICE will increase as demand increases, and symbionts that provide inadequate amounts of nutrients are selected against.

As another series of simulations, the symbiont selection and the carbon pool adjustment strategies were combined. For this combined strategy, the tree has an active role in modifying C allocation as well as probabilities of fungi remaining in cells. In these simulations, the tree calculates the optimum adjusted size of the carbon pools based on the previous step. Thus, the tree is able to pre-determine the C_F pool and maximize C_G. Next, the tree selects against the less favorable symbionts, creating vacant cells. Then, colonization occurs on vacant cells until the C_F pool is exhausted rather than utilizing the entire C_{TOTAL} pool. Often, there are vacant cells at the end of each step.

4.2.8 Selective Carbon Allocation

In the selective carbon allocation strategy, the tree alters c_f allocation to individual cells based on the performance of the fungi within the cell. The probability of fungi remaining in the cell is determined by $prob_f$, calculated using the c_f value of the occupied cell. The initial c_f transferred to each cell is initially determined by the fungus as in the

symbiont selection strategy as the amount of c_f transferred to newly colonized cells. Adjustments to c_f for each cell are administered in increments of 0.1 C units for the simulations of the selective carbon allocation strategy. The 0.1 increment level was chosen because it allowed for the finest scale carbon allocation adjustments given computational memory limitation.

As in the carbon pool adjustment and the symbiont selection strategies, the relationship between N_{ACQ} and N_{REQ} is the major deciding factor in the selective carbon allocation strategy. If $N_{ACQ} \geq N_{REQ}$, the tree will increase C_G by increasing c_f to lower priced symbionts and decreasing c_f to the higher priced symbionts, lowering the PRICE. Through successive steps under this condition, $prob_f$ for the higher priced symbionts will decrease such that the fungi will be unlikely to remain in the cell. If $N_{ACQ} < N_{REQ}$, c_f will be increased to the cells that have greater than average $n_{transfer}$ values and will be reduced in cells with lower than average $n_{transfer}$ values. The price per unit of nutrients is likely to increase under these situations.

4.2.9 Environmental Conditions

Simulations for all strategies were performed on stable homogeneous environments. Each cell in the simulation will have the same composition in regards to the amounts of inorganically and organically bound nutrients. In all simulations, each cell has a total of 4 units of nutrients. The environmental states that were simulated in the homogenous environments are 0.5: 3.5; 0.75:3.25; 1.0:3.0; 1.25:2.75; 1.5:2.5; 2:2; inorganic:organic, respectively.

4.3 RESULTS

To reach stabilization, the selective carbon allocation simulations went through 6000 discrete time steps and the other simulations underwent 450 discrete time steps. The carbon pool adjustment, symbiont selection, and combined carbon pool adjustment and symbiont selection strategies all reach stable nutrient acquisition levels with stable symbiont communities (Figure 4.2). Two hundred replicate simulations were performed for each strategy and environmental condition. Comparisons between strategies were based on the N_{ACQ}, C_G and C_{MIS} values at the stable plateau regions of the simulation curves. These values represent equilibriums achieved through iterative applications of the host strategies under stable environmental conditions.

When cells contain more than 1.5 units of inorganic nutrients per cell (the point at which the optimum nutrient acquisition can occur without the uptake of any organic nutrients), the results for each strategy do not differ from the results at the 1.5 inorganic nutrient units per cell. The results of the simulations performed in homogenous environments are summarized in Table 4.3.

The pool adjustment strategy is unsuccessful if inorganic nutrient availability is low (0.5 and 0.75 units per cell), and the average carbon cost per unit of nutrients is greater than 2.0. In these cases, the equilibrium quantity of nutrients acquired is below the minimum required nutrients (Figure 4.3). If inorganic nutrient availability is high ($\geq$1.5 units per cell), the pool adjustment strategy achieves the theoretically optimum conditions. In intermediate levels of inorganic nutrient availability (1.0 and 1.25 units per cell), the strategy is successful and is able to minimize C_{MIS}. At high inorganic nutrient availability, the symbiont community is monospecific of functional type 1. Species richness is higher in simulations at

TABLE 4.3. Plant-level simulation results at the equilibrium plateaus for all strategies in homogenous environments. The nutrient availability is indicated in the in_{cell}:on_{cell} column. More successful strategies acquire a sufficient amount of nutrients ($\geq N_{MIN,}$ which is 10000 units for the simulations) with the lowest C_{MIS} values.

Strategy	in_{cell}:on_{cell}	N_{ACQ}	C_F	C_G	C_{MIS}	PRICE	% occupied
Pool Adjustment	0.5:3.5	7498.2	20000	0	0	2.67	100
	0.75:3.25	9933.61	20000	0	0	2.01	100
	1.0:3.0	11249.6	18750.4	1249.6	1.4	1.67	100
	1.25:2.75	12763	17236.9	2763.1	0	1.35	100
	1.5:2.5	15000	15000	5000	0	1	100
Symbiont Selection	0.5:3.5	12498.9	20000	0	3998.6	1.6	83.3
	0.75:3.25	13792.9	20000	0	5499.1	1.45	92
	1.0:3.0	15000	19499.9	500.1	5849.9	1.3	100
	1.25:2.75	15000	17248.28	2751.72	2585.3	1.15	100
	1.5:2.5	15000	15000	5000	0	1	100
Symbiont Selection	0.5:3.5	11539.4	18460.6	1539.4	6.2	1.6	76.9
with	0.75:3.25	12245.1	17754.9	2245.1	0	1.45	82.4
Pool Adjustment	1.0:3.0	13043.6	16956.4	3043.6	0	1.3	87
	1.25:2.75	13953.5	16046.5	3953.5	1.3	1.15	93.03
	1.5:2.5	15000	15000	5000	0	1	100
Selective Carbon	0.5:3.5	11617.6	20000	0	2784.7	1.72	81.4
Allocation	0.75:3.25	13027.5	20000	0	4647.8	1.53	92.1
	1.0:3.0	14997	19498	502	5845	1.3	100
	1.25:2.75	13686.1	17026	2974	885.9	1.24	100
	1.5:2.5	15000	15997.9	4002.1	1064.3	1.07	100

low and intermediate inorganic nutrient levels (Figure 4.5A). The maintenance of lower priced, yet non-optimum nutrient providing fungi limits the success of this strategy.

The symbiont selection strategy is the best strategy for acquiring nutrients (Figure 4.4), but not for maximizing carbon use efficiency, as shown by relatively large quantities of carbon mismanaged at low and intermediate inorganic levels. When cell occupancy is below 100% and inorganic nutrient levels are less than 1.0 unit per cell the C_{TOTAL} pool is exhausted by the occupying symbionts. Thus, carbon is only allocated to growth if cells are 100% occupied by fungi and the carbon required to satisfy the symbiont community is less than $C_{TOTAL;}$ this is the case when inorganic nutrients are greater than 1.0 unit per cell. At equilibrium, the symbiont communities are all monospecific and made up of the optimum fungal functional type for the given environmental conditions (Figure 4.5B).

Combining the symbiont selection and the pool adjustment strategies results in optimum carbon use efficiency under all conditions. The results of simulations at inorganic nutrient levels ≥ 1.5 are identical to the results for the symbiont selection strategy operating solely. Furthermore, the symbiont communities are all monospecific at equilibrium, and comprised of the same fungal functional types as in the symbiont selection strategy. However, at low and intermediate inorganic nutrient levels, the strategies operating in concert result in lower levels of cell occupancy and thus lower levels of carbon required to maintain the symbiont community. The simulations utilizing both the symbiont selection and the pool adjustment strategies are able to maximize carbon use efficiency at all nutrient levels (Figure 4.4).

