Ectomycorrhizal Community Recovery in a *Quercus* Savanna Following Restoration from a *Rhamnus cathartica* Invasion

A THESIS SUBMITTED TO THE FACULTY OF THE PROGRAM IN PLANT BIOLOGY AND CONSERVATION

BY ROBERT D. HEVEY JR

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT BIOLOGY AND CONSERVATION FROM NORTHWESTERN UNIVERSITY AND THE CHICAGO BOTANIC GARDEN

APRIL 24, 2014

COMMENTS ON THESIS DEFENSE FOR MASTER OF SCIENCE IN PLANT BIOLOGY AND CONSERVATION NORTHWESTERN UNIVERSITY AND THE CHICAGO BOTANIC GARDEN

The student Robert D. Hevey Jr defended his/~~her~~ thesis, titled

Ectomycorrhizal Community Recovery in a Quercus Savanna Following Restoration from a Rhamnus Cathartica Invasion

on the date of 24 April 2014. The committee members have rendered the following decision:

☐ Pass with no revisions ☒ Pass with minor revisions

☐ Pass with major revisions ☐ Fail to pass

Committee members:

Printed name	Signature	Department/Affiliation
Louise Egerton-Warburton Major adviser		CBG
Greg Mueller		CBG
Neal Blair		Northwestern

In the case of revisions, specify below, on back, or on additional attached pages what they are and who on the committee (major adviser, or everyone) will review the revisions before the thesis is officially accepted as complete. After this approval has been met, the major adviser needs to sign again below and this form, along with a pdf file of the final approved thesis must be turned into the PBC program assistant. Students need to check the deadlines for June and December graduations with TGS to make sure these forms are completed on time.

- Methods section needs clarification of sampling numbers (in a flowchart) sequence numbers (total samples sequenced vs successful matches), root colonization by buckthorn
- Results - explore the species richness of samples, trees, & treatments, add time series data for species (if possible); also sampling errors

PTO →

Signature of major adviser confirming final approval — Date: May 13, 2014

This form, along with a pdf file of the final approved thesis, must be submitted to the PBC program assistant, Susan Black, in Hogan 2-144. The program director will then sign off on it and approval will be communicated to TGS.

Plant Biology and Conservation Program Director Signature — Date: 5/15/14

ABSTRACT

Once widespread throughout southern Wisconsin, *Quercus* savannas are now one of the most endangered communities due to fire suppression and increasing landscape fragmentation. These plant communities are also highly susceptible to invasions by non-native, exotic species. Understanding the mechanisms of exotic species invasion has been the focus of numerous studies. However, few studies have documented ecosystem recovery following invasive species' removal; even fewer have examined mycorrhizal community recovery. In this study, I examined the recovery of the ectomycorrhizal (ECM) community and changes in the soil factors carbon (C), nitrogen (N), and moisture over a three-year period following the removal of *Rhamnus cathartica* (buckthorn) in a *Quercus macrocarpa* (bur oak) savanna. I tested the hypotheses that the removal of the buckthorn would result in an increase in soil moisture and C/N ratio and would also result in an increase in species richness and diversity, and a modification in the community structure of the ECM community.

Soil factors and the ECM community were monitored under the canopies of eight bur oaks; four trees were located in areas where buckthorn had been manually removed, while the remaining four trees were located in an area that was heavily invaded by buckthorn. Soil cores (448 total) were collected from eight bur oaks in fall 2009 and during the early summer and early fall over the following three consecutive years (2010, 2011, 2012). Soil N and C were determined by combustion analysis, and soil moisture by gravimetric methods. Sequencing of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA was used to identify ECM species colonizing *Q. macrocarpa* roots. Sequence data were used to provide measures of species richness and diversity, and ECM community structure was summarized and linked to soil factors using non-metric multidimensional scaling (NMDS).

The soil analyses showed significant interannual and seasonal variations in soil moisture, and that soil moisture was higher in Cleared than Invaded areas. Similarly Soil C and N were significantly higher for the duration of the study however there was no significant time *x* treatment effect. In contrast, there was no significant difference in soil texture, available NO_3, NH_4, and P, and soil C/N ratio between Invaded and Cleared areas over time.

More than 3200 ECM root tips were sorted into 82 morphological categories comprising 279 root tip samples for analysis. From this analysis 210 sequences were identified using a Basic Local Alignment Search Tool (BLAST) query of GenBank and UNITE databases. A total of 162 sequences were determined to be ECM of which 26 were identified as unique species of Ascomycota and 38 unique species of Basidiomycota. The ECM community showed a strong, positive response to Clearing. Species richness and diversity measures were significantly greater in the Cleared area (44 species) than the Invaded area (31 species). In addition, the ECM community in the Invaded area was dominated by three taxa (*Scleroderma*, *Pachyphloeus*, and members of the Pezizales) which accounted for 68% of the Invaded area root tips. In the Cleared area, the principal genera were *Inocybe*, *Tuber*, *Cortinarius* and *Boletus* (54% of the Cleared area root tips). Over the three-year

period, only 11 ECM species occurred in both the Cleared and Invaded areas. The results of the NMDS analyses support these findings and show two distinct ECM communities: one associated with the Cleared area and one with the Invaded area. NMDS also indicated that ECM community structure was significantly influenced by soil C content (Cleared > Invaded).

The results did not support the first hypothesis. Carbon, nitrogen, and soil moisture in the Cleared area were consistently higher than in the Invaded area throughout the study and while there were significant differences between Cleared and Invaded areas for all three soil factors over time, there was no significant difference over time *x* treatment.

Analysis of ECM root tips collected from the Cleared and Invaded areas found distinct ECM communities between these areas, and indices of ECM richness and diversity were greater in the Cleared area than in the Invaded area supporting the second hypothesis of a favorable change in the Cleared ECM community.

While this study did not find any obvious effect of the measured environmental variables (C, N, and moisture) it suggests that possible significant soil factors remain unmeasured since there was an identified difference in the communities between the Cleared and the Invaded areas.

Acknowledgements

Were it not for my participation in the predecessor to the Plant Biology and Conservation (PBC) program, the Plant Conservation and Biology certificate program at Chicago Botanic Gardens, I very most likely would never have considered this program. So, my heartfelt thanks to Kay Havens, Louise Egerton-Warburton, Stuart Wagenius, and Pati Vitt for developing and presenting that outstanding program.

I want to thank my thesis committee: Louise, Greg Mueller, and Neal Blair for their substantial patience, understanding, and assistance. In particular Louise, who routinely scheduled around my horrendous work schedule and provided much needed guidance and support. And, a very special thank-you to Steve Finkelman for his lab assistance (particularly Leco maintenance) and his exceptionally dry humor.

I had help from so many and I will apologize now if I forget someone. Anne Nies; without whose help I would have struggled greatly to ever get my DNA extractions started and Thomas Wilson; who assisted with the extractions. Ben Morgan; who graciously ran my sequencing. Billy Levinson: who endured the frustration of running (and re-running) many soil samples on the then very temperamental Leco. Louise, Lauren Umek, Joe Gawronski-Salerno, Rachel Gross, and Becky Tonietto; who trusted me enough to help them with their lab work and who unknowingly assisted in pulling me into the PBC program. The entire soil group (Louise, Greg, Lauren, Ben, Anne, Corey Palmer, Rui Zhang, Chen Ning, Kathryn Schreiner, Nik Desai, Lynnaun Johnson, and Andy Wilson) who were all so graciously tolerant. And the always helpful assistance from Susan Black and Gail Kushino.

Warmest regards to Nyree Zerga, whom I met very shortly after her arrival, whose dedication to PBC is beyond fierce, whose contagious enthusiasm makes it nearly impossible to be anything less, and without whose encouragement I would not have applied.

To Constance who politely tolerated what ended up being a fourteen year odyssey (and to Eddie, Mick, and sadly departed Bailey and Logan), all of whom never complained about my early morning study sessions nor my countless weekends in lab and field.

TABLE OF CONTENTS

List of Tables

List of Figures

Appendices

BACKGROUND

Definition of invasive species

For the purposes of this thesis, an invasive species is defined following the guidelines of The Invasive Species Advisory Committee (ISAC): "a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health" (ISAC 2006). An expansion of that definition would include observations that invasive species reproduce rapidly forming stands excluding nearly all other plants, damaging natural areas, altering ecosystem processes, displacing native species, and supporting other non-native plants, animals and pathogens (Randall 1996).

Mechanisms of exotic species' invasion

Invasive species are a major threat to plant community composition and ecosystem processes worldwide (Pejchar and Mooney 2009) and while numerous studies have explored native species diversity reduction, few have documented any associated mechanisms of exotic plant invasions (Bennett *et al.* 2011, reviewed in Levine *et al.* 2003). Accumulating evidence suggests that the traits of an invasive species may include a large reproductive output, creation of a large and persistent seed bank, rapid germination, pre-emption of resources (light, space, nutrients), the presence of allelopathic root exudates, efficient dispersal mechanisms (particularly by animals), and the establishment of dense local populations with the capacity for rapid range expansion (Sakai *et a*l. 2001; Blumenthal 2005). For example, a long-term field experiment in limestone grassland found that invasive species showed rapid growth, high rates of reproduction, and efficient dispersal mechanisms in comparison to native plants, and could behave opportunistically

when resources became abundant (Burke and Grime 1996). These results suggest that plant communities may be most susceptible to invasion when intense disturbance events coincide with high levels of nutrient availability (Burke and Grime 1996). In addition, native species richness was found to decline with increased invasion intensity owing to decreased colonization by native species. This suggests that the impact of invasion may occur as soon as the new species become established (Yurkonis *et al.* 2005) and that high densities set the environmental context for persistence of exotic species (Denslow and Hughes 2004).

Bennett *et al.* (2011) mentions four potential mechanisms related to invasive species; Direct competition, Changes in soil community abundance and diversity, Indirect competition (herbivory), and Interference competition via allelopathy. Yurkonis *et al.* (2005) suggests the mechanisms of species displacement (invaders reduce diversity by displacement) and establishment limitation (reduction of resident species colonization).

Changing community conditions could also facilitate the establishment and spread of an invasive species (Blumenthal 2005). A resource hypothesis suggests that a plant invasion could coincide with the sudden increase in resources if a resident population declines (Davis *et al*. 2000); something that could occur with the collapse of a canopy tree that creates a sun lite gap and changes in nutrient and moisture levels.

Contribution of plant-soil feedbacks to exotic species' invasions

Recent studies on exotic plants have also revealed a wide variety of plant–soil interactions that might lead to plant invasiveness in new habitats. Inderjit and Van den Putten (2010) summarized three major pathways through which invasive species could modify the plant community: (1) plant–soil feedbacks; (2) the impact of exotic plants on

soil communities; and (3) responses of the native soil community to novel chemicals produced by the invasive species (allelopathy). There is also the phenomenon whereby exotic plants are released from specialist enemies usually found in their native environs; this has been termed the 'Enemy Release' hypothesis (Keane and Crawley 2002; Beckstead and Parker 2003).

Invasive species effects have also been classed as direct or indirect. Direct effects contributing to the success of an invasive species include influences on: local soil biota (see 1, below), or release from enemies. Indirect effects of soil communities contributing to the invasion success of an exotic plant are: accumulation of soil pathogens in the presence of an invasive species and disruption of mutualistic associations between native symbionts and their host plant (see 2 below); and release of allelochemicals by invasive plants or nutrient release from exotic litter (see 3 below).

1) *Plant-soil feedbacks*. Plant-soil feedbacks generated by invasive species can be either positive or negative. In biogeographical experiments in the native and non-native ranges of the invasive species, *Centaurea maculata*, Callaway *et al.* (2008) provided evidence that local soil microbial communities can promote the performance of an invasive exotic plant through positive soil feedbacks. In its native range, soil communities controlled *Centaurea* plant growth, whereas in the non-native range (U.S.), the soil communities tended to enhance plant growth. Similarly, plant–soil feedback of the exotic plant, *Prunus serotina*, was negative in its native range, but neutral to positive in the non-native range (Reinhart *et al.* 2003). On the other hand, Nijjer *et al.* (2007) found that the woody invasive, *Sepium sebiferum*, showed a reduction in growth when grown in soil collected from under *Sepium* plants in comparison to native plants.

2) *Impact of exotic plants on soil communities*. Exotic plants can accumulate local pathogens in their rhizosphere that, in turn, suppress native plants (Kourtev *et al.* 2002). This is known as the Accumulation of Local Pathogens hypothesis (Eppinga *et al.* 2006). For example, the invasion of *Chromolaena odorata* results in an accumulation of generalist soil pathogens that, in turn, negatively influence native plant growth. The Accumulation of Local Pathogens hypothesis can also be extended to include 'suppression of symbioses and mutualisms'. Notably, exotic species can influence the abundance and diversity of mycorrhizal symbioses so as to indirectly reduce the growth of native plants (Pringle *et al.* 2009). Several examples demonstrate the breadth of this effect. The establishment of the invasive *Ageratina adenophora* increased the abundance of arbuscular mycorrhizal fungi in the soil and soil fungal: bacterial ratios; these shifts facilitated the growth of *A. adenophora* and suppressed native plant establishment (Niu *et al.* 2007). In addition, the exotic invasive forb *Alliaria petiolata* (garlic mustard) indirectly inhibits native tree regeneration by suppressing the colonization of roots by arbuscular mycorrhizal fungi (Stinson *et al.* 2006; Callaway *et al.* 2008) and reducing ECM fungal abundance and diversity (Wolfe *et al.* 2008).

3) *Soil community responses to novel chemicals*. Certain invasive plant species are known to produce chemicals (allelochemicals) that are novel to native plants and their microbial communities within an invaded site. The novel-weapons hypothesis suggests that invasive plant species might become successful through production of allelopathic chemicals (Callaway and Ridenour 2004). Buckthorn has been found to produce allelochemicals (Vincent 2006) and ECM may be sensitive to allelochemicals (Rose *et al.* 1983).

Rhamnus cathartica as an invasive species in the Midwest

Buckthorn may have been introduced into the United States before 1800 for use in hedges and for wildlife habitat (Converse 1984). A number of traits enhance the invasive success of buckthorn including early leaf emergence, prolific fruiting (fruits of which are attractive to birds) and a high germination rate. Leaf emergence occurs in April and senescence in November (Godwin 1943). Early leaf emergence and late senescence are important traits: leaf out begins earlier in the season than many native species, and leaves tend to be held until well past first frosts (Harrington *et al.* 1989). The species is dioecious with female tress fruiting heavily and fruits are presumably taken by birds (Godwin 1943). All parts of the tree contain anthraquinones (emodin), which has a strong laxative effect (Randall 1996). Germination rate is high: Godwin (1943) found germination rates of 90 – 100% for undried fruit while Archibold (1997) found a mean emergence rate of 87.5 ± 1.7%. The entire root system is mycorrhizal and the association is endotrophic with arbuscular mycorrhizal fungi (AMF) (Godwin 1943).

As seedlings, buckthorn tolerates a wide variety of shade and soil conditions and establishes rapidly as an understory species. Plants tend to be shrub-like in youth, producing multiple stems (5- 10 cm diameter) that are heavily leaved. These stems actively grow towards any light pockets with the results that numerous intertwined branches form a dense canopy that can effectively preclude sunlight from entering an established buckthorn thicket. Consequently, there is reduced native plant germination and establishment, except near the edges of the thicket. As well as regenerating from seed, buckthorn plants are prolific re-sprouters when cut, especially if all stems are cut simultaneously, regardless of the location of the cut (personal observations).

The leaf litter of buckthorn is N rich (2.2 % N) in comparison to the leaf litter of native species (cottonwood, oak 1.4% N; wild cherry 0.6% N; Heneghan *et al.* 2004). Unfortunately, Heneghan et al. (2004) did not provide standard deviations for these total %N values. However, Miller (2010) documented a mean %N in buckthorn of 2.17% N ± 0.07% while Moreau *et al*. (2004) documented N levels in cottonwood (*Populus deltoids*) at 1.55% N ± 0.35%. Such high levels of N not only contribute to the rapid decomposition of buckthorn litter but also to the overall rates of litter decomposition of other plant species when buckthorn litter is present. The rapid litter decomposition contributes to modified soil properties including elevated soil N and pH (Heneghan *et al.* 2004). It is important to note that while elevated soil N was an expectation of this study when it was begun in 2009, a more recent study (Iannone 2013) found that buckthorn alters few soil properties and that differences are largely reflective of pre-invaded conditions.

In addition to elevated soil N, soil moisture may be higher under buckthorn than in adjacent areas, possibly due to a lower evaporative loss under the dense buckthorn canopy (Heneghan *et al.* 2004). Alternatively, buckthorn may selectively establish in wetter soils. In a southern Wisconsin forest, Mascaro and Schnitzer (2007) found that buckthorn is capable of invading and dominating sites with high water tables. In particular, the site with the highest relative buckthorn basal area also had the highest gravimetric soil moisture content among the eight buckthorn -dominated sites, and the third highest of the 16 sites studied. While both of these studies found buckthorn in high moisture areas, these studies did not specifically consider whether buckthorn was the cause of the higher soil moisture or simply the beneficiary.

Oak savannas in the Midwest

Oak savanna communities were established in the Midwest region ~5000 years ago, when the post-glacial climatic conditions became comparatively warm and dry, and were maintained by periodic drought and fire until European settlement (Abrams 1992). These oak savannas are now a globally threatened ecosystem and occur on just 0.02% of their pre-settlement acreage in the Midwestern United States (Nuzzo 1994). As a community, oak savannas are not prairies with trees: Curtis (1959) defined a savanna as "a plant community where trees are a component but where their density is so low that it allows grasses and other herbaceous vegetation to become the actual dominants of the community". Most savannas are characterized by an open canopy of widely dispersed oak trees and a continuous herbaceous layer in the understory. Soil moisture, nutrients (especially K, P) and organic matter levels are higher in savannas than surrounding grasslands (Ko and Reich 1993). Site water balance also differs between open savannas and forests because oaks have different patterns of water uptake than forest tree species (Asbjornsen *et al.* 2007).

Bur oak has historically comprised a significant component of the savanna community across the Midwest. Bur oaks exhibit physiological adaptations that facilitate plant survival in sites exposed to drought, fire, and nutrient-poor soils. Bur oak is one of the most fire-resistant oaks, with thick, corky bark (Abrams 1992), and is resistant to drought owing to the development of an extensive root system (Faber-Langendoen and Tester 1993; Farrar 1995). ECM fungi form essential connections with the soil and form typical ectomycorrhizas on members of the Fagaceae that colonize the majority of the fine root tips of the trees providing nutrients and water (Smith and Read 2008). In return for

soil-derived nutrients and water, the fungi receive photosynthetically derived carbon from the host plant. Because a large percentage of bur oak roots are colonized by ECM (up to 80%; Dickie *et al.* 2004), most of the nutrients and water used by the plant will be acquired by their ECM symbionts.

The decline of oak savannas with buckthorn invasion - the role of ectomycorrhizal fungi

A suite of factors including logging, disease, fire suppression and landscape fragmentation have widely reduced the dominance of oak savannas in the landscape. This is particularly true in Wisconsin, where the oak savanna, which was once the most widespread plant community type in southern Wisconsin, is now one of the most endangered (Leach and Givnish 1999). Further contributing to the endangerment of oak savannas are intrusions by *Prunus serotina* and invasions by buckthorn and the invasive forb, garlic mustard. Buckthorn has become the dominant woody plant in many savannas and woodlands in southern Wisconsin. This dominance far exceeds the levels noted in northeastern temperate forest studies (Mascaro and Schnitzer 2007). In Wisconsin, buckthorn has effectively formed an exotic dominated ecosystem that is structurally distinct from native savannas and woodlands.

Buckthorn may influence oak growth via its direct effect on soil properties, most notably through increases in N availability and moisture, and changes in soil pH (Heneghan *et al.* 2004). Another mechanism by which buckthorn might influence oak productivity is by disrupting ECM symbioses. It is possible that buckthorn-related changes, such as soil N and moisture availability, might influence ECM abundance, species richness, and community structure that could feedback to influence plant productivity.

It is widely recognized that N enrichment either through anthropogenic deposition or experimental fertilization reduces the abundance, diversity, and community composition of ECM fungi on root tips (e.g., Lilleskov *et al.* 2002; Avis *et al.* 2003; Parrent and Vilgalys 2007; Cox *et al.* 2010), and results in the shifts of specific fungal genera. For example, an increasing input of N reduced the abundance of *Cortinarius* species (Lilleskov *et al.* 2002; Avis *et al.* 2003; Toljander *et al.* 2006), but increased the abundances of *Lactarius* or *Russula* species (Lilleskov *et al.* 2002; Avis *et al.* 2003). Such shifts in composition also corresponded with changed ECM functioning: those ECM that responded positively to N enrichment were capable of using inorganic N sources (nitrophilic species), whereas species sensitive to N appeared to use organic N sources (Lilleskov *et al.* 2002).

There is also evidence that ECM may be sensitive to changes in soil moisture availability. Lodge (1989) found that ECM infection was greatest in moist but well drained soil while Slankis (1974) found that AMF were more abundant in dry soils.

Restoration after buckthorn

Restoration of a buckthorn invaded oak savanna involves more than just the removal of the established buckthorn. To minimize disruption to the soil, the buckthorn should be cut and the stumps treated with either a high concentrate glyphosate (>40%) or Garlon; repeat applications may be required. Buckthorn have a rather shallow root system with relatively thin roots that can dry out in two or three years allowing the stump to be gently removed if desired (personal observations).

