THE EFFECT OF VINEYARD GROUNDCOVER VEGETATION ON SOIL FUNGI AND PLANT-SOIL FEEDBACK

by

Eric Kraemer Vukicevich

A THESIS SUBMITTED IN PARTIAL FULLFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

The College of Graduate Studies

(Biology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Okanagan)

March 2019

The following individuals certify that they have read, and recommend to the College of Graduate Studies for acceptance, a dissertation entiled:

THE EFFECT OF VINEYARD GROUNDCOVER VEGETATION ON SOIL FUNGI AND PLANT-SOIL FEEDBACK

submitted by __Eric Kraemer Vukicevich__in partial fulfillment of the requirements of the degree of ___Doctor of Philosophy in Biology__ .

Dr. Miranda Hart, Irving K. Barber School of Arts and Sciences

Supervisor

Dr. D. Thomas Lowery, Agriculture and Agri-Food Canada, Summerland

Co-Supervisor

Dr. David Scott, Irving K. Barber School of Arts and Sciences

University Examiner

Dr. Dario Cantu, University of California, Davis

External Examiner

Additional committee members include:

Dr. Pat Bowen, Agriculture and Agri-Food Canada, Summerland

Supervisory Committee Member

Dr. José Ramon Úrbez-Torres, Agriculture and Agri-Food Canada, Summerland

Supervisory Committee Member

Abstract

Groundcover vegetation is managed in vineyards for many purposes including for soil quality, trafficability, and pest and fertility management. Because plants are major drivers of soil biota, groundcover identity could also cause changes in soil microbial communities that then influences vine health. Using greenhouse and field trials as well as a multi-year survey of Okanagan valley vineyards, I studied the effect of groundcover identity and management on soil fungi known to be important in influencing vine health and growth attributable to these plant-soil feedbacks.

Overall, groundcover vegetation influenced abundances of each of the studied guilds of soil fungi in the drive row, with plant effects on the entomopathogenic *Beauveria bassiana* being the most consistent. Under vine living mulches, however, did not affect these same groups of fungi. When soil trained by different groundcovers from the same field were used as microbial inoculant in the greenhouse, they led to differences in arbuscular mycorrhizal (AM) communities in vine roots, but differences in vine growth were only seen when a pathogen was also included.

Taken together, these results suggest that groundcover vegetation does influence soil fungi in drive rows of Okanagan vineyards. Certain groundcovers may provide ecosystem services such as conservation biological control of pest insects through increases in entomopathogenic fungi, improved carbon sequestration and soil structure through increases in AM fungi, and deterrence of soil-borne pathogens. However, feedback effects on vines in this dissertation were limited to abiotic competitive effects in the field and biotic responses in the greenhouse, suggesting a high degree of context-dependency of plant-soil feedbacks in this system.

Lay Summary

Because plants interact in specific ways with soil microorganisms, the types of plants in vineyard groundcovers have the potential to change the microorganisms in vineyard soils. Through a greenhouse experiment, a field trial, and a survey of Okanagan valley vineyards, we tested the hypothesis that different vineyard groundcovers would alter soil fungi and affect vine growth. Overall, I found that groundcovers altered several important groups of soil fungi and influenced grapevine growth, but only in a high pathogen environment. The identity of vineyard groundcovers may change soil fungi, but how these changes influence vine growth may be context dependent and may be relatively minor compared to direct competition between vines and groundcover vegetation.

Preface

A version of Chapter 1 has been published. Vukicevich E, Lowery T, Bowen P, Urbez-Torres JR, Hart M (2016) Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agronomy for Sustainable Development 36. doi: 10.1007/s13593-016-0385-7.

A version of Chapter 2 has been published: Vukicevich E, Lowery DT, Urbez-Torres JR, Bowen P, Hart M (2018) Groundcover management changes grapevine root fungal communities and plant-soil feedback. Plant and Soil 424: 419-433. doi: 10.1007/s11104-017-3532-2.

A version of Chapter 3 has been published: Vukicevich E, Lowery DT, Eissenstat, D, Hart M (2018) Changes in arbuscular mycorrhizal fungi between young and old *Vitis* roots grown with soils trained by distinct plant communities. Symbiosis. doi: https://doi.org/10.1007/s13199-019-00598-3.

A version of Chapter 4 has been accepted for publication pending revisions: Vukicevich E, Lowery DT, Bennett J, Hart M. Groundcover vegetation affects soil fungi in multi-year survey across five semi-arid vineyard sites. Frontiers in Ecology and Evolution.

A version of Chapter 5 has been submitted for publication: Vukicevich E, Lowery DT, Hart M. Effects of living mulch on young vine growth and soil in a semi-arid vineyard. Submitted to *Vitis,* October 15, 2018.

I wrote the review paper presented as chapter 1 with guidance and edits from Dr. Miranda Hart, Dr. D. Thomas Lowery, Dr. Pat Bowen and Dr. José Ramon Úrbez Torres.

The greenhouse experiments described in chapters 2 and 3 were designed in consultation with Dr. Miranda Hart, Dr. D. Thomas Lowery, and Dr. John Klironomos. The greenhouse portion of the experiments were run at Agriculture and Agri-food Canada's Summerland Research and Development Centre (AAFC-SuRDC) with the help of the laboratories of Dr. D. Thomas Lowery, Dr. Pat Bowen, and Dr. José Ramon Úrbez-Torres.

Chapter 3 stemmed from a collaboration project with visiting PhD candidate, Diana Morales (Universidade Federal de Santa Catarina, Brasil) and would not have existed without her help. Laboratory work was conducted in the Hart lab at the University of British Columbia, Okanagan (UBC-O) with the help of students Michelle MacDonald (UBC-O) and Diana Morales. Diana Morales optimized the protocol for and carried out staining and preparation of slides for viewing and quantifying AM fungi in root samples with the help of Michelle MacDonald.

Field sites for chapter 4 were chosen with the help of Dr. D. Thomas Lowery, Naomi DeLury, and several local vineyard managers. Experimental sites at AAFC-SuRDC were established and maintained by Dr. D. Thomas Lowery, Keith DeGlow, and co-op students. Co-op students also helped with sample collection. Michelle MacDonald and Haley Bessette (AAFC-SuRDC) helped with DNA extractions and Haley Bessette also helped with some of the ddPCR analysis. Dr. Jonathan Bennett wrote the R code and performed the statistical analysis.

The field trial described in chapter 5 was established with the help and guidance of D. Thomas Lowery at AAFC-SuRDC. Co-op students also helped with plot

maintenance on occasion. The field crew at AAFC-SuRDC led by Brian Fodey helped with irrigation, mowing, and spraying during the trial.

All figures are in-line with text for ease of reference.

Table of Contents

List of Tables

List of Figures

Acknowledgements

First and foremost, I am sincerely grateful to Dr. Miranda Hart and Dr. Tom Lowery for taking me on as a graduate student four years ago. Thank you for believing in my abilities, supporting me, and offering encouragement throughout my time as your student. Your dedication to the success of your student is admirable and appreciated.

I also want to thank all the great people at AAFC-Summerland for help with various aspects of my project. Dr. Pat Bowen, Carl Bogdanoff, Rob Brownlee, Brad Estergaard, Brian Fodey (and crew), Dr. José Úrbez-Torres, Paula Haag, Melanie Walker, Naomi DeLury, Dr. Tom Forge, and a host of co-op students all helped me considerably. Thank you to my committee members, Dr. Pat Bowen and Dr. José Ramon Úrbez-Torres for providing me with expertise in their fields, comments on my work, and access to resources at the research station.

This work, of course, would not be possible without interest and funding from both Agriculture and Agri-Food Canada and the BC Winegrape Council. I am especially grateful to the vineyard managers who cooperated with my sampling effort: Lisa Wambold, Andrew Stone, Matt Mavettee, and Erasmo Nunes. Hopefully there is something useful for you here.

I also want to thank several people who provided me the opportunity to gain experience in teaching: Dr. Bob Lalonde, Dr. Dan Durall, Dr. Brendan D'Souza, Dr. Michael Deyholos, and Dr. Miranda Hart at UBC-O; Dr. Gabriel Balint, Jonathan Rouse, and Gail Wolski at Okanagan College; and Dr. Stefan Seiter at Linn Benton Community College. Thank you also to Stefan Seiter for introducing me to the world of agricultural research and education.

I also want to thank my lab mates at UBC-O, who are supportive, insightful, and good friends: Antreas Pogiatzis, Vasiloes Kokkoris, Meike van der Heyde, Cameron Egan, Taylor Holland, Corrina Thomsen, Jon Bennett, and Andrew Richards. A special thanks to Michelle MacDonald, Haley Bessette and Alicia Tymstra for their help both at UBC-O and AAFC; and to Diana Morales, whose beautiful AMF slides I will probably keep forever.

Finally, thank you to my family who have supported me in whatever I do in life. I realize how fortunate I am to have these opportunities and am so grateful. I love you guys!

Dedication

For my grandfather, Dan Kraemer (Pop), a great farmer and a leader. He probably would have thought this is crazy.

1 Introduction

1.1 Background

Perennial crops often experience reduced productivity over time due to the accumulation of soil-borne pests and pathogens (Hamel et al. 2005; Mazzola and Manici 2012; Úrbez-Torres et al. 2014). This is particularly problematic in woody perennial systems where crop rotation is not possible and, ultimately, replanting is necessary to restore production levels. However, addressing this problem from an ecological perspective may lead to more sustainable solutions and avoidance of decline altogether.

An ecological concept that is useful for understanding crop decline is plant-soil feedbacks. This concept describes the reciprocal effects of plants and their associated soil microbial communities (Bever 1994). Negative soil feedback occurs when plants promote the colonization and growth of soil microorganisms that are deleterious to their own growth, contributing to the maintenance of plant coexistence in natural systems through density-dependent regulation of more dominant plant species (Bever et al. 2015). In perennial monocultures, however, negative feedback leads to crop decline and replant problems (e.g., Hamel et al. 2005; Mazzola and Manici 2012). That is, the deleterious soil microbial community cultured though this mechanism will suppress the growth of neighboring conspecifics or replants of the same crop.

The negative effect of soil microbial communities in monocultures is not altogether surprising, given the negative relationship between biodiversity and the frequency of parasitism (Civitello et al. 2015). In many systems, low levels of diversity will allow a parasite of the dominant host species to more easily find a suitable host, whereas a "dilution effect" is often seen with increasing diversity, making suitable hosts

more difficult to find and disease outbreaks less frequent (Keesing et al. 2010). For plant-soil ecosystems in particular, it is well established that increased soil microbial diversity decreases incidence of plant disease (Garbeva et al. 2004a; van Elsas et al. 2002) and improves plant productivity (van der Heijden et al. 1998, 2008).

So how can growers increase soil microbial diversity in perennial systems? While many factors contribute to forming a given soil microbial community, e.g., pH and soil nutrient status (Fierer and Jackson 2006; Lauber et al. 2008), there is an extensive body of literature that documents the ability of plants to train the soil microbial community (Badri and Vivanco 2009; Fanin et al. 2014; Hartmann et al. 2009; Rovira 1969) and this may be easily exploited in perennial systems. While growers are limited in their ability to manipulate the diversity of crop plants in their cropping system, cover crop identity and diversity can be an efficient way to increase soil microbial diversity and suppress soil-borne pests that cause crop decline (Garbeva et al. 2004a). Cover crops are already a common feature in many perennial systems (Fig. 1.1), but their potential impact on the biotic component of soils is often overlooked, leading to underutilization of this key management tool. For the purpose of this review, we define the term 'cover crop' as any form of managed vegetation grown between crop plant rows, including annual and perennial swards.

Fig. 1. 1 Examples of common cover cropping strategies and experimentation at the Summerland Research and Development Center, British Columbia. (left) A permanent cover of mixed grass species in a cherry orchard and (right) a long-term cover crop experiment in wine grapes evaluating mixtures and monocultures of native and introduced grasses.

Here, we incorporate ecological knowledge of plant-soil feedbacks into the context of perennial agriculture to explore the use of cover crops to increase microbial diversity and manage crop decline. Taking this approach, the specific aims of this review are to: 1) synthesize our current understanding of how plant communities influence soil microbial communities into the context of cover crops; 2) highlight key groups of beneficial soil microbes, their role in affecting crop decline, and their association with plant communities; and 3) present, from this perspective, some considerations for managing non-crop vegetation to mitigate decline in perennial agriculture.

1.2 Manipulating cover crops to influence soil microbial communities

1.2.1 Root exudates attract rhizosphere microbes

Perhaps the most well studied mechanism through which plants affect the soil microbial community is root exudates (Badri and Vivanco 2009; Broeckling et al. 2008; Rovira 1969). Root exudates contain C-rich compounds, including amino acids, organic

acids, sugars, phenolics, secondary metabolites, and proteins that are excreted mostly from root hairs and the cells immediately behind the penetrating root tip (Badri and Vivanco 2009). Root exudates attract and sustain a variety of rhizosphere microorganisms (Badri and Vivanco 2009), including arbuscular mycorrhizal (AM) fungi (Akiyama et al. 2005), entomopathogens in response to root herbivory (Rasmann 2005), and N-fixing bacteria (Long 2001). However, root exudates also attract host-specific pathogens (Nicol et al. 2003; Hamel et al. 2005; Hofmann et al. 2009). Thus, this phenomenon can result in both positive and negative soil feedback. Since exudation composition, quantity, and seasonality depend on host plant identity (Broeckling et al. 2008), thereby coupling distinct microbial communities with different hosts (Schweitzer et al. 2008), a cover crop that includes a variety of plants should be able to maintain greater diversity of root-associated microbes with higher overall benefits to crops (Bardgett and van der Putten 2014; Garbeva et al. 2004a)(Fig. 1.2).

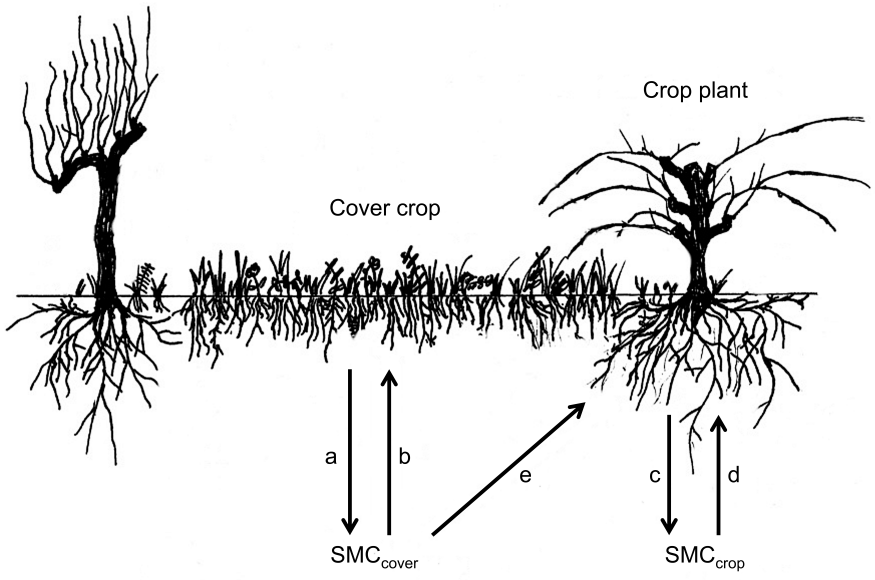

Fig. 1. 2 Depiction of how plant-soil feedbacks might influence crop decline in the context of a highly diverse permanent vineyard cover crop (adapted from Bever et al. 2010). (a) A diverse cover crop influences the soil microbial community through deposition of root exudates and litter creating a diverse microbial community (SMC_{cover}) which provides feedback (b) to the cover crop maintaining plant diversity. (c) The vine "trains" its own soil microbial community (SMC_{crop}), which may include pest and pathogen buildup due to a low diversity of plant-derived resources. (d) If plant diversity in the vineyard is limited to vines only, SMC_{crop} would feed back negatively on the vine leading to crop decline due to monoculture. (e) However, a highly diverse SMC_{cover} provides inoculum of microbial diversity that may interact with SMC_{crop} and the vine directly, increasing the microbial diversity available to roots of the vine and preventing decline due to negative feedback.

1.2.2 Plant litter affects decomposer community

While growers are familiar with the effect of soil organic material on disease suppression, e.g., compost addition (Hoitink and Boehm 1999), a similar effect can occur with cover crop plant litter. The identity of decomposing plant litter has been shown to affect both the activity (Bardgett and Shine 1999) and community structure of soil microbes (Fanin et al. 2014). Litter quality is now becoming more thoroughly

understood as a combination of both the ratio of carbon to nutrients and the proportions of different C chemistry, e.g., low molecular weight compounds (easily labile) and more complex forms such as lignin (more recalcitrant) (Cotrufo et al. 2013). The quality and quantity of decaying plant roots and above ground litter differs greatly among plant species (Cornelissen and Thompson 1997) and can determine which saprotrophic microorganisms are most prevalent in soil (Fanin et al. 2014). High quality plant litter (lower C:nutrient ratio, greater proportion of labile to recalcitrant C chemistry) is broken down more rapidly and thus favors faster-growing copiotrophic microbes, including disease-suppressive pseudomonads (Bastian et al. 2009), while oligotrophic microbes such as fungal decomposers (Holland and Coleman 1987) and Acidobacteria (Bastian et al. 2009) are largely responsible for the decomposition of low quality litter. Recently, Fanin et al. (2016) compared microbial communities from soils to which litter was added that differed in C:N ratio, lignin, and total nutrients and found that litter type had a strong effect on shaping soil microbial communities, surpassed only by the influence of soil type (Fanin et al. 2016). Other aspects of plant litter chemistry, such as glucosinolate content, can have a dramatic and long-term effect on the soil microbial community (Mazzola et al. 2015). Stimulating soil saprotrophic communities with a diverse composition of plant litter may reduce soil-borne pathogens, either through increased competition for resources (Fontaine et al. 2003) or by increasing the abundance of beneficial, pathogen antagonistic microbes (Garbeva et al. 2004b).

In addition to the identity of the plant material, the physical location of decomposing plant litter may also be an important factor for managing decline. Whether litter is left on the surface (as in a perennial cover crop) or incorporated into the soil via

tillage will affect the decomposer communities and thus many pathogens as well (Govaerts et al. 2007; Holland and Coleman 1987). When litter is left on the soil surface, as in a no-till system, saprotrophic microbial communities become more structured due to the stratification of different nutrients through the soil (Holland and Coleman 1987). This heterogeneity of resources leads to increased microbial diversity found in no-till systems (Govaerts et al. 2007) and could increase the ability of soils to suppress disease (Stirling et al. 2012), but this has not been investigated specifically in a cover cropped perennial system.

1.2.2 Plant cover affects soil moisture

Another way in which cover crops may influence the soil microbial community is by altering soil moisture dynamics (Bezemer et al. 2006; Lange et al. 2014). In addition to differences in water use through transpiration, plants differ in the proportion of the soil surface their canopy covers, affecting temperature and evaporation from the soil and therefore soil moisture (Lange et al. 2014). In dryland ecosystems, more water from the top 15 cm of soil is typically lost to evaporation than is used by plants (Loik et al. 2004). In semi-arid agricultural systems the maintenance of soil cover, as in a no-till system, can significantly decrease soil temperature and increase soil moisture retention at shallow depths compared to tilled soil (Pannkuk et al. 1997). Depending on traits of the ground cover vegetation (e.g., root system architecture, photosynthetic pathway, dormant states) slight changes in soil moisture can result from maintaining plant cover and this can be ecologically significance in terms of soil microbial functioning (Chowdhury et al. 2011; de Vries et al. 2012). This may result from microbial taxa differing in their tolerance of

stress associated with changes in water potential, meaning soil texture (affecting pore size distribution), and salt content (affecting water potential) are also key factors (Chowdhury et al. 2011). This threshold appears to be phylogenetically conserved for bacteria (Placella et al. 2012). Microorganisms that provide ecosystem services at shallower depths, such as entomopathogenic fungi, may benefit most from the preservation of moisture by plant cover (Pell et al. 2010). This mechanism has not been tested specifically, but there is some evidence for the persistence of natural populations of *Beauveria bassiana* when a cover is maintained (Shapiro-Ilan et al. 2012). Although plant cover and thus soil moisture can be an important driver of a soil microbial community (Lange et al. 2014), more work needs to be done to elucidate this effect on crop decline outcomes as some pathogens also benefit from increased soil moisture (Pieczarka and Abawi 1978; Kuan and Erwin 1982).

1.3 The role of beneficial microbes in crop decline

While discouraging negative feedback is a critical part of managing crop decline, promoting beneficial microbes may be as important. Because generalist pathogens may not be as affected by the diversity of plant community per se, and are common in some perennial systems (e.g., '*Cylindrocarpon*' in apple (Mazzola and Manici 2012), avocado (Vitale 2012), cut flowers (Lombard et al. 2013), and grape (Úrbez-Torres et al. 2014)), the ability of a cover crop to encourage beneficial microbes might be paramount to avoiding crop decline if these pathogens are prevalent. Microbial antagonists such as AM fungi, disease-protective fungi (e.g., *Trichoderma* spp.), and disease-suppressive bacteria (e.g., pseudomonads) are instrumental in preventing soil-borne disease whether

through competition for resources or niche space (e.g., Larsen and Bodker 2001), direct antibiosis (Haas and Defago 2005), or by induction of plant systemic resistance (Pozo et al. 2002). Entomopathogenic fungi provide protection from soil-dwelling insect pests, outbreaks of which are another form of negative feedback in these systems. Here we examine the role these beneficial microbes play in preventing crop decline and provide evidence for the influence of plant communities on driving populations of each group.

1.3.1 Mycorrhizal fungi

Most perennial crops are mycorrhizal, forming a root symbiosis with fungi (e.g., apple (Gnekow and Marschner 1989), citrus (Menge et al. 1978), olive (Róldan-Fajardo and Barea 1985), raspberry (Taylor and Harrier 2000), and grape (Trouvelot et al. 2015)). This mutualism confers many benefits to hosts, particularly nutritional and stress tolerance (Smith and Read 2008), but is threatened in most agricultural systems (Verbruggen et al. 2010) due to management activities that inhibit the fungi such as tillage (Brito et al. 2012), fungicide use (Graham et al. 1985), and some forms of weed control (Schreiner et al. 2001). Increasingly AM fungi (phylum Glomeromycota), which are the most widespread fungal mutualists, are considered pathogen antagonists in addition to nutritional symbionts (Azcón-Aguilar and Barea 1996; Cameron et al. 2013). Competition for root space is one way that AM fungi can limit root disease (Cameron et al. 2013). AM fungi may also inhibit pathogenic fungi through induced systemic resistance (ISR), by triggering an enhanced immune response in the host plant (Pozo et al. 2002). Finally, AM fungal hyphae in the soil cultivate their own diverse microbial communities (Scheublin et al. 2010) and microbes that inhabit this 'hyphosphere' can

include fungi and bacteria that play a role in protection from soil-borne pathogens (Filion et al. 1999).

Because some AM fungal taxa may offer more disease protection than others (Maherali and Klironomos 2007; Sikes et al. 2009), greater diversity of AM fungi could lead to broader disease suppression due to the increased probability of the presence of taxa effective in guarding against pathogens. Due to the tight link between plant diversity and AM fungal diversity (Hart et al. 2003; van der Heijden et al. 1998), using diverse cover crop mixes could increase the suite of AM fungal partners available to crop plants, with a greater chance of symbioses forming between crop plants and multiple AM fungi that are effective in alleviating both abiotic and biotic stresses.

1.3.2 Disease-protective fungi

Many saprotrophic fungi opportunistically colonize roots as endophytes and aid in controlling pathogenic fungi. A notable example is *Trichoderma,* which can parasitize other fungi using cell wall-degrading enzymes and antibiotic compounds (Harman et al. 2004). Non-pathogenic strains of *Fusarium oxysporum* have been observed to function in a similar way (Benhamou et al. 2002) and there are myriad other fungi that are also known to protect roots from pathogens, e.g., *Penicillium* (Hossain et al. 2007) and *Clonostachys* (Luongo et al. 2005). Because many of these fungi can also promote increased root and plant growth (Harman et al. 2004), they may also contribute to positive feedbacks similar to those experienced by plants associating with AM fungal mutualists (Avis et al. 2008).

Some disease-protective fungi are sold commercially as biocontrol agents (e.g., *Trichoderma* spp.), but they are naturally common in soil and may be affected by plant diversity as well as soil disturbance due to their additional roles as decomposers and endophytes. There is some empirical evidence for a plant host effect on populations of pathogen-antagonistic fungi. For example, Berg et al. (2005) found *Trichoderma* and other *Verticillium* antagonists such as *Penicillium* and *Monographella* to vary in diversity and abundance across sites and among host plant (strawberry and oilseed rape) rhizosphere soils. Recently, Taheri et al. (2016) reported different proportions of fungal antagonists found on wheat roots depending on the species of legume pulse crop grown in rotation with wheat. The authors speculated that this difference in prevalence of beneficial fungi due to the pulse crop was responsible for the yield increase seen in the wheat crop (Taheri et al. 2016). Although there is growing empirical evidence for a plant host effect on populations of antagonistic fungi, our current understanding of the mechanisms by which plant communities selectively amplify certain disease-protective fungi does not go beyond plant species and genotype-specific composition of root exudates (Hartmann et al. 2009). Cover crops may be paramount in determining the relative abundance of these fungi in an agroecosystem and further investigation into specific terms of association between host plants and endophytes would aid in better predicting disease outcomes.

1.3.3 Disease-suppressive bacteria

Root associated, disease-suppressive bacteria contribute another mechanism by which plants resist disease (Haas and Defago 2005; Sturz and Christie 2003). These

rhizosphere bacteria, which include fluorescent pseudomonads, inhibit bacterial and fungal pathogens, and even root-feeding nematodes through competition for C, N, and Fe resources as well as through production of antimicrobial compounds such as pyrrolnitrin (PRN) and 2,4-diacytlphloroglucinol (DAPG) (Haas and Defago 2005). Although the mode of action of some of these compounds is not completely understood, both PRN and DAPG appear to interfere with fungal respiration (de Souza et al. 2003; Tripathi and Gottleib 1969). Like AM fungi, pseudomonads can also offer indirect protection through ISR (van Loon et al. 1998; Haas and Defago 2005).

Plant genotype seems to have an effect on prevalence of DAPG-producing bacteria in rhizosphere soils (Hartmann et al. 2009; Mazzola et al. 2004) and there is evidence linking higher plant diversity with greater abundance of DAPG and PRN-producers and disease suppression (Latz et al. 2012), although the specific mechanisms responsible for this effect have not yet been elucidated. Alternative to the effect of plant diversity, increased abundances of disease-suppressive bacteria are often associated with the onset of disease suppressive soils after continuous monoculture of wheat (Raaijmakers and Weller 1998). Because pathogen outbreak is required for onset of soil suppressiveness and differences in level of disease suppression seem to depend on host plant genotype (Mazzola et al. 2004), this phenomenon seems to involve an intimate interaction between a specific host plant, fungal pathogen, and resident populations of pseudomonads (Weller et al. 2002). Although many details of bacteria-plant interactions have been elucidated (e.g., see review by Hartmann et al. 2009), the mechanisms by which certain plants tend to increase disease-suppressive bacteria remain in the realm of speculation. Despite this gap in knowledge, plant diversity as well as specific genotypes

within a cover crop may promote greater numbers of disease-suppressive bacteria and help to minimize decline of perennial crop plants and further assessment of commonly used cover crop species and mixes would be highly beneficial.

1.3.4 Entomopathogenic fungi

Entomopathogenic fungi (orders Entomophthorales and Hypocreales) are pathogens of many insects, including crop herbivores (Vega et al. 2009). These fungi attack insects by penetrating the exoskeleton with chitin degrading enzymes, eventually killing them by consuming nutrients in the hemolymph or through release of toxins (Clarkson and Charnley 1996). Because they spend most of their life cycle in the soil, these fungi may be affected by cover crops and help control insect-related problems associated with crop decline. The most well studied taxa of these entomopathogenic fungi include *Beauveria* and *Metarhizium* spp. (Meyling and Eilenberg 2007), both of which have been cultured and sold commercially as biocontrol agents. However, naturally occurring entomopathogens may be effective in reducing insect pests where the habitat encourages their persistence (Meyling and Eilenberg 2007; Pell et al. 2010).

Although their distribution was traditionally thought to be driven by the presence and movement of insect hosts, they are also found as root endophytes (Behie et al. 2015) and show rhizosphere competence (Wyrebek et al. 2011), suggesting their distribution likely depends, at least in part, on plant communities. Recently, Moonjely et al. (2016) showed that the genetic machinery responsible for insect pathogenicity evolved more recently in these fungi than the ability to live inside of plants (i.e., some entomopathogenic fungi evolved from pure endophytes), strengthening the case that plant

communities can drive entomopathogenic fungal communities. This endophytic growth strategy may be key in their additional potential role as fungal pathogen antagonists (Ownley et al. 2008). Although there has been an effort to establish a connection between habitat type and occurrence of these fungi on a landscape scale (e.g., Bidochka et al. 2002; Medo and Cagan 2011; Meyling et al. 2011), using cover crops for conservation biocontrol of entomopathogens has seen little experimentation (Meyling and Eilenberg 2007). Vegetation management strategies that encourage these beneficial fungi might also contribute to a reduction in crop decline caused by insect herbivore outbreaks, possibly even supplementing parasitism by beneficial insects.

1.4 Considerations for managing non-crop vegetation

Manipulating cover crops may be the simplest tool to help mitigate or reverse crop decline issues in perennial systems like orchards and vineyards as these systems are amenable to manipulation of plant species growing between crop rows. Different parameters, including species diversity, plant identity, and whether the plants are native or exotic, need to be considered when choosing a cover crop mix. Obviously, factors other than vegetation such as soil type, climate, management of water and nutrients, etc. need also to be considered if a certain mix is to be successful. While it is unlikely that there is a one-size-fits-all solution, certain fundamental properties of the new plant community may help in guiding management. The influence of different cover crop types on key microbial groups as reviewed and postulated here is summarized in Table 1.1.

Table 1. 1 Potential effects of cover crops on soil microbial communities involved in plant-soil feedbacks.

Cover crop type	Microbial effects	Species studied	System type	Reference
Legumes	Increases diversity of AM fungi	*Lotus corniculatus, Trifolium repens, Ononis repens*	Annual; Natural	Klabi et al. 2015; Scheublin et al. 2004
	Increases persistence of entomopathogenic fungi	*T. repens*	Perennial	Shapiro-Ilan et al. 2012
	Decreases abundance of DAPG and PRN-producing bacteria	*Lathyrus pratensis, Lotus corniculatus, Medicago lupulina, Medicago varia, Onobrychis viciifolia, Trifolium spp., Vicia cracca*	Experimental	Latz et al. 2012
C3 grasses	Increases abundance of DAPG and PRN-producing bacteria	*Lolium perenne*	Experimental	Latz et al. 2015
	Cultivar-specific disease-suppressive bacterial community	*Triticum* (different cultivars)	Perennial	Mazzola et al. 2004
	Low mycorrhizal response (less AM fungi?)	*Koeleria cristata, Bromus inermis, Festuca arundinacea, Lolium perenne, Agropyron smithii, Elymus cinereus*	Natural	Hetrick et al. 1988
C4 grasses	High mycorrhizal response (more AM fungi?)	*Andropogon gerardi, Panicum virgatum, Sorghastrum nutans, Bouteloua curtipendula*	Natural	Hetrick et al. 1988
Brassicas	Decreases fungal pathogens	*Brassica napus*/mustard green manure; *B. napus* seed meal	Annual; Perennial	Larkin et al. 2010; Mazzola et al. 2015
	Increases disease-suppressive bacteria	*B. napus* (living plant or seed meal)	Annual	Berg et al 2002; Hollister et al. 2013; Mazzola et al. 2015
	Favors *Trichoderma* and other disease-protective fungi	*B. juncea, B. napus* (crop rotation)*;* seed meal	Annual	Galletti et al. 2008; Kirkegaard, et al. 2004
	Alters microbial community	*B. napus* seed meal	Perennial	Mazzola et al. 2015
	Inhibits AM spore germination; decreases AM fungal diversity	*B. kaber, B. nigra; B. napus* seed meal	Experimental; Perennial	Schreiner and Koide 1993; Mazzola et al. 2015
Increased plant diversity	Increases disease-suppressive bacteria	N/A	Experimental; Annual/Natural	Latz et al. 2012; Garbeva et al. 2006
	Increases AM fungal diversity	N/A	Perennial/Natural	Holland et al. 2016

Cover crop type	Microbial effects	Species studied	System type	Reference
	Decreases overall negative feedback	N/A	Experimental	Maron et al. 2011; Schnitzer et al. 2011
	Increases diversity and abundance of entomopathogenic fungi	N/A	Annual/Natural	Meyling et al. 2009
Native species	Improved AM fungal diversity and plant benefit	N/A	Perennial/Natural; Experimental	Holland et al. 2016; Rúa et al. 2016

1.4.1 Plant diversity

It is well accepted that greater plant diversity leads to a suite of benefits of interest to growers including increased microbial diversity and ecosystem functioning (Bardgett and van der Putten 2014). Reliability of ecosystem services (including those that lead to disease suppression) also improves through functional redundancies associated with enhanced diversity (Naeem 1998; van Bruggen et al. 2006). In terms of plant-soil feedbacks, research shows that negative feedback is lessened with increasing plant diversity (Maron et al. 2011; Schnitzer et al. 2011) because highly diverse plant communities are less affected by soil-borne pathogens than are monocultures or low-diversity communities (Garbeva et al. 2006; Maron et al. 2011). In perennial systems, the permanent siting of crop plants and the spatial structuring of cover crops between crop rows means that growers can most easily manipulate plant diversity between rows. Increasing the diversity of cover crop species could enhance heterogeneity of soil microbial communities at multiple spatial scales leading to less negative feedback and decline of crop plants (*Figure* 2).

While the overall species richness of cover crop vegetation may be the ultimate driver of soil microbial diversity and thus soil feedback (Eisenhauer et al. 2011), plant functional group diversity may also be an important consideration (Milcu et al. 2013). Common cover crop mixtures may consist of a mix of any of the four main (non-woody) functional groups (namely C_3 and C_4 grasses, legumes, and non-leguminous forbs) and plants with different functional traits often grow well together due to temporal and spatial niche differentiation (Roscher et al. 2013). Similarly, greater diversity of plant functional groups also creates heterogeneity of niches inhabitable by soil microbes. In that case, a

cover crop with high plant functional group diversity should promote enhanced microbial diversity and subsequent ecosystem services such as disease suppression.

1.4.2 Considerations within plant functional groups

1.4.2.1 Legumes

Legumes can have significant effects on many soil microbes in addition to their nitrogen-fixing rhizobial partners. Abundance of AM fungi can be enhanced with the inclusion of legumes due to the high P cost of N_2 fixation and thus the need for AM symbionts at the nodulation sites of most legumes (Scheublin et al. 2004). The resulting high quality (high-N) litter of most leguminous plants would also be expected to attract copiotrophic microbes such as fluorescent pseudomonads (Bastian et al. 2009). However, there have been reports of a decrease in abundance of disease-suppressive bacteria when legumes are present in a mix (Latz et al. 2012, 2015) possibly because of plant defense mechanisms such as production of saponins more commonly found in legumes and other forbs than in most grasses (Osbourn 2003). Further study may incorporate the knowledge of the remarkable diversity of plant-protective secondary metabolites produced by different legumes (Wink 2013) to better understand how they affect relationships with soil microorganisms. This knowledge could aid in choosing legumes for building beneficial microbial populations in perennial systems.

1.4.2.2 C_3 and C_4 grasses

Within grasses, the photosynthetic pathway can also influence plant soil feedbacks. C_4 grasses have been shown to be highly dependent on AM fungi for

productivity (Hetrick et al. 1988; Wilson and Hartnett 1998) because they evolved in warm, arid, high light-intensity environments (Sage et al. 2011) where efficient water and P scavenging and uptake are crucial. C_3 grasses, on the other hand, are typically adapted to cooler climates or seasons with more available water. Although C_3 grasses do form associations with AM fungi, they are less reliant on them for survival due to highly fibrous root systems that are more effective at absorbing nutrients than are the coarser roots of C_4 grasses (Hetrick et al. 1988). C_3 grasses may offer other benefits, such as increasing the abundance of disease-suppressive bacteria (Latz et al. 2015) because they may have evolved a greater need for association with protective microorganisms due to their finely branched root systems, which are more susceptible to pathogen attack (Sikes et al. 2009). Some success has been seen using specific genotypes of wheat in building native populations of DAPG-producing bacteria in apple replant soils, thus mitigating negative feedback on newly-planted trees by pathogens that had built up in the orchard soil (Gu and Mazzola 2003). Therefore, given the right climate, inclusion of C_4 grasses in a cover crop mix might increase the abundance of AM fungi, while C_3 grasses could provide resources for more copiotrophic beneficial microbes such as disease-suppressive bacteria in the cooler months.

1.4.2.3 Mycorrhizal response and brassicas

Some common cover crop plants are not mycorrhizal, which may inhibit the mycorrhizal community associating with the crop plant (Karasawa et al. 2001; Stinson et al. 2006). For example, most of the Brassicaceae, are non-mycorrhizal and inhibit AM fungal spore germination due to the antifungal volatiles produced by their roots

(Schreiner and Koide 1993). There may also be a negative effect on the functioning of endemic populations of entomopathogenic fungi, as seen with *Allaria petiolata* (garlic mustard) invasion (Keesing et al. 2011). In some situations, however, brassicas can be beneficial, as they have been shown to inhibit pathogen growth and spore germination through the same volatile sulfur-containing isothiocyanates that inhibit AM fungi (Walker et al. 1937). Indeed, brassicas are often used as 'biofumigants' to kill fungal pathogens and decrease viability of their dormant spores (Smolinska and Horbowicz 1999).

Aside from decreasing fungal pathogens directly, brassicas may alter the soil microbial community in other ways that could be of use, especially in replant situations. The most illustrative study on this topic involved examination of the soil microbial community two years after the use of *Brassica napus* seed meal as a biofumigant (Mazzola et al. 2015). Replanted apple tree growth was improved in the seed meal treatment compared to chemical fumigation and a control despite significant reduction in diversity of bacteria, general fungi, and AM fungi. Most notably, with the brassica seed meal there was a shift in bacterial community favoring disease suppressive bacteria, an increase in Ascomycete fungi *Mortierella* and *Oidiodendron* spp., and elimination of almost all AM fungal taxa besides *Glomus* spp. (Mazzola et al. 2015). *Trichoderma* spp. were initially more prevalent in the seed meal treatment, but not after two years (Mazzola et al. 2015). The cause of this shift in communities could have been a selective bottleneck with the addition of the seed meal and subsequent dominance by more recalcitrant microbes that evolved the ability to use substrates rich in volatiles toxic to other organisms. There are other examples of fungi such as *Trichoderma* responding

positively to brassica cover crops (Kirkegaard et al. 2004) or persisting with the application of brassica seed meal (Galletti et al. 2008), further suggesting the ability of this genus to utilize glucosinolate-rich substrates. Berg et al. (2005) examined the effect of actively growing *B. napus* on fungal and bacterial community structure in the rhizosphere and found that disease-suppressive bacteria were enriched in the *B. napus* rhizosphere compared to that of strawberry, potato, and bulk soil. Although this study compared the rhizosphere populations among growing plants and thus is a different environment than a biofumigated soil, there may be an important correlation between brassicaceous cover crops and bacteria-induced disease suppression that goes beyond the biofumigation effect. It is important to note that brassicas are not necessarily broadly anti-fungal as they experience the same type of negative feedback from the accumulation of specific pathogens (e.g., *Olpidium brassicae*) when grown repeatedly in the same soil as do most plants (Bennett et al. 2014). Whether through inhibition of economically-important fungal pathogens or culturing of a specific rhizosphere bacterial community, brassicaceous cover crops may be a powerful tool in disease management in some cases, especially where soil pathogen load is high (e.g., replanting), but in the context of a permanent sward, benefits should be weighed against the potential negative effect on necessary AM fungal-host mutualisms of many perennial crop plants.

1.4.3 Native plant species in cover crops

Plant provenance may be an important aspect of cover crop management in order to maximize positive soil interactions (Rúa et al. 2016). Native plants, as opposed to exotics or cultivars, may be better adapted to the local climate, requiring fewer inputs

when employed as a cover crop (e.g., Costello 2010). For example, perennial grasses native to climates that typically experience long dry summers may be effectively dormant during those months and thus provide many ecosystem services associated with cover crops without risk of competition with the crop plant (Costello 2010). Local AM fungal populations may also benefit from native plants and vice-versa (Rúa et al. 2016), suggesting a better functioning mycorrhizal network that could be of benefit to crop plants. However, the use of native plants in agricultural settings is not well studied in relation to soil biotic factors, with the closest scenarios comparing neighboring natural grasslands with agronomic fields (e.g., Garbeva et al. 2006; Holland et al. 2016; Meyling et al. 2009). Garebeva et al. (2006) found greater disease suppression to the generalist pathogen *Rhizoctonia solani* AG3 in soil collected from a native grassland as compared to soil from the same site that had been converted to agricultural production. Similarly, Meyling et al. (2009) saw greater diversity of the entomopathogenic fungus, *Beauveria bassiana,* in a natural area bordering a cropping system. The increased fungal diversity and unique AM fungal community seen by Holland et al. (2016) may also have been driven by native plants in the natural sites adjacent to vineyard blocks. Agricultural habitats that more closely resemble the surrounding natural landscape may therefore benefit from the efficiency of co-evolved organisms, but it is unknown if these benefits would remain in the presence of other disturbances associated with agricultural management.

Establishment of native species can be difficult in disturbed settings and careful management (e.g., mowing frequency, weed management prior to planting) is paramount to successful stands (Penfold and Collins 2012). Knowledge of the native species'

resource requirements (water use, fertility and soil type preference) and growth habits (germination and flowering time, rhizome formation, etc.) are also helpful for establishment. Although native plants have not typically been used as cover crops in perennial systems, their potential to promote beneficial native soil biota while consuming few resources makes them an attractive option and warrants further investigation.

1.4.4 Management practices that disrupt cover crop-mediated soil feedbacks

Despite a healthy and diverse cover crop, benefits may be masked or even negated due to certain management practices. While any practice may alter microbial communities and thus ecosystem services provided by a cover crop (including irrigation, fertilization, mowing, compaction, etc.), frequent tillage, herbicide use, and fungicides may have the greatest known potential to negatively affect the soil microbial community in relation to disease outcomes.

1.4.4.1 Tillage

Perhaps more than any other practice, tillage negatively affects most soil microbes and their functioning (López-Pineiro et al. 2013; Lupwayi et al. 1998). This largely results from a decrease in soil fungi, especially AM species (Brito et al. 2012), through the disruption of mycelial networks and reduced colonization of roots and soil (Jasper et al. 1989). Furthermore, with frequent tillage, the AM fungal community often shifts with selection for species that sporulate efficiently but may invest fewer resources in extra-radical hyphae, perhaps conferring less benefit to host plants (Verbruggen and Keirs 2010). Bacterial functional diversity may also be greatly reduced through tillage

practices (Lupwayi et al. 1998). Although much of the literature on how tillage influences the soil microbial community is based on annual vegetable or grain cropping systems, a causal link exists between maintenance of permanent cover crops and increased soil microbial biomass and diversity in perennial systems such as grape (López-Pineiro et al. 2013) and citrus (Balota et al. 2011), though implications for disease outcomes have not been explored specifically in this context. Reduced or no-till cover crop management in perennial systems may also benefit from more than just the avoidance of soil disturbance. Because the effect of a plant community on the diversity and functioning of a soil microbial community likely depends on a more long term existence of that plant community (Eisenhauer et al. 2011), a permanent cover crop may be more advantageous for promoting microbe-mediated ecosystem services to crop plants than the incorporation of annual cover crops or clean cultivation. Of course, tillage can be necessary and beneficial in certain instances (e.g., replanting), especially if it involves distribution throughout the soil of disease-suppressive pseudomonads that have been amplified by certain cover crops such as wheat or brassicas (sections 4.2.2 and 4.2.3, respectively). However, the loss of diversity and heterogeneity of the soil microbial community that comes with frequent tillage may be counter productive in preventing negative feedbacks from dominating in perennial monocultures.

