**Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada**

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Title: Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada

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Abstract

Rhizosphere and root associated bacteria are key components of plant-microbiomes and influence crop production. In sustainable agriculture, it is important to investigate bacteria diversity in various plant species and how edaphic factors influence the bacterial microbiome. In this study, we used high-throughput sequencing to assess bacterial communities associated with the rhizosphere and root interior of canola, wheat, field pea, and lentil grown at four locations in Saskatchewan, Canada. Rhizosphere bacteria communities exhibited distinct profiles among crops and sampling locations. However, each crop associated with distinct root endophytic bacterial communities, suggesting that crop species may influence the selection of root bacterial microbiome. Proteobacteria, Actinobacteria and Bacteroidetes were the dominant phyla in the root interior, whereas Gemmatimonadetes, Firmicutes and Acidobacteria were prevalent in the rhizosphere soil. *Pseudomonas* and *Stenotrophomonas* were predominant in the rhizosphere and root interior, whereas *Acinetobacter*, *Arthrobacter*, *Rhizobium*, *Streptomyces*, *Variovorax* and *Xanthomonas* were dominant in the root interior of all crops. The relative abundance of specific bacterial groups in the rhizosphere, correlated with soil pH, silt and organic matter contents, however, there was no correlation between root endophytes and analyzed soil properties. These results suggest that the root microbiome may be modulated by plant factors rather than soil characteristics.

Key words: crop microbiome, root endophytic bacteria, rhizosphere bacteria, 16S rRNA high-throughput sequencing, soil properties
Introduction

Improving crop yield while optimizing the use of fertilizers, water and pesticides is a continuous challenge in agricultural production. In this context, interactions between crops and microbes are important for the improvement of plant health, nutrient uptake, disease control and stress resistance (Paul 2014). Most of the bacteria associated with crops originate from the bulk agricultural soil (Turner et al. 2013a), however, to exert beneficial effects on crops, they must be in a close relationship with the host plant (Vessey 2003). For example, the release of root exudates by the crops is an important source of substrates that can be available to a wide group of microorganisms in the rhizosphere (Zhang et al. 2017).

Microbial endophytes, are defined as microorganisms that colonize the plant interior during all or part of their life cycle without causing disease to the host (Wilson 1995). Recent studies demonstrate numerous benefits of root bacterial endophytes in crops. For example, endophytic asymbiotic diazotrophic bacteria fix atmospheric nitrogen in rice, sugarcane and canola (Elbeltagy et al. 2001; Boddey et al. 2003; Puri et al. 2016). Other authors reported that endophytic bacteria also can ameliorate abiotic stress conditions such as salinity stress on tomato and drought on maize (Ali et al. 2014; Sandhya et al. 2017). Endophytes also can control crop diseases such as wheat head blight, Verticillium wilts in olive and damping-off of tomato seedlings (Goudjal et al. 2014; Martínez-García et al. 2015; Herrera et al. 2016).

Crop type and soil characteristics may affect the composition of endophytic bacteria within the root (Sturz et al. 2000). Soil properties have a direct influence on microbial community structure by providing specific habitats when selecting distinct groups of microorganisms, or indirectly, by affecting the physiology of plant root (Garbeva et al. 2004). However, numerous
reports indicate that plant species and/or cultivar also are important factors for establishment of root microbiome (Germida et al. 1998; el Zahar Haichar et al. 2008; Ofek-Lalzar et al. 2014).

Saskatchewan agriculture is a great contributor to the Canadian food market, accounting for more than 40% of total field crop acreage in 2017 (Statistics Canada 2017). In the past, studies on crop-associated bacteria in Saskatchewan crops assessed the diversity of the rhizosphere and endophytic bacteria (Germida et al. 1998; Siciliano and Germida 1999; Dunfield and Germida 2001; Misko and Germida 2002). These studies concluded that a diverse group of bacteria associated with the rhizosphere and root interior of crops has the potential to improve crop yield and long term soil sustainability. However, it remains unclear how plant species and edaphic factors may influence bacteria communities colonizing commercial crops grown in distinct Chernozemic soils in Saskatchewan. The objective of this study was to determine the diversity of bacteria associated with rhizosphere and root interior of canola, wheat, pea and lentil grown in four agricultural soils in Saskatchewan using high-throughput sequencing.

Materials and methods

Sampling and processing

Canola (Brassica napus L.), wheat (Triticum aestivum L.), field pea (Pisum sativum L.) and lentil (Lens culinaris L.) were collected from farmers fields during the 2013 and 2014 growing seasons. Plants were harvested at the flowering stage from sites in Central Butte, Stewart Valley, Saskatoon, and Melfort in Saskatchewan. Information on GPS coordinates and crop cultivars sampled on each location are provided in Table 1. Mean precipitation and temperature recorded on the sampling locations during the growing season of 2013 and 2014 are reported in Table S1. Each crop was rotated within each location from 2013 and 2014. Four samples, consisting of 4 to 6 plants and adhering soil were taken from rows in each field by excavating at 15 cm depth (Figure
Plants and roots were collected 10 m apart in each row. Individual plant samples with attached soil (N=96) were kept in plastic bags, stored in ice coolers during transport and stored for a maximum of 24h at 4°C until processing at the laboratory.

**Analysis of soil physical and chemical properties**

Bulk soil was manually separated from each individual plant roots under aseptic conditions and sieved (<2mm). Four soil samples from plants collected at each field were pooled together (N=24) and stored at -20°C for physical and chemical characterization. Soil samples were sent to ALS Environmental Laboratory (Saskatoon, Saskatchewan) for basic soil nutrients (Table 2). Soil pH was measured in a 2:1 soil: water slurry. Soil organic matter (OM), was determined using the dry-ash method (McKeague 1978). Soil available nitrate was determined according to Laverty and Bollo-Kamara (1988). Available phosphorus and potassium in soil were determined using a modified Kelowna method (Qian et al. 1994). Available sulfate was measured by a calcium chloride extraction (McKeague 1978). The particle size was analyzed using the Mini-Pipet Method (Burt et al. 1993). Available nitrate, phosphorus, potassium and sulfate in soil were expressed in mg kg⁻¹ of soil, whereas particle size and organic matter content were expressed in %.

