Vineyard Soil Communities and Arbuscular Mycorrhizal Fungi Associating with

Grapevine Roots in Response to Irrigation Frequency

by

Taylor Craig Holland

B.Sc., Simon Fraser University, 2010

A THESIS SUBMITTED IN PARTIAL

FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF

Master of Science

in

THE COLLEGE OF GRADUATE STUDIES

Biology

THE UNIVERSITY OF BRITISH COLUMBIA

(Okanagan)

April 2013

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.

PREFACE

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 Marciniak (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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AM	Arbuscular Mycorrhizal
CSM	Constant Soil Moisture
DFS	Differential Fluorescent Staining
DI	Deficit Irrigation
FSM	Fluctuating Soil Moisture
LSU	Large Subunit
MID	Multiplex Identification
MUSCLE	Multiple Sequence Comparison by Log-Expectation
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
PCA	Principal Component Analysis
PCO	Principal Coordinate Analysis
QIIME	Quantitative Insights Into Microbial Ecology
RDP	Ribosomal Database Project
TDR	Time Domain Reflectometry
dbRDA	Distance Based Redundancy Analysis

ACKNOWLEDGMENTS

There are numerous people I must recognize for aiding me through this challenging and enlightening experience. First, my supervisor Dr. Miranda Hart for her guidance, understanding and reassurance throughout this entire process. My committee members Dr. P Bowen, Dr. L Nelson, Dr. D Scott and external examiner Dr. M Jones for taking time to go through my thesis and provide me with crucial feedback. The Klironomos and Hart labs for standing beside me as we look into the black box that is soil ecology and arbuscular mycorrhizae. The UBC Okanagan Biology Department for providing an excellent and exciting academic environment for conducting research within. Barb Lucente and Jennifer Janok for truly making all of this possible, taking care of all the lost graduate students, making sure we dot our i's and cross our t's while providing the most relaxing stress free atmosphere possible.

Without having a good cohort of graduate students surrounding me the last three years would have been a painful experience. For this, the biology graduate student body deserves a huge thank-you. This truly remarkable group of people was once small but has grown into a tight knit community, making everyone feel at home. They have provided a shoulder to lean on, an ear for exhausting rants and many social opportunities to remind ourselves that we are people and that life is more than just school and research. This includes my roommates and friends of 1410 Gordon Drive, the best group of housemates anyone could ask for always knowing what to say and what needs to be done in any situation. There are many laughs and memories to be shared in years to come.

Lastly, some of the most important people in my life, my support group back at home including my friends, family, bro-dog and the best parents anyone could ask for. For all of their enduring support, reminding me who I am, and reassuring me of what truly matters whenever I felt on the brink of insanity. Without them I would not be who I am today.

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 a. 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 at. 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?

5

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 6.4×10^{-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

Shiraz

Three vines, each sampled 3 times were combined to represent 1 experimental unit.

N

Merlot
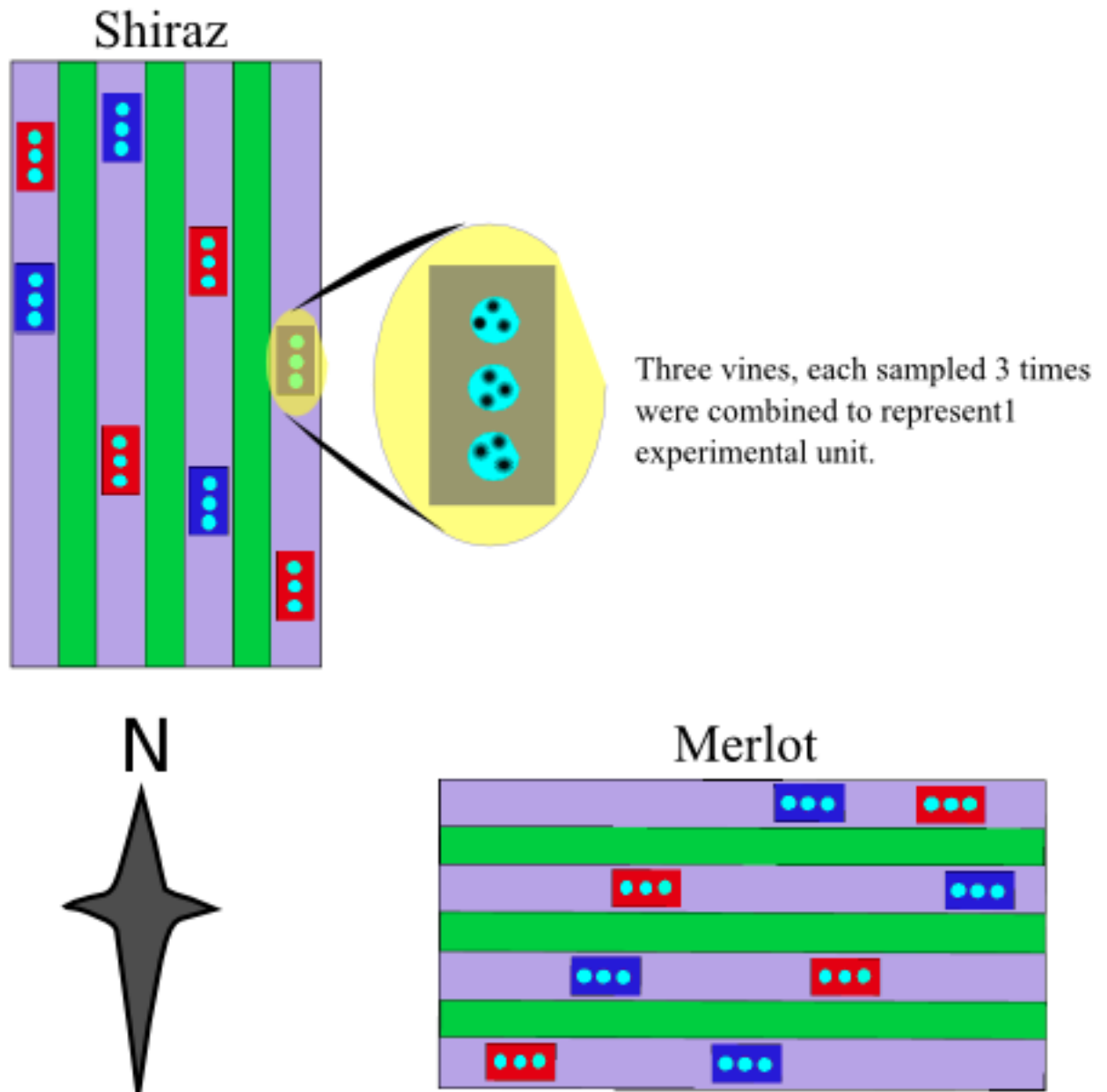
Figure 2.1 - The sampling design for both the two experimental blocks. Each colored rectangle (red and blue) represents an experimental unit, a composite of 9 soil cores (black dots) taken from 3 vines (turquoise). Red represents fluctuating soil moisture and blue the constant soil moisture profile.
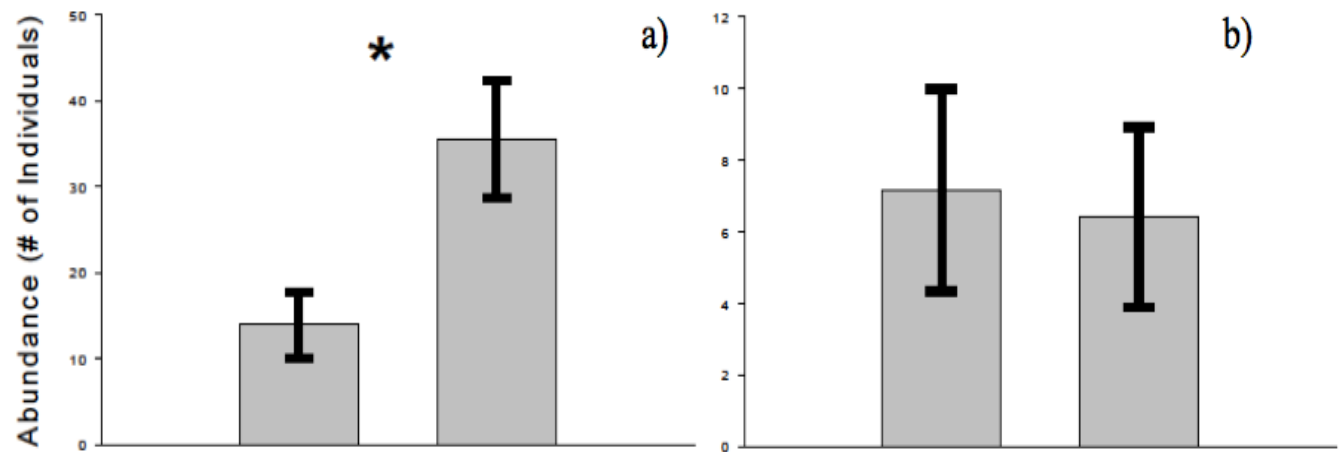
Figure 2.2 - Bacterial biomass (a) and fungal biomass (b) per gram of soil associated with all vines subjected to fluctuating (FSM) and constant (CSM) soil moisture in a vineyard in Osoyoos, BC Canada. Values represent mean ± SE, n=16. Asterisks represent significant difference for $p<0.1$
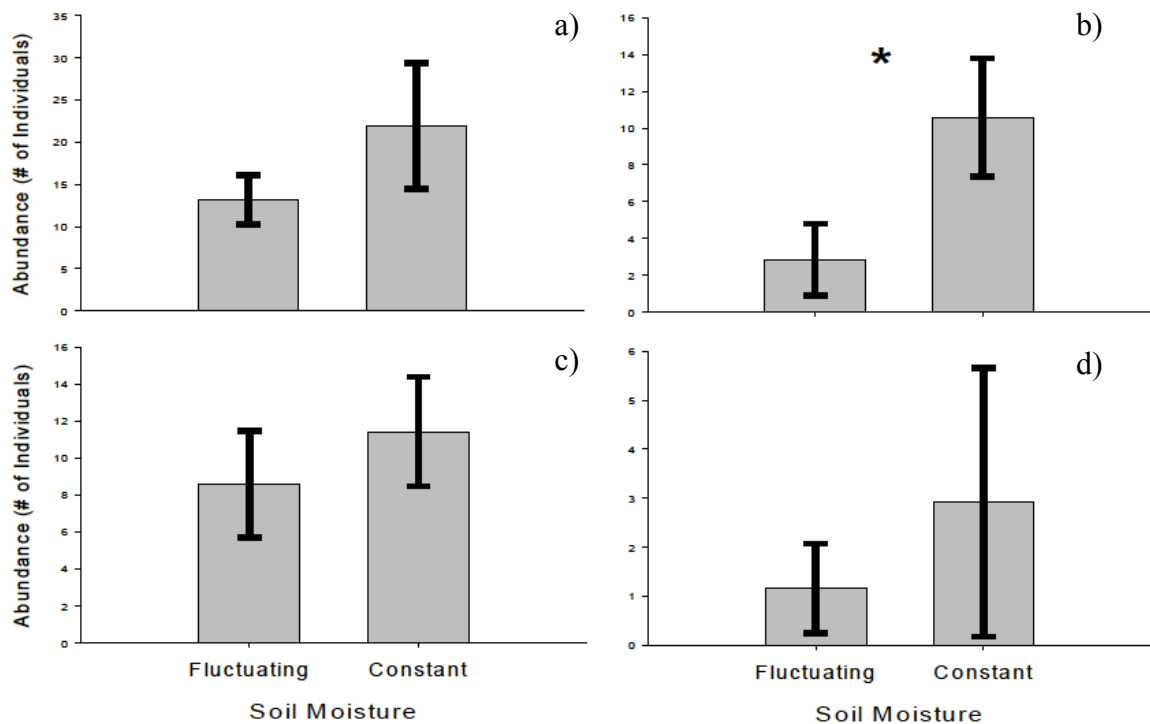
Figure 2.3 - The abundance of protozoa (a), collembola (b), nematodes (c), and mites (d) per gram of soil associated with Merlot vines grown in soils with FSM or CSM, Osoyoos, BC Canada. Values represent mean ± SE, n=8. Asterisks represent significant differences at $p<0.05$ using a t-test.

19
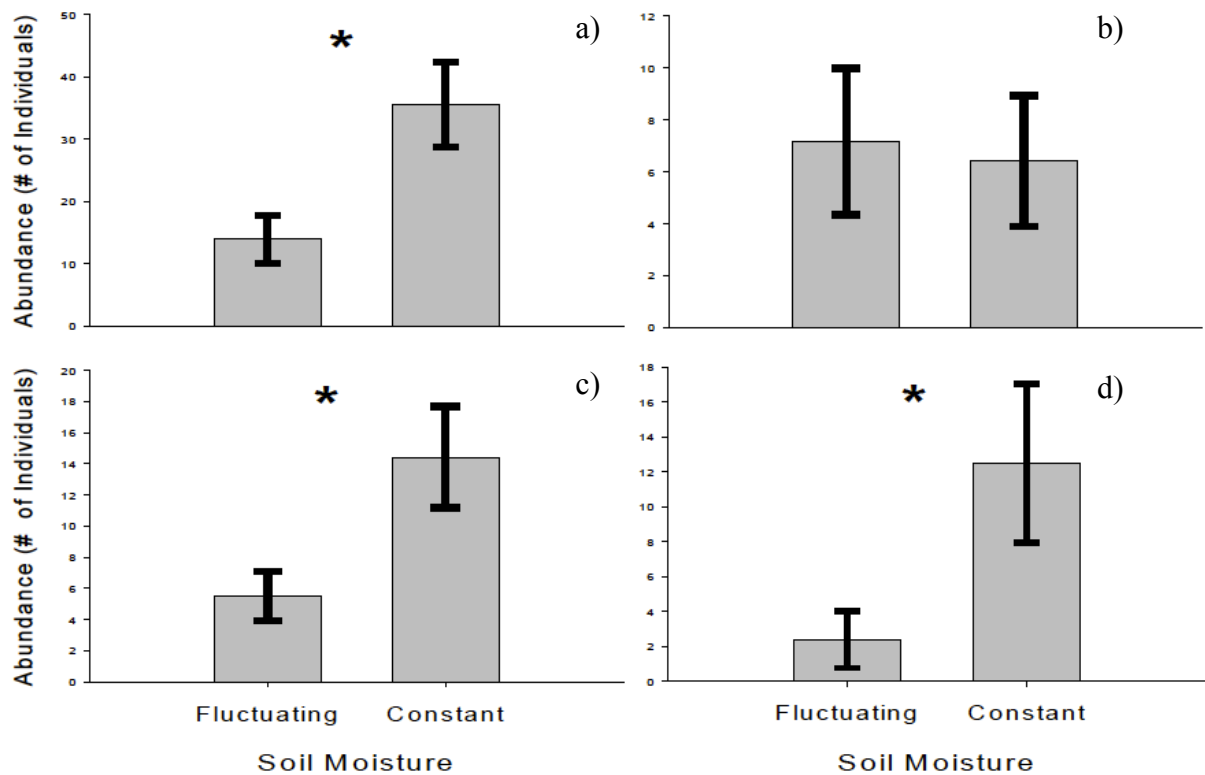
Figure 2.4 - The abundance of protozoa (a), collembola (b), nematodes (c), and mites (d) per gram of soil associated with Shiraz vines grown in soils with FSM or CSM, Osoyoos, BC Canada. Values represent mean ± SE, n=8. Asterisks represent significant differences at $p<0.05$ using a t-test.