1.4.4.2 Herbicide use

The herbicide glyphosate, which is ubiquitous in perennial agriculture, can negatively affect beneficial soil microbes. First, it can be directly harmful to soil bacteria that make use of the shikimate pathway in the production of amino acids (Steinrucken

and Amrhein 1980). There is also evidence that glyphosate formulations when used at field concentrations have a fungicidal effect against the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Morjan et al. 2002). Perhaps most alarming, glyphosate has been shown to affect woody perennials even when applied sparingly as a 1% solution to bark (Levésque and Rahe 1992), weakening the plant sufficiently to enable damage by weak pathogens such as '*Cylindrocarpon*' spp. that were present on roots but kept in check by costly plant defense mechanisms (Levésque and Rahe 1992). This suggests that glyphosate can disrupt the delicate balance of soil feedback and may contribute to decline of crop plants.

Alternative weed management strategies in crop rows include the use of cultivation equipment, flaming, mulching, and seeding living mulches (Krohn and Ferree 2005; Hartwig and Ammon 2002). While some growers take the approach of tolerating weeds in crop rows, this strategy can either be dangerous if weeds harbor generalist pathogens that also affect crop plants (e.g., Agustí-Brisach et al. 2011) or advantageous if the weeds are a source of beneficial microbes such as disease-suppressive bacteria (e.g., Sturz et al. 2001). The local weed community likely influences these outcomes. Since weed resistance to glyphosate is becoming more common (Ghanizadeh et al. 2015; Koger et al. 2004), repeated use of materials with this mode of action often results in few dominant weed species, which may contribute to decreased soil microbial diversity.

1.4.4.3 Fungicides

Fungicides are used extensively in many perennial systems to control foliar pathogens such as mildews and their non-target effects on soil microbes can be

significant (Bünemann et al. 2006). Even with woody perennials such as apples or grapes where foliar fungicides are directed up into the canopy, residual material still accumulates on the soil surface and has the potential to affect the soil microbial community (Mackie et al. 2012). This is especially problematic with inorganic materials such as copper and copper sulfate, which are widely used in both conventional and especially in organically managed systems (Mackie et al. 2012). Heavy metals like copper do not biodegrade and thus accumulate in agricultural soils with a history of copper fungicide use (e.g., Wang et al. 2009). Although some microbes can tolerate or adapt to high concentrations of heavy metals like copper (Hassen et al. 1998; Ezzouhri et al. 2009), the reduction in microbial diversity caused by elimination of copper-intolerant species can result in decreased ecosystem functioning in the presence of additional stressors (Tobor-Kaplon et al. 2005). Overall, copper contamination seems to have a negative effect on total soil microbial biomass and functioning (Fernandez-Calvino et al. 2010; Kandeler et al. 1996) and the potential for inhibiting specific groups of beneficial fungi, such as AM fungi, has also been demonstrated (Graham et al. 1985). Although foliar fungicide treatments are often a necessity in many perennial systems, the material used should be considered carefully as long-term effects on soil microbial functioning could be affected by accumulation of active ingredients in the soil.

1.5 Looking forward

Managing an agroecosystem to more closely resemble a natural system with maximized diversity and with minimal inputs and disturbance should, over time, favor microbe-mediated ecosystem services and less negative soil feedback that leads to crop

decline. Perennial cover crop mixtures with high plant diversity and, if feasible, locally adapted species may be the best choice for increasing microbial diversity and functioning in the soil. While these ideas have been well tested in the ecological sphere, there has been little work evaluating these concepts in perennial agriculture. Although much of the literature points to the role of increased plant and microbial diversity in preventing negative feedbacks and thus crop decline, documented success in altering soil microbial communities using whole-field monoculture cover crops and plant-based amendments for replant disease is sometimes associated with changes in the soil microbial community that are unrelated to diversity *per se* (Gu and Mazzola 2003; Mazzola et al. 2015). However, increased plant diversity can also suppress disease and increase the same beneficial microbes (e.g., Garbeva et al. 2004a, 2006; Latz et al. 2012; Larkin et al. 2010)) that can be amplified through cover crop monocultures (e.g., Gu and Mazzola 2003; Raaijmakers and Weller 1998). It could be that different scenarios require a different toolkit and the tools needed to successfully prevent crop decline may be very different from those needed to cure it once negative feedback has dominated the system. Still, the demonstrated potential in either case for alleviating this problem by manipulating cover crop diversity is encouraging for improving sustainability and profitability in perennial agriculture. Further improving our understanding of the role of vegetative diversity and cover crop identity on decline outcomes through directed research efforts could help create an effective and ecologically sound management tool for perennial agriculture.

1.6 Research objectives

Vineyards in the Okanagan Valley, British Columbia were used as a model system to investigate the relationship between groundcover management and feedbacks on vine health through plant-mediated changes in soil microbial ecology, specifically soil fungi. This was addressed through five main questions, each addressed in a chapter of this dissertation:

1.6.1 Do groundcovers affect vine resistance to disease through plant-mediated changes in soil microbes?

This question is addressed in chapter 2 of this dissertation. In this greenhouse study, I grew newly rooted grapevine rootstock plants in pots inoculated first with soils from different groundcover management schemes from a field trial. I then "challenged" the vines with a concentrated suspension of a soilborne pathogen to assess if these groundcovers inhibited or promoted vine growth and disease development. I isolated fungal communities from vine roots to see if groundcovers had led to different suites of root-associated fungi and if there was any relationship to growth responses. I expected groundcover treatments to lead to differences in vine disease resistance and therefore growth responses. Furthermore, I expected that these differences would also relate to root-associated fungal communities found in vine roots.

1.6.2 Do groundcovers alter fungal endophyte community in newly planted vine roots?

This question is addressed in chapter 3 of this dissertation, which was a greenhouse study conducted in parallel to and simultaneously with the study described in chapter 2. The only difference was that no pathogen was added and rather than disease suppressive ability of groundcovers, I focused on how they influenced both AM and saprotrophic root-associated fungal communities in vine roots without the influence of an introduced pathogen. I isolated saprotrophic fungi as in chapter 2 and AM fungal communities were determined using metabarcoding (amplicon sequencing). I expected groundcover identity to influence both AM and saprotrophic fungal endophyte communities in vine roots.

1.6.3 Do these fungal endophyte communities change as vine roots senesce and/or does this depend on groundcover?

This question was also addressed in chapter 3 in an attempt to understand root-fungal interactions with groundcover-trained soil communities on a finer scale. I separated young and old absorptive roots that are capable of being colonized by endophytic fungi and, in addition to determining communities as described above, I also stained roots and quantified AM fungal structures. This allowed me to answer the question of how the AM symbiosis changes over a root's life and if this differed with different groundcovers. I expected that AM fungi would change compositionally and/or morphologically as roots age due to a changing root environment. Saprotrophic root-associated fungi were expected to change compositionally as these roots aged as well.

Because I expected groundcovers to lead to different root-associated fungal communities, I suspected that different AM fungi might differ in the way they respond to an aging root due to differences in life history strategies among groundcover-trained cohorts of AM fungi.

1.6.4 Does groundcover vegetation affect populations of beneficial and pathogenic soil microbes in vineyards?

This question was addressed in chapter 4, which is a three-year survey that included six Okanagan vineyards. I selected sites that employed at least two different groundcover management strategies (e.g. as alternate vineyard rows or experimental randomized blocks). I sampled groundcover plant communities using quadrats and collected soil cores associated with each measured plant community. Using digital droplet PCR, I then quantified AM fungi, the entomopathogenic *Beauveria bassiana,* and the plant pathogenic *Ilyonectria* spp. I then used a model selection framework to determine the strength of plant community effects as well as soil properties on each of these three groups. Based on a review of the literature (chapter 1), several relationships might be expected to emerge, e.g. AM fungi may increase with the presence of highly dependent plants such as legumes or plants native to the semi-arid Okanagan valley, but I made no *a priori* hypotheses due to the lack of precedence of this type of study.

1.6.5 Does under vine weed management affect populations of beneficial and pathogenic soil microbes and vine growth?

This question was addressed in chapter 5, which describes a field trial conducted at AAFC - Summerland. I established four living mulch treatments, including Chewing's fescue (*Festuca rubra* ssp. comutata), buffalo grass (*Bouteloua dactyloides*), birdsfoot trefoil (*Lotus corniculatus*), and shepherd's purse (*Capsella bursa-pastoris*) along with herbicide and cultivation treatments as industry standards. In the second year of establishment I measured vine growth responses and collected soil samples, which I then used to analyze soil chemistry and quantities of each of the three fungal guilds studied in chapter 4. I expected differences in competition for abiotic resources among the living mulch treatments due to differences in temporal growth patterns and resource requirements. I expected to see negative effects of herbicide and cultivation treatments on AM fungi because they remove plant hosts. I also expected shepherd's purse to decrease all three groups of fungi due to anti-fungal volatiles produced by brassicaceous plants. Conversely, I expected birdsfoot trefoil and buffalo grass to increase AM fungi due to the dependence of legumes and C_4 grasses on the symbiosis.

2 Groundcover management changes grapevine root fungal communities and plant-soil feedback[1]

2.1 Background

Groundcover vegetation management is ubiquitous in vineyards where vines often cover only a fraction of the soil surface. Non-crop vegetation, such as annual cover crops or perennial groundcovers, is recognized for its ability to drive nutrient dynamics and improve soil structure and water infiltration (Hartwig and Ammon 2002), and can affect the success of beneficial insects in the context of conservation biological control (Eilers and Kline 2009; Simon et al 2010). Groundcover vegetation can have a similarly powerful effect on soil microbial communities (Whitelaw-Weckert et al. 2007a). With the increasing economic stress imposed by soil borne pathogens in vineyards (Agustí-Brisach and Armengol 2013), capitalizing on this phenomenon may be useful for increasing vine tolerance to pathogens and thus vineyard sustainability.

Due to the effects plants have on soil microbial communities, the identity of groundcover vegetation is likely important to grapevine soil-borne disease outcomes. Plant root exudates attract microorganisms to the rhizosphere (Rovira 1969; Badri and Vivanco 2009) with plant identity being especially important in shaping fungal communities (Grayston et al. 1998; Berg and Smalla 2009; Yang et al. 2012). The composition of root exudate compounds varies among plants at the genotype level (Broeckling et al. 2008), allowing plants to select for different fungal communities. Other factors that play a role in plant selection of soil fungi include litter quality and composition (Bardgett and Shine 1999; Fanin et al. 2014) as well as the plant

[1] A version of this chapter has been published as: Vukicevich E, Lowery DT, Urbez-Torres JR, Bowen P, Hart M (2018) Groundcover management changes grapevine root fungal communities and plant-soil feedback. Plant and Soil 424: 419-433. doi: 10.1007/s11104-017-3532-2.

community's effect on soil moisture dynamics (Bezemer et al. 2006; Lange et al. 2014). In this way, there exists some top-down control by plants on soil fungal communities.

The reciprocal effect of fungal communities on plant communities is referred to as plant soil feedback (Bever et al. 1994). Feedback can be either positive if the soil fungal community improves plant growth, or negative if such a community is deleterious to plant growth. Negative plant soil feedback is common in agriculture in the absence of crop rotation and is associated with an accumulation of plant pathogens (Nicol et al. 2003; Hamel et al. 2005). Crop rotation is not suited to perennial crop production systems such as vineyards, but manipulation of non-crop plant communities may serve a similar function. If specific groundcover plant communities can modify soil fungal communities in a beneficial way, vines may avoid negative plant soil feedback caused by pathogen accumulation (Vukicevich et al. 2016).

Although the outcome of plant soil feedbacks is difficult to predict, certain fungal taxa may serve as indicators of a more positive or negative outcome. For example, *Trichoderma* Pers. spp., *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, *Chaetomium* Kunze spp., and some *Penicillium* Link spp. promote plant growth by eliciting plant defense responses while growing and/or directly attacking plant-pathogenic fungi as mycoparasites (Harman et al. 2004; Hossain et al. 2007; Vinale et al. 2008; Karlsson et al. 2015). Similarly, arbuscular mycorrhizal (AM) fungi, which are obligate plant symbionts that help vines acquire nutrients and water in exchange for carbon (Smith and Read 2008), may confer pathogen protection to their plant hosts through competition for root space and resources (Whipps 2004), signaling the plant to increase inherent defense responses (Pozo et al. 2009), or some combination of these

effects (Cameron et al. 2013). In this way, many of these fungi are often thought of as plant 'beneficials' or even 'pathogen antagonists' and their proliferation may be associated with more positive soil feedbacks.

The ability of groundcover vegetation to ameliorate negative feedback on crop plants may rely on two non-mutually exclusive factors: increased abundance of beneficial fungi and increased fungal diversity. For example, certain plant functional groups are well known to respond positively to and thus promote reciprocal growth of AM fungi in the soil. These include plants that respond positively to mycorrhizal fungi such as legumes and C4 grasses (Hoeksema et al. 2010). Native plants, especially in arid regions, may also promote positive plant-soil feedbacks due to coevolved symbionts that are especially good at scavenging resources (Rúa et al. 2016). However, little is known regarding how plant communities might favor populations of other beneficial saprobe/endophytes such as *Trichoderma*.

Since plant diversity has been shown to minimize negative feedback and increase plant productivity by decreasing the success of pathogens (Maron et al. 2011; Wright et al. 2017), a more diverse fungal community may be ultimately responsible. This could stem from a dilution effect or by increasing the likelihood of effective competitors or pathogen antagonists due to a sampling effect (Wright et al. 2017). However, groundcover identity may also lead to negative soil feedback if it serves as a reservoir for generalist pathogens as was the case for hairy vetch (*Vicia villosa* Roth) growing in Minnesota prairie soils (Benitez et al. 2016) and certain weeds in Spanish vineyards (Agustí-Brisach et al. 2011). Thus, carefully selected groundcovers may ameliorate negative feedback on crop plants, but outcomes are likely context-dependent.

A greenhouse experiment was conducted using soils from different groundcover management schemes to assess feedback outcomes on grapevine and on fungal communities inhabiting vine roots. It was hypothesized that 1) groundcover treatments would alter vine growth when challenged with a soil borne pathogen and 2) growth responses would be reflected in root endophyte communities, with those treatments that promote either greater diversity of fungi or a greater proportion of plant beneficial fungi associated with greater plant growth.

2.2 Materials and methods

2.2.1 Study site and experimental design

A greenhouse study was conducted using soils collected from a groundcover field experiment established at Agriculture and Agri-Food Canada's Summerland Research and Development Centre (SuRDC) in Summerland, British Columbia, in the spring of 2014. The climate at the site is semi-arid with a mean annual precipitation of about 320 mm. The soil at this site is Osoyoos series loamy sand. Groundcover treatments were designed to compare strategies relevant to the Okanagan Valley viticultural area and included: i) exotic C_3 grasses; ii) exotic C_3 grasses with legumes; iii) native C_4 grasses; and iv) native C_4 grasses with non-leguminous forbs. Groundcover treatments were paired with appropriate irrigation schemes that permit the persistence of the respective groundcovers (Table 2.1). Vines in all treatments received equal amounts of water in the greenhouse supplied by drip irrigation.

Table 2. 1 Summary of species included in groundcover treatments as established in spring of 2014 at SuRDC and supplemental irrigation applied to each treatment.

Treatment	Seeded species	Supplemental irrigation applied in 2014*
Exotic grass (EG)	*Festuca trachyphylla* (Hack.) Krajina *Agropyron cristatum* L. Gaertn *Festuca rubra* subsp. *commutata* Gaudin *Lolium perenne* L.	Every 3 days for 4 hrs from July 24 to Oct 28 2014 (21.3 mm/event; 682 mm total)
Exotic grass plus legumes (EGL)	EG grass mixture + *Lotus corniculatus* L. *Medicago lupulina* L. *Trifolium repens* L.	Every 7 days for 2.5 hr from July 24 to Oct 28 (13.3 mm/event; 180 mm total)
Native grass (NG)	*Bouteloua dactyloides* (Nutt.) Columbus *Festuca idahoensis* Elmer *Pseudoroegneria spicata* (Pursh) A. Love *Boteloua gracilis* (Kunth) Lag. ex Griffiths	None
Native grass plus forbs (NGF)	NG grass mixture + *Nepeta racemosa* Lam. cv. 'Little Titch' *Origanum vulgare* L. cv. 'Compacta Nana' *Artemesia frigida* Willd. *Achillea millefolium* L. *Heterotheca villosa* (Pursh) Shinners *Erigonium neveum* Douglas ex Benth. *Erigeron filifolius* (Hook.) Nutt.	Every 7 days for 2.5 hr from July 24 to Oct 28 (13.3 mm/event; 180 mm total)

Treatment	Seeded species	Supplemental irrigation applied in 2014*
C	Soils pooled and autoclaved	N/A

*Supplemental irrigation using under vine micro-sprinklers is used to maintain ground cover plant communities that would otherwise not survive at the study site.

Soil collected from groundcover treatments was used as inoculum in pots planted to grapevine rootstocks. All pots were inoculated with a soil-borne fungal pathogen, *Ilyonectria liriodendri* (Halleen, Rego & Crous) Chaverri & C. Salgado, to measure the protective effect of groundcover-trained soil (details below). All four soils were pooled and autoclaved (121°C for 90 min) and used as a sterilized control representing the vine response to pathogen in the absence of soil biota. Treatments were applied in the greenhouse in a completely randomized design with 10 replicate vines per groundcover treatment and control (50 vines total).

2.2.2 Soil collection and plant community assessment

Soils for the greenhouse experiment were collected on April 22, 2015, by pooling 18 soil cores (15 cm depth) collected along a 4 m diagonal transect through the center of each of four replicate plots per treatment. The soil was homogenized and sieved to 2 mm then stored at -20°C until planting on May 21st. Chemical analyses (OM, Bray-P, Bicarbonate-P, NO_3-N, K, Mg, Ca, pH, and CEC) of all soils used as inoculum was performed by A&L laboratories (London, Ontario) to ensure there were no large differences in soil abiotic factors as a result of our treatments that may have influenced vine growth in the greenhouse.

Groundcovers in field plots were representative of treatments established the previous year, though weeds were present in all plots. In order to characterize the realized plant communities in each treatment, plant communities were assessed approximately one month after soil collection by estimating percent cover of each species in a 25 cm x 50 cm quadrat placed at four equidistant points within each replicate in the field. Data from these four quadrats were then averaged for each replicate plot to obtain one representative plant community assessment per replicate.

2.2.3 Vine preparation and planting

Dormant cuttings of rootstock 101-14 Mgt. (*Vitis riparia* Tomenteuse x *V. rupestris* Martin) were collected from a rootstock block at SuRDC in winter of 2014/2015. Cuttings were taken out of cold storage 45 days before planting, cut into two-bud segments, and placed in the dark in moist perlite until callus began to form around nodes. Cuttings were removed from the perlite 30 days before planting and rooted upright in trays of Turface (Turface Athletics) in a growth chamber, tented to maintain humidity and allow roots to develop to a length of 2-5 cm.

Vines were planted in 3.8 L pots containing 90 g of inoculum soil applied in a band on top of 2 kg of a 75% medium-fine sand/25% Turface mixture. Another 750 mL of the sand/Turface mixture was added to cover the plant roots. Finally, 180 mL of water was added as an initial irrigation. All beakers and surfaces were sterilized with a 0.6% sodium hypochlorite solution between treatments.

2.2.4 Growing conditions

Plants were watered using a drip system with one 2 L hr^{-1} pressure-compensating emitter per vine supplying approximately 117 mL of water every third day. Initially, vines were fertigated every two weeks with 50 ppm N in a low-P fertilizer (12.5-2-14) solution. Growth was assessed at this point and the fertilization was increased to encourage more growth. For the remainder of the experiment the vines were fertilized with 100 mL of 1.5 g L^{-1} solution of 20-20-20 (300 ppm N) as needed to maintain moderate vigor. On day 106 (Sep 4), the vines were treated for powdery mildew with a foliar spray of wettable Sulfur (Kumulus, BASF) after all soil surfaces were shielded thoroughly with plastic film to prevent fungicide contact with the growing media. Vines were trained up hanging strings 113 days after planting so shoot growth direction was the same for all vines and to allow for increased airflow and ease of data collection. Supplemental lighting was initiated on day 110 (Sep 8) to provide 14 hours of total light (combined ambient plus supplemental morning/evening artificial light) each day for the remainder of the experiment.

2.2.5 Pathogen challenge

All vines were inoculated with *I. liriodendri* isolate 'PARC 340', a causal agent of black foot disease of grapevine 63 days after planting. The pathogen was isolated from the grapevine rootstock '3309C' (*Vitis rupestris* x *riparia*) as part of a survey on grapevine trunk diseases in British Columbia (Úrbez-Torres et al. 2014) and maintained on potato dextrose agar stored at 4°C. Two weeks prior to inoculation, the culture was grown up on fresh potato dextrose agar (39 g L^{-1}) at 25°C. When conidia had formed,

each plate was flooded with a 0.5% Tween 80 (Sigma Aldrich) solution and a glass rod was used to gently separate conidia from hyphae. The concentration of conidia was then quantified using a hemocytometer and adjusted to 10^5 spores ml^{-1}. Twenty mL of this conidial suspension of *I. liriodendri* was then applied to each pot the same day. Conidia from the suspension were streaked onto fresh media and incubated at 25°C to confirm viability. Frequency and duration of watering was altered for two weeks post-inoculation to 40 mL every day in order to maintain a moist soil environment for establishment of *I. liriodendri*. Care was taken to not overwater and wash conidia through the potting media. No fertilizer was applied during those two weeks.

2.2.6 Growth responses

Plants in both experiments were grown for 256 days and destructively harvested on February 1, 2016, by cutting all shoots between the first and second bud. Above-ground biomass was dried at 65°C for 48 hours. Leaves were removed and shoot mass (pruning weight) was measured. Root systems were carefully extracted from the media and rinsed with tap water. Five randomly chosen plants for each treatment were processed on February 2 and their root systems were stored in 35% ethanol for further analyses (see below). The remaining plants were processed in an identical manner on February 5 but their root systems were oven dried at 65°C for 24 hours and weighed.

Root necrosis was assessed using a WinRHIZO (Regent Instruments) scanning system to measure the proportion of necrotic surface area of entire root systems of five randomly selected plants per groundcover treatment. Briefly, root systems were scanned against a blue background using an EPSON Expression 11000XL scanner. The color

analysis feature in WinRHIZO was used to define a set of colors for 'background', 'healthy', and 'necrotic.' Several scans with easily identifiable healthy and necrotic segments were used to set six shades of blue (background), eight shades of green to light brown (healthy roots), and 12 shades of dark brown to black (necrotic roots).

2.2.7 Identification of cultivable fungi

Differences among groundcover treatments in root-associated fungi were assessed using fungi isolated from equal proportions of healthy and necrotic roots at the time of harvest. The roots were surface sterilized using a technique optimized by Úrbez-Torres (pers. comm.). Briefly, roots were rinsed 3 times in deionized (DI) water to remove spores, submerged in 70% ethanol for 30 sec followed by 0.6% sodium hypochlorite for 1 min. Roots were then washed in DI water three times to remove bleach and kept in individual plastic bags at 4°C until plating on media within 48 hrs.

Surface sterilized roots were plated on potato dextrose agar (39g L^{-1}) amended with rose Bengal (30 mg L^{-1}) to slow fungal growth and thus encourage growth of a greater diversity of fungi. Ampicillin (100 mg L^{-1}) was added to inhibit bacterial growth. Two plates were used per replicate plant, one each for healthy and necrotic roots. Eight 1cm root segments were randomly chosen from the cassette of surface-sterilized roots and pushed into agar on each plate with sterile forceps. Plates were incubated in the dark at room temperature (21-22 °C). Fungi that differed in colony morphology were selected to obtain pure cultures and isolated from hyphal tips. Isolates were scored as present/absent for each plate using pure cultures as reference for identification of colonies on subsequent plates.

To determine the identity of each fungal colony, DNA was extracted from pure cultures using the MP Bio Fast Prep Spin Kit for Soil (MP Bio, CA). Amplification and sequencing of the internal transcribed spacer (ITS1-5.8S-ITS2) region of ribosomal DNA was carried out by Fragment Analysis and DNA Sequencing Services (University of British Columbia, Kelowna, BC) using primers ITS1/ITS4 (White et al. 1990). The following recipe was used for PCR (12.5 µL total reaction volume): 2.5 µL 5X Green GoTaq buffer, 0.5 µL dNTP mix (10 mM each), 0.25 µL forward primer ITS1 (10 µM), 0.25 µL reverse primer ITS4 (10 µM), 1.25 µL $MgCl_2$ (25 mM), 0.13 µL GoTaq DNA polymerase (5 U μL^{-1}), 1.5 µL DNA template, and 6.37 µL dH_2O. Reaction conditions were: initial denaturation at 94°C for 3 min, 34 cycles of 94°C for 40 sec, 54°C for 40 sec and 72°C for 50 sec, and finally 72°C for 10 min before holding at 4°C.

Some fungal isolates did not amplify (especially yeasts), thus the large ribosomal subunit primers NL1/NL4 (Kurtzman and Robnett 1998) were used for these samples. PCR product was cleaned using 2 µL of ExoSAP-IT (Applied Biosystems) per sample. Sequencing was carried out by the Fragment Analysis and DNA Sequencing Service (University of British Columbia) using a Big Dye Terminator kit (Applied Biosystems). Sequences were aligned to existing databases using BLAST (Altschul et al. 1990) and taxonomy was assigned to the level of species using the 'species hypothesis' (SH) feature of the UNITE database (Koljag et al. 2013) for most sequences and the 'TrichoKEY' feature of The International Subcomission on *Trichoderma* and *Hypocrea* (ISTH)(Druzhinina et al. 2005) database for *Trichoderma* ITS sequences.

Because *I. liriodendri* was not isolated from roots, a DNA-macroarray technique developed by Úrbez-Torres et al. (2015) was used to confirm colonization of roots by the

fungus. Briefly, genomic DNA was isolated from randomly sampled roots of five vines grown with treatment C soil by macerating roots in liquid nitrogen, then using the MP Bio Fast Prep Spin Kit for Soil (MP Bio, CA) according to the manufacturer's instructions. DNA was diluted 1 in 3 to prevent PCR inhibition. Procedures for amplification of the beta-tubulin (TUB) region and hybridization to probes on the macroarray followed those described previously (Úrbez-Torres et al. 2015).

2.2.8 Colonization of roots by arbuscular mycorrhizal fungi

Colonization of roots was assessed by quantification of recognizable AM fungal structures, including hyphae, spores, vesicles, and arbuscules. Approximately 1 g of roots (equal proportions of healthy and necrotic) were sampled from five randomly-selected root systems from each groundcover treatment and stored in root cartridges in 30% ethanol at 4°C. Staining followed a modified version of Vierheilig et al. (1998) optimized in our lab for *Vitis* roots. Roots were cleared in 10% KOH for 3 days at room temperature, followed by addition of 3% H_2O_2 to make a 3:2 ratio of 10% KOH to 3% H_2O_2 and heating in a water bath to 70°C for 10-15 minutes until roots became transparent. Roots were then stained in a 1% ink-vinegar solution (6% acetic acid) and again heated in the 70°C water bath for 10-15 minutes until stain had entered the hyphae. Clearing and staining was confirmed intermittently using a dissecting microscope. Roots were then mounted on slides in polyvinyl-lacto-glycerol. The magnified intersections method (McGonigle et al. 1990) was used to quantify percent colonization. Colonization was assessed at 200x magnification for four slides of 15 x 1 cm root segments per root system (two slides for healthy roots and two slides for necrotic roots).

2.2.9 Data Analysis

All analyses were performed in R (R core team 2016). Scripts and data files are available for viewing on the Open Science Framework using the following link: https://osf.io/dnpze/?view_only=76776a2559a54837855736abb9be3840

Plant communities in groundcover treatments were compared in terms of both functional composition as well as indicator plant species. Functional characteristics were compared using MANOVA with percent cover of C_3 grasses, C_4 grasses, legumes, non-leguminous forbs, native species, and Simpson's diversity index as response variables. Post-hoc ANOVA was used to assess which characteristics varied among groundcovers, with Tukey's honest significant difference used to separate treatments (Tukey 1949). P-values were adjusted using a Bonferroni correction in the case of multiple comparisons for each of the six functional characteristics assessed. Indicator plant species for groundcover treatments were identified using the R package 'indicspecies' (De Caceres et al. 2009). Plant community composition was visualized using non-metric multidimensional scaling (nMDS) with Bray-Curtis dissimilarity employing the R package vegan (Oksanen et al. 2016).

To compare feedback outcomes for vines grown with soils from groundcover treatments and challenged with pathogen, growth responses (dry pruning weight and dry root weight) were normalized relative to mean weight of control vines. This allowed for comparison of groundcover treatments in their ability to modify the expected negative feedback caused by the pathogen, *I. liriodendri.* These data were analyzed using MANOVA in the R base package with relative pruning weight and relative root weight as dependent variables and groundcover treatment as the independent variable. Post-hoc

ANOVA was used to assess which growth parameters contributed to differences, with Tukey's honest significant difference (Tukey 1949). P-values were adjusted using a Bonferroni correction in the case of multiple comparisons for pruning and root weight. Percent necrotic root surface area was determined using a subset of samples and was thus analyzed using a separate ANOVA.

A two-way ANOVA was used to test for differences in fungal operational taxonomic unit (OTU) richness among groundcover treatments and root type (healthy or necrotic). Fungal community composition was analyzed with a constrained analysis of principal coordinates (CAP) (Anderson and Willis 2003) based on Jaccard dissimilarity (for binary matrix data) using the 'capscale' function within the R package vegan (Oksanen et al. 2016). Significance of the constraining factors 'groundcover treatment' and 'root type' and any interaction were assessed using an ANOVA-like permutation test with 9,999 permutations. Indicator species analysis using the R package 'indicspecies' (De Caceres et al. 2009) was used as a post-hoc test to identify fungal OTUs that were drivers of any differences in community composition seen using CAP analysis.

Total colonization by AM fungi was compared using one-way ANOVA testing for the effect of groundcover-trained soil on percent colonization. Post-hoc analyses employed Tukey's honest significant difference (Tukey 1949). Because of the observation that AM fungi were common in necrotic roots, a linear regression was used to assess the significance of this relationship across groundcover treatments (excluding sterilized control where no AM fungi were found) with percent necrosis as a function of AM colonization (healthy and necrotic roots combined) and groundcover treatment as a random factor. The R package 'MuMln' (Nakagawa and Schielzeth 2013) was used to

determine R^2 values for the model including both the fixed effect (colonization) and fixed plus random effect (groundcover treatment).

2.3 Results

2.3.1 Plant communities and soil inoculum chemistry

Realized plant communities in groundcover field plots differed in all functional group characteristics except percent cover of legumes and non-leguminous forbs (Table 2.2). The native grass treatment (NG) had lower plant species diversity than the other three treatments. As expected, the exotic grass (EG) and exotic grass plus legumes (EGL) treatments had greater percent cover of C_3 grasses than NG or the native grass plus forbs (NGF) treatment, while NG and NGF had a greater proportion of native species. These differences were caused by the dominant planted species in these treatments, with the C_3 grasses *Lolium perenne* and *Festuca* spp. prevalent in the EG and EGL treatments and native C_4 *Bouteloua* spp. dominant in NG and NGF. EGL had a lower proportion of C_4 grasses than NG, NGF, or EG due to the dominance of *Bouteloua* spp. in NG and NGF and the presence of *Setaria viridis* (also C_4) as a weed in EG at the time of assessment.

Table 2. 2 Simpson's diversity and percent cover of plant functional groups and native species for each groundcover treatment in the field one month after soil collection. Treatments are: (EG) exotic grasses, (EGL) exotic grasses plus legumes, (NG) native grasses, and (NGF) native grasses plus forbs. Treatments were compared using MANOVA with each characteristic as response variables with post-hoc ANOVA and Tukey's Honest Significant Difference used to separate treatments.

Characteristic	EG	EGL	NGF	NG	*F*	*P-adj*
Diversity (1-*D*)	0.75±.03a*	0.74±.02a	0.72±.06a	.39±.27b	*10.19*	*0.008*
% C3G cover	29.7±4.9a	35.0±8.4a	0.6±.6b	0.6±.6b	*14.18*	*0.002*
% C4G cover	34.4±4.9a	10.0±1.5b	47.2±5.7a	35.6±5.8a	*10.51*	*0.007*
% Legume cover	15.6±9.6	8.1±1.9	0.9±.6	0±0	*2.2*	*0.846*
% Non-legume forb cover	9.8±1.3	23.4±5.3	29.1±5.0	24.1±11.7	*1.43*	*0.280*
% Native species cover	0±0b	0.6±.6b	35.3±7.9a	35.3±5.9a	*16.9*	*<0.001*

*mean ±standard error (*n*=4); letters indicate differences between treatments at a 95% confidence limit.

Plant community visualization using nMDS (Fig. 2.1) shows plant community composition among groundcover treatments in the field. Indicator species for treatment EG included *Trifolium repens* (*IndVal*=0.93, *P*=0.01) and *Setaria viridis* (*IndVal*=0.81, *P*=0.009) while *Lotus corniculatus* was an indicator species for treatment EGL (*IndVal*=0.95, *P*=0.002). There were no indicator species for NG or NGF because they either shared dominant species (*Bouteloua*) and/or species that were only found in one treatment were too rare to be considered indicator species, statistically (e.g., *Artemesia frigida* and *Pseudoroegneria spicata* in NGF).

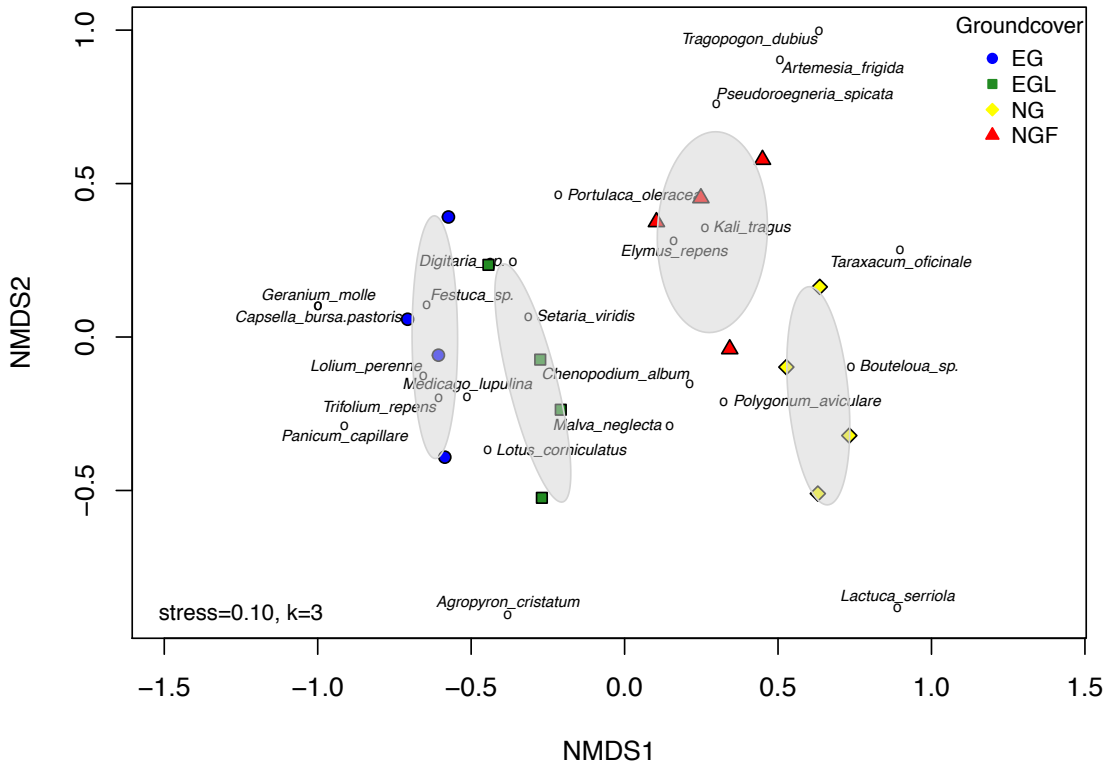

Fig. 2. 1 Plant communities in each groundcover treatment visualized using non-metric multi-dimensional scaling. Indicator species analysis was used to confirm which plants were associated with each treatment in the realized species mixes one month after soil collection (~14 months after establishment). Significant indicator species were: *Trifolium repens* and *Setaria viridis* in treatment EG (*IndVal*=0.93, *P*=0.01 and *IndVal*=0.81, *P*=0.009, respectively) and *Lotus corniculatus* in treatment EGL (*IndVal*=0.95, *P*=0.002). Treatments are: (EG) soil trained by exotic grasses, (EGL) soil trained by exotic grasses plus legumes, (NG) soil trained by native grasses, and (NGF) soil trained by native grasses plus forbs. Ellipses indicate 95% confidence limits of weighted means (centroids) for each treatment.

Soil chemistry was similar among groundcover treatments, with an expected slight decrease in pH and increase in NO_3-N for the autoclaved control (Table S1, Appendix A). Because a small amount of soil inoculum was used in this experiment and

fertilizer was applied, we assumed that any minute changes in soil chemistry caused by groundcover treatments did not directly affect vine growth in the greenhouse.

2.3.2 Effect of groundcover on vine growth responses

Groundcover treatment marginally affected vine growth relative to control (*Wilks Lambda*=0.727, *P*=0.094). Relative pruning weight differed among treatments (*F*=3.61, *P-adj*=0.046) with vines grown with EG soil producing more above-ground growth than vines grown with NGF soil (Fig. 2.2a). Root weight relative to control did not differ among groundcover treatments (*F*=0.29, *P-adj*=1.0) but vines in all groundcover treatments tended to have smaller root systems compared to control (Fig. 2.2b).

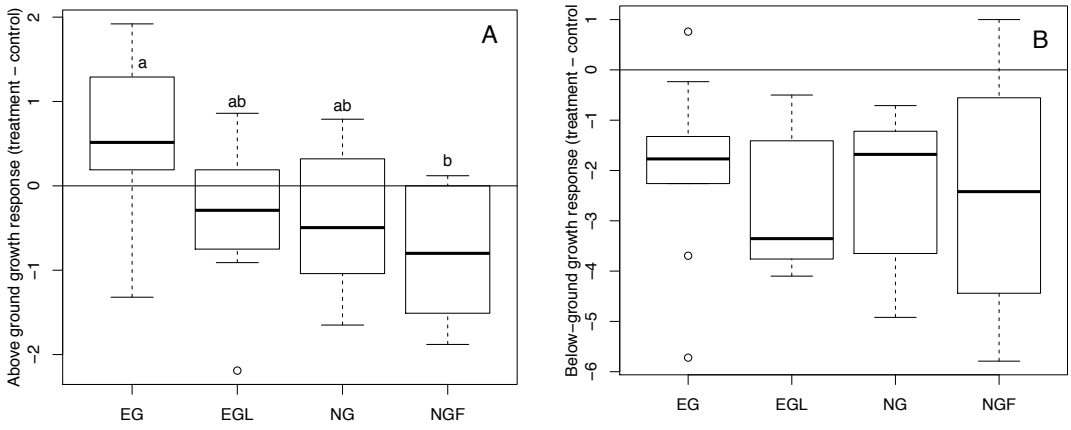

Fig. 2. 2 Growth response of vines grown with different groundcover trained soils and challenged with *I. liriodendri* relative to a sterile control. Values above the line indicate positive feedback effect (better growth than control) and values below the line indicate a negative feedback effect of soil biota trained under each groundcover treatment. Treatments are: (EG) soil trained by exotic grasses, (EGL) soil trained by exotic grasses plus legumes, (NG) soil trained by native grasses, and (NGF) soil trained by native grasses plus forbs. Letters indicate significant differences ($p<0.05$) among treatments. Above ground growth response (A) differed among groundcover treatments EG and NGF (*F*=3.61, *P-adj*=0.046) while below ground growth response (B) did not vary among treatments (*F*=0.29, *P-adj*=1.0).

Groundcover treatment influenced root necrosis (F=3.39, P=0.029) with vines grown with NGF soil having more necrotic surface area than those grown with EGL, NG, or control soil but not more than EG (Fig. 2.3). The proportion of root surface area measured as necrotic averaged 56.8±1.0% across all treatments.

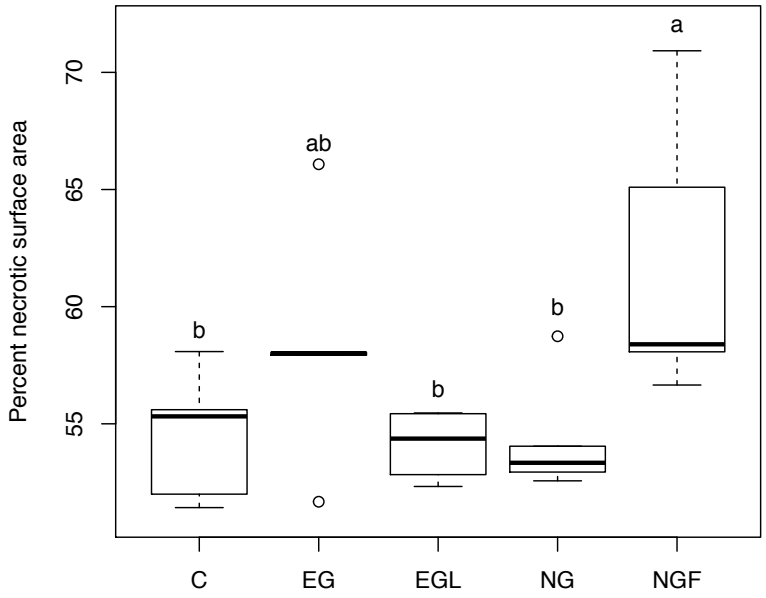

Fig. 2. 3 Percent necrotic root surface area of vines grown with different groundcover trained soils and sterile control soil. Treatments are: (C) sterilized control soil, (EG) soil trained by exotic grasses, (EGL) soil trained by exotic grasses plus legumes, (NG) soil trained by native grasses, and (NGF) soil trained by native grasses plus forbs. Letters indicate significant ($p<0.10$) differences among treatments defined by Tukey's honest significant difference. n = 5.

2.3.3 Effect of groundcover soil on endophyte community

In total, 43 unique morphotypes were isolated and sequenced from roots across all treatments, which yielded a total of 21 OTUs after taxonomy was assigned based on sequence identity (Table 2.3, see Table S2 in Appendix A for details). All sequences generated were deposited to Genbank under the accession numbers MF567494 to MF567528 and MF563967 to MF563968. OTU richness did not vary among

groundcover treatments (F=0.61, P=0.66) or root type (F=0.02, P=0.89) with a mean of 4.09±0.36 OTUs found per vine. The most frequently isolated species were *Penicillium levitum* Raper & Fennell and *Malassezia restricta* E. Guého, J. Guillot & Midgley, which were found on almost all plates and thus removed prior to community analysis. Other common OTUs included *Clonostachys rosea*, *Fusarium proliferatum* (Matsush.) Nirenberg*, Gibberella fujikuroi* (Sawada) Wollenw.*,* and *Trichoderma harzianum* Rifai. *Ilyonectria liriodendri* colonization was confirmed using the macroarray technique (Fig. S3, Appendix A).

Table 2. 3 Proportion of vines (healthy/necrotic roots) from which each OTU was isolated. Treatments are: (C) autoclaved soil, (EG) soil trained by exotic grasses, (EGL) soil trained by exotic grasses plus legumes, (NG) soil trained by native grasses, and (NGF) soil trained by native grasses plus forbs.

