Vineyard Soil Communities and Arbuscular Mycorrhizal Fungi Associating with Grapevine Roots in Response to Irrigation Frequency

by

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ABSTRACT

Arbuscular mycorrhizal fungi form a root symbiosis with most plants and are known to benefit plants in a variety of ways. These organisms could be valuable in agricultural settings, leading to increased crop production and quality. For instance, the economically important *Vitis vinifera* is a highly mycorrhizal plant, dependent on the fungi for tolerating harsh growing conditions. Deficit irrigation is a viticultural practice that enables *V. vinifera* to be grown in arid climates where water resources are scarce, but how this management practice affects soil communities is not understood. There are three parts to this thesis. In the first experiment, I studied the abundance of different soil organisms to determine if irrigation frequency affects soil communities in general. The two frequencies of irrigation resulted in soil moisture levels that were either constant or fluctuated in a three-day cycle. I found that the biomass of fungi increased with fluctuating moisture, but in general the animal groups were most abundant in constantly moist soils. Secondly, I focused solely on the response of AM fungal communities. These did not respond to irrigation frequency. Instead, results of this experiment indicated there was an environmental effect as the fungal communities differed between the blocks. Both plant physiology and soil chemistry were identified as contributing to observed variation. Arbuscular colonization increased in the fluctuating soil moisture treatment compared with constant soil moisture, indicating a possible functional change in AM fungi due to irrigation frequency. In the final experiment I determined whether grapevines had distinct AM fungal communities compared to plants in co-occurring communities in the adjacent interrows. I found support for my prediction that the vinerow and interrow plant communities supported different AM fungal communities. Overall, this thesis indicates that management practices do affect soil communities, either in abundance, composition, or function. This could be due to changes in soil moisture, chemistry, or indirectly through changes in plant eco-physiology. It also provides
reasoning that more research is needed to enhance our understanding how AM fungal communities function in this agricultural setting.
This study was carried out in the final year of a four-year study of irrigation regime impacts to grapevines cultivated at Sunrock Vineyard, Osoyoos, BC, conducted by Dr. Pat Bowen, Pacific Agri-Food Research Centre (PARC), Summerland, BC (Bowen et al. 2012b).

Chapter 2 – ‘The response of soil biota to water availability in vineyards’ has been published online in Pedobiologia (DOI: 10.1016/j.pedobi.2012.08.004). This was a collaboration among myself, Dr. Hart, Dr. Andrew Reynolds and Matthieu Marcinia (Brock University), Dr. Ralph Brown (University of Guelph), and Dr. Pat Bowen and Carl Bogdanoff (PARC Summerland). Dr. Bowen and Carl Bogdanoff designed the experiment and I performed the fieldwork. Organisms were measured at the Soil Chemical Analysis Center, University of California. I was responsible for analyzing all data, and writing the manuscript.

For Chapters 3 and 4, I was responsible for creating a sampling design, collecting soil samples, performing molecular and microscopy lab work, and performing statistical analyses. The soil chemical analysis was performed at the BC Ministry of Environment Technical Services Lab and the plant physiology measurements were made Bowen et al. 2012b. I wrote this thesis with guidance from Dr. Hart, and it was reviewed by the members of my supervisory committee: Dr. David Scott and Dr. Louise Nelson from the University of British Columbia (Okanagan), and Dr. Pat Bowen from PARC Summerland.
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<td>Arbuscular Mycorrhizal</td>
</tr>
<tr>
<td>CSM</td>
<td>Constant Soil Moisture</td>
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<tr>
<td>DFS</td>
<td>Differential Fluorescent Staining</td>
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<tr>
<td>DI</td>
<td>Deficit Irrigation</td>
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<tr>
<td>FSM</td>
<td>Fluctuating Soil Moisture</td>
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<td>LSU</td>
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DEDICATION

To those who challenge the man, explore the unknown and live with a smile.
CHAPTER 1 – General introduction

1.1 Microbial systems

Soil microbes are important for maintaining soil integrity, both physically and chemically. They are responsible for the majority of nutrient cycling, decomposition of organic matter and the release of mineral nutrients back into the soil, making them readily available for other organisms (Brussaard 1997; Dodd et al. 2000; Coleman et al. 2004). They can also affect the nutrient and water holding properties of soil by increasing its stability and aggregate characteristics (Rillig 2004; Wilson et al. 2009). Due to the important services these organisms perform, efforts have been made to better comprehend the dynamics of these communities.

Studies have shown that soil microbial community composition is sensitive to a variety of factors including moisture availability (Jenerette et al. 2008), nutrient status (Carrero-Colon et al. 2006, Orwin et al. 2006), temperature (Bell et al. 2008) and pH (Tobor-Kaplon 2006; Liu et al. 2010). It has been reported that plant species composition also contributes to microbial community structure (Orwin and Wardle 2005, Steenwerth et al. 2005). Although these factors have been reported to influence soil microbes, it is still poorly understood how they contribute to changes that occur in soil microbial community composition. In particular, there is little literature dealing with how fluctuations in climatic factors such as moisture and precipitation may be involved in determining the structure of these communities.

1.2 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi are a group of ubiquitous, microscopic fungi that make up the Phylum Glomeromycota (Smith and Read 2008). AM fungi are obligate symbionts, forming associations with up to 80% of the plant species found on earth (Smith and Read 2008). It is well known that AM fungi have multiple, beneficial effects on their host plant’s performance.
For instance, AM fungi have been shown to be important to plant communities, with the
diversity of these fungi being correlated to that of plants (van der Heijden 1998). AM fungi have
also been shown to influence host plant biomass (Linderman and Davis 2001), nutrient status
(Schreiner, 2007), pathogen resistance (Li et al., 2006; Song et al. 2010) and reproductive output
(Koide and Dickie, 2002). These fungi represent an excellent microbial system for studying the
impact of soil moisture dynamics on microbial communities because they are relatively species
poor when compared to other soil microbes, making it easier to detect changes within their
communities.

1.2.1 AM fungal responses to environmental factors

Although the importance of AM fungi in managed and natural landscapes has been
established, we still know very little about how these fungal communities assemble. There is
some evidence of host plant communities and soil chemistry characteristics leading to
differences in AM fungal communities. For instance, plant identity (Scheublin et al. 2004),
physiology (Bais et al., 2006; Broeckling et al., 2008; Hartmann et al., 2009) and diversity
(Chung et al., 2007) have been correlated with differences seen in AM fungal community
structure (De Deyn et al., 2010; Alguacil et al., 2011). There are also reports that suggest soil
physiochemical properties are capable of significantly affecting mycorrhizal fungal
communities, more so than the plants they co-exist with (Turner and Fiese 1998; Garcia and
Mednoza 2008; Li et al. 2010). These factors include soil pH, temperature, nutrients and
moisture (Augé 2001; Klironomos et al., 2001; An et al., 2008; Fitzsimons et al. 2008; Dumbrell
et al., 2010). Knowing how AM fungal communities and functioning respond to environmental
factors may be important if we are to ever realize the full potential of AM fungi in agricultural
systems.
1.2.3 AM fungal response to soil moisture dynamics

While we are beginning to understand how soil chemistry affects AM fungal community structure and functioning, we know little about how these communities respond to soil moisture, which is a significant aspect of landscape management. For instance, there are currently no published studies directly focused on the effects of irrigation on AM fungal communities. It has been shown however, that in natural systems soil moisture does affect both the abundance and diversity of AM fungi. Studies in wetlands have revealed that root colonization decreases when conditions are increasingly mesic, or anoxic (Stevens and Peterson, 1996). This coincides with a change in community identity (Miller and Bever, 1999) and species richness (Ipsilantis and Sylvia, 2007). In arid regions a similar trend is seen. That is, when conditions become more stressful (increasingly xeric in this case) there are reductions in fungal species richness (Jacobson 1997) and root colonization (Trent et al 1994; Clark et al 2009). While the quantity of water itself can impose stress on microbial communities (ie. from too much or too little), the frequency of water supply may also influence AM fungal communities.

1.3 Deficit Irrigation

Deficit irrigation (DI) is a common agricultural technique that imposes a regulated drought to the roots of a plant (Dodd 2009; Chaves et al. 2010). The purpose is to maintain a predetermined level of soil water content or transpiration by altering the timing, amount or spatial application of water (Dodd 2009; Chaves et al. 2010). The end result is an induced stress response by plants, leading to changes in the physiology and timing of fruit development (Du et al. 2010). These irrigation regimes however may also have major consequences on soil communities, as the moisture environment of soils will be greatly altered, either as a reduction in the amount of water available, or a change in the frequency of water influxes. This type of stress may also become more relevant in natural systems in the future since many Global Climate
Change models predict an increase in fluctuating weather patterns including changes in precipitation events (Walther et al. 2002).

1.3.1 Grapevines and deficit irrigation

Grapevines produce the highest quality in climates that have low precipitation (Bowen, Chapter 2 BCWGC and BCMAL 2010). As regulating the water status is important for these plants, there has been an increasing amount of research done on DI for these crops (Qian et al. 2009; Acevedo-Opazo et al. 2010; Chaves et a. 2010). Not only is this technique more sustainable due to the decreased amounts of water needed for irrigation, it can also contribute to changes in the chemical properties of berries that are produced (Qian et al. 2009; Acevedo-Opazo et al. 2010; Chaves et a. 2010). However this depends on the climate and soil water holding properties and only sometimes is this increased berry quality seen in the semi-arid Okanagan valley (Bowen et al. 2012b).

The frequency of irrigation may also be important. In a DI study by Bowen et al. (2012a,b) increased frequency of irrigation affected the chemical composition of grape berries. Applying irrigation daily compared with every three days, led to significant physiological changes within the grapevines including reduced stress, increased photosynthesis and transpiration, higher sugar and phenolics levels within the berries, and more fruit aromas and body in the wine.

The change in irrigation also causes a major difference in the soil moisture properties over the watering cycle (Bowen et al. 2012b). When watered daily, the soil moisture remains at a more constant level than when watered less frequently at a three-day interval. The latter regime allows for significant moisture depletion due to vine uptake between watering events.
1.4 AM fungi and *Vitis* grapevines

Grapevine roots are known to form associations with AM fungi (Linderman and Davis, 2001; Schreiner and Mihara, 2009; Balestrini et al. 2010) and these vines can be highly dependent upon this symbiosis (Menge et al. 1983). However there are few studies focused on the composition of AM fungal communities associated with *Vitis* roots, with most having contrasting results when looking at the abundance (Schreiner 2003) and identity (Schreiner and Mihara, 2009; Balestrini et al. 2010) of fungal species. As a result, there remain large gaps in our knowledge of the AM fungal communities associated with grapevines and how these assemble in response to common viticultural methods. It may be that altering irrigation regimes, such as changing the frequency of deficit irrigation, can impose selection pressure on AM fungal communities, which may result in altered functioning, but there are no reported studies that have investigated this.

1.5 Research Objectives

My goal was to determine how different irrigation frequencies affect the microbial communities associated with grapevines in a typical viticultural system in the Okanagan Valley. I used two frequencies, one resulting in a soil constant moisture profile and the other a fluctuating soil moisture profile. However, as so little is known about the symbiosis between grapevines, especially those on rootstocks, and AM fungi, my goal was also to ask basic questions about the AM fungal communities found in a viticultural system.

Specifically, I asked:

1) Does irrigation frequency affect microbial communities *in general*? That is, is the abundance of vineyard soil organisms (fungi, bacteria, protozoa, nematodes, arthropods) affected by the irrigation treatments?
I predicted that soil microbial communities would have higher biomass in soils of fluctuating moisture status than in constantly moist soils. This would be due to the increased niche range available with fluctuating soil moisture.

2) Is the AM fungal community, in particular, affected by the frequency of deficit irrigation? To do this, I looked at the communities associating with *Vitis* roots in more detail, to see if I could detect changes in abundance, diversity and taxonomic identity. I predicted that the abundance and diversity of AM fungi on roots would increase with the fluctuating moisture status. With a greater range of niches available with a fluctuating moisture status, more species should be able to coexist in this system. Also, I expected the two frequencies of irrigation to result in different taxonomic composition as different species should be better able to tolerate or thrive in the different environments.

3) I also asked whether local conditions, unrelated to irrigation, were significant drivers of the variation in AM fungal communities in this system. To do this I looked at a suite of soil chemical properties that are important for both plants and microbes, plus plant physiological measurements to see how they influenced observed variation in AM fungal communities.

*I predicted that soil chemistry and plant physiological traits would have a major influence on the composition of AM fungal communities. This is due to previous evidence that AM are sensitive to changes in the soil environment and their host plants.*
4) Finally, I asked whether *Vitis* associates preferentially with certain AM fungi compared with interrow plants. To do this, I measured AM fungal diversity and characterized the communities associating with *Vitis* and interrow vegetation, including the identity and abundance of different species present.