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 (eg. 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 ddH$_2$0, 5 μL 5x PCR buffer(Promega), 2 μL MgCl$_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° for 3 min followed by 35 cycles of (95 °C for 30secs, 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 dH_2O, cleared by autoclaving for 15

min in 10% KOH, further bleached in room temperature 3% H_2O_2 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 CH_3COOH + 0.25N NH_4NO_3 + 0.013N HNO_3 + 0.015N NH_4F + 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, PO_4, 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 phylogenic 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 ($F_{pseudo,1,15}$ =1.345, p=0. 244) or Unifrac values ($F_{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_{pseudo,1,15=}$3.194, p=0.028) and vine shoot length ($F_{pseudo,1,15}$=2.902, p=0.038) both explained a significant proportion of differences among AM fungal communities (Table 3.5). Photosynthesis ($F_{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

33

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.

PC	Eigenvalues	% Variation	Cum. % Variation
1	3.81	38.1	38.1
2	2.2	22.0	60.1
3	1.6	16.0	76.1
4	0.768	7.7	83.8
5	0.585	5.9	89.7

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.

PC	Eigenvalues	% Variation	Cum. % Variation
1	2.16	43.3	43.3
2	1.47	29.4	72.7
3	0.917	18.3	91.0
4	0.307	6.1	97.2
5	0.14	2.8	100.0

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.

Variable	PC1	PC2	PC3	PC4	PC5
Al	-0.432	0.162	-0.227	-0.235	0.202
B	0.229	-0.462	0.272	-0.028	0.113
C	-0.301	-0.231	0.456	-0.197	-0.304
Cu	-0.147	-0.219	-0.455	0.730	-0.281
Fe	-0.464	-0.052	-0.185	-0.205	0.177
Na	-0.447	-0.051	-0.039	0.123	0.410
S	0.290	-0.358	-0.292	-0.151	0.541
Zn	-0.338	-0.438	-0.001	-0.156	-0.331
pH	-0.181	-0.065	0.561	0.511	0.417
PO_4	0.050	-0.574	-0.153	-0.079	0.012

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.

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

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

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

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

AICc	R^2	# Variables	Selections
-91.997	0.1857	1	Carbon
-91.723	0.1717	1	Vine shoot length
-91.591	0.3109	2	Copper, Carbon
-91.367	0.3012	2	Copper, V. shoot length
-91.247	0.2960	2	Boron, V. shoot length
-91.182	0.1432	1	Photosynthesis
-91.172	0.2927	2	Copper, photosynthesis
-90.834	0.1243	1	Copper
-90.82	0.2769	2	Boron, Carbon
-90.798	0.1224	1	Sodium

3.6 Figures
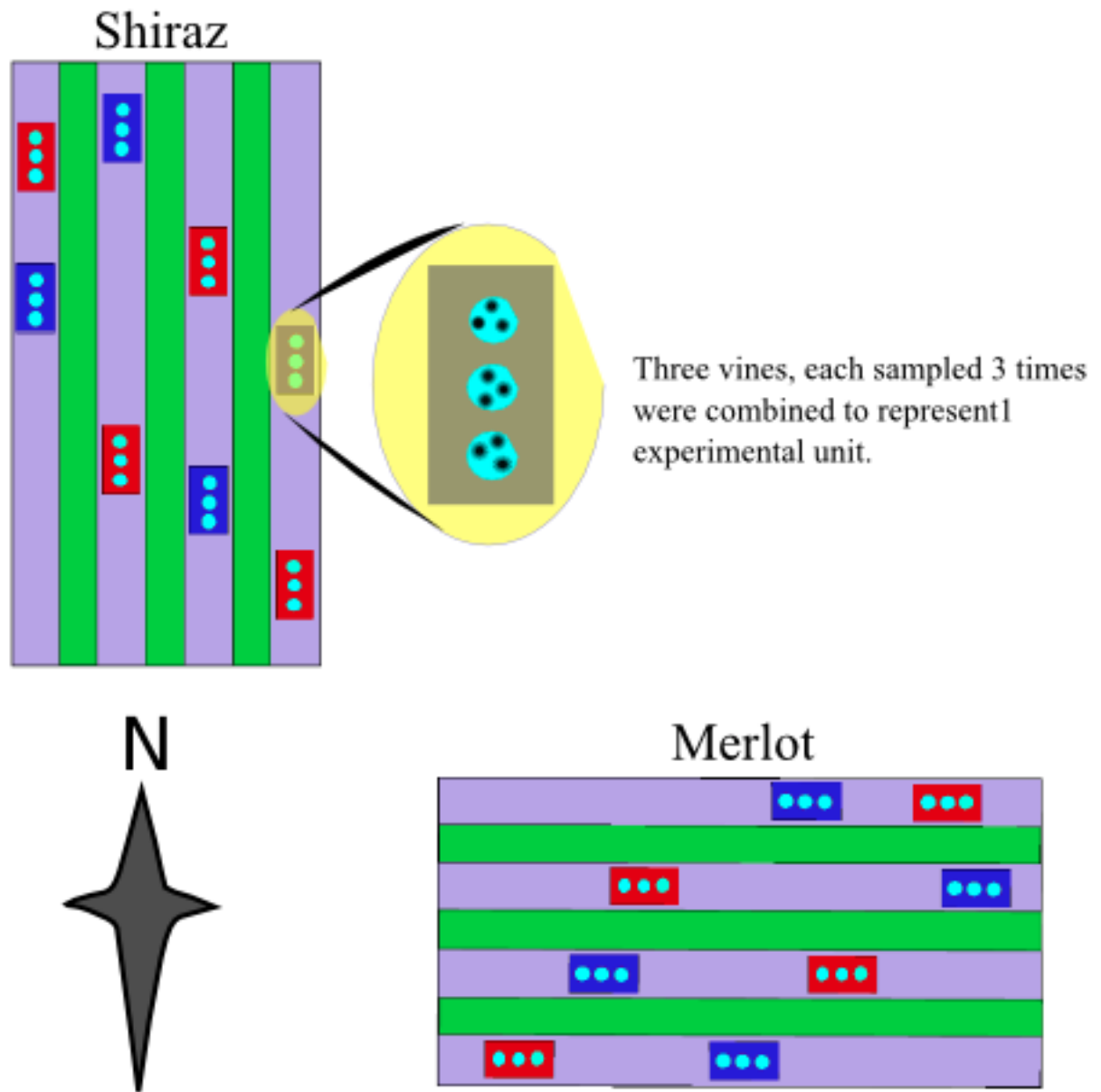
Figure 3.1 - Sampling design for the Shiraz and Merlot blocks. Each rectangle (red or blue) represents an experimental unit, a composite of 9 soil cores (black dots) taken over 3 vines (turquoise). Red represents fluctuating soil moisture and blue the constant soil moisture profile.

a)

