The impact of cover crops and crop residue removal on soil microbial community abundance, diversity and soil health in a medium-term cover crop field trial in Southwest Ontario.

by

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ABSTRACT

THE IMPACT OF COVER CROPS AND CROP RESIDUE REMOVAL ON SOIL MICROBIAL COMMUNITY ABUNDANCE, DIVERSITY AND SOIL HEALTH IN A MEDIUM-TERM COVER CROP FIELD TRIAL IN SOUTHWEST ONTARIO.

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Soil microorganisms are an important component of soil health and drive key biogeochemical processes. The objective was to assess the impacts of fall cover crops (CC), main crops (MC) and residue harvest on the abundance, diversity and function of bacteria and fungi over the growing season. A medium-term trial was established in a horticultural rotation with annual fall CCs. Microbial communities after winter wheat and tomatoes were examined. After MC harvest, CC treatments (none, oat, radish, rye, rye/radish mixture) were applied and plots were split for MC residue retention. Microbial abundances were quantified throughout the growing season with qPCR and sequenced annually with Illumina MiSeq. CCs significantly increased bacterial (rye, radish/rye) and fungal (radish, radish/rye) abundance compared to the no-cover control. PiCRUST and FUNGuild analysis found that bacterial and fungal diversity and functions were significantly impacted by MC species as well as increased by the inclusion of rye and radish.
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1.0 **General Introduction:**

1.1 **Soil Health and its role in agriculture.**

Agroecosystems benefit society through food and biomass production and play roles in important ecosystems services such as nutrient mineralization, water quality protection, biodiversity conservation and greenhouse gas reduction (carbon and nitrogen) (Stalman 2011). However, there is a tradeoff between production and ecosystem services which can impact society. Agricultural management practices chosen for short-term profits may have a harmful impact on the local environment. The concept of soil health is currently the subject of much interest to researchers, farmers, and consumers, with many people concerned about the land practices their food is grown on. Soils provide a range of supporting (e.g. nutrient cycling), provisioning (e.g. food supply), and regulating services (e.g. water quality and supply) (Haygarth and Ritz, 2009). Simply put, soil health is a term that reflects the combination of the physical, biological, and chemical properties of a soil which interact to influence aspects of soil quality resulting in an overall state of health as a functioning ecosystem. Soil health reflects the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals and humans (Lehman et al. 2015). Soil resilience, which is a characteristic of a healthy soil, is defined in terms of tolerance against stress, buffering capacity, and the ability to regenerate (Lal et al. 1997). However, we are still developing the knowledge of what constitutes a healthy soil and how we can compare between soil types, management methods and climates based on a given state of soil health. Soil ecologists are looking beyond the basic biological measures traditionally used and focusing on more recently developed techniques to fill in the gaps on specific species and communities associated with the trends we see in microbes (Jansson and
Hofmockel 2018). Soil microbes (specifically bacteria fungi and archaea communities) are the very definition of functional redundancy, with many soil functions conserved within and between communities. These microbes and their functions may shift in abundance and diversity based on slight variations in the soil microclimate. For example, tillage greatly reduces slow-growing organisms (e.g. fungi), leading to rapidly growing organisms becoming dominant during disturbance stress (e.g. bacteria) (Tiemann et al. 2015). Depending on fluctuations in environmental variables such as nutrients, substrate available, porosity and aggregate stability, certain microbial groups or populations may gain an advantage or disadvantage leading to a population diversity shift (Tiemann et al. 2015). This in turn impacts the functional capacity of the soil community, as a loss of certain groups may destabilize parts of nutrient cycles or allow for the influx of pathogenic competitors in the soil agroecosystem. Generally, though not always, with more overall abundance comes more potential for diversity and functional redundancy within groups.

This thesis examines the impacts of processing vegetable crop rotation in field crops with fall cover crops and crop residue retention on the seasonal abundance and annual diversity of soil microbes. The first chapter follows seasonal shifts in microbial abundance of bacteria, fungi, and archaea over the course of one full year under a tomato field crop following fall cover crops and straw residue retention. The second chapter compares the soil microbial diversity under fall cover crops between rotation years following wheat and tomato field crops. Our objective is to identify the relationships that exist between our chosen field crops, their rotation and the soil microbial community, with the goal of identifying trends that will aid in informed land management decisions.
2.0 Seasonal shifts in microbial abundance in a medium-term cover crop - residue retention management system in a horticultural crop rotation in Southwestern Ontario

2.1 Introduction

Land management practices and crop species can have significant impacts on belowground microbial communities (Buyer et al. 2017). For example, fall cover crops (CCs) and crop residue retention, when compared to artificial ground cover, were found to increase microbial abundance (Buyer et al. 2017). Increasing aboveground diversity, for example by adding different crops in rotation and CCs, has also been shown to significantly increase total soil nitrogen and carbon (McDaniel et al., 2014). Farmers and agronomists are becoming increasingly interested in promoting belowground diversity and soil health with diversified crop rotations.

2.1.1 Quality indicators as they relate to soil health

Since the term soil quality was introduced (Warkentin and Fletcher, 1977), scientists have attempted to assess soil quality through a variety of “indicators”, which are simply measurable soil properties that influence the capacity of a soil to perform a specified function (Acton and Padbury 1993). The development of a comprehensive set of quantifiable indicators of soil quality has been the focus of many studies (Doran and Parkin, 1996; Hussain et al., 1999; Idowu et al., 2009, Chahal and Van Eerd, 2018) and continues to be debated in the scientific community. Commercially or commonly used soil health indices include: the comprehensive assessment of soil health (CASH), Agriculture and Agri-Food Canada (AAFC) Soil Fingerprinting Framework, and the Agricultural Research Services Soil Test (Chahal and Van Eerd 2018).

The biological component of most tests (such as the CASH) only consider fungi to bacteria ratios, biomass, or overall soil organism respiration rates. While informative, for
example fungal dominated communities often have higher quality and quantity of soil organic matter (Six et al., 2006), this general data such as dominant community shifts do not represent the ecological potential or specific processes responsible for the observed soil quality composition. More advanced techniques such as quantitative polymerase chain reaction (qPCR), can provide this same information but more accurately in terms of microbial population (bacteria, fungi, archaea) and functional gene detection. High-throughput sequencing techniques and abundance measurements have become cost-effective for use in environmental soil microbial community analysis to allow for a clearer understanding of the impact of land management changes on the diversity and abundance of the soil microbial community (Jansson and Hofmockel 2018). Since a diverse soil community provides a range of functions and demonstrates greater resilience to disturbances (Finney et al., 2017), biodiversity assessments can be a potential key indicator of soil resilience and health. No commercial tests currently include specific measurements of soil biodiversity, and while no optimal composition of microbes may exist, we do know that certain microbial groups are associated with increased soil health. For example, large AMF communities can discourage pathogen establishment, improve nutrient uptake and increase plant drought tolerance (Rillig 2004). Therefore, we believe that genomic tools are a cost-effective method to measure total soil microbial communities including bacteria, archaea and fungi for the evaluation of field microbe responses to land management.

2.1.2 Microbial community shifts over the crop growing season

Over the growing season, we can expect to see large shifts in the different groups of microbes as the local climate and environment changes. It has been shown through previous research that seasonal effects will be the driving factors causing the bulk of fluctuations expected over the course of the growing season (Tiemann et al., 2015).
When a crop is first planted it initially stimulates primary growth and root development with a focus on carbon uptake (Austin et al. 2017). As the main crop grows and matures, focus within the plant switches to secondary growth and sexual organ development, which requires less carbon and more nitrogen uptake (Van Doorn et al. 2011). This corresponds with a change in type and complexity of nutrients being taken up and released by the plant, which impacts the soil microbial community at different life stages of the field crop (Austin et al. 2017). At crop maturity (around time of harvest) the plant goes into senescence, withdrawing vital nutrients out of the plant, excluding the fruit bearing portion, to the roots belowground. A field goes through major physical, biological and chemical changes over the course of a growing season, including the changing of belowground resources. Therefore, it is important to assess the microbial community at several points throughout the season, to fully understand its temporal shifts.

2.1.3 Cover crops and residue retention as best management techniques

Cover crops, residue retention, and intercropping are proposed sustainable management methods which have benefits in a variety of environments and soil types for different aspects of soil health. These management practices have been shown to influence a variety of important soil qualities such as: nitrogen cycling (Chahal and Van Eerd, 2018; Németh et al. 2015; Tiemann et al., 2015) microbial biomass (Njeru et al. 2014), and nutrient mineralization rates (Zhang et al., 2017). Measurement of these processes provides crucial information on specific ecological services and functions being performed within the field, but often lack resolution by not including direct sampling and measurement of the microbes.
2.1.4 Cover crops

A CC is to be planted during fallow periods, the time between field crops during which the field is traditionally left bare such as after summer harvest but before winter freeze, without any biomass or crop products removed for profit from the CC themselves. The goal of CCs is to introduce and maintain living plant and root associated microbial communities into the soil to maintain beneficial soil interactions and functions, often referred to as a green manure/fertilizer. For example, CC presence has been shown to increase soil organic carbon (SOC) (Chahal and Van Eerd 2018), which is a key indicator of soil health as it in turn influences other soil properties such as: aggregate stability, water infiltration rates, water holding capacity, and porosity (Lehman et al. 2015). Previous studies at the field site indicated that CCs did not decrease yield or total profits of field crops (O’Reilly et al. 2011), reduced the establishment and success of weeds (O’Reilly et al. 2011), and did not decrease yield or nitrogen mineralization of crop residues (Belfry and Van Eerd, 2016). Additionally, it was found that CCs, when compared to no CCs, displayed up to 22% more crop yield and up to 9.3% more soil organic carbon (Chahal and Van Eerd, 2018).

This study examined non-traditional CCs; including two cereal grains (rye, *Secale cereale* L., and oat, *Avena sativa* L.), a brassica spp., (oilseed radish (OSR), *Raphanus sativus* L. var. oleoferus Metzg. Stokes) and an intercropped mixture of OSR and rye. The intercropped mixture combines two different root types (highly branched in rye and deep tuber in OSR). *Brassica* species, through different life stages, emit biofumigants which act by killing off susceptible species, notably some common fungal pathogens as well as many arbuscular mycorrhizal fungi (AMF) (Sarwar et al. 1998). The brassica root structure does not promote as dense rhizosphere compared to cereal grains (e.g. oat and rye) which has been shown to be
associated with large populations of AMF in the rhizosphere (Cho et al., 2017). Additionally, cereal grains and legumes intercropped interact to decrease N cycling in the soil, so examining the combined effects of a grain species with a brassica could be a way to gain diversity benefits without impacting nutrient cycling (Zhao et al. 2017). It is interesting to note that in a previous study, under tomatoes, OSR increased soil mineral N into the growing season (Belfry et al. 2017) which could indicate that select CC under certain field crops could minimize the need for N fertilizer inputs. It is not clear how field crop species and rotations interact with species of CC to impact the belowground biodiversity and ecosystem functioning.