Buckthorn fruits prolifically however seed germination is high in the first year (Godwin 1943) resulting in a short-lived seedbank. Buckthorn seedlings are easily pulled

and Mundahl *et al.* (2010) found that pulling was the most suppressive treatment, although labor intensive. Burning is often prescribed in oak savanna restorations although burning alone has not proven successful in buckthorn control (Brock and Brock 2004, Maloney 1997, Mundahl *et al.* 2010).

Brock and Brock (2004) found that because buckthorn is allelopathic, it could take two or three years to re-establish herbaceous species. Maloney (1997) provides a list of understory species suitable for recoverable oak savanna and open oak woodland in southern Wisconsin and suggests use of both seedlings and seeding coupled with careful monitoring and weed removal in the first few years of recovery.

Objectives

The overarching objective of this study was to document changes in soil factors (C, N, moisture) and the recovery of the ECM community over three consecutive years (2010-2012) following the removal of buckthorn in a bur oak savanna. To do so, I monitored soil factors and the ECM community under the canopies of eight bur oak trees; four trees were located in areas where buckthorn had been manually removed, while the remaining four trees were located in an area that was heavily invaded by buckthorn. Total soil N and C were analyzed by combustion and soil moisture by gravimetric methods. ECM root tip samples were identified with ITS rRNA gene sequencing, assessed using measures of both species (richness, diversity indices) and community structure, and linked to soil factors using non-metric multidimensional scaling.

I used these data to test two hypotheses:

1.) The removal of the buckthorn will result in reduced nutrient levels and increased soil moisture and,

2.) The removal of buckthorn will result in an increase in ECM abundance, species richness, and diversity and a shift in ECM community composition.

MATERIALS AND METHODS

Study site

The study site is a 1-hectare remnant bur oak savanna in the Rock River Basin (Rock County, Wisconsin; Latitude 42.83525, Longitude -88.98245; Figure 1A). The site is raised several meters above the surrounding terrain to an elevation of approximately 240 meters (Figure 1B). The general area is near the edge of the Milton glacial margin of the Horicon Member of the Green Bay Lobe of the Laurentide Ice Sheet (Clayton *et al*. 2006). Soil in the study site is classified as the Zurich series (ZuB) surrounded by Palms muck (USDA 2011).

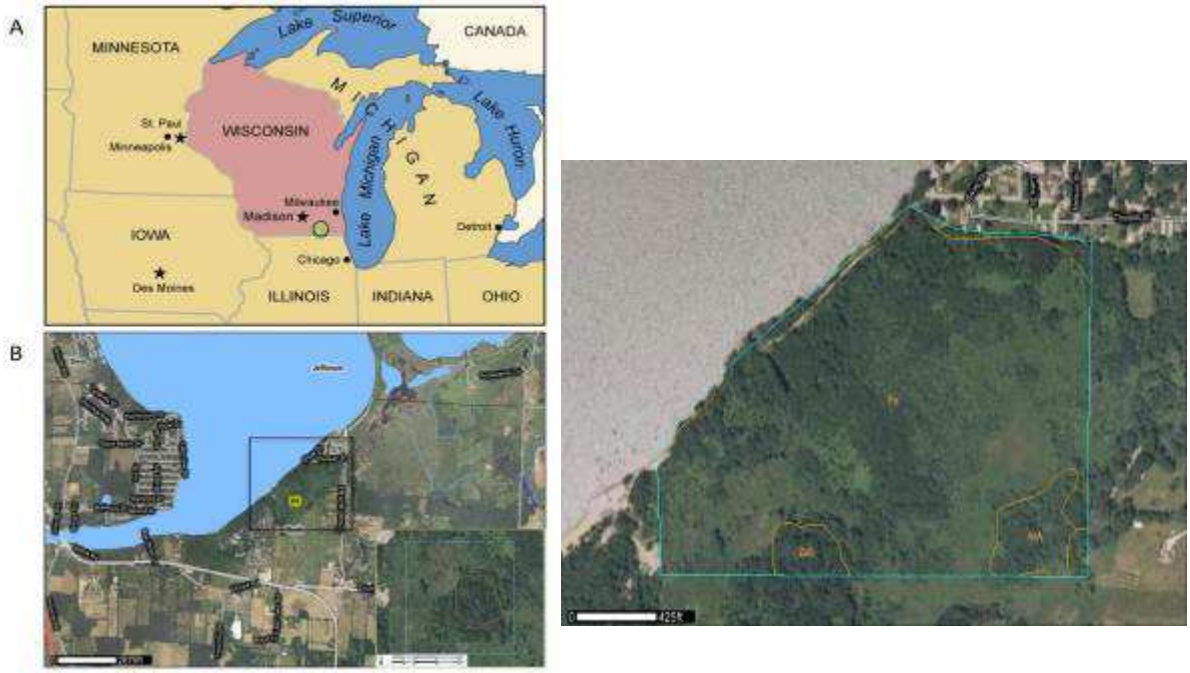


Figure 1. A). Location of study site within the U.S. Midwest region. B). Satellite view of site; WI designates approximate location of site. Inset shows higher resolution site view.

The climate is classified as humid continental and typified by large seasonal fluctuations in temperature; summers are typically warm and humid, and the winters cold. At the site, the four-year mean maximum temperature over the growing season from May

to September was 25.6 ˚C, and mean minimum was 13.3˚C (Figure 2; NOAA). Total rainfall from April to September during the study ranged from 378 mm (2012) to 746 mm (2010). In addition, average precipitation in the latter part of the season, i.e., July to September (325 mm) was consistently higher than precipitation during the early part of the season, from April through June (237 mm; Figure 3; NOAA).

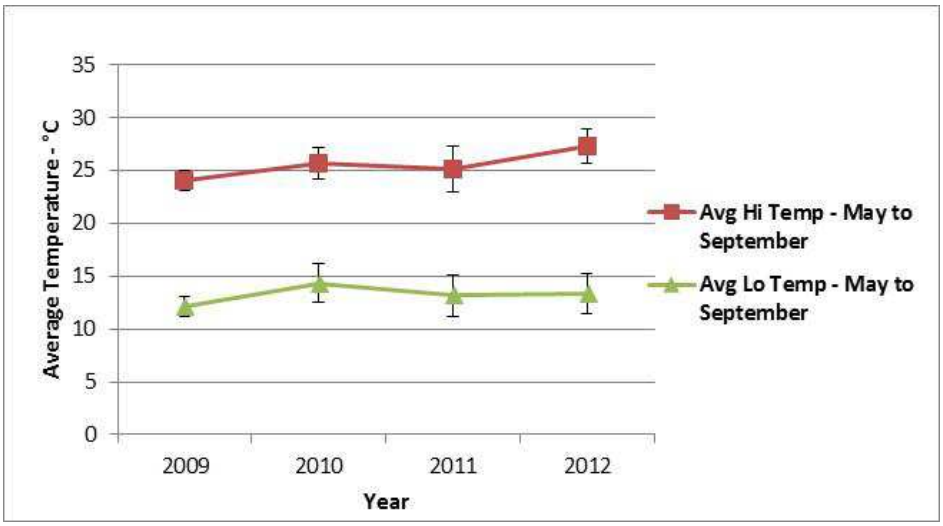


Figure 2. Average maximum and minimum temperatures at the study site from 2009 to 2012.

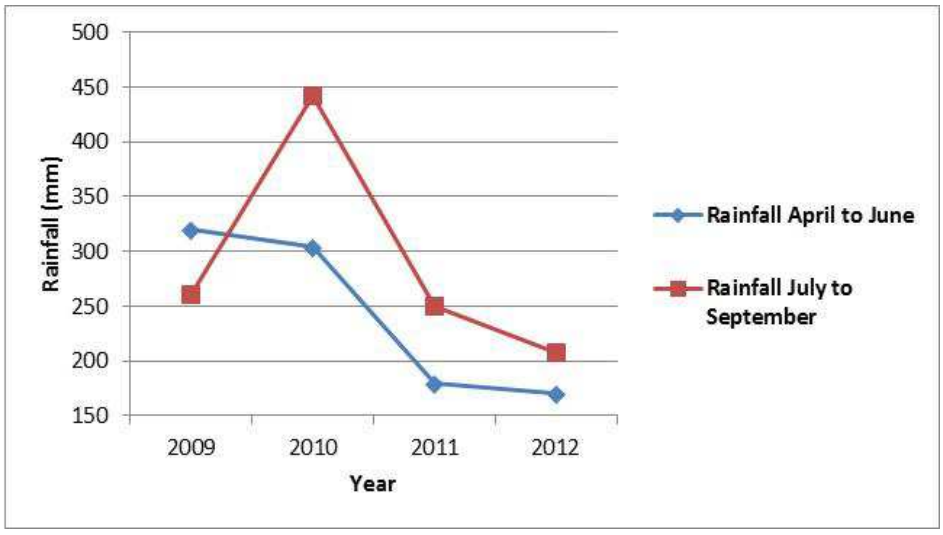


Figure 3. Average rainfall at the study site during early (April to June) and late (July to September) growing season, from 2009- 2012.

Soil factors and the ECM community were monitored under the canopies of eight (initially nine) mature bur oaks (DBH 0.7- 1.4m) from fall 2009 (pre-clearing, invaded state) to fall 2012 (three years after buckthorn removal). At the beginning of the study (2009), all bur oaks were surrounded by dense thickets of buckthorn (Figure 4). Buckthorn was cleared from under the canopy of four Trees (labeled 1, 5, 6 and 7) between fall 2009 and spring 2010 (Figure 5). Clearing was accomplished by cutting the buckthorn near the soil line and leaving the root structure intact to minimize disturbance to the soil. Resprouts were treated with a 25% (v/v) concentration of Glyphosate.

The remaining four Trees were located in an area that remained heavily invaded by buckthorn for the remainder the duration of the study (labeled 3, 4, 8 and 9). Tree 2, whose canopy overlapped Tree 3 was cleared on the side opposite Tree 3 in spring 2010 and included in the study after Tree 1 toppled in a winter 2009/2010 storm.

Figure 4). Study site prior to Buckthorn removal (2009).

Figure 5). Study site subsequent to Buckthorn removal (2010 onwards).

Additional buckthorn removal took place as needed throughout the study to maintain the Cleared treatment. Subsequent to removal of the buckthorn from the Cleared area, garlic mustard, which had been present in small populations mostly at the edges of the site, responded positively to the increase in available light and carpeted substantial portions of the area between Tree 2 (sectors C & D) and Tree 5 (sectors A & B) by Fall 2012. Efforts to control the spread of the garlic mustard by physical removal were made in 2011 and 2012 in areas outside of the dripline, but not within the sampling areas.

Soil sampling

Plots of a quasi-circular nature were established around each bur oak using the dripline as a guide (Figure 6). Each plot was then divided into four sectors for sampling purposes (A, B, C and D). Soil samples were taken with a bulb planter (7.5 cm deep, 5.5 cm diameter). Surface debris was removed prior to taking each sample, samples were

immediately placed into one-quart Zip-lock® plastic bags, and labeled with location and collection date. Samples were refrigerated upon return from the field, kept chilled during transport to the lab, and refrigerated until processed within two days of collection. Soil cores were collected in September 2009; May, August and October 2010; May, June, and September 2011; and June and September 2012. Samples were taken in late Spring/early Summer and late Summer/early Fall in an effort to capture any potential seasonal differences in ectomycorrhizal diversity and/or abundance.

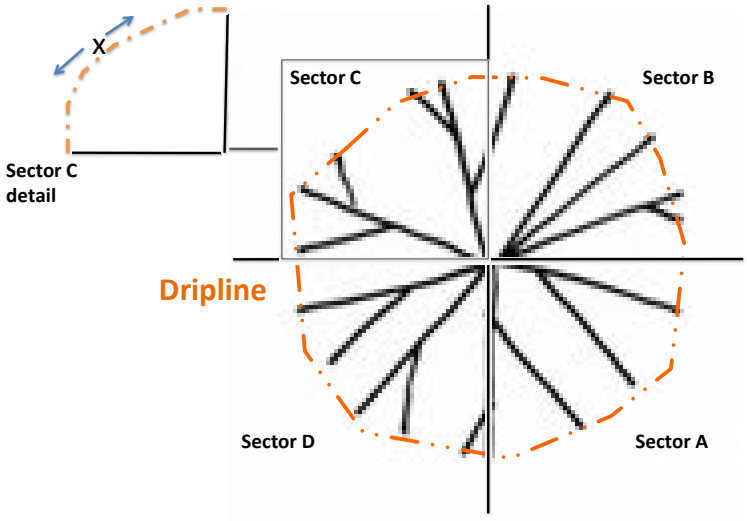


Figure 6. Schematic showing the location of the sectors, polypropylene braid, and sampling points.

Cores collected in 2009, 2010, and May 2011 were non-randomly selected and were taken along the approximate dripline near the approximate center of each sector. In spring 2011, a polypropylene hollow braid, marked off in one-quarter meter increments, was placed along the approximate dripline of each bur oak. Samples taken in 2011 and 2012 were randomly determined and taken along a five-meter section of the dripline from the approximate center point of each sector (Figure 6). Two samples were taken in each sector, one left and one right of the center point. Each sampling location was determined

by adding one to the last digit of a randomly selected number and counting that number of one-quarter meter segments left or right.

Within two days of collection, root structures were removed from each soil sample and carefully washed in a container of tepid tap water (~32° C), with fresh water used for each sample. Cleaned root structures for each sample were placed between a moistened and folded KimWipe in Zip-lock® plastic bag. Each bag was marked with location and collection date and was kept refrigerated (4 ˚C) until processed for morphotyping.

Soil analyses

Sub-samples of soil (~90g each) from each sampling location and date were tested for gravimetric soil water content following procedures as outlined in Robertson *et al.* (1999). The remaining soil samples were air-dried at room temperature (22 ˚C). Sub-samples of air-dried soil (~5 g) of each sampling location and date were ground to a fine powder with a mortar and pestle and analyzed for total % C and % N content using a LECO TruSpec CN Carbon Nitrogen combustion analyzer (LECO Corp., St. Joseph, MI).

Samples collected in 2009 and 2012 were also analyzed for N (as NH_4, NO_3) and P to determine if there was any effect of buckthorn removal on plant-available nutrients. Air-dried, sieved bulk soil was used for nutrient extraction using KCl. Five grams of soil was shaken with 50 mL of 1M KCl for 30 minutes on a medium to high speed (100 RPM) on a shaker platform. Samples were centrifuged for 5 minutes at 8,000x to pellet the soil, after which 10 mL of the supernatant was placed in clean vials for storage. Extracts were then analyzed for plant-available ammonium (NH_4), nitrate (NO_3), and orthophosphate (P) using microplate methods. The method chosen for NH_4 was from Weatherburn (1967). In this method, the ammonium reacts with salicylate in the presence of hypochlorite (oxidizer) and

nitroprusside (catalyst) to form an emerald green complex. Nitrate was measured colorimetrically using the protocol of Doane and Howrath (2003). In this method, any nitrate in the extract is reduced to nitrite with vanadium (III) chloride, which then reacts with Griess reagents (sulphanilamide; N-(1-naphthyl)-ethylenediamine dihydrochloride) to form a pink colored diazodye. Phosphorus levels were quantified using the malachite green method for orthophosphate (Baykov *et al.* 1988). This assay capitalizes on the strong absorption shifts created when P forms a stable malachite green-phosphomolybdate complex at low pH.

Morphological typing of ECM roots

Intact roots were viewed in water using a Motic DM143-FBGG digital stereomicroscope (magnification, 40*x*) for identification of ECM. Buckthorn roots were segregated first based on their color (black) and viewed for ECM infection of which none were found. Infected root tips were then characterized and typed using reference according to patterns of differentiation proposed by Agerer (2001) and concise descriptions per BCERN (Goodman *et al*. 2008). Mycorrhizal tips of the same morphology were classified and counted. The four morphological attributes used to distinguish different ECM morphotypes were: (a) the outer mantle layers in plain view (texture), (b) presence/ absence of rhizomorphs, (c) presence/ extent of emanating hyphae; and (c) color (Agerer 2006). Each morphological type was placed in an individually labeled 1.5 ml microfuge tube, covered with reverse-osmosis water, and frozen at -20° C until DNA extraction.

Molecular analysis of ECM root tips

To identify ECM root tips to species' level, molecular genetics techniques were used to sequence the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA

genes. ITS is a common barcode gene and provides sufficiently high resolution to separate most ECM species (Hughes *et al.* 2009).

DNA was extracted for each morphotype using the MO BIO PowerSoil DNA Isolation Kit following the manufacturer's instructions (MO BIO Laboratories, Inc., Carlsbad, California; http://www.mobio.com/). Final extraction products were stored at -20° C until analysis. Efficacy of DNA extraction was visualized on agarose gels (1.0 % agarose, 0.5M TE buffer; 100 V, 20 min) with 5 µl of 1kb DNA Ladder (Promega Corp., Madison, Wisconsin) as a standard, and stained with SYBR Green (Molecular Probes, Eugene, Oregon). All samples showed strong, positive DNA signals and were thus subsequently subjected to PCR to amplify the internal transcribed spacer (ITS) regions 1, 5.8s and 2 of rDNA.

The optimal DNA concentration for PCR reactions was determined using a dilution series in eight selected samples. Undiluted DNA extract and two dilutions (1/10 and 1/100 prepared using sterile nanopure water) of each DNA extract were subjected to PCR amplification as follows: 12.5 µl Promega mastermix (Promega), 9.5 µl ddH2O, 1.0 µl ITS1F primer (Gardes and Bruns 1993), 1.0 µl ITS4 primer (White *et al.* 1990) and 1 µl of DNA template.

Amplifications were performed in Eppendorf Thermal Cyclers as follows: 96° C for 2 minutes; followed by 35 cycles of 96° C for 30 sec, 50° C for 30 sec, and 72° C for 60 sec; and then 72° C for 10 minutes being subsequently held at 4° C until retrieval. Amplicons (5 µl each) were electrophoretically separated on agarose gels in 0.5 M TE buffer (1.0 % agarose; 100 V, 20 min) with 5 µl of 1kb DNA Ladder (Promega) as a standard, and stained with SYBR Green (Molecular Probes). Undiluted DNA extracts

produced consistent PCR amplification in all samples. All subsequent PCR reactions were then undertaken using undiluted DNA extracts.

Prior to cycle sequencing, all PCR products were cleaned using the Wizard SV Gel and PCR Clean-Up System (Promega). Cycle sequencing was performed in 96-well plates and 10 µl reactions contained the following: 1µl BigDye v. 3.1 (Life Technologies, Carlsbad, California), 3 µl BigDye Buffer, 0.5 µl primer (single direction sequencing with ITS1F primer for forward directions), 4.5 µl of ddH_2O, and 1 µl PCR product (DNA). The reactions took place in Eppendorf thermalcyclers as follows: initial denaturation of one minute at 96° C followed by 25 cycles of: 96° C for 10 sec, 50° C for 5 sec, and 60° C for 4 minutes being subsequently held at 4° C until retrieval.

Cycle sequencing products were cleaned using an ethanol precipitation protocol. Briefly, each PCR product was precipitated by the addition of 80 µl 75% (v/v %) ethanol to each well, mixed, and then pelleted by centrifugation (max speed = 2000 rpm, 30 minutes). The 75% ethanol solution was removed and the process repeated twice except using 100 µl 100% ethanol for each step and centrifuged for 15 min. After the final centrifugation, residual ethanol was evaporated from the samples by placing the uncovered plate into a thermal cycler programmed to run at 95° C for 5 minutes. DNA sequencing was performed at the Field Museum of Natural History in Chicago on the Applied Biosystems 3730 DNA Sequencer (Life Technologies), with 10µl of hi-di formamide (Invitrogen, Life Technologies) added to each sample.

The editing of raw DNA sequences was performed using 4Peaks (Griekspoor and Groothius 2010) and ambiguous regions at the ends were trimmed. Species' names were assigned to ECM root sequences using the approach of Smith *et al*. (2007). Initially,

individual internal transcribed spacer (ITS) sequences were grouped together into larger putative taxonomic groups (e.g. Boletales, Thelephorales) using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST), which allows query sequences to be matched against a large public database (GenBank). Within each taxonomic group, sequences were grouped into potential species-level operational taxonomic units (OTUs) with parameters set to a minimum of 20% overlap and 97% sequence similarity, a cut-off that has been found to correspond well with morphologically defined species in many fungal groups (Peay *et al.* 2010). Sequences were considered to represent the same operational taxonomic unit (OTU), a proxy for species, if they differed by <3% across the ITS region. Representative sequences from each OTU were then searched against the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) using BLAST or UNITE (http://unite.ut.ee/) and named to the lowest taxonomic rank possible based on the level of match. A small number of root tips were colonized by well-known non-mycorrhizal pathogenic, saprophytic, or endophytic species, e.g., *Trichoderma*, and were excluded from further analyses.

Table 1. Summary of soil core samples, analysis, morphology, sequences, and taxa

Count	Description
448	Soil cores between fall 2009 and fall 2012
384	Soil core samples tested for C, N, and moisture
96	Soil core samples tested for texture
>3200	Root tips extracted, washed, and morphotyped
82	Morphotype categories
279	Root tip samples (separated by morphotype) sequenced
210	Sequences identified to taxa
162	Sequences identified as ECM
64	Species identified via GenBank and United databases
	26 Ascomycota, 38 Basidiomycota
	44 species in Cleared area, 31 species in Invaded area

DATA ANALYSIS

The three objectives of the study were to: (1) determine the effects of buckthorn invasion and removal on soil factors (Hypothesis 1); (2) identify the magnitude and direction of changes in ECM species richness and diversity following the removal of buckthorn (Hypothesis 2); and (3) determine the shifts in ECM community composition following the removal of buckthorn and the contribution of soil factors to such shifts (Hypothesis 1, 2).

