Arbuscular Mycorrhizal Fungi of Abandoned Agricultural Land and their Implications for the Restoration of Puget Sound Prairies

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TABLE OF CONTENTS

LIST OF FIGURES

Figure Number Page

LIST OF TABLES

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Introduction

Arbuscular Mycorrhizal Physiology

Arbuscular Mycorrhizal Fungi (AMF), members of the Glomeromycota, are by far the most widespread of the mycorrhizal fungi (Brundrett 1991) occurring in 80% of all plant species (Smith 1997). Morphologically, these fungi are a network of hyphae that grow within the roots of plants and extend out into the soil. Unlike the ectomycorrhizal fungi, AMF actually penetrate the walls of root cells and form intracellular structures. They produce two distinct structures: the sac-like vesicles, which are thought to act as storage structures for lipids (Morton and Benny 1990); and densely branched or coiled hyphal masses called arbuscles, which act as the site of nutrient exchange between the plant and the fungus. These fungi were previously known as Vesicular Arbuscular Mycorrhizae. However, it has been shown that vesicles can be produced by non-mycorrhizal fungi and only arbuscles are unique to this group of fungi (McGonigle et al. 1990). There is evidence that the proportion of arbuscles to vesicles can be influenced by ambient nutrient levels and can act as an indicator of the level of benefit received by each partner of the symbiosis (Johnson et al. 2003).

While these fungi are generally considered to be obligate symbionts they have been shown to also have saprophytic capabilities (Hodge et al. 2001) and have limited spread and viability in the absence of a live host (Warner and Mosse 1980). Although hyphal spread through the soil is limited and no epigeous sporocarps are produced, long distance dispersal is possible through mycophagous mammals (Gerdemann 1974; Allen 1987). Only asexual reproduction has been observed in AMF (Kytoviita 2000).

Infection of roots by AMF generally instills many benefits upon the plant host including direct effects such as increased growth (Kemery and Dana 1995), reduced water stress (Allen and Allen 1986) and increased seedling establishment (van der Heijden 2004), as well as indirect effects such as protection from pathogens, decreased herbivory, increased pollination and increased interactions with other mutualists such as rhizobium bacteria (Hartnett and Wilson 2002). The direct effect of increased growth is attributed to the increased ability to uptake nutrients, primarily phosphorus, after infection (Bolan et al. 1987). The resistance to water stress is likely associated with the extraradical network of hyphae which are far more resistant to drought than fine roots and can resume growth immediately after drought conditions cease (Miller et al. 1995). However, some plants exhibit a mycorrhizal dependence that can not be explained by either of these mechanisms (Streitwolf-Engel et al. 1997).

The benefits of the mycorrhizal association are not without costs to the plant. This was demonstrated by the slower growth of mycorrhizal seedlings compared to seedlings provided with a comparable level of supplemental phosphorus (Yocom 1983). Additionally, interactions in the field are far more complex than greenhouse studies. Hetrick et al (1986) found that mycorrhizal growth response was significantly suppressed in non-sterile soils, suggesting three-way interactions with other soil organisms. The specific pairing of plant and fungal species is greatly important in determining the nature of the symbiosis. In a study by Klironomos (2003) pairing 64 plant species with 10 species of AMF, the relationship was equally often parasitic as mutualistic and all species of plants and AMF were capable of both extremes. Even within a given pairing of species the nature of the symbiosis can change throughout the

life span of the plant, with seedlings deriving a benefit from different AMF species than adult plants (van der Heijden 2004).

Patterns have been observed in the mycorrhizal dependence of plants. The most striking of these is along family lines with Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Polygonaceae, Portulacaceae and Proteaceae all lacking mycorrhizal infection (Read et al. 1976; Malajczuk et al. 1981; Pendleton and Smith 1983; Allen 1991). Non-mycorrhizal species tend to be weedy, but the inverse is not true (Pendleton and Smith 1983). C3 grasses tend to be far less responsive to mycorrhizal infection than either C4 grasses or forbs (Wilson and Hartnett 1998). Root architecture is also linked to mycorrhizal infection with coarse roots (0.2 - 1.0mm) being generally more infected than fine roots (0.075 – 0.2mm) (Reinhardt and Miller 1990; Miller et al. 1995). There is some evidence that AMF may hormonally influence the root structure of infected plants (Hetrick et al. 1992). Not only do plant species vary in their mycorrhizal dependence, but AMF species exhibit strong host preferences (Eom et al. 2000; Vandenkoornhuyse et al. 2003). Environmental factors such as light, temperature and phosphorus levels can also influence the infectivity of a given plant and fungus pair (Furlan and Fortin 1977; Hayman 1983).

AMF Taxonomy

Taxonomy within the arbuscular mycorrhizal fungi is difficult. There is a lack of morphological structures upon which to base taxonomic classification. Historically spore morphology has been used to develop this classification and spore abundance has been used to survey for communities of AMF; however, this method has some major

drawbacks. There is a seasonality to spore production, field collected spores can be difficult to identify, not all AMF have been found to sporulate, the density of spores is not necessarily related to the abundance of hyphae and there is a lack of relation between functional diversity and spore morphological diversity (Douds and Millner 1999). Often in order to facilitate identification of spores, field collected AMF samples are grown in pot culture and the spores are collected and identified. This allows adequate quantities of fresh spores for identification (Stutz and Morton 1996). These techniques, however, only allow identification of a subset of AMF that perform well with disturbed mycelial networks (Rillig 2004) and the diversity of spores changes as the pot culture is grown for successive generations (Stutz and Morton 1996). Molecular techniques have been employed and show promise but as of yet a comprehensive library of taxon specific probes does not exist and PCR products from field samples are often variable and unpredictable (Douds and Millner 1999). Given these limitations it has been suggested that there is currently no method of AMF identification that provides a useful representation of diversity and abundance in the field (Douds and Millner 1999) and that the true diversity of AMF is likely highly underestimated (Vandenkoornhuyse et al. 2002).

Despite the difficulties in understanding the complexity of natural communities of AMF there are about 155 identified species of AMF (Douds and Millner 1999). While it has been shown that different species of AMF have varying interactions with plants under identical conditions (Sanders et al. 1977; StreitwolfEngel et al. 1997; Stampe and Daehler 2003), the consistency of this interaction within an AMF species is questionable. At least at the family level, there has been shown some functional basis

for AMF taxonomy derived mainly from differing colonization strategies (Hart and Reader 2002). However, functional diversity exists within single species and even individuals of AMF. This can be partially explained by the multi-nucleated nature of AMF. Genetically divergent nuclei within the hyphae of an AMF can segregate themselves, creating a functional diversity across the body of a single AMF individual (Hart and Trevors 2005). While the worldwide genetic diversity of a single species of AMF is low, the genetic divergence of individuals within a locale is high, that is to say a local collection of many individuals of a single species will contain nearly the worldwide breadth of genetic diversity for that species (Koch et al. 2004). This suggests that caution is necessary when making generalizations about the function of a given species of AMF.

Ecology of Arbuscular Mycorrhizas

The varying responses of plants to mycorrhizal infection have a profound impact on plant interactions and community structure. AMF can dictate the dominant plants in a community by mediating competition between plants of differing mycotrophy (Yocom 1983; Allen and Allen 1986; Allen 1990; Hartnett et al. 1993; Hartnett and Wilson 2002). This can be extended to a model of succession where the mycotrophy of dominant plants changes as the community develops (Allen 1990; Johnson et al. 1991). The lack of mycorrhizal fungi during primary succession gives the advantage to non-mycorrhizal plants (Reeves et al. 1979). During this time of dominance by non-mycorrhizal plants, succession may be slowed or completely arrested until the establishment of facultative mycorrhizal plants begins to build a community of AMF.

This paves the way for the establishment of obligate mycorrhizal plants which then come to dominate the community (Koske and Gemma 1997). In this way the mycotrophy of the dominant plants can be used to indicate the successional trajectory of a community (Doerr et al. 1984). While this model is appealing in its simplicity, there are cases where AMF have been shown to slow succession by inhibiting non-mycotrophic plants that were important to facilitating secondary succession (Allen and Allen 1988).

Additionally complicating the situation is the discovery that nitrogen and phosphorus can be passed between plants of differing species via mycorrhizal fungi (Chiariello et al. 1982; Francis et al. 1986; Allen 1990; Hartnett and Wilson 2002). The implications of this AMF mediated sharing of nutrients could dramatically change our understanding of plant competition. There is evidence that this nutrient sharing allows the survival of subordinate seedlings which act as nutrient sinks to the dominant source plants (Grime et al. 1987; Grime et al. 1988). It has also been suggested that invasive plants can act as parasites, using the web of AMF to draw resources from native vegetation (Marler et al. 1999).

Whatever the case, it is clear that AMF affect competition and thereby influence the level of diversity found in plant communities. Plant diversity can either be increased or decreased by the presence of AMF depending on the mycotrophy of the dominant species (Urcelay and Diaz 2003). Mycorrhizas were shown to increase the diversity of a turf community with a weakly mycorrhizal dominant (Grime et al. 1987), whereas mycorrhizas decreased the diversity of a Kansas tallgrass prairie where the dominant plants were obligately mycotrophic C4 grasses (Hartnett and Wilson 1999).

To generalize, increased diversity and spatial heterogeneity of AMF will increase the diversity of plants when a variation in host plant mycotrophy is present (van der Heijden et al. 1998; Hartnett and Wilson 2002).

The effect of AMF on plant communities has been the focus of considerable research; however, the reciprocal can be explored as well. The diversity of AMF is affected by the diversity of the plant community (Eom et al. 2000). Monocultures of mycorrhizal plants may have lower AMF diversity than communities dominated by non-mycorrhizal plants (Johnson et al. 2004). While this may seem counter-intuitive at first, it is an analogue of the situation where AMF increase the competitive dominance of mycorrhizal plants in tall grass prairies, thereby reducing the overall diversity of the plant community. The successional status of the plant community also dramatically impacts AMF communities with an increase in AMF diversity along the successional gradient (Koske and Gemma 1997). As well as a change in the level of diversity, there is a change in species composition, with a different group of species of AMF being represented in an early successional community than in a late successional community (Johnson et al. 1991). The endomycorrhizal relationship is truly a two-way street and as of yet it is unclear whether the AMF is the driver or the passenger in this relationship, or whether this distinction can even be made (Hart et al. 2001).

Use of AMF in Ecological Restoration

Stochastic events, whether natural or human mediated, impact the diversity and abundance of AMF communities. Generally, disturbed and eroded soils have lowered mycorrhizal infectivity (Moorman and Reeves 1979; Powell 1980; Doerr et al. 1984).

Changing land management is reflected in altered AMF communities (Vandenkoornhuyse et al. 2002) as in the case of agricultural fields being abandoned and reverting to forest (Johnson et al. 1991). While it is clear that humans can effect changes in the AMF community and the AMF community exerts considerable influence on plant communities, it has been postulated that our ability to restore damaged plant communities may be dependent on the establishment of the appropriate mycorrhizal community (Reeves et al. 1979). This may often occur without our explicit intent (Koske and Gemma 1997; Bauer et al. 2003); however, in other cases direct manipulation of the AMF community may be essential or give an advantage to our restoration plantings.

In highly disturbed areas or challenging sites such as strip mines and salt marshes, it may be critical to preinoculate restoration materials with appropriate AMF (Allen and Allen 1980; Miller et al. 1985; Cooke and Lefor 1990; Noyd et al. 1995). In other situations such as prairie and dune restoration, AMF are likely already present but additional inoculation has been found to give a distinct advantage to restoration materials (Gemma and Koske 1997; Smith et al. 1998). In light of the earlier discussion on plant and fungal interactions, a diverse array of locally adapted AMF should be used for restoration purposes (Klironomos 2003).

AMF and Agriculture

Agricultural practices have dramatic impacts on soil and soil organisms, and AMF are no exception. A number of studies have shown that agriculture reduces the diversity of the AMF community (Helgason et al. 1998; Daniell et al. 2001; Oehl et al.

2003). This has been attributed to physical disturbance from tilling (Kabir et al. 1997; Jansa et al. 2003), the effects of supplemental fertilizers (Linderman and Davis 2004) and the use of fungicides and soil fumigants (Menge 1982), all of which reduce the abundance and or diversity of AMF. Interestingly, the use of some pesticides has been shown to increase the diversity of AMF, possibly due to a decline in mycophageous insects after treatment (Vandenkoornhuyse et al. 2003).

Some alternative agriculture methods have less of an impact on AMF communities. Generally low input and low till agricultural systems have a higher abundance and diversity of AMF than their traditional counterparts (Douds and Millner 1999; Galvez et al. 2001). Organic fertilizers can be less damaging to AMF functioning than chemical fertilizers (Linderman and Davis 2004) and one study found that an organically farmed system had a similar AMF diversity to a nearby native grassland (Oehl et al. 2003). It has also been shown that the presence of agricultural weeds can increase the abundance of beneficial AMF in fields (Vatovec et al. 2005).

Agricultural practices such as tilling and fertilizing not only cause a decline in AMF diversity, there is substantial evidence that they produce a shift in the AMF community composition (Boddington and Dodd 2000; Egerton-Warburton and Allen 2000; Jansa et al. 2003). These agriculturally adapted AMF have been shown to be slower to infect, faster to sporulate and to produces fewer arbuscles (Johnson 1993; Scullion et al. 1998; Oehl et al. 2003). These findings all suggest that the altered AMF community is drawing more resources from the plant while providing less in return.

The ramifications of these altered AMF communities may not be that great as long as the field is kept under agricultural production. However, in many areas of the

United States, historically farmed land is being abandoned for social or environmental reasons. In many cases land managers are seeking to restore native plant communities to these sites. The loss of AMF species diversity during agricultural use may result in a diminished biodiversity of the reestablishing plant community (van der Heijden et al. 1998). The AMF community may continue to be less beneficial to plants even 25 years after abandonment (Corkidi et al. 2002; Richter et al. 2002). Researchers have employed the use of AMF from remnant native sites to help reestablish the native flora on abandoned agricultural fields. This has been found to increase germination, growth and survivorship of restoration materials (Richter and Stutz 2002).

Geography of AMF Research

The studies cited above come from a diversity of ecosystems across the world with a particular breadth of research on grasslands and agricultural fields from Eastern Canada, Switzerland, the United Kingdom and the United States. Within the United States, arid and semi-arid systems have received the greatest attention and there is a clear lack of research into the AMF of the Pacific Northwest. While some AMF work has been done on post-eruption Mt. St. Helens (Allen 1987) and there was a taxonomic treatment of the Pacific Northwest AMF published some years ago (Gerdemann 1974), the understanding of the ecology of the AMF of the Pacific Northwest is based almost entirely on studies done elsewhere in the world. There is reason to believe that AMF may function differently in the cool and damp climate of the Pacific Northwest than they do in the tall grass prairies of Kansas, for example. Pendleton and Smith (1983) have suggested that the successional model of AMF interactions discussed above is only

relevant in semi-arid conditions. Additionally, environmental factors affect the nature of a given plant and fungus pair (Furlan and Fortin 1977; Hayman 1983). Given the functional variability exhibited by AMF (Klironomos 2003), assumptions about their ecological role must be made tentatively or tested explicitly. In the Pacific Northwest there is still much to be understood about these influential organisms.

Puget Sound Prairies

The prairie ecosystem of the Puget Sound region has been reduced to a tiny fraction of its historic extent. Primarily this was due to the conversion of the prairies to farms and housing and to a lesser extent the encroachment of Douglas-fir (*Pseudotsuga menziesii*) forest after the termination of regular burning by the native peoples. Much of the remaining prairie ecosystem has been protected by groups such as The Nature Conservancy, the Washington Department of Fish and Wildlife, the Washington Department of Natural Resources, and the Whidbey Camano Land Trust. Some sites of historic agricultural use have been abandoned and purchased by conservation organizations with the intent of restoring the native prairie communities. Given the work done by Richter and Stutz 2002 and Richter et al. 2002 on abandoned farms in Arizona, and Corkidi et al. 2002 in Colorado, there is concern that the AMF communities of abandoned agricultural sites around the Puget Sound may have been altered such that establishment of native plant species will be difficult. If this is the case then pre-inoculation of restoration materials with AMF from nearby extant prairies may be necessary for successful restoration.

Study Objectives

This study investigates the impact of AMF on the restoration of abandoned agricultural land in the Puget Sound area back to native prairie. The objectives of this study were to determine (1) what effect AMF from native prairies around the Puget Sound have on the germination and growth of yarrow (*Achillea millefolium*) and Roemer's fescue (*Festuca roemeri*), two common prairie restoration plants of differing mycotrophy (Pendleton and Smith 1983; Wilson and Hartnett 1998), (2) if the effects of AMF are consistent across the Puget Sound or are site dependent, and (3) if the effects of AMF from abandoned agricultural fields around Puget Sound differ from that of AMF from native prairies.