**Survey of rhizosphere and root endophytic bacterial communities**

A total of 96 samples from the rhizosphere and 96 samples from the root interior were obtained from the studied crops at four locations in Saskatchewan during 2013 and 2014. To collect the rhizosphere soil, plant roots (2 g) with adhering soil were placed in a 500 mL Erlenmeyer flask containing 200 mL of sterile PBS and placed on a rotary shaker (150 rpm) at 28°C for 25 min. Then, resulting soil slurry was transferred to 50 mL Falcon tubes and centrifuged (2000 × g for 5 minutes). The supernatant containing PBS buffer was discarded and the rhizosphere soil stored at -80°C for DNA extraction (Dunfield and Germida 2003). After removing rhizosphere soil, roots
were transferred into a 300 mL Erlenmeyer flask containing 100 mL NaClO (1.05% v·v⁻¹) in sterile PBS and placed on a rotary shaker (150 rpm) at 22°C for 15 min. Roots were rinsed 10 times with 100 mL sterile tap water and 0.1 mL of the final wash spread onto agar plates of 1/10 TSA to check for contamination. (Siciliano and Germida 1999). Root nodules from lentil and pea plants were removed after surface disinfection. Sterile roots were chopped aseptically into 2-3 mm and stored in sterile vials at -80°C for DNA extraction.

**Analysis of bacterial communities using 16S rRNA high-throughput sequencing**

Total genomic DNA was extracted from rhizosphere soil and surface sterilized roots using a soil and plant DNA extraction kit (MO BIO Laboratories, Inc.), respectively. DNA yield was quantified using Qubit R Fluorometric Quantitation (Invitrogen). DNA samples were submitted for high-throughput sequencing to the Génome Québec Innovation Centre, McGill University using Illumina technology. The PCR amplifications were conducted using the primers 520F (5’-AGCAGCCGCGGTAT-3’) and 799R2 (5’-CAGGGTATCTAATCCTGTT-3’) that amplifies the V4 region of the 16S rRNA gene (Edwards et al. 2007). Sample libraries were prepared according to the MiSeq reagent kit preparation guide (Illumina, San Diego, CA), and the sequencing protocol from Caporaso et al. (2010).

**Bioinformatics and statistical analyses**

Sequences derived from rhizosphere and endophytic root bacteria using high-throughput Illumina technology were analyzed using Mothur v.1.34.3 (Kozich et al. 2013). The standard operating procedure included the generation of contigs from the combination of forward and reverse reads and the removal of sequence errors and chimeras. Sequences from chloroplasts, archaea, eukaryotic organisms were also removed. Taxonomic classification was done with naive Bayesian classifier using SILVA database. Reads displaying at least 97% identity were clustered.
into operational taxonomic units (OTU). Relative abundance of a bacterial taxa in a sample was calculated as the percentage of sequence reads belonging to the bacterial taxa in relation to the total number of reads in a sample. Rarefaction curves values, Simpson’s reciprocal (1/D) (used as diversity estimator) and Chao 1 (used as species richness estimator) were generated using Mothur software at OTU cutoffs of 0.03 distance units using the number of observed OTUs. In order to obtain an equivalent sequencing depth for the estimation of the diversity indexes, each sample was rarefied to the size of the smallest library. Principal coordinate analysis (PCoA) based on Bray-Curtis distances was performed using QIIME (Quantitative Insights Into Microbial Ecology 1.9.1) (Caporaso et al. 2010) to explore differences in the bacterial community structure of the rhizosphere and root interior. The influence of plant compartment, growing season, sampling location and crop species on the bacterial communities was examined by permutational multivariate analysis of variance (PERMANOVA) of the OTU profiles using PCOrd software (McCune and Grace 2002). Heatmaps were conducted using the VEGAN package (version 2.0–7) in R version 2.15.2 (R Core team 2012). The soil properties, number of OTUs as well as richness and diversity indexes were subjected to analysis of variance (ANOVA) and Tukey post hoc test using SAS software version 9.6 (Copyright © 2002-2010 SAS Institute Inc. Cary, NC, USA.). Redundancy analysis (RDA) was performed in PCOrd software (McCune and Grace 2002) to examine the relationship among the rhizosphere bacterial communities and soil variables (pH, organic matter, texture and available N, P, S and K). Pearson correlation was conducted to determine the relationship between soil properties and bacterial genera abundance (SAS software version 9.6). The Bonferroni’s correction for multiple comparison was used in the correlation test. The sequence data can be accessed in NCBI under Genome Project ID 510213 (accession PRJNA510213).

Results
Community structure and diversity of bacterial communities associated with crops assessed by 16S rRNA high-throughput sequencing

Processing of 16S rRNA high-throughput Illumina sequencing generated 5,261,433 high-quality sequence reads. A total of 12,549 OTUs were detected, which corresponded to 11,932 OTUs and 3,491 OTUs in 96 rhizosphere and/or root samples, respectively (data not shown). Venn diagram representing OTU distribution in the rhizosphere and root interior of each crop, revealed that canola had the highest number of OTUs, followed by wheat, pea and lentil (Figure 2). The number of OTUs of rhizosphere bacteria in all crops was higher in relation to root endophytes. Venn diagram also revealed that number of OTUs that were detected only in the rhizosphere of wheat and canola was higher when compared to pea and lentil separately (Figure 2). Similarly, the number of OTUs detected only in the root interior of canola and wheat was higher when compared to pea and lentil (Figure 2). Additionally, some OTUs were shared between the rhizosphere and root interior. However, these OTUs represented a high percentage (97-99%) and (77-90%) of the sequence reads in the root interior and rhizosphere, respectively.