OTU	C	EG	EGL	NG	NGF
Alternaria sp.	0[a]/0[b]	0/0	0/0	0/10	0/0
Ascomycota	0/0	0/0	0/0	0/0	0/0
Ceratobasidiaceae	0/0	0/0	0/0	0/0	0/0
Chaetomium cupreum	11.1/0	11.1/0	0/0	0/0	0/0
Chaetomium grande	0/0	0	0/11.1	0/0	0/0
Clonostachys rosea	55.6/77.8	44.4/55.6	50/66.7	30/40	25/33.3
Clonostachys rosea (yeast)	11.1/33.3	11.1/11.1	12.5/0	20/10	0/0
Coniolariella hispanica	0/0	0/0	12.5/0	0/0	0/0
Fusarium proliferatum	55.6/33.3	11.1/22.2	12.5/33.3	30/50	50/33.3
Gibberella fujikuroi	33.3/55.6	55.6/55.6	12.5/33.3	60/30	50/11.1
Dactylonectria macrodidyma	11.1/0	44.4/11.1	12.5/11.1	20/0	12.5/11.1

OTU	C	EG	EGL	NG	NGF
Dactylonectria macrodidyma (yeast)	0/0	0/11.1	37.5/11.1	10/10	0/0
Penicillium sp.	0/0	0/0	12.5/11.1	0/0	0/11.1
Penicillium abidjanum	22.2/0	11.1/0	12.5/0	20/10	0/11.1
Penicillium astrolabium	0/0	11.1/0	0/0	10/0	0/0
Peziza ostracoderma	0/0	0/0	0/0	0/0	0/0
Rhizopus arrhizus	0/0	0/0	0/0	0/0	25/0
Sordariomycetes	0/0	22.2/0	12.5/0	10/0	0/0
T	22.2/0	0/33.3	12.5/11.1	20/30	12.5/44.4
Trichoderma virens	11.1/0	0/0	0/0	0/0	0/0
Trichoderma neorufum	0/0	0/0	25/11.1	10/30	0/0
Trichoderma harzianum	0/66.7	0/33.3	25/55.6	40/30	50/66.7

[a]Proportion of healthy roots; n=9 for treatments C, and EG, n=8 for EGL and NGF, n=10 for NG
[b]Proportion of necrotic roots; n=9 for C, EG, EGL, and NGF, n=10 for NG

Fungal community in vine roots differed among groundcover treatments (F=1.54, P= 0.023), with vines grown with NGF soil harboring a different composition of fungi than those grown with EG soil (Fig. 2.4a). Indicator species analysis comparing these two communities identified *Trichoderma harzianum* as an indicator species for treatment NGF (*IndVal*=0.68, P=0.01)(Fig. 2.4a). None of the communities found in groundcover treatments differed from the sterilized treatment, C. Among the endophytes isolated from vines grown with sterilized soil were *Chaetomium cupreum, Clonostachys rosea, Fusarium proliferatum, Giberella fujikoroi, Dactylonectria macrodidyma* (Halleen, Schroers & Crous)*, Penicillium abidjanum* Stolk*, P. levitum,* unknown yeast 'T', *Trichoderma virens* (J.H. Mill., Giddens & A.A. Foster) Arx*,* and *T. harzianum* (Table 2.3).

Fungal communities differed between healthy and necrotic roots (F=2.41, P=0.02) (Fig. 2.4b), but there was no interaction between groundcover treatment and the type of root sampled (healthy or necrotic) (F=0.98, P=0.45). *Trichoderma harzianum* was an indicator species for necrotic roots (*IndVal*=0.59, P=0.008). Constrained axes in CAP analysis explained 13.5% of the variation in community among samples, with 86.5% of variation left unexplained.

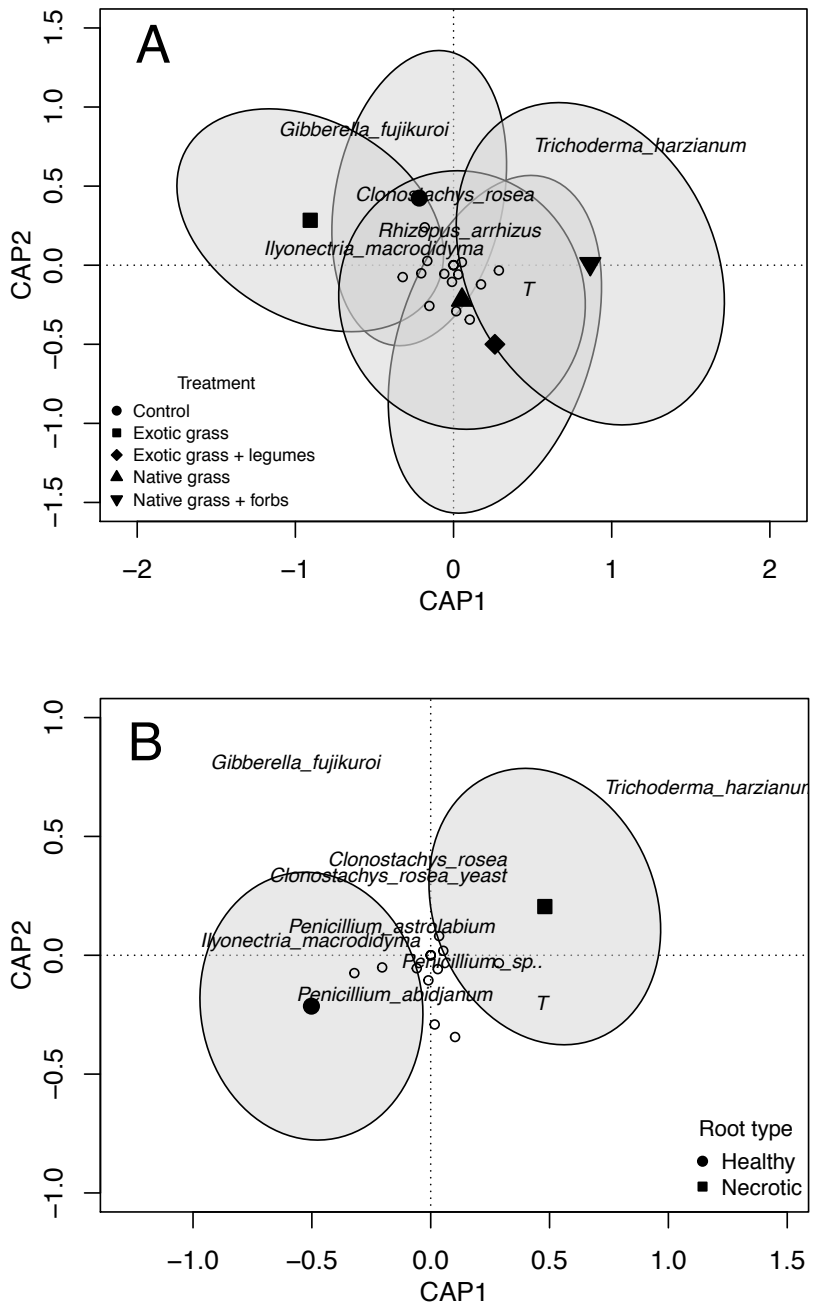

Fig. 2. 4 Constrained analysis of principal coordinates (CAP) of cultured fungal communities isolated from vine roots growing in each groundcover treatment (A) as well as from healthy and necrotic roots from all treatments (B). Composition of root-inhabiting fungal communities differed between exotic grass and native grass plus forbs treatments (F=1.54, P=0.02)(A) and between healthy and necrotic roots (F=2.41, P=0.02)(B). Symbols represent weighted means (centroids) for each treatment and ellipses represent 95% confidence intervals.

2.3.4 Effect of groundcover management on colonization by AM fungi

No difference in intensity of colonization by AM fungi was detected among groundcover treatments (F=0.93, P=0.44). Mean colonization was 45.8±5.9% across treatments EG, EGL, NG, and NGF. No AM fungal colonization was found for vines grown with sterilized control soil. Greater colonization was found in necrotic roots compared with healthy roots overall (F=16.4, P=0.0004), with no significant interaction between groundcover treatment and root type.

There was a positive linear relationship between percent necrosis and AM fungal colonization across the experiment fit by the following equation: necrosis = 0.14 x colonization + 50.7 ($R^2_{\textit{fixed effects}}$ = 0.22, $R^2_{\textit{fixed + random effects}}$ = 0.36, P=0.037)(Fig. 5). This indicates that AM fungal colonization was associated with roots measured as necrotic.

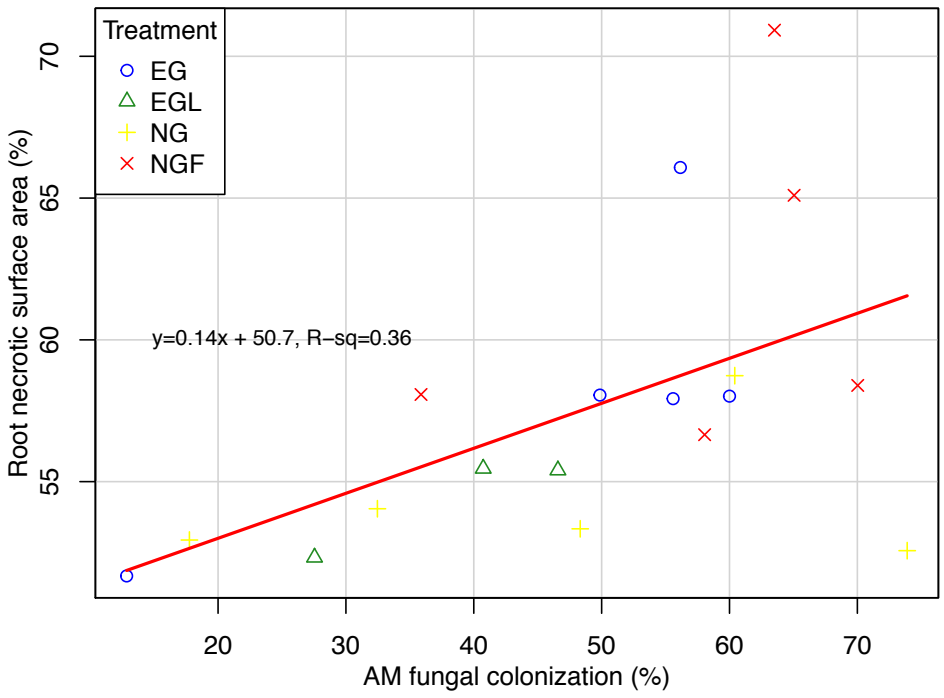

Fig. 2. 5 Positive relationship (P=0.037) between root necrosis and AM fungal colonization across all groundcover treatments excluding sterilized control for which there was no AM fungal colonization.

2.4 Discussion

2.4.1 Effect of groundcover on vine growth response

Confirming our hypothesis, vine growth response was affected by groundcover identity, but only for two treatments: exotic grass (EG) and native grass plus forbs (NGF). Soil trained by EG improved vine growth compared to NGF, indicating that EG may have promoted a more beneficial microbial community, capable of mitigating negative soil feedback on grapevine, at least compared to NGF.

Although these two treatments differed in terms of C_3 grass cover and native species cover, these differences also existed between EGL and NG, which did not differ in vine growth response. Therefore differences in plant functional group or plant provenance did not underlie the observed disparity in growth response between EG and NGF. Plant diversity did not drive growth responses either, as diversity did not differ between NGF and EG, and the only groundcover treatment with lower plant diversity than the other treatments, NG, did not alter feedback outcomes. Although there is some evidence for plant diversity *per se* increasing antagonistic microbial communities (Latz et al. 2012) and decreasing the success of pathogens (e.g., Maron et al. 2011), these effects can depend on the extent to which the added plant species increase the availability of limiting resources in the soil, thereby allowing for diversification of the soil community (Waldrop et al. 2006).

The results of this study can be explained by this enrichment of an otherwise stressful soil environment by the EG treatment, which contains plants and irrigation strategies that increase limiting resources at the study site, namely water and nitrogen. Much of this may have to do with the indicator species for that treatment, *Trifolium*

repens, which became established in most field replicates due to the use of sprinkler irrigation in those plots, and an abundance of seed in the seed bank at our site. *Trifolium repens* is noted in some biodiversity studies as a potential keystone species because it can dramatically increase soil microbial diversity and activity (Stephan et al. 2000) by increasing soil nitrogen pools as a prolific N_2 fixer (Carlsson and Huss-Danell 2003; Palmborg et al. 2005). In our N-limited soil, *T. repens* could have increased soil N enough to select for a more abundant, diverse and potentially competitive microbial community. In fact, although still very low, the NO_3-N in EG soil was higher than in the other treatments. This effect could have been further enhanced by the use of sprinkler irrigation, as microbial biomass and subsequent N-mineralization increases when water is applied in semi-arid climates (Austin et al. 2004). This is especially true of coarse textured soils, such as those at our site, where irrigation leads to increases in mineralization of labile soil organic matter with minimal buffering by more stable soil N pools as is the case in finer-textured soils (Austin et al. 2004). In this environment, an N_2-fixing legume coupled with consistent sprinkler irrigation could dramatically alter available resources and subsequent microbial diversification and competition, with alteration of feedback outcomes as a result. If these changes involve bacteria or non-culturable fungi, we would not have detected them with our methods. Future work could employ sequencing technologies to see if groundcover management also affects these other groups.

The NGF treatment produced vines with the highest mean root necrosis overall and the poorest growth, but levels of necrosis were not greater in response to NGF than to EG where a stronger growth response was seen. This could mean that necrotic surface

area might not be a good predictor of vine growth response in this case. Although this is the first study to explore the use of color analysis for determining root necrosis on grapevine, comparative methods used in banana pathosystems (Tabarant et al. 2011; Vermeire et al. 2011) provide some evidence that necrosis is a better predictor of plant growth in simplified systems with sterilized soil than for plants grown in live soil. Using sterile soil, Vermeire et al. (2011) reported a negative relationship between root necrosis and root weight with root pathogen challenge, but Tabarant et al. (2011) found no consistent trends involving a banana/nematode pathosystem in a living soil where organic amendment treatment effects dramatically altered this relationship. This could be due to complex interactions between soil microbial communities, pathogens, and plants in "necrotic" roots that have variable outcomes in terms of plant growth. For example, because AM fungal colonization was correlated with necrosis in our study, it could be that at least part of the necrosis measured was due to infection by these fungi. Indeed, AM fungi are known to enhance lignification of infected roots (Smith and Read 2008) in which case some of the darker roots may actually be responsible for growth promotion. On the other hand, AM symbioses are known to function along a continuum from mutualism to parasitism (Johnson et al. 1997) and thus could have been acting as parasites in some roots leading to a necrotic response. Parasitism by AM fungi can be found in the presence of high rates of plant-available nutrients such as phosphorous added as fertilizer (Hoeksema et al. 2010), which was the case in our experiment. Although there is some literature documenting the use of senescent roots by AM fungi for survival and sporulation (Mueller et al. 2017), our current understanding of mycorrhizas

in necrotic roots, whether caused by pathogens, root age, or the AM fungi themselves, is limited.

Contrary to expectations, we did not see strong symptoms of disease development in our study despite inoculation with *I. liriodendri*. Our eight-month timescale was likely long enough for disease development as greenhouse studies involving black foot pathogens typically show disease symptoms within four to six months (Alániz et al. 2007; Úrbez Torres et al. 2014). However, there is one report of delayed onset of disease into the second year of growth (Whitelaw-Weckert et al. 2007b). The events leading to development of black foot disease are not well known, but these fungi can exist as endophytes in asymptomatic vines (Halleen et al. 2003), which could have been the case in our study. Indeed we isolated another causal agent of black foot disease, *D. macrodidyma* from healthy roots in all treatments. The observed growth responses offer insight into the response of vines to fungal interactions in the early stages of infection rather than a comparison of disease-preventative effects *per se* afforded by different groundcover soil biota. It is not clear from our results if these feedback responses would be consistent over a longer timescale.

2.4.2 Effect of groundcover on root-associated fungi

The diversity and identity of fungi recovered in this study is consistent with previous surveys of grapevine-inhabiting fungal endophytes (Halleen et al. 2003; Casieri et al. 2009). For example, Halleen et al. (2003) isolated 22 OTUs from cuttings of four rootstock/scion combinations including fungi from the genera *"Cylindrocarpon"* (*Ilyonectria*), *Fusarium, Trichoderma, Alternaria,* and *Clonostachys*. Casieri et al.

(2009) isolated 66 OTUs from above-ground parts of five *V. vinifera* cultivars. In both studies, as in ours, the cultivable fungal community was dominated by the Ascomycota, with Sordariomycetes as the most abundant class of fungi. We also observed many known plant-protective fungi in our samples (e.g., *Trichoderma harzianum, Clonostachys rosea,* and *Cheatomium* spp.) (Inglis and Kawchuck 2002; Harman et al. 2004; Karlsson et al. 2015). It is possible that these fungi provided some protective effect against the introduced pathogen *I. liriodendri,* as seen in previous work, e.g. with *Trichoderma* (Fourie et al. 2001), thereby preventing rapid disease development. Because many of these studies, including this one, employed culturing on media, which limits the fungi isolated to saprotrophs, a complete picture of community interactions is difficult to elucidate.

As many of the taxa found in this study are commonly isolated from nursery propagation material and above ground woody tissues of vines (Halleen et al. 2003; Casieri et al. 2009; Gonzalez and Tello 2011) and are able to colonize different parts of a vine (Casieri et al. 2009), it is likely that some fungi colonized young roots from our cuttings, becoming established in roots before the application of treatment soil inoculant. This may explain why, although groundcover treatments EG and NGF led to divergent root associated fungal communities, no treatment differed from the sterilized control in terms of species richness or composition. Because infection by latent pathogens such as *I. liriodendri* can occur during nursery propagation (Gramaje et al. 2011) and planting in nursery fields (Halleen et al. 2003; Whitelaw-Weckert et al. 2013), the interaction between fungal communities in new vines and vineyard soil biota could determine

disease outcomes. The results of our study suggest that groundcover management can affect these interactions and thus may be crucial to vine disease tolerance over time.

The differences between fungal communities in treatments EG and NGF may have resulted from interactions among introduced soil biota, the pre-existing endophytes, and *I. liriodendri*. For example, the mycoparasite *T. harzianum* was found in very high numbers in the NGF treatment compared to EG. One explanation is that the NGF treatment increased the inoculum potential of *T. harzianum* in soil and thus roots in that treatment were more thoroughly colonized. However, since *T. harzianum* was also found in roots of control vines, we cannot rule out that *T. harzianum* was already present in vine roots (having colonized them from the dormant cuttings), but proliferated more when interacting with soil biota from NGF than EG. Contrary to reports of pathogen protection and growth promotion with *T. harzianum* (Harman et al. 2004; Vinale et al. 2008), it was found to be most prevalent in necrotic roots and in the treatment where vines grew most poorly. It could be that the recovered *T. harzianum* isolates were not acting as plant-protective endophytes but rather saprotrophs or mycotrophs feeding on dying root and fungal biomass. These findings highlight the challenge of predicting outcomes based on changes in native populations of these opportunistic fungi. It may be that groundcover management directly alters the community of plant-protective mycoparasites in roots or that these fungi respond to changes in root environment induced by other plant soil interactions.

No differences were found in the intensity of root colonization by AM fungi among groundcover treatments despite evidence that plant identity among groundcover treatments should affect AM fungal populations (Hoeksema et al. 2010). It is possible

that groundcover treatments altered the identity of AM fungi in vine roots without altering colonization intensity, which is largely determined by host and would only be affected by extremely different levels of inoculum density (McGonigle and Miller 2000). Since most plant species in the groundcover treatments were mycorrhizal, large differences in inoculum levels in each groundcover might not be expected. If groundcovers were to include a large proportion of species from the Brassicaceae, e.g., mustards, a decrease in AM fungal inoculum (Schreiner and Koide 1993) and subsequently lower colonization rates in vines might be expected. Other non-mycorrhizal weeds that were present in this trial such as *Chenopodium album* are not known to decrease AM fungal inoculum as would be expected from brassicas and thus would not be expected to affect vine root colonization if most of the other species in the groundcover are mycorrhizal.

3 Changes in arbuscular mycorrhizal fungi between young and old *Vitis* roots grown with soils trained by distinct plant communities[2]

3.1 Background

Arbuscular mycorrhizal (AM) fungi, perhaps the most ubiquitous root-associated fungi, are obligate biotrophs living within active cortical cells of the majority of land plants (Smith and Read 2008). They are known as mutualists that confer nutritional and plant protective benefits to their host in return for sugars derived from photosynthesis (Smith and Read 2008). As such, the availability of plant-derived C is paramount to the growth and reproduction of these fungi. In woody perennial hosts, absorptive roots that are colonized by AM fungi are ephemeral and produced throughout the growing season (Majdi et al. 2003), meaning that changes to the AM symbiosis must be occurring as colonized roots become less functional and eventually senesce. Although researchers working with these fungi have made anecdotal observations of how the symbiosis appears at different stages during a root's lifespan, there are not yet any reports that have explicitly examined how AM fungi respond as absorptive roots age.

Although our current understanding of the general process of senescence in plants is reasonably thorough (Wojciechowska et al. 2018), only recently have researchers begun to examine what happens to fine roots during this process (Bagniewska-Zadworna et al. 2014). Although evidence is limited, it seems that fine root aging and senescence may follow a similar process to that of aboveground tissues with programmed cell death and autophagy prominent in the process (Bagniewska-Zadworna et al. 2014). Fine roots are ephemeral (Pregitzer 2003) and as they age they lose their absorptive capacity

[2] A version of this chapter has been published as: Vukicevich E, Lowery DT, Eissenstat, D, Hart M. Changes in arbuscular mycorrhizal fungi between young and old *Vitis* roots. Symbiosis. doi: https://doi.org/10.1007/s13199-019-00598-3.

(Bouma et al. 2001; Volder et al. 2005) concomitantly with a decrease in metabolic activity (Comas et al. 2000) and sugar content (Kosola et al. 2002). Thus, from the perspective of an AM fungus, an aging root may become increasingly hostile in terms of the availability of plant-derived C and host-fungus communication.

Changes in colonization of host roots by AM fungi with decreasing plant-derived C has been proposed by the carbon limitation hypothesis, wherein fungal symbionts are expected to decrease as plant C becomes limiting. However, a meta-analysis by Barto and Rillig (2009) challenged the C limitation hypothesis by showing that for many types of host plants there is no overall effect of C limitation on AM fungal colonization. This hypothesis is based on studies of plant-wide effects in which the C limitation has been imposed by a reduction in leaf area. How AM fungi respond to the aging of ephemeral absorptive roots may be very different.

AM fungal response to an aging root may depend not only on the flow of plant C to the fungus, but also in the functionality of the aging root. The increase in secondary compounds such as tannins and decrease in metabolic activity (Comas et al. 2000) and absorptive capacity (Bouma et al. 2001; Volder et al. 2005) suggest potential declines in the complex exchange between host and symbiont, including the maintenance of a periarbuscular membrane (Luginbuehl and Oldroyd 2017). A decrease in the frequency of arbuscules, the site of nutrient exchange, could then be expected in older roots. But does this mean the fungus simply leaves the root altogether, resulting in lower colonization of older roots?

A senescing host environment for an obligate biotroph should cause a shift toward propagule formation favoring survival of the fungal species. The anecdotal observations

that AM fungi, especially species from the most common family, Glomeraceae, tend to form more intra-radical storage organs (ISOs) such as vesicles and/or spores as roots lose their ability to absorb nutrients (INVAM 2018) is consistent with this notion, though this has not been shown experimentally. Indeed ISOs formed in dead roots are used as propagules by these taxa (Biermann and Linderman 1983; Klironomos and Hart 2002; Muller et al. 2017). However, there are also many AM fungal taxa that do not form ISOs and instead may form spores outside the root (Hart et al. 2002). Due to these different life history strategies, it could be that older absorptive roots favor communities of AM fungal species more heavily represented by those capable of propagule formation inside roots compared with communities in younger roots. Exploring this relationship could give us a more mechanistic insight into how AM fungal communities are shaped over time in perennial hosts.

As part of a larger study examining the effects of vineyard groundcovers on soil fungi, the current study sought to explore how AM fungal communities change between young and old roots and if this is affected by groundcover vegetation that may have supported different soil microbial communities. This study adresses two main questions:

1. How do AM fungal species respond to root aging? Do they change compositionally and/or functionally between young and old roots?
2. Do different groundcover plant communities change the composition of AM fungi in a subsequent crop host? If so, do AM fungi respond differently to aging roots depending on the plant community in which fungal communities were assembled?

3.2 Materials and Methods

3.2.1 Study site and experimental design

This greenhouse experiment was part of a larger study at the Agriculture and Agri-food Summerland Research and Development Centre in Summerland, British Columbia looking at the effect of groundcover plant communities on soil fungi, which was described in chapter 2. Grapevine rootstocks (*Vitis riparia* x *rupestris* '101-14') were grown with whole soil inoculum collected from a field trial described previously (Vukicevich et al. 2018). Groundcover treatments represented a range of production practices: exotic grass mix with sprinkler irrigation (EG), exotic grass mix plus legumes with occasional sprinkler irrigation (EGL), native grass mix with no sprinkler irrigation (NG), and native grass mix plus forbs with occasional sprinkler irrigation (NGF). The plants used coupled with different amounts of irrigation in these groundcover treatments have the potential to lead to different soil environments and thus different suites of soil microbial communities (Vukicevich et al. 2016). This range in soil environments thus enabled us to test the consistency of any changes to AM fungi in young and old roots across potentially distinct fungal cohorts.

Five replicates of vines grown with soil from each groundcover treatment (20 vines total) were harvested after eight months. Root systems were subsampled by root type and young (light-colored) and old (darkly-pigmented) first- and second-order roots were processed to determine: a) AM fungal colonization, including total % root length colonized, proportion arbuscules, and proportion ISOs using microscopy (n=5 root systems per groundcover treatment) and b) AM fungal community composition by DNA extraction and Illumina metabarcoding (n=5 root systems per groundcover treatment).

3.2.2 Greenhouse establishment and growing conditions

Vines were rooted from two-bud cuttings taken from the previous season's growth and planted as described in a similar trial that tested the effects of groundcover identity on plant-soil feedback (chapter 2 of this thesis). The vines in this experiment were different than those used in the previous feedback study (Vukicevich et al. 2018). Vines were grown in 3.79 L pots (17.5 cm deep). Watering occurred every third day with ~117 mL applied to each pot. Initial fertilization with 50 ppm N using a 12.5-2-14 liquid formulation occurred three times during the first 52 days of growth, followed by 100 mL of a 300 ppm N solution using a 20-20-20 formulation as needed to maintain adequate, but not overly vigorous growth for the rest of the trial. In total, this higher rate was applied 11 times during the remaining 168 days of the trial. Supplemental lighting was applied beginning on day 110 and maintained as 14 hours of combined ambient plus supplemental light for the remainder of the trial.

3.2.3 Harvest and root subsampling

Vines were destructively harvested after 256 days. Pruning weight was measured after drying as described previously (Vukicevich et al. 2018). Root systems were carefully rinsed free of media. Subsamples of light and dark absorptive roots used for isolation of saprotrophic fungi were stored in individual Ziplock bags at 4°C. Root systems were then stored in 35% ethanol at 4°C for transport to UBC Okanagan, Kelowna BC.

Color classes were used to approximate young versus old absorptive roots as pigmentation is tightly correlated with metabolic activity and root age in grapevines

(Comas et al. 2000). Two sets of approximately 5 g (fresh weight) of light and dark absorptive first- and second-order roots were randomly sampled from each root system and stored in plastic root cartridges in 35% ethanol. One set of roots was used for quantification of AM fungal structures using microscopy and the other half was used for DNA extraction and subsequent downstream molecular sample preparation for sequencing and community analysis.

3.2.4 Staining and microscopy for AM fungal morphology

To measure AM fungal colonization, roots were cleared in KOH, stained using ink and vinegar, and mounted on slides using the protocol of Vierheilig et al (1998) modified for *Vitis* roots as described previously (Vukicevich et al. 2018). Colonization was assessed using the magnified intersections method (McGonigle et al. 1990), examining two slides of 15 ~1 cm root fragments for young roots and two slides for old roots. Scoring followed the method used by McGonigle et al. (1990). To better represent fungal allocation of resources to structures, proportion ISO and arbuscular colonization was calculated by dividing total ISO or arbuscular colonization by total colonization.

3.2.5 Preparation of samples for analysis of AM fungal communities

To determine AM fungal communities in young and old roots, roots were macerated in liquid N, extracted for genomic DNA using the FastPrep spin kit for soils (MP Bio, Carlsbad, CA) according to the manufacturer's directions, and then prepared for sequencing using a nested PCR protocol.

In the first step of the nested protocol, an 800 bp product was amplified in a 50µL total reaction volume using 10 µL 5x GoTaq PCR buffer (Promega, Madison, WI), 4µL 2.5mM dNTP mix, 3.5 µL 25mM MgCl2, 2µL of a 10µM solution of the primers AML1 and AML2 (Lee et al. 2008), 0.25 µL HotStart Taq polymerase, 26.25 µL nuclease-free water, and 2 µL template DNA. Thermocycler conditions for this first reaction were: 95°C for 2 min, 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by 72°C for 10 min. Presence of target was then confirmed by running the product on a 1% agarose gel and viewing under a UV light for comparison with a 100 bp ladder. This primer set is known to amplify exclusively Glomeromycotan DNA (Lumini et al. 2011; van Geel et al. 2014).

The second PCR amplified a ~550 bp fragment nested within the product from step one using 10 µL 5x GoTaq PCR buffer (Promega), 4µL 2.5mM dNTP mix, 3.5 µL 25mM MgCl2, 2µL of a 10µM solution of the primers WANDA (Dumbrell et al 2011) and AML2 (Lee et al. 2008), 0.25 µL GoTaq polymerase, 27.25 µL nuclease-free water, and 1 µL template DNA, which was product from reaction diluted 100x. Thermocycler conditions for the second reaction were: 95°C for 1 min, 15 cycles of 95°C for 30 sec, 60°C for 30 sec, and 68°C for 1 min, followed by 68°C for 5 min. Presence of target was assessed by running product on a 1% gel and visualizing with a UV light comparing to a 100 bp ladder. WANDA and AML2 were modified to include Fluidigm universal tags (Fluidigm, San Francisco, CA), which allowed subsequent barcode attachment as per Egan et al. (2018).

The final PCR that attached a unique set of barcodes to each sample, was carried out in 20 µL reaction volumes using 5 µL GoTaq buffer, 0.4 µL 10mM dNTP mix, 3.6

µL 25mM MgCl2, 0.6 µL 20 mg/mL BSA, 0.2 µL GoTaq polymerase, 0.5 µL each of a forward and reverse primer with unique barcodes, 9.2 µL nuclease-free water, and 1 µL DNA template, which was product from step two diluted 1 in 15. Thermocycler conditions for this final step were: 95°C for 1 min, 10 cycles of 95°C for 30 sec, 60°C for 30 sec, and 68°C for 1 min, followed by 68°C for 5 min.

Concentrations of product DNA were quantified using a fluorometer (Promega) and adjusted to 4nM by combining with the appropriate amount of nuclease-free water. Samples were then pooled and shipped to the University of Alberta's sequencing facility where they were sequenced using Illumina MiSeq (Illumina, San Diego, CA) both forward and reverse, allowing complete coverage of the 550 bp product.

3.2.6 Bioinformatics

Raw sequences were de-multiplexed at University of Alberta's sequencing lab using the software, MiSeq Reporter 2.5.1 (Illumina, San Diego, CA). Sequences were trimmed, de-noised and a feature table was constructed in QIIME 2.1 (http//:qiime2.org/) using DADA2 (Callahan et al. 2016). One sample was discarded at this step due to abnormally low sequence number (<1,000). Features were aligned and masked using the Mafft algorithm in QIIME (Katoh et al. 2002). A phylogenetic tree was constructed using the features with a midpoint root (Price et al. 2009). Taxonomy was assigned to features using version 5 of the MaarjAM database for AM fungal SSU sequences (Opik et al. 2010) and the naïve Bayes classifier algorithm in QIIME (Zhang 2004). All data was exported from QIIME2 for analysis in 'phyloseq' (McMurdie and Holmes 2013) where data were normalized by calculating simple proportions, creating a feature table of

relative abundances for each feature. Sequence data and Linux commands used for all QIIME 2.1 steps are available on the Open Science Framework using the following link: https://osf.io/ekrdf/?view_only=eaca88e109c641e79244c8abaf88030a

3.2.7 Statistical analysis

3.2.7.1 AM fungal colonization

To test if total AM fungal colonization as well as proportions of arbuscular and ISO colonization differed between young and old roots, a two-way ANOVA in the R base package (R core team 2013) was used. Groundcover treatment was included as an interaction term to see if a) these plant communities led to functional changes in AM fungal morphology and b) morphological changes between root age classes depended on groundcover.

3.2.7.2 AM fungal communities

A two-way *PERMANOVA* (Anderson 2001) including an interaction term with groundcover treatment was used to test if AM fungal community structure differed between young and old roots and if this depended on groundcover-trained soil. Weighted UniFrac distances were used to account for phylogenetic distance between samples as well as relative abundance of each sequence variant (Lozupone et al. 2011). Pairwise *PERMANOVA* using the R package 'RVAidememoire' (Hervé 2018) was employed as a *post hoc* test to discern which groundcover treatments differed. Differences in community structure were also visualized using principal coordinates analysis (PCoA) in the R package 'vegan' (Oksanen et al. 2018). In order to identify which sequence

variants more strongly associated with each root age or groundcover soil, indicator species analysis was performed using the R package 'indicpecies' (De Caceres et al. 2009).

3.3 Results

3.3.1 AM fungal colonization

Total AM fungal colonization was higher in old roots with 56.6 (±13.7)% of root area colonized versus young roots, which were colonized at a rate of 32.9 (±10.0)% (F=7.789, P<0.01)(Fig. 3.1a). There were no differences in total colonization of grapevine roots among groundcover soil inoculum (F=0.662, P=0.58) nor was there any interaction between root age and groundcover inoculum (F=0.581, P=0.63).

Arbuscules were more prevalent in young roots where they comprised 9.2(± 5.5)% of structures observed (F=5.477, P=0.03)(Fig. 3.1b). Arbuscules also varied with groundcover soil and were more frequently found in the NGF treatment than all other treatments (F=4.552, P=0.01)(Fig. 3.1c), but there was no interaction between root type and groundcover treatment (F=0.12, P=0.95).

Proportion of fungal colonization as storage structures (vesicles and/or spores, ISOs) was also higher in old roots, where ISOs made up 52.5(±14.0)% of AM fungal structures, than in young roots, where ISOs represented 30.9(±10.4)% of AM fungal structures (F=6.068, P=0.02)(Fig. 3.1d). There was no difference in proportion of ISOs among groundcover soil inocula (F=1.120, P=0.36), nor was there any interaction between root type and groundcover soil (F=0.067, P=0.98).

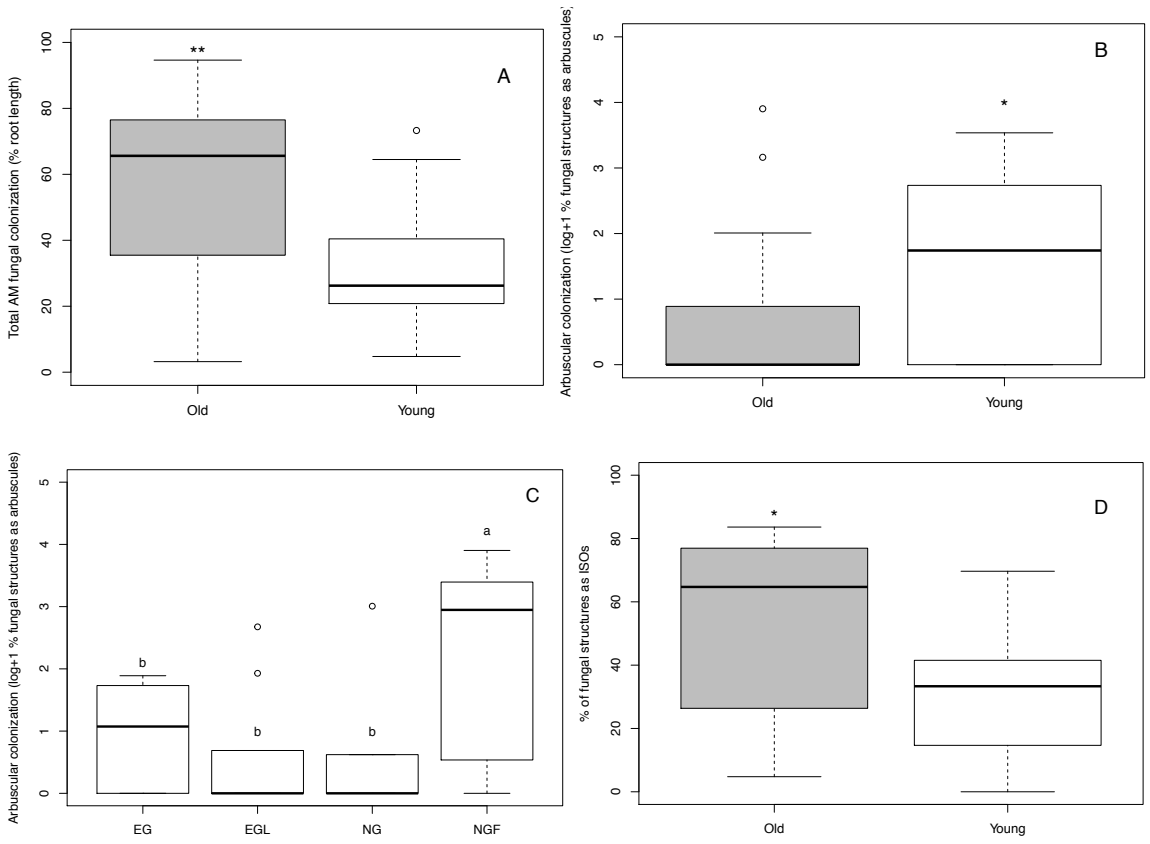

Fig. 3. 1 (A) Total colonization was greater in old roots (grey boxes) than in young roots (white boxes)(P<0.01). (B) Proportion of AM fungal structures that were arbuscules was greater in young versus old roots (P=0.02). (C) Proportion of AM fungal structures that were arbuscules was greatest in vines grown with soil from groundcover of native grass plus forbs (NGF)(P=0.01). (D) Proportion of AM fungal structures that were intra-radical storage organs (ISO) was also greater in old versus young roots (P=0.03). "*" and "**" indicate significant differences between root types at (P<0.05) and (P<0.01), respectively. Letters in (C) indicate significant (P<0.05) differences among treatments. Treatments abbreviations are: EG, exotic grasses; EGL, exotic grasses plus legumes; NG, native grasses; NGF, native grasses plus forbs.

3.3.2 AM fungal communities

In contrast to saprotrophic fungi, AM fungal communities in vine roots were not different between young and old roots (F_{pseudo}=0.35, P=0.76) but varied across groundcover treatments (F_{pseudo} =2.43, P=0.04). Pairwise *PERMANOVA* showed that the native grass treatment, NG, led to a different AM f

ungal community than exotic grasses plus legumes, EGL, and native grasses plus forbs, NGF (P=0.038 and P=0.044, respectively). Other treatments did not vary among each other. There was no interaction between groundcover soil used and root age class (F_{pseudo}=0.26, P=0.98)(Fig. 3.2).

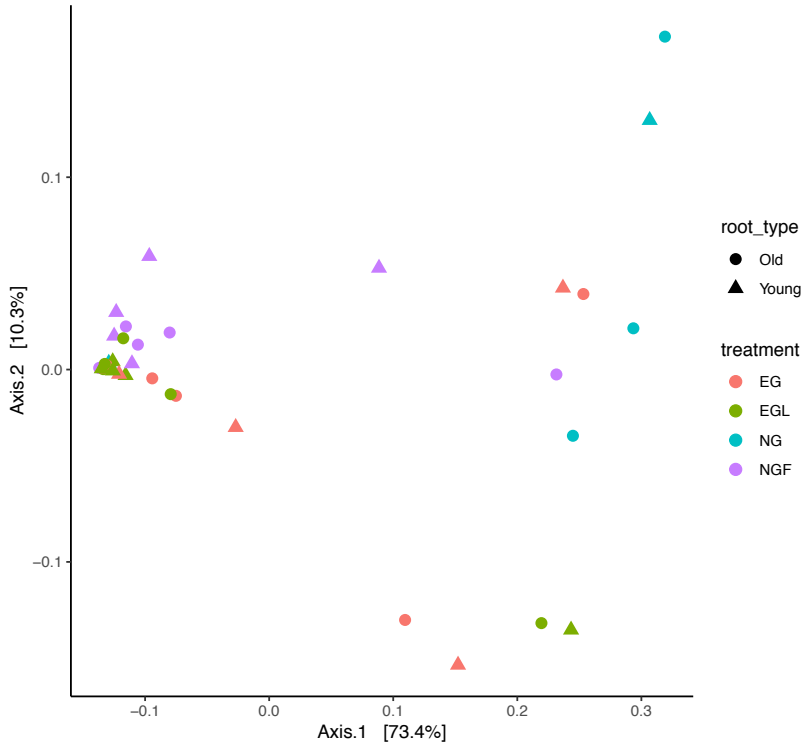

Fig. 3. 2 PCoA of AM fungal communities by groundcover (colors) in young (triangles) and old (circles) roots. *n=5* for each groundcover x root type combination except for groundcover NG, for which one old root sample failed to amplify (n=4) and three young root samples failed to amplify (n=2). Communities did not change with root age class (P=0.78) but varied by groundcover treatment (P=0.04). Treatment NG was significantly different from EGL and NGF. There was no interaction between root age and treatment (P=0.98). Treatments abbreviations are: EG, exotic grasses; EGL, exotic grasses plus legumes; NG, native grasses; NGF, native grasses plus forbs.

Most operational taxonomic units (OTUs) were classified as belonging to the family Glomeraceae with a mean relative abundance per sample of 90.3±4.7%. The next

most abundant family represented was Claroideoglomeraceae (7.5±4.3%), followed by Diversisporaceae (1.9±2.0%), Paraglomeraceae (0.17±0.25%) and Archaesporaceae (0.005±0.006%). The single most abundant OTU in our experiment, which made up on average 51.7±12.8% of sequences in each sample, was classified as VTX00067. This virtual taxon (VT) is most consistently associated with voucher specimens identified as *Funneliformis mosseae* (Nicholson & Gerd.) Walker & Schüßler, according to the MaarjAM database.

OTUs strongly associated with each groundcover treatment (indicator taxa) are given in Table B.1 (Appendix B). There were also two OTUs that were significant indicators of old roots. One was OTU #086472a2b3a3c45864359719ec753ec1 (*IndVal*=0.562, *P*=0.02), which was assigned to VTX00063, *Glomus* sp. at 85% confidence by the naïve Bayes classifier. The second was feature #39ba3d22322e5cbfabaee2751c37aca7 (*IndVal*=0.558, *P*=0.03), which was assigned only to the family level (Glomeraceae) with a 99.9% confidence level.

3.4 Discussion

3.4.1 Do AM fungi respond functionally or compositionally to aging roots?

Overall it was found that AM fungi responded functionally rather than by differential selection of different species in aging roots. The most prominent shifts were that older roots had higher colonization, fewer arbuscules, and more storage organs than younger roots. Despite groundcover identity leading to compositionally different AM fungal communities and a greater amount of arbuscules with one groundcover, the shifts in AM fungal morphology between root ages were consistent.

3.4.1.1 Effect of root age class on AM fungal colonization

Total AM fungal colonization was higher in old roots than young roots across all groundcover treatments. This trend has been seen in this symbiosis in hosts ranging from transformed root organ cultures (Bécard and Fortin 1988) to apple roots in the field (Resendes et al. 2008). However, as the metabolic activity of the host root, and presumably the efficiency of nutrient exchange within cortical cells, decreases with age (Comas et al. 2000), the proliferation of AM fungal biomass in older roots is initially counter-intuitive. How is the fungus able to access more plant-derived C if the root is less active?

