LEGACY EFFECTS OF COVER CROP MONOCULTURES AND MIXTURES ON SOIL INORGANIC NITROGEN, TOTAL PHENOLIC CONTENT, AND MICROBIAL COMMUNITIES ON TWO ORGANIC FARMS IN ILLINOIS

BY

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THESIS

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ABSTRACT

Cover crops can leave behind legacy effects on their soil environments by influencing soil inorganic nitrogen (N) pools, total phenolic content through the release of secondary compounds, and by altering soil microbial communities. I analyzed soils collected during a two-year field study and aimed to determine how spring-sown cover crops (grass, legume, or Brassica monocultures or diverse, five-way mixtures) influence these three aspects of the soil environment. Soils were collected in the spring of 2015 and 2016 on two different organic farms in Central and Northern Illinois, PrairiErth and Kinnikinnick, during the four weeks post-cover crop incorporation. The first part of this study addressed the influence of cover crops on soil inorganic N (nitrate, ammonium, and potentially mineralizable N, PMN) and total phenolic content intensity, as measured by the integrated area under the curve of the three sample dates plotted against time. I found that Brassica monocultures, the most productive cover crop treatment, resulted in the lowest soil nitrate intensities and greatest soil PMN intensities, but they did not affect the total phenolic content of the soil. Weedy contributions to total plant biomass were also important in determining soil inorganic nitrogen levels, and weed biomass was positively correlated with soil PMN intensity. The second part of this study addressed the changes in microbial community structure and α-diversity as a result of cover crop type as well as identified specific cover crop drivers that were associated with individual microbial taxa using partial least squares regression (PLSR) modeling. I found the greatest bacterial α-diversity under Brassicas and the lowest under the plant-free control plots. Fungal diversity, in contrast, was greatest under the plant-free control plots and lowest in the Brassica monocultures. Idagold mustard, weeds, and oat were the most influential cover crops in describing bacterial and fungal

taxa according to the PLSR models. Though taxa often displayed individualistic responses, I found that Idagold mustard biomass was positively associated with several pathogen-suppressive taxa and negatively associated with pathogenic taxa. Ammonia-oxidizing archaea were abundant among the top model results and appeared to be suppressed by Idagold mustard and oat at both farms. In conclusion, Brassicas were the most effective at reducing soil nitrate and increasing PMN concentrations, which reduces risks of nitrate leaching after cover crop termination and increases the potential inorganic N supply for subsequent crops. Brassicas also increased bacterial diversity and decreased fungal diversity, which could have implications for managing subsequent crop disease. Landowners should consider their cover crop goals (reducing soil nitrate leaching, increasing inorganic N for subsequent crops, or mitigating crop disease) when evaluating cover crop type and diversity.

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CHAPTER 1: GENERAL INTRODUCTION

Cover crops, which are planted during otherwise fallow periods, provide a variety of benefits to agroecosystems. These benefits have been well-studied, and include reduced soil erosion, increased soil organic matter, and weed suppression (Sainju et al., 2005; Ding et al., 2006; De Baets et al., 2011; Teasdale, 2013; Schmidt et al., 2018). After they are terminated and incorporated into the soil, cover crops continue to leave behind legacy effects on their soil environments (Madden et al., 2004; Larkin and Honeycutt, 2006; Pascault et al., 2010; Vukicevich et al., 2016; Brennan and Acosta-Martinez, 2017; Finney et al., 2017; Liu et al., 2017). These changes can occur as differences in nitrogen (N) availability, the presence of potentially allelopathic phenolic compounds, or alterations to soil microbial communities, and all of these changes can affect subsequent crop growth. In Illinois, approximately 20 million acres are cropland, with about 40,000 acres currently certified organic (USDA, 2017) and additional acres are in transition from conventional. As the popularity of organic farming and demand for organic produce increases, it will be vital to have a better understanding of the legacy effects of cover crops on organically-managed farms.

Cover crops can influence soil inorganic N pools through the decomposition of their residues and the timing of N release. N is one of the most important plant macronutrients, and the cycling of N in terrestrial systems is regulated by soil microorganisms. The carbon to nitrogen (C:N) ratios of plant tissue, as well as other organic material, influences the rate of microbial mineralization of organic N into inorganic, plant-available N (nitrate, NO_3^-, and ammonium, NH_4^+), and therefore the timing of inorganic N release into the soil. Since soil microorganisms have a threshold C:N ratio of approximately 26:1, plant residues with lower C:N

ratios result in net N mineralization, or faster release of inorganic N, while residues with higher C:N ratios result in net N immobilization, or slower release of inorganic N (Hu et al., 1997). The potentially mineralizable N (PMN) content of the soil is an important indicator of soil fertility during the growing season that reflects these differences in the timing of inorganic N release (Drinkwater et al., 1996; Curtin and Wen, 1999; Poudel et al., 2001; Steenwerth and Belina, 2008). Grasses like oat (*Avena sativa*) and wheat (*Triticum aestivum*) have high C:N ratios, so it takes longer for soil microorganisms to fully liberate soil N via N mineralization (Teasdale and Abdul-Baki, 1998). Legumes, on the other hand, have low C:N ratios (Stevenson and Cole, 1985; Wagger, 1989) and experience high PMN before rapid mineralization and quick release of organic N. Legume species such as field pea (*Pisum sativum*) and fava bean (*Vicia faba*) can provide significant N contributions to soil, more rapidly supplying N to subsequent crops than grasses (Wagger et al., 1998; Madden et al., 2004; Luscher et al., 2014). However, increased inorganic N pools in the soil without live crops can increase the risk of nitrate leaching, which is a major environmental concern in the Midwest (Ranells and Wagger, 1996).

In addition to the provision of inorganic N, plants can influence soil biology and chemistry by releasing allelopathic or anti-fungal chemicals into the soil during decomposition. Plants in the family *Brassicaceae*, which includes mustards and turnips, can influence the soil in this way (Brown and Morra, 1997; Haramoto and Gallandt, 2004). Though Brassica C:N ratios often fall below the 26:1 microbial threshold, the presence of glucosinolates, a class of potentially-allelopathic secondary metabolites, can suppress microbial decomposition and slow N release into the soil (Bending et al., 1998; Ohno and First, 1998; Kumar et al., 2009; Gao et al., 2016). During decomposition, soil microorganisms convert glucosinolates into isothiocyanates, which are known anti-fungal chemicals that can further shape soil microbial

communities (Kirkegaard et al., 1996; Larkin et al., 2010). All Brassicas, including Idagold mustard (*Sinapis alba*) and purple top turnip (*Brassica campestris*), produce glucosinolates, and we need to better understand the influences of these species on N release, the total soil phenolic content, and the resultant soil microbial communities.

Cover crops also influence their soil microbial environments, which can further influence crop growth due to changes in microbial diversity, resource availability, and the degree of plant disease or pathogen suppression (Carrera et al., 2007; van der Heijden et al., 2008; Fernandez et al., 2016). Cover crops can increase bacterial diversity, which can lead to greater functional redundancy and community resilience (Griffiths et al., 2003; Yin et al., 2010; Lehman et al., 2015). Soil microorganisms are vital to the terrestrial N cycling process of mineralization, as described above, and nitrification, the conversion of ammonium into nitrate (via nitrite). While mineralization can provide inorganic N necessary for plant uptake, nitrification is an important pathway for N loss in agricultural systems. Therefore, it is necessary to further our understanding of the legacy effects of cover crops on the microbial taxa relevant to these processes.

By promoting beneficial bacteria and fungi and decreasing fungal pathogens, cover crops can also reduce the incidence of soil-borne disease (Akemo et al., 2000b; Larkin and Honeycutt, 2006; Kumar et al., 2009; Vukicevich et al., 2016; Brennan and Acosta-Martinez, 2017). For example, the glucosinolates and isothiocyanates released from Brassica tissues during decomposition can act as fungicides, thereby altering resultant soil microbial communities (Larkin et al., 2010). Similar to the N cycling processes described, diverse microbial communities are more equipped to suppress pathogenic taxa (Reynolds et al., 2003; Brennan and Acosta-Martinez, 2017). Several beneficial, or pathogen antagonistic, soil bacteria and fungi have been identified as a result of cover crop use in agricultural systems, such as *Pseudomonas*

spp. and *Trichoderma* spp. (Larkin and Honeycutt, 2006; Brennan and Acosta-Martinez, 2017). However, cover crops can also increase rates of disease in subsequent crops by promoting environmental conditions such as soil moisture that can increase disease rates (Conklin et al., 2002). Further, Dabney et al. (1996) found higher rates of *Rhizoctonia solani* infection in sorghum seedlings after legume cover crop incorporation, suggesting that certain cover crop types can also influence plant disease. Cover crops can therefore have mixed effects on the incidence of crop disease in agricultural systems.

Weeds, though undesirable in agroecosystems, also tend to contribute substantial biomass in spring-sown cover cropping systems; Holmes et al. (2017) reported that total aboveground biomass of cover crop plots was 8-93% weeds. Just like any other plant or cover crop type, weeds can also influence soil microbial communities (Wortman et al., 2013a; Higo et al., 2014). Since the establishment of cover crops is important in determining the legacy effects they leave behind, it is therefore necessary to include weed biomass in the present study.

Traditional monoculture systems sometimes include winter cover crops to take up excess or residual nitrates from the soil over the winter (Brandi-Dohrn et al., 1997). In organic systems with multiple, shorter rotations, spring-sown cover crops are used suppress weeds (Akemo et al., 2000b; Kumar et al., 2009) and provide immediate inorganic N for subsequent vegetable crops. Legumes, grasses, and simple mixtures are most commonly used (Akemo et al., 2000a; Akemo et al., 2000b; Kumar et al., 2009), but Brassicas are a promising candidates for spring-sown cover cropping in the Midwest (Wortman et al., 2012b; Holmes et al., 2017). As cover crops leave behind species-specific legacy effects that are determined by the quality and quantity of their residues (Smalla et al., 2001; Alvey et al., 2003; Tiemann et al., 2015), it is important to

understand how these legacy effects on soil inorganic N, phenolic content, and microbial communities are realized within monocultures and diverse cover crop mixtures.

In the present field study, six cover crop species (two grasses, two legumes, and two Brassicas) were grown in monocultures and diverse, five-way mixtures in order to advance our understanding of their legacy effects on the soil environment. In Chapter 2, I focused on the role of cover crop type on soil inorganic N and total soil phenolic content. In order to measure this, I analyzed cover crop biomass data and soils collected during the four weeks post-cover crop incorporation on two organic farms in Illinois over two years. I measured soil nitrate, ammonium and PMN concentrations as well as the total soil phenolic content. I sought to answer the following questions: 1) how does cover crop type (grass, legume, Brassica, or mixture) and biomass affect inorganic soil N (nitrate, ammonium, and PMN) concentrations after termination; and 2) do Brassica species leave behind remnant phenolic compounds in the soil that may affect N mineralization?

In Chapter 3, I addressed the legacy effects of cover crop type on the soil bacterial and fungal communities present. DNA was extracted from the same soils as described above, and high throughput sequencing was performed on the bacterial 16S rRNA gene and the fungal ITS region in order to answer the following questions: 1) does cover crop type influence microbial community structure; and 2) how does cover crop type influence bacterial and fungal alpha-diversity? Using partial least squares regression (PLSR) modeling, I also sought to answer a third question: what are the specific cover crops drivers most responsible for the presence or absence of specific microbial taxa?

CHAPTER 2: LEGACY EFFECTS OF COVER CROP MIXTURES AND MONOCULTURES OF GRASSES, LEGUMES, AND BRASSICAS ON SOIL INORGANIC NITROGEN AND TOTAL PHENOLIC CONTENT ON TWO ORGANIC FARMS IN ILLINOIS

Abstract

Cover crops can leave behind legacy effects on the soil by altering soil inorganic nitrogen (N) content or through the release of allelopathic phenolic compounds. By comparing mixtures of grasses, legumes, and Brassicas with their component monocultures on two organic farms in Illinois, I aimed to evaluate the legacy effects of different cover crop types on soil inorganic N after cover crop incorporation. This field study was conducted on two organic farms in Illinois over two years, 2015 and 2016, in which soils were collected at three time points over the four weeks after cover crop incorporation. I determined the nitrate, ammonium, potentially mineralizable N (PMN), and total phenolic content of the soil using colorimetric assays, and intensity was calculated as the trapezoidal integration under the curve of the three sample dates plotted over time. Before termination, the most productive cover crops were the Brassicas monocultures, which resulted in decreased soil nitrate and increased soil PMN intensities compared to the plant-free control plots. The inclusion of Brassica monocultures did not increase total soil phenolic content, as compared to the plant-free control plots. Mixtures, which were as productive as monocultures, resulted in higher nitrate and lower PMN intensities, similar to those found under the control plots. Finally, I found that weedy contributions to total biomass were important in predicting soil inorganic N; cover crop treatments that were less able to establish

(such as the legumes) during the study period had higher proportions of weed biomass and therefore did not decrease soil nitrate or increase soil PMN intensities compared to the control plots. Landowners should consider their goals of including a cover crop (such as reduced soil nitrate leaching or increased soil N sequestration) when selecting a cover crop.

2.1. Introduction

Cover crops, like all plants, can have lasting effects on soil in agroecosystems. Such plant "legacy effects" can occur as changes to soil quality through the provision of nutrients or potentially allelopathic compounds, and these effects can persist in the soil after the plant dies (Buyer et al., 2010; van der Putten et al., 2013; Wurst et al., 2015). In agricultural systems traditionally under monoculture, negative legacy effects from cash crops, such as nutrient depletion and species-specific pathogen accumulation, can hinder future crop growth (Schnitzer et al., 2011; van der Putten et al., 2013). The inclusion of cover crops in a rotation can minimize these negative effects by increasing nitrogen (N) sequestration and soil organic matter content, among other benefits (Doran and Smith, 1991; Tonitto et al., 2006; Wortman et al., 2012b; van der Putten et al., 2013). While traditional monocultures employ winter cover crops to take up excess or residual nitrates in the soil over the winter (Brandi-Dohrn et al., 1997), spring-sown cover crops in organic systems are used to suppress weeds (Akemo et al., 2000b; Kumar et al., 2009), though they can also provide immediate inorganic N for subsequent vegetable crops. Spring-sown legume and grass cover crop mixtures and monocultures are most commonly used (Akemo et al., 2000a; Akemo et al., 2000b; Kumar et al., 2009), but the inclusion of Brassicas in this context not been extensively studied (Holmes et al., 2017), and their unique legacy effects warrant further research.

Through residue decomposition, cover crops can affect changes to their soil environment. N cycling is regulated by soil microorganisms, and the processes of mineralization and immobilization of organic N are influenced by the carbon to nitrogen (C:N) ratio of plant tissues. Soil microorganisms have a threshold C:N ratio of 26:1; plants with lower C:N ratios result in

net N mineralization while residues with higher C:N result in net N immobilization (Hu et al., 1997). The potentially mineralizable N (PMN) content of the soil, an important indicator of soil fertility and N availability during the growing season, is also influenced by cover crop residue composition (Drinkwater et al., 1996; Curtin and Wen, 1999; Poudel et al., 2001; Steenwerth and Belina, 2008). Legumes, which have low C:N ratios, decompose rapidly as organic N is mineralized into plant-available inorganic N (nitrate, NO_3^-, and ammonium, NH_4^+) once microbial N demand is satisfied (Table 2.1) (Stevenson and Cole, 1985; Wagger, 1989). Legume species such as field pea (*Pisum sativum*) and fava bean (*Vicia faba*) can provide significant N contributions to soil via rapid mineralization of residues and provision of inorganic N for subsequent crops (Wagger et al., 1998; Luscher et al., 2014). While increased soil inorganic N immediately post-cover crop incorporation is beneficial for crops, the timing of crop planting is important since increased soil nitrate concentrations in the spring can increase the risk of leaching (Ranells and Wagger, 1996).