b)
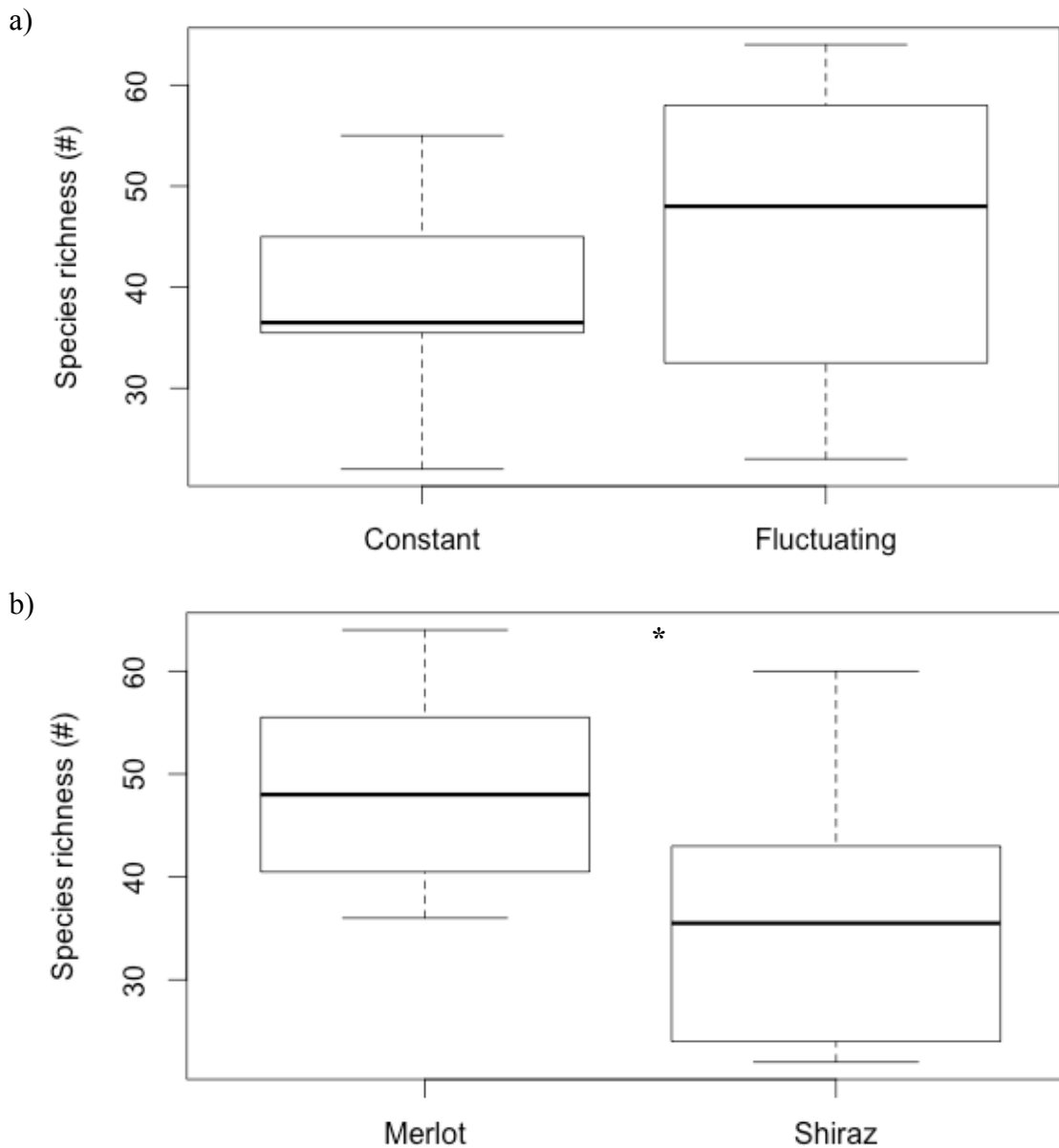
Figure 3.2 - AM fungal alpha-diversity in roots of *Vitis* rootstock 3309C in response to; a) constant and fluctuating soil moisture, and b) block at Sunrock vineyards, Osoyoos BC Canada. Values are based on species richness, defined as the number of OTUs (confidence level of 95%) per sample. (* signify significance at α= 0.05, n=16).
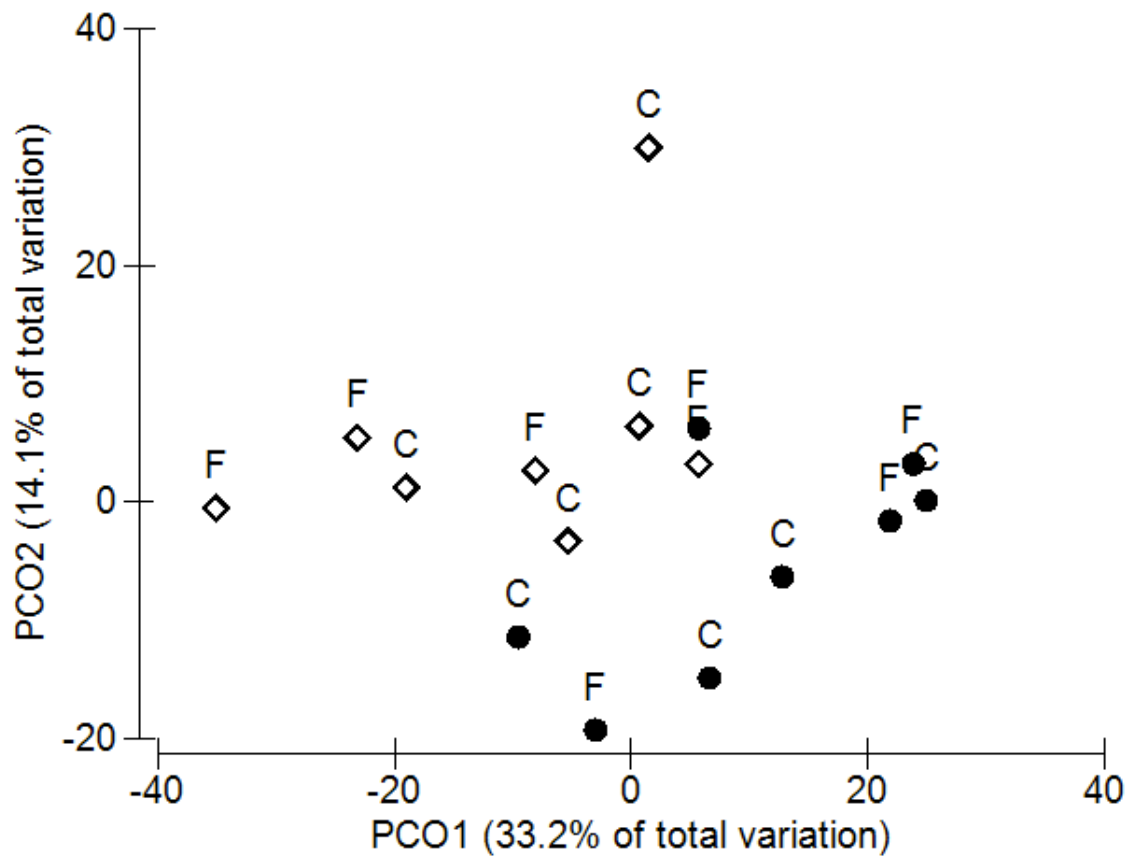
Figure 3.3 - The dispersion of AM fungal species for Merlot (open diamonds) and Shiraz (black circles) blocks that were subjected to fluctuating (F) and constant (C) soil moisture profiles at Sunrock vineyard, Osoyoos BC Canada. Each point represents a Sørensen distance value composed from OTU presence/absence data. n=22
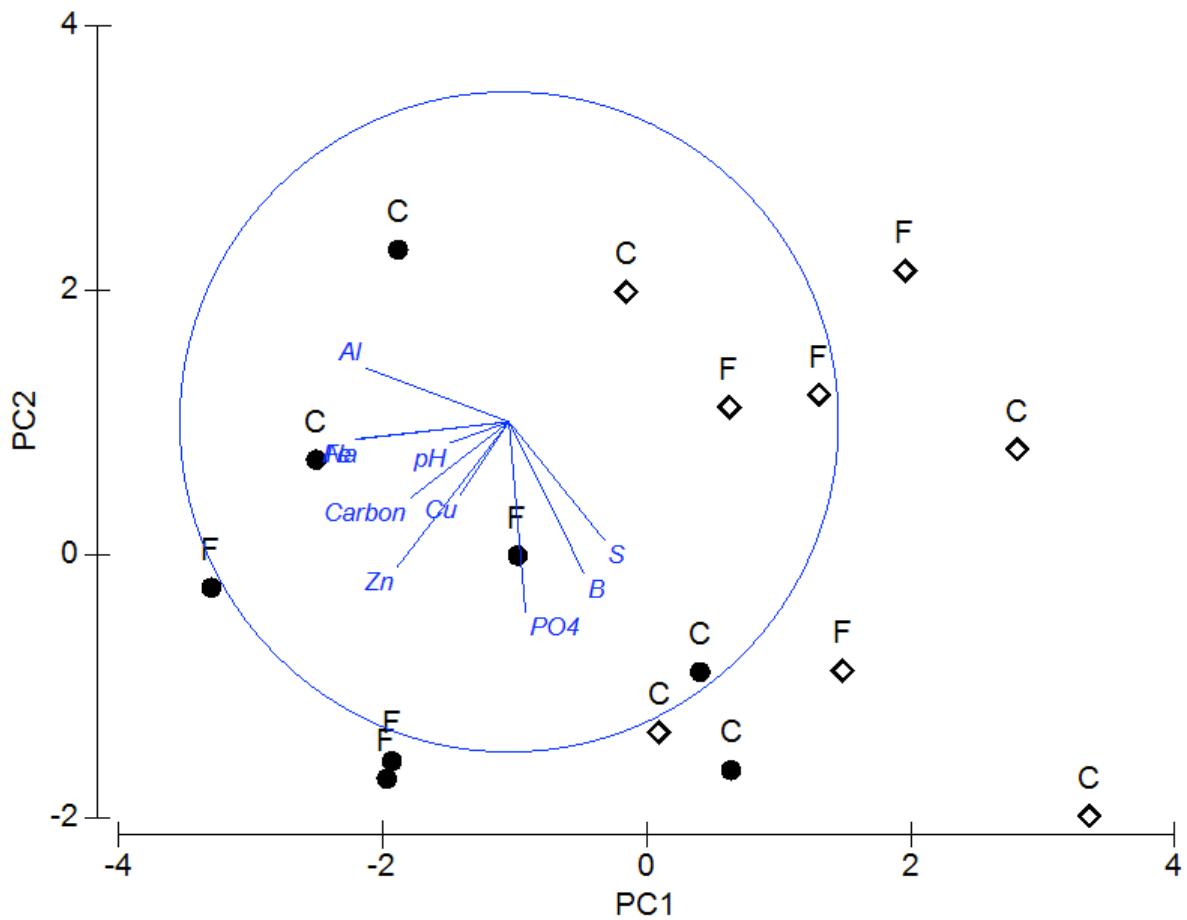
Figure 3.4 - Principal component analysis for soil chemistry characteristics measured for vines in the Merlot (open diamonds) and Shiraz (black circles) blocks that were subjected to fluctuating (F) and constant (C) soil moisture, Sunrock vineyard, Osoyoos BC Canada (n=16). Each value represents a mean 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, PO_4, 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.
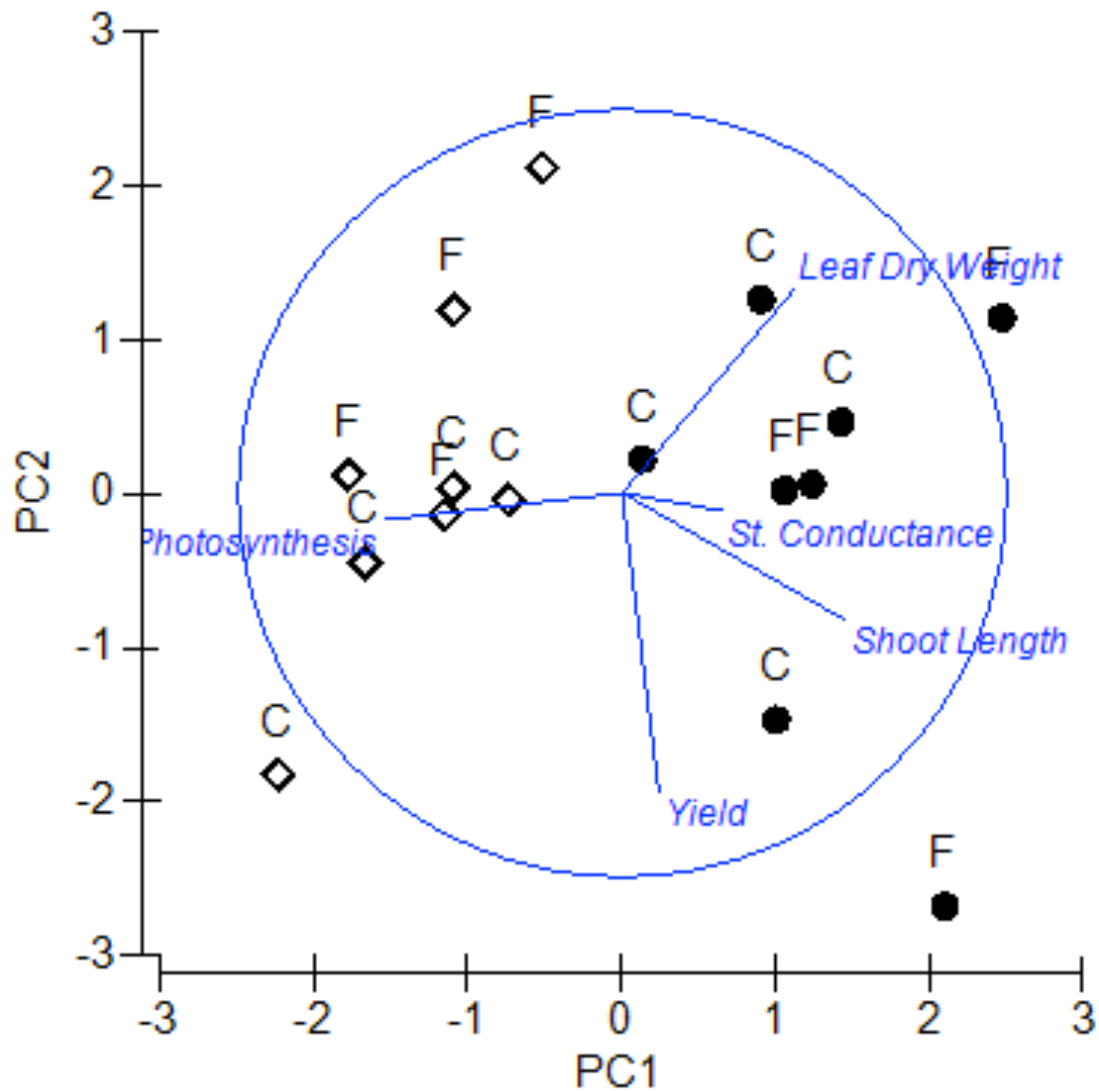
Figure 3.5 - Principal component analysis for plant physiological characteristics measured in the Merlot (open diamonds) and Shiraz (black circles) blocks that were subjected to fluctuating (F) and constant (C) soil moisture, Sunrock vineyard, Osoyoos BC Canada (n=16). Each value represents a mean value 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.