*I predicted that there would be significant differences in the composition of AM fungal communities present in the two host groups. Specifically, I thought Vitis roots would have a subset of the species present in interrow plants. This would be due to the preferential coexistence that has been shown between some fungal and host combinations.*

Question 1 is addressed in Chapter 2. Questions 2 and 3 are addressed in Chapter 3, while Question 4 is addressed in Chapter 4.
CHAPTER 2 – The response of soil biota to water availability in vineyards

2.1 Synopsis

Many agricultural practices have been developed in order to enhance aspects of farming such as crop yield, product quality, pest control and sustainability. These practices include the use and manipulation of fertilizers, pesticides/herbicides, tillage and irrigation (Drinkwater et al. 1995; Cassman, 1999; Raun and Johnson, 1999; Fereres and Soriano, 2007). While these practices can benefit production, the unintended consequences on soil ecosystems are not well understood. Ultimately, even small changes in soil community structure and functioning can improve or jeopardize crop health (Giller et al. 1997; Cassman 1999).

In viticulture, attaining high quality grapes for winemaking is a priority for growers, as crop value is highly dependent on quality. Soil moisture has been identified as a major factor influencing crop yields, fruit composition and overall wine quality (Seguin 1986). It is important that vines have sufficient water to allow for adequate vine and berry growth, but not excess amounts, which can lead to excess vigor and poor fruit composition and wine quality. Irrigation affects fruit characteristics including colour, sugar content, acidity, flavor and aroma (Chaves et al. 2007; Qian et al. 2009; Acevedo-Opazo et al. 2010; Chaves et al. 2010).

Depending on the terroir (biotic and abiotic characteristics) of a vineyard, soil moisture balance may be achieved through natural precipitation or may require irrigation.

2.1.1 The use of deficit irrigation in viticulture and possible indirect effects

Deficit irrigation (DI) is a technique widely adapted to viticultural systems and is used in viticulture to impose a regulated drought to plant roots (Dodd 2009; Chaves et al. 2010). This is achieved by altering the timing and/or amount of water applied to crops in order to maintain a predetermined level of soil water content or transpiration, allowing for optimal growing conditions (Cifre et al. 2005; Dodd 2009; Chaves et al. 2010). In arid regions where irrigation is
essential, DI is regularly implemented to conserve water, limit vigour and produce high yields of quality crops.

While irrigation is clearly beneficial for arid viticulture, the consequences on soil microbes have been largely unexamined. Because water availability is a major factor in determining the diversity, abundance and distribution of microorganisms in soils (Wardle 2002; Coleman et al. 2004; Steenworth et al. 2005; Landesman et al. 2010; Chowdhury et al. 2011), changes to the hydrology of arid soils are likely to have consequences for soil communities and processes. For example, bacterial abundance and functioning are known to be sensitive to changes in soil moisture and osmotic stress (Wardle 2002; Coleman et al. 2004). In contrast, fungi are more resistant to changes in soil moisture, and have various strategies for tolerating drought, including spore production, variable hyphal growth rates, and osmoregulation capabilities (Blomberg and Adler 1992; Wardle 2002; Coleman et al. 2004). Soil moisture can also indirectly influence soil organisms, by affecting processes and soil characteristics that are tightly linked to organism abundance, such as nutrient status (Carrero-Colon et al. 2006; Orwin et al. 2006), temperature (Bell et al. 2008) and pH of soils (Tobor-Kaplon 2006; Liu et al. 2010).

2.1.2 Soil communities in viticulture and how they may be affected by management practices

A functional soil community is important in viticultural and other systems. Soil organisms provide many essential ecosystem services, including nutrient cycling, (Brussaard 1997; Dodd et al. 2000; Coleman et al. 2004), protection against crop pests (Weckert 2004; Song et al. 2010), soil aggregation and stabilization (Wilson et al. 2009) and increased water retention (Rillig 2004). In the case of viticulture, it is possible that these communities may impart key aspects of terroir, by improving the soil conditions and imparting distinct, site-specific qualities.

Viticulture is unusual in that it is often conducted on sites considered unsuitable for most crops. These include arid to semi-arid sites with long, hot summers. The ability of grapevines
(often European *Vitis vinifera* grafted onto American *Vitis* spp. and hybrid rootstocks) to tolerate these environmental conditions will partially be dependent on the functioning of their soil community. There is considerable evidence that plants growing in stressful conditions (such as water stress) are more dependent, and derive more benefit from, their associated soil organisms than plants growing under more moderate conditions (Klironomos *et al.* 2001; Maestre *et al.* 2011). Any viticultural practice that affects soil communities, such as DI, has the potential to indirectly affect vines through their soil associates. Unfortunately, we know very little about how DI affects the composition and functioning of soil communities.

### 2.1.3 Experimental system and objective.

The goal of this study was to investigate the effects of variable soil moisture on soil communities in viticultural systems. To determine whether there were effects on the communities, I determined the abundance of major soil taxa including fungi, bacteria, protozoa, nematodes, collembola and mites. I looked at the abundance of soil organisms in a vineyard subjected to two irrigation frequencies (fluctuating (FSM) or relatively constant (CSM) soil moisture). I hypothesized that soil communities would benefit more from FSM than CSM because a fluctuating wet/dry cycle more closely mimics natural precipitation cycles. Thus, I hypothesized that the organisms would be better adapted to periods of depleted soil moisture, compared with a constantly moist environment as provided by the CSM, seen as a greater biomass in FSM.
2.2 Methods

2.2.1 Study site and experimental design

This study was composed of two separate experiments that had been underway for four years (2007 to 2010) at Sunrock Vineyard, Osoyoos BC, Canada (49.5N, 119.31W). The experiments differed only in grapevine scion, one trial using *V. vinifera* Merlot (clone 347) and the other *V. vinifera* Shiraz (clone 100), both grafted on rootstock 3309C, a hybrid of the American species *V. riparia* and *V. rupestris*. Two of six irrigation treatments were included for the experiments in this thesis, providing the same total water volume but at different application frequencies. These resulted in either fluctuating soil moisture (FSM) in response to 16 L of water applied every three days, or more constant soil moisture (CSM) in response to 5.33 L of water applied daily. Soil moisture content (% v/v) was measured hourly to a depth of 120 cm using time domain reflectometry (TDR) probes (Moisturepoint, ESI, Victoria, BC, Canada). Soil moisture fluctuated between 7 and 17%, (on average) in response to the 3-day irrigation interval, and between 11 and 13% under daily irrigation (Bowen, 2012a,b).

Both experimental blocks consisted of 4 rows of winegrapes, with every row containing one section of both the FSM and CSM, in a randomized design. For the sampling of this study, each section of irrigation treatment was sampled as an experimental unit, totaling 8 experimental units for each block (Figure 2.1). Each experimental unit consisted of three adjacent vines, all receiving the same irrigation regime. Following harvest October 2010, 3 soil cores (15.2 cm deep, 2.5 cm in diameter) were taken from each vine, 20 cm from the base, for a total of 9 soil cores per experimental unit. These were kept at -20°C until being processed. A subsample of each was sent to the Soil Chemical Analysis Center, University of California for soil organism abundance measurements to be taken.
2.2.2 Abundance determination of soil organisms

Bacterial and fungal abundance was estimated by differential fluorescent staining (DFS) following the procedure from Conners et al. (1994), adapted by Klironomos et al. (1996). For fungal counts, 200 mL of soil suspension was combined with 1 mL of DFS solution (a mixture of europium (III) thenoyl-trifluoroacetonate and a fluorescent brightener (Anderson and Westmoreland 1971)), stained for 1 hr, followed by filtration through nitrocellulose filter papers using a 50% ethanol wash. Filters were then mounted on microscope slides for visual inspection. For bacteria, smears were made using dilutions of soil, which were then stained with DFS for 1 hr. Again, a 50% ethanol wash was used to rinse slides. For both fungal and bacterial abundance counts, stained slides were viewed using UV light (620 nm) with active cells observed as red fluorescence. Biomass was then calculated using images taken and analyzed using computer imaging software. Fungal biomass was estimated using the hyphal length and published estimates of hyphal diameter (1.65 μm) (Kjøller and Struwe 1982), density (0.33 g cm$^{-3}$) (Van Veen and Paul 1979), and C content (45%) (Swift et al. 1979). Bacterial biomass was estimated using the assumption $6.4 \times 10^{-14}$ g C cell$^{-1}$ (Hunt and Fogel 1983).

2.2.2.1 Nematode abundance

The number of nematodes was determined using the wet-sieve sucrose centrifuge approach, as described in Klironomos et al. (1993). Briefly, samples were suspended in water and passed through a series of mesh sieves, decreasing in size from 1.0 mm to 45 pm, with the final product being centrifuged on top of a 60% sucrose solution. Nematodes were collected as part of the sucrose-water interface, and manually counted.
2.2.2.2 Microarthropods and protozoan abundance

A high efficiency canister-type soil arthropod extractor was used to extract microarthropods (mites and collembola) onto dishes containing picric acid as in Klironomos et al. (1996), first described by Lussenhop (1971). Each microarthropod was manually counted to obtain abundances. To estimate protozoan biomass, a series dilution was used as in Janssen and Heijmans (1998). Briefly, soils were dried and large litter removed. They were then reconstituted in demineralized water and centrifugally filtered. This extract was then used to make a series of 11 dilutions from the original collected soil, to eventually obtain a $4^{10}$ dilution. After two weeks of incubation the abundance of protozoa was calculated using the presence and distribution of ciliates, flagellates and amoebae throughout the dilution series, as described in Janssen and Heijmans (1998).

2.2.3 Statistical analysis

Differences in abundances were analyzed in JMP 9 using a significance value of $\alpha=0.05$ (SAS Institute, 2010). Two-sample t-tests were used to determine differences between irrigation treatments for bacterial and fungal abundances, which were normally distributed. The fungal and bacterial data for the Shiraz and Merlot experiments were first analyzed separately. Because a similar trend was observed for these responses, they were subsequently pooled and reanalyzed to increase the statistical power.

The Wilcoxon Two Sample Test, a non-parametric t-test equivalent, was used to analyze for differences in protozoa, nematode, collembola and mite abundances, along with the bacterial and fungal biomass ratio, all of which failed to meet the assumptions of normality. This was done separately for the two experiments, as they did not follow similar trends such as with the bacterial and fungal biomass above.
2.3 Results

2.3.1 Bacterial and fungal biomass

Bacterial biomass was unaffected by irrigation frequency in both the Shiraz and Merlot trials separately (data not shown), and when the two trials were grouped (Figure. 2.1a). However, fungal biomass did show slightly higher abundance with FSM in both the Merlot (t-ratio=1.855, df=19, P=0.079) and Shiraz experiments (t-ratio=1.920, df=19, P=0.070) (data not shown), and when the experiments were analyzed together (Figure. 2.1b).

2.3.2 Soil invertebrate abundance

For the cases of soil invertebrates, there was an increased abundance under CSM (Figures 2.3 and 2.4). However, there was also a significant difference in response between the experiments for the two varieties, so they were analyzed separately. Collembola abundance was higher (Z=-2.005, P=0.045) under CSM (Figure 2.3b) in the Merlot but not in the Shiraz experiment (Figure 2.4b). Conversely, the abundances of protozoa (Z=-2.686, P=0.007), mites (Z=-1.974, P=0.048) and nematodes (Z=-2.341, P=0.019) were higher under CSM in the Shiraz experiment (Figure 2.4 a,b,d), but not in the Merlot experiment (Figure. 2.3 a,b,d).

2.4 Discussion

2.4.1 Fungal and bacterial biomass in response to irrigation frequency

The results support my hypothesis that abundance of organisms is affected by soil moisture variability, as determined by the two irrigation treatments. Fungal biomass was higher in soils with cyclical fluctuations in moisture. The fluctuating conditions are more similar to those that naturally occur in arid climates; and it has similarly been shown through microcosm experimentation that species richness of fungi positively responds to fluctuating moisture conditions (McLean and Huhta, 2000). The reasons for this are not clear. It may be that a variety of soil water potentials allow fungi to adapt to specific soil moisture levels. Because
fungi are more resilient to osmotic stress (Vishnevetsky and Steinberger 1997; Wardle 2002; Coleman et al. 2004) than are many other soil organisms, the fungi although suited to particular water potential could survive during the drought periods. Thus, FSM may provide a greater range of niche space (opportunities) for fungi in viticultural soils, leading to the increased abundance, since the positive relationship between biodiversity and abundance is well established (Chapin III et al. 1997; Tilman et al. 1996, 1997; Tilman 1999; van der Heijden et al. 1998). Whether less frequent irrigation results in higher fungal diversity remains to be tested.

In the case of bacteria, their biomass levels indicate that they were able to exploit moisture conditions resulting from both irrigation regimes equally in this vineyard. Other studies have shown bacteria to be relatively resistant to water stress (Wilkinson and Anderson 2001; Fierer et al. 2003; Williams 2007). Whether this is due to physiological responses, or reflects the ability of natural populations to quickly respond to selection pressure is not known. Because soil bacterial diversity is usually vast, with a reservoir of genetic resources, adaptive genotypes may already exist, allowing for rapid adaptation to a variety of conditions (Fierer et al. 2003; Landesman and Dighton, 2010). If this is true, bacterial biomass should not be significantly affected by most irrigation regimes, including those that differ in frequency.