In contrast to legumes, grasses have high C:N ratios, and this can result in slower residue decomposition and net immobilization of N (Teasdale and Abdul-Baki, 1998). Grass species like oat (*Avena sativa*) and spring wheat (*Triticum aestivum*) can accumulate biomass more rapidly than legumes, while more effectively scavenging excess soil nitrate and reducing the risk of nitrate leaching (Brandi-Dohrn et al., 1997; Tonitto et al., 2006; Kaspar and Singer, 2011; Wortman et al., 2012a; O'Connell et al., 2015). The combination of high biomass and high C:N ratio results in less inorganic N made available for succeeding crops.

Plants of the family *Brassicaceae* are growing in popularity for their use as cover crops in the Midwest due to their ability to generate biomass quickly and thrive in cooler climates, as well as their potentially allelopathic properties (Brown and Morra, 1997; Haramoto and Gallandt,

2004). Though there is variation among Brassicas, the C:N ratios of their plant tissues are only slightly greater than legumes, and they often fall below the 26:1 microbial threshold (Table 2.1). However, the presence of potentially allelopathic secondary metabolites, specifically glucosinolates, can suppress microbial decomposition and N mineralization, as well as hinder future plant growth (Bending et al., 1998; Ohno and First, 1998; Kumar et al., 2009; Gao et al., 2016). During decomposition, soil microorganisms convert glucosinolates into isothiocyanates; both of these compounds contain a phenolic ring and can be measured in the total soil phenolic content as they are leached from residues into the soil (Fenwick et al., 1983; Fenwick et al., 1989). All Brassicas, including Idagold mustard (*Sinapis alba*) and purple top turnip (*Brassica campestris*), are known to produce these phenolic compounds. Their high biomass and slower rates of decomposition suggest that the inclusion of Brassicas as a cover crop can reduce nitrate leaching potential and increase a soil's PMN content (Jackson et al., 1993).

Planting diverse cover crop mixtures may allow us to take advantage of the known legacy effects associated with multiple plant types, and there is a growing popularity in the use of diverse cover crop mixtures, or "cocktails," in the organic farming community. While most research has historically focused on simple grass-legume bicultures, more research has started to address diverse mixtures (Akemo et al., 2000b; Creamer and Baldwin, 2000; Cardinale et al., 2007; Wortman et al., 2012a; Wortman et al., 2012b; Wortman et al., 2013b; Smith et al., 2014). Diverse plant communities increase the temporal and spatial diversity within an agroecosystem, can increase productivity, and allow for regulation of plant C:N ratios (Jensen, 1996; Tribouillois et al., 2015). For example, planting a grass-legume biculture can decrease the C:N ratio compared to that of a grass monoculture, allowing for a gradual and steady stream of inorganic N provision to the soil (Ranells and Wagger, 1996; Fageria and Baligar, 2005; Tonitto et al., 2006;

Stute and Posner, 2013). Furthermore, such alterations to the C:N ratio of residues results in an increased PMN content under grass-legume mixtures as compared to the component monocultures (O'Connell et al., 2015). It will be fruitful to learn how the inclusion of Brassicas, a productive family of plants with moderately low C:N ratios and potentially allelopathic compounds that may impact the rate of residue decomposition and N mineralization, will influence soil N when grown in diverse mixtures with legumes and grasses.

In the present study, six cover crop species (two grasses, two legumes, and two Brassicas) were grown in mixtures and monocultures in order to better understand their legacy effects on soil inorganic N content and total phenolic content. Further, I aimed to determine how the inclusion of Brassicas, specifically Idagold mustard, in cover crop mixtures influences the legacy effects of the soil environment. This field study was conducted on two organic vegetable farms in Illinois, PrariErth and Kinnikinnick, in 2015 and 2016. I measured soil nitrate, ammonium, and PMN concentration over four weeks after cover crops were terminated in order to answer the following questions: 1) how does cover crop type (grass, legume, Brassica, or mixture) and biomass affect inorganic soil N (nitrate and ammonium) concentrations after termination; 2) how does cover crop type and biomass affect soil PMN; and 3) do Brassica species leave behind remnant phenolic compounds in the soil that may affect N mineralization? I therefore had three hypotheses: 1) cover crops that generate the greatest biomass will result in reduced soil nitrate and increased soil PMN concentrations post-incorporation; 2) mixtures will be more productive than monocultures, and therefore have lower nitrate and higher PMN concentrations; and 3) Brassica monoculture decomposition will result in an increase in the total phenolic content of the soil due to the presence of glucosinolates and isothiocyanates.

2.2. Methods

2.2.1. Experimental design and study species

Two organic vegetable farms were sampled in 2015 and 2016: PrairiErth Farm in Atlanta, IL (40°13'N 89°13'W) and Kinnikinnick Farm in Caledonia, IL (42°27'N 88°52'W). The soils at both sites are a silt loam (Pecatonica silt loam, 2-5% slope at PrairiErth and Rozetta silt loam, 0-5% slope at Kinnikinnick). Cropping history at both sites was highly varied and included both vegetable and grain crops. Both farms were certified organic under the United States Department of Agriculture National Organic Program guidelines.

Six cover crop species were included in the study: two grasses (oat, *Avena sativa*, and spring wheat, *Triticum aestivum*), two legumes (field pea, *Pisum sativum* and fava bean, *Vicia faba*), and two Brassicas (Idagold mustard, *Sinapis alba* and purple top turnip, *Brassica campestris*). The weedy control received no cover crop seed but allowed volunteer weed growth, and the plant-free control contained no plants and was maintained by hand-pulling. Cover crops were planted in monocultures and all possible five-species mixtures for a total of six monocultures and six mixture treatments with two controls, 14 treatments in total. The spring-sown cover crops were planted in a randomized complete block design with four replicates of the 14 treatments. Each plot was 16 m^2, and a different experimental site within each farm was chosen in each year. For subsequent analyses, cover crop types refer to the following designations: grass, legume, Brassica, mixture, weedy, and plant-free control.

Following Wortman et al. (2012a) and Smith et al. (2014), and as described by Holmes et al. (2017), seeding rates were obtained by dividing the monoculture seeding rate by five, the

number of plant species in each mixture. Cover crops were planted by hand-broadcasting and seeds were lightly incorporated using gravel rakes and drag harrows. Cover crops grew for approximately two months before termination by mowing and rotavation to a depth of 15 cm. Cover crops were planted in early April at PrariErth, and in late April at Kinnikinnick, of each year. At PrairiErth in 2016, the prior cover crop of winter oat was not fully terminated, which resulted in the inclusion of only four cover crop treatments: the plant-free control plots, the weedy plots, and the purple top turnip and Idagold mustard monocultures.

2.2.2. Cover crop biomass determination

Aboveground cover crop biomass was measured from two random 1-m^2 quadrats immediately before termination. Weeds were separated from cover crops and weighed separately, but were treated as a single plant type and individual weed species were not identified. Dry weights were calculated for each cover crop species and converted to kg ha^{-1} for subsequent analyses. For 2015, biomass data were available for all Kinnikinnick plots and three of the four PrariErth blocks. For 2016, biomass data were only available for three of the four Kinnikinnick blocks. This resulted in a total of 141 experimental units (unique combinations of year, site, replicate, and cover crop treatment).

2.2.3. Sample collection

In 2015, soils from each plot were collected at 3, 7 and 34 days post-termination at PrariErth and 6, 18, and 32 days post-termination at Kinnikinnick. In 2016, samples were

collected 3, 17, and 33 days post-termination at PrairiErth and 5, 14, and 34 days post-termination at Kinnikinnick. Sample dates of 3-7 days post-termination were classified as "one week post-termination," 14-18 days as "two weeks post-termination," and 32-34 days as "four weeks post-termination." Approximately 12-16 cores, at a depth of 10 cm, were collected from each plot to generate a composite 600-700 g soil sample per plot. Soils were air-dried at room temperature and manually ground with a mortar and pestle for subsequent soil assays.

2.2.4. Soil inorganic N analyses

Soil inorganic N content was assessed through KCl-extraction followed by colorimetric quantification of nitrate and ammonium. For each sample, two subsamples of 10 ± 0.05 g were weighed into 50 mL centrifuge tubes. One subsample was incubated anaerobically to allow for mineralization of organic N (see below), while the other was processed immediately for inorganic N content. To each inorganic N sample, 40 mL 1 M KCl was added and samples were shaken at approximately 240 rotations per minute at room temperature for 50 minutes. A nitrate colorimetric reagent was prepared by mixing 2% sulfanilamide solution (in 1 M HCl), 0.2% N-(1-napththyl)-ethylenediamine dihydrochloride and saturated vanadium (III) chloride (in 1 M HCl) following protocols adapted from Doane and Horwath (2003). Nitrate concentration was measured by mixing equal parts KCl extract and reagent solution and left to incubate in the dark at room temperature for five hours. Two colorimetric reagents were made following protocols adapted from Weatherburn (1967) to evaluate ammonium concentration of KCl extracts: 1) a solution of sodium salicylate, sodium citrate, sodium tartrate, and sodium nitroprusside; and 2) a 2% bleach solution in 1.5 M sodium hydroxide solution. Ammonium concentration was

measured by mixing 2 parts KCl extract with 9 parts sodium salicylate reagent and 9 parts bleach-sodium hydroxide solution and allowed to incubate at room temperature for 50 minutes. Absorbance values were measured at 540 nm for nitrate and 650 nm for ammonium to colorimetrically quantify N concentrations using an Epoch BioTek plate reader (BioTek Instruments, Inc., Winooski, VT) and Gen5 software version 2.03.1. Standard curves of known concentrations of KNO_3 and $(NH_4)_2SO_4$ were used to measure nitrate and ammonium concentrations, respectively. For all N assay results, concentrations were converted to g N kg^{-1}.

Potentially mineralizable N (PMN) was measured following protocols adapted from Drinkwater et al. (1996) and Moebius-Clune et al. (2016). The second subsample of soil was combined with 10 mL ddH_2O and the headspace was cleared of O_2 with the addition of He gas to create a waterlogged, anaerobic environment in order to inhibit the oxidation of NH_4^+. Samples were left to incubate anaerobically at 37°C for seven days in order to accumulate mineralized NH_4^+. Total PMN was determined by measuring the ammonium concentration following the protocol described above. PMN was calculated as the difference in ammonium concentration after and before the seven-day incubation.

2.2.5. Phenolic content determination

The purpose of estimating total soil phenolic content is that it may represent the potential content of plant-derived allelochemicals (Inderjit, 1996). The protocol for determination of total phenolic content from dried soils was adapted from Villada et al. (2016), Ainsworth and Gillespie (2007), and Lou et al. (2016). To 50 mL centrifuge tubes, 3 ± 0.05 g dried soil was added and mixed with 30 mL of ultrapure water to obtain a 1:10 ratio of solids to liquids.

Samples were shaken for 3 hours at approximately 200 revolutions per minute. Samples were then centrifuged for 10 minutes at 5,000 rpm. Supernatant was filtered through Whatman No. 42 filter papers. To quantify phenolic content colorimetrically, 140 μL of sample or standard was mixed with 100 μL Folin-Ciocalteau reagent, 200 μL saturated Na_2CO_3 solution, and 300 μL ultrapure water on a 96-well microplate. Gallic acid (dissolved in water) was used to generate the standard curve. After a 20-minute incubation, the absorbance was read at 765 nm, using the same microplate reader and software as described above. Concentrations were converted to mg L^{-1} and are expressed as "mg gallic acid equivalents L^{-1}." In order to evaluate if the Brassica monocultures increased the phenolic content present in the soil after incorporation, this assay was performed on 96 samples, or 32 experimental units (unique combinations of site, year, treatment and replicate), from PrariErth in 2015 and 2016: the weedy plots, weed-free controls, the Idagold mustard monocultures, and the purple top turnip monocultures.

2.2.6. Data analysis

All data were analyzed using R software version 3.4.2 (R Core Team, 2017). "Experimental units" were designated as unique combinations of site, year, cover crop treatment, and replicate; there were 184 experimental units in total, but only 141 for which there was cover crop biomass data. Linear mixed models were first used to determine if and how cover crop type influenced cover crop biomass and weed biomass using the package *nlme* (Pinheiro et al., 2017). Cover crop and weed biomass were treated as response variables, cover crop type was a fixed effect, and year, site, and replicate were nested random effects. Models were fitted using the maximum likelihood approach. To test for mean differences between treatment groups, Tukey's

Honestly Significant Difference (HSD) post-hoc tests were applied using the package *lsmeans* (Lenth, 2016). Results of model tests and Tukey's test were considered significant at the level of alpha < 0.05. The mean and standard error estimates from the Tukey's HSD test were plotted using *ggplot2* (Wickham, 2009). An example mixed effects model structure is shown below:

Model = Response ~ Cover crop type + (Year/Site/Replicate)

In order to estimate the effects of cover crop composition on soil inorganic N and total phenolic content, I calculated N intensity (g N d kg^{-1}) separately for nitrate, ammonium, and PMN by applying the trapezoidal rule to integrate the area under the curve generated by the three sample dates plotted against time (Burton et al., 2008; Engel et al., 2010). Trapezoidal integration was carried out on all 184 experimental units using the packages *dplyr* and *pracma* (Wickham et al., 2017; Borchers, 2018). For the phenolic content assays, where only a subset of samples was used (32 experimental units) from PrariErth only, phenolic intensity was calculated in the same way as N intensity. Determining soil N and phenolic content in this way allows us to estimate soil N or phenolic content from one plot over the course of the four-week sampling period. I then used linear mixed models to investigate how cover crop type influenced measures of soil N intensity and soil phenolic intensity and followed with Tukey's HSD post-hoc tests, as described above.

To evaluate if the effects of cover crops on soil N was related to their biomass (or success of establishment), conditional inference trees were constructed using the function "ctree" in the package *partykit* (Hothorn et al., 2006; Hothorn and Zeileis, 2015). Site and year were also included, since both significantly affected soil N. Conditional inference trees are useful for examining the relationship between a single response variable and many potential predictors, especially when there is multicollinearity or multiple interactions between predictors, and they

reduce bias when splitting a continuous predictor variable; they also provide easy-to-interpret results and have been successfully utilized in agricultural research (Quinn and Keough, 2002; Zuur et al., 2007; Johnstone et al., 2014; Finney et al., 2015; Mourtzinis et al., 2018). Conditional inference trees differ from traditional Classification and Regression Trees (CART) in that they are Random Forest-based, where the algorithm generates a series of trees in order to determine the best fit (Hothorn et al., 2006). The algorithm for each individual tree works by recursively splitting data into groups, or nodes, with small within-group variations and large between-group variations. Only the 141 experimental units were included for which cover crop biomass data was complete.

2.3. Results

2.3.1. Cover crop biomass

The most productive cover crop types were the Brassicas and mixtures (Fig. 2.1A; Tukey HSD post-hoc test, $p < 0.05$). Grasses were intermediate in productivity, and legumes were the least productive. The opposite trend was observed for weed biomass within cover crop plots: weed biomass was lowest in the Brassicas, grasses, and mixtures and highest in the legume plots (Fig. 2.1B; Tukey HSD post-hoc test, $p < 0.05$).

2.3.2. Influence of cover crop type on measures of soil N intensity

The linear mixed models showed that soil nitrate intensity was lower under Brassicas than all other cover crop types, including the plant-free control plots (Table 2.2). The Tukey HSD test confirmed that Brassicas resulted in the lowest soil nitrate intensity, but further separated grasses and weeds as intermediate and legumes, mixtures, and the plant-free control plots as having the greatest soil nitrate intensity (Table 2.2). Soil PMN intensity was greatest under the Brassicas, weeds, and cover crop mixtures, but the Tukey HSD post-hoc test revealed no differences in soil PMN intensity across cover crop types. There were no differences in soil ammonium intensity by cover crop type, and ammonium levels were the same in cover cropped plots and plant-free plots.