The effects of buckthorn removal on soil %C, %N, and soil moisture were analyzed using repeated-measures analysis of variance (ANOVA) with treatment (Cleared, Invaded) and sampling date as fixed effects, and tree as a random effect. Significant variables were then compared using Fisher's Least Significant Difference test. Prior to analysis, all data sets were tested for normality and, where required, were transformed using ln (x), ln (x+1), or square root (x) to satisfy the assumptions of uni- and multivariate normality. Statistical analyses were performed using JMP 10.0.2 (SAS Institute Inc., Cary, NC).

To test the effects of buckthorn removal on the ECM community, I undertook three sets of analyses. First, a repeated-measure ANOVA was used to test whether ECM species richness per tree was altered by buckthorn removal over time. Next, I calculated the effects of buckthorn removal on ECM species richness and diversity using two non-parametric indices of richness, first-order jackknife ($Jack_1$) and $Chao_2$, and the Simpson's index of diversity and evenness. $Jack_1$ is the simplest jackknife richness estimator and a function of the number of ECM species that occur in only one sample (singletons). The $Chao_2$ estimator uses the observed number of ECM species combined with singletons and doubletons (species detected twice). Calculations of $Jack_1$, $Chao_2$, and Simpsons indices

were undertaken with EstimateS v9.1.0 for Windows (Colwell 2013) using 500 randomizations of sample order without replacement. Indices were calculated using an abundance matrix or a presence-absence matrix of ECM species by treatment by year.

Finally, shifts in ECM assemblage structure between treatments and over time were visualized using non-metric multi-dimensional scaling (NMDS). For this study, ECM root tip abundance data were ordinated using NMDS and the Manhattan similarity measure in PAST (Hammer *et al.* 2001). The Manhattan distance metric was used because it decomposes into the relative contributions made by each variable, i.e., ECM species. Nonparametric correlations (Spearman rank) were then used to examine the relationship between the measured environmental variables (soil C, N, moisture) and each of the axes of the final two-dimensional ordination space. For interpretability, the correlated environmental variables were plotted as vectors to show their relationships with the ECM community scores.

The effect of invasion and clearing on the ECM fungal community structure was analyzed using MRPP (Multiple Response Permutation Procedure) in BLOSSOM and using 999 randomized runs (Cade and Richards 2008). The MRPP is a nonparametric, multivariate procedure that tests the null hypothesis of no difference in ECM community between Cleared and Invaded areas. The test statistic (*T*) explains the separation between groups in multidimensional 'species' space; the more negative the *T*-value, the greater the separation between groups. The *P*-value quantifies separation between groups when compared with what is expected by chance and the agreement statistic *A* represents the chance-corrected within group agreement and is a measure of effect size (McCune & Grace, 2002).

RESULTS

Soil carbon and nutrients

Soil %C was significantly higher in Cleared than Invaded areas for the duration of the study (Figure 7; F= 6.2751, p =0.0179). Soil %C in the Cleared area averaged 7.6% (range 4.3% - 10.8%; Appendix A) in comparison to 6.4% (range 3.9 % - 11.8%; Appendix A) in the Invaded area. There were also significant differences in soil %C over time (F= 4.07, p <0.01) but no significant time *x* treatment effect (F=1.7382, p>0.05). The lack of a significant interaction between time and treatments indicates that the pattern of soil %C in each treatment was similar across time.

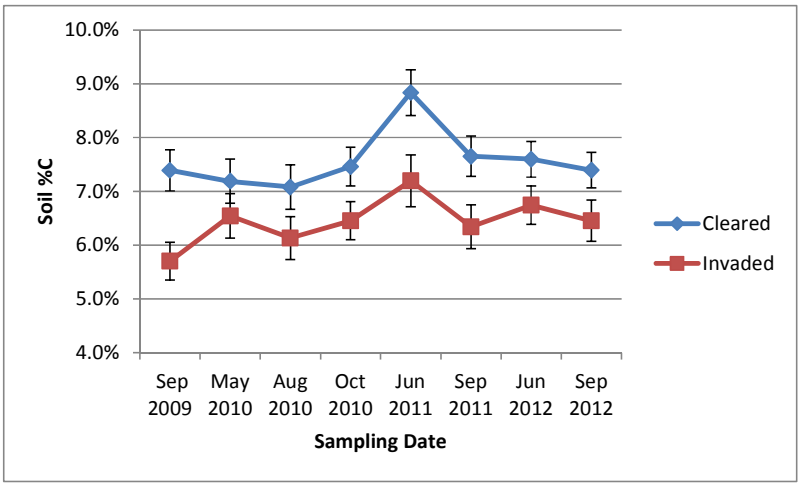


Figure 7. Patterns of soil %C in Cleared and Invaded areas before treatments were installed (2009) and in the three subsequent years (2010, 2011, 2012). Vertical bars indicate the standard error of the mean.

Similarly, soil %N was significantly higher in Cleared than Invaded areas over the three years of the study (Figure 8; F=9.031, p =0.0053). Soil %N in the Cleared area averaged 0.588% (range 0.313% - 0.942%; Appendix B) in comparison with 0.465% (range 0.230% - 0.465%; Appendix B) in the Invaded area. Analysis of variance also showed a significant difference in soil %N over time (F= 8.5623, p <0.0001), but no significant time x treatment effect (F=0.8286, p>0.05).

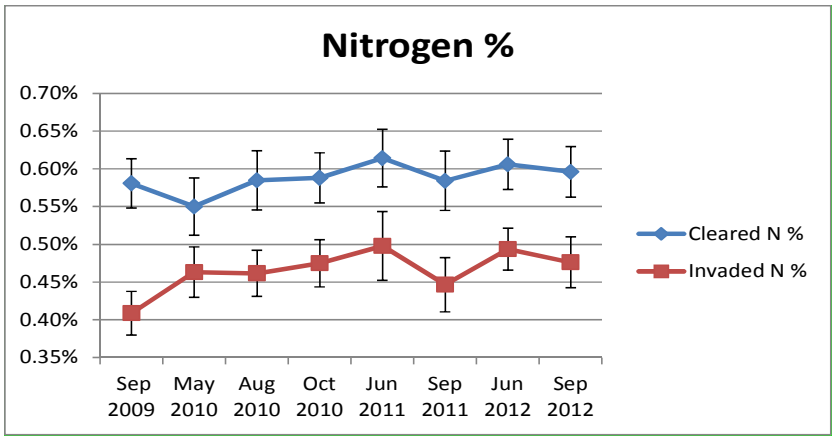


Figure 8. Patterns of soil %N in Cleared and Invaded areas before treatments were installed (2009) and in the three subsequent years (2010, 2011, 2012). Vertical bars indicate the standard error of the mean.

When %C and %N data were summarized as the ratio of C to N (C/N), there was a significant difference in C/N over time (Figure 9; F= 24.9102, p <0.0001) and between treatments (average Cleared C/N 13; average Invaded C/N 14; F=17.22, p=0.0003) however, there was no significant time *x* treatment effect (F=1.7336, p>0.05). Overall, average soil C/N was highest in June 2011 (C/N 14.5), and lowest in August 2010 (C/N 12).

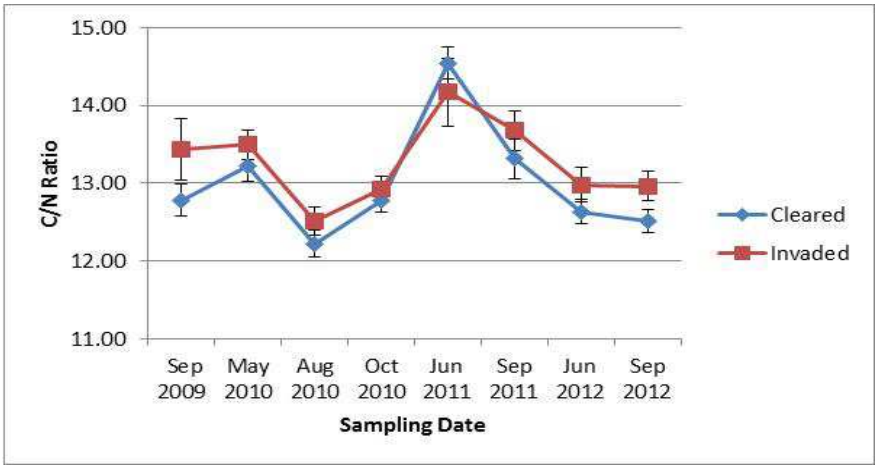


Figure 9. Patterns of soil C/N in Cleared and Invaded areas before treatments were installed (2009) and in the three subsequent years (2010, 2011, 2012). Vertical bars indicate the standard error of the mean.

A comparison of plant-available nutrients demonstrated that there was no significant difference in NH_4, NO_3 or P levels from Cleared and Invaded areas either before the buckthorn removal were undertaken (2009) or three years after buckthorn clearing (2012; $p > 0.05$, *t*-tests; Table 2). In addition, there was no significant difference in NH_4, NO_3 or P levels between 2009 and 2012. These similarities in soil nutrient levels suggest that any differences in the ECM communities between Cleared and Invaded areas were not related to these soil properties.

Table 2. Levels of plant-available NH_4, NO_3, and P in Cleared and Invaded areas in 2009 and 2012. Values represent the mean with standard error in parentheses.

Soil Attribute	Cleared – 9/20/09	Invaded - 9/20/09	Cleared – 9/14/12	Invaded – 9/14/12
Ammonium ($\mu g\ g^{-1}$ soil)	30 (5.6)	33 (3.7)	22 (3.3)	26 (3.5)
Nitrate ($\mu g\ g^{-1}$ soil)	28 (2.4)	25 (2.1)	29 (1.6)	26 (1.8)
Orthophosphate ($\mu g\ g^{-1}$ soil)	4.1 (0.4)	4.9 (0.2)	5.0 (0.4)	5.8 (0.4)

Soil moisture

Gravimetric soil moisture content varied significantly over time ($F = 125.8617$, $p < 0.0001$) and between Cleared and Invaded areas ($F = 0.8.4847$, $p = 0.0067$). However, there was no significant time *x* treatment effect ($F = 2.1124$, $p > 0.05$). Both Cleared and Invaded areas showed the same patterns of soil moisture: the highest levels were recorded in May 2010 and June 2011, while the lowest soil moisture content occurred during September 2011 and throughout the 2012 growing season (Figure 10). In addition, soil moisture in the Cleared area (mean 37.5%; range 17.5%- 75.2%; Appendix C) was significantly higher than in the Invaded area (mean 30.4%; range 10% - 85%; Appendix C).

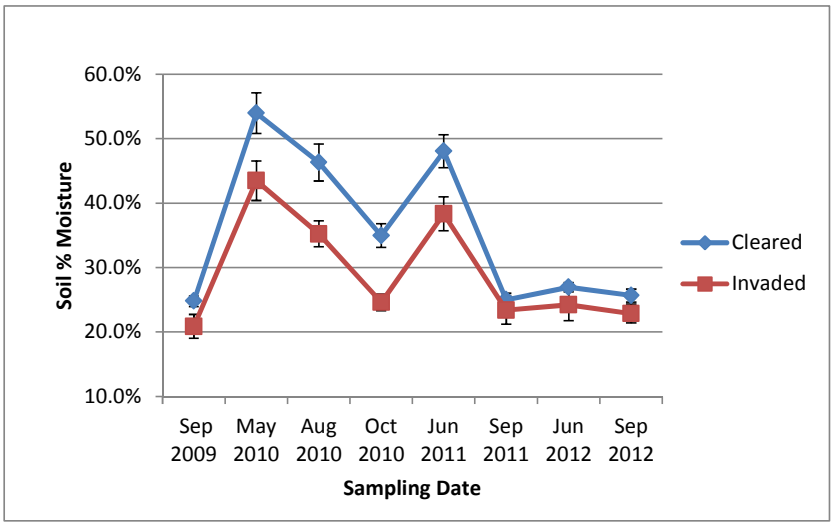


Figure 10. Patterns of soil moisture in Cleared and Invaded areas before treatments were installed (2009) and in the three subsequent years (2010, 2011, 2012). Vertical bars indicate the standard error of the mean.

Soil texture

Soil samples were also tested for texture following procedures as modified from Bouyoucos (1962) for samples taken prior to clearing (September 20, 2009) and for the final samples (September 14, 2012). These analyses demonstrated a broad overlap in the textural categories found in Cleared and Invaded areas. Using the soil texture triangle (Wunsch 2009), the 2009 and 2012 soil samples could be broadly classed as loams (sandy loam, sandy clay loams, loam; Figures 11 and 12). In addition, clay loams were identified in a limited number of 2009 samples, and silt loams in 2012 samples. Small differences in the amount of soil separates contributed to these differences in classification. Silt content declined from 32.7% to 30.4% in the Cleared area but increased from 35.9% to 38.1% in the Invaded area. The sand content in the Cleared area samples ranged from 46.0% (2009) to 47.2% (2012) while the sand content in the Invaded area samples averaged 40.6% (2009) to 38.8% (2012). Nevertheless, the similarities in soil texture classification coupled with

the small changes in soil separates suggest that any differences in the ECM communities between Cleared and Invaded areas were not related to these variables.

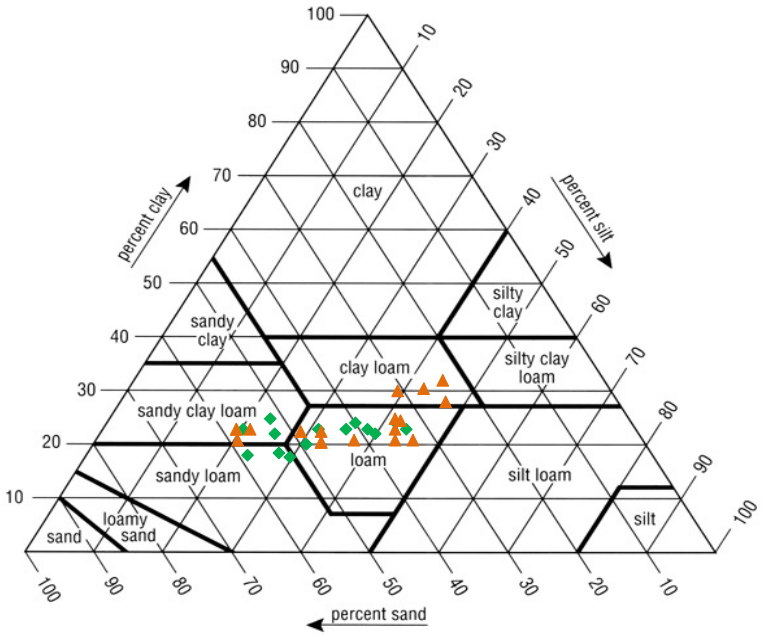


Figure 11. Results of soil texture analyses for September 2009 soil samples from Cleared (green diamond) and Invaded (gold triangle) areas dispersed in a soil textural triangle.

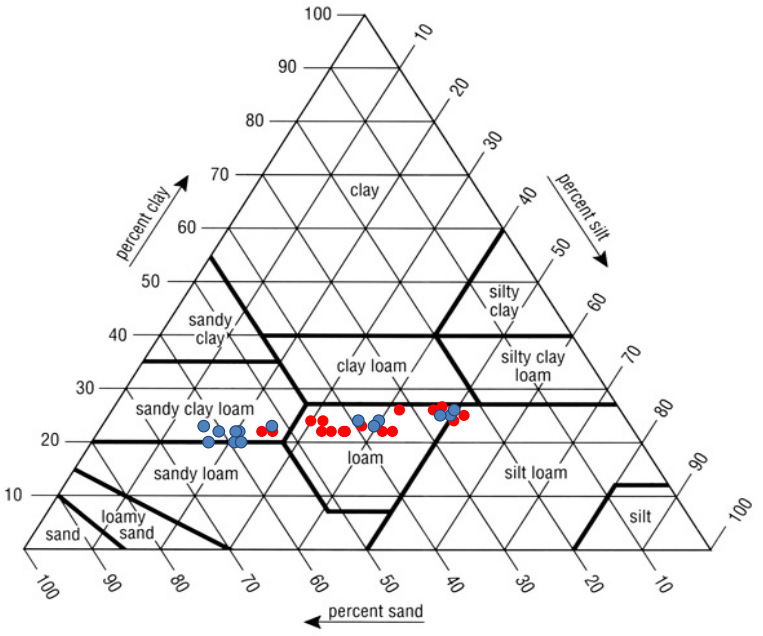


Figure 12. Results of soil texture analyses for September 2012 soil samples from Cleared (blue large circle) and Invaded (red small circle) areas dispersed in a soil textural triangle.

Ectomycorrhizal species richness and community structure

Soil cores (384 total) were collected from eight bur oaks during the early summer and early fall over three years (2010 to 2012). Over 3200 root tips were recovered from the soil core samples, sorted in to 82 morphological categories, and separated into 279 samples from which DNA was extracted to obtain nuclear ribosomal ITS sequences for identification. Sequences (210 total) were identified using a Basic Local Alignment Search Tool (BLAST) query of GenBank and UNITE databases of which 162 sequences were determined to be ECM.

The mean number of ECM species per tree showed significant treatment *x* time effects (Figure 13; F=6.7317, p=0.0293). Ectomycorrhizal species richness was similar between Cleared and Invaded areas in 2010 (first year after treatments), but was higher in Cleared than Invaded areas in the second and third year after treatments were initiated (2011, 2012; Figure 13). Over all years, however, there was no significant difference in species richness between Cleared (mean 6.4, range 1 - 13) and Invaded (mean 4.5 species, range 1 – 8) areas (F= 0.8798, p>0.05).

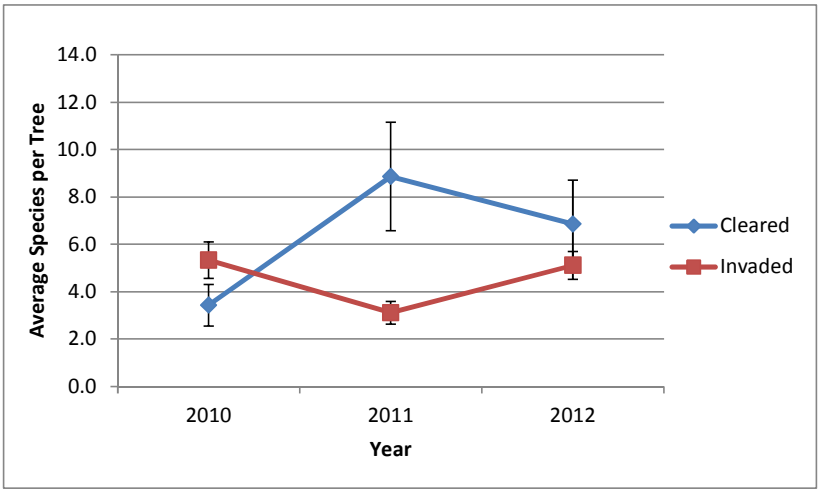


Figure 13. Mean number of ECM species per tree for Cleared and Invaded areas over the three years following buckthorn removal. Vertical bars indicate the standard error of the means.

The observed species richness (based on root tips alone) and estimates of richness ($Jack_1$, $Chao_2$) and diversity (Simpson's index) confirmed that the Cleared area showed significantly higher ECM species richness and diversity than the Invaded area (Table 3). The total ECM species richness over the three year study was greater in the Cleared area (44 species) than the Invaded area (31 species), (Table 3). This difference was largely generated by the number of singletons (unique species) present on root tips. However, both the $Jack_1$ and $Chao_2$ estimates of taxon richness indicated that the vast majority of ECM taxa were not captured during the study. The Simpson index of evenness, which denotes the distribution of individual root tips over ECM species, also differed between trees in Cleared and Invaded areas. Evenness was lower in Invaded than Cleared areas indicating that a limited suite of ECM species dominated the community in the Invaded area.

Table 3. Estimates of ECM species and diversity in Cleared and Invaded areas over three years. Values given as the mean standard deviation in parentheses.

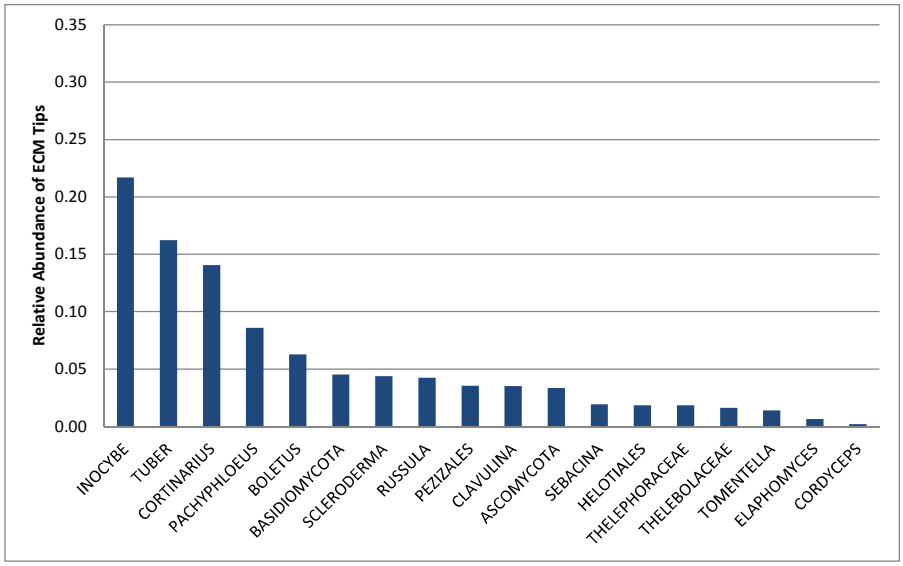
	Cleared	Invaded
Species observed	44 (6)	31 (5)
Species expected ($Chao_2$)	152 (66)	127 (70)
Species expected ($Jack_1$)	68 (7)	47 (5)
Diversity (Simpsons)	22.59	9.82
Evenness (Simpson's E)	0.514	0.317
Singletons	30	22
Doubletons	5	3

Table 4. Estimates of ECM species and diversity in Cleared and Invaded areas by tree over three years. Values given as the mean standard deviation in parentheses.