This study shows that arbuscules are more prevalent in young roots and decrease as roots age across groundcover treatments. Early work by Hepper (1985) showed that infection by AM fungi decreased with the age of roots but varied depending on plant host. This effect was attributed to senescing cortical cells as a root ages (Holden 1975; Henry and Deacon 1981), which would likely decrease the establishment of arbuscules by the obligate biotrophic AM fungi. Arbuscules are ephemeral, lasting from just a few days to two weeks (Smith and Read 2008), and the cellular processes that accompany their degeneration suggest a regulated process and maintenance of an active cell that could later be recolonized (Luginbuehl and Oldroyd 2017). A decrease in arbuscules as a root ages as seen in this study, however, is likely a result of natural processes of cortical cell maturation including loss of connectivity between cortex cells and vascular tissue (rendering an arbuscule non-functional), the development of a suberized hypodermis (Peterson 1989) (preventing new infections), and/or cortical cell senescence (Blee and

Anderson 1998). The capacity of a cell to undergo the complex process of periarbuscular membrane formation (Luginbuehl and Oldroyd 2017) could also be expected to decrease in an aging cortical cell. This finding of a lower proportion of arbuscules in older roots is congruent with the notion of arbuscules becoming obsolete in aging portions of roots that may be less active, suberized, or senescent.

As expected, intra-radical storage organs (ISOs) were more abundant in old roots. The increase in total colonization and proportion of ISOs in these older roots means that the end of the exchange phase does not lead to abandonment of the root by the fungi, at least for the communities in these soils. This is consistent with reports of the ability of ISO-forming fungi to use excised or senescent roots as launch points for future infection (Baermann and Linderman 1983; Klironomos and Hart 2002; Muller et al. 2017), although these data suggest that this process begins when roots are still intact and not necessarily dead as shown in these other studies. The issue of C allocation from plant to fungus, i.e., how all of that fungal biomass is acquired at this stage in the symbiosis, draws into question the conventional view of this evoluntionarily-stable mutualism. Three non-mutually exclusive hypotheses may place this trend of increased storage structures in aging perennial absorptive roots into the context of this symbiosis in perennial hosts:

1. The parasite hypothesis

It may be that the fungi switch to parasitism *sensu* Johnson et al. (1997) as host roots senesce. Colonization by some common AM fungi, especially *F. mosseae*, a ubiquitous agricultural taxon (Rosendahl et al. 2009) capable of forming ISOs (Biermann

and Linerman 1983; Muller 2017), could represent a significant C drain on the plant host as fungal biomass invested in ISOs increases (Johnson 1993). Excess fertilizer (Johnson 1993; Nijjer et al. 2010) and water application (García et al. 2008) could also exacerbate this effect. The proliferation of ISOs in older roots would indicate parasitism if it was based solely on resource exchange with the fungus giving less to the plant in old roots (fewer arbuscules) per unit C invested in the fungus (more ISOs).

2. The refuge hypothesis

Another hypothesis is that the proliferation of storage structures in old roots represents propagule formation and thus a natural progression of the symbiosis for these fungal taxa (Biermann and Linderman 1983; Valera-Cervero et al. 2016; Muller 2017). Many fungi in the Glomeraceae and Claroideoglomeraceae are able to use dead roots as infective propagules (Klironomos and Hart 2002; Valera-Cervero et al. 2016; Muller et al. 2017). The consistent shift to ISOs in older roots in this study could simply be an artifact of the community present in these soils, which was dominated by these fungi and especially by *F. mosseae*, which indeed completes its lifecycle in this manner (Muller et al. 2017). The mechanism responsible for the shift is unknown. It could involve fungal sensing of an increasingly stressful root environment (Jin et al. 2017), though this was not tested in this study. However, the loss of arbuscules and degeneration of the exchange phase of the symbiosis or some other mechanism might also be responsible for triggering the proliferation of vesicles as a root ages. AM fungi that are adapted to the frequent disturbances associated with agricultural soils would likely benefit from sporulation inside a host root where spore maturation could complete in a relatively safe environment

post-tillage as opposed to in the bulk soil where hyphal attachment is tenuous in anticipation of the next disturbance.

3. The saprotroph hypothesis

As AM fungi are known to proliferate in patches of organic matter in bulk soil (St. John et al.1983; Hodge et al. 2001) and may utilize N acquired there for its own metabolism (Hodge and Fitter 2010), less metabolically active old roots could represent a source of nutrition. In that case, the fungi with the greatest ability to break down organic compounds within roots would have the advantage over slower growing AM fungi and saprotrophs. Tissue-derived nutrients could then be incorporated into fungal biomass with subsequent growth and establishment of the symbiosis in new roots benefitting from a short-circuiting of soil nutrient cycling. There is some peripheral evidence for this in the literature. Eason et al. (1991) showed P transfer from dying roots of one plant to intact roots of a receiver plant through AM fungi. Recently, Pepe et al. (2018) showed that *F. mosseae* and *R. irregulare* extra-radical hyphae remained active and able to colonize neighboring plants up to five months after plant hosts had been killed and roots were no longer viable. More work is needed to establish if and how AM fungi might be able to utilize organic forms of nutrition from dead or dying roots and if this then gives them a competitive advantage over taxa incapable of this.

3.4.1.2 Effect of root age class on AM fungal community

The lack of change in community structure between young and old roots suggests that the same AM fungi that are initial colonizers persist in those roots as the roots age

and confirms that AM fungi responded functionally rather than compositionally to a changing root environment in this study. To our knowledge, there are no other reports on the dynamics of AM fungal communities as roots age.

A possible explanation for the consistency in community structure as roots age could be due to founder effects stemming from the relatively narrow window of time in which AM fungi typically colonize new absorptive roots (Resendes et al. 2008). As roots age and become less active (Comas et al. 2000), they are probably less attractive to would-be symbionts, precluding AM fungal succession in aging mycorrhizal roots. The initial colonizers may also leave little physical space for colonization by other fungi as they grow within the root. This may be especially true for the Glomeraceae-dominated communities in this experiment, which are known for rapid and thorough intra-radical colonization (Hart and Reader 2002) and could also be why these taxa are often the dominant constituents inside roots of many plants worldwide (Opik et al. 2013).

AM fungal communities are known, however, to shift over time in perennial plant hosts (Husband et al. 2002; Kil et al. 2010; Hart et al. 2014) and it was suspected that shifts during root aging might contribute to this phenomenon. It could be that other factors may drive communities over time at the root system level and these may be especially prevalent in the field compared to the greenhouse, as in this study. Also worth noting is that sufficient P fertilization and water were applied in this study to support moderate plant growth. Combined with the use of agricultural soil, these factors could have selected for cohorts of AM fungi dominated by taxa capable of sporulation inside of roots as these traits are common in taxa ubiquitous in agricultural soils (Rosendahl et al. 2009). This may have drowned out any community shifts that might have taken place in

a different environment and limit the interpretation of these results to the context of this study.

3.4.2 Do groundcovers change AM fungal communities in vine roots?

Groundcovers led to different AM fungal communities in vine roots, with the native grass treatment differing from both the exotic grass plus legumes and native grass plus forbs treatments. These differences could be due to selective pressures created by the plant-soil environment unique to each treatment in the field where communities were trained. Our groundcovers were comprised of distinct plant communities (Vukicevich et al. 2018) and plant identity alone could have been responsible for the changes to AM fungal community structure as reported elsewhere (Johnson et al. 2004; Hausmann et al. 2009; van de Voorde et al. 2010; De Deyn et al. 2011). These groundcovers, however, were also coupled with different irrigation regimes (Vukicevich et al. 2018), which created different abiotic soil environments that may also alter AM fungal communities (Deveautour et al. 2018).

Some indication of how these different groundcovers may have led to different AM fungal communities is indicated by the indicator species associated with each groundcover treatment. Indicator fungi found in the native grass treatment included several taxa within the family Claroideoglomeraceae, a recently described family formerly called '*Glomus* group B' within the Glomeraceae (Schüßler and Walker 2010). This groundcover treatment was the only one in this study where no irrigation water was applied to the drive row, thus representing the most similar environment to the natural conditions of the semi-arid region. The native C_4 grasses, *Bouteloua* and

Pseudoroegneria spp., that persist in this treatment likely rely on AM fungi for the provisioning of soil resources in the heat of the summer. It is possible that some of these indicator species represent more drought tolerant AM fungi, as fungal taxa are known to vary in their ability to confer drought tolerance (Ruiz-Lozano et al. 1995; Augé 2001).

Indicator species for the native grass plus forbs treatment, where significantly more arbuscules were found, included the common species *Rhizoglomus irregulare*, which has been observed in our lab to form many arbuscules (V. Kokkoris, pers. comm.), but it was also the only treatment to include as an indicator an OTU from the Paraglomeraceae. Although the greater prevalence of arbuscules in this treatment cannot be attributed to any particular indicator taxon, it is interesting to note that Paraglomeraceae was only found in this treatment. Regardless of the taxa responsible, the link found between community differences and functional differences in terms of arbuscule frequency hints at a potentially important role of herbaceous groundcover vegetation in influencing the AM symbiosis in perennial woody crop plants whose roots share the same soil.

Despite this difference between several of the groundcover treatments in AM fungal community and the greater proportion of arbuscules in the native grass plus forbs treatment, groundcover did not affect the functional shift from more arbuscules in young roots to higher colonization rates and more vesicles in older roots. In other words, for this study system, the functional changes that occur as roots age were consistent across different communities of fungal symbionts. However, it is unknown if these changes would be consistent among more disparate AM fungal communities in other soils.

4 Groundcover vegetation affects soil fungi in multi-year survey across five semi-arid vineyard sites[3]

4.1 Background

Perennial agriculture is characterized by crop rows alternating with drive rows to facilitate field and tractor work. This means that much of the land area in a perennial cropping system is not actually planted to crop plants, but subjected to floor management practices. Depending on regional climate patterns, pest pressure, nutrient challenges, and aesthetics, different strategies are used to manage drive rows. Perhaps the most common approach is the maintenance and management of vegetation in the drive row using cover crops or groundcovers that provide a host of ecosystem services, such as improved carbon sequestration, pest control, and soil fertility (Winter et al. 2018). With increasing interest in the linkage between soil microbial diversity and ecosystem functioning (Bardgett et al. 2014) and the potential for groundcover vegetation to affect crop plant health through plant-soil feedbacks (Vukicevich et al. 2018), a logical question is: how does groundcover vegetation affect key groups of soil biota?

Plants alter the spatial distribution of soil resources through rhizodeposition (Badri and Vivanco 2009) and litter decomposition (Fanin et al. 2014), creating unique nutrient rich patches that vary with plant species (Broeckling et al. 2008; De Deyn et al. 2011). In fact, specific plants have been used by farmers for millennia to affect changes in populations of soil microorganisms, e.g. through crop rotations (Bullock 1992). Because rotation of the crop plant is not possible in perennial vineyards and orchards, the drive row then provides an opportunity to introduce plant diversity and subsequently soil

[3] A version of this chapter is under consideration for publication as: Vukicevich E, Lowery DT, Bennett J, Hart M. Groundcover vegetation affects soil fungi in multi-year survey across five semi-arid vineyard sites. Frontiers in Ecology and Evolution.

microbial diversity to the system (Vukicevich et al. 2016). There is good evidence that cover crops or permanent groundcovers could have particularly pronounced effects on soil fungi, a guild that includes important crop pathogens and mutualists as soil fungi are strongly affected by plant identity (Lankau and Lankau 2004; De Bellis et al. 2007; De Deyn et al. 2011; Corneo et al. 2013; Detheridge et al. 2016).

One of the most abundant guilds of soil fungi in temperate agroecosystems are arbuscular mycorrhizal (AM) fungi. These are obligate plant symbionts that obtain carbon from their plant host and, in return, provision the host with hard-to-access soil nutrients (Smith and Read 2008). They are known to play an important role in soil aggregation (Jastrow et al. 1998; Rillig and Mummey 2006) and the abundance of their hyphae in soil represents a pool of soil carbon that contributes to carbon sequestration from the atmosphere (Wilson et al. 2009). They are also implicated in crop plant health, both through provisioning of resources in limiting conditions (e.g. Schreiner 2007), and also through pathogen protection (Petit and Gubler 2006). Because of their intimate association with and reliance on plant hosts and because some common groundcover plant species do not associate with AM fungi, their abundance may be greatly affected by groundcover management.

Another beneficial group of soil fungi of interest in agriculture are entomopathogenic (EP) fungi. EP fungi, typified by the well studied and commercially sold *Beauveria bassiana* and *Metarhizium anisopliae*, are naturally common in soils where they are responsible for regulation of insect pest communities and appreciated as biocontrol agents (Shah and Pell 2003; Pell et al. 2010). Living in close association with plants (Moonjely et al. 2016), they are even able to transfer N from infected insects to a

plant host in return for plant carbon (Behie et al. 2012; 2017). Because they may show rhizosphere specificity to some degree (Hu and St. Leger 2002; Behie et al. 2015) and are preferentially associated with certain types of habitats (Meyling et al. 2009), vineyard groundcover management might also affect the abundance of these beneficial fungi with potential consequences for regulation of soil dwelling insect pests of grapevine.

In addition to beneficial soil fungi, generalist soil borne plant pathogens that harm woody perennial crops may build up on certain alternate host plants. For example, Agusti-Brisach et al. (2011) found *Ilyonectria* spp., the causal agent of black foot disease of grape, living in various asymptomatic common vineyard weeds. Because some groundcover plants may be good hosts for these pathogens, there is potential for spillover onto grapevine roots that occupy the same soil space. The perceived benefit of increased microbial diversity through enhanced vegetative diversity may be negated if these generalist pathogens accumulate in non-crop vegetation and promote establishment of disease in vines. On the other hand, certain groundcover plants have been seen to decrease the prevalence of these fungi and improve replant outcomes in crops such as apple (Manici et al. 2015).

Understanding how to manage groundcover vegetation for beneficial fungi while deterring pathogenic fungi could improve the sustainability of perennial crop production (Vukicevich et al. 2016). Though some work has shown that vegetation management can increase overall microbial biomass and activity in vineyard soils (Ingels et al. 2005, Whitelaw-Weckert et al. 2007; Steenwerth and Belina 2008), how groundcovers may change key fungal guilds over time remains largely unknown.

Groundcover plant communities and associated soils were sampled from vineyards in the Okanagan valley, British Columbia. Because a variety of groundcover management practices already exist in vineyards in this region, this provided a good opportunity to test for effects on soil microbes in real world cropping scenarios. Across these vineyards, how groundcover vegetation affects the abundance of AM fungi, the common EP fungus, *Beauveria bassiana*, and plant pathogenic *Ilyonectria* spp were studied. Based on a previous review of the literature (Vukicevich et al. 2016), it was expected that plant functional traits such as functional group, life cycle, plant provenance, and mycorrhizal status would be related to abundances of these fungal guilds.

4.2 Methods

Plant community data and soil samples were collected during three growing seasons and a model-selection approach was used to identify which plant community characteristics, irrigation techniques, and soil factors had the greatest effect on the abundance of each of these fungal guilds as measured by digital droplet PCR (ddPCR) assays.

4.2.1 Field sites

All field sites were located in the southern Okanagan Valley (British Columbia, Canada) (from 49°33'52.44"N, 119°38'19.55"W south to 49° 4'44.02"N, 119°30'41.78"W). This region receives on average ~320 mm of precipitation annually in the form of snow in winter months and occasional rainfall in spring, summer, and fall. Vineyard sites were chosen that had different vegetation management schemes within the

same vineyard block, i.e., alternating rows or randomized complete block designs. Sites were chosen independent of soil type, which was controlled for statistically. Each site, including groundcover identity and sample collection detail, is described in Supplementary material file 1. Sites 1, 2, and 3 were sampled four times: summer 2015, spring 2016, summer 2016, and spring 2017. Site 4 was sampled in summer 2015 and spring 2016 only due to changes to management practices that eliminated groundcovers. Site 5 was added in summer 2016 and sampled again in spring 2017.

4.2.2 Plant community assessment

Plant communities were quantified using quadrats measuring 25 x 50 cm. Four quadrats were evenly spaced throughout four rows of each management scheme (in the case of alternating rows at sites 3, 4, and 5) or four replicate plots (in the case of randomized complete block designs at sites 1 and 2) making for 16 total quadrats for each management scheme at each sampling period. Details of sample collection at each site are given in supplementary materials. Vines adjacent to quadrat placement were marked to facilitate sample collection from the same location at each sampling event. Visual estimation of percent coverage of the quadrat by each plant species was used as a proxy for relative abundance.

4.2.3 Soil collection and processing

Three soil cores (2.5 cm x 20 cm) were collected per quadrat, pooled in sealed plastic bags and kept on ice for transport to a -20°C freezer. Samples were then weighed, oven dried at 60°C for 72 hours to ensure DNA extraction from equal quantities of soil in

each sample, and sieved to 2 mm to remove most roots and rocks and homogenize samples. A 0.5 g subsample was then taken for DNA extraction and quantification of fungi (below).

4.2.4 Soil abiotic factors

As soil abiotic factors may also be important determinants of fungal abundance in soils, a composite sample of post-processed (after drying and sieving) soil for each treatment was sent to Zenalytic Laboratories (Kelowna, BC) for analysis of organic matter (by loss on ignition)(Davies 1974), Total N (Kjeldhal)(Kjeldhal 1883), Total P (Mehlich-3 ICP)(Mehlich 1984), and pH (1:1 in water)(Jackson 1956).

4.2.5 Molecular analysis

Genomic DNA was extracted from bulk soil using the FastPrep spin kit for soils (MP Biomedical, Carlsbad, CA) following the manufacturer's instructions. Quantification of target fungal groups was then performed using digital droplet PCR (ddPCR). Each protocol was optimized through the use of dilution series and melt curve analysis in qPCR and annealing/extension temperature gradients with both positive pure culture controls and positive environmental samples in ddPCR.

To quantify beneficial AM fungi the primer set AMV4.5F/AMDGR (Sato et al. 2005) was used, which targets Glomeromycotan fungi at the phylum level. Of the AM fungal primers previously used that have product sizes appropriate for quantitative PCR, this primer set has the highest fidelity to the phylum (Lumini et al. 2010, van Geel et al. 2014) and has been used successfully to quantify AM fungi in soils (Dai et al. 2013, Hu

et al. 2013). The following recipe was used in a 20µL final reaction volume: 10µL QX200 ddPCR EvaGreen supermix (BioRad, Livermore, CA), 250 nM each primer, 2µL DNA template, and 7µL nuclease-free water. Droplets were prepared using BioRad droplet generation cartridges and a QX100 droplet generator. Reaction conditions were: initial denaturing at 95°C for 10 min followed by 40 cycles of denaturing at 95°C for 30 sec and annealing/extension at 56.5°C for 1 min, then 4°C for 5 min, 90°C for 5 min.

To quantify the EP fungus the primer set BB.fw/BB.rv (Landa et al. 2013) was used, which targets *B. bassiana* at the species level. The same recipe and reaction conditions described above were used with an annealing/extension step of 56°C for 2 min to accommodate the somewhat longer product size of this primer set.

To quantify the plant pathogenic *Ilyonectria* spp. the primer set YT2F (Tewoldemedhin et al. 2011) and CYLR (Dubrovsky and Fabritus 2007) was used, which targets *Ilyonectria* spp. (species complex) that cause Black foot disease of grape. This primer set has been used to evaluate abundance of *Ilyonectria* spp. (including *I. macrodidyma* and *I. liriodendri*) in nursery soils (Agustí-Brisach et al. 2014) as well as *I. macrodidyma, I. pauciseptatum, Cylindrocarpon destructans,* and *I. liriodendri* in diseased apple roots (Tewoldmedhin et al. 2011). Trials using this primer set in our lab indicate positive amplification also of *I. torresensis, I. europaea, I. ianthothele, I. gamsii, Dactylonectria pauciseptum*, *Clyindrocarpon cylindroides,* and *C. olidum, a*ll of which are known to cause Black foot disease of grape (Úrbez-Torres et al. 2015). The same recipe and reaction conditions were used as described above except for the annealing/extension step was 60°C for 1 min.

After PCR, droplets were read for fluorescence in a QX200 droplet reader (BioRad, Livermore, CA). Only samples with >10,000 droplets were used for analysis. Raw amplitude and cluster data was exported from Quantasoft version 1.7 (BioRad, Livermore, CA) and the open source software 'ddPCRquant' (Trypsteen et al 2015) was used to determine amplicon concentration of each sample.

4.2.6 Data analysis

The goal of this study was to assess the effect of plant community characteristics on each of the fungal guilds (AM fungi, *B. bassiana,* and *Ilyonectria* spp.) across all vineyard sites. As such, the focus of this paper is on plant effects as opposed to management strategies *per se* within individual sites, comparisons among which are presented in Supplementary material file 2. Data generated during this project can be viewed on the Open Science Framework, following this link: https://osf.io/cxesk/?view_only=f513573264b141389af7cf23e67200b0.

4.2.6.1 Quantification of plant community characteristics

To assess the effect of groundcover vegetation on abundance of the three fungal guilds, the abundance and species richness of the plant community, as well as the abundance of different functional traits within the community were calculated. Plant abundance was calculated as the total % cover of all vascular plants, whereas species richness was the sum of the number of species. Plant community functional traits included life history strategy (annual, annual/biennial, biennial, biennial/perennial, and perennial), origin (native/exotic), mycorrhizal status (+/-) and plant functional group

(grass, forb, or legume). For the life history strategies, the different strategies were coded ordinally by increasing length (1=annual, 2=annual/biennial, etc.), and the community weighted mean was calculated using the R package 'FD' (Laliberté and Legendre 2010). Origin and mycorrhizal status was also coded ordinally (0=native, 1=exotic and 0=non-mycorrhizal, 1=mycorrhizal) and community weighted means were calculated, resulting in indices representing the weighted abundance of exotic and mycorrhizal plants within each quadrat. Exotic plants included both seeded exotic groundcover species as well as exotic weedy species. Plant functional groups were not recoded as there was no obvious order among them. Instead, the community weighted mean of % cover of each category was calculated; however, forb abundance was not used in subsequent models to avoid extreme collinearity among the indicators.

4.2.6.2 Determination of effects using model selection

To determine which factors affect the abundance of fungal taxa, mixed models in the R package lme4 (Bates et al. 2015) along with model selection were used. Separate mixed models for each fungal group (AM fungi, *B. bassiana,* and *Ilyonectria* spp.) were used. Fixed effects within the mixed models included irrigation type, soil characteristics (phosphorus, pH, and organic matter) and plant community characteristics (total cover, species richness, plant life history strategy, mycorrhizal status, origin, and functional groups). Given that microbial abundance was quantified in multiple seasons and years, interaction terms between each of these predictors and the sampling period were also included. Block (vineyard row or experimental block) nested within site and sample

quadrat nested within block were included as random effects to account for spatial structuring of samples and inherent site differences.

A combination of model and variable selection were used to reduce the complexity of these models. First, all possible combinations of the models were run using the *dredge* function in the R package MuMIn (Bartoń 2017). These models were then ranked by their AICc score relative to the most parsimonious model (ΔAICc). Models with a ΔAICc score > 2 were considered uninformative and not considered further (Burnham and Anderson 2002). Using this subset of models, each variable was weighted using the sums of the ΔAICc scores for the models in which they were included using the *model.avg* function in 'MuMIn'. Variables with a weight > 0.7 were considered important and included in the final model. This procedure was repeated separately for each microbial group. Outputs from the *model.avg* function listing the average importance of all variables tested across all models run using the *dredge* function are given in Tables S1-S3. For the final models, R^2 values, partitioned between the fixed effects and fixed plus random (Nakagawa and Schielzeth 2013), were estimated as implemented in MuMIn.

To aid the interpretation of the effects of sampling periods, the estimated marginal means for each sampling period were calculated using the R package 'emmeans' (Lenth 2018). Additionally, the emtrends function within this package was used to compare the slopes between sampling periods in cases where there were significant interactions with the continuous predictors. To enable comparison among indicators, each indicator variable was scaled to a mean of zero and divided by the standard deviation prior to

calculating the trends. Estimated effects of variables were also visualized using the *effect* function in the R package 'effects' (Fox 2003).

Results are organized into three categories based on the three individual models run (AM fungi, *B. bassiana,* and *Ilyonectria* spp.) and then based on three subcategories ('plant effects', 'soil effects', and 'irrigation and time effects') for ease of interpretation and discussion. Full results for each model can be obtained from the Open Science Framework by following this link: https://osf.io/7qctx/?view_only=e0c567cad4f74a57a81185640b93d62a.

4.3 Results

4.3.1 Arbuscular mycorrhizal fungi

The best model to determine the effects of plant, soil, and irrigation on abundance of AM fungi included sample period, irrigation type, exotic plant cover, legume cover, plant life history strategy, total plant cover, soil P, organic matter, and pH, as well as interaction terms with sample period for all variables except soil P and pH (Table 1). Fixed factors in this model explained approximately 46% of the variation in AM fungal abundance and random factors (vineyard site and structure of sampling with sites) explained 5%. AM fungal abundance varied with sampling time, increasing from spring to summer in 2016 ($F_{3,755}$=13.72, P<0.001).

Table 4. 1 Effects of sampling date and biotic and abiotic factors in the model of AM fungal abundance as tested using Satterwaite type III approximation for degrees of freedom (Model AIC: 1217, R^2_{fixed}=0.46). Significant (P<0.05) P-values are in bold.

Category	Factor	F-value	P-value
Time	Sample period[a]	13.72	**<0.001**
Abiotic factors	Irrigation type[b]	3.49	**0.031**
	Soil P	7.45	**0.006**
	Organic matter	42.02	**<0.001**
	pH	1.18	0.277
Biotic factors	Exotics[c]	4.26	**0.039**
	Legumes[c]	0.28	0.596
	Total plant cover[c]	0.58	0.448
	Life history strategy[d]	26.51	**<0.001**
Interactions	Sample period x Irrigation type	23.54	**<0.001**
	Sample period x Organic matter	9.2339	**<0.001**
	Sample period x Exotics	7.97	**<0.001**
	Sample period x Legumes	8.91	**<0.001**
	Sample period x Life history strategy	4.03	**0.007**
	Sample period x Total plant cover	5.24	**0.001**

[a]'Sample period' indicates when the samples were collected (summer 2015, spring 2016, summer 2016, and spring 2017).
[b]'Irrigation type' includes drip (no supplemental irrigation applied to groundcover), dual (occasional watering of groundcover), and sprinkler (frequent watering of groundcover when vines are irrigated).
[c],'Exotics','Legumes, and 'Total plant cover' refers to % quadrat covered by: exotic (non-native) species, legume species, and all plant species, respectively.
[d] Plant 'Life history strategy' was dummy coded along a continuum from annual (1) to perennial (5).

4.3.1.1 Plant effects on AM fungi

Overall, AM fungal abundance was negatively related to percent cover of exotics and plant life history strategy ($F_{1,670}$=4.26, P=0.04 and $F_{1,662}$=26.51, P<0.001, respectively) but both of these effects also depended on sampling period (Fig. 1a). The relationship between exotics and AM fungal abundance was negative in summer 2015 and spring 2016, but positive in spring 2017 (Fig. 1a).

Plant life history strategy was negatively related to AM fungal abundance at all sample periods except summer 2016 when there was no effect (Fig. 1a). Percent cover of

legumes was unrelated to AM fungal abundance overall, varying widely among sample periods with a positive relationship in summer 2016 and a negative relationship in spring 2017 (Fig. 1a). Total cover did not affect AM fungal abundance overall, but showed a negative relationship with AM fungal abundance in summer 2016 and a positive relationship in spring 2017 (Fig. 1a).

4.3.1.2 Soil effects on AM fungi

AM fungal abundance increased with soil organic matter and P overall ($F_{1,45}$=42.02, P<0.001 and $F_{1,577}$=7.45, P=0.006, respectively)(Fig. 1b). The effect of soil organic matter depended on sampling period, with positive relationships seen in all but one sampling period (Fig. 1b), while soil P had a consistently positive effect (Fig. 1b). Soil pH had no consistent effect overall ($F_{1,305}$=1.18, P=0.28).

4.3.1.3 Irrigation effects on AM fungi

Irrigation type also affected AM fungal abundance ($F_{2,238}$=3.49, P=0.03), but there was a strong sample period interaction ($F_{6,694}$=23.54, P<0.001) with a decrease in abundance with dual and sprinkler irrigation in summer 2016 but the opposite effect in spring of 2017.

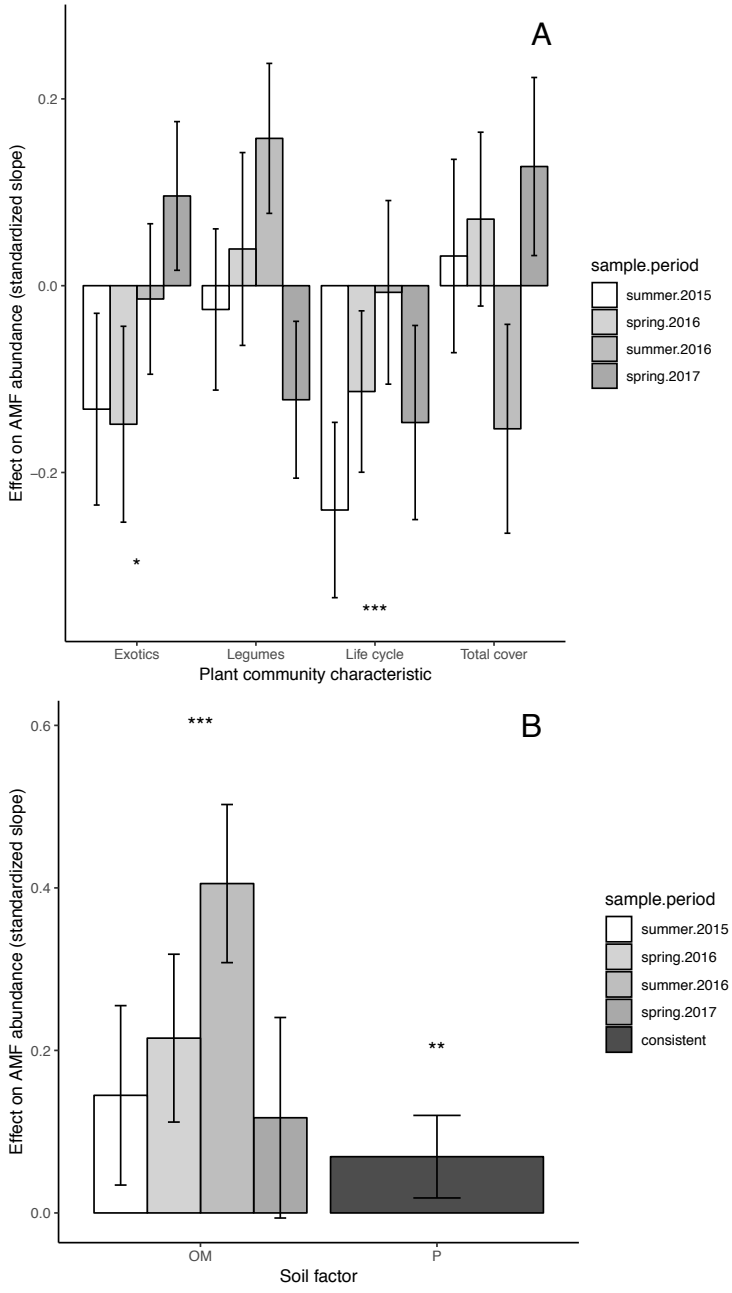

Fig. 4. 1 Effect of plant community characteristics (A) and soil properties (B) on AM fungal abundance. Positive values indicate a positive relationship (standardized slope) between a given factor and AM fungal abundance at that sampling point while negative values represent a negative relationship. Size of the bar indicates relative strength of each effect. Error bars represent 95% confidence limits. Error bars that do not cross 0 on the y-axis indicate significantly positive or negative relationships between soil factors and AM fungal abundance at a given sample period. "*", "**", and "***" indicate overall significant (P<0.05, 0.01, and 0.001, respectively) positive or negative effects of a factor on AM fungal abundance.

4.3.2 Beauveria bassiana

The final model for *B. bassiana* included sample period, irrigation type, plant life history strategy, exotic species, legumes, grasses, organic matter, and soil P, as well as sampling period interaction terms with irrigation type, grasses, legumes, and soil P (Table 2). The fixed effects in this model explained 19% of the variation in *B. bassiana* abundance, while 54% of the variation was explained by fixed plus random effects (vineyard site and spatial structuring of sampling). Sampling period affected the abundance of *B. bassiana* ($F_{3,717}$=21.93, P<0.001), with abundance increasing in spring 2017 compared to spring 2016.

Table 4. 2 Effects of sampling date and biotic and abiotic factors in the model of *Beauveria bassiana* abundance as tested using Satterwaite type III approximation for degrees of freedom (Model AIC: 2166, R^2_{fixed}=0.19). Significant (P<0.05) P-values are in bold.

Category	Factor	F-value	P-value
Time	Sample period[a]	21.93	**<0.001**
Abiotic factors	Irrigation type[b]	7.5333	**<0.001**
	Organic matter	9.70	**0.002**
	Soil P	0.01	0.90
Biotic factors	Exotics[c]	11.57	**<0.001**
	Legumes[c]	4.84	**0.028**
	Grasses	0.18	0.67
	Life history strategy[d]	3.91	**0.048**
Interactions	Sample period x Irrigation type	8.01	**<0.001**
	Sample period x Soil P	3.49	**0.015**
	Sample period x Grasses	8.50	**<0.001**
	Sample period x Legumes	2.51	0.058

[a]'Sample period' indicates when the samples were collected (summer 2015, spring 2016, summer 2016, and spring 2017).
[b]'Irrigation type' includes drip (no supplemental irrigation applied to groundcover), dual (occasional watering of groundcover), and sprinkler (frequent watering of groundcover when vines are irrigated).
[c],'Exotics' and 'Legumes' refers to % quadrat covered by: exotic (non-native) species and legume species, respectively.
[d] Plant 'Life history strategy' was dummy coded along a continuum from annual (1) to perennial (5).

4.3.2.1 Plant effects on *Beauveria bassiana*

The abundance of *B. bassiana* was consistently related to plant life history strategy, proportion of exotic species, and legumes (Table 2). *B. bassiana* decreased with average plant lifespan ($F_{1,586}$=3.91, P=0.048), increased with the proportion of native plant species ($F_{1,670}$=11.57, P<0.001), and increased with legume cover ($F_{1,667}$=4.84, P=0.03)(Fig. 2a). These plant effects were consistent across the experiment, i.e. did not depend on sampling period. The effect of grass cover was not significant overall, but was positively associated with *B. bassiana* abundance in spring of 2017.

4.3.2.2 Soil effects on *Beauveria bassiana*

Soil organic matter had a consistently positive effect on *B. bassiana* ($F_{1,662}$=9.70, P=0.002)(Fig. 2b). Soil P had no effect overall, but depended on sampling period ($F_{3,733}$=3.49, P=0.015) with a positive relationship seen in spring 2017 (Fig. 2b).

4.3.2.3 Irrigation effects on *Beauveria bassiana*

Irrigation type was also related to *B. bassiana* abundance with dual and sprinkler irrigation increasing *Beauveria* compared with drip irrigation ($F_{2,689}$=7.53, P<0.001), but effects were inconsistent among sampling periods ($F_{6,719}$=8.01, P<0.001)(Fig. 3).

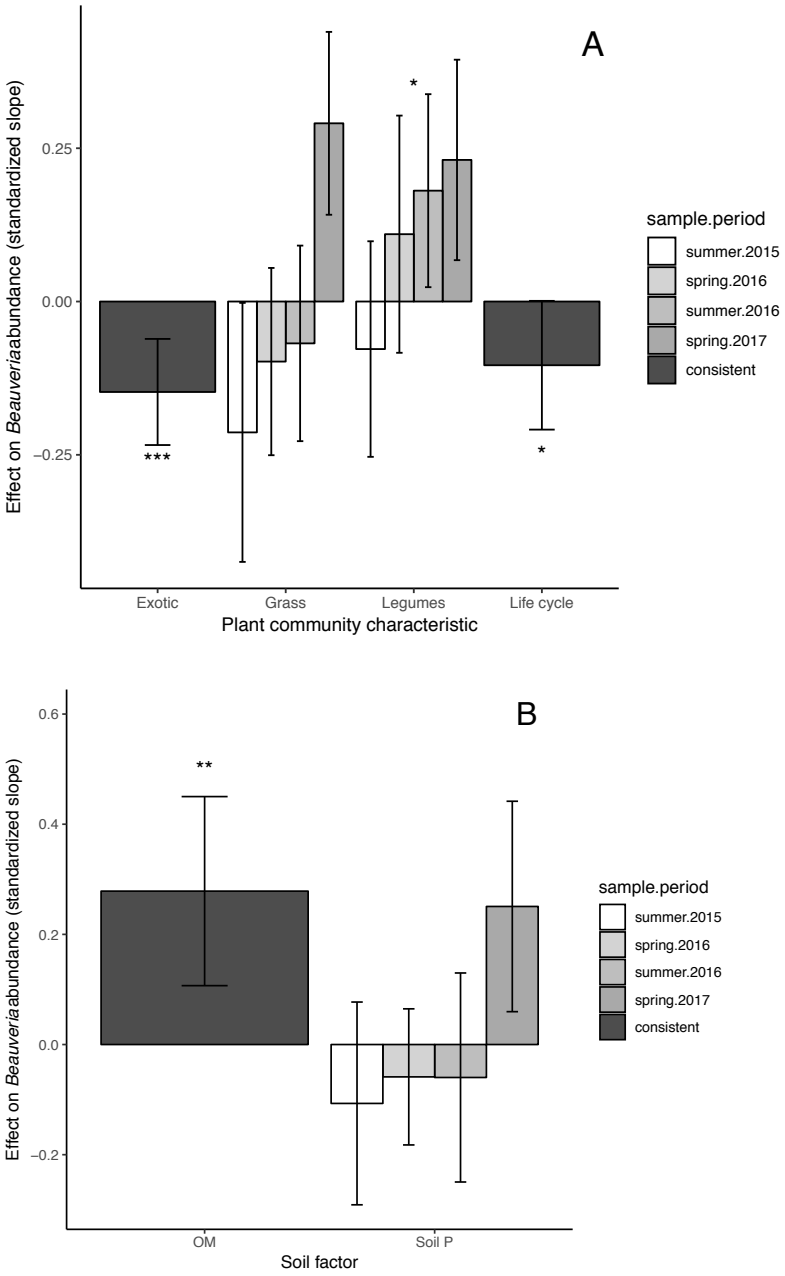

Fig. 4. 2 Effect of plant community characteristics (A) and soil properties (B) on *Beauveria bassisana* abundance. Positive values indicate a positive relationship (standardized slope) between a given factor and *B.bassiana* at that sampling point while negative values represent a negative relationship. Size of the bar indicates relative strength of each effect. Error bars represent 95% confidence limits. Error bars that do not cross 0 on the y-axis indicate significantly positive or negative relationships between soil factors and AM fungal abundance at a given sample period. "*", "**", and "***" indicate overall significant (P<0.05, 0.01, and 0.001, respectively) positive or negative effects of a factor on *B. bassiana* abundance.

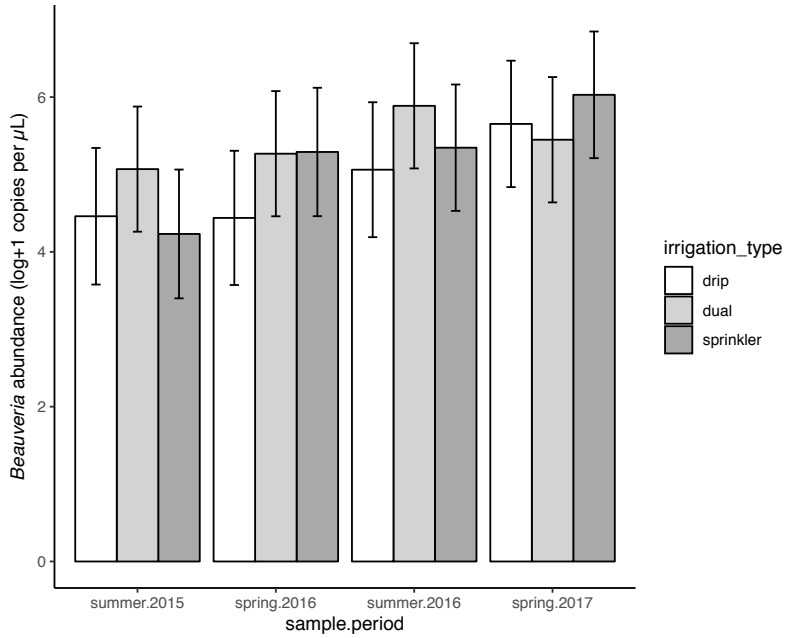

Fig. 4. 3 Effect of irrigation type (shades) on *Beauveria bassiana* abundance across all sites at four sampling periods. Error bars indicate 95% confidence limits based on likelihood of these estimates within the final model, i.e. accounting for all other factors included in the model.

4.3.3 *Ilyonectria* spp.

The final model for *Ilyonectria* spp. included sample period, irrigation type, organic matter, soil P, total plant cover, exotic plant cover, and grass cover, as well as interaction terms with sample period for all factors except grass cover and soil P. Fixed effects in this model explained 38% of the variation, with fixed plus random effects explaining 45%. *Ilyonectria* spp. abundance varied with sampling period ($F_{3,568}$=12.21, P<0.001), with a lower amount of *Ilyonectria* spp. detected in spring 2016 compared to the other sampling periods.

Table 4. 3 Effects of sampling date and biotic and abiotic factors in the model of *Ilyonectria* spp. abundance as tested using Satterwaite type III approximation for degrees of freedom (Model AIC: 2168, R^2_{fixed}=0.38). Significant (P<0.05) P-values are in bold.

Category	Factor	F-value	P-value
Time	Sample period[a]	12.21	**<0.001**
Abiotic factors	Irrigation type[b]	9.51	**<0.001**
	Organic matter	27.02	**<0.001**
	Soil P	1.77	0.184
Biotic factors	Grasses	22.38	**<0.001**
	Exotics[c]	11.25	**<0.001**
	Total plant cover[c]	3.09	0.079
Interactions	Sample period x Irrigation type	5.02	**<0.001**
	Sample period x Exotics	3.27	**0.021**
	Sample period x Total plant cover	5.14	**0.002**
	Sample period x Organic matter	3.06	**0.027**

[a]'Sample period' indicates when the samples were collected (summer 2015, spring 2016, summer 2016, and spring 2017).
[b]'Irrigation type' includes drip (no supplemental irrigation applied to groundcover), dual (occasional watering of groundcover), and sprinkler (frequent watering of groundcover when vines are irrigated).
[c],'Exotics' and 'Total plant cover' refers to % quadrat covered by exotic (non-native) species and all plant species, respectively.

4.3.3.1 Plant effects on *Ilyonectria* spp.

The abundance of *Ilyonectria* spp. was consistently negatively associated with grass cover ($F_{1,531}$=22.38, P<0.001)(Fig. 4a). Overall, *Ilyonectria* spp. increased with exotic plant cover ($F_{1,675}$=11.25, P<0.001), but this relationship varied with sampling period (Fig. 3a).

4.3.3.2 Soil effects on *Ilyonectria* spp.

Soil organic matter was positively related ($F_{1,47}$=27.02, P<0.001) at all sampling periods despite a weak interaction with sampling period ($F_{3,751}$=3.06, P=0.03)(Fig. 4b).

4.3.3.3 Irrigation effects on *Ilyonectria* spp.

Irrigation type also affected *Ilyonectria* spp. abundance ($F_{2,115}$=9.51, P<0.001), with both dual and sprinkler irrigation leading to greater abundance overall compared to drip irrigation, though the strength of this effect depended on sampling period (Fig. 5).