2.3.3. Conditional inference trees reveal influence site, year and weedy biomass on soil N

Site was the most important predictor of all three measures of soil N: nitrate, ammonium, and PMN intensities were all greater at PrariErth than Kinnikinnick (Fig. 2.2, 2.3, and 2.4). However, plant biomass data was only available in 2015 for PrariErth and included both years for Kinnikinnick. Since there were interesting patterns relating to year and plant biomass within the data from Kinnikinnick, I will only refer to this site's data for the remainder of this section. I saw that soil nitrate and PMN intensities were greater when the biomass of weeds exceeded 884 kg ha^{-1} (Fig. 2.2 and 2.3). Further, all cover crop treatments except for Idagold mustard monocultures experienced such weed "outbreaks." Plots with weed biomass less than 884 kg ha^{-1} experienced greater nitrate intensities in 2015 and greater PMN in 2016. Of note, I found that plots that did not experience a weed "outbreak" (i.e., weed biomass < 884 kg ha^{-1}) and had at least a minimal Idagold mustard establishment (> 25 kg ha^{-1}) experienced almost as great of PMN intensities (2.045 g N d kg^{-1}) as those plots under weed infestation (2.263 g N d kg^{-1}) and at PrariErth (2.330 g N d kg^{-1}). Additionally, weed biomass appeared to be positively associated with soil PMN: plots with high weed biomass had the highest soil PMN intensities (2.263 g N d kg^{-1}), plots with medium weed biomass had medium soil PMN intensities (1.240 g N d kg^{-1}), and plots with very low weed biomass had the lowest PMN intensities (1.702 g N d kg^{-1}). Soil ammonium was not influenced by cover crop biomass or year (Fig. 2.4).

2.3.4. Cover crop type did not influence soil phenolic content

Soil phenolic content assays were performed on a subsample of weedy plots, plant-free control plots and both Brassica monocultures (Idagold mustard and purple top turnip) at PrariErth farm. There was no significant effect of cover crop type on cumulative soil phenolic content (Table 2.3).

2.4. Discussion

In the present study, I sought to investigate the influence of cover crops, planted in mixtures or monocultures, on soil inorganic N pools and total phenolic content post-incorporation. I found that Brassica monocultures were the most productive cover crop and resulted in both the lowest soil nitrate and greatest PMN intensities post-incorporation. This provided support for my hypothesis that more productive cover crops would decrease soil nitrate and increase soil PMN. In contrast, legume monocultures were the least productive and resulted in nitrate and PMN intensities that were no different from the plant-free control plots. Mixtures were moderately productive and resulted in relatively high soil nitrate and PMN intensities post-incorporation, disproving my hypothesis that mixtures would be more productive than monocultures. Soil ammonium intensity, the third measure of inorganic N, was not affected by cover crop treatment. I also noticed that PrariErth had higher intensities of nitrate, ammonium, and PMN, which suggests differences in initial soil N levels before the start of sampling. In addition, Brassicas did not appear to increase the total soil phenolic content of the soil, which I would have expected to see given the presence of glucosinolates and isothiocyanates in Brassica tissues during decomposition.

2.4.1. Brassicas decreased soil nitrate intensity and increased soil PMN intensity, but did not affect soil phenolic content

Of the cover crops tested, Brassica monocultures were the most productive and resulted in the lowest soil nitrate intensity and greatest PMN intensity observed over the four weeks post-

incorporation. In fact, Brassicas were the only cover crop that decreased the soil nitrate compared to the plant-free control plots. Uptake of nitrate by Brassicas during the spring can decrease the risk of nitrate leaching and support greater PMN concentrations throughout the fallow period prior to subsequent crop establishment (Tribouillois et al., 2015), as well as potentially aid in weed suppression (Wortman et al., 2012a). However, reduced nitrate concentrations after termination also means lower soil inorganic N supply for meeting subsequent crop demand.

PrariErth farm consistently had greater soil PMN intensity than Kinnikinnick, and these PMN pools were further influenced by Idagold mustard biomass. The conditional inference trees revealed that plots from Kinnikinnick with at least 24 kg ha^{-1} of Idagold mustard had almost as great of PMN intensities (2.045 g N d kg^{-1}) as those soils from PrariErth (2.330 g N d kg^{-1}). Two potential explanations for this pattern are: 1) only a small amount of Idagold mustard biomass is necessary to achieve increased soil PMN concentrations, which translates into improved soil fertility compared to soils with lower Idagold mustard biomass (Drinkwater et al., 1996); and 2) Brassica biomass inhibited microbial decomposition of residues, which slowed the mineralization of N and resulted in a greater pool of PMN. Brassicas, and Idagold mustard specifically, can therefore play a significant role in supporting the potentially-available N pool to soil and N demands of subsequent crops. It is also important to note that the PMN values measured were in the lab under optimal anaerobic conditions, and reflect an unrealized inorganic N pool. If we saw PMN levels like this in the field, we'd expect to also see greater inorganic N concentrations under Brassicas, but that was not the case.

Idagold mustard residues have typical C:N ratios ranging from 10-20, while purple top turnip residues are between 10-30; since these ratios fall below the microbial threshold of 26:1,

one would expect more rapid residue decomposition and organic N mineralization (Chaves et al., 2004; USDA, 2013; Brennan and Smith, 2018). However, nitrate content is reduced under Brassicas, suggesting that rapid N mineralization is not occurring over the course of the four weeks post-termination. The high PMN concentrations observed under both Brassica species is therefore due to the high biomass of Brassica tissues with high N content. There is, therefore, some mechanism slowing down Brassica residue decomposition. It has previously been reported that phenolic compounds present in Brassica residues have inhibited N-cycling microorganisms (Bangarwa et al., 2012). However, I found no differences in soil phenolic content under Brassicas as compared to weeds or plant-free control plots, so I cannot attribute high PMN to slowed decomposition due to potentially allelopathic phenolic compounds released from Brassica residues. I cannot conclude whether there simply is no difference in soil phenolic content between the Brassicas and the plant-free controls or if the methods used were not adequate to capture the potentially allelopathic phenolic content. The non-specific assay used may have detected other sources of phenolic compounds in the soil, such as tannins, lignin, and remnant plant root exudates. Future research should address the effects of Brassica residue decomposition and N release from tissues in the presence of glucosinolates and isothiocyanates.

2.4.2. Diverse mixtures did not decrease soil nitrate, but did increase soil PMN

Diverse mixtures were moderately productive, and generated almost as much biomass as the Brassica monocultures, as was found in prior research (Wortman et al., 2012b). Cover crop mixtures resulted in high soil nitrate intensities, similar to the plant-free control plots, after cover crop incorporation. Greater nitrate content under soils following mixtures may be due to the

rapid mineralization of organic N from low-biomass legumes, and this is beneficial for supporting the growth of the subsequent crop in an organic vegetable agroecosystem. However, to increase the effectiveness of legume establishment and the availability of soil inorganic N post-incorporation, a more optimal cover crop species ratio is needed when seeding (Wagger et al., 1998).

Even though mixtures were as productive as Brassicas, I found that soil PMN concentrations were not as high under mixtures as under Brassica monocultures. Increased inorganic N is made available for subsequent crops during the sampling period, with less stored in tissues as organic N reserves. Since mixtures contained tissues with variable C:N ratios, different rates of decomposition were occurring during the four-week sampling period. Organic N mineralization from legumes occurred more rapidly, which would lower the overall PMN content when integrated over the 30-day window following termination. This positive relationship between N retention and plant C:N ratios, which reflects non-leguminous cover crops' ability to have greater N retention than legumes, may also explain the increased PMN as compared to legumes and the plant-free control plots (Tonitto et al., 2006; Finney et al., 2016). The failure of legumes to retain N as compared to grasses and Brassicas within mixtures likely contributed to increased soil nitrate and slightly decreased soil PMN as compared to the equally-productive Brassicas.

2.4.3. Weedy contributions to total plant biomass were important predictors of soil N

The effects of weeds on soil N intensities were more complex. The weedy plots decreased soil nitrate compared to the plant-free control plots, but weed biomass was positively

associated with soil nitrate and PMN according to the conditional inference trees. So, while my results are consistent with previous findings that weeds are effective at lowering soil nitrate compared to plant-free plots (Lindquist et al., 2017), cover crop plots that contain a substantial amount of weeds (greater than 884 kg ha^{-1}) actually result in increased soil nitrate and PMN. Since weeds are opportunistic and have relatively low C:N ratios, their presence in high enough biomass may lead to more rapid N mineralization than in plots with fewer weeds. The 31 plots that experienced weed outbreaks (great than 884 kg ha^{-1}) included all cover crop types except for the Idagold mustard monocultures and were all from Kinnikinnick in 2015. First, this supports that Idagold mustard was most successful in suppressing weed growth (Finney et al., 2016). It also means there must have been some variability between the two years that allowed a weed outbreak to occur at Kinnikinnick. Rainfall was much greater in May 2015 (123 mm) than 2016 (79 mm), which may have supported greater weed establishment than in 2016 (Holmes et al., 2017).

Legume monocultures, which were the least productive cover crop, were comprised of nearly 75% weeds by biomass and were therefore not true legume monocultures. If the legume plots were instead considered as mixtures of legumes and weeds, this would support prior findings that non-legumes tend to dominate in mixtures and can negatively impact less-productive legumes within such mixtures (Tribouillois et al., 2015). The lack of establishment of legumes, and specifically fava beans, in cover crop field studies was reported by Holmes et al. (2017) and was attributed to the large seed size and inadequate depth of planting of legumes compared to small-seeded grasses and Brassicas. Ensuring seeds are planted adequately is a hurdle that will need to be addressed in future field studies, especially regarding improved legume establishment.

Though mostly weeds, the legume plots resulted in higher soil nitrate and lower PMN intensities than the weedy plots. However, compared to the diverse mixtures, nitrate intensity levels were the same as legumes, though PMN was reduced. Legume cover crops have previously increased the availability of inorganic N compared to non-legumes or mixtures (Wagger et al., 1998; Madden et al., 2004; Kaspar and Singer, 2011; Detheridge et al., 2016). However, legumes have also increased soil PMN compared to grasses (O'Connell et al., 2015), which was not observed in the present study. The lower PMN intensities of the legume plots compared to the weedy plots could be due to the rapid mineralization of legume residues early in the sampling period, though closer inspection of legume PMN data revealed no peak in PMN concentrations in the early sampling dates (data not shown). N mineralization early in the sampling period would support the increased nitrate observed after legumes were incorporated.

2.5. Conclusion

Brassicas were the most productive cover crop and resulted in decreased soil nitrate compared to the plant-free control plots, as well as the highest PMN intensities of all cover crop treatments post-incorporation. Further, Brassica treatment did not affect the total soil phenolic content. Mixtures were slightly less productive than Brassicas and resulted in high soil nitrate and PMN intensities post-incorporation. Legumes were the least productive cover crop planted, with monocultures comprised by nearly 75% weeds by biomass. Soil nitrate and PMN intensities were no different between the plant-free control and legume plots. There was also a distinct site effect, as all measures of inorganic soil N were greater at PrariErth farm than at Kinnikinnick.

When planting a spring cover crop, organic farmers should prioritize their goals. If the objectives are to reduce potential N losses (via nitrate leaching) and increase the potentially mineralizable N content throughout the growing season, then a Brassica cover crop would achieve these goals by generating substantial biomass and maintaining a low C:N ratio. However, while lower soil nitrate concentrations post-cover crop incorporation may be beneficial for suppressing weed establishment, low inorganic N could potentially hinder crop growth if N demands are not met. Rather, if the objective of cover crop usage is to increase N fixation, then a mixture that includes legumes would allow for increased inorganic N available to subsequent crops while reducing the growth and establishment of weeds in legume monocultures. These decisions will continue to be important for landowners to address when prioritizing the goals of cover crops.

Table 2.1. Previously published C:N ratios of cover crop species used in this study.

Cover crop species	C:N ratio
Oat	33:1 (Hu et al., 1997)
Wheat	94:1 (Pascault et al., 2010)
Fava bean	10-15:1 (Yousef and Sprent, 1983)
Field pea	15:1 (Jensen, 1997)
Idagold mustard	10-20:1 (Chaves et al., 2004; Brennan and Smith, 2018)
Purple top turnip	10-31:1 (USDA, 2013)
Weeds	9-22:1 (Lindsey et al., 2013)
Microbial threshold	26:1 (USDA, 2011)

Table 2.2. Results of linear mixed models for the effect of cover crop type on cumulative soil nitrate, ammonium and PMN over the four-week sampling period (n = 184 experimental units). Cover crop type was treated as a fixed effect, and year, site and replicate were treated as nested random effects. Cumulative soil N is expressed as g N d kg^{-1}, and values have been integrated over the approximately 30-day sampling period. Model results are considered significant at the level of $p < 0.05$, meaning they are significantly different from the control, and are labeled with an asterisk. Non-significant results are no different from the control plots. Letters indicate significant differences in the means from Tukey HSD post-hoc test ($p < 0.05$).

Soil N measure	*Cover crop type*	*Mean ± Std. Error (g N d kg^{-1})*	*df*	*t*	*p*
Nitrate	Control (plant-free)	0.420 ± 0.071^{b}	159	5.96	0.000
	Brassica	0.341 ± 0.023^{a}	159	-3.41	0.0008*
	Grass	0.373 ± 0.025^{ab}	159	-1.86	0.064
	Legume	0.407 ± 0.025^{b}	159	-0.53	0.594
	Mixture	0.403 ± 0.022^{b}	159	-0.77	0.440
	Weeds	0.384 ± 0.026^{ab}	159	-1.38	0.169
Ammonium	Control (plant-free)	0.199 ± 0.015	159	13.31	0.000
	Brassica	0.190 ± 0.008	159	-1.16	0.249
	Grass	0.192 ± 0.008	159	-0.74	0.456
	Legume	0.186 ± 0.008	159	-1.41	0.159
	Mixture	0.194 ± 0.007	159	-0.66	0.510
	Weeds	0.191 ± 0.009	159	-0.83	0.411
PMN	Control (plant-free)	1.838 ± 0.174	159	10.55	0.000
	Brassica	2.074 ± 0.089	159	2.66	0.009*
	Grass	1.997 ± 0.096	159	1.66	0.098
	Legume	1.911 ± 0.096	159	0.76	0.447
	Mixture	2.010 ± 0.085	159	2.03	0.045*
	Weeds	2.103 ± 0.100	159	2.64	0.009*

Table 2.3. Results of linear mixed model for cumulative soil phenolic content over the four-week sampling period for a subset of samples (n = 32 experimental units). Cover crop type was treated as a fixed effect, and year, site and replicate were treated as nested random effects. Cumulative soil phenolic content is expressed as mg gallic acid equivalents d L^{-1}, and values have been integrated over the approximately 30-day sampling period. Model results are considered significant at the level of $p < 0.05$. Non-significant results indicate no difference from the control plots.

