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SPECIES COMPOSITION AND ABUNDANCE OF ENDORRHIZAL FUNGI IN *CAREX PENSYLVANICA* FROM WISCONSIN SAND PRAIRIES

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Samuel T. David

College of Science and Allied Health
Biology

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SPECIES COMPOSITION AND ABUNDANCE OF ENDORRHIZAL FUNGI IN *CAREX PENSYLVANICA* FROM WISCONSIN SAND PRAIRIES

By Samuel T. David

We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Master of Science in Biology

The candidate has completed the oral defense of the thesis.

Thomas Volk, Ph.D.
Thesis Committee Chairperson — Date: 6/14/2017

Todd Osmundson, Ph.D.
Thesis Committee Member — Date: 6/15/17

Meredith Thomsen, Ph.D.
Thesis Committee Member — Date: 6-15-17

Elisabeth Paluch, M.S.
Thesis Committee Member — Date: 6-22-17

Thesis accepted

Steven Simpson, Ph. D.
Graduate Studies Director — Date: 6/24/17

ABSTRACT

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Wisconsin sand prairies are rare habitats characterized by sandy soils and sparse vegetation. *Carex pensylvanica*, the most abundant sedge in Wisconsin sand prairies, has generally been considered non-mycorrhizal. To better understand plant-fungal associations that may promote *C. pensylvanica* colonization in sand prairies, we characterized the diversity of endorrhizal fungi using culture-based and molecular approaches. Plants were collected in pairs along sand prairie/oak barren ecotones. Culturing data revealed 70 morphotypes of fungi, while Illlumina sequencing of roots showed 362 OTUs, the most commonly isolated being *Acephala harenae nom. prov.* Cohabitation of dark septate endophytes, arbuscular mycorrhizal fungi, and ectomycorrhizae was determined within ~1cm root sections using light microscopy and Illumina sequencing. *Tomentella ferruginea, Russula,* and *Laccaria* were present in all samples sequenced. There was no significant difference in environmental factors or fungal community structure between tree and no tree sites. Soil data showed that pH and organic matter correlate with fungal community structure, the cause of which is unclear. To-date, this is the most in-depth survey of root associated fungi associated with any *Carex* species. Results provide insights into the diversity of fungi associated with sedges and the soil factors affecting fungal colonization in plants in sand prairie habitats.

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INTRODUCTION

One of the most prevalent and important symbioses on earth is between plants and mutualistic fungi called mycorrhizae. The majority (80-90%) of land plants require mycorrhizal fungi to acquire water and nutrients, primarily phosphorus and nitrogen, from soil (Brundrett 1991). In return for nutrients, the plant provides sugar to the fungus. If the majority of land plants form mycorrhizae, why don't all plants? Non-mycorrhizal plants are thought to have lost the need for a fungal mutualist because one or more of the following: they grow in wet places where getting nutrients is easy, they are parasitic or carnivorous, or they have cluster or dauciform roots (Brundrett 2008, Lamont 1974) that have specialized regions of long fine hairs for absorption that release compounds to help solubilize phosphorus and nitrogen (Shane *et al*. 2004). Some non-mycorrhizal plants don't have any of the above adaptations, but may form mutualistic relationships with other microbes, such as leguminous plants (Fabaceae) and nitrogen fixing bacteria. However, these are not mutually exclusive; plants with nitrogen fixing bacteria or other types of symbionts may also have mycorrhizae.

Mycorrhizal fungi are unable to inhabit areas that are too hot, cold, dry, disturbed, nutrient poor, saline, contaminated, or for some other reason harsh (Haselwandter and Read 1980, Brundrett and Abbott 2002). Mycorrhizae can persist as spores, but need living plants to grow, so habitats with little vegetation don't have as many

mycorrhizal species. As expected, harsh and scarcely vegetated habitats typically have a proportionately higher percentage of non-mycorrhizal plants (Jumpponen and Trappe 1998). However, just because some plants are non-mycorrhizal does not mean they don't have fungi in them.

Endophytes are a group of fungi that grow in all plants in all natural environments. The term endophyte, meaning "inside plants," can be used to describe any organism, such as a bacterium, growing in living plant tissues, but I will be using it just in reference to fungi. Fungi inhabit all plant tissues and can provide different benefits to the host plant (Rodriguez *et al*. 2009). One such benefit is growing like mycorrhizae, sharing nutrients and helping protect against pathogens. Because most endophytes can also decay dead plant material in the soil, they are able to reside in areas other mycorrhizae cannot (Jumpponen and Trappe 1998). Mycorrhizae, especially endomycorrhizae, are technically endophytes, but I will use the terms separately to highlight the differences in trophic status, morphology, and ecology. All types of root inhabiting fungi including ectomycorrhizae, arbuscular mycorrhizal fungi (AMF) and endophytes, can be collectively referred to as endorrhizal fungi.

The interaction between plant roots and mutualistic fungi that grow with them is a very intimate one, with each partner allowing for the other to be there. We often think of fungi as being ectomycorrhizal or endomycorrhizal, but plants definitely influence the morphology of the fungi. Plants also do not necessarily always associate with one type.

Rodriguez *et al*. (2009) composed an extensive review article about fungal endophytes and categorized them into four classes based on transmission, host specificity, how and where they grow in the plant, and benefits to the host plant. I will briefly summarize their work on classes 1, 2, and 3. More detail from other studies regarding class 4 will be included because that is the group this research was focused on. These classes provide a good way to think about the different types, but it is important to understand that the classes are static categories in a plastic world.

Class 1, or Clavicipitaceous endophytes, can be vertically transferred through seed or infect seedlings in the spring with spores. They grow systemically throughout grasses, sedges and rushes. Some class 1 endophytes synthesize alkaloid compounds that help protect the plant from herbivory (Clay 1988). Some of these fungi, including *Claviceps purpurea,* eventually harm the plant because they produce sclerotia in the ovaries thereby reducing the amount of viable seeds. However, unlike *C. purpurea,* species in the tribe Balansieae, including the genera *Balansia*, *Epichloë,* and *Acremonium*, do not form sclerotia within ovaries. Instead they form stromata on leaves and culms while still providing protection against insects and drought (White and Morgan-Jones 1996). Some endophytes, including *Epichloë* spp., can stimulate root hair growth and production of exudates that facilitate phosphorus absorption in grasses (Malinowski and Belesky, 2000).

Class 2 endophytes are transferred to new host plants through underground stems, fallen seed coats, or various types of plant litter. They grow in all types of land plants and grow throughout the plant. Class 2 endophytes provide resistance to various abiotic stresses such as heat, oil, salt, and chemical contaminants such as

heavy metals or pesticides. Furthermore, these endophytes provide site-specific adaptations, e.g. fungi from salty places provide salt tolerance (Rodriguez *et al*. 2008).

Class 3 endophytes grow only in above ground tissues, usually leaves, of all land plant types. Although class 3 endophytes only grow in a limited area of the plant, they can be highly diverse within even a single leaf. The benefits of class 3 endophytes come from a myriad of compounds made by different species as chemical defenses against competing bacteria, fungi, and viruses. These diverse compounds are a novel area for finding new medicines.

Class 4, also called dark septate endophytes (DSE), grow with the roots of all types of land plants. "Dark" refers to the melanized hyphae in many of the species, and "septate" means the hyphae have many segments divided by septa. Many DSE are Ascomycetes in the Helotiales, but DSE is a morphological grouping encompassing a broad phylogeny. Many are imperfect fungi, meaning they do not form sexual structures, and these fungi are nearly impossible to identify visually without sexual or asexual reproductive structures. Nondescript fungi can be identified using DNA sequencing, but they have to first be described and have the sequence in a database. Even with advancements in genetic sequencing, there have not been enough studies to fully evaluate their diversity.

The species *Phialophora fortinii* is the most studied DSE, probably due to its broad range in geography and host plants (Newsham 2011). Phylogenetically it fits within a group of closely related endophytes called the *Phialophora fortinii-Acephala applanata* species complex, a.k.a. PAC (Helotiales). They have been

found from a wide range of host plants from pine trees to liverworts, sometimes forming ectomycorrhizae (Grünig *et al.* 2006). Some of these species increase phosphorus uptake, especially when growing with AMF (Della Monica *et al.* 2015). In general they are believed to provide benefits to host plants, but do fall within the continuum of pathogen to mutualist. The nature of the interaction is highly dependent on fungal species/strain, host plant, and environmental conditions.

Unlike the other endophyte classes, DSE have hyphae that extend beyond the tissues of the plant, allowing for nutrient uptake from the soil. DSE mycelia can live in the soil without a plant host, an important distinction between them and most mycorrhizae, which allows for DSE to grow in more places. Many species exhibit biotrophism, changing how they acquire nutrients based on changes in the host plant, going from a mutualist to a saprotroph when the host plant dies (Sinclair and Cerkauskas 1996). This might be why DSE can be better mutualists, or at least more common, in nutrient deficient habitats where most of the carbon and nitrogen are bound in higher molecular weight organic forms that most plants are not able to uptake. Haselwandter and Read (1982) showed DSE can improve growth and phosphorus levels in sedges.

Dark septate endophytes can provide protection against harmful microbes and arthropods. Sometimes the host plant benefits because fungi occupy space that harmful pathogens could invade, but some fungi are known to act antagonistically against other fungi (Mejía 2008). Similar to class 2 endophytes, DSE increase tolerance to a wide range of stresses such as heat, pH, and metal contamination (Hutton *et al*. 1996, Likar and Regvar 2013, Zhang *et al*. 2008)

The frequency of DSE is higher than that of mycorrhizae in some harsh environments. For example, some Antarctica and arctic regions, as well as some alpine regions are too cold for mycorrhizae (Haselwandter and Read 1980, 1981, Upson 2009). Western Australia, the Great Hungarian Plains, and semi-arid grasslands of Venezuala are dry areas deficient in nutrients with high levels of DSE (Hubbard 1995, Kovacs and Szigetvari 2002, Knapp 2012, Loro *et al*. 2012). Recent studies have discovered new endophyte species growing in dry, sandy habitats in pine barrens in New Jersey (Luo *et* al. 2014, Walsh *et al*. 2015), and semi-arid grasslands in Europe (Knapp 2015).

Many experiments looking at the effects of DSE have inoculated sterile host plants with individual fungal species that were isolated from wild plants (Haselwandter and Read 1982, Carver 2013). Although such studies show the possible benefits DSE can provide to a plant, they do not address the issue that in a natural setting, there are potentially hundreds of species of fungi in a single plant (Arnold and Lutzoni 2007). Some of the fungi are common, some rare, and each fungus has a slightly different effect on the host-plant, along a continuum of parasite to mutualist (Sinclair and Cerkauskas 1996).

There are an estimated 1.5 to 5 million species of fungi and only about 90,000-100,000 are named (Hawksworth 1990). Endophytic fungi in understudied habitats have proven to be a rich source for novel fungi (Luo *et* al. 2014, Walsh *et al*. 2015, Arnold and Lutzoni 2007). There is still a lot of research to be done in naming and describing new endophytic species, considering there are thousands of endophytic fungi in thousands of plants. Plants growing in sand prairies of the Driftless region in

the United States are understudied niches with potential for novel species of fungi. Furthermore, the fungi in sedges growing in sand prairies have not been well studied. (Hastelwandter and Read 1982, Miller *et al.* 1999).

Sedges are a group of grass-like plants, in the family Cyperaceae. Although sedges are sometimes lumped in with grasses for functionality in ecosystems, they should be considered different from a restoration/management perspective because of the difference in fungal associations (Johnson et al. 2004). Other than *Kobresia* spp. forming ectomycorrhizae with *Cortinarius*, *Laccaria* and *Tomentella* (Qian and Yang 2010), sedges were previously thought to be non-mycorrhizal (Haselwandter and Read, 1981; Brundrett 1991). This may because sedges are often the dominant vegetation in various wetland habitats, and most fungi cannot grow in water. Terrestrial species have evolved various adaptations to acquire phosphorus and nitrogen from the soil. Some have dauciform roots that release chemicals into surrounding soil to solubilize phosphorus and increase surface area for absorption with long, fine hairs (Lamont 1974). Most plants can only take in nitrogen in the form of ammonia or nitrate, but several arctic sedge species can utilize forms of organic nitrogen including amino acids and peptides (Chapin *et al*. 1993).

The impressive ability of sedges to adapt to new and challenging environments facilitated speciation in *Carex* (Spalink *et al.* 2016). *Carex* is by far the largest genus in the sedge family Cyperaceae, with over 1,700 species. It is the fifth largest genus of plants. They grow in almost every terrestrial habitat on earth, including extremes such as Antarctica and alpine regions.

Two *Carex* species were found to associate with *Cortinarius* and other ectomycorrhizal fungal genera (Harrington and Mitchell 2002). Although these species were known to be ectomycorrhizal with other plants, they did not form a true Hartig net around roots of *Carex*. Miller *et al*. (1999) examined 23 *Carex* species in Illinois (USA) for mycorrhizae, collecting plants growing in a range from wet to dry. They found some species always form arbuscular mycorrhizae, some never do, and some do depending on conditions. The study also noted the presence of dark septate endophytes growing on the roots of three species growing in sandy upland habitats. Sedges as a whole are able to form various types of endorrhizal associations, so should not be lumped together in such a regard. This should also prompt further investigations into the fungi that associate with various sedge species. *Carex pensylvanica* forms both AMF and DSE and is also one of the most abundant plants found in the sand prairies of Fort McCoy, Wisconsin, my study area.

If you have taken a hike in a forest anywhere between Iowa and Nova Scotia, I would wager you have walked past *Carex pensylvanica*, also known as Penn sedge. Penn sedge is a plant that grows nearly everywhere and many people recognize, but nobody really pays attention to it. It only grows to be about 20cm tall, but forms intricate networks of rhizomes in the upper layer of soil. The flower has male and female florets separate, but one subtending the other on the culm. The base of the culm is fibrous and brick-red. Many botanists associate it with moist woodlands, but because of the exploratory nature of its rhizomes, *C. pensylvanica* is able to grow on rock out-croppings and other bleak strata. It is very common in our part of Wisconsin called the driftless area.

The driftless region in the Midwest United States is an area untouched by ancient glaciers that scraped the surrounding area. When the glaciers melted they formed lakes that drained into rivers that carved the driftless region into bluffs and coulees. The area served as a refugium for plants and animals, now containing endemic species not found elsewhere, such as the Pleistocene snail, *Discus macclintocki* (U.S. Fish and Wildlife Service accessed May 2, 2017). Habitats in the driftless include oak-hickory woodlands, sand prairies, old growth white pine forests, and spring-fed riparian habitats. Fort McCoy is an Army installation in Wisconsin that lies on the eastern edge of the driftless region. Beside the characteristic habitats of the driftless, Fort McCoy also has hardwood forests to the east, pine dominant forests in the north, and oak savannahs in the south.

In terms of wildlife, Fort McCoy is home to many rare, threatened, protected, or endangered species (Tim Wilder pers. comm.). There are bald eagles, grey wolves, Karner blue butterflies, red-tailed prairie leaf-hoppers, regal fritillaries, Blanding's turtles, wood turtles, glass lizards. Rare plants found on Fort McCoy include brittle prickly pear cactus, rough white lettuce, sundew, cream gentian, blue bog grass, and more. There is a lot of restoration work and natural resource management being done, such as invasive plant species management, habitat improvement for endangered butterflies, game fish management, and forestry. The goal of Fort McCoy's natural resources branch is to maintain resilient training grounds for the military, while supporting local biodiversity and wildlife.

Although annual rainfall is higher than that in desert and semi-arid regions, ~71 cm, plus ~99 cm of snow (Fort McCoy Integrated Natural Resources Management

Plan), the high percentage of large particles (sand) in the soil makes sand prairies very dry because the water drains rapidly. The sandy soils on Fort McCoy include military training areas of high disturbance and contamination from a range of metals, plastics, explosives, various chemicals and fuel spills. Despite disturbance and contamination, the military base is home to some of Wisconsin's most pristine remnant sand prairies.