The selective carbon allocation strategy is also successful at all nutrient levels, and reaches its optimal levels at inorganic nutrient levels ≥ 1.5 units per cell. However, this

optimal achieved level is lower than the theoretical optima achieved by the pool adjustment and symbiont selection strategies. Cell occupancy for all environmental conditions is approximately 90%, and populations of all functional types are maintained (Figure 4.5C). The maintenance of populations of higher priced functional types under this strategy increases the price paid for nutrients above the optimum levels reached in the symbiont selection strategy. Under all environmental conditions, this strategy is able to dedicate carbon to growth and reproduction; however, there are also moderate C_{MIS} pools indicating that growth and reproduction are nutrient limited. Functional type community stabilization requires up to 6000 time steps in the selective carbon allocation strategy depending on the environmental condition, thus the dynamics are much slower than the other strategies. This is due in part to the size of the incremental increases and decreases of carbon allocated to each cell. Smaller magnitudes of incremental adjustments require a greater number of time steps to reach community stabilization. Larger magnitudes reject less favorable functional types more rapidly. However, community stability is rarely achieved since favorable species may be deemed unfavorable if they are unable to reciprocate appropriate quantities of nutrients compared to the increased amount of carbon allocated to them. The community then "resets" to the random initial condition when all functional types are equally present in the community.

4.4 Discussion

The mutualism strategies presented here facilitate empirical research because they can be characterized through measurable, and therefore experimentally testable, plant and fungal physiological activities. The carbon pool adjustment strategy relates to whole plant carbon

allocation and determines how much of the excess carbon pool should be allocated to the root system, in particular to the fine roots, rather than stored for future use by the plant. Therefore, experiments or comparative studies tracing the fate of carbon in the plant under different soil nutrient conditions would be suitable for testing carbon pool hypotheses. The symbiont selection strategy may operate via selective root turnover or the rejection of the fungal hyphae at the plant-fungus interface. Thus, symbiont selection experiments should incorporate fine root growth and turnover, multiple fungal partners, and a wide range of nutrient availabilities. Selective carbon allocation requires sanctions where less beneficial symbionts receive a smaller portion of the host's carbon (adapted from rhizobia models proposed by Denison 2000, West et al. 2002, see also Nehls et al. 2007). Measuring the carbon exchange between a host and multiple fungal species under the same environmental condition or a single fungus under differing environmental conditions would assess selective carbon allocation potential (as suggested by Kummel and Salant 2006).

Functional diversity is a potential factor in maintaining highly diverse ectomycorrhizal fungal communities (Bruns 1995, Cairney 1999, Kernaghan 2005, Buée et al. 2007). Experiments investigating the effect of ectomycorrhizal diversity on host productivity suggest that increased functional diversity enhances nutrient uptake in culture (Jonsson et al. 2001, Baxter and Dighton 2001) and that the availability of nutrients influences the effect (Baxter and Dighton 2005). Furthermore, nutrient addition has been shown to differentially affect root colonization by ectomycorrhizal fungi with distinct life history strategies (Lilleskov and Bruns 2003). Our simulations clarify the conditions under which to expect the host to maintain diverse symbionts. Only the selective carbon allocation strategy is able to maintain a successful, diverse functional type community and only does so

when inorganic nutrient availability is low (Figure 4.5 C). The carbon pool adjustment strategy also results in diverse functional type communities at low inorganic nutrient availabilities (Figure 4.5 A), but these do not provide sufficient nutrients to the host (Figure 4.3 A). The simulations suggest that diverse communities enhance host productivity only if the appropriate functional types are present. Furthermore, the strategy utilized by the host plant greatly affects the ability to maintain associations with the appropriate fungal functional types.

The most effective mutualisms from the host's perspective (i.e., those with the highest C_G and lowest C_{MIS}) utilized strategies functioning at both the whole tree and individual root tip scales. The more basic strategies (pool adjustment and symbiont selection) both function optimally as long as the inorganic nutrient availability is sufficient. The limitations of these strategies are exposed when inorganic nutrient availability is below optimal, and the plant must acquire some amount of organically bound nutrients to satisfy its demands. Natural habitats where this is common include heaths (dominated by ericaceous species) and boreal and temperate forests with well developed organic soil horizons (Schlesinger 1977). The pool adjustment strategy fails to acquire sufficient nutrients at low inorganic nutrient availability due to its inability to reject lower cost symbionts that do not acquire sufficient quantities of organically bound nutrients. The symbiont selection strategy suffers high levels of C_{MIS} because it does not adequately manage plant level carbon pools. This may be less of a disadvantage than the nutrient limitation likely under pool adjustments because trees can allocate more carbon to root symbionts when they grow in high-light locations (Tweig et al. 2007).

Combining the pool adjustment and symbiont selection strategies results in the most favorable, albeit monospecific, symbiont community. But this is expected, since environmental conditions are homogenous. Given a heterogeneous environment, a tree should maintain several different symbiotic fungal types. As in the symbiont selection strategy, the fungal symbiont determines the amount of carbon it receives, and the tree selects for or against the symbiont based on the price per unit nutrient paid at each cell. The pool adjustment simply serves to control the total C_F pool, and effectively controls the number of symbiotic associations that are maintained. Since soils are almost universally heterogeneous at fine scales (e. g., Lechowicz and Bell 1991, Farley and Fitter 1999), under this strategy we may expect a tree to maintain a more diverse fungal community as soil nutrient heterogeneity increases. Although, the amount of soil heterogeneity that will result in a tree maintaining diverse symbionts will of course depend on the relative costs and efficiencies of the available suite of fungi.

The selective carbon allocation strategy operates at the scale of the single root tip, and like the symbiont selection strategy, the conditions at the whole plant level influence the outcomes at each root tip. The strategy results in optimal symbiont utilization in regards to nutrient acquisition and is able to maintain diverse symbiont communities in environments with lower mineralized nutrient content. However, since the whole plant carbon pools are not directly managed under this strategy, there is still a sizable C_{MIS} pool when mineralized nutrients are abundant. When inorganic nutrient levels are 1.0 unit per cell, and organic nutrients are 3.0 units per cell, the selective carbon allocation strategy stabilizes with a single fungal functional type (Figure 4.5). This result appears out of place when compared to the multiple fungal functional types maintained under environmental conditions containing 0.75

and 1.25 units of inorganic nutrients per cell. The condition arises because the optimum N_{ACQ} occurs at 1.5 units per cell, and fungal functional type 3 is able to provide the ideal price for nutrients given the environmental conditions. The same condition occurs in the symbiont selection strategy and the combined carbon pool allocation with symbiont selection strategy under the same environmental conditions. Therefore, one prediction is that a single fungal functional type should be maintained where an available fungal species provides an ideal price at a given nutrient availability.

Unlike the symbiont selection strategy, the selective carbon allocation strategy cannot be combined with the carbon pool allocation strategy as it is currently applied. The sizes of the carbon pools are adjusted following each step, modifying the available C_F pool for the next step. The modification of the carbon allocated to each root tip is applied in a random order, and to limit the carbon availability to the entire root system via pool adjustment would potentially sanction more beneficial symbionts simply based on the order at which the cell is assessed. However, the selective carbon allocation strategy is able to acquire nutrients in excess of the host requirements under all environmental conditions (Table 4.3). Plants in full sun, as these simulations are intended to model, are rarely carbon limited but are commonly nutrient limited. Thus, processes that maximize nutrient uptake at the cost of carbon may only rarely reduce the fitness of plants in full sun. Actively managing carbon pools would be more important for shaded seedlings and saplings. However, shaded plants may share common mycorrhizal networks with canopy trees that may ease their carbon deficiency (Simard et al., 1997).

In these simulations, the factors that limit establishment and continuation of a host-fungus association are colonization sites and carbon availability from the host. If there is no

carbon input from the host, there will be no mycorrhizal associations. Severe disturbances that destroy the host plant aboveground structure greatly reduce the amount of carbon available to mycorrhizal fungi, and thus result in lower diversity fungal communities even if fungal innocula are not destroyed. For example, unlike fire and clearcut logging (see Taylor and Bruns 1999 and Jones et al. 2003), wind disturbance does not remove or destroy fungal innocula from organic soil horizons, but disrupts the carbon transfer to the fungal symbionts. Following severe windthrow, ectomycorrhizal communities in the disturbed area are less diverse than those in adjacent undisturbed forest (Egli et al. 2002). If mineralized nutrients increase in abundance, as occurs with atmospheric nitrogen deposition, the host plants are likely to allocate less carbon to symbionts for the acquisition of nutrients, thus leading to lower symbiont diversity and abundance (as reported by Dighton et al. 2004). At the opposite extreme, if there is abundant carbon from the host - such that associations with even the most costly functional types do not reduce fitness - site availability would be the only factor affecting functional type diversity.