2.4.2 The abundance of soil invertebrates in response to irrigation frequency

There was an overall trend of increasing abundance of soil invertebrates under CSM. This suggests that the abundance of soil invertebrates was limited by soil moisture, and that these organisms are sensitive to desiccating conditions. Interestingly, the abundance of these animals did not correspond to the changes in their food sources, i.e. bacteria and fungi. The abundance of fungal feeders is expected to track the abundance of fungi. Since there were more fungi under FSM than CSM, FSM should support more feeders (i.e. nematodes, collembolans). Several studies have found changes in population of soil predators independent of microbial abundance
but related to abiotic changes in habitat (Yeates et al. 1999; Wardle et al. 2001; Papatheodorou et al. 2004). In my study, it may be that hyphal abundance was not limiting these populations. Rather, water was a more important limiting resource.

### 2.4.3 Block effects

It is interesting to note the differential responses between the Merlot and Shiraz blocks. This could be due to many factors, including differences among blocks in soil chemistry, aspect or isolation. Additionally, while I cannot attribute the differences to variety, results suggest that components of the scion physiology, such as exudation patterns (Broeckling et al. 2008, Hartmann et al. 2008) may mediate how the soil community responds to abiotic stress. The Merlot leaves transpired about 30% more on a leaf area basis than the Shiraz leaves, but the Shiraz vines produced more vegetative growth (equivalent to about 20% more pruning mass) (Bowen et al. 2012b). While it was not determined whether water consumption by the vines differed between experiments, the more vigorous Shiraz vines likely cast more shade reducing the amount of solar radiation received at the soil surface in that experiment. The direction of the vine rows was also perpendicular to one another, possibly increasing this heating affect. Granted, observed differences between communities are likely due to factors beyond differences in varietal physiology, but whatever the mechanism spatial effects on soil ecology in vineyards warrant more attention.
2.4.5 Summary

This study provides a starting point for further research on the effects of irrigation regimes on soil communities in viticultural systems. While I detected some changes in abundance, it is essential to determine whether these changes are reflected in community composition and in ecosystem functions associated with soil organisms.

In this study I showed that changing the frequency of irrigation could have large effects on the abundance of major soil taxa. Further work must be completed to fully understand the effect of crop management on the abundance and identity of associated organisms and how these changes subsequently affect vine growth and berry quality. Ultimately, growers must manage their crops considering both conditions optimal for berry quality, and for cultivating the most functional soil communities. Ostensibly, this will result in irrigation regimes that complement site specific conditions.
2.5 Figures

Figure 2.1 - The sampling design for both the two experimental blocks. Each colored rectangle (red and blue) represents an experimental unit, a composite of 9 soil cores (black dots) taken from 3 vines (turquoise). Red represents fluctuating soil moisture and blue the constant soil moisture profile.
Figure 2.2 - Bacterial biomass (a) and fungal biomass (b) per gram of soil associated with all vines subjected to fluctuating (FSM) and constant (CSM) soil moisture in a vineyard in Osoyoos, BC Canada. Values represent mean ± SE, n=16. Asterisks represent significant difference for p<0.1.

Figure 2.3 - The abundance of protozoa (a), collembola (b), nematodes (c), and mites (d) per gram of soil associated with Merlot vines grown in soils with FSM or CSM, Osoyoos, BC Canada. Values represent mean ± SE, n=8. Asterisks represent significant differences at p<0.05 using a t-test.
Figure 2.4 - The abundance of protozoa (a), collembola (b), nematodes (c), and mites (d) per gram of soil associated with Shiraz vines grown in soils with FSM or CSM, Osoyoos, BC Canada. Values represent mean ± SE, n=8. Asterisks represent significant differences at p<0.05 using a t-test.
CHAPTER 3 – AM fungi associated with grapevine roots under different frequencies of irrigation

3.1 Synopsis

3.1.1 The importance of AM fungi in vineyards

The importance of arbuscular mycorrhizal (AM) fungal communities in natural and agricultural soils is well established. These fungal symbionts associate with the roots of most plant species and are linked with increases in plant nutrient acquisition (Schreiner, 2007), soil aggregation (Rillig, 2004; Wilson et al., 2009), water uptake (Augé, 2001), and plant productivity (van der Heijden et al., 1998; Linderman and Davis, 2001). Grapevines (Vitis spp.) are known to form mycorrhizas, but we are only beginning to learn about how these associations are important for vine productivity, nutrition and berry quality (Schreiner, 2003).

Grapevines may be highly responsive to AM fungi, since plants growing under stressful conditions (e.g. nutrient limitations or drought, typical in many viticultural systems) experience large benefits from AM fungi (Hildebrandt et al., 2007; Bunn et al., 2009; Harris-Valle et al., 2009). Furthermore, plants with relatively low density and coarse root morphology, such as Vitis spp. (Mohr, 1996), tend to be more mycorrhizae dependent than plants with fine, highly branched roots (Hetrick, 1991; Newsham et al., 1995; Schweiger et al., 1995; Sikes, 2010). Grapevines show increased biomass (Linderman and Davis, 2001; Nogales et al., 2009), nutrient uptake (Schriener, 2007) and water use efficiency in association with AM fungi (Valentine et al., 2006). Furthermore, colonization by AM fungi can result in beneficial changes to the proteome of the grapevines (Cangahuala-Inocente et al., 2011). This suggests that AM fungi might also be important for biochemical changes in grape berries, such as increased levels of antioxidants seen in other host systems (Baslam et al., 2012). Changes to these compounds are important factors for determining wine quality.
3.1.2 AM fungal communities and management practices, in particular deficit irrigation

Though the potential benefits of AM fungi in viticulture are clear, there remains a gap in our understanding of how environmental factors affect AM fungal communities associating with grapevines. There is a wide range of practices in viticulture that vineyard managers use to maintain healthy vine growth and produce high quality berries, including vine training and pruning, pesticides and herbicides spraying, fertilizer addition and cover crop management (Jackson and Lombard, 1993). Most management decisions are based on grape and vine parameters, while effects on soil communities such as AM fungal symbionts are often overlooked.

In arid climates, the use of irrigation is a widespread management practice in viticulture. Deficit irrigation (DI) is a technique that allows growers to reduce water use while maintaining crop yields (Dodd, 2009; Acevedo-Opazo et al., 2010; Gasque et al., 2010). By altering the amount and timing of water application, plants are subjected to a water stress that leads to changes in physiology (Dodd, 2009; Chaves et al., 2010; Du et al., 2010). In winegrapes, this technique can also increase the quality of fruit and wine (Qian et al., 2009; Acevedo-Opazo et al., 2010; Chaves et al., 2010). By increasing the frequency of irrigation (but keeping the overall amount the same), Bowen et al. (2012a,b) found that they altered important berry properties such as the size, juice soluble solids, pH, acidity, anthocyanins and tannin levels. This led to wines that were described as having more fruit aroma and body when tasted by experienced winemakers.

With this change to the irrigation schedule imposed by Bowen et al. (2012 a,b) moisture in the soil profile fluctuated less and became more constant over time. How do these new conditions affect soil communities that are adapted to these dry soils? There is some evidence that altering irrigation regimes may affect the abundance of various soil organisms, including
microarthropods, nematodes, fungi and bacteria (Holland et al., 2013). In our previous study, there was a trend for higher biomass of fungi when subjected to fluctuating soil moisture, yet decreases in the other organisms. Changes to soil moisture have also been shown to change the amount of AM fungal colonization of Vitis spp. roots. This includes decreases in overall colonization under drought stress (Valentine et al., 2006), yet increases in arbuscular colonization of deficit-irrigated vine roots (Schreiner et al., 2007).

Water availability, both limiting and excess, has also shown to influence the diversity of AM fungi. That is, in increasing xeric (Jacobson, 1997) or mesic (Miller and Beaver 1999; Ipsilantis and Sylvia 2007) conditions there is a tendency for less diverse communities. This is likely due to differential tolerance among fungi. For instance, decreases in soil water availability have correlated with decreased hyphal growth and colonization of roots (Trent et al., 1994; Clark et al., 2009), yet Klironomos et al. (2001) found that in dry soil conditions AM levels varied depending on the AM fungal species tested.

In Okanagan vineyards, fluctuating dry/wet soil environment may allow a greater range of fungi to persist (i.e. both drought tolerant and mesic tolerant fungi will be able to establish and persist). Conversely, soils that are kept constantly moist will select for a specific subset of the community that is able to tolerate only those conditions. Thus I expect fluctuating DI to support AM fungal communities that are more diverse and have a different composition than constant DI.

3.1.3 Experimental system and objective

In this study two irrigation frequencies were applied to two blocks of winegrapes and I measured changes to the AM fungal communities. First, I predicted the fungal communities would respond to irrigation frequency. Specifically, I predicted that fluctuating soil moisture (FSM) would be associated with AM fungal communities that were more diverse with higher species richness compared with those exposed to constant soil moisture (CSM).
3.2 Methods

3.2.1 Experimental design and sampling

Soil samples were collected following harvest October 2010, from an existing irrigation study at Sunrock Vineyard, Osoyoos BC, Canada (49.5N, 119.31W). The study consisted of two experiments, which were designed identically except for the row direction and scion variety, Merlot or Shiraz, both grafted on 3309C rootstocks. For the purposes of this study, the two experiments were treated as two blocks. The experiments were established to test the effects of different irrigation frequencies on vine performance. The irrigation frequencies were: Constant Soil Moisture (CSM), which consisted of 5.33 L/daily, and Fluctuating Soil Moisture (FSM), which consisted of 16 L/every third day. These treatments translated to a change of 3% water content for the daily irrigation, or 17% water content over the three-day treatment (Bowen et al., 2012a,b).

For this randomized block design, the factor was soil moisture, either fluctuating or constant due to the irrigation regimes described above. Each block consisted of 8 experimental units (Figure 3.1). In this design, treatments were applied to three adjacent vines, which constituted 1 experimental unit. In each block these two treatments were randomized down four rows. At each experimental unit of 3 vines, I sampled in order to maximize my success at capturing all the variation inherent in the soil community. To do this, I took 3 soils cores (15.2 cm deep, 2.5 cm in diameter) from each of the three vines in each experimental unit. Cores were kept at -20 °C and processed independently until sequencing when they were pooled into 1 composite sample. This resulted in 8 experimental units for each block,
3.2.2 Community analysis

3.2.2.1 DNA extraction

*Vitis* roots were separated from soils and DNA was extracted in duplicate from 0.05 g of *Vitis* root tissue following the protocol outline in the MoBio PowerSoil kit (MoBio Laboratories Inc).

3.2.2.2 PCR conditions and 454 sequencing

From each sample triplicate PCR amplifications were performed using the Glomeromycotan specific primer FLR3/FLR4 (Gollotte et al., 2004), which amplifies a 400 bp fragment of the large subunit of rDNA. Primers were modified for 454 pyrosequencing with the addition of Roche ligating adaptor and multiplex identification (MID) regions. A 23 µL mixture of 13.25 µL ddH2O, 5 µL 5x PCR buffer(Promega), 2 µL MgCl\(_2\) (BioLabs), 0.5 µL dNPTs(Amresco), 1 µL BSA(BioLabs), 0.25 µL GoTaq(Promega) and 0.5 µL of each primer was used per reaction. To this two-1 µL aliquots of DNA template were added (1 µL from each DNA extraction), making a total volume of 25 µL. Cycling conditions were: 95°C for 3 min followed by 35 cycles of (95 °C for 30 secs, 52.5 °C for 30 secs, 72 °C for 60 secs), 72 °C for 10 min, and a hold at 4 °C. PCR products were standardized to 1.25 ng/µL using the Invitrogen SequalPrep kit before amplicon sequencing using Roche 454 pyrosequencing GS FLX+ Titanium chemistry (UBC Prostrate Center, Vancouver Canada).

3.2.2.3 Sequence analysis

The Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al., 2010) was used to analyze sequencing data, with the following modifications. Sequences were first filtered under the default parameters with the exceptions: 370-410 bp fragment length, a maximum of 6 homopolymers allowed, zero mismatches in MID tags and an average quality score of 30. Sequences were then organized into their respective samples using the MID
identifier tags, rarified to 1250 sequences per sample and then grouped into operational
taxonomic units (OTUs) based on 95% similarity using the UCLUST algorithm (Edgar, 2010).
Further, to ensure identified OTUs were not due to sequencing error, I required that each OTU
be represented by a minimum of 6 sequences, or that it occur in at least three separate samples in
order to be retained in my analysis. While I acknowledge that this might bias against rare taxa, I
felt it was important to take a conservative approach in order to ensure that OTUs in my analysis
were real entities.

3.2.3 AM fungal measurements

3.2.3.1 Root colonization

Percent colonization of the Vitis roots was measured using a modified version of
Klironomos et al. (1993). Briefly, roots were washed using dH₂O, cleared by autoclaving for 15
min in 10% KOH, further bleached in room temperature 3% H₂O₂ for 30 min, acidified in
boiling 10% HCl for 10 min and stained in 0.5% Trypan blue (5% lactic acid, 50% glycerol)
autoclaving for 15 min. Roots were then destained overnight and stored in lactoglycerol for
further use. The magnified intersections method (McGonigle et al., 1990) was applied to
quantify the proportion of roots that contained arbuscules, vesicles and hyphal colonization.