Results of these investigations address whether historic agricultural practices on sites that have been selected for prairie restoration in the Puget Sound area have altered the functionality of the AMF community in such a way as to make re-establishment of native prairie plants difficult. Furthermore, the need for pre-inoculation of plants with local native prairie AMF in the restoration of abandoned agricultural fields is assessed.

Methods

Inoculum Collection

Three prairies in the Puget Sound area (Au Sable, Naas Preserve and Scatter Creek) were identified as having extant native prairie in close proximity to abandoned agricultural land. These prairies served as my source for mycorrhizal inoculum. Twelve liters of soil were collected in April of 2006 from each of the six paired sites. Ten to twelve small holes were dug across each site to a depth of 15 cm. The location of the holes was chosen to spread the impact across the site while avoiding sensitive plants and margins of transition to other habitat types. Soil from all the holes at a given site was mixed together, cleaned of rocks and large root masses and stored in plastic bags in a cold room at 2° C.

Site Descriptions

Au Sable This site is part of the greater Smith Prairie on central Whidbey Island on the eastern side of Ebby's Landing National Historic Reserve at 60 m above sea level (Figures 1 & 2). The area was first settled during the 1850s. Agricultural was attempted on this site but the soils proved too dry to be productive and this use was soon abandoned. In 1947 the land was purchased by the Washington Department of Game and used for raising pheasants. In 1999 the land was purchased by the Au Sable Institute of Environmental Studies (Steve Erickson, Whidbey Environmental Action Network, pers. comm.). The 'prairie soil' treatment was collected from an area outside of the range of the game farming that was dominated by native prairie grasses and forbs.

The ‘abandoned agricultural field’ soil was collected from an adjacent area, also outside of the game farm, that was dominated by non-native, ruderal species, suggesting that the area had been tilled in the past. No plow horizon was observable in either area.

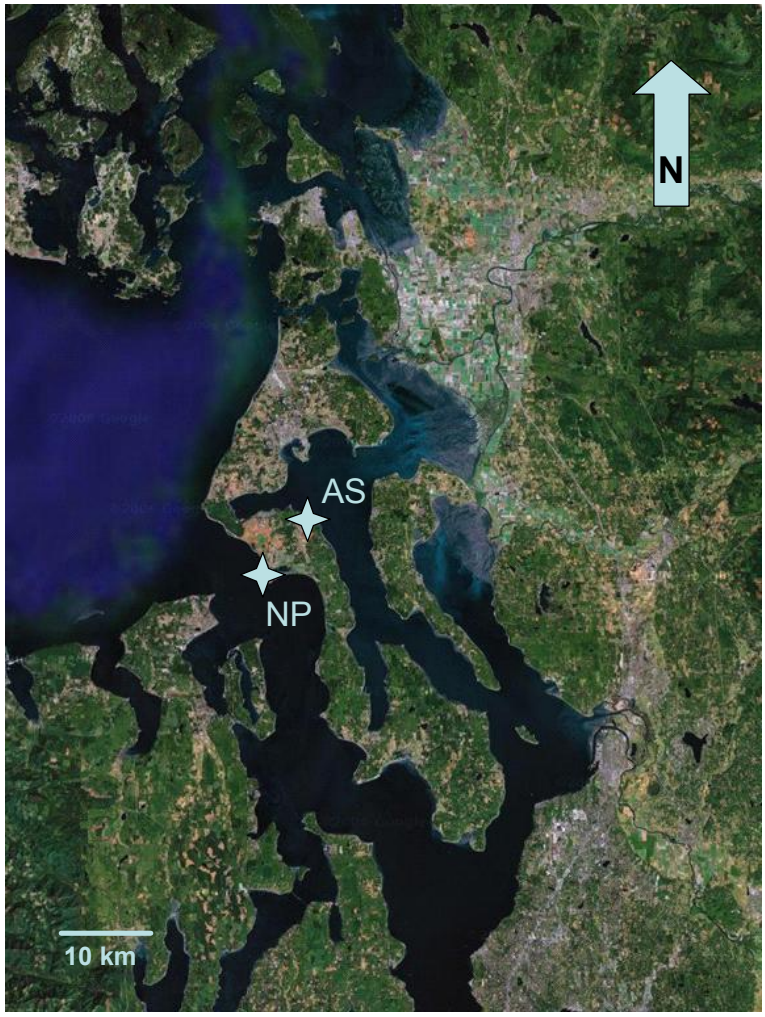

Figure 1 Aerial photograph of Whidbey Island showing the Au Sable (AS) and Naas Preserve (NP) soil collection sites

Naas Preserve This is a small remnant prairie on the southern edge of the Ebey’s Landing National Historic Reserve (Figures 1 & 2), preserved by the Whidbey Camano Land Trust for the conservation of a small population of golden paintbrush (*Castilleja levisecta*). It is sited 50 m above sea level. The foundation of a small military building remains on the site suggesting that some past disturbance was likely.

The ‘abandoned agriculture field’ soil was collected from the north end of the site near a fence line that separated the prairie from an active agricultural field. Aerial photos from 1957 show this area to be under active agricultural production. Another series of aerial photos from 1983 show the fence line moved to its current location and the area laying fallow. It is not known at what point during this 25 year window that the land was abandoned. The plant cover here was invasive annual grasses and a plow horizon was observable at a depth of 15 cm. The soil was dark and saturated with water. The ‘prairie soil’ was collected from the center of the site which was likely never heavily plowed. The military had used this area for grazing horses and it is possible that they lightly tilled this area to seed pasture grasses (Pat Powell, Whidbey Camano Land Trust, pers. comm.) The plant cover here was predominantly native grasses and forbs.

Figure 2 Detail of Au Sable and Naas Preserve soil collection sites

Scatter Creek This site, located in the southeast corner of Thurston County between the towns of Grand Mound and Rochester, sits at 55 m above sea level and is a South Puget Sound Wildlife Area managed by the Washington Department of Fish and Wildlife (Figures 3 & 4). This was the site of the first homestead in Thurston County and the historic barn is still remaining. The area from which the 'abandoned agriculture' collection was taken is generally known by local land managers to have been plowed at some point in the past prior to the 1960s. No definite dates are known. This area was characterized by a mix of native and ruderal species. At a depth of approximately 10 cm a layer of small rocks existed that was impenetrable with the soil core. The 'prairie soil' was collected from a site with no known history of tilling. Additionally, the cover was almost exclusively native species and the rocks were evenly distributed in the upper layer of the soil. A thick carpet of moss was interspersed with the native grasses.

Figure 3 Satellite photo of western Washington showing the Scatter Creek (SC) soil collection site

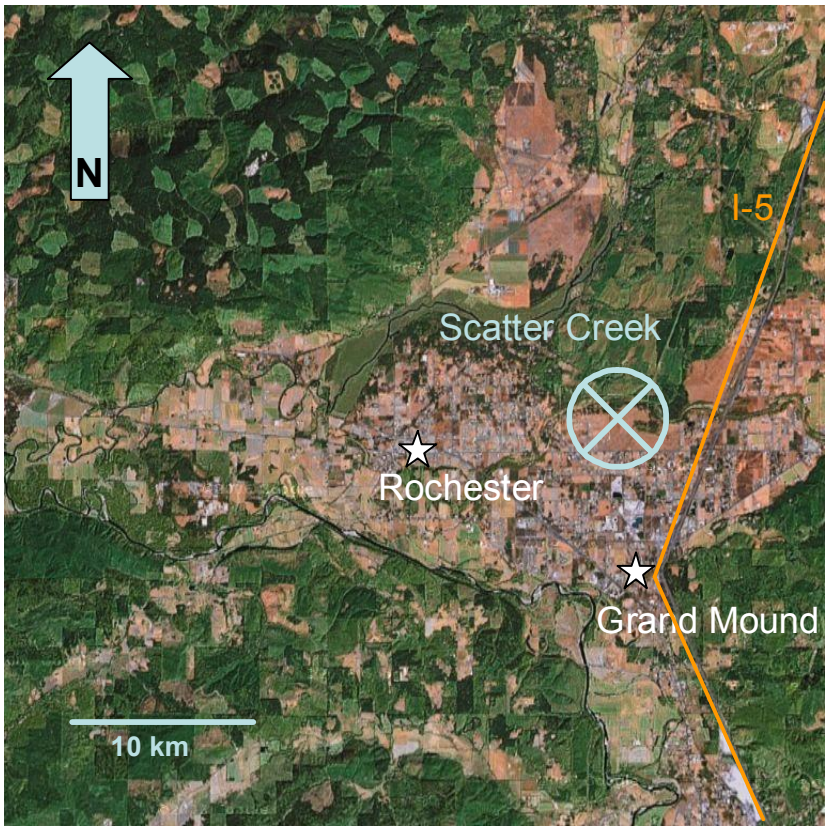

Figure 4 Detail of Scatter Creek soil collection site

Soil Analysis

Each of the six soils collected were analyzed for texture, pH, percent organic matter, total carbon and total nitrogen. For soil texture analysis, soil was sifted through a 2 mm screen. Sifted soil was added to a graduated cylinder up to the 70 ml mark and 60 ml of sodium hexametaphosphate (0.93% aqueous solution) was gradually added to the cylinder. The slurry was shaken for five minutes and allowed to settle overnight. The volumes of sand, silt and clay were then visually assessed and the soil texture class was determined using the soil texture triangle (Cahilly 2000).

Soil pH was determined by mixing one part soil with two parts de-ionized water, agitating and allowing to sit for thirty minutes. The slurry was agitated again immediately prior to taking the reading. An electric pH meter (VWR Scientific 2000) was used and recalibrated between each reading.

Percent organic matter was calculated based on loss on ignition. Approximately 18 g of soil was sifted to 2 mm, tamped into porcelain crucibles and dried at 105°C overnight. These dry samples were then weighed and placed in a muffle furnace at 556°C for two hours. They were subsequently cooled in the 105°C desiccator for 3.5 hours and reweighed (Heiri et al. 2001). Percent organic mater was calculated as (dry weight – ignited weight)/dry weight x 100%.

Soil samples were analyzed for total carbon and total nitrogen using a Perkin Elmer 2400 CHN analyzer.

Soil Treatments

The mycorrhizal soil treatments were developed using a base of Sunshine mix #2 (SUN GRO Horticulture Canada LTD.) that was sterilized by autoclaving at 260° F for 90 minutes. The soil was left to sit for at least two weeks before use to remove any toxic effects of autoclaving (Rovira and Bowen 1966). Raw soil from the prairie and agricultural sites was mixed with the sterilized potting soil in a 1:3 ratio (Kemery and Dana 1995) to provide the experimental mycorrhizal inoculations. Additionally, a control treatment was set up for each inoculum type by autoclaving half of the field soil at 260° F for 90 minutes (Smith and Smith 1981; Stutz and Morton 1996). After cooling, controls were reinoculated with the native, non-mycorrhizal, microbial

community by mixing 100 g of field soil with 300 ml water and passing it through a 63 μm sieve. These soil sievings were then added back to the appropriate sterilized control (Koide and Li 1989). A seventh control group with no field soil was established. These soil treatments were placed in 160 cc supercells (Ray Leach Conetainers, Stuewe and Sons Inc, Corvallis, OR) and were randomized across a large grid tray system, skipping every other row and column to avoid potential contamination (Figure 5). Non-experimental plants were used in the cones on the perimeter of the block to control for edge effect (Figure 6). Ten replicates for each of thirteen treatment/controls were used and repeated for two plant species for a total of 260 plants.

Figure 5 Photograph of planting design

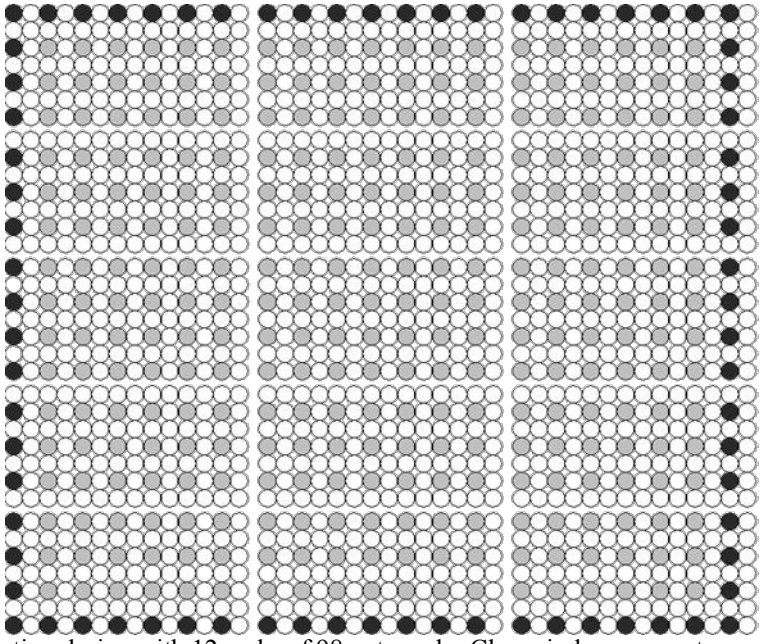

Figure 6 Planting design with 12 racks of 98 pots each. Clear circles are empty; gray circles are experimental plants, and black circles are non-experimental plants

Seedling Establishment

Wild collected seeds of *Festuca roemeri* and *Achillea millefolium* were obtained from Frosty Hollow Ecological Restoration, Whidbey Island, WA. These were surface sterilized in 5% bleach solution for 10 minutes (Johnson 1993) and sown five to a pot. Germination percentage and the time to first and last seedling emergence were recorded for each pot. All germination ceased at 14 days and at 18 days the pots were thinned to one seedling each. Plants were watered from a municipal water supply and fertilized weekly with a custom mixed, low phosphorus fertilizer (Miyasaka 2003).

Data Collection

After thirteen weeks a general health ranking was assigned to each plant. The health ranking for the fescue plants was assigned as follows: 0 = dead; 1 = at least 75% of leaves dead; 2 = at least 50% of leaves dead and live leaves discolored; 3 = mostly

healthy, some discoloration or dead leaves; 4 = healthy bright green. For the yarrow plants the ranks were assigned: 0 = dead; 1 = many dead leaves, browning on live leaves, heavily infested with insects; 2 = at least two dead leaves, some browning of live leaves and moderate infestation; 3 = one to two dead leaves, live leaves all green, little sign of insects; 4 = healthy, no senescence, no insects. Because all plants were subject to the same levels of insect predation pressure, the variation in observed predation was taken as a sign of inherent plant health.

Plant height was then measured and the above ground biomass was harvested, dried overnight at 65°C and weighed (Johnson 1993; Kemery and Dana 1995). The roots were washed clean of soil. Half of each root system was dried and weighed and the other half was stored in ethanol for later staining. Preserved roots were stained with Trypan Blue to visualize AMF infection (Moorman and Reeves 1979; Brundrett et al. 1984; Koske and Gemma 1989).

Because of evidence that infection level is not correlated to AMF functionality (Noyd et al. 1995; Wilson and Hartnett 1998), and because the accepted methods of infection level assessment are subject to investigator bias and are therefore not comparable between studies (McGonigle et al. 1990), no attempt was made to precisely quantify the level of AMF infection. Instead, a ranking system was employed that gives a general measure of AMF infection (Koske and Gemma 1997). The ranking system is as follows: 0 = no infection; 1 = AMF structures present in 1-10% of roots sampled; 2 = AMF structures present in 10-25% of roots sampled; 3 = AMF structures present in 26-50% of roots sampled; 4 = AMF structures present in >50% of roots sampled. Because non-mycorrhizal hyphae are not often present in mycorrhizal roots (Kormanik 1982) all

intraradical fungal hyphae were considered to be mycorrhizal if vesicles or arbuscles were observed in the root tissue. Figure 7 shows a photograph of AMF vesicles and figure 8 shows a photograph of arbuscular development in one of my root samples. While only arbuscles are uniquely AMF structures (McGonigle et al. 1990), they were rare and/or difficult to observe in our samples. However, the occurrence of vesicles in all but one of the live mycorrhizal treatments and the absence of vesicles in all but three of the non-mycorrhizal treatments, gave confidence that vesicles were an appropriate means of identification of AMF infection in my sample.