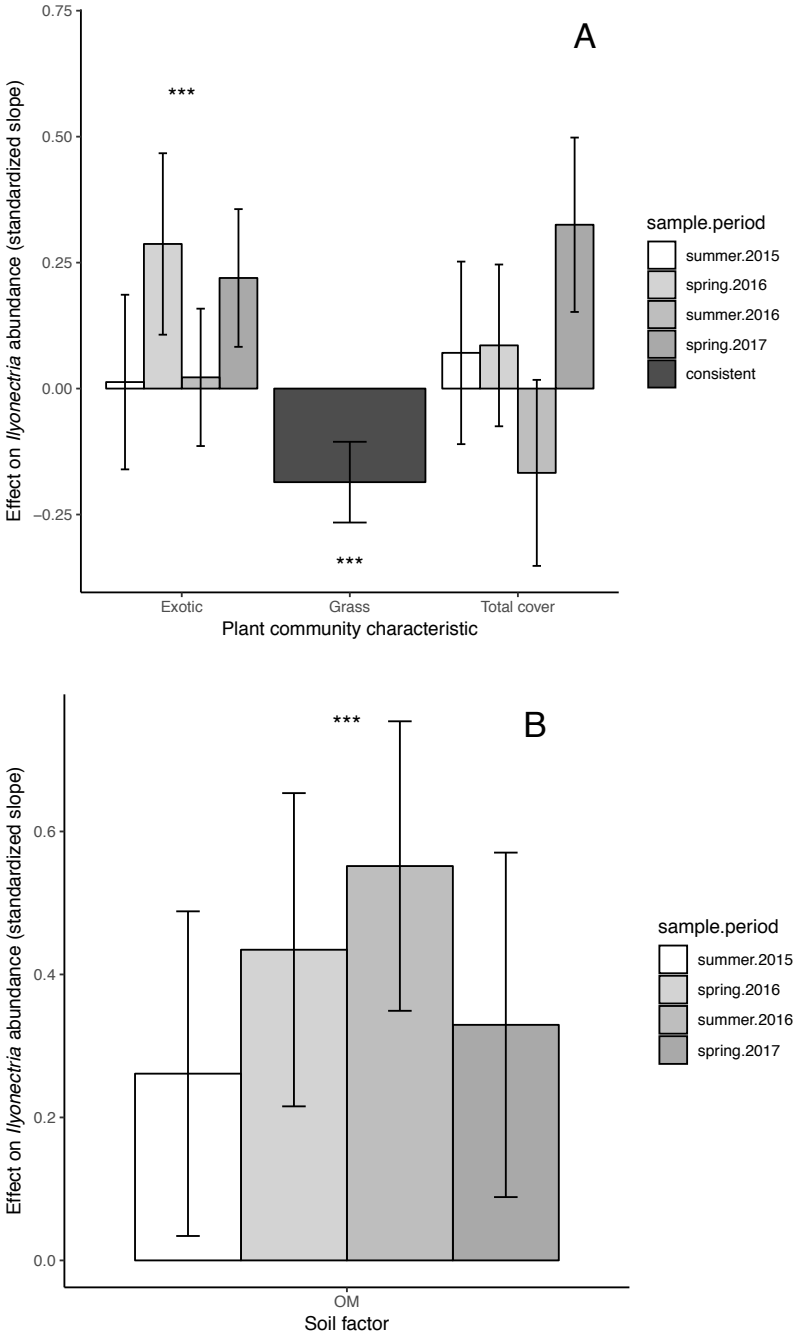

Fig. 4. 4 Effect of plant community characteristics (A) and soil properties (B) on *Ilyonectria* spp. abundance. Positive values indicate a positive relationship (slope) between a given factor and *Ilyonectria* spp. abundance at that sampling point while negative values represent a negative relationship. Size of the bar indicates relative strength of each effect. Error bars represent 95% confidence limits. Error bars that do not cross 0 on the y-axis indicate significantly positive or negative relationships between soil factors and *Ilyonectria* spp. at a given sample period. "*", "**", and "***" indicate overall significant (P<0.05, 0.01, and 0.001, respectively) positive or negative effects of a factor on *Ilyonectria* spp. across all sample periods.

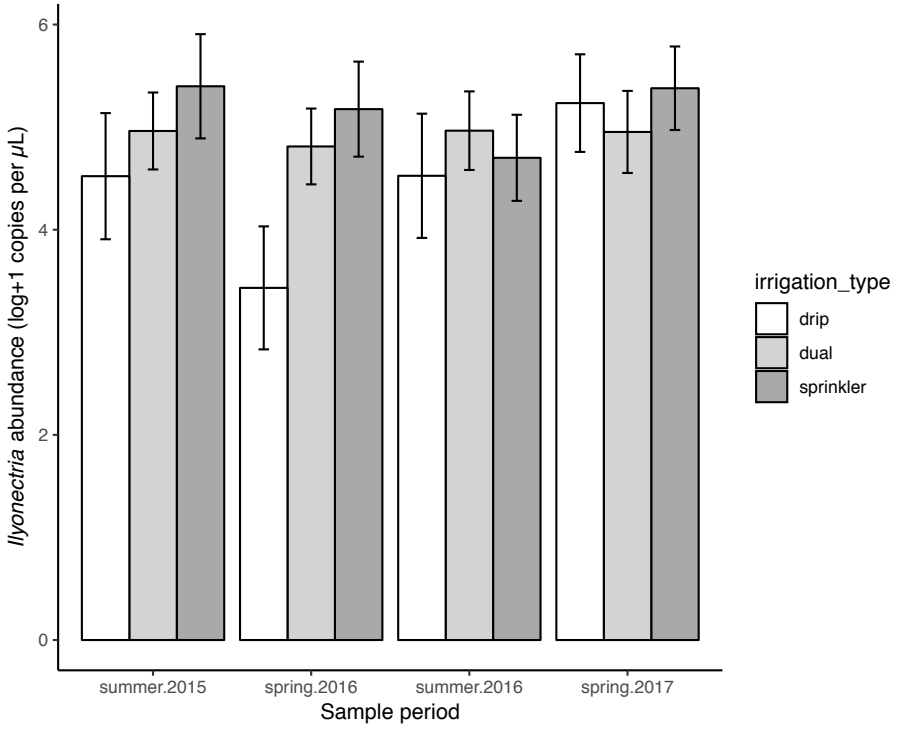

Fig. 4. 5 Effect of irrigation type (shades) on *Ilyonectria* spp. abundance across all sites at four sampling periods. Error bars indicate 95% confidence limits based on likelihood of these estimates within the final model, i.e. accounting for all other factors included in the model.

4.4 Discussion

Overall, groundcover vegetation was related to the abundance soil fungal guilds, though effects changed over time especially for AM fungi and *Ilonectria* spp. Groundcover and *B. bassiana* abundance, however, were highly correlated, which may suggest that conservation biological control of soil dwelling insect pests could be achieved through management of non-crop vegetation.

4.4.1 AM fungi

The abundance of AM fungi changed with plant and soil factors, but plant effects varied over time, making the link between groundcover management and AM fungi somewhat unpredictable.

4.4.1.1 Plant effects on AM fungi

In this study, annuals were associated with higher AM fungal abundance. This was surprising given that perennials are generally more dependent on the symbiosis (Boerner 1992). However, other studies have shown AM fungal colonization to be greater in annual plants, perhaps because an annual strategy necessitates fast resource acquisition while perennials are more conservative, investing C in construction of their own below-ground biomass (Roumet et al. 2006). Furthermore, ruderal plants and the disturbances that favor them in agriculture tend to promote ruderal AM fungi in these systems (Verbruggen and Kiers 2010). There was some management-scale evidence for this at Site 4 where a five-year stand of *Festuca ovina* had significantly less AM fungi than alternating rows that were recently tilled and had become very weedy (Supplementary material file 2).

Native plants had a positive effect on AM fungi, compared to exotic plants, perhaps because locally-adapted plants benefit more from AM fungi when growing in their native soils (Johnson et al. 2010, Rúa et al. 2016). The positive effect of native plants on AM fungi was consistent for the first two sampling periods but disappeared after that. This perhaps could have been due to the loss of site 4 and the addition of site 5 for the last two sampling periods because site 5 included a groundcover that was

dominated by *Achillea millefolium*, thus increasing the prevalence of native species in the data set (Supplementary file 2). If *A. millefolium* under sprinkler irrigation at that site did not enhance AM fungi in the same way that other native species did at predominantly drip irrigated sites where most native species were found, it could have contributed to this apparent change.

The inconsistency of plant effects on AM fungi may also be due to the gene region we targeted in our molecular assay. Although this site has correlated well with AM fungal biomass in several studies (Dai et al. 2013, Hu et a. 2013), it does not give full coverage of the Glomeromycota. For example, the families Paraglomeraceae and Diversisporaceae and Ambisporaceae may be under-represented (van Geel et al. 2014). Changes in the abundance of representatives of these families may not have been detected in this assay (Rosendahl et al 2009).

4.4.1.2 Soil effects on AM fungi

Organic matter was positively correlated with AM fungal abundance at most sampling periods. This is expected given the tight link between soil organic matter and soil microbes in general, whose remains contribute to stabile organic matter (Kallenbach et al. 2016). The increase in water-holding capacity that accompanies increases in organic matter (Hudson 1994) could also contribute to increased microbial biomass, in general. AM fungi, although not known to have the enzymatic capacity for saprotrophic growth, also tend to proliferate in patches of organic matter (Hodge 2014). In addition to the plant hosts' need for symbionts to more efficiently obtain nutrition from organic sources (Gosling et al. 2014), the tendency of AM fungi to proliferate in high organic

matter soils may also be beneficial for soil aggregation and C sequestration and may effectively be increased through management practices that concomitantly increase soil organic matter and aggregate stability (Tisdall and Oades 1982).

AM fungi also increased with soil P at all sampling periods, which may seem counter-intuitive given the common view that greater P availability decreases mycorrhizas (Smith and Read 2008). However, it has been reported that only very high levels of P fertilization inhibit AM development (Linderman and Davis 2004; van Geel et al. 2017). P levels were highest overall in the organically farmed Site 4 where compost was applied more often than other sites. Any negative effect of higher levels of soil P on AM fungi that might result from heavy compost application might be offset by the beneficial effect of organic matter discussed above and reported elsewhere (Gosling et al. 2006).

4.4.1.3 Irrigation effects on AM fungi

Irrigation had an inconsistent effect on AM fungal abundance. It could be that the plants used as sprinkler-irrigated groundcovers (e.g. cool-season plants) are simply more active in the spring vs. summer and thus may be providing more photosynthate to AM fungi then. It could also be that mowing, which is necessary with sprinkler irrigation in this region, negatively affects AM fungi in summer more than spring before plots are mown. There is some support for this effect with fescues, as *F. campestris* has been shown to decrease allocation of C to roots following defoliation (McInenly et al. 2010). Although one may expect supplemental irrigation to consistently increase AM fungi by increasing plant productivity in this semi-arid environment, the inconsistency of these

results indicates that irrigation type is not a reliable way to influence AM fungi in vineyards in this region *per se.*

4.4.2 Beauveria bassiana

B. bassiana was consistently responsive to plant community characteristics, increasing with proportions of native plants, annuals, and legumes as well as soil organic matter. This represents a new finding and perhaps lends some validity to the notion of conservation biocontrol of insect pests through habitat management for EP fungi (Pell et al. 2010).

4.4.2.1 Plant effects on *Beauveria bassiana*

B. bassiana abundance was enhanced consistently by several plant community characteristics, especially native plants. *B. bassiana* can be endophytic (Quesada-Moraga et al. 2014) having evolved from endophytes (Moonjely et al. 2016), and shows local adaptation to different climates, e.g. thermal growth preferences (Bidochka et al. 2002). Persistence of *B. bassiana* with locally adapted plant communities may therefore have a basis in co-adaptation. This is, to my knowledge, the first report of a preferential association between an EP fungus and native plant communities within an agricultural field. Preliminary data from a bioassay in this lab suggests that groundcovers comprised of greater proportions of native species increase the overall infectivity of the native EP fungal community. The inclusion of native species may therefore be one way to improve the effectiveness of natural soil-dwelling pest control, but more work needs to be done in this area.

Annuals were associated with more *B. bassiana* consistently among sites. Although there are no studies to date that examine the effect of plant functional traits on *B. bassiana* in soil, it is known that *B. bassiana* persists in annually cropped fields, unlike some other EP fungi that are more abundant in undisturbed natural habitats (Meyling et al. 2006; Meyling et al. 2009; Medo and Cagán 2011). Randhawa et al. (2018) recently showed that another common EP fungus, *Metarhizium robertsii*, occurs in higher numbers soon after disturbance and then declines with time since disturbance. Perhaps common EP fungi such as *B. bassiana* and *Metarhizium* spp. are most dominant in annual agricultural fields due to some unknown adaptation to physical disturbance, annual and weedy plant species, or the insect communities that occur in these disturbed habitats (e.g., ground-dwelling decomposers).

Legumes also increased *B. bassiana* abundance in this study. This is consistent with a previous finding that *B. bassiana* persists well in legume cover crops in orchards (Shapiro-Ilan et al. 2012), although that study only compared legumes to the absence of a cover crop. Because legumes had a positive influence on *B. bassiana* in our large dataset looking at many different plant traits, this hints at some functional attribute of legumes that is especially beneficial for the success of *B. bassiana.* Most EP fungi like *B. bassiana* are poor competitors as saprotrophs (Meyling et al. 2007), largely ruling out the effect of high litter quality associated with legumes. Most likely the benefits of a legume for EP fungi lie either in the attractiveness of the legume roots to soil herbivores (Schallhart et al. 2012), their suitability for endophytic colonization by EP fungi (Behie et al. 2015), protection from environmental stresses (Shapiro-Ilan et al 2012) or some

combination of these. The higher litter quality of legumes may also attract more soil-dwelling insects (House and Alzugaray 1989) and indirectly increase EP fungi.

4.4.2.2 Soil effects on *Beauveria bassiana*

Organic matter was positively associated with *B. bassiana* abundance at most sampling periods. As with AM fungi, a positive correlation between organic matter and the organisms that contribute to its formation can be expected (Kallenbach et al. 2016). There are several studies that show greater EP fungal isolation associated with higher organic matter soils (Ali-Shtayeh et al. 2003; Medo and Cagán 2011) and organic fertilization (Clifton et al. 2015). Because *Beauveria* are generally poor competitors as saprotrophs, it is unlikely that there is a direct effect of organic matter on their populations. Instead, higher organic matter is likely associated with greater biological activity in general, including more plant roots and insects, both of which are hosts for these fungi.

4.4.2.3 Irrigation effects on *Beauveria bassiana*

The effects of irrigation type on *B. bassiana* were largely inconsistent, although supplemental irrigation did increase these fungi overall. Although little is known about how frequency of irrigation might affect populations of entomopathogens in the soil, there is some indication that the effect of soil moisture on EP fungi depends on both soil texture and fungal isolate (Jaronski 2010). As *Beauveria bassiana* is known to be a genetically diverse species whose population dynamics are influenced by environmental conditions (Bidochka et al. 2002), it is possible that different strains persist at the

different sites in our study, perhaps explaining inconsistencies in irrigation effects. Some of these strains may be better adapted to increased frequency of soil wetting coupled with exotic plants that typify groundcover vegetation in sprinkler irrigated sites, while others are more adapted to the natural conditions in the region and thus persist in drip irrigated sites with locally adapted vegetation, though this has not been studied specifically. Further investigation is warranted to ascertain whether the addition of supplemental irrigation improves soil dwelling insect pest control outcomes through the activity of EP fungi.

4.4.3 *Ilyonectria* spp.

Although *Ilyonectria* spp. abundance changed with several plant community characteristics and tended to increase with supplemental irrigation, the strongest consistent plant effect was a decrease when grasses were more prevalent. As grasses are often dominant constituents of many perennial groundcover mixes, this may be good news for growers already employing perennial groundcovers in general. There was also a consistently positive influence of exotic species on *Ilyonectria* abundance in the spring, perhaps implicating annual exotic weedy species as alternate hosts for these pathogens.

4.4.3.1 Plant effects on *Ilyonectria* spp.

Grass cover was negatively correlated with *Ilyonectria* spp. abundance across all sampling periods, suggesting that grass-dominant groundcovers could lead to fewer of these pathogens in vineyard soils. Although a survey of vineyard weeds by Agustí-Brisach et al. (2011) found *Ilyonectria* spp. in many common weeds, only six species of

grass were included in that study, of which only one third hosted *Ilyonectria* spp.. In studies of replant disease of apple, Mazzola et al. (2004) were able to reduce damage caused by pathogens such as *Ilyonectria* spp. through stimulation of an antagonistic rhizobacteria population using a wheat cover crop. Other grasses, such as *Lolium perenne*, have also been implicated in promoting bacteria with fungistatic genes (Latz et al. 2015), suggesting that perhaps some grasses deter these generalist pathogens through culturing of an antagonistic rhizosphere community and thus are not good hosts. Although these potential mechanisms are speculative, the consistency of the results presented here suggest that grasses are somehow poorer hosts for *Ilyonectria* spp. in these vineyards than broadleaves and might then be utilized to decrease pathogen loads in vineyard soils.

Exotic plants tended to increase *Ilyonectria* spp. abundance overall, but this was due to the strong effects seen only during spring sampling periods. It could be that this pathogen thrives in cultivated plants and associated weedy species, which are mostly exotic species in our region. Work on invasive plant species has shown that generalist pathogens can build up on exotic species without any negative effects on those plants (Mangla and Callaway 2008). If *Ilyonectria* spp. accumulates on exotic plants, this may negatively affect vines sharing the same soil through a spillover effect. A possible explanation for the springtime effects of exotic plants on *Ilyonectria* spp. abundance could be that many of the exotic weedy species may be more active in the spring. This represents a unique finding that has implications for managing soil borne diseases in vineyard and nursery soils.

4.4.3.2 Soil effects on *Ilyonectria* spp.

As with the other two fungal groups, *Ilyonectria* spp. was positively related to soil organic matter, perhaps relating to the general microbial contribution to stabile organic matter (Kallenbach et al. 2016). This increase in *Ilyonectria* spp. with greater amounts of organic matter is not necessarily an indication of increased disease pressure for crop plants occupying this soil because increased microbial competition and antagonism also occurs with the use of organic amendments (Bonanomi et al. 2007; Watson et al. 2017).

4.4.3.3 Irrigation effects on *Ilyonectria* spp.

Ilyonectria spp. increased with dual and sprinkler irrigation overall. This could be expected given that *Ilyonectria* spp.-related diseases such as Black foot disease of grape tend to be more problematic with prolonged periods of excessive soil moisture (Halleen et al. 2006). It is also likely that the use of sprinkler irrigation leads to broader distribution of grapevine roots throughout the vineyard floor. As grapevines are good hosts for these fungi, the proximity of vine roots to the drive row sample plots could have also contributed to this effect in addition to the increased frequency of wetting.

5 Effects of living mulch on young vine growth and soil in a semi-arid vineyard[4]

5.1 Background

In a typical vineyard, as in many perennial cropping systems, management of non-crop vegetation is a significant consideration. Inter-rows are often planted to cover crops or permanent groundcovers to prevent soil erosion, manage fertility, and provide habitat for beneficial organisms (Hartwig and Ammon 2002). The area directly under the vine row, however, is typically maintained as bare soil through the use of herbicide applications or mechanical cultivation in order to minimize competition between weeds and grapevines (Hembree et al. 2013). Each of these approaches has notable drawbacks ranging from the development of herbicide resistant weed populations (Heap 2014) and environmental pollution (Louchart et al. 2001) to erosion and grape root and trunk damage (Hembree et al. 2013). With growing interest in environmentally sustainable production practices in many grape-growing regions, exploration of alternative strategies is warranted.

Recently the use of living mulch, or actively growing plants, underneath vine rows has emerged as an alternative weed management scheme (Centinari 2016). Although much of the work to date has focused on competitive effects with vines (e.g., Karl et al. 2016), and there has been some work on how living mulch might affect soil properties (Karl et al. 2016a), only recently has an effect on soil biota been demonstrated (Chou et al. 2018). The effect on soil biota has the potential to add another suite of ecosystem services to the use of living mulch through conservation biocontrol and resource provisioning to vines through symbioses. Soil biota may be especially sensitive

[4] This chapter is under consideration for publication as: Vukicevich E, Lowery DT, Hart M. Effects of living mulch on young vine growth and soil in a semi-arid vineyard. Submitted to *Vitis*.

to the identity of plants used as living mulch, and changes below ground may also contribute to vine growth outcomes (Vukicevich et al. 2018).

Because plants are known to change soil biota through rhizosphere (Badri and Vivanco 2009) and litter effects (Fanin et al. 2014), living mulch may change soil microbial communities in ways that promote or inhibit vine growth and resilience. Fungi are common constituents of plant rhizospheres and show some degree of plant host specificity as endophytes inhabiting roots (De Deyn et al. 2011; Agusti-Brisach et al. 2011; Behie et al. 2015). While there is a risk that living mulch plants could host fungal pathogens of grape, such as *Ilyonectria* spp. (Agusti-Brisach et al. 2011; Benitez et al. 2016), beneficial microbes may also be enhanced via living mulch plants. For example, plant identity is known to affect the abundance of arbuscular mycorrhizal (AM) fungi (De Deyn et al. 2011), which are important for maintaining soil structure (Rillig and Mummey 2006) and improving vine nutrient and water acquisition (Torouvelot et al. 2015). Entomopathogenic fungi (EPF), another beneficial group responsible for the regulation of soil dwelling grape insect pest populations (Kirchamair et al. 2004), also may be differentially abundant depending on plant identity (Behie et al. 2015).

Plant functional group may be important determinants of vine growth responses both through changes to soil abiotic properties and fungal guilds. For example, plants with extensive finely branched root systems such as grasses may hinder vine performance in N-limiting conditions because they are known to increase soil C:N ratios and scavenge soil N (Clark 2008). Legumes, on the other hand, may enhance vine growth in N-limiting situations by adding biologically fixed N to the system (Clark 2008) or reduce vine performance if they compete for water and other limiting nutrients such as P (Caradus

1980). Certain plant functional groups may also enhance or suppress AM fungi (Hetrick et al. 1988), soil borne pathogens (Benitez et al. 2016), or EPF (Behie et al. 2015). Notably, plants in the family Brassicaceae can decrease the abundance of soil fungi, including pathogens as well as beneficial AM fungi, due to volatiles released when tissues are damaged or decompose (Schreiner and Koide 1993).

In the present study, living mulch comprised of plants representing different functional groups was evaluated for effects on vine growth as well as soil abiotic factors and the abundance of beneficial and pathogenic soil fungi in a young vineyard in a semi-arid region. Plants were selected based on suitability to site and climate, including a warm- and cool-season grass, a legume, and a brassicaceous forb. The study addressed the question: Does living mulch identity influence a) growth of young vines, b) soil abiotic factors, and c) the abundance of AM fungi, the common EPF *Beauveria bassiana,* or the pathogenic *Ilyonectria* spp.?

5.2 Materials and Methods

5.2.1 Field site and vine establishment

This project was established in the field at the Summerland Research and Development Centre in Summerland, British Columbia, Canada, on Osoyoos series sandy loam. Mean annual precipitation at the site is approximately 320 mm yr^{-1}. The 0.08 ha experimental field that was previously planted to grapevines was left fallow for one year and tilled prior to planting to eliminate weeds. Dormant vines of *Vitis vinifera* cv. 'Sauvignon Blanc' grafted onto *Vitis riparia* x *rupestris* cv. '101-14' were planted at 1.22 by 2.44 m spacing (vine by row) on 23 June, 2015. Vines were trained to a vertically-

shoot positioned trellis system; pruning to trunks after the 2015 growing season, cordons after 2016, and spurs following the 2017 growing season. Standard viticulture practices for young vineyards were carried out, including spring shoot thinning, shoot positioning, leafing, and crop removal on young vines.

Vines were irrigated through a drip system with two 2 L hr^{-1} pressure-compensating emitters delivering water to either side of each vine. Irrigation was delivered as needed in 2015 and 2016. In the summer of 2017 irrigations were made on three and four-day intervals (twice per week), applying 12 L of water to each vine on each event and 16 L $vine^{-1}$ during the hotter weeks of August. Vines were not fertilized in 2015. In 2016, vines were fertilized using 20-20-20 'Plant-Prod' (Master Plant-Prod, inc., Brampton, ON) applying 8.3 g $vine^{-1}$ (2 kg total applied) on June 10, 16.6 g $vine^{-1}$ on June 30 and 16.6 g $vine^{-1}$ on August 2. In 2017, vines were fertilized with 34-0-0 Urea, applying 8.75 g $vine^{-1}$ on June 23 and 15 g $vine^{-1}$ on July 23. Each of these applications was applied through the drip irrigation system with sufficient water to deliver the majority of the solubilized fertilizer to the root zone of the vine.

5.2.2 Experimental design and establishment of living mulch treatments

Treatments were established in a six-by-six Latin square design to account for potential differences among or within vine rows. Each replicate plot was a panel of five vines with at least five vines at each end of each row to eliminate edge effects. In 2015, inter-rows were maintained by cultivation and 1% glyphosate herbicide was applied to the vine row to minimize weed competition with newly-planted vines. A permanent groundcover of *Festuca* spp. and *Lolium perenne* was seeded in March of 2016 in inter-

rows, leaving a 1 m bare strip under vine rows. In preparation for treatment establishment, weeds were removed by hand from the under vine areas in early March of 2016. The cleared area was then raked and seeded on March 16.

5.2.3 Living mulch plant identity

Living mulch treatments were chosen to include one representative of each of four plant functional groups: warm-season grasses, cool-season grasses, legumes, and non-legume forbs as they are expected to vary in their effects on soil properties and vine competition. Specific cultivars were chosen based on successful growth in other groundcover trials at the study site. We also included two industry standard under-vine management practices as comparison.

1) Buffalo grass (*Bouteloua dactyloides* (Nutt.) Columbus) is a warm-season grass native to the Great Plains of North America. It is used as a low-water alternative turf for residential lawns and establishes a virtually weed-free groundcover in inter-rows at the study site. Buffalo grass cv. Columbus '8315' was obtained from OSC seeds (Kitchener, Ontario, Canada) and seeded at a rate of 27 g m^{-2} with light raking.
2) Chewing's fescue (*Festuca rubra* subsp. *communtata* Gaudin) is a cool-season fine fescue native to Eurasia. It is commonly used as a residential turfgrass and is noted for shade tolerance, adaptation to poor soils, and drought tolerance (Cook 2011). Chewing's fescue cv. '7117' was obtained

from OSC seeds (Kitchener, Ontario, Canada) and seeded at a rate of 27 g m^2 and lightly raked.

3) Shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) is a brassicaceous forb that is a common winter annual weed in vineyards in Southern Interior British Columbia. It was selected for inclusion in this trial for several reasons: 1) it is a non-legume forb that should be minimally competitive due to spring and fall growth, 2) its presence as a weed in vineyards of the Pacific Northwest is welcome and even encouraged as it acts as cultural control for climbing cutworm pests (Lepidoptera:Noctuidae) (Mostafa et al. 2011), and 3) as a brassica, it may have beneficial (Mazzola 2015) or detrimental (Schreiner and Koide 1993) effects on soil fungi. Shepherd's purse was obtained from Richter's seeds (Goodwood, Ontario), mixed with fine sand and broadcast at a rate of 1 g m^{-2} using saltshakers for more even seeding.
4) Birdsfoot trefoil (*Lotus corniculatus* L.) is native to Eurasia but is well established and commonly used as forage in British Columbia. Of the legumes included in a previous inter-row groundcover trial at the study site, it persisted best under minimal irrigation (Lowery, pers. comm.). Birdsfoot trefoil was obtained from Northstar seeds (Neepawa, Manitoba) and seeded at a rate of 1 g m^2. Pursh's milkvetch (*Astragalus purshii* M.E. Jones var. tinctus), failed to germinate in the field in 2016, thus we replanted with *L. coniculatus* in 2017.
5) A cultivation treatment was maintained by hand hoeing three times in 2016 and three times in 2017. Timing of hoeing coincided with weeds approaching

flowering stage. Weed biomass was left on the surface to decompose as would be the case with most mechanical cultivation tools.

6) An herbicide treatment employed a hand-sprayed application of 1.5% Crush'R Plus (360 g L^{-1} glyphosate; AgWest Inc., Calgary, AB) twice yearly in mid-May and early August. Glyphosate was chosen because of its widespread and consistent use in vineyards. Glyphosate was also applied to the shepherd's purse treatment after seed set to eliminate summer weeds and allow for successful re-emergence of the shepherd's purse in the fall and spring of each year. This practice is consistent with management of this weed for cultural control of climbing cutworm in the region (Lowery, pers. comm.).

5.2.4 Plot maintenance

Under-vine microsprinklers delivered water to the entire experiment as needed to establish treatments in 2016 and spring of 2017. They were not used after May 2017 until September 27 of that year. Several hand-weeding passes were made to encourage establishment of treatments in the spring of 2016. Weeds were maintained in plots based on the particular needs of the focal plant. For example, Fescue and Buffalo grass treatments were mowed twice in 2016 with a weed eater, as trimming favors grasses over weedy forbs due to the ability of grasses to regenerate from the crown. The Birdsfoot trefoil treatment was hand weeded twice in the spring of 2017 to encourage establishment. The other treatments were not weeded in 2017 as they had established sufficient cover in 2016.

5.2.5 Measures of vine growth responses to living mulch treatments

Vine growth data was collected in the summer of 2017 once all treatments had established. Measurements included: leaf greenness, shoot length at bloom, leaf water potential, and dormant pruning weight.

5.2.5.1 Leaf greenness

Leaf greenness was determined using a *SPAD* chlorophyll meter (Spectrum Technologies, Aurora, Illinois), calculating the mean chlorophyll density for fifteen randomly selected, mature, mid-canopy leaves per vine for the center three vines in each replicate plot (five leaves per vine). *SPAD* readings are used as a proxy for leaf N content, as leaf tissue N concentration and *SPAD* readings are highly correlated in perennial crops such as grape (Porro et al. 2000). *SPAD* readings were taken on two occasions in 2017: June 23 and August 1.

5.2.5.2 Shoot length at bloom

As a representation of early season shoot growth, bloom shoot length was determined on June 21 by calculating the mean length of eight random shoots per vine for the center three vines of each plot (24 shoots total).

5.2.5.3 Leaf water potential

Leaf water potential was measured mid-season (July 27) on a warm, cloudless day at the end of an irrigation cycle (immediately before the next scheduled irrigation) for two mature, healthy, un-shaded leaves (one from each of two vines per replicate) using a

pressure chamber (PMI Instruments, Corvallis, Oregon). Readings were averaged by replicate.

5.2.5.4 Dormant pruning weight

Dormant pruning weight as a measure of final vine productivity was collected on November 24, 2017, by calculating the mean of total cane weight above the second bud from the cordon for each of the center three vines per plot. Vines were not hedged during the growing season.

5.2.6 Measures of soil responses to living mulch treatments

5.2.6.1 Soil moisture

Soil moisture was measured mid-season (July 10) during a warm week at the end of an irrigation cycle. A 2.5 cm corer was used to extract the top 0-30 cm as well as 30-60 cm of soil beneath four emitters between the five vines in each replicate plot. Cores were pooled and sealed in an airtight Ziplock bag, weighed, oven dried at 105°C for 48 hours and weighed again to calculate gravimetric water content.

5.2.6.2 Soil chemistry

A second set of cores (2.5 cm x 15 cm) was collected from beneath emitters on September 15. Although grapevines can be very deep rooted (Smart et al. 2006), this shallower depth for analysis of soil chemistry and biota was used because most of the biological activity is concentrated in the top 10-15 cm of most soils (Lavelle and Spain 2001). Four cores per plot were pooled and used to measure total soil C, total N, Bray-P,

pH, and abundance of soil fungi (see below). Soil chemistry was analyzed at the British Columbia Ministry of Environment Laboratory (Victoria, British Columbia). Briefly, pH was measured in water at a 1:1 soil:water ratio. Available P was measured as Bray P-1 of a 1:10 soil:water ratio using a one minute extraction and colorimetric analysis at 882 nm with a phospho-molydenum blue complex. Total C and N were analyzed by combustion elemental analysis with a Thermo Flash 2000 analyzer (Thermo fisher scientific).

5.2.6.3 Soil fungal responses

Abundances of the common grapevine pathogen, *Ilyonectria* spp., the EPF, *B. bassiana,* and AM fungi were determined in soil samples collected on September 15 (see above) using digital droplet PCR (ddPCR). Soils were first sieved to 2 mm following collection and *Vitis* roots were separated and stored in 35% ethanol at 4°C for later extraction. Soils were then dried at 60°C for 72 hours. Whole genomic DNA was extracted from 0.5 g of each soil sample using the FastPrep Spin Kit for Soils (MP Biomedical, Carlsbad, California) according to the manufacturer's directions. Whole genomic DNA was also extracted from 65 mg of fine (first and second order, <2mm) *Vitis* roots by crushing the roots in liquid N_2 and then using the above mentioned extraction kit according to the manufacturer's directions.

Both soil and root DNA were analyzed for abundance of *Ilyonectria* spp. using digital droplet PCR (ddPCR) with the internal transcribed spacer (ITS) primers YT2F and CYLR as per Agusti-Brisach et al. 2014. This primer set targets several species of the soil borne pathogen *Ilyonectria* (= "*Cylindrocarpon"*) known to cause black foot disease of grape (Agusti-Brisach et al. 2014). The following recipe was used in a 20µL final

reaction volume: 10µL QX200 ddPCR EvaGreen supermix (BioRad), 250 nM each primer, 2µL DNA template, and 7µL nuclease-free water. Reaction conditions were: initial denaturing at 95°C for 10 min followed by 40 cycles of denaturing at 95°C for 30 sec and annealing/extension at 60°C for 1 min, then 4°C for 5 min, 90°C for 5 min.

AM fungi were quantified using a similar protocol with the small subunit rRNA primers AMV4.5F and AMDGR (Sato et al. 2005) and an annealing/extension step of 56.5°C for 1 min. These primers target fungi within the phylum Glomeromycota and, of the AM fungal primers that yield products short enough for use with quantitative PCR methods, show the greatest specificity for this phylum (Lumini et al. 2010).

The abundance of the common EPF species *B. bassiana* was determined using the same approach with the ITS primers BB.fw and BB.rv (Landa et al. 2013) and an annealing/extension step of 56°C for 2 min.

After PCR amplification, droplets were read for fluorescence in a QX100 droplet reader compatible with EvaGreen dye (BioRad). Droplets were analyzed for fluorescence amplitude using QuantaSoft version 1.7 (BioRad) and raw amplitude and cluster data from each run were exported for threshold determination using 'ddpcRquant' (Trypsteen et al. 2015). All ddPCR assays were optimized by running temperature gradients with positive controls (fungal isolates) and environmental samples and selecting the highest annealing temperature at which there was good separation between positive and negative droplet clouds.

Data generated in this study will be made available on the Open Science Framework upon acceptance for publication.

5.2.7 Statistical analysis

5.2.7.1 Effects of living mulch identity on vine growth

To determine the effect of living mulch on vine growth responses (bloom shoot length, dormant pruning weight, *SPAD,* and pre-irrigation leaf water potential) a multivariate analysis of variance (MANOVA) was used comparing log-transformed values for each measurement. Univariate analysis of variance (ANOVA) and Tukey's honest significant difference (Tukey 1949) were employed as *post-hoc* tests to parse out differences among treatments for each parameter and growth stage measured. The R packages 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2017) with Satterwaite approximation of degrees of freedom were used to perform ANOVA, allowing inclusion of row and block as random factors to account for the Latin square design in the field.

5.2.7.2 Effects of living mulch identity on soil abiotic factors

In order to test if living mulch treatments altered soil factors, MANOVA was first used including log-transformed response variables pH, C:N ratio, C:P ratio, and soil moisture 0-30 cm and 0-60 cm pre-irrigation. Univariate ANOVA and Tukey's honest significant difference (Tukey 1949) were then used as described above to separate the effect of treatment on individual response variables.

5.2.7.3 Effects of living mulch identity on soil fungi

To test if living mulch identity altered abundance of AM fungi, *Ilyonectria* spp., or *B. bassiana*, a MANOVA was also used comparing log-transformed target copy

numbers g soil^{-1}. Individual ANOVA for each fungal group and post-hoc Tukey's honest significant difference (Tukey 1949) to determine which treatments differed for which fungal groups. Differences in target copy number of AM fungi g root^{-1} were assessed using ANOVA as described previously.

5.3 Results and Discussion

5.3.1 Effects of living mulch identity on vine growth

The identity of the living mulch led to noticeable differences in vine growth responses in this study (*Wilk's* λ=0.013, F=8.62, P<0.0001). Both the grasses and the legume treatments decreased vine growth significantly compared to the industry standard practices of cultivation or herbicides, with the fescue treatment causing the most severe growth depression in young vines at bloom (F=17.61, P<0.0001)(Fig. 5.1a) and at pruning (F=21.147, P=<0.0001)(Fig. 5.1b). The shepherd's purse treatment did not reduce vine growth compared to cultivation or herbicide treatments.

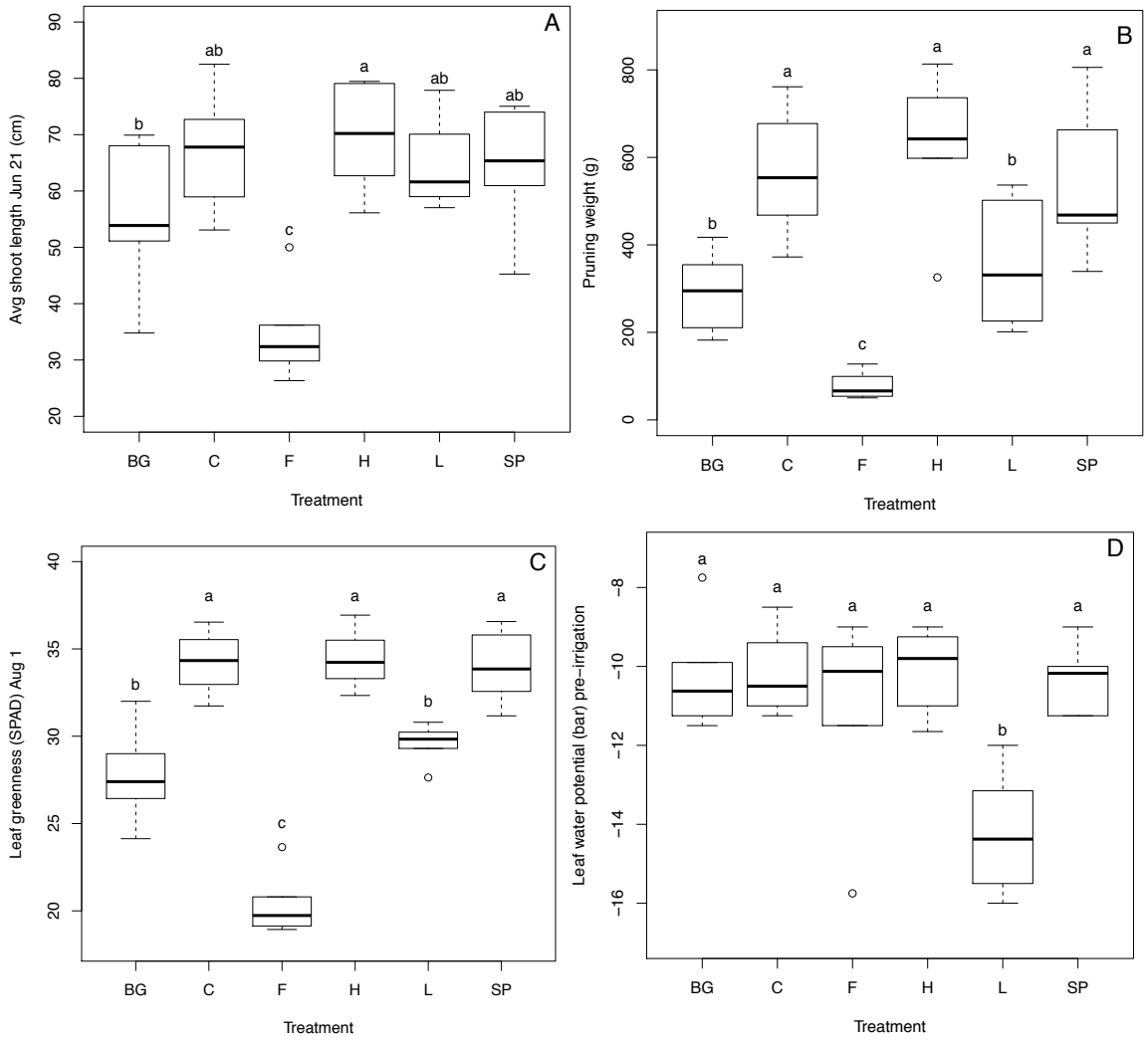

Fig. 5. 1 Vine growth and physiological responses to living mulch treatments. Responses varied by treatment in bloom shoot length (A), dormant pruning weight (B), leaf greenness (C), and leaf water potential (D). Treatments are: BG, buffalo grass; C, cultivation; F, Chewing's fescue, H, herbicide, L, birdsfoot trefoil, and SP, shepherd's purse. Letters indicate significant differences assessed at α=0.05.

Overall growth depressions were expected given the young age of the vines and previous reports of vine growth suppression when perennial cool-season grasses such as *F. rubra* (Hickey et al. 2016) and warm-season grasses (Muscas et al. 2017) are used as living mulch. For a two-year old vineyard at this site and with the quantities of water and

fertilizer applied in this study, both of the grasses and the birdsfoot trefoil led to unacceptable growth depressions compared to industry standards of cultivation or herbicides in this study. Because fertilizer and water application were applied to target balanced (i.e. not overly vigorous) growth in cultivation and herbicide treatments, there is potential for some treatments such as buffalo grass or birdsfoot trefoil to work well if inputs were increased or they were established under mature vines. The winter annual shepherd's purse, which has other benefits such as cultural control of climbing cutworm (Lepidoptera: Noctuidae)(Mostafa et al. 2011), seems not to inhibit vine growth at this site likely due to temporal asynchrony of growth with vines.

5.3.1.1 Effects of living mulch identity on vine nutrition

The growth depressions seen in this study can be attributed to competition with the vine over soil resources. As expected, the two grass treatments were more competitive for soil N, as was evident from large differences in leaf greenness in June (F=44.249, P<0.0001) and August (F=92.066, P<0.0001)(Fig. 5.1c). Fescue decreased leaf greenness more than any other treatment on both occasions, while buffalo grass produced vines that were less green compared to cultivation, herbicide, birdsfoot trefoil, and shepherd's purse treatments in June. This same pattern was seen in August, except for the birdsfoot trefoil also produced vines with intermediate greenness (Fig. 5.1c). Muscas et al. (2017) also found a reduction in vine N when a mixture of cool season grasses, *Dactylis glomerata* and *Lolium rigidum,* were used as living mulch in a 17-year old vineyard in a Mediterranean climate. In this study, the Chewing's fescue treatment was notably efficient at taking up fertilizer N, even producing salt deposits at hydathodes

at the tips of leaf blades the day after fertilization events in 2017. This suggests that the Chewing's fescue treatment was strongly competitive with vines for soil N as seen previously with this species of grass (Hickey et al. 2016). The buffalo grass treatment was not associated with the same degree of yellowing and stunted vine growth seen in the fescue treatment. Because *Bouteloua dactyloides* is native to the Great Plains where it persists in low fertility, droughty soils and N additions to buffalo grass range does not improve growth (Pettit and Fagan 1974), it could be expected that buffalo grass would not be as competitive for soil N.

5.3.1.2 Effects of living mulch identity on vine water status

Although the birdsfoot trefoil treatment also led to slight decreases in leaf greenness compared to industry standards in August (Fig. 5.1c), the data suggest that the competitive effects from this plant were largely related to water, as birdsfoot trefoil strongly decreased leaf water potential compared to all other treatments (F=11.140, P<0.0001)(Fig. 5.1d). Although this is the first study to use this particular legume as living mulch in vineyards, other perennial legumes such as *Trifolium repens* as living mulch can also compete with vines for water (Karl et al. 2016). Birsdsfoot trefoil has been shown to be a strong competitor for water in other systems, e.g. in jujube orchards (Pan et al. 2017) and may be more competitive than more drought sensitive perennial legumes such as *T. repens,* as it continues to produce biomass under drought conditions by forming a deep taproot (Peterson et al. 1992). This trait may be beneficial for persistence of this legume in dry climates such as in this study, but could lead to unacceptable levels of vine water stress or vineyard water use. Birsdfoot trefoil may be

better suited as part of a mixture of plants or as a drought-tolerant inter-row cover where direct competition with vines for irrigation water would be minimized.