3.2.4 Environmental measurements

3.2.4.1 Soil chemistry

To account for environmental factors that may have contributed to community variance
soil chemistry was considered. From each experimental unit, a subsample of the original soil
core was sent to the BC Ministry of Environment, Technical Services Lab (Victoria, British
Columbia), for chemical analysis of soil pH and mineral nutrients. To measure the total
extractable elements in the soil (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Zn), an inductively
coupled plasma-optical emission spectrometer was used in combination with the Mehlich III
extractant (0.2N CH₃COOH + 0.25N NH₄NO₃ + 0.013N HNO₃ + 0.015N NH₄F + 0.001M
EDTA). Soil pH was determined as the pH of a 1:1 mixture of soil:water measured using a pH electrode. Lastly, total C and N were quantified using combustion elemental analysis.

### 3.2.4.2 Plant physiology

In order to account for plant physiological effects on fungal communities data collected and reported previously (Bowen et al. 2012a,b) were acquired from Dr. Bowen including: leaf nitrogen and dry weight, leaf gas exchange parameters, pruning mass, shoot length, stomatal conductance, transpiration and berry yield. The leaf gas exchange rates (stomatal conductance, transpiration and photosynthesis) had been measured using a portable system (6400; Li-Cor, Lincoln, NB), equipped with an artificial light source (6400-02B) to provide a photosynthetic photon flux of 1000 µmol m$^{-2}$ s$^{-1}$. Shoot lengths were based on measurements of 5 randomly chosen shoots per vine in early July before vine hedging was completed. Percent leaf N was determined from a 0.25 g sample of dried leaves with a LECO FP-528 (Leco Corporation, St. Joseph, MI).

### 3.2.5 Data analysis

#### 3.2.5.1 AM fungal root colonization

To determine if irrigation and the varietal block affected mycorrhizal root colonization R-v.2.8.1 (R Development Core Team 2008) was used to perform a one-way blockd analysis of variance (ANOVA) with irrigation as the factor. This was done separately on both arbuscular and vesicular colonization.

#### 3.2.5.2 AM fungal alpha diversity

To determine whether AM fungal alpha diversity differed among the irrigation treatments, a one-way blocked analysis of variance (ANOVA) test (R, version 2.8.1R Development Core Team 2008) was performed. For this I used species richness, determined as the number of OTUs present in each sample.
3.2.5.3 **Effect of irrigation on AM fungal community structure: Beta-diversity**

To determine if irrigation affected the beta diversity of AM fungal communities, PERMADISP in PERMANOVA+ (Anderson *et al.* 2008) was used to detect differences in spread, or ‘dispersion’ of samples around treatment centroids (in this case, ‘irrigation frequency’). The magnitude of spread is related to changes in beta-diversity, that is, the greater the spread, the more change over between samples. Presence-absence data (based on OTUs) were used to create a Sørensen similarity distance matrix and PERMADISP analysis for 9999 permutations was run.

3.2.5.4 **Environmental effects on AM fungal communities**

To account for variables other than irrigation that might influence the AM fungal community soil chemistry (contents of Al, B, C, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, S, Zn and pH) and plant physiology (leaf nitrogen, leaf dry weight, photosynthesis, pruning mass, vine shoot length, stomatal conductance, transpiration and berry yield) were included as covariables in the analyses to determine whether they contributed to an indirect influence of the irrigation treatments on the AM communities. Soil texture was not included, as it did not vary throughout the vineyard. Data were first transformed in order to meet the conditions of normality. This involved log transforming soil S and Zn values. Values were then normalized, with each value being subtracted from the mean for that variable. Redundant variables were then removed by checking for correlations between each using draftsman plots in PERMANOVA+. The resulting dataset included soil variables: Al, B, C, Cu, Fe, Na, PO₄, S, Zn, and pH(Table A.1) and plant physiological values (yield, vine shoot length, leaf dry weight, photosynthesis and stomatal conductance).

Next, I determined if these variables differed between irrigation frequencies using PERMANOVA+ (Anderson *et al.* 2008). This was used as the data failed to meet assumptions
for MANOVA including having equal sample sizes and the observations being independent.

Briefly, I ran PERMANOVA on Euclidean distance matrices for both soil and plant physiology data. For PERMANOVA tests, irrigation frequency and block were fixed factors and 9999 unrestricted permutations were performed on raw data using type III sum of squares.

3.2.5.5 AM fungal community analysis

To calculate a measure of distance between AM fungal communities, I calculated weighted-UniFrac distances between samples. Weighted-UniFrac is a measure of community similarity based on the amount of phylogenetic overlap present between the taxa present in two samples taking abundance into account (Lozupone and Knight, 2005). I also created a Sørensen similarity distance matrix based on OTU presence/absence to give a measure of similarity without considering phylogenetic relationships or relative abundance.

To test for differences among AM fungal communities between irrigation treatments, I performed a PERMANOVA with irrigation and block as fixed factors. Because I knew that soil chemistry and plant physiology were also affected by irrigation treatments, these were used as covariates in my PERMANOVA (Anderson et al. 2008). Due to the large number of soil/physiological variables, I used the first PCA axis from the soil chemistry/plant physiological measurements (see above), to reduce the number of covariates in the analysis. I ran 9999 permutations on residuals under a reduced model with type I sums of squares. This was done for both the weighted-UniFrac and Sørensen distance matrices.

3.2.5.6 Testing for Environmental Effects

In order to break down the relative contribution of the environmental measures to observed variation I implemented DISTLM (Anderson et al. 2008), which partitions variance between environmental predictor variables using distance-based linear models. Soil characteristics (Al, B, C, Ca, Cu, Fe, K, Mg, Mn, N, Na, P, S, Zn and pH) along with plant
physiology traits (berry yield, vine shoot length, leaf weight, photosynthesis and stomatal conductance) were included in this analysis to determine if they correlated with changes in the AM fungal community.

The *Best* selection routine was used, along with AICc selection criterion on 9999 permutations. The *Best* selection routine samples all possible combinations of predictor variables and details the models that best explain variance between communities. The AICc selection criterion is stringent when adding variables and is often used when the ratio of samples to predictor variables is low as in this case. It uses a correction term so that the addition of a variable must significantly increase the explanatory power of the model. Marginal tests were also performed to individually examine the explanatory power of each variable. Using the ‘best’ DISTLM model, I conducted a Distance Based Redundancy Analysis (dbRDA). This depicts the community variance after taking into account the environmental variables in the model.

### 3.3 Results

#### 3.3.1 AM fungal community responses

##### 3.3.1.1 Irrigation frequency

Overall, I found no evidence to support my hypothesis that irrigation treatments affected AM fungal community composition. AM fungal communities subjected to different irrigation frequencies showed no difference in alpha diversity (measured as species richness)(Figure 3.2a)($F_{1,12} = 1.331$, $p = 0.271$). Similarly, when I looked at the beta diversity of samples, I could not detect a difference in dispersion (i.e. turnover between samples) associated with FSM and CSM (Figure 3.3) ($F_{1,14}=0.903$, $p=0.369$), indicating that treatments had similar levels of beta diversity.

When I looked at how the communities changed in terms of composition and abundance of taxa, I found no difference between communities associated with CSM and FSM irrigation.
regimes. This was found when I considered the distance between communities in terms of both a weighted phylogenetic measure of distance (weighted-UniFrac distances (Figure 3.3)(F_{pseudo, 1,15} =0.396, p=0.828), as well as a simple measure of fungal identity (Sørensen similarity based on presence or absence of each OTU per treatment) (F_{pseudo, 1,6} =0.698, p= 0.718). Neither soil chemistry (F_{pseudo 1,15} =0.716, p=0.647)(Figure 3.4), nor plant physiological traits (F_{pseudo 1,15} =2.541, p=0.057)(Figure 3.5) differed with irrigation frequency.

I did, however, find an effect of irrigation on AM fungal colonization. I found a significant increase in root arbuscular colonization (F_{1,28} = 6.324, p = 0.018)(Figure 3.6a) in FSM compared with CSM, but no change in the vesicles(F_{1,28} = 1.795, p = 0.208)(Figure 3.6b).

### 3.3.1.2 Community differences between blocks

While I could not detect an irrigation effect on AM fungal communities, I found differences between the two blocks. The roots of Shiraz vines had significantly fewer fungal species than the vines in the Merlot block (Figure 3.2b)(F_{1,12} =4.834, p = 0.048). When looking at community composition there was a block effect based both on Sørensen similarity (F_{pseudo, 1,15} =3.065, p= 0.007) and Unifrac values (F_{pseudo,1,15} =3.407, p=0.023). Figure 3.3 shows that between PCO1 (33.2% total variation) and PCO2 (14.1% total variation) the communities associated with the two blocks have minimal overlap, indicating they are different based on their taxonomic composition. In Figure 3.8 the difference in communities can be seen as the Shiraz block on average contains less of the *Rhizophagus* genus, but more of the unidentified Glomeraceae group.

When considering other factors that contributed to observed experimental block differences, both soil chemistry (F_{pseudo,1,15} =4.4326, p=0.002) (Figure 3.4) and vine physiology (F_{pseudo,1,15} = 9.708, p=0.0003) (Figure 3.5) differed between the blocks. Due to this result, I repeated the PERMANOVA, this time including these variables as covariates in order to account
for influences they may have on the communities. In order to reduce the overall number of variables in the model I used the first two PCA axes, for each set of data, to represent the variables (Tables 3.1 and 3.2). How the different variables contributed to these axes can be found in Table 3.3 and Table 3.4. I found differences in soil chemistry contributed to 60.1% of the variation (38.1% PC1 & 22% PC2) (Table 3.1), whereas plant physiology axes contributed to 72.7% of the observed variation (43.3% PC1 & 29.4% PC2) (Table 3.2). After including these factors as covariates in my PERMANOVA, I found that AM fungal community structure no longer differed between the two blocks based on either the Sørensen similarity ($F_{\text{pseudo},1,15} = 1.345$, $p = 0.244$) or Unifrac values ($F_{\text{pseudo},1,15} = 0.530$, $p = 0.711$).

### 3.3.2 Explaining community variance

To elucidate the relative importance of different soil and vine characteristics in explaining the community variance observed I performed a DISTLM test. Carbon ($F_{\text{pseudo},1,15} = 3.194$, $p = 0.028$) and vine shoot length ($F_{\text{pseudo},1,15} = 2.902$, $p = 0.038$) both explained a significant proportion of differences among AM fungal communities (Table 3.5). Photosynthesis ($F_{\text{pseudo},1,15} = 2.340$, $p = 0.074$), was not significant at $p = 0.05$, but indicates a role for plant primary production or vine vigour. When combining the effects of chemistry and plant variables to create models that further explain community variance, the best 10 models included combinations of these variables (soil carbon, vine shoot length and photosynthesis) and the micronutrients boron, copper and sodium (Table 3.6). The model that explained the most community variance (31%) was composed of soil carbon and copper. When these variables were incorporated into a distance based redundancy analysis (dbRDA) the resulting ordination (Figure 3.7) illustrates carbon is associated with dbRDA1 (58% fitted, 18.7% of total variation) and copper with dbRDA2 (39.3% fitted, 12.7% of total variation).
3.4 Discussion

Despite large differences in soil moisture profiles, I found AM fungal community composition to be insensitive to irrigation frequency. Although there were significant increases in arbuscular colonization of roots under fluctuating DI, differences in AM fungal communities were only apparent between the two experimental blocks.

3.4.1 Fungal response to irrigation

I found no difference in the AM fungal communities exposed to different irrigation frequencies. Thus there was no support for the prediction that when exposed to fluctuating moisture conditions, communities would have higher species richness and a different composition than communities in constantly moist soils. Previously, studies have shown differential growth (Blomberg and Adler, 1992; Wardle, 2002; Coleman et al., 2004), diversity (Jacobson, 1997; Miller and Bever, 1999; Ipsilantis and Sylvia, 2007) and abundance (Valentine et al., 2006; Schreiner et al., 2007) for AM fungi in response to water stress, but these studies did not consider community composition as done here. As the arid soils of the Okanagan represent an extreme growth habitat for AM fungi, it may be that soils are naturally so dry that any irrigation, regardless of the frequency, alleviates drought stress. Thus, differences may be apparent between arid and irrigated soils, rather than among different frequencies of irrigation.

I did however detect higher arbuscular colonization for fungi exposed to the fluctuating soil moisture. This signifies a possible functional change in these roots. Since arbuscules are known to be the site of carbon and nutrient exchange (Smith and Read, 2008), it may be that fungi altered the allocation of resources under the different DI regimes. Similar results have been seen both with grapevines and other host plants when dealing with soil moisture stress. Schreiner et al. (2007) observed that vines associating with DI had increased arbuscular levels compared to non-DI vines. A field study using Lythrum salicaria grown in a gradient of soil moisture conditions (Stevens and Peterson, 1996) also had the highest root colonization in plants
grown in the driest soils. And lastly this pattern was seen in *Boswellia papyrifera* subjected to irregular pulses of water instead of regular continual watering regimes (Birhane *et al*., 2012). It is still unclear whether this pattern results from the fungi being directly affected by the soil moisture, or if plant stress response to irrigation mediates this response. In any case, AM fungi have changed resource allocation, which may affect the quality of the symbiosis.