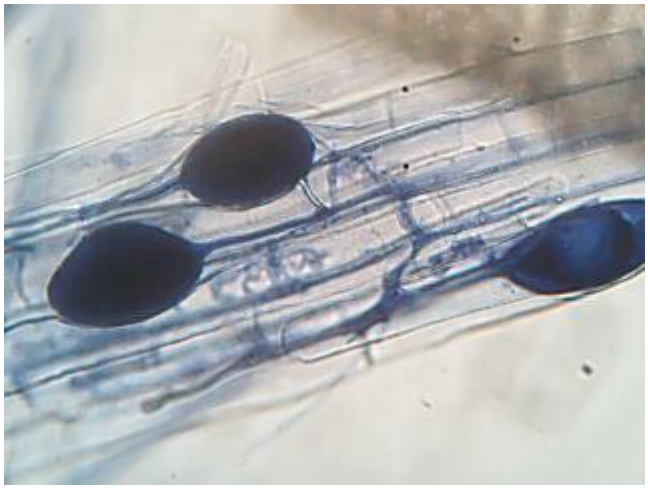

Figure 7 A cleared root showing vesicles of an AMF connected by hyphae (200x)

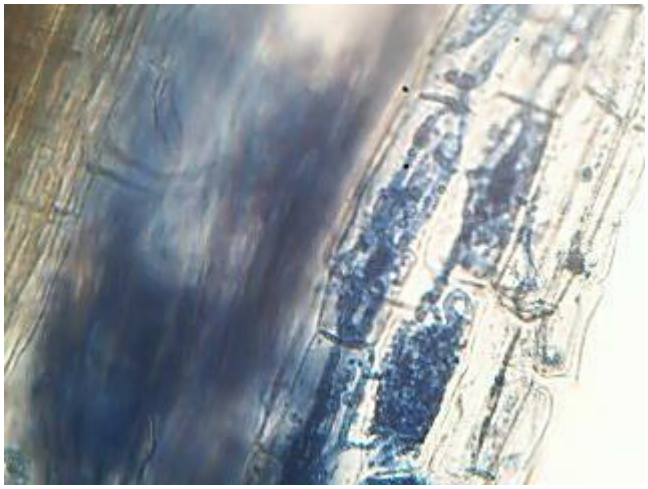

Figure 8 Root cells filled with branching hyphae showing different stages of arbuscle development (200x)

Statistical Analysis

The two plant species were analyzed separately. The effects of live mycorrhizal inoculum, the site of inoculum collection, and the land use history of the field soil within the site (extant prairie vs. abandoned agricultural field) were analyzed based on the following responses: Germination percentage, time to first seedling emergence, time to last seedling emergence, shoot dry weight, root dry weight, root/shoot ratio, plant health and level of mycorrhizal infection. Responses with continuous data (germination and weight data) were initially analyzed with a three-factor, type II ANOVA, which allows for dissimilar sample sizes. Significant three-way interactions ($\alpha = 0.05$) were explored with two-factor ANOVAs broken out by site. Significant two way interactions were analyzed using a Welch two sample T test which does not assume equal variance among groups. Significant main effects were evaluated utilizing Tukey's Honestly Significant Difference or the Welch two sample T test depending on whether there were two or three groups being compared.

Both the plant health and mycorrhizal infection responses were ordered categorical data. Multidimensional contingency tables were used to build log linear models of these data (Zar 1999). The smallest model yielding a non-significant likelihood ratio test statistic (lrt) was chosen as the best model and the included terms were considered to significantly affect the response variable. A weakness of this method is that it ignores the ordered nature of the categorical data and thus has a higher type II error rate (β) than necessary. These data were re-analyzed using the ANOVA approach described above which assumes that the response is continuous. If the distance between the ranked categories is equal then this assumption is not greatly

violated. This was not completely the case in the situation of the root infection ranking and is arguable in the case of the qualitative health ranking. Because of this I would expect a somewhat elevated type I error rate (α) using the ANOVA method. So my true, desired significance level of $\alpha = 0.05$ would lie somewhere between the two analysis methods. The results of the two analyses agreed in nearly all cases and any discrepancies were interpreted with caution.

The results of all statistical analyses are presented in Appendix 1.

Results

Soil Analysis

All of my soil collections were remarkably similar with a relatively consistent pH, carbon-to-nitrogen ratio and soil texture (Table 1). Scatter Creek soils stood out as being highly organic and consequently had the highest levels of total nitrogen. Additionally they were the most acidic soil collections. Interestingly, the Scatter Creek agricultural soil had a higher total nitrogen level than the Scatter Creek prairie soil but had a lower level of organic matter. In all other cases total nitrogen mirrored the levels of organic matter.

The Au Sable soils were very similar to each other in pH and texture but the prairie soil was more organic and thus had a higher total nitrogen level than the agricultural soil. The Naas Preserve soils were nearly identical to each other in all measurements. This was surprising considering this was the most recently, and likely most extensively, tilled of the abandoned agricultural sites.

Table 1 Analysis of soil collections used as AMF inoculum

	pH	% Organic Matter	% Total Carbon	% Total Nitrogen	C/N Ratio	%Sand	%Silt	%Clay	Soil Texture
Au Sable Prairie	5.14	12.84	6.973	0.559	12.474	62.1	34.5	3.4	Sandy Loam
Au Sable Ag	5.5	9.57	5.323	0.404	13.176	70	27	2.7	Sandy Loam
Naas Preserve Prairie	5.52	10.41	5.375	0.428	12.558	45	52	3	Silt Loam
Naas Preserve Ag	5.58	10.41	5.657	0.447	12.655	36	60	4	Silt Loam
Scatter Creek Prairie	4.82	18.06	9.704	0.703	13.804	58.2	39.1	2.7	Sandy Loam
Scatter Creek Ag	4.48	16.07	12.023	0.941	12.777	40.8	56.1	3.1	Silt Loam

Germination Data:

The various mycorrhizal treatments had little effect on germination of either fescue or yarrow. For yarrow, time to first and last seedling emergence (Figure 9) as well as total number of seedlings germinated (Figure 10) were similar across both soil types and with soil from all sites and were unaffected by the sterilization of mycorrhizal inoculum. The spike in the time to first germination seen in the sterilized Naas Preserve prairie soil treatment was due to one individual pot in which the first seedling emerged 49 days after the experiment was started. Excluding this individual from this group drops the mean from 9.9 to 5.6 days.

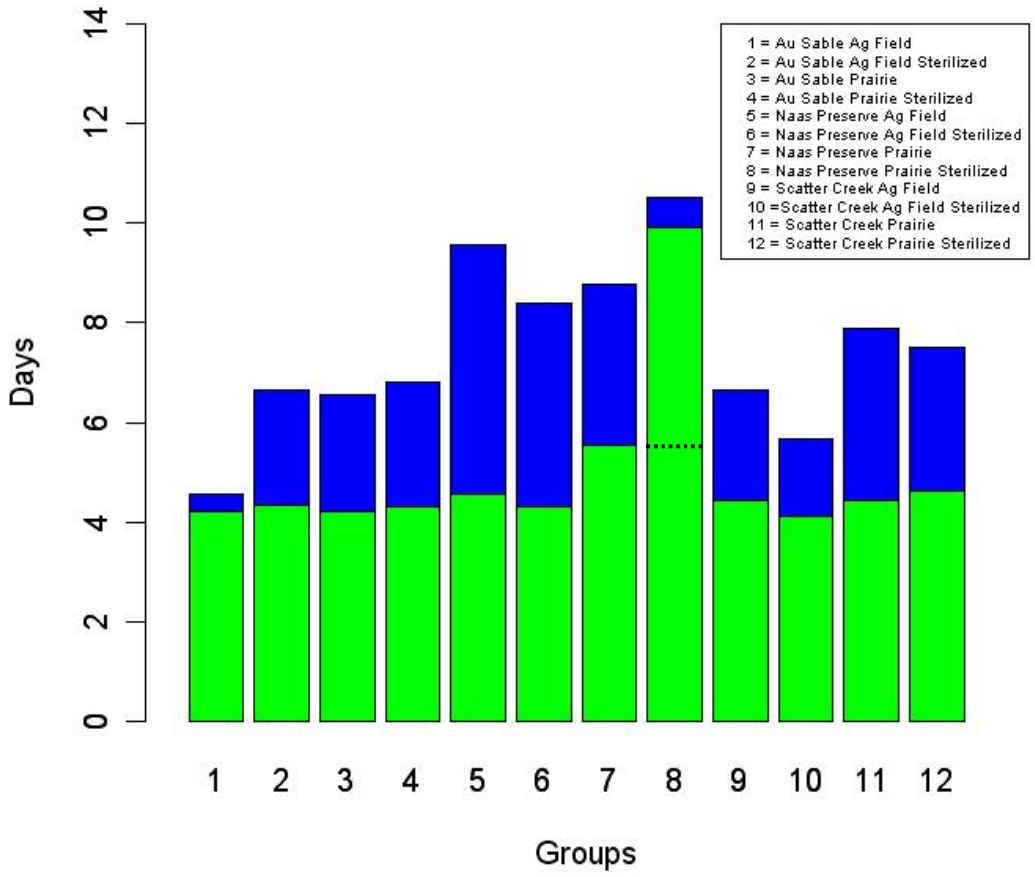

Figure 9 Average time to first seedling emergence (green) and last seedling emergence (blue) of yarrow by experimental group. The dotted black line shows the average for group 8 if the anomalous individual is removed

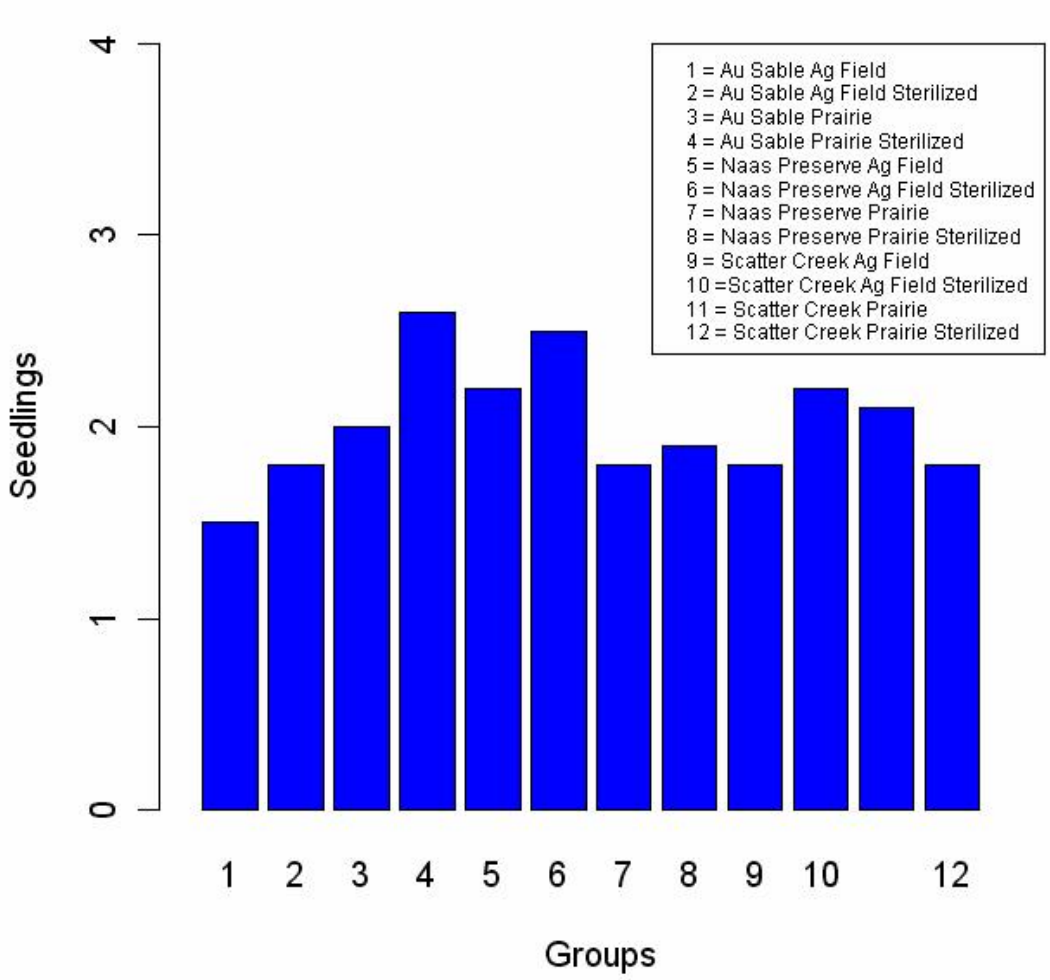

Figure 10 Average number of yarrow seedlings per pot by experimental group

Germination timing of fescue was not affected by the treatments (Figure 11). Live AMF reduced the total germination of fescue seeds in all sites ($p < 0.05$) (Figure 12). Total germination of fescue seeds varied across sites ($p < 0.05$) with a higher germination in Naas Preserve soil than in Scatter Creek soil (Figure 13). The total germination data for Au Sable treatments were not significantly different from the other two sites.

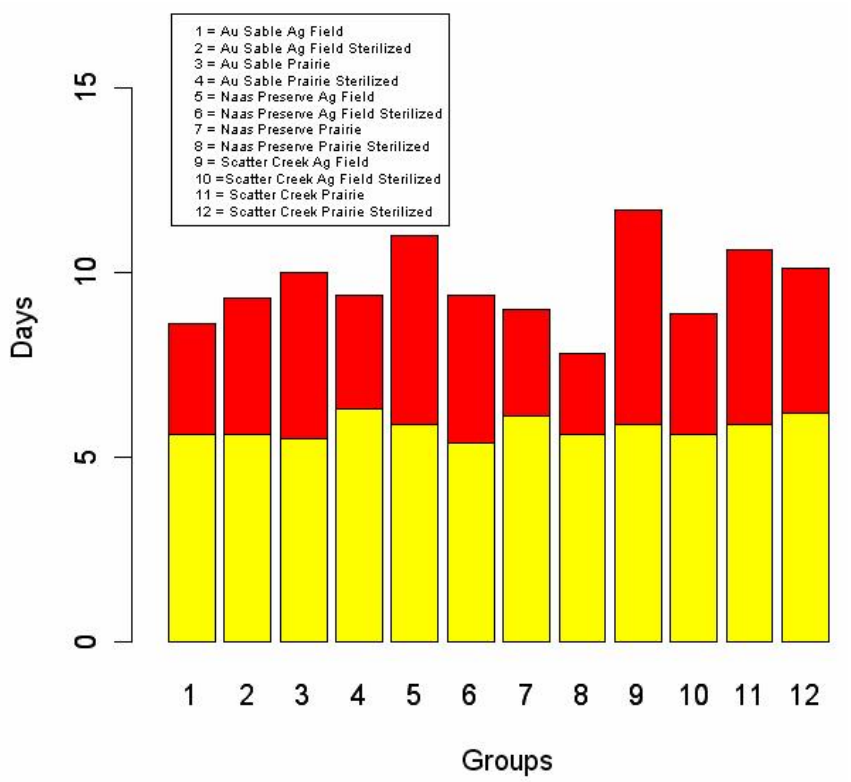

Figure 11 Average time to first germination (yellow) and last germination (red) of fescue by experimental group

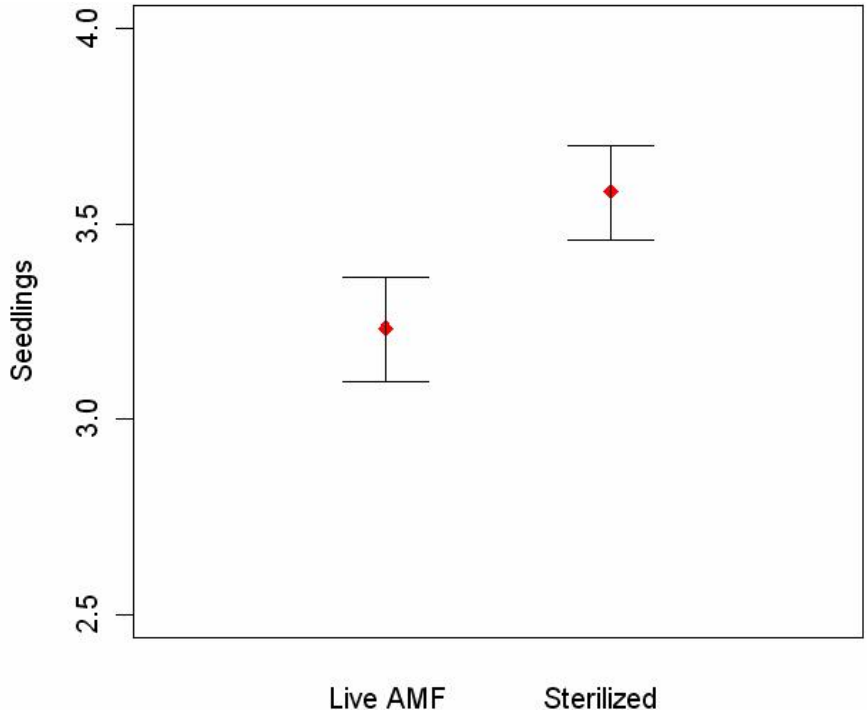

Figure 12 Average germination per pot of five fescue seeds with live or sterilized AMF inoculum. Bars show the standard error of the mean.