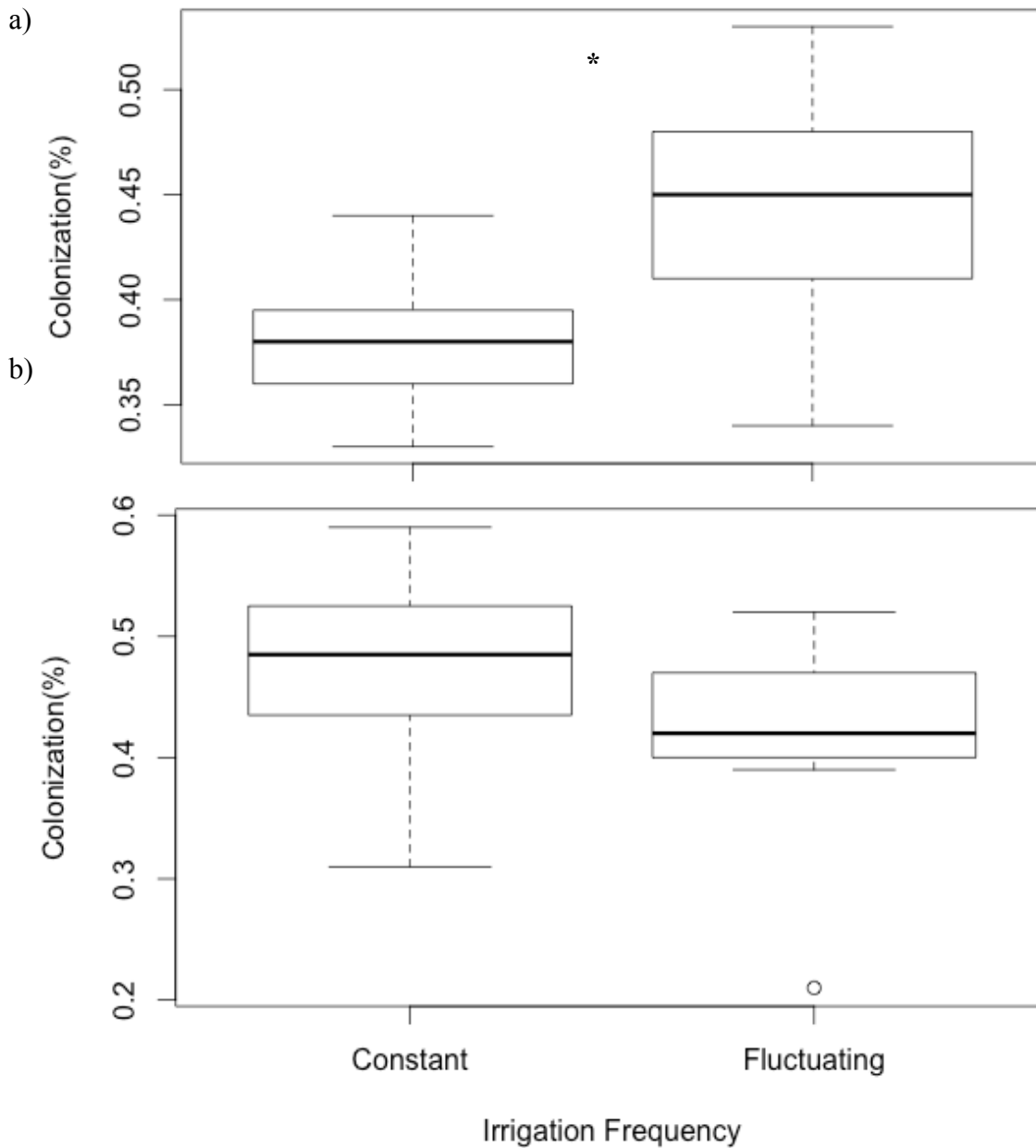
Figure 3.6 - Arbuscular (a) and vesicular (b) colonization in *Vitis* roots treated to either constant or fluctuating soil moisture. Measures represent gridline-intersect proportion for 100 fields of view. (* signify statistical significance with α=0.05, n=32)
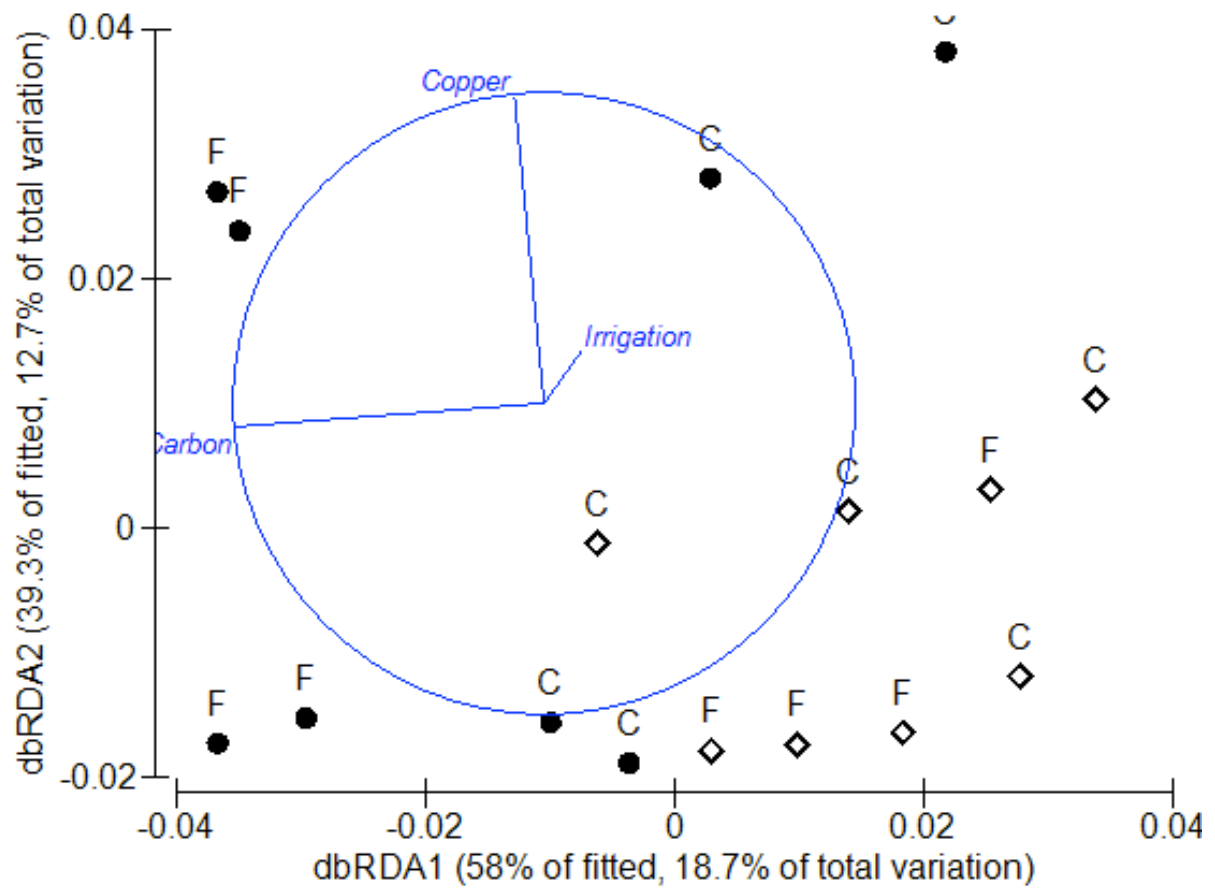
Figure 3.7 - A dbRDA plot depicting AM fungal community variance in vine roots of Merlot (open diamonds) and Shiraz (black circles) block that were subjected to fluctuating (F) and constant (C) irrigation moisture at Sunrock vineyard, Osoyoos BC Canada. Each point represents a Unifrac distance value 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.
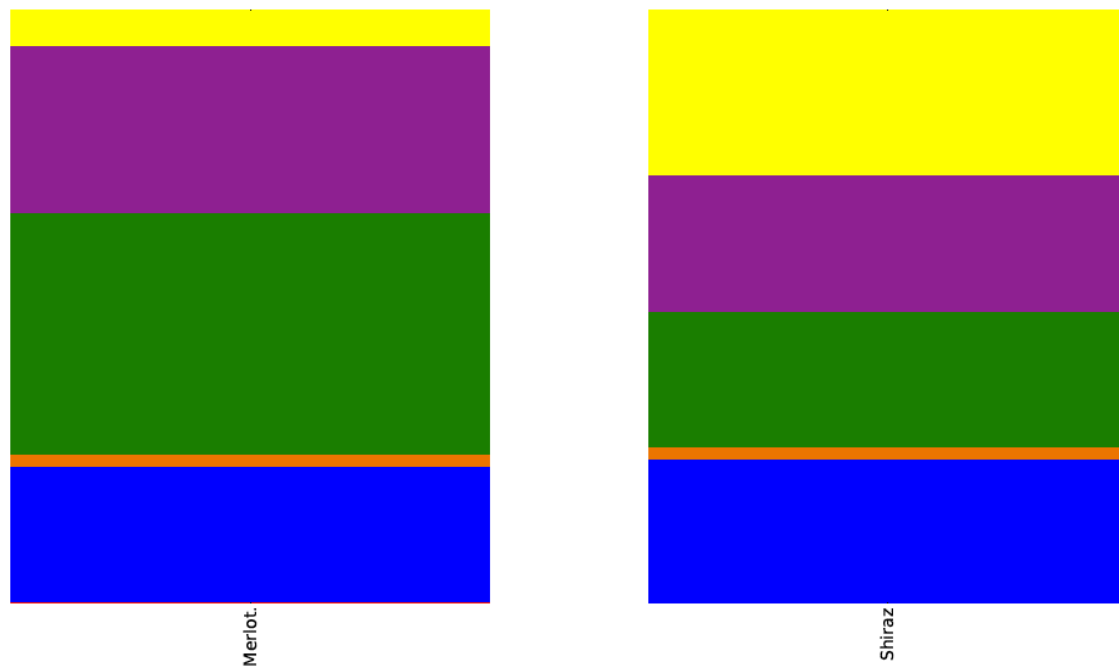
Figure 3.8 - The taxonomic distribution for AM fungal communities associating with two blocks at Sunrock vineyard, Osoyoos BC Canada. The distributions signify the number of OTUs representing each taxonomic group found in the different plant communities. Blue – *Funneliformis*, Orange – *Glomus*, Green – *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 dH_2O. 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 ddH_2O, 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. 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