### 3.4.2 Block differences

I observed significant differences in AM fungal communities between the two blocks. These differences were observed in the species richness and community composition of AM fungi. However, block differences were no longer significant after incorporating chemistry and vine physiological covariates, indicating that soil and host physiological differences played a large role in determining AM fungal community structure. Though this study cannot test for differences between varietals it is understood that grape varieties differ in both physiology and phenology (Coombe, 1960; Schriener, 2003; Costantini *et al*., 2008) and this was established for our system by Bowen *et al*. (2012a, b). Furthermore, in the DISTLM analysis, vine shoot length explained a significant proportion of the fungal community variance, and photosynthesis also associated with differences in the communities when creating a model to best explain this variance. These plant physiological traits could be proxies for the amount of sugar available for root allocation, which can affect fungal abundance and communities (Heinemeyer *et al*., 2003; Bever *et al*., 2009; Helgason and Fitter, 2009).

It is important to note that soil chemistry differed between the blocks as well (discussed below), and this can also lead to differences in plant physiology, indirectly affecting AM fungi (Jones *et al*., 2004; Hartmann *et al*., 2009; Johnson, 2010). For example, fungi able to tolerate specific conditions in the soil might have differential effects on host performance (Lekberg *et al*., 2007; Dumbrell *et al*., 2010; Lekberg *et al*., 2011). The relative role of host versus environment must
be further investigated if we are to understand the formation and function of AM fungal communities within vineyards.

Whether these physiological differences are due to varietal traits or resulted from environmental variation between the two blocks is not known, and is beyond the scope of this study. However, the relative role of host versus environment must be further investigated if we are to understand the formation and function of AM fungal communities within vineyards.

3.4.3 Spatial soil heterogeneity

It is possible that AM fungal communities differed simply due to spatial heterogeneity of the soil and niche differentiation (Ettema and Wardle, 2002; Lekberg et al., 2007; Fitzsimons et al., 2008; Dumbrell et al., 2010; Lekberg et al., 2011). This phenomenon, also referred to as ‘distance decay’, predicts that community similarity will decrease with increasing spatial distance among communities (Nekola and White, 1999; Green and Bohannan, 2006; Soininen et al., 2007; Davison et al., 2012). Since the blocks in my study were physically separated, it is likely that environmental conditions differed between the blocks, and I found support for this in terms of soil chemistry.

There is increasing evidence that physiochemical properties of the soil are important determinants of AM fungal communities (i.e., pH gradients (An et al., 2008; Lekberg et al., 2011), water status (Jacobson, 1997; Miller and Bever, 1999), and temperature (Klironomos et al. 2001)), but there is no consensus as to which factors are most influential. Such differences could exert strong selection on AM fungal taxa, which may or may not be well adapted to specific soil conditions (Niche theory). Here, carbon and the micronutrients iron, sodium, aluminum, and zinc were associated with axis one of the PCA (38% variation), while phosphorous, zinc, boron and sulphur were associated with the second axis (22%)(Figure 3.4)(Table 3.2). When performing a DISTLM analysis soil carbon was the only soil variable that explained a significant proportion of the community variation. While I was not able to
distinguish between various types of soil carbon, it is possible that this represents host exudates, which are significant drivers of AM fungal communities (Bais et al. 2006; Broeckling et al. 2008; Bever et al., 2009; Hartmann et al., 2009; Yang et al. 2011). It is also possible that soil carbon and the soil communities could be affected by another common factor not measured here, such as temperature or the surrounding plant community composition.

In addition to carbon, I also identified soil boron, copper and sodium as significantly contributing to variation among AM fungal communities. Micronutrients are rarely linked to changes in community structure, with most studies instead looking at phosphorous, nitrogen and carbon. (Treseder and Allen, 2002; Fitzsimons et al., 2008; Toljander et al., 2008; Alguacil et al., 2010; Johnson, 2010). Boron, copper and sodium are all essential plant nutrients (Arnon and Stout, 1938). There is evidence that AM fungi are able to mediate the uptake for all of these nutrients (Marschner and Dell, 1994; Clark and Zeto, 1996; Clark and Zeto, 2000; Liu et al., 2000; Clark, 2002; Schreiner, 2007; Audet and Charest, 2009; Tseng et al., 2009), and this influence on nutrient uptake may cause hosts to preferentially associate with fungi that can improve access to limiting nutrients (Bever et al., 2009; Helgason and Fitter, 2009; Long et al., 2010). Alternatively, fungi may be affected directly by micronutrient levels. In some extreme cases of sewage and heavy metal contaminated soils it has been shown that the infection rate of AM fungi is often lowered with increasing levels of Cu, Zn and other heavy metals (Gildon and Tinker, 1983; Boyle and Paul, 1988; Leyval et al., 1997; Karagiannidis and Nikolaos 2000). If certain fungal species can tolerate higher levels of micronutrients and heavy metals, environmental selection may result in changed community composition.
3.4.5 Summary

This study provides important information about factors that may influence AM fungal communities in vineyards. To date, we know little about how landscape management influences these important organisms, which provide many ecosystem services important to grape growing and soil maintenance (Linderman and Davis, 2001; Rillig, 2004; Schreiner, 2007). Here I observed that the frequency of deficit irrigation did not influence the composition of AM fungi, but there may be a long-term functional change, since irrigation frequency contributed to different levels of colonization by fungal functional structures (ie. arbuscule density), specifically colonization was higher in response to less frequent irrigation. I also found that differences in soil micronutrients and plant vigor and physiology were strong drivers of variation among communities. Further studies should emphasize the functional role of these fungi communities, how they are influenced by vineyard factors, and their possible influences on grapevine physiology that controls berry compositional quality. Also, there should be further investigations into how management practices alter soil chemistry and how soil micronutrients may affect AM fungal community composition.
3.5 Tables

**Table 3.1** - Results from a principal component analysis (PCA) for soil chemistry variables associated with *Vitis* roots. The first two Eigenvalues were used as proxies for plant physiology covariates in PERMANOVA analyses and the percent variation contributed by each PC axis is given.

<table>
<thead>
<tr>
<th>PC</th>
<th>Eigenvalues</th>
<th>% Variation</th>
<th>Cum. % Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.81</td>
<td>38.1</td>
<td>38.1</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>22.0</td>
<td>60.1</td>
</tr>
<tr>
<td>3</td>
<td>1.6</td>
<td>16.0</td>
<td>76.1</td>
</tr>
<tr>
<td>4</td>
<td>0.768</td>
<td>7.7</td>
<td>83.8</td>
</tr>
<tr>
<td>5</td>
<td>0.585</td>
<td>5.9</td>
<td>89.7</td>
</tr>
</tbody>
</table>

**Table 3.2** - Results from a principal component analysis (PCA) for vine physiological traits with the percent variation contributed by each PC axis. The first two Eigenvalues were used as proxies for plant physiology covariates in PERMANOVA analyses.

<table>
<thead>
<tr>
<th>PC</th>
<th>Eigenvalues</th>
<th>% Variation</th>
<th>Cum. % Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.16</td>
<td>43.3</td>
<td>43.3</td>
</tr>
<tr>
<td>2</td>
<td>1.47</td>
<td>29.4</td>
<td>72.7</td>
</tr>
<tr>
<td>3</td>
<td>0.917</td>
<td>18.3</td>
<td>91.0</td>
</tr>
<tr>
<td>4</td>
<td>0.307</td>
<td>6.1</td>
<td>97.2</td>
</tr>
<tr>
<td>5</td>
<td>0.14</td>
<td>2.8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Table 3.3** - Eigenvectors of soil chemistry variables included in principal component analysis (PCA). These values are coefficients in the linear combinations for the variables, showing their contribution to the PC axes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>-0.432</td>
<td>0.162</td>
<td>-0.227</td>
<td>-0.235</td>
<td>0.202</td>
</tr>
<tr>
<td>B</td>
<td>0.229</td>
<td>-0.462</td>
<td>0.272</td>
<td>-0.028</td>
<td>0.113</td>
</tr>
<tr>
<td>C</td>
<td>-0.301</td>
<td>-0.231</td>
<td>0.456</td>
<td>-0.197</td>
<td>-0.304</td>
</tr>
<tr>
<td>Cu</td>
<td>-0.147</td>
<td>-0.219</td>
<td>-0.455</td>
<td>0.730</td>
<td>-0.281</td>
</tr>
<tr>
<td>Fe</td>
<td>-0.464</td>
<td>-0.052</td>
<td>-0.185</td>
<td>-0.205</td>
<td>0.177</td>
</tr>
<tr>
<td>Na</td>
<td>-0.447</td>
<td>-0.051</td>
<td>-0.039</td>
<td>0.123</td>
<td>0.410</td>
</tr>
<tr>
<td>S</td>
<td>0.290</td>
<td>-0.358</td>
<td>-0.292</td>
<td>-0.151</td>
<td>0.541</td>
</tr>
<tr>
<td>Zn</td>
<td>-0.338</td>
<td>-0.438</td>
<td>-0.001</td>
<td>-0.156</td>
<td>-0.331</td>
</tr>
<tr>
<td>pH</td>
<td>-0.181</td>
<td>-0.065</td>
<td>0.561</td>
<td>0.511</td>
<td>0.417</td>
</tr>
<tr>
<td>PO₄</td>
<td>0.050</td>
<td>-0.574</td>
<td>-0.153</td>
<td>-0.079</td>
<td>0.012</td>
</tr>
</tbody>
</table>
Table 3.4 - Eigenvectors of vine physiological variables included in principal component analysis (PCA). These values are coefficients in the linear combinations for the variables, showing the strength of their contribution to the PC axes that were used in PERMANOVA analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berry Yield</td>
<td>0.095</td>
<td>-0.777</td>
<td>0.018</td>
<td>0.470</td>
<td>0.407</td>
</tr>
<tr>
<td>Vine Shoot Length</td>
<td>0.581</td>
<td>-0.331</td>
<td>0.196</td>
<td>-0.054</td>
<td>-0.715</td>
</tr>
<tr>
<td>Leaf Dry Weight</td>
<td>0.445</td>
<td>0.528</td>
<td>0.040</td>
<td>0.718</td>
<td>0.074</td>
</tr>
<tr>
<td>Photosynthesis</td>
<td>-0.622</td>
<td>-0.070</td>
<td>-0.190</td>
<td>0.505</td>
<td>-0.563</td>
</tr>
<tr>
<td>Stomatal Conductance</td>
<td>0.261</td>
<td>-0.046</td>
<td>-0.961</td>
<td>-0.072</td>
<td>-0.024</td>
</tr>
</tbody>
</table>

Table 3.5 - Marginal tests from DISTLM analysis showing the proportion of variation among AM fungal communities attributed to soil chemistry and plant physiology

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pseudo-F</th>
<th>P value</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum</td>
<td>1.172</td>
<td>0.315</td>
<td>7.72E-02</td>
</tr>
<tr>
<td>Boron</td>
<td>1.213</td>
<td>0.302</td>
<td>7.98E-02</td>
</tr>
<tr>
<td>Copper</td>
<td>1.988</td>
<td>0.120</td>
<td>0.12435</td>
</tr>
<tr>
<td>Iron</td>
<td>0.904</td>
<td>0.450</td>
<td>6.07E-02</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.952</td>
<td>0.118</td>
<td>0.12238</td>
</tr>
<tr>
<td>Sulphur</td>
<td>0.842</td>
<td>0.492</td>
<td>5.67E-02</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.729</td>
<td>0.157</td>
<td>0.10992</td>
</tr>
<tr>
<td>pH</td>
<td>1.532</td>
<td>0.204</td>
<td>9.87E-02</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.340</td>
<td>0.868</td>
<td>2.37E-02</td>
</tr>
<tr>
<td>Carbon</td>
<td>3.194</td>
<td>0.028</td>
<td>0.18575</td>
</tr>
<tr>
<td>Berry Yield</td>
<td>0.339</td>
<td>0.870</td>
<td>2.36E-02</td>
</tr>
<tr>
<td>Vine Shoot Length</td>
<td>2.902</td>
<td>0.038</td>
<td>0.1717</td>
</tr>
<tr>
<td>Leaf Dry Weight</td>
<td>1.203</td>
<td>0.315</td>
<td>7.91E-02</td>
</tr>
<tr>
<td>Photosynthesis</td>
<td>2.340</td>
<td>0.074</td>
<td>0.1432</td>
</tr>
<tr>
<td>Stomatal Conductance</td>
<td>1.110</td>
<td>0.344</td>
<td>7.35E-02</td>
</tr>
</tbody>
</table>

Table 3.6 - ‘Best’ models of soil chemistry and plant physiology traits for explaining community variance. Included is the approximate proportion that each model explains, represented by the $R^2$ values

<table>
<thead>
<tr>
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3.6 Figures