2.1.5 Residue retention/removal

Crop residue retention is a method of management that has been shown to influence aspects of soil health, especially SOC (Austin et al. 2017). By removing crop residue such as straw or corn stover for use off field, there is a corresponding decrease in amount of substrate for soil biological communities to metabolize, causing SOC depletion over time. Past research has highlighted the benefits of retaining residue, for example, showing more complete nitrogen metabolism and decreased GHG emissions during spring melt (Németh et al. 2015) increased moisture content and decreased erosion (Reicosky and Forcella 1998), increased SOC, and increased nutrient retention (Austin et al. 2017). It is interesting to note, that the belowground contributions of CC rather than aboveground biomass are what persists long-term in the soil C pool (roots vs shoots) (Austin et al. 2017). This means that when we remove field residue the carbon inputs from the field crop roots are still present, indicating we most likely will not see a large difference in our soil plough layer because of residue retention. Additionally, type and quality of residue may have a significant impact on soil microbial community. Chemical diversity and soil organic matter (SOM) input complexity of different residues have long-term
impacts on SOM dynamics (McDaniel et al. 2014). This was seen previously at our field site, where wheat straw residue increased tomato yield and profit but rye straw did not (Van Eerd et al., 2015)

2.1.6 Hypothesis and Objectives

This study identifies shifts in abundance of soil microbial groups associated with sustainable aboveground crop management strategies, field crop residue retention and fall cover crops. It is predicted that there will be an increase of microbial abundance in all three communities over the growing season because of increased SOC storage, porosity, aggregate stability, and decreased erosion associated with growing cover crops. Our study can be defined as a medium-term study (Chahal and Van Eerd, 2018), as our current rotation had been established since 2008, a long-term study would hopefully examine treatment impacts well over this time frame; many long-term experiments reflect multiple decades of consistent treatment and measurements (Knapp et al., 2012; Sessitsch et al. 2001). Microbial populations can shift significantly over the course of a single growing season (short-term) when new management practices are introduced, depending on climatic shifts as well as treatment influences (Richter et al. 2007). In order to better understand measured population shifts as a result of applied treatments, climatic influences can be separated from treatment impacts through consistent annual measurements over the long-term (Richter et al., 2007). The current study examines the impact of a full six-year crop rotation in one growing season of the rotation, with a single annual comparison of fall CC influences. The hypothesis of the study was that the use of fall cover crops and residue retention would impart benefits to the microbial community detectable through increased relative abundance of key microbial groups (bacteria, fungi and archaea) (Ch. 2) as well as increased general diversity and beneficial microbial functional groups (Ch. 3).
Our objectives were to: i) quantify general abundance of microbial communities (bacteria, fungi, and archaea) under cover crops ii) establish links between microbial abundance and experimental treatments and iii) identify seasonal trend impacts of our main crop paired with CC and residue retention treatments.

2.2 Methods

2.2.1 Site description and Experimental design

The research site was located at Ridgetown Campus, of the University of Guelph, Ontario, Canada (42.44° N, 81.88° W; 201 m elev.). In 2008, collaborators established a cover crop (CC) and rotation study in a diverse processing vegetable and grain system. The soil at the site was a sandy loam (Orthic Humic Gleysol) with 68:21:11% sand:silt:clay composition (Chahal and Van Eerd 2018). The field trial was a randomized complete block design (four replicates) of five cover crop treatments with a split plot residue presence/absence (R+/−) treatment overlaid (Fig. 1). Plots measured 6 m x 8 m (post-split). Cover crop treatment was the main plot factor where no cover, oat, rye, OSR and an OSR/rye mixture were the treatments in each block which were then split, and main crop residue was either removed or retained at harvest (R +/-). Main crop residue in 2015 (winter wheat) from the vegetable crop was removed from split plots at harvest in summer and cover crops were planted in August 2015. The fall planted cover crops overwinter and are incorporated under in the spring (April 2016) before tomato planting (May 2016) with cultivation before main and cover crop plantings to prepare seedbeds for field crop and cover crop planting. The field was treated with glyphosate (May 2016) several weeks before tomato planting to control weeds.
2.2.2 Soil DNA Sampling

Soil was sampled on 6 occasions for microbial analysis (Oct-2015, May-June-July-Sept-Nov-2016) within each split plot; 9 soil cores (0-10 cm depth; 2.5 cm diameter) were collected along 2 transects (forming an ‘X’ shape, Fig. 2) using aseptic collection techniques and bulked for each transect (Fig. 2). Soil samples were immediately homogenized by manual mixing within an airtight sample bag. Approximately 200-300 g of soil was collected total per plot. Samples were stored at 4°C for transport to Guelph and stored and processed within 24h for DNA extraction and moisture content analysis. Excess soil not used for DNA extraction or moisture content was placed into long-term storage at -20°C.

2.2.3 DNA Extraction from Soil

After transport to the lab, DNA was extracted from soil using MoBio Powersoil DNA kits (MOBIO Laboratories, Inc.) within 24 h of sampling and standard extraction protocols were followed according to kit guidelines. Soil was then stored at -20°C. DNA was eluted after extraction and 50 uL was aliquoted and stored at -20°C working stock and -80°C freezer stock DNA.

DNA concentration and purity were determined by nanodrop (Nanodrop 8000, Thermo Fisher Scientific, Waltham, MA, USA). All samples consistently tested within acceptable limits (260/280 = 1.8-2.0) for pure quality DNA suggesting that no further cleanup steps prior to molecular analysis were required.

2.2.4 Quantitative PCR

Quantitative PCR (qPCR) analysis took place on the Bio-Rad CFX detection system (Bio-Rad Laboratories, Inc.) and analyzed through Bio-Rad CFX manager 3.1 (Bio-Rad
Laboratories, Inc.). There are a variety of previously described conserved genes which allow for microbial community (relative) abundances to be readily quantified through qPCR. To target the three microbial groups of interest to our study (bacteria, fungi, and archaea) we selected the three gene targets most suitable to our study in terms of target specificity, reaction efficiency and base pair length for reliable quantification for bacteria (338F-518R: Bakke et al. 2011), fungi (FR1-FF390: Vanio and Hantula, 2000) and archaea (A364aF: Burggraf et al., 1997; A934b: Grosskopf et al., 1998).

For amplification of bacteria 16S DNA and archaeal 16S DNA markers, a 20 uL volume assay was used with BioRad Ssofast EvaGreen® Supermix, 2 uL template DNA, 400 nM primers and RNase/DNAse free water. The one step qPCR assays using Ssofast were conducted within an initial denaturation step of 2 min at 98 °C followed by 40 cycles of 98 °C for 5 s, a 5 s annealing at 55°C for bacteria and a 10 s annealing at 62°C for archaea. For amplification of fungal 18S DNA markers, a 20 uL volume assay was used with BioRad iQ™SYBR® Supermix, 2 uL template DNA, 400nM primers and RNase/DNase free water. The two step qPCR assays using SYBR®Green were conducted with an initial denaturation step of 5 min at 95°C followed by 40 cycles of 95°C for 15 s, a 30 s annealing at 50°C, and 45 s extension at 70°C. Random qPCR products containing each of the primers were run out on a 1% agarose gel to verify proper sequence size against a 1 kB ladder.

A subsequent melt curve analysis was done to verify specific target amplification with 41 cycles of 5 s at 65°C to 95°C. No-template controls were run with every assay to ensure that reagents were not contaminated.
2.2.5 Preparation of qPCR Standard Curve

Standard curves for qPCR quantification were constructed using serial dilutions of plasmid DNA containing the target genes (10^{1}-10^{8} copies per reaction). Bacterial 16S, archaeal 16S, and fungal 18S genes were cloned from PCR products amplified from Clostridium thermocellum spp., Methanosarcina mazei spp., and environmental fungal genomic DNA, respectively. PCR products were obtained for each gene target using PCR conditions as described above for soil DNA extracts, and amplicon specificity was screened by gel electrophoresis on 1% agarose gel. PCR amplicons were cloned into One Shot® DH5α™-T1R, Top10 E. coli competent cells (Life Technologies Corp.) using a TOPO TA Cloning® kit (Life Technologies Corp.). Cloned plasmid DNA was extracted using a PureLink™ Quick Plasmid Miniprep Kit (Life Technologies Corp.) and verified by sequencing on an Applied Biosystems ABI Prism 3720 (Life Technologies Corp.) to confirm target identity (Laboratory Services, University of Guelph, Guelph, ON). All qPCR assays included no template controls and were optimized to ensure reaction efficiencies of 95-110% and standard curve slopes of -3.1 to -3.6 with R^2 values between 0.990-1.000.

To obtain the amount of soil used in our individual soil DNA extractions, gravimetric dry weight was used to obtain moisture content of field collected soil samples. Approximately 10 g of soil was loaded onto tin trays and dried at 105 °C for 72 h until moisture loss had plateaued. Dried soil was weighed again to calculate mass of water evaporated out of the sample. The calculated moisture content was used to obtain the amount of dry soil represented in each tube of extracted soil sample DNA by subtracting the calculated moisture content from total soil mass used in the extraction process for each tube.
2.2.6 Inhibition testing

All DNA samples were diluted and tested for inhibition to find the most empirically suitable working concentration for analysis as described by Reardon et al. (2013). -20 °C working stocks were diluted for inhibition testing. Using M13 primers, we tested reaction efficiency to determine a 50x diluted sample removed all signs of inhibition (higher concentrations were believed to show inhibition due to excess template DNA rather than presence of actual inhibitors in our template; specifically, within bacterial 16S). To test for inhibition, we spiked various dilutions of pooled environmental samples with M13 template, which doesn’t naturally occur in the environment, using a standard 20 uL reaction volume, and found that at 50x dilution, no inhibition was detectable in pooled environmental samples compared to lab cultures.