The diversity of bacterial communities associated with crops was evaluated using Chao 1 estimator and Simpson’s reciprocal (1/D) index (Table 3). Bacterial communities of the rhizosphere from the crops exhibited significant higher (P<0.001) diversity and species richness compared to the root interior. Rhizosphere bacteria associated with crops were significantly influenced by the interaction of soil and crops based on Chao1 (P=0.01) and 1/D index (P=0.004) (Table 4). In the rhizosphere, the highest number of OTUs and diversity was observed in wheat collected in Central Butte. However, the lowest number of OTUs was observed in pea collected at Stewart Valley, but the diversity index was the lowest in the same crop collected in Central Butte (Table 3). Growing season influenced the species richness (P<0.01) of the rhizosphere
communities but not the diversity. Species richness of root bacterial endophytes based on Chao1 was significantly influenced by crop species (P<0.001) and location (P=0.0004) whereas bacterial diversity was only significantly different (P<0.001) between crop species (Table 4). In the root interior, the diversity and number of OTUs were higher in canola, followed by wheat, pea and lentil (Table 3). Growing season influenced the diversity (P<0.05) of the bacteria communities associated with the root interior but not the species richness.

The community structure of bacterial OTUs, determined using PCoA, indicated distinct pattern in the rhizosphere and root endophytic bacterial communities associated with the crops, with the first two axes explaining 33% and 69% of the total variation in the rhizosphere and root interior, respectively (Figures 3 and 4). Permanova analysis confirmed that bacteria community structure differed between the rhizo-compartments (P≤0.001) (Table 5). Rhizosphere bacteria exhibited high variability in the OTU profiles among all crops and locations studied. As a result, no clustering was identified by PCoA in response to these factors (Figure 3). Bacterial communities associated with the root interior were clustered in 3 regions using PCoA, corresponding to canola, wheat, and a cluster containing pea and lentil communities (Figure 4). However, no clustering was detected between endophytic communities from different field locations. Permanova analysis also indicated that crop species influenced (P≤0.001) both rhizosphere and root endophytic bacteria communities in all the sampling locations (Table 5). Similarly, bacterial communities in both rhizo-compartments were influenced (P≤0.001) by sampling locations, except for root endophytes associated with lentil (Table 5). Growing season influenced (P≤0.01) the bacterial community structure in the rhizosphere, but not in the root interior of the crops (Table 5).
Phylotype classification of the OTUs resulted in 21 bacterial phyla, from which 20 and 17 phyla were present in the rhizosphere (RZ) and root interior (RI), respectively (Figures S1 and S2). Rhizosphere soil associated with the crops exhibited similar phyla profiles, characterized by a high abundance of Proteobacteria and Actinobacteria followed by Bacteroidetes, Gemmatimonadetes, Firmicutes and Acidobacteria (Figure S1). The remaining phyla represented less than 1% of the total OTUs detected in the rhizosphere. However, there were notable differences in the phyla profiles of bacteria colonizing the root interior of the four crops studied. For example, Proteobacteria was the predominant phylum in lentil and pea, followed by canola and wheat. Actinobacteria and Bacteroides were also detected in wheat and canola, but their relative abundance was very low in pea and lentil (<0.02%). Relative abundance of the remaining phyla inside the roots was low. Furthermore, the phylum Fusobacteria was only observed in the root interior of canola (Figure S2).

Within the root endophytic Proteobacteria, the genus *Rhizobium* was predominant in the two legume species lentil and pea, accounting for 91-99% of the total population. Interestingly, *Rhizobium* was also detected in the interior of wheat and canola roots, accounting for up to 7% of the total endophytic population (Figure S3). Within the rhizosphere, the abundance of *Rhizobium* accounted for up to 38% and 10% in pea and lentil, respectively, opposed to only 2% of the total population detected in wheat and canola. Since the proportion of *Rhizobium* was noticeably inconsistent among the four crops, the genus *Rhizobium* was dropped of the dataset for the analysis of bacterial genera, thus facilitating visualization of rhizosphere and root endophytes community profiles.
Hierarchical clusters based on Bray–Curtis distance of the 1.5% most abundant genera, indicated that the rhizosphere bacteria communities grouped into five clusters and appeared to be influenced by locations and crops species, but a definitive trend was unclear (Figure 5). For example, cluster A, which included canola samples collected at Stewart Valley, Saskatoon and Melfort in 2014 consisted mostly of Stenotrophomonas (14%), Acinetobacter (8%), Pseudomonas (3%) and unclassified genera of Enterobacteriaceae (32%). Cluster B, which included lentil and pea samples, as well as canola samples collected at Saskatoon in 2013 mainly consisted of Pseudomonas (13%), Arthrobacter (4%) as well as unclassified genera of Enterobacteriaceae (6%) and Comamonadaceae (7%). In cluster C, which included wheat samples, as well as canola samples collected at Central Butte, Stewart Valley and Melfort, a prevalence of Gemmatimonas (5%), Gaiella (5%) and unclassified genera of Comamonadaceae (4%) and Rhizobiales (3%) were detected (Figure 5).