Unsurprisingly, Buffalo grass did not show signs of competing with vines for water as it has relatively low water requirements due to low evapotranspiration (ET) rates even in conditions of low water stress (Qian et al. 1997) and the ability to hydraulically redistribute water from lower depths (Huang et al. 1998). Similarly, the fescue living mulch did not induce water stress in vines as measured here. Cool season grasses such as *Festuca* spp., despite having higher ET rates, are known to evade drought stress with the help of foliar endopytes (Malinowski and Belesky 2000) and this could partly explain why little to no water competition has been found between vines and *F. rubra* living mulch in other studies (Giese et al. 2014; Hickey et al. 2016) and with other cool-season grasses (Bavougian and Read 2018). Although an alternate explanation for the lack of water competition in this treatment could be that the majority of active growth of the fescue is asynchronous with that of the vines, the use of irrigation in our study allows continued growth of fescues though the summer in our area, i.e. they are not truly summer dormant as with select species of *Dactylis glomerata* (Volaire and Norton 2006). In the present study, the lack of water competition observed in the fescue treatment could also be explained by the extreme reduction in vine size due to N competition and thus lower total vine water use requirements relative to other treatments.

5.3.2 Effects of living mulch identity on soil abiotic factors

Living mulch treatments led to changes in measured soil abiotic effects (*Wilk's* λ=0.137, F=2.25, P=0.003). Living mulch treatments varied in their effect on C:N ratio

(F=3.058, P=0.027), with buffalo grass leading to a higher soil C:N ratio than cultivation where weeds were mechanically removed (Fig. 5.2a). As soils inhabited by *B. dactyloides* have been shown to have higher microbial biomass C than many other prairie grasses (Bell et al. 2014), prolific shallow root production by this species (Derner et al. 2006) could have not only helped outcompete weeds, but also increased the soil C:N ratio through prolific root litter and exudation. The fescue treatment increased C:N ratio as well, probably due to efficient uptake of N as discussed previously, but also due to a thick thatch layer that developed over the course of the experiment. This thatch layer was very effective at keeping weeds out, but likely was responsible for the immobilization of any remaining soil N. Because of this, Chewing's fescue as living mulch may only be useful in managing vine vigor and weeds in overly fertile soils (Giese et al. 2014). Despite effective weed suppression and production of a healthy, green canopy about 30 cm tall, the birdsfoot trefoil did not reduce soil C:N ratio. This is probably indicative of effective symbiotic N_2 fixation and thus little N uptake from the soil solution by this legume.

Soil C:P ratio depended on living mulch identity as well (F=3.652, P=0.016) with higher C:P levels in the birdsfoot trefoil treatment than the cultivation and herbicide treatments (Fig. 5.2b). This can be explained by the fact that legumes tend to have a high P requirement due to the energy required at nodulation sites for symbiotic N_2 fixation (Sa et al. 1991) and tend to be more efficient at acquiring soil P compared to grasses (Caradus 1980). Because vine P was not measured in this study, it is impossible to tell if this alteration of soil C:P ratio contributed to the slight, but significant growth depression with birdsfoot trefoil as living mulch.

Consistent with lower leaf water potential discussed above, birdsfoot trefoil living mulch greatly decreased soil water content in the top 30 cm by the end of the irrigation cycle compared to any other treatment (F=12.822, P<0.0001)(Fig. 5.2c). This same trend was seen in the 30-60 cm soil depth as well (F=3.17, P=0.03)(Fig. 5.2d), indicating the effect of a deep rooting pattern that allows this legume to persist in semi-arid climates.

Soil pH was not affected by living mulch treatment (F=1.41, P=0.26).

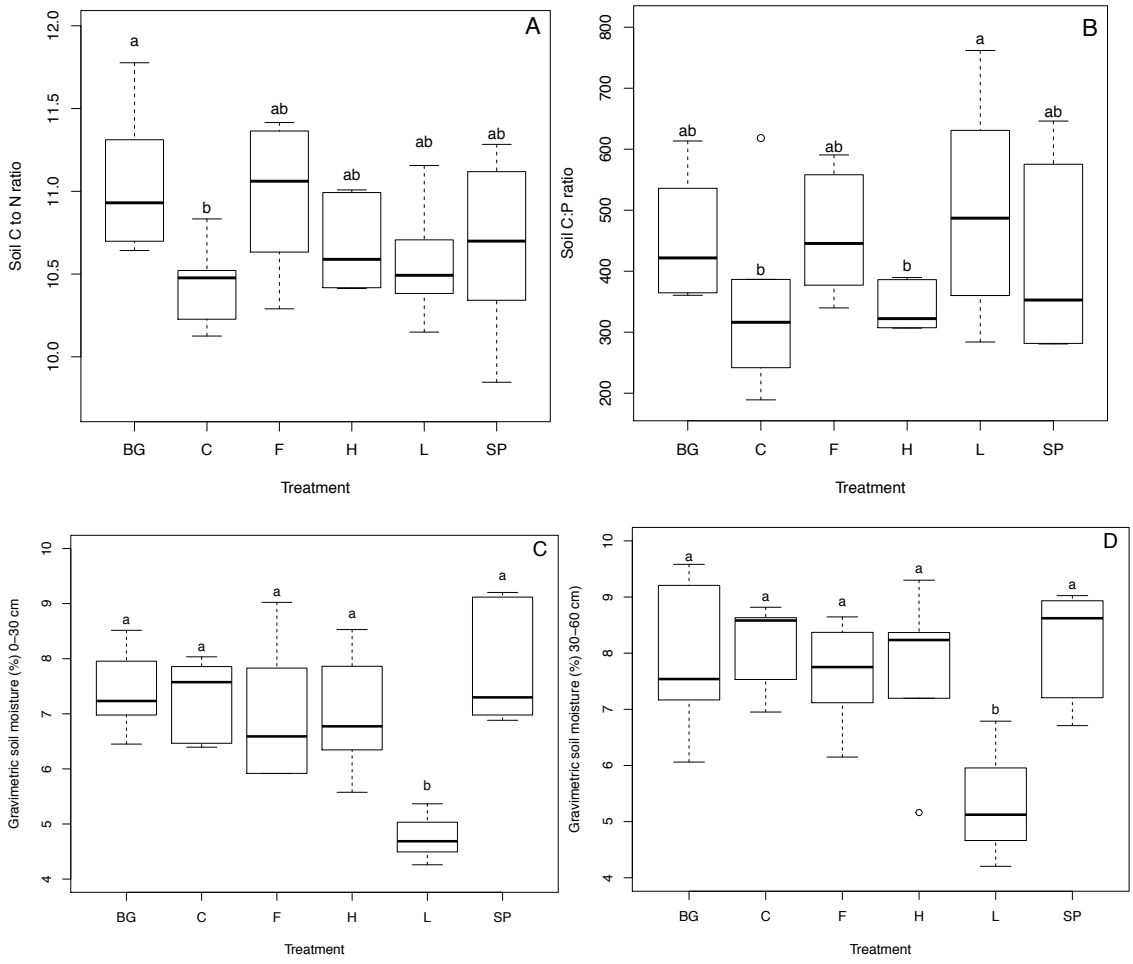

Fig. 5. 2 Living mulch effects on soil properties. Treatment effects were seen in soil C:N ratio (A), C:P ratio (B), soil moisture in the top 30 cm pre-irrigation (C), and soil moisture from 30-60 cm pre-irrigation (D). Treatments are: BG, buffalo grass; C, cultivation; F, Chewing's fescue, H, herbicide, L, birdsfoot trefoil, and SP, shepherd's purse. Letters indicate significant differences assessed at α=0.05.

5.3.3 Effects of living mulch on soil fungi

Living mulch treatment did not affect the abundance of any of the three fungal guilds in bulk soil (*Wilk's* λ=0.749, F=0.57, P=0.89)(Fig. 5.3a, 5.3b, and 5.3c). This was surprising given the reports of plant effects on populations of these soil fungi (Agusti-Brisach et al. 2011; De Deyn et al. 2011; Behie et al. 2015). Because each of these assays targeted functionally distinct fungi at various levels of taxonomic resolution, i.e.,

specificity, the reasons for the lack of effects seen may be best explored for each individual guild.

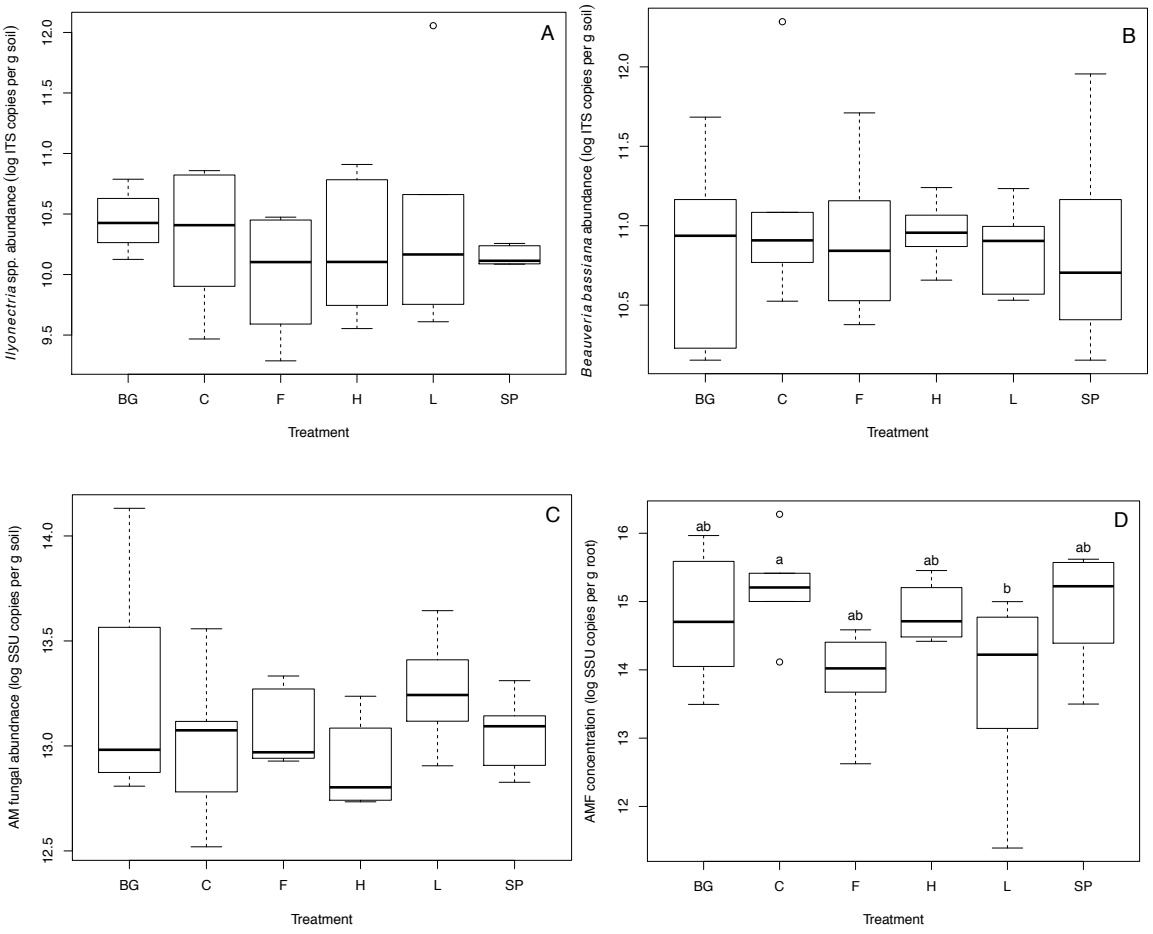

Fig. 5. 3 Effect of living mulch on abundance of *Ilyonectria* spp. (A), *Beauveria bassiana* (B), and AM fungi (C) in soil as well as AM fungi in vine roots (D). Treatment effects were only seen for AM fungi in roots. Treatments are: BG, buffalo grass; C, cultivation; F, Chewing's fescue, H, herbicide, L, birdsfoot trefoil, and SP, shepherd's purse. Letters indicate significant differences assessed at α=0.05.

5.3.3.1 Effects of living mulch on *Ilyonectria* spp.

The lack of treatment effects on *Ilyonectria* abundance may indicate that living mulch plants do not act as a good alternate host for these pathogens as has been seen with some vineyard weeds (Agusti-Brisach et al. 2011) and certain vetch cover crops in annual

cropping systems (Benitez et al. 2016). It was also surprising that shepherd's purse did not decrease *Ilyonectria* spp. abundance due to the anti-fungal volatiles produced by plants in the family Brassicaceae (Fahey et al. 2001). However, *Ilyonectria* spp. can survive as durable chlamydospores in soil (Halleen et al. 2006) and thus may be more resistant to these biofumigant effects (Stephens et al 1999). More work is needed to see if there are any temporal effects, i.e. suppression of soil fungi when shepherd's purse is actively growing in spring or shifts in communities of fungi if some fungi are more tolerant of these volatiles than others.

The quantity of *Ilyonectria* detected in this soil was several orders of magnitude lower than those reported previously in infested nursery fields using this primer set (Agusti-Brisach et al. 2014). Although an accurate threshold of these fungi in soils indicating disease potential has not been established, black foot pathogens such as *Ilyonectria* spp. are perhaps too infrequent at this site to warrant an assessment of the impacts of living mulch vegetation on disease potential. The coarse textured soil here could be a deterrent to the proliferation of these fungi as finer textured soils that hold more water are generally more problematic for development of black foot disease of grape (Berlanas et al. 2017).

5.3.3.2 Effects of living mulch identity on *Beauveria bassiana*

The EPF, *B. bassiana,* was similarly unaffected by living mulch treatment in this study. This was surprising given reports of plant host preference as a root endophyte for this species (Behie et al. 2015). However, because *B. bassiana* is a fungus with known intraspecific genetic diversity that varies along environmental gradients (Bidochka et al.

2002) and between natural and managed habitats (Meyling et al. 2009), it is possible that changes occurred at the population level in response to living mulch treatment that were not detected by measuring total abundance of *B. bassiana* at the species level as done here. Although *B. bassiana* is among the most common EPF isolated from Canadian agricultural soils (Bidochka et al. 1998), other EPF such as *Fusarium* spp. have been isolated more frequently at this study site and thus represent a target outside of the *Beauveria* assay used here that might have been affected by living mulch treatment. The development of a molecular assay targeting all EPF, perhaps using a functional gene, would be highly advantageous for future studies investigating the functional consequences of management practices such as living mulch on the infectivity of soil-dwelling insect pests by these natural enemies.

5.3.3.3 Effect of living mulch identity on AM fungi

Similarly, no differences were seen in abundance of AM fungi in bulk soil under living mulch treatments. It is surprising that the C_4 buffalo grass did not promote a greater abundance of AM fungi than other treatments as C_4 grasses are known to be strongly mycorrhizal (Hetrick et al. 1988) and are therefore expected to increase the quantity of AM fungi in surrounding soils. *Bouteloua* spp., however, are also known to be colonized extensively by dark septate endophytes that may also relieve abiotic stresses (Barrow 2003) while displacing AM fungi. It is possible that these plants may instead enhance a different group of soil fungi that do colonize *Vitis* (Likar et al. 2017) but have unknown effects on vines. The cultivar used here, a residential turfgrass selection, may also be adapted to higher resource environments as it stays greener during the heat of

summer compared to wild populations (Lowery, pers. comm.), perhaps indicating a trend toward less reliance on AM fungi commonly seen in cultivated plants when resources are provided (Martin-Robles et al. 2018).

Surprisingly, the shepherd's purse living mulch did not decrease AM fungal abundance compared to other treatments, which is contrary to reports in the literature of other brassicas having fungicidal properties, e.g., garlic mustard against AM fungi (Stinson et al. 2006). There is a wide range of glucosinilate profiles and quantities produced within the Brassicaceae (Fahey et al. 2001) and shepherd's purse may have relatively weak biofumigant effects relative to other species. In fact, shepherd's purse can be colonized by AM fungi at rates upwards of 30% if growing in proximity to good AM fungal host plants (DeMars and Boermer 1994). This could further decrease the competitive effects of this living mulch with vines as the shepherd's purse does not gain any benefit from colonization by AM fungi due to the absence of arbuscules (DeMars and Boermer 1994).

AM fungal abundance in vine roots, in contrast, varied among living mulch treatments (F=2.977, P=0.03), with more SSU target copies g root^{-1} detected in roots of vines from the cultivation treatment compared to those from the birdsfoot trefoil treatment (Fig. 5.3d). This is surprising because legumes are typically strongly mycorrhizal (Chalk et al. 2006) and might therefore be expected to increase the abundance of these fungi in neighboring host plants. Speculatively, the birdsfoot trefoil treatment may have reduced AM fungi in vine roots due to water stress-induced carbon limitation in the vine (Valentine et al. 2006). The decrease could also be due to changes in identity of the AM fungi colonizing vine roots with a selection towards drought-

tolerant fungal species that do not colonize roots as thoroughly and instead invest more in external soil hyphae (Hart and Reader 2002).

The physical disturbance of topsoil during hoeing in the cultivation treatment also might be expected to reduce AM fungi (Jasper et al. 1989), but this was not the case in this study. Although it is possible that the disturbance from hand hoeing was shallower than some other cultivation equipment, preventing overall damage to AM fungal hyphae in the top 20 cm of sampled soil, mechanical means of weed control common in organic vineyards may not be as harmful to AM fungal symbioses as perceived.

6 Conclusions

In this thesis I aimed to assess how groundcover vegetation affects plant-soil feedbacks on vine growth through changes to soil fungal communities. Overall, results show that groundcover vegetation can have a significant impact on important groups of soil fungi and, in turn, vine health outcomes. However, the latter was only seen in certain circumstances, i.e. only when vines were challenged with a pathogen. Although researchers and growers are already familiar with above ground effects of vegetative diversity in these systems, below ground effects are only beginning to be understood. The work depicted in this dissertation may help in facilitating several new directions for research as well as offer some useful insight into the effects of current practices on soil microbes and subsequent crop health in this region.

6.1 Do groundcovers affect vine resistance to disease through plant-mediated changes in soil microbes?

My research shows that groundcovers can, in fact, alter vine response to pathogen infection (chapter 2). Soils that had been trained by different groundcover vegetation led to different vine growth response when challenged with a soil borne pathogen. However, it should be noted that we saw little, if any, signs of disease development in that study, only differences in growth response. Because the treatments that differed in vine growth response also differed in root-associated saprotroph communities, this indicates that growth effects may have been due to microbial interactions stemming from unique groundcover-trained soil microbial communities.

It is important to note that results show only that the identity of groundcover or cover crop matters in determining these feedback outcomes. Because I was not able to test specific plant community characteristics, e.g. gradients of plant species richness or functional traits, independently from the groundcover treatments as a whole, more work would be needed in order to supply growers with specific recommendations if other groundcover mixes are desired.

6.2 Do groundcovers alter fungal endophyte community in newly planted vine roots?

I found that groundcovers alter AM fungal endophyte communities but not saprotrophic root-associated fungal communities in vine roots (chapter 3). The influence of groundcover identity on AM fungal community could have implications for managing new plantings as AM fungal identity and diversity may be important for vine health

(Trouvelot et al. 2015). However, I do not present any evidence in this thesis that these differences in AM fungal communities were responsible for any vine health outcomes. The particular conditions in which a plant host and a particular mycorrhizal community are engaged in the symbiosis can be crucial in determining which fungi are more beneficial to the plant (Hoeksema et al. 2010). It is thus difficult to link fungal community to plant benefit in a greenhouse trial lacking a gradient of resource availability or pathogen pressure that might show varying mycorrhizal benefit depending on conditions. Still, this finding could have implications for vine health if it holds true in the field where vines are more likely to experience conditions in which there may be a notable difference in mycorrhizal response depending on the particular groundcover-trained AM fungal community with which vines are associated.

Saprotrophic fungal communities in vine roots only differed when vines were challenged with a pathogen (chapter 2). In the absence of a pathogen challenge, however, these saprotrophic communities were unaffected by groundcover identity. It must be noted that many of these fungi likely were introduced with rootstock cuttings (chapter 2), meaning soil effects could have been secondary at this stage in vine development. Because we isolated many competitive and mycoparasitic fungi in these experiments, especially from older roots and from the pathogen challenge experiment (chapter 2), there could be an influence of groundcover-trained microbial community on fungal competition and pathogen antagonism.

Due to the experimental design in the greenhouse, I was not able to statistically compare results from these two parallel experiments (chapter 2 and 3). I was also unable to quantify the AM fungal community in the pathogen challenge experiment due to the

inadvertent loss of a number of root samples earmarked for AM fungal colonization and extraction. Therefore, I cannot infer if the different AM fungal communities introduced from different groundcovers were involved in the feedback outcomes seen with a pathogen challenge.

6.3 How do AM fungal communities change as roots age and does this depend on groundcover?

AM fungi changed morphologically, i.e. in terms of colonization patterns between young and old roots as described in chapter 3, though community structure did not change, indicating that most AM fungal symbionts remain in their host roots as roots move toward senescence. The shift to fewer arbuscules and more storage structures in old roots occurred independent of groundcover despite groundcovers leading to different AM fungal communities. Taken together, this means that AM fungi occurring at this site tend to alter their growth patterns in response to an aging root rather than turnover by adding and removing constituents of the fungal community. This is expected based on anecdotal observations made by mycorrhizal researchers (e.g. see INVAM website page on *Glomus*), but to my knowledge has not been demonstrated empirically until now. This finding thus represents a contribution to the mycorrhizal literature.

Although groundcover did not affect the overall shift from arbuscules to storage organs as roots aged, the groundcover consisting of native grass and forbs led to a greater proportion of arbuscules overall. Greater arbuscule formation is thought to indicate a more mutualistic symbiosis (Johnson et al. 1997), as arbuscules are necessary for flow of soil nutrients from the fungus to the plant host. The AM fungal community trained under

this groundcover might be more beneficial for vines when soil resources are limiting as it apparently has the capacity to continue to provide nutrition to the plant host longer than other groundcover-trained communities. However, I saw no indication of improved plant growth in this treatment in chapter 3, perhaps because I provided ample water and nutrients in the greenhouse, which may have diluted any differences in mycorrhizal response (Hoeksema et al. 2010).

6.4 Do existing groundcover management strategies and vegetation affect populations of beneficial and pathogenic soil microbes?

Different groundcover strategies altered the abundance of several guilds of soil fungi, including the beneficial AM fungi and *Beauveria bassiana,* as well as the pathogenic *Ilyonectria* spp. I found evidence of plant functional characteristics driving these changes (chapter 4). For example, *B. bassiana* was consistently more abundant when groundcovers included more native plants, legumes, or plants with annual life cycles. *Ilyonectria* spp. were consistently more abundant in areas where forbs were more plentiful and less abundant in groundcovers dominated by grass. *Ilyonectria* spp. were also more abundant overall under groundcovers with more exotic plants, though this varied over time. AM fungi were overall more abundant with native plants and annual plants, but this also varied over time.

These findings give support to the idea of managing habitat to favor EP fungi in conservation biological control of insect pests (Pell et al. 2010). In our study, the abundance of the common EP fungus, *B. bassiana*, increased with the relative abundance of native plants and legumes. Additionally, EP fungi trained under groundcovers

dominated by native species also increased rates of bait insect mortality in a laboratory bioassay not presented as part of this thesis (see Honors thesis by Michelle MacDonald). Future work should look into the use of vineyard-adapted native plants as reservoirs for naturally occurring biological control agents of soil-dwelling pests.

A plant community characteristic that was related to abundances of all three groups was the proportion of native vs. exotic plants, with natives increasing beneficial fungi (AM and EP fungi) and exotics increasing our pathogenic guild (*Ilyonectria* spp.). Taken broadly, this influence of native plants is interesting in the context of plant-soil feedback because positive feedbacks may result from more symbiotic fungi (AM fungi) and pathogens of herbivores (EP fungi) while negative plant-soil feedbacks due to pathogens such as *Ilyonectria* spp. may occur with the prevalence of exotic species in a groundcover. Further tests of feedbacks between specific native plants that persist in vineyards and grapevines through changes in soil biota may aid in making recommendations for native groundcover mixes in this region.

6.5 Does under vine weed management affect populations of beneficial and pathogenic soil microbes and vine growth?

Living mulch planted under vine rows did not affect the abundance of the three fungal guilds measured, but did produce strong vine growth effects. The lack of fungal effect was surprising given the stark differences in plant communities managed under vine in this field trial and the results from the survey in chapter 4. The herbicide glyphosate did not decrease any of the fungal guilds despite some indication in the literature that it might negatively impact some EP fungi (Morjan et al. 2002). Cultivation

or glyphosate did not decrease AM fungi despite eliminating potential host roots for infection. Also, we saw no detrimental effect of the brassica, shepherd's purse, on any of the fungal groups despite literature suggesting that isothiocyanate-producing plants from this family can be anti-fungal (Schreiner and Koide 1993). In all cases, the presence of vine roots in the vine row where samples were taken could have a positive effect on many fungi, including AM fungi, by providing plentiful root litter, exudates, and habitat for endophytes, thus negating any detrimental effects of certain under vine management tactics.

My aim in assessing both vine growth as well as fungal guilds in this field trial was to see if feedbacks on vine growth through the kind of changes to soil fungi seen in chapter 4 would be noticeable in the field. Because competitive effects between living mulch and vine growth were strong in most treatments and fungal guilds were not affected, this suggests that direct plant-plant competition may be more important for determining vine growth outcomes. However, the lack of changes to soil fungi seen in our living mulch treatments, along with the relatively short timescale of this trial does not allow me to determine if fungal effects would play a role in vine growth outcomes over time.

6.6 Concluding remarks

Based on a review of the literature, I expected several characteristics of groundcover plant communities to play a larger role in driving soil microbial communities and plant-soil feedback than I observed. For instance, I saw no effects of plant species richness on abundances of any fungal groups in the multi-season field

survey (chapter 4), although microbial diversity was not included as a response variable in that chapter. Species richness of groundcover plant communities also did not influence fungal richness in vine roots (chapters 2 and 3), though the groundcover treatments employed in those experiments were not designed to test plant species richness *per se.* I can therefore conclude that plant species richness likely does not drive abundances of soil fungi in these soils, though more work would be needed to fully test a link between plant species richness and fungal species richness.

Another surprise was that I saw no negative effect of non-mycorrhizal plants such as brassicas or chenopods on AM fungal abundance (chapters 4 and 5). Although there is evidence that non-mycorrhizal plants can reduce AM fungal inoculum potential in soil (Schreiner and Koide 1993; Stinson et al 2006), the AM fungal species most abundant in agricultural soils (Rosendahl et al. 2009) might not be affected as much and may even colonize these plants to some degree if only as refuge or to form propagules (e.g., Muller et al. 2017).

The most consistent groundcover effect overall was related to plant functional group and provenance, suggesting that the functional identity of plants in a groundcover could be an important force in driving soil microbial communities in the Okanagan valley. There has already been some very good work done looking at different types of vegetation, including native species, for use in vineyards in this region. This dissertation was timely because in utilizing experimental and commercial plantings established over the past 10 years, I was able to see the effects of plant communities that not only had time to drive changes in soil fungi but also were comprised of species capable of persisting at these sites. This means that there were very few instances in which the effects I detected

would be ephemeral, dying out with an ill-fated groundcover mix. Instead, these findings should be robust and useful for this region as a whole. The plants that do persist and were associated with beneficial fungi, such as native species (chapter 4), may persist as a result of the very changes they induce, via positive plant-soil feedback.

This thesis shows that vineyard groundcover management does have important implications for soil fungi and vine health, suggesting that the soil ecosystem can be managed to some degree by grower manipulation of non-crop vegetation. Vineyards in the Okanagan valley are uniquely positioned to garner the benefits of plant-induced changes to the soil ecosystem due to the relative infrequency of disturbance compared with other farming systems. As groundcover vegetation already exists in these perennial systems, our ability to manipulate and induce changes below ground adds an entirely new set of potential tools that can contribute to the sustainability of viticulture in this region and elsewhere.

Bibliography

Agustí-Brisach C, Armengol J (2013) Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies. Phytopathol Mediterr 52: 245-261

Agustí-Brisach C, Gramaje D, Leon M, Garcia-Jimenez J, Armengol J (2011) Evaluation of vineyard weeds as potential hosts of black-foot and petri disease pathogens. Plant Dis 95: 803-810. doi: 10.1094/pdis-12-10-0888.

Agusti-Brisach, C., Mostert, L., Armengol, J., 2014. Detection and quantification of *Ilyonectria* spp. spp. associated with black- foot disease of grapevine in nursery soils using multiplex nested PCR and quantitative PCR. Plant Pathology 63, 316-322.

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435: 824-827. doi: 10.1038/nature03608.

Ali-Shtayeh, M.S., Mara'i, A., Jamous, R.M., 2003. Distribution, occurrence and characterization of entomopathogenic fungi in agricultural soil in the Palestinian area. Mycopathologia 156, 235-244.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Molec Biol 215: 403-410

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austr Ecol 26: 32-46. doi: 10.1046/j.1442-9993.2001.01070.x.

Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. Ecology 84: 511-525

Anderson, R.C., Liberta, A.E., Dickman, L.A., 1984. Interaction of vascular plants and vesicular-mycorrhizal fungi across a soil moisture-nutrient gradient. Oecologia 64, 111-117.

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42. doi: 10.1007/s005720100097.

Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, Ravetta DA, Schaeffer SM (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141: 221-235

Avis TJ, Gravel V, Antoun H, Tweddell RJ (2008) Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol Biochem 40: 1733-1740. doi: 10.1016/j.soilbio.2008.02.013.

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - An overview of the mechanisms involved. Mycorrhiza 6: 457-464.

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32: 666-681. doi: 10.1111/j.1365-3040.2009.01926.x.

Bagniewska-Zadworna A, Stelmasik A, Minicka J (2014) From birth to death - *Populus trichocarpa* fibrous roots functional anatomy. Biol Plant 58: 551-560. doi: 10.1007/s10535-014-0433-6.

Balota EL, Martins Auler PA (2011) Soil microbial biomass under different management and tillage systems of permanent intercropped cover species in an orange orchard. Rev Brasileira Ciencia Solo 35: 1873-1883.

Bardgett RD, Shine A (1999) Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol Biochem 31: 317-321. doi: 10.1016/s0038-0717(98)00121-7.
Bardgett RD, van der Putten WH (2014) Belowground biodiversity and ecosystem functioning. Nature 515: 505-511. doi: 10.1038/nature13855.
Barrow JR (2003) Atypical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. Mycorrhiza 13: 239-247. doi: 10.1007/s00572-003-0222-0.
Barto EK, Rillig MC (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. J Ecol 98: 745-753. doi: 10.1111/j.1365-2745.2010.01658.x.
Bartoń, K. 2017. MuMIn: multi-model inference. R package version 1.40.0. R Foundation for Statistical Computing, Vienna, Austria.
Bastian F, Bouziri L, Nicolardot B, Ranjard L (2009) Impact of wheat straw decomposition on successional patterns of soil microbial community structure. Soil Biol Biochem 41: 262-275. doi: 10.1016/j.soilbio.2008.10.024.
Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. 2015 **67**:48.
Bavougian CM, Read PE (2018) Mulch and groundcover effects on soil temperature and moisture, surface reflectance, grapevine water potential, and vineyard weed management. Peerj 6. doi: 10.7717/peerj.5082.
Becard G, Fortin JA (1988) Early events of vesicular arbuscular mycorrhiza formation on RI T-DNA transformed roots. New Phytol 108: 211-218. doi: 10.1111/j.1469-8137.1988.tb03698.x.
Behie SW, Jones SJ, Bidochka MJ (2015) Plant tissue localization of the endophytic insect pathogenic fungi *Metarhizium* and *Beauveria*. Fungal Ecol 13: 112-119. doi: 10.1016/j.funeco.2014.08.001.
Behie, S.W., Moreira, C.C., Sementchoukova, I., Barelli, L., Zelisko, P.M., Bidochka, M.J., 2017. Carbon translocation from a plant to an insect-pathogenic endophytic fungus. Nature Communications 8.
Behie, S.W., Zelisko, P.M., Bidochka, M.J., 2012. Endophytic Insect-Parasitic Fungi Translocate Nitrogen Directly from Insects to Plants. Science 336, 1576-1577.
Bell C, Carrillo Y, Boot CM, Rocca JD, Pendall E, Wallenstein MD (2014) Rhizosphere stoichiometry: are C:N:P ratios of plants, soils, and enzymes conserved at the plant species-level? New Phytol 201: 505-517. doi: 10.1111/nph.12531.
Benhamou N, Garand C, Goulet A (2002) Ability of nonpathogenic *Fusarium oxysporum* strain Fo47 to induce resistance against *Pythium ultimum* infection in cucumber. Appl Environ Microbiol 68: 4044-4060. doi: 10.1128/aem.68.8.4044-4060.2002.
Benitez MS, Taheri WI, Lehman RM (2016) Selection of fungi by candidate cover crops. App Soil Ecol 103: 72-82. doi: 10.1016/j.apsoil.2016.03.016.
Bennett AJ, Hilton S, Bending GD, Chandler D, Mills P (2014) Impact of fresh root material and mature crop residues of oilseed rape (*Brassica napus*) on microbial communities associated with subsequent oilseed rape. Biol Fert Soils 50: 1267-1279. doi: 10.1007/s00374-014-0934-7.
Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. Fems Microbiol Ecol 68: 1-13

Berg G, Zachow C, Lottmann J, Gotz M, Costa R, Smalla K (2005) Impact of plant species and site on rhizosphere-associated fungi antagonistic to *Verticillium dahliae* Kleb. Appl Environ Microbiol 71: 4203-4213. doi: 10.1128/aem.71.8.4203-4213.2005.

Berlanas C, Lopez-Manzanares B, Gramaje D (2017) Estimation of viable propagules of black-foot disease pathogens in grapevine cultivated soils and their relation to production systems and soil properties. Plant Soil 417: 467-479. doi: 10.1007/s11104-017-3272-3.

Bever JD (1994) Feedback between plants and their soil communities in an old field community. Ecology 75: 1965-1977. doi: 10.2307/1941601.

Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M (2010) Rooting theories of plant community ecology in microbial interactions. Trends Ecol Evol 25: 468-478. doi: 10.1016/j.tree.2010.05.004.

Bever JD, Mangan SA, Alexander HM (2015) Maintenance of plant species diversity by pathogens. Annu Rev Ecol Evol Syst 46: 305-325. doi:10.1146/annurev-ecolsys-112414-054306

Bezemer TM, Lawson CS, Hedlund K, Edwards AR, Brook AJ, Igual JM, Mortimer SR, Van der Putten WH (2006) Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. J Ecol 94: 893-904. doi: 10.1111/j.1365-2745.2006.01158.x.

Bidochka MJ, Kasperski JE, Wild GAM (1998) Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Can J Bot 76: 1198-1204.

Bidochka MJ, Menzies FV, Kamp AM (2002) Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. Arch Microbiol 178: 531-537. doi: 10.1007/s00203-002-0490-7.

Biermann B, Linderman RG (1983) Use of vesicular arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. New Phytol 95: 97-105. doi: 10.1111/j.1469-8137.1983.tb03472.x.

Blee KA, Anderson AJ (1998) Regulation of arbuscule formation by carbon in the plant. Plant J 16: 523-530. doi: 10.1046/j.1365-313x.1998.00315.x.

Boerner, RE (1992) Plant life span and response to inoculation with vesicular-arbuscular mycorrhizal fungi. Mycorrhiza 1: 153-161

Bonanomi, G., Antignani, V., Pane, C., Scala, E., 2007. Suppression of soilborne fungal diseases with organic amendments. Journal of Plant Pathology 89, 311-324.

Bouma TJ, Yanai RD, Elkin AD, Hartmond U, Flores-Alva DE, Eissenstat DM (2001) Estimating age-dependent costs and benefits of roots with contrasting life span: comparing apples and oranges. New Phytol 150: 685-695. doi: 10.1046/j.1469-8137.2001.00128.x.

Brito I, Goss MJ, de Carvalho M, Chatagnier O, van Tuinen D (2012) Impact of tillage system on arbuscular mycorrhiza fungal communities in the soil under Mediterranean conditions. Soil Tillage Res 121: 63-67. doi: 10.1016/j.still.2012.01.012.

Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco JM (2008) Root exudates regulate soil fungal community composition and diversty. Appl Environ Microbiol 74: 738-744. doi: 10.1128/aem.02188-07.

Bullock, D.G., 1992. Crop-rotation. Critical Reviews in Plant Sciences 11, 309-326.
Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multi-Model Inference : A Practical Information-Theoretic Approach (2nd Edition). Springer, Secaucus, NJ, USA.
Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Method 13: 581-+. doi: 10.1038/nmeth.3869.
Cameron DD, Neal AL, van Wees SCM, Ton J (2013) Mycorrhiza-induced resistance: more than the sum of its parts? Trends Plant Sci 18: 539-545. doi: 10.1016/j.tplants.2013.06.004.
Caradus JR (1980) Distinguishing between grass and legume species for efficiency of phosphorus use. New Zealand J Agric Res 23: 75-81.
Carlsson G, Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. Plant Soil 253: 353-372
Casieri L, Hofstetter V, Viret O, Gindro K (2009) Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. Phytopathol Mediterr 48: 73-83
Centinari (2016) Impacts of under-trellis cover crops. Wines and Vines 2016: October. https://www.winesandvines.com/features/article/175449/Impacts-of-Under-Trellis-Cover-Crops Accessed 28 May 2018.
Chalk PM, Souza RD, Urquiaga S, Alves BJR, Boddey RM (2006) The role of arbuscular mycorrhiza in legume symbiotic performance. Soil Biol Biochem 38: 2944-2951. doi: 10.1016/j.soilbio.2006.05.005.
Chaudhary, V.B., Bowker, M.A., O'Dell, T.E., Grace, J.B., Redman, A.E., Rillig, M.C., Johnson, N.C., 2009. Untangling the biological contributions to soil stability in semiarid shrublands. Ecological Applications 19, 110-122.
Chowdhury N, Marschner P, Burns R (2011) Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant Soil 344: 241-254. doi: 10.1007/s11104-011-0743-9.
Civitello DJ, Cohen J, Fatima H, Halstead NT, Liriano J, McMahon TA, Ortega CN, Sauer EL, Sehgal T, Young S, Rohr JR (2015) Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proc Natl Acad Sci USA 112: 8667-8671. doi: 10.1073/pnas.1506279112.
Clark, A (2008) Managing Cover Crops Profitably. 3rd ed. Handbook Series 9. Sust Agric Net. Beltsville, MD. [Online] https://www.sare.org/content/.../file/Managing%20Cover%20Crops%20Profitably.pdf
Clarkson JM, Charnley AK (1996) New insights into the mechanisms of fungal pathogenesis in insects. Trends Microbiol 4: 197-203. doi: 10.1016/0966-842x(96)10022-6.
Clifton, E.H., Jaronski, S.T., Hodgson, E.W., Gassmann, A.J., 2015. Abundance of Soil-Borne Entomopathogenic Fungi in Organic and Conventional Fields in the Midwestern USA with an Emphasis on the Effect of Herbicides and Fungicides on Fungal Persistence. Plos One 10.
Comas LH, Eissenstat DM, Lakso AN (2000) Assessing root death and root system dynamics in a study of grape canopy pruning. New Phytol 147: 171-178. doi: 10.1046/j.1469-

8137.2000.00679.x.
Cook, T. 2011. The Fine Fescues. Oregon State Univ, Hort Crop Soil Sci. [Online] http://groups.hort.oregonstate.edu
Cornelissen JHC, Thompson K (1997) Functional leaf attributes predict litter decomposition rate in herbaceous plants. New Phytol 135: 109-114. doi: 10.1046/j.1469-8137.1997.00628.x.
Corneo, P.E., Pellegrini, A., Cappellin, L., Gessler, C., Pertot, I., 2013. Weeds influence soil bacterial and fungal communities. Plant and Soil 373, 107-123.
Costello MJ (2010) Grapevine and Soil water relations with nodding needlegrass (*Nassella cernua*), a California native grass, as a cover crop. Hortsci 45: 621-627.
Cotrufo MF, Wallenstein MD, Boot CM, Denef K, Paul E (2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biol 19: 988-995. doi: 10.1111/gcb.12113.
Dai, J., Hu, J.L., Lin, X.G., Yang, A.N., Wang, R., Zhang, J.B., Wong, M.H., 2013. Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. Journal of Soils and Sediments 13, 1-11.
Davies, B.E., 1974. Loss-on-ignition as an estimate of soil organic-matter. Soil Science Society of America Journal 38, 150-151.
De Bellis, T., Kernaghan, G., Widden, P., 2007. Plant community influences on soil microfungal assemblages in boreal mixed-wood forests. Mycologia 99, 356-367.
De Caceres M, Legendre P (2009) Associations between species and groups of sites: indices and statistical inference. Ecology 90: 3566-3574
De Deyn GB, Quirk H, Bardgett RD (2011) Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biol Lett 7: 75-78. doi: 10.1098/rsbl.2010.0575.
de Souza JT, Arnould C, Deulvot C, Lemanceau P, Gianinazzi-Pearson V, Raaijmakers JM (2003) Effect of 2,4-diacetylphloroglucinol on *Pythium*: Cellular responses and variation in sensitivity among propagules and species. Phytopathol 93: 966-975. doi: 10.1094/phyto.2003.93.8.966.
de Vries FT, Manning P, Tallowin JRB, Mortimer SR, Pilgrim ES, Harrison KA, Hobbs PJ, Quirk H, Shipley B, Cornelissen JHC, Kattge J, Bardgett RD (2012) Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol Lett 15: 1230-1239. doi: 10.1111/j.1461-0248.2012.01844.x.
Demars BG, Boerner REJ (1994) Vesicular-arbuscular mycorrhizal fungi colonization in *Capsella bursa-pastoris* (Brassicaceae). Amer Midland Nat 132: 377-380. doi: 10.2307/2426593.
Derner JD, Boutton TW, Briske DD (2006) Grazing and ecosystem carbon storage in the North American Great Plains. Plant Soil 280: 77-90. doi: 10.1007/s11104-005-2554-3.
Detheridge, A.P., Brand, G., Fychan, R., Crotty, F.V., Sanderson, R., Griffith, G.W., Marley, C.L., 2016. The legacy effect of cover crops on soil fungal populations in a cereal rotation. Agriculture Ecosystems & Environment 228, 49-61.
Deveautour C, Donn S, Power SA, Bennett AE, Powell JR (2018) Experimentally altered rainfall regimes and host root traits affect grassland arbuscular mycorrhizal fungal communities.

