THE EFFECT OF GRAZING ON ARBUSCULAR MYCORRHIZAL FUNGI IN TEMPERATE GRASSLANDS

by

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B.Sc., McGill University, 2013

A THESIS SUBMITTED IN PARTIAL

FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF

Master of Science

in

THE COLLEGE OF GRADUATE STUDIES

Biology

UNIVERSITY OF BRITISH

COLUMBIA (Okanagan)

September 2016

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THE UNIVERSITY OF BRITISH COLUMBIA

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The effect of grazing on arbuscular mycorrhizal fungi in temperate grasslands

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ABSTRACT

Managed grazing, involving large animals destined for human consumption, covers more than 25% of the land surface and has the capacity to alter ecosystems, often leading to desertification, woody encroachment, and deforestation (Asner et al 2014). Arbuscular Mycorrhizal (AM) fungi are ubiquitous root symbionts that colonize 80% of terrestrial plants and influence plant productivity and community composition. Despite the importance of AM fungi for plant communities, the effect of grazing on AM fungal communities is largely unknown. I used grazing exclosures of varying ages to compare AM fungal community and infectivity in grazed and ungrazed plots, as well as several environmental variables that may be affected by grazing. AM fungal community composition was not significantly different between grazed and ungrazed, but grazing increased spore density while decreasing soil hyphal length. This may be attributed to the plasticity of AM fungi in response to environmental conditions, flexibility allowed isolates to respond to grazing without shifting community composition. None of the environmental variables was related to the change in AM fungi, indicating that variables not measured may be responsible. Time since the exclosure was established was the only variable related to community dissimilarity between grazed and ungrazed. As the age of the exclosure increased, the dissimilarity also increased, highlighting the importance of long-term studies in furthering our understanding of grazer-fungi interactions.

PREFACE

This study was conducted using grazing exclosures established and maintained by the Ministry of Forests, Lands, and Natural Resource Operations in the Southern Interior British Columbia, Canada. Sampling was conducted with permission and assistance from Range Ecologist Dr. Rick Tucker, who provided the plant community data. I designed the experiment and performed the fieldwork with the help of members of the Hart lab. I was responsible for processing the samples and performing molecular work. The BC Ministry of Environment, Technical Services Lab (Victoria, British Columbia) did the soil chemical analysis, and IBEST Genomics Resources Core at the University of Idaho did the sequencing. I performed the sequence analysis and collaborated with Dr. Jonathan Bennet on the data analysis. I wrote my thesis with the guidance of my supervisor Dr. Miranda Hart, and it was reviewed by my supervisory committee: Dr. David Scott and Dr. Jason Pither from the University of British Columbia (Okanagan).

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Grasslands grazing and Arbuscular Mycorrhizal (AM) fungi

Grassland plants have a long evolutionary history with herbivores that allows them to tolerate high levels of grazing (Stebbins 1981). Humans depend on this coevolution to maintain pastures that are essential for food production across many cultures. Managed grazing, which involves large animals destined for human consumption, covers more than 25% of the global land surface and can result in desertification, woody encroachment, and/or deforestation (Asner et al. 2004). As an economically and ecologically important human impact, it is vital we understand all the factors influencing the productivity and biodiversity of these systems, including Arbuscular Mycorrhizal (AM) fungi.

AM fungi are soil microbes that colonize the roots of plants and form a symbiotic partnership with their hosts. These organisms make up the phylum Glomeromycota and colonize 80% of terrestrial plants (Smith and Read 2008). They are beneficial fungi capable of acquiring nutrients for the plant (Smith and Read 2008), protecting them from pathogens (Sikes et al. 2009), and helping plants deal with stress such as drought (reviewed in Augé 2001) and salt stress (Sharifi et al. 2007). In return, plants supply mycorrhizal fungi with carbon, without which the fungi cannot survive (Pearson and Jakobsen 1993). If the plant is carbon limited (i.e. from losing productive biomass to herbivores) then there is also less carbon available for the fungi.

Such grazing-induced limits on the AM symbiosis may have far reaching effects. AM fungi are important in ecosystems, capable of influencing plant biodiversity and productivity (van der Heijden, Bardgett, and van Straalen 2008; Köhl, Oehl, and van

der Heijden 2014). This is particularly true in grasslands because virtually all perennial plants in these systems form AM symbioses.

Despite the importance of AM fungi in grasslands, little is known about the effect of grazing on AM fungi in these systems. While the plant response to grazing is relatively well understood, the AM fungal response is not. Since grazing imposes changes in carbon- supply by removing productive biomass, it may be a selective force on AM fungal communities as well.

1.2 Ambiguous effects of grazing on AM fungi

There has been considerable research on AM fungi and grazing, but AM fungal responses to grazing are inconsistent and even contradictory among studies (Hokka et al. 2004; Klironomos et al. 2004; Mikola et al. 2005). This lack of consensus is not due lack of research: there have been 58 papers in this area since 1980, not including reviews and meta-analyses. Rather, the problem lies in the controversy over the effect of herbivory on the AM symbiosis

One theory has been proposed to explain the response of AM fungi to herbivory primarily in terms of carbon supply, the *carbon limitation hypothesis* (Wallace 1987; Gehring and Whitham 1994). The carbon limitation hypothesis predicts that herbivory should decrease fungal colonization overall as host limits the amount of carbon available to root symbionts (Wallace 1987). Overall, there is mixed support for the carbon limitation hypothesis in the literature, with considerable support among different systems and hosts (Gehring and Whitham 1994; Frank et al. 2003; Wardle et al. 2002; Barber et al. 2012) Many studies, however, show herbivory elicits either no response in root colonization

(Pietikäinen et al. 2005), or increased root colonization (Eom et al. 2001; Hokka et al. 2004; Wearn and Gange 2007; Keitaro et al. 2012; Techau et al. 2004; Nishida et al. 2009) or variable colonization responses (Bethlenfalvay and Dakessian 1984; Hokka et al. 2004; Klironomos et al. 2004; Piippo et al. 2011). A recent meta-analysis challenging the carbon limitation hypothesis (Barto and Rillig 2010) found little evidence for reduction in root colonization following herbivory, and suggested that other factors may determine fungal response to grazing.

Considering that grazing presents more that simply a carbon limitation to AM fungi may help to better understand disturbance effects on AM fungi and resulting plant communities. In addition to carbon limitation, grazing imparts a suite of changes, which can affect plant, fungus, and soil that may impinge upon the AM symbiosis. These include soil compaction (Wallace 1987), increases in Nitrogen (N) availability from root exudates (Hamilton et al. 2008), increased nutrient availability through feces and urine of herbivores (Huntly 1991) or plant mediated N-mineralization (Holland and Detling 1990; Hamilton et al. 2008), and/or changes to plant communities (Wikeem et al. 2012). Given that each of these factors may affect AM partners differentially, understanding the response of the AM symbiosis to grazing may be difficult using only single response variables.

1.3 Response to herbivory depends on response variable

AM fungi may be affected by grazing in multiple ways, including (but not limited to) changes in infectivity of root and soil, and changes in fungal community composition. Most studies, however, tend to gauge fungal response using only one variable, typically percent

root length colonization. By neglecting to measure multiple response variables, important grazing responses may be overlooked.

1.3.1 AM fungal infectivity

Percent root colonization, a measure of relative AM fungi abundance, is the most commonly reported response of AM fungi to grazing, and also perhaps the most variable. Although reduced percent root colonization following herbivory is predicted by the carbon limitation hypothesis, and is commonly reported (Gehring and Whitham 1994; Barber et al. 2012; Saravesi et al. 2014) there are many examples where herbivory leads to increased root colonization (Eom et al. 2001; Kula et al. 2005; Nishida et al. 2009; Wearn and Gange 2007; Keitaro et al. 2012).

Increases in AM fungal colonization following defoliation may be a response to increased resources associated with grazing (i.e. through urine and feces of grazing herbivores (Baron et al. 2001)), as plants may opportunistically invest more heavily in AM fungi to exploit additional nutrients and stimulate regrowth (Barto and Rillig 2010).

Counterintuitively, increases in AM colonization could result from increased carbon availability as defoliated plants briefly increase carbon exudation in the roots (Holland et al. 1996; Bazot et al. 2005; Hamilton et al. 2008). This pulse of carbon may be reflected in increases in fungal abundance overall, including hyphal length, root colonization, and spore density. Such 'pulses' of resources belowground may explain why the increases in colonization have been shown to decrease after two weeks (Nishida et al. 2009). It may also explain why only low frequency herbivory is associated with increases in root colonization: if the frequency of defoliation is too high, the plant has no carbon to "pulse" in the roots and the colonization decreases (Klironomos et al.

2004).

In addition to biological explanations, changes in AM fungal colonization after grazing may be related to the measure itself. Percent root length colonized may over- or underestimate fungal investment into roots and does not account for differences among fungal structures such as storage vesicles, intra-radical hyphae, and arbuscules (Hart and Reader 2002). Soil fungal structures such as extra-radical hyphae and spores are also ignored. Thus, AM fungal investment may be reduced following defoliation, but not perceived by % root length colonized. Similarly, as root biomass is almost uniformly reduced by herbivory (Holland et al. 1996; Thornton et al. 2016; Ferraro and Oesterheld 2002), total fungal colonization may decrease, even if there are no changes, or increases in
% root colonization. The more grazing reduces the root length, the more the absolute amount of fungal colonization will be overestimated.

1.3.2 AM fungal community structure

Herbivory may also result in changes to AM fungal communities. Since herbivory changes the plant community through grazing (Frank 2005), soil compaction (Shelton et al. 2014), and changes to soil fertility (Frank and Groffman 1998), these changes may translate into changes below ground, as AM fungi and plant communities are tightly linked (van der Heijden et al. 1998).

As with other response variables, there is no clear pattern to grazing response. AM
fungal diversity has been shown to both decline (Eom et al. 2001; Su and Guo 2007; Ba et al. 2012), and increase in response to grazing (Ba et al. 2012). While fewer in number, there are also studies showing compositional changes (Eom et al. 2001;

Murray et al. 2010; Yang et al. 2013), but not in all (Shelton et al. 2014). One explanation for varying results is the degrees of grazing intensity; Ba et al (2012) found increases in diversity at light

grazing, but reductions at higher grazing levels. Thus, compositional changes may only be apparent at high herbivory.

Compositional changes as a result of grazing suggest that there are different levels of grazing tolerance between AM fungi. Where AM fungal diversity was reduced with grazing, the AM fungi under grazing were predominantly Glomeraceae isolates (Eom et al. 2001; Su and Guo 2007; Ba et al. 2012). The fungi in this family have fast growth rates (de la Providenicia et al. 2005; Hart and Reader 2005), abundant, early sporulation (Oehl et al. 2009; Pringle and Bever 2002) and are often considered disturbance tolerant. This suggests the Glomeraceae may be the most grazing tolerant, but compositional changes because of grazing do not always result in increases in Glomeraceae (Murray et al. 2010; Yang et al. 2013). Glomeraceae can be sensitive to grazing (Su and Guo 2007; Ba et al. 2012) and non-Glomeraceae can increase with grazing (Eom et al. 2001; Murray et al. 2010).

The variability in response may be an artifact of community measurements; most

published reports used spore sievings to quantify AM fungal communities (Eom et al. 2001; Su and Guo 2007; Murray et al. 2010; Ba et al. 2012; Shelton et al. 2014). Studies using spores to identify communities are limited to the soil, and spores producing AM fungi only, possibly missing changes in AM fungi community. Only two studies have used a molecular approach (Yang et al. 2013; Guo et al. 2016). Of those, Yang et al (2013) found that grazing changed the communities in the roots only and Guo et al

(2016) found that responses depended on the steppe type and plant communities. It is well established that different sample types (ie root, soil, spores, sequences, etc.) result in significantly different AM

fungal communities (Hempel et al. 2009; Selosse et al. 2016). Thus both techniques and the nature of the sample may contribute to variation in observed responses.

1.4 Herbivory response depends on ecological context

Even if studies were able to measure the full suite of AM fungal responses, it is likely that the discord among studies would exist, albeit reduced. The context of grazing (i.e. plant identity, AM fungal identity, soil chemistry) may be as important as response variable in determining disturbance outcomes and may significantly alter the ability to detect, or change the direction of grazing response.

1.4.1 AM identity affects responses to herbivory

If AM taxa have differential tolerance to conditions associated with grazing, then it is likely that AM fungal community composition will affect grazing outcomes. While there are many reports in the literature about differential disturbance tolerance among the Glomeromycota (Cuenca et al. 1998; Jansa et al. 2002; Hart and Reader 2004; Schnoor et al. 2011), we have yet to fully understand how this variation is organized (i.e. whether or not it is phylogenetically conserved (Hart and Reader 2004) or a result of selection pressure (Maherali and Klironomos 2012)).

In terms of grazing, such variation could result in positive (if fungi are disturbance

tolerant) or negative feedback (if fungi are disturbance intolerant) between host and fungi. There is only indirect evidence of this feedback in the literature: we know that AM fungi differ in their capacity to improve compensatory growth capacity (Bennett et

al. 2009; Bennett and Bever 2007), or ability to stimulate herbivore defense (Nishida et al. 2009; Barber 2013), but there is yet no direct evidence for differential grazing tolerance among AM hosts attributable to AM identity. Due to the high levels of functional variation among

AM fungal isolates (Hart and Reader 2002b; Munkvold et al. 2004; Koch et al. 2004) it is not yet possible to determine which fungi, and under which contexts, AM fungi would lead to positive versus negative feedback for defoliated hosts.

1.4.2 Host plant/ plant community influence the effect of herbivory

Varying reports of AM fungal grazing response may also stem from differences in host identity. There is a history of research showing that response to herbivory is a continuum among plant taxa, with some intolerant, and others that are competitively dominant under grazing (Ellison 1960; Robertson 1971; Crawley 1990). If hosts are differentially tolerant of grazing, then it follows that fungi associated with tolerant hosts should be less affected by grazing. For example, the root biomass of grasses is more resistant to change following defoliation than annual plants (reviewed in Ferraro and Oesterheld 2002), which may explain why percent root colonization for annual plants decreases more than grasses and forbs (Barto and Rillig 2010). If, disturbance tolerance among plants results from compensatory growth (McNaughton 1983), this may also stimulate AM fungal activity in order to acquire the necessary nutrients. However, if plant grazing tolerance is due to plant traits conferring stress tolerance (eg. quiescence during grazing, perenniating structures below ground (Huhta et al. 2009), or unpalatable tissues (Diaz et al. 2007), AM fungal activity may be reduced (quiescence), or be unchanged (unpalatable plants) in response to grazing.