Sand prairies are geographically-limited habitats characterized by sparse vegetation, xeric soils, and periodic fires. Fire suppression from human development and land practices have reduced the amount and quality of these native habitats. The geographic restrictions and changes in land use have made oak barrens a globally imperiled based on rarity (S2) by the Wisconsin National Heritage Inventory. Sand prairies in Wisconsin are often surrounded by and scattered with oak trees (*Quercus ellipsoidalis*, *Q. velutina, Q. alba, Q. macrocarpa, Q. rubra*). Trees in grasslands influence the plant community found beneath them by increasing water retention in the soil with their roots and provide shade, and organic input via leaf litter (Weltzin and Coughenour 1990). Trees also attract animals that defecate and add nutrients into the area. In general, productivity of sub-canopy vegetation increases in hot and dry climates near tropics because the trees relieve drought stress, and decreases in cooler wetter temperate grasslands due to competition for nutrients (Dohn *et al.* 2013).

Trees may also affect the surrounding mycorrhizal community because plant-fungal interactions are influenced by soil nitrogen and phosphorus levels, organic matter, field capacity, and plant community (Treseder 2004). Plants act like energy

accountants that contract fungi to help scavenge soil nutrients. If the resources, like water, nitrogen and phosphorus, are abundantly available to the plant, it won't contract as many fungi to help. Arbuscular mycorrhizal fungal colonization can increase or decrease with more organic matter depending on what compounds make up the organic matter (Gryndler *et al.* 2009).

Plants species form mycorrhizae with certain fungal species, and fungal species form mycorrhizae with certain plant species. Plants with broader geographic ranges form relationships with more species of fungi, and may be the reason such plants can grow in many places. Alternately, some plants, like orchids, are very selective with fungal symbionts and are therefore restricted to growing in places where their fungi are also present (Davis *et al.* 2014). Because of its broad range, I hypothesize that *Carex pensylvanica* associates with a wide variety of fungal symbionts

There are also discrepancies in type of mycorrhizae formed in different larger groups of plants. For example, pine trees (Pineceae) are ectomycorrhizal (ECM) and maples (*Acer* spp.) are endomycorrhizal (Tom Volk pers. comm.). Some plants can form both such as Cottonwood (*Populus deltoides*), but not typically at the same time. Conversely, dark septate endophytes simultaneously cohabit roots with both AMF and ECM (Miller *et al.* 1999, Della Monica *et al.* 2015, Lukešová *et al.* 2015). Because many mycorrhizae propagate through the soil from root to root transfer, I hypothesize that the surrounding plant community influences the fungal community found in *Carex pensylvanica.*

To test the hypotheses that *C. pensylvanica* associates with a many species of fungi and its endorrhizal community is influenced by environmental factors, fungal

community and colonization was analyzed with data on surrounding plant community, organic matter, pH, total nitrogen, phosphorus, and water holding capacity of soils, in roots of *C. pensylvanica* growing in tree-influenced and tree-less sand prairie habitats.

Carex pensylvanica is a good species to study because it is abundant, found in 91% of plots on Fort McCoy sand prairies (Susan Vos, pers. comm.). It is also native, grows in a range of conditions from wet to xeric, does not form dauciform roots, and has been shown to contain dark septate endophytes (Miller *et al*. 1999). My research is in support of Fort McCoy's Integrated Natural Resources Management Plan, especially in reference to sections 3.6.6, 4.5.4.1, 4.5.3, 3.8, 2.6.2.2.1, 2.6.2.2, 3.1.10.1. Summed up, my proposed work supports research about sand prairies and oak barrens, provides insights into biodiversity and possible management efforts to restore disturbed areas resistant to plant establishment.

This study identified fungal species from a sedge species via direct culturing and high throughput Illumina sequencing. Results include root colonization levels of various endorrhizal fungi with insights into how soil conditions affect plant-fungal interactions. These data advance basic science because they provide insight into the mycorrhizal status of *Carex*. More broadly, these findings also shed light on plant-fungal interactions. New insights can also be applied to environmental management and restoration, especially in areas with high disturbance or sandy soils.

METHODS

Root Collection and Preparation

Sites were selected based on my previous knowledge of the area from working as a botanist at Fort McCoy for five years, and communication with one of the Federal Biologists working on Fort McCoy (Tim Wilder). Collections were done May 16, 2016, which is when *C. pensylvanica* flowers in Wisconsin. The 15 sites are sand prairies with surrounding oak barrens of similar age. Plants were collected in pairs, from directly under an oak tree canopy, and a little outside the oaks suspected root area (about 1.5 times the distance from the trunk to the drip line). Pairs were spaced by at least 50 m, and the sites span across 13 km (1 pair from each of 15 sites, n = 15, 30 plants total). Exact plants were determined by tossing a hula-hoop (Wham-o, inside diameter of 82 cm) and harvesting a *Carex pensylvanica* specimen within the hoop. *Carex pensylvanica* is so abundant the hoop almost always landed around at least one. All vegetation within the hula-hoop was identified to genus or species and overall percent ground cover recorded.

Carex ramets were dug out at a diameter of 25.4 cm and a depth of 15 cm, removed with surrounding soil and placed into gallon sized Ziploc bags. Excavated areas were filled in with adjacent plant material to counter disturbance and reduce the chance of invasion of unwanted species.

Samples were immediately transported back to UWL and stored at 4°C. The next morning soils were separated from the roots by hand carefully to avoid damaging the fine roots. The soils were air dried and stored in their collection bags for later analysis. Roots were further cleaned by gently dunking in a beaker of tap water, then rinsing in distilled water. After allowing to air dry, roots were cut into ~1cm segments. Segments were randomly divided into three portions; 20-30 segments per plant for culturing, 40-60 for staining, and 3-4 for Illumina sequencing. Root for Illumina sequencing were stored in silica gel at room temperature. The roots for culturing and staining were placed into VWR Premium Biopsy cassettes, a plastic mesh container. Roots to be stained were stored in 95% ethanol. Roots for culturing were stored at 4°C overnight before culturing the next day.

Direct Culturing

Root segments, still held in cassettes, were surface sterilized by submerging in 90% ethanol for 2 minutes, 5% Sodium hypochlorite (bleach) for 5 minutes, and 70% ethanol for 1 minute, then dunked in distilled water (Carver 2013). Root segments were air dried in a laminar flow hood, rolled on a PDA plate for contamination test, then plated on PDA supplemented with 100mg/L streptomycin (antibacterial) and 30mg/L rose bengal (retards fast-growing fungi, such as Zygomycota). Approximately 18 root segments 0.5-1.5cm long from each plant were plated for a total of 566 segments plated. Plates were examined daily for two weeks and root segments with growth were fully removed and plated onto PDA supplemented only with 100mg/L streptomycin. A total of 336 root segments were

transferred, some of which contained up to three visually different fungi, and allowed to grow for up to six weeks.

The number of fungal morphotypes was recorded for each individual plant and identical morphotypes were tallied to determine which ones were the most abundant. As a measure of isolation frequency, the number of plants in which a morphotype was found was used as opposed to the number of plates found on (e.g. found in three plants once is better than one plant thrice). Code names were given to the most commonly isolated morphotypes for convenience before identification could be made. The 17 morphotypes found in three or more plants (3 was chosen because it represents 10% of samples) were identified to species, visually and using DNA sequencing of the nuclear ribosomal internal transcribed spacer (ITS) region. DNA was extracted from pure cultures using the NaOH procedure of Wang *et al*. (1993) as modified by Osmundson *et al.* (2013). The rDNA ITS region was PCR amplified using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White *et al.* 1990) and sequenced by Sanger sequencing (Eton Bioscience NJ, USA). Sequences were quality trimmed and assembled into contigs using Geneious version 7 (Biomatters Inc.). Sequences were assigned to species using BLAST searches of the Genbank and UNITE (Kõjalg *et al.* 2005) databases.

Staining and Scoring Procedure

Root segments were kept in cassettes during staining for convenience. Staining procedure was modified from Barrow and Aaltonen (2003). Ethanol was rinsed off the roots with tap water before being soaked in 5% (w/v) KOH for 7 minutes at room temperature. The beaker holding the roots and KOH were then placed in a boiling

water bath (BWB) for 6 minutes. Roots were then triple rinsed in tap water before covering with 1% (w/v) HCl for 3 minutes. Roots were rinsed once with tap water before submerging in trypan blue stain (500mg trypan blue in 500ml glycerol, 450ml dH_2O, and 50ml HCl) for 2 minutes at room temp, followed by 3 minutes in BWB. The Trypan blue was rinsed off, and the sample was further washed twice in distilled water in BWB for 3 minutes. The root segments were soaked in Sudan IV at room temp for 2 minutes, then BWB for 3 minutes. To finish, samples were rinsed and stored in distilled water before being made into slides.

Four whole-mount slides were prepared for each plant. Each slide had 5 root segments approximately 1cm long, fixed in clear polyvinyl alcohol-lactic acid-glycerol (PVLG). Following a modified Magnified Intersection Method (McGonigle *et al*. 1990), root segments were pseudorandomly examined 3 times each for 60 observations per plant, and 1800 total. Slides were positioned so that a root section would be in the field of view. Once in place, observations were made through the eyepiece where the cross hairs intersected the root and recorded what the cross hair touched (no fungi, hyaline hyphae, dark septate hyphae, microsclerotia or other fungal structures). Observations were made with a compound light microscope at 400x and 1000x magnification.

DNA Extraction, PCR, and Illumina Sequencing

Root samples (3 random 1cm segments per plant) were removed from the silica and placed into reinforced 2 mL screw-cap tubes with 1 sterile 6 mm-diameter glass bead. Root segments were treated with liquid nitrogen and shaken in a mini bead-beater for one minute. At this point seven of the thirty samples were lost due to

breaking of the tubes. DNA was extracted using the phenol chloroform-GeneClean procedure described by Ivors *et al.* (2004). Samples were amplified using the tagged primer approach from Smith and Peay (2014) and assessed using agarose gel electrophoresis. Three samples were thrown out due to weak or no amplification. The remaining 20 samples were purified using Agencourt AMPure XP beads (Beckman Coulter) at a ratio of 0.7:1 beads to PCR product, quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific), and sequenced using Illumina 2 x 250 TruSeq Nano at the UW Biotechnology Center (Madison, WI).

Soil Tests

pH

For each soil sample collected with every plant, 10g of air dried soil was mixed with 10mL of distilled water, making a 1:1 ratio. Samples were then allowed to sit for 24 hours before remixed immediately before measuring pH with an Orion Star A111 pH meter (Thermo Scientific).

Organic Matter

Measured using Loss of Ignition. For all 30 soil samples collected (done in triplicate), approximately 2.1g air dried soil was weighed out in a small plastic dish. Soil samples were added to pre-weighed crucibles and combined weight recorded. Samples were then placed in an oven overnight at 105°C to evaporate all water, allowed to cool in a dessication chamber, then weighed. Evaporated samples were placed in an oven for 2 hours at 360°C to burn off organic matter. Samples were allowed to cool to room temperature inside the oven before being weighed. Organic matter was then calculated using the equation from Konen *et al*. (2002).

Water Holding Capacity

Water holding capacity was assessed by weight difference between the fully saturated and air dried soil sample. Funnels were lined with a coffee filter that was fully saturated in distilled water and weighed. Approximately 10g of air-dried soil from each sample was weighed and added to the filter lined funnel. Soil samples were then super-saturated to the point of pooling at the top before allowing excess water to drain. After water stopped dripping, the funnels were gently tapped onto a paper towel to remove the residual couple drops of water that stick to the funnel before a final weighing. Water holding capacity = (weight of saturated soil - weight of dry soil)/weight of dry soil.

Phosphorus and Total Nitrogen

Samples were sent to the University of Wisconsin Soils and Forage Lab to be tested for phosphorus via Bray and Kjeldahl total nitrogen. Total nitrogen was measured as an overall indicator of soil fertility. Fungi are able to utilize nitrogen sources plants cannot, so total nitrogen might be a stronger predictor of plant-fungal relationships than than ammonium and nitrate levels.

Data Analysis

Two-sample t-tests were performed using IBM SPSS Statistics 24 to compare means (pH, percent organic matter, water holding capacity, number of plants with presence of Basidiomycota, total fungal colonization, DSE colonization, hyaline hyphal colonization) between tree and no-tree sites. Linear regressions were run to analyze relationships among different soil properties and colonization rates across all sample sites treated independently.

Illumina sequences were quality trimmed and filtered, clustered into Operational Taxonomic Units (OTU; proxy for species in molecular analysis), and identified to taxonomic hierarchical level using Amplicon tool kit (AMPtk 0.4.6; https://github.com/nextgenusfs/amptk). Quality trimming and filtering of demultiplexed data were performed using the Illumina script with default settings. OTU clustering was performed using a 97% similarity threshold and expected error quality trimming with the default setting (1.0). Because sequencing error can result in creation of spurious OTUs, OTUs represented by less than 10 sequences across the entire dataset were omitted from further analysis. Sensitivity of OTU count to minimum sequence threshold was assessed by performing clustering using various minimum sequence representation values from 2 to 100. A steep drop in total OTUs was observed when using a threshold above the default of 2, but leveling out around 10-15 (Appendix A). The resulting OTU table was filtered using a 0.5% index bleed filter. Taxonomy was assigned to OTUs using USEARCH 9. The OTU and taxonomy tables from AMPtk were used as input for diversity analyses using QIIME (Caporaso *et al.* 2010). Alpha diversity was compared across samples using observed OTUs as the diversity metric. Beta diversity was compared using the binary Lennon metric and the resulting distance matrix was used to construct Principle Coordinate Analysis (PCoA) plots.

RESULTS

Direct Culturing

Surface sterilization was confirmed by the test plates (not shown). Seventy morphotypes were sorted out of the 366 fungal isolates from direct culturing. Of those 70, 18 were found in more than one plant. The 17 morphotypes that were found in 10% (3 plants) or more were identified (Fig. 1, Appendix B). *Fusarium oxysporum* was the species most commonly found in both the most plants and the most root segments. Most commonly cultured fungi morphotype code names and plant tally data are in Appendix B.

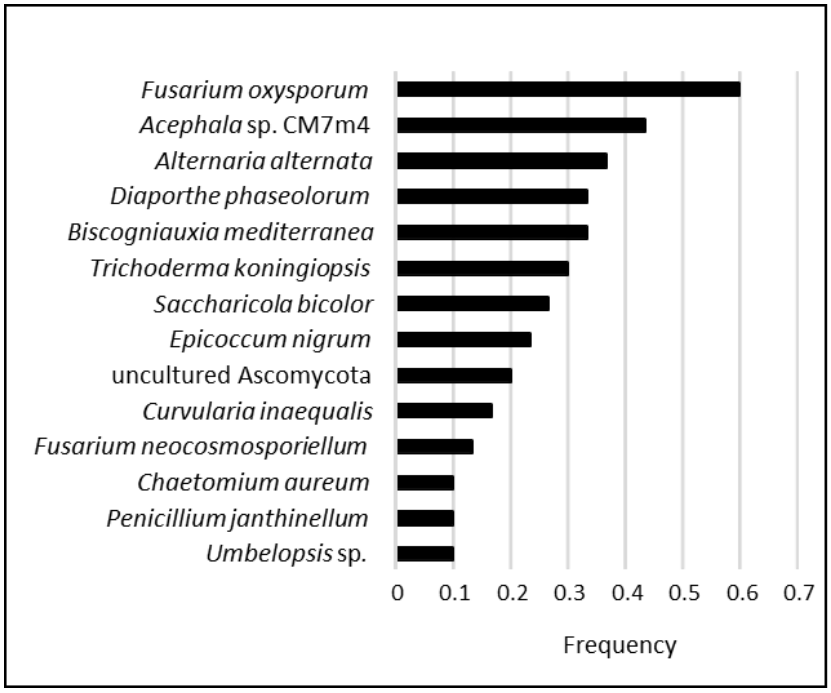

Figure 1. Frequency of most commonly isolated fungal root endophytes from *Carex pensylvanica* via direct culturing. Measured by number of plants found in/number of plants samples. Plants were collected from Fort McCoy, Wisconsin. (n=30)

New Species

Because of the occurrence of *Acephala* sp. CM7m4, I plan on naming and describing it as a new species: *Acephala harenae nom*. *prov*. It is a dematiaceous ascomycete in the Helotiales, closely related to the *Phialocephala fortinii-Acephala applanata* species complex. Microscopically in culture it is highly septate and forms numerous club-shaped hyphal projections, often containing oil drops (Fig. 2). These projections appear walled off from the hyphae and occur sometimes in opposite arrangement, indicating these are not branches. These projections can "germinate" while still attached and grow to anastomose, fusing with neighboring hyphae. The club-shaped projections may play a role in propagation similar to chlamydospores. They are not chlamydospores because they are not intercalary and nor do they have

thick walls. When grown on PDA, *A. harenae* produces a clear-tan exudate, possibly the cause of the warty appearance of some hyphae (Fig 2.d.). It was isolated from roots of graminoid plants in sandy grassland/savannah habitats in Wisconsin and New Jersey, but its distribution may be wider.