51

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 $(0.2N\ CH_3COOH + 0.25N\ NH_4NO_3 +$

$0.013N\ HNO_3 + 0.015N\ NH_4F + 0.001M\ EDTA)$. 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, PO_4, 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 ($F_{pseudo\ 1,21}$=3.603,

p=0.016) and blocks ($F_{pseudo\ 1,21}$=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 ($F_{pseudo\ 1,21}$=0.490,

p=0.874) compared to interrow AM fungal communities (Figure 4.3) nor were there differences

between the two blocks ($F_{pseudo\ 1,21}$=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

PC	Eigenvalues	% Variation	Cum. % Variation
1	6.45	46.1	46.1
2	3.75	26.8	72.8
3	1.4	10.0	82.8

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

Variable	PC1	PC2	PC3
Al	0.078	-0.461	0.058
B	0.309	0.281	-0.129
C	0.364	0.155	-0.014
Ca	0.354	-0.057	0.152
Cu	0.075	-0.111	-0.575
Fe	0.232	-0.353	-0.133
K	0.314	0.191	-0.296
Mg	0.214	-0.351	0.297
Mn	0.261	-0.355	-0.087
Na	-0.120	-0.460	0.040
S	-0.280	-0.008	-0.345
Zn	0.321	-0.073	-0.369
pH	0.335	0.098	0.128
PO$_4$	-0.247	-0.179	-0.389
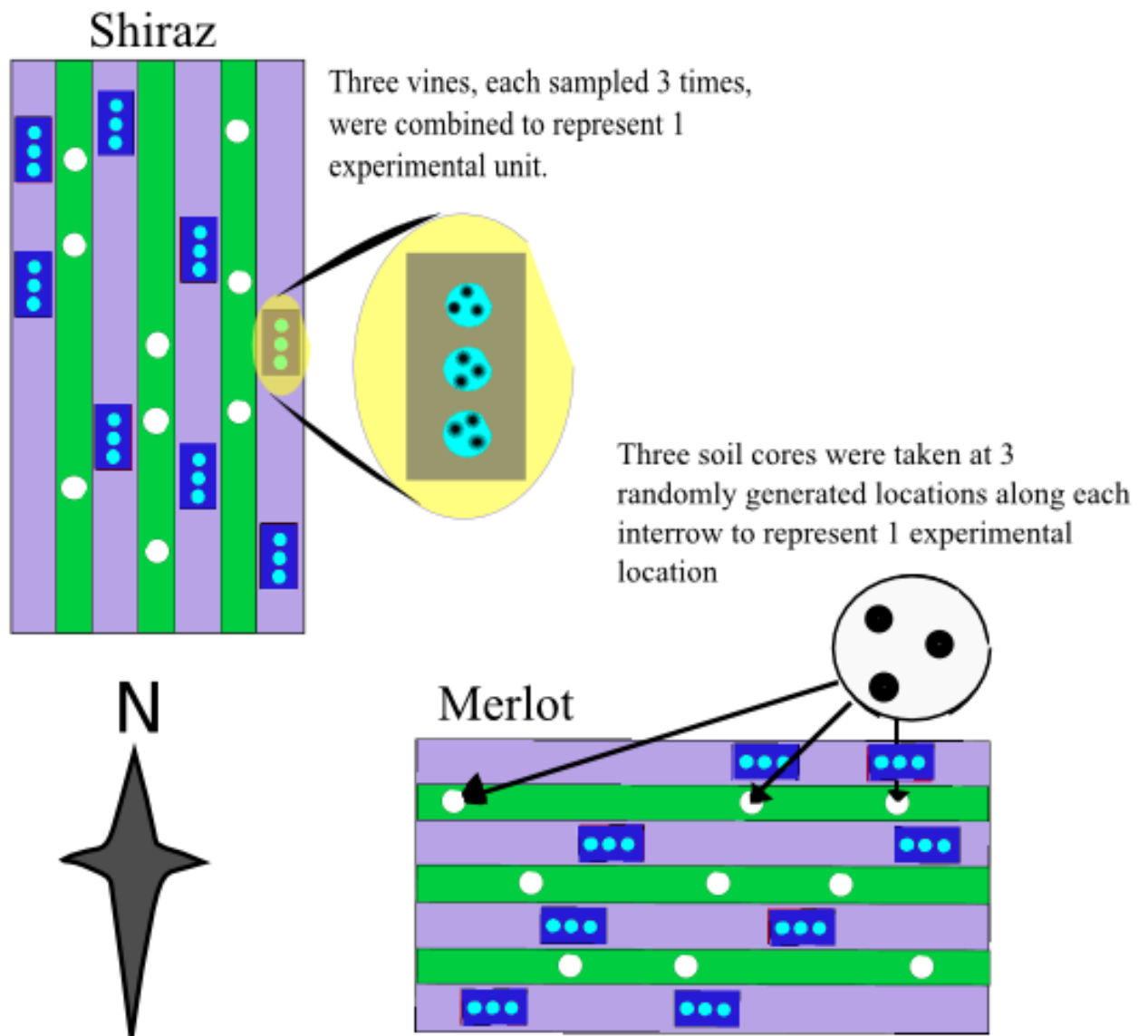
Figure 4.1 - Sampling design for the Shiraz and Merlot blocks. For vines, each experimental unit (blue rectangle) is represented by 9 soil cores taken from 3 adjacent vines (turquoise). Each interrow is represented by one experimental unit, composed of 9 soil cores, 3 taken at each of 3 randomly generated locations along the interrow (white circles).
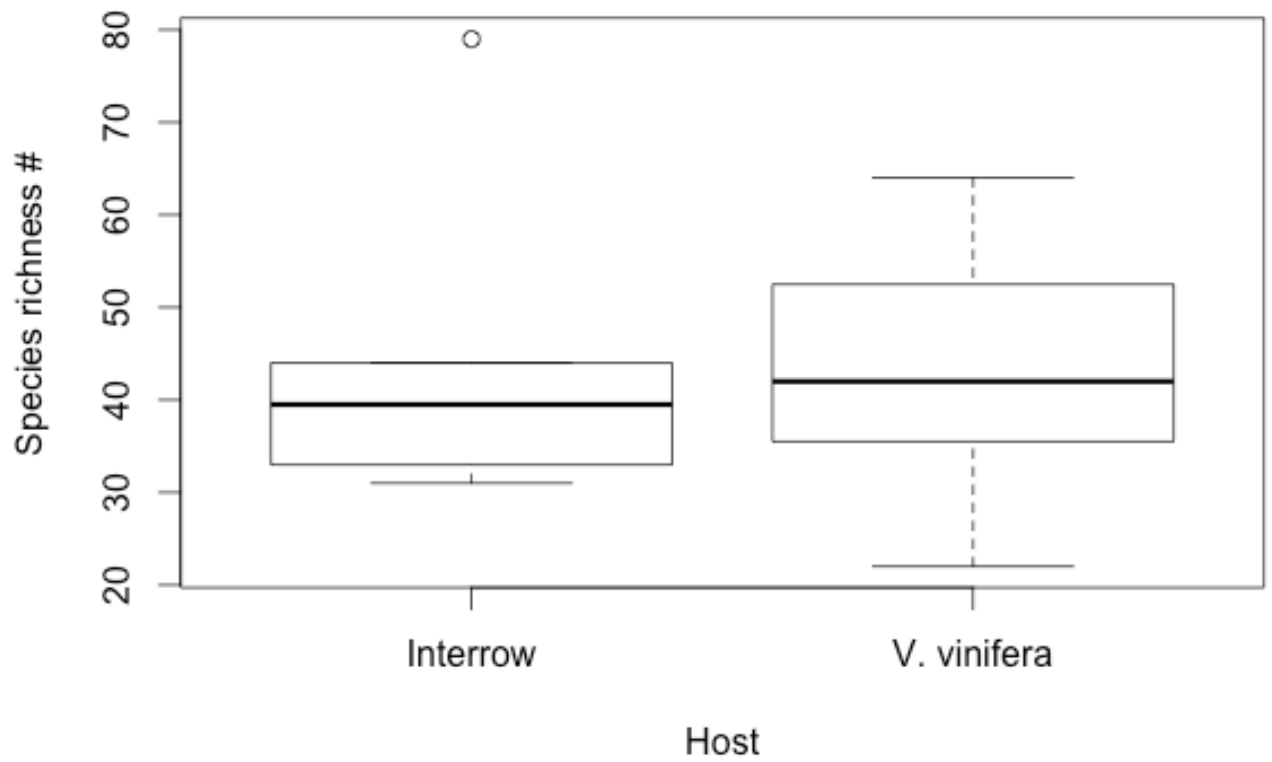
Figure 4.2 - Alpha-diversity of the AM fungi found in *Vitis* rootstock 3309C and interrow plant roots from Sunrock vineyard, Osoyoos BC Canada. Species richness, determined as the number of OTUs (confidence level of 95%) per sample, was used as a proxy for alpha-diversity. n=22
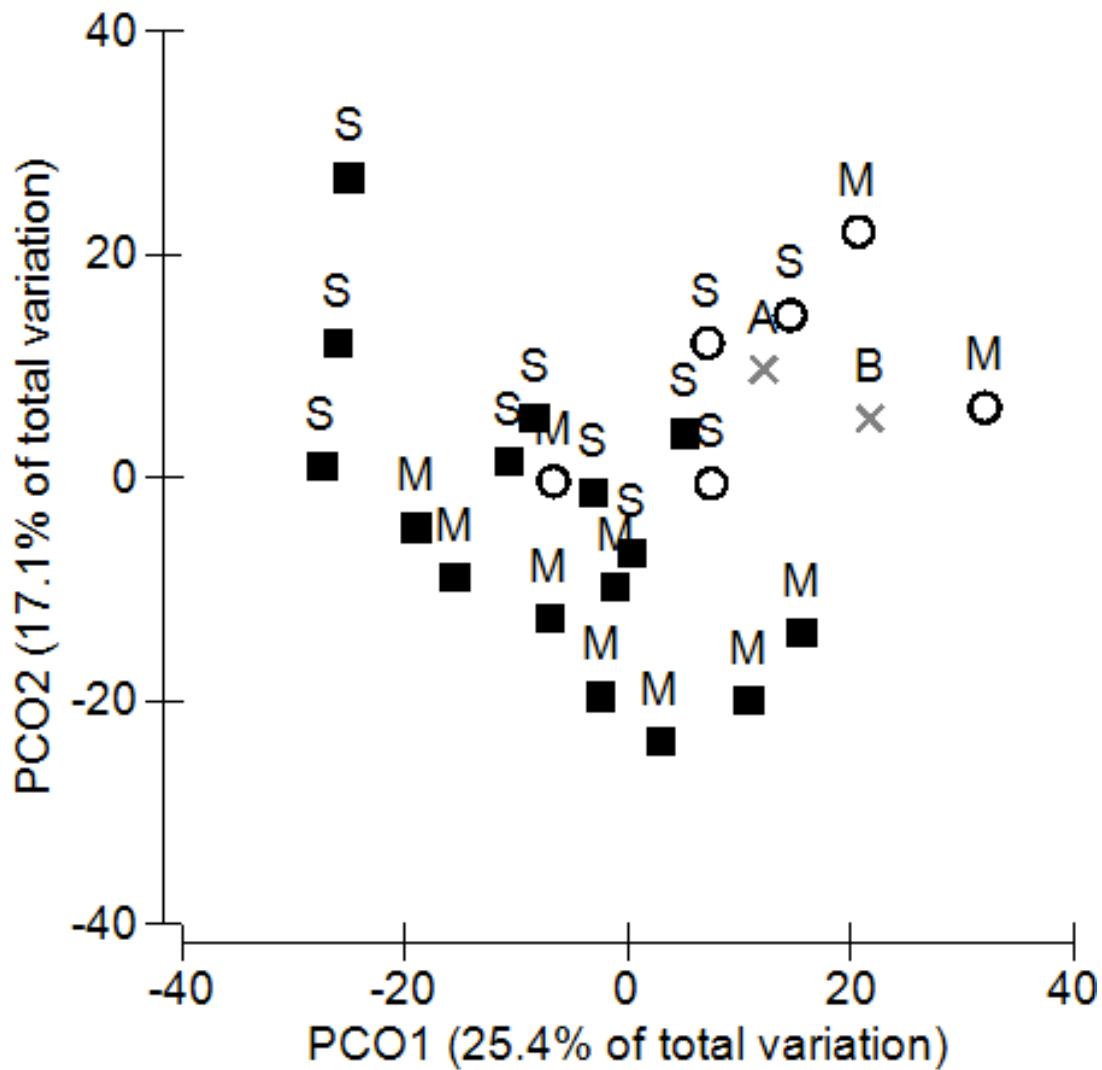
Figure 4.3 - Distribution of AM fungal species for *Vitis* rootstock 3309C(black squares), interrow grasses (open circles) and native (crosses) plant roots, from Merlot (M) or Shiraz (S) blocks and the surrounding natural area (Artemesia – A, Bromus – B) at Sunrock vineyard, Osoyoos BC Canada. Each point 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
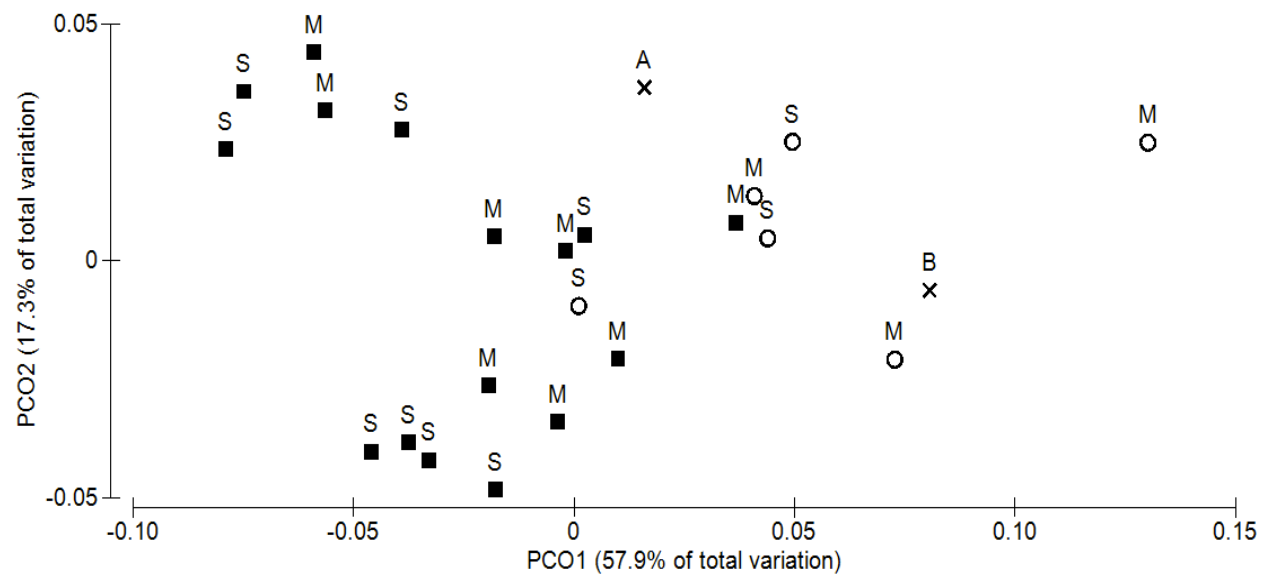
Figure 4.4 - AM fungal phylogenetic (UniFrac) community composition for the roots of *Vitis* (black squares), interrow (open circles) and natural (crosses) plants from Merlot (M) and Shiraz (S) blocks along with surrounding the natural area (Artemesia-A, Bromus-B) at Sunrock vineyard, Osoyoos BC Canada. n=24
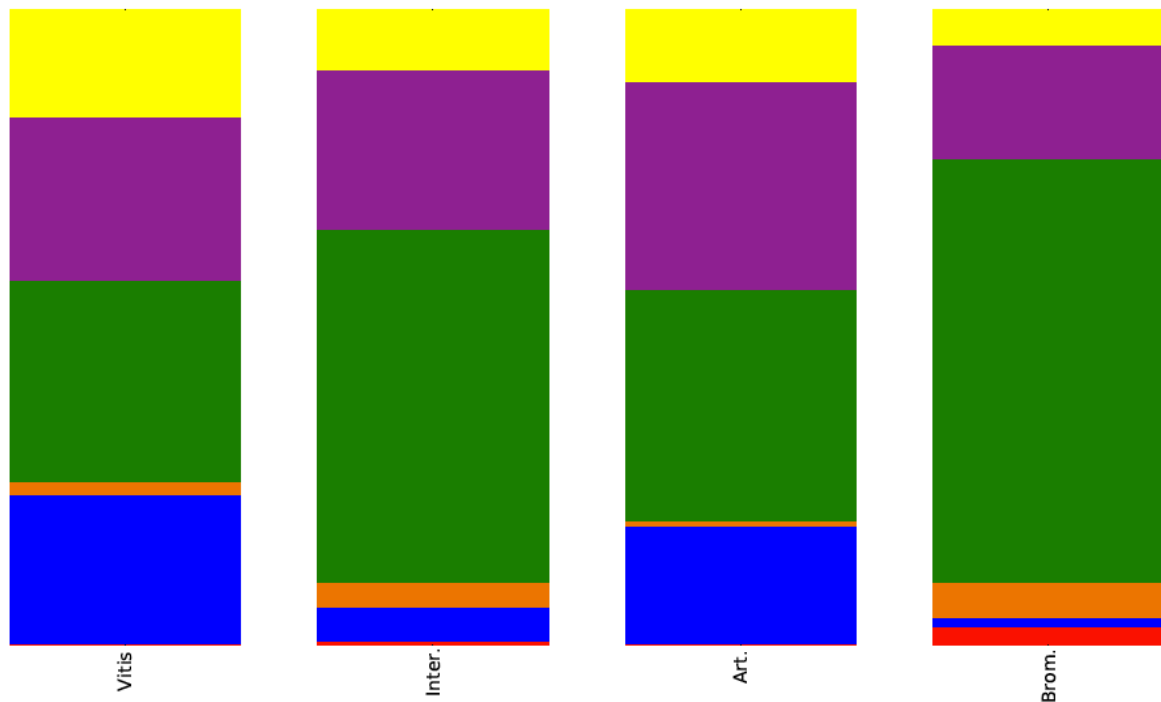
Figure 4.5 - Taxonomic distribution for AM fungal communities associating with *Vitis,* interrow and the surrounding natural plants (*Artemesia* and <u>*Bromus*</u>) at Sunrock vineyard, Osoyoos BC Canada. The distributions signify the number of OTUs representing each taxonomic group found in the different plant communities. Red – *Claroideoglomus*, Blue – *Funneliformis*, Orange – *Glomus*, Green – *Rhizophagus*, Purple – Unidentified *Glomeraceae* group B, Yellow – Unidentified *Glomeraceae* group A. n=24
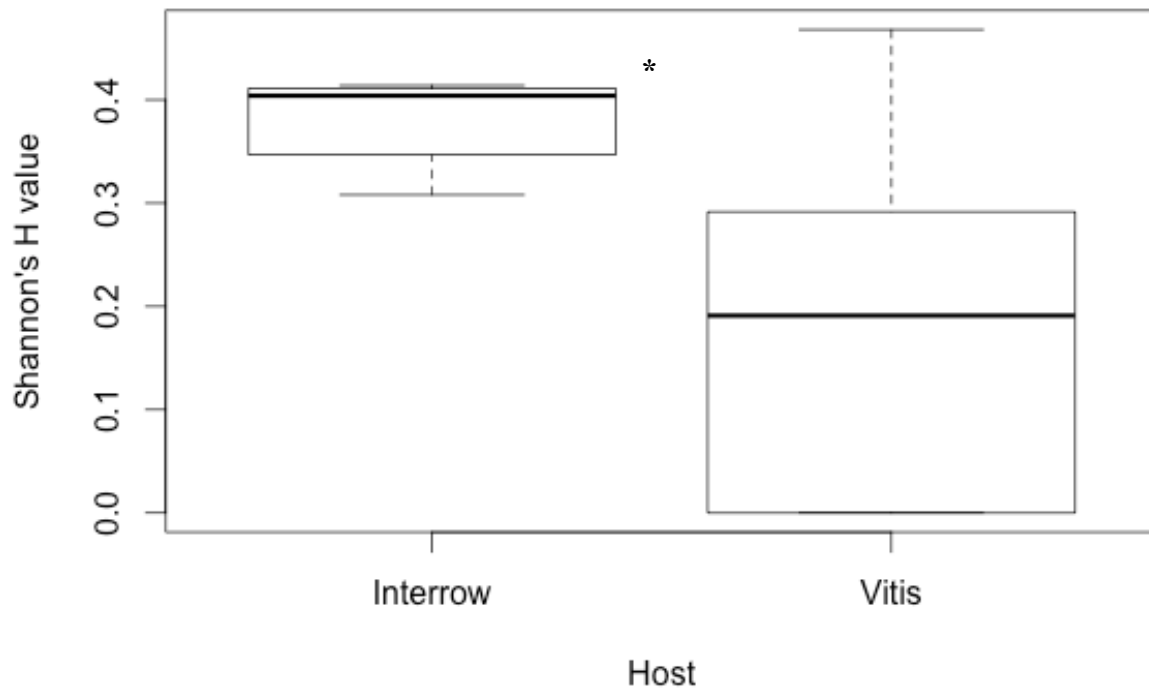
Figure 4.6 - Plant diversity in the *Vitis* rows and interrows at Sunrock vineyard, Osoyoos BC Canada. Values represent alpha diversity using Shannon's H index, calculated from percent coverage data (Appendix I). (*signifies significance at $\alpha=0.05$, n=46)
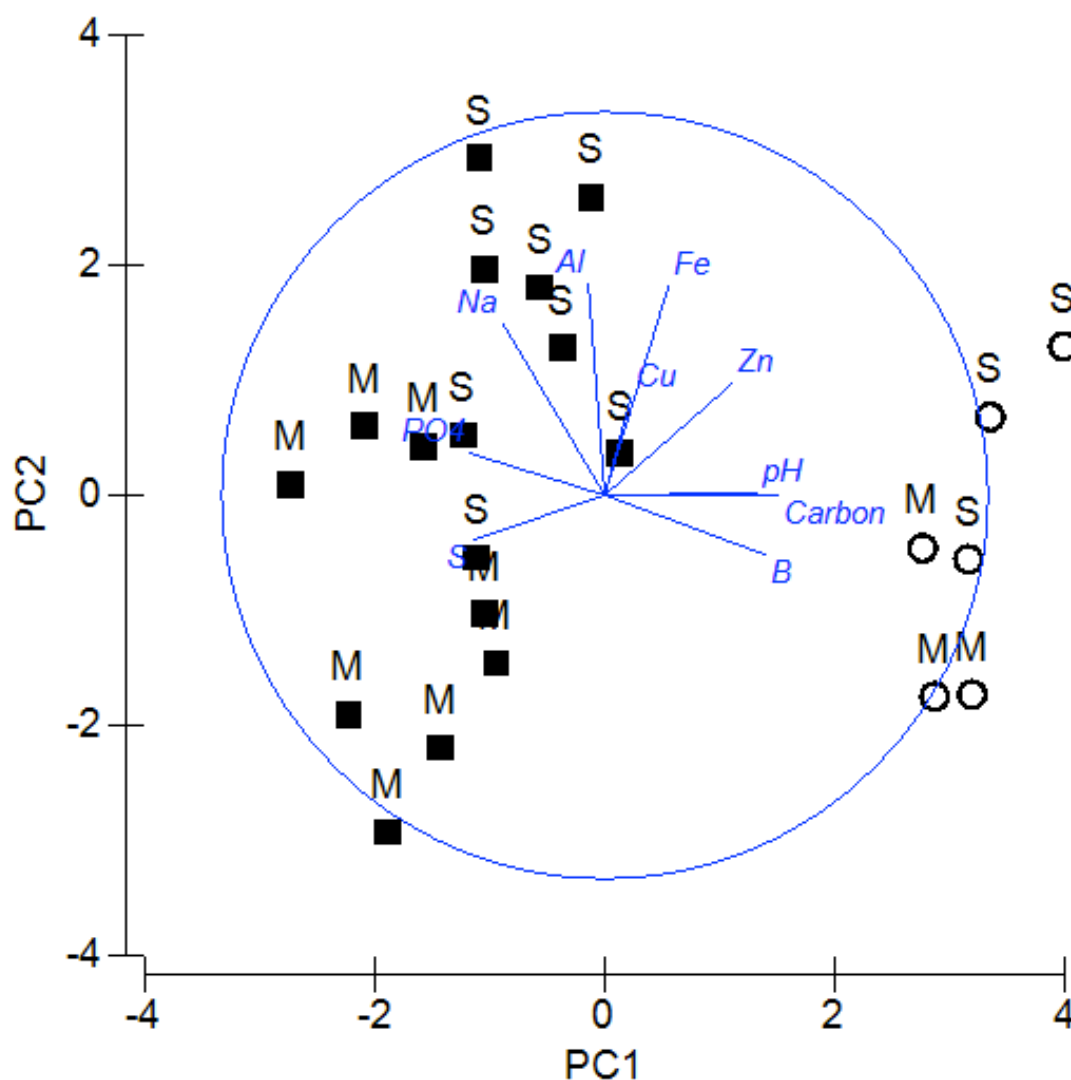
Figure 4.7 - Distribution of soil characteristics of *Vitis* (black squares) and interrow (open circles) plants from the Merlot (M) and Shiraz (S) experimental blocks at Sunrock vineyard, Osoyoos BC Canada. Each point represents a Euclidean distance value composed from soil Al, B, C, Cu, Fe, Na, PO4, 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.