4.5 Conclusions

Biological markets involve at least two interacting partners. In these simulations, one tree interacts with multiple mycorrhizal functional types, at most one per cell, from a 6-member symbiont pool. In previous theoretical biological market applications, the host has the responsibility of selecting its symbiont and the extent to which that symbiont is utilized (Kummel and Salant 2006). Here, the extent of utilization includes the number of interactions (occupied cells) and the allocation of carbon to each cell. Thus, the plant must employ strategies that can modify both aspects of utilization. In uniform static environments,

both symbiont selection and selective carbon allocation strategies (the strategies that make decisions at the single cell level) can organize stable functional type communities that maximize the host's ability to acquire nutrients.

Predictions concerning mycorrhizal communities based on these simulations include a decrease in fungal functional type diversity with decreased carbon input from the host as indicated in both the carbon pool adjustment and selective carbon allocation strategies when inorganic nutrient availability is high. Moreover, plants must invest more carbon with the mycorrhizal symbionts when inorganic nutrients are less abundant. Active control of carbon distribution by plants at the scale of the individual root is required to optimally structure and utilize functional type communities.

FIGURE 4.1. (A) Conceptual representation of N_{ACQ} and N_{REQ} values based on the partitioning of the C_{TOTAL} pool into the C_F and C_G subpools. The intersection of the N_{ACQ} and N_{REQ} lines represents the equilibrium condition where the host optimally manages C_F and C_G pools and $C_{MIS} = 0$. When $N_{REQ} < N_{ACQ}$, excess nutrients are acquired; conversely, when $N_{REQ} > N_{ACQ}$, too much carbon is allocated to C_G. In both cases, $C_{MIS} > 0$. (B) The effects of inorganic nutrient availability on the N_{ACQ} slope. The N_{ACQ} slope is inversely related to the average price per unit nutrient, and the different lines represent environments ranging from those with mostly inorganic nutrients to those with mostly organic nutrients. Hosts in environments with lower inorganic nutrient availability must allocate more carbon to C_F in order to acquire sufficient nutrient quantities. The plateaus are due to physiological constraints on the amount of nutrients the fungus is capable of transferring to the host over a period of time. Refer to Table 4.1 for the definitions of parameters.

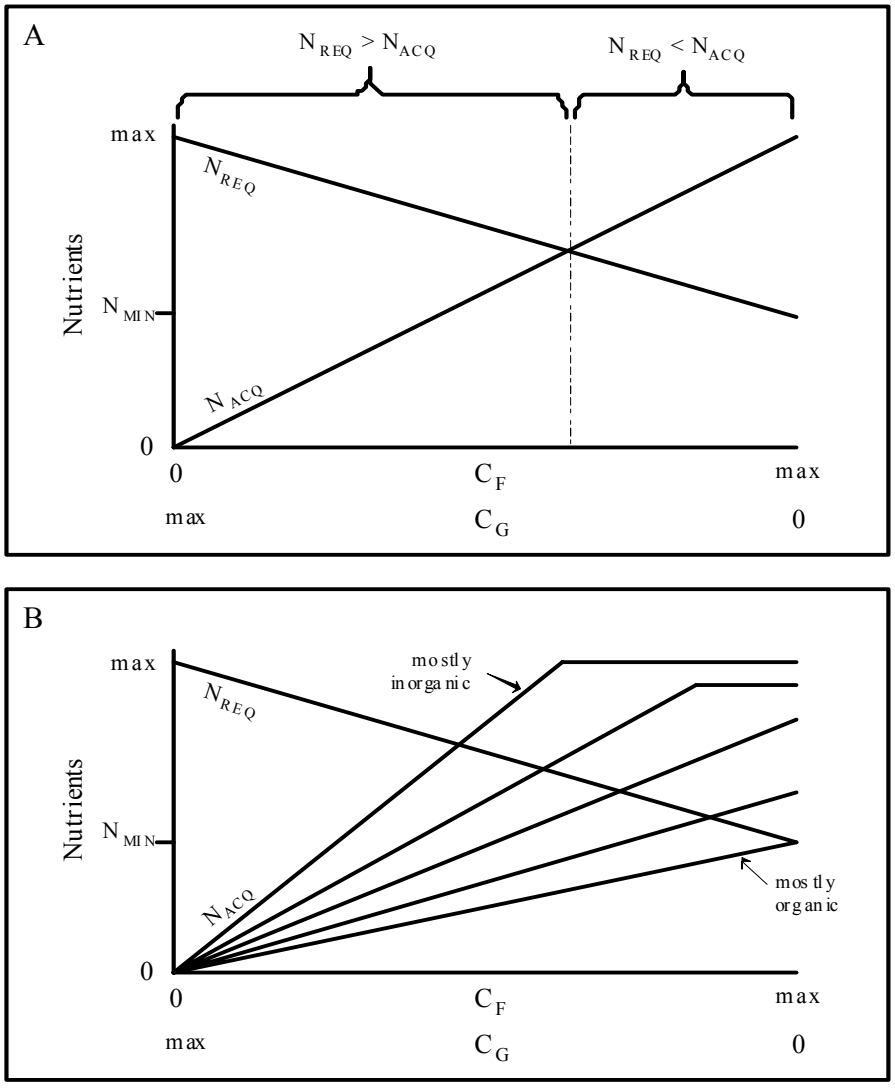


FIGURE 4.2. Nutrient acquisition results of the strategies simulated on a homogeneous environment with 1.5 inorganic units and 2.5 organic units of nutrients in each cell. The optimum condition of 15000 units acquired occurs after approximately 100 cycles in the carbon pool adjustment strategy (A) and symbiont selection strategy (B). A plateau emerges relatively quickly in the selective carbon allocation strategy, but the second and optimal plateau occurs after approximately 1400 steps (C). Error bars are +/- one standard deviation.