2.2.7 Statistical Analysis

Gene abundance values were normalized to gene copy number per gram of dry soil and log10 transformed. Variance Analysis of these data to test for differences between plots and changes over time were conducted in SAS 9.4 (Carlsbad, NC, USA) using a Generalized Linear Mixed Model (PROC GLIMMIX). The Shapiro-Wilks test was used to test for normality of data; student’s t-test for residuals confirmed the absence of outliers. Gene abundance data sets were log normal or highly skewed; values were normalized to gene copy number per gram dry soil and analyzed using a log normal distribution. ANOVA, and LS means were used for statistical analysis to detect any differences within and between time points. Data was log transformed to meet assumptions of normality and back transformed for presentation. To understand the differences in community abundance under our CC treatment effects evident in our ANOVA, we
performed multiple mean comparisons including Tukey’s pairwise comparison and Dunnett’s test comparing cover crops treatments to our no cover crop control plots. The Dunnett’s test, instead of looking for differences in all the treatments from each other, only the control (no CC) mean is used as a baseline to illustrate individual species effects on microbial communities.

Within each data set, sampling time was a continuous measure; independent and interactive fixed effects were associated with cover crop, and residue retention within plots, while field replicate and associated interactions were random effects.

The residual maximum likelihood method was employed to fit the model for all data sets. The variance components structure used was chosen based on convergence and bestfit model criteria. Individual treatment means within data sets were compared using post-hoc Tukey’s test for all pairwise comparisons. Distribution testing found log-normal to be the best fit for all communities. Significant differences among and between least-squares means were determined by P-values; the null hypothesis (Ho) was rejected at P ≤ 0.05.
Figure 2. 1 Plot map of the field experiment in Ridgetown, Ontario.
Figure 2. 2 Example transect locations (denoted by ‘X’) for soil probe sampling locations within each plot. Which were then pooled and homogenized to create a single sample for each plot.
2.3 Results

2.3.1 Cover crops and seasonal influences on microbial communities

Microbial communities showed significant differences associated with treatment for fungi and archaea (P < 0.05) but not bacteria (Table 2.1, Fig. 2.4). In addition, seasonal effects (timepoints) were significantly different in all three communities measured (Fig. 2.3). No significance differences were seen due to the presence of residue therefore, plot treatments (residue +/-) were grouped and averaged to examine CC species impacts on the microbial communities.
Table 2. Analysis of variance of fixed effects for cover crops and residue retention over the growing season using back calculated treatment means of relative abundances for bacteria fungi and archaea.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Num DF</th>
<th>Den DF</th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Archaea</th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Archaea</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC.</td>
<td>4</td>
<td>177</td>
<td>1.95</td>
<td><strong>2.44</strong></td>
<td>6.54</td>
<td>0.10</td>
<td><strong>0.04</strong></td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Residue</td>
<td>1</td>
<td>177</td>
<td>0.26</td>
<td>0.12</td>
<td>1.55</td>
<td>0.61</td>
<td>0.72</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>CC*Residue</td>
<td>4</td>
<td>177</td>
<td>0.92</td>
<td>1.41</td>
<td>0.90</td>
<td>0.45</td>
<td>0.23</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Timepoint</td>
<td>5</td>
<td>177</td>
<td><strong>13.11</strong></td>
<td><strong>33.84</strong></td>
<td><strong>22.08</strong></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Timepoint*CC</td>
<td>20</td>
<td>177</td>
<td>0.29</td>
<td>0.91</td>
<td>0.62</td>
<td>0.99</td>
<td>0.57</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Timepoint*Residue</td>
<td>5</td>
<td>177</td>
<td>0.84</td>
<td>0.21</td>
<td>0.30</td>
<td>0.52</td>
<td>0.96</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Timepoint*CC.*Residue</td>
<td>20</td>
<td>177</td>
<td>0.48</td>
<td>0.75</td>
<td>0.49</td>
<td>0.97</td>
<td>0.76</td>
<td>0.96</td>
<td></td>
</tr>
</tbody>
</table>

* Bolding denotes significance at p < 0.05
Figure 2. 3 Microbial gene abundance shifts of A) 16S DNA genetic markers specific to general soil bacteria, B) 18S DNA genetic markers specific to general soil fungi, and C) 16S DNA genetic markers specific to general soil archaea under fall CC and wheat residue treatments as measured by qPCR over the course of one full growing season from Oct. 2015 to Nov. 2016. Each CC treatment is averaged at each sample event under presence/absence of residue. Error bars represent a calculated standard error of the mean (α = 0.05, n = 4).
Figure 2. Annual average microbial gene abundance under cover crop treatments for A) general bacteria 16S DNA, B) general fungi 18S DNA, and C) general archaea 16S DNA as measured by qPCR over the course of the growing season from October 2015 to November 2016. Error bars represent a calculated standard error of the mean (α = 0.10, n = 48). Letter’s represent Tukey’s pairwise comparisons, no significance in A.
2.3.2 Cover crop species influences on microbial communities compared to no cover crop control.

Tukey’s analysis showed no significance in CC species impacts on the bacterial or fungal community, however, archaea showed significant effects of OSR treatments showing increased abundance (35.9% difference) compared to no CC (Table 2.2) (Fig. 2.4).

Table 2.2 Mean microbial gene copies per gram dry soil under cover crop species treatment. Bolded letters denote similarity between treatments within gene targets (Tukey’s pairwise comparison significance at P < 0.05).

<table>
<thead>
<tr>
<th>Cover Crop Spp</th>
<th>Bacteria (16S gene copies)</th>
<th>Fungi (18S gene copies)</th>
<th>Archaea (16S gene copies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Estimate x 10^8</td>
<td>Standard Error x 10^7</td>
<td>Mean Estimate x 10^5</td>
</tr>
<tr>
<td>No CC</td>
<td>1.33 a^z</td>
<td>1.67</td>
<td>3.94 a</td>
</tr>
<tr>
<td>Oat</td>
<td>1.36 a</td>
<td>1.70</td>
<td>4.21 a</td>
</tr>
<tr>
<td>OSR</td>
<td>1.27 a</td>
<td>1.60</td>
<td>5.11 a</td>
</tr>
<tr>
<td>Rye</td>
<td>1.59 a</td>
<td>1.99</td>
<td>4.66 a</td>
</tr>
<tr>
<td>Rye/OSR</td>
<td>1.50 a</td>
<td>1.88</td>
<td>5.18 a</td>
</tr>
</tbody>
</table>

^2 Bolding denotes significance at p < 0.05 level.

In a comparison with the microbial community abundance associated with CC species to the no-cover control treatment, our Dunnett’s test (p < 0.10) identified OSR and rye/OSR treatments having a significant impact by increasing abundance of archaea (35.9% for OSR and 31.4% for rye/OSR) and fungi (22.9% for OSR and 23.9% for rye/OSR) compared to our no CC control (Table 2.3).
Table 2. 3 Significant microbial abundances averaged under fall cover crops. Abundance of bacterial, fungal and archaea associated with crop species. Dunnett’s test results comparing CC species to the no CC control. Significance is denoted by bolding at the P < 0.05 level.

<table>
<thead>
<tr>
<th>Crop Species</th>
<th>Mean Estimate $x 10^8$</th>
<th>Standard Error $x 10^7$</th>
<th>P-value</th>
<th>Mean Estimate $x 10^5$</th>
<th>Standard Error $x 10^4$</th>
<th>P-value</th>
<th>Mean Estimate $x 10^5$</th>
<th>Standard Error $x 10^4$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CC control</td>
<td>1.33</td>
<td>1.67</td>
<td>-</td>
<td>3.94</td>
<td>4.77</td>
<td>-</td>
<td>1.28</td>
<td>1.88</td>
<td>-</td>
</tr>
<tr>
<td>Oat</td>
<td>1.35</td>
<td>1.70</td>
<td>0.99</td>
<td>4.21</td>
<td>5.10</td>
<td>0.93</td>
<td>1.50</td>
<td>2.19</td>
<td>0.34</td>
</tr>
<tr>
<td>OSR</td>
<td>1.27</td>
<td>1.60</td>
<td>0.97</td>
<td>5.11</td>
<td>6.18</td>
<td>$0.06^y$</td>
<td>1.50</td>
<td>2.01</td>
<td>2.91 $0.00$</td>
</tr>
<tr>
<td>Rye</td>
<td>1.58</td>
<td>1.99</td>
<td>0.18</td>
<td>4.66</td>
<td>5.64</td>
<td>0.34</td>
<td>1.56</td>
<td>2.29</td>
<td>0.15</td>
</tr>
<tr>
<td>Rye/OSR</td>
<td>1.50</td>
<td>1.88</td>
<td>0.50</td>
<td>5.18</td>
<td>6.27</td>
<td>$0.04^z$</td>
<td>1.87</td>
<td>2.74</td>
<td>$0.00$</td>
</tr>
</tbody>
</table>

$^y$ and bolding denote significance at the P < 0.10 level
$^z$ bolding denotes significance at the P < 0.05 level
2.3.2 Seasonal impact of cover crops on microbial communities after wheat straw residue management in tomato crop growth.

Our seasonal impacts were also found to be significant in pairwise comparison as well as ANOVA for all three microbial communities (Table 2.4) (Fig. 2.3). This shows how each community shifts over the course of the growing season. We see three unique patterns emerge in each of the communities. Overwinter, from Oct/15 to May/16, while fall CC residues were present, there was a significant increase (32.9%) of bacteria, the fungal community marginally decreased in abundance but not significantly, however May/16 is when we found the lowest measured abundance, while the archaeal population was highest in fall on Oct/15 with a 35.1% decrease in archaea overwinter.

During tomato growth, there was a significant decline (35.9%) in bacterial abundance in July/16 from the previous sampling date. This captures the community at peak primary growth of the tomato plant. This was followed by a significant increase (49.1%) of bacterial abundance found at tomato harvest in Sept/16. During fall CC growth in 2016, a marginal decrease of bacteria was seen from harvest but not significantly so. Fungi showed significant increase in abundance (70.7%) from annual comparison (fall CC sample points). Through the growing season the fungal community increased in abundance significantly by 37.6% when measured in June/16, after which the community did not change in abundance until tomato harvest when we saw another significant increase of 36.6%. Fall CCs in Nov/16 saw the highest measured fungal abundance with an increase of 33.4% from harvest. Annual comparison of the archaeal communities showed a significant decline between years of 60.7%, in a trend that continued through our next sample date around early tomato growth (June/16) with a 28.2% decrease in abundance. July/16 which saw a significant 41.7% increase in archaeal abundance corresponding
to peak primary growth of the tomato crop, making this date statistically similar to Oct/15 and May/15. After this increase, the archaeal population continued the previous trend of decline through tomato harvest (Sept/16) and fall CCs (Nov/16) with decreases of 41.7% and 14.6%, respectively; harvest and fall CCs in 2016 were statistically similar to June 2016, however 2016 fall CCs saw the lowest measured abundance of all time points.