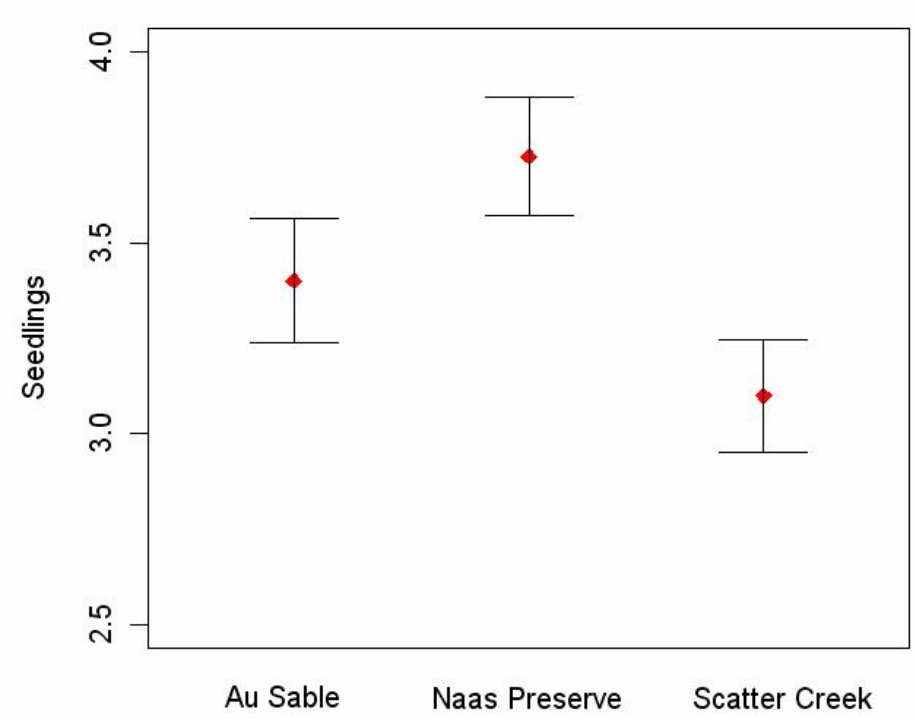

Figure 13 Average germination per pot of five fescue seeds by site of soil collection. Live and sterilized AMF are grouped together. Bars show the standard error of the mean.

Below Ground Dry Weight

Below ground dry weight deviated from the normal distribution for both species. The fescue data approximated a normal distribution after a 0.5 power transformation and the yarrow data were normalized after a 0.25 power transformation (Appendix 2).

The trend was for plants to have a less massive root system when grown with live AMF inoculum. Secondarily, plants had more massive root systems when grown with agricultural soil although there was variation in this trend (Figure 14). Yarrow plants grown with soil from the Naas Preserve site followed this pattern nicely with both sterilized inoculum and agricultural land use increasing the below ground dry weight ($p < 0.001$ and $p < 0.001$, respectively). Fescue grown in the same Naas

Preserve soil showed the same response with live AMF ($p < 0.001$). However, when the inoculum was sterilized the dwarfing effect of the prairie soil disappeared.

With soil from the Au Sable site, both plants had more massive root systems when the AMF inoculum was sterilized ($p < 0.001$ for both fescue and yarrow). In contrast to plants grown in Naas Preserve soil, fescue and yarrow grown in Au Sable soil showed identical root growth in both prairie and agricultural soil with live AMF. When the inoculum was sterilized, fescue continued to show no difference, whereas yarrow plants had more massive root systems with prairie soil ($p < 0.01$). This was the only incidence of roots in a prairie soil treatment being significantly larger than roots in the paired agricultural soil treatment.

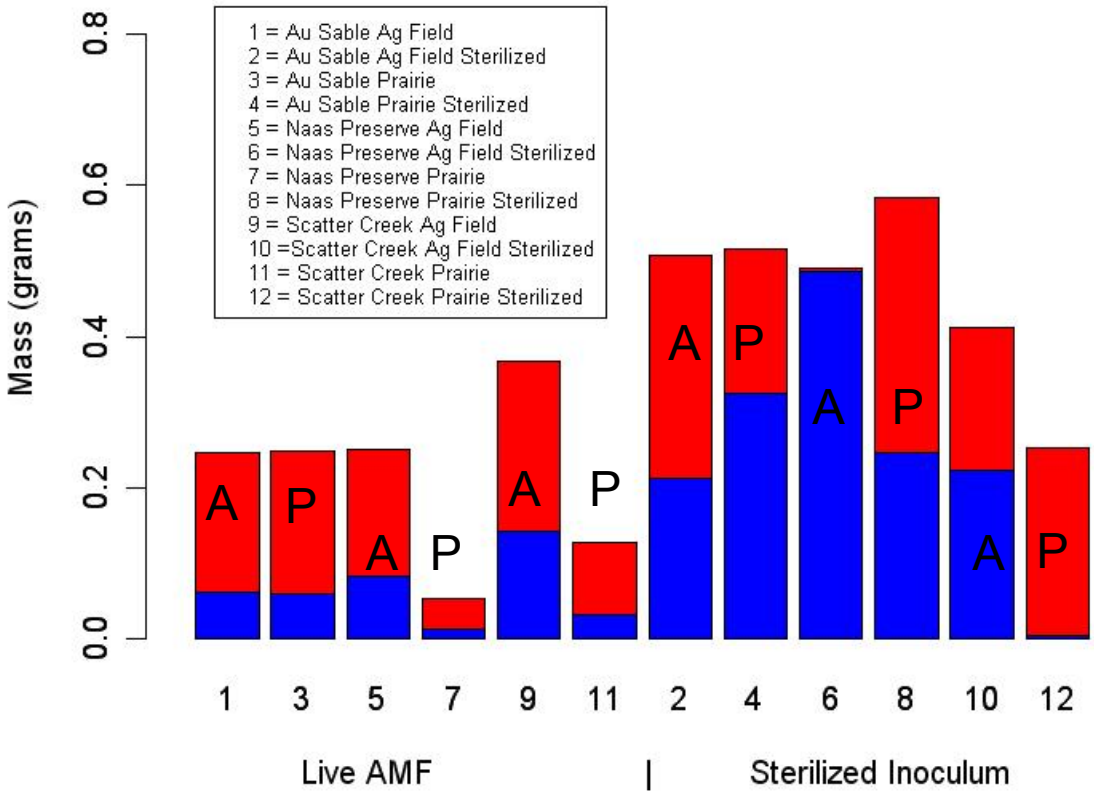

Figure 14 Below ground dry weight of yarrow (blue) overlaid on fescue (red) by experimental group. Note groups are reorganized with mycorrhizal treatments on the left. Paired agricultural soil treatments (A) and prairie treatments (P) are adjacent.

Fescue at Scatter Creek followed the expected pattern with agricultural treatments having more massive root systems than prairie treatments ($p < 0.001$) and live mycorrhizal treatments having less massive roots than sterilized treatments, although this effect was just shy of significant ($p = 0.054$). Yarrow with Scatter Creek soil showed an interesting deviation from the general pattern. While the treatments with agricultural soil were larger than prairie treatments ($p < 0.001$) and the sterilized agricultural treatment was more massive than the non-sterilized agricultural treatment ($p < 0.05$), the prairie soil treatment showed an increase in root growth with live AMF ($p < 0.01$) (Figure 14, groups 11 and 12, blue bars). This was the only treatment in which live AMF caused an increase in growth. The increase was not drastic but the minute size of the yarrow plants with sterilized Scatter Creek soil made the difference significant.

Also of note is the difference in growth experienced by both yarrow and fescue between the treatments with live inoculum from the Naas Preserve prairie and the sterilized inoculum from the Naas Preserve prairie (Figure 14, groups 7 and 8). This effect is especially prominent in the fescue plants in which the sterilized treatment represents the group with greatest growth and the live AMF treatment represents the group with the least growth of all fescue treatments.

Above Ground Dry Weight

The above ground dry weight deviated from a normal distribution for both species. These data were normalized for both species after a 0.5 power transformation (Appendix 2).

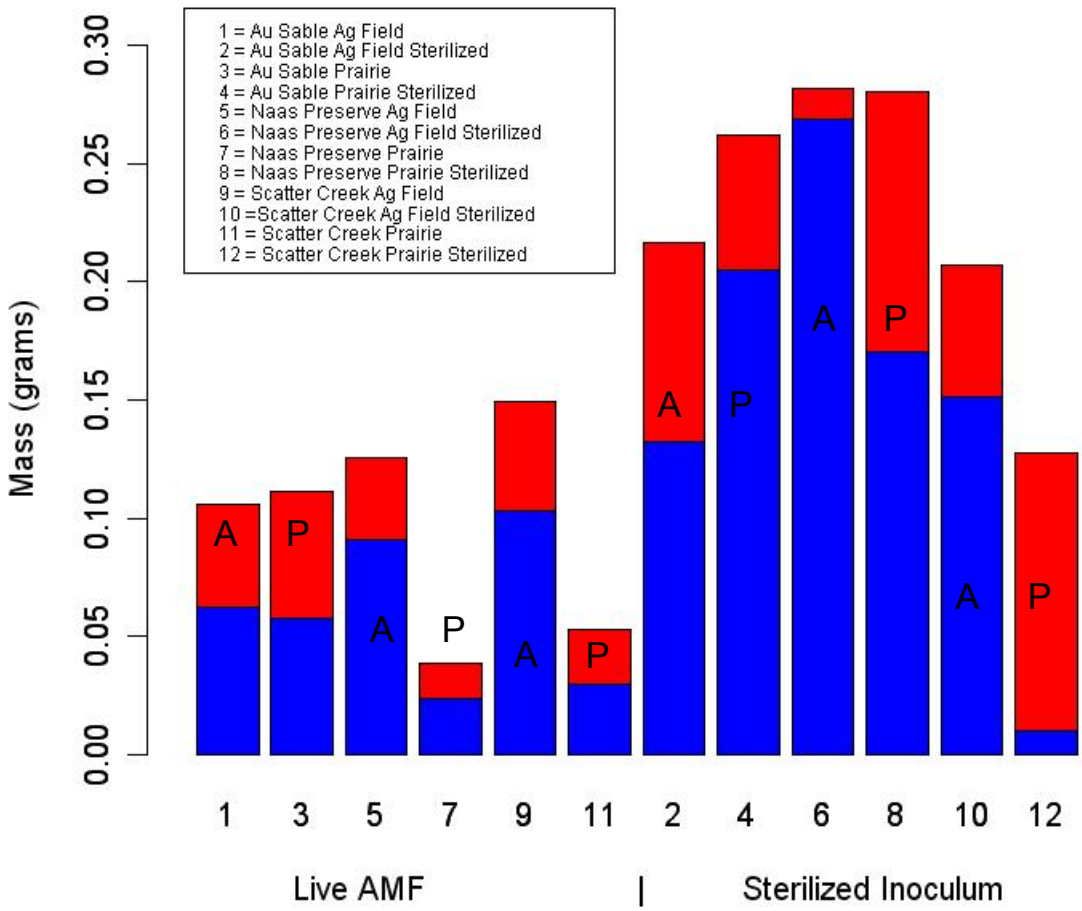

Figure 15 Above ground dry weight of yarrow (blue) overlaid on fescue (red) by experimental group. Note groups are reorganized with mycorrhizal treatments on the left. Paired agricultural soil treatments (A) and prairie treatments (P) are adjacent.

The above ground dry weight echoed exactly the patterns seen with the below ground dry weight (Figure 15). With soil from the Naas Preserve yarrow plants with live AMF had lower above ground dry mass than their sterilized counterparts ($p < 0.001$). Yarrow grown with soil from the Naas Preserve abandoned agricultural collection had a larger above ground dry mass than plants grown with the Naas Preserve prairie collection ($p < 0.001$). Fescue plants with sterilized Naas Preserve inoculum showed increased growth over those plants with live Naas Preserve inoculum ($p < 0.001$). As before, fescue with live Naas Preserve agricultural soil were larger than

fescue with live Naas Preserve prairie soil ($p < 0.001$) but when sterilized, prairie and agricultural inoculum were equivalent.

Sterilized inoculum from Au Sable caused greater above ground biomass in fescue ($p < 0.001$) and yarrow ($p < 0.001$) than live inoculum. There was no difference in growth between plants with agricultural or prairie inoculum for fescue with Au Sable soil. For yarrow there was also no difference when the inoculum was live. However, as with the root weight data, when the inoculum was sterilized yarrow plants showed the same anomalous response of greater growth with prairie inoculum ($p < 0.001$).

With Scatter Creek soil, fescue plants grew larger in agricultural soil ($p < 0.001$) and with sterilized AMF inoculum ($p < 0.001$). Yarrow treatments with agricultural soil were larger than prairie treatments ($p < 0.001$) and the sterilized agricultural treatment was larger than the non-sterilized agricultural treatment ($p < 0.01$). Consistent with the root weight data but in contrast to the overall trends, the prairie soil treatment showed an increase in shoot growth with live AMF ($p < 0.01$).

Root to Shoot Ratio

The root/shoot ratios for fescue more closely approximated a normal distribution after log transformation of the data. The yarrow root/shoot ratio data were best left untransformed (Appendix 2).

The root to shoot ratio of fescue plants was unaffected by any of the mycorrhizal inoculum treatments (Figure 16). This is not surprising considering the root and shoot data mirrored each other so precisely. There was an almost significant effect of site

with fescue plants grown in Naas Preserve soil having a smaller root to shoot ratio ($p = 0.068$).

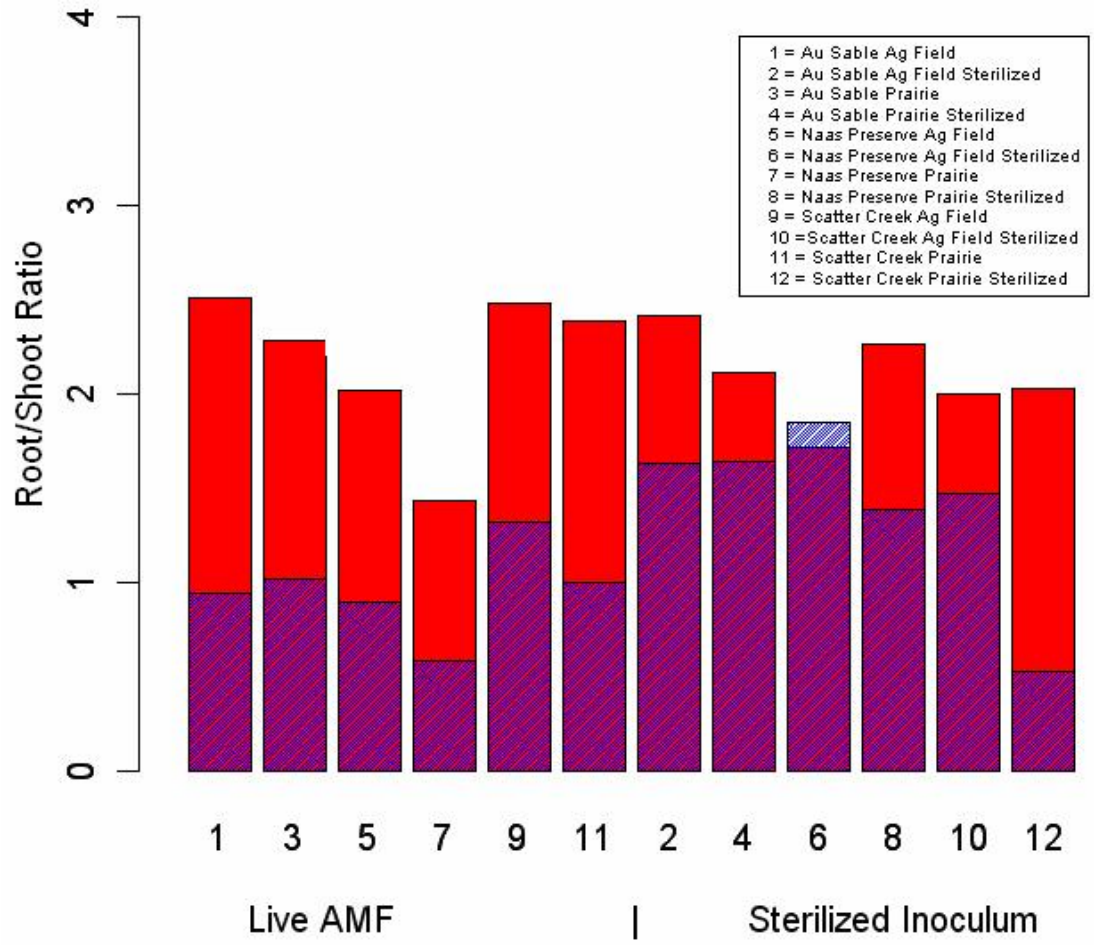

Figure 16 Root to shoot ratio (g/g) by group. Mycorrhizal groups on the left. Yarrow data (blue hashing) overlaid on fescue data (red).