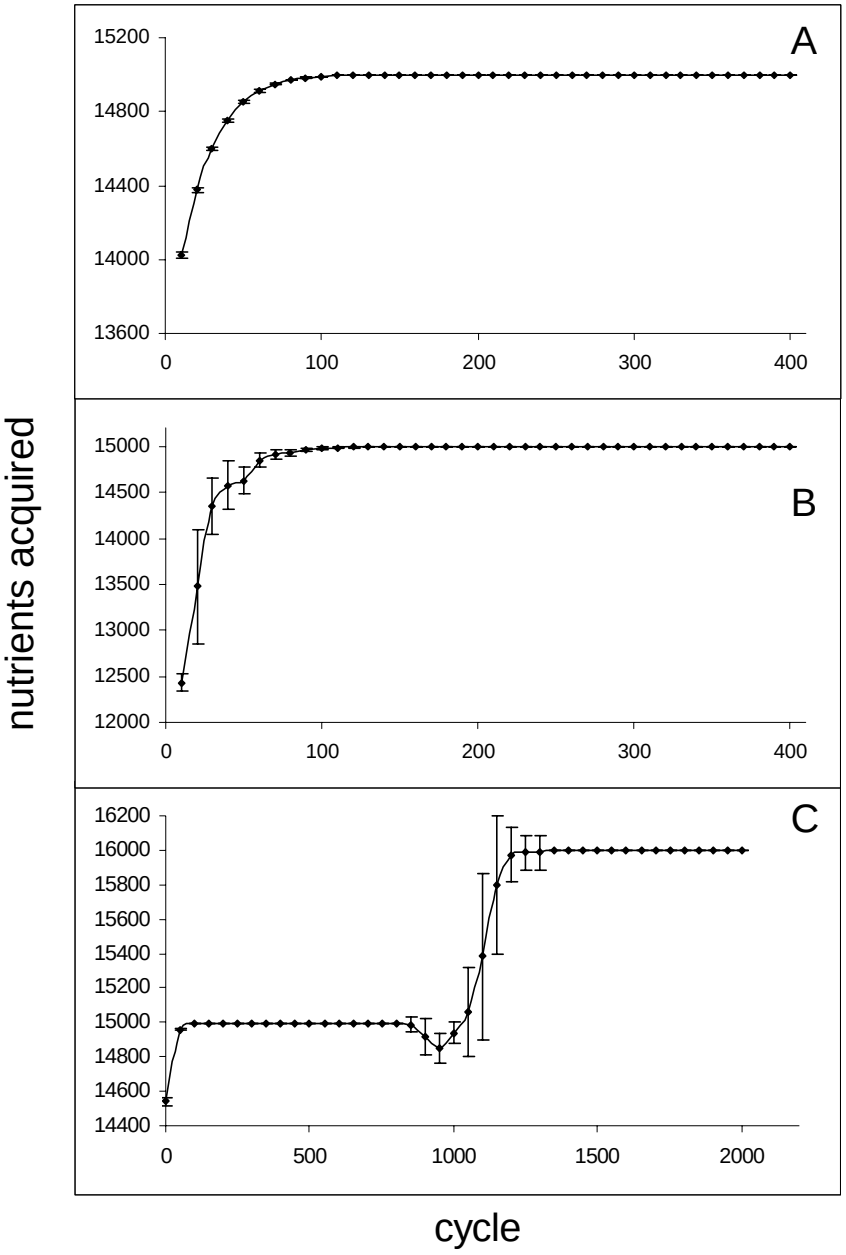


FIGURE 4.3. Nutrients available for growth based on the environmental conditions of a homogenous plot for (A) the carbon pool adjustment strategy, (B) the symbiont selection strategy, (C) the selective carbon allocation strategy, and (D) the combined carbon pool adjustment and symbiont selection strategy. The carbon pool adjustment strategy is insufficient at low levels of available inorganic nutrients (≤ 0.75 units per cell). Note the differences in nutrient acquisition under the symbiont selection strategy (B) and combined carbon pool adjustment and symbiont selection strategy (D) when inorganic nutrient availability is < 1.5 units per cell.

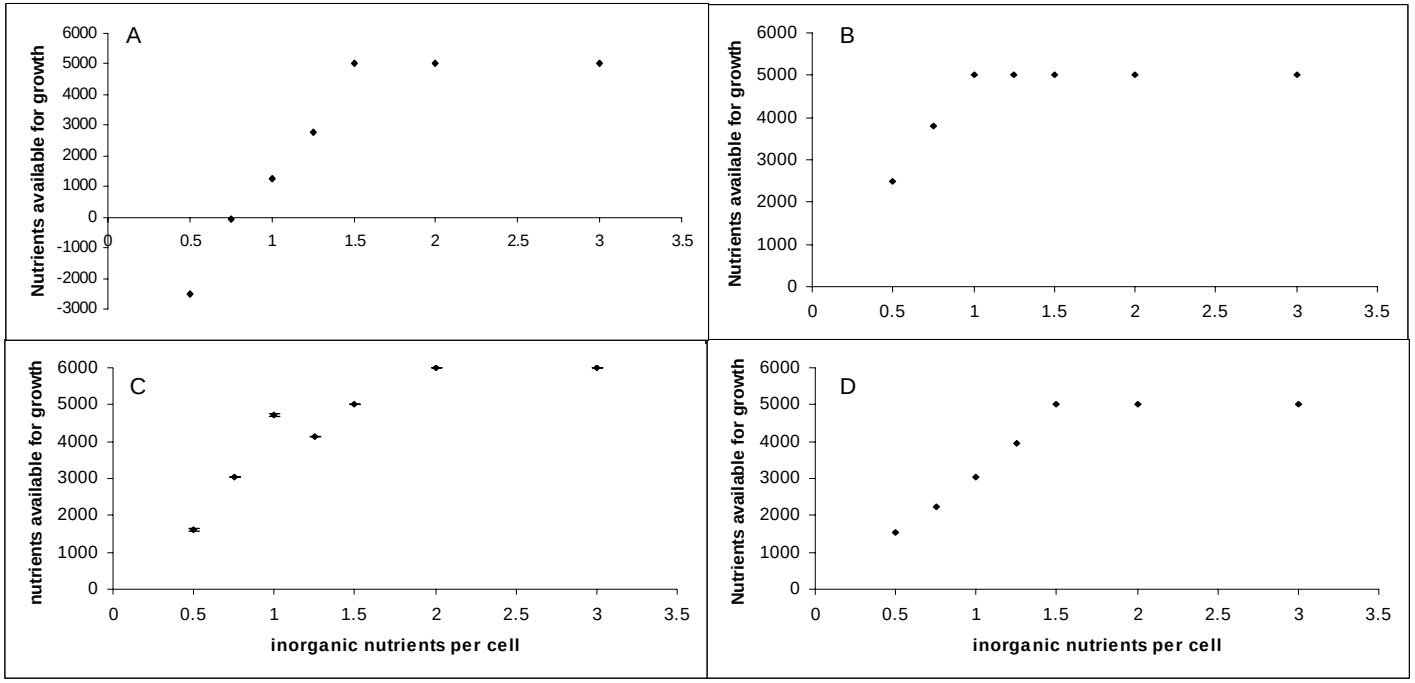


FIGURE 4.4. Carbon allocated to growth based on environmental conditions of a homogenous plot for (A) the symbiont selection strategy and (B) the combined carbon pool adjustment and symbiont selection strategy. Note that when inorganic nutrient availability is <1.5, the symbiont selection strategy does not adequately allocate carbon to growth. Although the symbiont selection strategy is able to acquire larger amounts of nutrients (see Figure 4.3), the combined strategy utilizes its carbon more efficiently.