Molec Ecol 27: 2152-2163. doi: 10.1111/mec.14536.
Druzhinina IS, Kopchinskiy AG, Komon M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. Fungal Genet Biol 42: 813-828
Dubrovsky, S., Fabritius, A.L., 2007. Occurrence of *Ilyonectria* spp.indrocarpon spp. in nursery grapevines in California. Phytopathologia Mediterranea 46, 84-86.
Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol 190: 794-804. doi: 10.1111/j.1469-8137.2010.03636.x.
Eason WR, Newman EI, Chuba PN (1991) Specificity of interplant cycling of phosphorus: the role of mycorrhizas. Plant Soil 137: 267-274.
Egan CP, Rummel A, Kokkoris V, Klironomos J, Lekberg Y, Hart M (2018) Using mock communities of arbuscular mycorrhizal fungi to evaluate fidelity associated with Illumina sequencing. Fungal Ecol 33: 52-64. doi: 10.1016/j.funeco.2018.01.004.
Eilers EJ, Klein AM (2009) Landscape context and management effects on an important insect pest and its natural enemies in almond. Biol Control 51: 388-394
Eisenhauer N, Milcu A, Sabais ACW, Bessler H, Brenner J, Engels C, Klarner B, Maraun M, Partsch S, Roscher C, Schonert F, Temperton VM, Thomisch K, Weigelt A, Weisser WW, Scheu S (2011) Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. Plos One 6. doi: 10.1371/journal.pone.0016055.
Ezzouhri L, Castro E, Moya M, Espinola F, Lairini K (2009) Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. Afr J Microbiol Res 3: 35-48.
Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochem 56: 5-51. doi: 10.1016/s0031-9422(00)00316-2.
Fanin N, Fromin N, Bertrand I (2016) Functional breadth and home-field advantage generate functional differences among soil microbial decomposers. Ecology 97: 1023-1037. doi: 10.1890/15-1263.1.
Fanin N, Haettenschwiler S, Fromin N (2014) Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil. Plant Soil 379: 79-91. doi: 10.1007/s11104-014-2051-7.
Fernandez-Calvino D, Soler-Rovira P, Polo A, Diaz-Ravina M, Arias-Estevez M, Plaza C (2010) Enzyme activities in vineyard soils long-term treated with copper-based fungicides. Soil Biol Biochem 42: 2119-2127. doi: 10.1016/j.soilbio.2010.08.007.
Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103: 626-631. doi: 10.1073/pnas.0507535103.
Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141: 525-533. doi: 10.1046/j.1469-8137.1999.00366.x.

Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial competition? Soil Biol Biochem 35: 837-843. doi: 10.1016/s0038-0717(03)00123-8.
Fourie P, Halleen F, van der Vyver J, Schreuder W (2001) Effect of *Trichoderma* treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines. Phytopathol Mediterr 40: S473-478
Fox, J., 2003. Effect Displays in R for Generalised Linear Models. Journal of
Galletti S, Sala E, Leoni O, Burzi PL, Cerato C (2008) *Trichoderma* spp. tolerance to *Brassica carinata* seed meal for a combined use in biofumigation. Biol Control 45: 319-327. doi: 10.1016/j.biocontrol.2008.01.014.
Garbeva P, Postma J, van Veen JA, van Elsas JD (2006) Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. Environ Microbiol 8: 233-246. doi: 10.1111/j.1462-2920.2005.00888.x.
Garbeva P, van Veen JA, van Elsas JD (2004a) Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42: 243-270. doi: 10.1146/annurev.phyto.42.012604.135455.
Garbeva P, Voesenek K, van Elsas JD (2004b) Quantitative detection and diversity of the pyrrolnitrin biosynthetic locus in soil under different treatments. Soil Biol Biochem 36: 1453-1463. doi: 10.1016/j.soilbio.2004.03.009.
Garcia I, Mendoza R, Pomar MC (2008) Deficit and excess of soil water impact on plant growth of Lotus tenuis by affecting nutrient uptake and arbuscular mycorrhizal symbiosis. Plant Soil 304: 117-131. doi: 10.1007/s11104-007-9526-8.
Ghanizadeh H, Harrington KC, James TK (2015) Glyphosate-resistant *Lolium multiflorum* and *Lolium perenne* populations from New Zealand are also resistant to glufosinate and amitrole. Crop Prot 78: 1-4. doi: 10.1016/j.cropro.2015.08.008.
Giese G, Velasco-Cruz C, Roberts L, Heitman J, Wolf TK (2014) Complete vineyard floor cover crops favorably limit grapevine vegetative growth. Sci Hort 170: 256-266. doi: 10.1016/j.scienta.2014.03.011.
Gnekow MA, Marschner H (1989) Role of VA mycorrhiza in growth and mineral-nutrition of apple (*Malus pumila* var domestica) rootstock cuttings. Plant Soil 119: 285-293. doi: 10.1007/bf02370421.
Gonzalez V, Luisa Tello M (2011) The endophytic mycota associated with *Vitis vinifera* in central Spain. Fungal Divers 47: 29-42
Gosling, P., Hodge, A., Goodlass, G., Bending, G.D., 2006. Arbuscular mycorrhizal fungi and organic farming. Agriculture Ecosystems & Environment 113, 17-35.
Gosling, P., Proctor, M., Jones, J., Bending, G.D., 2014. Distribution and diversity of Paraglomus spp. in tilled agricultural soils. Mycorrhiza 24, 1-11.
Govaerts B, Mezzalama M, Unno Y, Sayre KD, Luna-Guido M, Vanherck K, Dendooven L, Deckers J (2007) Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. Appl Soil Ecol 37: 18-30. doi: 10.1016/j.apsoil.2007.03.006.
Graham JH, Timmer LW, Fardelmann D (1985) Toxicity of fungicidal copper in soil to citrus seedlings and vesicular-arbuscular mycorrhizal fungi. Phytopathol 75: 1284-1284.

Gramaje D, Armengol J (2011) Fungal Trunk Pathogens in the Grapevine Propagation Process: Potential Inoculum Sources, Detection, Identification, and Management Strategies. Plant Dis 95: 1040-1055

Grayston SJ, Wang SQ, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30: 369-378

Gu YH, Mazzola M (2003) Modification of fluorescent pseudomonad community and control of apple replant disease induced in a wheat cultivar-specific manner. Appl Soil Ecol 24: 57-72. doi: 10.1016/s0929-1393(03)00066-0.

Haas D, Defago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3: 307-319. doi: 10.1038/nrmicro1129.

Halleen F, Crous PW, Petrini O (2003) Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australas Plant Pathol 32: 47-52

Halleen F, Fourie PH, Crous PW (2006) A review of black foot disease of grapevine. Phytopathol Mediterr 45: S55-S67.

Hamel C, Vujanovic V, Jeannotte R, Nakano-Hylander A, St-Arnaud M (2005) Negative feedback on a perennial crop: *Fusarium* crown and root rot of asparagus is related to changes in soil microbial community structure. Plant Soil 268: 75-87. doi: 10.1007/s11104-004-0228-1.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species - Opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2: 43-56. doi: 10.1038/nrmicro797.

Hart MM, Gorzelak M, Ragone D, Murch SJ (2014) Arbuscular mycorrhizal fungal succession in a long-lived perennial. Botany 92. doi: 10.1139/cjb-2013-0185.

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 153: 335-344. doi: 10.1046/j.0028-646X.2001.00312.x.

Hart MM, Reader RJ, Klironomos JN (2003) Plant coexistence mediated by arbuscular mycorrhizal fungi. Trends Ecol Evol 18: 418-423. doi: 10.1016/s0169-5347(03)00127-7.

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321: 235-257. doi: 10.1007/s11104-008-9814-y.

Hartwig NL, Ammon HU (2002) 50th Anniversary - Invited article - Cover crops and living mulches. Weed Sci 50: 688-699. doi: 10.1614/0043-1745(2002)050[0688:aiacca]2.0.co;2.

Hassen A, Saidi N, Cherif M, Boudabous A (1998) Resistance of environmental bacteria to heavy metals. Bioresour Technol 64: 7-15. doi: 10.1016/s0960-8524(97)00161-2.

Hausmann NT, Hawkes CV (2009) Plant neighborhood control of arbuscular mycorrhizal community composition. New Phytol 183: 1188-1200. doi: 10.1111/j.1469-8137.2009.02882.x.

Heap I (2014) Global perspective of herbicide-resistant weeds. Pest Management Science 70: 1306-1315. doi: 10.1002/ps.3696.

Hembree K, Lanini WT, DiTomaso J, Vargus R (2013) Weed management. In: Bettiga LJ (ed) Grape pest management, 3rd ed. Univ California Agric Nat res. Oakland, CA

Henry CM, Deacon JW (1981) Natural (non-pathogenic) death of the cortex of wheat and barley seminal roots, as evidenced by nuclear staining with acridiidine-orange. Plant Soil 60: 255-274. doi: 10.1007/bf02374110.
Hepper CM (1985) Influence of age of roots on the pattern of vesicular arbuscular mycorrhizal infection in leek and clover. New Phytol 101: 685-693. doi: 10.1111/j.1469-8137.1985.tb02874.x.
Hervé, M (2018). RVAideMemoire: Testing and plotting procedures for biostatistics. R package version 0.9-69-3.
Hetrick BAD, Kitt DG, Wilson GT (1988) Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass praire plants. Can J Bot 66: 1376-1380.
Hickey CC, Hatch TA, Stallings J, Wolf TK (2016) Under-Trellis Cover Crop and Rootstock Affect Growth, Yield Components, and Fruit Composition of Cabernet Sauvignon. Am J Enol Vit 67: 281-295. doi: 10.5344/ajev.2016.15079.
Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413: 297-299. doi: 10.1038/35095041.
Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Nat Acad Sci USA 107: 13754-13759. doi: 10.1073/pnas.1005874107.
Hodge, A., 2014. Interactions Between Arbuscular Mycorrhizal Fungi and organic Material Substrates. Advances in Applied Microbiology, Vol 89 89, 47-99.
Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Umbanhowar J (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Let 13: 394-407
Hofmann A, Wittenmayer L, Arnold G, Schicber A, Merbach W (2009) Root exudation of phloridzin by apple seedlings (*Malus domestica* Borkh.) with symptoms of apple replant disease. J Appl Bot Food Qual 82: 193-198.
Hoitink HAJ, Boehm MJ (1999) Biocontrol within the context of soil microbial communities: A substrate-dependent phenomenon. Annu Rev Phytopathol 37: 427-446. doi: 10.1146/annurev.phyto.37.1.427.
Holden J (1975) use of nuclear staining to assess rates of cell-death in cortices of cereal roots. Soil Biol Biochem 7: 333-334. doi: 10.1016/0038-0717(75)90077-2.
Holland EA, Coleman DC (1987) Litter placement effects on microbial and organic-matter dynamics in an agroecosystem. Ecol 68: 425-433. doi: 10.2307/1939274.
Holland TC, Bowen PA, Bogdanoff CP, Lowery TD, Shaposhnikova O, Smith S, Hart MM (2016) Evaluating the diversity of soil microbial communities in vineyards relative to adjacent native ecosystems. Appl Soil Ecol 100: 91-103. doi: 10.1016/j.apsoil.2015.12.001.
Hossain MM, Sultana F, Kubota M, Koyarna H, Hyakumachi M (2007) The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiol 48: 1724-1736. doi: 10.1093/pcp/pcm144.
House GJ, Alzugaray MD (1989) Influence of cover cropping and no-tillange practices on community composition of soil arthropods in a North-Carolina agroecosystem. Environmental Entomology 18: 302-307. doi: 10.1093/ee/18.2.302.

Hu, G., St Leger, J., 2002. Field studies using a recombinant mycoinsecticide (Metarhizium anisopliae) reveal that it is rhizosphere competent. Applied and Environmental Microbiology 68, 6383-6387.
Hu, Y.J., Rillig, M.C., Xiang, D., Hao, Z.P., Chen, B.D., 2013. Changes of AM Fungal Abundance along Environmental Gradients in the Arid and Semi-Arid Grasslands of Northern China. Plos One 8.
Huang B (1999) Water relations and root activities of *Buchloe dactyloides* and *Zoysia japonica* in response to localized soil drying. Plant Soil 208: 179-186. doi: 10.1023/a:1004472314631.
Hudson BD (1994) Soil organic-matter and available water capacity. Journal of Soil and Water Conservation 49: 189-194.
Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Molec Ecol 11: 2669-2678. doi: 10.1046/j.1365-294X.2002.01647.x.
Ingels, C.A., Scow, K.M., Whisson, D.A., Drenovsky, R.E., 2005. Effects of cover crops on grapevines, yield, juice composition, soil microbial ecology, and gopher activity. American Journal of Enology and Viticulture 56, 19-29.
Inglis GD, Kawchuk LM (2002) Comparative degradation of Oomycete, Ascomycete, and Basidiomycete cell walls by mycoparasitic and biocontrol fungi. Can J Microbiol 48: 60-70
INVAM (2018) Glomeraceae. In: International culture collection of arbuscular mycorrhizal fungi. Davis College of Agriculture, Natural Resources, and Design, University of West Virginia. Available via: http://fungi.invam.wvu.edu/the-fungi/classification/glomaceae.html. Accessed 22 Sep 2018.
Jackson, M.L., 1956. Soil Chemical Analysis, Prentice-Hall, Inc.
Jaronski ST (2010) Ecological factors in the inundative use of fungal entomopathogens. Biocontrol 55: 159-185. doi: 10.1007/s10526-009-9248-3.
Jasper DA, Abbott LK, Robson AD (1989) Soil disturbance reduces the infectivity of external hyphae of vesicular arbuscular mycorrhizal fungi. New Phytol 112: 93-99. doi: 10.1111/j.1469-8137.1989.tb00313.x.
Jastrow, J.D., Miller, R.M., Lussenhop, J., 1998. Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biology & Biochemistry 30, 905-916.
Jin L, Wang Q, Wang XJ, Gange AC (2017) Mycorrhizal-induced growth depression in plants. Symbiosis 72: 81-88. doi: 10.1007/s13199-016-0444-5.
Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Booth RE, Grime JP, Young JPW, Read DJ (2004) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytol 161: 503-515. doi: 10.1046/j.1469-8137.2003.00938.x.
Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae. Ecol Appl 3: 749-757. doi: 10.2307/1942106.
Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135: 575-586. doi: 10.1046/j.1469-8137.1997.00729.x.
Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A., Miller, R.M., 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses.

Proceedings of the National Academy of Sciences of the United States of America 107, 2093-2098.
Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications 7.
Kandeler E, Kampichler C, Horak O (1996) Influence of heavy metals on the functional diversity of soil microbial communities. Biol Fert Soils 23: 299-306. doi: 10.1007/s003740050174.
Karasawa T, Kasahara Y, Takebe M (2001) Variable response of growth and arbuscular mycorrhizal colonization of maize plants to preceding crops in various types of soils. Biol Fert Soils 33: 286-293. doi: 10.1007/s003740000321.
Karl A, Merwin IA, Brown MG, Hervieux RA, Vanden Heuvel JE (2016) Impact of undervine management on vine growth, yield, fruit composition, and wine sensory analyses in Cabernet franc. Am J Enol Vit 67: 269-280. doi: 10.5344/ajev.2016.15061.
Karl AD, Merwin IA, Brown MG, Hervieux RA, Vanden Heuvel JE (2016a) Under-vine management impacts soil properties and leachate composition in a New York state vineyard. Hortsci 51: 941-949.
Karlsson M, Durling MB, Choi J, Kosawang C, Lackner G, Tzelepis GD, Nygren K, Dubey MK, Kamou N, Levasseur A, Zapparata A, Wang JH, Amby DB, Jensen B, Sarrocco S, Panteris E, Lagopodi AL, Poggeler S, Vannacci G, Collinge DB, Hoffmeister D, Henrissat B, Lee YH, Jensen DF (2015) Insights on the evolution of mycoparasitism from the genome of *Clonostachys rosea*. Genome Biol Evol 7: 465-480
Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acid Res 30: 3059-3066. doi: 10.1093/nar/gkf436.
Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, Myers SS, Bogich T, Ostfeld RS (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468: 647-652. doi: 10.1038/nature09575.
Keesing F, Oberoi P, Vaicekonyte R, Gowen K, Henry L, Mount S, Johns P, Ostfeld RS (2011) Effects of garlic mustard (*Alliaria petiolata*) on entomopathogenic fungi. Ecoscience 18: 164-168. doi: 10.2980/18-2-3385.
Kil YJ, Eo JK, Lee EH, Eom AH (2014) Root age-dependent changes in arbuscular mycorrhizal fungal communities colonizing roots of *Panax ginseng*. Mycobiology 42: 416-421. doi: 10.5941/myco.2014.42.4.416.
Kirchmair M, Huber L, Porten M, Rainer J, Strasser H (2004) *Metarhizium anisopliae*, a potential agent for the control of grape phylloxera. Biocontrol 49: 295-303. doi: 10.1023/b:bico.0000025387.13747.74.
Kirkegaard JA, Simpfendorfer S, Holland J, Bambach R, Moore KJ, Rebetzke GJ (2004) Effect of previous crops on crown rot and yield of durum and bread wheat in northern NSW. Aust J Agric Res 55: 321-334. doi: 10.1071/ar03178.
Kjeldahl, J. (1883) "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern" (New method for the determination of nitrogen in organic substances), *Zeitschrift für analytische Chemie*, **22** (1) : 366-383.
Klironomos JN, Hart MM (2002) Colonization of roots by arbuscular mycorrhizal fungi using

different sources of inoculum. Mycorrhiza 12: 181-184. doi: 10.1007/s00572-002-0169-6.
Koger CH, Poston DH, Hayes RM, Montgomery RE (2004) Glyphosate-resistant horseweed (*Conyza canadensis*) in Mississippi. Weed Technol 18: 820-825. doi: 10.1614/wt-03-218r.
Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Duenas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Luecking R, Martin MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Poldmaa K, Saag L, Saar I, Schuessler A, Scott JA, Senes C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of fungi. Molec Ecol 22: 5271-5277
Kosola KR, Dickmann DI, Parry D (2002) Carbohydrates in individual poplar fine roots: effects of root age and defoliation. Tree Physiol 22: 741-746. doi: 10.1093/treephys/22.10.741.
Krohn NG, Ferree DC (2005) Effects of low-growing perennial ornamental groundcovers on the growth and fruiting of 'Seyval blanc' grapevines. Hortsci 40: 561-568.
Kuan TL, Erwin DC (1982) Effect of soil matric potential on *Phytophthora* root-rot of alfalfa. Phytopathol 72: 543-548. doi: 10.1094/Phyto-72-543.
Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek Int J Gen Molec Microbiol 73: 331-371
Laliberté, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology **91**:299-305.

Landa BB, Lopez-Diaz C, Jimenez-Fernandez D, Montes-Borrego M, Munoz-Ledesma FJ, Ortiz-Urquiza A, Quesada-Moraga E (2013) In-planta detection and monitorization of endophytic colonization by a *Beauveria bassiana* strain using a new-developed nested and quantitative PCR-based assay and confocal laser scanning microscopy. Journal of Invertebrate Pathology 114: 128-138. doi: 10.1016/j.jip.2013.06.007.
Lange M, Habekost M, Eisenhauer N, Roscher C, Bessler H, Engels C, Oelmann Y, Scheu S, Wilcke W, Schulze E-D, Gleixner G (2014) Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. Plos One 9. doi: 10.1371/journal.pone.0096182.
Lankau, E.W., Lankau, R.A., 2014. Plant species capacity to drive soil fungal communities contributes to differential impacts of plant-soil legacies. Ecology 95, 3221-3228.
Larkin RP, Griffin TS, Honeycutt CW (2010) Rotation and Cover Crop Effects on Soilborne Potato Diseases, Tuber Yield, and Soil Microbial Communities. Plant Dis 94: 1491-1502. doi: 10.1094/pdis-03-10-0172.
Larsen J, Bodker L (2001) Interactions between pea root-inhabiting fungi examined using signature fatty acids. New Phytol 149: 487-493. doi: 10.1046/j.1469-8137.2001.00049.x.
Latz E, Eisenhauer N, Rall BC, Allan E, Roscher C, Scheu S, Jousset A (2012) Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. J Ecol 100: 597-604. doi: 10.1111/j.1365-2745.2011.01940.x.

Latz E, Eisenhauer N, Scheu S, Jousset A (2015) Plant identity drives the expression of biocontrol factors in a rhizosphere bacterium across a plant diversity gradient. Funct Ecol 29: 1225-1234. doi: 10.1111/1365-2435.12417.

Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40: 2407-2415. doi: 10.1016/j.soilbio.2008.05.021.

LaveUe, P. & Spain, A. (2001). *SoilEcology.* Kluwer, Boston.

Lee J, Lee S, Young JPW (2008) Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. Fems Microbiol Ecol 65: 339-349. doi: 10.1111/j.1574-6941.2008.00531.x.

Lenth, R. (2018). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.1.3. https://CRAN.R-project.org/package=emmeans

Levésque CA, Rahe JE (1992) Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annu Rev Phytopathol 30: 579-602. doi: 10.1146/annurev.py.30.090192.003051.

Likar M., Regvar M. (2017) Arbuscular Mycorrhizal Fungi and Dark Septate Endophytes in Grapevine: The Potential for Sustainable Viticulture? In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza - Function, Diversity, State of the Art. Springer, Cham

Linderman, R.G., Davis, E.A., 2004. Evaluation of commercial inorganic and organic fertilizer effects on arbuscular mycorrhizae formed by Glomus intraradices. Horttechnology 14, 196-202.

Loik ME, Breshears DD, Lauenroth WK, Belnap J (2004) A multi-scale perspective of water pulses in dryland ecosystems: climatology and ecohydrology of the western USA. Oecologia 141: 269-281. doi: 10.1007/s00442-004-1570-y.

Lombard L, Van Der Merwe NA, Groenewald JZ, Crous PW (2014) Lineages in Nectriaceae: re-evaluating the generic status of *Ilyonectria* and allied genera. Phytopathol Mediterr 53: 515-532.

Long SR (2001) Genes and signals in the *Rhizobium*-legume symbiosis. Plant Physiol 125: 69-72. doi: 10.1104/pp.125.1.69.

Louchart X, Voltz M, Andrieux P, Moussa R (2001) Herbicide transport to surface waters at field and watershed scales in a Mediterranean vineyard area. J Environ Qual 30: 982-991. doi: 10.2134/jeq2001.303982x.

Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. ISME J 5: 169-172. doi: 10.1038/ismej.2010.133.

Luginbuehl LH, Oldroyd GED (2017) Understanding the arbuscule at the heart of endomycorrhizal symbioses in plants. Curr Biol 27: R952-R963. doi: 10.1016/j.cub.2017.06.042.

Lumini E, Orgiazzi A, Borriello R, Bonfante P, Bianciotto V (2010) Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. Environ Microbiol 12: 2165-2179. doi: 10.1111/j.1462-2920.2009.02099.x.

Luongo L, Galli M, Corazza L, Meekes E, De Haas L, Van der Plas CL, Kohl J (2005) Potential of fungal antagonists for biocontrol of *Fusarium* spp. in wheat and maize through competition in crop debris. Biocontrol Sci Technol 15: 229-242. doi: 10.1080/09583150400016852.

Lupwayi NZ, Rice WA, Clayton GW (1998) Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. Soil Biol Biochem 30: 1733-1741. doi: 10.1016/s0038-0717(98)00025-x.

Mackie KA, Mueller T, Kandeler E (2012) Remediation of copper in vineyards - A mini review. Environ Pollut 167: 16-26. doi: 10.1016/j.envpol.2012.03.023.

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316: 1746-1748. doi: 10.1126/science.1143082.

Majdi H, Pregitzer K, Moren AS, Nylund JE, Agren GI (2005) Measuring fine root turnover in forest ecosystems. Plant Soil 276: 1-8. doi: 10.1007/s11104-005-3104-8.

Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. Crop Sci 40: 923-940. doi: 10.2135/cropsci2000.404923x.

Mangla, S., Inderjit, Callaway, R.M., 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. Journal of Ecology 96, 58-67.

Maron JL, Marler M, Klironomos JN, Cleveland CC (2011) Soil fungal pathogens and the relationship between plant diversity and productivity. Ecol Lett 14: 36-41. doi: 10.1111/j.1461-0248.2010.01547.x.

Martin-Robles N, Lehmann A, Seco E, Aroca R, Rillig MC, Milla R (2018) Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species. New Phytol 218: 322-334. doi: 10.1111/nph.14962.

Mazzola M, Funnell DL, Raaijmakers JM (2004) Wheat cultivar-specific selection of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species from resident soil populations. Microb Ecol 48: 338-348. doi: 10.1007/s00248-003-1067-y.

Mazzola M, Hewavitharana SS, Strauss SL (2015) Brassica Seed Meal Soil Amendments Transform the Rhizosphere Microbiome and Improve Apple Production Through Resistance to Pathogen Reinfestation. Phytopathology 105: 460-469. doi: 10.1094/phyto-09-14-0247-r.

Mazzola M, Manici LM (2012) Apple Replant Disease: Role of microbial ecology in cause and control. Annu Rev Phytopathol, Vol 50 50: 45-65. doi: 10.1146/annurev-phyto-081211-173005.

McGonigle TP, Miller MH (2000) The inconsistent effect of soil disturbance on colonization of roots by arbuscular mycorrhizal fungi: a test of the inoculum density hypothesis. Appl Ecol 14: 147-155

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytol 115: 495-501

McInenly, L.E., Merrill, E.H., Cahill, J.F., Juma, N.G., 2010. Festuca campestris alters root morphology and growth in response to simulated grazing and nitrogen form. Functional Ecology 24, 283-292.

McMurdie PJ, Holmes S (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. Plos One 8. doi: 10.1371/journal.pone.0061217.

Medo J, Cagan L (2011) Factors affecting the occurrence of entomopathogenic fungi in soils of Slovakia as revealed using two methods. Biol Control 59: 200-208. doi: 10.1016/j.biocontrol.2011.07.020.

Mehlich A (1984) Mehlich 3 soil test extractant: A modification of the Mehlich 2 extractant. Commun Soil Sci Plant Anal 15:1409-1416

Menge JA, Johnson ELV, Platt RG (1978) Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. New Phytol 81: 553-559. doi: 10.1111/j.1469-8137.1978.tb01628.x.

Meyling NV, Eilenberg J (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. Biol Control 43: 145-155. doi: 10.1016/j.biocontrol.2007.07.007.

Meyling NV, Lubeck M, Buckley EP, Eilenberg J, Rehner SA (2009) Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. Mol Ecol 18: 1282-1293. doi: 10.1111/j.1365-294X.2009.04095.x.

Meyling NV, Thorup-Kristensen K, Eilenberg J (2011) Below- and aboveground abundance and distribution of fungal entomopathogens in experimental conventional and organic cropping. Biol Control 59: 180-186. doi: 10.1016/j.biocontrol.2011.07.017.

Meyling, N.V., Eilenberg, J., 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agriculture Ecosystems & Environment 113, 336-341.

Milcu A, Allan E, Roscher C, Jenkins T, Meyer ST, Flynn D, Bessler H, Buscot F, Engels C, Gubsch M, Koenig S, Lipowsky A, Loranger J, Renker C, Scherber C, Schmid B, Thebault E, Wubet T, Weisser WW, Scheu S, Eisenhauer N (2013) Functionally and phylogenetically diverse plant communities key to soil biota. Ecol 94: 1878-1885. doi: 10.1890/12-1936.1.

Moonjely S, Barelli L, Bidochka M (2016) Insect pathogenic fungi as endophytes. Adv Genet doi:10.1016/bs.adgen.2015.12.004

Morjan WE, Pedigo LP, Lewis LC (2002) Fungicidal effects of glyphosate and glyphosate formulations on four species of entomopathogenic fungi. Environ Entomol 31: 1206-1212. doi: 10.1603/0046-225x-31.6.1206.

Mostafa AM, Lowery DT, Jensen LBM, Deglow EK (2011) Host Plant Suitability and Feeding Preferences of the Grapevine Pest *Abagrotis orbis* (Lepidoptera: Noctuidae). Environmental Entomology 40: 1458-1464. doi: 10.1603/en11049.

Muller A, Ngwene B, Peiter E, George E (2017) Quantity and distribution of arbuscular mycorrhizal fungal storage organs within dead roots. Mycorrhiza 27: 201-210. doi: 10.1007/s00572-016-0741-0.

Muscas E, Cocco A, Mercenaro L, Cabras M, Lentini A, Porqueddu C, Nieddu G (2017) Effects of vineyard floor cover crops on grapevine vigor, yield, and fruit quality, and the development of the vine mealybug under a Mediterranean climate. Agric Ecosysy Environ 237: 203-212. doi: 10.1016/j.agee.2016.12.035.

Naeem S (1998) Species redundancy and ecosystem reliability. Conserv Biol 12: 39-45. doi: 10.1046/j.1523-1739.1998.96379.x.

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4: 133-142
Nicol RW, Yousef L, Traquair JA, Bernards MA (2003) Ginsenosides stimulate the growth of soilborne pathogens of American ginseng. Phytochem 64: 257-264. doi: 10.1016/s0031-9422(03)00271-1.
Nijjer S, Rogers WE, Siemann E (2010) The impacts of fertilization on mycorrhizal production and investment in western gulf coast grasslands. Am Midl Nat 163: 124-133. doi: 10.1674/0003-0031-163.1.124.
Oksanen J, et al. (2016). vegan: Community ecology package. R package version 2.4-1. https://CRAN.R-project.org/package=vegan
Oksanen, J, Blanchet, FG, Friendly, M, et al. (2018). vegan: community ecology package. R package version 2.4-6.
Olsson, P.A., Baath, E., Jakobsen, I., 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. Applied and Environmental Microbiology 63, 3531-3538.
Opik M, et al. (2013) Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. Mycorrhiza 23: 411-430. doi: 10.1007/s00572-013-0482-2.
Opik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier U, Zobel M (2010) The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol 188: 223-241. doi: 10.1111/j.1469-8137.2010.03334.x.
Osbourn AE (2003) Saponins in cereals. Phytochem 62: 1-4. doi: 10.1016/s0031-9422(02)00393-x.
Ownley BH, Griffin MR, Klingeman WE, Gwinn KD, Moulton JK, Pereira RM (2008) *Beauveria bassiana*: Endophytic colonization and plant disease control. J Invertebr Pathol 98: 267-270. doi: 10.1016/j.jip.2008.01.010.
Palmborg C, Scherer-Lorenzen M, Jumpponen A, Carlsson G, Huss-Danell K, Hogberg P (2005) Inorganic soil nitrogen under grassland plant communities of different species composition and diversity. Oikos 110: 271-282
Pan DL, Song YQ, Dyck M, Gao XD, Wu PT, Zhao XN (2017) Effect of plant cover type on soil water budget and tree photosynthesis in jujube orchards. Agric Water Manag 184: 135-144. doi: 10.1016/j.agwat.2017.01.009.
Pannkuk CD, Papendick RI, Saxton KE (1997) Fallow management effects on soil water storage and wheat yields in the Pacific Northwest. Agron J 89: 386-391.
Pell JK, Hannam JJ, Steinkraus DC (2010) Conservation biological control using fungal entomopathogens. Biocontrol 55: 187-198. doi: 10.1007/s10526-009-9245-6.
Penfold C, Collins C (2012) Native cover crops in viticulture. Australian Government/Grape and Wine Research and Development Corporation. http://research.wineaustralia.com/wp-content/uploads/2012/09/2012-05-FS-Native-Cover-Crops.pdf. Accessed 12 June 2016.
Pepe A, Giovannetti M, Sbrana C (2018) Lifespan and functionality of mycorrhizal fungal mycelium are uncoupled from host plant lifespan. Sci Rep 8. doi: 10.1038/s41598-018-28354-5.

Peterson C (1989) Significance of the exodermis in root function. In: Loughman BC et al. Stuctural and functional aspects of transport in roots, Kluwer.
Peterson PR, Sheaffer CC, Hall MH (1992) Drought effects on perennial forage legume yield and quality. Agron J 84: 774-779. doi: 10.2134/agronj1992.00021962008400050003x.
Petit, E., Gubler, W.D., 2006. Influence of Glomus intraradices on black foot disease caused by *Ilyonectria* spp.indrocarpon macrodidymum on Vitis rupestris under controlled conditions. Plant Disease 90, 1481-1484.
Pettit RD, Fagan RE (1974) Influence of nitrogen on irrigated buffalograss yield and protein-content. J Range Manag 27: 473-476. doi: 10.2307/3896726.
Pieczarka DJ, Abawi GS (1978) Influence of soil-water potential and temperature on severity of *Pythium* root-rot of snap beans. Phytopathol 68: 766-772.
Placella SA, Brodie EL, Firestone MK (2012) Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. Proc Natl Acad Sci USA 109: 10931-10936. doi: 10.1073/pnas.1204306109.
Porro D, Dorigatti C, Stefanini M, Ceschini A (2001) Use of SPAD meter in diagnosis of nutritional status in apple and grapevine. Proc Fourth Int Symp Mineral Nut Decid Fruit Crop: 243-252. doi: 10.17660/ActaHortic.2001.564.28.
Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcón-Aguilar C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. J Exp Bot 53: 525-534. doi: 10.1093/jexbot/53.368.525.
Pozo MJ, Verhage A, García-Andrade J, García JM, Azcón-Aguilar C (2009) Priming plant defence against pathogens by arbuscular mycorrhizal fungi. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (eds) Mycorrhizas—functional processes and ecological impact. Springer, Berlin, pp 123–135
Pregitzer KS (2003) Woody plants, carbon allocation and fine roots. New Phytol 158: 421-424. doi: 10.1046/j.1469-8137.2003.00766.x.
Price MN, Dehal PS, Arkin AP (2009) FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. Molec Biol Evol 26: 1641-1650. doi: 10.1093/molbev/msp077.
Qian YL, Fry JD (1997) Water relations and drought tolerance of four turfgrasses. J Am Soc Hort Sci122: 129-133.
Quesada-Moraga, E., Lopez-Diaz, C., Landa, B.B., 2014. The Hidden Habit of the Entomopathogenic Fungus Beauveria bassiana: First Demonstration of Vertical Plant Transmission. Plos One 9, 6.
R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
Raaijmakers JM, ; DM (1998) Natural plant protection by 2,4-diacetylphloroglucinol - Producing *Pseudomonas* spp. in take-all decline soils. Molec Plant-Microbe Interact 11: 144-152. doi: 10.1094/mpmi.1998.11.2.144.
Randhawa, P.K., Mullen, C., Barbercheck, M., 2018. Plant identity, but not diversity, and agroecosystem characteristics affect the occurrence of M. robertsii in an organic cropping system. Biological Control 124, 18-29.

Rasmann S, Kollner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434: 732-737. doi: 10.1038/nature03451.
Resendes ML, Bryla DR, Eissenstat DM (2008) Early events in the life of apple roots: variation in root growth rate is linked to mycorrhizal and nonmycorrhizal fungal colonization. Plant Soil 313: 175-186. doi: 10.1007/s11104-008-9690-5.
Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytologist 171: 41-53. doi: 10.1111/j.1469-8137.2006.01750.x.
Roldán-Fajardo BE, Barea JM (1985) Mycorrhizal dependency in the olive tree (*Olea europaea* L.). In: Gianinazzi-Pearson V, Gianinazzi S. (Eds.) Physiology and Genetical Aspects of Mycorrhizal. INRA, Paris, pp 323–326.
Roscher C, Schumacher J, Lipowsky A, Gubsch M, Weigelt A, Pompe S, Kolle O, Buchmann N, Schmid B, Schulze E-D (2013) A functional trait-based approach to understand community assembly and diversity-productivity relationships over 7 years in experimental grasslands. Perspect Plant Ecol Evol Syst 15: 139-149. doi: 10.1016/j.ppees.2013.02.004.
Rosendahl S, McGee P, Morton JB (2009) Lack of global population genetic differentiation in the arbuscular mycorrhizal fungus *Glomus mosseae* suggests a recent range expansion which may have coincided with the spread of agriculture. Molec Ecol 18: 4316-4329. doi: 10.1111/j.1365-294X.2009.04359.x.
Roumet, C., Urcelay, C., Diaz, S., 2006. Suites of root traits differ between annual and perennial species growing in the field. New Phytologist 170, 357-368.
Rovira AD (1969) Plant root exudates. Bot Rev 35: 35-&. doi: 10.1007/bf02859887.
Rúa MA, Antoninka A, Antunes PM, Chaudhary VB, Gehring C, Lamit LJ, Piculell BJ, Bever JD, Zabinski C, Meadow JF, Lajeunesse MJ, Milligan BG, Karst J, Hoeksema JD (2016) Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. Bmc Evol Biol 16
Ruizlozano JM, Azcon R, Gomez M (1995) Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance - Physiological and nutritional plant-responses. Appl Environ Microbiol 61: 456-460.
Sa TM, Israel DW (1991) Energy status and functioning of phosphorus-deficient soybean nodules. Plant Physiol 97: 928-935. doi: 10.1104/pp.97.3.928.
Sage RF, Christin P-A, Edwards EJ (2011) The C-4 plant lineages of planet Earth. J Exp Bot 62: 3155-3169. doi: 10.1093/jxb/err048.
Sato K, Suyama Y, Saito M, Sugawara K (2005) A new primer for discrimination of arbuscular mycorrhizal fungi with polymerase chain reaction-denature gradient gel electrophoresis. Grassland Sci 51: 179-181. doi: 10.1111/j.1744-697X.2005.00023.x
Schallhart, N., Tusch, M.J., Wallinger, C., Staudacher, K., Traugott, M., 2012. Effects of plant identity and diversity on the dietary choice of a soil-living insect herbivore. Ecology 93, 2650-2657.
Scheublin TR, Ridgway KP, Young JPW, van der Heijden MGA (2004) Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appl Environ Microbiol 70: 6240-6246. doi: 10.1128/aem.70.10.6240-6246.2004.

Scheublin TR, Sanders IR, Keel C, van der Meer JR (2010) Characterisation of microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi. Isme J 4: 752-763. doi: 10.1038/ismej.2010.5.
Schnitzer SA, Klironomos JN, HilleRisLambers J, Kinkel LL, Reich PB, Xiao K, Rillig MC, Sikes BA, Callaway RM, Mangan SA, van Nes EH, Scheffer M (2011) Soil microbes drive the classic plant diversity-productivity pattern. Ecol 92: 296-303. doi: 10.1890/10-0773.1.
Schreiner RP, Ivors KL, Pinkerton JN (2001) Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression. Mycorrhiza 11: 273-277.
Schreiner RP, Koide RT (1993) Mustards, mustard oils, and mycorrhizas. New Phytol 123: 107-113.
Schreiner, R.P., 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.
Schüßler A. and Walker C. 2010. The Glomeromycota, A species list with new families and new genera. Gloucester, UK, 58
Schweitzer JA, Bailey JK, Fischer DG, LeRoy CJ, Lonsdorf EV, Whitham TG, Hart SC (2008) Plant-soil-microorganism interactions: Heritable relationship between plant genotype and associated soil microorganisms. Ecol 89: 773-781. doi: 10.1890/07-0337.1.
Shah, P.A., Pell, J.K., 2003. Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology 61, 413-423.
Shapiro-Ilan DI, Gardner WA, Wells L, Wood BW (2012) Cumulative impact of a clover cover crop on the persistence and efficacy of *Beauveria bassiana* in suppressing the pecan weevil (Coleoptera: Curculionidae). Environ Entomol 41: 298-307. doi: 10.1603/en11229.
Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. J Ecol 97: 1274-1280. doi: 10.1111/j.1365-2745.2009.01557.x.
Simon S, Bouvier JC, Debras JF, Sauphanor B (2010) Biodiversity and pest management in orchard systems. A review. Agron Sustain Dev 30: 139-152
Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, New York.
Smolinska U, Horbowicz M (1999) Fungicidal activity of volatiles from selected cruciferous plants against resting propagules of soil-borne fungal pathogens. J Phytopathol 147: 119-124. doi: 10.1111/j.1439-0434.1999.tb03817.x. Statistical Software, 8(15), 1-27. <http://www.jstatsoft.org/v08/i15/>.
Steenwerth, K., Belina, K.M., 2008. Cover crops and cultivation: Impacts on soil N dynamics and microbiological function in a Mediterranean vineyard agroecosystem. Applied Soil Ecology 40, 370-380.
Steinrucken HC, Amrhein N (1980) The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic-acid 3-phosphate synthase. Biochem Biophys Res Commun 94: 1207-1212. doi: 10.1016/0006-291x(80)90547-1.
Stephan A, Meyer AH, Schmid B (2000) Plant diversity affects culturable soil bacteria in experimental grassland communities. Journal of Ecology 88: 988-998
Stephens PM, Davoren CW, Wicks T (1999) Effect of methyl bromide, metham sodium and the

biofumigants Indian mustard and canola on the incidence of soilborne fungal pathogens and growth of grapevine nursery stock. Australas Plant Pathol 28: 187-196. doi: 10.1071/ap99032.
Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. Plos Biol 4: 727-731. doi: 10.1371/journal.pbio.0040140.
Stirling GR, Smith MK, Smith JP, Stirling AM, Hamill SD (2012) Organic inputs, tillage and rotation practices influence soil health and suppressiveness to soilborne pests and pathogens of ginger. Australas Plant Pathol 41: 99-112. doi: 10.1007/s13313-011-0096-0.
St. John TV, Coleman DC, Reid CPP (1983) Association of vesicular-arbuscular myocrrhizal hyphae with soil organic particles. Ecology 64: 957-959. doi: 10.2307/1937216.
Sturz AV, Christie BR (2003) Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. Soil Tillage Res 72: 107-123. doi: 10.1016/s0167-1987(03)00082-5.
Sturz AV, Matheson BG, Arsenault W, Kimpinski J, Christie BR (2001) Weeds as a source of plant growth promoting rhizobacteria in agricultural soils. Can J Microbiol 47: 1013-1024. doi: 10.1139/cjm-47-11-1013.
Tabarant P, Villenave C, Risede JM, Roger-Estrade J, Dorel M (2011) Effects of organic amendments on plant-parasitic nematode populations, root damage, and banana plant growth. Biol Fertil Soil 47: 341-347
Taheri AE, Hamel C, Gan Y (2016) Cropping practices impact fungal endophytes and pathogens in durum wheat roots. Appl Soil Ecol 100: 104-111. doi: 10.1016/j.apsoil.2015.12.007.
Taylor J, Harrier L (2000) A comparison of nine species of arbuscular mycorrhizal fungi on the development and nutrition of micropropagated *Rubus idaeus* L. cv. Glen Prosen (Red Raspberry). Plant Soil 225: 53-61. doi: 10.1023/a:1026519431096.
Tewoldemedhin, Y.T., Mazzola, M., Mostert, L., McLeod, A., 2011. *Ilyonectria* spp.indrocarpon species associated with apple tree roots in South Africa and their quantification using real-time PCR. European Journal of Plant Pathology 129, 637-651.
Tisdall, J.M., Oades, J.M., 1982. Organic-matter and water stable aggregates in soils. Journal of Soil Science 33, 141-163.
Tobor-Kaplon MA, Bloem J, Romkens P, de Ruiter PC (2005) Functional stability of microbial communities in contaminated soils. Oikos 111: 119-129. doi: 10.1111/j.0030-1299.2005.13512.x.
Tripathi RK, Gottlieb D (1969) Mechanism of action of antifungal antibiotic pyrrolnitrin. J Bacteriol 100: 310-&.
Trouvelot S, Bonneau L, Redecker D, van Tuinen D, Adrian M, Wipf D (2015) Arbuscular mycorrhiza symbiosis in viticulture: a review. Agron Sustain Dev 35: 1449-1467. doi: 10.1007/s13593-015-0329-7.
Trypsteen W, Vynck M, De Neve J, Bonczkowski P, Kiselinova M, Malatinkova E, Vervisch K, Thas O, Vandekerckhove L, De Spiegelaere W (2015) ddpcRquant: threshold determination for single channel droplet digital PCR experiments. Annal Bioanal Chem 407: 5827-5834. doi: 10.1007/s00216-015-8773-4.