Cover crop type	*Mean ± Std. Error (mg gallic acid equivalents d L^{-1})*	*df*	*t*	*p*
Control (plant-free)	29.3 ± 3.0	22	9.77	0.000
Brassica	29.9 ± 2.9	22	0.20	0.847
Weeds	30.5 ± 3.3	22	0.38	0.709

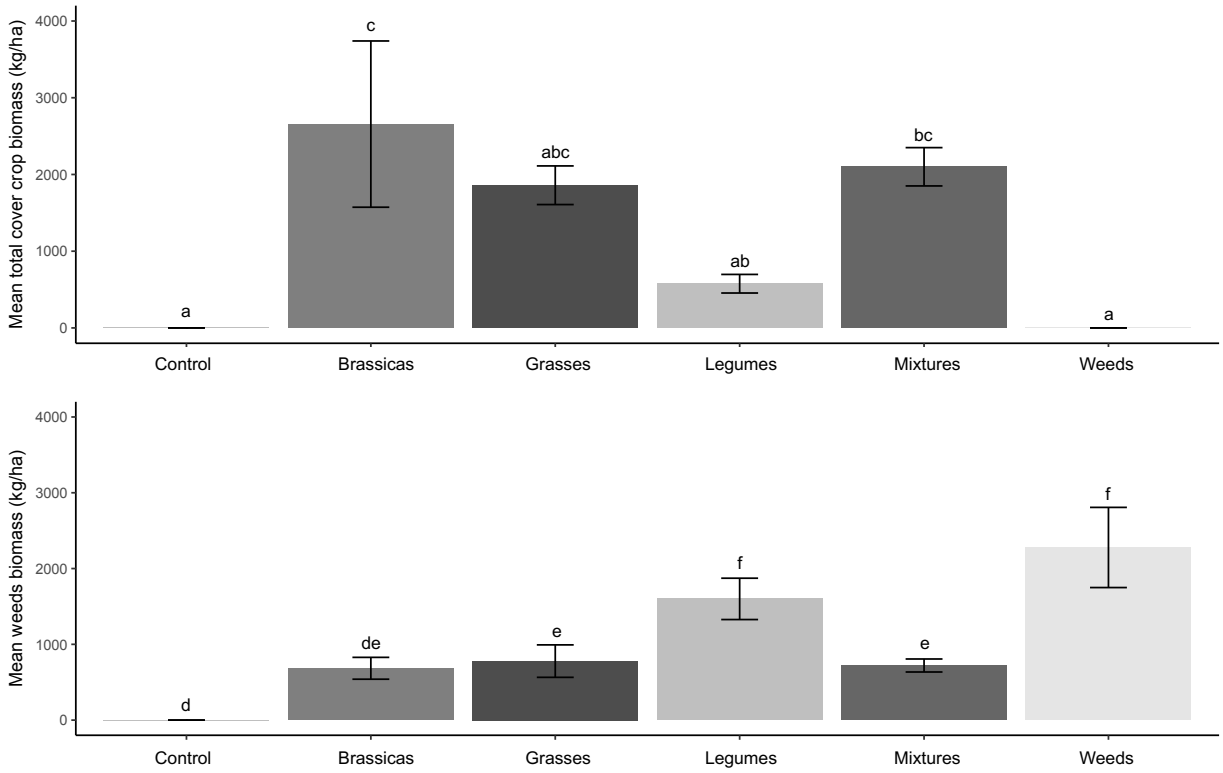

Figure 2.1. A) Mean total cover crop biomass across all cover types, and B) mean weed biomass within plots for each cover crop type. Letters indicate significant differences between total cover crop biomass for each of the cover crop types.

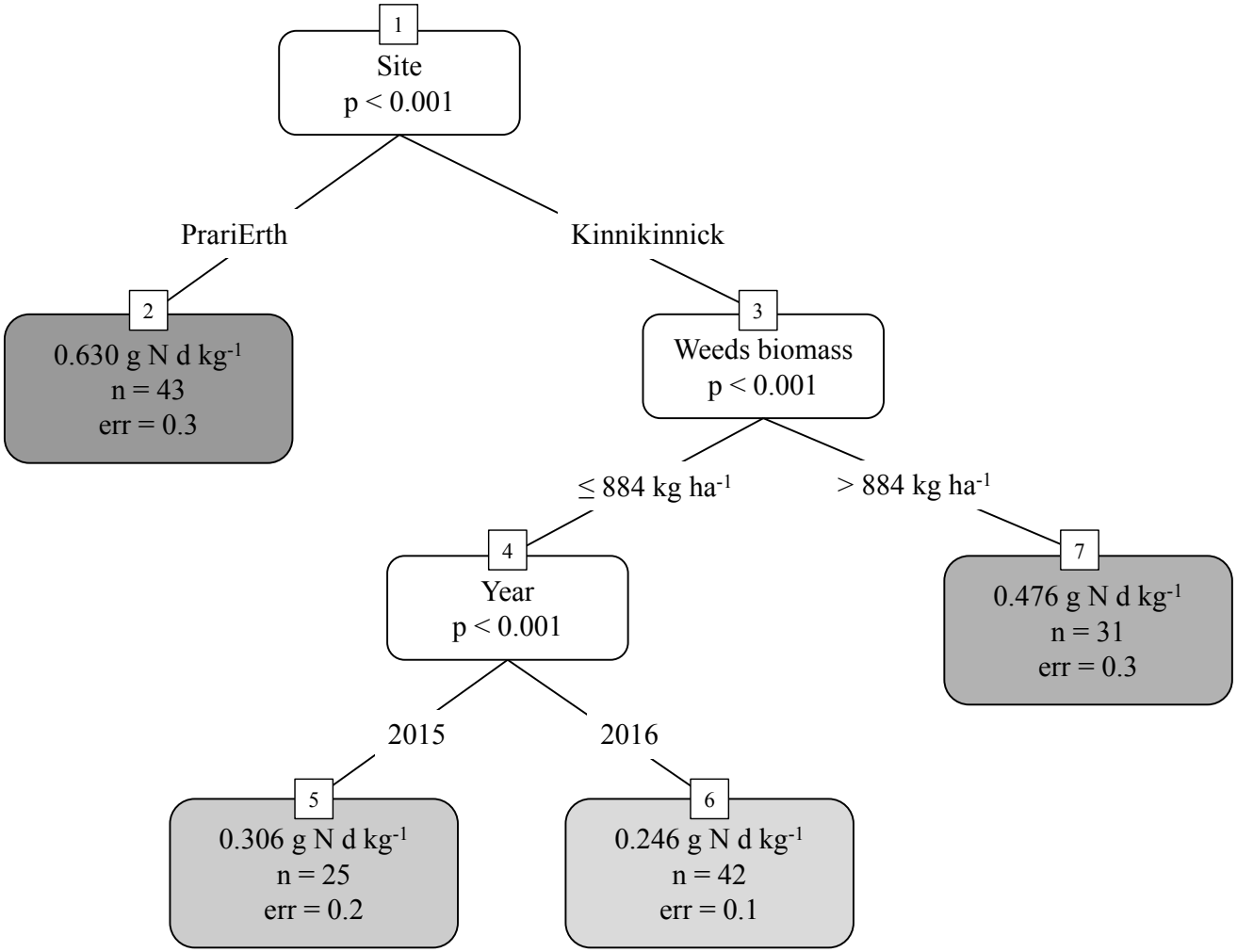

Figure 2.2. Conditional inference tree for cumulative soil nitrate concentrations as predicted by component cover crop biomass, site, year and cover crop type. Reported values are in N intensity (g N d kg^{-1}), n = number of samples included in each node, and err = sum of squares error of the prediction. Darker shades of grey indicate greater soil nitrate intensity.

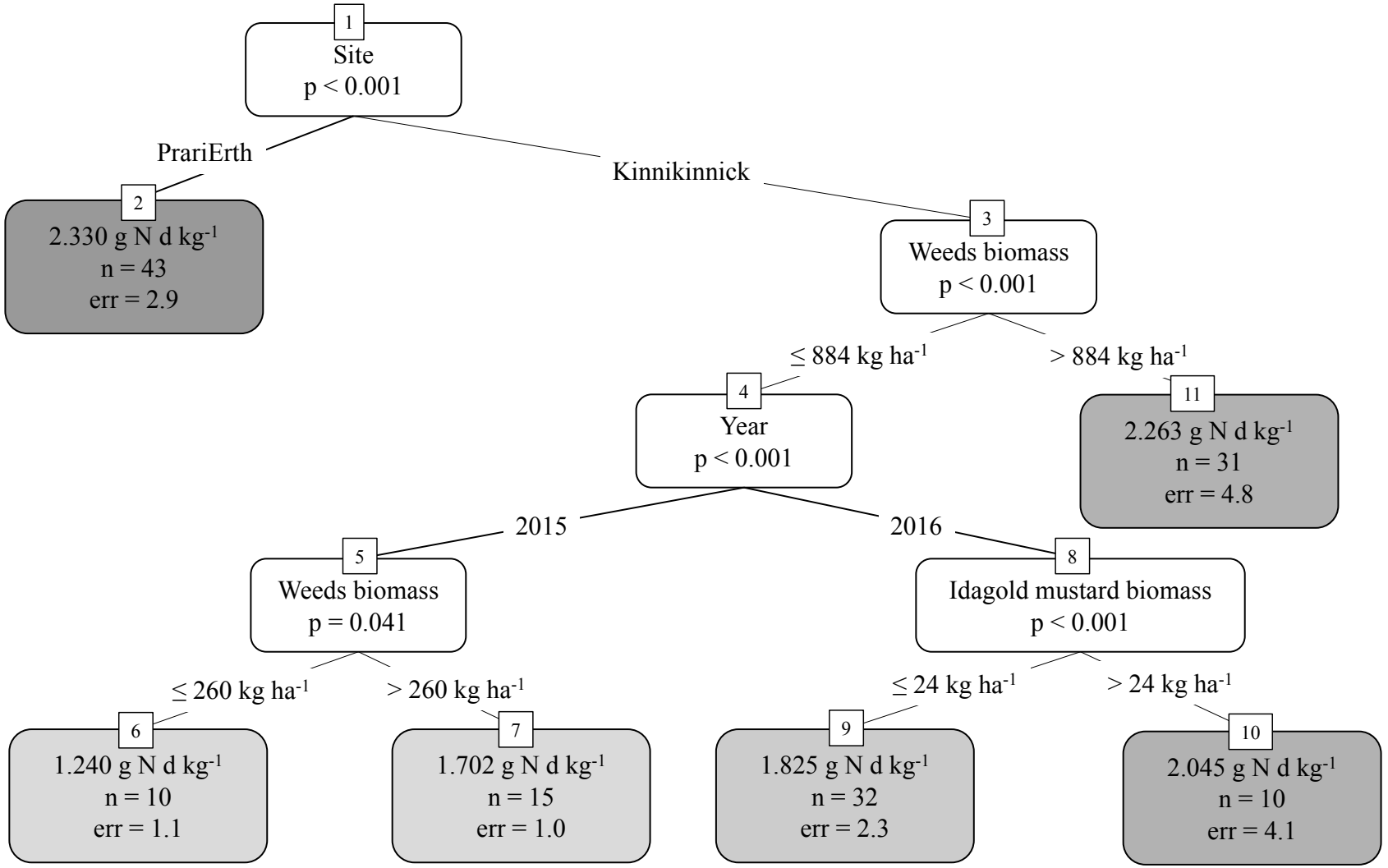

Figure 2.3. Conditional inference tree for cumulative soil nitrate PMN as predicted by component cover crop biomass, site, year and cover crop type. Reported values are in N intensity (g N d kg^{-1}), n = number of samples included in each node, and err = sum of squares error of the prediction. Darker shades of grey indicate greater soil PMN intensity.

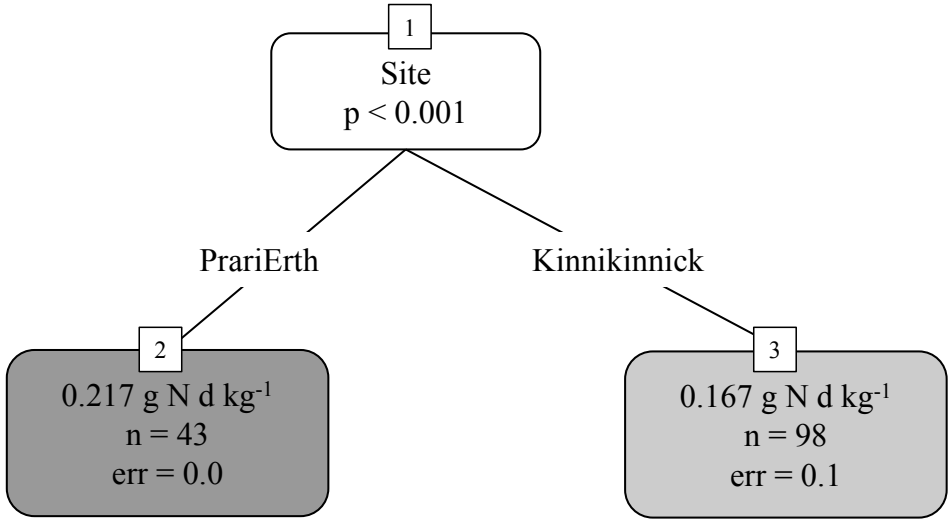

Figure 2.4. Conditional inference tree for cumulative soil ammonium concentrations as predicted by component cover crop biomass, site, year and cover crop type. Reported values are in N intensity (g N d kg^{-1}), n = number of samples included in each node, and err = sum of squares error of the prediction. Darker shades of grey indicate greater soil ammonium intensity.

CHAPTER 3: DETERMINATION OF THE LEGACY EFFECTS OF COVER CROPS ON THE SOIL MICROBIAL ENVIRONMENTS ON TWO ORGANIC FARMS IN ILLINOIS

Abstract

Cover crops can influence their soil microbial environments in a variety of ways, which can have implications for pathogen suppression or nutrient cycling in agricultural soils. In order to determine how cover crops (monocultures or mixtures of grasses, legumes, and Brassicas) influence soil microbial community composition, a two-year field study was conducted on two organic vegetable farms in Illinois. I also aimed to determine if any of the cover crop species included were dominant drivers in explaining the presence of certain bacterial and fungal taxa using partial least-squares regression (PLSR) modeling. Differences in community composition (β-diversity) across cover crop treatments at both sites and in both years were observed between fungal but not bacterial communities, indicating that fungal communities were more sensitive to changes in cover cropping treatment. Bacterial and fungal α-diversity showed opposite trends: bacterial diversity was greatest under Brassica monocultures and lowest under the plant-free control plots while fungal diversity was greatest under the plant-free control plots and lowest under Brassicas. Weedy plots resulted in high bacterial and fungal α-diversity, which may be due in part to the aboveground plant diversity within those plots, though individual weed species were not recorded. Finally, the PLSR revealed that Idagold mustard (*Sinapis alba*), weeds, and oat (*Avena sativa*) were the most important cover crop drivers in explaining the presence of certain microbial taxa. Idagold mustard promoted several taxa of pathogen-suppressive bacteria

and fungi but also suppressed *Actinobacteria*, a common and beneficial soil bacterial phylum responsible for many important soil processes. Among the top-loading OTUs isolated from the PLSR model results, ammonia-oxidizing archaea (AOA) of the genus *Candidatus Nitrososphaera* were the most common, and the negative associations between AOA and oat biomass at both farms may suggest suppression of these taxa by cover crops that include oat.

3.1. Introduction

Cover crops, like all plants, leave behind legacy effects that influence their soil microbial environments (Madden et al., 2004; Larkin and Honeycutt, 2006; Pascault et al., 2010; Vukicevich et al., 2016; Brennan and Acosta-Martinez, 2017; Finney et al., 2017; Liu et al., 2017). Alterations to a soil microbial community can affect subsequent crop growth via changes in nutrient pools, rates of residue decomposition, and relative abundance of plant mutualists and pathogens (Carrera et al., 2007; van der Heijden et al., 2008; Fernandez et al., 2016). The accumulation of species-specific pathogens can occur in agricultural systems traditionally under monoculture, but increasing the temporal diversity of a cropping rotation can help mitigate these negative effects by supporting beneficial microorganisms and suppressing potential plant pathogens (Larkin and Honeycutt, 2006; Vukicevich et al., 2016). In organic systems that do not use pesticides, cover crops can therefore be important for reducing the incidence of plant disease for subsequent crops.