	2C	2I	3I	4I	5C	6C	7C	8I	9I
Species observed	11(1)	10(3)	23(2)	12(2)	21(4)	23(3)	32(3)	16(2)	14(2)
Species expected ($Chao_2$)	7(2)	18(9)	22(7)	9(3)	63(33)	40(21)	32(8)	14(5)	16(7)
Species expected ($Jack_1$)	9(3)	14(3)	21(1)	10(1)	29(3)	23(4)	30(4)	14(2)	14(3)
Diversity (Simpsons)	3.66	8.33	8.96	5.53	15.20	11.75	13.12	4.92	5.44
Evenness (Simpson's E)	0.61	0.93	0.64	0.79	0.84	0.84	0.66	0.55	0.60
Singletons	4	8	10	4	16	7	15	7	7
Doubletons	1	1	2	1	1	5	1	0	1

A total of 64 putative ECM species from 25 genera were identified on root tips from both Cleared and Invaded areas. These represented 26 species of Ascomycota and 38 species of Basidiomycota. The dominant taxon in the Cleared area was *Inocybe* (22% root tips) while *Scleroderma* was the most abundant taxon in the Invaded area (34% root tips), (Figure 14A). Other taxa present in high abundance on root tips in the Cleared area were species of *Tuber,* notably *Tuber scruposum*, and *Boletus*. Notably, *Cortinarius, Clavulina*, *Sebacina,* Thelebolaceae, and Thelephoraceae were only identified on root tips from the Cleared area. In contrast, root tips from the Invaded area were largely colonized by *Scleroderma*, *Pachyphloeus* and members of Pezizales (Figure 14B). Only 11 species were shared between Invaded and Cleared areas and included species of *Boletus, Inocybe, Pachyphloeus, Scleroderma*, *Tuber,* and a member each of the Helotiales and Pezizales.

A



B

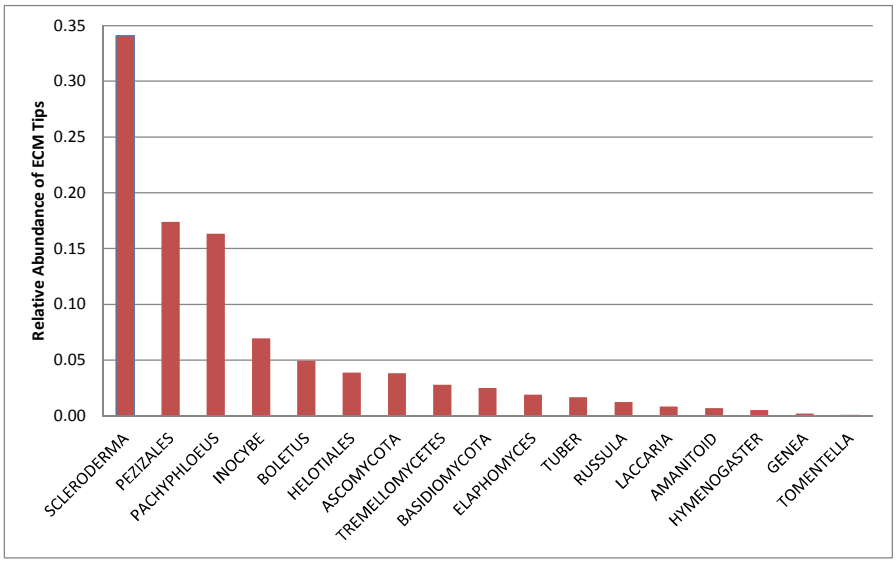


Figure 14. Relative abundance of ECM taxa over three years for A) Cleared and B) Invaded areas.

In the Cleared area, over the three year period of this study, there were species that increased in abundance and those that decreased. The three species with the largest increases were *Boletus*, *Russula* and *Clavulina* while those with the largest decreases were *Cortinarius*, *Inocybe*, and *Tuber* although *Inocybe* almost doubled in abundance in 2011 from 2010 before falling in 2012 to about half of its 2010 measure.

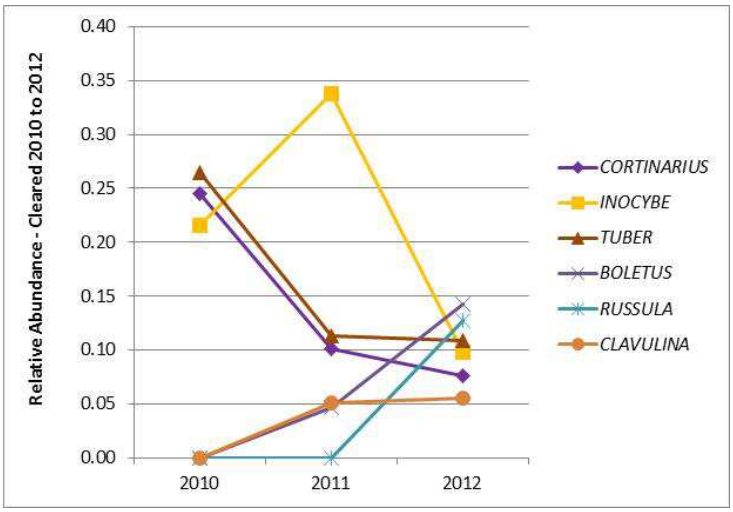


Figure 15. Relative abundance of ECM taxa in the Cleared area over three years with the largest increases and decreases in abundance.

In the Invaded area, over the three year period of this study, there were species that increased in abundance and those that decreased. Species that increased in abundance included *Inocybe*, *Boletus*, *Pachyphloeus*, and members of the *Pezizales*, and *Helotiales*, while *Scleroderma* had the largest decrease.

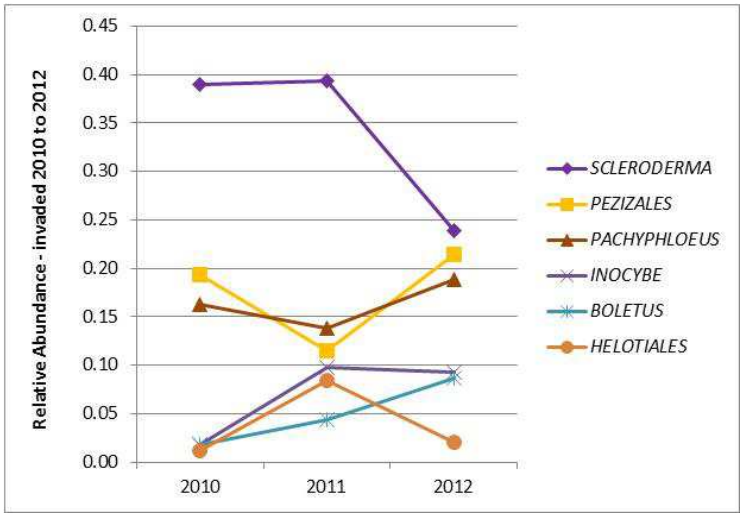


Figure 16. Relative abundance of ECM taxa in the Invaded area over three years with the largest increases and decreases in abundance.

A solution with two dimensions was achieved for non-metric multidimensional scaling (NMDS) of ECM abundance between treatments (final stress= 0.024; R^2 0.633).

These analyses illustrated that ECM communities from Cleared and Invaded areas were separated along the first dimension of the NMDS plot (Figure 17). The MRPP tests corroborated this finding, and indicated that the difference in ECM community structure between the Cleared and Invaded areas was not random ($A = -2.229$; $p = 0.0017$). The negative direction of the A value indicates a decrease in ECM community homogeneity with the transition from an Invaded to Cleared area. The MRPP analyses also indicated that the ECM community structure in the Invaded area was similar across years ($A= 0.371$, $p>0.05$), whereas there were significant differences in ECM community structure over time in the Cleared area ($A=-3.984$, $p=0.006$).

The potential contribution of soil properties and differences in ECM species' abundances to the separation of ECM communities in ordination space was also tested. These analyses showed a strong relationship between coordinate 1 and soil %C (Spearman r 0.943, $p<0.005$), and a marginally significant relationship with %N (Spearman r 0.771, $p=0.072$). The differences between Cleared and Invaded ECM communities were also related to shifts in the relative abundances of certain ECM taxa. Coordinate 1 was positively correlated with the relative abundance of *Inocybe* (Spearman r 0.886, $p=0.019$), *Tuber* (Spearman r 0.829, $p=0.042$) and *Cortinarius* (Spearman r 0.759 $p=0.080$). These were the dominant taxa (*Inocybe*) and those that increased significantly in abundance in the Cleared area (*Tuber*; *Cortinarius*; Figure 14A). Coordinate 1 was negatively correlated with the relative abundance of *Pachyphloeus* (Spearman r -0.990, $p<0.001$), Pezizales (Spearman r -0.829; $p=0.0416$), and *Scleroderma* (Spearman r -0.772, $p=0.070$). All three taxa showed a significant decline following the removal of buckthorn (Figure 17).

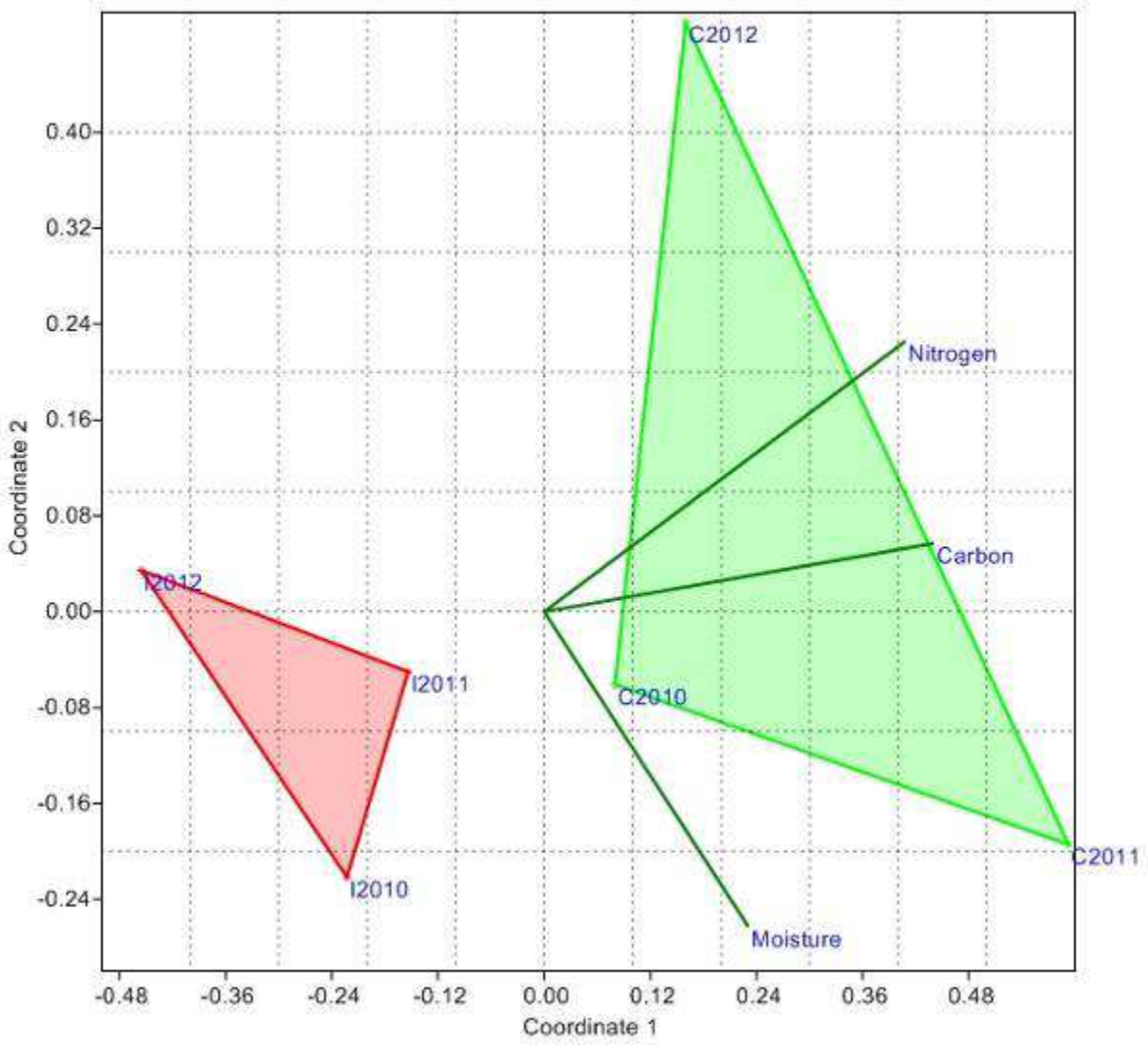


Figure 17. Nonmetric multidimensional scaling (NMDS) plots derived from pair-wise Manhattan distances between ECM communities with symbols coded by treatment (C= Cleared, I= Invaded) and year (2010, 2011, 2012). Minimum convex hulls show the vertices of each treatment. The first coordinate explained 61.7% of the ECM community composition variation, and the second coordinate, 14.7% of community composition variation.

DISCUSSION

Exotic species invasion are powerful disruptors of the mutualisms between plants and their mycorrhizal fungi, including ECM (see Wolfe *et al.* 2008). From the restoration perspective, a key question remains: can these effects be reversed by the removal of an invasive species? Here, I observed that ECM diversity increased, and community composition and structure clearly shifted with the removal of buckthorn. These shifts were positively correlated with soil C content but not soil N and P fertility or moisture content. To my knowledge, this is the first study to demonstrate that the negative effects of an invasive species on mycorrhizal mutualisms may be reversed.

The removal of buckthorn resulted in a significant increase in ECM diversity. That ECM species richness and diversity was lower in the Invaded versus Cleared areas was not surprising. A number of studies have established that invasive species can reduce ECM diversity and alter community composition (Wolfe *et al*. 2008, and references therein). More notably, the ECM community in the Invaded area was dominated by three taxa, *Scleroderma verrucosum*, *Pachyphloeus*, and members of the Pezizales that, together, comprised 68% of the community. Conversely, these taxa persisted in low abundance in the Cleared area. Most ECM communities comprise a few abundant species together with a large number of uncommon and rare species (e.g., Tedersoo *et al.* 2006). However, ECM communities dominated by a few taxa of fungi are characteristic of disturbed habitats. Indeed, *Scleroderma* is abundant in early successional environment (Nara 2006), while *Pachyphloeus* and most Pezizales appear to be well adapted to open or disturbed habitats (Colgan *et al.* 1999; Dickie and Reich 2005; Smith *et al.* 2006; Tedersoo *et al.* 2010), post-fire environments (Southworth 2011), and seasonally dry forests and woodlands (Gehring

et al. 2005; Smith *et al.* 2007). That *Scleroderma* and *Pachyphloeus* were clearly favored in the Invaded areas suggests that these dominant fungi are tolerant of or more competitive in the conditions created by buckthorn than other fungi.

The removal of buckthorn resulted in significant increases in ECM fungal diversity and a dramatic shift in ECM fungal community composition (Hypothesis 2). The observed increases in diversity are consistent with the patterns reported for ECM communities after disturbances such as fire (Visser 1995; Lazaruk *et al.* 2005), clear-cutting (Jones *et al*. 2003), reforestation (Mason *et al*. 1998), and along forest chronosequence (Kranabetter *et al.* 2005, Tweig *et al.* 2007). The ECM communities in the Cleared area were also comparably diverse and shared ECM taxa in common with other *Quercus* including *Q. douglasii* (Smith *et al.* 2007) and *Q. ellipsoidalis* (Avis *et al.* 2003). Similarly, many fungi on the roots were Ascomycota (~ 41%) as occurs in other *Quercus* savannas (Smith *et al.* 2007; Dickie *et al*. 2009), yet the cosmopolitan ascomycete, *Cenococcum geophilum*, was not detected. The absence of *Cenococcum* was very unexpected given that *Cenococcum* is tolerant of disturbance and stress. In fact, Horton and Bruns (2001) emphasize that there are virtually no studies in which this fungus was not detected. Nevertheless, this finding parallels an earlier report in which *Rhododendron* was found to suppress the abundance of *Cenococcum* (Walker *et al.* 1999). Buckthorn can produce a "buckthorn desert" owing to the presence of residual allelochemicals (Vincent 2006) for two (or more) years after clearing (Brock and Brock 2004). This raises the possibility that allelochemicals from buckthorn may have reduced the soil inoculum potential of *Cenococcum*.

One of the most striking results in the Cleared area was the appearance of new species in the Cleared treatment. Most notable were *Clavulina*, *Sebacina* and members of

the Thelephoraceae, which comprised on average 10% of the root tips. Conversely, taxa such as *Laccaria* and the Tremellomycetes were observed in root tips from trees in the Invaded area but never the Cleared. Because there were so many new species in the Cleared treatment, the overall effect was one of both diversification and a shift in assemblage structure. These results suggest that one of the main effects of buckthorn removal was to increase the number and diversity of viable ECM propagules available to colonize oak roots. There are four possible non-exclusive mechanisms that might explain these shifts. First, as mentioned earlier, buckthorn produces persistent allelopathic chemicals (Brock and Brock 2004; Vincent 2006), and ECM fungi demonstrate different levels of sensitivity to allelochemicals (Rose *et al.* 1983). For example, *Laccaria* was found to be relatively insensitive to allelochemicals and in this study, *Laccaria* was only found in the Invaded area. Thus, the dramatic increase in the diversity and number of colonized roots tips sampled in the Cleared area between 2010 and 2011 (102 vs. 495) might be related to the diminishing presence of allelochemicals.

Second, clearing may have released root competition between buckthorn and bur oaks and opened up patches for oak fine root growth and colonization, as is predicted for colonization- competition trade-offs (Tilman 1994). Buckthorn has a shallow root system with copious quantities of fine roots that occupy a similar spatial distribution as the fine roots produced by bur oaks. The four-fold increase in the number of ECM root tips after the removal of buckthorn (2010- 2011) supports this possibility. In a similar fashion, Southworth *et al.* (2011) found greater root tip abundance and ECM species richness after mechanical mastication. In addition, a study of root competition between beech and oak, Leuschner *et al.* (2001) showed that oak fine-root biomass was eight-times higher in

monolithic than mixed oak/beech woodlands.

Third, the removal of buckthorn may have acted as a signal for spore germination from soil spore banks. The increase in the abundance of *Inocybe* in the Cleared areas supports this possibility. Fox (1986) noted that *Inocybe* readily and consistently formed ectomycorrhizas from spores on birch seedlings. Similarly, Ishida *et al*. (2008) found a high germination of *Inocybe* spores with the host, *Salix reinii*. On the other hand, *Cortinarius* colonizes root tips from local patches of mycelia or mycorrhizal networks (Fox 1986). In this study, *Cortinarius* was found consistently, and exclusively, in only two adjacent sectors in the Cleared area. This result suggests that other processes, such as root colonization from mycorrhizal networks, may have contributed to the increases in ECM diversity. Future studies should therefore address the extent and composition of the ECM spore bank.

Fourth, the removal of buckthorn may have altered the competitive environment between ECM species as has been seen in paired ECM competitions elsewhere (Lilleskov and Bruns 2003). Removing the buckthorn appeared to have increased the abundance of bur oak fine roots and, as a result, provided new opportunities for ECM colonization (see above). This raises the possibility of priority effects (Kennedy *et al.* 2009), succession (Fleming *et al.* 1984), or direct root tip takeover and replacement by different ECM fungi (Wu *et al.* 1999). This study was not designed to test these possibilities. However, it is reasonable to hypothesize that the presence of one ECM species may have a significantly altered the ability of other ECM fungi to colonize based on to the proportion of the root system occupied by the first colonizer (Kennedy *et al*. 2009).

On the other hand, shifts in ECM community composition were not strongly

correlated with soil N or P fertility or moisture. This is especially interesting given what has been hypothesized about the effects of buckthorn on soil N fertility and moisture previously (Heneghan *et al*. 2004). Based on previous research, it was anticipated that soil %N would decline in the Cleared area after removal of the buckthorn with its N-rich, rapidly decomposing leaves (Heneghan *et al.* 2002, 2004), and, in turn, a decreasing N supply may have promoted ECM diversity (Avis *et al*. 2003). However, plant-available N and P did not differ between treatments, and soil %C, %N and moisture were consistently higher in the Cleared than Invaded area throughout the study (Hypothesis 1). Similarly, Swaty *et al*. (1998) found that differences in ECM community composition were not associated with variations in soil factors. While there were significant differences between Cleared and Invaded areas for all three soil factors over time, there was no significant difference over time *x* treatment. This does not preclude the possibility that fine-scale variations in soil moisture, temperature, or other parameters may have directly or differentially affected the growth and survival of some ECM fungi. Physiognomic differences between the treatment areas could also have contributed to the soil moisture results, e.g., the Invaded area was 5- 30 meters further from wetlands than the Cleared area. Nevertheless, soil factors other than those tested appeared to have influenced the renaissance of ECM communities after buckthorn removal.