Table 2.4 Mean microbial gene copies per gram dry soil over time averaged across treatments. Bolded letters denote similarity between treatments within gene targets (tukey’s pairwise comparison significance at p < 0.05).

<table>
<thead>
<tr>
<th>Sample Date (timepoint)</th>
<th>Bacteria (16S gene copies)</th>
<th>Fungi (18S gene copies)</th>
<th>Archaea (16S gene copies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Estimate x 10^8</td>
<td>Standard Error x 10^7</td>
<td>Mean Estimate x 10^5</td>
</tr>
<tr>
<td>Oct. 2015</td>
<td>1.17 bc</td>
<td>1.51</td>
<td>2.87 d</td>
</tr>
<tr>
<td>May 2016</td>
<td>1.74 a</td>
<td>2.25</td>
<td>2.78 d</td>
</tr>
<tr>
<td>June 2016</td>
<td>1.44 ab</td>
<td>1.86</td>
<td>4.45 c</td>
</tr>
<tr>
<td>July 2016</td>
<td>0.925 c</td>
<td>1.19</td>
<td>4.13 c</td>
</tr>
<tr>
<td>Sept. 2016</td>
<td>1.81 a</td>
<td>2.34</td>
<td>6.52 b</td>
</tr>
<tr>
<td>Nov. 2016</td>
<td>1.55 ab</td>
<td>2.00</td>
<td>9.81 a</td>
</tr>
</tbody>
</table>

2.3.3 Straw residue removal impacts on the microbial community.

When all treatments are averaged across time points and cover crop (CC) species (table 1), straw residue removed/retained (R-/R+) showed no significant differences in any microbial community. R- showed marginally (not significant) more microbial gene abundance compared to R+ trials, representing an approximate 2.9%, 2.3% and 7.5% increase for bacteria, fungi and archaea respectively (Table 2.1).
Table 2. Mean microbial gene copies per gram dry soil under residue retention treatment. Bolded letters denote similarity between treatments within gene targets (Tukey’s pairwise comparison significance at p < 0.05).

<table>
<thead>
<tr>
<th>Residue Treatment</th>
<th>Bacteria (16S gene copies)</th>
<th>Fungi (18S gene copies)</th>
<th>Archaea (16S gene copies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Estimate x 10^8</td>
<td>Standard Error x 10^7</td>
<td>Mean Estimate x 10^5</td>
</tr>
<tr>
<td>Residue Removed</td>
<td>1.42 a</td>
<td>1.64</td>
<td>4.65 a</td>
</tr>
<tr>
<td>Residue Retained</td>
<td>1.38 a</td>
<td>1.59</td>
<td>4.54 a</td>
</tr>
</tbody>
</table>
2.4 Discussion

2.4.1 Cover crop species impacts on microbial community abundances

Fungal and archaeal abundances were significantly higher in plots containing an OSR or Rye/OSR cover crop, compared to the no cover crop control. A previous 15-year study by Mitchell et al. (2017) in an arid irrigated field environment described the effects of a diverse CC mixture (including Juan triticale, rye, and vetch) including deep rooting species which were found to increase bacterial abundance by improving the microbial environment through increasing porosity, water infiltration and nutrient transport; however, no changes were seen in bacterial abundance in our current study. At our site, nitrogen mineralization has been shown to be higher under OSR treatments in our field trial (Belfry et al. 2017). Brassicaceae (i.e. OSR) species contain within their tissues glucosinolates, which are hydrolyzed into isothiocyanates, which are known to act as biofumigants and possess anti-fungal properties (Vierheilig et al. 2000). Due to the nature of OSR to produce fungal inhibiting isothiocyanate compounds as they decompose, we expected a notable decrease in fungal abundance, particularly arbuscular mycorrhizal fungi (Vierheilig et al. 2000; White and Weil 2010). Our results quantifying abundance of fungal communities did not support this hypothesis (Tables 2.3 and 2.4), however, it is possible that the diversity of fungal species will be altered in the OSR plots without impacting the total fungal abundance, this will be examined in Chapter 3.

Bacterial abundance showed no significant impact due to cover crops, contrary to the trend in archaeal and fungal communities (Fig. 2.2). There are numerous factors which may be responsible. Jorquera et al. (2008) highlighted the impact of crop treatments on functional group composition of rhizosphere bacteria in the short and long term. The authors (through PLFA)
noted increased proportion of phosphobacteria when rhizosphere cultures were grown on agar under oat and rye (3 years of consistent field treatment) treatments compared to other traditional CCs such as red clover or wheat. Following rye incorporation, Lundquist et al. (1999) noted that bacteria populations responded rapidly to rye residue incorporation by increasing abundance, a result which lasted over the course of their six-year (4-year rotation) experiment. Sampling bulk soil, or time of sampling may have led to lack of impact on bacterial communities seen in our study.

2.4.2 Straw residue impacts on microbial community abundances

The removal of crop residue resulted in no significant differences in gene abundance for bacteria, fungi or archaea communities (Table 3.3). This was consistent with previous studies, which showed that residue presence is associated with higher microbial abundance and activity resulting from a variety of factors such as increased moisture retention, warmer soil temperatures, and increased SOC and nutrient inputs (Németh et al. 2015). However, Turmel et al. (2015), highlighted that in some cases, residue retention can lead to a surplus of water retention and poor infiltration causing anoxic conditions and detrimental impacts on microbial communities and soil quality. While these conditions were not seen in the current study’s field conditions the non-significant decreases in relative abundances under CC R+ compared to CC R- split plots indicate that the microbial populations could be impacted by CC R+ interactions over the long-term. However, previous medium-term studies found no interaction between CCs and R+ over a range of soil and plant parameters (Belfry and Van Eerd 2017; Chahal and Van Eerd 2018) making long-term interactions between CC and R+ unlikely. In all R- plots winter wheat roots were left in the soil and only the above ground biomass was removed. A study by Buyer et al. (2010), noted that varying amounts of residue treatments applied produced significant
increases in microbial abundance. The authors noted that their CC residue treatments of root and shoot (only a few cm remained above the ground) vs total (root, shoot and stem) CC residue yielded less pronounced abundance increases but still similar trends were seen as in total residue presence, indicating that some CC residues could be removed for additional uses without sacrificing the benefits of the CC residue in whole.

Previous studies at our field site saw an increase of plant available mineralized nitrogen under tomatoes with straw residue under OSR (Belfry et al. 2017); which may be helping to drive the increase of fungi observed over the season, as the population was ‘primed’ overwinter by straw and CC residue and was able to capitalize on the introduction of mineral nitrogen over the growing season to outcompete others. This nitrogen mineralization also contributed to an increase of tomato production, noted in increased tomato biomass by Chahal and Van Eerd (2018). Our changing microbial populations could be a contributing factor to this increase of aboveground biomass and thus field productivity. Diversity analysis of microbes may reveal what type of substrate is contributing the most, being reflected in increased metabolism associated gene presence from previously identified microbes such as nitrogen, carbon and phosphorous metabolizers.

2.4.4 Seasonal changes in microbial abundance under tomato crop and fall cover crops

We would expect to see an increase in the species-specific associations between tomatoes and soil fungi, leading to the observed increase of fungi over the growing season. Both total soil fungal communities (18S) and soil bacterial (16S) populations increased over the growing season while archaeal (16S) showed a decrease over time. Archaea have been shown to decrease over time in a tomato cropping system by Maul et al. (2014), however the authors attributed this change equally to seasonal climatic influences, their ground cover treatments and plant-driven
changes (tomato influences). The authors believed that minor plant species interactions on the archaeal population were accentuated by the archaeal population being such a small portion of the microbial community overall that the effects of plant species were amplified as any change to their measured archaeal populations would be significant. Given that the current study observed a similar decrease over the season under tomatoes, it is recommended that further studies specifically track the archaeal community over time under tomatoes to identify any plant-microbe interactions that may explain this decrease in archaea. In regard to the increases observed in fungal and bacterial populations, the trend was much more pronounced in fungi compared to bacteria (64.5% increase compared to 20.5% for bacteria) (Fig. 2.2, Table 2.4). Tomatoes have been shown to increase abundance of arbuscular mycorrhizal fungi detected through morphological spore identification, (Njeru et al., 2014). We see the largest jump in fungal abundance in November 2016, coinciding with peak CC growth. Other studies have shown that fungi have beneficial associations with tomato leading to increased abundance of fungal colonies grown on agar based on the presence of field tomato crop and CCs (Buyer et al., 2010; Njeru et al., 2015); most significantly at tomato harvest compared to a significantly smaller amount found at the end of the CC cycle most likely due to seasonal and climatic variables as the end of CC cycle takes place in the harsher fall environment (Njeru et al., 2015).

Our CCs at Nov 2016 had the highest amount of fungi in all trials suggesting they are promoted by tomato litter after incorporation in Sept/16 or cover crop biomass which becomes incorporated in the spring as the seed bed is prepared for planting. Therefore, after summer harvest and during decomposition over the fall and winter, straw residue injects more fungal favoring poor-quality litter. Over winter, populations of fungi decreased and when tomatoes were
planted the populations rebounded quickly, indicating there may have been a priming effect from the straw residue for fungi that did not affect bacterial abundances.