The plot of root to shoot ratio for yarrow plants (Figure 16) closely approximates the plot of shoot or root weight. This means that as the overall dry mass of the plant increased so did the root/shoot ratio. Consequently, the same factors that caused an increase in yarrow dry weight caused an increase in yarrow root to shoot ratios. In Naas Preserve soil this ratio was increased with agricultural inoculum ($p <$

0.05) and with sterilized inoculum ($p < 0.001$). With soil from the Au Sable site, yarrow root/shoot ratio was increased by sterilization of the AMF inoculum ($p < 0.01$). And root/shoot ratio of yarrow plants was higher in agricultural soils than prairie soils ($p < 0.01$).

Health Rank

Health rank data were first evaluated using ANOVA. Findings were then corroborated using the more conservative log linear modeling approach. Fescue and yarrow responded differently to the various treatments but one clear trend emerged. Plants were healthier with live AMF. Fescue plants in particular were far healthier with live inoculum than when grown with sterilized inoculum ($p < 0.001$) (Figure 17). From our analysis it would appear that fescue plants with soil from the Naas Preserve site were less healthy than plants from the other two prairie sites ($p < 0.01$). However, this was entirely due to the extremely poor health of plants with sterilized Naas Preserve inoculum (Figure 17, groups 6 and 8). This interaction was not detected by our statistical analysis.

While the trend for yarrow plants with live AMF to be healthier is clear by examining Figure 18 and was shown through log linear modeling, ANOVA analysis revealed an interaction with site ($p < 0.05$). Further exploration of this interaction found that this trend is visible but not significant in soil from Au Sable. We must carefully interpret any discrepancies between the two statistical methods employed. However, this interaction appears to be a reality. The difference in average health rank between plants with live and sterilized AMF inoculum from the Au Sable site was approximately

0.2. The difference from the other two sites taken together was approximately 0.8, a four-fold difference. While this is not a statistically rigorous re-evaluation of the data it supports the findings of the ANOVA.

Additionally, yarrow plants were found to be less healthy with soil from the Scatter Creek site ($p < 0.05$). This is again likely due to the poor health of the sterilized inoculum treatments.

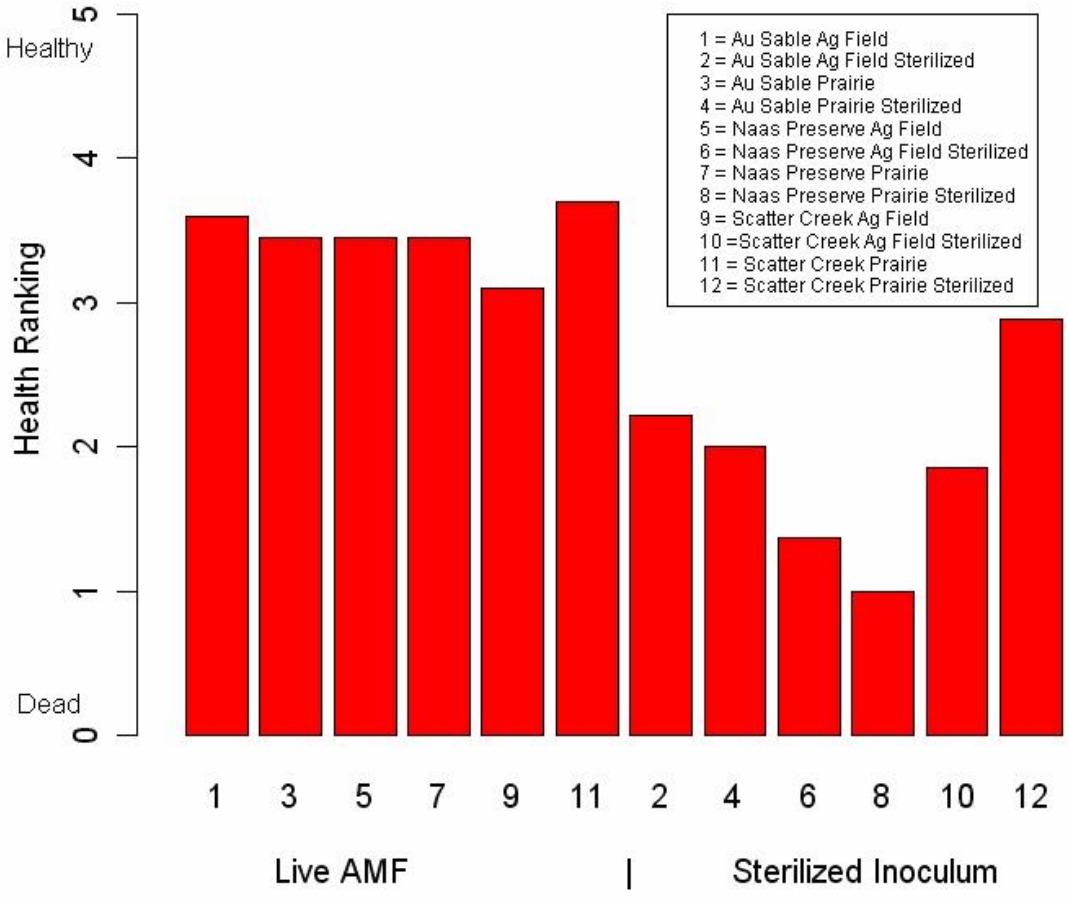

Figure 17 Average health rank of fescue plants by group. Mycorrhizal groups on the left

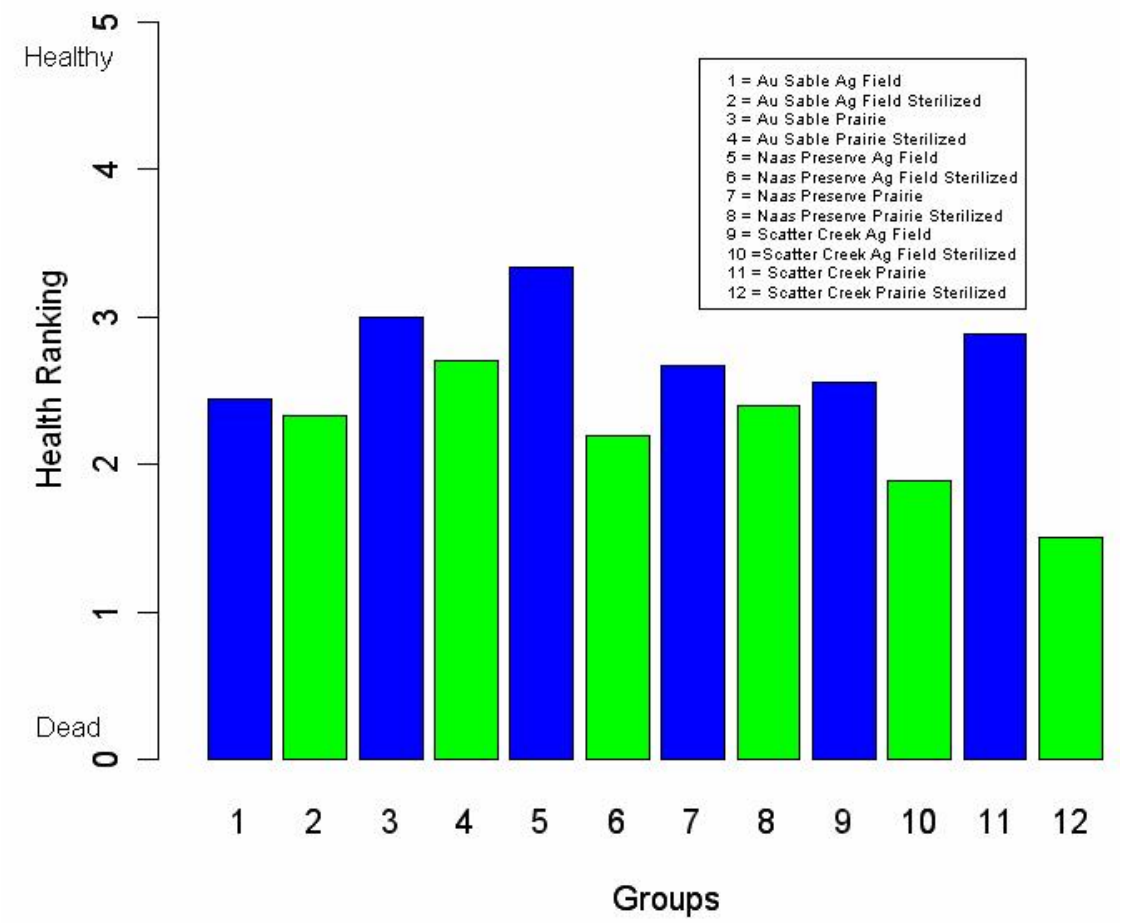

Figure 18 Average health rank of yarrow plants by experimental group. Mycorrhizal groups (blue) are placed next to their sterilized control (green).

Root Infection Rank

Root infection was primarily used to confirm that our treatments were effective at inoculating or sterilizing AMF as prescribed. All of the fescue plants receiving live AMF inoculum had detectable levels of root infection and only one of the yarrow plants receiving live AMF inoculum was lacking visible internal AMF structures. This one exception had a plethora of external fungal hyphae on the roots which may have impaired the ability to detect internal hyphae. Figure 19 shows an example of roots highly infected with AMF. All of the yarrow plants receiving sterilized AMF inoculum

were devoid of fungal root infection. Figure 20 shows an uninfected root tip from a control plant.

Figure 19 Roots highly infected with AMF. The dark blue staining is fungal tissue. Vesicles and hyphae are apparent (100x)

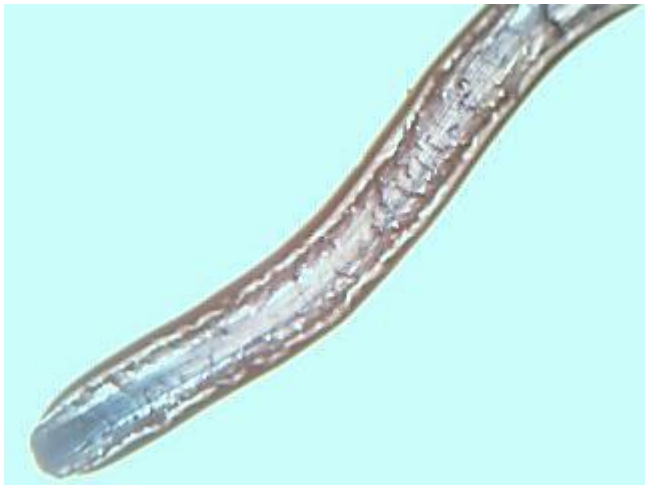

Figure 20 Root sample from a treatment receiving sterilized AMF inoculum, showing no AMF infection (100x)

Four of the 70 fescue plants receiving sterilized AMF unexpectedly had a small amount of presumably mycorrhizal mycelium infecting the roots. The contamination was not consistent through any given treatment and so was not a result of incomplete sterilization but instead likely occurred at some point during growth in the greenhouse or in the processing of the roots after harvest. These contaminated plants were not in

close proximity to each other in the block and so this was likely not the result of a single event. In two of the four cases the infection was localized to one highly infected root fragment which suggests that the root fragment may have come from a different plant during post-harvest root processing. In the third case internal fungal hyphae were found but not vesicles or arbuscles, and in the final case hyphae and vesicles were found infecting less than 10% of the root. In any case, the level of contamination was considered low enough to not alter the results of this study.

Level of root infection was evaluated using log linear modeling and ANOVA even though it is thought to have a negligible effect on the functionality of the mycorrhizal relationship (Klironomos 2003). No trends in infection level were found in yarrow plants. Fescue plants showed higher infection levels with prairie inoculum than with agricultural inoculum, but only from two of the three sites.

Discussion

It is clear from the data that mycorrhizal yarrow and fescue plants were significantly smaller than plants grown with sterilized mycorrhizal inoculum. This could be due to the growth inhibiting effects of other non-mycorrhizal organisms in the unsterilized field soil used as mycorrhizal inoculum (Hetrick et al. 1986; Koide and Li 1989). While I attempted to control for this by reinoculating the sterilized controls with the non-mycorrhizal soil community, any organisms that were greater than 2 μm, such as invertebrates or other fungal spores, were excluded from the non-mycorrhizal soil treatments. Alternatively, the mycorrhizal relationship itself may have had a stunting effect on the plants. AMF infection has been shown to cause a lag in plant growth in very young plants (Yocom 1983). However, the dwarfing experienced in my experiment was still very evident after thirteen weeks of growth. Under some environmental conditions, such as heavily fertilized agricultural fields, infection by AMF can decrease plant growth (Johnson 1993; Johnson et al. 2003). Presumably in these situations the plant receives all of the nutrients it needs in the absence of the AMF and so does not gain from the relationship, but still the fungus draws carbohydrates from the plant resulting in diminished plant growth. The field soil in this study was rich in organic matter and consequently had high total nitrogen. However, fertilization was kept extremely low and it is not likely that this experiment represented a nutrient saturated situation.

My results are in contrast to the generally accepted notion that mycorrhizas cause a positive growth response in infected plants. This axiom, however, is being

challenged. In a rigorous study by Klironomos (2003) ten plant species were grown with ten AMF species in all possible pairings. Above ground dry mass was measured after sixteen weeks of growth and compared against non-mycorrhizal controls. The results showed that a stunting of growth occurred as often as an increase in growth. Klironomos interpreted a negative growth response to indicate a parasitic relationship, concluding that arbuscular mycorrhizal relationships represent a spectrum from parasitic to mutualistic. Following this interpretation, my growth data would describe a relationship on the parasitic end of this spectrum. In contrast I propose that even with decreased growth, other benefits may be conferred to the plant and the relationship may yet be mutualistic.

This idea is supported by the health data which clearly show that despite being smaller both yarrow and fescue were generally healthier when grown with live mycorrhizal inoculum. For yarrow this meant that mycorrhizal plants had less leaf senescence as well as showing increased resistance to insect herbivory. While predation pressure was equal for all experimental yarrow plants, both aphid and thrip damage was far more extensive on non-mycorrhizal plants. Other researchers have similarly found that AMF reduces insect herbivory on infected plants (Rabin and Pacovsky 1985; Gange et al. 1994; Gange and West 1994). Two mechanisms have been provided to explain increased resistance to insect herbivory in mycorrhizal plants (Gehring et al. 1997). First, it has been suggested that AMF can improve the vigor of plants and herbivore performance is often negatively associated with plant vigor. This was clearly not the case in my experiment as the mycorrhizal plants were significantly less vigorous than non-mycorrhizal plants. Secondly, its has been proposed that AMF

infection can alter a plant's carbon-to-nutrient ratios and allow enhanced investment in antiherbivore defenses. While this experiment does not explicitly test this theory, it is a tempting explanation and is worthy of further research.

In the case of fescue, mycorrhizal plants had far less leaf senescence and did not discolor as the non-mycorrhizal plants did. Additionally, many of the non-mycorrhizal fescue plants developed a fungal infection at the crown that caused necrosis and even death in some plants. This infection developed in later stages of growth and was much less common on mycorrhizal fescue. It is recognized that AMF can alter a plant's interaction with other soil organisms including pathogenic fungi. These three way interactions are complex and difficult to study (Fitter and Garbaye 1994; Hartnett and Wilson 2002). It is possible that the AMF competitively excluded the pathogen from the soil. Alternatively, the mycorrhizal relationship may have allowed the plants themselves to become more resistant to the pathogen. This may be from a similar mechanism proposed above in which altered nutrient levels allow more production of plant defenses. Finally, the AMF itself may produce compounds that combat the pathogen. Further investigation is required to distinguish between these possibilities.

Another clear trend to emerge was the higher above ground dry mass in plants grown with abandoned agricultural field soil over prairie soil. This pattern was regardless of sterilization and so was likely an artifact of physical or chemical properties of the soil. Total nitrogen was higher in the agricultural soil from all sites except Au Sable, but not greatly. At Au Sable where the prairie treatment had higher total nitrogen the trend was non-existent except with yarrow in sterilized inoculum where the trend reversed following the nitrogen levels. This suggests the higher soil

nitrogen presumably from historic agricultural practices is at least partly responsible for the enhanced growth in the abandoned agricultural treatments.

Compared to the other two sites, I saw very little difference in any response between the agricultural and prairie treatments from the Au Sable site. I interpret this to mean that the agricultural practices at Au Sable were so minimal or so long ago that their effects on the soil and soil organisms have disappeared. If this was the case it is likely that the agricultural and prairie treatments would have a similar AMF community composition. A further study investigating the taxonomic diversity of AMF at our two Au Sable collections sites would help to verify this interpretation.