One critical soil factor may be the change in the level of organic N after the removal of buckthorn. Studies of soil N availability and exotic invasions have almost exclusively focused on mineral N. Yet, it is increasingly recognized that plants can take up organic N in the form of free amino acids at biologically important rates. Soil organic N levels can be modified by disturbances such as fire as well as stand age (LeDuc and

Rothstein 2010), but little is known about the changes in plant-available organic N forms during exotic species' invasions or recovery. This is an important deficit to address because organic N is the major N pool in forests, and oaks are dependent on ECM for the acquisition of organic N.

The significant inter-annual variations in the abundance of ECM taxa indicated that ECM (re-) colonization after buckthorn removal is a highly dynamic process. Only seven species were present in all three years (*Inocybe* GQ166872, *Scleroderma verrucosum,* *Boletus* GQ166883, *Pachyphloeus* EU543203, *Tuber scruposum,* Helotiales FN669205, and Pezizales GU256216), and there was high turnover from year to year. This is in general agreement with the patterns noted in other ECM studies (Tedersoo *et al*. 2006) and *Q. agrifolia* (Querejeta *et al*. 2009), but not *Q. douglasii* (Smith *et al*. 2007).

Some of the observed turnover may have arisen due to increases in fine root production by the bur oaks and subsequent colonization of roots by a variety of ECM fungi. An additional component of this variation may be due to the year-to-year variations in ambient temperature and precipitation during the study (Vogt *et al.* 1980). The year after the buckthorn removal (2010) showed the highest rainfall total from April to September of all three years (712 mm) but the lowest numbers of ECM root tips and species richness. In the subsequent year (2011), rainfall from April to September totaled 316 mm while ECM root tip abundance and species richness increased significantly. These data are more consistent with the hypothesis that increasing stress is associated with increased investment into ECM mutualists (Swaty *et al.* 1998) than precipitation effects. An alternative explanation is that the large increase in ECM colonization in 2011 was related to the leaching of any buckthorn allelochemicals in the Cleared area during 2010.

Implications

Changes in ECM diversity are likely important to the oak ecosystem processes. Because each ECM species differs in its physiological abilities and the benefits they provide to the host plant, an increase in ECM diversity could indicate a greater number of fungal symbionts are available to provide more varied functions (N, P, H_2O acquisition). Without further experimentation, there is no way to know if the ECM community shifts that I observed are favorable or unfavorable for the bur oaks. However, other studies show that increases in ECM fungal diversity, rather than overall root colonization, best explain variations in P uptake (Baxter and Dighton 2001; Jonsson *et al.* 2001). Another perspective on ECM diversity in this study could be related to oak decline. The subject oaks are all showing various stages of decline (fallen limbs and various areas of rot). Kovacs *et al*. (2000) found a difference in species composition between healthy and declining oaks suggesting that differing ECM species that provide a protective role against abiotic stress and against pathogens could colonize oaks in decline.

It is tempting to consider the change in the ECM community within the Cleared area as a successional shift from 'early' to 'late' stage fungi, even though the oak savanna is well established. For example, *Inocybe*, *Laccaria*, and members of the Thelephoraceae are considered early stage ECM that may colonize from spores (Fleming *et al.* 1984); both *Inocybe* and *Thelephora* were abundant in the Cleared area. In contrast, taxa such as *Russula*, *Cortinarius* and *Boletus* are late successional ECM that may colonize root tips from common mycorrhizal networks. While the increasing abundance of these taxa after clearing supports this division, is it more likely that the ECM species detected and subsequent community structure reflect the age of the root systems more than the age of the

individual oak tree. Categorizations could also be made among ECM fungi using the concepts of contact *versus* long-distance mycorrhizal strategies (Agerer 2001), or mineral *versus* organic N users (Lilleskov *et al*. 2002). Although informative, such classifications overlook the considerable ecological and physiological differentiation that exists within, as well as among, ECM species, and the variable benefits they impart to their hosts. Future research should be directed to better understanding the functional roles of the different ECM and interactions with oaks after the removal of buckthorn.

Conclusion

The invasion of plant communities by non-native, exotic species is recognized as a major threat to many native ecosystems (Denslow and Hughes 2004). Yet, there have been few studies that have documented the mechanisms of invasion (Levine *et al.* 2003). One of the most endangered communities in southern Wisconsin is the oak savanna (Leach and Givnish 1999) and one of the exotic invaders, buckthorn, can form an exotic dominated ecosystem that is structurally distinct from a native ecosystem (Mascaro and Schnitzer 2007). One structural distinction of a buckthorn-dominated ecosystem compared to an oak savanna is that buckthorn form AMF associations while mature oak form ECM associations. A change in the mycorrhizal community from ECM to AMF may be a contributing factor to a successful and sustained buckthorn dominated ecosystem. This is of particular significance because, unlike easily observable changes in leaf litter or canopy coverage, belowground changes in the mycorrhizal community are less readily observable but could occur more rapidly than any aboveground changes.

This study did not find any obvious effect of the measured environmental variables (C, N, and moisture), which suggests that any possibly significant soil factors remain

unmeasured. What the study did find was that there were marked differences in the ECM communities between the Cleared and the Invaded areas. The Cleared area was more diverse with three dominate genera, a large number of rare species, and strong year to year variation in ECM community composition while the Invaded area was consistently dominated by one species (*Scleroderma verrucosum*) with few rare species and less year to year species variability.

An unexpected variable during this study was the invasion of garlic mustard into portions of the Cleared area. Both Wolfe *et al.* (2008) and Castellano (2008) found that garlic mustard inhibits the growth of ECM. Castellano (2008) also found significantly lower ECM species richness and diversity in garlic mustard-invaded sites. While garlic mustard's presence in the Invaded area was zero to minimal, its presence in certain sectors of the Cleared area could have contributed to the minimal samples in those sectors, especially sector 5a. A summary is presented in Table 5.

Table 5. Annual root tips sampled in sectors invaded by garlic mustard.

Sector	2010 Root Tips	2011 Root Tips	2012 Root Tips
2c	9	34	0
2d	0	26	20
5a	0	0	0
5b	0	8	40

Follow-up study considerations would include:

- Installation of continual soil moisture and temperature monitoring equipment by tree.
- Measurement of soil organic N dynamics and factors, such as pH and Ca, that have been implicated in other studies of invasive species;
- Measurement of leaf litter;

- Burning in the previously cleared area;
- Removal of garlic mustard (to extent possible) in the Cleared area and removal in Invaded area if/as necessary.

REFERENCES

Abrams, M.D. (1992) Fire and the development of oak forests. *BioScience* 42:346-353.

Agerer, R. (2006) Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress* 5:67-107.

Agerer, R. (2001) Exploration types of ectomycorrhizae – A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107-114.

Archibold, O.W., Brooks, D., Delanoy, L. (1997) An investigation of the invasive shrub European Buckthorn, *Rhamnus cathartica* L., near Saskatoon, Saskatchewan. *Canadian Field-Naturalist* 111:617-621.

Asbjornsen, H., Mora, G., Helmers, M.J. (2007) Variation in water uptake dynamics among contrasting agricultural and native plant communities in the Midwestern U.S. *Agriculture Ecosystems & Environment* 121:343-356.

Avis, P.G., McLaughlin, D.J., Dentinger, B.C., Reich, P.B. (2003) Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. *New Phytologist* 160:239-253.

Baykov, A.A., Evtushenko, O.A., Avaeva, S.M. (1988) A malachite green procedure for orthophosphate determination and its use in alkaline phosphatase-based enzyme immunoassay. *Analytical Biochemistry* 171:266-270.

Baxter, J.W., Dighton, J. (2001) Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host-symbiont culture conditions. *New Phytologist* 152: 139-149.

Beckstead, J. and Parker, I. M. (2003) Invasiveness of *Ammophila arenaria*: release from soil-borne pathogens? *Ecology* 84:2824-2831.

Bennett, A.E., Thomson, M., Strauss, S.Y. (2011) Multiple mechanisms enable invasive species to suppress native species. *American Journal of Botany* 98:1086-1094.

Blumenthal, D. (2005) Interrelated causes of plant invasion. *Science* 310:243-244.

Bouyoucos, G.J. (1962) Hydrometer method improved for making particle size analyses of soils. Agronomy Journal 54:464-465.

Brock, T.D. and Brock, K.M. (2004) Oak savanna restoration: A case study. *Proceedings of the North American Prairie Conference 19*. pp. 178-183.

Burke, M.J.W. and Grime, J.P. (1996) An experimental study of plant community invisibility. *Ecology* 77:776 – 790.

Cade, B.S. and Richards, J.D. (2008) Blossom Statistical Software. Version W2008.04.02. Fort Collins Science Center (FORT), U.S. Geological Sciences.

Callaway, R.M., Cipollini, D., Barto, K., Thelen, G.C., Hallett, S.G., Prati, D., Stinson, K., Klironomos, J. (2008) Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology* 89:1043-1055.

Callaway, R.M. and Ridenour, W.M. (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2:436-443.

Castellano, S.M. (2008) Effect of *Alliaria petiolata* invasion on ectomycorrhizal colonization of *Quercus rubra*. Master's Thesis. Miami University Department of Biology. 65 pp.

References (continued)

Clayton, L., Attig, J.W., Mickelson, D.M., Johnson, M.D., Syverson, K.M. (2006) Glaciation of Wisconsin. *Educational Series 36 – 3rd Edition*. Wisconsin Geological and Natural History Survey.

Colgan III, W., Carey, A.B., Trappe, J.M., Molina, R, Thysell, D. (1999) Diversity and productivity of hypogenous fungal sporocarps in a variably thinned Douglas-fir forest. Canadian Journal of Forest Restoration 29:1259-1268.

Colwell, R.K. (2013) Statistical Estimation of Species Richness and Shared Species from Samples. *EstimateS_910_Windows*.

Converse, C.K. (1984) Element Stewardship Abstract for *Rhamnus cathartica, Rhamnus frangula* (syn. *Frangula alnus*). The Nature Conservacy. Arlington, VA. 17 pp.

Cox, F., Barsoum, N., Lilleskov, E.A., Bidartondo, M.I. (2010) Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. *Ecology Letters* 13:1103-1113

Curtis, J.T., (1959) Savanna. In: The Vegetation of Wisconsin An Ordination of Plant Communities. The University of Wisconsin Press, Madison, WI pp. 325-327.

Davis, M.A., Grime, J.P., Thompson, K. (2000) Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88:528-534.

Denslow, J.S. and Hughes, R.F. (2004) Exotic plants as ecosystem dominants. *Weed Technology* 18:1283-1287.

Dickie, I.A., Detinger, B.T.M, Avis, P.G. McLaughlin, D.J, Reich, P.B. (2009) Ectomycorrhizal fungal communities of oak savanna are distinct from forest communities. *Mycologia* 101:473-483.

Dickie, I.A. and Reich, P.B. (2005) Ectomycorrhizal fungal communities at forest edges. *Journal of Ecology* 93:244-255.

Dickie, I.A., Guza, R.C., Krazewski, S.E., Reich, P.B. (2004) Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknelli*) and oak (*Quercus*) seedlings. *New Phytologist* 164:375-382.

Doane, T.A., Horwath, W.R. (2003) Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters* 36:2713-2722.

Eppinga, M.B., Rietkerk, M., Dekker, S.C., De Ruiter, P.C. (2006) Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. *Oikos* 114:168-176.

Faber-Langendoen, D. and Tester, J.R. (1993) Oak mortality in sand savannas following drought in east-central Minnesota. *Bulletin of the Torrey Botanical Club* 120:248-256.

Farrar, J.L. (1995) Group 10 - Leaves alternate, simple; edges lobed. Trees of the Northern United Sates and Canada. Fitzhenry & Whiteside Limited. Markham, Ontario. pp. 258-259.

Fleming, L.V., Deacon, J.W., Last, F.T. (1984) Influence of propagating soil on the mycorrhizal succession of birch seedlings transplanted to a field site. *Transactions of the British Mycological Society* 84:707-711.

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* 87:371-380.

References (continued)

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

Gerhing, C., Denich, M., Vlek, P.L.G. (2005) Resilience of secondary forest regrowth after slash-and-burn agriculture in central Amazonia. *Journal of Tropical Ecology* 21:519-529.

Godwin, H. (1943) *Rhamnus cathartica* L. *Journal of Ecology* 31:69-76.

Goodman, D.M., Durall, D.M., Trofymow, J.A., Berch, S.M., editors (2008) *Concise descriptions of North American ectomycorrhizae.* British Columbia Ectomycorrhizal Research Network (BCERN). http://forestry-dev.org/biodiversity/bcern/cde/index_e.html

Griekspoor, A. and Groothuis, T. (2010) 4 Peaks Software; version 1.7.1. http://nucleobytes.com/index.php/4peaks/

Hammer, O., Harper, D.A.T., Ryan, P.D. (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electonica* 4: 9 pp.

Harrington, R.A., Brown, B.J., Reich, P.B. (1989) Ecophysiology of exotic and native shrubs in Southern Wisconsin: I. Relationship of leaf characteristics, resource availability, and phenology to seasonal patterns of carbon gain. *Oecologia* 80:356-367.

Heneghan, L., Ruaschenberg, C., Fatemi, F., Workman, M. (2004) European buckthorn (*Rhamnus cathartica*) and its effects on some ecosystem properties in an urban woodland. *Ecological Restoration* 22:275-280.

Heneghan, L., Clay, C., Brundage, C. (2002) Rapid decomposition of buckthorn litter may change soil nutrient levels. *Ecological Restoration* 20:108-111.

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

Hughes, K.W., Petersen, R.H, Lickey, E.B. (2009) Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. *New Phytologist* 182:795-798.

Iannone III, B.V. (2013) Utilizing Multiple approaches to Understand the Ecology of *Rhamnus cathartica* L. Invasion and Management. Thesis. Doctor of Philosophy in Biological Sciences. Graduate college of the University of Illinois at Chicago. 178 pp.

ISAC - Invasive Species Advisory Committee (Definitions Subcommittee) (2006) Invasive species definition clarification and guidance white paper. The National Invasive Species Council. 11 pp.

Ishida, T.A., Nara, K., Tanaka, M., Kinoshita, A., Hogetsu, T. (2008) Germination and infectivity of ectomycorrhizal fungal spores in relation to their ecological traits during primary succession. *New Phytologist* 180: 491-500.

Inderjit and Van der Putten, W.H. (2010) Impacts of soil microbial communities on exotic plant invasions. *Trends in Ecology and Evolution* 25:512-519.

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

References (continued)

Jonsson, L.M., Nilsson, M., Wardle, D.A., Zackrisson, O. (2001) Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *OIKOS* 93: 353-364.

Keane, R.M. and Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *TRENDS in Ecology & Evolution* 17:164-170.

Kennedy, P.G., Peay, K.G., Bruns, T.D. (2009) Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? *Ecology* 90: 2098-2107.

Ko, L.J. and Reich, P.B (1993) Oak tree effects on soil and herbaceous vegetation in savannas and pastures in Wisconsin. *American Midland Naturalist* 130:31-42.

Kourtev, P.S., Ehrenfeld, J.G., Haggblom, M. (2002) Exotic plant species alter the microbial community structure and function in the soil. *Ecology* 83:3152-3166.

Kovacs, G., Pausch, M., Urban, A. (2000) Diversity of ectomycorrhizal morphotypes and oak decline. *Phyton (Austria) Special Issue: "Root-soil interactions"* 40: 109-116.

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

Lazaruk, L.W., Kernaghan, G., Macdonald, S.E., Khasa, D. (2005) Effects of partial cutting on the ectomycorrhizae of *Picea glauca* forests in northwestern Alberta. *Canadian Journal of Forest Restoration* 35:1442-1454.

Leach, M.K., Givnish, T.J. (1999) Gradients in the composition, structure, and diversity of remnant oak savannas in Southern Wisconsin. *Ecological Monographs* 69:353-374.

LeDuc, S.D., Rothstein, D.E. (2010) Plant-available organic and mineral nitrogen shift in dominance with forest stand age. *Ecology* 91: 708-720.

Leuschner, C., Hertel, D., Coners, H., Büttner, V. (2001) Root competition between beech and oak: a hypothesis. *Oecologia* 126: 276-284.

Levine, J.M., Vila, M, D'Antonio, C.M., Dukes, J.S., Grigulis, K, Lavorel, S. (2003) Mechanisms underlying the impact of exotic plant invasions. *Proceedings of The Royal Society B: Biological Sciences* 270:775-781.

Lilliskov, E.A., Hobbie, E.A., Fahey, T.J. (2002) Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytologist* 154:219-231.

Lilliskov, E.A., Bruns, T.D. (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.

Lodge, D.J. (1989) The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in Populus and Salix. *Plant and Soil* 117:243-253.

Maloney, J.N. (1997) Oak savanna restoration techniques. *Restoration and Reclamation Review* 2:1-7.

Mascaro, J., Schnitzer, S.A. (2007) *Rhamnus cathartica* L. (Common Buckthorn) as an ecosystem dominant in Southern Wisconsin forests. *Northeastern Naturalist* 14:387-402.

References (continued)

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

McCune, B. and J. B. Grace. (2002) MRPP (Multi-response Permutation Procedures) and Related Techniques. In: Analysis of Ecological Communities. MJM Software Design, Gleneden Beach, Oregon, USA. Page 190.

Miller, S.N. (2010) Effects of *Rhamnus cathartica* (common buckthorn) stand age on decomposition. Undergraduate Thesis. Carthage College – Environmental Science: Conservation and Ecology. 26 pp.

Moreau, B., Gardiner, E.S., Stanturf, J.A., and Fisher, R.K., (2004) Estimating leaf nitrogen of eastern cottonwood trees with a chlorophyll meter. *Proceedings of the 12th biennial southern silvicultural research conference Gen. Tech. Rep. SRS–71*. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. pp. 487-491.

Mundahl, N., Eaton, E., Brutt, S., Peterson, K. (2010) Experimental management of common buckthorn on a dry bluff savanna restoration site. *Proceedings of the North American Prairie Conference 21*. pp. 126-131.

Nara, K. (2006) Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytologist 169:169-178.

NOAA - U. S. Department of Commerce, National Oceanic and Atmospheric Administration (NOAA). National Weather service. Station Index numbers 47-5473-08 and 47-2869-08.

Nijjer, S., Rogers, W.E., Siemann, E. (2007) Negative plant-soil feedbacks may limit persistence on an invasive tree due to rapid accumulation of soil pathogens. *Proceedings of the Royal Society B: Biological Sciences* 274:2621-2627.

Niu, H., Liu, W., Wan, F., Liu, B. (2007) An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant and Soil* 294:73-85.

Nuzzo, V.A. (1994) Extent and status of Midwest oak savanna; presettlement and 1985. *1994 Proceedings North American Conference on Savannas and Barrens*. 20 pp.

Parrent, J.L., Vilgalys, R. (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO_2 and nitrogen fertilization. *New Phytologist* 176:164-174.

Peay, K.G.**,** Kennedy, P.G., Davies, S.J., Tan S., Bruns, T.D. (2010) Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone. *New Phytologist* 185:529-542.

Pejchar, L. and Mooney, H.A. (2009) Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution* 24:497-504.

Pringle, A., Bever, J.D., Gardes, M., Parrent, J.L., Rilling, M.C., Klironomos, J.N. (2009) Mycorrhizal symbioses and plant invasions. *Annual Review of Ecology, Evolution, and Systematics* 40:699-715.

Querejeta, J.I., Egerton-Warburton, L.M., Allen, M.F. (2009) Topographic position modulates the mycorrhizal response of oak trees to interannual rainfall variability. *Ecology* 90:649-662.

References (continued)

Randall, P.M. (1996) Plant Invaders – How non-native species invade & degrade natural areas. In: Invasive Plants: Weeds of the Global Garden. Eds. P.M. Randall, and J. Marinelli. Brooklyn Botanic Garden Publications. Brooklyn, NY. pp. 9, 64.

Reinhart, K.O., Packer, A., Van der Putten, W.H., Clay, K. (2003) Plant-soil biota interactions` and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters* 6:1046-1050.

Robertson, G.P, Coleman, D.C., Bledsoe, C.S., Sollins, P., editors (1999) Soil Physical Properties. Standard soil methods for long-term ecological research. Oxford University Press. New York, NY. pp. 55-85.

Rose, S.L., Perry, D.A., Pilz, D., and Schoeneberger, M.M. (1983) Allelopathic effects of litter on the growth and colonization of mycorrhizal fungi. *Journal of Chemical Ecology* 9:1153-1162.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J. *et al*. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* 32:305-332.

Slankis, V. (1974) Soil factors influencing formation of mycorrhizae. *Annual Review of Phytopathology* 12:437-457.

Smith, M.E., Douhan, G.W., Rizzo, D.M. (2007) Ectomycorrhizal community structure in a xeric *Quercus* woodland based on rDNA sequence analysis of sporocarps and pooled roots. *New Phytologist* 174:847-863.

Smith, M.E., Trappe, J.M., Rizzo, D.M. (2006) *Genea*, *Genabea* and *Gilkeya* gen. nov.: ascomata and ectomycorrhiza formation in a *Quercus* woodland. *Mycologia* 98:699-716.

Smith, S.E. and Read, D.J. (2008) Structure and development of ectomycorrhizal roots. In: Mycorrhizal Symbiosis, 3rd ed. Academic Press. London, UK. pp. 192-197.

Southworth, D., Donohue, J., Frank, J. and Gibson, J. (2011) Mechanical mastication and prescribed fire in conifer-hardwood chaparral: differing responses of ectomycorrhizae and truffles. *International Journal of Wildland Fire* 20:888-896.