2.5 Conclusion

No interactions between CC and R- were detected, which may be attributable to this study only being a medium-term experiment (only the second time through the crop rotation) as well as the presence of belowground residue in R- plots causing the similar observed microbial populations under R+/-. CCs in general influenced all microbial communities in our experiment, but there were significant species-specific influences at work which highlighted that CC species selection can influence the microbial populations. OSR and rye/OSR mixtures promoted fungal and archaeal communities compared to a no CC control. The shifts in total microbial abundance could be beneficial or detrimental to plant growth depending on the specific microbial groups within each community being stimulated. Increased levels of beneficial plant-growth promoting organisms, such as AMF, or groups which are associated with nutrient metabolism, such as carbon fixation (allowing for increased SOC), would be valuable information for the best use of these management practices. Likewise, if the abundance increase that was observed is associated with detrimental microbial groups, such as plant pathogens, this information would also be extremely valuable for the recommendation or discouragement of the use of these particular management practices. For this reason, it is important to examine and identify the microbial communities in order to understand the full impact on the agroecosystem of the observed abundance shifts. Therefore, the next chapter will look at identification and impacts on ecological functional groups with next-generation sequencing.
The growing season has significant influences on the microbial communities, which are likely attributed to the natural progression of growth stages in our field tomato crop; different microbial associations develop over the season including uptake and release of metabolites by the plant directly influencing the belowground root and microbial environment. Due to the large variations in microbial abundances over the course of the field season, and clear impacts of CCs on the microbial community, we chose to examine the microbial diversity, through next-generation sequencing, in an annual comparison which focuses on the microbial community under fall CCs (comparing fall CC time points between years).
3.0 Bacterial and fungal diversity associated with fall cover crops in a vegetable crop rotation

3.1 Introduction:

The diversity of soil microbial communities is currently being debated as a suitable indicator of soil health. Srivastava and Vellend (2005) point to the standard measure of ecological processes (i.e. soil quality indicators) as being more reliable, cheaper and useful in terms of land management decisions compared to diversity analysis. There are many commercial tests currently available for evaluating soil quality, but no test currently includes a specific measure of soil biodiversity. Now that genomics tools are a cost-effective method of measuring total soil biodiversity, there is evidence that biodiversity in addition to relative community abundance may be used as an objective verifiable biological indicator of soil quality and overall soil health. Soil is widely recognized as one of the most diverse environments on earth, containing an immense abundance of microorganisms whose functions and impacts within the soil environment and associated ecosystem services are not well understood (Decaens 2010; Bardgett and Van der Putten, 2014). High diversity is often associated with good soil quality, which allows for increased substrate use and nutrient availability (Bending et al., 2004; Liu et al., 2009). Microbial diversity changes have been associated with land management changes, such as long-term conventional to no-tillage systems (Strohm 2015), but it is still unclear if the shifts are only temporary (as the field equilibrates to the new treatment) or due to local environmental conditions. Genomics tools, such as high throughput sequencing (HTS) targeting particular microbial groups, have shown how agricultural practices, such as crop residue removal, can influence the bacterial communities and diversity associated with ecological processes (Banerjee et al., 2019) and nitrogen cycling (Bent et al., 2016).
Soil health and sustainable management techniques may impact the success and yield of crops, through a variety of mechanisms such as cover crop suppression of weeds, directly impacting crop yield through competition for critical resources. Genetic analysis has enabled researchers to use more comprehensive analysis (qPCR and HTS) of biodiversity to identify the links between soil health, sustainable agriculture, and belowground species composition.

It is the objective of this study to identify any changes over time in diversity of bacterial and fungal communities in a horticultural-cover cropping system, as described in Chahal and Van Eerd (2019). Sequencing at similar growth stages and plant compositions between years will identify any CC species specific interactions with the soil microbial populations.

3.1.2 Barcoding as a tool for diversity measurement

The sequencing of conserved targets such as the small subunit ribosomal RNA genes, sometimes referred to as DNA barcoding, is considered to be one of the best techniques for obtaining the soil microbial community profile (Kim et al. 2013). The 16S DNA region is used to identify bacteria and archaea while the internal transcribed spacer (ITS) region is used for fungi. Barcodes are clustered according to DNA sequence similarity (often 97% similarity or higher for general community measurements) and assigned to operational taxonomic units (OTUs) which can be identified (Blaxter et al., 2005) with a suitable reference database. This barcoding allows for taxonomic identification and comparison of microbial groups often to the microbial family or genus levels but, in the case of previously identified and well-studied taxonomies, identification can be down to the species level based on the targeted barcode sequence of standardized genes (Hebert et al. 2003; Tautges et al. 2016).

Metabarcoding is used to barcode all available organisms contained within a given sample; by targeting highly conserved community gene targets, this allows us to gain a picture of
the total biodiversity contained within a sample. As such, it is suitable to facilitate the measurement and monitoring of soil microorganisms for the evaluation of our experimental conditions and land management practices. Many studies have used barcode sequences to explore the diversity of a range of organisms, such as fungi (Schmidt et al., 2013), nematodes (Sapkota and Nicolaisen, 2015) and eukaryotes (Yang et al., 2014), highlighting the potential for its use to evaluate agricultural land practices and changing soil conditions. Our study examines influence of crop residues and cover crop species on the diversity of the microbial population. Through using known ecological diversity metrics, we will analyze the HTS data to evaluate the impact our experimental treatments have on soil microbial processes through differences in community diversity.

3.1.3 Ecological databases and their use to evaluate cover crop impacts

Metabarcoding makes genetic sequences, phylogenetic similarities, and diversity metric analysis of ecosystems possible; however, traditionally, this information has not provided evidence about the ecological impact and significance of the community. Now that genetic analysis is cost effective for biodiversity studies, ecological database tools have been developed to pair genetic analysis results (specifically OTU tables) with identified ecological functions and niches from previous studies to gain an understanding of the ecological functions present within a given sample. For bacteria, the phylogenetic investigation of communities by reconstruction of unobserved states (PiCRUST) database is used to estimate the functional-gene profile for a given sample. This allows us to gain ecological relevance from both identified OTUs and unidentified OTUs by assigning their most likely ecological role based on phylogenetic similarity to known organisms.
Management practices have been evaluated with PiCRUST, which shows the prediction of functional profiles, that bacterial populations noticeably fluctuate depending on: tillage type (Hariharan et al. 2017), plant type and farming intensity (Chen et al. 2018), organic amendment types (Ling et al. 2018), presence of soil and root pathogens (Wu et al. 2016), soil pH (Lewis et al. 2017), and nitrogen fertilizer addition (Wang et al., 2018). From these studies, we expect that there will be detectable functional profile differences between cover crops due to different root types (ex. OSR is non-mycorrhizal, as well as different nutrient contributions as green fertilizer) and our no cover crop control, as well as residue management (due to organic amendment differences between R+ and R- plots).

Fungi often change how they act in the ecosystem throughout their life stages, which makes identifying their ecological role, referred to as guilds, challenging. FUNGuild breaks down all detected OTUs into ecological guilds based on previous identifications, giving each assignment a rating of how probable it is based on the strength of the previous studies classifying it (Nguyen et al. 2016). It is recommended to use the rating of probable and highly probable classifications in comparisons (Nguyen et al. 2016), introducing order to avoid bias or over-estimation errors (Nie et al. 2018). Fungal guilds have been shown to shift depending on a range of soil, plant and land management factors including soil pH (Rigg et al. 2016), fertilizer regime (Wang et al. 2018)), plant growth stage (Wang et al. 2018), local environmental conditions (Zhang et al. 2017), rhizosphere vs bulk soil (Zhang et al. 2017), spatial heterogeneity (Kivin and Hawkes, 2016), pedogenesis (Courty et al. 2018), organic matter removal (Wilhelm et al. 2017) and disturbance frequency (Cho et al. 2017). The aboveground plant species has often been shown to effect fungal communities (Amma et al. 2018, Kivin and Hawkes, 2016; Leff et al. 2018; Urbina et al, 2018) through: initial fungal population inoculum (Rigg et al. 2016), invasive
plants forging new fungal-plant associations (Gornish et al. 2016), plant community diversity and root structure diversity driving plant-fungi associations (Leff et al. 2018), regulating frequency of pathogenic soil fungi (Liang et al. 2016), and legacy effects of cover crops showing lasting fungal populations in following years (Detheridge et al. 2016). We expect our treatments of cover crops to show species specific guild differences from each other and from no cover crop treatments. Due to the ability of organic matter removal to influence fungal diversity, but not necessarily functional profiles (Wilhelm et al. 2017), we would expect a difference in general diversity which may be detectable in FUNGuild analysis.

Through the use of applicable diversity metrics (which obtain information on differences of populations and diversity between samples, paired with functional prediction tools (PiCRUST and FUNGuild, for bacteria and fungi respectively) we will be able to quantify the different ecological impacts of 5 cover crop treatments (no cover crop, oat, rye, OSR, and rye/OSR mix) and crop residue removal (in our rotation we examined wheat residue in 2015 and tomato in 2016) on the below-ground soil microbial community diversity and function.

3.2 Methods

3.2.1 Site description and Experimental design

The research site was located at Ridgetown Campus (University of Guelph, Ontario, Canada (42.44° N, 81.88° W; 201 m elev.) research station, and in 2007 and repeated in 2008 collaborators established a medium-term cover crop (CC) rotation in a diverse vegetable crop rotation (Belfry et al. 2017; Chahal and Van Eerd 2018). Microbial sampling occurred October 19, 2015 and November 4, 2016. The field trial was a randomized complete block design (four replicates) of five cover crop treatments with a split plot residue presence/absence (R+/-) treatment overlaid (Fig. 2.1) (note, R- refers to aboveground biomass, belowground biomass and
its microbial associations are still present). Plots measured 6 m x 8 m (post-split). Based on our first round of sequencing, no impact was detectable of residue presence/absence, thus the second round utilized only plots with residue present to reduce project cost of sequencing; meaning removal of residue was eliminated from our diversity analysis for between year comparison and in 2016 sequencing analysis.

Cover crop treatment was the main plot factor where no cover, oat, cereal rye, oilseed OSR and an OSR/rye mixture were the treatments in each block which were then split, and main crop residue was either removed or retained at harvest before planting of fall CCs (5 crop treatments split into residue +/-). Winter wheat was planted in fall of 2014, overwintered, and harvested in early August of 2015 when straw was removed. Total straw residue removal took place on the 20 designated plots (Fig. 2.1) and cover crops planted after light tillage to prepare the seed bed. Fall cover crops were planted Aug. 16, 2015, after which CC residues were incorporated in spring. Tomatoes were planted May 21, 2016 and harvested Sept. 8, 2016. In 2016 Fall CCs were planted Sept. 12, 2016. Fall Cover crops were sampled Oct. 19, 2015 and Nov. 4, 2016 for DNA sampling, as described in Chapter 2. Samples were sequenced winter 2016 and 2017 for Oct. 2015 and Nov. 2016 samples respectively.