Most abundant bacterial genera in the root interior (>0.5%) of all crops studied grouped into four clusters, which were mainly influenced by crop species (Figure 6). For example, cluster A, which consisted of wheat samples collected at Central Butte in 2013 was characterized by high abundance of Stenotrophomonas (45%), as well as Acinetobacter (5%) and Pseudomonas (5%). In contrast, in cluster B, which also included wheat samples collected in Central Butte, Saskatoon and/or Melfort, consisted mostly of Pseudomonas (13%), Stenotrophomonas (4%), Streptomyces (5%), Xanthomonas (5%) and unclassified genera of Enterobacteriaceae (9%). In addition, cluster C, which included only canola samples, exhibited high abundance of Pseudomonas (45%), Stenotrophomonas (7%), Acinetobacter (5%), Variovorax (3%) and unclassified genera of Enterobacteriaceae (4%). Finally, in samples collected from pea and lentil (cluster D), unclassified
Rhizobiales (32%), Rhizobiaceae (15%), as well as Pseudomonas (27%) and Stenotrophomonas (7%), Variovorax (3%) and unclassified Enterobacteriaceae (5%) were the dominant genera.

Influence of soil properties on the relative abundance of rhizosphere and root endophytic bacteria

Soil physical and chemical analysis revealed that soil pH, available nitrate, texture and organic matter varied according to locations (Table 2). The redundancy analysis results indicate that soil pH, organic matter and silt content explained 33.1% of the total variation in the bacterial communities of the rhizosphere (Figure 7). The samples from different locations were separated by the first component (RDA 1). From all sampling locations, the rhizosphere soil from Melfort exhibited the lowest pH and highest organic matter and silt contents and also exhibited a large abundance of Firmicutes, Bradyrhizobium and Gaiella (Figure 7). Correlations between soil properties with abundance of bacterial phyla and/or most abundant genera were examined, and only significant values are reported (Table 6). In the rhizosphere, abundance of the phylum Firmicutes was positively correlated with the organic matter content. The genera Bradyrhizobium and Gaiella correlated negatively with soil pH, but positively with the organic matter and silt content. No significant correlations were detected between diversity indexes (Chao1 and 1/D) and soil properties in the rhizosphere (data not shown). Conversely, within the root interior, abundance of bacterial genera (>0.5%) and diversity indexes were not significantly correlated with any soil physical characteristics or chemical parameters.

Discussion

In this study, bacterial communities associated with the rhizosphere and root interior of wheat, canola, pea and lentil were characterized using high-throughput sequencing. Results revealed that a high proportion of the OTUs were present in both the rhizosphere and the root interior, suggesting
that most of root endophytes originate from the rhizosphere. In addition, diversity (1/D) and species richness (Chao1) of the bacterial communities associated with the root interior of the crops were less diverse compared to the rhizosphere bacteria. These findings support the idea that endophytic bacterial communities are a subset of the rhizosphere microbiome (Germida et al. 1998; Bulgarelli et al. 2013; Edwards et al. 2015). However, in our study, some OTUs were found only in the root interior of the crops at flowering stage and not detected in the rhizosphere. This result suggests that these bacterial OTUs may have colonized the root interior from the rhizosphere at the vegetative stage of the crop growing season, however, at flowering these bacteria were only found in the root interior of the crops. Additional suggested pathways of bacterial colonization of the root interior may include the vertical transmission through the seeds to the root endosphere during the early growth of the crop (Frank et al. 2017).

Analysis of community structure, species richness and diversity indicated that root bacterial endophytes were mainly influenced by the host crop, whereas the rhizosphere bacterial communities varied among crop species and/or sampling locations. The lack of correlation between the abundance of bacterial endophyte genera and soil physical and chemical parameters suggests that root endophytes may be influenced by factors related to the host plant rather than soil characteristics. In fact, previous studies indicate that during the colonization process, crops may select specific groups of endophytic bacteria from the soil by actively changing the composition of roots exudates (Garbeva et al. 2004; Jones et al. 2009; Bulgarelli et al. 2013). Root exudates may act as chemo-attractants that can mediate the interaction between plant roots and bacteria (el Zahar Haichar et al. 2008). Additional factors affecting the selection of specific bacterial endophytes include differences in root morphology and/or the presence of wounds that may favor the penetration of bacteria into the host plant roots (Gaiero et al. 2013). Similarly, plant species
and/or cultivars, plant growth stage, and plant health also are known to influence the interactions that occur between host crop and bacterial endophytes (Garbeva et al. 2004). Furthermore, endophytic bacteria may exhibit colonization traits that allow their establishment within the tissues and adaptation to the root environment (Compant et al. 2010).

The phyla Proteobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Firmicutes and Acidobacteria were the dominant rhizosphere bacteria in the four crops studied. A similar phyla profile was previously reported by Turner et al. (2013b) and Donn et al. (2015) in studies of rhizosphere communities in wheat and pea. Similarly, in winter wheat, the rhizosphere bacterial communities were enriched with Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria and Gemmatimonadetes (Mahoney et al. 2017). Other cereal plants such as barley, wild oat and rice are known to exhibit high abundance of Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria (DeAngelis et al. 2009; Knief et al. 2012; Bulgarelli et al. 2015). When canola was grown in agricultural soils from Ottawa, Canada, (Monreal et al. 2017) rhizosphere of the plants were colonized by phyla similar to those observed in our study including the presence of Proteobacteria, Actinobacteria and Gemmatimonadetes. In contrast, winter *Brassica napus* was dominated by Proteobacteria, Actinobacteria and Bacteroidetes (Gkarmiri et al. 2017; Rathore et al. 2017). A high abundance of Proteobacteria, Actinobacteria, Bacteroidetes, and Acidobacteria was also detected in legumes, such as soybean and alfalfa (Xiao et al. 2017).

In the current study, we found that the root interior of the four crops studied was also enriched with Proteobacteria, Actinobacteria and Bacteroidetes. However, contrary to rhizosphere soil, roots exhibited a lower abundance of Gemmatimonadetes and Firmicutes, thus suggesting a crop selection for specific bacterial phyla during the root endophytic colonization. The higher abundance of Proteobacteria and Bacteroidetes in the rhizosphere and/or root endosphere,
compared to bulk soil, has been attributed to their fast-growing capacity and higher efficiency in metabolizing root exudates (Fierer et al. 2007; García-Salamanca et al. 2012; Peifer et al. 2013). These attributes may allow classifying these Proteobacteria and Bacteroidetes as r-strategists (Fierer et al. 2007; Peifer et al. 2013). In contrast, the phylum Actinobacteria are commonly classified as K-strategists due to their low growth rates and high persistency in soils, even under low nutrient availability (Van Elsas et al. 2006).