1.4.3 Soil Chemistry

Soil chemistry can mask the effects of herbivory on AM fungal colonization, (Techau et al. 2004; Ruotsalainen and Eskelinen 2011) plant benefit (Aguilar-Chama and

Guevara 2012) and mycorrhizal community (Murray et al. 2010). This is not surprising as not only are plants better able to tolerate herbivory when there are abundant resources (McNaughton 1983), but high soil fertility is also known to suppress AM fungi (Treseder and Allen 2002). Simply put, there are situations, particularly at high nutrient availability, where AM fungi respond to soil fertility more than of herbivory (Techau et al. 2004), and this may be compounded by AM fungal specific differences in their tolerance to soil nutrients (Murray et al. 2010). In such cases, herbivory effects may only be apparent, or may change directions, under different nutrient regimes.

Other soil variables like pH can reverse the effect of herbivory entirely (Ruotsalainen and Eskelinen 2011) possibly by affecting the plant and AM fungal community composition. Routslainen and Eskilinen (2011) showed grazing reduced root colonization only in acidic soil, while in ungrazed soil, root colonization increased with acidity. Thus, studies showing changes in AM fungal community composition (Murray et al. 2010; Ba et al. 2012) may be the result of low pH rather than grazing itself. Understanding how soil chemistry interacts with herbivory is vital to determining whether the AM fungal responses general responses to herbivory or due to concomitant changes in soil conditions.

1.4.4 Time

Time is also very important and little understood factor in determining AM fungal responses to herbivory. Some AM fungal responses, such as percent colonization,

can occur almost instantly but may be equally short lived (Nishida et al. 2009). Others, such as changes in community composition may take years to develop and years to recover from (Shelton et al. 2014). Variation among published studies in time frame, both between the herbivory event and the AM fungal measurement, but also in the length of time the plants

were exposed to herbivory treatments, may significantly change the outcome. Perhaps it takes time to affect the AM fungal community because grazing also takes time to affect the soil condition and plant community, and possibly decades for them to recover (Pyke et al. 2016; Krzic et al. 2012; Shelton et al. 2014).

1.5 Research Objectives

My goal is to determine how grazing affects the AM fungal community in temperate grasslands. The literature on grazing and AM fungi is inconsistent due to several factors such as host identity, AM identity, sampling bias, and ecological context. To address these shortcomings I measured multiple indicators of AM fungal infectivity, including community composition. While I could not completely control for ecological context, I was able to measure several soil and plant variables to understand what changes in response to grazing and whether these changes affect the AM fungal communities. I used an established, long term grazing exclosure chronosequence, to answer the following questions:

1) Does grazing affect AM fungal infectivity (root colonization, spore density, and hyphal length), and/or AM fungal community composition?

- *I predicted that AM fungal infectivity would remain the same as grasses*

and forbs are habituated to grazing and are able to maintain their symbionts.

- *However, I expected there would be shifts in AM fungal community composition as the grazed and ungrazed plots are under different selection pressures.*

2) Does grazing affect the mycorrhizal environment? That is, does grazing affect factors known to influence the AM fungal community such as soil chemistry, soil density, plant community, and plant biomass?

I predicted that grazing would reduce plant biomass and shift the plant community by selecting for more grazing tolerant species.

- *I predicted that grazing would alter the chemical profile of the soil because grazing may also increase nutrient availability through defecation/urination.*
- *Finally, I predicted grazing would lead to increased soil compaction compared to soil in exclosures due to the activity of herbivores.*

3) Are changes in AM fungal infectivity/community related to grazing induced changes in soil environment and/or age of the exclosure?

- *I predicted changes in AM fungal infectivity would be related to changes in soil nutrient availability and/or plant biomass, as those variables affect how much carbohydrates are available for the AM fungi.*
- *I predicted changes in plant community and soil nutrients to be the strongest predictors for AM fungal community composition because both are known to cause shifts in community composition through host preference and nutrient stress.*

- *I also predicted the differences between grazed and ungrazed would increase with the age of the exclosure, as the communities would have more time to differentiate.*

CHAPTER 2: METHODS

2.1 Study Site

I selected nine grazing exclosures established by the Ministry of Forests, Lands, and Natural Resource Operations in the Southern Interior British Columbia, Canada. Sites were selected based on similar vegetation types (grasslands), elevation (~1100m), and grazing (cattle and horses). Seven of the sites were located in the Hamilton Mountain area about 25-30km east of Merritt, BC. The remaining two were located near Tunkwa Provincial Park, BC. Soils at all sites were loam Black Chernozems that developed from glacial till deposits of volcanic rock and limestone (Young et al 1992). The area has a semi-arid climate with hot, dry summers. Each exclosure was fenced off using barbed wire fencing to prevent any grazing within. The exclosures varied in age from 17 to 85 years (See Table 1). Each exclosure ranged from 0.5-1 ha, and were paired with a grazed site directly adjacent.

2.2 Sampling

2.2.1 Soil

Samples were collected in May 2015, every 10 m along a 70m transect (10m from the fence). Twenty soil samples at each site (10 grazed, 10 exclosure) were taken, 5 at the surface (0-15cm) and 5 at the subsurface (15-30cm). In total, I collected 9 sites x 2 grazing treatments x 2 depths x 5 = 180 samples to be used for AM fungal analyses.

To collect subsurface samples, I first dug a small hole to 15 cm and then used a soil corer to collect deeper soil. The samples were kept cool in the field until they could be frozen, at most 48 hours from time of collection. They were dried for two days at 60 °C, homogenized with 500μm sieve, and subsampled for DNA extraction. Then samples were

pooled by plot because there was not enough soil to quantify the soil chemistry and mycorrhizal infectivity of each sample separately. By pooling the samples I was able to get values for the plot as a whole, but not the heterogeneity within plot. In all, 36 samples from 18 grazed/ungrazed plots were used to determine AM fungal abundance and soil properties.

In June 2015 further sampling was undertaken to determine soil bulk density. Five undisturbed cores were taken in each plot near the original samples, except they were 5m from the fence instead of 10m. The samples were taken closer to the fence in June to minimise the destructive effect of biomass harvesting and core sampling on the integrity of the exclosures, and their future uses. Again, cores were taken at the surface (0-15cm) and subsurface (15-30cm). The samples were dried for five days at 40 °C and weighed. As one site (Muscrat) was too rocky for coring, I ended up with 160 bulk density cores that were kept separate until they were weighed. The average bulk density of the five samples in each plot was used as the plot bulk density in future analysis.

2.2.2 AM fungal infectivity

Root segments in dried soil samples were randomly selected from dried soil samples for staining. This selection likely covered only the most common plant species. Washed root segments were cut into 1cm pieces and stored in 50% ethanol before being stained with Chlorazol Black E (Brundrett et al 1984). Percent colonization was determined using the magnified intersection method (McGonigle et al 1990) to determine what proportions of the roots were colonized by AM fungi. Only the surface samples contained enough root segments to accurately quantify percent colonization. Spores were extracted from soil samples using a wet-sieving technique (Klironomos et al. 1993) to calculate spore abundance. Hyphal length was determined using 200 mL of soil suspension was

combined with 1 mL of a mixture of europium (III) thenoyl-trifluoroacetonate and a fluorescent brightener (Anderson and Westmoreland 1971). The suspensions were then stained for 1 hr, and then filtered through nitrocellulose filter papers using a 50% ethanol wash. The filters were mounted on microscope slides and computer-imaging software was used to estimate hyphal length.

2.2.3 Soil Chemistry

The pooled soil samples from each plot were sent to the BC Ministry of Environment, Technical Services Lab (Victoria, British Columbia) for chemical analysis. Soil Phosphorous was extracted using the Bray-P1 method (Bray et al. 1945) and quantified using a visible spectrophotometer. A 1:1 mixture of soil:water was used to measure soil pH using an ion meter. Total C an N were quantified using combustion elemental analysis.

2.2.4 Plant Community

The aboveground biomass for ten 0.5 m^2 quadrats per site was removed, dried at 60°C for three days and weighed. In order to obtain a better measure of the productive biomass in each plot, only live biomass was included and litter was removed prior to harvest. Five plots were harvested inside the exclosures and the other five outside, located adjacent to where soil samples were collected and 5 m from the fence to minimize the destructive effect on the study sites.

Plant communities inside and outside the exclosures were determined using a random number generator to select 50 Daubenmire (50 x 20cm) quadrats along five parallel 30m transects spaced 5m apart. Percent cover was estimated at each quadrat for each plant species before all fifty quadrats were combined to give a description of the plant community of the plot as a whole (Daubenmire 1959). The Ministry of Forests, Lands, and

Natural Resource Operations completed this in 2014 for 5 sites (Tunkwa New, Tunkwa Old, Repeater, Muscrat, Goose), and in 2015 for 4 sites (Stipa Rich, Stipa Nel, Bluegrass, and Aspen). I assisted with surveys at Stipa Rich, but most work was done by range ecologists at the Ministry. These plant surveys are usually conducted every two decades to monitor changes in community, as differences can take decades to develop. Therefore a one-year difference between sampling should not be enough to noticably alter plant communities.

2.3 Community Analysis

2.3.1 Molecular analysis

DNA was extracted from 125mg soil following the protocol of the FastDNA-96™ Soil Microbe DNA (MP Biomedicals) after first drying, sieving and homogenizing soil. Nested PCR was performed on the samples, first to amplify, then to attach barcodes for Illumina sequencing. In the first PCR, Glomeromycota specific primers WANDA (Dumbrell et al 2010) and AML2 (Lee et al 2007) were used to amplify a 500bp fragment of the small subunit of rDNA. Samples were amplified in a 20 µL reaction consisting of 11.75 µL ddH_20, 5 µL 5x PCR buffer (Promega), 1 µL $MgCl_2$ (BioLabs), 0.5 µL dNPTs (Amresco), 0.25 µL BSA (BioLabs), 0.5 µL GoTaq (Promega), and 0.5 µL of each primer. This mixture was heated for 2 minutes at 95°C then underwent 34 cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, finishing with 72°C for 5 minutes. Barcodes were added to PCR products in a 20 µL reaction of 9.2 µL ddH_20, 4 µL 5x PCR buffer (Promega), 3.6 µL $MgCl_2$(BioLabs), 0.4 µL dNPTs(Amresco), 0.6 µL BSA(BioLabs), 0.2 µL GoTaq(Promega), and 0.5 µL of each barcode. Cycling conditions were: 1 min at 95°C, 10 cycles of 30 secs at 95°C, 30 secs at 60°C, 1 min at 68°C, and ending with 5 min at 68°C. Samples were frozen and sent to the IBEST Genomics Resources Core at the University of Idaho for sequencing using Illumina

Sequencing Technology (Bennet 2004).

2.3.2 Sequence Analysis

Raw sequences were processed using QIIME (Caporoso et al. 2011). The raw sequence files contained approximately 4.2 million sequences, 3.2 million of which were retained after low quality (Q , 3) , and reads with more than 3 N characters were removed. We used USEARCH (Edgar 2010) and a 97% identity threshold to pick Operational Taxonomic Units (OTUs) using maarjAM database (Opik et al. 2010) as a reference database. This resulted in 28927 OTUs, which was reduced to 351 OTUs after filtering out those comprising less than 0.01% of the data set. Representative sequences of these OTUs were aligned using MUSCLE (Edgar 2004), then BLASTed (Alschul et al. 1990) against both the maarjAM database (Opik et al. 2010) and the NCBI database (Geer et al. 2010) to ensure consistent taxonomic assignment. In total, 11 non-Glomeromycotan OTUs were removed, and OTUs matching identical MaarjAM virtual taxon accessions were merged to give a final number of 62 OTUs with an average of 14000 sequences per sample. The resulting OTU table was rarefied to 1718 sequences/sample, as that was the lowest sequencing depth for all samples. The final table was log transformed before analysis because of the skewed distribution of sequences.

2.4 Data Analysis

Data analysis was done using R v3.2.1 (R Core Team 2015). Because of my limited sample size, I am considering p-values of less than 0.1 significant.

2.4.1 Does grazing affect the AM fungal community?

2.4.1.1 AM fungal infectivity (% root col, spore density, hyphal length) responses to grazing

I tested the effect of grazing on each AM fungal infectivity variable using the paired Wilcox signed rank test for non-parametric data from the R package MASS (Venables and Ripley 2002). The samples were paired by site comparing infectivity values at grazed and ungrazed. The soil layers were tested separately, as they were not independent, and not paired for this test.

2.4.1.2 AM fungal community responses to grazing

To determine if there were differences in AM fungal community composition resulting from grazing, I used non-metric multidimensional scaling in the R package 'vegan' to create an ordination based on the Bray Curtis distance metric (Oksanen et al 2015). The dimensionality of the ordination was increased until model stress dropped below 0.2 – the resultant model had 4 axes with a stress of (0.1745). We tested each axis separately for response to grazing using a variation on the split plot mixed model above. However, as we took multiple samples per plot, we also included a random factor, representing sample as a repeated measure within plot. Note that because these tests are not independent, alpha should be adjusted from 0.1 to 0.1/3=0.033 for statistical significance.

For subsequent AM fungal community analyses, we pooled the data by plot. We then calculated AM fungal richness and evenness (Pielou 1966) and tested the effect of grazing

using the paired Wilcox signed rank test. In addition, we conducted indicator specie analysis (Dufresne and Legendre 1997) to identify AM fungal families and isolates that were indicators of grazing using 'indicspecies' in R (De Caceres and Legendre 2009).

2.4.2 Does grazing affect the mycorrhizal environment?

Plant and soil responses to grazing

The soil and plant variables measured were tested with the same paired Wilcox signed rank tests with the two soil layers being tested separately.

Are changes in AM fungal community related to grazing induced changes in soil environment and/or age of exclosure?

To determine how environmental changes and age of exclosure might affect the AM fungal community response to grazing, I compared environmental variables to the changes in AM fungal infectivity and community. I calculated the change in AM fungal infectivity, soil chemistry, and plant community as:

Change in infectivity =infectivity in grazed plots-infectivity in ungrazed.

I used a multi-model inference approach in the R package MuMin (Barton 2016) to tests all possible combinations of the predictors (changes in environmental variables and age of exclosure) on changes in spore density, and hyphal length, and then ranked the resultant models by AICc scores, which represent model fit penalized by the number of parameters (Burnham and Anderson 2002). Variable weights were subsequently derived from the model weights as the sum of AICc scores for all models in which that variable appeared. Only variables with the highest weights were plotted. Multicollinearity of predictors were tested using the variance inflation factor in the R package 'car' (Fox and Weisberg 2011).

To test whether changes in environmental variables or exclosure age explain changes in AM fungal community composition, we calculated the Bray-Curtis dissimilarity between Hellinger transformed (Legendre and Gallagher 2001) grazed and ungrazed plots at each site. Then we used the same multi-model inference approach introduced previously to determine which grazing induced changes in the soil and plant are responsible for the changes AM fungal community composition, and how age of exclosure ranks as a predictor.