Stinson, K.A., Campbel, S.A., Powel, J.R., Wolfe, B.E., Callaway, R.M., Thelen, G.C., Hallett, S.G., Prati, D., Klironomos, J.N. (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biology* 4:727-731.

Swaty, R.L., Gehring, C.A., Van Ert, M., Theimer, T.C., Keim, P., Whitman, T.G. (1998) Temporal variation in temperature and rainfall differentially affects ectomycorrhizal colonization at two contrasting sites. *New Phytologist* 139: 733 – 739.

Tedersoo, L., May, T.W., Smith, M.E. (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza* 20:217-263.

Tedersoo, L. Suvi, T, Larsson, E., Koljalg, U. (2006) Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. *Mycological Research* 110:734-748.

Tilman, D. (1994) Competition and biodiversity in spatially structured habitats. *Ecology* 75: 2-16.

References (continued)

Toljander, J.F., Eberhardt, U., Toljander, Y.K., Paul, L.R., Taylor, A.F.S. (2006) Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytologist* 170:873-884.

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

USDA Natural Resources Conservation Service. (2011) http://websoilsurvey.nrcs.usda.gov/app/

Vincent, M. (2006) Allelopathic effect of the fruit of European Buckthorn, *Rhamnus cathartica* L. Honors Thesis. The University of Winnipeg – Department of Biology. 49 pp.

Visser, S. (1995) Ectomycorrhizal fungal succession in Jack Pine stand following wildfire. *New Phytologist* 129:389-401.

Vogt, K.A., Edmonds, R.L., Grier, C.C., Piper, S R. (1980) Seasonal changes in mycorrhizal and fibrous-textured root biomass in 23- and 180-year-old Pacific silver fir stands in western Washington. *Canadian Journal of Forest Research* 10: 523-529.

Walker, J.K., Miller Jr, O.K., Let, T., Semones, S., Nilsen, E., Clinton, B.D. (1999) Suppression of ectomycorrhizae on canopy tree seedlings in *Rhododendron maximum* L. (Ericaceae) thickets in the southern Appalachians. *Mycorrhiza* 9: 49-56.

Weatherburn, M.W. (1967) Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry* 39:971-974.

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. Eds., M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, Inc., New York. pp. 315-322.

Wolfe, B.E., Rodgers, V.L., Stinson, K.A., Pringle, A. (2008) The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology* 96:777-783.

Wu, B., Nara, K. Hogetsu, T. (1999) Competition between ectomycorrhizal fungi colonizing *Pinus densiflora*. *Mycorrhiza* 9: 151-159.

Wunsch, Assaf (2009) Soil texture utility - http://inside.mines.edu/~awunsch/awunsch.html Colorado School of Mines. Golden, CO.

Yurkonis, K.A., Meiners, S.J., Wachholder, B.E. (2005) Invasion impacts diversity through altered community dynamics. *Journal of Ecology* 93:1053 – 1061.

APPENDIX A

				Soil Carbon					
	Sample Date:	9/20/09	5/24/10	8/5/10	10/1/10	6/19/11	9/15/11	6/22/12	9/14/12
Sample	Treatment								
2a	Uncleared	5.02%	8.18%	5.67%	5.84%	6.99%	5.89%	5.81%	5.26%
2b	Uncleared	5.01%	5.67%	5.22%	5.99%	6.36%	5.37%	5.83%	5.43%
2c	Cleared	5.49%	4.64%	4.26%	5.69%	6.96%	6.06%	5.96%	6.09%
2d	Cleared	4.79%	5.94%	6.86%	6.36%	7.69%	6.23%	6.12%	5.98%
3a	Uncleared	6.16%	6.04%	5.99%	6.06%	7.00%	5.84%	6.98%	5.97%
3b	Uncleared	4.38%	5.75%	5.99%	5.95%	6.27%	5.32%	6.56%	5.34%
3c	Uncleared	4.83%	6.26%	4.51%	5.24%	7.51%	6.03%	5.19%	6.05%
3d	Uncleared	4.83%	7.48%	4.85%	5.95%	7.85%	5.78%	9.73%	7.89%
4a	Uncleared	5.77%	4.53%	4.08%	4.33%	5.00%	4.55%	4.47%	4.71%
4b	Uncleared	3.86%	4.43%	4.32%	4.94%	5.77%	4.34%	5.07%	4.85%
4c	Uncleared	5.08%	5.79%	4.49%	5.96%	6.42%	5.52%	6.59%	5.44%
4d	Uncleared	4.47%	6.19%	5.26%	5.49%	5.54%	5.26%	5.95%	5.61%
5a	Cleared	6.11%	4.91%	6.21%	6.63%	6.21%	5.62%	6.80%	6.46%
5b	Cleared	6.88%	6.03%	9.15%	5.80%	7.13%	6.85%	6.06%	5.20%
5c	Cleared	9.92%	9.49%	6.56%	6.74%	9.12%	7.53%	6.61%	7.78%
5d	Cleared	8.29%	7.44%	9.18%	9.88%	8.44%	6.71%	9.26%	7.07%
6a	Cleared	7.11%	8.06%	6.38%	7.58%	8.40%	6.93%	7.73%	7.27%
6b	Cleared	7.95%	6.42%	5.29%	8.29%	9.41%	7.39%	7.78%	7.35%
6c	Cleared	8.71%	8.18%	5.83%	6.67%	10.83%	9.37%	7.41%	8.75%
6d	Cleared	7.80%	9.35%	8.36%	8.32%	12.31%	9.52%	9.61%	9.61%
7a	Cleared	6.43%	6.86%	9.42%	6.53%	9.40%	8.62%	7.33%	8.94%
7b	Cleared	9.02%	8.30%	7.72%	7.51%	9.58%	9.39%	7.60%	7.97%
7c	Cleared	8.40%	8.55%	7.45%	9.04%	9.68%	9.67%	8.79%	8.21%
7d	Cleared	6.60%	6.44%	6.45%	9.39%	8.55%	7.24%	9.28%	6.84%
8a	Uncleared	8.80%	11.20%	8.11%	9.82%	11.60%	11.82%	8.37%	10.74%
8b	Uncleared	8.45%	8.24%	10.14%	9.07%	7.71%	7.12%	9.07%	7.53%
8c	Uncleared	8.21%	7.30%	7.59%	6.88%	8.03%	7.49%	8.18%	7.91%
8d	Uncleared	7.39%	8.99%	8.64%	8.87%	12.95%	8.37%	8.98%	9.36%
9a	Uncleared	5.66%	6.52%	7.84%	8.11%	6.37%	6.72%	6.51%	6.63%
9b	Uncleared	4.99%	5.23%	5.53%	5.59%	5.50%	5.52%	6.29%	5.34%
9c	Uncleared	4.57%	4.85%	5.43%	6.00%	5.91%	5.81%	5.60%	6.01%
9d	Uncleared	5.16%	5.15%	6.73%	6.10%	6.78%	7.39%	6.24%	6.07%

APPENDIX B

				Soil Nitrogen					
	Sample Date:	9/20/09	5/24/10	8/5/10	10/1/10	6/19/11	9/15/11	6/22/12	9/14/12
Sample	Treatment								
2a	Uncleared	0.487%	0.576%	0.397%	0.419%	0.439%	0.412%	0.469%	0.406%
2b	Uncleared	0.427%	0.396%	0.389%	0.420%	0.395%	0.355%	0.426%	0.383%
2c	Cleared	0.469%	0.333%	0.313%	0.406%	0.460%	0.418%	0.443%	0.463%
2d	Cleared	0.379%	0.400%	0.512%	0.480%	0.501%	0.423%	0.479%	0.453%
3a	Uncleared	0.446%	0.408%	0.469%	0.445%	0.435%	0.387%	0.464%	0.443%
3b	Uncleared	0.331%	0.393%	0.455%	0.443%	0.411%	0.389%	0.498%	0.396%
3c	Uncleared	0.365%	0.457%	0.383%	0.388%	0.473%	0.415%	0.383%	0.431%
3d	Uncleared	0.344%	0.545%	0.382%	0.422%	0.432%	0.348%	0.598%	0.504%
4a	Uncleared	0.344%	0.296%	0.309%	0.306%	0.288%	0.278%	0.323%	0.329%
4b	Uncleared	0.230%	0.291%	0.339%	0.347%	0.344%	0.280%	0.359%	0.335%
4c	Uncleared	0.321%	0.390%	0.345%	0.430%	0.409%	0.370%	0.465%	0.402%
4d	Uncleared	0.292%	0.505%	0.411%	0.398%	0.344%	0.358%	0.433%	0.406%
5a	Cleared	0.456%	0.351%	0.506%	0.528%	0.407%	0.385%	0.545%	0.495%
5b	Cleared	0.502%	0.467%	0.753%	0.437%	0.450%	0.497%	0.445%	0.394%
5c	Cleared	0.737%	0.774%	0.559%	0.530%	0.624%	0.565%	0.496%	0.636%
5d	Cleared	0.616%	0.569%	0.811%	0.826%	0.581%	0.500%	0.782%	0.564%
6a	Cleared	0.550%	0.602%	0.543%	0.607%	0.555%	0.499%	0.619%	0.571%
6b	Cleared	0.630%	0.470%	0.416%	0.656%	0.665%	0.561%	0.620%	0.573%
6c	Cleared	0.731%	0.661%	0.479%	0.527%	0.784%	0.764%	0.583%	0.751%
6d	Cleared	0.674%	0.748%	0.716%	0.687%	0.942%	0.792%	0.836%	0.817%
7a	Cleared	0.480%	0.522%	0.803%	0.484%	0.679%	0.711%	0.566%	0.756%
7b	Cleared	0.761%	0.632%	0.626%	0.604%	0.684%	0.780%	0.619%	0.657%
7c	Cleared	0.662%	0.695%	0.624%	0.716%	0.691%	0.759%	0.694%	0.678%
7d	Cleared	0.482%	0.475%	0.525%	0.745%	0.575%	0.523%	0.756%	0.534%
8a	Uncleared	0.661%	0.867%	0.623%	0.756%	1.032%	0.913%	0.664%	0.903%
8b	Uncleared	0.628%	0.576%	0.784%	0.752%	0.607%	0.523%	0.734%	0.580%
8c	Uncleared	0.605%	0.530%	0.516%	0.495%	0.636%	0.550%	0.625%	0.614%
8d	Uncleared	0.491%	0.616%	0.667%	0.663%	0.919%	0.666%	0.685%	0.683%
9a	Uncleared	0.393%	0.456%	0.604%	0.635%	0.483%	0.496%	0.460%	0.491%
9b	Uncleared	0.310%	0.351%	0.387%	0.411%	0.390%	0.380%	0.464%	0.377%
9c	Uncleared	0.309%	0.324%	0.380%	0.401%	0.429%	0.403%	0.383%	0.428%
9d	Uncleared	0.372%	0.359%	0.469%	0.418%	0.495%	0.515%	0.453%	0.461%

APPENDIX C

				Soil Moisture						
	Sample Date:	9/20/09	5/24/10	8/5/10	10/1/10	5/27/11	6/19/11	9/15/11	6/22/12	9/14/12
Sample	Treatment									
2a	Uncleared	15.68%	59.60%	34.25%	23.63%	37.23%	34.30%	25.69%	18.67%	22.88%
2b	Uncleared	23.88%	30.07%	29.60%	18.77%	38.37%	35.20%	18.43%	16.87%	19.11%
2c	Cleared	23.88%	33.44%	29.58%	30.57%	45.76%	36.80%	23.57%	24.35%	23.43%
2d	Cleared	21.48%	40.43%	43.74%	29.96%	38.33%	36.94%	21.21%	25.84%	26.43%
3a	Uncleared	29.88%	38.34%	40.05%	26.06%	37.60%	35.58%	21.43%	22.27%	21.29%
3b	Uncleared	18.23%	42.33%	36.54%	24.68%	37.62%	32.80%	17.53%	19.75%	17.12%
3c	Uncleared	13.24%	38.79%	32.47%	23.21%	43.50%	42.97%	22.89%	20.26%	23.34%
3d	Uncleared	20.12%	45.30%	29.00%	21.65%	42.81%	45.33%	22.47%	27.46%	34.48%
4a	Uncleared	19.24%	30.10%	22.50%	14.51%	24.48%	23.69%	13.71%	13.74%	13.94%
4b	Uncleared	10.01%	30.18%	25.52%	19.42%	27.64%	24.68%	11.95%	13.97%	14.76%
4c	Uncleared	16.49%	40.60%	27.71%	25.32%	38.23%	34.98%	20.64%	24.89%	19.82%
4d	Uncleared	14.44%	41.84%	26.14%	17.38%	27.48%	32.68%	21.59%	19.31%	20.97%
5a	Cleared	19.89%	34.55%	37.96%	22.82%	34.38%	31.40%	19.29%	27.02%	26.10%
5b	Cleared	25.70%	45.23%	36.32%	25.95%	40.11%	40.47%	24.67%	29.07%	17.49%
5c	Cleared	31.12%	69.95%	34.55%	31.09%	44.31%	47.23%	22.72%	30.07%	26.13%
5d	Cleared	26.39%	52.22%	61.26%	41.00%	49.54%	45.24%	22.20%	28.76%	30.29%
6a	Cleared	21.48%	66.74%	48.43%	42.88%	57.38%	53.10%	23.25%	26.25%	21.82%
6b	Cleared	25.75%	54.01%	49.83%	39.15%	45.53%	44.61%	26.82%	23.99%	23.55%
6c	Cleared	22.67%	54.71%	36.65%	29.53%	48.82%	49.38%	24.61%	25.49%	29.60%
6d	Cleared	24.29%	62.01%	59.56%	45.53%	65.36%	60.00%	29.70%	30.68%	26.22%
7a	Cleared	22.67%	53.19%	50.21%	41.75%	75.19%	65.36%	29.68%	21.06%	30.11%
7b	Cleared	28.11%	66.73%	63.96%	35.72%	66.52%	54.30%	32.41%	27.43%	29.44%
7c	Cleared	26.17%	60.93%	54.04%	36.72%	63.06%	54.26%	27.62%	30.24%	27.02%
7d	Cleared	27.46%	61.00%	42.28%	36.70%	55.24%	53.49%	22.33%	27.01%	21.75%
8a	Uncleared	41.64%	76.96%	42.31%	27.94%	84.78%	65.93%	35.56%	59.40%	35.58%
8b	Uncleared	32.17%	57.64%	53.76%	34.42%	53.74%	46.39%	24.55%	34.68%	27.75%
8c	Uncleared	26.60%	49.45%	48.59%	32.12%	50.17%	42.56%	25.29%	30.41%	28.39%
8d	Uncleared	23.88%	61.87%	41.28%	30.13%	50.32%	61.36%	53.88%	30.82%	24.60%
9a	Uncleared	17.31%	40.49%	43.60%	32.43%	35.52%	31.03%	22.27%	23.35%	21.37%
9b	Uncleared	14.10%	31.49%	27.96%	20.45%	33.72%	28.21%	16.68%	16.86%	17.26%
9c	Uncleared	16.76%	32.82%	35.89%	26.14%	32.22%	35.00%	18.97%	23.03%	19.62%
9d	Uncleared	22.27%	34.74%	37.28%	24.78%	40.78%	37.51%	27.89%	20.71%	29.65%

APPENDIX D

Sample ID	Blast Accession	Blast Max ID %	Blast - Order / Family / Genus	Blast Species	Sample Date	Capture	Count
1	lost sample		bad data		**5/24/10**	2a1	7
2	FN669205.1	76%	Helotiales	sp. P224	**5/24/10**	2a2	2
3	lost sample		bad data		**5/24/10**	2b1	4
4	EU543203.1	95%	Pachyphloeus	carneus	**5/24/10**	2c1	9
5	lost sample		bad data		**5/24/10**	2d1	4
6	lost sample		bad data		**5/24/10**	2d2	9
7	lost sample		bad data		**5/24/10**	3a1	3
8	lost sample		bad data		**5/24/10**	3b1	3
9	lost sample		bad data		**5/24/10**	3d1	5
10	lost sample		bad data		**5/24/10**	5b1	1
11	GU327498.1	83%	Ceratobasidium	clone R77p5 18S	**5/24/10**	5d1	5
12	GQ166872.1	98%	Inocybe	sp. PRL7420 18S	**5/24/10**	7a1	6
13	KC007320.1	96%	Neonectria	sp. O_2_BESC_883e	**5/24/10**	7d2	6
14	GQ166872.1	99%	Inocybe	sp. PRL7420 18S	**5/24/10**	7d1	7
15	EU543206.1	97%	Pachyphloeus	carneus	**5/24/10**	8a1a,b,c	25
16	EU543206.1	99%	Pachyphloeus	carneus	**5/24/10**	8a2	4
17	EU819518.1	98%	Scleroderma	areolatum	**5/24/10**	8d1a,b	25
18	EU819438.1	96%	Scleroderma	areolatum	**5/24/10**	8d2	25
19	JF908018.1	74%	Genea	fragrans	**5/24/10**	9a1	2
20	GQ166883.1	88%	Boletus	rubellus	**5/24/10**	9a2	1
21	JX630841.1	96%	Tomentella	uncultured clone AR931	**5/24/10**	9b1	1
22	EU543203.1	72%	Pachyphloeus	carneus	**5/24/10**	9c1	25
23	lost sample		bad data		**5/24/10**	9c2	4
24	JF419276.1	96%	Tuber	mexiusanum	**5/24/10**	9d1	2
27	FN669205.1	82%	Helotiales	sp. P224	**8/5/10**	2a1	4
28	no sample		bad data		**8/5/10**	2d1	2
29	FN669205.1	86%	Helotiales	sp. P224	**8/5/10**	3a1	4
30	EU819438.1	99%	Scleroderma	areolatum	**8/5/10**	3a2	25
31	EU754979.1	97%	Tetracladium	clone B1 d ITS1F	**8/5/10**	3b2	3
32	DQ189228.1	99%	Geomyces	pannorum	**8/5/10**	3b1	25
33	lost sample		bad data		**8/5/10**	3d1a	4
34	lost sample		bad data		**8/5/10**	4a2a	4
35	bad data		bad data		**8/5/10**	4a1a	1
36	GQ166883.1	99%	Boletus	rubellus	**8/5/10**	4c2	2
37	lost sample		bad data		**8/5/10**	4c1	25
38	AJ509866.1	96%	Geomyces	pannorum	**8/5/10**	4d1	4
39	bad data		Non-ECM		**8/5/10**	4d2	25
40	lost sample		bad data		**8/5/10**	5c2	1
41	EU819525.1	81%	Tuber	scruposum	**8/5/10**	5c1	25
42	lost sample		bad data		**8/5/10**	6a1	9
43	EU523591.1	71%	Inocybe	sp. CIF205-302 18S	**8/5/10**	6a2	3
44	JX030288.1	90%	Scleroderma	areolatum	**8/5/10**	6a4	6
45	lost sample		bad data		**8/5/10**	6a3	5
46	lost sample		bad data		**8/5/10**	6b2	25
47	GQ483644	95%	Thelebolus	microsporus	**8/5/10**	6b3	5
48	EU754979.1	95%	Tetracladium	clone B1 d ITS1F	**8/5/10**	6b1	5
49	lost sample		bad data		**8/5/10**	7b2b	3
50	lost sample		bad data		**8/5/10**	7b1	4
51	AJ302000.1	83%	Myrothecium	leucotrichum	**8/5/10**	7b3	2
52	lost sample		bad data		**8/5/10**	7b2a	4
53	JQ408760.1	76%	Inocybe	lanatodisca	**8/5/10**	7c1	4
54	lost sample		bad data		**8/5/10**	7d3	1
55	bad data		bad data		**8/5/10**	7d4	1
56	lost sample		bad data		**8/5/10**	7d2	25
57	AY052493.1	98%	Cystofilobasidium	capitatum	**8/5/10**	7d1	25