When analyzing the crops for bacterial communities, all four crops also exhibited distinct phyla profiles. That is, Proteobacteria dominated the root endophytic communities in lentil and pea (mainly represented by Rhizobium). In other legumes, such as red clover, a high dominance of Proteobacteria, i.e., 90% of the total bacterial profile, have been reported (Hartman et al. 2017), whereas in soybean and alfalfa, Proteobacteria and Actinobacteria have been reported as the most dominant phyla (Xiao et al. 2017). In contrast to pea and lentil, which were dominated mostly by Proteobacteria, in the current study, wheat and canola exhibited a high abundance of Proteobacteria, Actinobacteria and Bacteroidetes. Similarly, Yang et al. (2012), Ofek et al. (2014) and Rascovan et al. (2016) reported a high abundance Proteobacteria and Actinobacteria in the root interior of wheat. Other cereals, such as barley and rice exhibited high abundance in the root interior of Proteobacteria, Actinobacteria and Bacteroidetes (Sessitsch et al. 2012; Bulgarelli et al. 2015). Similarly, studies conducted by de Campos et al. (2012), Gkarmiri et al. (2017) and Rathore et al. (2017) demonstrated that a high abundance of Proteobacteria, Actinobacteria and Bacteroidetes, was detected in the root interior of winter Brassica napus and canola. These differences in bacteria community profiles among crops, indicate that individual plant species may be colonized by distinct bacteria consortia at a broad taxonomic level such as at the phylum level. Interestingly in the current study, the phylum Fusobacteria, represented by Fusobacterium, was
only detected in the root interior of the canola. Fusobacteria are human pathogens, obligate anaerobes and non-spore forming G- bacilli (Bennet and Eley 1993). The phylum Fusobacteria also has been found in the roots of winter *Brassica napus* and potato (Manter et al. 2010; Gkarmiri et al. 2017). The presence of *Fusobacterium* in the root interior, but its absence in the rhizosphere, suggest that *Fusobacterium* may have been horizontally transmitted to the roots from aboveground plant canola organs, and/or vertically transmitted from the seeds.

As indicated by high-throughput sequencing, *Pseudomonas* and *Stenotrophomonas* were highly abundant in the rhizosphere soil and root interior of canola, wheat, pea and lentil. These results suggest that *Pseudomonas* and *Stenotrophomonas* were widely distributed in all four soils studied, hence their high abundance in all the four studied crops. Other studies assessing culturable bacteria associated with canola roots also detected *Pseudomonas* as a common genus associated with plants collected in agricultural soils from Belgium, Brazil, Canada, China France and Scotland (Bertrand et al. 2001; Misko and Germida 2002; Farina et al. 2012; Croes et al. 2013; Rathore et al. 2017; Zhao et al. 2017). Studies on wheat root endophytes in Argentina, Canada and Israel, also detected *Pseudomonas* as the dominant genera (Germida and Siciliano 2001; Ofek et al. 2014; Rascovan et al. 2016). Other authors also reported *Pseudomonas* as a predominant genus in the rhizosphere of wheat and canola (Croes et al. 2013; Ofek et al. 2014; Donn et al. 2015).

Additionally, *Stenotrophomonas* also was reported in canola grown in agricultural soils in Brazil (de Campos et al. 2012). Similarly, *Pseudomonas* and *Stenotrophomonas* are reported as the most abundant genera in the root interior of legumes such as red clover (Hartman et al. 2017). Bacterial endophytes, such as *Pseudomonas* and *Stenotrophomonas* are often deemed as generalists and potentially produce beneficial effects to numerous plant species (Compant et al. 2005; Khan et al. 2012). For example, *Pseudomonas* spp. isolated from canola and wheat produced indole
compounds, antibiotics and siderophores, solubilized phosphorus and exhibited biocontrol activity against pathogenic fungi (Thomashow et al. 1990; Germida and Walley 1996; Alström 2001; Bertrand et al. 2001; Farina et al. 2012). _Stenotrophomonas maltophilia_ is reported to increase resistance against biotic and abiotic stress in wheat, whereas it has been demonstrated to stimulate plant growth in canola (Banerjee 1995; Singh and Jha 2017). Generalist bacterial endophytes are hypothesized to be horizontally transmitted since they can be found in a variety of plant species. Frank (2017) reported that generalist endophytes distribution is more correlated to environmental factors rather than to the host plant.

Although _Pseudomonas_ and _Stenotrophomonas_ were associated with all four crops studied, additional bacterial genera were predominant only in certain crops. For example, in canola, high-throughput sequencing detected high abundance of _Variovorax_. Similar studies have reported a high abundance of _Variovorax_ in the rhizosphere and root interior of canola plants (de Campos et al. 2012; Croes et al. 2013). In our study, high-throughput sequencing analysis of bacteria communities in wheat revealed a predominance of _Xanthomonas, Streptomyces_ and _Arthrobacter_. _Xanthomonas_ was also reported in several wheat cultivars grown in Saskatchewan (Germida and Siciliano 2001). Previous studies assessing culturable endophytic Actinobacteria in wheat roots grown in Australia also detected _Arthrobacter, Streptomyces_ and _Mycobacterium_ among the most abundant genera (Coombs and Franco 2003; Conn and Franco 2004). Similarly, Kumar et al. (2014) also have reported that an _Arthrobacter_ sp. was able to fix N\textsubscript{2}, solubilize phosphate and promote wheat growth in a growth chamber and field conditions. As expected, high-throughput sequencing confirmed that _Rhizobium_ was the most dominant genus in pea and lentil. Symbiosis between rhizobia and legumes have been extensively investigated because of their key role on the evolution and ecology of leguminous plants such as pea and lentil (Masson-Boivin et al. 2009;
Lindström et al. 2010). Interestingly in the current study, the genus *Rhizobium* was also detected in wheat and canola roots, which accounted for 2% of the total bacterial population. Endophytic rhizobia have been previously reported in wheat and canola (Lupwayi et al. 2004). Although there was no conclusive evidence that this association resulted in symbiotic nitrogen fixation, Sharma et al. (2005) identified a *Rhizobium* in the root interior of wheat plants, which produced indole acetic acid and increased seedling shoot and root length. Our results revealed that specific bacterial genera were predominant in each crop species, suggesting that specific interactions may occur between crop and bacterial endophytes during root colonization.