CHAPTER 3: RESULTS

3.1 Does grazing affect the AM fungal community?

3.1.1 AM fungal infectivity responses to grazing

Percent Root Colonization

Grazing did not affect percent root colonization (p=0.930) 95% CI [-16.0, 10.5], (Figure 1). Average percent colonization across all plots was 31.8 (SE 3.12)

Spore density

Spore density tended to be higher under grazing than in the exclosure at the surface (p = 0.054) 95% CI [-8.0, 236.5] (Figure 1). The subsurface soil showed less of a response (p = 0.359) 95% CI [-71.0, 169]. At the surface, ungrazed soil had an average spore density of 194.9 spores/10g (SE 41.71), while the average in grazed soil was 302.6 spores/10g (SE 26.15). In the B layer, spore density was lower, with an average of 108 spores/10g (SE 32.19) in ungrazed and 156 spores/10g (SE 26.90) under grazing.

Soil hyphal length

Grazing is associated with reduced soil hyphal length in the surface soil (p=0.039) 95% CI [-1.85, -0.05] (Figure 1). In the subsurface, there was no association with grazing (p=0.160) 95% CI [-1.9, 0.3]. Average hyphal length in the surface soil was 3.26 mm/g (SE 0.27) in ungrazed and 2.32 mm/g (SE 0.24) under grazing, while in the subsurface average hyphal length was 1.84 mm/g (SE 0.31) in ungrazed and 0.99 mm/g (SE 0.133).

3.1.2 AM fungal Community responses to grazing

AM fungi Alpha diversity

Neither AM fungal richness (surface p=0.938, subsurface p=0.875) nor AM fungal evenness (surface p=0.734, subsurface p=0.301) appears affected by grazing (Figure 2). AM

fungal richness was 35.8 (SE 1.07) at the surface and 30.1 (SE 1.23). Average evenness was 0.678 (SE 0.012) at the surface and 0.643 (SE 0.018) in the subsurface soil.

AM fungal community changes

Across all sites, we identified 62 OTUs from 8 families: 2 Ambisporaceae, 4 Archeaosporaceae, 3 Acaulosporaceae, 7 Diversisporaceae, 1 Pacisporaceae, 8 Claroideoglomeraceae, 33 Glomeraceae, 3 Paragomeraceae, and 1 Glomeromycotan OTU that did not match any AM fungal family in the databases.

There was no significant effect of grazing on AM fungal community composition (P= 0.28, 0.45, 0.46, 0.20 for each axis) (Figure 3). The low marginal R-squared values for each axis (0.0056, 0.0048, 0.0031, and 0.014 respectively) demonstrate how little of the variation could be explained by grazing alone. The conditional R-squared values were considerably higher (0.1208, 0.1884, 0.3111, and 0.1696 respectively) indicating the importance of accounting for site differences. Several isolates were significant as indicators of either grazed or ungrazed plots (Table 3): two *Glomus* (p=0.0031 and p=0.0856) a *Paraglomus* (p=0.0817), and an *Ambispora* (p=0.084), isolate were positively associated with grazing, while three *Diversispora* isolates were found more often in ungrazed plots (p=0.0388, p=0.0974, and p=0.1024). None of the AM fungal families were indicators of grazing exposure (Table 3).

3.2 Does grazing affect the mycorrhizal environment?

3.2.1 Plant and soil responses to grazing

Plant community

Biomass and percent cover

Aboveground biomass was higher inside exclosures (p <0.001) 95% CI [25.52,

54.18]. Both treatments were dominated by grasses, which had an average percent cover of 71.6 (SE 4.9), while forbs had an average percent cover of 26.9 (SE 4.1). Dominant species (>50% percent cover) were *Festuca campestris* Rydb. and *Poa pratensis* L. Other plants that were found in most sites (at least 7 out of 9) include the grasses *Koeleria macrantha* Ledeb., *Stipa nelsonii* Scribn., and forbs *Achilllea millefolium* L., *Agoseris glauca* Pursh., *Aster campestris* Nuttall., *Astragalus miser* Hook., *Geum triflorum* Pursh, *Taraxacum officinale* L., and *Tragopogon dubius* Scop.

Alpha diversity

Plant richness was unchanged by grazing (21.4 plants (SE 0.71) in grazed and 18.5 plants (SE 1.89) in ungrazed plots (P=0.44). Plant evenness, however, was significantly higher in grazed plots 0.592 (SE 0.053) compared to ungazed plots 0.458 (SE 0.050) (p=0.048) (Table 2). Differences in evenness were largely due the reduced the abundance of *Festuca campestris* in grazed plots (by 89.3 % (Goose) 50% (Repeater, Muscrat, Tunkwa Old) or 25% (Aspen, Stipa Nel, Tunkwa New)(See Table 3). One site (Bluegrass) had no *Festuca campestris* inside or outside the exclosure. *Festuca* campestris was identified as an indicator of ungrazed plots (P=0.0081) in our indicator species analysis, and *Tragopogon dubius* was an indicator of grazing (P=0.011).

Soil chemistry

Phosphorous tended to be a little higher under grazing for both surface (Adjusted p=0.37) and subsurface (Adjusted p=0.27) soil layers (See Table 2 for details). Total nitrogen was also higher under grazing at the surface (p=0.195) but not in the subsurface (p=1.000). Although p-values do not indicate significance because they had to be adjusted for multiple comparison, there seems to be a trend towards higher soil nitrogen and phosphorous under grazing. Total Carbon was not significantly different under grazing at

the surface (p=0.31)or subsurface (p=1.00) neither was pH (surface p=1.00, subsurface p=1.00). Soil density was also higher under grazing in the surface soil (p=0.039), with an average of 0.818 g/cc (SE 0.0188) under grazing and 0.676 g/cc (SE 0.0314) in the exclosures. In the subsurface, there was no significant difference between grazed an ungrazed (p=1.00).

3.3 Are changes in AM fungal community related to grazing induced changes in soil environment and/or age of exclosure?

Because percent root colonization, subsurface hyphal length, and subsurface spore density did not change with grazing; they were not included in this analysis.

Spore Density

The multi-model inference approach to identify which environmental variable was related to the change in spore density identified plant evenness as the variable with the most weight. As plant evenness increased, so did spore density (standardized estimate = 0.313, weight=0.53). However, as the standard error (0.381) is greater than the estimate, and the 95% CI [-0.196, 1.375] overlaps with 0, this relationship is not significant (Table 5).

Hyphal length

Similar to spore density, none of the changes in environmental variables was significantly related to the changes in hyphal length with grazing. Age of exclosure was the most important variable explaining changes in soil hyphal length (estimate = 0.118, weight=0.31). Again, the standard error (0.265) was greater than the estimate and the 95% CI [-0.468, 1.220] overlaps with 0, indicating little importance of this variable

AM fungal community composition

Time since exclosure was the most important variable explaining differences

between the AM fungal communities inside and outside the exclosures. This is true for both the surface (estimate=0.526, weight=0.73) and the subsurface (estimate=0.604, weight=0.81). With increasing age of the exclosure, the dissimilarity between grazed/ungrazed AM fungal communities increased (Fig 4). The correlation coefficient was 0.66 for the surface and 0.64 in the subsurface. No other variables contributed to variation among AM fungal communities.

CHAPTER 4: DISCUSSION

In this study I showed that grazing affects AM fungal infectivity more than community composition. Further, a single measure of AM fungal infectivity is not sufficient to determine AM fungal responses to grazing, as different measures gave contrasting results. Despite significant changes in soil fertility, plant community, and soil density, none of these factors were related to changes in AM fungal infectivity or AM fungal community. Rather, the most the most important driver of changes in AM fungal communities was time, as dissimilarity between grazed and ungrazed plots increased over time.

4.1 Does grazing affect AM fungal infectivity?

Percent Colonization

As predicted, grazing did not affect percent root colonization. This result is consistent with the literature for field experiments, which find either no change (Bethlenfalvay and Dakessian 1984; Pietikäinen et al. 2005; Yang et al. 2013) or an increase in percent colonization (Eom et al. 2001; Kula et al. 2005). Studies which show decreases in percent colonization with herbivory (Barber et al. 2012; Saravesi et al. 2014) typically occur only under greenhouse conditions. While it has been thought that carbon limitation from the plant following herbivory would result in a decrease in fungal abundance (Gehring and Whitham 1994), this is not always the case, especially for fungi associated with grasslands, which are adapted to grazing (Barto and Rillig 2010). In these systems, plants may be able to maintain the same level of colonization, through defoliation induced carbon exudates (Hamilton et al. 2008), compensatory photosynthesis in residual foliage

(Piippo et al. 2011) or through the action of a secondary metabolite which could stimulate mycorrhization (Landgraf et al. 2012).

Alternatively, while I was unable to detect a change in fungal colonization among grazing treatments, this may be due to the methods used to estimate fungal abundance. Percent colonization is a relative measure, thus if root biomass is reduced by grazing and percent colonization remains the same, there would be a net reduction in total fungal colonization. While I was not able to measure root biomass in this study, grazing is known to reduce root biomass (Ferraro and Oesterheld 2002), and I observed much less dense root mats under grazing. Therefore, it is possible that while plants were able to maintain relative colonization levels in roots, the total abundance of fungi decreased in grazing treatments. This conclusion would match results of Nadian et al. (1997) who also found that soil compaction had no effect on percent root colonized, but decreased total root colonization because root length was greatly reduced.

Spore Density

Unlike root colonization, spore density tended to be higher under grazing, at least at the surface. This result supports results of Frank et al (2003); however, studies have also found decreases in spore density with grazing (Su and Guo 2007; Murray et al. 2010), or no changes at all (Yang et al. 2013). The difference may be associated with the intensity of grazing: Su and Guo (2007) showed decreased sporulation in severely overgrazed areas of the Mongolian Steppe where plant richness and cover was also reduced. Similarly, Ba et al (2012) found that grazing increased sporulation, with the largest increases at light grazing intensities, with less of an increase at higher grazing intensities. In my study, grazing intensity was not intense, as I did not detect any changes in plant richness, thus my study supports studies showing increased sporulation under mild to moderate grazing pressure.

The reasons for increases in sporulation associated with moderate grazing are not clear, but may be a fungal response to stress. For example, it has been shown that sporulation is stimulated among the Glomeromycota when fungi are carbon limited (von Alten et al. 1993). Presumably this is an adaptive strategy to improve survival (in the form of resistant propagules) during difficult times.

Soil Hyphal Length

Soil hyphal length decreased with grazing, more noticeably in the surface than the subsurface. A reduction in SHL has been shown before by some (Miller et al. 1995), but not others (Eom et al. 2001; Yang et al. 2013). Soil hyphal length is not used as often as spore density and percent colonization to measure AM fungal infectivity because it is not a discrete measure of AM fungal abundance; other fungi, including saprotrophs and parasites are included in this measure (Hart and Reader 2002a). However, it is considered a robust relative measure of AM fungal abundance in the soil (Newman 1966; Miller et al. 1995; Hart and Reader 2002a).

Reduced SHL under grazing could lead to reduced host tolerance to grazing as AM fungi are thought to help plants recover from defoliation by increasing their nutrient absorption (Walling and Zabinski 2006) and a reduction in hyphal length would theoretically reduce nutrient acquisition by the plant (Walling and Zabinski 2004). But since I detected concurrent increases in N in grazed plots, reduced SHL may decrease simply fungi may require less hyphae to access the same amount of nutrients. Alternatively, higher nutrient levels in grazed plots may inhibit SHL: high levels of P and N are well known to reduce mycorrhizal activity (Treseder 2004; Jansa et al. 2006). In my study, although I detected reduced SHL and increased N in grazed plots, these changes were not correlated, suggesting that it is another factor of grazing that is causing the changes, not the

related soil nutrient changes.

The concomitant changes in sporulation and hyphal length may have implications for the stability of the AM symbiosis under grazing. Extra-radical mycelia are the nutrient acquisition structures, and AM fungi with larger mycelia are thought to confer greater plant benefit (Hart and Reader 2002). Under grazing an extensive external mycelium may represent too much of a carbon drain on hosts that are already experiencing carbon limitation, particularly when nutrients are not limiting (Hart and Reader 2002; Piippo et al. 2011a). Increased spore production combined with the reduced nutrient acquisition capacity suggests that the symbiosis may not be as beneficial under grazing. In such situations, the fungi and plant may act as competing carbon sinks (Piippo et al. 2011b).

Considering all fungal traits, it is important to highlight that all three variables (percent colonization, spore density and soil hyphal length) gave conflicting measures of fungal abundance. This result speaks to the importance of measuring multiple measures of fungal abundance.

4.2 Does grazing affect AM fungal community composition?

Contrary to my expectations, AM fungal communities did not change in response to grazing. In some studies, grazing has reduced AM fungal diversity (Ba et al. 2012; Bai et al. 2013; Eom et al. 2001; Su and Guo 2007), caused shifts in relative abundance of AM fungi (Eom et al. 2001; Murray et al. 2010) or had no effect on the soil AM fungal community (Yang et al. 2013; Shelton et al. 2014). The lack of consensus is not surprising given the multiplicity of methods and approaches used to quantify "AM fungal communities". In general, researchers use a wide variety of approaches from spore counts to molecular inventories to obtain AM fungal species inventories, making comparisons difficult (Hart et

al. 2016?). In general, studies looking at the effect of disturbance on AM fungal communities often find only changes in relative abundance (Jansa et al. 2002; Jansa et al. 2003; Schnoor et al. 2011; Saito et al. 2004; Murray et al. 2010; Hassan et al. 2013; Sharmah and Jha 2014), rather than changes in identity. In this study, it is likely that the co-evolution of grassland AM fungi and grazing over millennia has made these communities robust to moderate grazing pressure. In this sense, Lekberg et al (2012) suggested that the lack of AM fungal community response to disturbance may be the result of strong community resilience when disturbed.

The fact that we found no community response to grazing in AM fungi may speak to the generalist nature and/or plasticity of AM fungi. If the changes in AM fungal infectivity were the results of changes in community composition, then it should have been possible to detect phylogenetic changes as well – i.e. the community shifting from fungi that invest primarily in extra radical mycelium like Gigasporaceae (Hart and Reader 2002b) to those that sporulate more like Glomeraceae (Oehl et al. 2009). Instead, I found that while the relative abundance of AM fungal structures changed, the identity did not. This may indicate that changes in AM fungal infectivity result from phenotypic plasticity in response to a change in grazing regime. Because AM fungi harbour genetically different nuclei they are able to rapidly produce variable offspring with different phenotypic characteristics (Angelard et al. 2014). This flexibility may allow them to rapidly respond to any disturbance without radical changes in community composition

Despite the lack of significant change in overall community composition, some AM fungal isolates occurred preferentially in grazed or ungrazed. In my study, *Glomus* isolates, plus *Paraglomus* (Paragloemraceae) and *Ambispora (*Ambisporaceae*)*, were indicators of grazing. Not much research has been done on traits of Paraglomeracae and

Ambisporaceae compared to Glomeraceae, but the latter is widely considered the more disturbance tolerant AM fungal family. Previous studies have found that AM fungi in the genus *Glomus* are particularly tolerant of grazing (Eom et al. 2001; Su and Guo 2007; Ba et al. 2012), perhaps due to their fast growth rates (Hart and Reader 2005) abundant and frequent sporulation (Oehl et al. 2009). While my data support these findings, I also show that, the majority of Glomeraceae OTUs showed no preference between grazed or ungrazed.