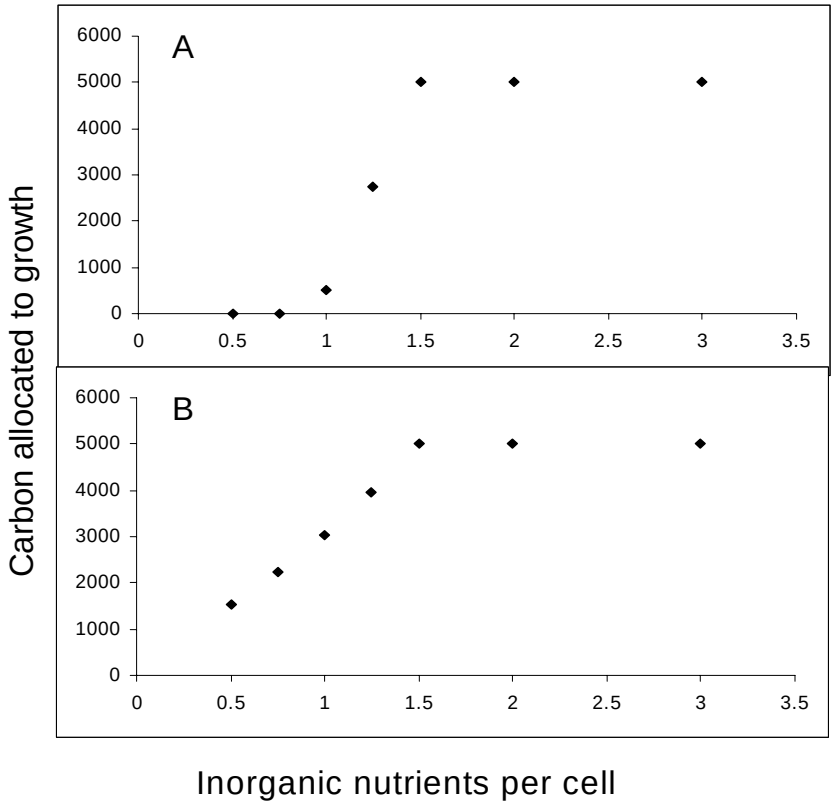
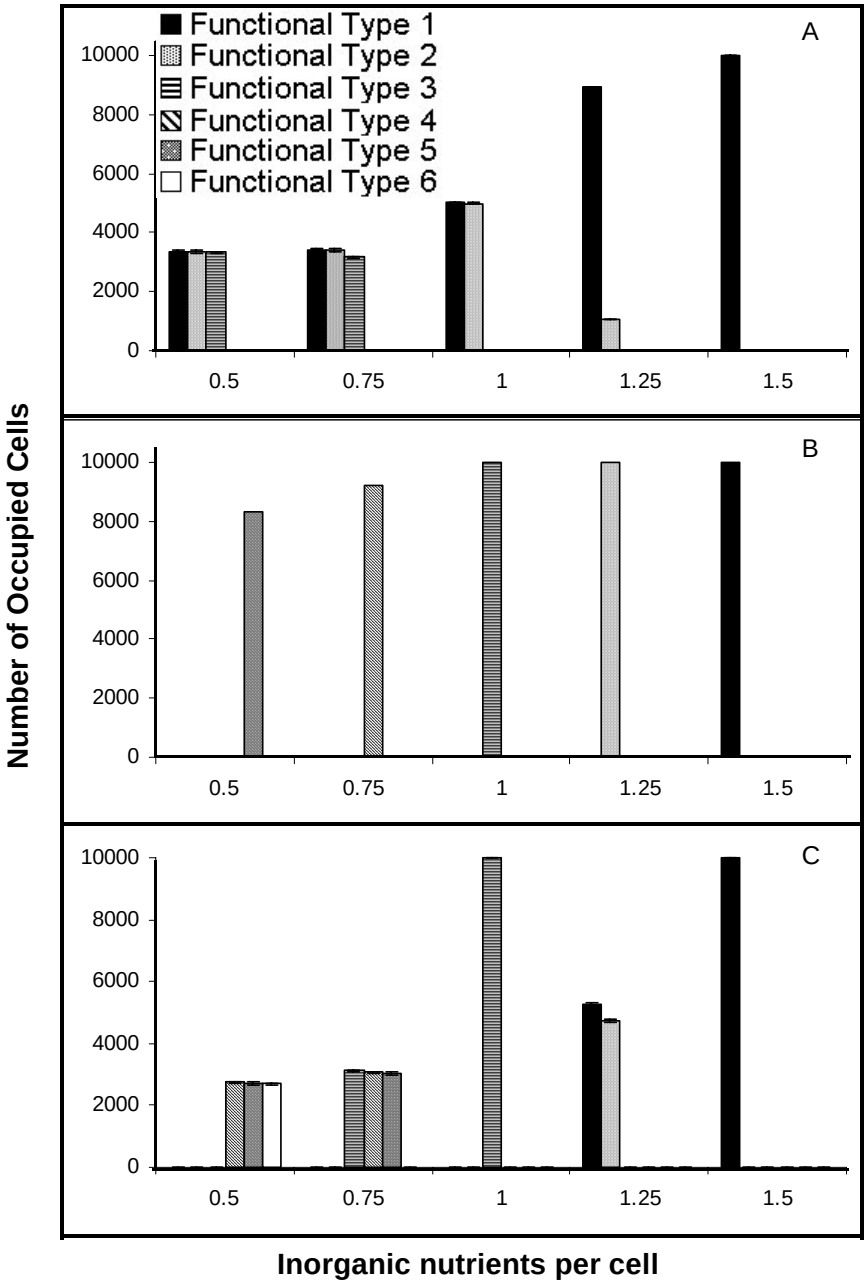


FIGURE 4.5. Functional Type community composition under various homogenous inorganic nutrient availability levels in (A) the pool adjustment strategy, (B) the symbiont selection strategy, and (C) the selective carbon allocation strategy. Pool adjustment never contains the most costly functional types (4-6), symbiont selection results in single functional type communities, and selective carbon adjustment results in communities with favorable functional types more abundant.



CHAPTER 5

CONCLUSIONS

This dissertation integrates ectomycorrhizal fungal (EMF) community ecology and large-scale wind disturbance in temperate forests. The results of the field- and greenhouse-based surveys (Chapters 2 and 3) indicate that wind disturbance can contribute to increased β-diversity in a forested landscape - the EMF community composition in the disturbance gap is different from the EMF community in nearby undisturbed forests. Furthermore, the computer simulation presented in Chapter 4 is useful in developing hypotheses to explore mechanisms that underlie the high diversity evident at small scales (< 1.0 Ha) in EMF communities. These studies provide insight on EMF community dynamics in forests and may prove useful in restoration efforts following intensive logging or catastrophic forest disturbance.

Advancements in molecular identification techniques have rejuvenated the field of EMF community ecology, enabling rapid and accurate identification of the large numbers of samples necessary to adequately describe diverse communities. Numerous studies have reported on the effects of forest fires (e.g., Visser 1995, Dahlberg 2002, Grogan et al. 2002) and logging (reviewed by Jones et al. 2003) on EMF communities, indicating the importance of changes in the inoculum availability and environmental

conditions. Although wind disturbance is common in forests, its effects on EMF communities have remained almost entirely uninvestigated (Egli et al. 2002).

The loss of inocula and disturbance to the soil conditions in wind disturbed forests are minimal in comparison to both burned and logged forests; thus, compositional shifts in the EMF communities in windthrow gaps are due to reduction in carbon availability from the host tree to EMF symbionts. The tornado gap in the Dawsonville Wildlife Management Area (DWMA) had few EMF hosts following the disturbance and little EMF host regeneration over the duration of the study. For this reason, the EMF fungal community in the gap was significantly less diverse than the nearby undisturbed forest (Chapter 2, Figure 2.4). Carbon availability to EMF fungi was similarly reduced in the 2-year-old gap near the Tionesta Scenic Area (TSA), and the EMF community in this gap was also significantly less diverse than that in the nearby undisturbed forest (Chapter 3).

Our results indicate a disturbance threshold that must be surpassed in order for wind disturbance to alter EMF community composition: the disturbance that caused the 11-year-old forest gap removed only 38% of the total forest basal area, and the EMF communities in the 11-year-old gap and the undisturbed forests are remarkably similar. Moreover, we found no decrease in EMF diversity as forests mature (Chapter 3). Our results contradict the Intermediate Disturbance Hypothesis (Connell 1978) and early models of EMF community succession (Last et al. 1987), both of which predict diversity to peak at an intermediate stage of forest development or recovery followed by a decrease in diversity as forests mature to old-growth status. Not surprisingly, compositional shifts in the host community lead to similar shifts in the EMF community as seen in the 20-year-old, *Betula* dominated gap at TSA, and EMF diversity in this gap is similar to that in

the old-growth forest (Chapter 3). Our findings suggest that EMF communities are stable up to a threshold level of disturbance, beyond which community organization changes, similar to models of forest reorganization following catastrophic disturbance described by Frelich and Reich (1999).

Whereas the field and greenhouse studies show that disturbance can maintain landscape-scale EMF diversity, our biological market based simulation describes within plot, or local-scale EMF diversity. The computer simulation developed for this dissertation is a novel approach to modeling and generating hypotheses in EMF community ecology. The trade of carbon for nutrients is central to the ectomycorrhizal symbiosis. Therefore, we incorporated existing economic models modified for the ectomycorrhizal symbiosis (Schwartz and Hoeksema 1998, Hoeksema and Schwartz 2003, Hoeksema and Kummel 2003, Kummel and Salant 2006) and three different plant strategies for allocating carbon to roots. The simulation generates patterns of EMF functional type communities based on the carbon allocated to the symbionts, the availability of inorganically and organically bound nutrients, and the ability of different EMF functional types to acquire organically bound nutrients. The three plant strategies tested were (1) carbon pool adjustment, in which the tree distributes carbon equally to all roots, but modifies the total carbon pool allocated to the symbionts; (2) symbiont selection, in which the plant selects against less favorable symbionts; and (3) selective carbon allocation, in which the plant incrementally increases or decreases the amount of carbon allocated to each root tip based on the cost of nutrients.

Strategies were tested over various nutrient availabilities (the amount of inorganically and organically bound nutrients). Success was defined on the basis that

minimized carbon expended for nutrient acquisition because this would allow more carbon to be utilized for growth and reproduction. In all cases, the symbiont selection and selective carbon allocation strategies were able to meet the nutritional requirements of the plant, but did not necessarily optimize carbon use. The carbon pool adjustment strategy is the only strategy that does not operate at the individual root tip scale, and the strategy was not successful when inorganic nutrients were scarce since there is no mechanism to exclude suboptimal symbionts. Functionally diverse EMF communities were maintained only in the selective carbon allocation strategy, and then only when inorganically bound nutrients were scarce.