The central question of this experiment was to determine if there was a differential effect of AMF from the abandoned agricultural fields and from the extant prairie on native prairie plants. This has been previously documented as slower infection, increased spore production and decreased arbuscle production in AMF from agricultural fields compared with AMF from nearby native grasslands (Johnson 1993; Oehl et al. 2003). All of these responses are associated with either an increased draw of plant resources or a decreased supply of fungal resources and thus a less beneficial mycorrhizal association for the plant. Additionally, AMF from native grasslands has been shown to increase plant growth more than AMF from active or abandoned agricultural fields (Scullion et al. 1998; Richter and Stutz 2002).

The results of my experiment do not show a consistent differential effect of agricultural and prairie AMF. With only two exceptions, AMF from the abandoned agricultural fields had an effect on the plants that was indistinguishable from the effect of AMF from the paired extant prairie. The similarity in plant responses to prairie and

abandoned agricultural AMF suggest that these two environs have functionally similar AMF communities. It could be that the agricultural production on these sites was minimal enough to not have had a dramatic effect on the AMF. However, practices were extensive enough to have a lasting effect on some soil attributes, as evident by the generally greater growth of plants in the abandoned agricultural soil. A second explanation is that the AMF communities have been altered but are functionally equivalent for the two plant species tested. While this is contrary to the studies cited above, all of the evidence for the functional shift in AMF after agricultural use comes from regions outside of the Pacific Northwest. It is known that environmental factors affect the outcome of arbuscular mycorrhizal relationships (Klironomos 2003) and so observed trends in some parts of the world may not be applicable everywhere else (Pendleton and Smith 1983). Given this and the lack of AMF studies in the Pacific Northwest, one must be careful attempting to fit our data to patterns observed elsewhere.

As a third explanation for the lack of functional difference between my abandoned agricultural AMF and extant prairie AMF, the time since abandonment may have been sufficient that the agriculturally altered fungal communities have restored themselves to pre-disturbance compositions. This is a likely possibility. The proximity of these sites to native prairies means that there was a ready source of propagules of the pre-disturbance AMF. Researchers have found that AMF communities can remain altered for at least 25 years (Corkidi et al. 2002) whereas my study sites have all been abandoned for longer than this and in the case of Au Sable it may have been five or six times this long since the fields were last tilled. An evaluation of the AMF species

composition of these sites would go a long way towards clarifying the reasons for the observed functional similarity.

Conclusions and Recommendations

Arbuscular mycorrhizal fungi from the three Puget Sound prairies had a profound effect on the growth and health of fescue and yarrow plants, but almost no effect on germination. When grown with AMF from extant prairies, fescue and yarrow plants were smaller but more resistant to predators and pathogens than controls. This response was consistent with soil from all three prairie sites suggesting that this may be a common phenomenon and not site dependent. Furthermore, nearly identical responses were observed when plants were instead grown with AMF from the nearby abandoned agricultural fields. There was no differential effect of AMF from native prairies versus abandoned agricultural fields, and thus it appears that the AMF communities in these abandoned agricultural fields are functionally equivalent to the AMF communities in the native prairies of the area.

Land managers interested in re-establishing native prairie plants on these types of abandoned agricultural fields can feel secure that a well functioning community of AMF is in residence. Growers of restoration plants for these fields need not go to pains to inoculate plants with AMF from the extant prairies as the plants will likely become infected with a comparable suite of AMF upon planting in the restoration site. Based on the increased susceptibility to herbivory and pathogens observed in my control plants, I would recommend against using completely sterilized potting media. With this in mind, it is my conclusion that restoration of long abandoned agricultural fields in the Puget Sound area back to native prairie can proceed without the concern of altered, dysfunctional AMF communities hindering the restoration efforts.

While I am confident in my recommendations based upon the data collected, there is still much to be understood about the role of AMF in these prairie systems. The arbuscular mycorrhizal relationship is a complicated one that is not easily quantified or deconstructed. Simplistic responses such as differential growth under greenhouse conditions are not likely to capture the true nature of the relationship. There are costs and benefits to both partners that change based on seasonality and environmental conditions as well as the identity and life stage of the participants. Three-way interactions between the plant, mycorrhizal fungus and other organisms such as predators, pathogens and pollinators are common and influential. Traditional biological terms such as parasite or mutualist fall short of describing the intricacy of this relationship.

While the reductionist approach has made great strides in elucidating the physiology of this symbiosis, new approaches and further research are necessary to truly understand the ecology of AMF. This was made clear during my study. The accepted measure of mycorrhizal dependence, determined by the increase in above ground dry mass of mycorrhizal plants compared to above ground dry mass of non-mycorrhizal controls, suggested a strongly negative effect for my experimental plants. However, the relationship was more complex. Benefits to the plants occurred that were not expressed by simple growth measures. While I was able to describe some differences in predation resistance and health, I wonder what other effects of AMF infection were missed in this experiment. For example, what other differences would arise under environmental stresses? How would reproduction have been affected? Would there be competition effects or resource sharing? Perhaps the smaller above

ground dry mass observed in mycorrhizal plants has some adaptive advantage in the native environment of these plants.

As we continue to struggle to understand the ecology of this important and prevalent phenomenon, we need to expand our interpretation of costs and benefits. We need to be flexible enough in our experimental design to observe and document unexpected responses. And above all we need to focus on *in situ* studies that allow the expression of complex ecological patterns.

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Appendix 1 Results of Statistical Tests

FESCUE

Anova Tables (Type II tests)

Response: Transformed Fescue Shoot Weight (^0.5)

	Sum Sq	Df	F value	Pr(>F)
SITE	0.04415	2	8.488	0.000429
AUTOCLAVED	0.55993	1	215.291	< 2.2e-16
SITE:SOIL.TYPE	0.23420	3	30.016	2.043e-13
SITE:AUTOCLAVED	0.06482	2	12.461	1.743e-05
SITE:SOIL.TYPE:AUTOCLAVED	0.04546	3	5.826	0.001125
Residuals	0.22627	87		

Anova Tables (Type II tests)

Response: Fescue Transformed Shoot Weight (^0.5) at Scatter Creek

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.151095	1	55.2986	1.838e-08
AUTOCLAVED	0.084956	1	31.0926	3.716e-06
SOIL.TYPE:AUTOCLAVED	0.007240	1	2.6498	0.1134
Residuals	0.087435	32		

Means:	Agricultural Field	Prairie Soil	Not Autoclaved	Autoclaved
	0.4111299	0.2871728	0.3057854	0.3956115

Anova Tables (Type II tests)

Response: Fescue Transformed Shoot Weight (^0.5) at Au Sable

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.005366	1	1.5972	0.2160
AUTOCLAVED	0.198096	1	58.9662	1.448e-08
SOIL.TYPE:AUTOCLAVED	0.003242	1	0.9652	0.3337
Residuals	0.100784	30		

Means:	Not Autoclaved	Autoclaved
	0.3267515	0.4790298

Anova Tables (Type II tests)

Response: Fescue Transformed Shoot Weight (^0.5) at Naas Preserve

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.07774	1	51.077	1.723e-07
AUTOCLAVED	0.34169	1	224.510	5.373e-14
SOIL.TYPE:AUTOCLAVED	0.03497	1	22.980	6.359e-05
Residuals	0.03805	25		

Welch Two Sample t-test for
Fescue Transformed Shoot Weight in Naas Preserve Prairie Soil across Autoclaving

t = 7.0524, df = 2.22, p-value = 0.01467

95 percent confidence interval: (0.1467587, 0.5138226)

Means:	Autoclaved	Not Autoclaved	
	0.5252026	0.1949119	Difference: 0.3302907

Welch Two Sample t-test for Fescue Transformed Shoot Weight
in Naas Preserve Agricultural Soil across Autoclaving

t = 10.4801, df = 13.591, p-value = 6.887e-08

95 percent confidence interval: (0.1398886, 0.2121348)

Means:	Autoclaved	Not Autoclaved	
	0.5291611	0.3531494	Difference: 0.1760117

Welch Two Sample t-test for
Fescue Transformed Shoot Weight in Autoclaved Naas Preserve soils across Soil Type

t = -0.0833, df = 2.35, p-value = 0.9402

95 percent confidence interval: (-0.1818563, 0.1739392)

Means:	Prairie Soil	Agricultural Soil
	0.5252026	0.5291611

Welch Two Sample t-test for
Fescue Transformed Shoot Weight in Sterilzed Naas Preserve soils across Soil Type

t = -10.7317, df = 15.983, p-value = 1.031e-08

95 percent confidence interval: (-0.1894980, -0.1269770)

Means:	Prairie Soil	Agricultural Soil
	0.1949119	0.3531494

Anova Table (Type II tests)

Response: Fescue Transformed (^0.5) Root Weight

	Sum Sq	Df	F value	Pr(>F)
SITE	0.12374	2	4.0125	0.021534
AUTOCLAVED	0.84397	1	54.7361	8.124e-11
SITE:SOIL.TYPE	0.49003	3	10.5937	5.242e-06
SITE:AUTOCLAVED	0.18743	2	6.0779	0.003383
SITE:SOIL.TYPE:AUTOCLAVED	0.20629	3	4.4597	0.005811
Residuals	1.34144	87		

Anova Table (Type II tests)

Response: Fescue Transformed Root Weight at Scatter Creek

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.32203	1	18.4107	0.0001537
AUTOCLAVED	0.06976	1	3.9884	0.0543774
SOIL.TYPE:AUTOCLAVED	0.03582	1	2.0477	0.1621271
Residuals	0.55973	32		

Means:	Agricultural Field	Prairie Soil
	0.6014273	0.4171042

Anova Table (Type II tests)

Response: Fescue Transformed Root Weight at Au Sable

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.00273	1	0.1341	0.7167784
AUTOCLAVED	0.33177	1	16.2867	0.0003461
SOIL.TYPE:AUTOCLAVED	0.00197	1	0.0966	0.7580729
Residuals	0.61112	30		

Means:	Not Autoclaved	Autoclaved
	0.491752	0.689908

Anova Table (Type II tests)

Response: Fescue Transformed Root Weight in Naas Preserve Soil

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.16526	1	24.219	4.569e-05
AUTOCLAVED	0.62986	1	92.305	7.148e-10
SOIL.TYPE:AUTOCLAVED	0.16850	1	24.694	4.035e-05
Residuals	0.17059	25		

Welch Two Sample t-test for
Transformed Fescue Root Weight in Naas Preserve Prairie Soil across Autoclaving

t = 11.7843, df = 2.306, p-value = 0.004135

95 percent confidence interval: (0.3610031, 0.7045849)

Means:	Autoclaved	Not Autoclaved	
	0.7612676	0.2284736	Difference: 0.532794

Welch Two Sample t-test for Transformed Fescue Root Weight
in Naas Preserve Agricultural Soil across Autoclaving

t = 3.8606, df = 10.014, p-value = 0.003149

95 percent confidence interval: (0.08211942, 0.30619338)

Means:	Autoclaved	Not Autoclaved	
	0.6904743	0.4963179	Difference: 0.1941564

Welch Two Sample t-test for
Transformed Fescue Root Weight in Autoclaved Naas Preserve soil across Soil Type

t = 1.1231, df = 6.517, p-value = 0.3011

95 percent confidence interval: (-0.08052537, 0.22211191)

Means:	Prairie Soil	Agricultural Soil	
	0.7612676	0.6904743	Difference: 0.0707933

Welch Two Sample t-test for Transformed Fescue Root Weight
in Non Autoclaved Nass Preserve soil across Soil Type

t = -10.9328, df = 12.512, p-value = 9.141e-08

95 percent confidence interval: (-0.3209822, -0.2147064)

Means:	Prairie Soil	Agricultural Soil	
	0.2284736	0.4963179	Difference: -0.2678443

Anova Table (Type II tests)

Response: Fescue Root/Shoot Ratio

	Sum Sq	Df	F value	Pr(>F)
SITE	5.932	2	3.2802	0.04232
AUTOCLAVED	0.737	1	0.8153	0.36905
SITE:SOIL.TYPE	0.859	3	0.3168	0.81319
SITE:AUTOCLAVED	0.998	2	0.5519	0.57784
SITE:SOIL.TYPE:AUTOCLAVED	1.963	3	0.7237	0.54054
Residuals	78.669	87		

Anova Table (Type II tests)

Response: Fescue Transformed Root/Shoot Ratio

	Sum Sq	Df	F value	Pr(>F)
SITE	0.19193	2	2.7723	0.06805
AUTOCLAVED	0.05851	1	1.6902	0.19700
SITE:SOIL.TYPE	0.03853	3	0.3710	0.77411
SITE:AUTOCLAVED	0.04644	2	0.6708	0.51394
SITE:SOIL.TYPE:AUTOCLAVED	0.11583	3	1.1154	0.34734
Residuals	3.01147	87		

Means:	Au Sable	Naas Preserve	Scatter Creek
	0.3291290	0.2272724	0.3114875

Anova Table (Type II tests)

Response: Fescue Time to First Germination

	Sum Sq	Df	F value	Pr(>F)
SITE	0.600	2	0.4513	0.63802
AUTOCLAVED	0.033	1	0.0501	0.82324
SITE:SOIL.TYPE	2.200	3	1.1031	0.35121
SITE:AUTOCLAVED	4.067	2	3.0585	0.05105
SITE:SOIL.TYPE:AUTOCLAVED	2.500	3	1.2535	0.29411
Residuals	71.800	108		

Anova Table (Type II tests)

Response: Fescue Time to Last Germination

	Sum Sq	Df	F value	Pr(>F)
SITE	27.35	2	1.3839	0.25501
AUTOCLAVED	30.00	1	3.0360	0.08428
SITE:SOIL.TYPE	38.05	3	1.2835	0.28377
SITE:AUTOCLAVED	16.85	2	0.8526	0.42915
SITE:SOIL.TYPE:AUTOCLAVED	17.85	3	0.6021	0.61500
Residuals	1067.20	108		

Anova Table (Type II tests)

Response: Fescue Total Germination

	Sum Sq	Df	F value	Pr(>F)
SITE	7.817	2	4.2593	0.01658
AUTOCLAVED	3.675	1	4.0050	0.04787
SITE:SOIL.TYPE	3.825	3	1.3895	0.24994
SITE:AUTOCLAVED	0.650	2	0.3542	0.70256
SITE:SOIL.TYPE:AUTOCLAVED	5.925	3	2.1524	0.09788
Residuals	99.100	108		

Means:	Not Autoclaved	Autoclaved
	3.233333	3.583333

Tukey's HSD between Au Sable and Naas Preserve (Groups 1 and 2)
Critical: 0.5220737
Observed: 0.325
CI: (-0.8470737, 0.1970737)
Not significant

Tukey's HSD between Au Sable and Scatter Creek (Groups 1 and 3)
Critical: 0.5220737
Observed: 0.3000
CI: (-0.2220737, 0.8220737)
Not significant

Tukey's HSD between Naas Preserve and Scatter Creek (Groups 2 and 3)
Critical: 0.5220737
Observed: 0.625
CI: (0.1029263, 1.147074)
Significant at $\alpha = 0.05$

Scatter Creek Au Sable Naas Preserve

Fescue Health Ranking
Log-linear Model comparison

Model:	Likelihood Ratio	df	p
1:2 + 1:4 + 1:3 %in% 4 + 1:2:4 + 1:3:2 %in% 4	0	0	1
1:2 + 1:4 + 1:3 %in% 4 + 1:2:4	14.385	15	0.497
1:2 + 1:4 + 1:3 %in% 4	25.733	25	0.422
1:2 + 1:4	67.128	40	0.0046
1:4 + 1:3 %in% 4	92.407	30	2.82e-08

Anova Table (Type II tests)

Response: Fescue Health Rank

	Sum Sq	Df	F value	Pr(>F)
Site	12.950	2	6.2887	0.002609
Autoclaved.	104.533	1	101.5252	< 2.2e-16
Site:Soil.Type	14.850	3	4.8076	0.003504
Site:Autoclaved.	4.717	2	2.2905	0.106116
Site:Soil.Type:Autoclaved.	3.050	3	0.9874	0.401633
Residuals	111.200	108		

Means:

Au Sable	Naas Preserve	Scatter Creek	Not Autoclaved	Autoclaved
2.475	1.900	2.675	3.283	1.417

Tukey's HSD between Au Sable and Naas Preserve (Groups 1 and 2)
Critical: 0.5386294
Observed: 0.575
CI: (0.0363706, 1.1136294)
Significant at $\alpha = 0.05$