BIBLIOGRAPHY

Acevedo-Opazo C., Ortega-Farias S. and Fuentes S. 2010. Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: An irrigation scheduling application to achieve regulated deficit irrigation. Agricultural Water Management. 97: 956-964.

Alguacil, M., Lozano Z., Campoy M.J. and Roldan A. 2010. Phosphorous fertilization management modifies the biodiversity of AM fungi in a tropical savanna forage system. Soil Biology and Biochemistry 42(7): 1114-1122

Alguacil M.M., Torres M.P., Torrecillas E., Diaz G. and Roland A. 2011. Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere after revegetation of a degraded, semiarid land. Soil Biology and Biochemistry. 43(1): 167-173

Alguacil M.M., Torrecillas E., Roldan A., Diaz G. and Torres M.P. 2012. Perennial plant species from semiarid gypsum soils support higher AMF diversity in roots than the annual *Bromus rubens*. Soil Biology & Biochemistry. 49: 132-138

Allen E.B., Allen M.F., Helm D.J., Trappe J.M., Molina R. and Rincon E. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant and Soil. 170(1): 47-62

An G.H., Miyakawa S., Kawahara A., Osaki M. and Ezawa T. 2008. Community structure of arbuscular mycorrhizal fungi associated with pioneer grass species *Miscanthus sinensis* in acid sulfate soils: Habitat segregation along pH gradients. Soil Science and Plant Nutrition 54: 517-528

Anderson M.J., Gorley R.N. and Clarke K.R. 2008. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods.* PRIMER-E: Plymouth, UK.

Anderson J.R. and Westmoreland D. 1971. Direct counts of soil microorganisms using a fluorescent brightener and a europium chelate. Soil Biology & Biochemistry. 3: 85-87.

Arnon D.I. and Stout P.R. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiol. 14(2): 371–375.

Audet P. and Charest C. 2009. Contribution of arbuscular mycorrhizal symbiosis to in vitro root metal uptake: from trace to toxic metal conditions. Botany-Botanique 87(10): 913-921.

Augé R.M. 2001 Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42

Bais H.P., Weir T.L., Perry L.G., Gilroy S. and Vivanco J.M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review Plant Biology 57: 233-266

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

Balestrini R., Magurno F., Walker C., Lumini E. and Bianciotto V. 2010. Cohorts of arbuscular mycorrhizal fungi (AMF) in *Vitis vinifera,* a typical Mediterranean fruit crop. Environmental Microbiology reports. 2(4): 594 - 604.

Bell C., McIntyre N., Cox S., Tissue D. and Zak J. 2008. Soil microbial responses to temporal variations of temperature and moisture in a Chihuahuan desert grassland. Microbial Ecology. 56: 153-167.

Bever J.D., Richardson S.C., Lawrence B.M., Holmes J. and Watson M. 2009 Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecology Letters 12: 13-21

Blomberg A. and Adler L. 1992. Physiology of osmotolerance in fungi. Advances in Microbial Physiology. v.33 Academic Press Limited. San Diego, California.

Bowen P. 2010 British Columbia Wine Grape Council and British Columbia Ministry of Agriculture and Lands. Best Practises Guide for Grapes for British Columbia Growers. Chapter 2, Chapter 4.4

Bowen, P., C. Bogdanoff, K. Usher, B. Estergaard, M. Cliff and K. Stanich. 2012a. Irrigation frequency and rate effects on vine growth, leaf gas exchange, berry composition, and wine sensory quality in red winegrapes. Technical Abstracts, 63rd Annual ASEV Conference, June 18-22, 2012, Portland, OR, pp 77-78.

Bowen, P., C. Bogdanoff, K. Usher, T. Lowery, M. Cliff and G. Neilsen. 2012b. Irrigation regimes affect soil wetting patterns, leaf gas exchange, berry composition, and wine quality in Merlot, Syrah and Cabernet Sauvignon. Report to AAFC and BCWGC for MII project A07344 (published on line at www.bbcgc.org) 23 pages.

Boyle M. and Paul E.A. 1988. Vesicular-arbuscular mycorrhizal associations with barley on sewage-amended plots. Soil Biology & Biochemistry 20(6): 945-94

Brihane E., Sterck F.J., Fetene M., Bongers F. and Kuyper T.W. 2012. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169(4): 895-904.

Broeckling C.D., Broz A.K., Bergelson J., Manter D.K. and Vivanco J.M. 2008. Root exudates regulate soil fungal community composition and diversity. Applied and Environmental Microbiology. 74: 738-44.

Brussaard L. 1997. Biodiversity and ecosystem functioning in soil. Ambio. 26: 563-570.

Bunn R., Lekberg Y. and Zabinski C. 2009. Arbuscular mycorrhizal fungi ameliorate temperature stress in thermophilic plants. Ecology 90(5): 1378-1388.

Cangahuala-Inocente G.C., Da Silva M.F., Johnson J., Manga A., van Tuinen D., Henry C., Lovato P.E. and Dumas-Gaudot E. 2011. Arbuscular mycorrhizal symbiosis elicits proteome responses opposite of P-starvation in SO4 grapevine rootstock upon root colonization with two *Glomus* species. Mycorrhiza. 21: 473–493

Caporaso G.J., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello EK., Fierer N., Pena A.G., Goodrich J.K., Gordon J.I., Huttley G.A, Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J, Yatsunenko T., Zaneveld J. and Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data**.** Nature Methods; doi:10.1038/nmeth.f.303

Carrero-Colon M., Nakatsu C.H. and Konopka A. 2006. Effect of nutrient periodicity on microbial
community dynamics. Applied and Environmental Microbiology. 72: 3175-3183.

Cassman K.G. 1999. Ecological intensification of cereal production systems: Yield potential, soil quality and precision agriculture. Proceedings of the National Academy of Science 96: 5952-5959.

Chapin III F.S., Walker B.H., Hobbs R.J., Hooper D.U., Lawton J.H., Sala O.E. and Tilman D. 1997. Biotic control over the functioning of ecosystems. Science 277: 500-504.

Chaves M.M., Zarrouk O., Francisco R., Costa J.M., Santos T., Regalado A.P., Rodrigues M.L. and
Lopes C.M. 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. Annals of Botany 105: 661-676.

Chaves M.M., Santos T.P., Souza C.R., Ortuño M.F., Rodrigues M.L., Lopes C.M., Maroco J.P. and Pereira J.S. 2007. Deficit irrigation in grapevine improves water-use efficiency while controlling vigor and production quality. Annals of Applied Biology. 150: 237-252.

Chowdhury N., Marschner P. and Burns R.G. 2011. Soil microbial activity and community composition: Impact of changes in matric and osmotic potential. Soil Biology & Biochemistry. 43: 1229-1236.

Chung H., Zak D.R., Reich P.B., and Ellsworth D.S. 2007, Plant species richness, elevated CO2, and atmospheric nitrogen deposition alter soil microbial community composition and function. Global Changes 13: 980-989

Cifre J., Bota J., Escalona J.M., Medrano H. and Flexas J. 2005. Physiological tools for irrigation scheduling in grapevine (Vitis vinifera L.). An open gate to improve water-use efficiency? Agriculture, Ecosystems and Environment. 106: 159-170.

Clark R.B. 2002. Differences among mycorrhizal fungi for mineral uptake per root length of switch grass grown in acidic soil. Journal of Plant Nutrition 25: 1753–1772.

Clark R.B. and Zeto S.K. 1996. Mineral acquisition by mycorrhizal maize grown on acid and alkaline soil. Soil Biology & Biochemistry 28(10-11): 1495-1503

Clark R.B. and Zeto S.K. 2000. Mineral acquisition by arbuscular mycorrhizal plants. Journal of Plant Nutrition. 23(7): 867-902

Clark N.M., Rillig M.C. and Nowak R.S. 2009. Arbuscular mycorrhizal fungi abundance in the Mojave Desert: Seasonal dynamics and impacts of elevated CO2. Journal of Arid Environments 73: 834-843

Coleman D.C. Crossley, Jr. D.A. and Hendrix P.F 2004 Fundamentals of soil ecology. Second Edition. Elsevier Academic Press. San Diego, CA

Conners, K., Zink, T., Bainbridge, D., Allen, M. and Morris, S. 1994. Europium staining for soil ecosystems disturbance evaluation (California). Restoration Management Notes. 12: 211-212.

Coombe B.G. 1960. Relationship of growth and development to changes in sugars, auxings and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiology 35(2): 241-250

Costantini L., Battilana J., Lamaj F., Fanizza G. and Grando M.S. 2008. Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): From Quantitative Trait Loci to underlying genes. BMC Plant Biology 8(38) doi:10.1186/1471-2229-8-38

Daft M.J. and El Giahmi A.A. 1978. Effects of arbuscular mycorrhiza on plant growth. VIII. Effects of defoliation and light on selected hosts. New Phytologist 80: 365-372

Daubenmire R. 1957 A canopy coverage method of vegetational analysis. Northwest Science 33(1): 43-64

Davison J, Opik M, Zobel M, Vasar M, Metsis M, *et al.* (2012) Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. PLoS ONE 7(8): e41938.

De Deyn G.B., Quirk H. and Bardgett R.D. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters. 7(1): 75-78

Dhillion S.S. 1992. Evidence for host mycorrhizal preference in native grassland species. Mycological Research 96: 359-362

Dodd I. 2009 Rhizosphere manipulations to maximize 'crop per drop' during deficit irrigation. Journal of Experimental Botany. 60: 2454-2459.

Dodd J.C., Boddington C.L., Rodriguez A,, Gonzalez C. and Mansur I. 2000. Mycelium of arbuscular mycorrhizal fungi from different genera: form, function and detection. Plant and Soil. 226: 131-151.

Drinkwater, L.E., Letourneau D.K., Workneh F., van Bruggen A.H.C., and Shennan C. 1995. Fundamental differences between conventional and organic tomato agroecosystems in California. Ecological Applications. 5: 1098-1112.