Predictions concerning mycorrhizal communities based on our simulations include a decrease in fungal functional type diversity with decreased carbon input from the host as indicated in both the carbon pool adjustment and selective carbon allocation strategies when inorganic nutrient availability is high. Moreover, plants must invest more carbon with the mycorrhizal symbionts when inorganic nutrients are less abundant. Lastly, active control of carbon distribution by plants at the scale of the individual root is required to optimally structure and utilize functional type communities.

Our results contribute to better understanding of the processes that result in between-patch EMF community diversity (β-diversity) and within-patch diversity (α-diversity). The immediate effect of wind disturbance on EMF fungal communities occurs via reduced carbon availability, rather than inoculum loss or changes in edaphic conditions. Long-term changes in the EMF community following wind disturbance occur following severe disturbance resulting in altered host plant communities. At a finer scale,

carbon availability to individual symbionts - based on nutrient supply and demand - also affects EMF community composition.

The field based and computationally based research for this dissertation provides a strong foundation for future research in EMF community ecology. Further field experimentation concerning disturbance severity on the EMF community (utilizing natural or simulated wind disturbance) is necessary to address our hypothesis of a threshold disturbance severity. Long-term effects of wind disturbance on EMF communities can be investigated by repeat sampling of the TSA gaps, or by sampling older gaps. Experimentally testing the assumptions of the computer simulation, and thereby refining the simulation, is a crucial next step. As it stands, the simulation generates hypotheses that may be tested with EMF community data and be utilized to design experiments; however, a better understanding of EMF functional types is still necessary. We thus propose more extensive descriptions of EMF physiology in order to advance the field.

REFERENCES

Abuzinadah, R.A. and Read, D.J. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. 1. Utilization of peptides and proteins by ectomycorrhizal fungi. New Phytologist **103:** 481-493.

Agerer, R. 1991. Characterisation of ectomycorrhiza. Methods in Microbiology **23**: 25-73.

Aguilar, R. 1981. Soil-landscape relationships of the Tionesta Research Natural Area, Pennsylvania - MS thesis, Cornell Univ., Ithaca, NY.

Amaranthus, M. P. and Perry, D.A 1994. The functioning of ectomycorrhizal fungi in the field - linkages in space and time. Plant and Soil **159**: 133-140.

Arnolds, E. 1991. Decline of ectomycorrhizal fungi in Europe. Agriculture Ecosystems and Environment **35**: 209-244.

Axelrod, D.I. 1986. Cenozoic history of some western American pines. Annals of the Missouri Botanical Gardens **73**: 565-641.

Axelrod, R., and Hamilton, W. D. 1981. The evolution of cooperation. Science **211**: 1390-1396.

Bâ, A.M., Garbaye, J., and Dexheimer J. 1991. Influence of fungal propagules during the early stage of the time sequence of ectomycorrhizal colonization on *Afzelia africana* seedlings. Canadaian Journal of Botany **69**: 2442-2447

Baxter, J. W., and Dighton, J. 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host-symbiont culture conditions. New Phytologist **152**: 139-142.

Baxter, J. W., and Dighton, J. 2005. Phosphorus source alters host plant response to ectomycorrhizal diversity. Mycorrhiza **15**: 513-523.

Bennett, L.T. and Adams, M.A. 2004. Assessment of ecological effects due to forest harvesting: approaches and statistical issues. Journal of Applied Ecology **41**: 585-598.

Berbee, M.L. and Taylor, J.W. 1993. Dating the evolutionary radiations of the true fungi. Canadian Journal of Botany. **71**: 1114-1127.

Bjorkbom, J. C. and Larson, R.G.1977. The Tionesta Scenic and Research Natural Areas. U.S. Department of Agriculture Forest Service General Technical Report NE-31, 25pp.

Boddy, L. 1993. Saprotrophic cord-forming fungi - warfare strategies and other ecological aspects. Mycological Research **97**: 641-655.

Boddy, L. and A. D. M. Rayner. 1983. Ecological roles of basidiomycetes forming decay communities in attached oak branches. New Phytologist **93**:77-88.

Bonfante, P., L. Lanfranco, V. Cometti, and A. Genre. 1997. Inter- and intraspecific variability in strains of the ectomycorrhizal fungus Suillus as revealed by molecular techniques. Microbiological Research **152**: 287-292.

Boucher, D. H., S. James, and K. H. Keeler. 1982. The ecology of mutualism. Annual Review of Ecology and Systematics **13**: 315-347.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. TREE **9:** 214-217.

Bronstein, J. L. 2001. The costs of mutualism. American Zoologist **41**: 825-839.

Brundrett M. C. and L. K. Abbott. 1994. Mycorrhizal fungus propagules in the jarrah forest.1. Seasonal study of inoculum levels. New Phytologist **127**: 539-546.

Bruns, T. D. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant and Soil **170**: 63-73.

Buée, M, P. E. Courty, D. Mignot and J. Garbaye. 2007. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. Soil Biology and Biochemistry **39**: 1947-1955.

Cairney, J. W. G. 1999. Intraspecific physiological variation: implications for understanding functional diversity in ectomycorrhizal fungi. Mycorrhiza **9**: 125-135.

Cline M. L., R. C. France, and C. P. P Reid. 1987. Intraspecific and interspecific growth variation of ectomycorrhizal fungi at different temperatures. Canadian Journal of Botany **65**: 869-875.

Connell, J. H. 1978. Diversity in tropical rainforests and coral reefs - high diversity of trees and corals is maintained only in a non-equilibrium state. Science **199**: 1302-1310.

Dahlberg, A. 2001. Community ecology of ectomycorrhizal fungi: and advancing interdisciplinary field. New Phytologist **150**: 555-562.

Dahlberg, A. 2002. Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests. Silva Fennica **36**: 69-80.

Dahlberg, A., I. Jonsson, and J. E. Nylund. 1997. Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. Canadian Journal of Botany **75**:1323-1335.

Dahlberg, A., J. Schimmel, A. F. S. Taylor, and H. Johannesson. 2001. Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. Biological Conservation **100**: 151-161.

Danielson, R. M. 1984. Ectomycorrhizal associations in hack pine stands in north-eastern Alberta. Canadian Journal of Forest Research **19**: 1412-1421.

Danielson, R. M. and S. Visser. 1989. Host response to inoculation and behaviour of introduced and indigenous ectomycorrhizal fungi of jack pine grown on soil-sands tailings. Canadian Journal of Forest Research **19**: 1412-1421.

DeBellis, T., G. Kernaghan, R. Bradley and P. Widden. 2006. Relationships between stand composition and ectomycorrhizal community structure in boreal mixed-wood forests. Microbial Ecology **52**: 114-126

de Romain, M. and A. M. de Miguel. 2005. Post-fire, seasonal and annual dynamics of the ectomycorrhizal community in a Quercus ilex L. forest over a 3-year period. Mycorrhiza **15**: 471-482.

Deacon, J. and L. Fleming. 1992. Interactions of ectomycorrhizal fungi. Mycorrhizal Functioning (ed, MA Allen), pp 249 - 300. Chapman & Hall, New York

Denison, R. F. 2000. Legume sanctions and the evolution of symbiotic cooperation by rhizobia. American Naturalist **156**: 567-576.

Didham R.K., J. Ghasoul, N. E. Stork and A. J. Davis. 1996. Insects in fragmented forests: A functional approach. TREE **11**: 255-260.

Dighton, J. and P. A. Mason. 1985. Mycorrhizal dynamics during forest tree development. In: Developmental Biology of Higher Fungi (eds. Moore, D., L. A. Casselton, D. A. Wood and J. C. Frankland). Cambridge University Press, Cambridge, U.K. pp. 117-139.

Dighton, J., J. M. Poskitt and D. M. Howard. 1986. Changes in occurrence of basidiomycete fruit bodies during forest stand development: with specific reference to mycorrhizal species. Transactions of the British Mycological Society **87**: 163-171.