Despite the presence of distinct genera profiles in the root interior of each crop studied, the rhizosphere bacterial microbiome associated with canola, wheat, pea and lentil varied greatly among crops and sampling locations. These results suggest that soil properties may have influenced the diversity of rhizosphere bacteria in the crops. For example, abundance of *Firmicutes, Bradyrhizobium* and *Gaiella*, in the rhizosphere was significantly correlated with soil pH, silt and organic matter content across sampling locations. Previous studies by Garbeva et al. (2004) indicated that soil properties can influence bacterial communities composition, not only in the bulk soil, but also communities in the rhizosphere. Soils have the ability to provide specific habitats for distinct groups of microorganisms, or to affect the physiology of the plant root, which indirectly influences the rhizosphere microbiome (Garbeva et al. 2004). Soil texture is known to influence bacterial community structure mainly by modulating water content and movement within the soil matrix (Carson et al. 2010). Other authors have reported that soil pH is the major factor determining bacterial diversity and the phyla composition in different soils including agricultural fields in Canada, in soils from across North and South America, Great Britain, and in polar soil ecosystems (Fierer and Jackson 2006; Lauber et al. 2009; Griffiths et al. 2011; Li et al. 2012;
Siciliano et al. 2014). Lauber et al. (2009) suggested that soil pH influences bacterial communities directly by imposing physiological limitations for bacteria in the soil, which may modulate competition among species or alter the dominance of certain taxa. Alternatively, Lauber et al. (2009) reported that soil pH influences nutrient availability, salinity and organic carbon, thus regulating the physiology of the microbial community in the soil. In agreement, studies conducted by Ferguson et al. (2013), indicated that *Bradyrhizobium* spp. are generally tolerant to acidic conditions in soil, which may explain their higher abundance in locations with lower soil pH in the current study. Similarly, Albuquerque et al. (2011) reported that *Gaiella* have optimal pH for growth between 6.5 and 7.5, which suggests that the abundance of *Gaiella* in the rhizosphere also may be influenced by the soil pH levels. The genus *Gaiella* was previously reported as a prevalent bacterial group in the rhizosphere of canola and legumes (Monreal et al. 2017; Xiao et al. 2017).

In the current study, the higher abundance of Firmicutes in the rhizosphere of crops grown at Melfort, i.e., a location with high organic matter content, may be related with the rapid growth of this phylum on readily available carbon sources (Van Elsas et al. 2006). In addition, Firmicutes can survive in the soil for prolonged periods of time due to their capacity to form endospores (Van Elsas et al. 2006).

In summary, analysis of bacterial communities associated with canola, wheat, pea and lentil using high-throughput sequencing revealed that crop species were colonized by specific bacterial consortia within their roots. In contrast, rhizosphere bacteria communities greatly varied among crop species and agricultural locations in Saskatchewan. These results reflect the fact that endophytic bacteria establish closer associations than rhizosphere bacteria communities with host crops. Furthermore, soil properties such as pH, silt and organic matter content influenced bacterial populations in the rhizosphere of the four crops studied. In contrast, the abundance of root
endophytes was not correlated with any of the soil properties. Collectively these findings reveal
that plants from the same crop species crops grown on different agricultural soils will associate
with similar endophytic communities, suggesting that the root microbiome is modulated mainly
by plant factors rather than soil characteristics. Therefore, the bacteria × plant interactions
occurring in the root interior may have important implications for crop growth and for practical
applications in agricultural production.

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### Tables

**Table 1.** Site GPS coordinates, soil order and crop cultivars collected at Saskatchewan agricultural fields.

| Location             | Central Butte | Stewart Valley | Saskatoon | Melfort |
|----------------------|---------------|----------------|-----------|---------|
| GPS coordinates      | (50°43'N, 106°25'W) | (50°37'N, 107°39'W) | (52°10'N, 106°30'W) | (52°49'N, 104°36'W) |
| Soil order           | Brown Chernozem | Dark Brown Chernozem | Dark Brown Chernozem | Black Chernozem |
| Crop cultivars       |               |                |           |         |
| Canola               |               |                |           |         |
| 2013                 | Invigor L150  | Clearfield     | Invigor L130 | Canterra 1990 |
| 2014                 | Invigor L151  | Clearfield     | Invigor L131 | Canterra 1991 |
| Wheat                |               |                |           |         |
| 2013                 | Waskeda       | -              | CDC Utmost | Unity    |
| 2014                 | Waskeda       | -              | CDC Utmost | Unity    |
| Pea                  |               |                |           |         |
| 2013                 | CDC Meadow    | CDC Meadow     | CDC Meadow | CDC Meadow |
| 2014                 | CDC Meadow    | -              | CDC Meadow | CDC Meadow |
| Lentil               |               |                |           |         |
| 2013                 | CDC Maxim     | CDC Impress    | IBC-605   | -        |