Sample ID	Blast Accession	Blast Max ID %	Blast - Order / Family / Genus	Blast Species	Sample Date	Capture	Count
58	lost sample		bad data		**8/5/10**	8c3	2
59	GQ166873.1	80%	Boletus	rubellus	**8/5/10**	8c1	3
60	lost sample		bad data		**8/5/10**	9b1	5
61	EU516822.1	91%	Tetracladium	clone IVP2--12 18S	**8/5/10**	9c2	1
62	AJ608960.1	98%	Geomyces	sp. FFI 30 5.8s	**8/5/10**	9c1	25
64	FN669205.1	97%	Helotiales	sp. P224	**10/1/10**	2a1	25
65	EU819438.1	99%	Scleroderma	areolatum	**10/1/10**	2a2	25
66	no sample		bad data		**10/1/10**	2b2	2
67	JF320819.1	74%	Geomyces	pannorum	**10/1/10**	2b3	25
68	JQ857024.1	97%	Cryptococcus	gastricus	**10/1/10**	2b1	2
69	bad data		Non-ECM		**10/1/10**	3b5	3
70	JF691212.1	70%	Helotiales	Uncultured clone FM109.4	**10/1/10**	3b4	8
71	FN669205.1	99%	Helotiales	sp. P224	**10/1/10**	3b6	25
72	JQ408760.1	72%	Inocybe	lanatodisca	**10/1/10**	3b7	4
73	KC311507.1	91%	Cryptosporiopsis	radicicola	**10/1/10**	3b8	25
74	FM213352.1	97%	Scleroderma	areolatum	**10/1/10**	3b2	25
75	HF558659.1	78%	Cystofilobasidium	capitatum	**10/1/10**	3b3	7
76	HF558655.1	70%	Cryptococcus	terricola	**10/1/10**	3b1	25
77	bad data		bad data		**10/1/10**	3c1	1
78	JQ408760.1	99%	Inocybe	lanatodisca	**10/1/10**	4b1	2
79	EU819493.1	99%	Russula	pectinatoides	**10/1/10**	4c1	2
80	EU057070.1	81%	Cortinarius	selandicus	**10/1/10**	4d1	4
81	bad data		Non-ECM		**10/1/10**	5a2	3
82	bad data		bad data		**10/1/10**	5a1	5
83	bad data		Non-ECM		**10/1/10**	5b1	13
84	FN669195.1	97%	Elaphomyces	sp. B337	**10/1/10**	5c1	2
85	bad data		bad data		**10/1/10**	5c2	2
86	EU543206.1	99%	Pachyphloeus	carneus	**10/1/10**	5c3	3
87	JQ857019.1	85%	Leucosporidiella	creatinivora	**10/1/10**	6a1	4
88	EF495232.1	98%	Podospora	sp. Ppf7 18S	**10/1/10**	6b1	25
89	EF495232.1	99%	Podospora	sp. Ppf7 18S	**10/1/10**	6b2	25
90	JX135042.1	97%	Peziza	clone U2 18S	**10/1/10**	7a1	3
91	JF419276.1	99%	Tuber	mexiusanum	**10/1/10**	7b2	2
92	JF311913.1	83%	Geomyces	pannorum	**10/1/10**	7b1	5
93	JQ408760.1	95%	Inocybe	lanatodisca	**10/1/10**	7d2	2
94	JN995638.1	82%	Trichocladium	opacum	**10/1/10**	7d3	25
95	JQ711811.1	94%	Cortinarius	sp. 5 RT-2012	**10/1/10**	7d1	25
96	bad data		bad data		**10/1/10**	8c1	25
97	bad data		Non-ECM		**10/1/10**	8d2	25
98	JX030288.1	97%	Scleroderma	areolatum	**10/1/10**	8d1	2
99	JX030288.1	90%	Scleroderma	areolatum	**10/1/10**	9b1	2
100	KC455910.1	85%	Mrakia	gelida	**10/1/10**	9c1	3
105	bad data		Non-ECM		**5/27/11**	3c1	3
106	JX030282.1	75%	Scleroderma	areolatum	**5/27/11**	3c2	25
107	HQ637328.1	99%	Mortierella	minutissima	**5/27/11**	5b1	5
108	EF417799.1	97%	Clavulina	clone B4BD1 18S	**5/27/11**	5d1	25
109	bad data		bad data		**5/27/11**	7c2	3
110	JQ711811.1	93%	Cortinarius	sp. 5 RT-2012	**5/27/11**	7c1	25
111	JF735314.1	94%	Neonectria	ramulariae	**5/27/11**	7d3	3
112	bad data		bad data		**5/27/11**	7d2	1
113	JF419276.1	99%	Tuber	mexiusanum	**5/27/11**	7d1	6
114	GQ166876.1	99%	Boletus	rubellus	**5/27/11**	7d4	5
115	JQ868435.1	81%	Neonectria	faginata Strain Nf75A1 18S	**5/27/11**	8a1	1
116	HF934029.1	98%	Mrakia	cf. frigida 52b	**5/27/11**	8b1	2
117	lost sample		bad data		**5/27/11**	8c2	2
118	lost sample		bad data		**5/27/11**	8c1	2

Sample ID	Blast Accession	Blast Max ID %	Blast - Order / Family / Genus	Blast Species	Sample Date	Capture	Count
119	AJ509866.1	99%	Geomyces	pannorum	**6/19/11**	2cL1	3
120	KF339224.1	86%	Paraconiothyrium	sporulosum	**6/19/11**	2cL2	6
121	FM213352.1	98%	Scleroderma	areolatum	**6/19/11**	2cL3	4
122	GQ379727.1	99%	Tuber	sp. GB-1 isolate 37b 5.8S	**6/19/11**	2cR3	25
123	KC592278.1	75%	Trypethelium	aeneum	**6/19/11**	2cR2	12
124	FJ627262.1	96%	Perenniporia	medulla-panis	**6/19/11**	2cR1	5
125	KC312634.1	72%	Trichoderma	asperellum	**6/19/11**	2dL1	25
126	FJ627262.1	87%	Perenniporia	medulla-panis	**6/19/11**	2dL2	3
127	bad data		Non-ECM		**6/19/11**	2dL3	2
128	EU523591.1	84%	Inocybe	sp. CIF205-302 18S	**6/19/11**	3cL1	25
129	GQ911549.1	98%	Mrakiella	cryoconiti	**6/19/11**	3dL1	25
130	no sample		bad data		**6/19/11**	4aL2	5
131	EU819438.1	99%	Scleroderma	areolatum	**6/19/11**	4aL1	25
132	GQ911549.1	98%	Mrakiella	cryoconiti	**6/19/11**	4aR1	25
133	KC455910.1	97%	Mrakia	gelida	**6/19/11**	4cR1	25
134	FN669205.1	87%	Helotiales	sp. P224	**6/19/11**	4cR2	16
135	AJ496629.1	89%	Phaeosphaeria	eustoma	**6/19/11**	5aL1	25
136	HF558655.1	97%	Cryptococcus	terricola	**6/19/11**	5bL1	25
137	JQ408760.1	98%	Inocybe	lanatodisca	**6/19/11**	5cL1	25
138	JQ408760.1	78%	Inocybe	lanatodisca	**6/19/11**	5cR1	25
139	FJ552720.1	96%	Agaricomycetes	clone LTSP EUKA P1B07 18S	**6/19/11**	5dL1	25
140	bad data		Non-ECM		**6/19/11**	5dR1	23
141	bad data		Non-ECM		**6/19/11**	6aL1	25
142	KC694147.1	81%	Chaetomium	clone 57 18S	**6/19/11**	6aR1	19
143	FJ552720.1	93%	Agaricomycetes	clone LTSP EUKA P1B07 18S	**6/19/11**	6bR1	25
144	bad data		Non-ECM		**6/19/11**	6cR1	25
145	EU819474.1	97%	Inocybe	cf. soriora	**6/19/11**	6dR2	5
146	FJ627262.1	92%	Perenniporia	medulla-panis	**6/19/11**	6dR1	4
147	GQ166872.1	94%	Inocybe	sp. PRL7420 18S	**6/19/11**	7bL1	3
148	FJ627262.1	95%	Perenniporia	medulla-panis	**6/19/11**	7bL2	9
149	JQ408760.1	91%	Inocybe	lanatodisca	**6/19/11**	7cL1	25
150	FJ552720.1	98%	Agaricomycetes	clone LTSP EUKA P1B07 18S	**6/19/11**	7cR3	17
151	JQ408782.1	76%	Inocybe	sororia	**6/19/11**	7cR1	10
152	FJ627262.1	75%	Perenniporia	medulla-panis	**6/19/11**	7cR2	4
153	bad data		Non-ECM		**6/19/11**	7dL1	25
154	JF735314.1	97%	Neonectria	ramulariae	**6/19/11**	8aL2	25
155	EU543206.1	88%	Pachyphloeus	carneus	**6/19/11**	8aL1	21
156	JQ857024.1	100%	Cryptococcus	gastricus	**6/19/11**	8aR1	25
157	AF444417.1	99%	Guehomyces	pullulans	**6/19/11**	9cL1	6
186	UE819438.1	99%	Scleroderma	areolatum	**9/15/11**	2aL1	25
187	bad data		Non-ECM		**9/15/11**	2bL1	3
188	bad data		Non-ECM		**9/15/11**	2bR1	7
189	GQ166876.1	79%	Boletus	rubellus	**9/15/11**	2dL1	3
190	EU543206.1	98%	Pachyphloeus	carneus	**9/15/11**	2dL3	10
191	FM213352.1	99%	Scleroderma	areolatum	**9/15/11**	2dL2	10
192	bad data		Non-ECM		**9/15/11**	3aR2	15
193	FN669205.1	93%	Helotiales	sp. P224	**9/15/11**	3aR1	25
194	EU819472.1	77%	Inocybe	calospora	**9/15/11**	3bR2	4
195	EU543203.1	98%	Pachyphloeus	carneus	**9/15/11**	3bR1	20
196	HF558656.1	99%	Trichosporon	porosum	**9/15/11**	3dR1	14
197	AF145323.1	99%	Cryptococcus	gastricus	**9/15/11**	4aL1	12
198	no sample		bad data		**9/15/11**	4aR1	3
199	GQ166888.1	83%	Boletus	rubellus	**9/15/11**	4bL1	4
200	bad data		Non-ECM		**9/15/11**	4bL2	10
201	GQ166888.1	87%	Boletus	rubellus	**9/15/11**	4bR1	9
202	FN669205.1	98%	Helotiales	sp. P224	**9/15/11**	4cL1	18

Sample ID	Blast Accession	Blast Max ID %	Blast - Order / Family / Genus	Blast Species	Sample Date	Capture	Count
203	EU375713.1	89%	Tomerntella	uncultured clone TRFLP 26 18S	**9/15/11**	5bR1	8
204	no sample		bad data		**9/15/11**	5cL1	6
205	GQ166876.1	96%	Boletus	rubellus	**9/15/11**	5cR1	15
206	bad data		bad data		**9/15/11**	5dR2	14
207	JN847456.1	80%	Cortinarius	clone WME11 18S	**9/15/11**	5dR4	11
208	EU543203.1	98%	Pachyphloeus	carneus	**9/15/11**	5dR6	13
209	HM347666.1	78%	Boletus	queletii	**9/15/11**	5dR1	8
210	bad data		Non-ECM		**9/15/11**	5dR5	16
211	EF619824.1	83%	Tomentella	uncultured clone 6S1.11.S05 18S	**9/15/11**	5dR3	5
212	bad data		Non-ECM		**9/15/11**	6cL1	20
213	EU819474.1	98%	Inocybe	cf. soriora JMP0032	**9/15/11**	6dL1	14
214	JQ408758.1	94%	Inocybe	lanatodisca	**9/15/11**	7aL1	5
215	JQ408758.1	96%	Inocybe	lanatodisca	**9/15/11**	7aL2	10
216	JX030293.1	74%	Tomentella	aff. Badia	**9/15/11**	7aR2	8
217	JQ408760.1	84%	Inocybe	lanatodisca	**9/15/11**	7aR1	6
218	JX030220.1	93%	Peziza	sp. SGT-2012	**9/15/11**	7aR3	25
219	EU523559.1	87%	Inocybe	sp. CIF205-302 18S	**9/15/11**	7bL1	20
220	JF419269.1	90%	Tuber	mexiusanum	**9/15/11**	7bL2	25
221	bad data		bad data		**9/15/11**	7bR1	5
222	JQ711811.1	83%	Cortinarius	sp. 5 RT-2012	**9/15/11**	7cR1	25
223	HQ630340.1	99%	Mortierella	gamsii	**9/15/11**	8aL1	25
224	bad data		bad data		**9/15/11**	8bL2	25
225	AF444418.1	99%	Guehomyces	pullulans	**9/15/11**	8bL1	25
226	JX030288.1	76%	Scleroderma	areolatum	**9/15/11**	8cR2	10
227	EU819438.1	99%	Scleroderma	areolatum	**9/15/11**	8cR1	25
228	JF419272.1	90%	Tuber	mexiusanum	**9/15/11**	8dR2	13
229	FM213352.1	99%	Scleroderma	areolatum	**9/15/11**	8dR1	2
230	EU819518.1	97%	Scleroderma	areolatum	**9/15/11**	9cR1	5
248	HE649377.1	86%	Chaetomium	piluliferum	**6/22/12**	2aR1	25
249	GQ166872.1	95%	Inocybe	sp. PRL7420 18S	**6/22/12**	2aR2	25
250	FN669244.1	98%	Russula	sp. B181	**6/22/12**	2bR2	14
251	EU543206.1	96%	Pachyphloeus	carneus	**6/22/12**	2bR1	25
252	EU819438.1	98%	Scleroderma	areolatum	**6/22/12**	2dR1	6
253	EU819438.1	99%	Scleroderma	areolatum	**6/22/12**	2dR3	7
254	EU819518.1	74%	Scleroderma	areolatum	**6/22/12**	2dR2	7
255	EU819460.1	86%	Boletus	rubellus	**6/22/12**	3aR1	25
256	EU819478.1	93%	Laccaria	laccata var. pallidifolia	**6/22/12**	3aR2	11
257	FN669205.1	98%	Helotiales	sp. P224	**6/22/12**	3bL	10
258	EU819438.1	97%	Scleroderma	areolatum	**6/22/12**	3bR	25
259	EU819438.1	98%	Scleroderma	areolatum	**6/22/12**	3dR	25
260	FN669205.1	81%	Helotiales	sp. P224	**6/22/12**	4cR1	25
261	JX029132.1	79%	Tetracladium	sp. WMM-2012a 211 18S	**6/22/12**	5bR1	2
262	bad data		Non-ECM		**6/22/12**	5dL1	25
263	bad data		Non-ECM		**6/22/12**	6aL2	4
264	KC007130.1	99%	Neonectria	sp. BESC 103a	**6/22/12**	6aL1	25
265	GU189709.1	93%	Sebacina	Clone 10361 18S	**6/22/12**	6aR1	25
266	bad data		Non-ECM		**6/22/12**	6bR1	10
267	GQ166872.1	98%	Inocybe	sp. PRL7420 18S	**6/22/12**	6bR2	3
268	EF114392.1	85%	Cryptosporiopsis	ericae	**6/22/12**	7bL1	25
269	AJ312123.1	91%	Mycocalicium	victoriae	**6/22/12**	8aL1	4
270	JF908084.1	89%	Hypoxylon	rutilum	**6/22/12**	8bL1	8
271	JX243901.1	93%	Mortierllacaea	sp. PDKB9	**6/22/12**	9aR1	25
272	EU543206.1	86%	Pachyphloeus	carneus	**6/22/12**	9cL1	25
273	FM213352.1	76%	Scleroderma	areolatum	**6/22/12**	9cR1	5
282	JQ408760.1	96%	Inocybe	lanatodisca	**9/14/12**	2aR1	2
283	EF114392.1	95%	Cryptosporiopsis	ericae	**9/14/12**	2bR1	15

Sample ID	Blast Accession	Blast Max ID %	Blast - Order / Family / Genus	Blast Species	Sample Date	Capture	Count
284	bad data		bad data		**9/14/12**	2cL1	13
285	JX976131.1	73%	Mortierella	elongata	**9/14/12**	2cL2	20
286	JF735314.1	74%	Neonectria	ramulariae strain CBS 182.36 18S	**9/14/12**	2cR1	1
287	EU543205.1	99%	Pachyphloeus	carneus	**9/14/12**	3aR2	8
288	bad data		bad data		**9/14/12**	3aR1	25
289	AF325635.1	94%	Hymenogaster	sp. Trappe 20345	**9/14/12**	3cR2	7
290	GQ166872.1	87%	Inocybe	sp. PRL7420 18S	**9/14/12**	3cR1	8
291	FN669205.1	93%	Helotiales	sp. P224	**9/14/12**	3dL1	25
292	FN669205.1	81%	Helotiales	sp. P224	**9/14/12**	3dR1	9
293	FN669205.1	99%	Helotiales	sp. P224	**9/14/12**	4aL1	2
294	FN669205.1	99%	Helotiales	sp. P224	**9/14/12**	4dL1	7
295	KC007320.1	89%	Neonectria	sp 0_2_BESC_883e	**9/14/12**	5aR1	1
296	EU598186.1	98%	Russula	pulverulenta	**9/14/12**	5bR1	15
297	GQ166872.1	99%	Inocybe	sp. PRL7420 18S	**9/14/12**	5bR2	25
298	EU375713.1	97%	Tomentella	uncultured clone TRFLP 26 18S	**9/14/12**	5cL1	25
299	bad data		Non-ECM		**9/14/12**	5cL2	5
300	EU819493.1	95%	Russula	pectinatoides	**9/14/12**	5cL3	25
301	EF417799.1	98%	Clavulina	clone B4BD1 18S	**9/14/12**	5dL1	25
302	EU819493.1	93%	Russula	pectinatoides	**9/14/12**	5dR1	17
303	EU543203.1	99%	Pachyphloeus	carneus	**9/14/12**	5dR3	25
304	GQ166872.1	95%	Inocybe	sp. PRL7420 18S	**9/14/12**	6aR2	4
305	JF419276.1	99%	Tuber	mexiusanum	**9/14/12**	6aR1	8
306	EF114392.1	78%	Cryptosporiopsis	ericae	**9/14/12**	6bL2	10
307	GQ166876.1	81%	Boletus	rubellus	**9/14/12**	6bL1	15
308	EU394704.1	99%	Tuber	lyonii	**9/14/12**	6cL1	1
309	FJ748910.1	99%	Tuber	lyonii	**9/14/12**	6cL2	14
310	HM036602.1	78%	Neonectria	macrodidyma	**9/14/12**	6cR2	25
311	GQ166888.1	87%	Boletus	rubellus	**9/14/12**	6cR5	6
312	JN225891.1	69%	Phaeomoniella	sp. 1 ICMP 18935	**9/14/12**	6cR3	4
313	GQ166883.1	85%	Boletus	rubellus	**9/14/12**	6cR4	4
314	GQ166883.1	80%	Boletus	rubellus	**9/14/12**	6cR1	3
315	JF419276.1	99%	Tuber	mexiusanum	**9/14/12**	6dL1	1
316	EU819474.1	99%	Inocybe	cf. soriora	**9/14/12**	6dL2	5
317	EU543203.1	97%	Pachyphloeus	carneus	**9/14/12**	6dR2	5
318	EU819442.1	98%	Sebacina	incrustans	**9/14/12**	6dR3	1
319	EU543203.1	99%	Pachyphloeus	carneus	**9/14/12**	6dR1	12
320	EU819474.1	99%	Inocybe	cf. soriora	**9/14/12**	6dR4	7
321	bad data		Non-ECM		**9/14/12**	7aR1	10
322	bad data		Non-ECM		**9/14/12**	7aR3	3
323	bad data		bad data		**9/14/12**	7aR2	7
324	GQ166883.1	95%	Boletus	rubellus	**9/14/12**	7cL1	11
325	FN669205.1	97%	Helotiales	sp. P224	**9/14/12**	7cL3	12
326	GQ166883.1	97%	Boletus	rubellus	**9/14/12**	7cL2	25
327	JF419256.1	99%	Tuber	sp. 36 GB-2010	**9/14/12**	7cR4	25
328	JQ711811.1	95%	Cortinarius	sp. 5 RT-2012	**9/14/12**	7cR1	25
329	FN669205.1	95%	Helotiales	sp. P224	**9/14/12**	7cR2	25
330	JQ711811.1	95%	Cortinarius	sp. 5 RT-2012	**9/14/12**	7cR3	9
331	bad data		Non-ECM		**9/14/12**	7dR1	25
332	FN669205.1	92%	Helotiales	sp. P224	**9/14/12**	8aL1	25
333	bad data		bad data		**9/14/12**	8aR1	2
334	EU543206.1	77%	Pachyphloeus	carneus	**9/14/12**	8aR2	25
335	GQ166883.1	90%	Boletus	rubellus	**9/14/12**	9aR1	13
336	EU819438.1	99%	Scleroderma	areolatum	**9/14/12**	9bR1	25
337	GU134499.1	80%	Russula	clone Z3 18S	**9/14/12**	9cL1	6
338	EU819438.1	99%	Scleroderma	areolatum	**9/14/12**	9cR1	25
369	DQ494374.1	85%	Dactylella	spermatophaga	**9/14/12**	5dR2	4