Figure 3.1 - Sampling design for the Shiraz and Merlot blocks. Each rectangle (red or blue) represents an experimental unit, a composite of 9 soil cores (black dots) taken over 3 vines (turquoise). Red represents fluctuating soil moisture and blue the constant soil moisture profile.
Figure 3.2 - AM fungal alpha-diversity in roots of *Vitis* rootstock 3309C in response to; a) constant and fluctuating soil moisture, and b) block at Sunrock vineyards, Osoyoos BC Canada. Values are based on species richness, defined as the number of OTUs (confidence level of 95%) per sample. (* signify significance at $\alpha = 0.05$, n=16).
Figure 3.3 - The dispersion of AM fungal species for Merlot (open diamonds) and Shiraz (black circles) blocks that were subjected to fluctuating (F) and constant (C) soil moisture profiles at Sunrock vineyard, Osoyoos BC Canada. Each point represents a Sørensen distance value composed from OTU presence/absence data. n=22
Figure 3.4 - Principal component analysis for soil chemistry characteristics measured for vines in the Merlot (open diamonds) and Shiraz (black circles) blocks that were subjected to fluctuating (F) and constant (C) soil moisture, Sunrock vineyard, Osoyoos BC Canada (n=16). Each value represents a mean value of 3 vines, for which each had 3 soil cores taken. For each soil sample, I determined soil concentration of 14 minerals and soil pH (see Appendix I), however to reduce redundancy only Al, B, C, Cu, Fe, Na, PO₄, S, Zn, pH are included here. Vectors depict the direction of each variable and its contribution to the axes, with variables closer to the circle parameter indicating stronger contribution.
Figure 3.5 - Principal component analysis for plant physiological characteristics measured in the Merlot (open diamonds) and Shiraz (black circles) blocks that were subjected to fluctuating (F) and constant (C) soil moisture, Sunrock vineyard, Osoyoos BC Canada (n=16). Each value represents a mean value for 3 vines, for which each had physiological measures taken. These included berry yield, vine shoot length, leaf dry weight, leaf gas exchange and stomatal conductance (Bowen et al. 2012b). Vectors depict the direction of each variable and its contribution to the axes, with variables closer to the circle parameter indicating stronger contribution.
Figure 3.6 - Arbuscular (a) and vesicular (b) colonization in *Vitis* roots treated to either constant or fluctuating soil moisture. Measures represent gridline-intersect proportion for 100 fields of view. (* signify statistical significance with $\alpha=0.05$, $n=32$)
Figure 3.7 - A dbRDA plot depicting AM fungal community variance in vine roots of Merlot (open diamonds) and Shiraz (black circles) block that were subjected to fluctuating (F) and constant (C) irrigation moisture at Sunrock vineyard, Osoyoos BC Canada. Each point represents a Unifrac distance value after incorporating carbon, copper and irrigation in the community variance. n=22 Vectors depict the direction of each variable and its contribution to the axes, with variables closer to the circle parameter indicating stronger contribution.
Figure 3.8 - The taxonomic distribution for AM fungal communities associating with two blocks at Sunrock vineyard, Osoyoos BC Canada. The distributions signify the number of OTUs representing each taxonomic group found in the different plant communities. Blue – *Funneliformis*, Orange – *Glomus*, Green – *Rhizophagus*, Purple – Unidentified *Glomeraceae* groupB, Yellow – Unidentified *Glomeraceae* groupA. n=16
CHAPTER 4 – AM fungi in grapevines and interrow host communities.

4.1 Synopsis

4.1.1 AM fungi interaction with grapevines

*Vitis vinifera* (L.) is an economically important crop grown throughout many regions of the world. Unlike many agricultural systems the crop value of winegrapes can be affected more by fruit quality than yield. This quality depends on climatic conditions, provision of soil nutrients and irrigation water, and other management practices to which vines are subjected (Seguin, 1986; BCWGC, 2010). Growers are increasingly aware of the impact soil biota may have on vine and berry quality, but unfortunately we know little about how soil biota affect winegrape production.

One group of soil organisms, arbuscular mycorrhizal (AM) fungi, may be particularly important for winegrape growers. The importance of AM fungi in ecosystems has been well documented, and is linked to increases in plant productivity (van der Heijden *et al.*, 1998; Linderman and Davis, 2001), soil aggregation (Rillig, 2004; Wilson *et al.*, 2009) and nutrient uptake (Schreiner, 2007) to name a few of their services. In theory, AM fungi should be very important for *Vitis*. Because grapevines develop a relatively low density of roots and root hairs (Smart and Coombe, 1983; Mohr, 1996; Schreiner and Linderman, 2004; Schreiner, 2005a; Schreiner, 2005b;) vine growth is likely dependent on AM fungi to a high degree (Menge *et al.*, 1983). Yet, we know little about the grapevine/AM fungal symbiosis. To date AM fungi have been shown to improve *Vitis* characteristics such as water use efficiency during drought (Valentine *et al.*, 2006), vine biomass (Linderman and Davis, 2001), protection of replant vines (Nogales *et al.*, 2008), and soil nutrient uptake (Schreiner, 2007). They have also been shown to be involved in important changes to the proteome of *Vitis* (Cangahuala-Inocente *et al.*, 2011) and could be involved in the production of antioxidants such as phenolics (Baslam and Goicoechea,
2012), which are important compounds in winemaking. With a better understanding of this association, growers may be able to incorporate AM fungi into viticulture management, potentially decreasing inputs, and improving both berry yields and quality.

4.1.2 AM fungal host relations

Although AM fungi may prove invaluable for the production of grapes, there is still a lot to discover about their interaction with grafted grapevines. For instance, it has been shown that the responsiveness of the association depends on the fungal and *Vitis* genotypes involved (Linderman and Davis, 2001), yet we do not know if rootstocks of different genotypes prefer certain fungal partners to others. Host identity is known to play a large role in AM fungal community composition. While most plants are able to associate with most AM fungi in pot culture (Klironomos, 2000; Smith and Read, 2008; Opik *et al.*, 2009), plants in natural communities have AM fungal communities that are distinct from those of neighboring plants (Scheublin *et al.*, 2004; Fitter, 2005; Opik *et al.*, 2009; Torrecilla *et al.*, 2012). For example, Torrecilla *et al.* (2012) found that in a semi-arid prairie region, six plant species belonging to different families contained AM fungal communities that differed in identity and abundance of AMF taxa. While it seems that plants are not strict specialists, some plants appear to ‘select’ specific cohorts of AM fungi from the environment (specialists) more than others (generalists). There have been previous reports that suggest host preference between *Vitis* and AM fungi exist, but that soil characteristics are also important in this association (Schreiner and Mihara, 2009). This continuum will have significant implications for the wine industry if grapevine performance is linked to specific communities of AM fungi.
4.1.3 Research Objective

Here I look at the AM fungal communities found in vineyards. These were associated with the grapevines (containing rootstock *Vitis* 3309C and two scions of *V. vinifera*), plus the surrounding interrow vegetation (Table A.2). I asked whether the AM fungal community associating with grapevines was distinct from those associating with other plants in the vineyard.

4.2 Methods

4.2.1 Experimental design and sampling

Soil samples were collected in October 2010, from an existing irrigation experiment at Sunrock Vineyard near Osoyoos BC, Canada (49.5N, 119.31W). The study consisted of two experiments that were identical except for the scion variety, Merlot or Shiraz both grafted onto 3309C *Vitis* rootstocks. For the purposes of this thesis, these experiments were treated as two varietal blocks as in Chapter 3.

For each block there were 8 experimental *Vitis* units and 3 experimental interrow plant units. Each *Vitis* experimental unit was composed of 9 soil cores (15.2 cm deep and 2.5 cm in diameter) as in Chapter 3. Three soil cores were taken 20 cm from the base of 3 adjacent vines (Figure 4.1). For interrow plants, 9 soil cores were taken along each interrow (Figure 4.1). At three randomly generated locations three soil cores were taken. Soil samples were kept at -20 °C and processed separately until the sequencing stage, where they were combined resulting in 1 experimental unit representing each interrow, or 3 vines.

Lastly, to get an understanding of the AM fungal communities that are native to the vineyard and surrounding area two naturally occurring plants were sampled. Soil cores were taken surrounding 3 *Artemesia tridentata* (Nutt.) and 3 *Bromus tectorum* (L.) plants and combined into a single aggregate sample for each species. All soil samples were stored at -20 °C until further processing. Though the sampling of these plants does not allow for statistical
comparisons, it allows for discussion of the native AM fungal communities and how these compare to the fungi present in the vineyard.

### 4.2.2 Community analysis

#### 4.2.2.1 DNA extraction

Roots were separated from soil samples and washed in dH₂O. DNA extractions were performed in duplicate following the protocol outline in the MoBio PowerSoil kit (MoBio Laboratories Inc).

#### 4.2.2.2 PCR conditions and 454 sequencing

From each sample triplicate PCR amplifications were performed using the Glomeromycotan specific primer FLR3/FLR4 (Gollotte et al. 2004), which amplifies a 400 bp fragment of the large subunit of rDNA. Primers were modified for 454 pyrosequencing with the addition of Roche ligating adaptor and multiplex identification (MID) regions. Each 23 µL reaction mixture was composed of 13.25 µL ddH₂O, 5 µL 5x PCR buffer(Promega), 2 µL MgCl₂(BioLabs), 0.5 µL dNPTs(Amresco), 1 µL BSA(BioLabs), 0.25 µL GoTaq(Promega) and 0.5 µL of each primer. To this two-1 µL aliquots of DNA template were added (1 µL from each of the duplicate DNA extractions), for a total volume of 25 µL. Cycling conditions were: 95 °C for 3 min, followed by 35 cycles of (95 °C for 30 s, 52.5 °C for 30 s, 72 °C for 60 s), 72 °C for 10 min, and held at 4 °C. Using the Invitrogen SequalPrep kit, amplification products were standardized to 1.25 ng/µL before being sequenced using Roche 454 pyrosequencing GS FLX+ Titanium chemistry (UBC Prostrate Center, Vancouver Canada). It was at this standardization step that samples were combined to result in the final experimental units.

#### 4.2.2.3 Sequence analysis

Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al. 2010) was used to analyze sequences. Sequences were first filtered based on default specifications with the
following exceptions: fragment length of 370-410 bp, a maximum of 6 homopolymers, a minimum average quality score greater than 30 and zero primer MID mismatches. Sequences were organized into their respective samples using MID tags, rarified to 1250 sequences per sample and then clustered into operational taxonomic units (OTUs) based on a similarity threshold of 95% using the UCLUST algorithm (Edgar, 2010). Further, to ensure identified OTUs were not due to sequencing error, I required that each OTU be represented by a minimum of 6 sequences, or each OTU needed to be present in at least 3 samples to be retained. While I acknowledge that this might bias against rare taxa, I felt it was important to take a conservative approach. OTUs were assigned taxonomy to the family level (and genus when possible) using the RDP classifier and a custom reference database. The database was composed of sequences from Krüger et al. (2012), which had been deposited in the EMBL database (accession numbers: AM114274, AM713432, FR750012–FR750095, FR750101–FR750117, FR750126–FR750127, FR750134–FR750217, FR750220–FR750228, FR750363–FR750376, FR750526–FR750544, FR772325, FR773142–FR773152, FR774917, and HE610426-HE610427).

4.2.3 Vineyard measurements

4.2.3.1 Soil chemistry

To account for environmental factors that may have contributed to community variance I tested for differences in soil chemistry both within and between blocks. A subsample of the soils taken from each experimental unit was sent to the BC Ministry of Environment Technical Services Lab for analysis. To measure the total extractable elements in the soil (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Zn), an inductively coupled plasma-optical emission spectrometer was used in combination with the Mehlich III extractant (0.2N CH₃COOH + 0.25N NH₄NO₃ + 0.013N HNO₃ + 0.015N NH₄F + 0.001M EDTA). Soil pH was determined as the pH of a 1:1 mixture of soil:water measured using a pH meter. Lastly, total C and N were quantified using combustion elemental analysis.
4.2.3.2 Plant identity and diversity

Plant diversity was determined for both vinerows and interrows using a 20 cm by 50 cm daubenmire plot (Daubenmire 1957). For the vinerows 16 plots were sampled per variety, adjacent to experimental vines. For interrows 15 plots were sampled per variety at randomly generated locations, 5 along each of the three interrows. For each plot, the percent cover of each plant species present was determined and used to calculate a Shannon-Wiener H diversity index (Tables A.2 & A.3).

4.2.4 Data analysis

4.2.4.1 Alpha diversity of AM fungi – species richness

As a measure of alpha-diversity a species richness index based on the number of OTUs per sample was used. To test for differences between hosts, these values were compared using a one-way blocked analysis in the statistical program R-version 2.8.1 (R Development Core Team 2008), with host as a fixed factor.

4.2.4.2 Community similarity

To test for differences among AM fungal communities, I used a weighted-UniFrac distance matrix to measure community similarity. UniFrac is a distance-based measure based on phylogenetic overlap between samples (Lozupone and Knight, 2005). In addition, a Sørensen similarity matrix was created, using presence-absence data based on the OTUs present in each sample. This was done in order to emphasize the change in identity of AM species present between hosts.