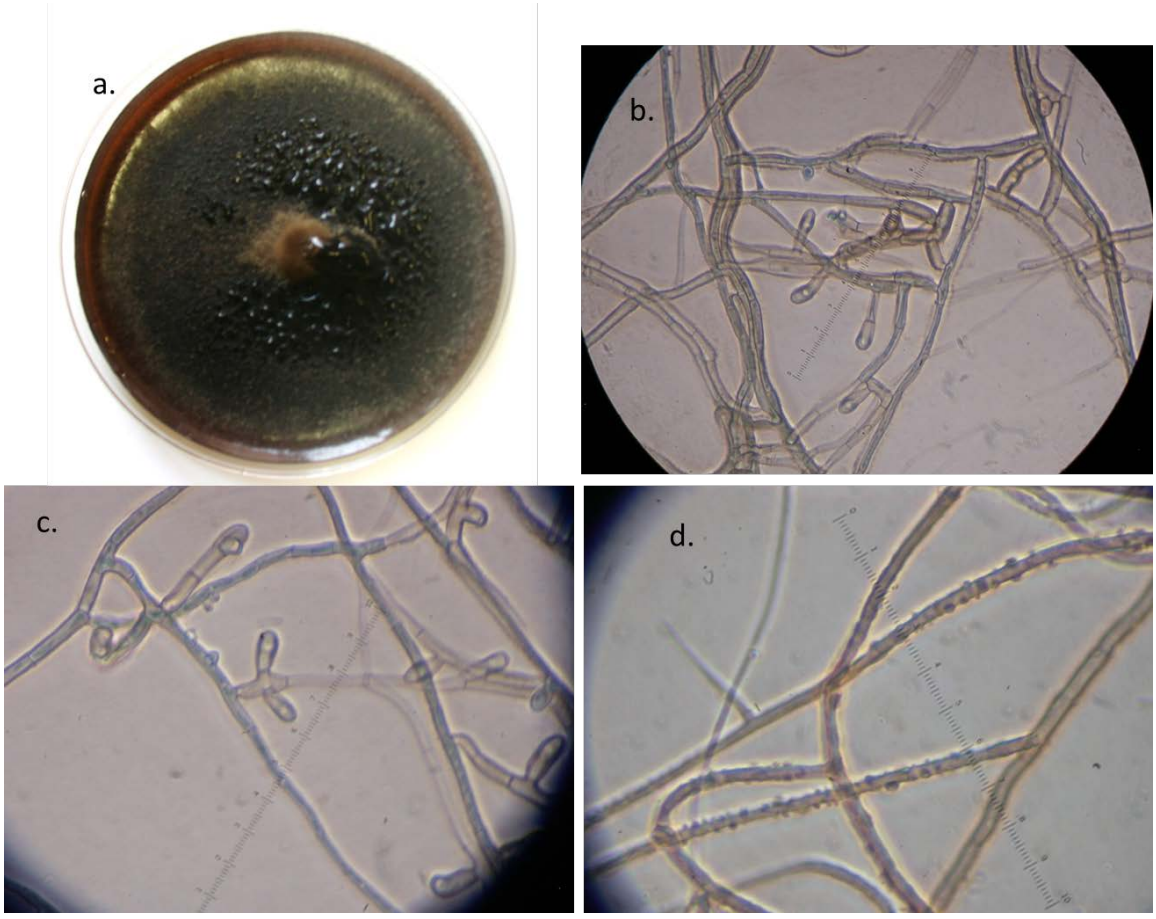

Figure 2. *Acephala harenae nom. prov.*. a. Colony on PDA after 30d. b. Hyphae and club-shaped projections, c. Opposite branching of club-shaped projections. d. Warty hyphae. The "warts" are thought to be an exudate. Images b, c, and d photographed under 400x magnification (1 micrometer tic mark=2.5µm).

Microscopic Root Colonization

About 58% (1061/1837) of root intersections examined had presence of fungal structures (Fig. 3). Dark septate hyphae were the most abundant fungal structures,

outnumbering hyaline hyphae almost two fold (656/325). Surprisingly, clamped pseudo-ectomycorrhizae (Fig. 4) were found in 1.4% of intersections examined (Fig. 3) and from 9 of the 30 plants. Six of the nine plants with pseudo-ectomycorrizae were tree sites. The hyphae were visually identified as *Tomentella* sp. by Thomas Volk and Scott Redhead, and supported by DNA sequence (Illumina) data. Cohabitation of AMF, DSE and ECM was observed (Fig. 5 & 6).

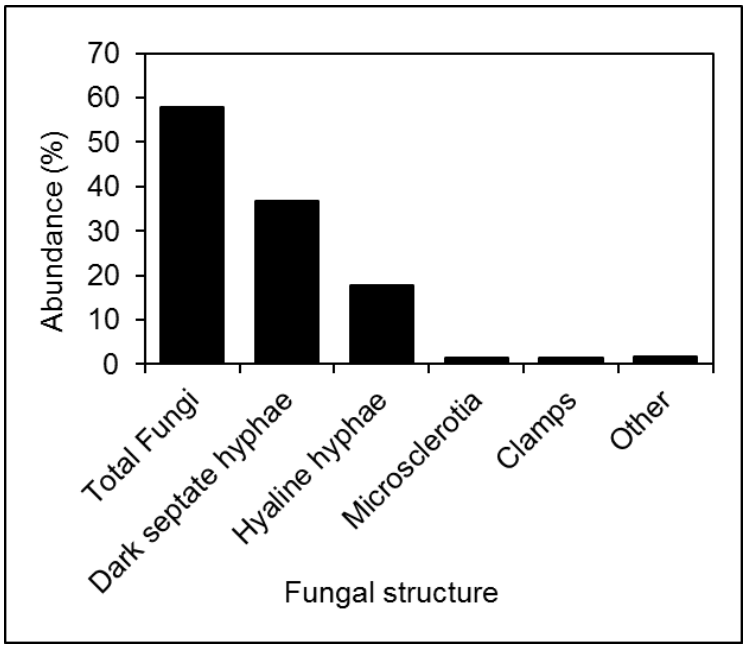

Figure 3. Abundance of various fungal structures in *Carex pensylvanica* roots collected from Fort McCoy, Wisconsin. Thirty plants were observed 60 times each, totaling 1800 observations. Abundance measured as total number of times observed over total number of observations, then converted to percentage.

Figure 4. Pseudo-ectomycorrhizae surrounding root of *Carex pensylvanica* collected from Fort McCoy, Wisconsin. Image on right taken at 1000x magnification (1 micrometer tic mark=1µm) to show clamp connections indicative of Basidiomycota, probably *Tomentella*.

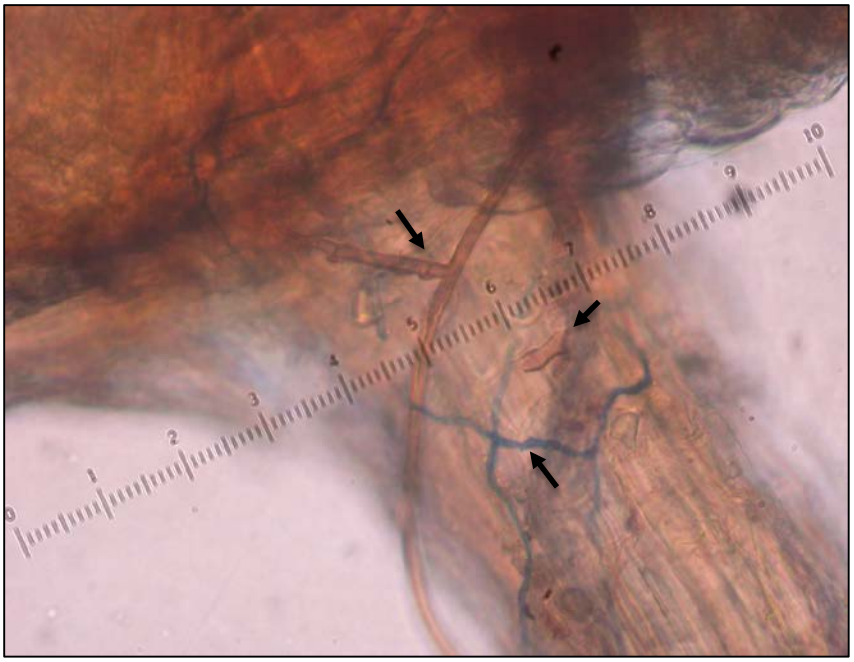

Figure 5. Root of *Carex pensylvanica* showing cohabitation of pseudo-ectomycorrhizae (upper arrow), dark septate endophytes (middle arrow), and arbuscular mycorrhizal fungi (lower arrow). Roots were dual stained with Trypan blue and Sudan IV. Image taken under 400x magnification (1 micrometer tic mark=2.5µm).

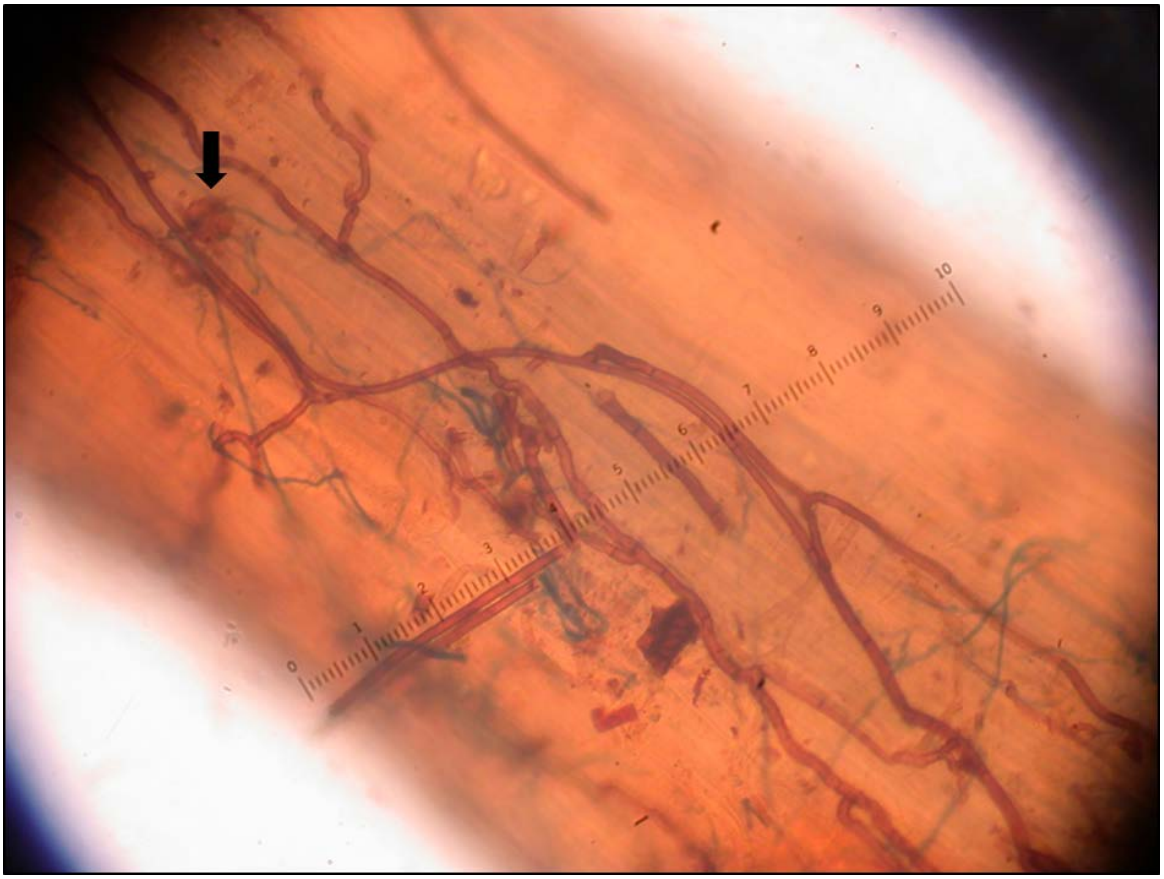

Figure 6. Root section of *Carex pensylvanica* with abundant fungal colonization. Both dark septate endophytes (brown hyphae) and arbuscular mycorrhizae (blue hyphae) are present. Arrow pointing at a vesicle. Photographed under 400x magnification (1 micrometer tic mark=2.5µm).

Statistical Tests

There was no significant difference between tree and no tree sites for soil pH, water holding capacity, percent organic matter, total nitrogen or phosphorus (Table 1; Fig. 7). Raw soil data is in Appendix C. There were no significant difference between tree and no tree sites for total fungal colonization (t_{14}=0.803, P=0.435), DSE colonization (t_{14}=0.003, P=0.998), or hyaline hyphae colonization (t_{14}=1.684, P=0.114).

Table 1. Results of paired t-test of soil factors comparing tree and no tree sites. Soils were collected from sand prairies on Fort McCoy, Wisconsin, May 2016.

Factor	t_{14}	P value
pH	0.734	0.475
organic matter	1.185	0.256
total nitrogen	1.008	0.330
phosphorus	1.356	0.197
water holding capacity	0.262	0.797

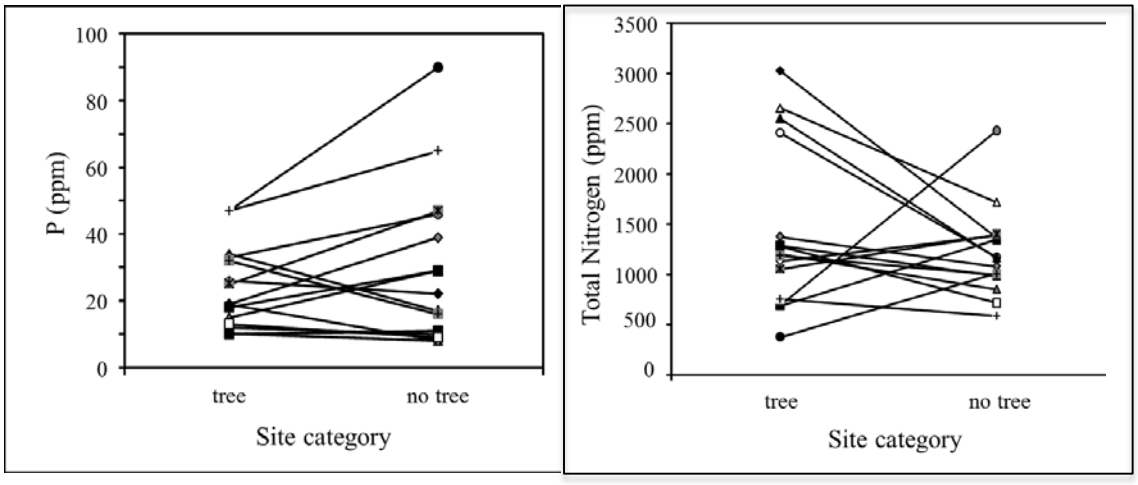

Figure 7. Soil phosphorus and total nitrogen levels in paired tree vs. no tree sites on Fort McCoy, Wisconsin. Pairs differ in how they compare. Soils were collected from just under an oak tree, and just past the estimated root zone. (n=15)

Total fungal colonization levels did not significantly correlate with any soil factors. Water holding capacity had a borderline significant positive relationship with fungal colonization, but the R^2 was low ($y = 77.811x + 27.718$, $t = 1.761$, $n = 30$, $P = 0.089$, $R^2 = 0.0998$) (Fig. 8). Although they only accounted for about 14% of the variability each, pH increased and organic matter decreased hyaline hyphae colonization. (pH: $y = -0.865x + 0.6762$, $t = -2.166$, $n = 30$, $P = 0.039$, $R^2 = 0.1435$)

(OM: y = 0.0282x + 0.138, t = 2.089, n = 30, P = 0.046, R^2 = 0.1349) (Fig. 9). Compared to hyaline hyphae, dark septate endophyte colonization showed an opposing, weakly negative trend with pH (y = 0.0966x – 0.1512, t = 1.775, n = 30, P = 0.087, R^2 = 0.1012) and was unaffected by organic matter (y = 0.0022x + 0.3633, t = -0.116, n = 30, P= 0.908, R^2 = 0.0005) (Fig. 10). None of the measured types of fungal colonization were shown to be significantly influenced by phosphorus, total nitrogen or water holding capacity (not shown).