I found *Diversispora* isolates that were indicators of ungrazed plots. The Diversisporaceae family is less studied than the Glomeraceae, but taxa in this family have been found to be both indicators of undisturbed and disturbance sites (Eom et al. 2001; Moora et al. 2014). Other members of the order Diversisporales are known to have slower growth rates (Hart and Reader 2002b; Hart and Reader 2005) and later, less frequent sporulation (Pringle and Bever 2002; Oehl et al. 2009) than the Glomeraceae, possibly making them less competitive. Unfortunately, with the lack of research into *Diversispora*, traits of this genus are relatively unknown, making it very difficult to determine why taxa in this genus might be vulnerable to grazing.

4.3 Does grazing affect the mycorrhizal environment? Plant and soil responses to grazing

AM fungal grazing responses were unrelated to environmental responses to grazing even though grazing induced several changes to plant and soil characteristics.

Plant community

In agreement with my hypothesis, grazing reduced plant biomass and changed plant evenness, although plant richness was not affected. These findings support other studies that found no changes in species richness with grazing, especially in dry and nutrient poor

sites (Lkhagva et al. 2013; Frank 2005). Like other studies before, plant evenness increased with grazing (Frank 2005; Ba et al. 2012; Krzic et al. 2012). This may be explained by trade offs among plant taxa in competitive ability and grazing tolerance (van Velzen and Etienne 2015). For example, in our study, ungrazed plots were dominated by *Festuca*, which are highly palatable, but grazing intolerant (Mack and Thompson 1982). They are well known as a 'decreasers' (Wikeem et al. 2012; van Ryswyk et al. 1966) and display several traits, such as height and tussock architecture, that are not favoured by grazing (Diaz et al. 2007). In the absence of grazing, this plant was able to outcompete many others and dominate the exclosures, resulting in a decrease in plant evenness in ungrazed plots.

Are plant community changes responsible for changes in AM fungal infectivity/community?

That changes in plant community was not related to changes in AM fungal community is surprising, as other grazing studies have linked changes in AM fungal community with grazing to plant richness (Yang et al. 2013), and plant community (Valyi et al. 2015) responses. In fact, plant community composition is generally the most important driver for changes in AM fungal communities (Davison et al. 2011; Opik et al. 2014). This is largely believed to result from preference among AM taxa and their hosts (Opik et al. 2014; Torrecillas et al. 2012). In our study, changes in plant community were due to changes in eveness, not species turnover. Further, this was largely due to one species (*Festuca*). Thus, preferred hosts were still available for AM fungi, even under grazing. Additionally, since plants typically host multiple fungi, and fungal isolates are connected to multiple plants (Vandenkoornhuyse et al. 2003), the fungi associated with *Festuca* in grazed plots were likely already well adapted to other, co-occuring taxa..

Soil Nutrients

As predicted, grazing increased soil nutrients (available P and total N, minimally affected soil carbon). The increase in soil N with grazing is consistent with the literature (Wallace 1987; Seagle et al. 1992; Shariff et al. 1994; Frank and Evans 1997). Increased soil N may be explained by the return of N through defecation and urine (Frank and Evans 1997; Holland et al. 1992) as well as feedbacks between plant responses to grazing and N cycling (Holland et al. 1992; Frank and Groffman 1998; Hamilton et al. 2008). Grazing causes an increase in N mineralization, which under grazing can be double that of ungrazed plots (Frank and Groffman 1998). Hamilton et al (2008) demonstrated that defoliation increased carbon exudation in the roots, which led to increased N-mineralization in the rhizosphere by stimulating soil microbes. This link between carbon exudation and N-mineralization may explain why total C and N were so highly correlated.

While there are many studies examining the effects of grazing on the N cycle (Holland and Detling 1990; Frank and Evans 1997; Olofsson et al. 2001) available P responses seem understudied. Likely, this is because most grasslands are N-limited (Vitousek and Howarth 1991) making P availability relatively unimportant. Of the few that have examined the response of available P to grazing, one found a decrease (Xie and Wittig 2004), and another found no change (Baron et al. 2001), unlike my results which indicate a weak trend for increased P levels under grazing.

Soil compaction

As predicted, I found that soils were compacted by grazing. My results agree with others who found higher bulk density under grazing down to 15cm (Krzic et al. 2012). In the deeper soils I found no significant differences between grazed and ungrazed, most likely because compaction affects the surface layers most (Xie and Wittig 2004; Krzic et al.

2012). Soil compaction is a well-known consequence of grazing (Chanasyk and Naeth 1995; Greenwood and McKenzie 2001; Xie and Wittig 2004), though few studies focused on microbial communities choose to measure it.

Are changes in soil environment causing changes in AM fungal infectivity/community?

Despite the significant effect of grazing on soil properties, none of these factors were related to AM fungal grazing responses. Other studies have linked grazing induced changes in AM fungal community composition to soil fertility (Murray et al. 2010; Yang et al. 2013), or soil electrical conductivity (Ba et al. 2012); but in my study soil properties had no effect. This is supported by Shelton et al (2014), who also found no difference in AM fungal communities despite significant changes in soil density. In addition, the soil bulk density under grazing (0.818 g/cc), though higher than the average bulk density in ungrazed (0.676g/cc) does not reach levels (~1.4g/cc) known to impede root growth (Atwell 1993).

Ultimately, that AM fungal responses were unrelated to plant and soil changes indicates that 1) AM fungi are resistant/tolerant of these changes, and/or 2) AM fungal changes are more related to environmental variables I did not measure (i.e. root biomass).
1) *AM fungi resist/tolerate changes to soil environment* supports the idea that the AM fungi are generalist and have high phenotypic plasticity (Angelard et al 2014). This would explain why the community composition did not change significantly – community members were able to adapt to new conditions. The genotypic and phenotypic flexibility of AM fungi means that isolates would be able to shift functioning as necessary to survive the pressure of grazing in an area (Angelard et al. 2014). This may be related to high levels intra-isolate variation found in AM fungi (Munkvold et al. 2004), but more research is needed to understand the link between genetic and trait plasticity in AM fungi.
2) *AM fungi are driven by unaccounted for factors* That there were significant changes in

spore density and hyphal length to grazing means that AM fungi were affected by *something*, but it may not be plant community and the soil properties measured in my study. One such variable may be root length. Reduced root length means less total root colonization, which may explain the decrease in soil hyphal length with grazing, since smaller root mycelium may mean fewer resources for external mycelium production. Decreased root availability may also lead to increased spore abundance because spores can stay dormant if there is no root to colonize (Smith and Read 2008). This is one possible variable that was not measured but may help explain the AM fungal responses to grazing.

4.4 The effect of time

As predicted, the age of the exclosure was the most important factor explaining grazing related changes in AM fungal community composition. That this was not true for measures of AM fungal infectivity implies that AM fungal infectivity responses to grazing happen relatively quickly; quicker than changes to the plant communities, which may take upwards of 50 years to recover from grazing (Pyke et al. 2016; Krzic et al. 2012). The relationship between age of exclosure and AM fungal dissimilarity suggest a succession-like change over time. In order to show true evidence of succession, I would need to show that the AM fungal communities were moving in a certain direction, which was not possible with my data set. The change in fungal communities has been a topic of interest for several decades (Janos 1980; Johnson et al. 1991; Boerner, DeMars & Leicht 1996; Treseder et al. 2004; Davison et al. 2011), but most studies are limited to short time scales or conflate site with time. In our study, the paired design allowed us to compare grazing effects among communities *of the same age*. The fact that the age of the exclosure is the only factor related to fungal community changes, *and* the dissimilarity increases with age underlines the

importance of long-term studies (over six decades in this case).

CHAPTER 5: CONCLUSION

My goal was to determine how grazing affect AM fungi in temperate grasslands, and with this study I was able to conclude that grazing affects AM fungal infectivity more than it changes the community composition. Further, I provided evidence of long term differentiation in AM fungal communities, in that grazed plots become more dissimilar from ungrazed plots until over time.

5.1 AM fungal infectivity

I showed that discovering the changes in AM fungal infectivity requires multiple measures, and reliance on percent colonization in the literature may cause researchers to miss important changes. These changes may also have functional implications; the decrease in hyphal length and increase in spore density may be detrimental to the plant but this hypothesis requires further study. Unfortunately, I was not able to link the changes in AM fungal infectivity with any environmental changes, which is most likely because other factors may be driving the differences in infectivity between grazed and ungrazed plots (i.e. root biomass).

5.2 AM fungal community

The AM fungal community composition was surprisingly resilient to grazing pressure. Considering the changes in AM fungal infectivity, I had assumed the community would shift from AM taxa with high levels of SHL (i.e. Gigasporaceae) to those taxa known to be prolific sporulaters (i.e. Glomeraceae). That this taxonomic shift does not occur indicates the AM fungal communities maybe have high trait plasticity, and are able to respond to grazing

without turnover of taxa. This idea is reinforced by the lack of relationship between changes in plant and soil characteristics and AM fungal community composition.

5.3 The Effect of Time

I show robust evidence that time plays a role in the change of AM fungal communities with grazing. I found a strong increase in AM fungal community dissimilarity with time; meaning communities inside and outside the exclosures became more dissimilar the longer the exclosure has been in place. This highlights the importance of long-term studies in order to understand the relationship between grazing and AM fungal communities.

FIGURES

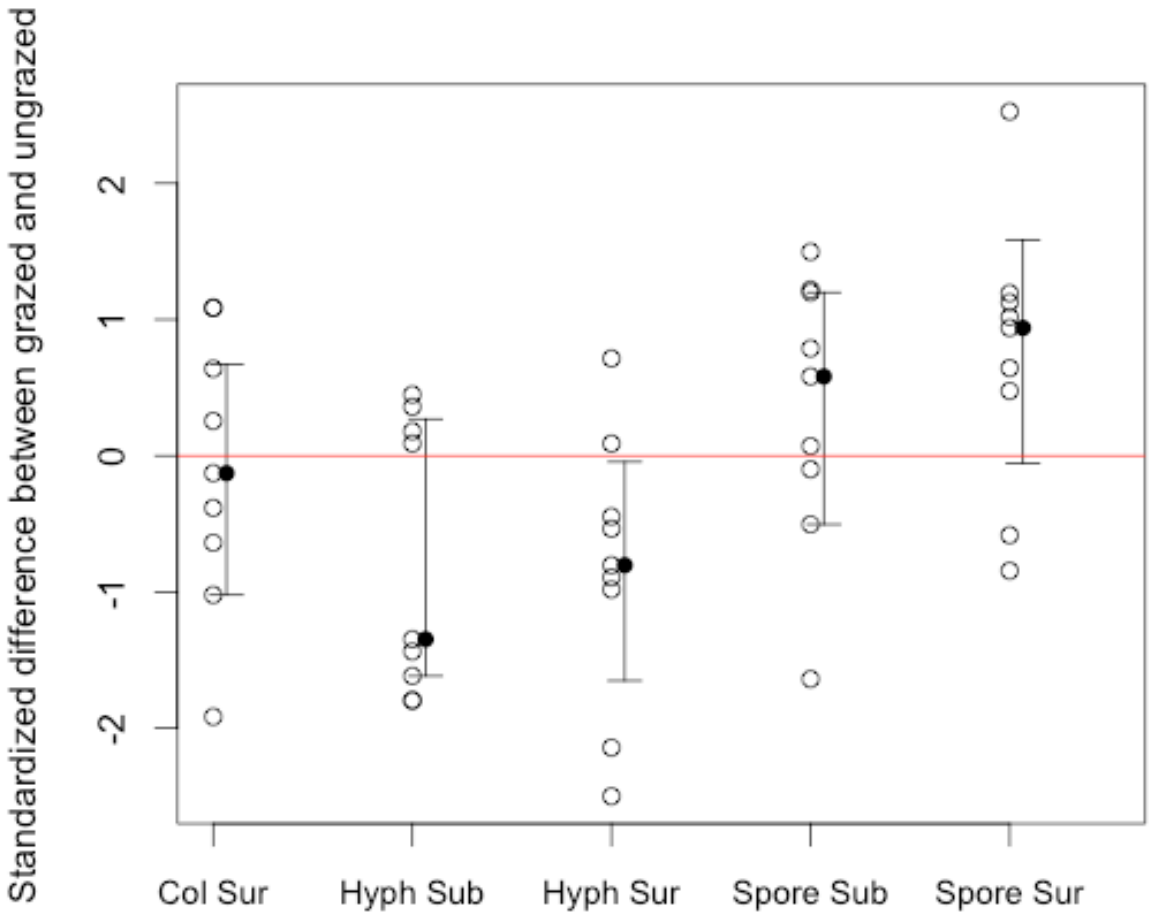

Figure 1: The standardized change in AM fungal infectivity with grazing. Change = (infectivity in grazed) – (infectivity in ungrazed). The solid red line indicates no difference between grazed and ungrazed. Open circles show the change at each site, closed circles represent the standardized median, and error bars show the 95% confidence intervals. Col = Colonization, Hyph = Hyphal length, Spore = Spore density, Sur = surface soil, Sub = subsurface soil. P-values of the paired Wilcox signed rank test were as follows: Col Sur (p-value=0.930), Hyph Sub (p-value = 0.160), Hyph Sur (p-value = 0.039), Spore Sub (p-value=0.359), Spore Sur(p-value=0.055). N=9

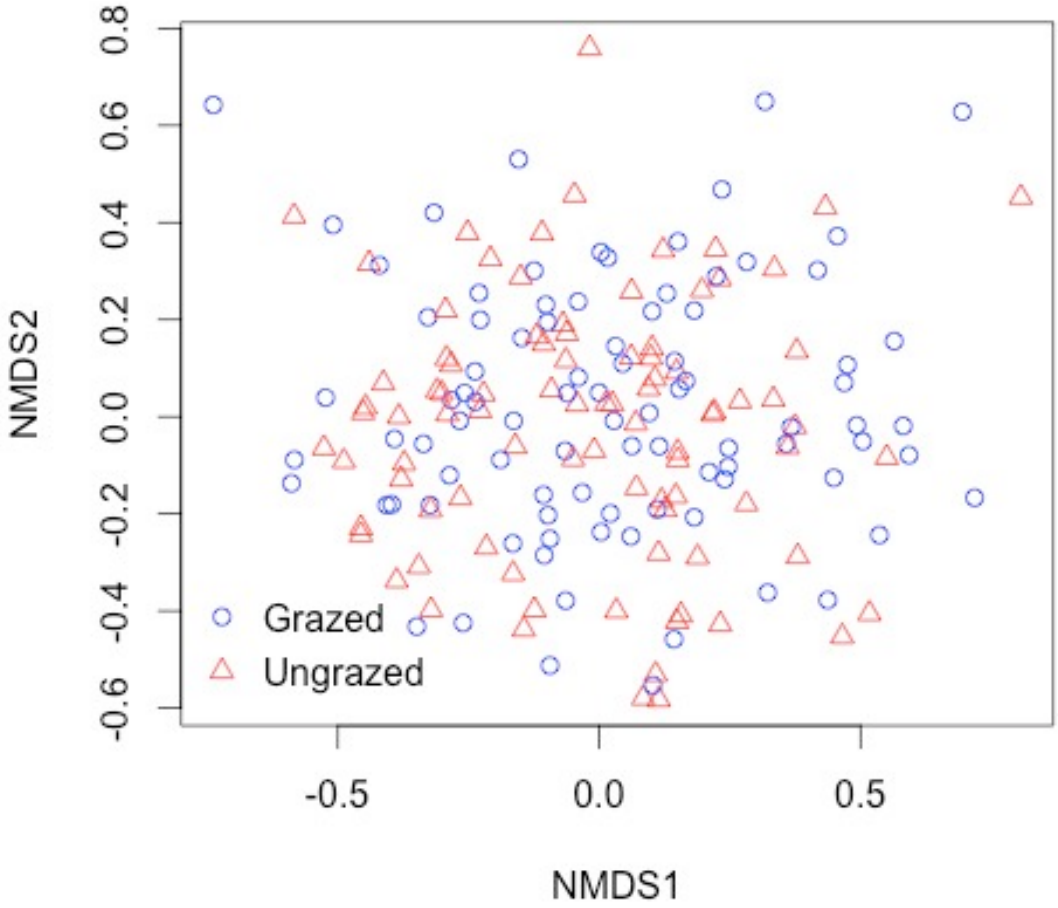

Figure 2: Nonmetric multidimensional scaling (NMDS) showing distance between AM fungal communities from grazed and ungrazed plots for both soil depths (data pooled). Ordination axes were calculated using Bray-Curtis distance metric (Bray and Curtis 1957), with 4 axes and a stress value of 0.1746. Grazing was not a significant factor any of the axes. An ordination of each layer separately also finds no significant effect of grazing on any of the axes.