Tukey's HSD between Au Sable and Scatter Creek (Groups 1 and 3)
Critical: 0.5386294
Observed: 0.200
CI: (-0.3386294, 0.7386294)
Not significant

Tukey's HSD between Naas Preserve and Scatter Creek (Groups 2 and 3)
Critical: 0.5386294
Observed: 0.775
CI: (0.2363706, 1.3136294)
Significant at $\alpha = 0.05$

Sites:	Naas Preserve	Au Sable	Scatter Creek

Welch Two Sample t-test for Fescue Health in Scatter Creek soil across Soil Type

t = 2.5743, df = 36.456, p-value = 0.01425

95 percent confidence interval: (0.2018945, 1.6981055)

Means:	Prairie Soil	Agricultural Soil
	3.15	2.20

Welch Two Sample t-test for Fescue Health in Au Sable soils across Soil Type

t = -1.4754, df = 37.121, p-value = 0.1485

95 percent confidence interval: (-1.5425287, 0.2425287)

Means:	Prairie Soil	Agricultural Soil
	2.15	2.80

Welch Two Sample t-test for Fescue Health in Naas Preserve soils across Soil Type

t = -0.7877, df = 37.197, p-value = 0.4359

95 percent confidence interval: (-1.4287843, 0.6287843)

Means:	Prairie Soil	Agricultural Soil
	1.7	2.1

Fescue Root Infection
Log Linear Model Comparison

Model	Likelihood Ratio	df	p
1:3 + 1:2 %in% 3	0	0	1
1:3	21.45649	12	0.0441

Anova Table (Type II tests)

Response: Fescue Root Infection

	Sum Sq	Df	F value	Pr(>F)
SITE	0.8535	2	0.7247	0.489507
SITE:SOIL.TYPE	10.6842	3	6.0477	0.001351
Residuals	29.4444	50		

Welch Two Sample t-test for
Fescue Root Infection Rank in Scatter Creek soil across Soil Types

t = 2.6107, df = 13.837, p-value = 0.0207

95 percent confidence interval: (0.1834644, 1.8832023)

Means:	Prairie Soil	Agricultural Soil
	3.700000	2.666667

Welch Two Sample t-test for
Fescue Root Infection Rank in Au Sable soils across Soil Type

t = 0.3696, df = 13.919, p-value = 0.7173

95 percent confidence interval: (-0.5875114, 0.8319559)

Means:	Prairie Soil	Agricultural Soil
	3.222222	3.100000

Welch Two Sample t-test for
Fescue Root Infection Rank in Naas Preserve soils across Soil Type

t = 3.1623, df = 8, p-value = 0.01335

95 percent confidence interval: (0.3008638, 1.9213584)

Means:	Prairie Soils	Agricultural Soil
	4.000000	2.888889

YARROW

Anova Tables (Type II tests)

Response: Yarrow Transformed Shoot Weight (^0.5)

	Sum Sq	Df	F value	Pr(>F)
SITE	0.17648	2	28.703	1.543e-10
AUTOCLAVED	0.52510	1	170.803	< 2.2e-16
SITE:SOIL.TYPE	0.61015	3	66.155	< 2.2e-16
SITE:AUTOCLAVED	0.25549	2	41.553	8.538e-14
SITE:SOIL.TYPE:AUTOCLAVED	0.06570	3	7.124	0.0002228
Residuals	0.30128	98		

Anova Tables (Type II tests)

Response: Yarrow Transformed Shoot Weight (^0.5) at Scatter Creek

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.41092	1	222.6711	1.110e-15
AUTOCLAVED	0.00013	1	0.0697	0.7936
SOIL.TYPE:AUTOCLAVED	0.04180	1	22.6502	4.278e-05
Residuals	0.05721	31		

Welch Two Sample t-test for
Yarrow Transformed Shoot Weight in Scatter Creek Prairie Soil across Autoclaving

t = -3.6526, df = 13.553, p-value = 0.002743

95 percent confidence interval: (-0.1072070, -0.0277277)

Means:	Autoclaved	Not Autoclaved
	0.0994973	0.1669646

Welch Two Sample t-test for Yarrow Transformed Shoot Weight
in Scatter Creek Agricultural Soil across Autoclaving

t = 3.2504, df = 12.005, p-value = 0.006948

95 percent confidence interval: (0.02339122, 0.11849901)

Means:	Autoclaved	Not Autoclaved
	0.3876917	0.3167466

Anova Tables (Type II tests)

Response: Yarrow Transformed Shoot Weight (^0.5) at Au Sable

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.016116	1	8.8374	0.005477
AUTOCLAVED	0.251545	1	137.9370	2.494e-13
SOIL.TYPE:AUTOCLAVED	0.020388	1	11.1801	0.002069
Residuals	0.060180	33		

Welch Two Sample t-test for
Yarrow Transformed Shoot Weight in Au Sable Prairie Soil across Autoclaving

t = 10.7652, df = 13.559, p-value = 5.077e-08

95 percent confidence interval: (0.1686858, 0.2529457)

Means:	Autoclaved	Not Autoclaved	
	0.4497832	0.2389674	Difference: 0.2108158

Welch Two Sample t-test for
Yarrow Transformed Shoot Weight in Au Sable Agricultural Soil across Autoclaving

t = 6.0129, df = 14.467, p-value = 2.771e-05

95 percent confidence interval: (0.07527717, 0.15836546)

Means:	Autoclaved	Not Autoclaved	
	0.3622207	0.2453994	Difference: 0.1168213

Welch Two Sample t-test for
Yarrow Transformed Shoot Weight in Autoclaved Au Sable soil across Soil Type

t = 4.2387, df = 15.19, p-value = 0.0006964

95 percent confidence interval: (0.04357902, 0.13154593)

Means:	Prairie Soil	Agricultural Field
	0.4497832	0.3622207

Welch Two Sample t-test for
Yarrow Transformed Shoot Weight in Non-Autoclaved Au Sable soil across Soil Type

t = -0.3518, df = 12.83, p-value = 0.7307

95 percent confidence interval: (-0.04597993, 0.03311602)

Means:	Prairie Soil	Agricultural Field
	0.2389674	0.2453994

Anova Table (Type II tests)

Response: Yarrow Transformed Shoot Weight (^0.5) at Naas Preserve

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.18311	1	33.8546	1.482e-06
AUTOCLAVED	0.52892	1	97.7912	1.552e-11
SOIL.TYPE:AUTOCLAVED	0.00352	1	0.6502	0.4256
Residuals	0.18390	34		

Means:	Agricultural Field	Prairie Soil	Not Autoclaved	Autoclaved
	0.4147065	0.2758734	0.2209292	0.4572146

Anova Table (Type II tests)

Response: Yarrow Transformed Root Weight

	Sum Sq	Df	F value	Pr(>F)
SITE	0.23708	2	17.0616	4.387e-07
AUTOCLAVED	1.01167	1	145.6095	< 2.2e-16
SITE:SOIL.TYPE	1.19537	3	57.3499	< 2.2e-16
SITE:AUTOCLAVED	0.60703	2	43.6846	2.731e-14
SITE:SOIL.TYPE:AUTOCLAVED	0.12876	3	6.1776	0.0006872
Residuals	0.68089	98		

Anova Table (Type II tests)

Response: Yarrow Transformed Root Weight at Scatter Creek

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.85287	1	135.3310	7.684e-13
AUTOCLAVED	0.00335	1	0.5311	0.4716122
SOIL.TYPE:AUTOCLAVED	0.09654	1	15.3187	0.0004638
Residuals	0.19537	31		

Welch Two Sample t-test for
Yarrow Transformed Root Weight in Scatter Creek Prairie Soil across Autoclaving

t = -3.2653, df = 12.494, p-value = 0.006441

95 percent confidence interval: (-0.2129327, -0.0429428)

Means:	Autoclaved	Not Autoclaved	
	0.2603573	0.3882950	Difference = -0.1279377

Welch Two Sample t-test for Yarrow Transformed Root Weight
in Scatter Creek Agricultural Soil across Autoclaving

t = 2.3206, df = 13.025, p-value = 0.03717

95 percent confidence interval: (0.005705289, 0.159122704)

Means:	Autoclaved	Not Autoclaved	
	0.681187	0.598773	Difference = 0.082414

Anova Table (Type II tests)

Response: Yarrow Transformed Root Weight at Au Sable

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.00481	1	0.8394	0.36621
AUTOCLAVED	0.56772	1	98.9761	1.841e-11
SOIL.TYPE:AUTOCLAVED	0.02432	1	4.2401	0.04744
Residuals	0.18929	33		

Welch Two Sample t-test for
Yarrow Transformed Root Weight in Au Sable Prairie Soil across Autoclaving

t = 7.252, df = 11.125, p-value = 1.541e-05

95 percent confidence interval: (0.2076330, 0.3882283)

Means:	Autoclaved	Not Autoclaved	
	0.7495337	0.4516031	Difference = 0.2979306

Welch Two Sample t-test for
Yarrow Transformed Root Weight in Au Sable Agricultural Soil across Autoclaving

t = 3.3197, df = 16.395, p-value = 0.004219

95 percent confidence interval: (0.04133878, 0.18662859)

Means:	Autoclaved	Not Autoclaved	
	0.6930878	0.5791042	Difference = 0.1139836

Welch Two Sample t-test for
Yarrow Transformed Root Weight in Autoclaved Au Sable soil across Soil Type

t = 3.439, df = 16.373, p-value = 0.003277

95 percent confidence interval: (0.02802475, 0.11766545)

Means:	Prairie Soil	Agricultural Soil	
	0.7495337	0.6766886	Difference = 0.0728451

Welch Two Sample t-test for
Yarrow Transformed Root Weight in Non-Autoclaved Au Sable soil across Soil Type

t = -0.6491, df = 14.402, p-value = 0.5265

95 percent confidence interval: (-0.12807725, 0.06844719)

Means:	Prairie Soil	Agricultural Soil	
	0.4516031	0.4814181	Difference = -0.029815

Anove Table (Type II tests)

Yarrow Transformed Root Weight at Naas Preserve

	Sum Sq	Df	F value	Pr(>F)
SOIL.TYPE	0.33768	1	38.7571	4.395e-07
AUTOCLAVED	1.04763	1	120.2400	1.046e-12
SOIL.TYPE:AUTOCLAVED	0.00790	1	0.9069	0.3477
Residuals	0.29624	34		

Means: Agricultural Field	Prairie Soil	Not Autoclaved	Autoclaved
0.6899175	0.5013822	0.4206287	0.7531688

Anova Table (Type II tests)

Response: Yarrow Root/Shoot Ratio

	Sum Sq	Df	F value	Pr(>F)
SITE	0.7943	2	1.4505	0.2394356
AUTOCLAVED	6.1554	1	22.4830	7.198e-06
SITE:SOIL.TYPE	4.8026	3	5.8472	0.0010231
SITE:AUTOCLAVED	5.3193	2	9.7145	0.0001416
SITE:SOIL.TYPE:AUTOCLAVED	0.9138	3	1.1126	0.3478108
Residuals	26.8306	98		

Welch Two Sample t-test for
Yarrow Root/Shoot Ratio in Scatter Creek soil across Soil Type

t = -3.135, df = 23.811, p-value = 0.004521

95 percent confidence interval: (-1.0209450, -0.2101268)

Means:	Prairie Soil	Agricultural Field
	0.7803922	1.3959281

Welch Two Sample t-test for
Yarrow Root/Shoot Ratio in Au Sable soil across Soil Type

t = 0.2304, df = 28.028, p-value = 0.8195

95 percent confidence interval: (-0.4230885, 0.5303347)

Means:	Prairie Soil	Agricultural Field
	1.345127	1.291504

Welch Two Sample t-test for
Yarrow Root/Shoot Ratio in Naas Preserve soil across Soil Type

t = -2.2256, df = 34.577, p-value = 0.03266

95 percent confidence interval: (-0.74232731, -0.03393964)

Means:	Prairie Soil	Agricultural Field
	1.008849	1.396982

Welch Two Sample t-test for
Yarrow Root/Shoot Ratio in Scatter Creek soil across Autoclaving

t = -0.63, df = 32.601, p-value = 0.5331

95 percent confidence interval: (-0.5821824, 0.3069786)

Means:	Autoclaved	Not Autoclaved
	1.026187	1.163789

Welch Two Sample t-test for
Yarrow Root/Shoot Ratio in Au Sable soil across Autoclaving

t = 3.0767, df = 26.243, p-value = 0.004851

95 percent confidence interval: (0.2169167, 1.0890260)

Means:	Autoclaved	Not Autoclaved
	1.6367015	0.9837302

Welch Two Sample t-test for
Yarrow Root/Shoot Ratio in Naas Preserve Soil across Autoclaving

t = 7.7952, df = 34.456, p-value = 4.126e-09

95 percent confidence interval: (0.6506661, 1.1092616)

Means:	Autoclaved	Not Autoclaved
	1.6197406	0.7397767

Anova Table (Type II tests)

Response: Yarrow Time to First Germination

	Sum Sq	Df	F value	Pr(>F)
SITE	79.13	2	2.0113	0.1393
AUTOCLAVED	13.86	1	0.7044	0.4034
SITE:SOIL.TYPE	111.73	3	1.8932	0.1357
SITE:AUTOCLAVED	25.88	2	0.6579	0.5202
SITE:SOIL.TYPE:AUTOCLAVED	50.69	3	0.8590	0.4652
Residuals	1927.86	98		

Anova Table (Type II tests)

Response: Yarrow Time to Last Germination

	Sum Sq	Df	F value	Pr(>F)
SITE	202.7	2	2.7730	0.06738
AUTOCLAVED	1.9	1	0.0516	0.82076
SITE:SOIL.TYPE	35.3	3	0.3224	0.80918
SITE:AUTOCLAVED	15.5	2	0.2120	0.80932
SITE:SOIL.TYPE:AUTOCLAVED	28.5	3	0.2597	0.85428
Residuals	3581.6	98		

Anova Table (Type II tests)

Response: Yarrow Total Germination

	Sum Sq	Df	F value	Pr(>F)
SITE	0.293	2	0.1445	0.8656
AUTOCLAVED	0.814	1	0.8031	0.3724
SITE:SOIL.TYPE	5.774	3	1.8993	0.1347
SITE:AUTOCLAVED	0.673	2	0.3321	0.7183
SITE:SOIL.TYPE:AUTOCLAVED	0.670	3	0.2203	0.8821
Residuals	99.300	98		

Yarrow Health Ranking
Log-linear Model comparison

Model:	Likelihood Ratio	df	p
1:2 + 1:4 + 1:3 %in% 4 + 1:2:4 + 1:3:2 %in% 4	0	0	1
1:2 + 1:4 + 1:3 %in% 4 + 1:2:4	5.804	12	0.926
1:2 + 1:4 + 1:3 %in% 4	16.43	20	0.690
1:2 + 1:4	35.346	32	0.313
1:2	56.673	40	0.042
1:4	58.199	36	0.011

Anova Table (Type II tests)

Response: Yarrow Health Rank

	Sum Sq	Df	F value	Pr(>F)
Site	4.206	2	4.4490	0.01414
Autoclaved.	11.076	1	23.4317	4.827e-06
Site:Soil.Type	2.367	3	1.6692	0.17862
Site:Autoclaved.	2.998	2	3.1712	0.04629
Site:Soil.Type:Autoclaved	2.999	3	2.1151	0.10320
Residuals	46.322	98		

Means:

Au Sable	Naas Preserve	Scatter Creek	Not Autoclaved	Autoclaved
2.622	2.632	2.229	2.815	2.196

Tukey's HSD between Au Sable and Naas Preserve (Groups 1 and 2)
Critical: 0.3774039
Observed: 0.009957
CI: (-0.3674466, .387361226)
Not Significant

Tukey's HSD between Au Sable and Scatter Creek (Groups 1 and 3)
Critical: 0.3853007
Observed: 0.3930502
CI: (0.0077495, 0.7783509)
Significant at $\alpha = 0.05$

Tukey's HSD between Naas Preserve and Scatter Creek (Groups 2 and 3)
Critical: 0.3828283
Observed: 0.4030075
CI: (0.0201792, .7858358)
Significant at $\alpha = 0.05$

Sites: Scatter Creek <u>Au Sable Naas Preserve</u>

Welch Two Sample t-test for
Yarrow Health Rank in Scatter Creek soils across Autoclaving

t = -4.5039, df = 27.231, p-value = 0.0001135

95 percent confidence interval: (-1.4791636, -0.5535162)

Means:	Autoclaved	Not Autoclaved
	1.705882	2.722222

Welch Two Sample t-test for
Yarrow Health Rank in Au Sable soils across Autoclaving

t = -0.9279, df = 34.282, p-value = 0.3599

95 percent confidence interval: (-0.6248222, 0.2330093)

Means:	Autoclaved	Not Autoclaved
	2.526316	2.722222

Welch Two Sample t-test for
Yarrow Health Rank in Naas Preserve soils across Autoclaving

t = -2.6977, df = 30.706, p-value = 0.01124

95 percent confidence interval: (-1.2294210, -0.1705790)

Means:	Autoclaved	Not Autoclaved
	2.3	3.0

Yarrow Root Infection Rank
Log-Linear Model Comparison

Model	Likelihood Ratio	df	p
1:3 + 1:2 %in% 3	0	0	1
1:3	13.55428	15	0.5596

Anova Table (Type II tests)

Yarrow Root Infection Rank

	Sum Sq	Df	F value	Pr(>F)
SITE	3.704	2	2.3669	0.10463
SITE:SOIL.TYPE	5.500	3	2.3432	0.08478
Residuals	37.556	48		

Appendix 2 Transformation Graphs

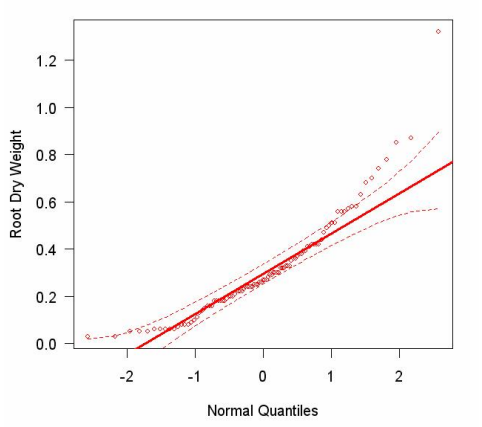

Figure 21 q-q plot of non-transformed root dry weight of fescue plants. Dotted lines are a 95% confidence envelope.