Dighton, J., A. R. Tuininga, D. M. Gray, R. E. Huskins and T. Belton. 2004. Impacts of atmospheric deposition on New Jersey pine barrens forest soils and communities of ectomycorrhizae. Forest Ecology and Management **201**: 133-144.

Douglas RB, Parker VT, Cullings KW (2005) Belowground ectomycorrhizal community structure of mature lodgepole pine and mixed conifer stands in Yellowstone National Park. Forest Ecology and Management **208**: 303-317.

Egli S., M. Peter, and S. Falcato. 2002. Dynamics of ectomycorrhizal fungi after windthrow. Forest Snow and Landscape Research **77**: 81-88.

Evans, A. M., A. E. Camp, M. L. Tyrrell, and C. C. Riely. 2007. Biotic and abiotic influences on wind disturbance in forests of NW Pennsylvania, USA. Forest Ecology and Management **245**: 44-53.

Everham, E. M. and N. V. L. Brokaw. 1996. Forest damage and recovery from catastrophic wind. Botanical Review **62**: 113-185.

Farley, R. A. and A. H. Fitter. 1999. Temporal and spatial variation in soil resources in a deciduous woodland. Journal of Ecology **87**: 688 -696

Finlay, R. D., Å. Frostegård, and A-M. Sonnerfeldt.1992. Utilisation of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex. Loud. New Phytologist **120**: 105-115.

Fox, F. M.1986. Groupings of ectomycorrhizal fungi of birch and pine, based on establishment of mycorrhizas on seedlings from spores in unsterile soils. Transactions of the British Mycological Society **89**: 551-560.

Foster, D. R., J. D. Aber, J. M. Melillo, R. D. Bowden, and F. A. Bazzaz. 1997. Forest response to disturbance and anthropogenic stress. Bioscience **47**: 437-445.

Frank, A. 1894. Die Bedeutung der Mykorrhiza-pilz für die gemeine Kiefer. Forstwissenschaftliches Centralblatt **16**: 185-190.

Frelich, L. E. 2002. Forest dynamics and disturbance regimes: Studies from temperate evergreen-deciduous forests. Cambridge University Press, Cambridge, UK.

Frelich, L. E. and P. B. Reich. 1999. Neighborhood effects, disturbance severity, and community stability in forests. Ecosystems **2**: 151-166.

Gadgil, M., O. T. Solbrig. 1972. The concept of r- and K-selection: evidence from wild flowers and some theoretical considerations. American Naturalist **106**: 14-31.

Gardes, M. and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology **2**: 113-118.

Gardes, M. and T. D. Bruns. 1996. Community structure of a ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Canadian Journal of Botany **74:** 1572-1583.

Gibson, F., and J. W. Deacon. 1990. Establishment of ectomycorrhizas in aseptic culture: effects of nitrogen and phosphorus in relations to successions. Mycological Research **94**: 166-172.

Givnish, T. J. 2002 Adaptive significance of evergreen vs. deciduous leaves: solving the triple paradox. Silva Fennica **36**: 703-743.

Grime, J. P. 1977. Evidence for existence of 3 primary strategies in plants and its relevance to ecological an evolutionary theory. American Naturalist **111**: 1169-1194.

Grogan, P., J. Baar and T. D. Bruns. 2002. Below-ground ectomycorrhizal community structure in a recently burned bishop pine forest. Journal of Ecology **88**: 1051-1062.

Guidot, A., J-C. Debaud, A. Effosse and R. Marmeisse. 2003. Below-ground distribution and persistence of an ectomycorrhizal fungus. New Phytologist **161**: 539-547.

Hagerman, S. M., M. D. Jones, G. E. Bradfield, M. Gillespie, and D. M. Durall. 1999. Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Canadian Journal of Forest Research **29**: 124-134.

Hagerman, S. M., M. D. Jones, G. E. Bradfield, and S. M. Sakakibara. 1999. Ectomycorrhizal colonization of *Picea engelmannii x Picea glauca* seedlings planted across cut blocks of different sizes. Canadian Journal of Forest Research **29**: 1856-1870.

Harvey, A. E., M. F. Jurgensen and M. J. Larsen. 1980. Clear-cut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in western Montana. Canadian Journal of Forest Research **10**: 300-303.

Henrion, B., F. Le Tacon and F. Martin. 1992. Rapid identification of genetic variation of ectomycorrhizal fungi by amplification of ribosomal RNA genes. New Phytologist **122**: 289-298.

Helms, J. A. 1998. The dictionary of forestry. Bethesda, MD, USA; The Society of American Foresters.

Hoeksema, J. D. and M. Kummel. 2003. Ecological persistence of the plant-mycorrhizal mutualism: A hypothesis from species coexistence theory. American Naturalist **162**: S40-S50.

Hoeksema, J. D. and M. W. Schwartz. 2003. Expanding comparative-advantage biological market models: contingency of mutualism on partner' resource requirements and acquisition trade-offs. Proceedings of the Royal Society of London B **270**: 913-919.

Horton, T. R. and T. D. Bruns. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black box. Molecular Ecology **10**: 1855-1871.

Horton, T. R., E. Cazares and T. D. Bruns. 1998. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. Mycorrhiza **8**: 11-18.

Hough, A. F. and R. D. Forbes. 1943. The ecology and silvics of forests in the high plateaus of Pennsylvania. Ecological Monographs **13**: 299-320.

Hutchison, G. E. 1961. The paradox of the plankton. American Naturalist **93**: 145-159.

Izzo, A., J. A. Agbowo and T. D. Bruns. 2005 Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytologist **166**: 619-630.

Johnson, D., M. Ijdo, D. R. Genney, I. C. Anderson and I. J. Alexander. 2005. How do plants regulate the function, community structure, and diversity of mycorrhizal fungi? Journal of Experimental Botany **56**: 1751-1760.

Johnson, N.C., J. D. Hoeksema, J. D. Bever, V. B. Chaudhary, C. Gehring, J. Klironomos, R. Koide, R. M. Miller, J. Moore, J., P. Moutoglis, M. Schwartz, S. Simard,

W. Swenson, J. Umbanhowar, G. Wilson, G., and C. Zabinski. 2006. From Lilliput to Brobdingnag: extending models of mycorrhizal function across scales. Bioscience **56**: 889-900.

Jones, M. D., D. M. Durall, and J. W. G. Cairney. 2003. Ectomycorrhizal fungal communities in young forest stands regenerating after clear-cut logging. New Phytologist **157**: 399-422.

Jonsson, I, A. Dahlberg, M. C. Nilsson, O. Zackrisson and O. Karen. 1999. Ectomycorrhizal fungal communities in late-successional Swedish boreal forests, and their composition following wildfire. Molecular Ecology **8**: 205-215.

Jumponen, A. and L. M. Egerton-Warburton. 2005. Mycorrhizal fungi in successional environments: A community assembly model incorporating host plant, environmental, and biotic filters. In: Dighton, J., J. F. White Jr. and P. Oudemans (eds.) The fungal community: Its organization and role in the ecosystem, 3rd ed. CRC Press, Boca Raton, FL. Pp 139-168.

Kennedy, P. G., S. Hortal, S. E. Bergemann and T. D. Bruns. 2007. Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. Journal of Ecology **95**: 1338-1345.

Koide, R. and G. Elliot. 1989. Cost, benefit and efficiency of the vesicular arbuscular

mycorrhizal symbiosis. Functional Ecology **3:** 252-255.

Kranabetter, J. M. 1999. The effect of refuge trees on a paper birch ectomycorrhiza community. Canadian Journal of Botany **77**: 1523-1528.

Kranabetter, J. M., J. Friesen , S. Gamiet, and P. Kroeger. 2005. Ectomycorrhizal mushroom distribution by stand age in western hemlock - lodgepole pine forests of northwestern British Columbia. Canadian Journal of Forest Research **35**: 1527-1539.

Krueger, L. M. and C. J. Peterson. 2006. Effects of white-tailed deer on *Tsuga canadensis* regeneration: Evidence of microsites as refugia from browsing. American Midland Naturalist **156**: 353-362.

Kummel, M. and S. W. Salant. 2006. The economics of mutualisms: optimal utilization of mycorrhizal mutualistic partners by plants. Ecology **87**: 892-902.