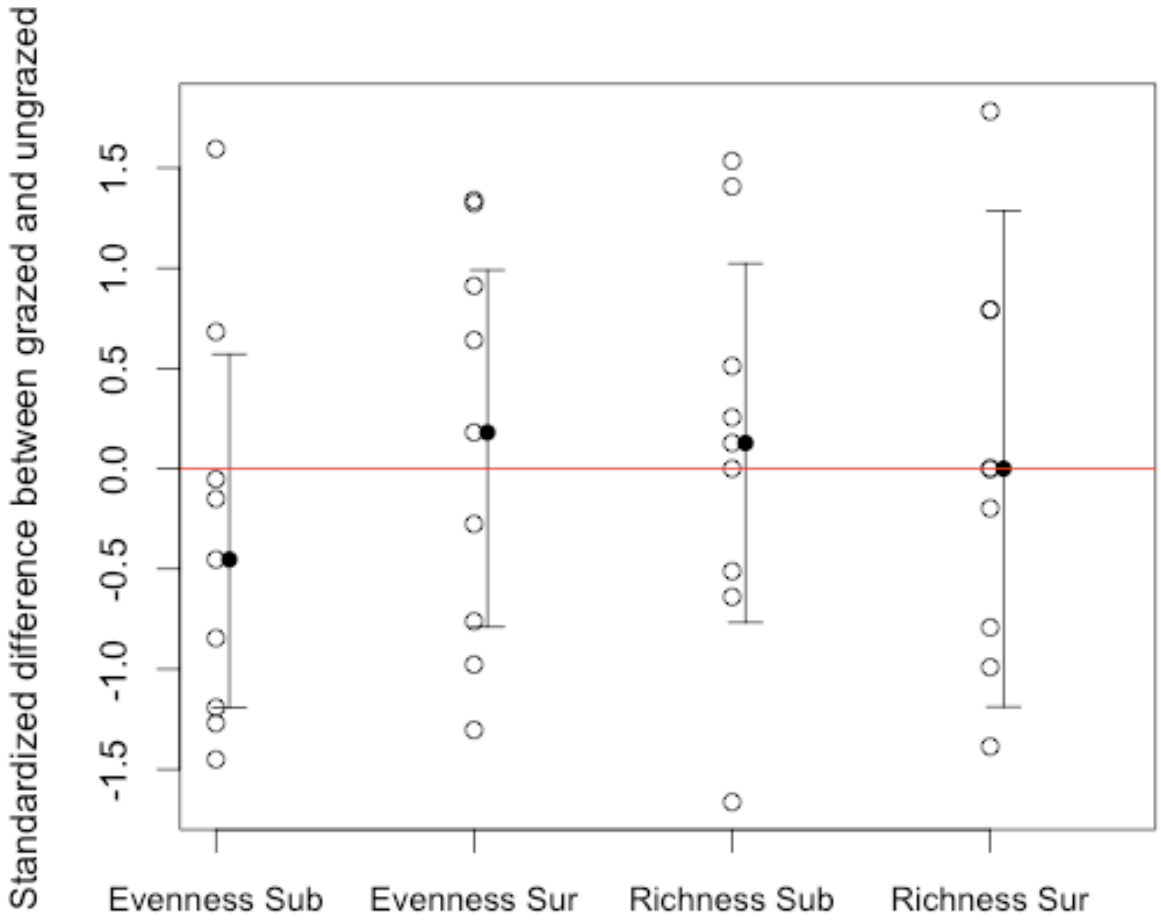

Figure 3: The standardized change in AM fungal richness and evenness with grazing. The solid red line indicates no difference between grazed and ungrazed. Open circles show the change at each site, closed circles represent the standardized mean, and error bars show the 95% confidence intervals. Sur = surface soil, Sub = subsurface soil. P-values for the paired Wilcox signed rank test were as follows: Evenness Sub (p-value=0.301), Evenness Sur (p-value = 0.735), Richness Sub (p-value = 0.875), Richness Sur (p-value = 0.938). N=9

A B

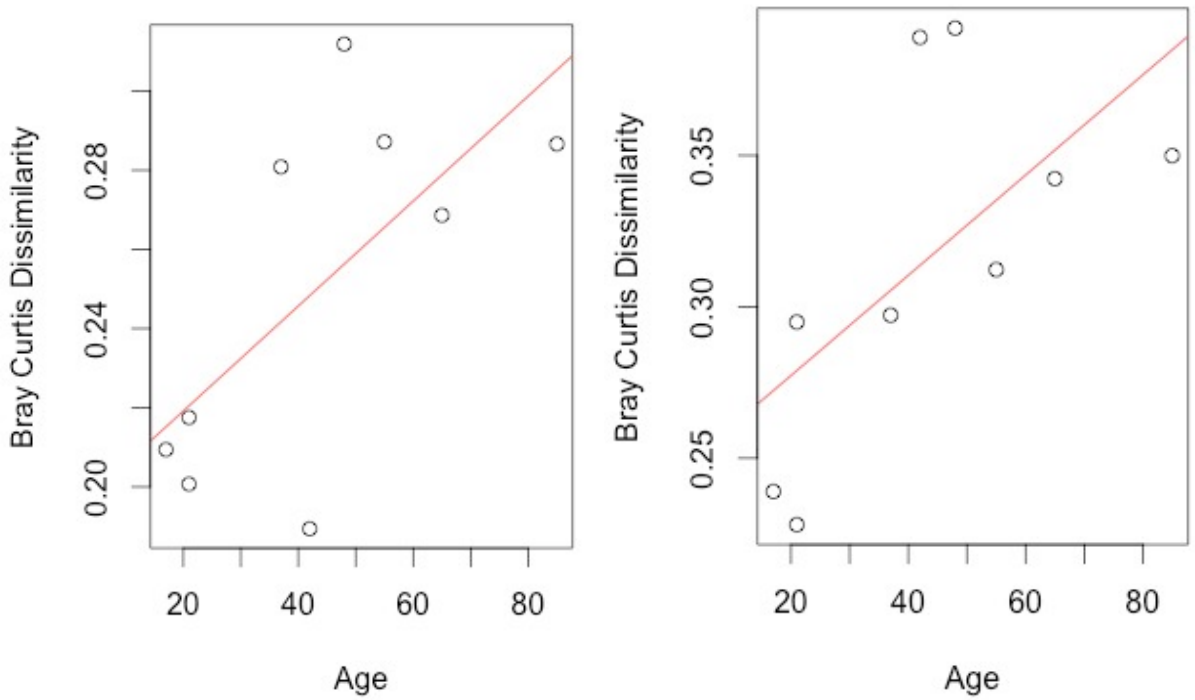

Figure 4: The relationship between Age of the exclosures and the dissimilarity between AM fungal communities in grazed and ungrazed. Plot A represents the surface communities and plot B shows the subsurface communities. Surface r=0.66, subsurface r=0.64.

TABLES

Table 1: Description of the nine exclosures used in this study.

Site	Latitude (N)	Longitude (W)	Elevation (m)	Slope	Age*
Muscrat Lk	50° 8'2.15"	120°26'30.45"	1025	30	17
Tunkwa New	50°35'53.45"	120°51'55.15"	1150	4	21
Bluegrass	50° 4'45.27"	120°28'2.70"	1000	0	21
Aspen	50° 4'54.14"	120°25'55.74"	1200	7	37
Stipa rich	50° 3'58.90"	120°26'51.29"	1125	8	42
Stipa nel	50° 4'43.39"	120°26'58.88"	1000	5	48
Tunkwa Old	50°35'58.03"	120°51'57.96"	1150	0	55
Repeater	50° 4'32.08"	120°25'30.02"	1306	0	65
Goose Lk	50° 6'11.59"	120°27'55.19"	1160	5	85

*Age indicates age of exclosure

Table 2: Biotic and abiotic responses to grazing at nine grazing exclosures. Differences in plant and soil variables between grazed and ungrazed were tested using Wilcoxon signed rank tests. 95% confidence intervals than did not overlap with 0 and p-values less than 0.1 are bolded as an indication that there was some difference between grazed and ungrazed.

	Depth	Effect size	95% CI	P value	Adjusted[2] P value
Plant Community					
Plant Evenness		0.145	**0.005, 0.258**	**0.039**	0.117
Plant Richness		3.25	-3.5, 9.5	0.414	1.000
Plant Biomass		-36.2	**-61.6, -24.1**	**0.004**	**0.012**
Soil Properties					
Total C	Surface	0.535	-0.24, 1.18	0.0742	0.370
(mg/kg)	Subsurface	-0.045	-0.42, 0.27	0.8203	1.000
Total N	Surface	0.056	**0.007, 0.106**	**0.039**	0.195
(mg/kg)	Subsurface	-0008	-0.033, 0.0185	0.5703	1.000
P	Surface	4.465	-1.770, 28.765	**0.0742**	0.370
(mg/kg)	Subsurface	1.305	-0.07, 35.79	**0.0547**	0.273
pH	Surface	0	-0.19, 0.155	0.992	1.000
	Subsurface	-0.0225	-0.38, 0.35	0.6406	1.000
Bulk Density[1]	Surface	0.133	**0.0935, 0.205**	**0.0078**	**0.039**
(g/cc)	Subsurface	0.0347	-0.0268, 0.0936	0.1953	1.000

[1] Bulk densities were collected from 8 sites only because one of the sites was too rocky

[2] Adjusted P values were calculated using the bonferroni correction for multiple comparisons.

Table 3: The change in percent cover with grazing of the most abundant plant species. Only plants that were found in most sites (at least 7/9) and made up a significant proportion (at least 10%) of the plant community were included. Change was calculated as = (% cover in grazed)-(%cover in ungrazed)

Plant	G*	A	B	S	T	O	H	M	R
Bromus inermis	0	0	1	0	-5.1	-11.55	0.3	0	0
Elymus spicata	12	0	0	0.35	-0.6	0	0	37.75	0
Festuca campestris	-89.3	-36.4	0	-38.5	-26.05	-62.5	-1.05	-55.3	-75.4
Koeleria macrantha	4.8	13.2	0	-0.15	5.25	5.95	0	-1	1.9
Phleum pratense	0	0	13.55	0	0	0	0	0	0
Poa pratensis	-4.3	18.45	-3.2	34.25	-14.7	20.8	18.6	0	6.5
Poa secunda	16.2	1.25	0	0	3.3	-12.1	0	5.2	4.1
Stipa nelsonii	7.3	1.25	-0.05	10.55	-4.4	-1.05	-0.7	0	0.8
Stipa richardsonii	0	0	0	0.45	14.8	22.6	6.75	0.05	0.2
Achillea millefolium	-8.9	-3.1	1.5	6.5	3.1	12.7	0.9	0.3	-1
Agroseris glauca	-13.2	-0.1	-0.65	-3.05	0	0	-0.65	0	-4.4
Antennaria dimorpha	2.5	-1.15	0	0	-2.95	0.05	0	0.55	0.1
Antennaria microphylla	2.2	0	0	0	1.95	2	0	3.6	16.9
Aster campestris	0.5	2.65	-0.4	-0.35	-0.9	2.85	0.55	-0.05	-1.1
Astragalus miser	0.1	0.3	-0.3	1.25	0	-0.7	-0.1	0.15	1.9
Cerastium arvense	0	-1	0.9	-0.1	9.6	17.5	0.4	0	0
Fragaria virginiana	-9.5	0	0	0	-0.1	-0.4	0.3	0	0
Geum triflorum	0	-0.35	-0.15	-2.25	-1.3	0	-0.45	0.05	-0.8
Taraxacum officinale	-0.1	-0.2	25.9	0	1.45	2.1	0.05	0.45	0.1
Tragopogon dubius	0	0	0.35	0.25	0.65	0.7	0.25	0.6	0.1

* Site names are abbreviated as follows: G for Goose, A for Aspen, B for Bluegrass, S for Stipanel, T for Tunkwa New, O for Tunkwa Old, H for Stiparich, M for Muscrat and R for Repeater.

Table 4: Blocked indicator species analysis of AM fungal Families and isolates as indicators of grazed or ungrazed plots. Only Families with more than 2 isolates were included in the family analysis. Only the nine strongest indicator isolates were shown.