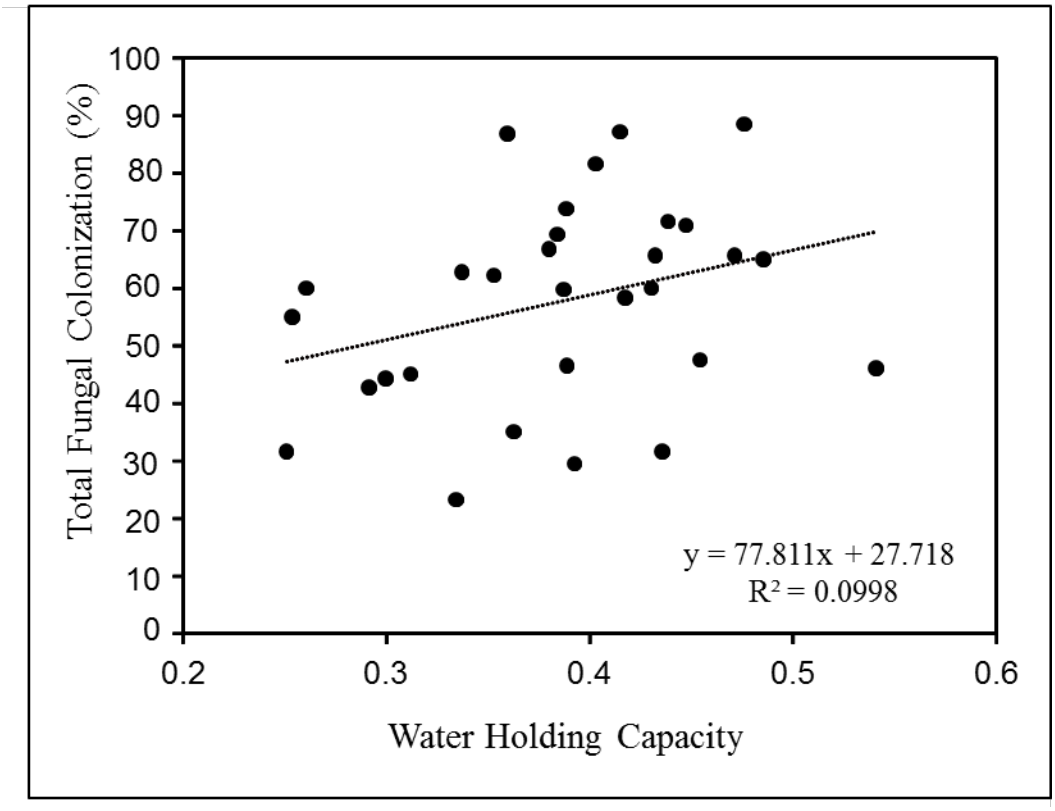

Figure 8. Total fungal colonization (number of intersections found in/number observed) of *Carex pensylvanica* roots and water holding capacity measured as grams of water held per gram of soil. Plants and soil collected from Fort McCoy, Wisconsin (P=0.089)

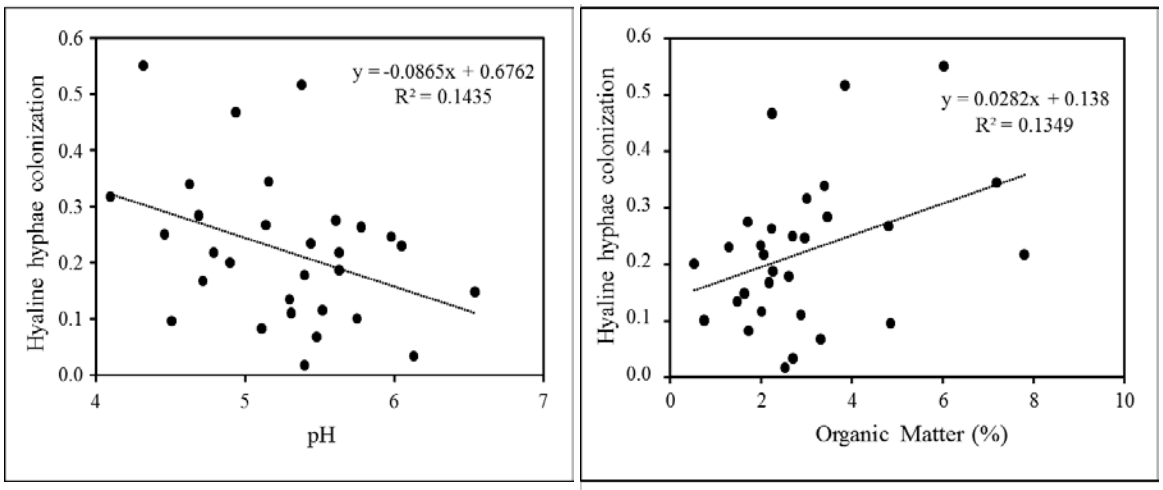

Figure 9. Hyaline hyphae colonization (number of intersections found in/number of observations) of *Carex pensylvanica* roots collected from Fort McCoy, Wisconsin compared to soil pH (left, P=0.039) and soil organic matter (right, P=0.046).

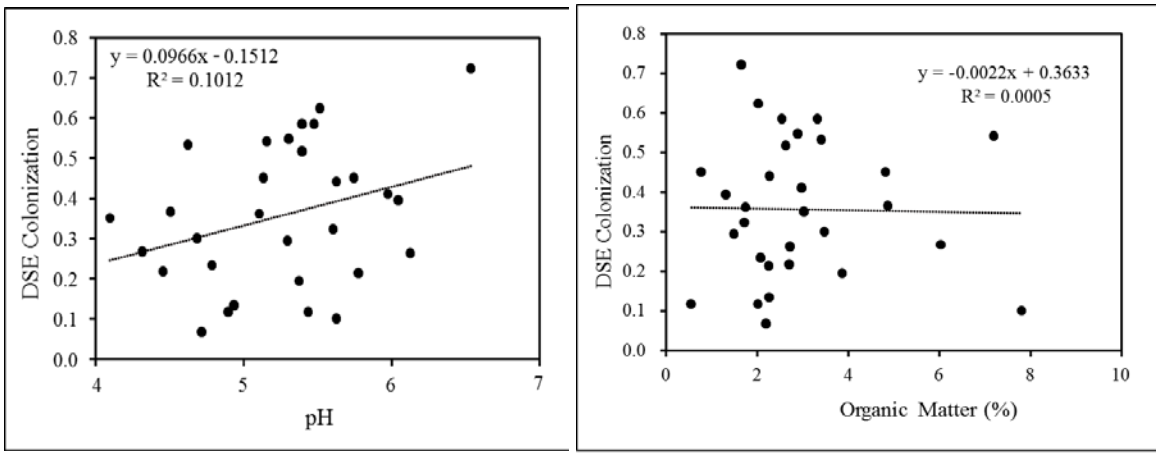

Figure 10. Dark septate endophyte (DSE) colonization (number of intersections found in/number of observations) of *Carex pensylvanica* roots collected from Fort McCoy, Wisconsin compared to soil pH (left, P=0.087) and soil organic matter (right, P=0.908).

Illumina Sequencing

We received data on only 18 of the 20 sample we sent in. A total of 363 OTUs were recovered using the minimum sequence representation threshold of 10.

Fourteen OTUs were found in all plants (Table 2), including OTU10=*Acephala harenae nom*. *prov*. Seventy-five of the 362 Fungal OTUs were found in 50% of plants sampled (Appendix D). Surprisingly, species in the ectomycorrhizal genera *Russula*, *Laccaria*, and *Tomentella*, were each found in all plants sampled. Assigned taxonomy and plant-presence count for all 362 OTUs analyzed are in Appendix D. No significant clustering was shown when comparing tree to no-tree sites (Fig. 11) or pH (Fig. 11). Site 3B was an outlier in all statistical PCoA charts examined (not shown).

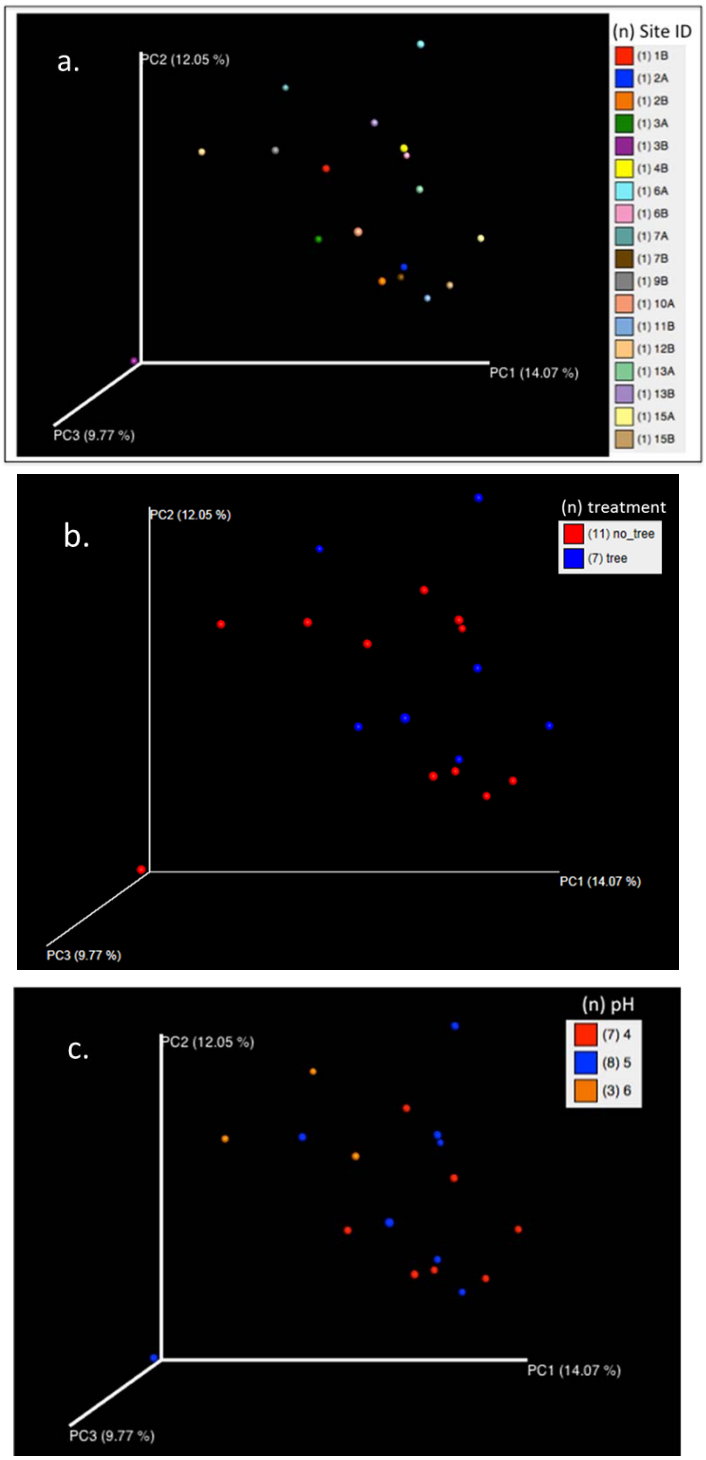

Figure 11. Principle Coordinate Analysis (PCoA) plots comparing beta diversity of endorrhizal fungi from *Carex pensylvanica* roots from Wisconsin sand prairies based on: a. site ID, b. tree vs. no tree treatment, c. soil pH. Distances calculated from presence-absence matrix of operational taxonomic units determined from the Illumina sequence dataset using UClust 9, implemented in AMPtk. PCoA conducted using QIIME.

Table 2. Taxonomy and nutritional mode of endorrhizal fungi found in all *Carex pensylvanica* sampled from Wisconsin sand prairies via Illumina sequencing.

OTU #	Taxonomy	Nutritional mode
OTU1	Dothideomycetes	?
OTU2	Helotiales	?
OTU3	Fungi	?
OTU4	*Marasmiellus tricolor*	saprotrophic
OTU5	Helotiales	?
OTU6	Pleosporales	?
OTU7	*Tomentella ferruginea*	ectomycorrhizal
OTU10	*Acephala harenae nom. prov.**	root-endophyte
OTU13	Ascomycota	?
OTU16	Hyaloscyphaceae	saprotrophic
OTU20	Fungi	?
OTU73	Ascomycota	?
OTU267	Atheliaceae	saprotrophic
OTU303	Fungi	?

