EFFECTS OF COVER CROPS ON THE SOIL MICROBIOME AND CARRY OVER IMPACT ON FRCR IN BARLEY AND SOYBEAN

By

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Dalhousie University is located in Mi'kma'ki,
the ancestral and unceded territory of the Mi'kmaq.
We are all Treaty people.

Dedication

This thesis is dedicated to the memory of my beloved grandmother Shyamala Amma.

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Abstract

This study explored the influence that different cover crops have on the soil microbiome (Chapter 2) and the impact on Fusarium root and crown rot (FRCR) of barley and soybean (Chapter 3). Field trials were conducted with cover crops planted in the first year, and barley and soybean no-till seeded in the second year. Cover crop effects on microbial communities were characterized using amplicon sequencing and carryover effect on FRCR was studied. Controlled inoculated greenhouse trials were conducted to study the potential disease suppressive nature of certain cover crop soils. Choice of cover crop significantly influenced soil microbial community structure and composition by changing the abundance of beneficial and pathogenic microorganisms, thereby having an impact on FRCR in barley and soybean. Soybean and barley FRCR were positively associated with soil pathotroph abundance. FRCR suppressive abilities of soil after growing sorghum-sudangrass were confirmed in both field and greenhouse trials.

Keywords: Alfalfa, crimson clover, annual ryegrass, sorghum-sudangrass, brown mustard, oilseed radish, buckwheat, phacelia, barley, soybean, soil microbiome, Fusarium root and crown rot (FRCR), FUNGuild, FAPROTAX

List of Abbreviations Used

16S rRNA – 16S ribosomal RNA

DSI – Disease severity index

FRCR – Fusarium root and crown rot

ITS – Internal transcribed spacer

OTU – Operational taxonomic unit

PCA – Principal component analysis

SSG – Sorghum-sudangrass

Tef-1α – Translational elongation factor 1

Tukey's HSD – Tukey's honestly significant difference

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Chapter 1 - Introduction

1.0 Crop rotation

Crop rotation refers to the practice of growing different crops sequentially on the same agricultural field (Bullock, 1992). This practice has been used globally for more than 3000 years (MacRae and Mehuys, 1985). Growing diverse crop families in rotation with one another is known to have several benefits including increased soil organic matter, increased microbial biomass, reduced weed pressure, and reduced disease incidence (Robinson, 1966; Campbell et al., 1991; Cardina et al., 2002). Robinson (1966) found that rotating sorghum (*Sorghum bicolor* (L.) Moench) with corn (*Zea mays* L.) or sunflower (*Helianthus* spp. (L.)) with soybean (*Glycine max* (L. Merr)) had significantly better yield outcomes compared to continuous cropping. They found a negative correlation between yield and the abundance of pathogenic fungi in the soil with growing continuous sunflower (Robinson, 1966). Cardina et al. (2002), found that there were significantly more weed seeds in the seedbank after growing continuous corn under no-till conditions compared to crop rotations including corn-soybean or corn-oats-hay. They also found that crop rotations influenced the dominant weed species and predicted that it was because of changes in soil quality (Cardina et al., 2002). Campbell et al. (1991), found an increase in carbon associated with microbial biomass when crop rotations included legumes such as clover (*Trifolium* spp.) and alfalfa (*Medicago sativa* L.), and this in turn led to an increase in soil organic matter. They suggested that the increase in soil organic matter was directly a result of retaining plant material after harvest, also referred to as crop residue (Campbell et al., 1991). As such, the positive effects of crop rotations can be broadly attributed to improved soil health (Congreves et al., 2015).

As defined by the United States Department of Agriculture [USDA], soil health is "the capacity of the soil to function as a vital living ecosystem to sustain plants, animals, and humans" (Stott, 2019). Soil health is greatly affected by the associated chemical, physical, and biological properties (Mann et al., 2019). This study was conducted in Prince Edward Island (PEI), where the soil type is primarily well drained sandy loam with high silt, low organic matter content, and low pH, classified as Orthic Humo-Ferric Podzol (Soil Classification Working Group, 1998). Though this soil type is suitable for agriculture, it is also highly susceptible to soil erosion, which could lead to pesticide-contamination in the surrounding water, nitrate leaching, and other major long-term issues, such as loss of agricultural land area (Jatoe et al., 2008). Potatoes (*Solanum tuberosum* L.) are the most important crop grown in PEI. Continuous potato production leads to soil erosion, loss of soil organic matter, and build-up of pathogenic microorganisms (Jatoe et al., 2008). Therefore, many land-use programs and policies such as the Integrated Pest Management (IPM) program, and the PEI Agricultural Crop Rotation (ACR) Act, have been implemented as intervention strategies (Jatoe et al., 2008). The ACR Act, which prohibits growers from planting any one row crop more than once in three consecutive years, was established in 2002 in response to pesticide-related fish kills caused by soil erosion (Jatoe et al., 2008). In order to comply with these regulations, crops such as barley (*Hordeum vulgare* (L.)) and soybean are commonly used in rotation with potatoes to improve soil health. Barley and soybean, two of the most important crops in Canada, are the key crops of interest in this study (Table 1.1; Statistics Canada, 2019).

Table 1.1 Canadian barley and soybean production summary for 2020 (AIMIS, 2021).

Region	Barley (metric tonnes)	Soybean (metric tonnes)
Canada	10,740,600	6,355,900
Eastern Canada	324,300	5,124,300
Ontario	113,100	3,908,700
Quebec	125,900	1,159,700
PEI	**65,200**	**39,300**
New Brunswick	17,600	5,400
Nova Scotia	2,500	11,200

1.1 Barley

Barley is one of the most important grain crops grown globally with an average annual production of 159 million metric tonnes (FAOSTAT, 2019). The earliest record of barley cultivation dates to 7000 BC in the fertile crescent of the Middle East (Smith, 1995). Barley is a cereal crop in family Poaceae which is primarily produced for human consumption as malt products, and for animal feed (Baik and Ullrich, 2008). PEI is the largest producer of barley in the Maritime provinces of Canada (Table 1.1). Barley, in PEI, is commonly grown in rotation with potatoes in order to scavenge excess nitrogen, help restore soil structural stability, and break disease cycles (Celetti et al., 1990; Peters et al., 2004; Carter, 2005; Jatoe et al., 2008). AAC Synergy, a hulled two-row spring malting barley variety, was used in this study (Legge et al., 2014). This cultivar was found to be resistant to spot-form net blotch which is caused by the fungal pathogen *Pyrenophora teres* f. sp. *maculata* (Drechs.), and moderately resistant to net-form net blotch caused by *P. teres* f. sp. *teres*. It is also considered intermediately susceptible to common root rot caused by *Bipolaris sorokiniana* ((Sacc.) Shoemaker; synonym:

Helminthosporium sativum Pammel, King & Bakke, *H. sorokinianum* Sacc. in Sorokin, and *Drechslera sorokiniana* (Sacc.) Subramanian & Jain; teleomorph: *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur), as well as smut caused by *Ustilago nuda* ((Jens.) Rostra.), and stem rust caused by *Puccinia graminis* f. sp. t*ritici* (Pers.), as well as moderately susceptible to Fusarium head blight (FHB), which is commonly caused by *Fusarium graminearum* (Schwabe; teleomorph: *Gibberella zeae* (Schweinitz) Petch.) (Legge et al., 2014).

Susceptibility to root diseases, such as those caused by *Fusarium* spp., are often ignored when assessing barley crop quality. Agricultural practices such as crop rotation and tillage are known to affect root disease caused by *Fusarium* spp., sometimes referred to as Fusarium root and crown rot (FRCR) (Sturz and Carter, 1995; Peters et al., 2004). FRCR often goes unnoticed as the symptoms may be difficult to detect and the impact on yield in one year may not be significant (Oliver, 2019). Smiley et al. (2005), through field trials with wheat (*Triticum aestivum* L.) and barley, found a 13 to 15% reduction in yield associated with FRCR, even though there was no measurable difference in symptoms between inoculated and non-inoculated treatments. Furthermore, disease may be hard to predict as the pathogen population in soil may not always correlate with the disease incidence. Sturz and Carter (1995), found that conservation tillage practices did not affect fungal pathogen populations in the soil or in left-over plant debris, but did reduce the level of barley root infection by *Fusarium oxysporum* ((Schlecht.) emend. Snyder & Hansen), *F. avenaceum* ((Fr.) Sacc.; teleomorph: *Gibberella avenacea* Cook), and *Rhizoctonia solani* (Kühn; teleomorph: *Thanatephorus cucumeris* Donk). This was attributed to high levels of beneficial fungi and increased soil bulk density (Sturz and Carter, 1995).

1.2 Soybean

Soybean is an annual legume in the family Fabaceae, thought to have originated in China more than 5000 years ago (Grassini et al., 2021). It is globally recognized for its many uses including production of tofu, soy sauce, soy milk, extractable phytohormones, as well as its use as animal feed and biodiesel. In 2019, there was a global annual soybean production of 333 million metric tonnes (FAOSTAT, 2019). As a legume crop, it is known to fix atmospheric nitrogen through symbiotic interactions with soil bacteria (Grassini et al., 2021). As such it is commonly used in rotational cropping systems to improve soil health. PEI is the largest producer of soybean in the Maritime provinces of Canada (Table 1.1). The two soybean varieties used in this study were DH401 (Sevita International, Ontario) in the first trial and 25-10RY (Dekalb) in the second trial. DH401 is a non-GMO tofu variety which is marketed as being tolerant to white mould caused by *Sclerotinia sclerotiorum* ((Lib.) de Bary) as well as brown stem rot caused by *Cephalosporium gregata* (Allington & Chamb.; synonyms: *Cadophora gregata* (Allington & Chamb.) T.C. Harr. & McNew and *Phialophora pregata* (Allington & Chamb.) W. Gams). It is also moderately resistant to Phytophthora root rot. According to seed company recommendations, this variety is ideal for use in conventionally tilled fields. 25-10RY is a glyphosate resistant variety that is marketed as having excellent no-till adaptability in all soil types. This variety contains the gene Rps1c which confers resistance to root and stem rot caused by *Phytophthora* spp. (de Bary) 25-10RY is also marketed as having good tolerance to white mould and brown stem rot.

Root diseases caused by *Fusarium* spp. have a significant negative impact on soybean crop quality and yield (Leath and Carroll, 1985). There is contradicting evidence in literature regarding the impact of FRCR on soybean yield. Leath and Carroll (1985) suggested there could be up to 56% reduction in yield attributed to FRCR; meanwhile, Díaz Arias et al. (2013), showed

that despite measurable differences in root rot symptoms, there may be no significant impact on actual yield. As such, more research is required to better understand the impact of FRCR on soybean and the strategies that can be taken to mitigate this disease.

1.3 Fusarium root and crown rot (FRCR)

FRCR can be caused by several different *Fusarium* spp., often with a dominant causal agent specific to host species and environment. The genus *Fusarium* is phylogenetically classified within phylum Ascomycota, order Hypocreales, family Nectriaceae, with teleomorphs in the genus *Gibberella*, *Haematonectria*, and *Albonectria (*Leslie and Summerell, 2008). Genus *Fusarium* consists of several pathogenic species known to cause disease in plants, humans, and animals (Leslie and Summerell, 2008). Several species including *F. graminearum, F. sporotrichioides* ((Sherb.) Bilai)*, F. avenaceum, F. culmorum* ((Wm.G.Sm.) Sacc.)*, F. oxysporum, F. cerealis* ((Cooke) Sacc.; synonym: *F. crookwellense* (Burgess) Nelson & Toussoun)*, F. pseudograminearum* (O'Donnell and Aoki; teleomorph: *Gibberella coronicola*) and *F. equiseti* ((Corda) Sacc.), among many others are known to cause root disease in barley and soybean (Chakraborty et al., 2006; Zhang et al., 2013). To further complicate the identity of the causal agent of this disease, organisms such as *F. oxysporum* are considered a species complex, with over 100 *formae specialis*, which cannot be differentiated based on morphology, but are often only pathogenic to a specific host (Bosland, 1988; Edel-Hermann and Lecomte, 2019).

Fusarium spp. are characterised by their production of some or all of the following types of spore bodies, including three types of asexual spores, known as microconidia, macroconidia, and chlamydospores, and one type of sexual spore, referred to as ascospores (Leslie and

Summerell, 2008). Micro- and macroconidia are produced by spore-forming structures called conidiophores on the mycelium (Leslie and Summerell, 2008). They play an important role in spreading inoculum during a single growing season. Chlamydospores are thick-walled spores produced either singly or in sets within a strand of hyphae or within a macroconidium Ascospores are formed within a sac called an ascus, in sets of eight (Beckett, 1981). Asci are contained within a flask-shaped perithecium, which forcibly ejects ascospores once there is a pressure build-up (Ingold, 1971; Leslie and Summerrell, 2008). Perithecia and chlamydospores are referred to as resting structures as they can survive in soil and crop residue for long periods of time, even withstanding winter temperatures (Smith and Snyder, 1975; Manzo and Claflin, 1984; Gordon and Okamoto, 1990; Cotten and Munkvold, 1998). Pathogen load, as defined here, is the amount of contamination of field environment by resting structures that can lead to infection and disease when the appropriate host is present (Peters et al., 2003).

A diagram depicting a generalized disease cycle for FRCR can be found in Fig 1.1. When the weather becomes warmer after winter, the dormant resting structures germinate and produce hyphae which can spread in the soil and infect plant tissue (Kazan and Gardiner, 2018). Once inside the root tissue, the fungal hyphae can spread up the stem, disrupting water and nutrient uptake (Knight and Sutherland, 2016). Fungal metabolism in the initial stages of infection is dedicated to mycelial growth and suppression of plant defence (Oliver, 2019). As such, the crop may not show visible symptoms while still having reduced quality and yield due to lack of resources. Once infection has been established, some *Fusarium* spp., may redirect energy to support a more necrotrophic lifestyle, causing visible symptoms, such as discoloration and lesions in the crown and root, sometimes even leading to plant death (Fernandez and Conner, 2011; Kazan and Gardiner, 2018). Once the plant is harvested or mowed, the mycelium left in

the soil and residue will produce more resting structures as inoculum for the subsequent year (Kazan and Gardiner, 2018). Ascospores which are forcibly released by perithecia have the potential to travel to other fields and spread inoculum (Trail et al., 2002). These airborne or splash-dispersed spores can infect other parts of the plant causing different diseases (Fig. 1.1). Several abiotic and biotic factors play a role in incidence and severity of FRCR including crop resilience, pathogen load, and environmental conditions such as drought (Fernandez and Conner, 2011). Plant material, infected with *Fusarium* spp., when left in the field, may increase pathogen load in the soil, leading to more disease in subsequent years (Gordon and Okamoto, 1990). The pathogen may also be transferred to other fields through contamination of soil or equipment (Fig. 1.1).

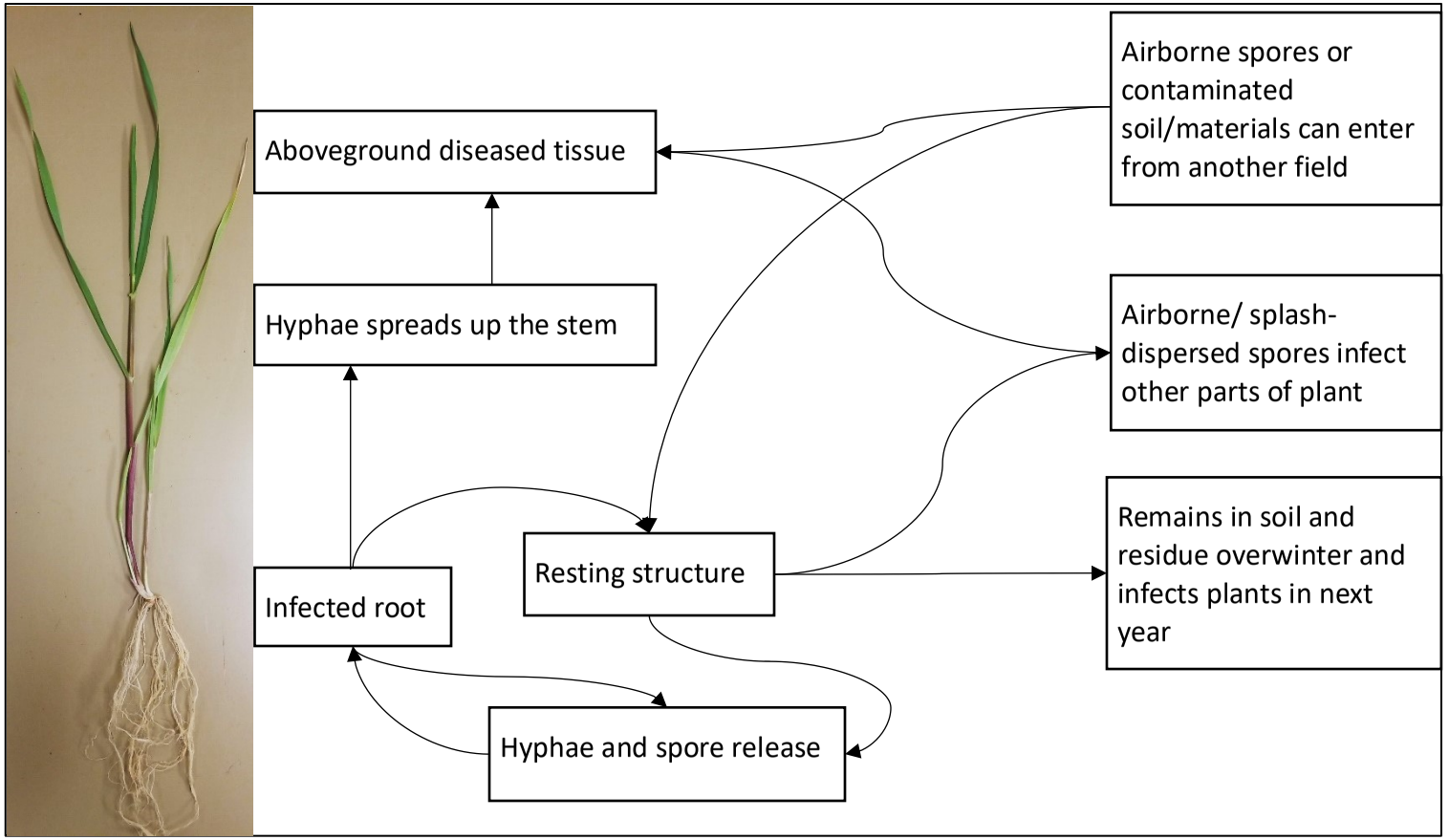

Fig. 1.1 Diagram of disease life cycle of Fusarium root and crown rot in a generic plant.

1.3.1 FRCR disease management

The major challenge to managing FRCR is that the symptoms are often hard to detect until there has already been a major impact on crop quality and yield (van Alfen, 2014; Oliver, 2019). FRCR disease management must involve preventative measures such as applying chemical fungicides, growing resistant plants and using appropriate agronomic practices, such as diversifying crop rotations and tillage practices to ensure that conditions are not favourable for disease development (Oliver, 2019). Also, monitoring symptoms of FRCR is difficult but an essential part of determining what management strategies need to be taken to ensure optimal agricultural productivity, and to assess if the strategies are working.

Fungicide seed treatments are the most common and cost-effective ways to reduce damage by soil-borne and seed-borne pathogens. Seed treatments provide early protection for the crops allowing them to establish and protect themselves through innate defense responses. Seed treatments are often used to protect against soil-borne pathogens such as *Pythium* spp. (Trow), and *Rhizoctonia solani*, *Phytophthora* spp. (de Bary), *Fusarium* spp. which can cause seedling blight and damping-off (Hall and Xue, 1995; Xue et al., 2007). Fungicides can be systemic, which means they move within the plant and provide a longer period of protection. Contact fungicides play an important role in preventing initial infection, and only function at the site of treatment. Seed treatments often include a combination of contact and systemic fungicides. For example, Vitaflo 280 (Chemtura, Connecticut, USA), which is marketed for protection against a wide range of pathogens including *Fusarium* spp., consists of carbathiin, a systemic fungicide, as well as thiram, a contact fungicide (Hwang, 1994). Carbathiin is a Group 7 fungicide, for which the primary mode of action is inhibition of succinate dehydrogenase activity, thus affecting a vital part of fungal mitochondrial respiration (Damicone, 2014). As a systemic fungicide,

carbathiin may also protect the plant from post-infection damage. However, due to the site-specific mode of action, there is a risk of pathogens developing resistance against carbathiin (Damicone, 2014). Thiram is a Group M fungicide, which has a multi-site mode of action, primarily involved in preventing infection (Hwang, 1994; Damicone, 2014). Fludioxonil, (Syngenta, Basel, Switzerland), is another commonly used contact fungicide (Damicone, 2014). As a Group 12 fungicide, it is a chemical derivative of the antifungal compound phenylpyrrole, that protects against seedling diseases caused by *Fusarium* spp., (Damicone, 2014). Broders et al. (2007), found that Fludioxonil, under laboratory and greenhouse settings inhibits mycelial growth of *Fusarium* spp., thereby significantly reducing its ability to cause infection. Fungicide seed treatments may also prevent infection caused by both soil-borne and seed-borne *Fusarium* spp. (Martin and Johnston, 1982). However, fungicide seed treatments are only effective for a short period of time and do not protect against infection occurring later in the growing season (Martin and Johnston, 1982). Based on their review of current literature, Paulitz et al. (2002), suggested that fungicides have almost no effect on FRCR, especially under drought conditions.

Tillage strategies are also an integral part of disease management strategies. Deep tillage was identified as an optimal way to bury soil-borne pathogens and reduce pathogen load (Schroeder and Paulitz, 2008; Scala et al., 2016; Oliver, 2019). However, intensive soil management practices like tillage reduce soil structural stability, moisture retention, and soil organic matter content (Nyiraneza et al., 2017). Furthermore, Sturz and Carter (1995), found no-till or conservation tillage did not significantly increase the risk of infection by soil-borne pathogens such as *Fusarium* spp., in barley and soybean. Therefore, the beneficial impact of no-till systems on soil health may outweigh the risk of increased disease incidence (Peters et al., 2004).

Promotion of disease suppressive soils is another way to control soil-borne pathogens; however, there is currently little information about the development of disease suppressive soils for FRCR control. Disease suppressive soil is loosely defined as an environment where the pathogen is not able to survive and/or cause disease (Baker and Cook, 1974). Studies related to this topic have often focused on a single species or group of organisms known to have antagonistic effects against a specific pathogen (Xue et al., 2017; El-Meleigi et al., 2017; Gimeno et al., 2019; Moreno-Velandia et al., 2019). These organisms are often referred to as biocontrol agents and maybe artificially cultivated and applied as treatment. *Clonostachys rosea* ((Link) Schroers; synonym: *Gliocladium roseum*), is a common soil-borne fungal species also classified under order Hypocreales, which has been studied for its ability to control various plant pathogens including *Fusarium* spp., through mycoparasitism (Gimeno et al., 2019). Jensen et al. (1999), reported that *C. rosea* (strain IK726) which was isolated in Finland, reduced barley seedling infection by *F. culmorum* when used as seed treatment. Disease suppressive soils can include higher abundance of biocontrol agents such as *C. rosea* and lower abundance of pathogens. Recently, studies have focused on understanding the mechanisms behind disease suppressive soil systems, and the potential replicability in field settings through adaptive agricultural management practices (Andrade et al., 1994; Peters et al., 2003; Wen et al., 2017).

Crop rotation also has a major impact on soil microbial structure and plays an important role in FRCR disease management (Bonanomi, et al., 2018; Lopes and Fernandes, 2020). Many studies have identified that planting different crop families in rotation with the main crop can reduce disease incidence when following conservation agricultural practices (Paulitz et al., 2002; Manici et al., 2018). Crop rotations that are three years or longer, including diverse crop groups, have better results for reducing pathogen load, when compared to shorter rotations (Peters et al.,

2004; Bainard et al., 2017). Peters et al. (2004), also found that longer crop rotations and choice of crop sequence can have a bigger effect on root diseases such as FRCR, than tillage.

1.4 Cover crops

Cover crops are crops grown as ground cover but not harvested, with the primary purpose of decreasing soil erosion, absorbing excess soil nutrients, adding soil organic matter, and increasing soil structural stability (Sarrantonio and Gallandt, 2003; Parr et al., 2011; Poeplau and Don, 2015; Veloso et al., 2019). Cover crops include a wide range of crops grown for restoring soil health, for pasture, or for green manure. Cover crops are terminated either by mowing, applying herbicides, or winter-killed by naturally occurring frost. The decomposing plant material, referred to here as crop residue, is left on the field to cover the ground or tilled into the soil (Sarrantonio and Gallandt, 2003). Retention of crop residue is known to help with moisture conservation and preventing soil erosion caused by snow melts (Paulitz, 2006). Tillage and residue management also plays an important role in soil health by affecting the soil structural stability and potential risk of increasing pathogen inoculum (Paulitz, 2006). Conservation agriculture is a type of soil management strategy that involves reduced tillage cropping systems with minimal soil disturbance and retention of crop residue and is primarily used as a soil and water conservation technique (Chekali et al., 2019; Flower et al., 2019).

1.4.1 Benefits of cover cropping

Economically important factors such as yield, and crop quality are greatly affected by abiotic and biotic environmental stressors (Kanianska et al., 2016). Major abiotic factors in the soil include moisture levels, salinity, availability of different micronutrients, availability of usable organic

matter, and soil structure, whereas biotic factors include pests, such as weeds, insects, pathogenic microorganisms, and small animals (Jatoe et al., 2008; Kanianska et al., 2016; Mann et al., 2019). Maintaining a balanced environment, with minimal stressors, is crucial to improving agricultural productivity. Recently, research has focused on the use of cover crops to improve agricultural productivity, such as through alternative pest management strategies and a way to reduce the amount of synthetic inputs in agriculture (Wittwer et al., 2017; Peralta et al., 2018). Increasing the biodiversity of crops in rotational cropping systems has been shown to promote the disease-suppressive nature of the soil microbial community and help reduce pathogen load (Peters et al., 2003; Peralta et al., 2018).

Agricultural management practices associated with cover crop residue, and tillage greatly influence crop quality and yield (Peters et al., 2003). Long-term global meta-analysis studies have shown that cover crop residue left in fields has the potential to increase soil organic matter content and that proper management can be used as an effective climate change mitigation strategy (Poeplau and Don, 2015). Crop residue left in the field has also been found to help reduce evaporation and decrease soil erosion (Meyer et al., 2020).

1.4.2 Drawbacks of cover cropping

Use of cover crops in Canadian climatic conditions poses an obvious drawback. As cover crops are not harvested, the growers do not gain any profits in one year (Snapp et al., 2005). However, the benefits of growing cover crops such as improved soil health, reducing input costs of fertilizers, and potential yield boost for subsequent crops, may offset the economic loss (Evers et al., 1997; Plaza-Bonilla et al., 2017). The retention of crop residue from cover crops has important benefits by raising soil organic matter but can also act as a nutrient source for

pathogenic microorganisms such as *Rhizoctonia solani*, and different *Fusarium* spp. complexes, hence leading to increased disease incidence (Flower et al., 2019; Chekali et al., 2019). As such, there is contradictory information about how conservation agricultural management can impact disease in subsequent years (Chekali et al., 2019).

1.4.3 Cover crops selected for study

Different crop groups are categorized and known for a varied set of beneficial effects although the crop species may not be phylogenetically closely related (Fig. 1.2). Beneficial effects of certain cover crop groups such as legumes and brassicas have been recognized and studied for a long time, as far back as the 5th century BC (Sarrantonio and Gallandt, 2003). However, little information is available about some individual crops such as phacelia (*Phacelia tanacetifolia* Benth.), especially with regards to their use as cover crops in no-till systems. A brief summary of the known beneficial effects of the different cover crops used in this project is described in this section.

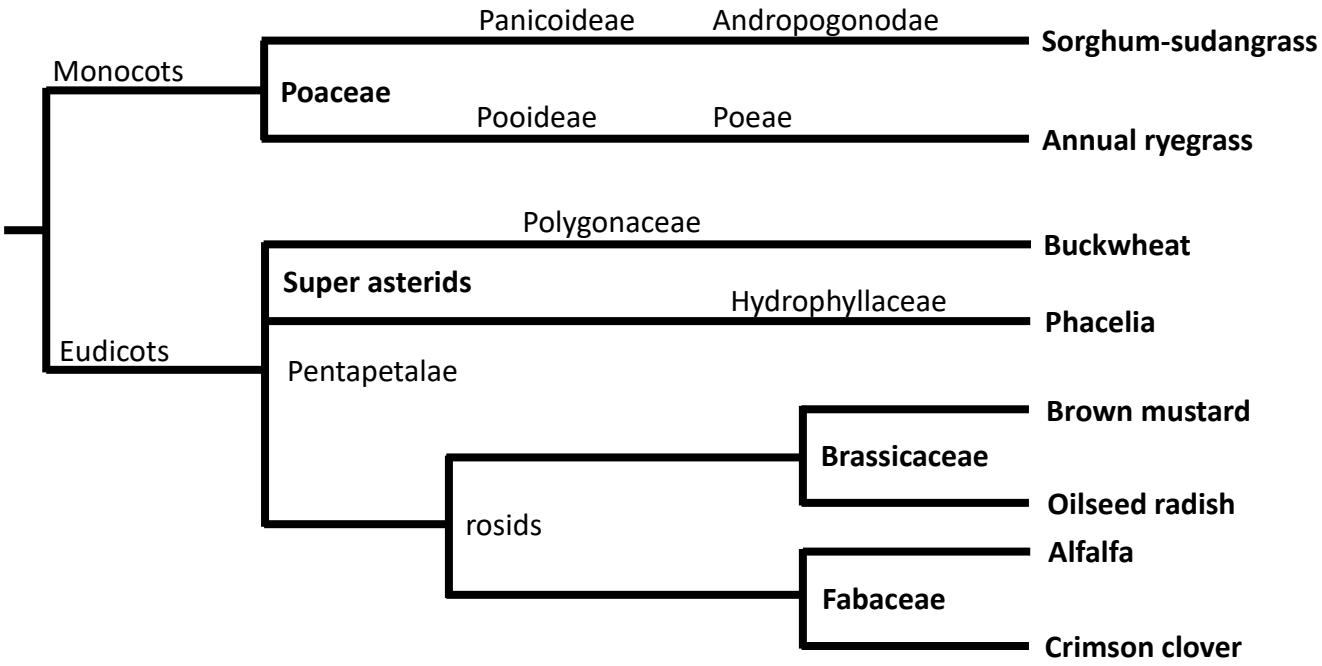

Fig. 1.2 Phylogenetic relationship between cover crop species studied in this project.