AM Family[1]	**Grazed**	**Ungrazed**	**Stat**	**P value**
Archeaosporaceae.4	0	1	0.4401	0.9165
Acaulosporaceae.3	0	1	0.2883	0.4312
Diversisporaceae.8	0	1	0.7112	0.1791
Claroideoglomeraceae.8	0	1	0.7017	0.8275
Glomeraceae.33	1	0	0.7080	0.7954
Paraglomeraceae.3	1	0	0.6062	0.1342
AM OTU				
Glomus VTX00342	1	0	0.5586	0.0031
Diversispora VTX00054	0	1	0.5937	0.0388
Paraglomus brasilianum VTX00239	1	0	0.4419	0.0817
Ambispora leptoticha VTX00242	1	0	0.2934	0.084
Glomus VTX00144	1	0	0.3375	0.0856
Diversispora VTX00062	0	1	0.7142	0.0974
DiversisporaVTX00347	0	1	0.4591	0.1024
Septoglomus furcatum VTX00064	1	0	0.7174	0.1251
Glomus VTX00172	0	1	0.5457	0.1392

[1] Numbers after each fungal family indicate how many OTUs belonged to each family

Table 5: Summary of Multimodel Inference Statistics. Italicized descriptions are the dependent values for the global models. Model summaries are in the Appendix

Variable	Stdized Estimate	Std. Error	95% CI	Relative Importance
Difference in Soil Hyphal Length between grazed and ungrazed in the surface soil				
ΔN	0.118	0.265	-0.468, 1.220	0.31
ΔP	0.078	0.229	-0.567, 1.187	0.25
ΔPlant evenness	0.012	0.164	-0.865, 1.007	0.17
Age	0.012	0.159	-0.843, 0.983	0.17
Plant dissimarity	-0.009	0.16	-0.979, 0.874	0.16
Difference in Spore Density between grazed and ungrazed in the surface soil				
ΔPlant evenness	0.313	0.381	-0.196, 1.375	0.53
ΔP	0.083	0.231	-0.454, 1.24	0.21
Plant dissimilarity	0.011	0.176	-1.005, 1.167	0.13
ΔN	0.02	0.137	-0.713, 1.065	0.11
Age	-0.011	0.127	-1.006, 0.792	0.11
AM fungal dissimilarity between grazed and ungrazed in the surface soil				
Age	**0.526**	**0.391**	**0.099, 1.344**	**0.73**
ΔN	0.194	0.299	-0.088, 1.144	0.37
Plant dissimilarity	0.06	0.203	-0.345, 1.314	0.12
ΔP	-0.057	0.205	-1.375, 0.388	0.12
ΔPlant evenness	0.006	0.103	-0.898, 1.150	0.05
AM fungal dissimilarity between grazed and ungrazed in the subsurface soil				
Age	**0.604**	**0.379**	**0.095, 1.386**	**0.81**
ΔPlant evenness	-0.108	0.233	-1.090, 0.168	0.23
ΔN	-0.040	0.164	-1.187, 0.437	0.11
Plant dissimilarity	-0.051	0.180	-1.226, 0.340	0.11
ΔP	0.026	0.128	-0.443, 1.078	0.08

BIBLIOGRAPHY

Aguilar-Chama A., and Guevara R. 2012. "Mycorrhizal Colonization Does Not Affect Tolerance to Defoliation of an Annual Herb in Different Light Availability and Soil Fertility Treatments but Increases Flower Size in Light-Rich Environments." *Oecologia* 168 (1): 131–39. doi:10.1007/s00442-011-2066-1.

Alexandra K., Brockhoff, P. B. and Christensen, R. H. B. (2015). lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-29. http://CRAN.R-project.org/package=lmerTest

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990 Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Angelard, C., Tanner, C.J., Fontanillas, P., Niculita-Hirzel, H., Masclaux, F., and Sanders, I. R. 2014. Rapid Genotypic Change and Plasticity in Arbuscular Mycorrhizal Fungi Is Caused by a Host Shift and Enhanced by Segregation. *The ISME Journal* 8 (2): 284–94. doi:10.1038/ismej.2013.154.

Asner, G. P., Elmore, A. J., Olander, L. P., Martin, R. E., and Harris, A. T. 2004. Grazing Systems, Ecosystem Responses, and Global Change. *Annual Review of Environment and Resources* 29 (1): 261–99. doi:10.1146/annurev.energy.29.062403.102142.

Atwell, B. 1993. Response of roots to mechanical impedance. *Env. and Exp. Bot.* 33(1): 27-40

Augé, R. M. 2001. Water Relations , Drought and Vesicular-Arbuscular Mycorrhizal Symbiosis. *Mycorrhiza* 11: 3–42.

Ba, L., Ning, J., Wang, D., Facelli, E., Facelli, J. M., Yang, Y., and Zhang, L. 2012. The Relationship between the Diversity of Arbuscular Mycorrhizal Fungi and Grazing in a Meadow Steppe. *Plant and Soil* 352 (1-2): 143–56. doi:10.1007/s11104-011-0985-6.

Bai, G., Bao, Y., Du, G., and Qi, Y. 2013. Arbuscular Mycorrhizal Fungi Associated with Vegetation and Soil Parameters under Rest Grazing Management in a Desert Steppe Ecosystem. *Mycorrhiza* 23 (4): 289–301. doi:10.1007/s00572-012-0468-5.

Barber, N. A. 2013. Arbuscular Mycorrhizal Fungi Are Necessary for the Induced Response to Herbivores by Cucumis Sativus. *Journal of Plant Ecology* 6 (2): 171–76. doi:10.1093/jpe/rts026.

Barber, N. A., Adler, L.S., Theis, N., Hazzard, R. V., and Kiers, E.T. 2012. Herbivory Reduces Plant Interactions with above- and Belowground Antagonists and Mutualists. *Ecology* 93 (7): 1560–70. doi:10.1890/11-1691.1.

Baron, V. S., Dick A. C., Mapfumo, E, Malhi, S. S., Naeth, M.A., Baron, V.S., Dick, A.C. et al. 2001. Grazing Impacts on Soil Nitrogen and Phosphorus under Parkland Pastures. *Journal of Range Management* 54 (6): 704–10.

Barto, E. K., and Rillig, M. C. 2010. Does Herbivory Really Suppress Mycorrhiza? A Meta-Analysis. *Journal of Ecology* 98 (4): 745–53. doi:10.1111/j.1365-2745.2010.01658.x.

Bartoń, K. (2016). MuMIn: Multi-Model Inference. R package version 1.15.6. http://CRAN.R-project.org/package=MuMIn

Bazot, S., J. Nguyen, M. C., and Robin, C. 2005. Defoliation-Induced Changes in Carbon Allocation and Root Soluble Carbon Concentration in Field-Grown Lolium Perenne Plants: Do They Affect Carbon Availability, Microbes and Animal Trophic Groups in Soil? *Functional Ecology* 19 (5): 886–96. doi:10.1111/j.1365-2435.2005.01037.x.

Bennett, A.E., and Bever, J.D. 2007. Mycorrhizal Species Differentially Alter Plant Growth and Response to Herbivory. *Ecology* 88 (1): 210–18. doi:10.1890/0012-9658(2007)88[210:MSDAPG]2.0.CO;2.

Bennett, A.E., Bever, J.D., and Bowers, M.D. 2009. Arbuscular Mycorrhizal Fungal Species Suppress Inducible Plant Responses and Alter Defensive Strategies Following Herbivory. *Oecologia* 160: 771–79. doi:10.1007/s00442-009-1338-5.

Bethlenfalvay, G., and Dakessian, S. 1984. Grazing Effects on Mycorrhizal Colonization and Floristic Composition of the Vegetation on a Semiarid Range in Northern Nevada. *Journal of Range Management* 37 (4): 312–16. http://www.jstor.org/stable/3898701.

Boerner, R. E. J., DeMars, B.G., and Leicht, P.N. 1996. Spatial Patterns of Mycorrhizal Infectiveness of Soils Long a Successional Chronosequence. *Mycorrhiza* 6 (2): 79–90. doi:10.1007/s005720050111.

Bray, R. H. and Kurtz, L. T. 1945. Determination of total, organic, and available forms of phosphorous in soils. Soil Science, 59: 39-45

Caporaso J. G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F. D., Costello E. K., Fierer N., Peña A. G., Goodrich J. K., Gordon J. I., Huttley G. A., Kelley S., Knights D., Koenig J. E., Ley R. E., Lozupone C. A., McDonald D., Muegge B. D., Pirrung M., Reeder J., Sevinsky J. R., Turnbaugh P. J., Walters W. A., Widmann J., Yatsunenko T., Zaneveld J., Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7: 335-336

Chanasyk, D. S., and Naeth, M.A. 1995. Grazing Impacts on Bulk Density and Soil Strength in the Foothills Fescue Grasslands of Alberta, Canada. *Canadian Journal of Soil Science* 75 (4): 551–57. doi:10.4141/cjss95-078.

Crawley, M J. 1990. Rabbit Grazing, Plant Competition and Seedling Recruitment in Acid Grassland. *Journal of Applied Ecology* 27: 803–20.

Cuenca, G., De Andrade, Z., and Escalante, G. 1998. Diversity of Glomalean Spores from Natural, Disturbed and Revegetated Communities Growing on Nutrient-Poor Tropical Soils. *Soil Biology and Biochemistry* 30 (6): 711–19. doi:10.1016/S0038-0717(97)00191-0.

Daubenmire, R. (1959). A canopy-coverage method of vegetational analysis. *Northwest Science*, *33*(1), 43-64.

Davison, J., Öpik, M., Daniell, T.J., Moora, M., and Zobel, M. 2011. Arbuscular Mycorrhizal Fungal Communities in Plant Roots Are Not Random Assemblages. *FEMS Microbiology Ecology* 78 (1): 103–15. doi:10.1111/j.1574-6941.2011.01103.x.

De Caceres, M., Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. Ecology, URL http://sites.google.com/site/miqueldecaceres/

De la Providenicia, I.E., de Souza, F.A., Fernandez, F., Delmas, N.S., and Declerck, S. 2005. Arbuscular Mycorrhizal Fungi Reveal Distinct Patterns of Anastomosis and Hyphal Formation Mechanisms Healing between Different Phylogenic Groups. *New Phytologist* 165 (1): 261–71.

Diaz, S., Lavorel, S., McIntyre, S., Falczuk, V., Casanoves, F., Milchunas, D.G., Skarpe, C., et al. 2007. Plant Trait Responses to Grazing - a Global Synthesis. *Global Change Biology* 13 (2): 313–41. doi:10.1111/j.1365-2486.2006.01288.x.

Ellison, L. 1960. Influence of Grazing on Plant Succession of Rangelands. *The Botanical Review* 26 (1): 1–78.

Edgar, R. C. (2004), MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Research* 32(5), 1792-97.

Edgar, R. C. 2010 Search and clustering orders of magnitude faster than BLAST, *Bioinformatics* 26(19): 2460-2461.

Eom, A-H, Wilson, G., and Hartnett, D.C. 2001. Effects of Ungulate Grazers on Arbuscular Mycorrhizal Symbiosis and Fungal Community Strucutre in Tallgrass Prairie. *Mycologia* 93 (2): 233–42.

Ferraro, D. O., and Oesterheld, M. 2002. Effect of Defoliation on Grass Growth. A Quantitative Review. *Oikos* 98: 125–33. doi:10.2307/3547619.

Frank, D. A. 2005. The Interactive Effects of Grazing Ungulates and Aboveground Production on Grassland Diversity. *Oecologia* 143 (4): 629–34. doi:10.1007/s00442-005-0019-2.

Frank, D. A., and Evans, R. D. 1997. Effects of Native Grazers on Grassland N Cycling in Yellowstone National Park. *Ecology* 78 (7): 2238–48.

Frank, D. A., Gehring, C. A., Machut, L., and Phillips, M. 2003. Soil Community Composition and the Regulation of Grazed Temperate Grassland. *Oecologia* 137 (4): 603–9. doi:10.1007/s00442-003-1385-2.

Frank, D. A., and Groffman, P.M. 1998. Ungulate vs. Landscape Control of Soil C and N Processes in Grasslands of Yellowstone National Park. *Ecology* 79 (7): 2229–41. doi:10.1890/0012-9658(1998)079[2229:UVLCOS]2.0.CO;2.

Fox, J., and Weisberg, S. 2011. An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Geer L. Y., Marchler-Bauer A., Geer R. C., Han L., He J., He S., Liu C., Shi W., Bryant S. H. 2010 The NCBI BioSystems database. *Nucleic Acids Res.* 2010 38: 492-6. [PubMed PMID: 19854944]

Gehring, C. A., and Whitham, T.G. 1994. Interactions between Aboveground Herbivores and the Mycorrhizal Mutualists of Plants. *Trends in Ecology and Evolution* 9 (7): 251–55. doi:10.1016/0169-5347(94)90290-9.

Greenwood, K. L., and McKenzie, B.M. 2001. Grazing Effects on Soil Physical Properties and the Consequences for Pastures: A Review. *Australian Journal of Experimental Agriculture* 41 (8): 1231–50.

Guo, Y., Du, Q., Li, G., Ni, Y., Zhang, Z., Ren, W., and Hou, X. 2016. Soil Phosphorus Fractions and Arbuscular Mycorrhizal Fungi Diversity Following Long-Term Grazing Exclusion on Semi-Arid Steppes in Inner Mongolia. *Geoderma* 269: 79–90. doi:10.1016/j.geoderma.2016.01.039.

Hamilton, E. W., Frank, D.A., Hinchey, P.M., and Murray, T.R. 2008. Defoliation Induces Root Exudation and Triggers Positive Rhizospheric Feedbacks in a Temperate Grassland. *Soil Biology & Biochemistry* 40 (11). Elsevier Ltd: 2865–73. doi:10.1016/j.soilbio.2008.08.007.

Hart, M. M., and Reader, R. J. 2004. Do Arbuscular Mycorrhizal Fungi Recover from Soil Disturbance Differently? *Tropical Ecology* 45 (1): 97–111.

Hart, M. M., Reader, R. J., and Klironomos, J. N. 2001. Life-History Strategies of Arbuscular Mycorrhizal Fungi in Relation to Their Strategies Successional Dynamics. *Mycologia* 93 (6): 1186–94.

Hart, M. M., and Reader, R.J. 2002a. Does Percent Root Length Colonization and Soil Hyphal Length Reflect the Extent of Colonization for All AMF? *Mycorrhiza* 12 (6): 297–301. doi:10.1007/s00572-002-0186-5.

———. 2002b. Taxonomic Basis for Variation in the Colonization Strategy of Arbuscular Mycorrhizal Fungi. *New Phytologist* 153 (2): 335–44. doi:10.1046/j.0028- 646X.2001.00312.x.

———. 2005. The Role of the External Mycelium in Early Colonization for Three Arbuscular Mycorrhizal Fungal Species with Different Colonization Strategies. *Pedobiologia* 49 (3): 269–79. doi:10.1016/j.pedobi.2004.12.001.

———. 2002. Host Plant Benefit from Association with Arbuscular Mycorrhizal Fungi: Variation due to Differences in Size of Mycelium. *Biology and Fertility of Soils* 36 (5): 357–66. doi:10.1007/s00374-002-0539-4.

Hassan, S. E. D., Liu, A., Bittman, S., Forge, T.A., Hunt, D.E., Hijri, M., and St-Arnaud, M. 2013. Impact of 12-Year Field Treatments with Organic and Inorganic Fertilizers on Crop Productivity and Mycorrhizal Community Structure. *Biology and Fertility of Soils* 49: 1109–21. doi:10.1007/s00374-013-0813-7.