*new species from this study ?=Taxon too broad to determine

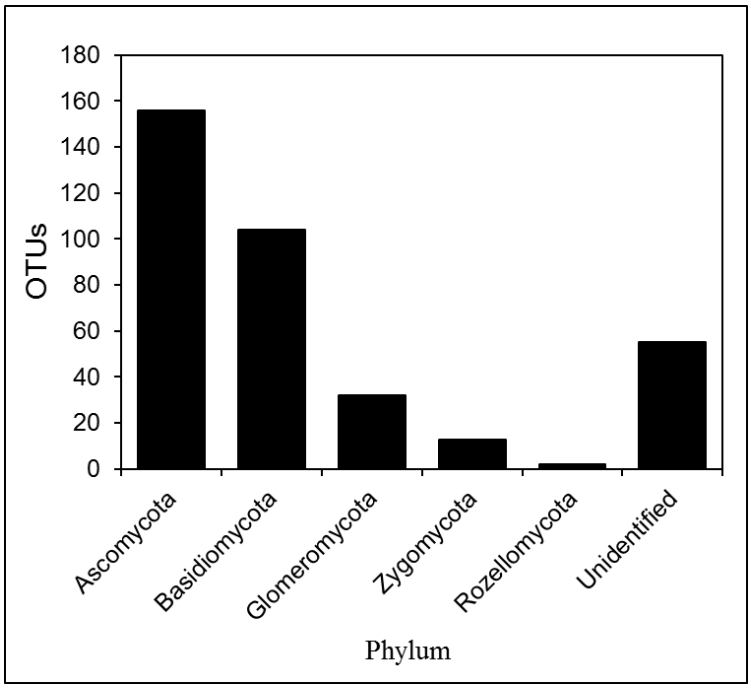

Figure 12. Total number of OTUs in each fungal phylum found in *Carex pensylvanica* roots from sand prairies in Fort McCoy, Wisconsin via Illumina sequencing. (n=18)

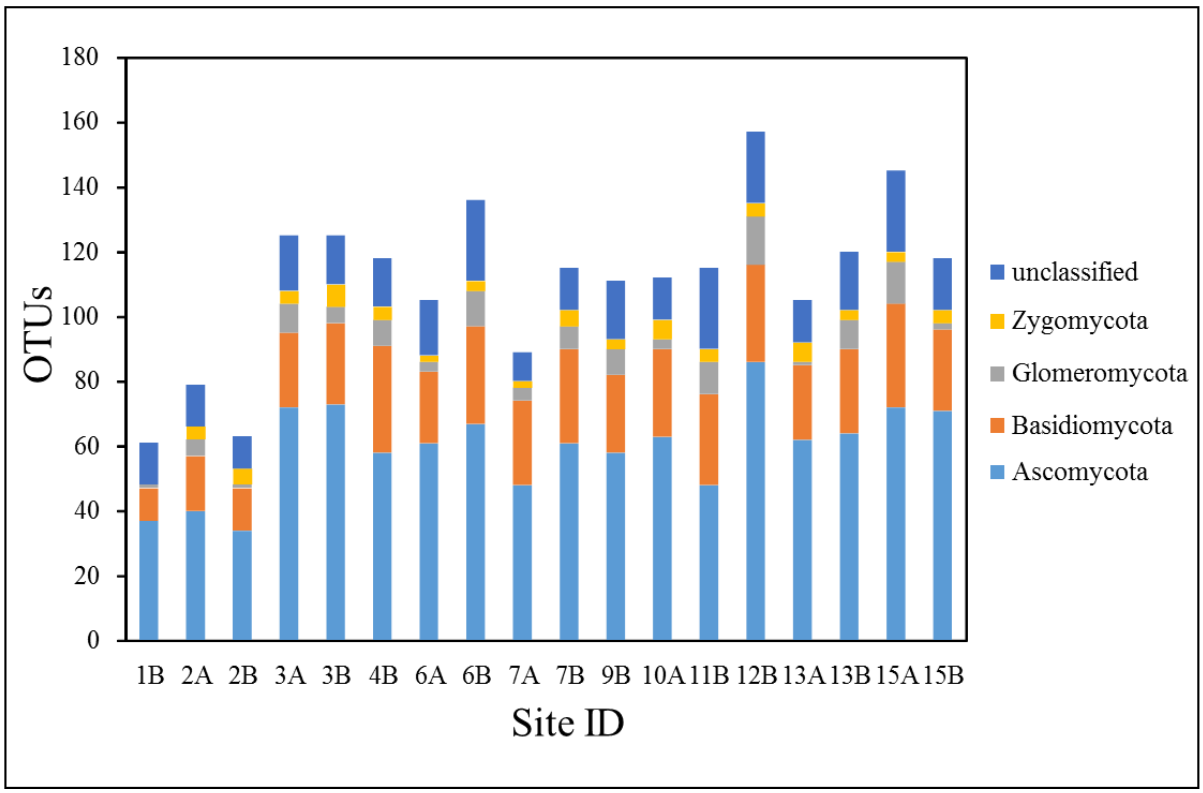

Figure 13. Fungal phyla OTU counts found in each individual plant (1 plant per site, A=tree, B=no tree). Data from Illumina sequencing of *Carex pensylvanica* roots from sand prairies in Fort McCoy, Wisconsin.

DISCUSSION

Wisconsin sand prairies are rare habitats characterized by sandy soils and sparse vegetation. Previous studies indicate that arbuscular mycorrhizae positively influence plant growth in soils with high sand content; however, *Carex pensylvanica*, the most abundant sedge in sand prairies on Fort McCoy Wisconsin, has generally been considered facultatively mycorrhizal with low colonization frequency. To better understand plant-fungal associations that may promote *C. pensylvanica* colonization in sand prairies, we characterized the diversity of endorrhizal fungi using culture-based and molecular approaches, as well as microscopy. Plants were collected in pairs along sand prairie/oak barren ecotones on Fort McCoy Army Installation. Culturing data revealed 70 morphotypes of fungi, while Illlumina sequencing of roots showed 362 OTUs, the most common species being *Acephala harenae nom. prov.* Cohabitation of dark septate endophytes, arbuscular mycorrhizal fungi, and ectomycorrhizae was determined within ~1cm root sections using light microscopy and Illumina sequencing. *Tomentella ferruginea, Russula,* and *Laccaria* were present in all samples sequenced. Ascomycota consisted mainly of Helotiales. There was no significant difference in environmental factors or fungal community structure between tree and no tree sites. Soil data show that pH and organic matter may have some weak influence on fungal community structure, the cause of which is unclear. To-date, this is the most in-depth

survey of root-associated fungi in any *Carex* species. Results provide insights into the diversity of fungi associated with sedges and the soil factors affecting fungal colonization in plants in sand prairie habitats.

Fungi from Direct Culturing

It is not surprising that *Fusarium oxysporum* was the most commonly isolated endophyte (Fig. 1). This is because *F. oxysporum* has been found to be endophytic in a wide variety of plants from across the globe, including lawn grass in Malaysia (Zakaria and Ning 2013), *Populus* species across the USA (Bonito *et al.* 2016), and in bananas in Uganda (Athman *et al.* 2006). *Fusarium oxysporum* was also the most commonly isolated endophyte from 23 plant species growing in sandy soils on the Mediterranean coastline (Maciá-Vicente 2008). In addition, three of the morphotypes I separated based on texture, subtle colors and sexual reproductive structures aligned with BLAST matches for *F. oxysporum,* but with slightly different accuracy. Such species are considered to be in a "species complex," which is a group of closely related species with closely conserved regions of DNA. *Fusarium* species are especially difficult to distinguish using the ITS region (David Geiser, pers. comm.), which is the region we used in this study. The true abundance of *F. oxysporum* may be lower than results from direct culture indicate (Bonito *et al.* 2016), but the frequency was recorded as number of plants isolated from, not number of times isolated. In contrast *Fusarium* was not the most prevalent taxon found in the Illumina sequence dataset, suggesting over-representation in culture data because it grows well in culture, or underrepresented in sequence data due to poor amplification.

Endophytic *F. oxysporum* is being used as an inexpensive way to help protect bananas against nematode damage, but it can be pathogenic on other plant species (Michielse and Rep 2009). As is true with most endophytes, it spans the continuum of pathogen to mutualist depending on plant host and growing conditions. It is particularly difficult to ascertain the relationship between *C. pensylvanica* and the fungi within it because this sedge grows in high abundance in a relatively large geographic range. Plants growing in high abundance typically have better resistance to pathogens (Klironomos 2002). The fungi found in *Carex pensylvanica* could be pathogens that are being held in check by the plant.

The second most common fungal isolate, codename Medusa, was *Acephala harenae nom. prov.* (a.k.a. *Acephala.* sp. CM7m4. (Luo *et al*. 2014)) It was originally found in Switch grass (*Panicum virgatum*) roots in New Jersey pine barrens (Luo *et* al. 2014), a sandy habitat similar to sand prairies. Nine of the 13 plants it was isolated from in my study were no tree sites. Illumina sequencing detected the same species in all 18 plants sequenced. These data suggest that this undescribed species is associated with graminoid roots in sandy savannah-like habitats. *Acephala* is related to *Phialocephala* in the PAC, which are generally believed to be beneficial endophytes in a wide variety of plants (Grünig *et al.* 2006, Lukešová *et al.* 2015). All of these factors lead me to think this species acts as a mutualist. *Phialocephala fortinii* was also found in 10 of the 18 plants sequenced.

Some of the most commonly isolated fungi have been found in various plants from around the globe. *Fimetariella rabenhorstii* was found in roots of diseased white fir (Jankowiak,R., Bilanski,P. and Paluch,J., unpublished work cited in the

NCBI flatfile for GenBank accession KU516462). *Trichoderma koningiopsis* was isolated from *Theobroma* in Ecuador (Evans *et al.* 2003). *Diaporthe phaseolorum* causes disease in soybean (Sinclair and Cerkauskas 1996). *Saccharicola bicolor* was found in roots of *Phragmites australis* (a grass) in the Great Lakes region, and as a root endophyte in the same New Jersey pine barren study where *A. harenae* was first isolated (Clay *et al.* 2016, Luo *et al.* 2014). Many of these species are known to cause disease in the plants they were found in, but that does not mean they are pathogenic on *C. pensylvanica*.

One of the more commonly isolated fungi, *Biscognauxia mediterranea*, is an oak pathogen causing Charcoal disease (Luchi *et al.* 2005) and was more common in tree sites (6 of 9 from tree sites). Charcoal disease is not of much concern on Fort McCoy (Charles Menzel, pers. comm.), but this insight should make foresters consider alternative plant hosts as reservoirs for disease. A common technique for managing tree pathogens, such as oak wilt, is digging trenches around trees to prevent root-root spreading. If other plant species contain the pathogen, the trench lines might need to be planned more carefully.

Further tests would have to be done to determine the true nature of the relationship between *Carex pensylvanica* and the fungi found in it. Greenhouse experiments could be done growing *C. pensylvanica* in sterile soils and soils inoculated with different endophyte isolates and tested for a number of factors including growth, root/shoot biomass, shoot phosphorus levels, pathogen resistance, and more. The endophytes could also be grown with other plant species to see how they interact with other host plants.

I would be most curious to see how *Acephala harenae nom. prov.* affects growth in other grasses. For agricultural purposes, I would try growing it with corn, and for ecological restoration purposes I would use *Andropogon gerardii* (big blue stem) and *Schizachyrium scoparium* (little blue stem). Some species in the *Phialocephala fortinii-Acephala applanata* species complex (PAC) form ectomycorrhizae with various trees (Lukešová *et al.* 2015). Therefore it is possible *A. harenae nom. prov.* associates with all types of plants but so far has only been found in graminoid plant roots.

Microscopic Root Colonization

When measuring the level of abundance, I chose to look at number of sections found in and not try to visually assess the amount of colonization at each observation. Collecting tally data is not exactly a measurement of percent colonization because one hypha scores the same as many hyphae. However, it is a fairly unbiased way to compare abundance of various fungi.

The categories I used when recording fungal colonization were no fungi, hyaline hyphae, dark septate endophytes, microsclerotia and other. “Other” included clamped hyphae, vesicles, spores or unidentified fungal structures, all of which were recorded by category when tallied. Hyaline hyphae represent AMF, but likely included non-dark septate endophytes that could potentially be pathogens. Although some of these fungi may be pathogens, only healthy plants in bloom were collected to avoid diseased roots. With this caveat, I analyzed hyaline hyphae as if they were AMF, or at least a type of endorrhiza distinct from DSE. This is supported by

opposing trends in colonization levels of hyaline hyphae and DSE with different edaphic factors (Fig. 5-8).

We suspected there would be relatively high levels of fungal colonization in this habitat because depauperate soils usually correlate with increased plant reliance on mycorrhizae. Our suspicions were correct; the average total fungal colonization was 57.8% (Fig. 3). Precisely 1061/1837 random cross sections examined had some sort of fungal structure, many times with lots of hyphae (Fig. 4, 5, & 6).

Dark septate endophytes were the most abundant fungal structures found within *C. pensylvanica* roots from Wisconsin sand prairies (Fig. 2). The average level of DSE colonization was 36.7% and ranged from 10-72% within individual plants. We collected from sand prairies in particular because the study from Miller *et al.* (1999) reported finding DSE in sandy upland habitats. My data confirm that DSE are prevalent in sandy grasslands.

Fungal communities change throughout the growing season, so collection timing can have an impact on results. DSE communities do not fluctuate much, but AMF levels increase in the summer and are lower in spring and fall (Lingfei *et al.* 2015). I collected during late spring, and I speculate the community might differ slightly by having more AMF if we sampled mid-summer. We timed collections to the flowering period of *Carex pensylvanica* for help with plant identification and as a metric only harvesting healthy mature plants.

One of the most significant and interesting findings from this research was discovering the presence of pseudo-ectomycorrhizal Basidiomycota in *Carex pensylvanica* for the first time. The term pseudo-ectomycorrhizae has been used

before to describe similar fungal morphology without formation of a Hartig net (Pacioni *et al.* 2014). Plants were collected in the spring, it is possible that this morphology is early stage development of a mantle and Hartig net (Tom Volk pers. comm.). The ectomycorrhizal taxa found in all plants sequenced, *Russula*, *Laccaria* and *Tomentella*, typically associate with oaks (Binion *et al.* 2008). Therefore it is likely that these fungi would form true ectomycorrhizae on oak roots.

Previous studies looking at fungi in roots of sedges have sometimes suggested cohabitation of multiple types of mycorrhizae, but not confirmed. I observed cohabitation of dark septate endophytes, arbuscular mycorrhizal fungi and ectomycorrhizae (Fig. 5) demonstrating that sedges can form several types of endorrhizae, sometimes all at the same time. This has interesting implications because plants are typically considered being either ecto- or endomycorrhizal, sometime switching, but usually not together at the same time. These results also add to previous data showing DSE cohabitation with both AMF and ECM in other plants (Della Monica *et al*. 2015, Lukešová *et al.* 2015).

Ectomycorrhizal fungi were more common in tree sites, suggesting that sedges can be colonized oak mycorrhizae. Of the nine plants where ECM were visually observed, seven were tree sites. It is tempting to extrapolate the scope of inference of these findings and say oaks and sedges share fungi, or fungi might act as nutrient conduit between oaks and sedges, but further studies using radiolabeled carbon would have to be done to confirm this idea.

Trends in Soil Data

When I compared tree to no tree sites there was no significant differences between soil factors or colonization levels of various endorrhizae (Table 1). The variability in soils is more likely due to heterogeneity in the landscape, not influence from oak trees. For example there were often dramatic differences between tree and no tree sites, but different locations had opposing trends (Fig. 7). This lack of consistent differences in soil conditions between tree and no tree sites was unexpected. Lack of significant trends between tree and no tree sites led us to examine each sample as an independent site. We looked for correlations between specific soil factors and colonization levels of various fungi.

Of all the soil factors analyzed, pH showed the most significant correlations with hyaline hyphae colonization and DSE colonization (Fig. 9 & 10). Dark septate endophyte colonization increased with pH and hyaline hyphae (representing AMF) colonization tended to decrease as pH increased (Fig. 9 & 10). These data suggest that the fungal community shifts from more DSE to more AMF as pH goes from acidic to neutral.

This hypothesis could be tested by comparing endorrhizal fungi in plants grown in soils with different pH levels. One could start with homogenized soil and treat different batches with sulfur or lime to manipulate pH levels ranging from acidic to basic (pH from 4-8 is typical for soils). Then after growing in a greenhouse for a certain amount of time, maybe 60-90 days, harvest and measure amounts of DSE and AMF colonization.

Organic matter (OM) is a mixture of various biological compounds that have been partially degraded. A previous study in Fort McCoy sand prairies showed that available nitrogen accounted for only about 2% of total nitrogen (Harried 2016). Thus organic matter is the major reservoir of nitrogen in soils. Organic matter also helps soil retain water, influencing water holding capacity (data not shown). Organic matter was only significantly correlated with hyaline hyphae levels (Fig. 9). Because of its influence on other soil factors, organic matter should be considered an important factor influencing soil-plant-fungus interactions.

Water holding capacity of soil affects the water availability for plants. Water availability is one of the factors influencing fungal colonization in roots. Certain *Carex* species form mycorrhizae when growing in dry habitats and no mycorrhizae when growing in wet habitats (Miller *et al.* 1999). My data showed fungal colonization increasing with water holding capacity (Fig. 8), but fungal colonization compared to the full spectrum of soil moisture is more of a bell curve where too dry or too wet do not harbor as many fungi.