3.2.2 High throughput sequencing

DNA samples stored in the -80°C freezer, as described in Chapter 2, and samples from October 2015 and November 2016 were selected for high throughput sequencing in order to identify microbial community differences under cover crops following a field crop growing season. Sequencing was performed on an Illumina MiSeq high throughput sequencer (Genome Quebec, McGill University, Montreal, Quebec) according to Genome Quebec’s standard protocols. Raw sequence data files were obtained for subsequent analysis. Amplicon libraries of
bacterial *16S* and fungal *ITS* were prepared from DNA samples obtained from all cover crop and residue plots in 2015, and R+ plots containing winter wheat straw in 2016. We found no significant diversity variation between residue presence/absence within plots in 2015, so in order to reduce project cost it was decided to only sequence the R+ plots in 2016 to determine the identities and relative proportions of bacterial and fungal phylotypes. Amplicon preparation was performed by Genome Quebec according to their standard protocols. For bacteria, amplicons were prepared using primers 341F and 805R (Herlemann et al. 2011), which target the V3-V4 region of the *16S* DNA gene, while fungal targeted the *ITS* region using the ITS1-F (forward) and ITS 2 (reverse) primers (Bellemain et al. 2010).

### 3.2.3 Bioinformatics

The bioinformatics pipeline, which was performed by Dr. E. Bent (University of Guelph, School of Environmental Sciences, Guelph, ON.), involved overlapping paired read ends which were assembled using pair end read merge (PEAR) software (Zhang et al. 2014). Primers and poor-quality sequences and sequence ends were removed using cutadapt software (Martin, 2011). For amplicons, USEARCH pipeline was used to cluster sequences into operational taxonomic units (OTUs) (Edgar, 2013). This involved chimera filtering, as well as generation of OTUs and OTU centroid sequences. The reads were then mapped to the centroid OTUs to generate OTU tables as well as taxonomic affiliations for each OTU based on its centroid sequence. Threshold for OTU clustering for *ITS* (fungi) and *16S* (bacteria) was 97% similarity. Taxonomic affiliations were generated using centroid sequences through UNITE (*ITS*) and RDP (*16S*) databases. Reads per OTU were standardized by total number of reads to allow comparison between samples. Transformed OTU table data was imported into PC-ORD software (MjM Software, Gleneden Beach, OR USA) for alpha diversity calculations (e.g. richness, evenness,
Shannon’s and Simpson’s diversity indices, and beta diversity (nonmetric multidimensional scaling (NMS) ordinations, as well as multi-response permutation procedure (MRPP) and blocked MRPPs comparisons of OTU profiles between plot treatments).

Student’s t-tests and ANOVA (Tables 3.1 and 3.2) were used to determine differences in sample means of Simpson’s diversity, Shannon’s diversity, richness and evenness between plots and years using SAS 9.1 software. NMS ordinations were graphed using Sigmaplot® 11 software (Systat Software Inc., 2008).

OTU tables were uploaded to PiCRUST and FUNGuild databases to obtain functional profiles of gene targets. Results were summarized into relevant functional processes (bacteria) and guilds (fungi). PiCRUST predicted functional gene profiles were transformed into ratios of associated functional genes under our cover crop species compared to the no cover crop control, as demonstrated by Hariharan et al. (2018). FUNGuild output was left as proportional abundance of OTUs in ecological guilds under individual crop species, as demonstrated by Nie et al. (2018). Graphs for PiCRUST and FUNGuild data were generated using GraphPad Prism Software (San Diego, CA)

3.3 Results

3.3.1 Soil microbial community compositions under fall cover crops

Bacterial and fungal composition were compared for cover crop species between Fall 2015 and Fall 2016. Trends in each year were very different, therefore they were considered independent of each other. ANOVA found no interactions between CC and R+/-. The bacterial community (2015), showed strong selection in the presence of rye, as seen in NMS plots (Fig. 3.1), in which bacterial community from rye and rye/OSR plots clustered together. Although
these plots are similar in composition, we see changes in alpha diversity through: richness, Shannon’s and Simpson’s diversity, and lower evenness between CC treatments (Table 3.1). Specifically, in 2015, oat was significantly lower in richness compared to rye and rye/OSR, and oat had higher species evenness, compared to 2015 rye and rye/OSR.

In 2016, fewer differences were detected between bacterial communities than the previous year; no significance was detected in: richness, evenness, Shannon’s and Simpson’s diversity between CC treatments (Table 3.1). For Shannon’s diversity, within 2015, oat was the only different species displaying lower diversity compared to rye and rye/OSR, and no differences were observed within 2016 crop treatments. Between years, 2016 rye, OSR and rye/OSR were different from 2015 oat and OSR; the exception being 2016 rye was similar to 2015 OSR. For Simpson’s diversity, within 2015, oat was significantly lower compared to no CC, rye and rye/OSR. Again, no differences were detected in diversity within any 2016 crop treatments. Between years, all treatments in 2016 were different from 2015 oat, with the addition that 2016 OSR was also different from 2015 OSR.

For the fungal community (Table 3.2), no differences within years were detected for species richness, but 2016 had significantly more richness in all treatments compared to 2015. For species evenness, no differences of crop treatments were apparent in any 2016 crop treatments. Within 2015 evenness, rye/OSR was significantly higher compared to rye and oat, and oat was significantly lower than OSR treatments. There were significant differences between annual crop treatments in 2015 and 2016 with 2016 showing significantly higher species evenness. Shannon’s diversity displayed no crop treatment differences within years, but between years held significant differences, with 2016 diversity being higher than 2015’s crop treatments.
3.3.2 Diversity of the microbial communities.

Nonmetric multidimensional (NMS) scaling ordinations for each community gene target exhibited differences in community compositions between crop species as well as sampling years (Fig 3.1 and 3.2). Blocked multi-response permutation procedure (MRPP) comparisons of community compositions showed several significant differences between crop species. At the \( p < 0.05 \) level, no significant differences are seen for bacteria for either 2015 or 2016 (Table 3.3). However, at the \( p < 0.10 \) level, 2015 showed differences in communities between rye/OSR and no CC, oat, and OSR. In 2016, significant differences in the composition of the bacterial communities were identified between rye/OSR and oat, as well as OSR and oat. In 2015, the fungal communities associated with Rye/OSR were significantly different from oat, and the communities associated with the no CC plots was significantly different from rye and OSR containing plots. In 2016, fungal communities associated with the no CC plots were different from the rye/OSR plots, and the rye plots, in addition there were differences between the rye/OSR and oat plots. From this we can see that the inclusion of OSR and rye have different impacts on microbial diversity compared to oat and significantly more than no CC.

Some distinct populations appeared through clustering (Fig. 3.1), but less distinct communities were observed under rye in 2016 compared to 2015. NMS assessment (Fig. 3.1) showed community clustering in the no CC, oat and rye/OSR plots. Statistical significance showed community differences between OSR and oat as well as rye/OSR and oat and no CC (Table 3.3). MRPP analysis showed that rye/OSR mixtures most strongly create distinct communities throughout years compared to no CC and oat plots.
3.3.3 PiCRUST predicted bacterial functions

When predicted bacterial functions of the microbial communities in cover crop plots were compared to no cover crop plots, ratios greater than 1 were considered to have higher functional capabilities, while ratios less than 1 were considered to have lower functional capabilities. The quality scores (NSTI) showing predictive accuracy of PiCRUST algorithm is presented in supplementary information (App. Table 1). Due to the poor-quality NSTI scores, we were unable to make fine-scale statistical comparisons between functional profiles under cover crops for our bacteria population. However, general conclusions within and between years are possible and presented in Fig. 3.3. In both years, plots containing OSR and Rye/OSR had higher functional abundance than control plots. In 2015, oat had lower functions than under no CC. OSR plots exhibited similar functional profiles to no CC. In 2016, oat and OSR caused similar functional profiles to the no CC control group.

3.3.4 FUNGuild: fungal ecological guild comparison of CC species

The fungal guilds in our field responded uniquely within and between sampling years (Fig. 3.4). Arbuscular mycorrhizae made up less of the population in 2016 than 2015, endophytes decreased in rye and rye/OSR in 2016 while no CC increased population proportion, fungal pathogens significantly decreased in 2016 in all crop treatments except OSR, plant pathogens increased in proportional abundance in 2016 (roughly double in all treatments except rye), and all saprotrophs decreased in proportional abundance in 2016 (most significantly in litter saprotrophs). For crop species effects, we observed that: rye-containing plots have the highest arbuscular mycorrhizae compared to other crop treatments, endophytes under oat and OSR were the most stable proportion compared to other crop species, fungal pathogens were highest under
rye containing plots while OSR maintained relatively low pathogen proportions, plant saprotrophs significantly increased in abundance between years in all treatments but the most pronounced was under rye/OSR, litter saprotrophs were initially highest in rye/OSR but almost non-existent in 2016, leaf saprotrophs were consistent in all treatments in 2015 except rye/OSR, both litter and leaf saprotrophs were essentially non-existent in the 2016 sampling under all treatments besides no CC.

In 2015, fungi showed minimal community similarity through cluster analysis (NMS) (Fig. 3.2), showing clustering under OSR only, with differences in community diversity (Table 3.4) between OSR and rye, as well as rye/OSR from no CC, OSR, and oat. We saw differences in evenness, oat significantly less than OSR and rye/OSR and Simpson’s diversity index only between oat and rye/OSR, with oat being less than rye/OSR, while no differences existed between richness and Shannon’s diversity for any crop species treatment (Table 3.2). In 2016, the fungal community showed species specific clustering in rye and rye/OSR plots (Fig. 3.2), with differences in community diversity detected between several crop treatments: communities under rye and oat were different from all other crop treatments except oat being similar to no CC, and rye/OSR was different from oat (Table 3.4). Diversity metrics, like bacteria, showed no differences between communities for: richness, evenness or Shannon’s and Simpson’s diversity indices (Table 3.2).
Table 3. Average richness, evenness, Shannon’s diversity and Simpson’s diversity of bacterial communities in 2015 and 2016 under fall cover crop treatments. Lower case letters following data in columns across years indicates significant differences (ANOVA, Tukey’s test, p < 0.05).