Diverse microbial communities are more suited for a broad range of functional capabilities and experience a greater degree of resilience (Griffiths et al., 2003; Yin et al., 2010; Lehman et al., 2015). In agricultural systems, nitrogen (N) cycling is especially important since N is an essential macronutrient for plant growth. Soil microorganisms are vital to terrestrial N cycling processes such as N mineralization, the conversion of organic N from plant residues into inorganic, plant-available sources like nitrate and ammonium, and nitrification, the conversion of ammonium into nitrate. While mineralization can provide inorganic N necessary for plant uptake, nitrification is an important process for N loss. Understanding the microbial taxa that are

relevant to these processes and their relationships to specific cover crops is an important aspect of the legacy effects that cover crops leave behind.

Furthermore, cover crops can reduce the incidence of soil-borne pathogens by promoting beneficial microbes and decreasing fungal pathogens (Akemo et al., 2000b; Larkin and Honeycutt, 2006; Kumar et al., 2009; Vukicevich et al., 2016; Brennan and Acosta-Martinez, 2017). Similar to the N-cycling processes described above, more diverse microbial communities are more equipped to suppress pathogenic taxa (Reynolds et al., 2003; Brennan and Acosta-Martinez, 2017). Many beneficial, pathogen-antagonistic, soil bacteria and fungi have been identified in agricultural systems that respond to organic inputs or cover cropping (Larkin and Honeycutt, 2006; Vukicevich et al., 2016; Brennan and Acosta-Martinez, 2017). For example, antagonistic fungal communities have responded differently to legumes (Taheri et al., 2017), while weeds have also increased the abundance of beneficial arbuscular mycorrhizal fungi in agricultural soils (Wortman et al., 2013a).

Traditional monocultures with a single growing season often employ winter cover crops in order to remove excess nitrate over the otherwise fallow period (Brandi-Dohrn et al., 1997). In contrast, spring-sown cover crops, which are more often used in organic vegetable systems with multiple, shorter growing seasons, are primarily used for weed suppression (Akemo et al., 2000b; Kumar et al., 2009). Legumes, grasses, and simple mixtures are most commonly used (Akemo et al., 2000a; Akemo et al., 2000b; Kumar et al., 2009), but plants from the *Brassicaceae* family, such as Idagold mustard (*Sinapis alba*) or purple top turnip (*Brassica campestris*), are becoming more popular options. Brassicas have proven to generate biomass rapidly and thrive cooler temperatures, making them a useful candidate for spring-sown cover cropping in the Midwest (Wortman et al., 2012b; Holmes et al., 2017).

Cover crops can also leave behind species-specific legacy effects that are determined by the quality and quantity of their residues (Smalla et al., 2001; Alvey et al., 2003; Tiemann et al., 2015). Wheat has also been found to enrich fungal diversity and reduce pathogen populations compared to oat (Benitez et al., 2016), though both are grasses with high C:N ratios and viewed as low quality. In contrast, legumes have high-quality, low C:N tissues, and have previously been shown to be a key species driving overall soil microbial diversity and disproportionate effecting changes to microbial activity (Fornara and Tilman, 2008; Tiemann et al., 2015). Brassicas, which also have relatively low C:N ratios, contain secondary compounds called glucosinolates, which undergo conversion by soil bacteria into isothiocyanates; these compounds can act as fungicides, thereby influencing the microbial communities present as a result (Kirkegaard et al., 1996). In addition, weeds often contribute substantial biomass in spring-sown cover cropping systems and can influence soil microbial communities (Wortman et al., 2013a; Higo et al., 2014). As diverse cover crop mixtures are increasingly used by organic farmers, it is important to better understand how such mixtures influence their soil microbial environments.

The present study aimed to investigate the microbial composition of soils from two organic farms post-cover crop incorporation. In particular, I wanted to evaluate if there were certain cover crops that were important drivers in predicting the presence or abundance of soil microbial taxa, and what individual microbial taxa were associated with the presence of these cover crops. By looking at six species of cover crops grown in monocultures and diverse, five-species mixtures, I aimed to answer the following questions: 1) do bacterial and fungal communities respond to differences in cover crop composition; 2) how does the diversity within each bacterial and fungal community change with cover crop type; and 3) what cover crops are important in determining the presence of particular microbial taxa? To answer these questions, I

sampled soils during the four weeks post-cover crop incorporation, extracted DNA from collected soils, and performed high throughput sequencing of the bacterial 16S ribosomal RNA and fungal internal transcribed spacer (ITS) regions to determine what microorganisms were present in the soil. I hypothesized that: 1) bacterial and fungal communities would respond to differences in cover crop type (grass, legume, Brassica, or mixtures); and 2) Idagold mustard, with its proven ability to generate rapid biomass and release potentially-suppressive secondary compounds, would be an important driver influencing the presence of beneficial, pathogen-suppressive bacterial and fungal taxa.

3.2. Methods

3.2.1. Field study design and sample collection

Two organic vegetable farms were sampled in 2015 and 2016: PrairiErth Farm in Atlanta, IL (40°13'N 89°13'W) and Kinnikinnick Farm in Caledonia, IL (42°27'N 88°52'W). The soils at both sites were a silt loam (Pecatonica silt loam, 2-5% slope at PrairiErth and Rozetta silt loam, 0-5% slope at Kinnikinnick). Cropping history at both sites was highly varied, including both vegetable and grain crops. Both farms were certified organic under the United States Department of Agriculture National Organic Program guidelines.

Six cover crop species were included in this field study study: two grasses (oat, *Avena sativa*, and spring wheat, *Triticum aestivum*), two legumes (field pea, *Pisum sativum* and fava bean, *Vicia faba*), and two Brassicas (Idagold mustard, *Sinapis alba* and purple top turnip, *Brassica campestris*). The weedy control received no cover crop seed but allowed volunteer weed growth, and the plant-free control contained no plants and was maintained by hand-pulling. Cover crops were planted in monocultures and all possible five-species mixtures for a total of six monocultures, six mixture treatments, and two controls, for a total of 14 treatments. The spring-sown cover crops were planted in a randomized complete block design with four replicates of the 14 treatments. Each plot was 16 m^2, and a different experimental site within each farm was chosen in each year. For subsequent analyses, cover crop types refer to the following designations: grass, legume, Brassica, mixture, weedy, and plant-free control.

Seeding rates were as described by Holmes *et al.* (Holmes et al., 2017). Cover crops were planted by hand-broadcasting and seeds were lightly incorporated using gravel rakes and drag

harrows. Cover crops grew for approximately two months before termination by mowing and rotavation to a depth of 15 cm. Cover crops were planted in early April at PrariErth, and in late April at Kinnikinnick, of each year. At PrairiErth in 2016, the prior cover crop of winter oat was not fully terminated, which resulted in the inclusion of only four cover crop treatments: the plant-free control plots, the weedy plots, and the purple top turnip and Idagold mustard monocultures.

3.2.2. Cover crop biomass determination

Aboveground cover crop biomass was measured from two random 1-m^2 quadrats immediately before termination, as described by Holmes et al. (2017). Weeds were treated as a single "species" and were separated from cover crops and weighed separately. Dry weights were calculated for each cover crop species and used for subsequent analyses. For 2015, biomass data were available for all Kinnikinnick plots and three of the four PrariErth blocks. For 2016, biomass data were only available for three of the four Kinnikinnick blocks. This resulted in a total of 141 experimental units (unique combinations of year, site, replicate, and cover crop treatment).

3.2.3. Soil sample collection

In 2015, soils from each plot were collected at 3, 7, and 34 days post-termination at PrariErth and 6, 18, and 32 days post-termination at Kinnikinnick. In 2016, samples were collected 3, 17, and 33 days post-termination at PrairiErth and 5, 14, and 34 days post-termination at Kinnikinnick. Sample dates of 3-7 days post-termination were classified as "one

week post-termination," 14-18 days as "two weeks post-termination," and 32-34 days as "four weeks post-termination." Approximately 12-16 cores, at a depth of 10 cm, were collected from each plot to generate a composite 600-700 g soil sample per plot. A subsample of approximately 20 g was frozen at -20°C for DNA extraction.

3.2.4. DNA extraction, sequencing, and analysis

Whole-community microbial DNA was extracted from frozen soil samples using the FastDNA spin kit for soil (MP Biomedicals, Solon, OH) following the manufacturer's protocol. Extracted DNA was purified at 65°C for 15 minutes with 1% cetyl-trimethylammonium bromide (CTAB) to remove humic acids. Samples were further extracted with 24:1 chloroform:alcohol to remove residual impurities, which could potentially inhibit PCR. DNA was precipitated and washed three times with ethanol, then dried in a vacuum concentrator and dissolved in 1 x Tris-EDTA buffer. The purified DNA was adjusted to approximately 20 ng/µL and stored at -40˚C until further analysis.

To prepare samples for sequencing, 10 µL of each sample was added to a 96-well PCR plate and sequenced on a single flow cell using the Illumina MiSeq V3 platform at W. M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. For bacteria and archaea, the V4-V5 region of 16S rRNA was sequenced using primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3') (Caporaso et al., 2011). For fungi, the internal transcribed spacer (ITS) region between the 18S and large subunit rRNA genes was sequenced using primers ITS3-F (5'-GCATCGATGAAGAACGCAGC-3') and ITS4-R (5'-

TCCTCCGCTTATTGATATGC-3') (White et al., 1990). Samples for 2015 and 2016 were sequenced separately and combined for downstream analyses. A total of 22,722,058 raw reads were obtained from samples in 2015 and 21,685,014 in 2016 from both bacterial and fungal sequences. Library size ranged from 3,979 to 112,830 sequences per sample for the fungal ITS region with a mean of 16,997 sequences per sample, and 3,551 to 102,839 sequences per sample from the bacterial V4 region with a mean of 12,280 sequences per sample.

Sequence files were obtained as fastq files. Paired-end 16S sequences were merged using Fast Length Adjustment of Short reads (FLASH) software (Magoč and Salzberg, 2011). Quality filtering of fastq files was performed using the FASTX-Toolkit software; sequence reads with a quality score of less than 30 and with fewer than 90% of bases were removed (Gordon and Hannan, 2010). Sequences were binned into discrete operational taxonomic units (OTUs) based on 97% similarity using usearch (Edgar, 2010). Quantitative Insight into Microbial Ecology (MacQIIME version 1.9.2) was used for aligning and assigning sequences (Caporaso et al., 2010). Sequences were aligned using the basic local alignment search tool (BLAST), and taxonomy was assigned based on the Greengenes reference database for bacteria and archaea and the UNITE database for fungi (Altschul et al., 1990; DeSantis et al., 2006; Urmas et al., 2013). Sequences identified as plants, protists, chloroplasts, and mitochondria were removed. Read counts were rarefied to 5,100 for bacterial sequences and 2,900 for fungal sequences. After rarefying, I was left with 527 samples from which 16,069 unique bacterial and 112 unique archaeal OTUs and were detected from the 16S rRNA gene. For the fungal sequences, I had 560 samples from which 4,932 fungal OTUs were identified from the ITS region after rarifying.

3.2.5. Analysis of α-diversity and community composition (β-diversity)

All data analyses were performed using R software version 3.4.2 (R Core Team, 2017). First, nonmetric multidimensional scaling (NMDS) was performed on bacterial and fungal OTU data to evaluate patterns observed in community composition as influenced by site, year, sample date, and cover crop type. OTU data were square-root transformed and the function "rankindex" from the package *vegan* was used to determine the best method for calculating a distance matrix (Oksanen et al., 2017). The original OTU data were then converted into distance matrices using the Bray-Curtis method, the NMDS was run using the package *vegan*, and outputs were plotted using *ggplot2* (Wickham, 2009; Oksanen et al., 2017).

Permutational multivariate analysis of variance (PERMANOVA) was conducted to first evaluate the effects of site, year, and their interaction on microbial community composition using the package *vegan* (Oksanen et al., 2017). Since site, year, and sample date were nested effects, I performed a stratified PERMANOVA analysis: when testing for effects of sample date, I stratified within site and year; when testing for effects of cover crop type, I stratified within site, year, and sample date. Distances were calculated from OTU tables using the Bray-Curtis distance method. I tested 999 permutations, and results were considered significant at the level of $p < 0.05$.

In order to test how soil microbial community α-diversity changed as a result of the cover crop type, the Shannon α-diversity index for each sample was calculated from raw OTU tables using the package *phyloseq* (McMurdie and Holmes, 2013). Shannon α-diversity was averaged over the three sample dates, so each experimental unit (the unique combination of site, year, cover crop treatment and replicate) had one corresponding value of α-diversity. Linear mixed

models were then performed on the mean Shannon indices using the package *nlme* (Pinheiro et al., 2017). Cover crop type was treated as a fixed effect and site, year, and replicate were nested random effects. Models were calculated using the method of maximum likelihood method and results were considered significant at the level of alpha < 0.05. Finally, Tukey's Honestly Significant Difference (HSD) post-hoc tests were used to evaluate mean differences estimated by the models, and results were considered significant at the level of alpha < 0.05. In total, there were 166 experimental units included in the bacterial α-diversity analyses and 184 in the fungal analyses.

3.2.6. Partial least squares regression (PLSR) analysis

To identify how cover crop biomass influenced specific bacterial or fungal taxa, partial least squares regression (PLSR) analyses were carried out using the package *pls* version 2.6-0 (Mevik and Wehrens, 2007). The goal was to understand which microbial taxa responded most strongly (both positively and negatively) to particular cover crops (based on cover crop biomass). PLSR is a bilinear modeling method that allows for examining the relationship between two multivariate data sets: the predictor (cover crop biomass) and response (bacterial or fungal OTU abundances) variables. PLSR is useful when there is collinearity among predictor variables (Carrascal et al., 2009). In PLSR, predictor variables are projected onto orthogonal "latent variables," similar to principal components analysis (PCA), which are calculated to maximize the covariance between the response and predictor variables (Carrascal et al., 2009). In this case, the latent variables were based on the biomass at cover crop termination, with weeds included as a single species.

Microbial OTU data (reads per sample) were Hellinger-transformed. Within each model, data sets were divided into training (75% of samples) and test (remaining 25% of samples) sets. A ten-fold cross-validation method was applied, and seven latent variables were tested since there were seven crop species in this study, including weeds. I found that three latent variables were able to summarize approximately 90% of the variance in cover crop biomass at each farm. I then selected the top five bacterial and fungal taxa based on their positive and negative loading values for each of these three latent variables, allowing me to identify prominent taxa that were associated with the presence of the three cover crops.

For the PLSR analysis, a total of 387 samples were included for which I had bacterial sequences, fungal sequences, and cover crop biomass data. There were 16,062 unique bacterial OTUs and 112 unique archaeal OTUs detected from the 16S ribosomal RNA gene and 4,925 unique fungal OTUs detected from the internal transcribed spacer (ITS) region that were included in the PLSR analysis. Since I detected influences of site and year on microbial community composition and α-diversity (Table 3.1), I ran individual models for each site from 2015 only. I therefore had four PLSR models: Kinnikinnick bacteria, PrariErth bacteria, Kinnikinnick fungi and PrariErth fungi. I highlighted the 30 top-loading microbial OTUs from the PLSR model results for bacteria and fungi at each farm and I conducted a literature review to determine the potential ecological role of each OTU. While using separate models for each site lowers the generalizability of the model, it was necessary in order to address the profound site effects observed on both bacterial and fungal community composition.

3.3. Results

3.3.1. Overarching patterns in bacterial and fungal community composition

Site, year, and the site-year interactions were significant predictors of soil bacterial and fungal community composition (Table 3.1). Sampling date was a significant predictor of soil bacterial and fungal community composition when stratified within site and year. Lastly, cover crop type was a significant predictor of fungal community composition but not bacterial community composition when stratified within site, year, and sample date.