Sample ID	Color - BK, BR, WH, YE, OR, TN, CPR, GLD	Color Mod - Dark, Light	Root Branching - Pinnate, Pyramidal, Coralloid, Irregular, NotBranched	Tip Shape - Tortuous, Bent, Straight, Club-Shape	Texture - Smooth, Grainy, Felty, Velvety, Cottony, Stringy	Surface Sheen - Matte, Shiny, Reflective	External Mycelial - Restricted, Flat Angle, Hyphal Fan
1	OR	Light	Pyramidal	Straight	Smooth	Shiny	Flat Angle
2	OR, TN	Light	NotBranched	Bent	Grainy	Matte	
3	BR	Dark	Pyramidal	Straight	Smooth	Matte	
4	OR	Dark	Coralloid	Bent	Grainy	Shiny	
5	OR	Light	NotBranched	Bent	Smooth	Shiny	
6	OR, TN	Dark	Pyramidal	Straight	Grainy	Matte	
7	OR, TN	Light	Pinnate	Straight	Smooth	Matte	
8	OR	Light	Pyramidal	Straight	Smooth	Shiny	
9	OR	Light	Pyramidal	Straight	Smooth	Matte	
10	WH, OR	Light	NotBranched	Club	Smooth	Matte	
11	OR, TN	Light	Pyramidal	Bent	Grainy	Matte	Flat Angle
12	BR	Dark	Pyramidal	Straight	Grainy	Matte	
13	BK	Light	Pyramidal	Bent	Grainy	Matte	
14	BK	Dark	NotBranched	Straight	Grainy	Matte	
15	OR	Light	Coralloid	Tortuous	Grainy/Cottony	Shiny	Hyphal Fan
16	OR	Light	Pyramidal	Straight	Velvety	Shiny	Hyphal Fan
17	OR	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
18	OR	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
19	OR	Light	Pinnate	Bent	Cottony	Matte	Flat Angle
20	OR	Light	NotBranched	Straight	Grainy	Matte	
21	OR	Light	NotBranched	Straight	Grainy	Matte	
22	OR	Light	Coralloid	Tortuous	Velvety	Reflective	Flat Angle
23	OR	Light	Pyramidal	Bent	Grainy	Matte	
24	OR	Light	Pyramidal	Straight	Grainy	Matte	
27	OR	Light	NotBranched	Straight	Smooth	Matte	
28	OR, WH	Light	NotBranched	Bent	Grainy	Shiny	Restricted
29	OR	Light	Irregular	Bent	Smooth	Matte	
30	OR, WH	Light	Irregular	Tortuous	Velvety	Shiny	Hyphal Fan
31	OR	Light	Irregular	Bent	Smooth	Matte	
32	OR, WH	Light	Irregular	Tortuous	Velvety	Shiny	Hyphal Fan
33	OR	Light	NotBranched	Straight	Grainy	Matte	Flat Angle
34	OR	Light	Pinnate	Bent	Stringy	Matte/Shiny	Hyphal Fan
35	TN	Light	NotBranched	Bent	Stringy	Matte/Shiny	Hyphal Fan
36	OR	Light	NotBranched	Bent	Velvety	Reflective	
37	TN	Light	Irregular	Bent	Smooth	Matte	
38	BR	Dark	NotBranched	Bent	Smooth	Matte	
39	OR	Light	Pinnate	Bent	Velvety	Matte/Reflective	
40	BR	Dark	Pinnate	Straight	Smooth	Matte	
41	OR, YE	Light	Coralloid	Tortuous	Velvety/Cottony	Matte/Reflective	Hyphal Fan
42	BR	Dark	NotBranched	Bent	Smooth	Matte	
43	BR	Dark	Pyramidal	Bent	Smooth	Matte	
44	BR	Dark	Pyramidal	Bent	Smooth	Matte	
45	OR	Light	NotBranched	Bent	Smooth	Matte	
46	OR	Light	Irregular	Bent	Grainy	Matte	
47	OR, YE	Light	Pinnate	Bent	Grainy	Matte	
48	OR, YE	Light	NotBranched	Bent	Grainy	Matte/Shiny	
49	BK	Dark	NotBranched	Bent	Smooth	Matte	
50	BR	Dark	Pyramidal	Straight	Grainy	Matte	
51	CPR	Dark	NotBranched	Bent	Smooth	Matte	
52	OR	Light	NotBranched	Straight	Stringy	Matte	
53	OR	Light	Irregular	Bent	Smooth	Matte	
54	BR	Dark	NotBranched	Straight	Smooth	Matte	Hyphal Fan
55	BR	Dark	NotBranched	Straight	Smooth	Matte	
56	OR, WH	Light	Coralloid	Tortuous	Velvety	Reflective	Hyphal Fan
57	OR, WH	Light	Coralloid	Tortuous	Velvety/Cottony	Reflective	Hyphal Fan

Sample ID	Color - BK, BR, WH, YE, OR, TN, CPR, GLD	Color Mod - Dark, Light	Root Branching - Pinnate, Pyramidal, Coralloid, Irregular, NotBranched	Tip Shape - Tortuous, Bent, Straight, Club-Shape	Texture - Smooth, Grainy, Felty, Velvety, Cottony, Stringy	Surface Sheen - Matte, Shiny, Reflective	External Mycelial - Restricted, Flat Angle, Hyphal Fan
58	YE	Light	NotBranched	Bent	Grainy	Reflective	
59	YE	Light	NotBranched	Bent	Grainy	Reflective	
60	WH	Light	Pyramidal	Bent	Felty	Reflective	
61	BR	Dark	NotBranched	Straight	Smooth	Matte	
62	OR, WH	Light	Coralloid	Tortuous	Felty	Reflective	
64	TN, WH	Light	Irregular	Tortuous	Velvety	Shiny/Reflective	Hyphal Fan
65	WH	Light	Irregular	Tortuous	Velvety	Shiny/Reflective	Hyphal Fan
66	OR	Light	NotBranched	Bent	Grainy	Matte	
67	OR, WH	Light	Coralloid	Tortuous	Cottony	Shiny	Hyphal Fan
68	OR, WH	Light	NotBranched	Bent	Velvety	Shiny	
69	BR	Dark	Pyramidal	Bent	Smooth	Matte	Restricted
70	OR, WH	Light	Irregular	Tortuous	Velvety/Cottony	Matte/Shiny	Hyphal Fan
71	OR, WH	Light	Irregular	Tortuous	Cottony	Shiny/Reflective	Hyphal Fan
72	OR, WH	Light	Irregular	Tortuous	Velvety/Cottony	Shiny	Restricted
73	OR, WH	Light	Coralloid	Tortuous	Velvety/Cottony	Shiny/Reflective	Restricted
74	WH	Light	Irregular	Bent	Cottony	Shiny	Hyphal Fan
75	WH	Light	Pyramidal	Bent	Velvety	Shiny	Flat Angle
76	WH, OR	Light	Coralloid	Tortuous	Velvety/Cottony	Shiny	Hyphal Fan
77	BR	Dark	NotBranched	Straight	Grainy	Matte	
78	OR	Light	NotBranched	Straight	Grainy	Matte	Flat Angle
79	OR	Light	NotBranched	Bent	Grainy	Matte	
80	OR, WH	Light	NotBranched	Bent	Grainy/Velvety	Shiny/Reflective	Restricted
81	OR	Light	Pinnate	Bent	Grainy	Matte	
82	OR, YE	Light	Pinnate	Bent	Grainy	Matte	
83	OR	Light	Pyramidal	Bent	Grainy	Matte	
84	OR	Light	NotBranched	Straight	Grainy	Matte	
85	TN	Light	Pyramidal	Straight	Grainy	Matte	
86	TN	Light	Pyramidal	Straight	Grainy	Matte	
87	OR	Light	Pyramidal	Bent	Grainy	Matte	
88	OR, YE	Light	Coralloid	Tortuous	Grainy/Velvety	Matte/Reflective	
89	OR, YE	Light	Coralloid	Tortuous	Grainy/Velvety	Matte/Reflective	
90	TN	Light	NotBranched	Straight	Grainy	Matte	
91	OR	Light	NotBranched	Straight	Grainy	Matte	
92	TN	Dark	Pyramidal	Straight	Smooth	Matte	
93	TN	Dark	NotBranched	Straight	Grainy	Matte	
94	TN, WH	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
95	TN, WH	Light	Coralloid	Tortuous	Cottony	Matte/Reflective	
96	TN, WH	Light	Coralloid	Tortuous	Velvety	Matte/Reflective	Hyphal Fan
97	OR, WH	Light	NotBranched	Bent	Grainy/Velvety	Matte/Reflective	Restricted
98	WH	Light	NotBranched	Straight	Velvety	Reflective	Restricted
99	WH	Light	NotBranched	Bent	Velvety	Reflective	
100	OR, WH	Light	NotBranched	Bent	Grainy	Matte	Hyphal Fan
105	BR	Dark	Pyramidal	Bent	Grainy	Matte	
106	OR	Light	Coralloid	Tortuous	Cottony	Reflective	
107	OR	Light	Pinnate	Bent	Cottony	Reflective	
108	OR	Light	NotBranched	Bent	Smooth	Matte	
109	OR	Dark	NotBranched	Bent	Stringy	Matte	
110	OR, WH	Light	Coralloid	Tortuous	Cottony	Reflective	
111	OR	Light	Irregular	Tortuous	Cottony	Reflective	
112	OR	Light	NotBranched	Straight	Grainy	Matte	
113	OR	Light	Pyramidal	Straight	Smooth	Matte	
114	OR, YE	Light	NotBranched	Bent	Felty	Reflective	
115	OR	Light	NotBranched	Bent	Grainy	Matte	
116	BR	Dark	NotBranched	Bent	Smooth	Matte	
117	OR	Dark	NotBranched	Bent	Grainy	Matte	
118	YE	Light	NotBranched	Bent	Grainy	Matte	

Sample ID	Color - BK, BR, WH, YE, OR, TN, CPR, GLD	Color Mod - Dark, Light	Root Branching - Pinnate, Pyramidal, Coralloid, Irregular, NotBranched	Tip Shape - Tortuous, Bent, Straight, Club-Shape	Texture - Smooth, Grainy, Felty, Velvety, Cottony, Stringy	Surface Sheen - Matte, Shiny, Reflective	External Mycelial - Restricted, Flat Angle, Hyphal Fan
119	OR	Light	NotBranched	Bent	Grainy	Matte	Restricted
120	OR	Light	NotBranched	Bent	Grainy	Matte	
121	OR, WH	Light	Coralloid	Tortuous	Velvety	Reflective	
122	BR	Dark	Irregular	Bent	Grainy	Matte	
123	OR	Light	Pinnate	Bent	Grainy	Matte	
124	TN	Dark	NotBranched	Bent	Grainy	Matte	
125	CPR	Dark	Pyramidal	Bent	Grainy	Matte	
126	OR	Light	Pinnate	Straight	Velvety	Shiny	
127	WH	Light	NotBranched	Bent	Velvety	Reflective	
128	BR	Dark	Pinnate	Tortuous	Grainy	Matte	
129	OR	Light	NotBranched	Bent	Grainy	Matte	
130	BR	Dark	NotBranched	Straight	Grainy	Matte	
131	OR, WH	Light	Coralloid	Tortuous	Velvety	Reflective	
132	OR	Light	NotBranched	Bent	Grainy	Matte	
133	OR, WH	Light	Coralloid	Tortuous	Velvety	Reflective	
134	TN	Light	NotBranched	Straight	Grainy	Matte	
135	OR	Light	NotBranched	Bent	Grainy	Matte	
136	TN	Light	NotBranched	Bent	Grainy	Matte	
137	OR	Light	Pinnate	Bent	Grainy	Matte	
138	OR	Light	Pyramidal	Bent	Grainy	Matte	Flat Angle
139	WH	Light	Pyramidal	Bent	Smooth	Matte	
140	BR	Dark	NotBranched	Straight	Grainy	Matte	
141	OR	Dark	Pyramidal	Straight	Grainy	Matte	
142	OR	Light	Pyramidal	Bent	Grainy	Matte	
143	OR	Light	NotBranched	Straight	Grainy	Matte	
144	BR	Dark	Irregular	Bent	Grainy	Matte	
145	BR	Dark	Pyramidal	Straight	Grainy	Matte	
146	OR	Light	NotBranched	Straight	Grainy	Matte	
147	BR	Dark	NotBranched	Straight	Grainy	Matte	
148	BR	Dark	Pyramidal	Straight	Grainy	Matte	
149	BR	Dark	Irregular	Bent	Grainy	Matte	
150	BR	Dark	NotBranched	Straight	Grainy	Matte	
151	BR	Dark	Pyramidal	Straight	Grainy	Matte	
152	GLD	Light	Pyramidal	Straight	Grainy	Matte	
153	BR	Dark	Pyramidal	Bent	Grainy	Matte	
154	BR	Dark	NotBranched	Bent	Grainy	Matte	
155	OR, WH	Light	Irregular	Bent	Felty	Reflective	Hyphal Fan
156	OR	Light	Irregular	Bent	Grainy	Matte	
157	OR	Light	Pinnate	Bent	Grainy	Matte	
186	OR, WH	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
187	GLD	Dark	Pyramidal	Straight	Grainy	Matte	
188	BR	Dark	NotBranched	Bent	Felty	Shiny	Restricted
189	OR	Light	Pyramidal	Bent	Grainy	Shiny	Restricted
190	OR	Light	Pyramidal	Bent	Grainy	Matte	
191	WH	Light	NotBranched	Bent	Cottony	Reflective	Hyphal Fan
192	OR, WH	Light	Irregular	Bent	Cottony	Reflective	Flat Angle
193	OR, WH	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
194	CPR	Light	NotBranched	Bent	Grainy	Matte	
195	OR, WH	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
196	WH	Light	Pinnate	Bent	Cottony	Reflective	Restricted
197	WH	Light	Irregular	Bent	Velvety	Reflective	Hyphal Fan
198	OR	Light	NotBranched	Bent	Cottony	Reflective	Hyphal Fan
199	BR	Dark	Pyramidal	Bent	Velvety	Reflective	
200	TN	Light	Irregular	Bent	Grainy	Matte	
201	WH	Light	NotBranched	Bent	Felty	Matte	Restricted
202	TN, WH	Light	Irregular	Bent	Stringy	Reflective	Restricted

Sample ID	Color - BK, BR, WH, YE, OR, TN, CPR, GLD	Color Mod - Dark, Light	Root Branching - Pinnate, Pyramidal, Coralloid, Irregular, NotBranched	Tip Shape - Tortuous, Bent, Straight, Club-Shape	Texture - Smooth, Grainy, Felty, Velvety, Cottony, Stringy	Surface Sheen - Matte, Shiny, Reflective	External Mycelial - Restricted, Flat Angle, Hyphal Fan
203	BR	Dark	Pyramidal	Straight	Grainy	Matte	Hyphal Fan
204	OR	Light	Coralloid	Bent	Velvety	Reflective	
205	YE	Light	Irregular	Bent	Velvety	Reflective	
206	BR	Dark	Irregular	Bent	Velvety	Reflective	Hyphal Fan
207	BR	Dark	Pyramidal	Bent	Grainy	Matte	
208	OR	Light	Irregular	Bent	Cottony	Reflective	Hyphal Fan
209	OR	Light	Irregular	Bent	Velvety	Reflective	Hyphal Fan
210	OR	Light	Irregular	Bent	Grainy	Matte	Restricted
211	OR	Light	NotBranched	Bent	Velvety	Reflective	Restricted
212	BR	Dark	Irregular	Bent	Grainy	Matte	
213	WH	Light	Irregular	Bent	Smooth	Matte	
214	GLD	Dark	Irregular	Bent	Grainy	Matte	
215	OR	Light	NotBranched	Bent	Grainy	Matte	
216	BK	Dark	Irregular	Bent	Grainy	Matte	
217	GLD	Light	NotBranched	Bent	Grainy	Shiny	
218	OR	Light	Irregular	Bent	Grainy	Matte	
219	CPR	Dark	Irregular	Bent	Grainy	Matte	
220	OR	Light	Irregular	Bent	Grainy	Matte	
221	BR	Dark	NotBranched	Bent	Grainy	Matte	
222	OR, WH	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
223	OR	Light	Pyramidal	Bent	Smooth	Matte	Restricted
224	OR	Light	Coralloid	Tortuous	Grainy	Matte	Restricted
225	WH	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
226	OR	Light	Irregular	Bent	Grainy	Matte	Restricted
227	WH	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
228	TN	Light	Pyramidal	Bent	Grainy	Matte	
229	WH	Light	Irregular	Bent	Velvety	Reflective	Restricted
230	OR	Light	Coralloid	Tortuous	Cottony	Reflective	Hyphal Fan
248	BR	Dark	Coralloid	Bent	Grainy	Matte	
249	BR	Dark	Coralloid	Bent	Grainy	Matte	
250	OR	Light	Pinnate	Bent	Smooth	Matte	
251	OR	Light	Coralloid	Tortuous	Cottony	Reflective	
252	WH, OR	Light	Irregular	Bent	Velvety	Reflective	Flat Angle
253	WH, OR	Light	Irregular	Bent	Velvety	Reflective	Flat Angle
254	WH, OR	Light	Irregular	Bent	Velvety	Reflective	Restricted
255	WH, OR	Light	Irregular	Bent	Velvety	Reflective	Restricted
256	WH, OR	Light	Pinnate	Bent	Velvety	Shiny	
257	WH, OR	Light	Irregular	Bent	Velvety	Matte/Shiny	
258	WH	Light	Coralloid	Bent	Velvety	Reflective	
259	WH	Light	Coralloid	Bent	Velvety	Reflective	Restricted
260	OR	Light	NotBranched	Straight	Grainy	Matte	
261	OR	Light	NotBranched	Straight	Grainy	Matte	
262	OR	Dark	Pinnate	Bent	Grainy	Matte	
263	CPR	Dark	Pyramidal	Straight	Grainy	Matte	
264	OR	Light	Pyramidal	Straight	Grainy	Matte	
265	BR	Dark	Pyramidal	Straight	Grainy	Matte	
266	CPR	Dark	Irregular	Bent	Grainy	Matte	
267	OR	Light	Pinnate	Bent	Grainy	Matte	
268	BR	Dark	NotBranched	Straight	Grainy	Matte	
269	OR	Light	Pyramidal	Straight	Grainy	Matte	
270	OR	Light	Pinnate	Straight	Grainy	Matte	Restricted
271	OR	Light	Pyramidal	Straight	Grainy	Matte	
272	WH	Light	Coralloid	Tortuous	Velvety	Reflective	Hyphal Fan
273	OR	Light	NotBranched	Straight	Velvety	Reflective	Hyphal Fan
282	GLD	Dark	NotBranched	Bent	Grainy	Matte	
283	TN	Light	Irregular	Bent	Grainy	Matte	

Sample ID	Color - BK, BR, WH, YE, OR, TN, CPR, GLD	Color Mod - Dark, Light	Root Branching - Pinnate, Pyramidal, Coralloid, Irregular, NotBranched	Tip Shape - Tortuous, Bent, Straight, Club-Shape	Texture - Smooth, Grainy, Felty, Velvety, Cottony, Stringy	Surface Sheen - Matte, Shiny, Reflective	External Mycelial - Restricted, Flat Angle, Hyphal Fan
284	CPR	Dark	Pyramidal	Straight	Grainy	Matte	
285	OR	Light	Irregular	Straight	Grainy	Matte	
286	TN	Light	NotBranched	Bent	Grainy	Matte	
287	TN	Light	NotBranched	Straight	Grainy	Matte	Hyphal Fan
288	WH	Light	Coralloid	Tortuous	Cottony	Reflective	
289	OR	Light	NotBranched	Bent	Grainy	Matte	
290	OR	Light	Pyramidal	Club	Grainy	Matte	
291	WH	Light	Coralloid	Bent	Velvety	Reflective	
292	OR	Light	NotBranched	Bent	Velvety	Matte	
293	WH	Light	NotBranched	Bent	Velvety	Reflective	Restricted
294	WH, OR	Light	Irregular	Bent	Velvety	Reflective	
295	OR	Light	NotBranched	Straight	Grainy	Matte	
296	OR, YE	Light	Pyramidal	Bent	Grainy	Reflective	
297	TN	Light	Irregular	Bent	Grainy	Matte	
298	BR	Dark	Pyramidal	Straight	Grainy	Matte	
299	OR	Light	NotBranched	Club	Grainy/Velvety	Matte/Reflective	
300	OR, WH	Light	Coralloid	Bent	Velvety	Reflective	
301	TN	Light	Irregular	Bent	Grainy	Matte	
302	OR, WH	Light	Irregular	Bent	Velvety	Reflective	
303	OR, WH	Light	Coralloid	Tortuous	Velvety	Reflective	
304	BR	Dark	Pyramidal	Bent	Grainy	Matte	
305	OR	Light	Pyramidal	Bent	Grainy	Matte	
306	BR	Dark	Coralloid	Bent	Grainy	Matte	
307	YE	Light	Pyramidal	Bent	Velvety	Reflective	Hyphal Fan
308	BR	Dark	NotBranched	Bent	Grainy	Matte	
309	OR	Light	Pyramidal	Bent	Grainy	Matte	
310	BR	Dark	Irregular	Bent	Grainy	Matte	
311	BR	Dark	NotBranched	Bent	Grainy	Matte	
312	OR	Light	NotBranched	Bent	Grainy	Matte	
313	YE	Light	NotBranched	Bent	Velvety	Reflective	
314	YE	Light	Pyramidal	Bent	Velvety	Reflective	
315	OR	Light	NotBranched	Bent	Grainy	Matte	
316	WH	Light	NotBranched	Straight	Smooth	Matte	
317	OR	Light	Irregular	Bent	Grainy	Matte	
318	OR	Light	NotBranched	Straight	Grainy	Matte	
319	OR, WH	Light	Irregular	Bent	Velvety	Reflective	
320	OR, YE	Light	Irregular	Bent	Velvety	Reflective	
321	OR	Light	Pyramidal	Straight	Grainy	Matte	
322	OR	Light	Pyramidal	Straight	Stringy	Matte	
323	TN	Light	Pyramidal	Straight	Grainy	Matte	
324	OR	Light	Pyramidal	Bent	Velvety	Reflective	
325	TN	Light	Pyramidal	Bent	Stringy	Matte	
326	WH, OR	Light	Pyramidal	Bent	Velvety	Reflective	
327	OR	Light	Coralloid	Tortuous	Stringy	Matte	
328	OR, WH	Light	Coralloid	Tortuous	Cottony	Reflective	Restricted
329	TN	Light	Pyramidal	Bent	Grainy	Matte	
330	WH	Light	Irregular	Bent	Velvety/Cottony	Reflective	Hyphal Fan
331	TN	Light	Pyramidal	Bent	Grainy	Matte	
332	OR, YE	Dark	Irregular	Bent	Felty	Matte/Reflective	
333	CPR	Dark	NotBranched	Straight	Grainy	Matte	
334	WH, OR	Light	Coralloid	Tortuous	Felty	Reflective	Hyphal Fan
335	YE	Light	Pyramidal	Bent	Velvety	Reflective	
336	WH, OR	Light	Irregular	Bent	Velvety	Reflective	
337	CPR	Dark	Pyramidal	Bent	Grainy	Matte	
338	WH, OR	Light	Coralloid	Tortuous	Velvety	Reflective	
369	YE	Light	NotBranched	Straight	Velvety	Reflective	