Last, F. T., J. Dighton and P. A. Mason. 1987. Successions of sheathing mycorrhizal fungi. TREE **72**: 157-161.

Le Page, B. A., R. Currah, R. Stockey and G. W. Rothwell. 1997. Fossil ectomycorrhiza from the middle Eocene. American Journal of Botany **84**: 410-412.

Lechowicz, M. J. and G. Bell. 1991. The ecology and genetics of fitness in forest plants. II. Microspatial heterogeneity of the edaphic environment. Journal of Ecology **79**: 687-696.

Lilleskov, E. A. and T. D. Bruns. 2003. Root colonization dynamics of two ectomycorrhizal fungi of contrasting life history strategies are mediated by addition of organic nutrient patches. New Phytologist **159**: 141-151.

Lopez, B., S. Sabate, and C. A. Gracia. 2001. Annual and seasonal changes in fine root biomass of a Quercus ilex L. forest. Plant and Soil **230**: 125-134.

Mason, P. A., J. Wilson, and F. T. Last. 1983. The concept of succession in relation to the spread of sheathing mycorrhizal fungi in inoculated tree seedlings growing in unsterile soils. Plant and Soil **71**: 247-256.

McIver, J. D. and L. Starr. 2001. A literature review on the environmental effects of postfire logging. Western Journal of Applied Forestry **16**:159-168.

Menkis, A., J. Allmer, R. Vasiliauskas, V. Lygis, J. Stenlid, and R. Finlay. 2004. Ecology and molecular characterization of dark septate fungi from roots, living stems, coarse and fine woody debris. Mycological Research **108**: 965-973.

Molina, R., H. Massicotte and J. M.Trappe. 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: Mycorrhizal Functioning. Allen MF (ed.) Chapman and Hall, London pp. 357-423.

Nehls, U., N. Grunze, M. Willmann, M. Reich H. and Küster. 2007. Sugar for my honey: Carbohydrate partitioning in ectomycorrhizal symbiosis. Phytochemistry **68**: 82-91.

Newman, G. S., M. A. Arthur, and R. N. Muller. 2006. Above- and belowground net primary production in a temperate mixed deciduous forest. Ecosystems **9**: 317-329.

Noë, R. and P. Hammerstein. 1994. Biological markets: supply and demand determine the effect of partner choice in cooperation, mutualism and mating. Behavioral Ecolology and Sociobiology **35**: 1-11.

Oliver, C. D. 1981. Forest development in North-America following major disturbances. Forest Ecology and Management **3**: 153-168.

Pacala, S. W., C. D. Canham and J. A. Silander. 1993. Forest models defined by field-measurements. 1. the design of a northeastern forest simulator. Canadian Journal of Forest Research **23**: 1980-1988.

Pacala, S. W., C. D. Canham, J. Saponara, J. A. Silander, R. K. Kobe and E. Ribbens. 1996. Forest models defined by field measurements: Estimation, error analysis and dynamics. Ecological Monographs **66**: 1-43.

Parsons, W. F. J., S. L. Miller and D, H. Knight. 1994. Root-gap dynamics in a lodgepole pine forest - ectomycorrhizal and nonmycorrhizal fine-root activity after experimental gap formation. Canadian Journal of Forest Research **24**: 1531-1538.

Perry, D. A., R. Molina and M. P. Amaranthus. 1987. Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Canadian Journal of Forest Research **17**: 929-940.

Peterson, C. J. 2000. Damage and recovery of tree species after two different tornadoes in the same old growth forest: a comparison of infrequent wind disturbances. Forest Ecology and Management **135**: 237-252.

Peterson, C. J. and S. T. A. Pickett. 1990. Microsite and elevational influences on early forest regeneration after catastrophic windthrow. Journal of Vegetation Science **1**: 657-662.

Peterson, C. J. and S. T. A. Pickett. 1991. Treefall and resprouting following catastrophic windthrow in and old-growth hemlock hardwoods forest. Forest Ecology and Management **42**: 205-217.

Petraitis, P. S., R. E. Latham and R. A. Niesenbaum. 1989. The maintenance of species-diversity by disturbance. Quarterly Review of Biology **64**: 393-418.

Pickett, S. T. A., S. L. Collins and J. J. Armesto. 1987. A hierarchical consideration of causes and mechanisms of succession. Vegetatio **69**: 109-114.

Pickett, S. T. A. and P. S. White. 1985. The ecology of natural disturbance and patch dynamics. Academic Press, Orlando, FL, USA.

Read, D. J. and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytologist **157**: 475-492.

Remy, W., T. N. Taylor, H.Haas and H. Kerp 1994. Four hundred-million-year-old vesicular-arbuscular mycorrhizae. Proceedings of the National Academy of Sciences **91**: 11841-11843.

Richardson, M. J. 2002. The coprophilous succession. Fungal Diversity **10**: 101-111.

Roberts, M. R. 2004. Response of the herbaceous layer to natural disturbance in North American forests. Canadian Journal of Botany **82**: 1273-1283.

Roiloa, S. R., P. Alpert, N. Tharayil, G. Hancock and P. C. Bhowmik. 2007. Greater capacity for division of labour in clones of *Fragaria chiloensis* from patchier habitats. Journal of Ecology **95**: 397-405.

Roughgarden, J. 1975. Evolution of marine symbiosis – a simple cost-benefit model. Ecology **56**: 1201-1208.

Schlesinger, W. H. 1977. Carbon balance in terrestrial detritus. Annual Review of Ecology and Systematics **8**:51-81.

Schwartz, M. W. and J. D. Hoeksema. 1998. Specialization and resource trade: biological markets as a model of mutualisms. Ecology **79**: 1029-1038.

Simard, S. W., D. A. Perry, M. D. Jones, D. D. Myrold, D. M.Durall, and R. Molina. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. Nature **388**: 579 - 582.

Smith, S. E. and D. J. Read. 1997. Mycorrhizal Symbioses, 2nd edn. Academic Press, San Diego, USA.

Stendell, E. R., T. R. Horton and T. D. Bruns. 1999. Early effects of prescribed fire on the structure of the ectomycorrhizal fungus community in a Sierra Nevada ponderosa pine forest. Mycological Research **103**: 1353 - 1359.

Stuefer, J. F., H. DeKriib and J. H. During. 1996. Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. Functional Ecology **10**: 328-334.

Tannenbaum, E. 2007. When does division of labor lead to increased system output? Journal of Theoretical Biology **247**: 413-425.

Taylor, A. F. S. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. Plant and Soil **244**: 19-28.

Taylor, D. L. and T. D. Bruns. 1999. Community structure of ectomycorrhizal fungi in a Pinus muricata forest: minimal overlap between the mature forest and resistant propagule communities. Molecular Ecology **8**: 1837-1850.

Trappe, J. M., 1977. Selection of fungi for ectomycorrhizal inoculation in nursuries. Annual Review of Phytopathology **15**: 203-222.

Turner, M. G., V. H. Dale and E. H. Everham. 1997. Fires, hurricanes, and volcanoes: Comparing large disturbances. Bioscience. **47**: 758-768.

Turner, M. G., W. L. Baker, C. J. Peterson and R. K. Peet.1998. Factors influencing succession: Lessons from large, infrequent natural disturbances. Ecosystems **6**: 511-523.

Twieg, B. D., D. M. Durall and S. W. Simard. 2007. Ectomycorrhizal fungal succession in mixed temperate forests. New Phytologist **176**: 437-447.

Visser, S. 1995. Ectomycorrhizal fungal succession in hack pine stands following wildfire. New Phytologist **129**: 389-401.

West, A. A., E. T. Kiers and E. L. Simms. 2002. Sanctions and mutualism stability: why do rhizobia fix nitrogen? Proceedings of the Royal Society of London B. **269:** 685-694.

Wilkinson, D. M. and T. N. Sherrat. 2001. Horizontally acquired mutualisms, and unsolved problem in ecology? Oikos **92:** 377-384.

Wong, K. K. Y. and J. A. Fortin. 1990. Root colonization and intraspecific mycobiont variation in ectomycorrhiza. Symbiosis **8**: 197-231.