1.4.3.1 Fabaceae

Fabaceae cover crops, commonly referred to as legume cover crops, are known to work with root-associated bacteria to convert atmospheric nitrogen into a usable form and increase the available soil organic carbon content (Phillips, 1980; Veloso et al., 2019). *Fabaceae* cover crops were shown to significantly reduce nitrogen fertilizer and pesticide requirement (Corak et al., 1991; Duc et al., 2015; Plaza-Bonilla et al., 2017). The two legume crops selected for this study were alfalfa (*Medicago sativa* L.; variety CRS 1001) and crimson clover (*Trifolium incarnatum* L.; variety Common #1) (Fig 1.2). Alfalfa, with its deep roots, is known to reduce soil compaction, which can be an issue with no-till cropping systems (Meek et al., 1990). The rhizomatous alfalfa variety CRS 1001 was developed for its tolerance to extreme climatic conditions such as drought, and flooding. Alfalfa variety CRS 1001 produces underground

stems, also referred to as rhizomes, which can branch out underground and produce new plants (Bélanger et al., 2017). Being the only perennial cover crop in this study, it has a high potential to reappear in the subsequent year as a weed, especially in no-till systems. Crimson clover is an annual crop, which grows rapidly in early spring, providing abundant ground cover. Its vibrant scarlet flowers attract beneficial insects such as honeybees (*Apis* spp. (L.)) and predatory insects such as *Orius tristicolor* (White), *Geocoris punctipesi* (Say) (Tillman et al., 2004).

1.4.3.2 Brassicaceae

Brassicaceae cover crops, commonly known as brassicas, are known for their ability to produce isothiocyanates (ITC), as reviewed by Stoewsand (1995). ITCs are secondary metabolites produced from the hydrolysis of glucosinolates, which are toxic compounds known to reduce population of pests including wireworms (Coleoptera: *Elateridae*), weeds, and microbial pathogens, present in the soil (Stoewsand, 1995). ITCs are known to inhibit mycelial growth of soil-borne pathogens, acting primarily as fungi-static compounds unless present in high concentrations (Smolinska et al., 2003). The biofumigant effects of *Brassicaceae* cover crops are most effective when incorporated as green manure, as they can increase soil nutrient content, and release ITCs to supress soil-borne pathogens and pests (Brown and Morra, 1995; Cohen et al., 2005). For example, crop rotations including *Brassicaceae* cover crops were shown to reduce disease in important cash crops caused by soil–borne pathogens including *Rhizoctonia solani*, *Phytophthora* spp., *Pythium* spp., *Scelrotinia* spp., and *Fusarium* spp. (Larkin and Griffin, 2007). However, not all *Brassicaceae* produce the same type of ITCs and not all pathogenic fungi are susceptible to all types of ITCs. Smolinska et al., (2003) found that pathogenic fungi such as *F. oxysporum* are better controlled with allyl and ethyl ITCs. Brown mustard (*Brassica juncea* L.;

cv. Centennial) and oilseed radish (*Raphanus sativus* L.) were the two cover crops from the *Brassicaceae* family used in this study (Fig 1.2). Brown mustard was found to produce higher concentrations of ethyl ITCs in comparison to oilseed radish (Smolinska et al., 2003).

1.4.3.3 Poaceae

Grasses, from the family *Poaceae*, when grown in rotation with plants from other crop groups, have been shown to significantly reduce soil-borne diseases by decreasing the abundance of certain pathogens and increasing biodiversity of other microorganisms, thereby breaking disease cycles (Larkin et al., 2011; Lawes et al., 2013). Grasses are taxonomically distinct from the other crop groups studied in this project as they are monocotyledons, whereas the other crops are dicotyledons (Fig 1.2). The main difference between the two is that monocotyledons have fibrous root systems that spread in the top layer of the soil, whereas dicotyledons have taproots that extend deeper into the ground (Clark and Barraclough, 1999). As such, the access to resources and susceptibility to pathogens are different between the two groups. Annual ryegrass (*Lolium multiflorum* Lam.) and sorghum-sudangrass a hybrid of *Sorghum bicolor* (L.) Moench x *Sorghum Sudanese* (P.) Stapf, were the two grass cover crops selected for this project (Fig 1.2).

Annual ryegrass is a small grass crop in the subfamily *Pooideae* (Fig 1.2). It is known to establish quickly and is suitable for no-till systems (Cuomo and Blouin, 1997). It has a highly branched fibrous root system, which increases soil structural stability, and absorbs large amounts of excess nitrogen, thereby reducing potential for leaching, as stated in the review by Evers et al. (1997). When left in the field as crop residue, it also increases soil organic matter content (Evers et al., 1997).

Sorghum-sudangrass is a tall grass crop in the subfamily *Panicoideae*, with fibrous roots which can significantly increase soil organic matter, and soil structural stability (Sattell et al., 1998). Unlike all the other cover crops studied in this project which are C3 plants, sorghum-sudangrass is a C4 plant (Ning et al., 2020). As a C4 grass, it is known to be more drought tolerant, which is important to consider for crop rotation, as climate change leads to increased incidence of extreme weather events (Schittenhelm and Schroetter, 2014; Ning et al., 2020). Sorghum-sudangrass is also known to produce phytoinhibitory chemicals such as *p*-hydrozybenzoic acid and *p*-hydroxybenzaldehyde which play a major role in weed suppression (Weston et al., 1989). Interestingly, *p*-hydrobenzoic acid has been found to influence soil microbial communities and has been reported to inhibit the growth of the pathogenic fungi *F. oxysporum* (Wu and Wang, 2006; Liu et al., 2011; Zhou et al., 2012). Wu et al. (2009), found that mycelial growth and conidia germination in *F. oxysporum* was suppressed when grown in liquid media amended with varying concentrations of *p*-hydrobenzoic acid.

1.4.3.4 Forbs (Super-asterids)

A forb is defined as a broadleaf flowering plant that does not fit into the above-mentioned three major crop groups. In this study, the two crops categorized as forbs include buckwheat (*Fagopyrum esculentum* Moench.) and phacelia (*Phacelia tanacetifolia* Benth.), which can be phylogenetically classified as *Super-asterids* (Fig. 1.2). Buckwheat is a pseudo-cereal, primarily used as a cover crop for its ability to grow quickly, increase organic matter, and improve soil structural stability (Small, 2017). It is also reported to suppress weeds through secretion of allelopathic chemicals such as, 4-hydroxyacetophenone (Kalinova et al., 2007; Szwed et al., 2019). Buckwheat plays an important role in solubilisation of inorganic phosphorus, making it

bioavailable to subsequent crops; however, the mechanism is still unclear (Teboh and Franzen, 2011). Incorporation of this crop as green manure has been found to suppress seedling blight and early root rot caused by *Rhizoctonia solani* and *Pythium* spp. (Abbasi et al., 2019). Recent studies found a promising use of buckwheat cover crops to reduce wireworm population, known to cause significant damage and yield loss of important cash crops such as potatoes (Noronha, 2011; Mills et al., 2019).

Phacelia is an herbaceous crop which produces bright purple flowers, primarily used to support pollinator populations (Williams and Christian, 1991). This is a fast-growing crop with abundant foliage, thereby suppressing weeds, and increasing organic matter through accumulation of biomass (Stivers-Young, 1998). Williams and Christians (1991) found that variability in flowering date allowed for phacelia to act as a forage plant for honeybees (*Apis mellifera* L.) and bumble bees (*Bombus* spp. (Latreille) and *Psithyrus* spp.) for a longer period. Phacelia is not closely related to other agricultural crop species, therefore potentially breaking disease cycles by acting as a non-host to a wide-range of soil-borne pathogens (Fig. 1.2).

1.4.4 Multi-species crop mixes

Growing multi-species crop mixes is often used in attempt to harness all the positive effects of the different crops grown (Fox et al., 2020). Planting multi-species crop mixes also reduces the risk of poor establishment of any one crop due to less-than-ideal environmental conditions. Aside from the eight individual cover crops, three crop-mixes were also studied in this project, including buckwheat + crimson clover (Mix-1), phacelia + brown mustard (Mix-2) buckwheat + crimson clover + brown mustard (Mix-3). Buckwheat grown along with crimson clover would be expected to have better weed suppressive ability, retention of soil organic matter, and increased

soil nitrogen fixation, compared to growing either crop individually. Similarly, Mix-3 would be expected to have increased soil-borne pathogen and pest suppression as well as the other benefits. As brown mustard does not have abundant biomass it would not add anything to the soil organic matter content when grown alone but Mix-2 would mitigate that issue. Furthermore, increasing crop diversity through intercropping or crop rotation has been shown to increase microbial diversity (Lange et al., 2015). This increases microbial community evenness, thereby potentially suppressing pathogen population through competition for resources. However, there is little research looking at the effects of multi-species crop mixes used a cover crops on root disease in subsequent years, especially in no-till cropping systems.

1.4.5 Role of cover crops in FRCR management

Certain cover crops have been used as bioactive plant products in disease suppressive soil management studies (Stoewsand, 1995; Alam et al., 2018). As mentioned earlier, it has been well established that ITCs produced by several Brassicaceae species have biofumigant effects that can reduce overall microbial abundance in the soil, including pathogens such as *Fusarium* spp. (Cohen et al., 2005). Manici et al. (1997), found that some Brassicaceae species have pathogen suppressive effects despite low ITC levels. Cohen et al. (2005), observed an increase in nitrifying bacteria in the soil after the use of *Brassica napus* (L.) seed meal as a soil amendment and suggested that the increased oxidation of nitrogen in the soil potentially stimulated plant defense pathways. However, they also noted an increase in pathogenic oomycete species in the genus *Pythium*, after the use of *B. napus* seed meal (Cohen et al., 2005). Similarly, Bełkot and Pięta (2004), found that soybeans planted after phacelia had lower FRCR symptoms and cultured fewer *F. oxysporum* isolates from these soybean roots compared to monoculture soybean. They

associated their results to a shift in soil microbial community to favour more beneficial organisms such as *Trichoderma* spp. (Pers.), associated with growing phacelia (Bełkot and Pięta, 2004). Furthermore, it is known that changes in soil microbial composition as a result of conservation agriculture and long-term crop rotation can lead to an increase in beneficial plant-microbe interactions and not only reduce pathogen load through competition for resources, but also increase plant resistance to pathogen infection (Peters et al., 2003). This further emphasizes the need for more research into understanding the role of plant-microbe interactions in disease suppressive soil systems.

1.5 Role of microorganisms in the soil

1.5.1 Soil microbiome

Various studies have shown that increasing cropping diversity can increase the biodiversity of the soil microbiome, which is the sum of all microorganisms in the soil environment (Venter et al., 2016; Schmidt et al., 2019). The soil microbiome includes fungi, bacteria, protists, and other microorganisms, which can be beneficial, pathogenic, or neutral. Soil microorganisms have a major impact on plant productivity and are mutually affected by the plant and soil environment in which they live (Reth et al., 2005; Cardinale, et al., 2015; Kunert, et al., 2016; Wiegmann, et al., 2019). The soil microbiome has an important role in sustaining soil health by carrying out several important eco-system functions including, nitrogen fixation, carbon sequestration, bioremediation of pollutants, and regulating pest populations, as reviewed by Garbisu and Alkorta (2003) and Chaparro et al., (2012). Anthropogenic activities, especially in terms of agriculture, have a lasting impact on the soil microbiome (Kim et al., 2020; Fox et al., 2020).

However, the resulting impact on soil health, especially in terms of resilience to abiotic and biotic stressors, is highly debated (Kim et al., 2020).

Plants have a strong influence on shaping the soil microbiome (Wortman et al., 2013). Using traditional culturing techniques, Patkowska and Konopinski (2014), compared commonly found bacteria including, *Pseudomonas* spp. (Migula), and *Bacillus s*pp. (Cohn) in the soil after mulching with oats (*Avena sativa* L.), hairy vetch (*Vicia villosa* Roth) and phacelia. They found that all three cover crops increased the population of these organisms in the soil compared to when no cover crops were used (Patkowska and Konopinski, 2014).

Growing diverse crop groups in rotation can increase soil microbial diversity, which is often considered an important indicator of soil health, but the effects of different crops on microbial community structure and function and the resulting impact on soil and plant health is still unclear (Venter et al., 2016; Bainard et al., 2017; Peralta et al., 2018). Bainard et al. (2017), found that increased microbial diversity in terms of the number of microorganisms present is not always an indicator of good soil health. High numbers of plant pathogenic microorganisms can appear as an increase in microbial diversity, while contributing to increased disease and reduced soil health (Bainard et al., 2017). Microbial community composition is possibly a more important factor to consider when assessing soil health (Bainard et al., 2017).

1.5.2 Soil beneficial fungal communities

Arbuscular mycorrhizal fungi (AMF) are one of the most studied beneficial fungal organisms associated with the soil and root environment. As reviewed by Douds and Millner (1999), AMF are known to maintain symbiotic relationships with a wide range of plant species, where they benefit the plant by increasing its below ground surface area to uptake water and nutrients. They

also prevent infection by pathogenic microorganisms by acting as competition for space and resources (Janeeshma and Puthur, 2020). As such, AMF play an integral role in reducing the impact of abiotic and biotic stress in plants (Munkvold et al., 2004; Janeeshma and Puthur, 2020). AMF primarily belong to the subphylum Glomeromycotina and can be found as endophytes in most terrestrial plants (Munkvold et al., 2004; Thonar et al., 2012). Plant health is known to positively correlate with the presence and abundance of AMF (Munkvold et al., 2004).

The soil is also home to fungal biocontrol agents which are fungi that have antagonistic effects against pathogenic microorganisms and other pests, through various biological or chemical mechanisms. Biocontrol agents have been extensively studied for their potential use as seed treatments. For example, *Clonostachys* spp. has been shown to have antagonistic effects against various important plant pathogens including *Fusarium* spp. (Jensen et al., 2000; Demissie et al., 2020), *Bipolaris sorokiniana* (Knudsen et al., 1995), *Botrytis cinerea* (Pers.) (de Morais et al., 2019), and *Pythium* spp. (Xue, 2003). *Clonostachys rosea* is a fungal biocontrol agent from the family *Bionectriaceae,* that is known to survive in the soil as chlamydospores, as well as colonize plant tissue (reviewed in Sun et al., 2020). It is known to attack plant pathogenic fungi by secreting cell-wall degrading enzymes and parasitizing the fungi (Sun et al., 2020). *C. rosea* f. *rosea* has been patented as a seed treatment for its use against *Botrytis cinerea*, the causal agent of grey mold disease in strawberries (de Morais et al., 2019). *C. rosea* is also known to parasitize insects and nematodes (Sun et al., 2020).

Entomopathogenic fungi, such as *Beauveria* spp. (Vuill.) and *Metarhizium* spp. (Sorokīn), are another type of biocontrol agent that cause disease in insects (Samson et al., 2013). They play an important role in controlling insect pests that affect plant productivity (Meyling and Eilenberg, 2006). Entomopathogenic fungi primarily act by parasitizing the insects

and secreting chemicals that paralyze and kill insects (Shah and Pell, 2003). *Beauvaria bassiana* ((Bals.-Criv.) Vuill.) are marketed for controlling aphids (Superfamily Aphidoidea (Geoffroy)), thrips (Order: Thysanoptera (Haliday)), whiteflies (Family: Aleyrodidae), and several other insects that affect field crop production (Meadow et al., 2000). Ansari et al. (2009), found that *Metarhizium anisopliae* was effective in controlling wireworms in laboratory assays.

1.5.3 Soil beneficial bacterial communities

The most common group of beneficial soil bacteria studied are plant growth promoting rhizobacteria (PGPR), which are often closely associated with root tissue. Olanrewaju et al. (2017), mentioned in their review that PGPR mechanisms include secreting chemicals directly which promote plant growth, making nutrients more bioavailable, inhibiting pathogenic microorganisms by producing antibiotic compounds, and by triggering plant defense response through quorum sensing. Noreen et al. (2012), found that some *Pseudomonas* spp. are known to produce auxins and other enzymes that played a role in increasing root and shoot length. Several genera of PGPR, such as *Azospirillum* spp., are known to solubilize inorganic phosphorous by synthesizing organic acids (Rodriguez et al., 2004). Nitrogen-fixing bacteria, the most studied PGPR, are known to maintain a symbiotic relationship with legume roots (El-Meleigi et al., 2017). *Bradyrhizobium japonicum* ((Kirchner) Jordan), is the most abundant species found in this group (Ren et al., 2019). Bacterial communities can also prime plant defense against abiotic and biotic stressors by secreting compounds such as flavonoids (Mabrouk et al., 2018). Some bacteria, such as certain species within genus *Pseudomonas*, have also been shown to improve soil health through biodegradation of xenobiotic or toxic compounds (Jain et al., 2005).

1.5.4 Plant pathogens

The soil environment is also home to many pathogenic microorganisms that can cause disease when the appropriate host and environment are present. Plant diseases are caused by several different types of organisms including bacteria, fungi, oomycetes, nematodes, protists and other infectious agents such as viruses and viroids (Strange, 2003; Scholthof et al., 2011; Dean et al., 2012; Mansfield et al., 2012; Jones et al., 2013; Kamoun et al., 2015; Schwelm et al., 2018).

Soil-borne pathogens generally cause seedling and root disease in associated crops. Bacterial pathogens tend to be opportunistic and primarily cause disease when there is tissue damage caused by insects, small animals, or wind and rain (Wei et al., 2019). *Pectobacterium carotovorum* ((Jones) Waldee), is a bacterial pathogen found in the soil, known to cause disease in a wide range of cash crops, including blackleg in potatoes, stalk rot of corn, and stem rot of tomato (Sabet et al., 1964; Czajkowski et al., 2009; Rosskopf and Hong, 2016). *Streptomyces scabies* (Lambert & Loria) causes common scab on potatoes, which makes them unmarketable (Goyer et al., 1998). *S. scabies* is also known to produce phytotoxin thaxtomin A, which inhibits seed germination (Leiner et al., 1996).

Fungal pathogens such as *Fusarium* spp., *B. sorokiniana*, and *Rhizoctonia solani*, all causes root rot in a wide range of crops leading to some of the most significant economic losses (Chakraborty et al., 2006; Bonanomi, et al., 2018; Wei, et al., 2019). *Fusarium* spp. are one of the major root disease pathogens in Canada, with species causing yield loss and reduced crop quality in a wide range of host crops, including barley and soybean (Carter et al., 1988; Zhang et al., 2013). Soil-borne pathogens may be manipulated to have beneficial side-effects. For example, some fungal pathogens play an important role in reducing plant competition by causing disease in weed plants (van der Putten and Peters, 1997; Schnick et al., 2002). This is another

reason why rotations with different crop groups would be more beneficial when compared to rotation with crops of the same group.

Oomycetes are fungal-like organisms that have spores, called zoospores, which are motile in water. *Phytophthora infestans* ((Mont.) de Bary) is the infamous oomycete pathogen that causes potato late blight, which led to the Irish Potato Famine in 1840s (Kamoun et al., 2014). *Phytophthora sojae* (Kaufm. & Gerd.) is known to cause damping-off and stem rot of soybeans, leading to annual losses of over a million US dollars, worldwide (Tyler, 2007; Kamoun et al., 2014). *Pythium ultimum* (Trow) is another oomycete pathogen that causes damping-off and root rot in a wide range of crops including barley and soybean (Kamoun et al., 2014).

Plant parasitic nematodes also cause considerable economic losses to a wide range of crops. A review by Jones et al. (2013), suggested that there are approximately 4100 known species of plant parasitic nematodes, that cause a total global loss of more than $80 billion (USD) annually. Some economically important representatives include *Meloidogyne* spp. (Göldi), which causes root rot in a wide range of vascular plants, *Heterodera glycines* (Ichinohe), which causes soybean cysts, and *Pratylenchus* spp. (Filipjev & Schuurmans Stekhoven), which causes root lesions in cereal crops (Jones et al., 2013).

Protists are another group of plant pathogens found in the soil environment. A review by Schwelm et al. (2018), describes protists as a phylogenetically diverse groups of eukaryotes known to cause economically devastating plant diseases. The most common protists associated with agricultural crops include *Plasmodiophora brassicae* (Woronin), and *Spongospora subterranea* (Wallr.), which are both classified under order Plasmodiophorida. *P. brassicae* is an obligate parasite which causes clubroot disease in almost all Brassicaceae crops (Hwang et al.,

2012). *S. subterranea,* is the causal agent of powdery scab on potato tubers, making the potatoes unmarketable (Schwelm et al., 2018). This protist is known to act as a vector for viruses such as potato mop top virus (genus *Pomovirus* in family Virgaviridae; Tamada and Kondo, 2013).

Insects can also act as vectors carrying disease causing viruses and viroids. Barley yellow dwarf virus is one of the most studied viruses known to cause disease in a wide range of small grains (Oswald and Houston, 1951; Miller et al., 2002). The disease is caused by several strains of viruses classified in the *Luteovirus* genus of family Luteoviridae (Miller et al., 2002). It is primarily transmitted by aphids such as *Rhoplosiphum padi* (L.), *Sitoban avenae* (F.), and *Schizaphis graminum* (Rond.) (Plumb, 1983; Miller et al., 2002). Infection by this virus can lead to stunted growth and significant loss of yield. Reducing the population of aphid vectors is the primary management strategy for controlling these viral strains (Miller et al., 2002). Soybean mosaic is another economically devastating disease caused by viruses in the genus *Potyvirus,* in family Potiviridae (Hajimorad et al., 2018; Widyasari et al., 2020). These viruses are also transmitted by aphid vectors as well as contaminated seeds (Hajimorad et al., 2018). The host range is restricted to six different plant families including Fabaceae, and infection can lead to leaf mottling, stunted growth, and plant death (Hajimorad et al., 2018; Widyasari et al., 2020).

Viroids are unencapsulated pieces of RNA, which are also transmitted primarily by insect vectors (Strange, 2003). The term viroid was first used to describe the causal agent of potato spindle tuber disease in 1971 (Diener, 1971). Since then, viroid-based diseases have been identified in several other crops such as tomatoes and cucumber (Diener, 2012)

The soil microbial communities are interconnected, and they are influenced by interactions with the surrounding environment, as described in a review by Chaparro et al. (2012). As such, management targeting one specific pathogen or group of pathogens may lead to

different problems caused by a shift in the microbial composition. This emphasizes the importance of understanding the impact of agricultural management practices on the soil microbiome.

1.6 Studying microbiomes

1.6.1 Culture-dependent approach

It may take multiple steps to achieve a pure culture of the target organism when dealing with soil and other environments that host millions of different species (Yang, et al., 2018). The traditional approach of culturing microorganisms in the laboratory using selective media is still widely used to study microorganisms (de Almeida Lopes et al., 2016). This is an integral step for isolating pathogens and endophytes from field samples and identifying causal agents of disease to satisfy Koch's postulates (Koch, 1877; Byrd and Segre, 2016). Once organisms have been isolated and cultured, they can be identified using morphological indicators such as type of resting structures. However, this process may lead to changes in phenotype through mutations and epigenetic changes in gene expression and can deter certain survival mechanisms used by the organism in nature (de Almeida Lopes et al., 2016; Smith et al., 2018).

Some organisms are known to be slow growing and can take months to grow under normal laboratory settings. Various plant pathogens such as *Blumeria graminis* f. sp. *tritici* ((DC.) Speer), which causes powdery mildew of wheat, are biotrophic and cannot be grown without the presence of a live host (Hückelhoven and Panstruga, 2011). In addition, according to "The Great Plate Count Anomaly" and other related studies, only 1% of all microorganisms present in nature can be cultured in-lab using the current protocols (Staley and Kanopka, 1985; Locey and Lennon, 2016).

Several microscopic diagnostic structures have been recorded for morphological identification of different species. For example, Leslie and Summerell (2008), describe techniques for isolation and characterization of different species in the genus *Fusarium*. Cultures can be grown on selective media under different conditions to produce the different diagnostic structures (Leslie and Summerell, 2008).

1.6.2 Molecular approach

Several culture independent microbial profiling techniques have been developed for studying microbial communities in environmental samples (Osborn et al., 2005). Genomic fingerprinting techniques such as quantitative PCR (qPCR) and fluorescence in-situ hybridization (FISH) use primers and probes for amplification of DNA extracted from specific known organisms (Osborn et al., 2005). With species-specific qPCR, absolute abundance of the organism in the sample can be quantified (Lazarevic et al., 2016). This method is especially useful for comparing pathogen load in different soil environments.

After the advent of Sanger sequencing in the 1970s, there has been a boom in phylogenetic analysis of microbial communities (Sanger et al., 1977). Genomes of several organisms have been sequenced and characterized using Sanger sequencing. This together with Carl Woese' proposed method of using primers from housekeeping genes such as the 16S rRNA gene also referred to as "reference genes" or "barcoding genes", to sequence and identify different organisms, has become one of the standard protocols for molecular identification of microorganisms (Woese and Fox, 1977). DNA templates can be extracted from cultured organisms or environmental samples, amplified using primers for barcoding genes or specific target species, sequenced, and identified by matching the sequence to a previously established

database. Barcoding genes have both regions which are conserved among several species, and hypervariable regions which differ between different taxa (Fig. 1.3). Internal transcribed spacer (ITS) regions and 16S ribosomal RNA (16S rRNA) are commonly used to analyze fungal and bacterial communities, respectively (White et al., 1990; Schoch et al., 2012; Starke et al., 2014). ITS1 and ITS2 are the hypervariable regions present between eukaryotic large ribosomal subunits 18S, 5.8S, and 28S (Fig. 1. 3), with a sequence length ranging from 260 bp to ~1800 bp (Yang et al., 2018). The 16S rRNA gene contains nine hypervariable regions referred to as V1-V9 (Fig. 1.3), with an average sequence length of approximately 1490 bp (Starke et al., 2014). These genes can be amplified using conventional PCR, using the primers listed in Fig. 1.3 and sequenced (Hong, et al., 2015). Primer sequences commonly used to study fungi are ITS-1F: CTTGGTCATTTAGAGGAAGTAA and ITS-2: GCTGCGTTCTTCATCGATGC (Op De Beeck et al., 2014). For amplification of the whole16S rRNA gene to study bacteria, primer sequences are 27F: AGRGTTYGATYMTGGCTCAG and 1492R: RGYTACCTTGTTACGACTT (Callahan et al., 2019). Furthermore, the sequences from various organisms can be aligned to assess their phylogenetic relationship.

Fusarium spp. are often identified by sequencing the ITS1 and ITS2 regions, as well as the translation elongation factor (Tef-1α) gene which is referred to as a secondary barcode (Geiser et al., 2004; Leslie and Summerell, 2008). Geiser et al. (2004), developed a web-based database specifically curated for identification of *Fusarium* spp., using these sequences.

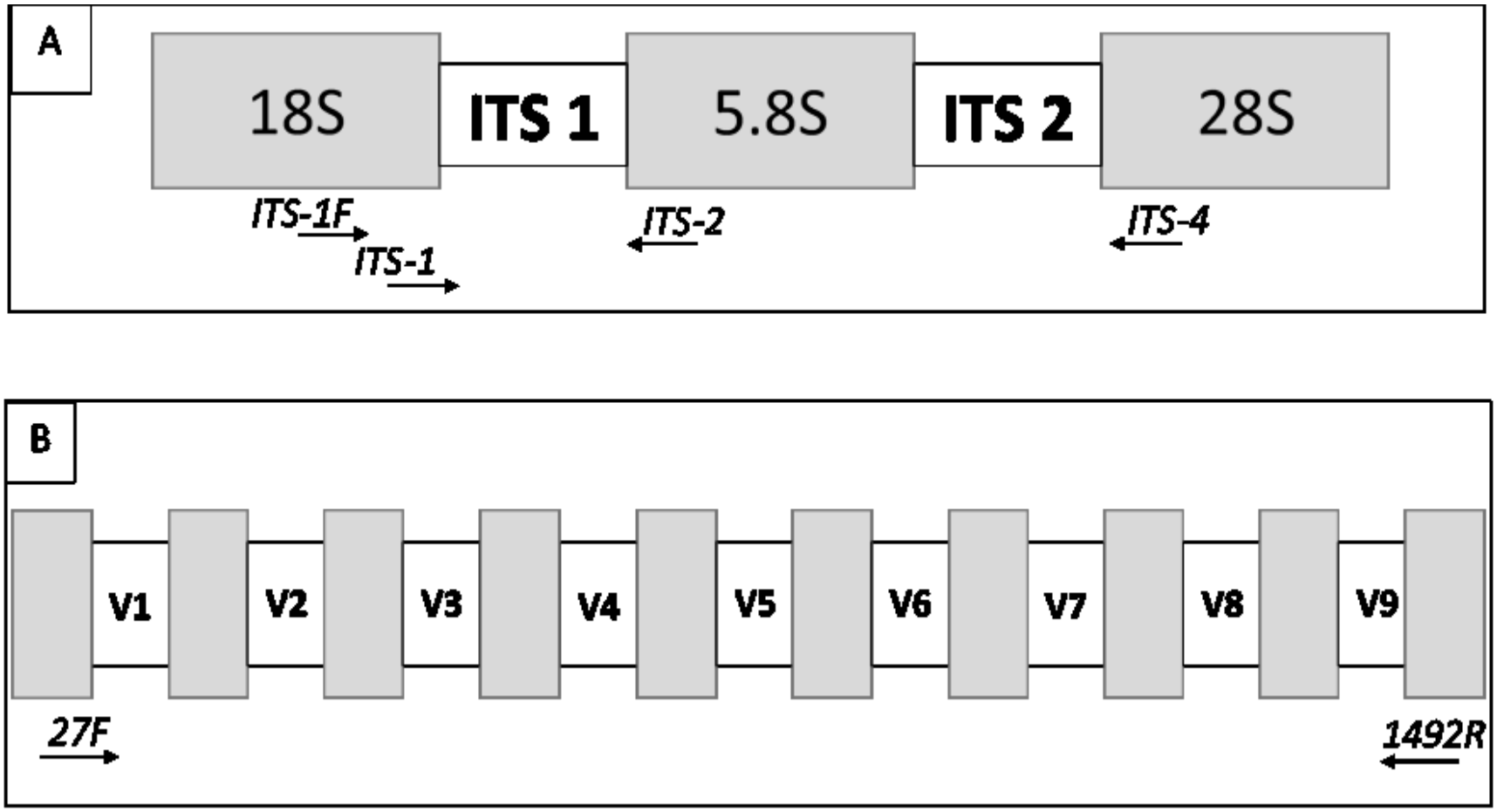

Fig. 1.3 A – Diagram of hypervariable internal transcribed spacer regions ITS1 and ITS2 (in white), between eukaryotic large ribosomal subunits 18S, 5.8S and 28S (in gray). B – Diagram of hypervariable regions V1-V9 (in white) and conserved regions (in gray) within the 16S ribosomal RNA gene. Primers used to amplify hypervariable regions are italicized with arrows depicting their direction.