The bacterial NMDS plots show a clear separation between sites along axis 2, with a stress level of 0.164 (Fig. 3.1). At both sites, bacterial communities from 2016 were tightly clustered, compared to a greater spread among communities observed in 2015. The fungal NMDS plots did not show as strong of a site separation despite the fact that site explained 13.1% of the variance in fungal populations (Fig. 3.2, Table 3.1). Rather, the NMDS plot shows that fungal communities at PrariErth in 2015 and Kinnikinnick in 2016 form distinct clusters, while PrariErth in 2016 and Kinnikinnick in 2015 largely overlap. The higher stress level of 0.214 in the fungal NMDS plot may be due to difficulties in capturing the variation in fungal communities in a two-dimensional plot.

3.3.2. α-diversity of bacterial and fungal communities

Bacteria: I found that the Brassica monocultures and weedy plots had greater bacterial α-diversity than all other treatments, including the plant-free control plots (Table 3.2). The Tukey HSD post-hoc test, a more conservative test of mean differences, revealed no differences in mean α-diversity estimates across cover crop types.

Fungi: Fungal α-diversity was greatest under the plant-free control and grass monoculture plots (Table 3.2; Tukey HSD post-hoc test, $p < 0.05$). The lowest fungal α-diversity was found under the Brassicas, legumes, mixtures, and weedy plots.

3.3.3. Bacterial, archaeal and fungal taxa associated with cover crop biomass

Three latent variables were able to express approximately 89-95% of cover crop biomass at the two farms (Table 3.3). Each latent variable was primarily driven by one or more cover crop species: Idagold mustard, weeds, oat, and/or wheat. These cover crops were the most influential drivers of microbial community variation. For each latent variable, the top five bacterial and fungal OTUs were selected based on positive and negative loading values. This resulted in a total of 30 bacteria and 30 fungi identified from each model, and it is this subset of OTUs that is discussed below.

Bacteria/Archaea: The top 30 OTUs were highlighted from the PLSR model results, with 29 unique OTUs from PrariErth and 30 OTUs from Kinnikinnick (Tables 3.4 and 3.5). OTU 6, an ammonia-oxidizing archaeon (AOA) identified as *Candidatus Nitrososphaera SCA1145*, was the only top-loading OTU found at both sites. Archaeal taxa from this genus, which were the

most common taxa from the top-loading OTUs highlighted, were found at both farms to be positively and negatively associated with Idagold mustard biomass, negatively associated with weed biomass, and both positively and negatively associated with oat biomass. Specifically, *Candidatus Nitrososphaera* SCA1170, OTU 17862, was negatively associated with Idagold mustard and oat at PrariErth; OTU 11804 and OTU 283 were also negatively associated with oat at Kinnikinnick. There was also a positive association between Idagold mustard and both AOA and potentially ammonia-oxidizing bacteria (AOB), *Planctomycetes* and *Alphaproteobacteria*. Idagold mustard biomass was negatively associated with *Actinobacteria* at both farms (family *Gaiellaceae* at Kinnikinnick and order *Actinomycetales* at PrariErth). Weed biomass was positively associated with potential pathogens in genus *Flavobacterium* (phylum: *Bacteroidetes*) as well as several AOB, but not AOA. Two Rhizobacterial taxa (class: *Alphaproteobacteria*), which include N-fixing rhizobia, were both positively associated with weed biomass and negatively associated with Idagold mustard biomass.

Fungi: The top 30 fungal OTUs were highlighted from the PLSR model results, with 27 unique OTUs at each of the farms (Tables 3.6 and 3.7). At PrairiErth, one OTU identified as the fungal plant pathogen *Monographella cucumerina* (phylum: *Ascomycota*) was a top-loading taxon for all three latent variables; it was positively associated with weeds and negatively associated with oat and Idagold mustard (Palm et al., 1995). Several OTUs from the class *Dothideomycetes* were also highlighted from the model results, and this class contains a variety of fungal plant pathogens that displayed individualistic responses to cover crop biomass at both farms. Of note, OTU 10 (genus: *Phoma*) was negatively associated with Idagold mustard and weed biomass at Kinnikinnick and positively associated with Idagold mustard biomass at PrariErth. *Minimedusa polyspora* (phylum: *Basidiomycota*), which produces antifungal

compounds, was positively associated with Idagold mustard biomass (Beale and Pitt, 1992). There were five fungal OTUs identified from the PLSR models from both farms: OTU 13 (*Zygomycota*, *Mortierella* sp., non-pathogenic); OTU 10 (*Ascomycota*, *Phoma* sp., pathogenic); OTU 59 (*Ascomycota*, *Hypocreales* sp., includes plant pathogens and insect parasites); OTU 15 (*Ascomycota*, *Chaetomiaceae* sp., includes benign and pathogenic species); and OTU 4 (*Ascomycota*, *Lasiosphaeriaceae* sp., includes benign and pathogenic species) (Vega et al., 2009; A'Hara, 2015; Chowdhary et al., 2015).

3.4. Discussion

In the present study, I sought to improve our understanding of the legacy effects of different cover crops, planted in mixtures or monocultures, on soil bacterial and fungal communities post-incorporation. I hypothesized that bacterial and fungal community composition and α-diversity of the bulk soil would respond to differences in cover crop type (grass, legume, Brassica, or mixtures). Interestingly, I found that bacteria and fungi displayed opposite patterns in terms of α-diversity: bacterial α-diversity was greatest under weeds and Brassicas and lowest under the plant-free control plots, while fungal α-diversity was greatest under grasses, weeds, and the plant-free control plots and lowest under Brassicas. Since the weedy plots allowed all volunteer plant growth, and I did not record the identity or number of species present, I cannot comment further on plant diversity in the weedy plots, and whether increased aboveground diversity contributed to greater belowground diversity.

Based on prior findings, I also hypothesized that Idagold mustard, since it generates biomass rapidly in a short growing season (Wortman et al., 2012b; Holmes et al., 2017) and releases potentially-suppressive secondary compounds as it decomposes, would be an important driver influencing the presence of beneficial, pathogen-suppressive bacterial and fungal taxa. Indeed, the PLSR analyses revealed that Idagold mustard, weeds, and oat were the most important cover crops in explaining total plant biomass and predicting the presence of certain microbial taxa.

3.4.1. Idagold mustard improved plant pathogen suppression, but also suppressed Actinobacteria

Bacterial and fungal α-diversity differed by cover crop type, but differences in community composition (β-diversity) between cover crop types were only observed among fungal communities (Table 3.1). This suggests that fungal communities were more sensitive to cover crop treatment than bacterial communities. Previously, plant-fungal relationships have been found to be stronger than plant-bacterial relationships, since fungi tend to be more directly dependent on plant products (Broeckling et al., 2008; Millard and Singh, 2010). However, bacterial communities have been also altered by cropping treatments and agricultural management practices in previous field studies (Esperschütz et al., 2007; Liu et al., 2017). The noise caused by differences in sites, soil types, or years of cropping treatment can often mask the immediate effects of current cropping treatment on bacterial community composition (Jangid et al., 2011; Jiang et al., 2011), and may help explain the lack of response observed within the bacterial communities in this study. It is also possible that there simply was little or no detectable effect of cover crop type on the bacterial community or there was high variance between treatments. A meta-analysis by Venter et al. (2016) found that bacterial species richness in the bulk soil increased by 15.1% and diversity by 3.6% when the diversity of crop rotations was increased. This would support the greater bacterial diversity observed under cover cropped plots compared to fallow, as well as suggest that the inclusion of cover crop, regardless of type or diversity, is enough to increase bacterial diversity.

The greatest bacterial α-diversity and lowest fungal α-diversity were found in soils under previous Brassica monocultures. High bacterial diversity may be beneficial for pathogen

resistance, and glucosinolates released from Brassica tissues during decomposition can further promote disease-suppressive bacteria and reduce the incidence of fungal disease (Reynolds et al., 2003; Vukicevich et al., 2016; Brennan and Acosta-Martinez, 2017). The gradual liberation of these anti-fungal compounds may act as a filter on the fungal community, and help explain the low fungal α-diversity observed in Brassica plots (Pascault et al., 2010; Hollister et al., 2013). Furthermore, the PLSR model results showed that Idagold mustard was positively associated with several potentially disease-suppressive bacteria and fungi. The family *Bacillaceae* (phylum: *Firmicutes*) contains many pathogen-suppressive taxa (Mandic-Mulec et al., 2015), and was positively associated with both Idagold mustard and oat. Taxa from the order *Mycococcales* (class: *Deltaproteobacteria*) are known to produce and secrete antibiotic compounds into the soil (Reichenbach, 2001), which could potentially suppress plant pathogens. *Minimedusa polyspora* (phylum: *Basidiomycota*) also has antifungal properties, and can suppress pathogens associated with root rot (Beale and Pitt, 1992); this OTU was also positively associated with Idagold mustard biomass. *Phaeosphaeria* (phylum: *Ascomycota*), a relatively cosmopolitan genus, includes model organisms used for industrial fungicide development as well as potential wheat pathogens (Hane et al., 2007), which could have obvious harmful effects on subsequent wheat crops. In general, the results of the PLSR models suggest that the inclusion of Idagold mustard as a cover crop may help reduce plant disease (Larkin and Griffin, 2007; Bensen et al., 2009) by supporting pathogen-suppressive bacteria and fungi (Smolinska, 2000; Vukicevich et al., 2016).

To further support Idagold mustard's disease-suppressive capabilities, several fungal pathogens were also among the top-loading OTUs from the PLSR model that were negatively associated with Idagold mustard biomass. This indicates that the pathogens discussed below may be suppressed by the presence of Idagold mustard. *Monographella cucumerina* (phylum:

Ascomycota) is a fungal plant pathogen that can cause fruit, root, and collar rot (Palm et al., 1995; Carlucci et al., 2012). *Myrothecium verrucaria* (phylum: *Ascomycota*) is a plant pathogen that has been formulated for chemical control of nematodes and weeds (Clarke et al., 2007). *Metarhizium anisopliae* (phylum: *Ascomycota*) is a soil-borne fungus that acts as a parasitoid in insects and could be potentially beneficial in agroecosystems by suppressing insect pests (Zimmermann, 2007). Idagold mustard was also positively associated with *Phoma* sp. and *Nectriaceae* (phylum: *Ascomycota*), which both include potential plant and insect pathogens (A'Hara, 2015). Overall, while the PLSR model results showed that Idagold mustard was negatively associated with some potential fungal plant pathogens, other pathogenic taxa persisted in plots with high Idagold mustard biomass.

An additional observation of note regarding Idagold mustard was the strong negative association with *Actinobacteria* (orders *Actinobacteria* and *Thermoleophilia*) at both farms. *Actinobacteria* are abundant in soils and are involved in a myriad of processes, such as ammonium fixation or decomposition of more recalcitrant organic materials (de Boer et al., 2005; Bhatti et al., 2017). Due to the range of secondary metabolites these bacteria produce, *Actinomycetes* are also thought to potentially inhibit plant pathogens (Sprusansky et al., 2005; Jeffrey et al., 2007; Jose and Jha, 2016). The mechanism behind *Actinobacteria* suppression under high Idagold mustard biomass is unclear, and results from the present study conflict with previous findings that Brassicaceous seed meal, including that of Idagold mustard and other mustard species, actually increased soil *Actinobacteria* (specifically, genus: *Streptomyces*) (Hollister et al., 2013; Ren et al., 2018). The suppression of *Actinobacteria* observed in this field study may therefore have negative consequences on the decomposition and release of nutrients from plant tissues, as well as the suppression of plant pathogens in the soil.

3.4.2. Ammonia-oxidizing archaea dominated the bacterial PLSR models

The most abundant taxa among the top-loading bacterial OTUs from the PLSR models were ammonia-oxidizing archaea (AOA) of the genus *Candidus Nitrosphaera*. Several potential ammonia-oxidizing bacteria (AOB) from the phyla *Proteobacteria* (class *Alpha*- and *Gammaproteobacteria*) and *Planctomycetes* were also among the top OTUs. While individual AOA displayed individualistic responses to cover crop biomass, they were associated with all latent components in the model. For example, an AOA taxon (OTU 17862) was negatively associated with both Idagold mustard and oat biomass at PrariErth but was not among the top-loading OTUs at Kinnikinnick. A second AOA taxon (OTU 6) was a top-loading OTU that was positively associated with Idagold mustard at both farms. In a concurrent study at this site, similar concentrations of soil ammonium across all cover crop types were reported (Lucadamo et al., *in prep*), so detection of ammonia-oxidizing microorganisms across the three cover crops was not surprising. Though archaea made up less than 1% of the total "bacterial" 16S rRNA sequences that were analyzed in this study, they are ubiquitous in soils and are generally resistant to changing environmental conditions (Simon et al., 2000; Bates et al., 2011; Maul et al., 2014). Their abundance in the top-loading OTUs from the PLSR models also suggest that AOA are very responsive to changes in cover crop biomass.

Both AOA and AOB are responsible for the first step of nitrification, the conversion of ammonium to nitrite. This pathway is particularly important in agricultural systems, where N loss via nitrification decreases the pool of available inorganic N for subsequent crop uptake (van der Heijden et al., 2008). The three cover crops (Idagold mustard, weeds, and oat) that explained

the greatest variance in microbial community composition, as determined by the PLSR model, also happened to be among the plots with the lowest soil nitrate concentrations after cover crop termination (Lucadamo et al., *in prep*). The generally negative association between these cover crops (specifically oat and weeds) and AOA and AOB, along with the lower soil nitrate concentrations found in these plots, may suggest that these species, when at high biomass, suppress ammonia-oxidizing bacteria and archaea. This may important implications in the ongoing efforts to reduce inorganic N losses in agroecosystems.

3.4.3. Opposing trends observed among bacterial and fungal α-diversity in response to cover crop treatments

Bacterial and fungal community α-diversity displayed opposing trends in response to cover cropping (Table 3.2). Among bacteria, α-diversity was greatest under Brassica monocultures and weeds and lowest under the plant-free control plots, grasses, legumes, and mixtures. Conversely, fungal α-diversity was greatest under the plant-free control plots, intermediate under grasses and weeds, and lowest under Brassicas, legumes, and mixtures. Previous studies showed that higher fungal α-diversity was observed in soils with low crop yields (Hagn et al., 2003), as well as under grasses like wheat (Benitez et al., 2016). It is possible, then, that the changes in bacterial and fungal diversity observed in this study were the result of differences in plant productivity in the treatment plots: the plant-free control had the lowest plant biomass and greatest fungal α-diversity, while the Brassicas and mixtures had the greatest biomass and lowest fungal α-diversity (Lucadamo et al., *in prep*).

The differential responses of bacteria and fungi may also be due to differences in cover crop residue quality and chemical composition. Fungi tend to favor lower quality (high C:N ratio) residues such as grasses, while bacteria favor higher quality (low C:N ratio) tissues (Bossuyt et al., 2001), and could explain the greater fungal α-diversity observed under grasses in the present study. Furthermore, Brassica species can have mixed effects on soil microbial communities due to the release of glucosinolates and the subsequent conversion to anti-fungal isothiocyanates, both decreasing fungal pathogens (Larkin et al., 2010) and increasing disease-suppressive bacteria (Hollister et al., 2013). The release of such compounds from Brassica residues may pose an additional a challenge to fungi while simultaneously providing increasingly diverse resources for bacteria.

Regardless of the mechanism, the altering of soil bacterial and fungal communities by cover crops can have short-term effects on the soil that can be felt by subsequent crops. Soil microorganisms are vital for decomposition of residues and release nutrients for the following crop. Increased bacterial diversity under cover cropped soils result in soil communities that are also more resilient to disease (Brussaard et al., 2007; Lehman et al., 2015). In contrast, fungi tend to cause more damage to agricultural crops than bacteria (Brussaard et al., 2007), so increasing fungal pathogen resistance is especially relevant to agroecosystems. In contrast to increased bacterial diversity being beneficial for agricultural crops, decreased fungal diversity may lead to decreased pressure from fungal pathogens.