Tukey JW (1949) Comparing individual means in the analysis of variance. Biom 5: 99-114. doi: 10.2307/3001913.
Úrbez-Torres JR, Haag P, Bowen P, Lowery T, O'Gorman DT (2015) Development of a DNA macroarray for the detection and identification of fungal pathogens causing decline of young grapevines. Phytopathol 105: 1373-1388
Úrbez-Torres JR, Haag P, Bowen P, O'Gorman DT (2014) Grapevine trunk diseases in British Columbia: Incidence and characterization of the fungal pathogens associated with black foot disease of grapevine. Plant Dis 98: 456-468. doi: 10.1094/pdis-05-13-0524-re.
Valentine AJ, Mortimer PE, Lintnaar A, Borgo R (2006) Drought responses of arbuscular mycorrhizal grapevines. Symbiosis 41: 127-133.
van Bruggen AHC, Semenov AM, van Diepeningen AD, de Vos OJ, Blok WJ (2006) Relation between soil health, wave-like fluctuations in microbial populations, and soil-borne plant disease management. Eur J Plant Pathol 115: 105-122. doi: 10.1007/s10658-005-1250-8.
van de Voorde TFJ, van der Putten WH, Gamper HA, Hol WHG, Bezemer TM (2010) Comparing arbuscular mycorrhizal communities of individual plants in a grassland biodiversity experiment. New Phytol 186: 746-754. doi: 10.1111/j.1469-8137.2010.03216.x.
van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11: 296-310. doi: 10.1111/j.1461-0248.2007.01139.x.
van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396: 69-72.
van Elsas JD, Garbeva P, Salles J (2002) Effects of agronomical measures on the microbial diversity of soils as related to the suppression of soil-borne plant pathogens. Biodegrad 13: 29-40. doi: 10.1023/a:1016393915414.
Van Geel M, Busschaert P, Honnay O, Lievens B (2014) Evaluation of six primer pairs targeting the nuclear rRNA operon for characterization of arbuscular mycorrhizal fungal (AMF) communities using 454 pyrosequencing. J Microbiol Methods 106: 93-100. doi: 10.1016/j.mimet.2014.08.006.
Van Geel, M., Verbruggen, E., De Beenhouwer, M., van Rennes, G., Lievens, B., Honnay, O., 2017. High soil phosphorus levels overrule the potential benefits of organic farming on arbuscular mycorrhizal diversity in northern vineyards. Agriculture Ecosystems & Environment 248, 144-152.
van Loon LC, Bakker P, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36: 453-483. doi: 10.1146/annurev.phyto.36.1.453.
Varela-Cervero S, Lopez-Garcia A, Barea JM, Azcon-Aguilar C (2016) Differences in the composition of arbuscular mycorrhizal fungal communities promoted by different propagule forms from a Mediterranean shrubland. Mycorrhiza 26: 489-496. doi: 10.1007/s00572-016-0687-2.
Vega FE, Goettel MS, Blackwell M, Chandler D, Jackson MA, Keller S, Koike M, Maniania NK, Monzon A, Ownley BH, Pell JK, Rangel DEN, Roy HE (2009)

Fungal entomopathogens: new insights on their ecology. Fungal Ecol 2: 149-159. doi: 10.1016/j.funeco.2009.05.001.
Verbruggen E, Kiers ET (2010) Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. Evol Appl 3: 547-560. doi: 10.1111/j.1752-4571.2010.00145.x.
Verbruggen E, Roling WFM, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MGA (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytol 186: 968-979. doi: 10.1111/j.1469-8137.2010.03230.x.
Vermeire M-L, Kablan L, Dorel M, Delvaux B, Risede J-M, Legreve A (2011) Protective role of silicon in the banana-*Cylindrocladium spathiphylli* pathosystem. Eur J Plant Pathol 131: 621-630
Vierheilig H, Coughlan AP, Wyss U, Piche Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64: 5004-5007.
Vierheilig H, Coughlan AP, Wyss U, Piche Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64: 5004-5007.
Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) *Trichoderma*-plant-pathogen interactions. Soil Biol Biochem 40: 1-10
Vitale A, Aiello D, Guarnaccia V, Perrone G, Stea G, Polizzi G (2012) First report of root rot caused by *Ilyonectria* (=*Neonectria*) *macrodidyma* on avocado (*Persea americana*) in Italy. J Phytopathol 160: 156-159. doi: 10.1111/j.1439-0434.2011.01869.x.
Volaire F, Norton M (2006) Summer dormancy in perennial temperate grasses. Annal Bot 98: 927-933. doi: 10.1093/aob/mcl195.
Volder A, Smart DR, Bloom AJ, Eissenstat DM (2005) Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. New Phytol 165: 493-501. doi: 10.1111/j.1469-8137.2004.01222.x.
Vukicevich E, Lowery DT, Urbez-Torres JR, Bowen P, Hart M (2018) Groundcover management changes grapevine root fungal communities and plant-soil feedback. Plant Soil 424: 419-433. doi: 10.1007/s11104-017-3532-2.
Vukicevich E, Lowery T, Bowen P, Urbez-Torres JR, Hart M (2016) Cover crops to increase soil microbial diversity and mitigate decline in perennial agriculture. A review. Agron Sust Dev 36. doi: 10.1007/s13593-016-0385-7.
Waldrop MP, Zak DR, Blackwood CB, Curtis CD, Tilman D (2006) Resource availability controls fungal diversity across a plant diversity gradient. Ecol Let 9: 1127-1135
Walker JC, Morell S, Foster HH (1937) Toxicity of mustard oils and related sulfur compounds to certain fungi. Am J Bot 24: 536-541. doi: 10.2307/2437076.
Wang Q-Y, Zhou D-M, Cang L (2009) Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide. Soil Biol Biochem 41: 1504-1509. doi: 10.1016/j.soilbio.2009.04.010.
Watson, T.T., Nelson, L.M., Neilsen, D., Neilsen, G.H., Forge, T.A., 2017. Soil amendments influence Pratylenchus penetrans populations, beneficial rhizosphere

microorganisms, and growth of newly planted sweet cherry. Applied Soil Ecology 117, 212-220.
Weller DM, Raaijmakers JM, Gardener BBM, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Ann Rev Phytopathol 40: 309-+. doi: 10.1146/annurev.phyto.40.030402.110010.
Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82: 1198-1227
Whitelaw-Weckert MA, Nair NG, Lamont R, Alonso M, Priest MJ, Huang R (2007b) Root infection of Vitis vinifera by *Cylindrocarpon liriodendri* in Australia. Australas Plant Pathol 36: 403-406
Whitelaw-Weckert MA, Rahman L, Appleby LM, Hall A, Clark AC, Waite H, Hardie WJ (2013) Co-infection by Botryosphaeriaceae and *Ilyonectria* spp. fungi during propagation causes decline of young grafted grapevines. Plant Pathol 62: 1226-1237
Whitelaw-Weckert, M.A., Rahman, L., Hutton, R.J., Coombes, N., 2007. Permanent swards increase soil microbial counts in two Australian vineyards. Applied Soil Ecology 36, 224-232.
Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. Amer J Bot 85: 1732-1738. doi: 10.2307/2446507.
Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., 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.
Wink M (2013) Evolution of secondary metabolites in legumes (Fabaceae). S Afr J Bot 89: 164-175. doi: 10.1016/j.sajb.2013.06.006.

Winter (2018) Effects of vegetation management intensity on biodiversity and ecosystem services in vineyards: A meta-analysis. J appl Ecol 55: 2484-2495

Wojciechowska N, Sobieszczuk-Nowicka E, Bagniewska-Zadworna A (2018) Plant organ senescence - regulation by manifold pathways. Plant Biol 20: 167-181. doi: 10.1111/plb.12672.
Wright AJ, Wardle DA, Callaway R, Gaxiola A (2017) The Overlooked Role of Facilitation in Biodiversity Experiments. Trend Ecol Evol 32: 383-390
Wyrebek M, Huber C, Sasan RK, Bidochka MJ (2011) Three sympatrically occurring species of Metarhizium show plant rhizosphere specificity. Microbiol-Sgm 157: 2904-2911. doi: 10.1099/mic.0.051102-0.
Yang H, Zang Y, Yuan Y, Tang J, Chen X (2012) Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: evidence from ITS rDNA sequence metadata. Bmc Evol Biol 12
Zhang, H (2004) The optimality of Naive Bayes. Proc. FLAIRS. <http://www.cs.unb.ca/~hzhang/publications/FLAIRS04ZhangH.pdf>

Appendices

Appendix A: Supplementary material for Chapter 2

Table A. 1 Soil chemical analysis for inoculant soil conducted by A&L Laboratories, London, Ontario.

Treatment	OM	P-bicarb	P-Bray	NO3-N (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	pH	CEC
C	1.9	48	106	5	195	200	1420	7.1	10.2
EG	1.9	41	86	2	170	195	1380	7.6	9
EGL	1.8	49	101	1	224	210	1290	7.5	8.8
NG	1.6	43	98	1	184	220	1580	7.7	10.2
NGF	1.8	40	80	1	179	170	1150	7.4	7.7

Table A. 2 Taxa isolated from the entire experiment with taxonomy assigned using UNTIE and ISTH for *Trichoderma* sequences. Taxonomy marked with '*' denotes sequences for which no SH was assigned. In these cases taxonomy was assigned to the level of genus based on 100% sequence identity.

Morphological classification	Best match accession #	% Identity	Unite species hypothesis	Database/SH code	Possible ID (when no SH)	Sequenced region
A	KR296874	99	*Penicillium levitum* Raper & Fennell, 1948	UNITE SH005485.07FU		ITS
AB	KU203330	99	*Rhizopus arrhizus* Cunningham, 2008	UNITE SH012761.07FU		ITS
AC	KR995110	100	*Trichoderma harzianum* Rifai, 1969	ISTH		ITS
AF	KJ434051	99	*Gibberella fujikuroi* (Sawada) Wollenw, 1931	UNITE SH026899.07FU		ITS
AH	KU556487	100	*Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, 1999	UNITE SH005597.07FU		ITS
AN	KF719198	99	*Coniolariella*	UNITE		ITS

Morphological classification	Best match accession #	% Identity	Unite species hypothesis	Database/SH code	Possible ID (when no SH)	Sequenced region
			hispanica Checa, Arenal & Rogers 2008	SH018645.07FU		
AU	LN833556	100	*Trichoderma virens* (Mill, Giddens & Foster) Arx, 1987	ISTH		ITS
B	FJ948135	99	Sordariomycetes	UNITE SH029831.07FU		ITS
C	KT759298	99	*Ilyonectria macrodidyma* (Halleen, Schroers & Crous) P. Chaverri & C. Salgado, 2011	UNITE SH005668.07FU	Yeast morph	LSU
D	KU164607	98	*Malassezia restricta* Guého, Guillot & Midgley, 1996	UNITE SH001484.07FU		ITS
E	KF428710	99	*Ilyonectria macrodidyma*	UNITE		ITS

Morphological classification	Best match accession #	% Identity	Unite species hypothesis	Database/SH code	Possible ID (when no SH)	Sequenced region
			(Halleen, Schroers & Crous) Chaverri & Salgado, 2011	SH005668.07FU		
I	LT558963	100	*Talaromyces* sp.*	UNITE	*T. pinophilus*	ITS
J	KT215192	93	*C. rosea*	UNITE SH005597.07FU	Yeast morph	LSU
K	AF033433	99	*Penicillium abidjanum* Stolk, 1968	UNITE SH027144.07FU		LSU
M	LC133875	100	*Penicillium* (Link) sp*	UNITE	*P. brasilianum*	ITS
O	KT895345	100	*Chaetomium grande* Asgari & Zare, 2011	UNITE SH013920.07FU		ITS
P	X94176	100	*Fusarium proliferatum* (Matsush.) Nirenberg, 1976	UNITE SH031260.07FU		ITS
S	JX186515	99	*Chaetomium cupreum* Ames, 1950	UNITE SH013927.07FU		ITS

Morphological classification	**Best match accession #**	**% Identity**	**Unite species hypothesis**	**Database/SH code**	**Possible ID (when no SH)**	**Sequenced region**
T	na	na	na	na	Unknown yeast	
U	X93981	100	*Trichoderma kongiopsis* Samuels, Suárez & Evans, 2006	ISTH		ITS
X	KU059938	100	*Alternaria* sp.*		*Alternaria* sp.	ITS
Z	KM265447	100	*Penicillium astrolabium* Serra & Peterson, 2007	SH055406.07FU		ITS

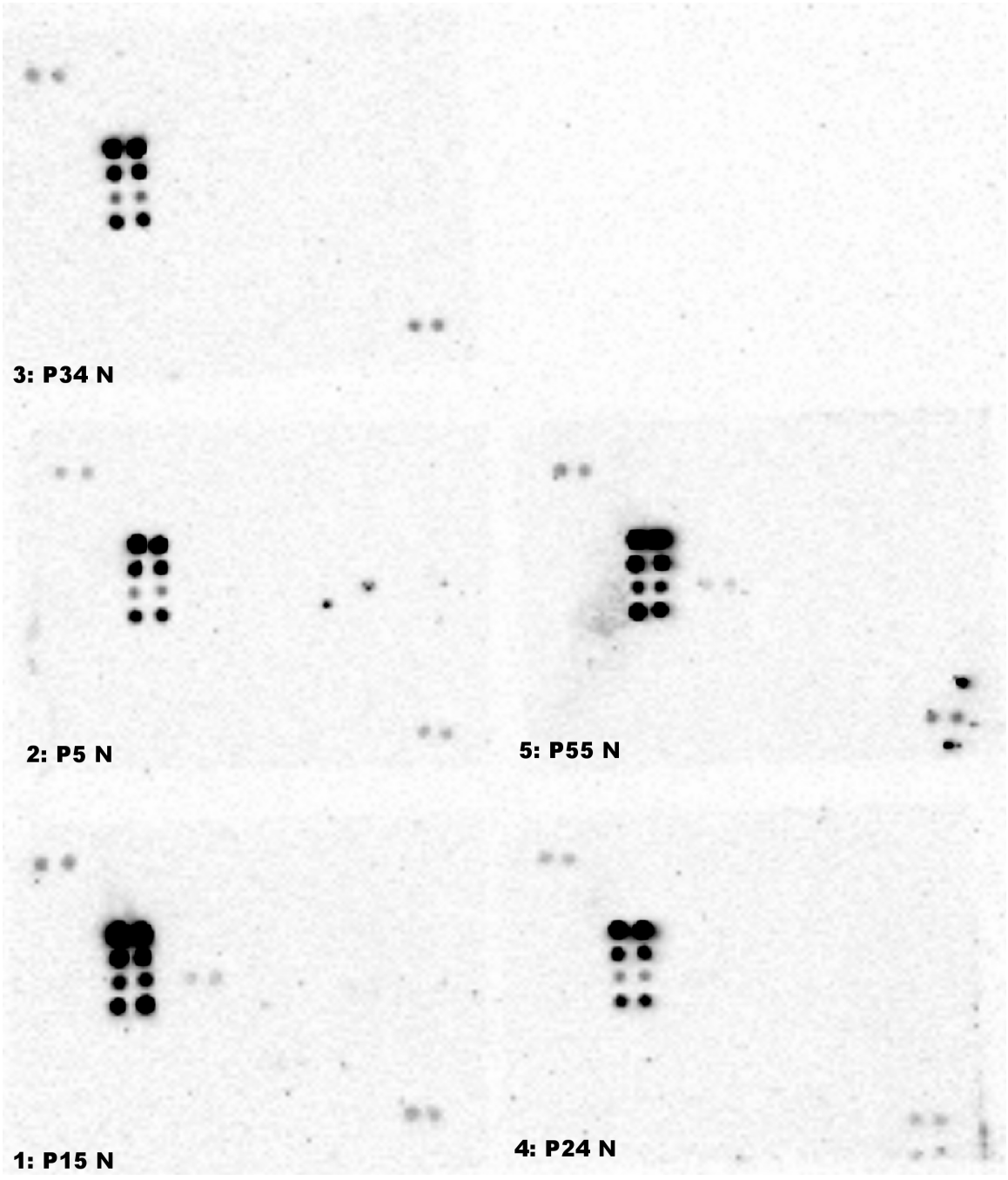

Fig. A. 1 Macroarray membrane photo showing positive reactions for *Ilyonectria liriodendri*. The set of eight dots at top left of membrane fluoresce when the probe specific to *I. liriodendri* is hybridized to the target.

Appendix B: Supplementary material for Chapter 3

Changes in AM fungi between young and old *Vitis* roots grown with soils trained by distinct plant communities

Table B. 1 Indicator taxa of AM fungi associated with each groundcover treatment. *Indicator value* statistics are given as defined in De Caceres et al. (2010). Groundcover treatments are: EG, exotic grasses; EGL, exotic grasses plus legumes; NG, native grasses; and NGF, native grasses plus forbs.

Groundcover	OTU ID	Family	Genus	Virtual Taxon	*Indicator value*	*P*
EG	0f3799fe1eb8ffe458126b77914d0698	Glomeraceae	N/A	N/A	0.754	<0.001
EG	372b149d1a8de19ea0ff04d17e6c22e8	Glomeraceae	N/A	N/A	0.752	0.001
EG	779ca079fb4cb939c28c35e7c5371f13	Glomeraceae	N/A	N/A	0.738	0.004
EG	8b6810c5abcb5beaff2204c44f2ac8a7	Glomeraceae	N/A	N/A	0.732	0.007
EG	4b74c4ff521eb3358988f7411c492409	Glomeraceae	N/A	N/A	0.73	0.004
EG	98ef9a8f694ca8a482b6ffdbcf5a6978	Glomeraceae	N/A	N/A	0.728	0.004
NG	b2c2cfc27aac638fdeb8b11e44d063ac	Claroideoglomeraceae	N/A	N/A	0.877	0.002
NG	93045bd371afdbdc4dd8d8be7ab8689d	Glomeraceae	N/A	N/A	0.709	0.003
NG	0f310c17dcee7f364	Claroideoglomeraceae	N/A	N/A	0.656	0.04

Groundcover	OTU ID	Family	Genus	Virtual Taxon	*Indicator value*	*P*
NG	e88abcae27a2a9f42b4810ea5d9cf0fbcdcbfbce42ae073	Claroideoglomeraceae	N/A	N/A	0.654	0.01
NG	39ba3d22322e5cbfabaee2751c37aca7	Glomeraceae	N/A	N/A	0.65	0.02
NG	f4a5f7f2aed9f1d7393c14459d1a6b60	Claroideoglomeraceae	N/A	N/A	0.631	0.02
NGF	133afa2cb131c120e74d9a48aa2cd353	Glomeraceae	Glomus	Glomus_irregulare_VTX00114	0.751	0.01
NGF	300b240ead365e3f50ab839510e3a8d3	Paraglomeraceae	N/A	N/A	0.632	0.02
NGF	fae9227bdf1ea3401ca336a772cabea7	Glomeraceae	Glomus	Glomus_irregulare_VTX00114	0.631	0.02
NGF	8e504ab0bfff30c1aadb947f0e05a226	Glomeraceae	Glomus	Glomus_Glo G6_VTX00067	0.631	0.05

Appendix C: Supplementary material file 1 for Chapter 4

Soil fungi are influenced by vineyard groundcover vegetation in multi-year survey across five sites

Description of vineyard sampling sites

Site 1

This experimental site is located near Summerland and was described in a previous study (Vukicevich et al. 2018). The soil at this site is Osoyoos loamy sand.

Four groundcover management schemes were present at site 1 representing plant communities paired with irrigation strategies optimized for growing conditions in the southern Okanagan. These management schemes were laid out in a randomized complete block design with four replicates of each (Fig. C.1). Management schemes were established in 2014 as described previously (Vukicevich et al. 2018) and included:

1. An exotic grass blend (EG) paired with under vine microsprinkler irrigation, i.e. vines were only watered with sprinklers thereby delivering water to the groundcover each time vines were irrigated (Approximately 700 mm supplemental irrigation applied annually, classified as 'sprinkler' irrigation).
2. The same exotic grass blend with the inclusion of a perennial legume, *Lotus corniculatus* L. (EGL) paired with occasional under vine microsprinkler irrigation, i.e. vines were watered via a drip system and sprinklers were used intermittently to keep the groundcover alive (Approximately 180 mm supplemental irrigation applied annually, classified as 'dual' irrigation).
3. A native grass blend (NG) with no supplemental irrigation, i.e. vines were drip irrigated and no under vine microsprinklers were in place (Classified as 'drip' irrigation).
4. The same native grass blend with the inclusion of perennial non-legume forbs (NGF) and intermittent microsprinkler irrigation to keep the forbs alive (Approximately 180 mm supplemental irrigation applied annually, classified as 'dual' irrigation).

Samples were collected on four occasions at Site 1: summer 2015, spring 2016, summer 2016, and spring 2017.

Site 2

This experimental site is located adjacent to site 1 described above. It was planted to *Vitis vinifera* L. var. Chardonnay clone '96' on rootstock 3309C (*Vitis rupestris* Martin x *riparia* tomenteus) in 2009 and spacing is 1.22 m x 3.7 m (vine x row). Soil is also Osoyoos loamy sand. The four groundcover schemes present at site 2 were established in 2011 and original plantings included:

1. Birdsfoot trefoil (BFT): *Lotus corniculatus* (L.) seeded at a rate of 7.25 g m^{-2}; native grass mix consisting of *Bouteloua dactyloides* (Nutt.) Columbus*, Festuca idahoensis* Elmer*, Poa secunda* J. Presl*, Sporobolus cryptandrus* (Torr.) A. Gray*, Pseudoroegneria spicata* (Pursh) A. Love*, Oryzopsis hymenoides* (Roem. & Schult.) Ricker ex Piper*,* and *Hesperostipa comata* (Trin. & Rupr.) Barkworth subsp. *comata* seeded at a rate of 8.4 g m^{-2}.
2. Orchard grass mixture (OG): "Orchard Mate non-irrigated" (Grower's Supply, Penticton, BC); 30% *Festuca trachyphylla* (Hack.) Krajina, 25% *Agropyron cristatum* (L.) Gaertn, 25% *Festuca rubra* subsp. commutata Gaudin, 20% *Lolium perenne* (L.). Seeding rate was 2.15 g m^{-2}.
3. Tillage (T): Cultivated using disk 2-3 times per season to maintain bare soil.
4. Buffalo grass (BG): *Bouteloua dactyloides* (Nutt.) Columbus (Sagebrush Nursery, Oliver, B.C.) seeded at a rate of 8.4 g m^{-2}.

All four treatments received no supplemental irrigation ('Drip' irrigation). Site 2 was sampled in summer 2015, spring 2016, summer 2016, and spring 2017.

Site 3

This commercial vineyard was planted to Merlot and is located near the town of Oliver, BC. Soil at this site is a Ponderosa series gravelly loam developed from coarse-textured fluvial fan deposits (Wittneben 1986). Two groundcover schemes were established in 2009 at this site including:

1. Flower mix (F): 50% *Cichorium intybus* L., 20% *Phacelia tanacetifolia* Benth., 10% *Lobulaira maritima* L. (Desv.), and 20% Interior wildflower mix. This scheme was planted to encourage beneficial insects in the vineyard. It was allowed to flower each year and thus was mown less frequently than the resident vegetation, below.
2. Resident grass-dominant vegetation (G) consisting mostly of *Dactylis glomerata* L., *Poa compressa* L., *Elymus repens* L., as well as a host of weedy forbs. This scheme was mowed more frequently than F to facilitate vineyard work.

Under vine microsprinklers were used to supply supplemental irrigation ('dual' irrigation) from the establishment of the groundcovers in 2009 until late summer of 2016 after which drip irrigation only was used ('drip' irrigation). Samples were collected on four occasions consistent with sites 1 and 2.

Site 4

This commercial vineyard was planted to own-rooted Chardonnay in 1990. Vine spacing is 1.25 m x 2 m. The site is located near the town of Okanagan Falls, BC. According to surveys, soil at this site is classified as Parkill loamy sand developed from deep, sandy, fluvioglacial deposits (Wittneben 1986). There are two groundcover management schemes in place at site 4:

1. A mono-dominant sheep fescue, *Festuca ovina* L., groundcover (Z) was originally planted as part of a cover crop mix in 2010.
2. The alternate rows were planted in spring of 2015 to a mix (M) of *Secale cereale* L. (5kg ha^{-1}), *Trifolium repens* L. (5 kg ha^{-1}), *Trifolium pratense* L. (5 kg ha^{-1}), *Medicago lupulina* L. (5 kg ha^{-1}), *Brassica napus* L. (1-2 kg ha^{-1}), and *Capsella bursa-pastoris* (L.) Medik (1-2 kg ha^{-1}). These plants did not establish well and annual weeds dominated the plant community of in these rows (see supplementary tables for plant community data).

The entire block at site 4 is sprinkler-irrigated. Samples were collected in summer of 2015 and spring of 2016 after which incorporation of both schemes prevented further sample collection at this site.

Site 5

Site 5 sits just northeast of Osoyoos lake, BC on Osoyoos series loamy sand. There are a total of four groundcover schemes at this site in two adjacent blocks, each arranged in alternating rows as per site 4:

1. Alfalfa, *Medicago sativa* L. (A) mixed with *Lolium perenne.*
2. *Festuca* spp. blend (F)
3. Yarrow, *Achillea millefolium* L. (Y)
4. Grass blend dominated by fescues (G)

Vines are sprinkler irrigated with overhead sprinklers, providing water to ground cover vegetation as well during each irrigation event. This site was sampled in summer 2016 and spring 2017.

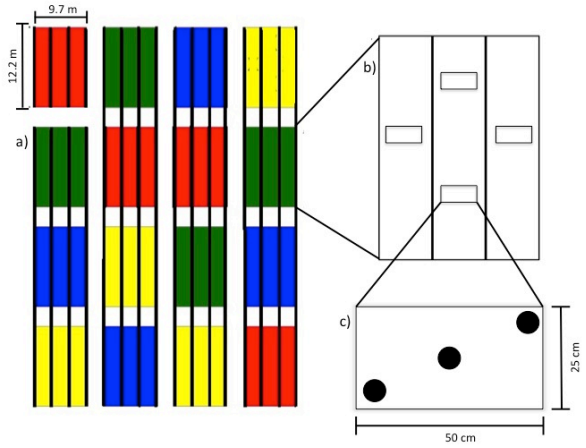

Fig. C. 1 Sample collection scheme for site 1. a) Randomized complete block design of experimental groundcover trial. Each plot was 9.1 m x 12.2 m and comprised three drive rows. Thick, black lines represent vine rows. Groundcover identity: Red = native grass plus forbs (NGF); Green = Exotic grass (EG); Blue = exotic grass plus legumes (EGL); Yellow = native grass (NG). b) Close up of a single field replicate showing the four locations of sampling by quadrat, and c) Close up of one quadrat (50 cm x 25 cm), showing locations of the three cores (black dots) which were taken along a diagonal transect within the quadrat and pooled for each quadrat. A total of 64 samples (4 quadrats x 4 replicates x 4 schemes) were taken at each sampling period.

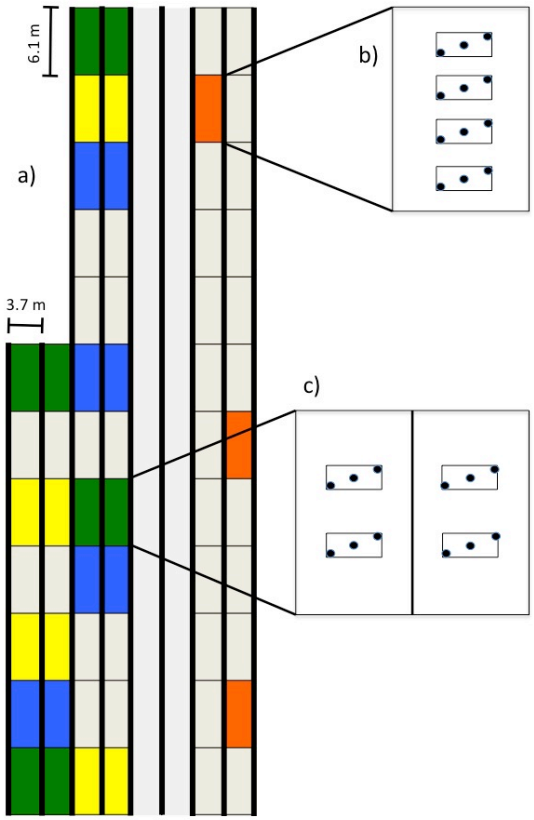

Fig. C. 2 Sample collection scheme for site 2. a) Randomized complete block design of groundcover trial with groundcovers: Green = exotic grass (G); Yellow = birdsfoot trefoil (BFT); Blue = tillage (T); and Red = buffalo grass (BG). b) Close-up of one BG plot. Three core samples (black dots) were taken along a diagonal transect across each of four 25 cm x 50 cm quadrats spaced 1.22 m apart down the center of the plot (one drive row) and were pooled for analysis. c) Close-up of a treatment plot for G, BFT, or T. The same procedure for taking core samples was used within each quadrat, except the four quadrats were placed in a rectangle with two in each drive row spaced 2.44 m apart (two rows per replicate for G, BFT, and T). A total of 60 samples were collected on each sampling occasion (4 quadrats x 3 treatments x 4 replicates + 4 quadrats x 1 treatment x 3 replicates).

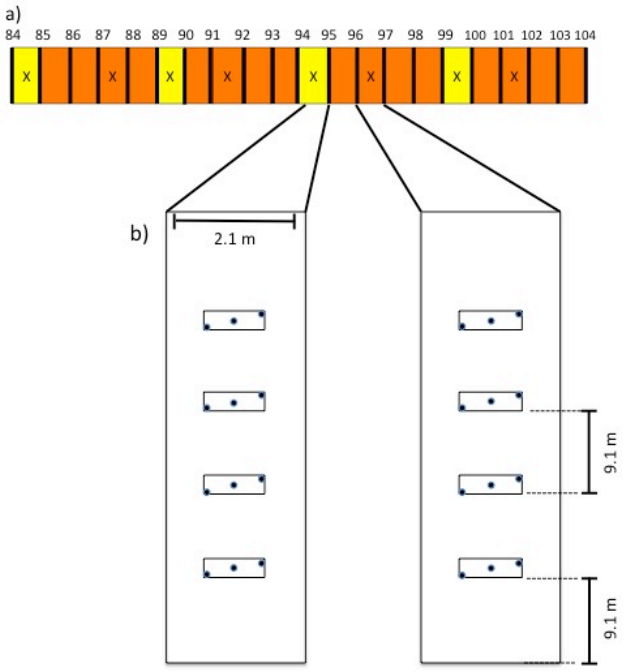

Fig. C. 3 Sample collection at site 3 with two groundcover schemes: Yellow = flowering mix (F); Red = grass-dominant resident vegetation (G). a) Rows 84 through 104 were used to make four replicates of each treatment. Rows sampled are marked with an "X" and vine rows are signified by thick, black lines between replicates. b) Close-up of two replicates (entire rows). Within each row we assessed four plant communities in 25cm x 50cm quadrats and collected and pooled three cores per quadrat (black dots). The first quadrat was aligned 9.1 m (10 vines) in from the block edge and each subsequent quadrat was placed 9.1 m from the last.

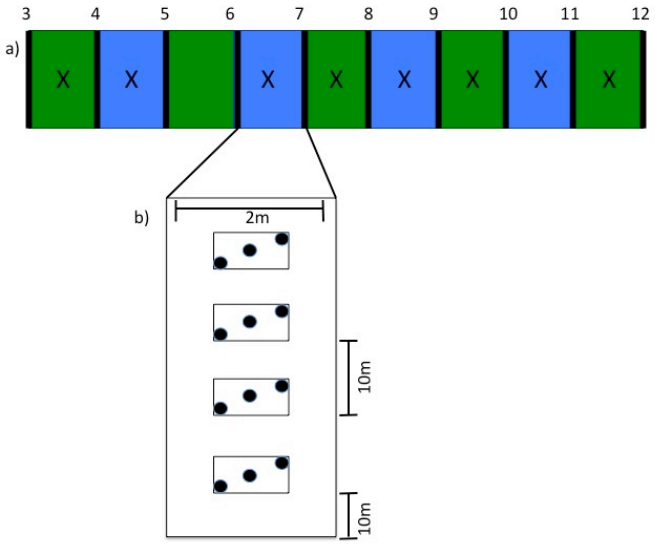

Fig. C. 4 Sampling scheme for site 4. a) Groundcovers include: Green = *Festuca ovina* L. monoculture (Z); Blue = mixed planting (M). Rows sampled are marked with an "X" and row numbers are given above. b) Close-up of a sampled replicate (entire row). Rows are 2 m wide and we sampled from four 25 cm x 50 cm quadrats per row, with each quadrat spaced 10 m apart and the first one 10 m from the field edge.

Model averaging to determine which variables to retain in final models

Table C. 1 Model averaging output for AM fungi showing average importance of each variable and the number of candidate models in which they occurred. For AM fungi, there were only 3 models with ΔAIC <2. Variables with average importance >7 that were included in the final model are in *italics.*

Variable	Importance	# containing models
cycle	1	4
Exotic	1	4
Form_Legume	1	4
irrigation_type	1	4
MelichP	1	4
OM	1	4
samp.period	1	4
Total_cov	1	4
cycle:samp.period	1	4
Exotic:samp.period	1	4
Form_Legume:samp.period	1	4
irrigation_type:samp.period	1	4
OM:samp.period	1	4
samp.period:Total_cov	1	4
pH	0.7	3
pH:samp.period	0.5	2
MelichP:samp.period	0.14	1
cycle	1	4

Table C. 2 Model averaging output for *Beauveria bassiana* showing average importance of each variable and the number of candidate models in which they occurred. For AM fungi, there were 18 models with ΔAIC <2. Variables with average importance >7 that were included in the final model are in *italics.*

Variable	Importance	# containing models
Exotic	1	19
Form_Grass	1	19
Form_Legume	1	19
irrigation_type	1	19
OM	1	19
samp.period	1	19
Form_Grass:samp.period	1	19
irrigation_type:samp.period	1	19
MelichP	0.94	18
MelichP:samp.period	0.89	17
cycle	0.79	14
Form_Legume:samp.period	0.73	13
Richness	0.67	12
Mycorrhizal	0.46	10
OM:samp.period	0.39	7
pH	0.08	2
Exotic	1	19
Form_Grass	1	19
Form_Legume	1	19

Table C. 3 Model averaging output for *Ilyonectria* spp. showing average importance of each variable and the number of candidate models in which they occurred. For *Ilyonectria* spp. there were 17 models with ΔAIC <2. Variables with average importance >7 that were included in the final model are in *italics.*

Variable	Importance	# containing models
Exotic	1	50
Form_Grass	1	50
irrigation_type	1	50
log(OM)	1	50
samp.period	1	50
Total_cov	1	50
Exotic:samp.period	1	50
irrigation_type:samp.period	1	50
samp.period:Total_cov	1	50
log(OM):samp.period	0.82	40
log(MelichP)	0.78	39
log(MelichP):samp.period	0.69	34
Form_Legume	0.66	33
Form_Legume:samp.period	0.62	30
pH	0.52	25
cycle	0.5	23
Mycorrhizal	0.21	12
pH:samp.period	0.16	7
Richness	0.13	8
Form_Grass:samp.period	0.08	4

Appendix D: Supplementary file 2 for Chapter 3

Groundcover vegetation affects soil fungi in multi-year survey across five semi-arid vineyard sites

Effects of different groundcover management schemes within vineyard survey sites

Data analysis

Data was subset according to vineyard site (1 through 5). Separate linear mixed models using the R package 'lme4' were run for each fungal guild at each site to determine if the two to four different management schemes at that site had any effect on fungi. Sample period was included as a random factor to account for changes over time (equivalent to repeated measures ANOVA). The package 'lmerTest' was used to assess significance. Tukey's honest significant difference was used *post-hoc* to determine how management schemes differed. Only significant effects are reported.

Results

Site 1

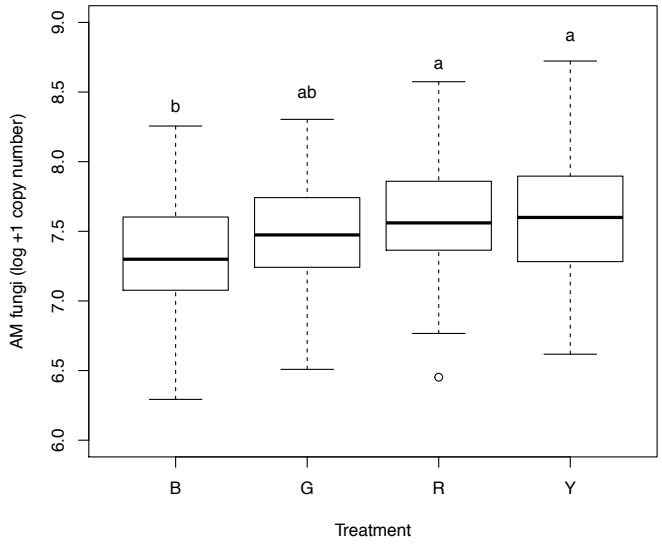

Fig. D. 1 Effect of groundcover scheme on AM fungi at site 1. Treatments are: B, exotic grasses; G, exotic grasses plus legumes; R, native grasses plus forbs; and Y, native grasses. Groundcovers affected AM fungal abundance at site 1 (*F=6.73, P<0.001*). Letters indicate significant differences (*P<0.05*) among groundcovers.

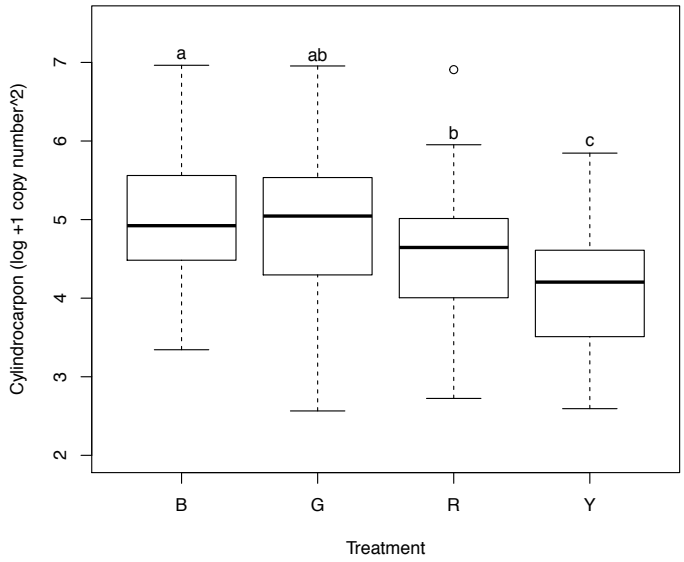

Fig. D. 2 Effect of groundcover scheme on *Ilyonectria* spp. at site 1. Treatments are: B, exotic grasses; G, exotic grasses plus legumes; R, native grasses plus forbs; and Y, native grasses. Groundcovers varied in their effect on *Ilyonectria* spp. ($F=16.07$, $P<0.001$). Letters indicate significant differences ($P<0.05$) among groundcovers.

Site 2

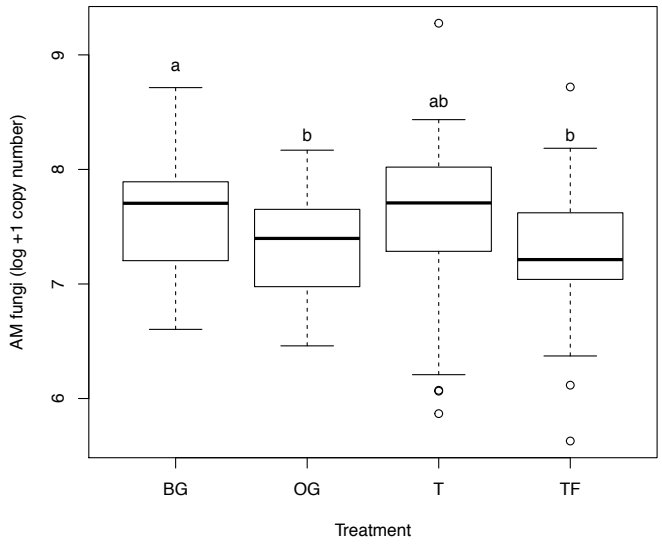

Fig. D. 3 Effect of groundcover scheme on AM fungi at site 2. Treatments are: BG, buffalo grass; OG, orchard grass mix; T, tillage; and TF, birdsfoot trefoil mix. Groundcovers affected AM fungal abundance at site 2 ($F=4.43$, $P=0.005$). Letters indicate significant differences ($P<0.05$) among groundcovers.

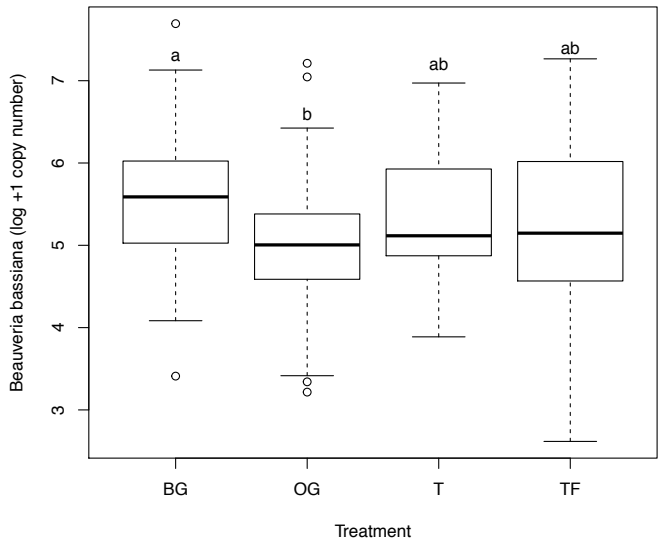

Fig. D. 4 Effect of groundcover scheme on *Beauveria bassiana* at site 2. Treatments are: BG, buffalo grass; OG, orchard grass mix; T, tillage; and TF, birdsfoot trefoil mix. Groundcovers affected *Beauveria bassiana* abundance at site 2 (*F=5.64, P<0.001*). Letters indicate significant differences (*P<0.05*) among groundcovers.

Site 3

There were no significant effects of management scheme on any of the three fungal guilds measured at site 3.

Site 4

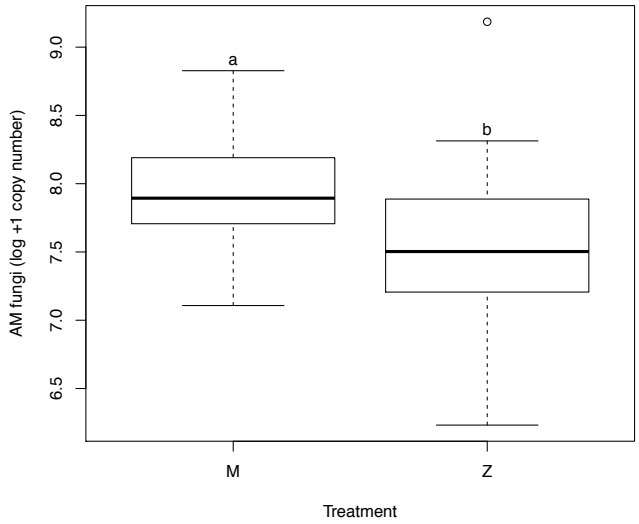

Fig. D. 5 Effect of groundcover scheme on AM fungi at site 4. Treatments are: M, mixed vegetation; Z, monodominant sheep fescue. Groundcovers affected AM fungal abundance at site 4 (*F=11.16, P=0.001*). Letters indicate significant differences (*P<0.05*) among groundcovers.

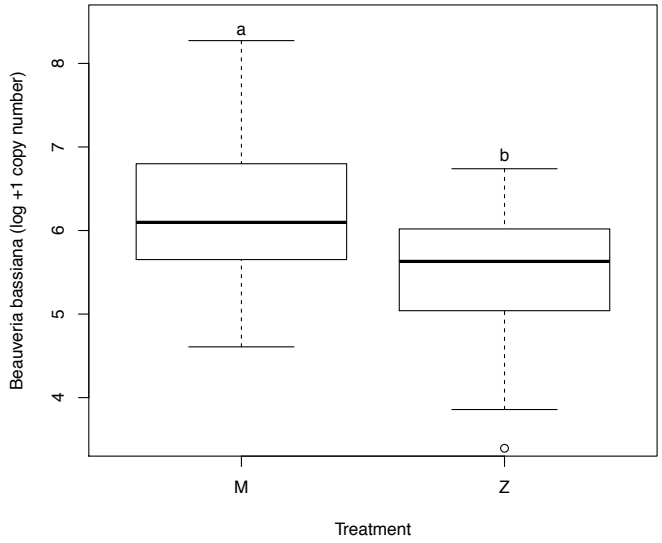

Fig. D. 6 Effect of groundcover scheme on *Beauveria bassiana* at site 4. Treatments are: M, mixed vegetation; Z, monodominant sheep fescue. Groundcovers affected *Beauveria bassiana* abundance at site 4 (*F=21.32, P<0.001*). Letters indicate significant differences (*P<0.05*) among groundcovers.

Site 5

There were no significant effects of management scheme on any of the three fungal guilds measured at site 5.