Nitrogen is typically the limiting nutrient for plants in terrestrial systems. Not only is the amount of nitrogen important, but the form of nitrogen plays a big role in plant-fungal-soil interactions. Plants can acquire some forms of nitrogen from soils alone, but through mycorrhizae they are able to utilize a wider variety of nitrogen sources (Shah *et al.* 2016). We looked at total nitrogen, not available nitrogen (ammonium and nitrate), as an overall indicator of soil fertility. Total fungal colonization, DSE colonization and hyaline hyphae were not significantly correlated with total nitrogen ($P=0.197$, $P=0.876$, $P=0.122$).

Although phosphorus levels are believed to influence plants reliance on mycorrhizae, my data showed no significant correlation between phosphorus level and fungal colonization (Total fungi P=0.959; DSE P=0.291; Hyaline hyphae P=0.181). Phosphorus might not be a limiting factor in the sand prairies sampled in this study. Data showed trends similar to pH, but that is expected because phosphorus availability is dictated by pH. Phosphorus adsorbs to soil particles very tightly in acidic and basic soils (pH<3.5 and pH>8), but is much more bioavailable to plants and other organisms at a pH around 6.5.

Illumina Sequencing

Due to issues from tubes breaking (7/30) and poor amplification/detection (3/30), only 20 of the 30 plants sampled were Illumina sequenced for fungi. We only received data on 18 of the 20 samples we sent in, further restricting our analysis. Only seven of the original fifteen paired samples remain (Sites 2, 3, 4, 6, 7, 13, 15). Some paired samples, including 3A and 3B, had similar fungal community at the phylum level (Fig. 13), indicating that fungal community maybe spatially influenced. Concurrently, tree sites did not have significantly more similar fungal communities than no tree sites (Fig. 11.b), as expected because there were no significant differences between tree and no tree sites for anything else measured. When choosing an OTU minimum sequence representation threshold two main factors need to be considered. The threshold needs to be high enough to disregard chimeric abnormalities not representing actual species. On the other hand, if the threshold is too high you lose rare or poorly PCR amplified species that are actually present. For our OTU clustering threshold we choose to disregard sequences found

less than 10 times in all samples sequenced. This gave us a total of 363 OTUs, one of which was thrown out because it was assigned to a Cercozoan taxon, which is not fungal. The assigned taxonomy and plant presence count for all 362 OTUs analyzed are in Appendix D.

A primary goal of this study was to determine fungal species associated with *C. pensylvanica* roots in sand prairies. Although the results provide an in-depth fungal profile with several unexpected results, a number of questions remain. One of these questions is the degree to which the fungi isolated in this study are associated specifically with *C. pensylvanica*, sand prairie habitats, or both. The fungi could be more broadly associated with *Carex*, or grass-like plants, or all plant types. It is also likely some of the fungi are more associated with sandy grasslands in general. In fact, endophytes are known to confer site-specific stress tolerance to host plants (Rodriguez *et al*. 2008). I think that *C. pensylvanica* probably associates with similar genera of ectomycorrhizal fungi, *Tomentella*, *Laccaria*, *Russula*, and *Cortinarius*, across its geographic range, but species within those genera are site specific. For example certain *Laccaria* species are known to grow in sandy habitats (Greg Mueller pers. comm.). Based on previous studies of prior locations where these fungi were found, I conclude that most of the fungi I isolated are more tightly associated with sand prairies, but some of these fungi also typically associate with *C. pensylvanica.* All plants had Ascomycota, Basidiomycota, Glomeromycota and Zygomycota (Fig. 13). I believe the fungi we found in *C. pensylvanica* are more of a product of the habitat than the plant species. Plants can favor colonization of specific fungi, but the fungi have to be present. Basically, they take what they can get, and beggars can't be

choosers. Luckily for the plants I sampled, Fort McCoy has some of the most pristine grasslands and forests in the state, so the fungi that reside there are likely to be diverse and to have a greater distribution of functional groups.

Ascomycota, which we expected to be the dominant phylum, represented 54% of the taxa found (Fig. 12). The Ascomycota taxa were dominated by Helotiales, which are common root endophytes (Jumpponen and Trappe 1996). There were also many Dothidiomycetes and Sordariales. Secondary metabolites made by Sordariales have proven to be sources for novel medicinal compounds (Higginbotham *et al.* 2014). The functional roles of the Ascomycota taxa include mutualist, endophyte, saprotroph, and pathogen. At least eight of the 14 OTUs found in all samples were Ascomycota (3 OTUs were just "Fungi").

Finding the Ascomycete *Minutisphaera aspera* in 13 of 18 samples was a surprise, because it was only previously known as an aquatic wood decay fungus. So why, how and what is it doing in sand prairies inside *C. pensylvanica* roots? Finds like this raise more questions than they answer and provide ideas for future studies. We were surprised to see Basidiomycota making up such a large portion of the OTUs recovered (Fig. 12). There were some saprotrophic Basidiomycota found that one would expect from prairie habitats, including *Marasmiellus tricolor* which was found in all 18 plants sequenced (Table 2, Appendix D). Other common saprotrophic taxa include various Aphyllophorales (polypores), *Agrocybe pediades* (13 plants), *Tetraprygos*, *Deliculata*, *Marasmius,* and *Mycena*. These fungi may be growing in the senesced root tissue from the previous year's growth. This is not necessarily harmful to the plant because that part of the plant was dead anyway. Conversely,

these fungi might be releasing nutrients back into the area to facilitate new growth of the plant.

Three ectomycorrhizal basidiomycete genera, *Russula*, *Laccaria* and *Tomentella*, were found in all plants sequenced. I have noticed *Laccaria* fruiting near scrub oaks in really sandy soils. I have also observed many *Russula* spp. fruiting around oaks and pines growing in savannahs. Although I have not noticed any *Tomentella* above ground in the area, it likely corresponds to the clamped hyphae I recorded when measuring fungal abundance microscopically (Fig 4). Other common ectomycorrhizal taxa recovered include, *Hortiboletus rubellus*, *Cortinarius* sp. and *Astraeus morganii*.

If we really did collect sedges from beyond the roots of the oaks, how did these ectomycorrhizal fungi get to the roots? Perhaps the oak roots didn't extend as far as we estimated, but maybe the mycorrhizae on them did. Are the fungi acting as a conduit between these sedges and other plants, including the oaks? Oak trees bud relatively late in the spring, perhaps they benefit from the earlier photosynthetic activity of *Carex pensylvanica* because the fungi are spreading the love. Even if the sedges are just helping the mycorrhizae associated with oaks, they could benefit oaks indirectly. If the roots are not directly connected, they got to the sedges by spore dispersal. But how did the spores travel there, and do they prefer growing with these sedges or are they just making do with what they have? In other words, can they develop mushrooms when growing with these sedges?

The spores of the ectomycorrhizal taxa found could be air or water dispersed, but they could also be animal or insect dispersed. There are lots of white-tailed deer on

Fort McCoy that love to eat mushrooms, *Russulas* in particular (Kevin Luepke, pers. comm.). It is known that flying squirrels play a role in truffle dispersal, but there should be more research done on the role of deer on spore dispersal. By selectively eating certain mushrooms, deer can alter distribution. I think there should also be investigations into the role insects play in spore or mycelial dispersal. The most commonly known example of insect dispersal is that of stinkhorns attracting flies with the putrid smell characteristic of the group. Springtails (Collembola) have been shown to eat and pass viable *Tomentella* spores (Lilleskov and Bruns 2005). So all in all, we don't know how these ectomycorrhizae got to these sedge roots.

Glomeromycota made up a lower percentage of the OTUs, but this would be expected as there are far fewer species of Glomeromycota than Asco- and Basidiomycota. Operational Taxonomic Units represent what was in the sample, but because of bias in PCR and other human and mechanical bias, we cannot accurately use sequence data to say one OTU was more abundant in an individual sample. Similar to how frequency of cultured fungi was assessed, we used a tally count to say an OTU is more common because we found it in more samples. Thus, even though Glomeromycota comprised fewer taxa, they could be growing in the same, more, or less abundance on the roots as the other phyla. Root colonization data showed AMF are likely less abundant than DSE (Ascomycota mostly), but more abundant than ECM (Basidiomycota in this study), demonstrating how multiple approaches provide valuable insights that might otherwise be speculative. Glomeromycota were found in all the plants sequenced, so we can definitively say

that *Carex pensylvanica* associates with arbuscular mycorrhizal fungi in Fort McCoy sand prairies.

Other fungal lineages represented included Zygomycota and Rozellomycota. Zygomycota are not generally considered common endophytes, but *Umbelopsis* was found abundantly in culture (Fig. 1) and sequence data. Zygomycota are typically saprophytic or plant pathogens, causing damping off in seedlings. Rozellomycota taxa were recovered in low amounts. Rozellomycota are a basal fungal lineage that were discovered in many environmental samples but, until recently, were not described (James *et al.* 2006).

Restoration Insights

Organic matter appears to be an important overall factor influencing mycorrhizal community and abundance of *C. pensylvanica* in these sand prairies. This is in part because OM influences water holding capacity and total nitrogen. Organic matter was sometimes really low at 1% or less (Fig. 9 & 10, Appendix C). For restoration in these depauperate areas, addition of organic matter might be an effective way to increase mycorrhizal abundance, which in turn will improve soil fertility and plant growth (Johnson 1998). Addition of inorganic nitrogen or phosphorus fertilizers increases plant growth, but decreases mycorrhizal colonization (Treseder 2004), creating a system in which plants rely on anthropogenic addition of nutrients instead of acquiring symbionts to aid in growth. Sand prairies are naturally low in nutrients, so precautions and exact management goals would need to be assessed before dramatic additions of organic matter to restoration sites on Fort McCoy. Organic matter typically needs to be broken down by microbes before plants can use it.

Illumina data showed that there are numerous species of normally saprotrophic fungi that reside in the roots of this widespread sand prairie sedge. It is possible that saprotrophic and mycorrhizal fungi benefit from growing in close proximity. Fungi are sloppy eaters, so the mycorrhizae could scavenge some of the predigested substrate from saprotrophs. To test this, one could grow plants inoculated with a mycorrhiza alone, a saprotroph alone, and both together and compare growth rates among the three treatments. In such and experiment, organic matter type (wood chips, compost, liquid extracted OM) and relative amount should be carefully determined or better yet, various levels and types tested.

The ability of *Carex pensylvanica* to colonize virgin soils and persist in late successional plant communities may be influenced by the variety of fungal symbionts it can associate with. Many early successional or "weedy" plant species, such as *Chenopodium*, get outcompeted by other plants as the community matures. Pioneer plant species that are able to form AMF and ECM, such as *C. pensylvanica*, may act as reservoirs for fungal inoculum in soils, facilitating establishment of late-successional species. Late successional plants are often seeded in prairie restoration in attempts to shortcut the natural plant community progression. Maybe land managers should also seed plant species that are more transitional between early and late succession.

Enigmatic Sample 1B

Sample 1B, the first no tree site, is the most interesting individual site in terms of results. It had the highest pH (6.54), the highest phosphorus levels (Fig. 7), the highest amount of DSE colonization (72.1%), the second highest number of

morphotypes (8), and had clamped hyphae. Despite high colonization levels, sample 1B had the least number of OTUs recovered from Illumina sequencing which suggests lower diversity (Fig. 13). It makes sense that the highest pH, also has the most phosphorus because there is the most available phosphorus at a pH range of about 6-7 (Busman 2002). Organic matter and water holding capacity were on the lower end of the sample sites. The combination makes site 1B sort of an outlier when comparing phosphorus, pH, organic matter, and water holding capacity.

Conclusions

The vast majority of the environment factors we tested, from surrounding vegetation to soil conditions, did not significantly explain variability or trends among endorrhizal fungal species composition or abundance in *Carex pensylvanica* roots from Wisconsin sand prairies. There was no significant difference between tree and no tree sites, but we did see some trends when analyzing samples independently. Soil data showed that pH and organic matter correlate with fungal community structure, the cause of which is unclear. Soil conditions could be driving fungal community and fungal community could be altering soil conditions, but it is more likely that both are happening. Fungal colonization tended to be higher as water holding capacity and organic matter increased, demonstrating another example of soil factors playing a role in plant-fungal interactions.

Fusarium oxysporum cultured well, but was not recovered abundantly from Illumina. Based on these data and previous studies, *F. oxysporum* is an abundant endophyte, especially in sandy habitats, that associates with most or all plants, but isn't necessarily favored by *Carex pensylcanica*. Conversely, *Acephala harenae no.*

prov. was the second most cultured species, found in 13 plants and 30 isolations total. It was also found in all 18 plants that were Illumina sequenced. These data and previous studies of closely related dark septate endophytes (PAC species) indicate *Acephala harenae nom. prov.* is a mutualistic symbiont with *Carex pensylvanica*.

Dark septate endophytes were the most abundant type of endorrhizal fungi on *Carex pensylvanica* growing in these sand prairies. Although seemingly favored by *C. pensylvanica*, sand prairies are also likely to promote DSE associations. *Tomentella ferruginea* was found in all plants sequenced and likely represents the clamped hyphae observed microscopically. These data suggest *T. ferruginea* associates with *Carex pensylvanica.* Representatives in the ectomycorrhizal genera *Russula* and *Laccaria* were also found in all plants sequenced. These ectomycorrhizal taxa also probably associate with the oak trees in the area. *Carex pensylvanica* does form arbuscular mycorrhizal associations, adding to the wide range of host plants known to associate with Glomeromycota. The cohabitation of dark septate endophytes, arbuscular mycorrhizal fungi and ectomycorrhizal fungi, demonstrate that *C. pensylvanica* can form multiple types of endorrhizae. More broadly, these findings show that three types of endorrhizal fungi do coexist within the roots of individual plants. To date, this is the most in depth survey of root-associated fungi in any *Carex* species and the most extensive investigation of fungi from Fort McCoy, Wisconsin.

Carex pensylvanica might not be the prettiest or most interesting looking plant, but it sure does have some interesting things growing in its roots. So next time you're walking in a forest, I hope you look twice at Penn sedge.