(a) Following the Canadian System of Soil Classification (Soil Classification Working Group 1998).
Table 2. Physical and chemical properties of soil samples collected at Saskatchewan agricultural fields. Reported values were obtained from pooled bulk soil samples (N= 24).

| Location      | Crop | Year | pH  | sand | silt | clay | OM  | NO₃⁻ | SO₄²⁻ | PO₄³⁻ | K⁺  | Available (mg kg⁻¹ soil) |
|---------------|------|------|-----|------|------|------|-----|------|-------|-------|-----|-------------------------|
|               |      |      | (%) |      |      |      |     |      |       |       |     |                         |
| Central Butte | Canola | 2013 | 7.6 | 42   | 40   | 18   | 3.8 | 25.3 | 10.1  | 73.9  | 603 |                         |
|               |      | 2014 | 8.0 | 51   | 38   | 11   | 3.4 | 12.4 | 6.8   | 23.5  | 586 |                         |
|               | Wheat | 2013 | 8.1 | 68   | 22   | 9    | 2.1 | 3.0  | 6.9   | 39.0  | 367 |                         |
|               |      | 2014 | 7.6 | 65   | 25   | 10   | 3.1 | 5.5  | 6.6   | 28.6  | 584 |                         |
|               | Pea  | 2013 | 6.9 | 44   | 41   | 16   | 3.3 | 12.5 | 8.5   | 34.5  | 435 |                         |
|               |      | 2014 | 7.9 | 43   | 46   | 11   | 4.3 | 6.8  | 4.2   | 47.3  | 1640|                         |
|               | Lentil | 2013 | 6.9 | 46   | 38   | 17   | 3.4 | 14.2 | 14.2  | 34.9  | 373 |                         |
|               |      | Average | 7.6 | 51   | 36   | 13   | 3.3 | 11.4 | 8.2   | 40.2  | 655 |                         |
| Stewart Valley| Canola | 2013 | 7.5 | 12   | 35   | 54   | 4.7 | 5.0  | 9.5   | 30.1  | 525 |                         |
|               |      | 2014 | 7.4 | 9    | 35   | 56   | 3.1 | 6.3  | 13.0  | 24.1  | 748 |                         |
|               | Pea  | 2013 | 7.5 | 11   | 31   | 58   | 4.4 | 5.2  | 8.9   | 10.0  | 331 |                         |
|               |      | 2014 | 7.6 | 10   | 30   | 59   | 4.6 | 8.1  | 12.3  | 32.2  | 525 |                         |
|               | Lentil | 2013 | 7.5 | 11   | 33   | 57   | 4.2 | 6.2  | 10.9  | 24.1  | 532 |                         |
|               |      | Average | 7.5 | 11   | 33   | 57   | 4.2 | 6.2  | 10.9  | 24.1  | 532 |                         |
| Saskatoon     | Canola | 2013 | 7.8 | 15   | 43   | 43   | 4.5 | 19.5 | 13.8  | 26.0  | 389 |                         |
|               |      | 2014 | 6.9 | 17   | 44   | 39   | 5.6 | 22.0 | 3.8   | 48.5  | 660 |                         |
|               | Wheat | 2013 | 6.8 | 27   | 50   | 24   | 4.9 | 20.1 | 12.5  | 60.2  | 510 |                         |
|               |      | 2014 | 7.4 | 17   | 39   | 44   | 4.3 | 14.0 | 3.3   | 42.6  | 566 |                         |
|               | Pea  | 2013 | 7.7 | 12   | 41   | 48   | 5.0 | 23.4 | 15.8  | 45.9  | 519 |                         |
|               |      | 2014 | 7.2 | 23   | 46   | 31   | 5.2 | 24.6 | 4.4   | 33.5  | 505 |                         |
|               | Lentil | 2013 | 7.2 | 31   | 41   | 28   | 4.1 | 30.4 | 11.5  | 16.2  | 358 |                         |
|               |      | Average | 7.3 | 20   | 43   | 37   | 4.8 | 22.0 | 9.3   | 39.0  | 501 |                         |
| Melfort       | Canola | 2013 | 6.5 | 11   | 55   | 34   | 11.5 | 17.0 | 11.4  | 54.5  | 425 |                         |
|               |      | 2014 | 6.5 | 9    | 61   | 30   | 11.8 | 17.9 | 5.2   | 79.7  | 838 |                         |
|               | Wheat | 2013 | 6.5 | 11   | 59   | 30   | 10.0 | 44.0 | 11.4  | 20.1  | 384 |                         |
|               |      | 2014 | 6.1 | 15   | 56   | 30   | 14.6 | 15.9 | 7.1   | 10.7  | 624 |                         |
|               | Pea  | 2013 | 6.2 | 12   | 54   | 34   | 10.4 | 25.1 | 7.6   | 30.5  | 476 |                         |
|               |      | 2014 | 6.3 | 7    | 72   | 21   | 11.4 | 14.5 | 4.3   | 32.1  | 303 |                         |
|               | Average | 6.3 | 11 | 60 | 30 | 11.6 | 22.4 | 7.8 | 37.9 | 508 |     |                         |

Note: Different letters indicate significant differences between sampling locations at α=0.05 using Tukey’s post hoc test. Soil sulphate, phosphate and potassium were not significant.
Table 3. Richness (Chao 1) and diversity (1/D) of rhizosphere (N=96) and root endophytic bacteria (N=96) in canola, wheat, pea and lentil at flowering, grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan.