4.2.4.3 Community dispersion

To determine if the beta diversity of AM fungal communities differed between hosts I used PERMADISP in PERMANOVA+ (Anderson et al. 2008) which tests for differences in the dispersion of samples from group centroids. Highly dispersed samples indicate greater Beta
diversity compared with samples that cluster more tightly around the centroid. First, presence-absence data (based on OTUs found per sample) were used to create a Sørensen similarity distance matrix. With this matrix, I executed the PERMADISP analysis based on ‘host species’, performing 9999 permutations.

4.2.4.4 Environmental data analysis

To account for other factors besides host identity that may influence the AM fungal community I measured soil chemistry and plant diversity for both interrow and vinerow plants. I tested soil chemistry and plant diversity for differences between the vine and interrow plant communities using PERMANOVA+ (Anderson et al. 2008).

Soil data were transformed in order to meet the conditions of normality. This involved log transforming soil S and Zn values. Values were then normalized. I then reduced the number of variables by testing for correlation among environmental variables using draftsman plots in PERMANOVA+. The resulting datasets included soil Al, B, C, Cu, Fe, Na, PO₄, S, Zn and pH (Table A.1), which were used to create Euclidean resemblance matrices. To determine if either soil chemistry or plant diversity differed with host, I used a one-way blocked design in PERMANOVA, where host and block were fixed factors and 9999 unrestricted permutations were performed on raw data using type III (sequential) sum of squares.

4.2.4.5 Testing for host effect

I tested for differences between host plants using a one-way blocked design PERMANOVA, with ‘plant host identity’ and ‘block’ as fixed factors. To determine if spatial changes in soil chemistry and/or background vegetation were driving observed differences in AM fungal communities, I used soil chemistry and plant diversity as covariates in my PERMANOVA model (Anderson et al. 2008). Due to the large number of soil chemistry variables, even after removing highly correlated variables, I used principal component analysis
(PCA) to reduce the number of covariates in my analysis. Thus, for the soil chemistry covariate I used the values associated with the first and second PCA axes of the soil chemistry data (Table 4.1). For plant effects, Shannon’s H diversity index values were also incorporated into the model as a covariate. Using the above model, I ran PERMANOVA using 9999 permutations on residuals under a reduced model with type I sums of squares.

4.3 Results

4.3.1 AM fungal community analyses

I found no differences in alpha diversity measurements for AM fungal communities between interrow and grapevine roots ($F_{1,21} = 0.118$, p-value=0.736) (Figure 4.2), or between the blocks ($F_{1,21} = 0.804$, p-value=0.382). There were also no differences in beta diversity among the two different host plant communities. That is, there was a similar amount of AM fungal species ‘turnover’ among samples, regardless of host identity ($F_{1,20} = 0.000$, p=0.998) (Figure 4.3).

When I looked at UniFrac values, however, I found that hosts ($F_{\text{pseudo} 1,21} = 3.603$, p=0.016) and blocks ($F_{\text{pseudo} 1,21} = 2.599$, p=0.049) (Figures 4.4 & 4.5) had significantly different AM fungal communities. Figure 4.4 illustrates this, with the fungal communities found in vines and interrows grouping separately over PCO1, which explains 57.9% of the variation in distances among samples. On average, interrow communities were largely composed of *Rhizophagus spp.*, whereas the vine-row communities had a more even distribution of *Rhizophagus spp.*, *Funneliformis spp.* and the two unidentified groups of Glomeraceae (Figure 4.5). These results were not consistent when I used the Sørensen Index as a distance metric; communities associated with grapevines were not significantly different ($F_{\text{pseudo} 1,21} = 0.490$, p=0.874) compared to interrow AM fungal communities (Figure 4.3) nor were there differences between the two blocks ($F_{\text{pseudo} 1,21} = 1.125$, p=0.353). Though some separation is seen in Figure
4.3 based on these two factors, all the points are tightly grouped along the PCO axes (17.1% axis 1 and 25.4% axis 2).

4.3.2 Contribution of environmental variables to differences in AMF communities

Plant diversity and soil chemistry were both significant covariables in the PERMANOVA. When I tested these variables separately for differences, plant diversity was higher in the interrows compared to vinerows ($F_{\text{pseudo df } 1,21}=11.054$, $p=0.004$)(Figure 4.6) with interrows being mainly composed of grasses (Tables A.2 & A.3), whereas soil chemistry only differed between the blocks ($F_{\text{pseudo df } 1,21}=7.517$, $p=0.009$). Figure 4.7 shows how both factors varied between samples, with PC1 explaining 46.1% and PC2 explaining 26.8% of the variation among samples (Table 4.2).

4.4 Discussion

4.4.1 AM communities in response to interrow or grapevine hosts

In this study, I showed that the AM fungal communities associated with grapevines differed compared to those associating with interrow vegetation. Despite our knowledge of the importance of AM fungi in agriculture and grapevines being highly mycorrhizal plants, there has been little investigation into the AM communities that form with this economically important crop. Here, I witnessed a strong host effect, which could be important to future studies and applications. This was only found when I measured distances between communities using weighted-UniFrac and not when I used the Sørensen index. These indices give slightly different information about changes in communities. Whereas Sørensen’s diversity index emphasizes changes in taxonomic identity, the weighted UniFrac index includes information about the relative abundance of each taxa present. Thus, my results indicate the change in AM fungal communities is due to a shift in abundance rather than the taxonomic identity, or taxa present, with the different hosts.
Teasing out the drivers of observed variation in AM fungal communities is not straightforward. There is evidence in the literature for both plant diversity (Chung et al., 2007; Deyn et al., 2010) and identity (Johnson et al., 1992; Scheublin et al., 2004; Torrecilla et al., 2012) affecting AM fungal diversity. In general, plant and AM fungal diversity have a positive relationship (van der Heijden et al., 1998; Alguacil et al., 2011). My results did not follow this trend and did not support my hypothesis that the more diverse interrows in terms of plant species would have higher AM species richness. The interrows, had higher plant diversity compared to vine rows, but had the same AM fungal species richness as vinerows. These results could be due to the close proximity of the plants in question. It is important to note that during soil processing, grapevine roots were found in interrow soil cores, indicating spatial overlap of the two root systems. This intimacy may have resulted in a rhizosphere containing a cosmopolitan distribution of AM fungal species, leading to the similar species richness found in both roots. Perhaps a study with more discrete “vine” and “interrow” treatments would be able to detect a difference in AM fungal species richness.

In my study, the most pronounced host effect was on the relative abundance of AM fungal taxa. For example, I saw that interrow communities were largely composed of *Rhizophagus spp.*, whereas the vine-row communities had a more even distribution of *Rhizophagus spp.*, *Funneliformis spp.* and the two unidentified groups of Glomeraceae (Figure 4.5). Previous studies have shown different hosts can lead to differences in AM species identity and diversity (Johnson et al., 1992; Dhillon, 1992; Allen et al., 1995; Helgason et al., 2002; Johnson et al., 2004; Scheublin et al., 2004; Alguacil et al. 2011; Torrecilla et al. 2012), but the mechanisms for this are not yet clear. It may be that certain plants partner best with a select few fungal species (host specialization) (Allen et al., 1995; Smith and Read 2008; Vogelsang and Bever 2009), but there is limited evidence for this.
Although this experiment was not designed to directly test the differences between vineyard and natural AM fungal communities, it is interesting to note how the fungal communities associating with naturally occurring vegetation (A. tridentate and B. tectorum) differed from Vitis and interrow plants. The AM communities associated with A. tridentate and Vitis resembled each other, as did those associated with B. tectorum and interrow communities (Figure 4.5). These comparisons indicate that not only host identity, but also the plant life history strategy may be important for determining AM fungal community composition. For example, in my study, the interrows were composed of few dominant grass species (Bromus tectorum L., Sporobolus cryptandrus Torr., Elymus repens L., Agropyron spicata Pursh., and Agropyron cristatum L.). These grasses associated primarily with the AM fungal genus Rhizophagus. Of these grass species B. tectorum, E. repens, and A. cristatum are exotic, ruderal species often found in disturbed areas. There is some evidence in the literature that invasive/exotic plants can alter local AM fungal community structure by their low degree of mycorrhizal dependency, or alternatively by associating with a subset of the available fungi compared to native plants (Helgason et al., 2002; Mummey and Rillig, 2006; Pringle et al., 2009; Seifert et al., 2009; Vogelsang and Bever, 2009; Wilson et al., 2012). Therefore the dominance of a few invasive interrow grasses could have highly influenced these fungal communities, leading to the results seen in my study.

I also found that AM fungal communities in A. tridentate and Vitis were more similar compared to each other compared to interrow grasses. Both of these plants are woody perennials. The fact that A. tridentate’s communities were more similar to that of Vitis suggests that Vitis may select for native fungi preferentially, or perhaps woody perennials associate with a broader range of taxa, but there are little data on the community dynamics of AM fungi associating with woody perennials. Opik et al. (2009) observed that plants typical of forests had similar AM fungal communities, compared with co-occurring vegetation within the same community, and
that these fungal communities were different to those found in the roots of generalist plant species found over a wide range of habitats. Differences such as these have also been found between annual grasses and perennial plants (Alguacil et al., 2012) along with legumes and non-legumes (Scheublin et al., 2004). Therefore it is possible that the needs of grapevines grafted onto *Vitis* spp. and *A. tridentate* to tolerate the semi-arid conditions of the Okanagan are different than those of *B. tectorum* and the other interrow species, leading to the distinct fungal communities found with these different plants. However, it is important to emphasize that this design cannot test for differences among native and vineyard vegetation. Rather, these data are included only to provide discussion points for future research.

### 4.4.2 Block effect on AM fungal community composition

It is interesting to note that my study was able to consistently detect differences in AM fungal communities between blocks (represented by Shiraz and Merlot scions on the 3309C rootstocks in each case). It is important not to over interpret this finding since my study was not specifically designed to test for scion varietal differences, since blocks represent environmental variation on many axes, and differences in environmental conditions could also induce differences among varietals. For example, I did detect a difference between blocks in soil chemistry (see Chapter 3). Nevertheless, this is an interesting result, considering that there was no change in the microbiota associating with interrow vegetation between the varietal blocks. One could hypothesize if environmental differences between blocks led to observed differences in my AMF communities, then there should be corresponding differences among block interrow vegetation, which was not the case. Only the grapevines showed different microbiota by block. This suggests that varietal effects may be an important source of variation in the *Vitis* root microbiota. There is little research into this phenomenon, though there is evidence for differences among rootstocks in terms of colonization (Schreiner, 2003) and growth responses to different AM fungi (Linderman and Davis, 2001). There is considerable evidence for
physiological, biochemical and ecological differences among scions, and these may lead to
community level changes in root microbiota. For example, it has been shown that scions differ
in terms of the development and physiological characteristics in terms of the climatic conditions
they tolerate (Coombe, 1960; Schreiner, 2003; Costantini et al., 2008; BCWGC, 2010). This
includes phenology, which further influences the timing of physiological events throughout the
growing season. Since we know that AM fungal communities are very responsive to
physiological changes in the host (Heinemeyer et al., 2003; Bais et al., 2006; Bever et al., 2009),
it may be that physiological differences exist between these two scions that contribute to the
unique AM fungal communities observed. Important to note is that soil chemistry was a
significant covariate when considering the differences in community composition observed
between the two blocks, but again, this is beyond the scope of my design and requires further
investigation.

4.4.3 Summary

In my study, the variation among AM fungal communities associating with *Vitis* was
driven largely by host identity (*Vitis* versus interrow vegetation). This indicates the potential for
growers to manipulate aspects of their cropping systems using below ground communities, but
we have much to learn about how AM fungi function in agrosystems. As we learn more about
the role of AM fungi in viticultural systems, the benefits that AM fungi provide their hosts will
become more apparent. As it is, there is a need to better understand these fungal communities,
both in organization and function, this study provides important evidence showing that a *Vitis*
rootstock does form unique communities. This could have implications for further analyses of
AM fungi in *Vitis*, along with future vineyard practices such as vine inoculants and interrow
management.
4.5 Tables

**Table 4.1** - Principal component analysis (PCA) for variation among soil samples in terms of soil chemistry (Al, B, C, Cu, Fe, Na, PO$_4$, S, Zn, and pH). The first two PC values were used as a proxy for soil chemistry and included in PERMANOVA tests as covariates.

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**Table 4.2** - Eigenvectors of soil chemistry variables included in principal component analysis (PCA). These values are coefficients in the linear combinations for the variables, showing their contribution to the PC axes.