1.6.3 Massively parallel sequencing

Recent advances in technology have paved the way for high throughput analysis of genetic material collected directly from the environment, often referred to as metagenomics. Metagenomics studies use massively parallel sequencing to characterize communities within the soil microbiome with limited bias. There are two major types of metagenomics sequencing, including amplicon sequencing and shotgun sequencing as reviewed by Escobar-Zepeda et al. (2015). Amplicon sequencing can be used to selectively identify a subset of microorganisms in a given environmental DNA sample. This technique involves amplifying barcoding genes of all the organisms in the environmental DNA sample, sequencing the amplicons, and matching the sequences to an appropriate database to identify the organisms that are present (Lundberg et al., 2013; Escobar-Zepeda et al., 2015). The individual organisms can be identified based on the difference in sequence of hypervariable regions (Lundberg et al., 2013). Alpha diversity is defined as the number of different organisms in a sample (richness) and how evenly their abundance is distributed (evenness) (Whittaker, 1960). Functional and network analysis of microbial communities in any given environment is better accomplished using shotgun sequencing results (Escobar-Zepeda et al., 2015). Various sequencing platforms have been established for metagenomics sequencing, such as Illumina MiSeq (Illumina Inc, California, USA), and PacBio Sequel (Pacific Biosciences of California Inc., California, USA).

1.6.4 Illumina MiSeq

The Illumina MiSeq platform uses sequencing-by-synthesis technology with fluorescence labelled dNTPs. The platform uses solid-phase bridge amplification where the template is first immobilized on a flow-cell by attaching to an adapter sequence. One nucleotide is incorporated

in each step with a 3' reversible terminator, and the imaged fluorophores record which dNTP was added. The 3' terminator is then removed, and the cycle is repeated. According to a review by Werner et al. (2012), this platform can produce up to 1.5 billion short read-length sequences, that can produce approximately 250 bp reads with the paired-end approach, which provides adequate sequencing depth with lower error rates but not enough information to obtain taxonomic resolution at the species level for bacteria and some fungi (Caporaso et al., 2012).

1.6.5 Pacific BioScience (PacBio)

PacBio uses Single Molecule Real Time (SMRT) sequencing with circular consensus sequences, which allow for long-read length sequencing (Quail, et al., 2012). SMRTbell, hairpin adapters are ligated to each amplicon to produce circular consensus sequences, which can range from 3,000 to 15,000 bp in length. Replication, sequencing, and imaging occurs in zero-mode waveguides, which are nanostructures which allow for fluorescence tagged nucleotides to be added and imaged real-time without the use of reversible terminators (Levene et al., 2003). With PacBio, the whole 16S rRNA gene can be sequenced for all organisms in environmental DNA samples, allowing for more taxonomic resolution. However, classification at species level is still limited due to inadequate reference databases.

1.6.6 Bioinformatics

QIIME (Quantitative Insight Into Microbial Ecology; pronounced "chime") and mothur (spelt in all lowercase as per author's artistic choice) are the two main open-source software used for bioinformatics analysis of metagenomics sequencing data (Schloss et al., 2009; Caporaso et al. 2010). mothur is a highly accessible, open-source, tool used to automate analysis of large

microbial sequencing data files. It was first developed by Dr. Patrick Schloss in 2002 and has been continuously adapted to resolve newer problems that arise with advancements in sequencing technology (Schloss et al., 2009). QIIME is another widely used open-source tool for bioinformatics analysis and visualizations of large datasets (Caporaso et al., 2010). A review of the studies using both software suggested that they provide similar, reproducible results (López-García, et al., 2018). There are also commercially available software packages such as CLC Genomics Workbench Microbial Module (QIAGEN) that incorporates many open-source and proprietary metagenomics software packages.

1.6.7 Taxonomic assignment

The sequenced reads can either be clustered into operational taxonomic units (OTU) based on a predetermined threshold for sequence similarity or be classified as amplicon sequence variants (ASV), which stringently separates sequence that differ even by a single nucleotide (Kopylova et al., 2016; Callahan et al., 2017). There is a lot of controversy about the ideal method for grouping sequences as described by Callahan et al. (2017), however the method of choice greatly depends on analysis of interest. Unlike OTUs, ASVs may allow for taxonomic classification at the species-level but are prone to bias through amplification and sequencing error. When using barcoding genes such as the ITS1 region to study fungal communities, it is not possible to resolve all sequences down to the species level as the sequences may be identical between closely related species. In this case, the OTU clustering method is more ideal, as it allows for adequate filtering of low-quality sequences, without discarding large portions of the raw data (Kopylova et al., 2016). Resolution of taxonomic assignment at the species-level is also highly

dependent on the accuracy of the reference database used (Quast et al., 2012; Nilsson, et al., 2019).

1.6.8 Reference databases

SILVA and UNITE are the most used reference databases for 16S rRNA bacterial sequences and ITS fungal sequences, respectively (Quast et al., 2012; Nilsson, et al., 2019). The SILVA database consists of more than 3 million rRNA sequences, which contain manually curated taxonomic assignments where the sequences are classified using a phylogenetic tree-based process (Quast et al., 2012). UNITE is a publicly available, curated database containing approximately 1 million fungal sequences, which can be matched at 97-100% as OTUs (Nilsson et al., 2019). The OTUs are assigned taxonomic classifications by comparing the representative sequences to the ones in the reference databases which are 97% similar and can then be used for down-stream statistical analysis (Edgar, 2018).

1.6.9 Statistical analysis of sequence data

Amplicon sequencing is generally used to study the structure and composition of microbial communities (Escobar-Zepeda et al., 2015). The Simpson's index and Shannon diversity index are the most used metrics for measuring alpha diversity by comparing the relative abundance of OTUs in a sample (Keylock, 2005). However, these indices are inherently biased by confidence in taxonomic assignment and the accuracy of reference databases. Faith's phylogenetic diversity (PD) uses a phylogenetic tree to define a measure of taxonomically different organisms present in a sample (Faith, 1992). PD also considers common ancestry or evolutionary history between OTUs, therefore defining diversity based on variation in branch lengths on a phylogenetic tree.

This further avoids over-sensitivity of species identification based solely on sequence variation (Faith and Baker, 2006). It is also a better representation of functional diversity if it is assumed that closely related species have similar traits (Faith and Baker, 2006). Furthermore, it is argued that it is not possible to assume normal distribution for statistical analysis of alpha diversity without transforming the dataset, due to the large number of zero values present in an OTU table (Hugerth and Andersson, 2017). Kruskal-Wallis test is a one-way non-parametric analysis of variance which can be used for testing significance when comparing phylogenetic diversity between samples, without requiring normal distribution of sample set (McKight and Najab, 2010; Hugerth and Andersson, 2017).

Beta diversity refers to the difference in community composition between two samples, often measured using dissimilarity matrices and visualized using ordination plots (Whittaker, 1960). Bray-Curtis, Jaccard, and Euclidean are the most common methods for producing dissimilarity matrices (Beals, 1984; Anderson et al., 2006). Recently, weighted UniFrac matrices have become more popular for microbial diversity analysis, as they account for phylogenetic differences (Chang et al., 2011). Permutational Multivariate Analysis of Variance (PERMANOVA) can be used for significance testing of beta diversity measures. PERMANOVA is a non-parametric analysis used for multiple comparison (Anderson, 2001; Hugerth and Andersson, 2017). PERMANOVA uses the pseudo-F statistic, which is the ratio of the variance between sample groups and the variance within sample groups, to test for dissimilarity between samples (Anderson, 2001). Higher pseudo-F statistic values indicate greater dissimilarity between sample groups.

Differential abundance analysis can be used to assess whether specific microorganisms increase or decrease in abundance over time, when associated with different sample types

(Paulson et al., 2013). This provides more in-depth information about whether the increase or decrease in diversity and community composition in a sample will have a positive or negative effect on soil health, especially when considering the abundance of certain bioindicators. Furthermore, the organisms can be matched to functional groups based on taxonomic assignment using programs such as FUNGuild (http://www.funguild.org/) for fungi and FAPROTAX (https://pages.uoregon.edu/slouca/LoucaLab/archive/FAPROTAX/lib/php/index.php?section=Instructions) for bacteria, to assess the impact of the cover crop treatment on eco-system functions provided by the microbial community (Louca et al., 2016; Nguyen et al., 2016).

1.7 Formulation of research goals and hypothesis

Although there is a substantial amount of information on the effects of cover cropping on soil physiochemical properties, little information is available regarding the impacts of cover cropping on the soil microbial communities and their associated ecosystem functions. Kim et al. (2020), conducted the first major meta-analysis on cover cropping effects on the soil microbiome. They suggested that there is need for more research looking at the carryover effects of cover crops on the soil microbial communities, in order to better understand their complex interactions and the potential to improve soil health. The soil microbiome is known to include non-pathogens, beneficial organisms, as well as pathogens that cause plant diseases such as FRCR. The impact of changes in the soil microbial composition on *Fusarium* spp. pathogen load, disease suppressive effects of the soil and the carryover effect on FRCR of barley and soybean planted in subsequent seasons is unknown. The main goal of this study was to explore the influence of commonly used cover crops on the soil microbiome and consequential impact on FRCR in barley and soybean planted the following year. The main hypothesis in this study was that some cover crops will influence FRCR disease incidence and severity in barley and soybean through changes in the bacterial and fungal communities within the soil microbiome. This study will provide a better understanding of changes in microbial community composition and their ecosystem functional profiles as a result of planting different cover crops. Furthermore, the results will contribute to understanding the role of soil microbial communities in FRCR susceptibility in barley and soybean.

1.8 Main hypotheses

1- Bulk soil microbial community structure (alpha diversity) will not change significantly based on cover crops in a single year.

2- FRCR in barley and soybean will positively correlate with *Fusarium* spp. taxa abundance in the field residue and soil.

3- Barley and soybean grown after cover crops that increase the abundance of specific beneficial bacterial and fungal taxa known to suppress *Fusarium* spp. in soil and residue will have reduced FRCR.

4- In a controlled greenhouse study, cover crops that increase abundance of beneficial bacterial and fungal taxa in soil and residue will increase soil disease suppressive potential even under high *Fusarium* spp. pressure.

1.9 Research Objectives

In order to test the above-mentioned hypotheses, fungal and bacterial communities in bulk soil and residue were first characterized through amplicon sequencing. Microbial community metrics including community structure (alpha diversity), community composition (beta diversity), functional groups, and differentially abundant taxa, were determined. This work is addressed in Chapter 2. The work in Chapter 2 was primarily done to determine the effects of selected cover crops on the microbial community metrics, and to define a set of beneficial and pathogenic fungal and bacterial communities that may influence plant health in the subsequent year. FRCR was measured in barley and soybean planted in the subsequent year, and the results were compared to the previously measured community metrics, in order to determine the impact of choice of cover crops on FRCR through changes in the soil microbial communities. Chapter 3 relies on results from Chapter 2 and addresses all four of the above-mentioned hypotheses.

Chapter 2 Research Objective: Assessing effects of selected cover crops on alpha diversity, beta diversity, functional groups, and differential abundance analysis of fungal and bacterial communities using amplicon sequencing of DNA from bulk soil during the cover crop growing season as well as in the subsequent year. The specific hypotheses being tested are as follows.

1. Cover crops will not affect soil fungal and bacterial community structure (alpha diversity) in the subsequent growing season.
2. Choice of cover crop will influence soil fungal and bacterial community composition (beta diversity) by the end of the growing season, and this trend will carry over to the soil in the subsequent year.

3. Different cover crops will have increased abundance of specific fungal and bacterial functional groups.
4. The abundance of fungal and bacterial taxa which may have an influence on plant health in the subsequent year (such as *Fusarium* spp.) will be differentially affected by various cover crops.

Chapter 3 Research Objective: Determining the impacts of specific cover crop treatments on severity of FRCR of barley and soybean in the subsequent growing season using visual rating, molecular analysis of bulk soil and cover crop residue, and a controlled inoculation experiment. The specific hypotheses being tested are as follows.

1. Choice of cover crops will influence FRCR in barley and soybean in the subsequent year.
2. FRCR will positively correlate with pathogen load in the soil and residue.
3. Barley and soybean grown in soil with increased abundance of beneficial microbial taxa will have less FRCR incidence.

Chapter 2 - Choice of cover crop influences soil fungal and bacterial communities in Prince Edward Island, Canada

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2.0 Abstract

Fungal and bacterial soil communities play various roles in agroecosystems and are significantly influenced by agricultural management practices. Currently, little is known about the effects of selected cover crops in no-till systems on soil fungal and bacterial communities. In this study, eight cover crops, three mixed crops, and an unmanaged fallow control were evaluated over two years for their effects on the soil microbiome. ITS1 and16S rRNA amplicon sequencing was performed to characterize fungal and bacterial communities in the soil during the cover crop growing season, and in the subsequent year. Fungal and bacterial alpha diversity significantly increased over time and were different by cover crop in the subsequent growing season. Some fungal and bacterial trophic and functional groups were also affected by crop choice. Fungal pathotroph abundance was positively associated with oilseed radish, alfalfa and phacelia, but negatively associated with sorghum-sudangrass. Beneficial symbiotrophic fungi and nitrification related bacterial functional groups were also associated with sorghum-sudangrass and buckwheat. These findings suggest that choice of cover crops influence the soil microbial community composition and may impact plant health in the subsequent crops.

Keywords: Cover crops, soil microbiome, no-till, amplicon sequencing, oilseed radish, phacelia, alfalfa, sorghum-sudangrass, fungal trophic groups, bacteria functional groups

2.1 Introduction

Crop rotation is vital for preventing soil erosion and increasing soil organic matter in the sandy loam soils of Prince Edward Island (PE), Canada. Cover crops are commonly used in crop rotations as plants grown, but not harvested, for the primary purpose of decreasing soil erosion (Sarrantonio and Gallandt, 2003). Cover crops are also known to improve soil and plant health through interactions with certain microbial communities in the soil (Schipanski et al., 2014). Microbial communities present in agricultural soils are affected by many factors including nutrient and water cycling, pest management, soil quality, and the associated crops (Bartram et al., 2014; Schipanski et al., 2014; Bainard et al., 2017; Mann et al., 2019). Certain crops such as brassicas, including brown mustard (*Brassica juncea* L.) and oilseed radish (*Raphanus sativus* L.), are known to affect microbial diversity through the biofumigation process (Kirkegaard and Sarwar, 1998; Nallanchakravarthula et al., 2021). With increased crop diversity by including diverse crop groups in rotation or using multi-species crop mixes, an increase in microbial biomass and diversity has been reported (Venter et al., 2016; Schmid et al., 2019; Schmidt et al., 2019; Xu et al., 2021). Increased microbial diversity is commonly associated with increased soil health as there will be more multi-functionality, making the soil more resilient to environmental changes (Allison and Martiny, 2008).

The manipulation of the microbial community by growing certain crops has been studied for many decades (Kim et al., 2020). Peters et al. (2003) found that long term crop rotations can increase the disease suppressive ability of the soil and suggested changes in soil microbial communities as potential mechanisms. It was also reported that the choice of cover crops influences the abundance of specific microbial communities (Martínez-García et al., 2018; Bainard et al., 2020). The recent development of high throughput sequencing technologies

allows for more detailed characterization of the soil microbial communities associated with specific crops (Bartram et al., 2014; Bainard et al., 2017; Bainard et al., 2020; Hannula et al., 2020). Metagenomics, including amplicon and shotgun sequencing, of DNA extracted from environmental samples have been used in microbiome research to study microbial communities and their interactions (Caporaso et al., 2012; Schloss et al., 2020). Functional annotation packages such as FUNGuild and FAPROTAX are used to further categorize the amplicon sequencing results into ecologically relevant functional groups and to better characterize the effects of environmental variables on agroecosystem functions (Louca et al., 2016; Nguyen et al., 2016). So far, the influence that choice of cover crops has on soil fungal and bacterial community structure and function in Atlantic Canada has not been studied.

The purpose of this study was to determine how the choice of cover crop affects microbial diversity (alpha and beta diversity) and the functional profiles of fungal and bacterial communities in the soil. We hypothesized that 1) cover crops will not affect soil fungal and bacterial community structure (alpha diversity) in the subsequent growing season; 2) Choice of cover crop will influence soil fungal and bacterial community composition (beta diversity) in one growing season and the changes will carry-over to the subsequent year; and 3) the choice of cover crop will differentially influence the abundance of specific fungal and bacterial functional groups which may potentially affect the health of subsequently grown plants. Using amplicon sequencing of the internal transcribed spacer region (ITS) and 16S ribosomal RNA (rRNA) gene, we show an increased fungal and bacterial alpha diversity over time and a positive association of fungal pathotroph abundance with oilseed radish, alfalfa, and phacelia.

2.2 Materials and Methods

2.2.1 Plant materials

A total of eight cover crops, with two each from grass, forb, legume, and brassica groups along with three cover crop mixtures were chosen for this study (Table 2.1). Cover crops were planted in the first year of trials. In the subsequent year, cash crops barley (*Hordeum vulgare* L.) and soybean (*Glycine max* (L.) Merr.) were planted in each half of the cover crop plots. Cover crop seeds were not treated with fungicides or bacterial inoculum. Both barley and soybean seeds were treated with Vitaflo 280 (Chemtura, Connecticut, USA).

2.2.2 Field trials

The experiments were conducted at the Agriculture and Agri-Food Canada (AAFC) Harrington Experimental Farm (46°20'47.4"N 63°10'25.5"W; PE, Canada) in fine sandy loam soil, classified as Orthic Humo-Ferric Podzol soil (Soil Classification Working Group, 1998). Two duplicated research trials were conducted from 2018 to 2020. Prior to planting cover crops, fields were tilled, and fertility was applied at a rate of 100 kg ha^{-1} $K_2Mg_2(SO_4)_3$ supplemented with 100 kg ha^{-1} NH_4NO_3 in 2018 or 75 kg ha^{-1} urea in 2019. Cover crops were planted in 15.24 cm row spacing in a randomized complete block design with 2m × 10m plots in three replications. In late summer/early fall cover crops were flail mowed, leaving all plant residues in the field. Field trial details are summarized in Table S1 including planting date, harvest date, and sample collection dates.

Table 2.1 Summary of cover crops studied in this project.

Cover crop	Scientific name	Group	Variety	Seeding rate (kg ha^{-1})
Crimson clover	*Trifolium incarnatum* (L.)	Legume	Organic Common #1	15
Alfalfa	*Medicago sativa* (L.)	Legume	CRS 1001	20
Brown mustard	*Brassica juncea* (L.)	Brassica	Centennial	6
Oilseed radish	*Raphanus sativus* (L.)	Brassica	Organic tillage	10
Buckwheat	*Fagopyrum esculentum* (Moench.)	Forb	Mancan	50
Phacelia	*Phacelia tanacetifolia* (Benth.)	Forb	Organic lacy	10
Sorghum-sudangrass	*Sorghum* × *drummondii* (Nees ex. Steud.) Millsp. & Chase	Grass	CFSH-30	40
Annual ryegrass	*Lolium multiflorum* (Lam.)	Grass	Lemtal	20
B + C		Mix-1		$15^{B}+7^{C}$
P + M		Mix-2		$5^{P}+3^{M}$
B + C + M		Mix-3		$15^{B}+7^{C}+3^{M}$
Unmanaged		Control	Fallow	-

The seeding rate for each cover crop in a mix is indicated by the following letters: buckwheat (B), crimson clover (C), brown mustard (M), phacelia (P). The variety for the crops in mixes are same as individual crops.

2.2.3 Sample collection

Bulk soil cores were collected at eight or six random locations inside plots during the cover crop phase or the cash crop phase, respectively. Cores were 2cm in diameter and taken at a depth of 0 to 15cm. Soil cores were collected between the inner rows to avoid confounding effects from neighboring plots at four time points (Table S1). To prevent cross contamination, soil probes were cleaned using 70% ethanol solution or quaternary ammonia. The soil cores from each plot were collected in a plastic bag, hand-mixed to obtain a representative composite sample and stored at -80 ℃ until further processing.

2.2.4 DNA extraction

Soil samples were lyophilized at less than 100 µbar for 24 h using a VirTis Freezemobile 12ES Freeze Dryer (SP Scientific, New York, USA), and then disrupted for 5 min at 1500 rpm with a GenoGrinder (SPEX, Metuchen, New Jersey, USA). Total DNA was extracted from 250 mg of each disrupted soil sample using the DNeasy PowerLyzer PowerSoil kit (QIAGEN, Hilden, Germany). DNA was quantified using a NanoDrop One (Thermo Fisher Scientific, Massachusetts, USA) and samples were run on a 1% 0.5x Tris-borate-EDTA (TBE) agarose gel amended with 1x SYBR Safe DNA Gel stain (Thermo Fisher) to check DNA quality. Gel electrophoresis was conducted at 100 V for 20 min and visualized on E-Gel Imager (Invitrogen, Waltham, Massachusetts, US).

2.2.5 Amplicon sequencing

Library preparation and sequencing was performed at the Genome Quebec Nanuq Sequencing Center (Montreal, Quebec, Canada) and the Integrated Microbiome Resource Center of Dalhousie University (Halifax, NS, Canada) for ITS and 16S sequencing, respectively, following manufacturers' recommended protocols. Paired-end sequencing of the ITS1 region targeting

fungal communities was performed using the Illumina MiSeq PE250 platform at the Genome Quebec Nanuq Sequencing Centre targeting 90,000 reads per sample. Primers used to amplify ITS1 were ITS1F: CTTGGTCATTTAGAGGAAGTAA and ITS2: GCTGCGTTCTTCATCGATGC (Op de Beeck et al., 2014). All samples with less than 50,000 reads were re-sequenced and combined. Bacterial communities were characterized by sequencing the 16S rRNA gene, with the PacBio Sequel platform at the Integrated Microbiome Resource of Dalhousie University using the primers 27F: AGRGTTYGATYMTGGCTCAG and 1492R: RGYTACCTTGTTACGACTT to target the bacterial communities (Callahan et al., 2019). PacBio sequencing targeted 5,000 circular consensus sequences per sample, with an expected length of approximately 1500 bp per sequence. All samples with less than 1,000 reads were re-sequenced and combined.

2.2.6 Bioinformatics analysis

2.2.6.1 Trimming and Quality control

Bioinformatics analysis was primarily performed using the CLC Genomics Workbench Microbial Package version 21.04 (CLC, QIAGEN, Hilden, Germany). For Illumina data, quality trimming removed adapter sequences, low quality sequences with a Phred quality score limit of 0.05 and read ends with more than two ambiguous nucleotides. ITS chimera screening was done based on default settings, with a chimera crossover cost of three and kmer size of six. PacBio reads were quality trimmed to remove adapter sequences, reads with less than 1,300 bp and more than 1,600 bp, read ends with more than eight ambiguous nucleotides and homopolymer G sequences at both 5’ and 3’ ends. Chimeric PacBio reads were removed using a chimera crossover cost of 60 and kmer size of seven.

2.2.6.2 OTU Clustering

Trimmed non-chimeric reads were aligned and clustered into operational taxonomic units (OTUs) with taxonomic differences resolved at 97% sequence identity for ITS reads, and 99% sequence identity for 16S rRNA reads. ITS reads were aligned to the UNITE dynamic database version 8 (February 4, 2020) and assigned taxonomic classification (Nilsson et al., 2019). The SILVA database version 132 SSU Ref NR 99 (Quast et al., 2012) was used to assign taxonomic classification for 16S rRNA reads (Yilmaz et al., 2014). OTUs not assigned a taxonomic classification of target at kingdom level and not classified at phylum-level, were removed from the table. Low abundance OTUs, defined as those with a minimum of 100 reads for ITS and 10 reads for 16S datasets, were removed. The 16S dataset was further filtered to remove any OTUs that were associated with chloroplast or mitochondria. A phylogenetic tree was built using maximum likelihood (Faith, 1992) and OTUs were further aligned using MUSCLE (v3.8.31; Edgar, 2004) with a minimum combined abundance of 10 and maximum number of sequences of 100.

2.2.6.3 Alpha and beta diversity

Alpha and beta diversity were analyzed using CLC Genomics Workbench Microbial Package. Prior to alpha diversity analysis, samples were rarified to the minimum number of reads per sample to include all samples in the analysis. Alpha diversity on the rarified datasets was calculated using phylogenetic diversity index for both bacterial and fungal samples using the corresponding phylogenetic tree, built during OTU clustering (Faith, 1992). Beta diversity for the different variables, including cover crop and soil sample collection time point, were determined using the weighted UNIFRAC dissimilarity matrix (CLC, QIAGEN; Chang et al.,

2011). Weighted UNIFRAC considers the phylogenetic tree branch length as well as the relative abundance of those taxa.

2.2.6.4 Functional and trophic groups

To explore the cover crop effects on predicted fungal and bacterial functional groups, OTUs were classified into fungal trophic groups and bacterial functional groups, using FUNGuild and FAPROTAX, respectively (Louca et al., 2016; Nguyen et al., 2016). FUNGuild uses an online community-annotated database to assign predicted functional trophic groups or guilds to OTUs based on taxonomy (github.com/UMNFuN/FUNGuild).

Similarly, FAPROTAX uses a database constructed from metabolic profiles obtained through shotgun sequencing to assign potential ecosystem functions to OTUs based on affiliated taxonomy (loucalab.com/archive/FAPROTAX). The sum of relative abundance per sample of OTUs assigned to a specific function was used as the proportional abundance value for the functional group. Functional groups to which no OTUs were assigned were not considered.

Differential abundance analysis was conducted using a negative binomial general linear model (GLM) to find organisms that were differentially abundant in cover crop soils with the CLC Genomics Workbench Microbial Package. Likelihood ratio test was used to test significance of OTUs differentially abundant by cover crop. Data for the cash crop time point from both trials was combined to study the carryover effects of previous crops on fungal trophic groups. The differentially abundant fungal OTUs that were common in soil samples collected from the cash crop phase in both trials and that were assigned trophic groups by FUNGuild were grouped. Fold change was calculated by comparing relative abundance of OTUs from each cover crop soil compared to unmanaged soil.

2.2.7 Soil chemical analysis

Changes in soil chemical parameters are known to influence microbial composition (Mann et al., 2019). Soil chemical analysis was performed using a subsample of bulk soil from each time point. Available Mg, K, Ca, P, B, and Cu were extracted using Mehlich-III extraction (Mehlich, 1984) and quantified using inductively coupled plasma mass spectrometer (820MS ICP-MS, Varian, California, USA). Samples collected in 2018 were analyzed by the AAFC regional chemistry lab and samples from 2019 and 2020 were analyzed by the PEI Analytical Laboratories using standard protocols. AAFC regional chemistry lab conducted total N and organic C analysis using the dry combustion method on an automated macro Vario Max Cube analyzer (Elementar Analysensysteme, Hanau, Germany). Conversion factor of 1.72 was used to calculate soil organic matter (OM) content from %C (Van Bemmelen, 1890). Soil pH was also measured using a 1:1 soil/water solution for all soil samples (Hendershot et al., 2008).

2.2.8 Statistical analysis

All statistical analysis was performed using JMP 16 (SAS Institute, North Carolina, USA) unless otherwise stated. Alpha diversity indices at different sample collection time points and cover crop treatments were compared. Datasets were transformed using sinh arcsinh (SHASH) to ensure normal distribution when necessary. Significance was tested by mixed linear model using restricted maximum likelihood (REML) and post hoc Tukey's HSD test at $\alpha \leq 0.05$, with trial, cash crop and replicate considered as random effects. Significance was defined as $\alpha \leq 0.05$, and $\alpha \leq 0.01$ considered highly significant. Cover crop and time point effects on fungal and bacterial community composition in soil were tested for significance using permutation multivariate analysis of variance (PERMANOVA) using the weighted UNIFRAC distance matrices with the CLC Genomics Workbench Microbial Package (Anderson, 2001) and differences were

considered significant at $\alpha \leq 0.05$ and weakly significant at $\alpha \leq 0.10$. Spearman's rank correlation analysis was conducted to compare the relationship between fungal trophic groups and bacterial functional groups. Effects of cover crop selection and sample collection time point on soil chemical parameters, bacterial functional group abundance and abundance of fungal trophic groups, with replicate considered as a random effect, was determined by mixed linear model using REML and post hoc Tukey's HSD test at $\alpha \leq 0.05$. Box-Cox transformations were performed to ensure normal distribution of the data sets. Principle component analysis (PCA) was conducted using least square means of fungal trophic groups and bacteria functional groups, which were significantly affected by cover crop selection in the cash crop phase.

2.3 Results

2.3.1 Sequencing and OTU Clustering

ITS1 sequencing produced a total of 69,005,920 reads from 360 samples. After trimming and chimera screening, a total of 28,407,646 clean reads were clustered into 23,576 OTUs, of which 1,356 OTUs remained after filtering out unclassified and low abundance OTUs. Of the 1,356 OTUs, 752 were predicted based on reference database and 604 were *de novo* annotated. The most abundant fungal taxa identified at family-level was *Plectosphaerellaceae* (Fig. 2.1A). Differences in abundance of fungal OTUs at different sample collection time points was observed at family-level (Fig. 2.1B). An increase in abundance of *Nectriaceae* in the cash crop phase of the second trial was also observed (Fig. 2.1B).

Bacterial communities were characterized by sequencing the 16S rRNA gene resulting in a total of 1,284,705 reads and 1,029,658 filtered and non-chimeric reads, with an average read length of 1451 bp, which clustered into 62,204 OTUs. After filtering based on low abundance and taxonomy, a total of 10,799 OTUs remained, of which 9,967 were predicted based on

reference database whereas 832 were *de novo* annotated. *Bacillaceae* was the most abundant bacterial family in the soil (Fig. 2.2A). Unlike with fungi, bacterial community relative abundance between sample collection time points was highly conserved at the family-level (Fig. 2.2B).