Hempel, S., Stein, C., Unsicker, S.B., Renker, C., Auge, H., Weisser, W.W., and Buscot, F. 2009. Specific Bottom-up Effects of Arbuscular Mycorrhizal Fungi across a Plant-Herbivore-Parasitoid System. *Oecologia* 160 (2): 267–77. doi:10.1007/s00442-009-1294-0.

Hokka, V., Mikola, J., Vestberg, M., and Setälä, H. 2004. Interactive Effects of Defoliation and an AM Fungus on Plants and Soil Organisms in Experimental Legume–grass Communities. *Oikos* 106: 73–84. doi:10.1111/j.0030-1299.2004.12963.x.

Holland, E. A., Parton, W.J., Detling, J.K., and Layne, D. 1992. Physiological Responses of Plant Populations to Herbivory and Their Consequences for Ecosystem Nutrient Flow. *The American Naturalist* 140 (4): 685–706.

Holland, E. A., and Detling, J.K. 1990. Plant Response to Herbivory and Belowground Nitrogen Cycling. *Ecology* 71 (3): 1040–49.

Holland, J. N., Cheng, W., and Crossley, D.A. 1996. Herbivore-Induced Changes in Plant Carbon Allocation: Assessment of below-Ground C Fluxes Using Carbon-14. *Oecologia* 107 (1): 87–94. doi:10.1007/BF00582238.

Huhta, A.-P., Rautio, P., Hellstrom, K., Saari, M., and Tuomi, J. 2009. Tolerance of a Perennial Herb, Pimpinella Saxifraga, to Simulated Flower Herbivory and Grazing: Immediate Repair of Injury or Postponed Reproduction? *Plant Ecology* 201 (2): 599–609. doi:10.1007/sl.

Huntly, N. 1991. Herbivores and the Dynamics of Communities and Ecosystems. *Annual Review of Ecology and Systematics* 22: 477–503.

Janos, D. P. 1980. Mycorrhizae Influence Tropical Succession Mycorrhizae Influence Tropical Succession. *Biotropica* 12 (2): 56–64.

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R., and Frossard, E. 2002. Diversity and Structure of AMF Communities as Affected by Tillage in a Temperate Soil. *Mycorrhiza* 12 (5): 225–34. doi:10.1007/s00572-002-0163-z.

Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R., and Frossard, E. 2003. Soil Tillage Affects the Community Structure of Mycorrhizal Fungi Inmaize Roots. *Ecological Applications* 13 (4): 1164–76. doi:10.1890/1051-0761(2003)13[1164:STATCS]2.0.CO;2.

Jansa, J., Wiemken, A., and Frossard, E. 2006. The Effects of Agricultural Practices on Arbuscular Mycorrhizal Fungi. *Geological Society, London, Special Publications* 266 (1): 89–115. doi:10.1144/GSL.SP.2006.266.01.08.

Johnson, N. C., Zak, D. R., Tilman, D., and Pfleger, F.L. 1991. International Association for Ecology Dynamics of Vesicular-Arbuscular Mycorrhizae during Old Field Succession. *Oevologia* 86 (3): 349–58.

Keitaro, T., Shinpei, S., Koji, U., Hideki, M., Takashi, N., Tomonobu, T., Tetsuya, M., Satoru, S., Tadao, W., and Hironori, Y. 2012. Leaf Herbivory by Spodoptera Litura Increases Arbuscular Mycorrhizal Colonization in Roots of Soybean. *Soil Science and Plant Nutrition* Vol.58 (No.4): 445–49. doi:10.1080/00380768.2012.704520.

Klironomos, J. N., McCune, J., and Moutoglis, P. 2004. Species of Arbuscular Mycorrhizal Fungi Affect Mycorrhizal Responses to Simulated Herbivory. *Applied Soil Ecology* 26 (2): 133–41. doi:10.1016/j.apsoil.2003.11.001.

Koch, A. M., Kuhn, G., Fontanillas, P., Fumagalli, L., Goudet, J., and Sanders, I.R. 2004. High Genetic Variability and Low Local Diversity in a Population of Arbuscular Mycorrhizal Fungi. *Proceedings of the National Academy of Sciences* 101 (8): 2369–74. doi:10.1073/pnas.0306441101.

Köhl, L., Oehl, F., and van der Heijden, M.G.A. 2014. Agricultural Practices Indirectly Influence Plant Productivity and Ecosystem Services through Effects on Soil Biota. *Ecological Applications* 24 (7): 1842–53. doi:10.1890/13-1821.1.

Krzic, M., Lamagna, S. F., Newman, R. F., Bradfield, G., and Wallace, B. M. 2012. Long-Term Grazing Effects on Grassland Soil Properties in Southern British Columbia. *Canadian Journal of Soil Science* 92: 685–93. doi:10.4141/cjss2011-070.

Kula, A. A. R., Hartnett, D.C., and Wilson, G.W.T. 2005. Effects of Mycorrhizal Symbiosis on Tallgrass Prairie Plant-Herbivore Interactions. *Ecology Letters* 8 (1): 61–69. doi:10.1111/j.1461-0248.2004.00690.x.

Landgraf, R., Schaarschmidt, S., and Hause, B. 2012. Repeated Leaf Wounding Alters the Colonization of Medicago Truncatula Roots by Beneficial and Pathogenic Microorganisms. *Plant, Cell and Environment* 35 (7): 1344–57. doi:10.1111/j.1365-3040.2012.02495.x.

Lekberg, Y., Schnoor, T., Kjøller, R., Gibbons, S.M., Hansen, L.H., Al-Soud, W.A., Sørensen, S.J., and Rosendahl, S. 2012. 454-Sequencing Reveals Stochastic Local Reassembly and High Disturbance Tolerance within Arbuscular Mycorrhizal Fungal Communities. *Journal of Ecology* 100: 151–60.

Lkhagva, A., Boldgiv, B., Goulden, C. E., Yadamsuren, O., and Lauenroth, W.K. 2013. Effects of Grazing on Plant Community Structure and Aboveground Net Primary Production of Semiarid Boreal Steppe of Northern Mongolia. *Grassland Science* 59 (3): 135–45. doi:10.1111/grs.12022.

Mack, N.R., and Thompson, J.N.. 1982. Evolution in Steppe with Few Large , Hooved Mammals. *The American Naturalist* 119 (6): 757–73.

Maherali, H., and Klironomos, J.N. 2012. Phylogenetic and Trait-Based Assembly of Arbuscular Mycorrhizal Fungal Communities. *PLoS ONE* 7 (5): e36695. doi:10.1371/journal.pone.0036695.

McNaughton, S J. 1983. Compensatory Plant Growth as a Response to Herbivory. *Oikos* 40 (3): 329–36.

Mikola, J., Nieminen, M., Ilmarinen, K., and Vestberg, M. 2005. Belowground Responses by AM Fungi and Animal Trophic Groups to Repeated Defoliation in an Experimental Grassland Community. *Soil Biology & Biochemistry* 37: 1630–39. doi:10.1016/j.soilbio.2005.01.027.

Miller, R. M., Reinhardt, D.R., and Jastrow, J.D. 1995. External Hyphal Production of Vesicular-Arbuscular Mycorrhizal Fungi in Pasture and Tallgrass Prairie Communities. *Oecologia* 103 (1): 17–23.

Moora, M., Davison, J., Opik, M., Metsis, M., Saks, U, Jairus, T., Vasar, M., and Zobel, M. 2014. Anthropogenic Land Use Shapes the Composition and Phylogenetic Structure of Soil Arbuscular Mycorrhizal Fungal Communities. *FEMS Microbiology Ecology* 90 (3): 609–21. doi:10.1111/1574-6941.12420.

Munkvold, L, Kjller, R., Vestberg, M., and Rosendahl, S. 2004. Functional Diversity within Species of Arbuscular Mycorrhizal Fungi. *New Phytologist* 164 (2): 357–64.

Murray, T. R., Frank, D. A., and Gehring, C.A. 2010. Ungulate and Topographic Control of Arbuscular Mycorrhizal Fungal Spore Community Composition in a Temperate Grassland. *Ecology* 91 (3): 815–27. doi:10.1890/09-0209.1.

Nadian, H., Smith, S.E., Alston, A.M., and Murray, R.S. 1997. Effects of Soil Compaction on Plant Growth , Phosphorus Uptake and Morphological Characteristics of Vesicular-Arbuscular Mycorrhizal Colonization of Trifolium Sub Terraneum. *The New Phytologist* 135 (2): 303–11.

Nishida, T., Izumi, N., Katayama, N., and Ohgushi, T. 2009. Short-Term Response of Arbuscular Mycorrhizal Association to Spider Mite Herbivory. *Population Ecology* 51 (2): 329–34. doi:10.1007/s10144-008-0116-2.

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Wiemken, A., and Boller, T. 2009. Distinct Sporulation Dynamics of Arbuscular Mycorrhizal Fungal Communities from Different Agroecosystems in Long-Term Microcosms. *Agriculture, Ecosystems & Environment* 134 (3-4): 257–68. doi:10.1016/j.agee.2009.07.008.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H. (2015). vegan: Community Ecology Package. R package version 2.2-1. http://CRAN.R-project.org/package=vegan

Olofsson, J., Kitti, H., Rautiainen, P., Stark, S., Oksanen, L., and Effects, L. 2001. Effects of Summer Grazing by Reindeer on Composition of Vegetation , Productivity and Nitrogen Cycling. *Ecography* 24 (1): 13–24.

Opik, M., Daniell, T.J., Zobel, M., and Moora, M. 2014. Large-Scale Parallel 454 Sequencing Reveals Host Ecological Group Specificity of Arbuscular Mycorrhizal Fungi in a Boreonemoral Forest. *New Phytologist* 184 (2): 424–37.

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, Ü., Zobel, M. 2010. The online database Maarj*AM* reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* 188: 223- 241.

Pearson, J. N., and Jakobsen, I. 1993. Symbiotic Exchange of Carbon and Phosphorus between Cucumber and Three Arbuscular Mycorrhizal Fungi. *New Phytologist* 124: 481–88.

Pietikäinen, A., Kytöviita, M.-M., and Vuoti, U. 2005. Mycorrhiza and Seedling Establishment in a Subarctic Meadow: Effects of Fertilization and Defoliation. *Journal of Vegetation Science* 16 (2): 175–82. doi:10.1658/1100-9233(2005)016[0175:MASEIA]2.0.CO;2.

Piippo, S., Huhta, A.P., Rautio, P., Markkola, A, and Tuomi, J. 2011a. Grazing Tolerance and Mycorrhizal Colonization: Effects of Resource Manipulation and Plant Size in Biennial Gentianella Campestris. *Flora: Morphology, Distribution, Functional Ecology of Plants* 206 (9): 808–13. doi:10.1016/j.flora.2011.03.001.

Piippo, S., Markkola, A., Härmä, E., and Tuomi, J. 2011b. Do Compensatory Shoot Growth and Mycorrhizal Symbionts Act as Competing Above- and Below-Ground Sinks after Simulated Grazing? *Plant Ecology* 212 (1): 33–42. doi:10.1007/s11258-010-9799-5.

Pringle, A., and Bever, J.D. 2002. Divergent Phenologies may Facilitate the Coexistence of Arbuscular Mycorrhizal Fungi in a North Carolina Grassland. *American Journal of Botany* 89 (9): 1439–46.

Pyke, D. A., Herrick, J.E., Shaver, P., Pellant, M. 2002. Rangeland Health Attributes and Indicators for Qualitative Assessment. *Society for Range Management* 55 (6): 584–97.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Robertson, J H. 1971. Changes on a Sagebrush-Grass Range in Nevada Ungrazed for 30 Years. *Journal for Range Management* 24 (5): 397–400.

Ruotsalainen, A. L., and Eskelinen, A. 2011. Root Fungal Symbionts Interact with Mammalian Herbivory, Soil Nutrient Availability and Specific Habitat Conditions. *Oecologia* 166 (3): 807–17. doi:10.1007/s00442-011-1928-x.

Saito, K., Suyama, Y., Sato, S., and Sugawara, K. 2004. Defoliation Effects on the Community Structure of Arbuscular Mycorrhizal Fungi Based on 18S rDNA Sequences. *Mycorrhiza* 14 (6): 363–73. doi:10.1007/s00572-003-0286-x.

Saravesi, K., Ruotsalainen, A.L., and Cahill, J. F. 2014. Contrasting Impacts of Defoliation on Root Colonization by Arbuscular Mycorrhizal and Dark Septate Endophytic Fungi of Medicago Sativa. *Mycorrhiza* 24 (4): 239–45. doi:10.1007/s00572-013-0536-5.

Schnoor, T. K., Lekberg, Y., Rosendahl, S., and Olsson, P.A. 2011. Mechanical Soil Disturbance as a Determinant of Arbuscular Mycorrhizal Fungal Communities in Semi-Natural Grassland. *Mycorrhiza* 21 (3): 211–20. doi:10.1007/s00572-010-0325-3.

Seagle, S. W., McNaughton, S. J., and Ruess, R.W. 1992. Simulated Effects of Grazing on Soil Nitrogen and Mineralization in Contrasting Serengeti Grasslands. *Ecology* 73 (3): 1105–23.

Selosse, M.-A., Vincenot, L., and Öpik, M. 2016. Data Processing Can Mask Biology: Towards Better Reporting of Fungal Barcoding Data? *New Phytologist*, doi:10.1111/nph.13851.

Shariff, A. R., Biondini, M. E., and Grygiel, C. E. 1994. Grazing Intensity Effects on Litter Decomposition and Soil Nitrogen Mineralization. *Journal of Range Management* 47 (6): 444–49.

Sharifi, M., Ghorbanli, M., and Ebrahimzadeh, H.. 2007. Improved Growth of Salinity-Stressed Soybean after Inoculation with Salt Pre-Treated Mycorrhizal Fungi. *Journal of Plant Physiology* 164 (9): 1144–51. doi:10.1016/j.jplph.2006.06.016.

Sharmah, D., and Jha, D. K. 2014. Diversity of Arbuscular Mycorrhizal Fungi in Disturbed and Undisturbed Forests of Karbi Anglong Hills of Assam, India. *Agricultural Research* 3 (September): 229–38. doi:10.1007/s40003-014-0110-1.

Shelton, A. L., Henning, J.A., Schultz, P., and Clay, K. 2014. Effects of Abundant White-Tailed Deer on Vegetation, Animals, Mycorrhizal Fungi, and Soils. *Forest Ecology and Management* 320: 39–49. doi:10.1016/j.foreco.2014.02.026.

Sikes, B. A., Cottenie, K., and Klironomos, J. N. 2009. Plant and Fungal Identity Determines Pathogen Protection of Plant Roots by Arbuscular Mycorrhizas. *Journal of Ecology* 97 (6): 1274–80. doi:10.1111/j.1365-2745.2009.01557.x.

Smith, S., and Read, D. 2008. *Mycorrhizal Symbiosis, 3rd Edition*. Elsevier Science Ltd, London.