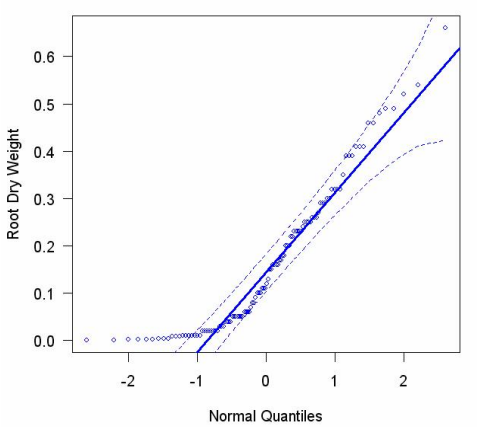

Figure 22 q-q plot of non-transformed root dry weight of yarrow plants. Dotted lines are a 95% confidence envelope.

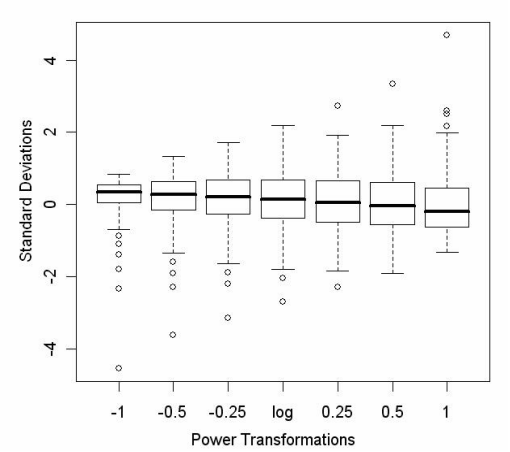

Figure 23 Comparative box plots of power transformations for fescue root dry weight.

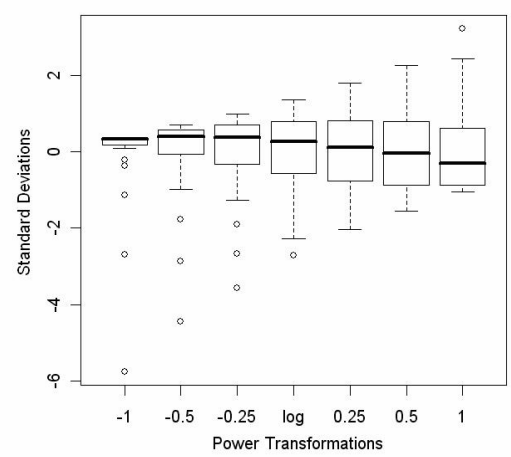

Figure 24 Comparative box plots of power transformations for yarrow root dry weight.

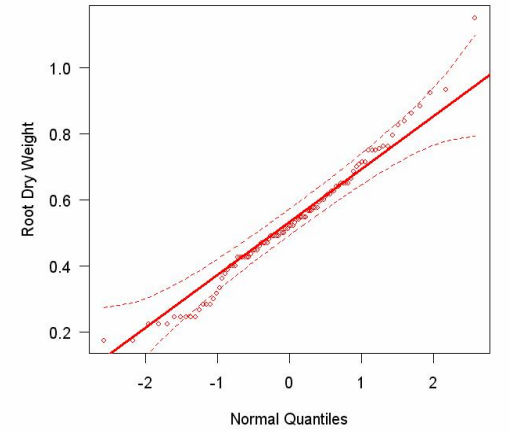

Figure 25 q-q plot of 0.5 power transformed root dry weight of fescue plants. Dotted lines are a 95% confidence envelope.

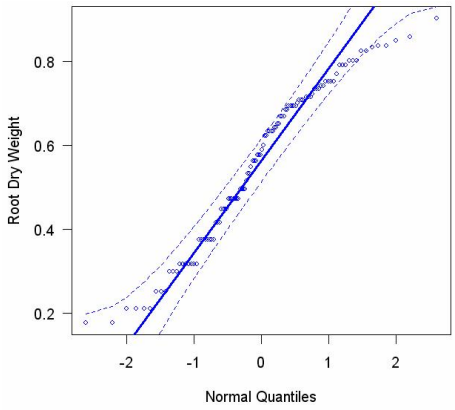

Figure 26 q-q plot of 0.25 power transformed root dry weight of yarrow plants. Dotted lines are a 95% confidence envelope.

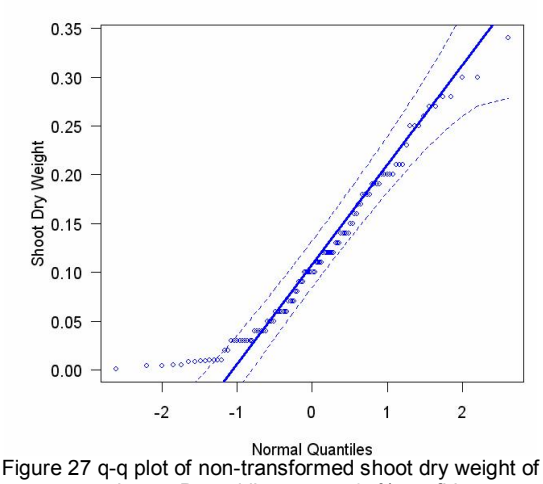

Figure 27 q-q plot of non-transformed shoot dry weight of yarrow plants. Dotted lines are a 95% confidence envelope.

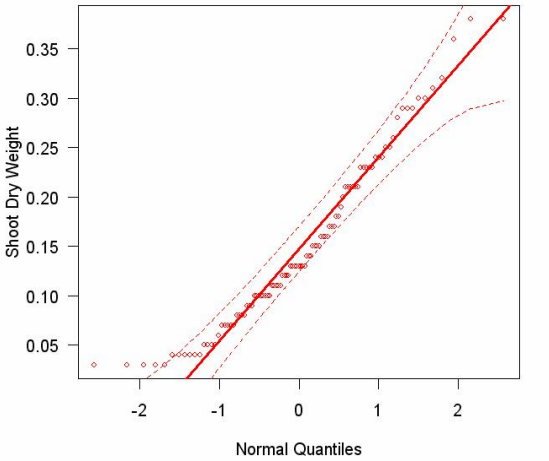

Figure 28 q-q plot of non-transformed shoot dry weight of fescue plants. Dotted lines are a 95% confidence envelope.

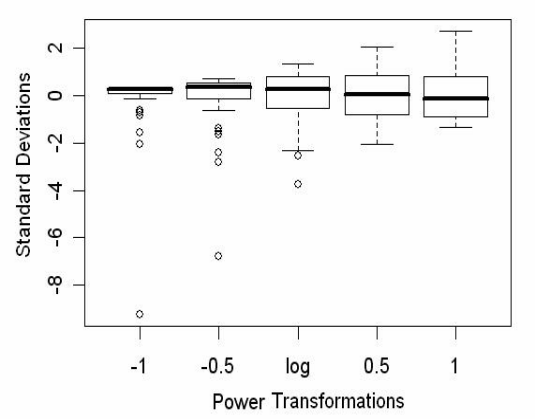

Figure 29 Comparative box plots of power transformations for yarrow shoot dry weight.

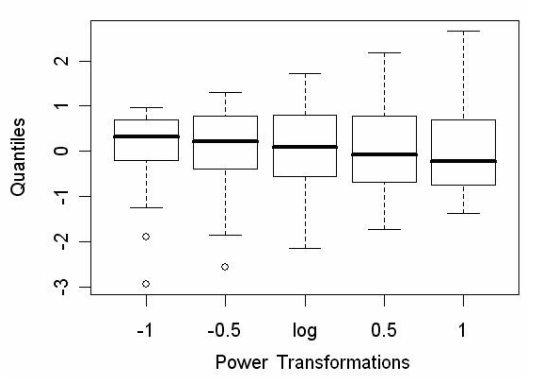

Figure 30 Comparative box plots of power transformations for fescue shoot dry weight.

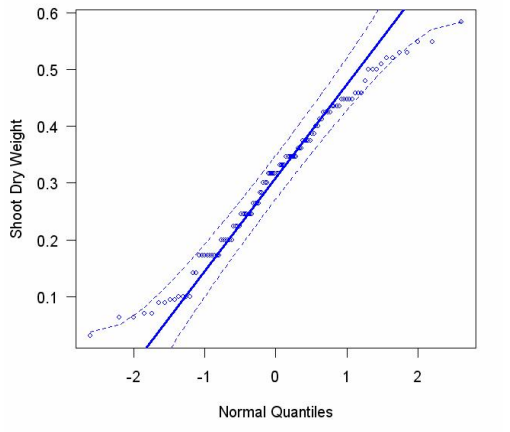

Figure 31 q-q plot of 0.5 power transformed shoot dry weight of yarrow plants. Dotted lines are a 95% confidence envelope.

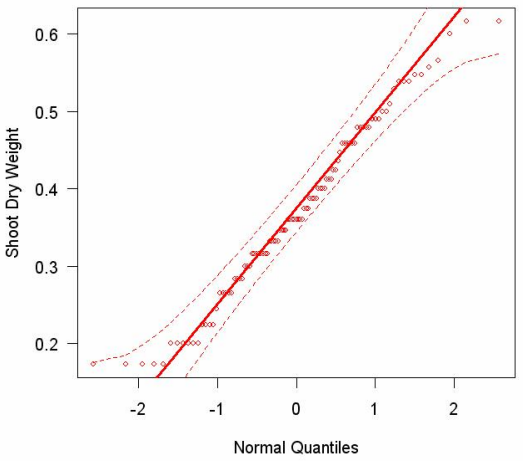

Figure 32 q-q plot of 0.5 power transformed shoot dry weight of fescue plants. Dotted lines are a 95% confidence envelope.

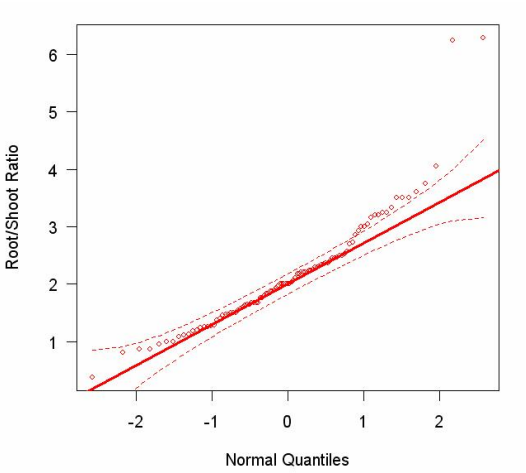

Figure 33 q-q plot of non-transformed root/shoot ratio of fescue plants. Dotted lines are a 95% confidence envelope.

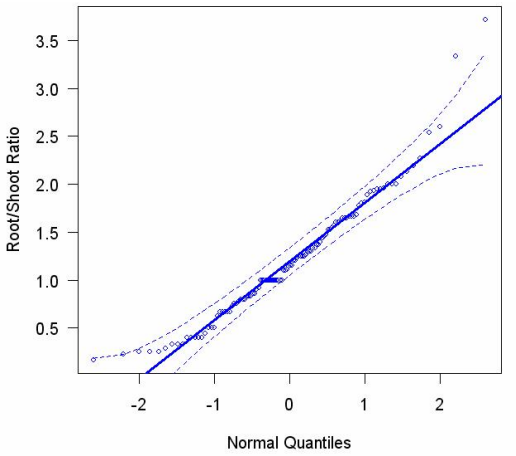

Figure 34 q-q plot of non-transformed root/shoot ratio of yarrow plants. Dotted lines are a 95% confidence envelope.

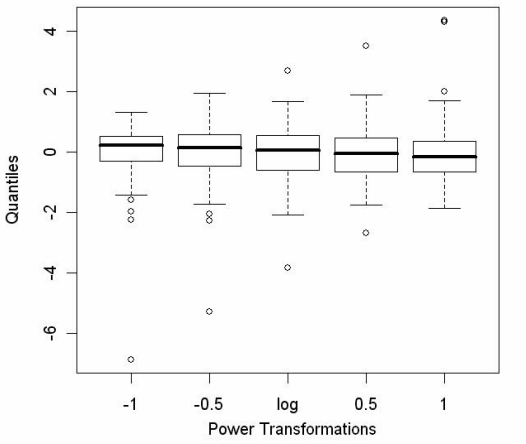

Figure 35 Comparative box plots of power transformations for fescue root/shoot ratio.

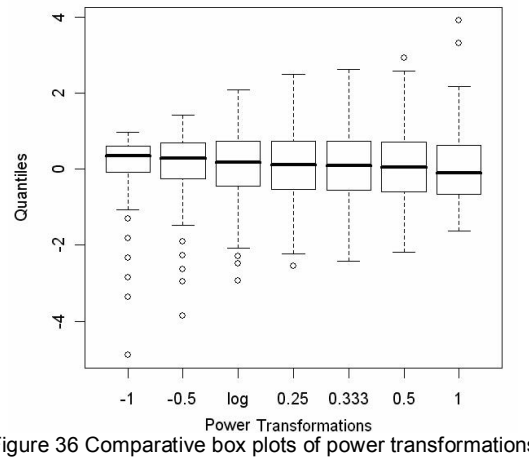

Figure 36 Comparative box plots of power transformations for yarrow root/shoot ratio.

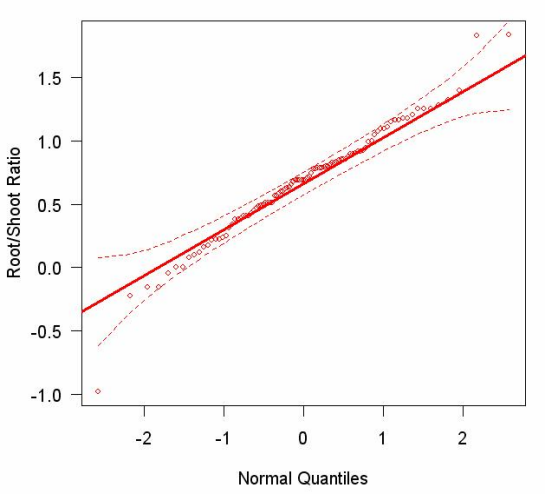

Figure 37 q-q plot of log transformed root/shoot ratio of fescue plants. Dotted lines are a 95% confidence envelope.

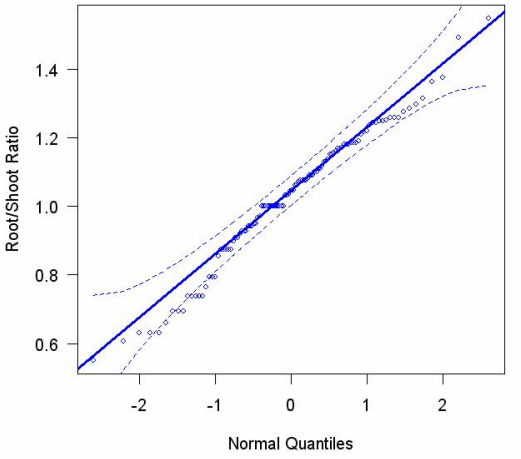

Figure 38 q-q plot of 0.333 power transformed root/shoot ratio of yarrow plants. Dotted lines are a 95% confidence envelope.