<table>
<thead>
<tr>
<th>Cover crop</th>
<th>Richness</th>
<th>Evenness</th>
<th>Shannon’s diversity</th>
<th>Simpson’s diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CC</td>
<td>3444.25 bcd</td>
<td>0.9935 ab</td>
<td>8.0885 bcd</td>
<td>0.9997 ab</td>
</tr>
<tr>
<td>Oat</td>
<td>3028.75 d</td>
<td>0.9945 a</td>
<td>7.9155 d</td>
<td>0.9996 c</td>
</tr>
<tr>
<td>OSR</td>
<td>3382.50 dc</td>
<td>0.9933 abc</td>
<td>8.0585 cd</td>
<td>0.9997 bc</td>
</tr>
<tr>
<td>Rye</td>
<td>4276.25 abc</td>
<td>0.9925 bcd</td>
<td>8.2763 abc</td>
<td>0.9997 ab</td>
</tr>
<tr>
<td>Rye/OSR</td>
<td>4125.00 abc</td>
<td>0.9923 bcd</td>
<td>8.2385 abc</td>
<td>0.9997 ab</td>
</tr>
<tr>
<td>2016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CC</td>
<td>4525.75 a</td>
<td>0.9913 d</td>
<td>8.3440 a</td>
<td>0.9997 ab</td>
</tr>
<tr>
<td>Oat</td>
<td>4447.50 a</td>
<td>0.9915 cd</td>
<td>8.3228 ab</td>
<td>0.9997 ab</td>
</tr>
<tr>
<td>OSR</td>
<td>4418.00 a</td>
<td>0.9913 d</td>
<td>8.3205 ab</td>
<td>0.9998 a</td>
</tr>
<tr>
<td>Rye</td>
<td>4394.00 ab</td>
<td>0.9910 d</td>
<td>8.3095 abc</td>
<td>0.9997 ab</td>
</tr>
<tr>
<td>Rye/OSR</td>
<td>4576.25 a</td>
<td>0.9913 d</td>
<td>8.3550 a</td>
<td>0.9997 ab</td>
</tr>
</tbody>
</table>
Table 3. 2 Average richness, evenness, Shannon’s diversity and Simpson’s diversity of fungal communities in 2015 and 2016 under fall cover crop treatments. Lower case letters following data in columns indicates significant differences (ANOVA, Tukey’s test, p < 0.05).

<table>
<thead>
<tr>
<th>Cover crop</th>
<th>Richness</th>
<th>Evenness</th>
<th>Shannon’s diversity</th>
<th>Simpson’s diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2015</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CC</td>
<td>420.25 a</td>
<td>0.9733 bcd</td>
<td>5.2903 b</td>
<td>0.9942 bc</td>
</tr>
<tr>
<td>Oat</td>
<td>347.00 a</td>
<td>0.9708 d</td>
<td>5.0880 b</td>
<td>0.9930 c</td>
</tr>
<tr>
<td>OSR</td>
<td>359.75 a</td>
<td>0.9783 bc</td>
<td>5.2980 b</td>
<td>0.9946 bc</td>
</tr>
<tr>
<td>Rye</td>
<td>388.00 a</td>
<td>0.9713 cd</td>
<td>5.1735 b</td>
<td>0.9936 bc</td>
</tr>
<tr>
<td>Rye/OSR</td>
<td>363.0 a</td>
<td>0.9788 b</td>
<td>5.3437 b</td>
<td>0.9948 b</td>
</tr>
<tr>
<td><strong>2016</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CC</td>
<td>232.50 b</td>
<td>0.9883 a</td>
<td>5.9343 a</td>
<td>0.9971 a</td>
</tr>
<tr>
<td>Oat</td>
<td>194.50 b</td>
<td>0.9897 a</td>
<td>5.7853 a</td>
<td>0.9968 a</td>
</tr>
<tr>
<td>OSR</td>
<td>227.00 b</td>
<td>0.9900 a</td>
<td>5.8105 a</td>
<td>0.9968 a</td>
</tr>
<tr>
<td>Rye</td>
<td>211.75 b</td>
<td>0.9872 a</td>
<td>5.8533 a</td>
<td>0.9969 a</td>
</tr>
<tr>
<td>Rye/OSR</td>
<td>237.75 b</td>
<td>0.9883 a</td>
<td>5.8163 a</td>
<td>0.9969 a</td>
</tr>
</tbody>
</table>
Table 3. Results of blocked multi-response permutation procedure (MRPP) analysis for bacterial communities (refer to fig. 3.1 for NMS ordinations). Comparisons were made between 2015 and 2016 annual sampling dates within all field crop treatments within each year. T=test statistic, more negative = more separation between communities and more positive = less separation between communities. Significance is denoted by bolding at p < 0.10.

<table>
<thead>
<tr>
<th>Crop Comparison</th>
<th>T</th>
<th>p</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CC vs rye/OSR</td>
<td>-1.577</td>
<td>0.060</td>
<td>-1.640</td>
<td>0.068</td>
</tr>
<tr>
<td>No CC vs OSR</td>
<td>-0.488</td>
<td>0.329</td>
<td>-0.617</td>
<td>0.278</td>
</tr>
<tr>
<td>No CC vs rye</td>
<td>-0.725</td>
<td>0.226</td>
<td>0.995</td>
<td>0.845</td>
</tr>
<tr>
<td>No CC vs oat</td>
<td>0.481</td>
<td>0.645</td>
<td>0.979</td>
<td>0.843</td>
</tr>
<tr>
<td>Rye/OSR vs OSR</td>
<td>-1.325</td>
<td>0.093</td>
<td>-0.946</td>
<td>0.160</td>
</tr>
<tr>
<td>Rye/OSR vs rye</td>
<td>-0.048</td>
<td>0.435</td>
<td>-0.628</td>
<td>0.221</td>
</tr>
<tr>
<td>Rye/OSR vs oat</td>
<td>-1.553</td>
<td>0.076</td>
<td>-1.706</td>
<td>0.061</td>
</tr>
<tr>
<td>OSR vs rye</td>
<td>-0.060</td>
<td>0.459</td>
<td>0.611</td>
<td>0.721</td>
</tr>
<tr>
<td>OSR vs oat</td>
<td>-0.286</td>
<td>0.325</td>
<td>-1.481</td>
<td>0.079</td>
</tr>
<tr>
<td>Rye vs oat</td>
<td>0.295</td>
<td>0.547</td>
<td>0.541</td>
<td>0.668</td>
</tr>
</tbody>
</table>
Table 3. 4 Results of blocked multi-response permutation procedure (MRPP) analysis for fungal communities (refer to fig. 3.2 for NMS ordinations). Comparisons were made between 2015 and 2016 annual sampling dates within all field crop treatments within each year. \( T = \) test statistic, more negative = more separation between communities. Significance is denoted by bolding at \( p < 0.10 \).

<table>
<thead>
<tr>
<th>Crop Comparison</th>
<th>2015 T</th>
<th>p</th>
<th>2016 T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CC vs rye/OSR</td>
<td>-1.542</td>
<td>0.067</td>
<td>-2.084</td>
<td>0.035</td>
</tr>
<tr>
<td>No CC vs OSR</td>
<td>-1.036</td>
<td>0.148</td>
<td>-0.736</td>
<td>0.238</td>
</tr>
<tr>
<td>No CC vs rye</td>
<td>0.597</td>
<td>0.706</td>
<td>-1.936</td>
<td>0.040</td>
</tr>
<tr>
<td>No CC vs oat</td>
<td>-0.157</td>
<td>0.465</td>
<td>-0.962</td>
<td>0.167</td>
</tr>
<tr>
<td>Rye/OSR vs OSR</td>
<td>-1.356</td>
<td>0.069</td>
<td>-0.897</td>
<td>0.172</td>
</tr>
<tr>
<td>Rye/OSR vs rye</td>
<td>-0.182</td>
<td>0.390</td>
<td>-1.593</td>
<td>0.068</td>
</tr>
<tr>
<td>Rye/OSR vs oat</td>
<td>-2.005</td>
<td>0.037</td>
<td>-2.112</td>
<td>0.037</td>
</tr>
<tr>
<td>OSR vs rye</td>
<td>-1.727</td>
<td>0.052</td>
<td>-1.646</td>
<td>0.064</td>
</tr>
<tr>
<td>OSR vs oat</td>
<td>-1.333</td>
<td>0.100</td>
<td>-1.643</td>
<td>0.055</td>
</tr>
<tr>
<td>Rye vs oat</td>
<td>0.342</td>
<td>0.590</td>
<td>-1.604</td>
<td>0.037</td>
</tr>
</tbody>
</table>
Figure 3. Nonmetric multidimensional scaling (NMS) ordinations for bacterial communities under each crop treatment and sampling year. Significant (p < 0.10) T value ranges for multi-response permutation procedure (MRPP) analysis contrasting cover crop plots and sampling year within the bacterial community are reported in Table 3.3.
Figure 3. Nonmetric multidimensional scaling (NMS) ordinations for fungal communities under each crop treatment and sampling year. Significant ($p < 0.10$) T value ranges for multi-response permutation procedure (MRPP) analysis contrasting cover crop plots and sampling year within the bacterial community are reported in Table 3.4.
Figure 3.5 Combined ratio graphs of fall cover crops in Oct. 2015 and Nov. 2016, depicting bacterial PICRUST predicted function associated genes (x-axis) (KEGG pathways) under different cover crop species compared to a no cover crop control (Cover crop spp. / no cover crop, y-axis).
Figure 3. 7 FUNGuild assigned guilds of identified fungal OTUs (probable and highly probable assignments) under cover crops in Oct. 2015 and Nov. 2016 for relevant ecological guilds. Proportional abundance of unique OTUs contained within samples (y-axis) averaged under cover crop species (x-axis).


3.4 Discussion

In this study, we collected soils from agroecosystems characterized by contrasting cover crops (CCs) and residue removal systems in a vegetable cropping environment to explore the relationships between soil microbial communities and agricultural management. Cover crop species introduce living roots into the soil during traditional fallow periods, providing habitat, nutrients (exudates and green manure), and increasing porosity.