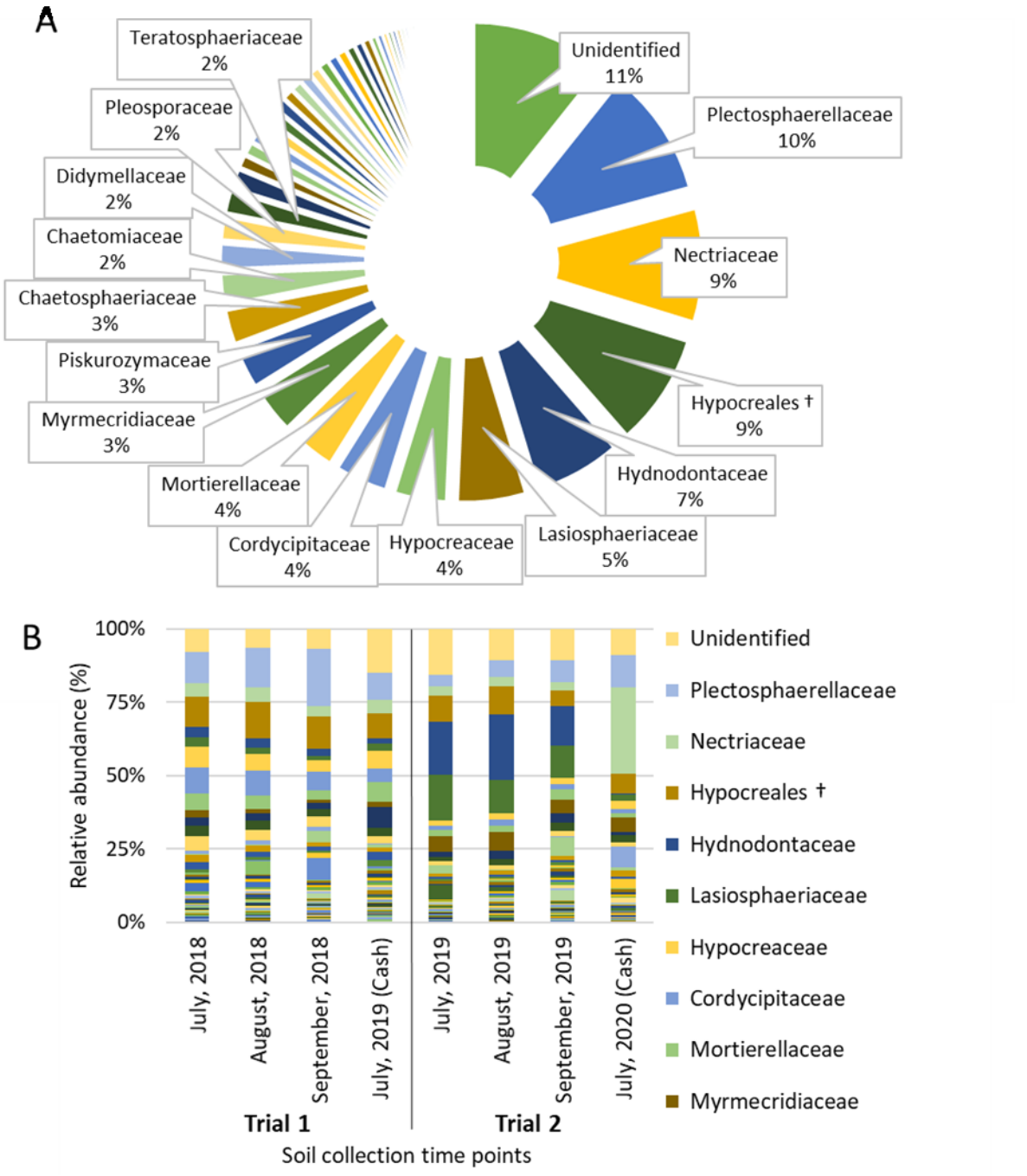


Fig. 2.1 Relative abundance of fungal families in soil samples. (A) Pie chart presenting the average relative abundance of fungal families in all soil samples, n=360; (B) Stacked bar chart presenting the changes in the relative abundance of fungal families over time in both trials. Soil samples were collected at three time points during the cover crop phase (Jul, Aug, Sep), n=36, after which soil samples were collected in July of the cash crop phase, n=72.† represents enigmatic taxa within the highlighted order.

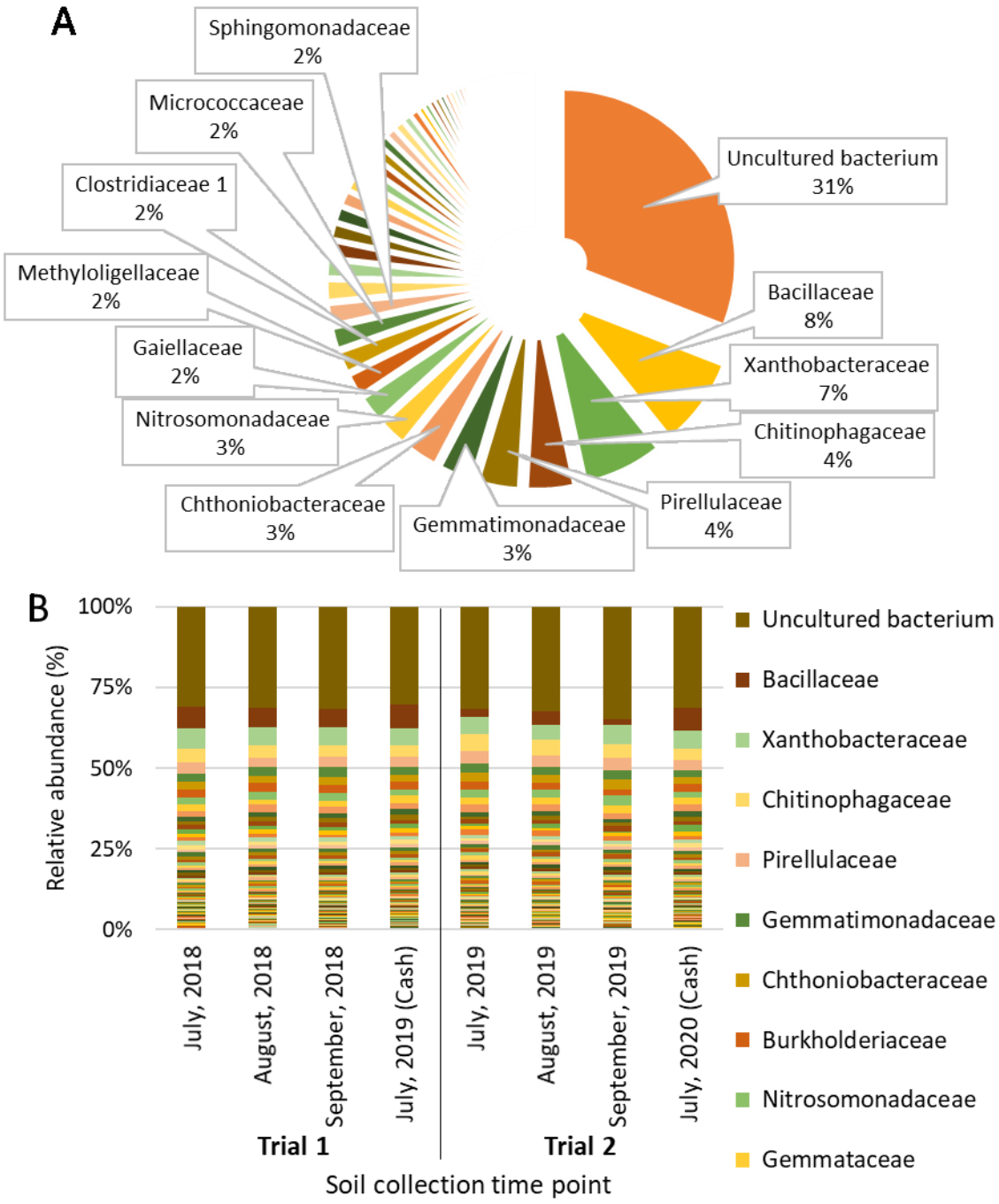


Fig. 2.2 Relative abundance of bacterial families in soil samples. (A) Pie chart presenting the average relative abundance of bacterial families in all soil samples, n=360. (B) Stacked bar chart presenting the changes in the relative abundance of bacterial families over time in both trials. Soil samples were collected at three time points during the cover crop phase (Jul, Aug, Sep), n=36, after which soil samples were collected in July of the cash crop phase, n=72.

2.3.2 Alpha Diversity

Fungal alpha diversity was calculated, with a dataset rarified to 19,729 reads per sample (Fig. S1), using the Faith's phylogenetic diversity index. The dataset was transformed using SHASH on JMP 16, as fungal alpha diversity was not normally distributed. Soil collected from the cash crop phase of the second trial had significantly higher fungal alpha diversity compared to that from the first trial (p-value < 0.001). Fungal alpha diversity slowly increased throughout the growing season and the soil collected in the cash crop phase had significantly higher diversity than the soil from the start of the trial. Statistically significant differences in soil fungal alpha diversity between cover crops were observed in the cash crop phase (Table 2.2). Soil fungal alpha diversity after growing sorghum-sudangrass and Mix-1 was significantly higher than alpha diversity after Mix-2 (Fig. 2.3A).

Bacterial alpha diversity was calculated with the dataset rarified to 824 reads per sample (Fig. S2). Choice of cover crops significantly influenced soil bacterial alpha diversity in the cash crop phase (Table 2.2). Soil after growing alfalfa and crimson clover had the lowest overall bacterial alpha diversity, while soil after growing buckwheat, phacelia, sorghum-sudangrass and Mix-1 had significantly higher bacterial alpha diversity (Fig. 2.3B).

Table 2.2 The effects of cover crop treatments at different time points, and effect of individual sample collection time points, on fungal and bacterial alpha and beta diversity in the soil.

			Alpha Diversity				Beta Diversity			
			Fungi		Bacteria		Fungi		Bacteria	
Variable	Trial	Time point	F	p-value	F	p-value	p-F	p-value	p-F	p-value
Cover crop	1	Jul-2018 [a]	1.11	0.40	0.68	0.74	1.02	0.39	1.12	0.25
		Aug-2018 [a]	0.86	0.59	1.05	0.44	1.09	0.29	1.05	0.40
		Sep-2018 [a]	0.78	0.65	1.57	0.18	**3.28**	**<0.01***	0.98	0.53
		Jul-2019 [b]	1.56	0.14	**2.77**	**0.01**	**2.37**	**<0.01***	**2.19**	**<0.01***
	2	Jul-2019 [a]	1.01	0.47	0.48	0.90	0.76	0.90	0.70	0.96
		Aug-2019 [a]	1.27	0.31	1.87	0.10	0.81	0.77	0.74	0.84
		Sep-2019 [a]	1.86	0.10	0.60	0.81	1.40	0.07	0.97	0.56
		Jul-2020 [b]	**4.85**	**<0.01***	**3.02**	**<0.01***	**2.09**	**<0.01***	**1.48**	**0.01**
Time point	1	2018-2019	2.38	0.07	**2.89**	**0.04**	**17.3**	**<0.01***	**9.59**	**<0.01***
	2	2019-2020	**6.17**	**<0.01***	**3.41**	**0.02**	**38.3**	**<0.01***	**31.6**	**<0.01***

Cover crop effects with p-value ≤ 0.05 are considered significant and are bolded and those with p-value < 0.01 are strongly significant and are highlighted with "*". Superscript "a" indicates time points in cover crop phase and superscript "b" indicates cash crop phase. F-ratio (F) was used to measure effect size for alpha diversity. Pseudo-F statistic (p-F) was used to measure effect size for beta diversity.

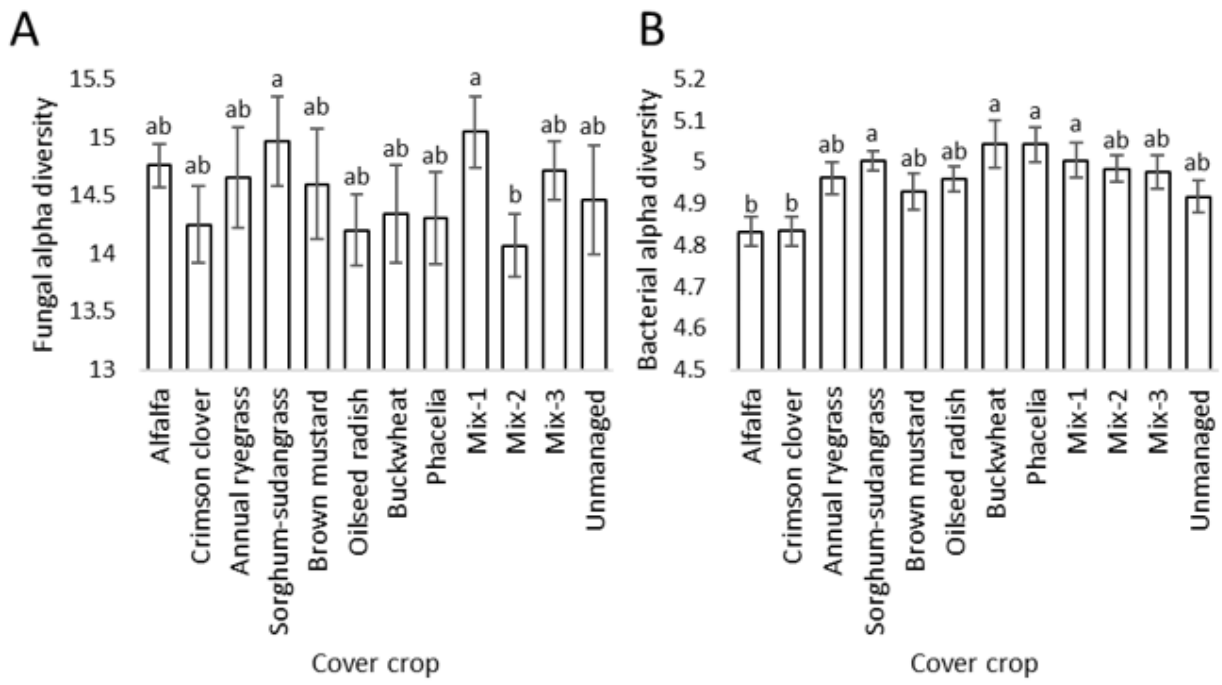


Fig. 2.3 Bar graphs representing average fungal alpha diversity, rarified at 19,729 reads (A) and average bacterial alpha diversity, rarified at 824 reads (B) for the cash crop phase; n=12. Different letters indicate statistical significance. Mix-1: Buckwheat + crimson clover; Mix-2: Phacelia + brown mustard; Mix-3: Buckwheat + crimson clover + brown mustard. Connecting letters for fungal alpha diversity were determined using Tukey's HSD test with sinh arcsinh transformed dataset; p-value ≤ 0.05. Error bars indicate standard error.

2.3.3 Beta Diversity

Beta diversity analysis indicated that fungal communities became increasingly dissimilar between cover crops as the growing season progressed in both trials. By September, the fungal community composition was significantly affected by cover crop and stayed different in the subsequent year (Table 2.2). Fungal community composition between Mix-1 and Mix-3 were conserved in both trials. There were no significant differences between soil fungal community composition between oilseed radish and phacelia in both trials. However, the fungal community composition in soil after growing oilseed radish and phacelia was significantly dissimilar from every other crop in the first trial. In the second trial, oilseed radish and phacelia associated soil fungal communities were only dissimilar from alfalfa and sorghum-sudangrass. Oilseed radish associated fungal community was also dissimilar from that of Mix-2 in the second trial. In the first trial, the fungal community in Mix-2 soil, which was composed of phacelia and brown mustard, was more similar to brown mustard soil compared to phacelia in both the cover crop and cash crop phases. This trend was the same in the cover crop phase of the second trial but was the opposite in the cash crop phase.

Bacterial community composition was significantly affected by cover crop choice in the cash crop phase the following growing season of both trials (Table 2.2). In the first trial, annual ryegrass associated soil bacterial community was significantly different from that of every other cover crop, and the bacterial community associated with alfalfa was significantly dissimilar to that of all other cover crops except crimson clover. However, in the second trial, annual ryegrass associated bacterial communities were only significantly different from that of unmanaged, while alfalfa associated bacterial communities were significantly dissimilar from that of phacelia, oilseed radish and sorghum-sudangrass. Like fungal communities, bacterial community

composition between buckwheat, Mix-1 and Mix-3 were highly conserved in both trials. Bacterial community composition in unmanaged soil was significantly dissimilar from that of alfalfa, crimson clover, annual ryegrass, sorghum-sudangrass, phacelia and Mix-3, in both trials.

2.3.4 Fungal trophic groups

FUNGuild was used to taxonomically parse fungal OTUs into groups corresponding to their predicted ecosystem functions. A total of 950 fungal OTUs were classified into one or more of the three functional trophic groups in FUNGuild; 517 OTUs were classified as pathotroph, 378 OTUs as saprotrophs, and 55 OTUs as symbiotrophs. Fungal OTUs were also subdivided into guilds representing agroecosystem functions performed by the microorganisms. Major guilds included plant pathogens, animal pathogens, fungal parasites and mycorrhizal fungi.

Fungal pathotroph abundance was significantly affected by the choice of cover crop by the end of the growing season in both trials (Table 2.3). In September of both trials, oilseed radish and phacelia soil had the highest pathotroph abundance, and this trend carried over to the subsequent year. In September 2019 (Trial 2), annual ryegrass soil also had high pathotroph abundance. Saprotroph and symbiotroph abundance were significantly influenced by cover crop choice in September of the first trial but not the second (Table 2.3). In September 2018 (Trial 1), Mix-3 soil had the highest saprotroph abundance and sorghum-sudangrass soil had the highest symbiotroph abundance.

All three fungal trophic groups were significantly affected by the choice of cover crop in the cash crop season of both trials. Out of 599 differentially abundant OTUs, 204 were identified as pathotroph using genus level classification in FUNGuild (Fig. 2.4A). These OTUs included several well-known plant pathogens such as *Fusarium* spp. (Link), *Septoria* spp. (Desm.),

Bipolaris spp. (Shoemaker), *Diaporthe* spp. (Nitschke), *Rhizoctonia* spp. (Kühn) and *Colletotrichum* spp. (Corda). The two most abundant *Fusarium* OTUs were differentially affected by cover crops. However, the two OTUs were both higher in alfalfa soil and lower in crimson clover, brown mustard, oilseed radish and Mix-2, compared to the unmanaged soil (Fig. 2.4A). Both *Septoria* OTUs were lower in all cover crop soils than unmanaged except for oilseed radish, where one was higher in abundance compared to unmanaged soil (Fig. 2.4A).

Insect pathogenic fungi and fungal biocontrol agents *Isaria* spp. (Berk.) Lloyd), *Metarhizium* spp. (Sorokin)*, Beauvaria* spp. (Vuil.)*, Clonostachys* spp. (Corda) and *Trichoderma* spp. (Pers.) were also classified as pathotrophs. Three *Clonostachys* OTUs, two *Trichoderma* OTUs and one *Isaria* OTU were among the top 40 most abundant pathotrophs (Fig. 2.4A). *Clonostachys* OTUs were more abundant in alfalfa, crimson clover, sorghum-sudangrass, buckwheat and Mix-2 soils compared to unmanaged, meanwhile they were less abundant or variable in all other cover crop soils (Fig. 2.4A). *Trichoderma* OTUs were consistently more abundant in brown mustard, phacelia and Mix-2 soils compared to unmanaged soil. *Isaria* spp. was less abundant in alfalfa, crimson clover, phacelia and Mix-2 soils, and more abundant in all other cover crop soils compared to unmanaged soil (Fig. 2.4A). A total of 33 differentially abundant OTUs were classified as symbiotroph, including several known arbuscular mycorrhizal fungi such as *Funneliformis* spp. (Walker and Schüßler), *Glomus* spp. (Tul. and Tul.), and *Paraglomus* spp. (Blaszk.) (Fig. 2.4B). Most *Funneliformis* OTUs were more abundant in all cover crop soils compared to the unmanaged soil, except for buckwheat. *Glomus* spp. was only lower in Mix-2 soil, meanwhile *Paraglomus* spp. was lower in all cover crop soils compared to unmanaged.

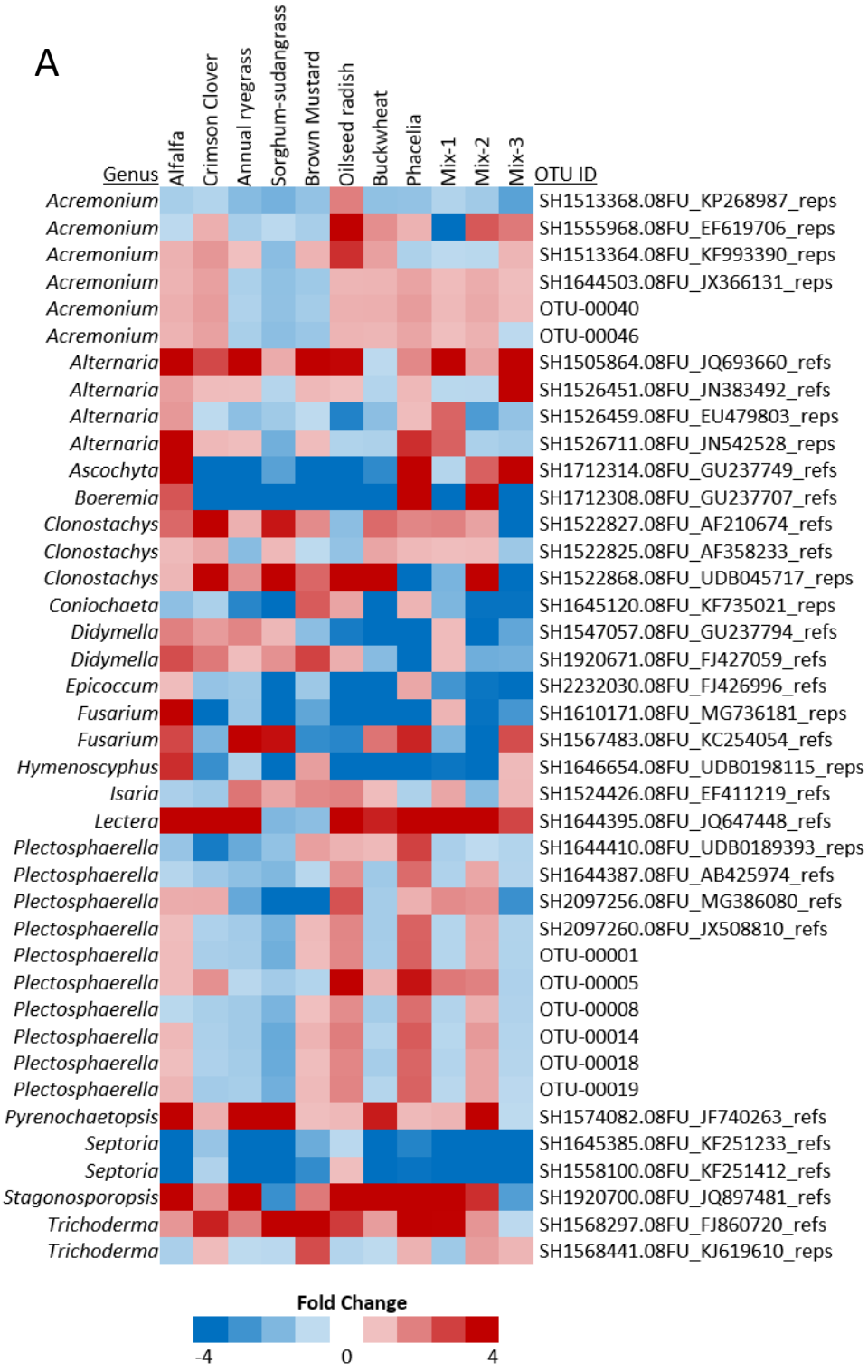
Finally, 190 OTUs were classified as saprotrophs, with the top 40 most abundant OTUs listed in Fig. 2.4C. Choice of cover crop significantly influenced saprotrophic fungi in the subsequent year, however the trends were highly variable.

The individual guilds were not normally distributed as many of the OTUs were not evenly present in all sample groups, leading to high variance in the results. Choice of cover crop had no significant effect on the abundant fungal guilds during the cover crop phase. However, in the cash crop phase, all major fungal guilds were significantly different between trials and affected by cover crop, with alfalfa in the second trial having the overall highest abundance of fungal plant pathogens (Table S2). Similarly, relative abundance of fungal parasites, which includes fungi which act as biocontrol agents by parasitizing other fungi, significantly increased over the cover crop growing season in both trials. This guild was significantly higher in abundance in the sorghum-sudangrass soil than oilseed radish, phacelia, and annual ryegrass soil.

Table 2.3 Effects of cover crop soil on fungal trophic groups at different soil collection time points.

		Pathotroph		Symbiotroph		Saprotroph	
Trial	Time point	F	p-value	F	p-value	F	p-value
1	Jul-2018[a]	0.63	0.78	1.35	0.26	0.40	0.94
	Aug-2018 [a]	1.00	0.47	0.99	0.49	0.94	0.52
	Sep-2018 [a]	**4.42**	**<0.01***	**2.89**	**0.02**	**3.21**	**0.01**
	Jul-2019 [b]	**4.10**	**<0.01***	**3.22**	**<0.01***	**5.71**	**<0.01***
2	Jul-2019 [a]	0.68	0.74	1.69	0.14	0.43	0.93
	Aug-2019 [a]	0.72	0.71	0.46	0.91	0.72	0.71
	Sep-2019 [a]	**3.57**	**<0.01***	0.96	0.50	1.98	0.08
	Jul-2020 [b]	**4.86**	**<0.01***	**4.16**	**<0.01***	**4.22**	**<0.01***

Cover crop effects with p-value ≤ 0.05 are considered significant and are bolded and those with p-value < 0.01 are strongly significant and are highlighted with "*". Superscript "a" indicates time points in cover crop phase and superscript "b" indicates cash crop phase. F-ratio (F) was used to measure effect.


A
Alfalfa
Crimson Clover
Annual ryegrass
Sorghum-sudangrass
Brown Mustard
Oilseed radish
Buckwheat
Phacelia
Mix-1
Mix-2
Mix-3
Genus
OTU ID
Acremonium SH1513368.08FU_KP268987_reps
Acremonium SH1555968.08FU_EF619706_reps
Acremonium SH1513364.08FU_KF993390_reps
Acremonium SH1644503.08FU_JX366131_reps
Acremonium OTU-00040
Acremonium OTU-00046
Alternaria SH1505864.08FU_JQ693660_refs
Alternaria SH1526451.08FU_JN383492_refs
Alternaria SH1526459.08FU_EU479803_reps
Alternaria SH1526711.08FU_JN542528_reps
Ascochyta SH1712314.08FU_GU237749_refs
Boeremia SH1712308.08FU_GU237707_refs
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Clonostachys SH1522868.08FU_UDB045717_reps
Coniochaeta SH1645120.08FU_KF735021_reps
Didymella SH1547057.08FU_GU237794_refs
Didymella SH1920671.08FU_FJ427059_refs
Epicoccum SH2232030.08FU_FJ426996_refs
Fusarium SH1610171.08FU_MG736181_reps
Fusarium SH1567483.08FU_KC254054_refs
Hymenoscyphus SH1646654.08FU_UDB0198115_reps
Isaria SH1524426.08FU_EF411219_refs
Lectera SH1644395.08FU_JQ647448_refs
Plectosphaerella SH1644410.08FU_UDB0189393_reps
Plectosphaerella SH1644387.08FU_AB425974_refs
Plectosphaerella SH2097256.08FU_MG386080_refs
Plectosphaerella SH2097260.08FU_JX508810_refs
Plectosphaerella OTU-00001
Plectosphaerella OTU-00005
Plectosphaerella OTU-00008
Plectosphaerella OTU-00014
Plectosphaerella OTU-00018
Plectosphaerella OTU-00019
Pyrenochaetopsis SH1574082.08FU_JF740263_refs
Septoria SH1645385.08FU_KF251233_refs
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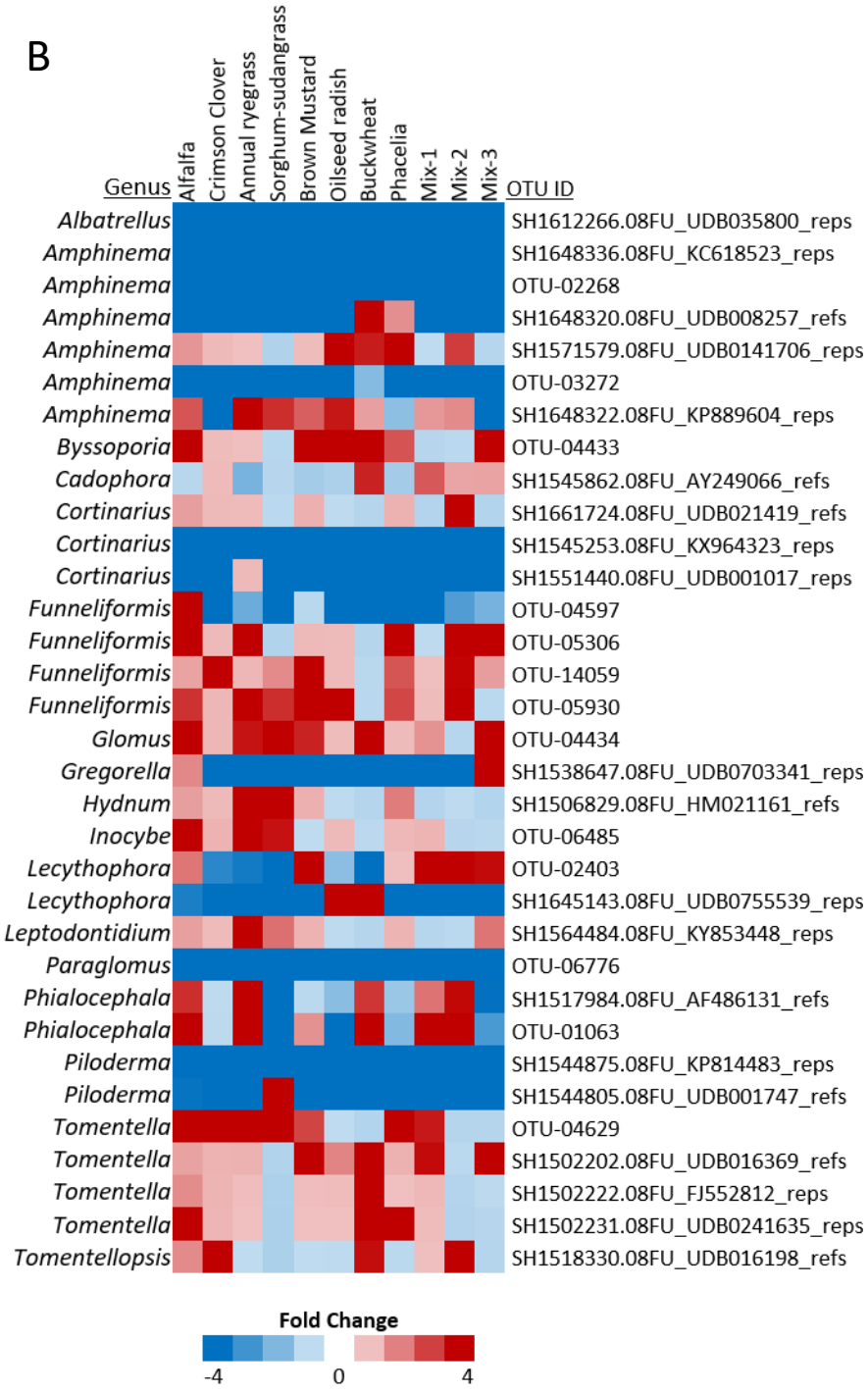
0
4