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<td>0.297</td>
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<td>-0.355</td>
<td>-0.087</td>
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<tr>
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<td>-0.460</td>
<td>0.040</td>
</tr>
<tr>
<td>S</td>
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<td>-0.008</td>
<td>-0.345</td>
</tr>
<tr>
<td>Zn</td>
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<td>-0.369</td>
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<td>0.128</td>
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<td>-0.179</td>
<td>-0.389</td>
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4.6 Figures

**Figure 4.1** - Sampling design for the Shiraz and Merlot blocks. For vines, each experimental unit (blue rectangle) is represented by 9 soil cores taken from 3 adjacent vines (turquoise). Each interrow is represented by one experimental unit, composed of 9 soil cores, 3 taken at each of 3 randomly generated locations along the interrow (white circles).
Figure 4.2 - Alpha-diversity of the AM fungi found in *Vitis* rootstock 3309C and interrow plant roots from Sunrock vineyard, Osoyoos BC Canada. Species richness, determined as the number of OTUs (confidence level of 95%) per sample, was used as a proxy for alpha-diversity. n=22
**Figure 4.3** - Distribution of AM fungal species for *Vitis* rootstock 3309C (black squares), interrow grasses (open circles) and native (crosses) plant roots, from Merlot (M) or Shiraz (S) blocks and the surrounding natural area (Artemesia – A, Bromus – B) at Sunrock vineyard, Osoyoos BC Canada. Each point represents a Sørensen index value composed from OTU presence/absence data. Dispersion is represented by the amount of spread between points of a similar treatment, rather than the grouping of points from similar treatments, which shows community similarity. n=22
Figure 4.4 - AM fungal phylogenetic (UniFrac) community composition for the roots of *Vitis* (black squares), interrow (open circles) and natural (crosses) plants from Merlot (M) and Shiraz (S) blocks along with surrounding the natural area (Artemesia-A, Bromus-B) at Sunrock vineyard, Osoyoos BC Canada. n=24
**Figure 4.5** - Taxonomic distribution for AM fungal communities associating with *Vitis*, interrow and the surrounding natural plants (*Artemesia* and *Bromus*) at Sunrock vineyard, Osoyoos BC Canada. The distributions signify the number of OTUs representing each taxonomic group found in the different plant communities. Red – *Claroideoglomus*, Blue – *Funneliformis*, Orange – *Glomus*, Green – *Rhizophagus*, Purple – Unidentified *Glomeraceae* group B, Yellow – Unidentified *Glomeraceae* group A. n=24
Figure 4.6 - Plant diversity in the *Vitis* rows and interrows at Sunrock vineyard, Osoyoos BC Canada. Values represent alpha diversity using Shannon’s H index, calculated from percent coverage data (Appendix I). (*signifies significance at $\alpha=0.05$, n=46)
Figure 4.7 - Distribution of soil characteristics of *Vitis* (black squares) and interrow (open circles) plants from the Merlot (M) and Shiraz (S) experimental blocks at Sunrock vineyard, Osoyoos BC Canada. Each point represents a Euclidean distance value composed from soil Al, B, C, Cu, Fe, Na, PO₄, S, Zn and pH levels (Appendix II). n=22 Vectors depict the direction of each variable and its contribution to the axes, with variables closer to the circle parameter indicating stronger contribution.
CHAPTER 5 - Conclusions

5.1 General discussion

The goal of my thesis was to gain a better understanding of the soil communities associated with cultivated grapevines and vineyard management. To do this I focused on AM fungal communities subjected to two frequencies of deficit irrigation, which resulted in two distinct dynamics of soil moisture. This was done first looking at the biomass for a range of communities including: fungi, bacteria protozoa, nematodes, collembola and mites. I then focused solely on the AM fungal communities under these two irrigation regimes. I also investigated if there were distinct AM fungal communities associated with grafted Vitis roots, comparing these to communities associated with interrow grasses. I found evidence to support some, but not all of my hypotheses:

1) Microbial communities will have higher biomass in soils with fluctuating moisture than in constantly moist soils.

I found partial support for this hypothesis. Fungal biomass increased in fluctuating moisture conditions as predicted, whereas the other organisms had greater biomass in constantly moist soils. Bacterial however was not affected by the irrigation frequency.

2) There will be a greater abundance and species richness of AM fungi in FSM, along with a different taxonomic composition compared to CSM.

I rejected this hypothesis as no differences were detected in AM fungal species richness, abundance or community composition. I did find, however, that a functional change as more arbuscules were present with the low-frequency irrigation regime.
3) Local conditions, including soil chemistry and plant physiology measures will be associated with AM fungal community structure.

I failed to reject this hypothesis, as certain traits (both soil and plant) were found to explain a significant amount of the variation in AM fungal communities.

4) Finally, I predicted that *Vitis* and surrounding interrow plants would be associated with different AM fungal communities.

I failed to reject this hypothesis. The communities were different between these two treatments when comparing the weighted-UniFrac values.

Overall, the work presented in this thesis indicates that host identity, physiology, and soil chemistry are more important in shaping AM fungal communities than differences in irrigation frequency. This thesis provides essential insight into a rarely studied but potentially very important symbiosis between AM fungi and *Vitis*. Below, I highlight the most important contributions from my thesis:

5.1.1 Environmental factors influencing AM fungal community composition

The importance of both plant physiology and soil physiochemistry has been well documented in relation to the AM fungal community assembly (Johnson *et al*., 1992; Klironomos *et al*., 2001; Augé 2001; Heinemeyer *et al*., 2003; Broeckling *et al*., 2008; Fitzsimons *et al*., 2008; An *et al*., 2008; Bever *et al*., 2009; Hartmann *et al*., 2009; Dumbrell *et al*., 2010). However, my results are novel in that I showed variation in soil micronutrients such as boron, copper and sodium may be important in accounting for the observed variation in AM fungal communities. Though high concentrations of certain micronutrients (mainly heavy metals) have been shown to limit the growth of AM fungi (Gildon and Tinker, 1983; Boyle and
Paul, 1988; Leyval et al., 1997; Karagiannidis and Nikolaos 2000; Tseng et al., 2009), to the best of my knowledge these nutrients have only once been indicated as determinants of community composition affecting the diversity of AM fungi (Hassan et al., 2011). The mechanism driving this relationship is unclear – as I did not directly test or control for these variables and was unable to ascertain whether high or low levels of these nutrients were driving changes. If high levels of these nutrients are driving changes, then it is possible that management practices such as the use of fertilizers, pesticides and herbicides, which contain these micronutrients, could be responsible for observed variation in AM communities.

Plant physiology is another factor that has affected the composition of AM fungal communities in this study. I found that vine shoot length and photosynthesis was correlated with a significant proportion of the variation in AM fungal communities. As both photosynthesis and vine shoot length are indictors of host productivity, it makes sense they would affect AM fungi that obligately rely on host photosynthate. For example, if less photosynthate is available to fungi, it is likely that AM fungal communities will select for those taxa best able to function under low resource availability. This has been shown in past studies where carbon limitation has led to decrease in fungal abundance, increased species richness and preferential allocation to specific partners (Hayman 1974; Daft and El Giahmi 1978; Heinemeyer et al., 2003; Smith and Read, 2008; Bever et al., 2009). Increases in host carbon availability have been shown to have the opposite effect on AM fungal communities, leading to lower diversity and different community composition (Klironomos et al., 2005; Smith and Read 2008). This supports the idea that plant photosynthesis and productivity are important drivers of AM fungal community structure.
5.1.2 AM fungal species identification in community characterization

Although I was able to detect strong relationships between the above environmental factors and AM fungal communities at a broad level of taxonomy, the resolution of this study was limited by my inability to identify AM fungi to the species using the large ribosomal subunit (LSU rDNA). While this region is widely used for describing AM fungal communities (Gollotte et al., 2004; Mummey and Rillig, 2007; Kruger et al. 2009), and is ideal for next generation sequencing approaches due to the short size of its amplicon, it does not contain enough information to resolve finer levels of taxonomy in this group and has been shown to preferentially amplify taxa in the Glomeraceae (Gamper et al., 2009). Though my sequence data still allow community level changes to be detected, they do not allow species-level inferences to be made. It is unlikely that this would affect the results presented here. Rather, it would allow us to differentiate communities at a finer scale.

5.2 AM fungi and Vitis grapevines

Prior to this research, there was little ecological information about the AM symbioses in grapevines. What existed pertained largely to the increases in beneficial vine traits such as biomass, nutrient uptake and water acquisition that result from AM fungal colonization (Menge et al., 1983; Karagiannidis and Nikolaos, 2000; Linderman and Davis, 2001; Weckert, 2004; Schreiner 2005a; Valentine et al., 2006; Schreiner 2007; Nogales et al., 2009; Cangahuala-Inocente et al., 2011). Alternatively, studies have focused on the levels of root colonization when in different soil conditions (Schreiner, 2003; Schreiner, 2005b; Schreiner et al., 2007). There was little, however, dealing with AM fungal community structure (Schreiner, 2003; Schreiner and Mihara 2009; Balestrini et al., 2010) and from these limited studies it is known that grapevines are a highly mycorrhizal plant, hosting a wide range of AM fungal taxa.
In this research, I showed that the AM fungal communities associated with grapevines represent a subset of the AM fungal taxa available in the vineyard, and differ significantly from interrow plants. What my research also showed is that the varietals studied here, though grafted on the same rootstock, hosted different AM fungal communities despite the fact that their associated interrow vegetation did not. This indicates that host specificity in grapevines may trickle down to the physiological differences in varietals, but there could be other variables involved in the differences observed, and this was beyond the scope of this research.

5.3 Future Directions

This thesis provides the basis for much needed research into AM symbioses in viticultural systems. My study shows that AM fungi, which are important components of viticultural systems, respond to several factors that comprise typical vineyard management. However, we need to know more about the following.

5.3.1 Vitis specificity

I found evidence in my study that *Vitis* may preferentially associate with particular AM fungal taxa. Is this trend universal or limited to this particular study site and does it span different varietals? If this trend is found in more regions and across different varieties of *Vitis*, it could have impacts on future management and inoculant practices. There are past reports of differential responses of rootstocks and AM fungi (Linderman and Davis, 2001; Schreiner, 2003). These studies found that in different combinations of plant genotypes and fungal partners, there were varying levels of AM colonization and plant responses. This could indicate that preference does exist and there is a further need to understand the response of grapevines to AM fungi.
5.3.2 AM fungal functioning in vineyards

Perhaps the next step is to understand what these AM fungi do in vineyards. AM fungi are known to increase nutrient uptake (Karagiannidis and Nikolaos, 2000; Schreiner, 2005b; 2007) and have been associated with the production of secondary metabolites in plant hosts (Cangahuala-Inocente et al., 2011; Baslam and Goicoechea, 2012). It is therefore likely that AM fungi may affect not only vine traits such as growth (Menge, 1983; Linderman and Davis, 2001) and water use efficiency (Valentine et al. 2006), but also important berry chemistry characteristics that depend on water and nutrient uptake (Schreiner, 2005b). This is a topic that deserves further research as it could have implications for vineyard management and overall berry quality.


Baslam M. and Goicoechea N. 2012. Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. Mycorrhiza 22: 347–359


Hildebrandt U., Regvar M., and Bothe H. Arbuscular mycorrhiza and heavy metal tolerance. 2007. Phytochemistry 68(1): 139-146


Miller S.P. and Bever J.D 1999 Distribution of arbuscular mycorrhizal fungi in stands of the wetland grass Panicum hemitomon along a wide hydrologic gradient. Oecologia 199: 586-582


Schreiner RP 2005b Spatial and temporal variation of roots, arbuscular mycorrhizal fungi, and plant and soil nutrients in a mature Pinot noir (Vitis vinifera L.) vineyard in Oregon, USA. Plant Soil 276: 219–234

Schreiner P.R. 2003 Mycorrhizal colonization of grapevine rootstocks under field conditions. American Journal of Enology and Viticulture 54: 599-611


### APPENDIX A: Environmental Data

**Table A.1** - Soil chemistry values for soils collected from Merlot and Shiraz blocks vines exposed to FSM or CSM, along with the interrow and surrounding natural plants in Sunrock Vineyard, Osoyoos BC Canada.

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<th>%C</th>
<th>%N</th>
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<th>B</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>K</th>
<th>Mg</th>
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<th>S</th>
<th>Zn</th>
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<td>149.3</td>
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<td>27.4</td>
<td>110.9</td>
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<td>305.9</td>
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<td>31.7</td>
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</table>
Table A.2 - Plant species occurring in vine and interrows in Sunrock Vineyard, Osoyoos BC Canada.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
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<tbody>
<tr>
<td>Bromus Tectorum</td>
<td>Cheatgrass</td>
</tr>
<tr>
<td>Sporobolus cryptandrus</td>
<td>Dropseed</td>
</tr>
<tr>
<td>Elymus repens</td>
<td>Quackgrass</td>
</tr>
<tr>
<td>Elymus spicatus</td>
<td>Bluebunch wheatgrass</td>
</tr>
<tr>
<td>Agropyron cristatum</td>
<td>Crested wheatgrass</td>
</tr>
<tr>
<td>Elymus cicutarium</td>
<td>Stork’s bill</td>
</tr>
<tr>
<td>Heterotheca villosa</td>
<td>Golden aster</td>
</tr>
<tr>
<td>Cardaria draba</td>
<td>Hoary cress (mustard)</td>
</tr>
<tr>
<td>Polygonum Convolvulus</td>
<td>Wild buckwheat</td>
</tr>
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</table>

Table A.3 - Average percent cover of plants found in the vine and interrows in Sunrock Vineyard, Osoyoos BC Canada.

<table>
<thead>
<tr>
<th></th>
<th>B. tectorum</th>
<th>S. cryptandrus</th>
<th>E. repens</th>
<th>C. draba</th>
<th>E. spicatus</th>
<th>E. cicutarium</th>
<th>H. villosa</th>
<th>A. cristatum</th>
<th>P. convolvulus</th>
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