However, fungi are also important in agricultural systems for improving nutrient cycling and water-holding capacity (Lehman et al., 2015), and fungal-dominated soils can experience improved soil organic matter content (Six et al., 2006). Higher fungal:bacterial ratios have been observed under diverse cover crop mixtures that include legumes, grasses, and Brassicas, with

cover crops leading to greater fungal dominance compared to fallow soils (Finney et al., 2017). The fungal:bacterial ratio may also be important for improving agricultural sustainability through improved C storage and regulation (Brennan and Acosta-Martinez, 2017), and there is a documented positive relationship between fungal:bacterial and C:N ratios of plant tissues on a global scale (Fierer et al., 2009). While the patterns in α-diversity that I documented here do not necessarily translate to shifts in the ratios of fungal:bacterial biomass, they do indicate that different cover crops had differential effects on communities of soil bacteria and fungi. These opposing shifts warrant further research into the impacts these community shifts have on soil functionality in organic agroecosystems.

3.5. Conclusion

In the present study, soil fungal communities were found to be more responsive to changes in cover crop type than bacterial communities. In addition, the diversity of bacterial and fungal communities responded to cover crop treatments in opposing ways. The greatest soil bacterial α-diversity was found under the Brassica monocultures and weedy plots, while the lowest was under legumes and the plant-free control plots. In contrast, the greatest fungal α-diversity was under the plant-free control plots and lowest was under the Brassica monocultures. Weedy plots contained high α-diversity of both bacterial and fungal taxa, and the diversity of plant species present in these plots may have explained this observed pattern, though weed species present were not recorded. Brassica monocultures resulted in the highest α-bacterial diversity and lowest fungal α-diversity observed across all cover crop treatments, which lends support for Brassicas as suppressive of fungal pathogens. According to the PLSR analyses, Brassicas (specifically Idagold mustard), weeds, and oat were the most important cover crops in expressing associations with individual microbial taxa. Idagold mustard biomass was important in explaining the presence of certain beneficial microbial taxa and was negatively associated with several fungal pathogens and *Actinobacteria*, which are responsible for a range of soil functions in agricultural systems. The most common taxa among the top-loading OTUs in the PLSR models were ammonia-oxidizing archaea (AOA), which were relatively uniform across the three cover crops identified by the PLSR model. They were negatively associated with oat at both farms, which may suggest a potential suppression of nitrification in plots with high oat biomass.

Table 3.1. Results of PERMANOVA tests that evaluated the influences of site, year, sample dates, and cover crop type on bacterial and fungal community composition. The Bray-Curtis distance method was applied to community data. There was a total of 527 bacterial samples and 560 fungal samples. df = degrees of freedom: numerator, total; F = F statistic; R^2 = R^2-value; p = p-value. Results were considered significant at the level of alpha < 0.05 and are indicated with an asterisk.

	Bacterial community				Fungal community			
	df	*F*	R^2	*p*	*df*	*F*	R^2	*p*
Site	1, 526	31.67	0.051	0.001*	1, 559	96.11	0.131	0.001*
Year	1, 526	32.02	0.052	0.001*	1, 559	50.50	0.069	0.001*
Site x Year	1, 526	29.03	0.047	0.001*	1, 559	31.29	0.043	0.001*
Sample Date *stratified by site and year	2, 526	4.13	0.016	0.001*	2, 559	13.37	0.046	0.001*
Cover crop type *stratified by site, year and sample date	5, 216	1.46	0.014	0.091	5, 559	2.11	0.019	0.001*

Table 3.2. Linear mixed models were carried out to determine if cover crop type influenced the observed α-diversity (Shannon index) in bacterial and fungal communities. Shannon indices were averaged over the three sampling dates. Cover crop type was treated as the fixed effect and site, year, and replicate were nested random effects. There were 167 experimental units for bacterial and 184 for fungal communities. Results were considered significant at the level of alpha < 0.05 and are indicated with an asterisk. Letters indicate significant differences in means (Tukey's HSD post-hoc test, $p < 0.05$).

	Cover crop type	*Estimate ± Std. Error*	*df*	*t*	*p*
Bacteria	Control (plant-free)	6.99 ± 0.03	146	231.18	0.000
	Brassica	7.02 ± 0.02	146	1.99	0.049*
	Grass	7.01 ± 0.02	146	0.85	0.397
	Legume	7.01 ± 0.02	146	0.90	0.367
	Mixture	7.01 ± 0.02	146	0.82	0.411
	Weeds	7.04 ± 0.02	146	2.01	0.046*
Fungi	Control (plant-free)	4.33 ± 0.08^{a}	163	53.57	0.000
	Brassica	4.16 ± 0.06^{b}	163	-3.07	0.003*
	Grass	4.23 ± 0.06^{ab}	163	-1.74	0.084
	Legume	4.12 ± 0.06^{b}	163	-3.45	0.001*
	Mixture	4.16 ± 0.05^{b}	163	-3.18	0.002*
	Weeds	4.19 ± 0.06^{ab}	163	-2.19	0.030*

Table 3.3. The top three latent variables from the four PLSR models. Percent variance in X (cover crop biomass) and cumulative percent variance explained by each of the three latent variables is listed. The cover crop associated with positive or negative X loadings is also listed. Blank loading associations indicate no association with a particular species. Bolded plant names indicate the primary driver of that latent variable.

	Latent variable	% variance in X explained	Cumulative %variance in X explained	Positive loading associations	Negative loading associations
Bacteria PrariErth	1	84.1%%	84.1%%	---	**Mustard (-0.998)**
	2	7.2%%	91.3%%	Oat (0.254), Wheat (0.159)	**Weeds (-0.959)**
	3	2.1%	95.2%%	**Oat (0.931)**, Weeds (0.139)	Wheat (-0.338)
Bacteria Kinnikinnick	1	33.3%	33.3%	**Mustard (1.000)**	---
	2	44.4%	77.7%	**Weeds (0.959)**	Oat (-0.266), Wheat (-0.162)
	3	10.8%	88.5%	Wheat (0.160)	**Oat (-1.020)**, Weeds (-0.195)
Fungi PrariErth	1	84.1%	84.1%	---	**Weeds (-1.008)**, Mustard (-0.116)
	2	7.2%	91.3%	Weeds (0.280)	**Mustard (-0.959)**
	3	3.6%	94.9%	Wheat (0.146)	**Oat (-0.986)**
Fungi Kinnikinnick	1	32.2%	32.2%	**Weeds (0.984)**, Mustard (0.222)	---
	2	45.8%	78.0%	Weeds (0.351), Oat (0.111)	**Mustard (-0.929)**
	3	10.6%	88.6%	Wheat (0.103)	**Oat (-0.988)**, Mustard (-0.105)

Table 3.4. Top five bacterial OTUs from the PLSR model at PrariErth farm for each of the three latent variables listed in Table 3.3. The latent variables from the PLSR model were used to determine what cover crops, at high biomass, were important drivers for individual bacterial OTUs. Bolded cover crop species indicate the main driver of that latent variable.

[a] Indicates taxa that may include ammonia-oxidizing archaea or bacteria

[b] Indicates taxa that have potential disease-suppressive capabilities

[c] Indicates taxa that are potential pathogens

[d] Indicates bacteria with N-fixing capabilities

Cover crop biomass associations	Bacterial/Archaeal OTU
Idagold mustard (-)	Actinobacteria, Actinobacteria, Actinomycetales, Microbacteriaceae (OTU 265)
	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae, *Chitinophaga* sp. (OTU 604)
	Actinobacteria, Actinobacteria, Actinomycetales, Micrococcaceae (OTU 15455)
	Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, *Rhodoplanes* sp. (OTU 379)[a,d]
	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU 17862)[a]
Idagold mustard (+)	Planctomycetes, Planctomycetia, Pirellulales, Pirellulaceae (OTU 1652)[a]
	Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae (OTU 192)[a]
	Planctomycetes, Phycisphaerae, WD2101 (OTU 1370)[a]
	Chloroflexi, Anaerolineae, H39 (OTU 111)
	Firmicutes, Bacilli, Bacillales, Bacillaceae (OTU 31)[b]
Oat and wheat (+)	Archaea, *Candidatus Nitrososphaera SCA1145* (OTU 6)[a]
Weeds (-)	Acidobacteria, iii1-8, 32-20 (OTU 340)
	Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae (OTU 137)[a]
	Actinobacteria, Actinobacteria, Actinomycetales, Nocardioidaceae (OTU 314)
	Acidobacteria, [Chloracidobacteria], RB41 (OTU 4131)
Weeds (+)	Bacteroidetes, Flavobacteriia, Flavobacteriales, *Flavobacterium succinicans* (OTU 112)[c]
Oat and wheat (-)	Bacteroidetes, Sphingobacteriia, Sphingobacteriales, Sphingobacteriaceae, *Sphingobacterium multivorum* (OTU 361)
	Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae (OTU 91)[a]
	Bacteroidetes, Flavobacteriia, Flavobacteriales, *Flavobacterium* sp. (OTU 451)[c]
	Alphaproteobacteria, Caulobacterales, Caulobacteraceae (OTU 3980)[a]

Table 3.4. cont.

Oat and weeds (+)	Deltaproteobacteria, Myxococcales, Polyangiaceae, *Sorangium cellulosum* (OTU 295)[b]
Wheat (-)	Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae (OTU 2332)[a]
	Verrucomicrobia, [Spartobacteria], [Chthoniobacterales], [Chthoniobacteraceae] (OTU 9481)
	Chloroflexi, Anaerolineae, CFB-26 (OTU 272)
	Firmicutes, Bacilli, Bacillales (OTU 6739)[b]
Wheat (+)	Betaproteobacteria, MND1 (OTU 13155)
Oat and weeds (-)	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU 17862)[a]
	Archaea, *Candidatus Nitrososphaera gargensis* (OTU 2608)[a]
	Actinobacteria, Actinobacteria, Actinomycetales, Pseudonocardiaceae, *Pseudonocardia* sp. (OTU 115)[b]
	Acidobacteria, [Chloracidobacteria], RB41 (OTU 22)

Table 3.5. Top five bacterial OTUs from the PLSR model at Kinnikinnick farm for each of the three latent variables listed in Table 3.3. The latent variables from the PLSR model were used to determine what cover crops, at high biomass, were important drivers for individual bacterial OTUs. Bolded cover crop species indicate the main driver of that latent variable.
[a] Indicates taxa that may include ammonia-oxidizing archaea or bacteria
[b] Indicates taxa that have potential disease-suppressive capabilities
[c] Indicates taxa that are potential pathogens
[d] Indicates bacteria with N-fixing capabilities

Cover crop biomass associations	Bacterial/Archaeal OTU
Idagold mustard (+)	Archaea, *Candidatus Nitrososphaera SCA1145* (OTU 6)[a]
	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae (OTU 14)
	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae, *Flavisolibacter* sp. (OTU 61)
	Archaea, *Candidatus Nitrososphaera* sp. (OTU 151)[a]
	Deltaproteobacteria, Myxococcales, Myxococcaceae, *Anaeromyxobacter* sp. (OTU 206)[b]
Idagold mustard (-)	Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae (OTU 366)
	Actinobacteria, Actinobacteria, Actinomycetales (OTU 374)
	Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae (OTU 1704)
	Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae (OTU 738)
	Verrucomicrobia, [Spartobacteria], [Chthoniobacterales, [Chthoniobacteraceae], *DA101* (OTU 3)
Weeds (+)	Chloroflexi, Anaerolineae, SBR1031, A4b (OTU 4695)
Oat and wheat (-)	Acidobacteria, [Chloracidobacteria], RB41 (OTU 78)
	Verrucomicrobia, [Pedosphaerae], [Pedosphaerales], auto67_4W (OTU 242)
	Acidobacteria, [Chloracidobacteria], 11-24 (OTU 246)
	Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, *Rhodoplanes* sp. (OTU 10565)[a,d]
Oat and wheat (+)	Bacteroidetes, Flavobacteriia, Flavobacteriales, Flavobacteriaceae, *Flavobacterium* sp.(OTU 451)[c]
Weeds (-)	Actinobacteria, Thermoleophilia, Solirubrobacterales (OTU 199)
	Gammaproteobacteria, Alteromonadales, Alteromonadaceae, *Cellvibrio* sp. (OTU 2349)[a]
	Gemmatimonadetes, Gemmatimonadetes, KD8-87 (OTU 316)
	Archaea, *Candidatus Nitrososphaera* sp. (OTU 77)[a]

Table 3.5. cont.

Wheat (+)	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU 11804)[a]
Oat and weeds (-)	Deltaproteobacteria, Syntrophobacterales, Syntrophobacteraceae (OTU 1864)
	Archaea, *Candidatus Nitrososphaera SCA1170* (OTU 283)[a]
	Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae (OTU 128)
	Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae (OTU 47)
Oat and weeds (+)	Gemmatimonadetes, Gemm-1 (OTU 720
Wheat (-)	Archaea, *Candidatus Nitrososphaera* sp. (OTU 66)[a]
	Chloroflexi, Ktedonobacteria, JG30-KF-AS9 (OTU 707)
	Archaea, *Candidatus Nitrososphaera* sp. (OTU 1084)[a]
	Bacteroidetes, [Saprospirae], [Saprospirales], Chitinophagaceae (OTU 10492)

Table 3.6. Top five fungal OTUs from the PLSR model at PrariErth farm for each of the three latent variables listed in Table 3.3. The latent variables from the PLSR model were used to determine what cover crops, at high biomass, were important drivers for individual fungal OTUs. Bolded cover crop species indicate the main driver of that latent variable.
[a] Indicates potential fungal plant pathogen
[b] Indicates fungal taxa with anti-fungal properties
[c] Indicates fungal insect pathogen

Cover crop biomass associations	Fungal OTU
Weeds and Idagold mustard (-)	Ascomycota, Incertae sedis, *Chalara* sp. (OTU 4176)
	Ascomycota, Pezizomycetes, Pezizales, Pyronemataceae (OTU 170)
	Ascomycota (OTU 86)
	Ascomycota, Pezizomycetes, Pezizales, Pyronemataceae, *Pseudaleuria* sp. (OTU 110)
	Ascomycota, Dothideomycetes, Pleosporales, Phaeosphaeriaceae (OTU 94)[a]
Weeds and Idagold mustard (+)	Fungi (OTU 54)
	Ascomycota, Sordariomycetes, Xylariales, Incertae sedis, *Monographella cucumerina* (OTU 5)[a]
	Zygomycota, Incertae sedis, Mortierellales, Mortierellaceae, *Mortierella* sp. (OTU 13)
	Ascomycota, Sordariomycetes, Incertae sedis, Glomerellaceae, *Colletotrichum anthrisci* (OTU 71)[a]
	Ascomycota, Sordariomycetes, Sordariales, Lasiosphaeriaceae (OTU 18)
Weeds (+) **Idagold mustard** (-)	Basidiomycota, Agaricomycetes, Cantharellales, Ceratobasidiaceae (OTU 44)[a]
	Ascomycota, Dothideomycetes, Pleosporales, Pleosporaceae, *Alternaria eichhorniae* (OTU 8)[a]
	Ascomycota, Sordariomycetes, Hypocreales (OTU 59)[a,c]
	Ascomycota, Sordariomycetes, Xylariales, Incertae sedis, *Monographella cucumerina* (OTU 5)[a]
	Ascomycota, Sordariomycetes (OTU 6)
Idagold mustard (+) Weeds (-)	Basidiomycota, Tremellomycetes, Cystofilobasidiales, Cystofilobasidiaceae, *Guehomyces pullulans* (OTU 25)
	Ascomycota, Leotiomycetes (OTU 26)
	Ascomycota, Dothideomycetes, Pleosporales, Sporormiaceae, *Preussia flanaganii* (OTU 81)
	Ascomycota, Dothideomycetes, Pleosporales, Incertae sedis, *Phoma* sp. (OTU 10)[a]
	Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae (OTU 1)[a,c]

Table 3.6. cont.