Du T., Kang S., Sun J., Zhang X. and Zhang J. (2010), An improved water use efficiency of cereals under temporal and spatial deficit irrigation in north China. Agricultural Water Management 97: Pages 66-74

Dumbrell A.J., Nelson M., Helgason T., Dytham C. and Fitter A.H. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. The ISME Journal 4: 337-345

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

Ettema C.H. and Wardle D.A 2002. Spatial soil ecology. Trends in Ecology & Evolution 17(4): 177-183

Fereres E. and Soriano M.A. 2007 Deficit irrigation for reducing agricultural water use. Journal of
Applied Botany. 58: 147-159.

Fierer N., Schimel J.P. and Holden P.A. 2003 Influence of drying-rewetting frequency on soil bacteria community structure. Microbial Ecology 45: 63-71.

Fitter A.H. 2005. Darkness visible: reflections on underground ecology. Journal of Ecology. 95(2): 231-243

Fitzsimons M.S., Miller R.M. and Jastrow J.D. 2008 Scale-dependent niche axes of arbuscular mycorrhizal fungi. Oecologia 158: 117-127

Gamper H, Walker C, Schüssler A. 2009. *Diversispora celata* sp. nov.: molecular ecology and phylotaxonomy of an inconspicuous arbuscular mycorrhizal fungus. New Phytologist 182: 495–506.

Garcia I.V. and Mendoza R.E. 2008. Relationships among soil properties, plant nutrition and arbuscular mycorrhizal fungi-plant symbioses in a temperate grassland along hydrologic, saline and sodic gradients. FEMS Microbiology Ecology 63(3): 359-371

Gasque M., Granero B., Turegano J.V., and Gonzaez-Altozano P. 2010. Regulated deficit irrigation effects on yield, fruit quality and vegetative growth of 'Navelina' citrus trees. Spanish Journal of Agricultural Research 8: S40-51

Gildon A. and Tinker P.B. 1983. Interactions of vesicular arbuscular mycorrhizal infection and heavy-metals in plants. 1. The effect of heavy-metals on the development of vesicular arbuscular mycorrhizas. New Phytologist 95(2): 247-261

Giller K.E., Breare M.H., Lavelle P., Izac I.M.N. and Swift M.J. 1997. Agriculture intensification, soil biodiversity and agroecosystem function. Applied Soil Ecology. 6: 3-16.

Gollotte A., van Tuinen D. and Atkinson D. 2004. Diversity of arbuscular mycorhizal fungi colonizing roots of the grass species *Agrostis capillaries* and *Lolium perenne* in a field experiment. Mycorrhiza 14: 111-117

Green J. and Bohannan J.M. 2006. Spatial scaling of microbial biodiversity. Trend in Ecology and Evolution 21(9): 501-507

Harris-Valle C., Esqueda M., Valenzuela-Soto E.M., and Castellanos A.E. Revista. 2009. Water stress tolerance in plant-arbuscular mycorrhizal fungi interaction: Energy metabolism and physiology. Fitotecnia Mexicana 32(4): 265-271.

Hartmann A., Schmid M., van Tuinen D. and Berg G. 2008. Plant-driven selection of microbes. Plant and Soil. 321: 235-257.

Hassan S.E.D., Boon E., St-Arnaud M. and Hijri M. 2011. Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. Molecular Ecology 20(16): 3469-3483

Hayman D.S. 1974. Plant growth responses to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature. New Phytologist 73: 71-80

Heinemeyer A., Ridgway K.P., Edwards E.J., Benham D.G., Young P.W. and Fitter A.H. 2003 Impact of soil warming and shading on colonization and community structure of arbuscular mycorrhizal fungi in roots of a native grassland community. Global Change Biology 10: 52–64,

Helgason T, and Fitter AH, 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). Journal of Experimental Botany 60: 2465–2480.

Helgason T., Merryweather J.W., Denison J., Wilson P., Young J.P.W. and Fitter A.H. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. Journal of Ecology 90(2): 371-384

Hetrick B.A.D. 1991. Mycorrhizas and root architecture. Experientia 47: 355-362

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

Holland T.C., Reynolds A.G., Bowen P.A., Bogdanoff C.P., Marciniak M., Brown R.B. and Hart M.M. 2013. The response of soil biota to water availability in vineyards. Pedobiologia 56(1): 9-14

Hunt, G.A. and Fogel, R. 1983. Fungal hyphal dynamics in a western Oregon Douglas fir stand. Soil Biology & Biochemistry 15: 641-649.

Ipsilantis I. and Sylvia D.M. 2007 Interactions of assemblages of mycorrhizal fungi with two Florida wetland plants. Applied Soil Ecology 35: 261-271

Jackson DI, Lombard PB (1993) Environmental and management practices affecting grape composition and wine quality—a review. American Journal of Enology & Viticulture 44: 409–430

Jacobson K.M. 1997. Moisture and substrate stability determines VA-mycorrhizal fungal community distribution and structure in an arid grassland. Journal of Arid Environments 35: 59-75

Janssen, M.P.M. and Heijmans, G. 1998 Dynamics and stratification of protozoa in the organic layer of a Scots pine forest. Biology of Fertility of Soils. 26: 285-292.

Jenerette G.D., Scott R.L. and Huxman T.E. 2008. Whole ecosystem metabolic pulses following precipitation events. Functional Ecology 22: 924-930

Johnson, N.C. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New phytologist 185: 631–47.

Johnson N.C., Tillman D. and Wedin D. 1992. Plant and soil controls on mycorrhizal fungal communities. Ecology 73(6): 2034-2042

Johnson D., Vandenkoornhuyse P.J., Leake J.R., Gilbert L., Booth R.E., Grime J.P., Young J.P.W. and Read D.J. 2004. Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytologist 161(2): 503-515

Jones D.L., Hodge A. and Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163(3): 459-480

Karagiannidis N. and Nikolaos N. 2000 Influence of arbuscular mycorrhizae on heavy metal (Pb and Cd) uptake, growth and chemical composition of *Vitis vinifera* L. (cv. Razaki). American Jounral of Enology and Viticulture 51(3): 269-275

Klironomos J.N., Allen M.F., Rillig M.C., Piotrowski J., Makvandi-Nejad S., Wolfe B.E. and Powell J.R. 2005. Abrupt rise in atmospheric CO2 overestimates community response in a model plant-soil system. Nature 433(7026): 621-624

Klironomos J.N., McCune J., Hart M. and Neville J. 2000. The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecology Letters 3(2): 137-141

Klironomos J.N., Rillig M.C. and Allen M.F. 1996. Below-ground microbial and microfaunal responses to *Artemesia tridentata* grown under elevated CO2. Functional Ecology 10: 527-534.

Klironomos J.N., Moutoglis, P., Kendrick B. and Widden, P. 1993. A comparison of spatial heterogeneity of vesicular arbuscular mycorrhizal fungi in two maple-forest soils. Canadian Journal of Botany 71: 1472-1480.

Klironomos J.N., Hart M.M., Gurney J.E. and Moutoglis P. 2001. Interspecific differences in the tolerance of arbuscular mycorrhizal fungi to freezing and drying. Canadian Journal of Botany 79: 1161-1166.

Kjøller A. and Struwe S. 1982. Microfungi in ecosystems: fungal occurrence and activity in litter and soil. Oikos. 39: 391–422.

Krüger M., Krüger C., Walker., Stockinger H. and Schüßler A. 2012. Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. New Phytologist 193: 970–984

Landesman W.J., and Dighton J. 2010. Response of soil microbial communities and the production of plant-available nitrogen to a two-year rainfall manipulation in the New Jersey Pinelands. Soil Biology & Biochemistry 42: 1751-1758.

Landesman W.J., Treonis A.M. and Dighton J. 2010. Effects of a one-year rainfall manipulation on soil nematode abundances and community composition. Pedobiologia 54: 87-91

Latch, G.C.M. 1993. Physiological interactions of endophytic fungi and their hosts . Biotic stress tolerance imparted to grasses by endophytes. Stress 44: 143-156.

Lau, J.A. and Lennon, J.T. 2011. Evolutionary ecology of plant-microbe interactions: soil microbial structure alters selection on plant traits. New phytologist 192: 215–24.

Lekberg Y., Koide R.T., Rohr J.R., Aldrich-Wolfe L, Morton J.B. 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. Journal of Ecology 95: 95–105.

Lekberg Y., Meadow J., Rohr J.R., Redecker D., Zabinski C.A. 2011. Importance of dispersal and thermal environment for mycorrhizal communities: Lessons from Yellowstone National Park. Ecology 92: 1292–1302.

Leyval C., Turnau K. and Haselwandter K. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7(3): 139-153

Li H.Y., Yang G.D., Shu H.R., Yang Y.T., Ye B.X., Nishida I. and Zheng C.C. 2006. Colonization by the arbuscular mycorrhizal fungus Glomus versiforme induces a defense response against the root-knot nematode *Meloidogyne incognita* in the grapevine (*Vitis amurensis* Rupr.), which includes transcriptional activation of the class III chitinase gene VCH3. Plant and Cell Physiology. 47(1): 154-163

Linderman R.G. and Davis A.E 2001. Comparative response of selected grapevine rootstocks and cultivars to inoculation with different mycorrhizal fungi. American Journal of Enology and Viticulture 52: 8-11

Liu W., Xu W., Hong J. and Shiqiang W. 2010. Interannual variability of soil microbial biomass and respiration in responses to topography, annual burning and N addition in a semiarid temperate steppe. Geoderma. 158: 259-267.

Long L.K., Qing Y., Guo J., Yang R.H., Huang Y.H., Zhu H.H. 2010 Molecular community analysis of arbuscular mycorrhizal fungi associated with five selected plant species from heavy metal polluted soils. European Journal of Soil Biology. 46: 288-294

Lozupone, C. A., and R. Knight. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71: 8228–8235.

Lussenhop, J. 1971. A simplified canister-type soil arthropod extractor. Pedobiologia 11: 40-45.
Maestre, F.T., Bowker M.A., Canton Y., Castillo-Monrow AP., Cortina J., Escolar C., Escudero A., Lazaro R. and Martinez I. 2011. Ecology and functional roles of biological soil crusts in semi-arid ecosystems of Spain. Journal of Arid Environments. 75: 1282-1291.

Marschner H. and Dell B. 1992. Nutrient-uptake in mycorrhizal symbiosis. Plant and Soil 159(1): 89-102.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., and Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115: 495-501.

McLean M.A. and Huhta V. 2000. Temporal and spatial fluctuations in moisture affects humus microfungal community structure in microcosms. Biol. Fertil. Soils. 32: 114-119.

Menge J.A., Raski D.J., Lider L.A., Johnson E.L.V., Jones N.O., Kissler J.J., Hemstreet C.L. 1983. Interactions between mycorrhizal fungi, soil fumigation and growth of grapes in California. American Journal of Enology and Viticulture 34:117–121

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

Mohr H.D. 1996. Periodicity of root tip growth of vines in the Moselle valley. Viticulture and Enology Science 51: 83–90

Mummey D.L. and Rillig M.C. 2007. Evaluation of LSU rRNA-gene PCR primers for analysis of arbuscular mycorrhizal fungal communities via terminal retrction fragment length polymorphism analysis. Journal of Microbiology Methods. 70(1): 200-204

Mummey DL, Rillig MC. 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant Soil. 288: 81–90

Nekola, JC; White, PS. 1999. The distance decay of similarity in biogeography and ecology. Journal of Biogeography 26:4 867-878

Newsham K, Fitter A, Watkinson A. 1995. Multifunctionality and biodiversity in arbuscular mycorrhizas. Trends in Ecology and Evolution 10: 407-411.

Nogales A., Luque J., Estaún V., Camprubi A., Garcia-Figueres F. and Calvet C. 2009. Differential growth of mycorrhizal field-inoculated grapevine rootstocks in two replant soils. American Journal of Enology and Viticulture. 60(4): 484-489

Opik M., Metsis M., Daniell T.J., Zobel M. and Moora M. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New phytologist. 184:2 424-437

Orwin K.H. and Wardle D.A. 2005. Plant species composition effects on belowground properties and the resistance and resilience of the soil microflora to a drying disturbance. Plant and Soil 278(1-2): 205-221

Orwin K.H., Wardle D.A. and Greenfield L.G. 2006. Context-dependent changes in the resistance and resilience of soil microbes to an experimental disturbance for three primary plant chronosequences. Oikos. 112: 196-208

Papatheodorou E.M., Argyropoulou M.D. and Stamou G.P. 2004. The effects of large and small scale differences in soil temperature and moisture on bacterial functional diversity and the community of bacterivorous nematodes. Applied Soil Ecology 25: 37-49.

Pringle A., Bever J.D., Gardes M., Parrent J.L., Rillig M.C. and Klironomos J.C. 2009. Mycorrhizal Symbioses and Plant Invasions. Annual Review of Ecology Evolution and Systematics. 40: 699–715

Qian M.C., Fang Y. and Shellie K. 2009. Volatile composition of merlot wine from different vine water status. Journal of Agricultural Food Chemistry. 57: 7459-7463

R Development Core Team. 2008. R version 2.8.1. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.orgi

Raun W.R. and Johnson G.V. 1999. Improving nitrogen use efficiency for cereal production. Agronomy Journal. 91: 357-363.

Rillig M.C. 2004 Arbuscular mycorrhizae and terrestrial ecosystem processes Ecology Letters. 7: 740-754.

Rodriguez, R. & Redman, R. (2008). More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. Journal of Experimental Botany, 59: 1109–14.

Scheublin T.R., Ridgway K.P., Young J.P.W. and van der Heijden M.G.A. 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Applied and Environmental Microbiology 70(10): 6240-6246

Schreiner P.R. 2005a. Mycorrhizas and Mineral Acquisition in Grapevines. Proceedings of the Soil Environment and Vine Mineral Nutrition Symposium., L.P. Christensen D.R. Smart (Eds). American Society for Enology and Viticulture.