B
Genus
Alfalfa
Crimson Clover
Annual ryegrass
Sorghum-sudangrass
Brown Mustard
Oilseed radish
Buckwheat
Phacelia
Mix-1
Mix-2
Mix-3
OTU ID
Albatrellus
Amphinema
Amphinema
Amphinema
Amphinema
Amphinema
Amphinema
Byssoporia
Cadophora
Cortinarius
Cortinarius
Cortinarius
Funneliformis
Funneliformis
Funneliformis
Funneliformis
Glomus
Gregorella
Hydnum
Inocybe
Lecythophora
Lecythophora
Leptodontidium
Paraglomus
Phialocephala
Phialocephala
Piloderma
Piloderma
Tomentella
Tomentella
Tomentella
Tomentella
Tomentellopsis
SH1612266.08FU_UDB035800_reps
SH1648336.08FU_KC618523_reps
OTU-02268
SH1648320.08FU_UDB008257_refs
SH1571579.08FU_UDB0141706_reps
OTU-03272
SH1648322.08FU_KP889604_reps
OTU-04433
SH1545862.08FU_AY249066_refs
SH1661724.08FU_UDB021419_refs
SH1545253.08FU_KX964323_reps
SH1551440.08FU_UDB001017_reps
OTU-04597
OTU-05306
OTU-14059
OTU-05930
OTU-04434
SH1538647.08FU_UDB0703341_reps
SH1506829.08FU_HM021161_refs
OTU-06485
OTU-02403
SH1645143.08FU_UDB0755539_reps
SH1564484.08FU_KY853448_reps
OTU-06776
SH1517984.08FU_AF486131_refs
OTU-01063
SH1544875.08FU_KP814483_reps
SH1544805.08FU_UDB001747_refs
OTU-04629
SH1502202.08FU_UDB016369_refs
SH1502222.08FU_FJ552812_reps
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Fold Change
-4
0
4

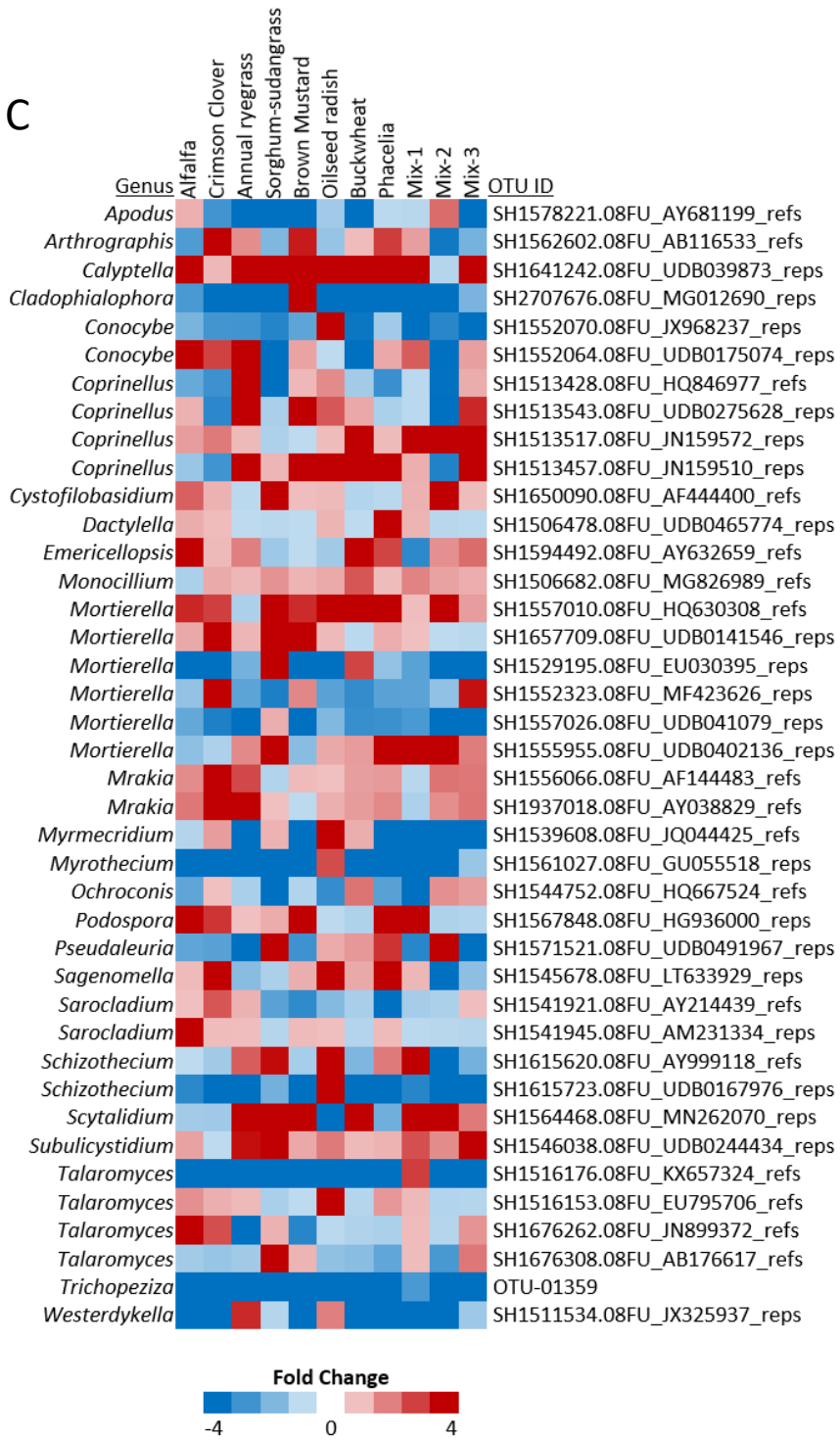


Fig. 2.4 Differences in abundance of fungal OTUs classified as pathotroph (A), symbiotroph (B), and saprotroph (C) present in the cash crop phase; n=12. Heatmaps present fold change comparison of the top 40 most abundant fungal pathotrophs and saprotrophs and the most abundant symbiotrophs found to be significantly different by cover crop. The relative abundance of fungal OTUs in soil from each cover crop was normalized against that of unmanaged soil. Genus-level taxonomic identification listed on the left with corresponding OTU reference ID listed on the right. Mix-1: Buckwheat + crimson clover; Mix-2: Phacelia + brown mustard; Mix-3: Buckwheat + crimson clover + brown mustard.

2.3.5 Bacterial functional groups

A total of 3,195 bacterial OTUs were classified into 59 functional groups using the FAPROTAX package on python (Louca et al., 2016). Cover crop effects on most bacterial functional groups in the cash crop phase significantly differed between the two trials, apart from methanol oxidation, methylotrophy, manganese oxidation and hydrocarbon degradation. Choice of cover crops significantly affected the abundance of bacteria involved in methanol oxidation, methylotrophy, ammonia oxidation, nitrification, sulfate respiration, cellulolysis, manganese oxidation, phototrophy and chemoheterotrophy, in the cash crop phase (Table S2). Bacteria involved in methylotrophy and methanol oxidation were more abundant after growing alfalfa and lowest after annual ryegrass. Cellulolytic bacteria were also most abundant after alfalfa, but least abundant after oilseed radish. Chemoheterotrophic bacteria were found in significantly higher abundance in soil collected after alfalfa compared to unmanaged soil. Ammonia oxidation and nitrification related bacteria were found in highest abundance after buckwheat and lowest after Mix-3. Sulfate respiration bacteria were more abundant in soil collected after brown mustard, and less abundant after phacelia. Manganese oxidation related bacteria were more abundant after Mix-2 and less abundant after annual ryegrass.

2.3.6 Soil chemical parameters

Soil chemical parameters were not significantly affected by cover crop during the cover crop growing season (Table S4A). Mg was the only soil chemical parameter that was influenced by cover crop in the cash crop phase, with the highest amount observed in soil collected after growing Mix-3 and lowest after annual ryegrass (Table S4B). Soil pH significantly increased over time and K and Mg decreased over time in both trials.

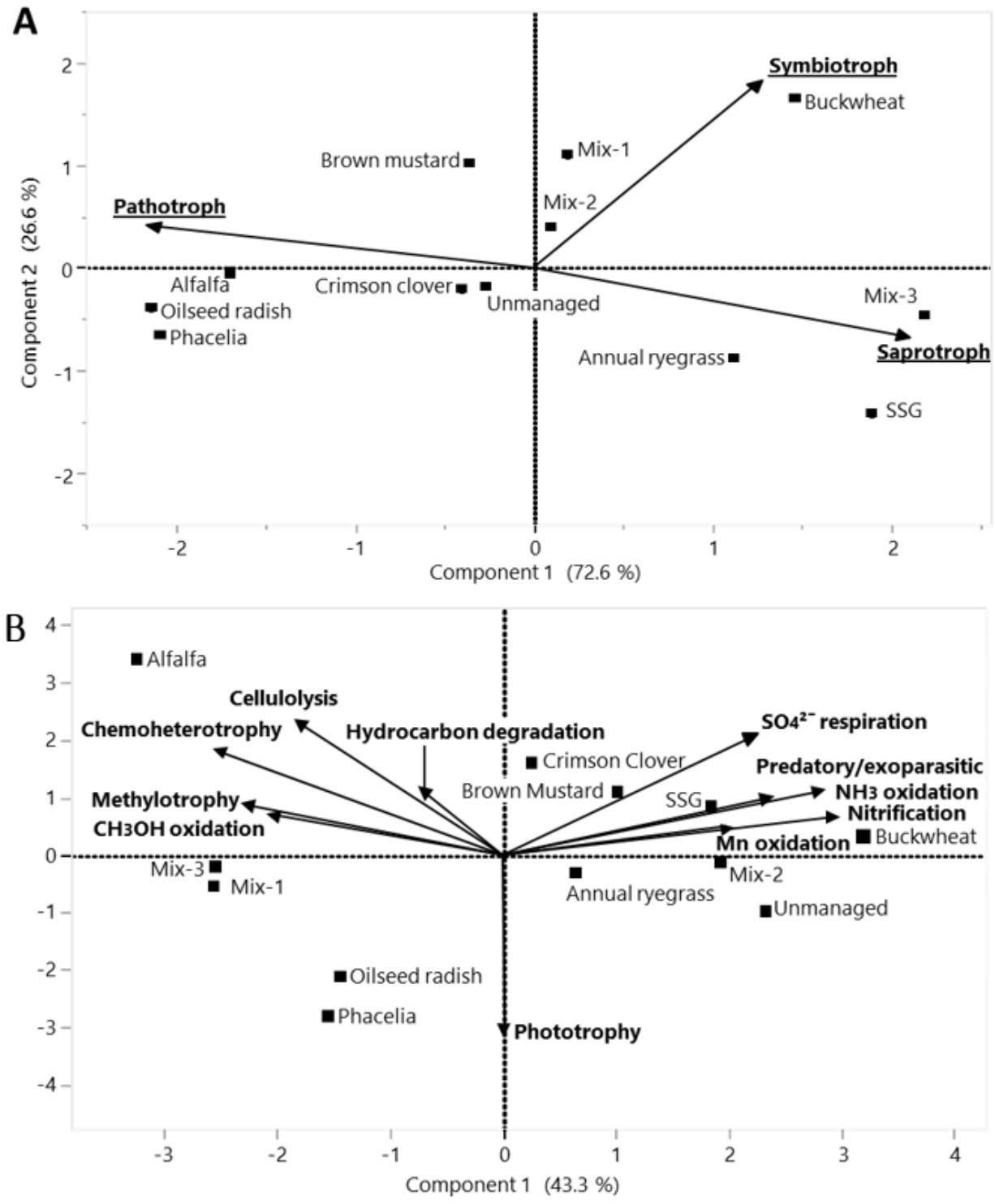
Some soil chemical parameters significantly correlated with specific fungal and bacterial functional groups in the cash crop year (Table S5). The parameters P, Ca, OM, and pH positively correlated with symbiotrophic and saprotrophic fungi and negatively correlated with fungal pathotrophs. Nitrogen fixation related bacteria negatively correlated with Ca, P and pH, while ammonia oxidation had the opposite trend. Ca content is also negatively correlated with chemoheterotrophic bacteria. Hydrocarbon degradation and methanotrophy positively correlated with P and OM, respectively (Table S5).

2.3.7 Principal component analysis

The first two principal components of all three PCA plots explained more than 50% of the variation in the dataset (Fig. 2.5). Principal component analysis with fungal trophic groups alone showed that crops including oilseed radish, phacelia, and alfalfa continued to associate closely with fungal pathotrophs in the cash crop phase (Fig. 2.5A). Whereas high biomass crops such as Mix-3, sorghum-sudangrass and annual ryegrass grouped closely with saprotrophs and buckwheat grouped closely with symbiotrophs (Fig. 2.5A). Bacterial functional groups including methanol oxidation, methylotrophy, chemoheterotrophy, and cellulolysis, clustered closely with alfalfa, oilseed radish, phacelia, Mix-3, and Mix-1 (Fig. 2.5B). Buckwheat, Mix-2, sorghum-sudangrass and unmanaged soils grouped closely with manganese oxidation, predatory or exoparasitic bacteria, and bacteria involved in nitrification and ammonia oxidation (Fig. 2.5B).

When comparing bacterial functional groups and fungal trophic groups, pathotrophs were found to group with methanol oxidation, methylotrophy, chemoheterotrophy, cellulolysis, hydrocarbon degradation, and phototrophy related bacteria (Fig. 2.5C). Meanwhile, symbiotroph and saprotroph groups clustered closely with predatory or exoparasitic bacteria, as well as

bacteria involved in sulfate respiration, ammonia oxidation, manganese oxidation, and nitrification (Fig. 2.5C).


A
Symbiotroph
Buckwheat
Brown mustard
Mix-1
Mix-2
Pathotroph
Alfalfa
Crimson clover
Unmanaged
Oilseed radish
Phacelia
Mix-3
Annual ryegrass
Saprotroph
SSG
Component 2 (26.6 %)
Component 1 (72.6 %)
B
Alfalfa
Cellulolysis
Chemoheterotrophy
Hydrocarbon degradation
SO4²⁻ respiration
Crimson Clover
Predatory/exoparasitic
Brown Mustard
SSG
NH3 oxidation
Methylotrophy
Nitrification
CH3OH oxidation
Buckwheat
Mn oxidation
Mix-3
Mix-2
Mix-1
Annual ryegrass
Unmanaged
Oilseed radish
Phacelia
Phototrophy
Component 1 (43.3 %)

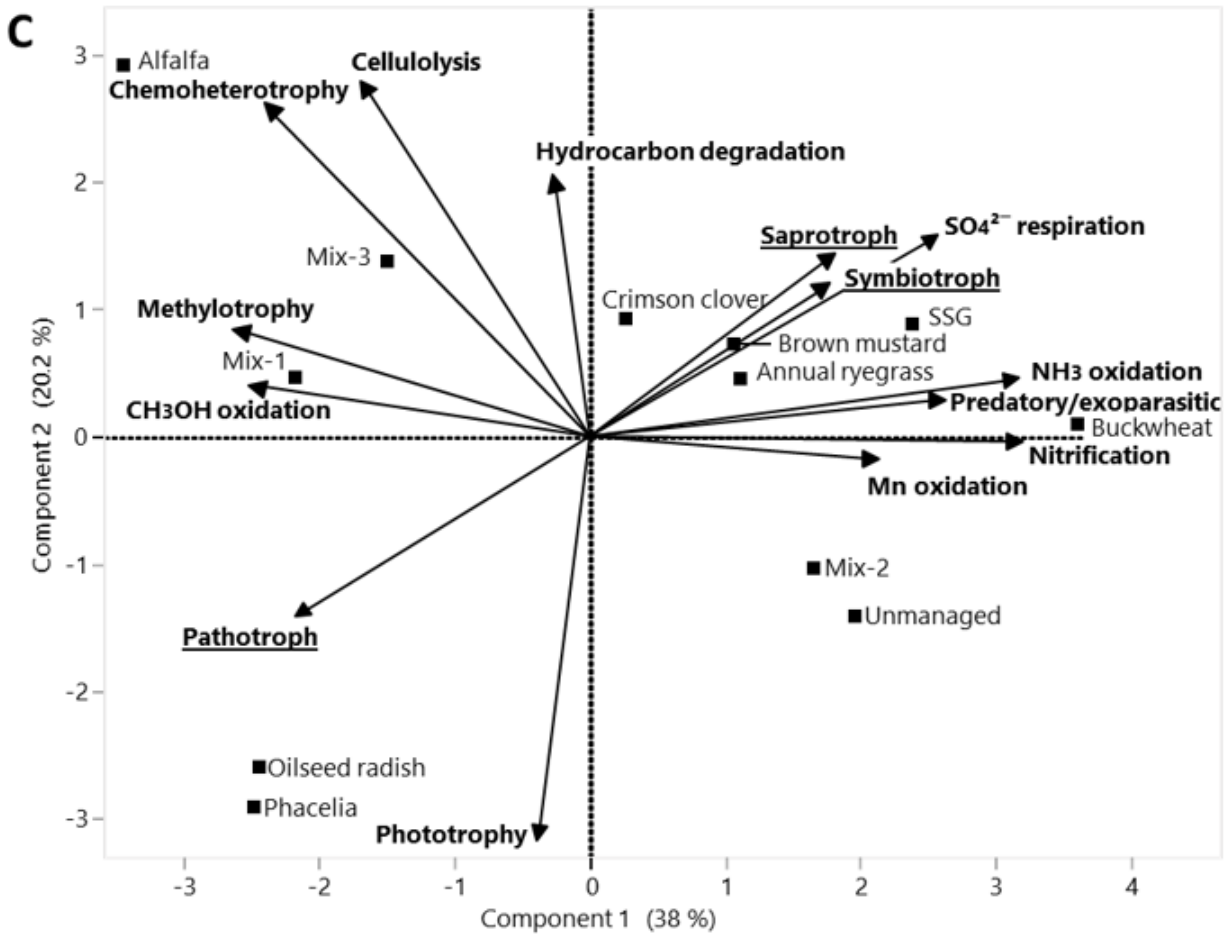


Fig. 2.5 Principal component analysis (PCA) biplots. Principal components 1 and 2 were produced from the least square means table and represent the bacterial functional (bolded) and fungal trophic group (bold underlined) relationship with cover crop groups. The trophic group relationship is based on the data collected in the cash crop phase. SSG: Sorghum-sudangrass; Mix-1: Buckwheat + crimson clover; Mix-2: Phacelia + brown mustard; Mix-3: Buckwheat + crimson clover + brown mustard. PCA biplot with fungal trophic groups (bolded and underlined) compared to different cover crops (A). PCA biplot with bacterial functional groups (bolded) compared to different cover crops (B). Combined PCA biplot with both bacterial and fungal groups in comparison with different cover crops (C).

2.4 Discussion

The role of cover crops on modifying the soil microbiome to influence agroecosystem functions is currently of interest (Kim et al., 2020). In this study, after a single growing season, the choice of cover crops significantly affected alpha diversity of soil microbial communities. Fungal and bacterial alpha diversity in bulk soil increased gradually through the growing season and were influenced by the cover crops planted in the previous year. Habekost et al. (2008), found soil microbial communities change slowly over time and may take as long as four years to respond to changes in crop diversity. However, in this study both fungal and bacterial alpha diversity were significantly influenced by the choice of cover crop in a single growing season. Soil bacterial alpha diversity was lowest after growing crimson clover and alfalfa in the previous year. This may be explained by a field history of soybeans planted in the three years prior to the field trials. It is well documented that closely related crops have similar associated microbial communities (Venter et al., 2016). However, fungal alpha diversity did not follow this same trend after growing these legumes.

Significantly higher fungal alpha diversity was observed in the cash crop phase of the second trial compared to the first trial. This may be due to the high wind speeds from post-tropical storm Dorian (September 8th, 2019) causing the movement of airborne spores, soil and plant residues. Alvarez-Manjarrez and Garibay-Orijel (2021) observed a similar increase in fungal richness and change in fungal community composition as a result of Hurricane Patricia (2015) and suggested that these changes may be explained by the intermediate disturbance hypothesis. This hypothesis suggests that environmental heterogeneity created by disturbances that are intermediate in frequency and intensity will lead to an increase in biodiversity (Connell,

1978). Fungi in the family *Nectriaceae* dramatically increased in relative abundance the year after post-tropical storm Dorian. As this family contains several plant pathogenic species, such as *Fusarium* spp., this may have consequences for different crops susceptible to root and crown diseases planted in subsequent seasons. Alvarez-Manjarrez and Garibay-Orijel (2021) also observed an increase in plant pathogen diversity after Hurricane Patricia, particularly noting an increase in *Fusarium* spp. abundance. High speed winds and heavy rainfall from post-tropical storm Dorian caused major damage to the agriculture industry, especially to corn growers in PE (Russell, 2020). Crops damaged by the storm are more susceptible to infection by microorganisms which may also explain the increase in plant pathogen abundance. Changes in microbial communities due to extreme weather conditions are known to have an impact on agricultural productivity (Unger et al., 2009; Lesk et al., 2016). This is important to consider as climatic conditions such as drought and flooding become more common as a consequence of climate change (Lesk et al., 2016).

Brassicaceae species are known to affect soil microbial alpha diversity through biofumigation. However, many factors influence the biofumigation ability of *Brassicaceae* species including incorporation of crop residue and the timing of incorporation (Kirkegaard and Sawar, 1998). In this study, cover crops were not incorporated into the soil so no biofumigation would be expected and neither oilseed radish nor brown mustard had a significant effect on soil fungal alpha diversity compared to other cover crops. Of note though, the lowest soil fungal alpha diversity was observed in the spring following the poly crop mix of brown mustard and phacelia, but this was not significantly different from alpha diversity after growing these crops as mono crops.

Dissimilarity in both fungal and bacterial community composition in bulk soil increased overtime and was significantly affected by the choice of cover crop at the end of the growing season. These changes in community composition are possibly a response to the adaptability of certain microbial taxa to the micro-environment created by differences in root structure and root exudate profiles of specific cover crops (Dudenhöffer et al., 2016; Fitzpatrick et al., 2018; Hannula et al., 2020). Fernandez et al. (2016), showed that bulk soil bacterial communities are more affected by soil amendments and soil type than the associated crop. This may explain why bacterial community composition in the present study was conserved during the cover crop growing season.

Choice of cover crop also significantly influenced certain fungal and bacterial trophic and functional groups in soil collected from the cash crop phase. Bainard et al. (2020), found that there was lower pathotroph abundance in the soil after oats monoculture compared to when other crops were planted. They suggested that the choice of crops may have a bigger influence on pathotroph population than crop rotation (Bainard et al., 2020). This is further validated by the findings in this study as choice of cover crops differentially influenced the pathotroph populations in the soil. There was an overall increase in pathotroph population in the soil after planting oilseed radish, phacelia and alfalfa, and a decrease after planting sorghum-sudangrass. This contrasted with findings by Schmidt et al. (2019) in a study examining effects of cover crops on fungal trophic groups where they showed that the fungal communities were not significantly affected by cover crops. However, their study focused on samples collected at one time point, whereas this study was able to capture changes in microbial community composition over the duration of the cover crop growing season as well as in the subsequent year.

Changes in soil fungal plant pathogen communities due to the crops planted may influence grower decisions about which cover crop to use in rotations. The most abundant pathotrophs were identified as species in the genus *Fusarium*, such as *F. poae* (Peck (Wollenw.)) and *F. sporotrichioides* (Sherb.) which are known to cause different diseases in a wide range of hosts. An increase in their pathogen load could lead to increased disease pressure for crops grown in future growing seasons (Booth, 1971). Sorghum-sudangrass lowered the relative proportion of pathotrophs in the soil and may decrease the risk of disease in future crops compared to other cover crops, however, one *Fusarium* spp. OTU did increase in the soil after growing this crop. An increase in beneficial symbiotrophs, which included arbuscular mycorrhizal fungi, was also observed after growing sorghum-sudangrass. Some symbiotrophs can increase plant productivity by increasing water and nutrient uptake, and by priming plant systemic defense (Miozzi et al., 2019).

Pathotrophs, as defined by Nguyen et al. (2016), include not only plant pathogenic fungi, but also fungi which are known to control animal, insect, nematode, and fungal pests. The highest relative abundance of insect pathogenic fungi was in the buckwheat soil. Buckwheat is known to reduce crop damage due to insect pests. Bohorquez Ruiz et al. (2019), showed that buckwheat reduced wireworm (Coleoptera: Elateridae) populations through the production of allelopathic chemicals that deter feeding and increase wireworm mortality with prolonged exposure times. These results suggest that buckwheat may directly increase fungal insect pathogens in the soil by acting as a host or modifying soil conditions for these organisms. Insect pathotrophic fungi may also increase in the soil indirectly by infection of insects that are injured after feeding on buckwheat. OTUs differentially affected by buckwheat included known entomopathogens such as *Clonostachys* spp., *Metarhizium* spp. and *Isaria* spp., as well as

nematode pathogen *Monocillium* spp. (Saksena) (Samson et al., 2013; Ashrafi et al., 2017; Sun et al., 2020). The role of the interaction between buckwheat and these soil-borne organisms may be key area of future study for developing new pest management strategies.

Poly cropping did not have a major effect on bulk soil fungal or bacterial alpha or beta diversity compared to monocrops during or in the next growing season. Effects of cover crop mixes were more similar to that of one or more of the individual crops in the mix, rather than the number of crops. This supports the findings of Fox et al. (2020), which suggest that plant species identity is the primary driver of changes in soil microbial communities. However, the tested poly crop mixes were associated with changes in bacterial functional groups. Cover crop mixes that contained buckwheat were associated with bacterial carbon cycling processes that were not associated with any of the monocrops in the mixes. These results suggest that individual crop species in the mixes may have a synergistic effect on some bacterial functional groups. It was suggested that different plant species may influence the microbiome to interact with the other plants, and this could involve carbon cycling bacteria (Wardle and Nicholson, 1996).

It may be expected that cover crops tested in family *Fabaceae* would influence the relative abundance of nitrogen cycle related bacterial functional groups, more than other cover crops. However, the only nitrogen cycle related functional groups that were significantly affected by the choice of cover crops tested were nitrification and ammonia oxidation. This is in line with findings by Graham et al. (2016), which indicated that nitrification is the only nitrogen-cycle related process which is significantly affected by environmental variables. Ammonia oxidation is a major step in the nitrification process and is highly conserved to a small group of bacteria known to be involved in obligate chemolithotrophic metabolism (Schimel, 1995; Schimel and Gulledge, 1998). In the cash crop phase, these groups were found in highest abundance in

sorghum-sudangrass and buckwheat soils. Other nitrogen cycle related processes, such as denitrification, are managed by a wide range of facultative anaerobic microorganisms. This is an example of functional redundancy where changes in microbial taxa have a negligible effect on the specific ecosystem function (Allison and Martiny, 2008). However, Sansupa et al. (2021) identified some nitrogen fixation related taxa that were not assigned a functional classification by FAPROTAX. As classification databases improve in the future, a re-examination of this study may find additional cover crop effects on bacterial functional groups.

Many soil microorganisms are known to be affected by abiotic factors (Fernandez et al., 2016; Bakker et al., 2018; Schmidt et al., 2019. In this study, soil chemical parameters were not affected by the choice of cover crop, and all samples tested were within the adequate ranges for most crops commonly grown in PE. Thus, the effects of cover crops on fungal and bacterial communities were independent of the soil parameters tested. There were some correlations between soil chemical parameters, fungal trophic group, and bacterial functional group abundance. Of note, Ca content negatively correlated with fungal pathotrophs and positively correlated with symbiotrophs and saprotrophs. Soil Ca gradually decreased over time, while fungal pathotrophs increased in relative abundance, however there is no clear causal relationship.

There is limited information available about the functional interaction between fungal and bacterial groups in the soil microbiome, and their potential effects on soil and plant health in subsequent years (Xu et al., 2021). This study is one of few which assessed the correlation between fungal and bacterial functional groups, providing better insight into the interconnectedness of the two communities. Fungal saprotrophs and symbiotrophs positively correlated with bacterial groups involved in sulfate respiration, ammonia oxidation, nitrification, and manganese oxidation, as well as predatory and exoparasitic bacteria. These groups were also

strongly associated with sorghum-sudangrass and buckwheat. This further indicates that microbial community interactions may add to the beneficial effects associated with use of certain cover crops.

2.5 Conclusion

The results from this study indicate that choice of cover crops have a significant influence on soil microbial community composition. The cover crop influence on soil microbial communities was found to carry over to the soil in the subsequent year, where it could potentially influence plant health. Choice of cover crop is important as it can affect soil health by manipulating the fungal community composition. The high proportion of plant pathotrophic fungi observed after growing oilseed radish, phacelia, and alfalfa may be an indication of higher pathogen load that could lead to increased root disease pressure in subsequently planted crops. Alternatively, the choice of sorghum-sudangrass as the cover crop may lead to disease suppressive soils and buckwheat towards pest suppressive soils. These results verified the hypothesis regarding beta diversity, as well as functional and trophic group analysis. However, we hypothesized that cover crops would not affect soil fungal and bacterial alpha diversity in a single growing season, but results did not support this. This shows that the choice of cover crops will have a profound influence on the soil microbiome in a single growing season, thereby influencing soil health.