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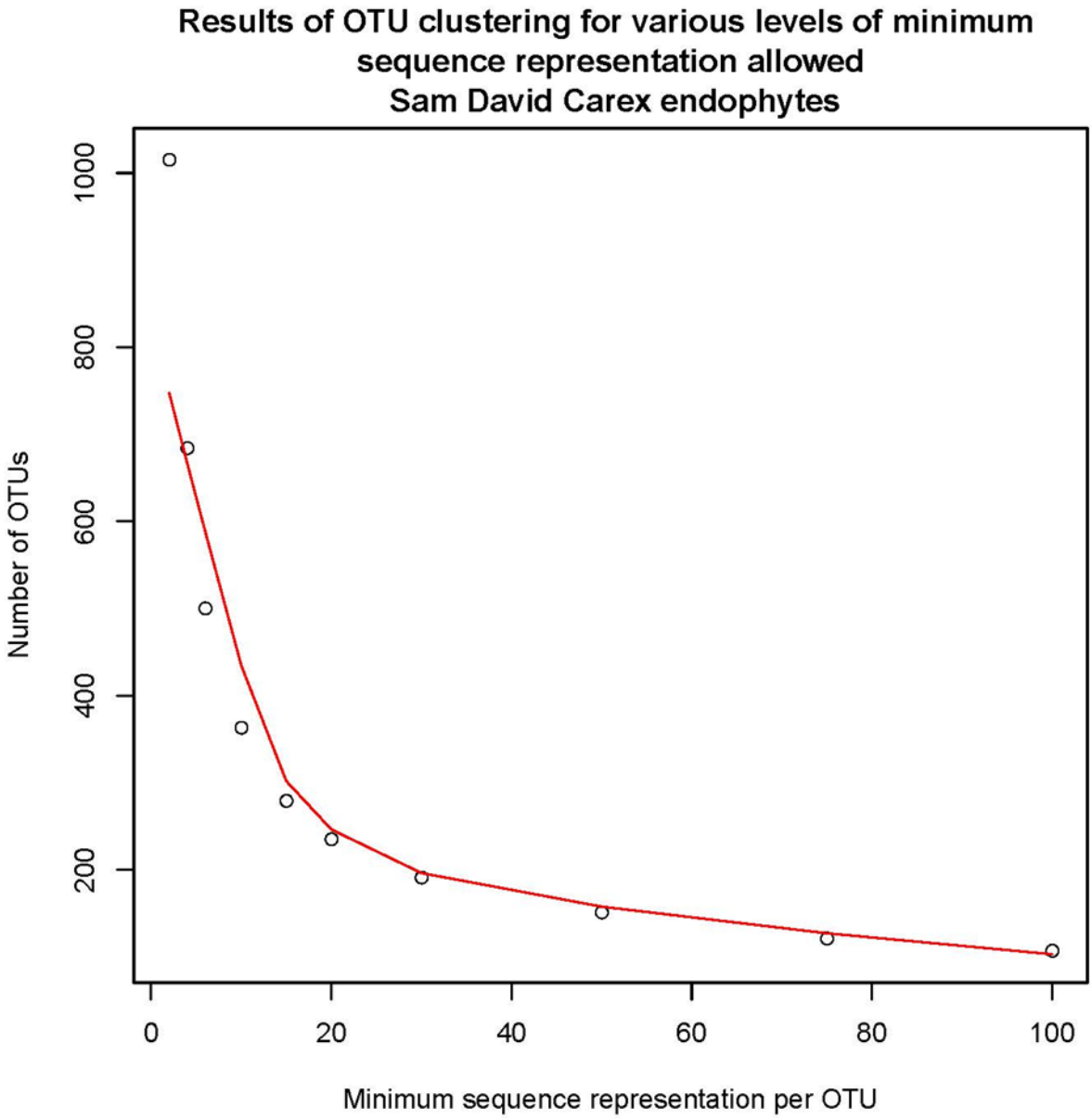
APPENDIX A

RESULTS OF OTU CLUSTERING FOR VARIOUS LEVELS OF MINIMUM SEQUENCE REPRESENTATION ALLOWED


Results of OTU clustering for various levels of minimum sequence representation allowed
Sam David Carex endophytes
Number of OTUs
1000
800
600
400
200
0
20
40
60
80
100
Minimum sequence representation per OTU

APPENDIX B

MOST COMMON CULTURED FUNGI TALLY DATA

Most Common Cultured Fungi Tally Data

Morphotype code	Species	# of Plants	# of Isolates	Plants found in
Pink Bear	Fusarium oxysporum (100%)	16	44	12B, 6A, 6B, 2B, 1B, 10A, 10B, 9A, 15A, 15B, 4A, 14A, 13B, 8A, 8B, 12A
Medusa	Acephala sp. CM7m4	13	30	9B, 8B, 15A, 15B, 14A, 14B, 13A, 11B, 7B, 4B, 3B, 1B, 2A
Lawn Boy	Alternaria alternata	11	26	15A, 11A, 8B, 4B, 14B, 12B, 9A, 7A, 6A, 2B, 1B
Speckles	Diaporthe phaseolorum	10	21	15B, 13A, 12A, 12B, 10B, 8A, 8B, 6B, 3B, 1A
Mammoth	Biscogniauxia mediterranea	10	15	*8A, 14A, 13A, 12A, 9B, 7A, 7B, 4A, 4B
Mr. Green	Trichoderma koningiopsis	9	20	15B, 10A, 5B, 3A, 3B, 2A, 14A, 13A, 12B
Agatha	Saccharicola bicolor	8	18	13A, 13B, 12A, 12B, 6B, 5B, 4B, 2B
Yellow Bear	Fusarium oxysporum (99.81%)	8	19	15B, 14A, 9A, 8A, 8B, 6A, 1A, 1B
Stringy	Fusarium oxysporum(99.801%)	8	10	15B, 14B, 12A, 10A, 8A, 6A, 6B, 13B
Olivia	Epicoccum nigrum	7	9	4B, 14B, 13A, 12A, 6B, 7A, 12B
Natalie	uncultured Ascomycota	6	7	5A, 15B, 12A, 7A, 12B, 9A
Matt	Curvularia inaequalis	5	5	11A, 9A, 13B, 14B, 1B
Salmon Ring	Fusarium neocosmosporiellum	4	4	14B, 11B, 11A, 7B
Ron Burgandy	Chaetomium aureum	3	11	*1B, 15A,
Banana peel	Penicillium janthinellum	3	3	*15A, 14A
Marshmallow	Umbelopsis sp.	3	15	3B, 8B, 1B
Spider	Fimetariella rabenhorstii	2	6	13B, 4A
Flakey	Mortierella	1	1	5A
				*1 ADDED BECAUSE FOUND IN PRELIMINARY SAMPLING

APPENDIX C

SOIL TEST RAW DATA

Soil Test Raw Data

Site ID	pH	% Org Mat	water/soil	P(ppm)	Total N(ppm)
1A	4.90	0.5424	0.250988142	47	375.4
1B	6.54	1.6529	0.359882006	90	1010.8
2A	5.14	4.8151	0.438834951	12	2410.7
2B	4.69	3.4759	0.41749503	10	1168.1
3A	4.10	3.0195	0.380239521	15	1194.6
3B	5.44	2.0088	0.362919132	29	847.4
4A	5.16	7.1920	0.476143141	34	2653.4
4B	5.38	3.8623	0.447674419	17	1714.0
5A	5.63	7.8002	0.435770751	26	3029.2
5B	5.98	2.9735	0.432379072	22	1362.4
6A	5.40	2.6251	0.384231537	19	1373.5
6B	5.52	2.0204	0.388724036	39	1078.6
7A	6.05	1.3060	0.353174603	33	695.3
7B	4.51	4.8631	0.541015625	46	2430.9
8A	4.32	6.0297	0.403369673	19	2551.3
8B	4.46	2.7065	0.389162562	8	1161.4
9A	5.78	2.2462	0.454545455	10	1132.1
9B	5.48	3.3206	0.48574238	8	1389.1
10A	5.61	1.7175	0.387512389	10	685.4
10B	5.31	2.8882	0.471568627	11	1345.7
11A	5.4	2.5435	0.430404738	13	1279.0
11B	5.11	1.7422	0.299800797	9	711.8
12A	5.63	2.2703	0.337301587	25	1051.0
12B	6.13	2.7221	0.392752204	47	1397.7
13A	4.63	3.4039	0.415338645	18	1286.5
13B	4.94	2.2598	0.260869565	29	986.3
14A	5.30	1.4910	0.291625616	47	751.8
14B	5.75	0.7627	0.253952569	65	582.7
15A	4.79	2.0719	0.312315271	32	1185.9
15B	4.72	2.1917	0.334637965	16	998.4
mean	5.26	2.95	0.39	26.87	1328.01

APPENDIX D

OTU COUNT AND IDENTITY FOR 362 AMPTK-ANALYZED SEQUENCES FROM ILLUMINA

OTU Count and Identity for 362 AMPtk-Analyzed Sequences from Illumina

count	#OTUID	taxonomy
18	OTU1	SINTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
18	OTU2	GQ219892;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
18	OTU3	EU490117;k:Fungi
18	OTU4	KJ188733;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Omphalotaceae,g:Marasmiellus, s:Marasmiellus tricolor
18	OTU5	HM136626;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
18	OTU6	SINTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales
18	OTU7	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Thelephorales,f:Thelephoraceae,g:Tomentella, s:Tomentella ferruginea
18	OTU10	JQ684858;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
18	OTU13	UTAX;k:Fungi,p:Ascomycota
18	OTU16	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae
18	OTU20	SINTAX;k:Fungi
18	OTU73	KT728323;k:Fungi,p:Ascomycota
18	OTU267	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Atheliales,f:Atheliaceae
18	OTU303	SINTAX;k:Fungi
17	OTU11	AB986370;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae
17	OTU15	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales
17	OTU22	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
17	OTU29	EU888622;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales
17	OTU34	JX043045;k:Fungi
17	OTU57	UTAX;k:Fungi,p:Ascomycota
17	OTU80	KJ188691;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
17	OTU137	SINTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes
16	OTU19	SINTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales,f:Mycosphaerellaceae
16	OTU23	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae
16	OTU24	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes
16	OTU32	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae,g:Lachnum, s:Lachnum virgineum
16	OTU43	GU062257;k:Fungi,p:Ascomycota,c:Leotiomycetes,f:Myxotrichaceae,g:Pseudogymnoascus, s:Pseudogymnoascus pannorum
15	OTU8	EU880226;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula
15	OTU9	UTAX;k:Fungi,p:Ascomycota
15	OTU30	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales
15	OTU31	SINTAX;k:Fungi
15	OTU33	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae,g:Lachnum
15	OTU36	KR673538;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Tricholomataceae,g:Delicatula, s:Delicatula integrella
15	OTU64	EU877757;k:Fungi,p:Zygomycota,o:Mortierellales,f:Mortierellaceae,g:Mortierella
15	OTU125	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula

15	OTU127	GU327639;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Hypocreales,f:Nectriaceae,g:Fusarium
15	OTU129	EF433978;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Hydnangiaceae,g:Laccaria
14	OTU17	UTAX;k:Fungi,p:Ascomycota,c:Pezizomycetes,o:Pezizales,f:Sarcosomataceae,g:Pseudoplectania, s:Pseudoplectania nigrella
14	OTU21	JF907840;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Psathyrellaceae,g:Coprinopsis
14	OTU49	KC966138;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
14	OTU153	KM067844;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Hydnangiaceae,g:Laccaria, s:Laccaria pumila
13	OTU12	KP309989;k:Fungi,p:Ascomycota,c:Dothideomycetes,g:Minutisphaera,s:Minutisphaera aspera
13	OTU18	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Strophariaceae,g:Agrocybe, s:Agrocybe pediades
13	OTU35	EU292532;k:Fungi,p:Ascomycota,c:Leotiomycetes,g:Meliniomyces,s:Meliniomyces bicolor
13	OTU46	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
13	OTU47	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae, g:Cladophialophora
13	OTU66	AB986329;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Hysteriales,f:Gloniaceae,g:Cenococcum, s:Cenococcum geophilum
13	OTU94	GU446638;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae, g:Cladophialophora
12	OTU14	HF674810;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
12	OTU58	SINTAX;k:Fungi
12	OTU82	SINTAX;k:Fungi
11	OTU28	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
11	OTU65	HM036645;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Niaceae,g:Flagelloscypha, s:Flagelloscypha minutissima
11	OTU191	EU661886;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales,f:Polyporaceae,g:Trametes, s:Trametes pubescens
10	OTU26	KF251179;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Phaeosphaeriaceae, g:Parastagonospora,s:Parastagonospora poae
10	OTU27	EU888624;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Vibrisseaceae,g:Phialocephala, s:Phialocephala fortinii
10	OTU37	SINTAX;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Magnaporthales,f:Magnaporthaceae
10	OTU59	KF617790;k:Fungi
10	OTU61	KP053824;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Trechisporales
10	OTU63	FJ553528;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales
10	OTU72	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Chaetothyriaceae
10	OTU83	DQ420848;k:Fungi,p:Zygomycota,o:Mortierellales,f:Mortierellaceae
10	OTU120	GQ179993;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Sordariales,f:Chaetomiaceae,g:Trichocladium, s:Trichocladium opacum
10	OTU121	EU035406;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae, g:Cladophialophora,s:Cladophialophora chaetospira
10	OTU149	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Cortinariaceae,g:Cortinarius
9	OTU25	KC965516;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
9	OTU40	KM042015;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae

9	OTU55	KJ188723;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales
9	OTU62	UTAX;k:Fungi,p:Ascomycota
9	OTU85	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales
9	OTU123	LN882168;k:Fungi
9	OTU135	KF800100;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales,f:Ganodermataceae
9	OTU147	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes
9	OTU171	UTAX;k:Fungi,p:Zygomycota,o:Mucorales,f:Umbelopsidaceae,g:Umbelopsis
9	OTU175	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Dothideales,f:Dothioraceae
8	OTU39	HF674809;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
8	OTU41	KC966164;k:Fungi
8	OTU45	SINTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
8	OTU52	SINTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales
8	OTU53	SINTAX;k:Fungi
8	OTU69	UTAX;k:Fungi,p:Ascomycota
8	OTU81	FJ378850;k:Fungi,p:Ascomycota,g:Chalara
8	OTU96	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Pleosporaceae
8	OTU100	SINTAX;k:Fungi,p:Ascomycota,c:Pezizomycetes,o:Pezizales,f:Pezizaceae
8	OTU134	SINTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae,g:Exophiala
8	OTU190	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
7	OTU42	KT933954;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula, s:Russula amoenolens
7	OTU48	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula
7	OTU50	UTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
7	OTU51	KJ012010;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Cantharellales,f:Ceratobasidiaceae, g:Ceratobasidium,s:Ceratobasidium cereale
7	OTU54	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Niaceae,g:Flagelloscypha
7	OTU56	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes
7	OTU68	UTAX;k:Fungi,p:Ascomycota
7	OTU70	KJ188730;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Massarinaceae,g:Saccharicola, s:Saccharicola bicolor
7	OTU74	KM041912;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
7	OTU89	SINTAX;k:Fungi,p:Ascomycota,c:Pezizomycetes,o:Pezizales,f:Pezizaceae
7	OTU110	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Pleosporaceae
7	OTU133	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes
7	OTU151	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae,g:Cladophialophora
7	OTU154	UTAX;k:Fungi,p:Zygomycota,o:Mortierellales,f:Mortierellaceae,g:Mortierella,s:Mortierella cystojenkinii
7	OTU157	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae,g:Lachnum
7	OTU183	SINTAX;k:Fungi
7	OTU194	SINTAX;k:Fungi
7	OTU202	UTAX;k:Fungi,p:Ascomycota
7	OTU226	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales

7	OTU238	UTAX;k:Fungi,p:Basidiomycota
6	OTU38	JX276903;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
6	OTU77	SINTAX;k:Fungi
6	OTU78	DQ421112;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Boletales,f:Diplocystidiaceae,g:Astraeus, s:Astraeus morganii
6	OTU84	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
6	OTU86	JX043029;k:Fungi,p:Ascomycota,g:Chalara
6	OTU91	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes
6	OTU138	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
6	OTU139	UTAX;k:Fungi,p:Ascomycota
6	OTU141	U57496;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae,g:Arachnopeziza, s:Arachnopeziza aurata
6	OTU152	JX270454;k:Fungi,p:Ascomycota,c:Leotiomycetes,g:Geomyces
6	OTU156	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Atheliales,f:Atheliaceae,g:Piloderma
6	OTU165	FJ666349;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,g:Cadophora
6	OTU168	UTAX;k:Fungi,p:Zygomycota,o:Mucorales,f:Umbelopsidaceae,g:Umbelopsis,s:Umbelopsis dimorpha
6	OTU188	SINTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
6	OTU212	SINTAX;k:Fungi,p:Zygomycota,o:Mortierellales
6	OTU214	DQ421257;k:Fungi
6	OTU233	UTAX;k:Fungi,p:Ascomycota
6	OTU295	GQ302684;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Pleosporaceae,g:Alternaria
6	OTU335	SINTAX;k:Fungi
5	OTU79	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Sebacinales,f:Sebacinaceae,g:Sebacina
5	OTU90	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Cucurbitariaceae,g:Pyrenochaetopsis
5	OTU99	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
5	OTU118	UTAX;k:Fungi,p:Ascomycota
5	OTU119	SINTAX;k:Fungi,p:Ascomycota,c:Sordariomycetes
5	OTU124	KC131409;k:Fungi
5	OTU126	SINTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales
5	OTU159	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae
5	OTU161	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes
5	OTU173	FJ552720;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales
5	OTU193	UTAX;k:Fungi,p:Ascomycota
5	OTU196	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Helotiaceae
5	OTU198	SINTAX;k:Fungi,p:Ascomycota
5	OTU199	FJ536208;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,g:Periconia,s:Periconia macrospinosa
5	OTU236	AB073265;k:Fungi,p:Basidiomycota,c:Microbotryomycetes,o:Sporidiobolales,g:Rhodotorula, s:Rhodotorula toruloides
5	OTU255	SINTAX;k:Fungi,p:Zygomycota
5	OTU256	SINTAX;k:Fungi
5	OTU279	EU833650;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Agaricaceae,g:Bovista, s:Bovista aestivalis