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

Schreiner P.R. 2007. Effects of native and nonnative arbuscular mycorrhizal fungi on growth and nutrient uptake of 'Pinot noir' (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus. Applied Soil Ecology 36: 205-215

Schreiner P.R. and Mihara K.L. 2009. The diversity of arbuscular mycorrhizal fungi amplified from grapevine roots (*Vitis vinifera* L.) in Oregon vineyards is seasonally stable and influenced by soil and vine age Mycologia, 101: 599–611

Schreiner P.R., Tarara J.M. and Smithyman R.P 2007. Deficit irrigation promotes arbuscular colonization of fine roots by mycorrhizal fungi in grapevines (*Vitis vinifera* L.) in an arid climate. Mycorrhiza. 17: 551–562

Schreiner R.P. and Linderman R.G. 2005. Mycorrhizal colonization in dryland vineyards of the Willamette Valley, Oregon. Small Fruits Review 4: 41–55.

Schweiger P.F., Robson A.D. and Barrow N.J. 1995. Root hair length determines beneficial effect of a glomus species on shoot growth of some pasture species. New Phytologist. 131(2): 247-254

Seguin G. 1986. 'Terroirs' and pedology of wine growing. Experientia. 42: 861-873.

Seifert E.K., Bever J.D. and Maron J.M. 2009. Evidence for evolution of reduced mycorrhizal dependence during plant invasion. Ecology. 90: 1055–1062

Sikes B.A. 2010. When do arbuscular mycorrhizal fungi protect plant roots from pathogens? Plant Signaling and Behavior 5(6): 763-765

Smart RE, Coombe BG (1983) Water relations of grapevines. In: Kozlowski TT (ed) Water deficits and plant growth. Academic, New York, pp 137–196

Smith, S.E. and D.J. Read. 2008. Mycorrhizal symbiosis. Third edn. Academic Press. London.

Soininen J., McDonald R. and Hillebrand H. 2007. The distance decay of similarity in ecological communities. Ecography, 30(1): 3-12

Song Y.Y., Zeng R.S., Jian F.X., Li J., Shen X. and Yihdego W.G. 2010. Interplant communication of tomato plants through underground common mycorrhizal networks. PLOS ONE 5(10): e13324

Steenwerth K.L., Jackson L.E., Calderon F., Scow K.M. and Rolston D.E. 2005. Response of microbial community composition and activity in agricultural and grassland soils after a simulated rainfall. Soil Biology and Biochemistry. 37: 2249-2262.

Stevens K.J. and Peterson R.L 1996. The effect of a water gradient on the vesicular-arbuscular mycorrhizal status of *Lythrum salicaria* L. (purple loosestrife). Mycorrhiza 6: 99-104

Swift, M.J., Cook, A.G. and Perfect, T.J. 1979. Decomposition in Terrestrial Ecosystems. Blackwell
Scientific Publication, Oxford, UK.

Tilman D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. Ecology. 80: 1455-1474.

Tilman D., Wedin D. and Knops J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. Nature. 379: 718-720.

Tilman D., Knops J., Wedin D., Reich P., Ritchie M. and Siemann E. 1997. The influence of functional diversity and composition on ecosystem processes. Science. 277: 1300-1302.

Tobor-Kaplon M.A., Bloem J. and de Ruiter P.C. 2006. Functional stability of microbial communities from long-term stressed soils to additional disturbance. Environmental Toxicology and Chemistry. 25: 1993-1999.

Toljander, J.F., Santos-González, J.C., Tehler, A. & Finlay, R.D. (2008). Community analysis of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilization trial. FEMS Microbiology Ecology, 65: 323–38

Torrecillas E., Alguacil M. M. and Roldán A. 2012. Host preferences of arbuscular mycorrhizal fungi colonizaing annual herbaceous pant species in semiarid Mediterranean prairies. Applied Environmental Microbiology. 768(17) 6180 DOI: 10.1128/AEM.01287-12.

Trent J.D., Svejcar T.J. and Blank R.R. 1994 Mycorrhizal colonization, hyphal lengths, and soil moisture associated with two *Artemisia tridentate* subspecies. The Great Basin Naturalist. 54: 291-300

Treseder K.K. and Allen M.F. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytologist 155:3 507-515

Tseng C.C., Wang J.Y. and Yang L. 2009 Accumulation of Copper, Lead, and Zinc by In Situ Plants Inoculated with AM Fungi in Multicontaminated Soil. Communications in Soil Science and Plant Analysis. 40(21): 3367-3386

Turner S.D. and Friese C.F. 1998. Plant-mycorrhizal community dynamics associated with a moisture gradient within a rehabilitated prairie fen. Restoration Ecology. 6(1): 44-51

Valentine A.J., Mortimer P.E., Lintnaar M. and Borgo R. 2006. Drought responses of arbuscular mycorrhizal grapevines. Symbiosis 41:3 127-133

Vandenkoornhuyse P., Ridgway K.P., Watson I.J., Fitter A.H. and Young J.P.W 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. Molecular Ecology 12:11 3085-3095

Van der Heijden M.G.A., Klironomos J.N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., Wiemken A. and Sanders I.R. 1998, Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature. 396: 69-72.

van der Heijden, M.G.A, Steitworlf-Engel R., Riedl R., Siegrist S., Neudecker A., Ineichen K., Boller T., Wiemken A. and Sanders I.R. 2006. The mycorrhizal contribution to plant producitivity, plant nutrition and soil structure in experimental grassland. New Phytologist 172:4 739-752

Van Veen, J.A. and Paul, E.A. 1979. Conversion of biovolume measurements of soil organisms grown under various moisture tensions to biomass and their nutrient content. Applied Environmental Microbiology. 37: 686-692.

Vishnevetsky S. and Steinberger Y. 1997. Bacterial and fungal dynamics and their contribution to microbial biomass in desert soil. Journal of Arid Environments. 37: 83-90.

Vogelsang KM, Bever JD. 2009. Mycorrhizal densities decline in association with non-native plants and contribute to plant invasion. Ecology. 90:399–407

Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., Fromentin J.M., Hoegh-Guldberg O. and Bairlein F. 2002. Ecological responses to recent climate change. Nature 416: 389-416

Wardle D.A. 2002 Communities and Ecosystems – Linking the Aboveground and Belowground Components. Princeton University Press, Princeton, NJ.

Wardle D.A., Yeates G.W., Bonner K.I., Nicholson K.S. and Watson R.N. 2001. Impacts of ground vegetation management strategies in a kiwifruit orchard on the composition and functioning of soil biota. Soil Biology and Biochemistry. 33: 893-905.

Weckert M. 2004. In vitro inhibition of grapevine root pathogens by vineyard soil bacteria and actinomycetes. Proceedings from the Third Australian Soilborne Diseases Symposium 129-130

Wilkinson S.C. and Anderson J.M. 2001. Spatial patterns of soil microbial communities in a Norway spruce (Picea abies) plantation. Microbial Ecology 42: 248-255.

Williams M.A. 2007. Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. Soil Biology and Biochemistry. 39: 2750-2757.

Wilson G.W.T., Rice C.W., Rillig M.C., Springer A. and Hartnett D.C. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecology Letters 12: 452-461.

Wilson G.W.T., Hickman K.R. and Williamson M.M. 2012. Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. Mycorrhiza 22: 327-336

Yang H.S., Yuan Y.G., Zhang Q., Tang J.J., Liu Y. Chen X. 2011. Changes in soil organic carbon total nitrogen and abundance of arbuscular mycorrhizal fungi along a large-scale aridity gradient. Catena 87(1): 70-77

Yeates G.W., Wardle D.A. and Watson R.N. 1999. Responses of soil nematode populations, community structure, diversity and temporal variability to agricultural intensification over a seven-year period. Soil Biology and Biochemistry. 31: 1721-1733.

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.

Block	Treat.	pH	PO$_4$	%C	%N	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
Merlot	Fluct.	6.97	83.5	42.41	3.82	389.8	0.145	746.5	3.3	225.8	149.3	121.6	68.9	27.4	110.9	7.5	5.5
Merlot	Const.	7.33	55.3	41.28	3.75	451.9	0.071	924.7	2.5	305.9	118.8	168.5	94.5	35.1	96.0	3.5	4.3
Merlot	Fluct.	7.07	57.2	34.61	3.48	510.7	0.036	817.9	2.5	364.4	136.1	167.7	109.2	38.2	87.4	5.0	3.8
Merlot	Const.	6.86	50.3	37.45	3.55	425.5	0.043	711.1	2.6	288.1	89.8	128.3	73.6	34.5	76.7	4.8	3.2
Merlot	Fluct.	7.18	82.8	37.44	3.48	450.2	0.082	736.3	3.6	388.5	173.2	142.6	101.9	35.9	117.9	6.3	7.2
Merlot	Const.	6.67	72.3	35.02	3.45	466.6	0.076	629.6	4.2	392.9	174.0	130.8	94.5	30.6	104.3	30.9	6.1
Merlot	Fluct.	7.14	54.2	32.21	3.41	420.0	0.104	682.6	4.3	276.8	145.1	124.9	68.6	27.3	87.4	6.6	3.9
Merlot	Const.	7.12	51.9	39.61	3.79	425.5	0.092	699.0	2.6	330.6	139.4	131.1	86.2	32.1	79.8	2.4	4.3
Shiraz	Fluct.	7.05	62.6	34.01	3.49	453.6	0.11	747.6	6.5	355.2	157.7	138.2	91.5	35.5	95.4	6.9	7.4
Shiraz	Const.	7.28	64.4	49.07	4.4	479.1	0.143	927.0	2.7	448.7	174.2	166.6	112.7	45.6	101.1	6.9	8.5
Shiraz	Fluct.	7.2	74.9	43.93	4.22	440.3	0.117	904.7	2.2	354.8	174.2	140.0	91.4	33.4	134.1	8.9	7.8
Shiraz	Const.	7.16	54.1	50.88	4.59	478.4	0.108	939.7	2.5	372.6	154.2	159.3	96.7	33.1	101.0	1.5	8.1
Shiraz	Fluct.	7.02	50.6	42.52	4.15	497.7	0.01	857.1	1.9	426.0	137.9	156.0	102.4	35.0	78.5	0.0	7.2
Shiraz	Const.	7	72.0	48.75	4.51	478.9	0.066	879.0	5.8	400.5	141.3	154.1	112.7	41.2	101.3	5.1	9.9
Shiraz	Fluct.	6.92	61.4	38.96	3.78	515.1	0.045	797.7	5.7	452.7	128.0	158.2	105.4	42.3	87.4	1.0	7.2
Shiraz	Const.	7.44	65.4	49.13	4.58	484.5	0.027	950.8	6.0	443.4	126.1	179.0	113.0	43.7	100.7	1.4	7.7
Merlot	Inter	7.38	36.3	68.01	5.52	424.2	0.278	908.9	3.3	420.7	200.8	153.0	99.1	29.3	64.7	0.1	8.2
Merlot	Inter	7.47	37.5	72.76	5.94	433.5	0.397	978.8	2.9	352.1	222.4	149.7	93.3	21.8	67.6	1.4	8.1
Merlot	Inter	7.51	37.9	65.20	5.45	423.5	0.326	913.1	4.1	337.3	216.2	152.3	95.1	20.8	65.9	1.5	8.5
Shiraz	Inter	7.56	41.9	72.22	6.18	472.9	0.386	1016.5	4.4	452.1	250.6	152.1	120.0	27.8	71.3	1.9	10.1
Shiraz	Inter	7.6	45.1	72.72	6.23	480.8	0.357	1071.2	4.5	431.5	239.0	158.7	114.2	28.7	70.8	0.1	16.4
Shiraz	Inter	7.4	43.2	72.19	6.18	429.0	0.274	969.8	5.3	374.5	231.1	145.6	99.5	23.3	66.5	0.1	10.4
Art.	Natural	7.73	32.4	57.85	5.05	483.6	0.452	1672.0	2.6	333.9	298.1	149.3	103.7	20.3	90.6	0.9	2.7
Brom.	Natural	7.64	23.5	48.70	3.95	427.5	0.144	2060.3	3.6	471.7	177.3	124.0	112.4	31.7	56.6	0.1	3.8

Table A.2 - Plant species occuring in vine and interrows in Sunrock Vineyard, Osoyoos BC Canada.

Scientific Name	Common Name
Bromus Tectorum	Cheatgrass
Sporobolus cryptandrus	Dropseed
Elymus repens	Quackgrass
Elymus spicatus	Bluebunch wheatgrass
Agropyron cristatum	Crested wheatgrass
Erodium cicutarium	Stork's bill
Heterotheca villosa	Golden aster
Cardaria draba	Hoary cress (mustard)
Polygonum Convolvulus	Wild buckwheat

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

	B. tectorum	S. cryptandrus	E. repens	C. draba	E. spicatus	E. cicutarium	H. villosa	A. cristatum	P. convolvulus
M.interrow1	30.00	17.33	2.67	0.00	0.00	1.67	0.00	0.00	0.00
M.interrow2	4.00	1.33	14.33	10.00	0.00	1.67	0.00	0.00	0.00
M.interrow3	23.33	22.00	2.33	7.33	0.00	1.00	0.00	0.00	0.00
S.interrow 1	0.00	21.67	2.33	0.00	0.00	1.00	1.33	0.33	0.00
S.interrow 2	2.00	15.67	8.33	0.33	0.00	0.00	0.00	0.00	0.00
S.interrow 3	2.33	5.00	20.33	0.33	0.00	0.00	0.00	0.00	0.00
Shiraz 1	3.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.50
Shiraz 2	2.50	0.00	0.00	10.00	0.00	0.00	0.00	0.00	2.00
Shiraz 3	2.00	0.00	0.00	14.00	1.00	0.00	0.00	0.00	3.00
Shiraz 4	2.00	0.00	0.00	17.50	0.00	0.00	0.00	0.00	1.50
Merlot 1	2.50	0.00	0.00	0.00	14.00	0.00	0.00	0.00	0.00
Merlot 2	0.00	0.00	0.00	0.00	7.50	1.50	0.00	0.00	0.00
Merlot 3	1.00	0.00	0.00	0.00	32.00	0.00	0.00	0.00	0.00
Merlot 4	7.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00