3.4.1 Bacterial community diversity under fall cover crops

From all our cover crops, the plots containing rye showed the most significant differences. In both years, a statistical difference in MRPP was detected, showing distinct bacterial communities associated with the rye/OSR and the no CC control plots. In addition, bacterial communities in the rye/OSR plots were different from all other non-rye containing plots. Research by Nair and Ngouajio (2012) found genetic diversity (via Shannon-Wiener index) and functional diversity (via Biolog-EcoPlate) increased significantly under cover crops containing rye in an organic tomato farming system. In their study, it was found that rye and rye-vetch treatments acted similarly to cause increases in soil quality as measured by increased diversity. Crop yields can be used as a measure of overall soil quality (Gaudin et al. 2015). Other research at the field site found that cover crops did not negatively impact yield, with moderate impacts on soil mineralized nitrogen, where OSR had increasing effects compared to other CCs that were examined (Belfry et al. 2017). Further research showed CC impacts on soil organic carbon and several other quality indicators (Chahal and Van Eerd 2018), with rye and OSR having particularly beneficial impacts. This was attributed to the larger biomass produced by OSR in 2015 as well as the ability of rye to overwinter (App. Fig. 3) (Chahal and Van Eerd
2018) which was consistent with studies at our CC site (Belfry et al. 2017; O’Reilly et al. 2011, 2012). Additionally, tomato yields under CCs were found to be equal or higher than in no CC plots which is likely due to improvements in soil health, from CC residue incorporation into the soil, and microbial activity (Chahal and Van Eerd 2018). This is supported by what we saw in our study which found that in 2015, when rye was mixed with OSR, microbial diversity was increased compared to OSR and oat alone, but not from rye monoculture cover crops (Table 3.1). Functional prediction, using PiCRUST, indicated that in 2015 all metabolic profiles examined were higher in plots containing rye compared to the control plots, however, this trend was not as clear in 2016. Oat and OSR consistently showed no significant differences from no CC controls in our experiment for diversity or functional capacity of bacterial populations (Figure 3.3). Together, this research highlights that under some conditions, rye, as a fall cover crop in a vegetable cropping rotation has significant beneficial effects on bacterial diversity and may be further increased by adding OSR intercropped with rye. Differences seen between years are most likely attributable to seasonal impact of the previous main crop (winter wheat in 2015, and tomato in 2016), climatic differences between years (longer growing season in 2015 compared to 2016), as well as CC plant growth stage and development, which have significant effects on the structure and composition of microbial communities (Wang et al. 2018).

The seasonal impact of tomato on the bacterial population may also be a large contributor to the observed changes in the microbial community. In 2016, we saw lower ratios of bacterial functional profiles of our cover crops over the no CC plots. This could result from tomato’s ability to stimulate the bacterial community. This is particularly true in the no CC treatment, where the increase in bacterial diversity from tomato would be more pronounced leading to the lower observed functional profile ratios. Additionally, we should note that the 2015 samples
showed higher cover crop impacts in rye, which may be due to the ability of the 2015 winter wheat field crop to stimulate bacterial populations. It may be that the communities developed under wheat were more species specific, causing lower functional profile ratios under the cover crops most different from wheat, such as OSR and oat which had significantly less functionality compared to plots containing rye. Due to the similarity of wheat and rye, the rye containing plots were able to sustain the bacterial populations developed under the wheat while OSR and oat containing plots caused the bacterial functional profiles to mirror the no CC.

It is important to note that there were opportunistic weeds and volunteer wheat that were not planted in the no CC which provided significant ground cover and may have acted to sustain the bacterial populations in the no CC plots (App. Fig. 1 + 2). The lateness of planting CCs in 2016 compared to 2015 highlights the impact of CC timing, where the later 2016 cover crops developed significantly less biomass by the time of sampling. The large biomass difference between CC plots and no CC in 2015 (App. Fig. 2) indicates that there were likely CC driven impacts on the bacterial community. The biomass similarity between CC and no CC in 2016 (App. Fig. 1) may have allowed for the similarity of CC plots to the no CC plots. From this, we can recommend that CCs should be planted as close to field crop harvest as possible in order to allow maximum CC growth and ensure the most benefits.

Increased bacterial diversity decreases disease incidence rates and pathogen populations (Wang et al. 2018), increases community stability and functional resilience (Girvan et al. 2005), and nutrient cycling potential (DuPont et al. 2000). Partnered with the benefits of CCs in general (i.e. decrease soil erosion, compaction, pest and disease incidence, and increase soil moisture retention) the inclusion of rye in fall cover crops has a large benefit for soil bacterial communities, and soil microbial diversity. However, seasonal impacts of field crops must be
considered in management decisions, as timing of CCs and field crop species have significant impacts on the bacterial community.

3.4.2 Fungal community diversity under fall cover crops

Fungal communities from Rye/OSR cover crops were consistently different than no cover crop control plots (Table 3.4). Fungal communities were more responsive to cover crops and were unique to cover crop species planted in 2016. Ecological guild comparisons of our fungal communities revealed little about any consistent effects of cover crop species on ecological guilds over time within our experiment (Fig. 3.4). It was predicted that we would see a notable decrease of arbuscular mycorrhizae in OSR plots compared to others, as OSR have been identified as a non-host to AMF (White and Weil, 2010) and due to their potential to produce antifungal biofumigants as they decompose over winter (White and Weil, 2010). Contrary to what we predicted, AMF did not decrease under OSR over time. White (2009) also found that proportional abundance and colonization of AMF was not significantly decreased by OSR. Interestingly, we did observe consistently low amounts of fungal parasites in OSR, which supports the findings of White and Weil (2010) that susceptible fungal pathogens can be managed by the inclusion of OSR in a CC system, but the general fungal community will be largely unaffected. Additionally, Rye has been noted to be associated with increased amounts of AMF and fungal pathogens (White and Weil, 2010), which is supported by FUNGuild analysis in this study (Figure 3.4). While the use of Rye may be beneficial to plants to increase bacteria and AMF, it opens the door for increased fungal parasites which could negatively impact soil health and crop yields. The combination of rye and OSR showed similar benefits to the AMF fungi, while marginally decreasing fungal pathogens over time. Therefore, a diverse CC, with the
inclusion of both rye and OSR, will capture the most benefits to the functionality of the soil microbial populations

In general, there were climatic differences between years as well as different application timing of cover crop species (August planted in 2015 vs September planted in 2016; biomass comparisons App. Fig. 3), which led to sequencing sampling occurring at different life stages of our cover crops. As outlined by Wang et al. (2018), plant life stage has a very significant impact on the soil pant-microbe interactions, particularly in fungi, causing changes in abundance, diversity and community structure. As the plants become more developed, so too do their roots, and as they age and become closer to maturity, a different variety of nutrients are released from their roots causing stimulus of different parts of the fungal community as a greater diversity of exudates and metabolites are released allowing for the diversification of the fungal community who can specialize and capitalize on the diversity of metabolites. Thus, it makes sense that we see less diversity significance in our 2016 sampling, as the cover crops were much less mature than at sampling in 2015. We particularly see this influence in saprotrophs, where litter and leaf saprotrophs in all treatments in 2016 are virtually non-detectable (Figure 3.4), likely due to the lack of available litter and leaves for soil saprotrophs to metabolize. In 2016, the no CC shows minimal functional capacity, likely due to early die off of volunteer weed species, or low weed population. A greater amount of plant pathogens was observed in 2016 in all treatments (Fig 3.4). This is believed to be a result of early life stages of plants being more susceptible to pathogens (Wang et al. 2018), which are probably more easily handled by plants later in their growth cycle.
3.5 Conclusion

It was consistently found that the use of any CC species was more beneficial to our soil microbial abundance measurements than no CC. However, communities associated with CC following tomatoes compared to winter wheat showed lower functionality and diversity, suggesting that the main crop and CC growing season length significantly impacted both the microbial population abundance and diversity. These impacts may have been created by main crop effects on microbial communities or the time of harvest and differences in timing of CC planting. Overall, there were cover crop species-specific results, detected in community structure and functional profiling. The use of rye/OSR mixtures show the most promise of our tested CC species for aiding in: general diversity, bacterial functional diversity, increasing crop yields (Chahal and Van Eerd 2018) as well as decreasing fungal pathogens and promoting beneficial AMF.
4.0 General conclusion

In all cases, cover crops (CC’s) proved to increase microbial abundances compared to the no cover plots. Slight decreases in abundance and diversity in all microbial groups occurred when both CCs and R+ were used together and were found to have marginally less yields than the use of CCs alone (R-) (Chahal and Van Eerd 2018). The increase measured in fungal abundance, the decrease in archaeal abundance and the maintenance of bacterial abundance could be a result of climatic variables, main crop-microbe interactions (winter wheat vs tomato impacts), or biomass differences (resulting from different CC growing season lengths) between years. All these must be considered for determining the desired effect that fall CCs will have over time in agroecosystems. The inclusion of rye is of interest for increasing bacterial abundance and diversity while the use of OSR had similar effects for fungi. We did not see decreases in essential fungal guilds such as AMF as was hypothesised from the inclusion of OSR in the cover crop rotation. We did see a slight decrease over time in the abundance of fungal pathogens present in OSR containing plots, however more research needs to be conducted to clarify if this resulted from our cover crop selection or from seasonal impacts of the main crop. There were pronounced differences in our communities following the different main crops, where lower functionality was seen following field tomatoes compared to winter wheat. Following both crops, rye/OSR showed the highest functionality in bacterial and fungal communities. The lower functionality displayed in all microbial groups and treatments in 2016 was influenced by the significantly shorter CC growing season, as there was less time for functions to develop in response to CC species impacts, in addition to the main crop species impacts on the microbial community prior to CC planting.
LITERATURE CITED


APPENDICES

Appendix A: PiCRUST NSTI values

Table A1. Sample read quality NSTI values of all sequenced samples from 2015 and 2016. As detailed by PiCRUST a NSTI value > 0.20 is considered unreliable and not suited for quantitative cross sample comparisons.

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Appendix B: Plot pictures

Figure 1. Nov. 2016 cover crops at Ridgetown field site (Ridgetown, ON) A) no cover B) rye/radish C) radish D) rye E) oat
Figure 2. Oct. 2015 cover crops at Ridgetown field site (Ridgetown, ON) A) no cover B) rye/radish C) radish D) rye E) oat
Figure 3. Comparison of fall cover crops between annual sampling at Ridgetown field site (Ridgetown ON). A) Oct. 2015 B) Nov 2016 C) Nov. 2016 adjacent field of cover crops planted one month earlier than studied plots.
Figure 4. Field crop tomatoes planted in 2016 and sampled for seasonal influences on microbial abundance at Ridgetown field site (Ridgetown ON). Flags indicate crop portion removed for biomass and nutrient content analysis A) July 2016 B) Sept. 2016
Figure 5. Field crop tomatoes from 2016 at Ridgetown field site (Ridgetown ON). A) no cover B) rye/radish C) radish D) rye E) oat F) Soil sampling on a nine-point soil core plot transect.