5	OTU283	UTAX;k:Fungi,p:Basidiomycota,c:Cystobasidiomycetes,o:Erythrobasidiales,g:Erythrobasidium, s:Erythrobasidium hasegawianum
5	OTU288	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales
5	OTU305	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales
5	OTU316	UTAX;k:Fungi,p:Ascomycota
5	OTU346	KU903000;k:Fungi
5	OTU355	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Stereaceae,g:Stereum, s:Stereum sanguinolentum
4	OTU60	EU819433;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula
4	OTU75	JX043030;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
4	OTU92	KP171108;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Inocybaceae,g:Inocybe
4	OTU95	UTAX;k:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,g:Candida
4	OTU97	SINTAX;k:Fungi
4	OTU102	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Sebacinales
4	OTU105	SINTAX;k:Fungi
4	OTU108	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
4	OTU109	HQ667799;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Cantharellales,f:Ceratobasidiaceae,g:Thanatephorus
4	OTU111	JX381584;k:Fungi
4	OTU113	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Sebacinales
4	OTU117	UTAX;k:Fungi,p:Ascomycota
4	OTU131	UTAX;k:Fungi,p:Zygomycota,o:Mortierellales,f:Mortierellaceae,g:Mortierella,s:Mortierella humilis
4	OTU140	UTAX;k:Fungi,p:Ascomycota
4	OTU146	HQ608098;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Eurotiales,f:Trichocomaceae,g:Talaromyces, s:Talaromyces verruculosus
4	OTU155	UTAX;k:Fungi,p:Ascomycota
4	OTU162	KF617727;k:Fungi,p:Ascomycota,c:Dothideomycetes
4	OTU172	KF296826;k:Fungi
4	OTU179	SINTAX;k:Fungi
4	OTU210	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Thelephorales,f:Thelephoraceae,g:Tomentella
4	OTU215	SINTAX;k:Fungi
4	OTU217	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae
4	OTU232	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
4	OTU234	KF251270;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Phaeosphaeriaceae,g:Stagonospora
4	OTU244	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes
4	OTU274	DQ421101;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Agaricaceae,g:Calvatia
4	OTU277	UTAX;k:Fungi,p:Ascomycota
4	OTU308	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes
4	OTU341	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
4	OTU343	SINTAX;k:Fungi
4	OTU354	UTAX;k:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,g:Cyberlindnera, s:Cyberlindnera jadinii

3	OTU44	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
3	OTU67	LT612868;k:Fungi,p:Ascomycota
3	OTU76	EU645602;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Cantharellales,f:Ceratobasidiaceae
3	OTU104	UTAX;k:Fungi,p:Ascomycota
3	OTU106	DQ778612;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Capnodiales,f:Mycosphaerellaceae
3	OTU107	SINTAX;k:Fungi
3	OTU112	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
3	OTU115	AB986449;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Hyaloscyphaceae
3	OTU122	SINTAX;k:Fungi
3	OTU132	UTAX;k:Fungi,p:Ascomycota
3	OTU150	UDB000020;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula, s:Russula vesca
3	OTU158	GQ166906;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Entolomataceae,g:Entoloma, s:Entoloma abortivum
3	OTU164	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,f:Helotiaceae
3	OTU167	EU490097;k:Fungi,p:Ascomycota,c:Lecanoromycetes
3	OTU182	KJ869110;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Phaeosphaeriaceae,g:Stagonospora, s:Stagonospora trichophoricola
3	OTU186	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Thelephorales,f:Thelephoraceae,g:Tomentella
3	OTU197	SINTAX;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Diaporthales,f:Diaporthaceae
3	OTU204	JX343510;k:Fungi
3	OTU206	AJ633598;k:Fungi,p:Ascomycota,c:Pezizomycetes,o:Pezizales,f:Pezizaceae
3	OTU207	KC884359;k:Fungi,p:Basidiomycota,c:Microbotryomycetes,o:Leucosporidiales
3	OTU208	HQ154357;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Sebacinales,f:Sebacinaceae
3	OTU213	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
3	OTU216	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes
3	OTU219	KT697970;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula, s:Russula cremeirosea
3	OTU220	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Sporormiaceae,g:Preussia
3	OTU221	JX031356;k:Fungi
3	OTU222	JF944993;k:Fungi
3	OTU224	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales,f:Fomitopsidaceae,g:Ischnoderma, s:Ischnoderma resinosum
3	OTU231	SINTAX;k:Fungi
3	OTU243	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Atheliales,f:Atheliaceae
3	OTU248	UTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
3	OTU251	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
3	OTU258	AB520603;k:Fungi,p:Basidiomycota,c:Wallemiomycetes,o:Geminibasidiales,f:Geminibasidiaceae, g:Geminibasidium
3	OTU259	KP068771;k:Fungi,p:Ascomycota,c:Saccharomycetes,o:Saccharomycetales,g:Candida, s:Candida tropicalis
3	OTU260	UTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae,g:Glomus

3	OTU261	GQ268659;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales,f:Meruliaceae,g:Phlebia
3	OTU262	HQ271355;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Entolomataceae,g:Entoloma
3	OTU265	SINTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
3	OTU266	EU252551;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,g:Hannaella,s:Hannaella luteola
3	OTU275	JX368582;k:Fungi
3	OTU284	KP769834;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Magnaporthales,f:Magnaporthaceae, g:Pseudophialophora,s:Pseudophialophora whartonensis
3	OTU285	AM902072;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Physalacriaceae,g:Armillaria
3	OTU310	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Marasmiaceae,g:Rectipilus, s:Rectipilus idahoensis
3	OTU314	AY854075;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Agaricaceae,g:Lycoperdon, s:Lycoperdon pyriforme
3	OTU326	SINTAX;k:Fungi
3	OTU332	SINTAX;k:Fungi,p:Ascomycota
3	OTU340	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes
3	OTU344	FJ553143;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
3	OTU349	UTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
3	OTU350	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
3	OTU351	KF251260;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Phaeosphaeriaceae,g:Stagonospora, s:Stagonospora pseudocaricis
3	OTU358	SINTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Leotiales,f:Leotiaceae,g:Pezoloma,s:Pezoloma ericae
3	OTU359	KF036587;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,f:Bulleraceae,g:Genolevuria, s:Genolevuria armeniaca
2	OTU71	FJ439582;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Hypocreales,f:Clavicipitaceae,g:Metapochonia, s:Metapochonia suchlasporia
2	OTU87	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Thelephorales,f:Thelephoraceae,g:Tomentella
2	OTU93	KP814185;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Lachnocladiaceae,g:Asterostroma
2	OTU98	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU101	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU103	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU114	HQ331006;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Sebacinales,f:Sebacinaceae
2	OTU116	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
2	OTU128	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Boletales,f:Sclerodermataceae,g:Pisolithus
2	OTU136	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Corticiales
2	OTU142	SINTAX;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Hypocreales
2	OTU143	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Phaeosphaeriaceae
2	OTU144	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales
2	OTU145	UTAX;k:Fungi,p:Ascomycota,c:Orbiliomycetes,o:Orbiliales,f:Orbiliaceae
2	OTU148	SINTAX;k:Fungi
2	OTU163	SINTAX;k:Fungi
2	OTU166	FJ528712;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
2	OTU169	GQ166887;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Boletales,f:Gyroporaceae,g:Gyroporus, s:Gyroporus castaneus

2	OTU174	UDB008725;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Cantharellales,f:Ceratobasidiaceae,g:Ceratobasidium
2	OTU176	UTAX;k:Fungi,p:Ascomycota
2	OTU178	UTAX;k:Fungi,p:Ascomycota
2	OTU181	UTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae
2	OTU187	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales
2	OTU189	SINTAX;k:Fungi
2	OTU192	SINTAX;k:Fungi
2	OTU201	AY969840;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Trechisporales,f:Hydnodontaceae,g:Luellia,s:Luellia recondita
2	OTU205	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Thelephorales,f:Thelephoraceae,g:Tomentella
2	OTU209	SINTAX;k:Fungi,p:Ascomycota
2	OTU211	UTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU225	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes
2	OTU228	UTAX;k:Fungi,p:Ascomycota
2	OTU230	SINTAX;k:Fungi,p:Ascomycota
2	OTU235	UTAX;k:Fungi,p:Ascomycota
2	OTU237	JX321182;k:Fungi
2	OTU239	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU240	KT269529;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Phaeosphaeriaceae,g:Phaeosphaeria
2	OTU241	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales
2	OTU242	UDB017590;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Marasmiaceae,g:Marasmius,s:Marasmius oreades
2	OTU246	FJ757926;k:Fungi
2	OTU249	JX319694;k:Fungi
2	OTU250	SINTAX;k:Fungi
2	OTU257	UTAX;k:Fungi,p:Ascomycota
2	OTU264	SINTAX;k:Fungi
2	OTU271	UTAX;k:Fungi,p:Zygomycota,o:Mortierellales,f:Mortierellaceae,g:Mortierella
2	OTU273	UTAX;k:Fungi,p:Zygomycota
2	OTU276	UTAX;k:Fungi,p:Ascomycota
2	OTU278	SINTAX;k:Fungi
2	OTU282	SINTAX;k:Fungi
2	OTU289	GU189689;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Sebacinales,f:Sebacinaceae
2	OTU290	AY292439;k:Fungi,p:Basidiomycota,c:Pucciniomycetes,o:Helicobasidiales,f:Helicobasidiaceae,g:Helicobasidium
2	OTU293	JQ760106;k:Fungi,p:Ascomycota,c:Dothideomycetes
2	OTU298	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU299	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales
2	OTU300	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula
2	OTU301	LT608818;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Entolomataceae
2	OTU302	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales

2	OTU306	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU311	UTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU317	AY634121;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Cantharellales,f:Ceratobasidiaceae
2	OTU318	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU319	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes
2	OTU322	SINTAX;k:Fungi,p:Ascomycota,c:Eurotiomycetes,o:Chaetothyriales,f:Herpotrichiellaceae
2	OTU325	KJ735021;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,g:Cryptococcus
2	OTU330	SINTAX;k:Fungi,p:Ascomycota,c:Pezizomycetes,o:Pezizales,f:Pezizaceae
2	OTU331	SINTAX;k:Fungi
2	OTU333	AM942468;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU334	SINTAX;k:Fungi
2	OTU336	SINTAX;k:Fungi
2	OTU337	SINTAX;k:Fungi,p:Ascomycota
2	OTU338	AB545810;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,g:Dioszegia,s:Dioszegia rishiriensis
2	OTU345	UTAX;k:Fungi,p:Rozellomycota
2	OTU347	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
2	OTU356	UTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
2	OTU357	KJ869113;k:Fungi,p:Ascomycota,c:Dothideomycetes,o:Pleosporales,f:Massarinaceae,g:Keissleriella, s:Keissleriella trichophoricola
2	OTU361	SINTAX;k:Fungi,p:Basidiomycota
2	OTU363	FJ389447;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae
1	OTU88	UTAX;k:Fungi,p:Basidiomycota,c:Tremellomycetes
1	OTU130	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Atheliales,f:Atheliaceae,g:Byssocorticium, s:Byssocorticium atrovirens
1	OTU160	FJ758464;k:Fungi
1	OTU170	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales
1	OTU177	EU624338;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales
1	OTU180	AF145318;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Tremellales,g:Cryptococcus, s:Cryptococcus elinovii
1	OTU184	GQ166883;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Boletales,f:Boletaceae,g:Hortiboletus, s:Hortiboletus rubellus
1	OTU185	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae,g:Russula,s:Russula mariae
1	OTU195	SINTAX;k:Fungi
1	OTU200	AF444557;k:Fungi,p:Basidiomycota,c:Microbotryomycetes,f:Chrysozymaceae,g:Chrysozyma, s:Chrysozyma griseoflava
1	OTU203	UTAX;k:Fungi,p:Basidiomycota,c:Tremellomycetes,o:Holtermanniales
1	OTU218	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes
1	OTU223	KF800622;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales
1	OTU227	UTAX;k:Fungi,p:Ascomycota,c:Orbiliomycetes
1	OTU229	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Sebacinales,f:Sebacinaceae
1	OTU245	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes
1	OTU247	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Hygrophoraceae,g:Hygrocybe

1	OTU252	KJ705165;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Entolomataceae,g:Entocybe, s:Entocybe vinacea
1	OTU253	UTAX;k:Fungi,p:Ascomycota
1	OTU254	JX504092;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Hydnangiaceae,g:Laccaria
1	OTU263	UTAX;k:Fungi,p:Zygomycota
1	OTU268	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Thelephorales,f:Thelephoraceae
1	OTU269	UTAX;k:Fungi,p:Zygomycota,o:Mortierellales,f:Mortierellaceae,g:Mortierella
1	OTU270	AM942468;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
1	OTU272	FJ554240;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Clavariaceae,g:Clavaria
1	OTU280	UTAX;k:Fungi,p:Ascomycota
1	OTU281	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,g:Scytalidium,s:Scytalidium circinatum
1	OTU286	SINTAX;k:Fungi
1	OTU287	UTAX;k:Fungi,p:Ascomycota,c:Dothideomycetes
1	OTU291	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes
1	OTU292	UTAX;k:Fungi,p:Basidiomycota,c:Microbotryomycetes,o:Sporidiobolales,g:Sporobolomyces, s:Sporobolomyces roseus
1	OTU294	SINTAX;k:Fungi,p:Ascomycota
1	OTU296	SINTAX;k:Fungi,p:Ascomycota,c:Sordariomycetes,o:Hypocreales
1	OTU297	SINTAX;k:Protista,p:Cercozoa
1	OTU304	FJ553302;k:Fungi,p:Ascomycota,c:Leotiomycetes,g:Meliniomyces
1	OTU307	UTAX;k:Fungi,p:Glomeromycota
1	OTU309	KM065566;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Cantharellales,f:Ceratobasidiaceae,g:Rhizoctonia
1	OTU312	FJ196944;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Russulales,f:Russulaceae
1	OTU313	KJ183186;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Polyporales,f:Polyporaceae,g:Trametopsis, s:Trametopsis cervina
1	OTU315	SINTAX;k:Fungi
1	OTU320	SINTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Omphalotaceae,g:Gymnopus
1	OTU321	UTAX;k:Fungi,p:Rozellomycota
1	OTU323	SINTAX;k:Fungi
1	OTU324	UTAX;k:Fungi,p:Basidiomycota,c:Agaricomycetes
1	OTU327	SINTAX;k:Fungi,p:Ascomycota,c:Orbiliomycetes,o:Orbiliales,f:Orbiliaceae
1	OTU328	SINTAX;k:Fungi
1	OTU329	UTAX;k:Fungi,p:Zygomycota,o:Mortierellales,f:Mortierellaceae,g:Mortierella
1	OTU339	AJ716324;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
1	OTU342	SINTAX;k:Fungi
1	OTU348	FN396102;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Psathyrellaceae,g:Coprinellus, s:Coprinellus xanthothrix
1	OTU352	UTAX;k:Fungi,p:Ascomycota,c:Leotiomycetes,o:Helotiales,g:Tetracladium
1	OTU353	JF519455;k:Fungi,p:Basidiomycota,c:Agaricomycetes,o:Agaricales,f:Mycenaceae,g:Mycena
1	OTU360	SINTAX;k:Fungi,p:Glomeromycota,c:Glomeromycetes,o:Glomerales,f:Glomeraceae
1	OTU362	SINTAX;k:Fungi,p:Ascomycota,g:Pseudorobillarda