Stebbins, G L. 1981. Coevolution of Grasses and Herbivores. *Annals of the Missouri Botanical Garden* 68 (1): 75–86.

Su, Y. Y., and Guo, L. D. 2007. Arbuscular Mycorrhizal Fungi in Non-Grazed, Restored and over-Grazed Grassland in the Inner Mongolia Steppe. *Mycorrhiza* 17 (8): 689–93. doi:10.1007/s00572-007-0151-4.

Techau, M. E. C., Bjørnlund, L., and Christensen, S. 2004. Simulated Herbivory Effects on Rhizosphere Organisms in Pea (Pisum Sativum) Depended on Phosphate. *Plant and Soil* 264 (1-2): 185–94. doi:10.1023/B:PLSO.0000047756.09364.3a.

Thornton, B., and Millard, P. 2016. Society for Range Management Effects of Severity of Defoliation on Root Functioning in Grasses. *Society for Range Management* 49 (5): 443–47.

Torrecillas, E., Alguacil, M. M., and Roldán, A. 2012. Host Preferences of Arbuscular Mycorrhizal Fungi Colonizing Annual Herbaceous Plant Species in Semiarid Mediterranean Prairies. *Applied and Environmental Microbiology* 78 (17): 6180–86. doi:10.1128/AEM.01287-12.

Treseder, K. K. 2004. A Meta-Analysis of Mycorrhizal Responses to Nitrogen, Phosphorous and Atmospheric Carbon in Field Studies. *New Phytologist* 164 (2): 347–55.

Treseder, K. K., Mack, M.C., and Cross, A. 2004. Relationships among Fires, Fungi, and Soil Dynamics in Alaskan Boreal Forests. *Ecological Applications* 14 (6): 1826–38.

Treseder, K. K., and Allen, M. F. 2002. Direct Nitrogen and Phosphorus Limitation of Arbuscular Mycorrhizal Fungi: A Model and Field Test. *New Phytologist* 155 (3): 507–15. doi:10.1046/j.1469-8137.2002.00470.x.

Valyi, K., Rillig, M.C., and Hempel, S. 2015. Land-Use Intensity and Host Plant Identity Interactively Shape Communities of Arbuscular Mycorrhizal Fungi in Roots of Grassland Plants. *New Phytologist* 205 (4): 1577–86. doi:10.1111/nph.13236.

van der Heijden, M. G. A., Bardgett, R. D., and van Straalen, N. M. 2008. The Unseen Majority: Soil Microbes as Drivers of Plant Diversity and Productivity in Terrestrial Ecosystems. *Ecology Letters* 11 (3): 296–310. doi:10.1111/j.1461-0248.2007.01139.x.

van der Heijden, M. G. A., Boller, T., Wiemken, A., and Sanders, I.R. 1998. Different Arbuscular Mycorrhizal Fungal Species Are Potential Determinants of Plant Community Structure. *Ecology* 79 (6): 2082–91.

van Ryswyk, A. L., Alastair, M., and Marchand, L. S. 1966. The Climate, Native Vegetation, and Soils of Some Grasslands at Different Elevations in British Columbia. *Canadian Journal of Plant Science* 46 (17): 35–50.

van Velzen, E., and Etienne, R. S. 2015. The Importance of Ecological Costs for the Evolution of Plant Defense against Herbivory. *Journal of Theoretical Biology* 372: 89–99. doi:10.1016/j.jtbi.2015.02.027.

Vandenkoornhuyse, P., Ridgway, K. P., Watson, I. J., Fitter, A. H., and Young, J. P. W. 2003. Co-Existing Grass Species Have Distinctive Arbuscular Mycorrhizal Communities. *Molecular Ecology* 12 (11): 3085–95. doi:10.1046/j.1365-294X.2003.01967.x.

Vitousek, P. M., and Howarth, R. W. 1991. Nitrogen Limitation on Land and in the Sea : How Can It Occur ? *Biogeochemistry* 13 (2): 87–115.

von Alten, H., Lindemann, A., and Schonbeck, F. 1993. Stimulation of Vesicular-Arbuscular Mycorrhiza by Fungicides or Rhizosphere Bacteria. *Mycorrhiza* 2 (4): 167–73. doi:10.1007/BF00210586.

Wallace, L. L. 1987. Mycorrhizas in Grasslands: Interactions of Ungulates, Fungi and Drought. *New Phytologist* 105 (4): 619. doi:10.1111/j.1469-8137.1987.tb00900.x.

Walling, S. Z., and Zabinski, C. A. 2004. Host Plant Differences in Arbuscular Mycorrhizae: Extra Radical Hyphae Differences between an Invasive Forb and a Native Bunchgrass. *Plant and Soil* 265 (1-2): 335–44. doi:10.1007/s11104-005-0606-3.

Walling, S. Z., and Zabinski, C. A. 2006. Defoliation Effects on Arbuscular Mycorrhizae and Plant Growth of Two Native Bunchgrasses and an Invasive Forb. *Applied Soil Ecology* 32 (1): 111–17. doi:10.1016/j.apsoil.2005.02.017.

Wardle, D. A., Bonner, K. I., and Barker, G. M. 2002. Linkages between Plant Litter Decomposition , Litter Quality , and Vegetation Responses to Herbivores. *Functional Ecology* 16 (5): 585–95.

Wearn, J. A., and Gange, A. C. 2007. Above-Ground Herbivory Causes Rapid and Sustained Changes in Mycorrhizal Colonization of Grasses. *Oecologia* 153 (4): 959–71. doi:10.1007/s00442-007-0789-9.

Wikeem, B. M., Ross, T. J., and Newman, R. F. 2012. Long-Term Effects of Cattle and Wildlife Grazing on Grassland Plant Community Recovery in the East Kootenay Region of British Columbia. *Prov. B.C., Vicotria, B.C. Tech Rep 072*.

Xie, Y., and Wittig, R. 2004. The Impact of Grazing Intensity on Soil Characteristics of Stipa Grandis and Stipa Bungeana Steppe in Northern China (Autonomous Region of Ningxia). *Acta Oecologica* 25 (3): 197–204. doi:10.1016/j.actao.2004.01.004.

Young, G., Fenger, A. M. and Luttmerding, H. A. 1992. Soils of the Ashcroft map area. BC Soil Survey Rep. 26. BC Environ- ment, Integrated Management Branch, Victoria, BC. 233 pp.

Yang, W., Zheng, Y., Gao, C., He, X., Ding, Q., Kim, Y., Rui, Y., Wang, S., and Guo, L. D. 2013. The Arbuscular Mycorrhizal Fungal Community Response to Warming and Grazing Differs between Soil and Roots on the Qinghai-Tibetan Plateau. *PloS One* 8 (9): e76447. doi:10.1371/journal.pone.0076447.

Zobel, M., and Öpik, M. 2014. Plant and Arbuscular Mycorrhizal Fungal (AMF) Communities - Which Drives Which? *Journal of Vegetation Science* 1-8. doi:10.1111/jvs.12191.

APPENDIX A: SUPPLEMENTARY MATERIALS

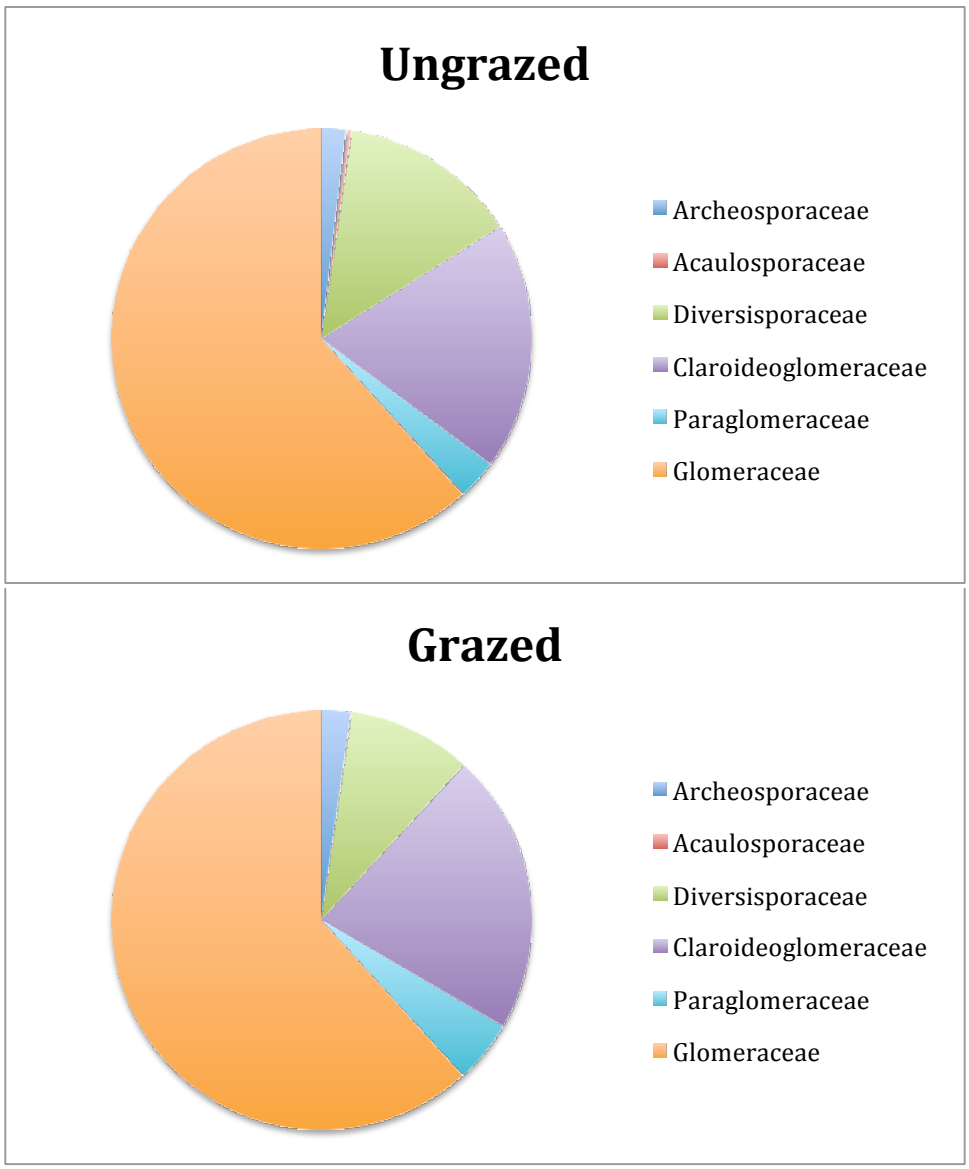

Figure 5: Abundance of AM fungal families in grazed and ungrazed plots. All sites are pooled together for this figure.

Statistical Models in R

AM fungal Community

comm.ord = metaMDS(comm.t[,5:ncol(comm.t)], k = 4, trymax = 50)

axis1.mod = lmer(NMDS1~Description+ (1|Site:Description)+ (1|Site), data = comm.char)

axis2.mod = lmer(NMDS2~Description+ (1|Site:Description)+ (1|Site), data = comm.char)

axis3.mod = lmer(NMDS3~Description+ (1|Site:Description)+ (1|Site), data = comm.char)

axis4.mod = lmer(NMDS4~Description+ (1|Site:Description)+ (1|Site), data = comm.char)

Multimodel inference Models

Parameter codes: 1=Age, 2= Change in N, 3 = Change in P, 4= Plant dissimilarity, 5=Change in plant evenness

Global Model: AMF.test = lm(difference in AMF~ Age+ change in N + change in P + plant.diss + change in plant evenness, data = data, na.action = na.fail)

Table 6: Summary of Model Selection Statistics for the Surface Soil Change in Hyphal Length. Models showing ΔAICc less than 10 are shown here.

Model	df	logLik	AICc	ΔAICc	weight
2	3	-12.57	35.95	0	0.28
1	3	-12.81	36.42	0.47	0.22
4	3	-13.23	37.27	1.32	0.15
5	3	-13.25	37.29	1.35	0.14
3	3	-13.25	37.31	1.36	0.14
12	4	-12.46	42.92	6.97	0.01
25	4	-12.47	42.95	7	0.01
23	4	-12.53	43.05	7.11	0.01
24	4	-12.57	43.14	7.19	0.01
15	4	-12.6	43.2	7.25	0.01
14	4	-12.74	43.47	7.52	0.01
13	4	-12.76	43.52	7.57	0.01
34	4	-13.13	44.25	8.31	0
35	4	-13.22	44.44	8.49	0
45	4	-13.22	44.45	8.5	0

Table 7: Summary of Model Selection Statistics for the Surface Soil Change in Spore Density. Models showing ΔAICc less than 10 are shown here.

Model	df	logLik	AICc	ΔAICc	weight
4	3	-55.5	121.79	0	0.46
1	3	-56.44	123.67	1.88	0.18
3	3	-57.07	124.94	3.14	0.1
2	3	-57.1	125	3.2	0.09
5	3	-57.25	125.3	3.51	0.08
34	4	-54.77	127.54	5.75	0.03
45	4	-55.21	128.42	6.62	0.02
14	4	-55.43	128.85	7.06	0.01
24	4	-55.49	128.97	7.18	0.01
13	4	-56.32	130.64	8.85	0.01
15	4	-56.4	130.79	9	0.01
12	4	-56.44	130.87	9.08	0
35	4	-56.82	131.65	9.85	0

Table 8: Summary of Model Selection Statistics for the AM fungal dissimilarity in the Surface Soil. Models showing ΔAICc less than 10 are shown here.

Model	df	logLik	AICc	delta	weight
1	3	18.1	-25.39	0	0.43
12	4	21.11	-24.21	1.18	0.24
4	3	16.82	-22.85	2.55	0.12
2	3	16.23	-21.66	3.73	0.07
3	3	15.79	-20.78	4.61	0.04
5	3	15.66	-20.53	4.86	0.04
14	4	18.36	-18.71	6.68	0.02
23	4	18.3	-18.61	6.78	0.01
13	4	18.14	-18.28	7.11	0.01
15	4	18.1	-18.19	7.2	0.01
45	4	17.47	-16.94	8.46	0.01
34	4	17.47	-16.93	8.46	0.01
24	4	17.44	-16.87	8.52	0.01

Table 9: Summary of Model Selection Statistics for the AM fungal dissimilarity in the Subsurface Soil. Models showing ΔAICc less than 10 are shown here.

Model	df	logLik	AICc	ΔAICc	weight
5	3	16.24	-21.68	0	0.47
45	4	18.9	-19.8	1.88	0.18
35	4	18.1	-18.2	3.48	0.08
2	3	14.5	-18.2	3.48	0.08
15	4	17.69	-17.39	4.29	0.05
4	3	13.86	-16.92	4.76	0.04

1	3	13.43	-16.05	5.63	0.03
25	4	16.93	-15.87	5.81	0.03
3	3	13.31	-15.81	5.87	0.02
24	4	15.61	-13.22	8.47	0.01