2.6 Appendix

Data availability: Raw amplicon sequencing data available on NCBI. SRA Bioproject ID: PRJNA781138

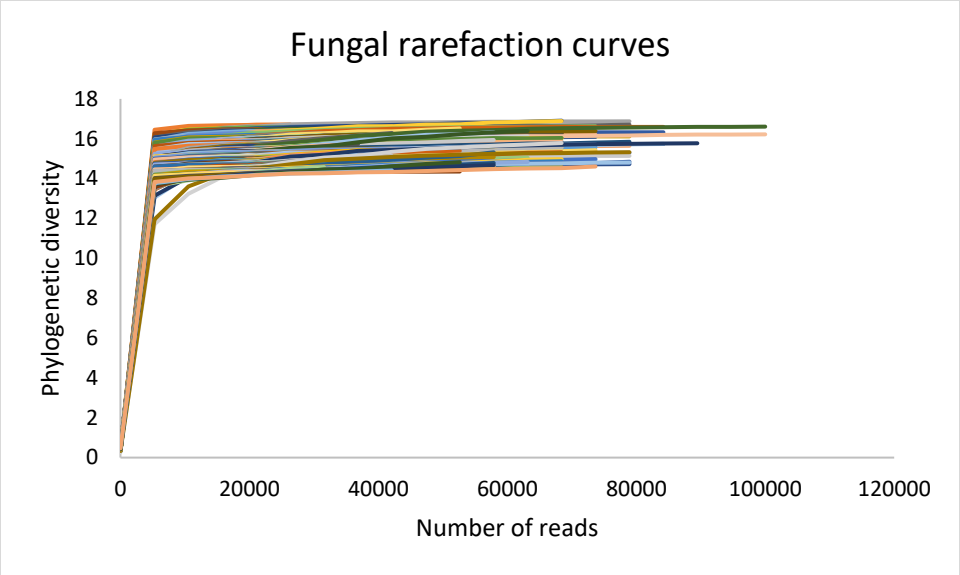


Fig. S1 Rarefaction curve for phylogenetic diversity based on ITS reads; N=360.

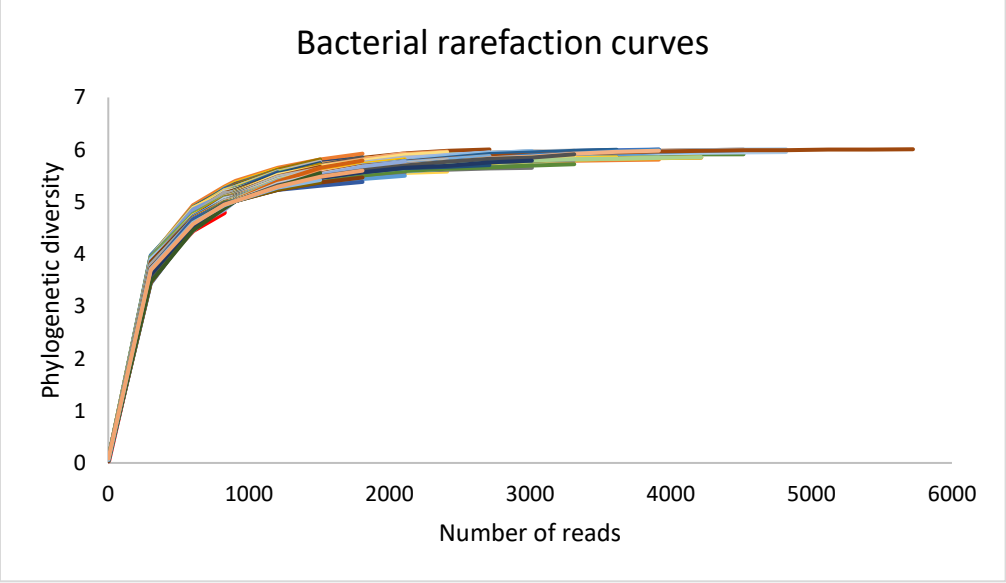


Fig. S2 Rarefaction curve for phylogenetic diversity based on 16S reads; N=360.

Table S1 Field trial planting, harvesting and sampling dates.

Trial	Year	Plot size	Planting date			Date mowed	Harvest date		Sample collection date		
		(m × m)	Cover crops	Barley	Soybean	Cover crops	Barley	Soybean	Soil		
1	2018	2 × 10	July 5	-	-	Sept. 5	-	-	July 25	Aug. 13	Sept. 17
	2019	2 × 3	-	June 17	June 25	-	Sept. 30	Oct. 30	July 16	-	-
2	2019	2 × 10	June 25	-	-	Oct. 11	-	-	July 17	Aug. 13	Sept. 18
	2020	2 × 3	-	June 19	June 19	-	Oct. 1	Oct. 16	July 10	-	-

Table S2 Summary of bacterial functional group and fungal trophic group data from cash crop year tested for cover crop carryover effects.

			P-value			
Variable	R^2	RMSE	Cover crop	Trial	Cover crop*Trial	Replicate
Methanol oxidation	0.26	0.001	**<0.01**	0.92	0.12	**<0.01**
Methylotrophy	0.25	0.002	**0.03**	0.67	0.14	0.94
Ammonia oxidation	0.36	0.006	**0.01**	**<0.01**	0.29	0.49
Nitrification	0.30	0.007	**0.01**	**<0.01**	0.34	0.57
Sulfate respiration	0.42	0.002	**<0.01**	**<0.01**	0.48	0.53
Cellulolysis	0.09	0.002	**<0.01**	**0.01**	0.86	**<0.01**
Manganese oxidation	0.24	0.018	**0.01**	0.11	0.81	0.70
Hydrocarbon degradation	0.20	0.001	0.06	0.82	0.68	0.74
Predatory or exoparasitic	0.20	0.004	0.07	**0.01**	0.96	0.88
Phototrophy	0.33	0.009	**0.02**	**<0.01**	0.26	0.94
Chemoheterotrophy	0.37	0.018	**<0.01**	**<0.01**	0.13	0.66
Fungi - Pathotroph	0.71	0.09	**<0.01**	**<0.01**	**<0.01**	0.70
Fungi - Symbiotroph	0.71	0.60	**<0.01**	**<0.01**	**<0.01**	0.42
Fungi - Saprotroph	0.60	0.25	**<0.01**	**<0.01**	**<0.01**	0.54
Fungi - Animal pathogens	0.50	0.31	**0.03**	**<0.01**	0.38	0.85
Fungi - Plant pathogens	0.77	0.09	**<0.01**	**<0.01**	**<0.01**	0.62
Fungi - Mycorrhizae	0.42	0.62	**<0.01**	**<0.01**	**0.01**	0.57
Fungi - Fungal parasite	0.60	0.35	**0.01**	**<0.01**	0.08	0.70

p-values <0.05 (bolded) were considered significant.

Table S3 Spearman's rank correlations between important bacterial functional groups and fungal trophic or major guilds.

	Saprotroph		Pathotroph						Symbiotroph	
			Fungi - Animal pathogens		Fungi - Plant pathogen		Fungi - Fungal parasite			
Bacterial groups	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|
Ammonia oxidation	**0.41**	**<0.01***	**-0.37**	**<0.01***	**-0.43**	**<0.01***	**-0.47**	**<0.01***	**0.22**	**<0.01***
Nitrification	**0.39**	**<0.01***	**-0.33**	**<0.01***	**-0.37**	**<0.01***	**-0.43**	**<0.01***	**0.24**	**<0.01***
Denitrification	**0.2**	**<0.01***	**-0.12**	**0.03**	**-0.17**	**0.01**	**-0.12**	**0.02**	0.1	0.08
Nitrogen fixation	**-0.12**	**0.03**	**0.15**	**0.01**	**0.13**	**0.02**	-0.1	0.12	0.07	0.18
Nitrate/ nitrite ammonification	**-0.16**	**<0.01***	**0.15**	**0.01**	**0.13**	**0.02**	**0.16**	**<0.01***	-0.09	0.1
Nitrite respiration	**0.16**	**<0.01***	-0.08	0.13	**-0.13**	**0.02**	-0.1	0.08	0.07	0.2
Nitrate reduction	**0.25**	**<0.01***	**-0.18**	**<0.01***	**-0.21**	**<0.01***	**-0.15**	**0.03**	0.09	0.1
Ureolysis	**-0.13**	**0.02**	**0.12**	**0.02**	**0.14**	**0.01**	0.05	0.33	**-0.16**	**<0.01***
Methanotrophy	-0.03	0.59	-0.01	0.87	-0.04	0.49	-0.04	0.4	0.02	0.65
Methanol oxidation	-0.07	0.17	-0.1	0.15	0.05	0.38	-0.04	0.41	-0.08	0.15
Methylotrophy	-0.08	0.14	-0.1	0.15	0.02	0.72	-0.1	0.16	-0.05	0.39
Hydrocarbon degradation	-0.1	0.06	0.1	0.24	0.06	0.26	0.01	0.78	-0.06	0.28
Intracellular parasites	**0.19**	**<0.01***	**-0.14**	**0.01**	-0.1	0.06	**-0.15**	**0.04**	-0.08	0.13
Cellulolysis	**-0.35**	**<0.01***	**-0.33**	**<0.01***	**0.32**	**<0.01***	**0.21**	**<0.01***	**-0.14**	**0.01**
Plant pathogen	**-0.11**	**0.03**	**-0.14**	**0.01**	0.1	0.05	0.1	0.08	-0.09	0.1
Predatory or exoparasitic	**0.23**	**<0.01***	**-0.14**	**0.01**	**-0.21**	**<0.01***	**-0.16**	**<0.01***	**0.11**	**0.05**
Invertebrate parasites	**-0.12**	**0.02**	0.1	0.09	**0.14**	**0.01**	**0.13**	**0.01**	0.09	0.1
Animal parasites or symbionts	**-0.18**	**<0.01***	0.05	0.31	0.16	0.002	0.07	0.21	0.02	0.75
Chitinolysis	0.05	0.35	**-0.04**	**0.41**	-0.02	0.69	0.04	0.43	-0.04	0.4
Sulfate respiration	**-0.11**	**0.04**	0.06	0.27	**0.11**	**0.03**	0.08	0.13	0.01	0.9
Manganese oxidation	**-0.34**	**<0.01***	**0.35**	**<0.01***	**0.35**	**<0.01***	**0.35**	**<0.01***	0.1	0.1

"ρ" represents Spearman's rank correlation. Prob>|ρ| values ≤ 0.05 were considered significant and are bolded and values < 0.01 were considered highly significant and are highlighted with "*".

Table S4A Summary statistics table presenting mean and standard error by cover crop for each soil chemical parameter measure in July in the cover crop season of both trials combined. Chemical parameters with significant differences by cover crop bolded.

	Nitrogen (%)		Organic matter (%)		pH		Phosphorous (ppm)		Potassium (ppm)		Magnesium (ppm)		Calcium (ppm)	
CoverCrop	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
Alfalfa	0.2	0.0	2.9	0.1	7.0	0.1	116.1	9.2	117	5.3	169.7	20.8	1543	97.1
Annual ryegrass	0.2	0.0	3.1	0.1	7.1	0.1	104.7	5.1	102	8.1	167.6	20.5	1696	69.7
Brown Mustard	0.2	0.0	2.9	0.2	7.0	0.1	112.0	10.0	111	4.7	164.9	24.4	1544	48.2
Buckwheat	0.2	0.0	3.0	0.1	7.1	0.1	108.2	8.2	100	9.5	185.6	20.1	1756	123.6
Crimson Clover	0.2	0.0	3.1	0.1	7.1	0.1	109.3	6.4	108	6.9	182.4	16.3	1481	94.4
Oilseed radish	0.2	0.0	3.0	0.1	7.1	0.1	110.8	8.0	125	24.0	196.3	30.3	1606	55.5
Phacelia	0.2	0.0	3.0	0.1	7.0	0.1	115.1	8.4	113	8.8	172.5	11.0	1626	47.4
Sorghum-sudangrass	0.2	0.0	3.1	0.1	7.2	0.1	104.3	9.0	103	11.6	217.0	54.5	1822	107.8
Mix-1	0.2	0.0	3.1	0.1	7.1	0.0	121.5	9.0	105	7.6	154.1	22.1	1361	260.8
Mix-2	0.2	0.0	3.1	0.1	7.0	0.1	121.8	7.4	140	35.1	201.2	36.4	1614	74.2
Mix-3	0.2	0.0	3.0	0.1	7.1	0.1	111.2	6.8	111	6.5	188.5	22.3	1560	60.4
Unmanaged	0.2	0.0	3.0	0.1	7.1	0.1	110.7	5.0	127	12.8	193.3	22.7	1659	64.1

Table S4B Summary statistics table presenting mean and standard error by cover crop for each soil chemical parameter measure in July of the cash crop season in both trials combined. Chemical parameters with significant differences by cover crop bolded.

	Nitrogen (%)		Organic matter (%)		pH		Phosphorous (ppm)		Potassium (ppm)		Magnesium (ppm)		Calcium (ppm)	
CoverCrop	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
Alfalfa	0.2	0.0	3.1	0.1	7.2	0.1	117.3	5.1	87.4	4.1	**113.3**	**5.4**	1511	57.8
Annual ryegrass	0.2	0.0	2.9	0.3	7.3	0.1	109.4	6.2	79.7	5.3	**104.0**	**4.6**	1470	33.2
Brown Mustard	0.2	0.0	2.9	0.1	7.3	0.1	113.0	6.0	81.8	4.3	**105.3**	**5.6**	1490	77.6
Buckwheat	0.2	0.0	2.9	0.1	7.3	0.1	112.7	7.2	83.6	3.9	**116.2**	**3.3**	1625	125.6
Crimson Clover	0.3	0.1	3.0	0.1	7.3	0.1	116.6	7.3	74.4	4.4	**114.3**	**6.1**	1519	131.1
Oilseed radish	0.2	0.0	3.0	0.0	7.3	0.1	110.5	8.2	96.7	4.1	**123.2**	**6.6**	1520	70.2
Phacelia	0.2	0.0	3.1	0.1	7.3	0.1	117.5	7.5	84.6	4.8	**111.7**	**3.4**	1529	94.3
Sorghum-sudangrass	0.2	0.0	3.0	0.1	7.4	0.1	108.1	7.6	84.2	6.9	**119.5**	**8.6**	1588	91.3
Mix-1	0.2	0.0	3.1	0.1	7.3	0.1	118.4	5.7	87.8	3.7	**108.2**	**2.9**	1494	71.9
Mix-2	0.2	0.0	3.2	0.1	7.2	0.1	116.2	6.4	84.4	4.1	**116.7**	**3.5**	1449	119.6
Mix-3	0.2	0.0	3.1	0.1	7.3	0.1	115.6	6.2	81.5	4.7	**130.5**	**4.2**	1462	96.3
Unmanaged	0.2	0.0	3.0	0.1	7.3	0.1	116.4	6.1	81.1	4.0	**115.3**	**6.0**	1521	51.4

Table S5 Spearman's rank correlation between soil chemical parameters and bacterial functional groups or fungal trophic groups.

	CA (ppm)		K (ppm)		MG (ppm)		OM (%)		P (ppm)		pH	
Microbial functional group	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|	ρ	Prob>|ρ|
Ammonia oxidation	**0.40**	**<0.01***	0.04	0.77	-0.10	0.40	0.05	0.70	0.12	0.34	**0.30**	**0.01**
Cellulolysis	-0.20	0.09	0.01	0.92	0.09	0.45	-0.20	0.10	0.16	0.17	-0.19	0.12
Chemoheterotrophy	**-0.25**	**0.03**	0.16	0.18	0.16	0.19	-0.05	0.67	0.04	0.73	-0.18	0.13
Chitinolysis	-0.15	0.22	-0.02	0.87	**0.23**	**0.05**	-0.25	0.04	-0.10	0.41	-0.05	0.66
Denitrification	0.01	0.90	-0.06	0.63	-0.03	0.80	-0.08	0.50	-0.14	0.25	-0.01	0.91
Hydrocarbon degradation	0.13	0.28	0.06	0.63	-0.05	0.68	0.16	0.18	**0.24**	**0.04**	0.12	0.32
Intracellular parasites	0.04	0.75	-0.02	0.86	0.12	0.31	0.17	0.15	-0.01	0.94	0.04	0.73
Manganese oxidation	0.04	0.74	-0.16	0.17	-0.07	0.59	**0.31**	**0.01**	0.17	0.15	0.03	0.78
Methanol oxidation	-0.01	0.91	0.12	0.31	-0.04	0.72	0.04	0.73	0.05	0.70	0.01	0.95
Methanotrophy	0.01	0.91	0.02	0.86	0.07	0.57	**0.24**	**0.04**	0.20	0.09	0.08	0.52
Methylotrophy	0.04	0.73	0.07	0.55	-0.03	0.83	0.22	0.07	0.15	0.20	0.08	0.53
Nitrate ammonification	-0.21	0.07	-0.11	0.37	-0.13	0.29	0.05	0.68	0.01	0.95	**-0.28**	**0.02**
Nitrification	0.22	0.06	-0.03	0.80	-0.09	0.47	0.04	0.72	0.07	0.57	0.09	0.47
Nitrogen fixation	**-0.36**	**<0.01***	-0.06	0.60	-0.20	0.09	-0.02	0.84	**-0.27**	**0.02**	**-0.40**	**<0.01***
Nitrogen respiration	-0.14	0.25	-0.13	0.27	-0.08	0.53	-0.19	0.10	**-0.23**	**0.05**	-0.12	0.30
Phototrophy	**0.29**	**0.01**	0.04	0.76	-0.06	0.64	-0.04	0.72	0.15	0.21	**0.29**	**0.01**
Sulfate respiration	**-0.32**	**0.01**	-0.21	0.08	-0.06	0.64	-0.05	0.67	**-0.40**	**<0.01***	**-0.42**	**<0.01***
Ureolysis	-0.14	0.25	0.16	0.19	-0.06	0.60	0.10	0.39	-0.05	0.66	**-0.29**	**0.01**
Pathotroph (Fungi)	**-0.62**	**<0.01***	-0.03	0.79	0.03	0.78	**-0.29**	**0.01**	**-0.32**	**0.01**	**-0.60**	**<0.01***
Saprotroph (Fungi)	**0.53**	**<0.01***	0.02	0.88	0.03	0.81	**0.24**	**0.04**	**0.26**	**0.02**	**0.56**	**<0.01***
Symbiotroph (Fungi)	**0.68**	**<0.01***	-0.05	0.66	0.03	0.82	**0.23**	**0.05**	**0.42**	**<0.01***	**0.73**	**<0.01***

"ρ" represents Spearman's rank correlation. Prob>|ρ| values ≤ 0.05 were considered significant and are bolded and values < 0.01 were considered highly significant and are highlighted with "*"

Chapter 3 - Effects of previous crop on Fusarium root and crown rot of no-till barley and soybean in Prince Edward Island, Canada

3.0 Abstract

Barley and Soybean are significantly affected by root and crown rot caused by soil-borne *Fusarium* spp. (FRCR). Growing certain cover crops influences the composition of soil and crop residue fungal communities including *Fusarium* spp., thereby having the potential to influence FRCR in subsequent crops. This study focuses on the carry over effects of cover crops on FRCR in subsequently planted barley and soybean. Cover crops were grown in randomized complete block design with barley and soybean planted in each half of the plots in the subsequent year. Research trials were conducted in duplicate over two years in AAFC Harrington Experimental Farm (PE, Canada). Roots were assessed for FRCR through visual disease rating. Pathogens were isolated from diseased tissue and FRCR was confirmed though molecular identification of *Fusarium* spp., as the major pathogen and verification of Koch's postulates. Amplicon sequencing was conducted to assess cover crop effects on plant pathogenic fungi in cover crop residue. qPCR was used to quantify the abundance of certain *Fusarium* spp. of interest. Cultures isolated from diseased root tissue indicated that *F. cerealis* and the *F. oxysporum* species complex were the major causal agents of root disease in barley and soybean, respectively. Both barley and soybean planted after oilseed radish had high incidence of FRCR, and low incidence after sorghum sudangrass. However, damping off caused by *Pythium* spp. and *Rhizoctonia solani* showed the opposite trend with higher incidence when the previous crop was sorgrhum-sundangrass. The results suggest that sorghum-sudangrass manipulates the associated soil microbial composition by increasing beneficial microorganisms, thus creating a soil environment resilient to FRCR.

Keywords: Fusarium root and crown rot (FRCR), amplicon sequencing, cover crops, soil microbiome, barley, soybean

3.2 Introduction

Root and crown rot caused by *Fusarium* spp. (FRCR) impacts a wide range of economically important crops, including barley and soybean, by causing significant decreases in crop quality and yield (Leath and Caroll, 1985; Smiley et al. 2005). Barley (*Hordeum vulgare* L.) and soybean (*Glycine max* (L.) Merr.) are both important crops in Prince Edward Island (PE), Canada. Several species including *F. oxysporum* ((Schlecht.) emend. Snyder & Hansen), *F graminearum* (Schwabe; teleomorph: *Gibberella zeae* (Schweinitz) Petch.), *F. sporotrichioides* ((Sherb.) Bilai), *F. cerealis* ((Cooke) Sacc.; synonym: *F. crookwellense* (Burgess) Nelson & Toussoun), *F. equiseti* ((Corda) Sacc.), *F. pseudograminearum* (O'Donnell and Aoki; teleomorph: *Gibberella coronicola*), and *F. culmorum* ((Wm.G.Sm.) Sacc.), are known to cause FRCR in barley and soybean (Sturz and Johnston, 1985; Backhouse et al., 2004; Ivic, 2014; Knight and Sutherland, 2017; Abdelmagid et al., 2018; Abdelmagid et al., 2021). FRCR is often hard to detect as the *Fusarium* spp. may infect the crop, reducing ability to uptake water and nutrients, without causing obvious visible symptoms (Knight and Sutherland, 2016).

Fusarium resting structures, are known to survive harsh winter conditions in soil and crop residues, potentially increasing the susceptibility of subsequently planted crops to FRCR, especially under no-till practices (Smith and Snyder, 1975; Cotton and Munkvold, 1998). As such, FRCR may have a significant impact on crop quality and yield, and disease management strategies should involve early preventative measures. This commonly includes the use of fungicide seed treatments to allow crops to establish without being affected by the pathogen early in the growing season (Martin and Johnston, 1982). However, fungicide seed treatments are ineffective in protecting the plants against pathogen infection later in the growing season (Paulitz, 2006). Furthermore, severe weather conditions such as drought are known to increase

plant susceptibility to FRCR and fungicide treatments may not be as effective (Wegulo et al., 2013). As climate change increases the frequency of such extreme weather events, conservation agriculture practices must be adopted to ensure sustainable and cost-efficient crop production (Howden et al., 2007; Li et al., 2009).

Conservation agricultural practices, along with growing diverse crops in rotation, is encouraged in PEI to reduce soil erosion, increasing soil organic matter, and breaking disease cycles (Jatoe et al, 2008). Increased crop diversity using cover crops can break disease cycles by including non-host crops, reduce pathogen loads through competition for resources and by secreting anti-microbial compounds as root exudates (Shah et al., 2021). Previously, it was identified that the choice of cover crop used in rotation has a significant influence on fungal and bacterial community composition, with an increase in abundance of plant pathogenic fungi after growing crops such as oilseed radish, alfalfa, and phacelia (Chapter 2). However, little information is available on the relationship between *Fusarium* abundance in soil and residue and FRCR in subsequently planted crops. Crop rotation can also promote disease suppressive soils by manipulating the microbial communities in the soil to increase abundance of antagonistic microorganisms (Peters et al., 2004). Previously, it was found that choice of cover crop may influence the disease suppressive potential of the soil environment, with crops such as sorghum-sudangrass and buckwheat increasing the abundance of certain beneficial fungal taxa in the soil including symbiotrophic fungi, as well as mycoparasitic fungi such as, *Clonostachys* spp. ((Link) Schroers; synonym: *Gliocladium roseum*) and *Trichoderma* spp. (Pers.) (Chapter 2).

The primary objectives in this study include assessing the effects of selected cover crops on the *Fusarium* abundance in the soil and residue, and how that affects FRCR in subsequently planted barley and soybean. Furthermore, the potential disease suppressive effects of certain

cover crop soils were also tested under high *Fusarium* pressure. It was hypothesized that 1) choice of cover crops will influence FRCR in barley and soybean in the subsequent year; 2) FRCR will positively correlate with pathogen load in the soil and residue; and 3) barley and soybean grown in soil with increased abundance of beneficial microbial taxa will have lower FRCR here.

3.3 Material and Methods

3.3.1 Field Trial set up and sample collection

Effects of cover crops on FRCR in barley and soybean were studied in two duplicate field trials conducted using standard grower practices by Dr. Aaron Mills and Dr. Andrew McKenzie-Gopsill's research teams at Agriculture Agri-Food Canada in Harrington Experimental Research Farms (46°20'47.4"N 63°10'25.5"W; PE, Canada) managed by Atlantic Grains Councils. Field trial set up and microbiome analysis was described previously in Chapter 2. Selected cover crops included oilseed radish [*Raphanus sativus* (L.)], brown mustard [*Brassica juncea* (L.)], alfalfa [*Medicago sativa* (L.)], crimson clover [*Trifolium incarnatum* (L.)], buckwheat [*Fagopyrum esculentum* (Moench.)], phacelia [*Phacelia tanacetifolia* (Benth.)], annual ryegrass [*Lolium multiflorum* (Lam.)], sorghum-sudangrass [*Sorghum* × *drummondii* (Nees ex. Steud.) Millsp. & Chase], as well as three crop mixes and an unmanaged fallow control, with no weed management. Cover crops were planted in a randomized complete block design with three replicates in the first year of each trial, in 2m × 10m plots. In the subsequent year barley and soybean were planted no-till into each half of the cover crop plots (2m × 3m, with 2 meters between cash crop plots) in 12 rows. Certified cash crop seeds that were available in the market were used in each trial. AAC Synergy barley (Legge et al., 2014) was planted in both trials at a seeding rate of 300 seeds m^{-2}. As for soybean, DH401 (Sevita International) was used in the first

trial and 25-10 RY (Dekalb) was used in the second trial, due to DH401 not being available, both planted at seeding rate of 55 seeds m^{-2}. Vitaflo 280 (Chemtura, Connecticut, US) was the seed treatment used for both cash crops. Seeds were treated to minimize any impact of seed-borne diseases, thus ensuring that majority of the observed symptoms were caused by soil-borne microorganisms.

Cover crop residue was collected in the second year of each trial, before planting barley or soybean (Table S1). Plant residues laying loosely over the soil surface from the previous growing season, were collected from five random locations in each plot and pooled in plastic bags to produce approximately 500 mL of composite sample per plot. Soil samples were collected in July of the second year after barley and soybean were planted, as described in (Chapter 2; Table S1). Field samples were subsampled into 50mL conical tubes and stored at -80℃ until further processing.

Monthly rainfall and average temperature information for the three trial years (2018-2020) were provided by the Environment Canada Harrington CDS weather station available at climate.weather.gc.ca (Table 3.1). Any missing values were replaced with data from nearby weather station (Charlottetown A) information.

Table 3.1 Summary of weather data from the three trial years compared to the 10 year average (Environment Canada).

	Monthly Rainfall (mm)						Mean temp (°C)					
Year	5	6	7	8	9	S	5	6	7	8	9	S
2018	78	154	14	111	87	444	9.0	12.7	20.6	20.2	14.4	15.4
2019	86	143	49	80	147	506	6.5	14.4	19.0	19.2	13.3	14.5
2020	70	30	45	37	96	278	8.9	16.1	19.3	19.4	15.2	15.8
10 year mean	78	91	63	120	100	453	9.4	14.2	19.2	18.9	14.9	15.3

May = 5; June = 6; July = 7; August = 8; September = 9; S = growing season.

3.3.2 Field disease assessment

Barley and soybean were destructively sampled in random 30 cm rows from each plot for disease assessment. In the first trial, six representative barley and soybean roots with high disease symptoms were saved for pathogen isolation. For the second trial, one root from each plot was plated for identification of primary pathogen population. Yield data in kg ha^{-1} was also collected after barley and soybean were harvested.

The effects of cover crops on FRCR of barley and soybean in the subsequent year were assessed through visual root disease rating. Roots were washed with soapy water to remove soil before rating visual symptoms based on an ordinal scale modified from Chekali et al. (2019) and Ellis et al. (2011), described as 0: no visual symptoms; 1: discoloration of 1-25% of the root and crown; 2: 26-50% discoloration; 3: 51-75% discoloration; 4: 76-99% discoloration; and 5: 100% discoloration or plant death. Disease severity index was calculated using the equation below, as described by Chiang et al. (2017).

$$\text{DSI\%} = \frac{\sum(\text{class frequency} \times \text{score of rating class})}{\text{total number of plants} \times \text{maximal disease index}} \times 100$$

Twelve representative barley and soybean plants from the first trial and one barley and soybean plant per plot from the second trial were saved for pathogen isolation. Tissue samples were surface sterilized by first washing in 10% bleach or soap water, then sterile water, then 70% ethanol, and finally sterile water. Samples were then plated on potato dextrose agar (PDA) media amended with tetracyclin [100µg mL^{-1}] and cefotaxime [100 µg mL^{-1}] and incubated at room temperature. Approximately two days after initial plating, individual isolates were transferred onto fresh PDA plates amended with tetracyclin [100µg mL^{-1}] and cefotaxime [100 µg mL^{-1}], in

order to obtain pure cultures. After 2 to 3 days of growth, isolates were categorized morphologically as *Fusarium* using the descriptions from Leslie & Summerell (2008) and molecular identification was conducted as described below.

Damping off in barley and soybean in the second trial was measured using a 0-10 scale, with 10% intervals. Some plants with disease symptoms were destructively sampled and sent to Marlene Clark at the Provincial Disease Diagnostics Lab (PE, Canada) for culturing and identification of pathogens responsible for damping off in barley and soybean. Only select samples were cultured due to site access restriction related to COVID-19.