Wheat (+)	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae, *Penicillium* sp. (OTU 38)
Oat (-)	Ascomycota, Sordariomycetes, Coniochaetales, Coniochaetaceae, *Lecythophora* sp. (OTU 32)
	Ascomycota, Sordariomycetes, Xylariales, Incertae sedis, *Monographella cucumerina* (OTU 5)[a]
	Ascomycota, Dothideomycetes, Pleosporales (OTU 75)[a]
	Basidiomycota, Agaricomycetes, Agaricales (OTU 223)
Oat (+)	Ascomycota, Sordariomycetes, Sordariales, Lasiosphaeriaceae (OTU 4)
Wheat (-)	Basidiomycota, Agaricomycetes, Corticiales, Corticiaceae, *Waitea circinata* (OTU 4637)
	Ascomycota, Sordariomycetes, Sordariales, Chaetomiaceae (OTU 15)
	Chytridiomycota (OTU 466)
	Ascomycota, Dothideomycetes, Pleosporales, Pleosporaceae, *Alternaria eichhorniae* (OTU 8)[a]

Table 3.7. Top five fungal OTUs from the PLSR model at Kinnikinnick farm for each of the three latent variables listed in Table 3.3. The latent variables from the PLSR model were used to determine what cover crops, at high biomass, were important drivers for individual fungal OTUs. Bolded cover crop species indicate the main driver of that latent variable.
[a] Indicates potential fungal plant pathogen
[b] Indicates fungal taxa with anti-fungal properties
[c] Indicates potential fungal insect pathogen

Cover crop biomass associations	Fungal OTU
Weeds and Idagold mustard (+)	Zygomycota, Incertae sedis, Mortierellales, Mortierellaceae, *Mortierella* sp. (OTU 13)
	Ascomycota, Sordariomycetes, Hypocreales, Clavicipitaceae, *Metarhizium anisopliae* (OTU 14)[c]
	Basidiomycota, Agaricomycetes, Agaricales, Psathyrellaceae (OTU 27)
	Ascomycota, Sordariomycetes, Xylariales, Incertae sedis (OTU 128)
	Ascomycota (OTU 19)
Weeds and Idagold mustard (-)	Ascomycota, Eurotiomycetes, Eurotiales, Trichocomaceae, *Aspergillus niger* (OTU 132)[a]
	Basidiomycota, Agaricomycetes, Agaricales (OTU 192)
	Ascomycota, Dothideomycetes, Pleosporales, Incertae sedis, *Phoma* sp. (OTU 10)[a]
	Ascomycota, Leotiomycetes, Helotiales, Incertae sedis, *Tetracladium* sp. (OTU 4836)
	Ascomycota, Sordariomycetes, Hypocreales (OTU 59)[a,c]
Weeds and oat (+) **Idagold mustard** (-)	Ascomycota, Dothideomycetes, Pleosporales, Incertae sedis, *Phoma* sp. (OTU 10)[a]
	Ascomycota, Dothideomycetes, Pleosporales (OTU 134)[a]
	Ascomycota, Sordariomycetes, Sordariales (OTU 62)
	Ascomycota, Sordariomycetes, Hypocreales, Incertae sedis, *Myrothecium verrucaria* (OTU 12)[a]
	Ascomycota (OTU 20)
Idagold mustard (+) Weeds and oat (-)	Basidiomycota, Agaricomycetes, Cantharellales, Incertae sedis, *Minimedusa polyspora* (OTU 122)[b]
	Ascomycota, Dothideomycetes, Pleosporales, Phaeosphaeriaceae, *Phaeosphaeria* sp. (OTU 47)[a]
	Ascomycota (OTU 21)
	Basidiomycota, Agaricomycetes, Agaricales, Psathyrellaceae (OTU 27)
	Ascomycota, Sordariomycetes, Incertae sedis, Glomerellaceae, *Colletotrichum anthrisci* (OTU 71)[a]

Table 3.7. cont.

Wheat (+)	Basidiomycota, Agaricomycetes, Cantharellales, Ceratobasidiaceae (OTU 44)[a]
Oat and Idagold	Zygomycota, Incertae sedis, Mortierellales, Mortierellaceae, *Mortierella humilis* (OTU 22)
mustard (-)	Ascomycota, Sordariomycetes, Sordariales, Chaetomiaceae (OTU 15)
	Ascomycota, Sordariomycetes, Sordariales, Lasiosphaeriaceae (OTU 4)
	Basidiomycota, Agaricomycetes, Agaricales, Psathyrellaceae (OTU 27)
Oat and Idagold	Ascomycota, Sordariomycetes, Sordariales, Lasiosphaeriaceae (OTU 253)
mustard (+)	Ascomycota, Leotiomycetes, Helotiales (OTU 127)
Wheat (-)	Ascomycota, Dothideomycetes, Pleosporales (OTU 134)[a]
	Ascomycota, Sordariomycetes, Sordariales, Chaetomiaceae, *Trichocladium asperum* (OTU 3930)
	Zygomycota, Incertae sedis, Mortierellales, Mortierellaceae, *Mortierella* sp. (OTU 13)

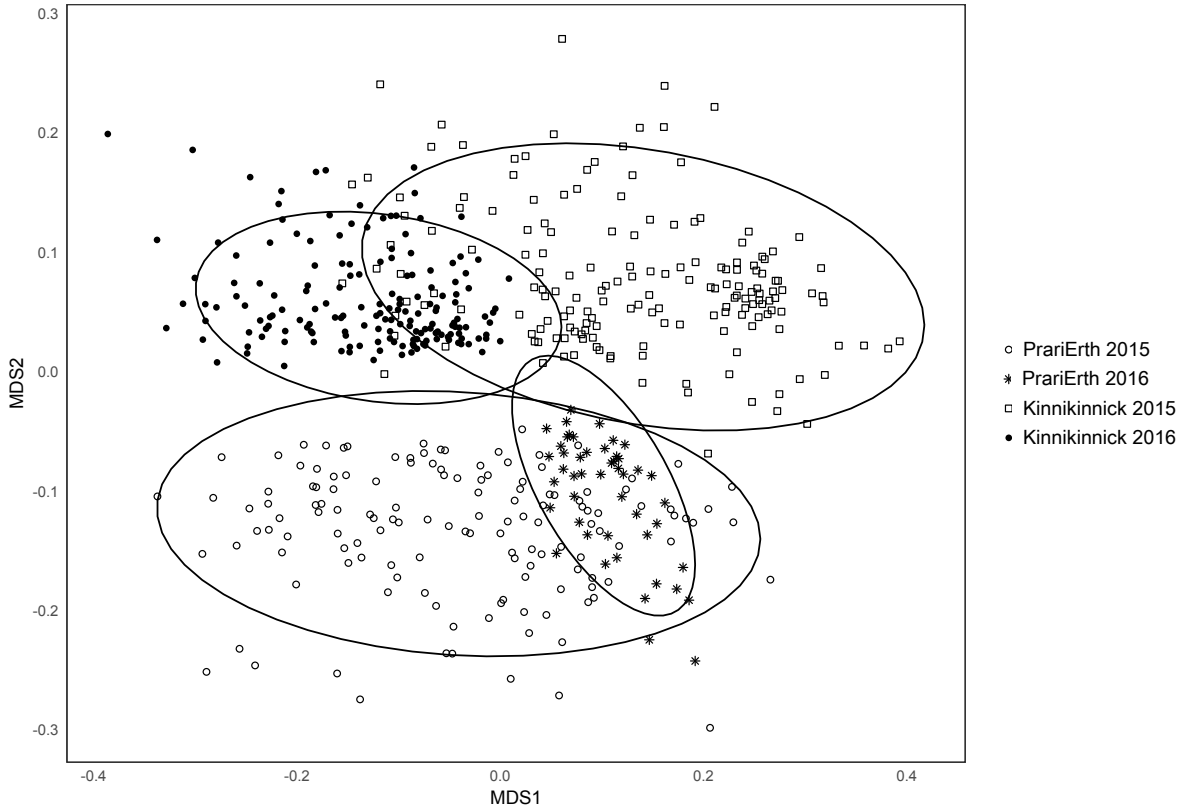

Figure 3.1. NMDS plot of bacterial communities representing all cover crop treatments. Each point represents the bacterial community from a single soil sample. Due to the significant effects of site and year, points are labeled by their site-year interactions. The Bray-Curtis distance method was used to perform the NMDS, with a stress level of 0.164. Ellipses represent the 95% confidence interval around the centroid for the given site-year.

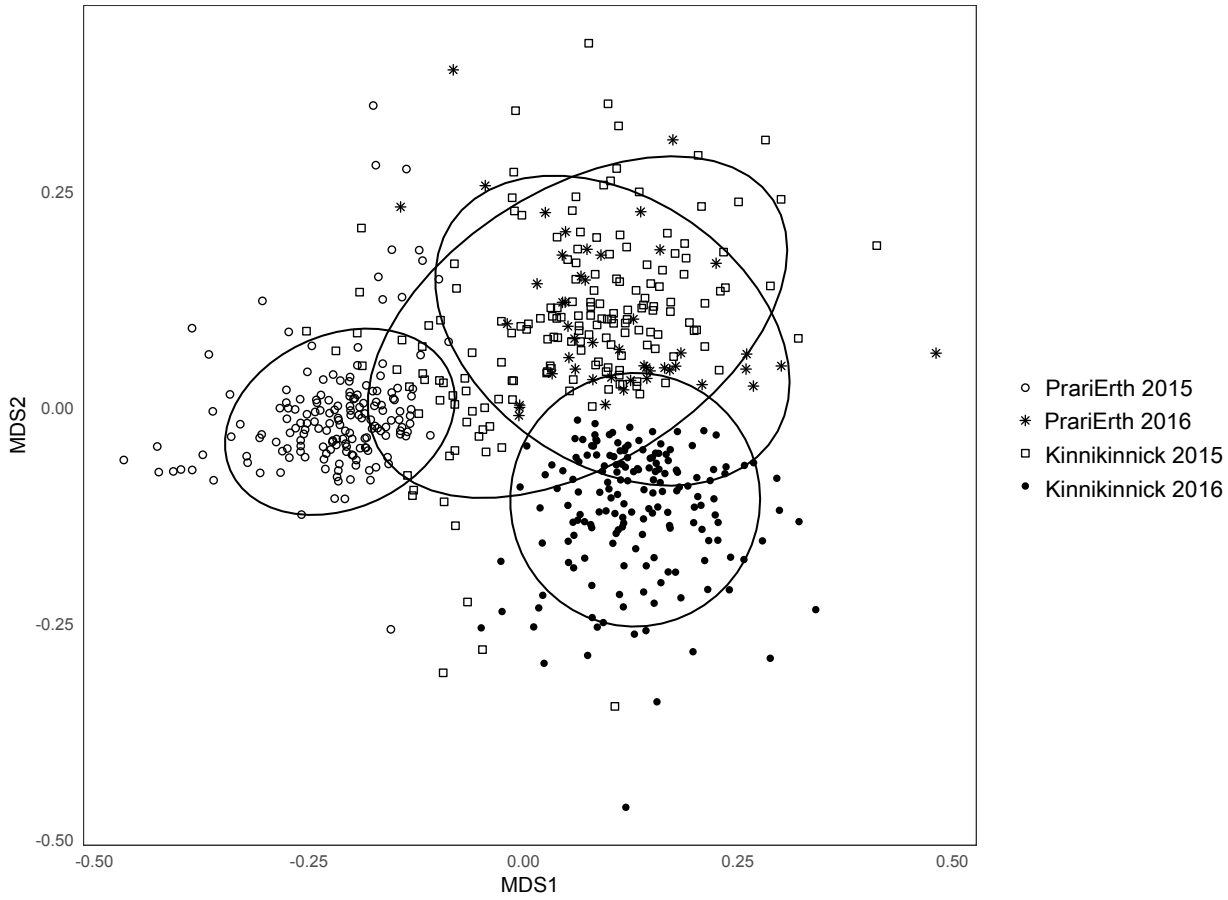

Figure 3.2. NMDS plot of fungal communities representing all cover crop treatments. Each point represents a fungal community from a single soil sample. Due to the significant effects of site and year, points are labeled by their site-year interactions. Bray-Curtis distances were used to perform the NMDS with a stress level of 0.214. Ellipses represent the 95% confidence interval around the centroid for the given site-year.

GENERAL CONCLUSIONS

In the present study, I sought to improve our understanding of the legacy effects of different cover crops, planted in mixtures or monocultures, on several measures of the soil chemical and biological environment. In the first part of my study (Chapter 2), I focused my attention on the legacy effects of cover crops on soil inorganic nitrogen (N) pools and total soil phenolic content. I found that Brassica monocultures were the most productive cover crop and both decreased soil nitrate and increased soil potentially mineralizable N (PMN) intensities. Legumes were the least productive and resulted in high soil nitrate and low soil PMN intensities, similar to the plant-free control plots. The diverse five-species mixtures were intermediate in productivity, and while soil PMN was increased as a result of these plots, soil nitrate levels remained high. I also did not find that Brassica monocultures caused any increase in total phenolic content compared to the plant-free control plots. The findings from this portion of my study suggest that Brassicas, which generated the greatest biomass, were most effective at both reducing soil nitrate pools and soil PMN. While I did not detect noticeable differences in total soil phenolic content as a result of Brassica cover crops, the combination of biomass and recalcitrant residues likely led to the increased PMN throughout the four weeks post-cover crop incorporation.

In the second part of my study (Chapter 3), I focused on the legacy effects of cover crops on soil microbial communities. Specifically, I sought to determine how cover crop type (grass, legume, Brassica, or mixture) influenced soil community composition (β-diversity) and bacterial and fungal α-diversity, as well as identify important cover crop species driving the presence or absence of certain microbial taxa using partial least squares regression (PLSR) modeling. I found

that fungal communities were more sensitive to changes in cover crop type than bacterial communities. Patterns in α-diversity were also opposite: fungal α-diversity was greatest under the plant-free plots and lowest under Brassicas and weeds, while bacterial α-diversity was greatest under Brassicas, weeds, legumes, and mixtures, and lowest under grasses and the plant-free plots. Finally, using PLSR, I identified Idagold mustard, weeds, and oat biomass as the primary cover crop drivers in expressing the presence or absence of certain microbial taxa. Idagold mustard enhanced several potentially pathogenic taxa and suppressed other fungal pathogens, but also suppressed *Actinobacteria*, an important bacterial phylum responsible for a range of soil functions in agricultural systems. The most commonly identified taxa belonged to the archaeal genus *Candidatus Nitrososphaera*. Thse ammonia-oxidizing archaea and are key players in the process of nitrification, and were negatively associated with oat biomass at both farms, suggesting the potential suppression of nitrification in plots with substantial grass biomass.

In conclusion, the legacy effects I measured varied with cover crop type, and such effects will need to be considered when farmers consider their cover crop goals. If the aim is to reduce the potential risk of nitrate leaching and increase the soil PMN content, the inclusion of a Brassica monoculture would be the most effective means of doing so. In contrast, if the aim is to increase the pool of inorganic N for subsequent crop uptake, legumes maintained high soil nitrate levels but were not very effective at establishing during this field study. If the aim is to reduce species-specific fungal pathogen pressures, Idagold mustard was effective at suppressing several pathogenic taxa and promoting other disease-suppressive bacteria. Though not detected in this study, the presence and release of glucosinolates and isothiocyanates may be the cause of these observed patterns. Finally, if a farmer's aim is to control weed populations, it will be important

to use a cover crop type (grass, mixture, or Brassica) that can successfully establish during the intended growing season and generate sufficient biomass to outcompete weeds. Though weeds were often a substantial portion of total aboveground biomass and weedy plots supported relatively high bacterial and fungal diversity, weeds were also associated with fungal pathogens that could cause harm to subsequent crops.

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