3.3.3 Identification of *Fusarium* spp.

Molecular identification of root isolates was done using universal primers for barcoding genes including internal transcribed spacer region (ITS) for all samples and translational elongation factor (Tef-1α) for suspected *Fusarium* isolates (Table 3.1). Template DNA preparation for conventional PCR, included transferring some mycelium from the pure culture to a 2 mL microcentrifuge tube with 200 µL AE buffer from the Plant Mini DNeasy kit (QIAGEN), with a small scoop of silicon dioxide beads. The samples were microwaved for 30 seconds, then ground twice using Bead Mill 24 Homogenizer (Fisherbrand, Pittsburgh, Pennsylvania, USA) at speed 6 for 15 s with 5 s pause in between cycles. Conventional PCR was then conducted using 4 µL of this suspension as template, along with 20 µL of 2x Phusion High-Fidelity Standard master mix (ThermoFisher), 2 µL of each primer, and 12 µL of molecular grade water, per reaction. Reactions were conducted in the SimpliAmp thermal cycler (Thermo Fisher) with the conditions listed in Table 3.2. Amplification was confirmed through agarose (1%) gel electrophoresis using 4 µL of PCR product. Remaining 36 µL of PCR product was purified using the MinElute PCR Purification Kit (QIAGEN) according to manufacturer's recommendations,

with an elution volume of 30 µL, before sending 10 µL of product for Sanger sequencing (Eurofins Genomics, Toronto, ON, Canada). Ambiguous nucleotides were trimmed from sequences before running BLAST to match them to reference sequences in the NCBI standard (nr/nt) database for Fungi. Representative Tef-1α sequences from *Fusarium* spp. isolated from root tissue were chosen for the phylogenetic tree. Sequences were aligned using MUSCLE and trimmed to 500 bp in order to remove ambiguous bases and to normalize the sequence length. The tree was built using Jukes-Cantor Neighbour joining method with 1000 bootstrap replicates. One sequence identified as *Trichoderma* spp. was used as the outgroup comparison.

Table 3.2 Primer sequences and PCR conditions for taxonomic identification of *Fusarium* isolates.

Primer	Sequence (5'-3')	Locus	Conditions
ITS1F	CTT GGT CAT TTA GAG GAA GTA A	ITS1	5 min - 95 ℃ ----40 cycles---- Denaturation: 30 s - 94 ℃ Annealing: 30 s - 52 ℃ Elongation: 1 min - 72 ℃ 8 min - 72 ℃
ITS4	TCCTCCGCTTATTGATATGC		
EF1	ATG GGT AAG GA(A/G) GAC AAG AC	TEF-1α	8 min - 95 ℃ ----- 35 cycles----- Denaturation : 30 s - 95 ℃ Annealing: 60 s - 53 ℃ Elongation: 1 min - 72 ℃ 5 min - 72 ℃
EF2	GGA (G/A)GT ACC AGT (G/C)AT CAT GTT		

ITS1 primers were designed by White et al., 1990. Tef-1α primers were designed by O'Donnell et al., 1998.

3.3.4 Metagenomic analysis

Soil samples processing and DNA extraction methods were described in detail in Chapter 2. Residue samples were first lyophilized at approximately 100 µbar (VirTis Freezemobile 12ES Freeze Dryer, SP Scientific, New York, USA), for at least 72 hrs, to remove moisture and ease tissue disruption. Subsamples were coarsely ground using a Coffee Grinder (Simplicite, Canadian Tire, Toronto, Canada) for 1 min. The resulting powder was then finely ground using the GenoGrinder for 5 min at 1500 *rpm* in presence of 3 mm diameter stainless steel beads. The coffee grinder was cleaned thoroughly between samples using 70% ethanol-soaked paper towel to remove any traces of sample residues and prevent cross contamination. Total DNA was extracted from 20 mg of ground plant residues using the DNeasy Plant Mini kit (QIAGEN, Hilden, Germany) for residue samples. DNA concentrations were normalized to approximately 10ng μL^{-1} and amplicon sequencing of the ITS1 region was conducted using Illumina MiSeq (Genome Quebec) to study fungal communities in residue, with the same methods as described for soil samples in Chapter 2. Quality trimming and chimera screening for amplicon sequencing results was done using CLC (QIAGEN), with details described in Chapter 2.

Sequences with 97% similarity were grouped into operational taxonomic units (OTUs) which were assigned taxonomic classifications based on the UNITE dynamic reference database version 8 (February 4, 2020; Nilsson et al., 2019). Bioinformatics analysis of metagenomics sequencing was described in detail in Chapter 2.

Differential abundance analysis was conducted using a negative binomial general linear model (GLM) to find organisms that were present in different cover crop residue compared to the residue from unmanaged plots (CLC, QIAGEN, Hilden, Germany). Likelihood ratio test was used to test significance OTU by cover crop. Trophic groups for these OTUs were assigned using

FUNGuild (Nguyen et al., 2016). Results were visualized in a heatmap created using fold change comparison of OTUs present in the cover crop residue compared to unmanaged.

3.3.5 qPCR on soil and residue

Abundances of *F. oxysporum*, *F. graminearum*, *F. sporotrichioides*, and *F. avenaceum* were quantified in DNA extracted from soil samples and *F. oxysporum* and *F. graminearum* were quantified in DNA from residue samples, using primers listed in Table 3.3. DNA concentration was normalized to 10 ng μL^{-1} and 4 µL were added to reaction. Each reaction contained 10 µL of 2x Prime Time Master Mix (Integrated DNA Technologies, Coralville, Iowa, US) 1µL each of primers (0.5 mM) and probes (0.125 mM), as well as molecular-grade water. Reactions were conducted in duplicate with the BioRad thermal cycler BR003847 (Hercules, CA, US), using the conditions listed in Table 3.3. Total *Fusarium* spp. (ng) per gram of soil or residue was calculated using the DNA concentration after extraction, elution volume, and amount of field sample that DNA was extracted from.

Table 3.3 Primer sequences and qPCR conditions for quantification of *Fusarium* spp. in environmental DNA.

Primer	Sequence (5'-3')	Probe (5'-3')	Organism	Conditions
Fef1-F	TAGTCACTTTCCCTTCAATCG	SYBR Green	[1]*F. oxy*	2 min - 98°C ---39 cycles--- 10 s - 98°C 15 s - 57°C
Fef1-R	CTCAAGTGGCGGGGTAAGT			
Spo-F	TTTTTTACGGCTGTGTCGTGA	TGATAGTGGGGCTCATACCC	[2]*F. sporo*	3 min - 95°C ---39 cycles --- 10 s - 95°C 10 s - 57°C 30 s - 72°C
Spo-R	CGGCTTCCTATTGACAGGTG			
Ave-F	GCTTATCTGCACTCGGAACC	CGACAAGCGAACCATCGAGA	[2]*F. ave*	
Ave-R	CGCGTAATCGAAGGGATATT			
Fg16	Unpublished	Unpublished	[3]*F. gram*	3 min - 95°C ---39 cycles--- 5 s - 95°C 30 s - 62°C

1 – *F.oxy = F. oxysporum,* amplified using primers designed by Haegi et al., 2013
2 – *F. sporo = F. sporotrichioides* and *F. ave = F. avenaceum,* primers and probes were designed by Zitnick-Anderson et al., 2018
3 – *F. gram = F. graminearum* primers and probe sequences were designed by Hafez et al., [Unpublished]

3.3.6 Greenhouse trials set up

Effects of certain cover crop soils on FRCR in barley (AAC Synergy) and soybean (25-10 RY) under high and low pathogen load were tested through greenhouse trials. Soil was collected from cover crop plots in November 2020, after the cover crops were flail-mowed. Treatments included soil collected after growing brown mustard, alfalfa, phacelia, sorghum-sudangrass, buckwheat, as well as soil collected from a managed weed-free plot referred to here as "no-crop", and autoclaved field soil. *Fusarium* inoculum for high pathogen load treatments were prepared on PDA media on standard 90 mm Petri dishes, overlain with sterilized Whatman #1 (85mm) filter papers. *F. oxysporum* (soybean root isolate SB112-1) was used as pathogen inoculum for soybean and *F. graminearum* spring wheat spike isolate 20-35 (Johnstone et al., 2021) was used for barley. *Fusarium* cultures were grown on the filter paper for 7 days until they were approximately 70 mm in diameter. Filter papers with the mycelium were placed in corresponding soil filled pots and covered with approximately 2 cm layer of soil. Pots were set up in a randomized complete block design with four replicates. Each replicate included seven cover crop treatments in duplicate, which were inoculated with *Fusarium* spp. or plain filter paper. Five seeds were planted per pot approximately 1 cm above filter paper. Soybean seeds were treated with soybean peat inoculum (Cell-Tech NS, Saskatoon, SK, Canada). Drip watering was used to maintain consistent moisture levels throughout experiment. Plants were fertilized one week after emergence until flowering, with 30mL of 1% solution 20:20:20 N:P:K according to manufacturer recommendations.

3.3.6.1 Greenhouse FRCR and crop quality assessment

Four seeds per pot were destructively sampled after emergence to re-isolate pathogens, with one plant remaining per pot. Pieces were cut 5 mm above and below the crown and plated

on PDA amended with antibiotics. *Fusarium* isolates were identified based on morphology, five days after plating. Remaining plants were monitored for above ground symptoms and FRCR was rated at harvest using the ordinal 0-5 scale as described previously. Number of tillers, number of nodes, number of heads or pods, number of seeds, seed weight, plant height and above ground biomass data were collected to assess crop quality.

3.3.7 Statistical analysis

All statistical analyses were conducted using JMP 16 (SAS Institute) unless stated otherwise. Cover crop effects on damping off ratings in barley and soybean were assessed with replicate considered as a random effect, using GLIMMIX with normal distribution and identity link. Significance was tested using Tukey's HSD with a p-value ≤ 0.05. Choice of cover crop effects on FRCR disease severity in barley and soybean, yield, as well as *Fusarium* spp. abundance by qPCR were significance tested using mixed linear models with the restricted maximum likelihood (REML) method and post-hoc Tukey's HSD test with p-value ≤ 0.05, with replicates and trial considered as random effects. Correlation between FRCR in each cash crop and *Fusarium* spp. abundance by qPCR was measured using Pearson's correlation with significance defined as p-value ≤ 0.05. Effects on root isolates and were tested using GLIMMIX with Log link Poisson distribution and post-hoc Tukey's HSD test. For the greenhouse trials, effects of soil treatment and pathogen load were also significance tested using mixed linear models with the restricted maximum likelihood (REML) method, with replicates considered as random effects. Principal component analysis was done to compare agronomic data and disease from the two greenhouse trials.

3.4 Results

3.4.1 FRCR in soybean and barley

Choice of cover crop significantly influenced FRCR in soybean. Soybean planted after oilseed radish had significantly high FRCR severity compared to soybean after buckwheat, phacelia, sorghum-sudangrass and the unmanaged control. Alfalfa, in the first trial, associated with relatively high FRCR in soybean, but the opposite trend was observed in the second trial (Fig. 3.1A).

Cover crops did not significantly influence damping off in soybean (Fig. 3.1C). *Rhizoctonia* spp., *Pythium* spp., and *Fusarium* spp. were isolated from diseased tissue sent to the Provincial Disease Diagnostics Lab (PE, Canada), and were the likely causal agents of damping off in soybean.

Yield data was for the first trial was inconclusive as the crops were harvested after post-tropical storm Dorian (September 8th, 2019), resulting in loss of all crops. Soybean planted after annual ryegrass and the unmanaged control had significantly higher yield compared to soybean after Mix-1 and buckwheat. Soybean grown after annual ryegrass had an average yield of 1658 kg ha^{-1}, which was significantly higher than from soybean planted after buckwheat with an average of 833 kg ha^{-1} (Fig. S3) (Mills, 2020 [Unpublished]).

FRCR in barley was observed in high levels in all crops, and cover crops still significantly influenced the disease severity. Barley grown after alfalfa, Mix-2 and sorghum-sudangrass had significantly lower FRCR compared to barley grown after oilseed radish (Fig. 3.1B). Barley grown after annual ryegrass associated with significantly high FRCR in the first trial but low FRCR in the second trial. In the second trial, barley yields were extremely low, likely due to FRCR damage.

Damping off in barley was also significantly influenced by the choice of cover crops (Fig. 3.1D). There was significantly more damping off with barley planted after Mix-2 compared to barley after phacelia and crimson clover (Fig. 3.1D). *Pythium* spp., *Rhizoctonia* spp., and *Fusarium* spp., were also isolated from barely tissue and were the likely causal agents of damping off. Damping off in barley and soybean significantly correlated with $R^2 = 0.31$ and p-value ≤ 0.05.

In 2020, barely planted after alfalfa had the highest yield with an average of 465 kg ha^{-1}, and lowest yield was after Mix-2 with an average of 36.4 kg ha^{-1} (Fig. S3.) (Mills, 2020 [Unpublished]). Other major factors that influenced barley yield measurement was the high variability in growth stage at harvest, resulting in a large number of green plants, as well as a large number of buckwheat seeds collected from certain plots due to high abundance of buckwheat weeds.

Principal component analysis showed that barley yield significantly correlated with FRCR and damping off in soybean with the first two principal components explaining more than 80% of the variation in the dataset. Mix-1 and buckwheat strongly associated with damping off in soybean, while oilseed radish and Mix-3 closely associated with FRCR (Fig. 3.2A). Similar to soybean results, principal component analysis showed that barley yield negatively correlated with both FRCR and damping off (Fig. 3.2B). This indicated that the extremely low yields were likely due to a combination of stressors including damping off and FRCR in both barley and soybean.

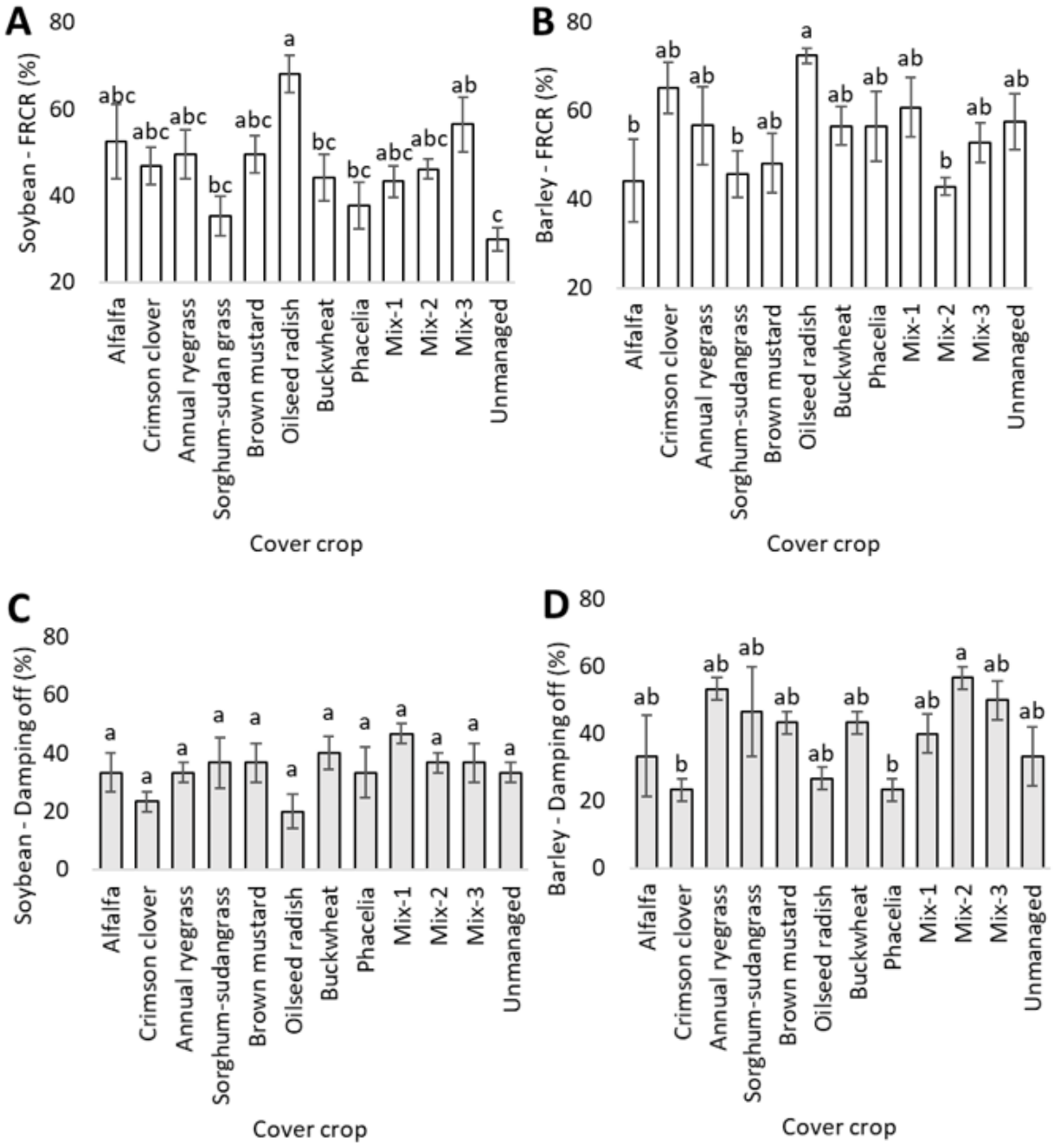


Fig. 3.1 Effects of previously planted cover crops on soybean (A) and barley (B) FRCR disease severity index (%), with data from 2019 and 2020; n=6. Effects of previously planted cover crops on damping off in soybean (C) and barley (D), with data from 2020; n=6. Mix-1: buckwheat + crimson clover; Mix-2: phacelia + brown mustard; Mix-3: buckwheat + crimson clover + brown mustard. Different letters represent statistically significant differences at p-value ≤ 0.05 by Tukey's HSD.

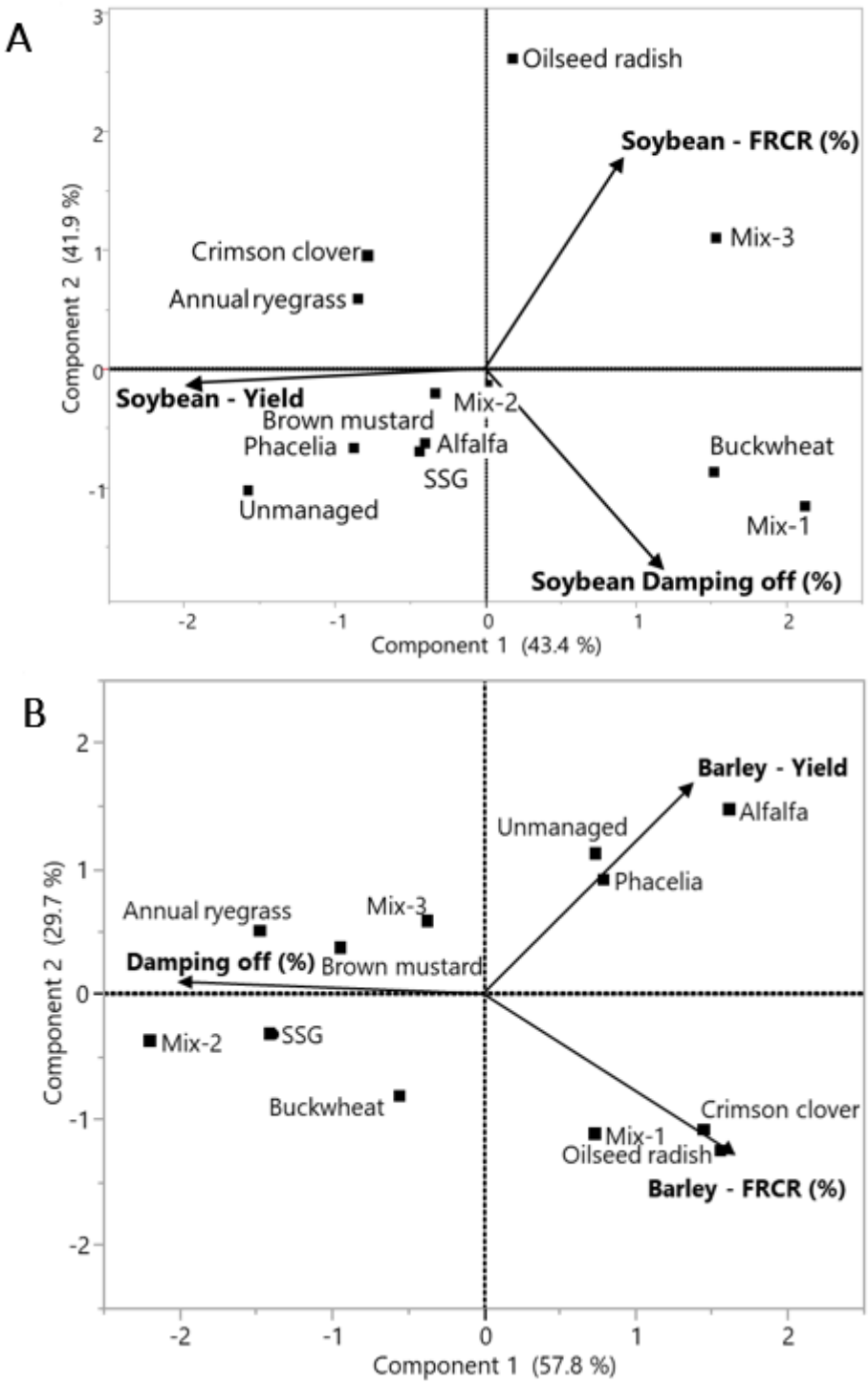
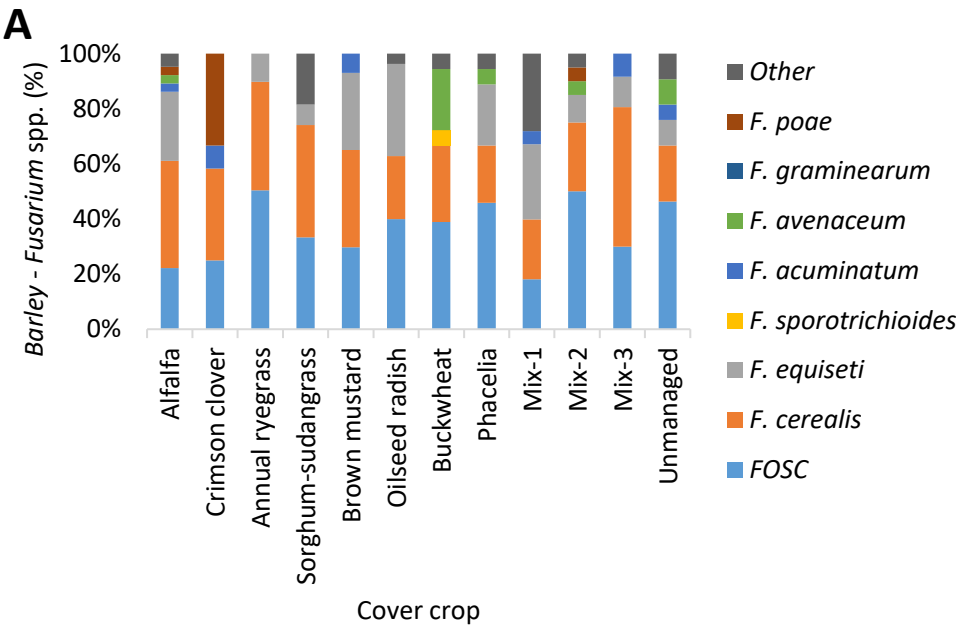


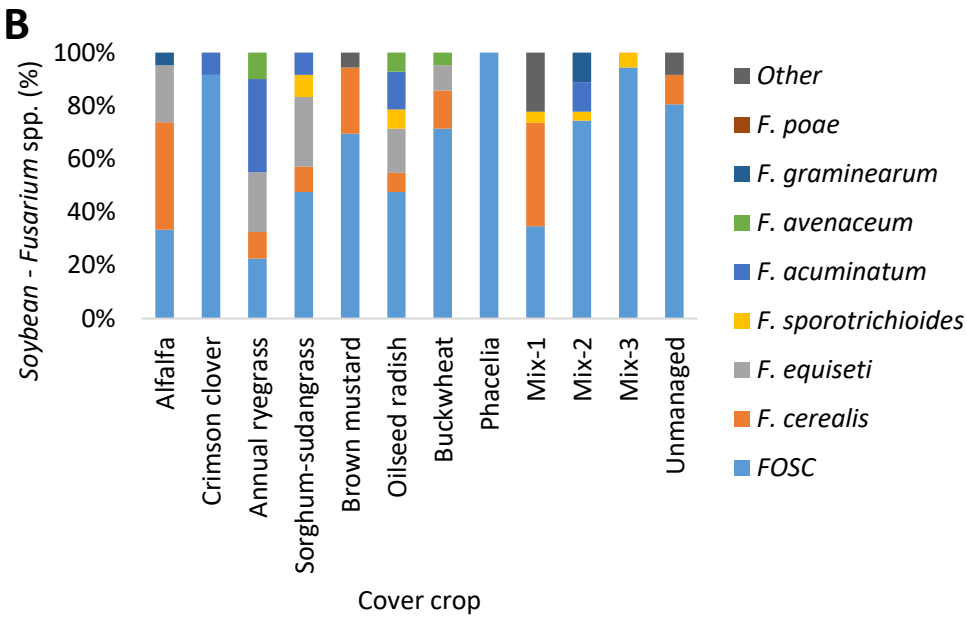
Fig. 3.2 Principal component analysis comparing damping off, FRCR DSI%, and yield from the 2020 for barley (A) soybean (B). FRCR: Fusarium root and crown rot; SSG: sorghum-sudangrass; Mix-1: buckwheat + crimson clover; Mix-2: phacelia + brown mustard; Mix-3: buckwheat + crimson clover + brown mustard.

3.4.2 Pathogen isolation and phylogenetic analysis

F. oxysporum was the most commonly isolated pathogen from representative barley and soybean roots from the first trial, and it was confirmed to be one of the causal agents of FRCR in both crops, through verification of Koch's postulates. A total of 379 root isolates from the second trial were identified as *Fusarium* spp., with 227 from barley roots and 152 from soybean roots. Isolates were identified as *F. oxysporum* species complex (FOSC)*, F. cerealis, F. equiseti, F. graminearum, F. avenaceum*, *F. acuminatum* (Ellis & Everhart; teleomorph: *Gibberella acuminate* (Wollenw.)), *F. poae* (Peck (Wollenw.)), and *F. sporotrichioides. F. cerealis,* FOSC and *F. equiseti* were the most abundant isolates from barley roots, making up 33%, 31% and 19% of all *Fusarium* isolated from barley, respectively (Fig. 3.3A). FOSC, and *F. cerealis* were the most abundant isolates from soybean comprising of 63% and 16% of *Fusarium* isolated, respectively (Fig. 3.3B). Previous crop did not have an effect on the number of *Fusarium* isolates or any of the species. Highest number of *Fusarium* spp. were isolated from barley and soybean planted after brown mustard.

All sequences used to build the phylogenetic tree were from isolates collected in the second trial (Fig. 3.3C). After removing low quality sequences, a total of 44 sequences were aligned. Sequences ends were trimmed to removed ambiguous bases, to a final size of 500bp. The *F. oxysporum* species complex (FOSC) was represented by three clades of *F. oxysporum* and one clade of *F. commune,* FOSC grouped separately from the other *Fusarium* spp. The second major group was *F. cerealis* and *F. graminearum*. *F. equiseti* was the next closely related group, along with *F. sporotrichioides.* The other two clades included *F. solani* and *F. avenaceum* (Fig. 3.3C). The *F. sporotrichioides* and *F. graminearum* isolates were from soybean roots while *F. avenaceum* was only isolated from barley roots.


A
Barley - Fusarium spp. (%)
100%
80%
60%
40%
20%
0%
Alfalfa
Crimson clover
Annual ryegrass
Sorghum-sudangrass
Brown mustard
Oilseed radish
Buckwheat
Phacelia
Mix-1
Mix-2
Mix-3
Unmanaged
Cover crop
Other
F. poae
F. graminearum
F. avenaceum
F. acuminatum
F. sporotrichioides
F. equiseti
F. cerealis
FOSC


B
Soybean - Fusarium spp. (%)
100%
80%
60%
40%
20%
0%
Alfalfa
Crimson clover
Annual ryegrass
Sorghum-sudangrass
Brown mustard
Oilseed radish
Buckwheat
Phacelia
Mix-1
Mix-2
Mix-3
Unmanaged
Cover crop
Other
F. poae
F. graminearum
F. avenaceum
F. acuminatum
F. sporotrichioides
F. equiseti
F. cerealis
FOSC

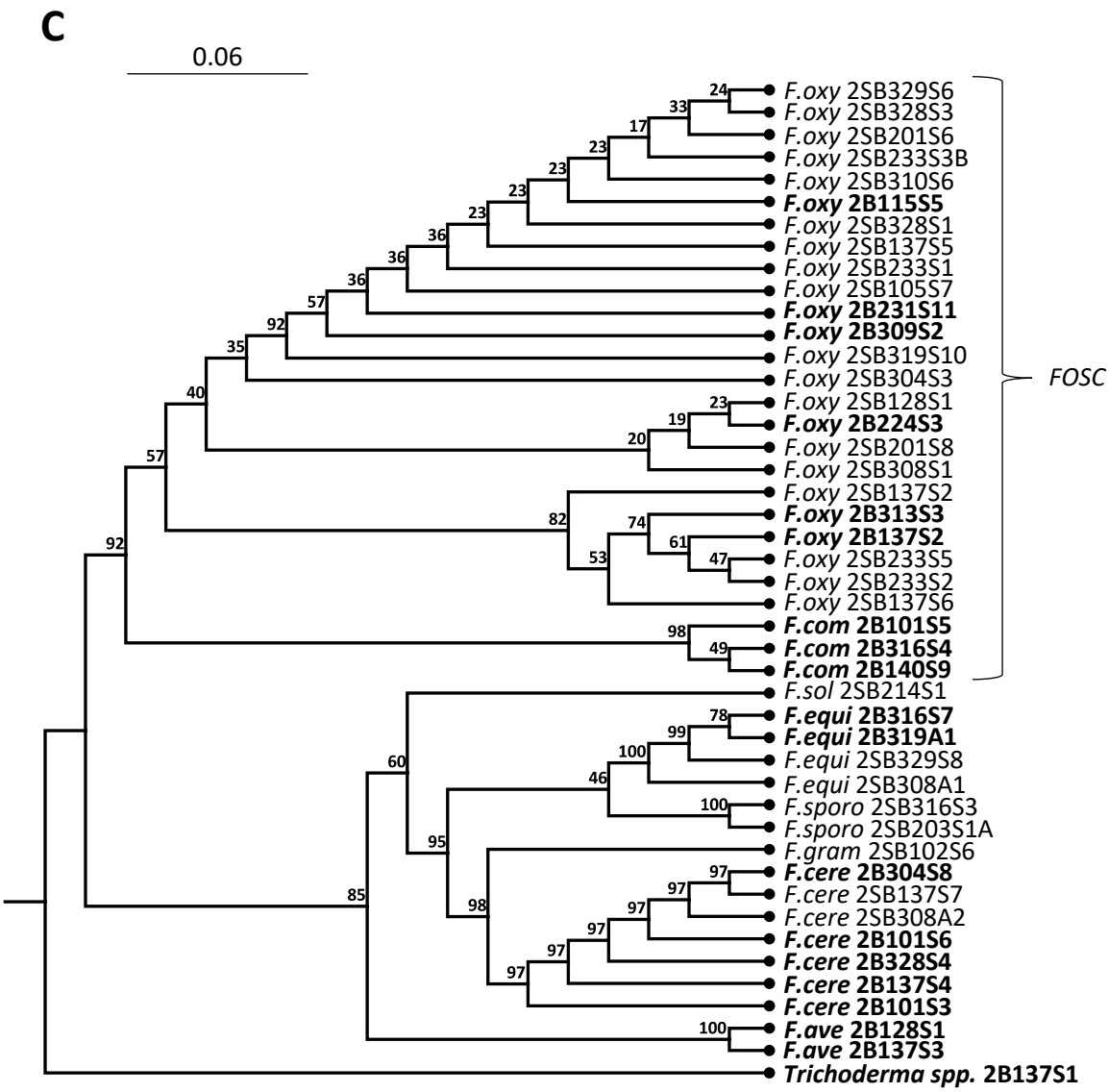


Fig. 3.3 *Fusarium* isolates from barley and soybean roots collected from the second trial. Proportions of *Fusarium* spp. isolates by the total number of *Fusarium* isolates per cover crop, for barley (A) and soybean (B) presented in stacked bar graphs; n=6. Rooted phylogenetic tree cladogram with sequences from the eight main *Fusarium* spp. isolated and a *Trichoderma* spp. Tef-1α sequence set as the outgroup (C); N=45. The labels list a short-form of the species name along with the unique isolate ID, with barley isolates bolded. *F.oxy* is short for *F. oxysporum*, *F.com = F. commune*, *F.sol = F. solani, F.equi = F. equiseti*, *F.sporo = F. sporotrichioides*, *F.gram = F. graminearum, F.cere = F. cerealis*, *F.ave = F. avenaceum. FOSC = F. oxysporum* species complex.

3.4.3 *Fusarium* abundance in soil and residue

PERMANOVA results indicated that the choice of cover crop had a significant effect on fungal community composition in the residue in the first trial (p-value < 0.01) but not the second (p-value = 0.41). Higher abundance of *Fusarium* OTUs was found in the residue collected from the second trial. In data from both trials combined, 142 residue fungal OTUs were identified as pathotroph, 63 as saprotroph and 5 as symbiotroph. The most abundant fungal pathotrophs were *Alternaria* spp., *Plectosphaerella* spp., *Colletotrichum* spp. and *Fusarium* spp. A total of 6 *Fusarium* spp. were differentially abundant by cover crop (Fig. 3.4). *F. oxysporum* and *F. poae* were the only OTUs which significantly increased in abundance as a response to growing certain cover crop, when compared to unmanaged. The *F. oxysporum* OTU was higher in abundance compared to unmanaged in every cover crop except annual ryegrass and Mix-2, in which it had the opposite trend. The *F. poae* OTU was higher in sorghum-sudangrass, brown mustard, buckwheat, phacelia and Mix-3 residue compared to unmanaged.

Quantitation of DNA markers for different *Fusarium* spp. using qPCR did not show that cover crops influence *Fusarium* abundance in soil or residue. The *Fusarium* spp. abundances measured by qPCR and the relative abundance of individual *Fusarium* OTUs did not significantly correlate with FRCR in either barley or soybean.

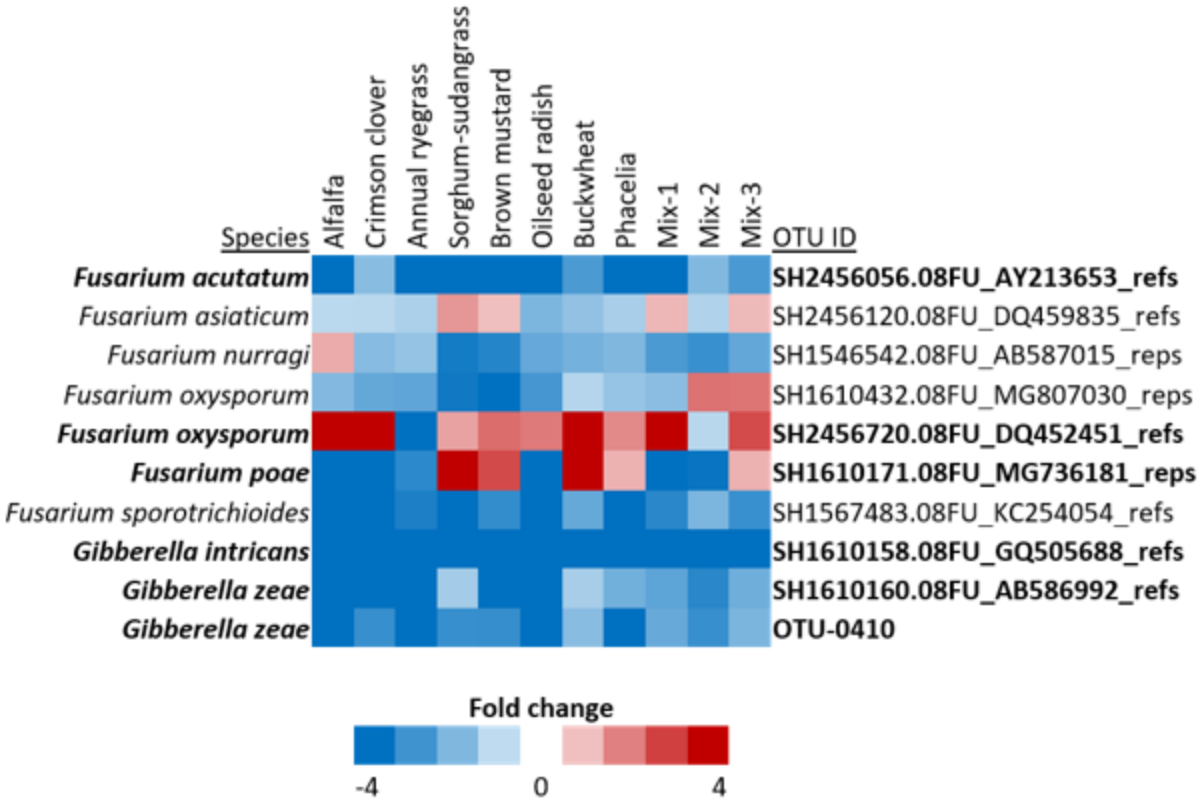


Fig. 3.4 Differentially abundant *Fusarium* OTUs in cover crop residue from both trials. Heatmap present fold change in relative abundance of *Fusarium* OTUs from each cover crop residue compared to unmanaged residue; n=6. Species names are listed on the left and OTU IDs listed on the right. OTUs with FDR p-value ≤ 0.05 were considered to have significant fold change and are bolded.

3.4.5 FRCR in greenhouse barley

F. graminearum was re-isolated from all destructively sampled roots collected early in the trial. Cover crop soils differentially influenced FRCR in barley based on visual symptoms assessed at harvest. Pathogen inoculation significantly increased FRCR severity (Fig. 3.5A). Plants growing in the brown mustard, and alfalfa soils had relatively high FRCR disease severity, while barley in the no-crop, and sorghum-sudangrass soils had relatively low disease severity regardless of inoculum. Barley grown in phacelia soil had the most seeds, whereas barley grown in brown mustard soil had the least seeds, regardless of inoculum. The effect of cover crop soils, as well as the effect of cover crop by inoculum on the seed weight was significant (p-value < 0.05). The seed weight from barley grown in no-crop soil (weed-free) was significantly higher than that from brown mustard soil, regardless of inoculum. Barley grown in phacelia, and sorghum-sudangrass soils with inoculum had significantly heavier seeds than those without inoculum. The first two components of the PCA comparing agronomic measurements and FRCR severity in the inoculated treatment, represented more than 80% of the variance (Fig. 3.6A). The PCA indicated that FRCR negatively correlated with all other agronomic measurements except for number of tillers. FRCR strongly associated with brown mustard soil while negatively associating with phacelia, sorghum-sudangrass and tilled soils.

3.4.6 FRCR in greenhouse soybean

F. oxysporum was re-isolated from all roots destructively sampled after emergence. Pathogen inoculum did not have a significant effect on FRCR in soybean or any of the other agronomic measurements, even in the autoclaved soil (Fig. 3.5B). However, cover crop soils did influence disease severity. Soybeans grown in alfalfa soil with pathogen inoculum had significantly more disease than without inoculum. Above-ground biomass was significantly

different by cover crop soil, where soybean grown in no-crop soil had the most biomass, and soybeans grown in brown mustard soil had significantly less biomass. The first two principal components in Fig. 3.6B represent more than 90% of the variance. FRCR severity negatively correlated with all other agronomic measurement, with the strongest effect being on seed weight. FRCR severity strongly associated with alfalfa, and negatively associated with sorghum-sudangrass and no crop soil.

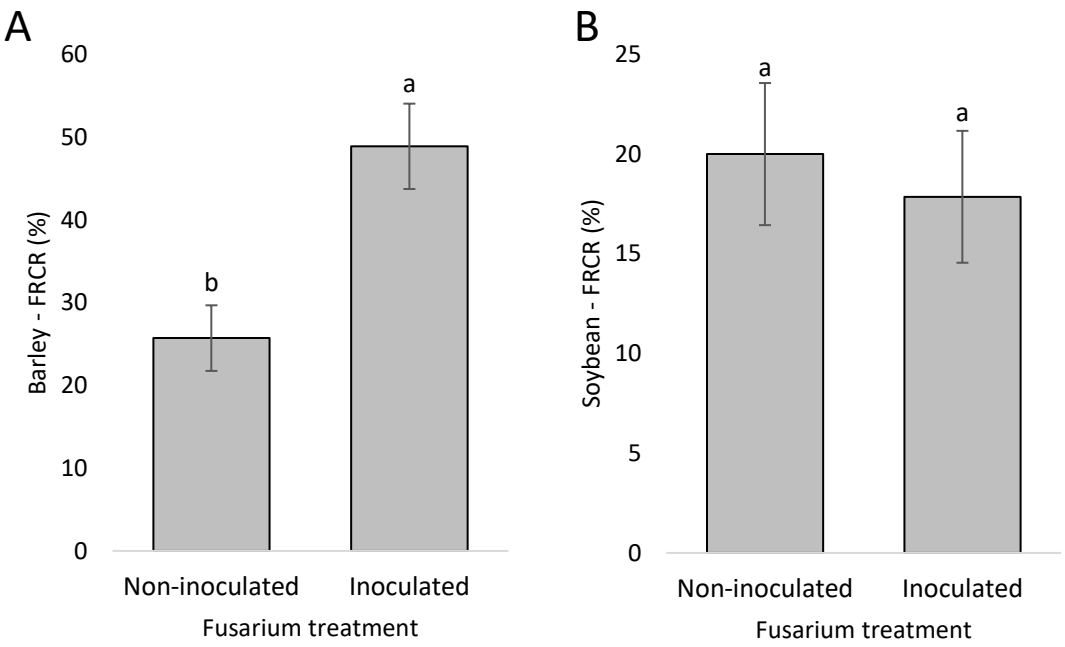


Fig. 3.5 Bar graphs represent FRCR in barley (A) and soybean (B) by *Fusarium* inoculum; n=28. Connecting letters represent significant differences with p-value ≤ 0.05, by Tukey's HSD test.

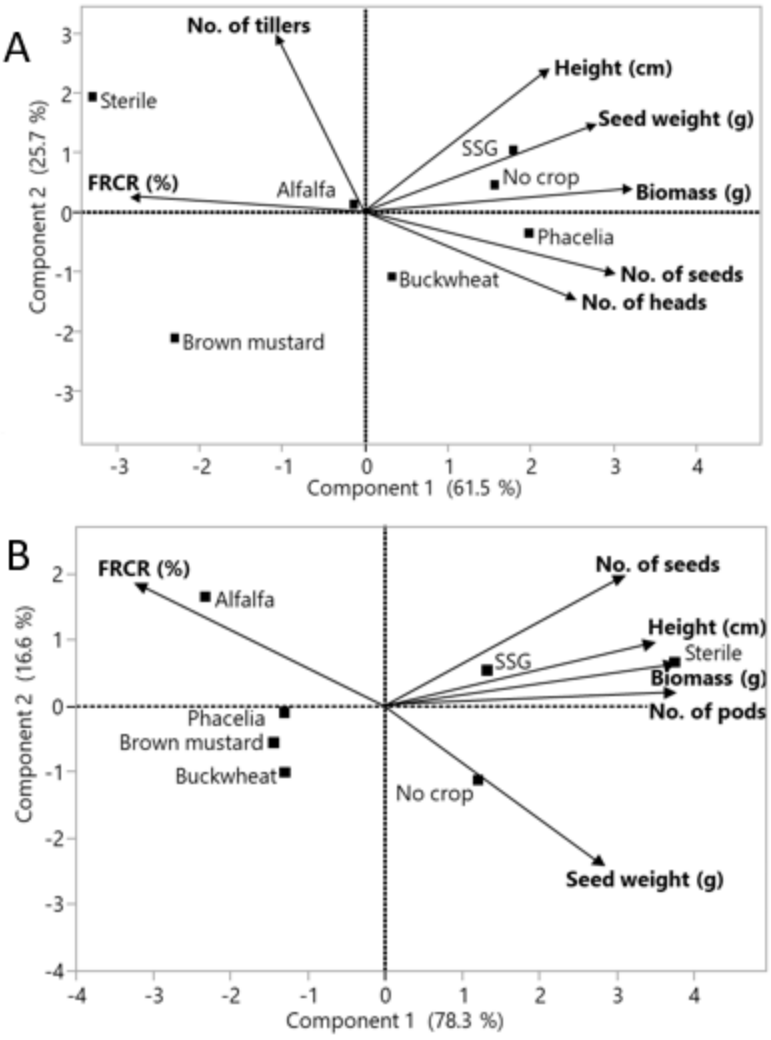


Fig. 3.6 Principal component analysis comparing disease severity and agronomic data collected from barley (A) and soybean (B) greenhouse trials, with *Fusarium* inoculum treatment. FRCR = Fusarium root and crown rot; SSG = sorghum-sudangrass.

3.5 Discussion

The choice of cover crop was found to influence *Fusarium* load in the soil, and therefore influenced FRCR severity in subsequent crops. High levels of FRCR were observed in both barley and soybean, and the disease severity was significantly influenced by previously planted cover crop. Barley and soybean planted after oilseed radish had the overall highest observable FRCR symptoms. Field trials and greenhouse trials also indicated that sorghum-sudangrass associated with lower FRCR in both barley and soybean.

Oilseed radish and brown mustard, like most *Brassicaceae*, produce isothiocyanates (ITC) which are known to have toxic effects against soil-borne microorganisms (Smolinska et al., 2003). However, these crops were associated with high FRCR in both barley and soybean, likely because the most commonly isolated pathogen was *F. oxysporum.* Smolinska et al., (2003), found that *F. oxysporum* was more resistant to ITCs, with inhibition of spore germination observed only in the presence of higher concentration of certain ITCs such as ethyl, benzyl, and phenethyl. Furthermore, their biofumigant abilities are less effective under no-till conditions. Brown mustard soil led to increased FRCR in barley and soybean in the greenhouse trials. This is possibly because the residue was mixed with the soil leading to a reduction of beneficial microorganisms such as mycorrhizal fungi, while having little effect on the *Fusarium* spp. that were also present in the soil. This is similar to findings by Nallanchakravarthula et al. (2021).

Pathogen load in cover crop residue in no-till cropping systems play an important role in root disease development (Fernandez et al., 2008). Differential abundance analysis identified several pathogenic fungal OTUs that were affected by cover crop including 6 *Fusarium* OTUs. *F. oxysporum* and *F. poae* were higher in abundance in cover crop residue compared to unmanaged residue. These two taxa were also differentially abundant by cover crop in soil samples, based on

results from Chapter 2 (F.g 2.4A). Differences in residue fungal community composition may be attributed to differences in residue structure, both above and below ground. The amount of plant material and the size of the residue pieces may change the rate of decomposition, thereby creating different microenvironments for the associated microorganisms (Cotten and Munkvold, 1998).

Residue fungal community composition in the second trial were not significantly different by cover crop indicating that post-tropical storm Dorian (September 8th, 2019) likely moved the crop residue into neighbouring fields, introducing high variance. An increase in *Fusarium* OTU abundance in the residue collected after the storm was also observed. Heavy winds may have also transported new fungal spores and infected plant material, thereby increasing the pathogen load (Alvarez-Manjarrez and Garibay-Orijel, 2021). Extreme weather events including post-tropical storms, flooding and drought are becoming more frequent, requiring growers to adopt more sustainable agricultural practices (Howden et al., 2007). Osmotic stress is known to increase pathogen biomass and susceptibility root disease, especially in barley (Liu and Liu, 2016). This may also explain the high levels of FRCR observed in this study, as both trials were conducted in drought years, with 2020 setting record high agricultural damages (Table 3.1; Robertson, 2020).

Lower yields in barley and soybean strongly correlated with damping off and FRCR, based on the data collected in the second trial. The cash crop phase of the second trial was conducted in 2020, with high drought stress (Table 3.1). This indicates that the crops that survived damping off caused by *Pythium* spp. and *Rhizoctonia* spp. likely got infected by *Fusarium* spp. later in the season, as they were weakend by osmotic stress. *Pythium* spp. and *Rhizoctonia* spp. are both known to cause damping off in barley and soybean (Ingram and Cook, 1990; Ogoshi et al., 1990;

Nelson et al., 1996; Navi et al., 2019). Future studies focused on the effects of certain cover crops on pathogen complexes involved in early season disease may be beneficial to better understand their impacts on plant production.

Higher FRCR in barley and soybean was associated with increased *Fusarium* load in the soil and residue with different species identified as dominant causal agents of disease in the two crops. Other pathogens including *Bipolaris sorokiniana* ((Sacc.) Shoemaker; teleomorph: *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. ex Dastur) were also isolated but less frequently, therefore it is possible that not all the disease observed was caused by *Fusarium* spp. However, it was assumed that most of the damage was done by the different *Fusarium* spp. which were more commonly isolated from barley and soybean roots. As different cash crops host different *Fusarium* species as major pathogens, the choice of cover crops should reflect their impact on individual *Fusarium* species in the soil. *F. oxysporum* was the more abundant pathogen isolated from soybean while, *F. cerealis, F. oxysporum* and *F. equiseti* were commonly isolated from barley. *F. oxysporum* is a well-known soybean pathogen, but less frequently associated with FRCR in barley (Ellis et al., 2014; Gentosh et al., 2020).

Fusarium spp. are ubiquitous and hard to eradicate; however, different management practices can be used to suppress certain pathogenic species, or promote *Fusarium* suppressive soil, which can include secretion of toxic metabolites and increasing the abundance of beneficial soil microbes (Alabouvette, 1999). Sorghum-sudangrass closely associated with less FRCR in both barley and soybean in the field. Sorghum-sudangrass is known to produce *p*-hydrobenzoic acid which has anti-fungal effects against *F. oxysporum* (Weston et al., 1989). Previously, sorghum-sudangrass was also found to increase the abundance of symbiotrophic fungi including important arbuscular myccorrhizal fungi (Chapter 2). As such, the results presented here suggest that

sorghum-sudangrass used as cover crop in no-till systems may have disease suppressive effects against FRCR in subsequently planted crops. It is possible that sorghum-sudangrass manipulated the soil microbiome thereby conferring disease suppressive abilities to the associated soil. Results indicate that sorghum-sudangrass can suppress *Fusarium* spp. in the soil, however it was not clear whether it has disease suppressive effects against other pathogens such as *Pythium* spp., and *Rhizoctonia* spp. Further research with on-farm data with different field history would be required to better understand the underlying mechanisms involved in disease suppression by sorghum-sudangrass soil.

In the greenhouse trial, *F. graminearum* inoculum caused significantly more disease in the sterile soil, however *F. oxysporum* inoculum did not have an effect on FRCR in soybean. This may be due to the *F. oxysporum* single spore isolate loosing virulence after repeated culturing or due to accumulation of mutations (de Almeida Lopes et al., 2016; Smith et al., 2018). Other soil treatments did influence soybean FRCR severity, likely due to the differential abundance of *Fusarium* spp. already present in the soil. The no crop soil treatment consistently associated with low FRCR in both barley and soybean, even with pathogen inoculum.

Amplicon sequencing and qPCR present a good way to identify pathogen load in the soil prior to planting, which related to disease in subsequent plants. Moya-Elizondo et al. (2011) found that qPCR was a robust technique for detecting the distribution of *Fusarium* spp. in the soil, and that it correlated closely with traditional culturing techniques. Amplicon sequencing provides a broad overview of pathogen population in the soil and residue, which can provide information about which groups of pathogens to target for disease management. Molecular techniques using *Fusarium* specific primers, of samples collected from grower fields could provide more insight into choice of cover crop and other preventative measures required to

manage potential root disease (Boutigny et al., 2019). Furthermore, models such as the one developed by Zitnick-Anderson et al. (2020) may be used to predict the likelihood of abiotic and biotic factors leading to increase in soil pathogen community.

3.6 Conclusion

Results from this study clearly indicate that FRCR in both barley and soybean is highly influenced by the previously planted cover crop. The effect is related not only to the increased pathogen population but also to the changes in overall microbial composition. Crops planted after oilseed radish had significantly higher incidence of FRCR while crops planted after sorghum-sudangrass had significantly lower FRCR incidence. This was predicted based on results from amplicon sequencing of DNA extracted from soil samples (Chapter 2).

3.7 Appendix

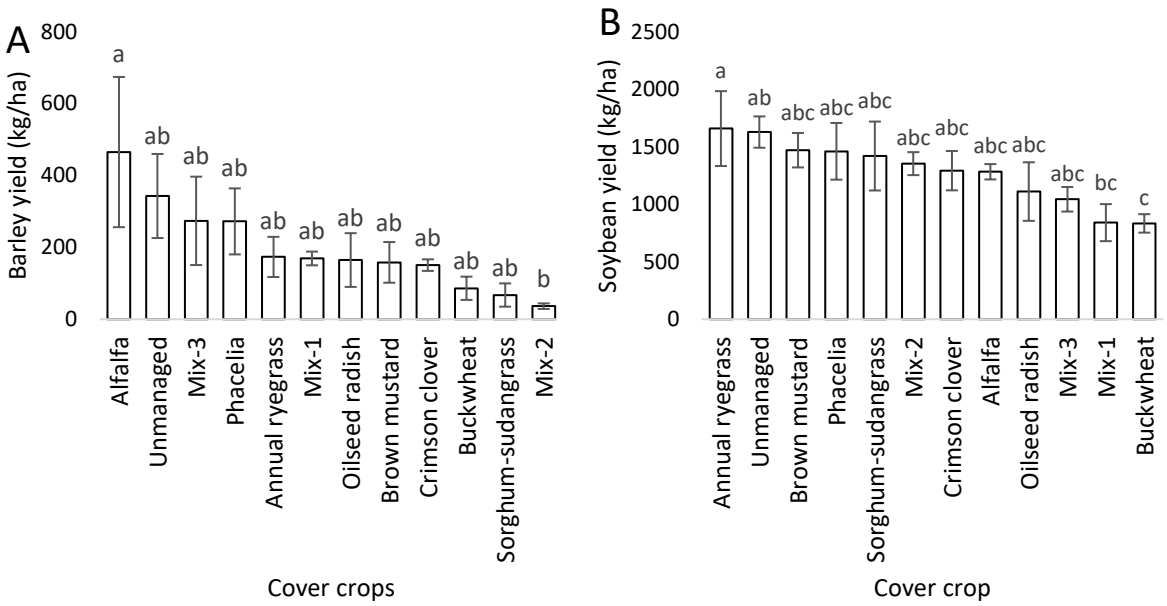


Fig. S3 Mills, 2020 [Unpublished] yield (kg ha^{-1}) for barley (A) and soybean (B) from the second trial; n=3. Connecting letters represent significant differences with p-value ≤ 0.05, by Tukey's HSD test. Mix-1: Buckwheat + Crimson clover; Mix-2: Phacelia + Brown mustard; Mix-3: Buckwheat + Crimson clover + Brown mustard.

Chapter 4 - Conclusion

This study assessed the effects of cover crops on fungal and bacterial communities in soil and residue and how this carries over to barley and soybean in the subsequent year to impact Fusarium root and crown rot (FRCR). The results clearly indicate that choice of cover crop significantly affects alpha and beta diversity of soil fungal and bacterial communities as well as certain soil microbial ecosystem functions. Cover crop induced changes in the soil microbiome carried over to the subsequent year and had a direct impact on FRCR in both barley and soybean.

4.1 Review of Chapter 2 hypotheses and findings:

1. **Cover crops will not affect soil fungal and bacterial community structure (alpha diversity) in the subsequent growing season.**

Findings: This hypothesis was not supported as alpha diversity was significantly affected by choice of cover crop in one growing season (Fig. 2.3)

2. **Choice of cover crop will influence soil fungal and bacterial community composition (beta diversity) by the end of the growing season, and this trend will carry over to the soil in the subsequent year.**

Findings: This hypothesis was supported as both fungal and bacterial beta diversity were significantly different by choice of cover crops in subsequent growing season (Table 2.2)

3. **Different cover crop soil will have increased abundance of specific fungal and bacterial functional groups.**

Findings: This hypothesis was supported as some cover crops increased the abundance of different fungal trophic groups as well as bacterial functional groups. For example, sorghum-sudangrass and buckwheat increased symbiotrophic fungi, pest biocontrol agent fungi, as well as predatory and exoparasitic bacteria and nitrification related bacterial functional groups (Fig. 2.5)

4. **The abundance of fungal and bacterial taxa which may have an influence on plant health in the subsequent year (such as Fusarium spp.) will be differentially affected by various cover crops.**

Findings: This hypothesis was partially supported as several fungal OTUs including some *Fusarium* spp. were differentially abundant by cover crop in the cash crop phase of the subsequent year. However, bacterial OTUs that might have an influence on soil and plant health were not significantly different by cover crop (Fig. 2.4)

4.2 Review of Chapter 3 hypotheses and findings:

1. **Choice of cover crops will influence FRCR in barley and soybean in the subsequent year.**

Findings: This hypothesis was supported as FRCR disease severity in both barley and soybean was different by cover crop with crops planted. For example, sorghum-sudangrass consistently associated with low FRCR in both barley and soybean in field and greenhouse settings (Fig. 3.1; Fig. 3.6)

2. **FRCR will positively correlate with pathogen load in the soil and residue.**

Findings: This hypothesis was not supported as FRCR in barley or soybean did not positively correlate with any individual *Fusarium* spp. abundances.

3. **Barley and soybean grown in soil with increased abundance of beneficial microbial taxa will have less FRCR incidence.**

Findings: This hypothesis was supported by greenhouse results evaluating the effects of soils collected following cover crops that increased beneficial and symbiotic organisms, on FRCR in barley and soybean under high *Fusarium* pressure (Fig. 3.6).

4.3 Future research

High levels of FRCR in both barley and soybean were observed in this study. FRCR may be controlled by reducing pathogen load using tillage (Steinkellner and Langer, 2004). This study was conducted under no-till with cash crops directly seeded into previous crop residue. Effects of the cover crops studied here may vary under different tillage conditions. Exploring the combined effects of the cover crops studied here under different tillage practices, on FRCR in barley and soybean would be of interest. Incorporation of crop residue with tillage may also enhance the biofumigation effects of oilseed radish and brown mustard (Cohen et al., 2005).

Amplicon sequencing identified several *Fusarium* OTUs that may be involved in the FRCR disease complexes of barley and soybean. Molecular techniques such as amplicon sequencing with *Fusarium* specific primers may be used to better understand the FRCR disease complexes in different crops. Boutigny et al. (2019) developed *Fusarium* specific primers based on the translation elongation factor (Tef1-α) sequence for amplicon sequencing analysis of the pathogen population. Amplicon sequencing of soils collected from grower fields with different

agricultural management practices may also provide insight into the effects of environmental variables on the species involved in FRCR disease complexes.

Fusarium spp. was highly abundant in both field trials, likely due to several factors. For example, cover crops were mowed at the end of the growing season and the cash crops were direct seeded into the crop residue. *Fusarium* spp. can survive as resting structures over winter in crop residue (Cotten and Munkvold, 1998). Vogelgsang et al. (2011) found that mulching the residue using a field shredder reduced *F. graminearum* inoculum and suggested that it may be due to the higher decomposition rate of mulched residue. Studying the effects of mulching the residue from the different cover crops tested in this study may provide more information for FRCR management.

Higher abundance of *Fusarium* OTUs were observed in the residue collected in the second field trial, indicating that this effect may be related to post-tropical storm Dorain. Surface residue may also host air-borne *Fusarium* spp. spores, thereby increasing the pathogen load (Cotten and Munkvold, 1998; Vogelgsang et al., 2011). Spore traps may be used to study the risk of air-borne inoculum increasing pathogen load in the residue. It may also be beneficial to identify whether the different cover crop residues have varied response to the air-borne inoculum, as this could impact FRCR in subsequent crops.

Sorghum-sudangrass was found to increase the abundance of beneficial soil-borne microorganisms and consistently associated with lower FRCR in both barley and soybean. Based on this it can be hypothesized that sorghum-sudangrass manipulates the microbial community to increase soil resiliency against FRCR. Recently, Paudel et al. (2021) found that sorghum-sudangrass also leads to suppression of plant parasitic nematodes, especially when the residue is incorporated. The mechanisms by which sorghum-sudangrass influences the microbial

composition, especially in no-till cropping systems, are unclear and should be a focus for future research.

Buckwheat was also found to increase the population of certain insect pathogens including *Isaria* spp., and *Metarrhizium* spp. This crop is known to reduce the population of pests such as wireworms (Bohorquez Ruiz et al., 2019). It can be hypothesized that along with the direct reduction of insect pests through the secretion of allelopathic chemicals, buckwheat also recruits insect pathogenic fungi, thus making the soil more pest-suppressive. Potential for using buckwheat cover crop to manipulate the soil microbiome to manage economically important insect pests is another area of interest for future research.

Finally, extreme weather events such as major droughts (2018 and 2020) and a post-tropical storm (Hurricane Dorian in September 2019), as well as the COVID-19 pandemic, severely impacted this project. Climate change will likely continue to affect the agriculture industry in the future as such events become more frequent. Conservation agriculture is important as it can minimize the damage caused by extreme weather events. It is well documented that FRCR in many crops increases with osmotic stress created by drought (Paulitz et al., 2002). This was also observed in this study. Studying the effectiveness of different fungicide seed treatments under drought stress may provide more information for developing disease management strategies under these conditions.

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