| Crop  | Location | Chao1 | 1/D  | Chao1 | 1/D  |
|-------|----------|-------|------|-------|------|
|       |          |       | Chao1 | 1/D   | Chao1 | 1/D  |
| Canola| CB       | 2084  | ab   | 97.1  | bc   | 350  | n.s. | 5.9  | n.s. |
|       | SV       | 2105  | ab   | 72.6  | cd   | 293  | n.s. | 6.8  | n.s. |
|       | SN       | 1998  | ab   | 33.5  | ef   | 174  | n.s. | 2.7  | n.s. |
|       | MF       | 1561  | b    | 54.0  | de   | 332  | n.s. | 4.2  | n.s. |
|       | Average  | 1937  | AB   | 64.3  | B    | 288  | A    | 4.9  | A    |
| Wheat | CB       | 2673  | a    | 140.4 | a    | 413  | n.s. | 5.3  | n.s. |
|       | SN       | 2201  | ab   | 113.9 | ab   | 277  | n.s. | 3.4  | n.s. |
|       | MF       | 2098  | ab   | 111.7 | ab   | 371  | n.s. | 5.0  | n.s. |
|       | Average  | 2324  | A    | 122.0 | A    | 354  | A    | 4.6  | A    |
| Pea   | CB       | 1742  | b    | 6.8   | f    | 206  | n.s. | 1.1  | n.s. |
|       | SV       | 1422  | b    | 9.6   | f    | 135  | n.s. | 1.0  | n.s. |
|       | SN       | 2236  | ab   | 30.8  | ef   | 118  | n.s. | 1.0  | n.s. |
|       | MF       | 1669  | b    | 55.5  | de   | 147  | n.s. | 1.0  | n.s. |
|       | Average  | 1767  | B    | 25.7  | C    | 151  | B    | 1.0  | B    |
| Lentil| CB       | 1548  | b    | 29.6  | ef   | 135  | n.s. | 1.0  | n.s. |
|       | SV       | 1789  | b    | 33.6  | def  | 118  | n.s. | 1.1  | n.s. |
|       | SN       | 1929  | ab   | 25.9  | ef   | 112  | n.s. | 1.0  | n.s. |
|       | Average  | 1755  | B    | 29.7  | C    | 122  | B    | 1.0  | B    |

**Note:** Different letters indicate significant differences at α=0.05 using Tukey’s post hoc test. n.s., not significant. Small letters indicate difference between sampling locations, whereas capitalized letters indicate differences between individual crop species.
Table 4. ANOVA of richness (Chao 1) and diversity (1/D) of rhizosphere and root endophytic bacteria in canola, wheat, pea and lentil at flowering, grown in Saskatchewan agricultural fields.

| Source of variation | Chao 1          | 1/D          |
|---------------------|-----------------|--------------|
|                     | dF  | F      | P    | F      | P    |
| Rhizo-compartment   | 1   | 2685.87 | ***  | 459.21 | ***  |
| Rhizosphere         |     |        |      |        |      |
| Root interior       |     |        |      |        |      |
| Growing season      | 1   | 10.15  | **   | 0.03   | n.s. |
| Crop                | 3   | 13.43  | ***  | 7.16   | ***  |
| Location            | 3   | 8.50   | ***  | 58.59  | ***  |
| Crop*Location       | 7   | 3.03   | **   | 2.10   | n.s. |

**Note:** *, **, ***; significant at P ≤ 0.05, 0.01, 0.001, respectively. n.s., not significant.
Table 5. Effect of rhizo-compartment, growing season, crop species and sampling location on bacterial OTU community compositions assessed with permutational multivariate analysis of variance (PERMANOVA). The influence of crop species was determined at each location, whereas the influence of sampling location was determined for each crop species.

| Source of variation | Rhizo-compartment | Growing season | Crop | Location |
|---------------------|-------------------|----------------|-----------------|----------|
|                     | dF    | F    | P    | dF    | F    | P    | dF    | F    | P    | dF    | F    | P    |
| Rhizo-compartment   | 1     | 65.83 | *** |       |       |       |       |       |       |       |       |       |
| Rhizosphere         |       |       |      |       |       |       |       |       |       |       |       |       |
| Root Interior       |       |       |      |       |       |       |       |       |       |       |       |       |
| Growing season      | 1     | 2.36  | **   | 1.18  | n.s. |       |       |       |       |       |       |       |
| Crop                |       |       |      |       |       |       |       |       |       |       |       |       |
| Central Butte       | 6     | 11.91 | ***  | 10.85 | ***  |       |       |       |       |       |       |       |
| Stewart Valley      | 3     | 2.99  | ***  | 9.82  | ***  |       |       |       |       |       |       |       |
| Saskatoon           | 6     | 3.14  | ***  | 12.75 | ***  |       |       |       |       |       |       |       |
| Melfort             | 5     | 4.13  | ***  | 11.78 | ***  |       |       |       |       |       |       |       |
| Location            |       |       |      |       |       |       |       |       |       |       |       |       |
| Canola              | 7     | 3.79  | ***  | 2.94  | ***  |       |       |       |       |       |       |       |
| Wheat               | 5     | 4.99  | ***  | 2.22  | ***  |       |       |       |       |       |       |       |
| Pea                 | 6     | 4.48  | ***  | 2.72  | ***  |       |       |       |       |       |       |       |
| Lentil              | 2     | 2.3   | ***  | 1.21  | n.s. |       |       |       |       |       |       |       |

Note: *, **, *** significant at P ≤ 0.05, 0.01, 0.001, respectively. n.s., not significant.
Table 6. Pearson correlation coefficients between soil properties (pH, organic matter and silt content) and relative abundance of rhizosphere bacteria associated with canola, wheat, pea and lentil at flowering, grown in agricultural soils in Saskatchewan. The Bonferroni’s correction for multiple comparison was used in the correlation test.

| Classification | pH    | OM   | silt  |
|----------------|-------|------|-------|
| Firmicutes     | -0.37 | n.s. | 0.57  **| 0.45  n.s. |
| *Bradyrhizobium* | -0.68 *** | 0.69 *** | 0.69 *** |
| Gaiella        | -0.48  * | 0.79 *** | 0.66 *** |

Note: *, **, *** significant at p ≤ 0.05, 0.01, 0.001, respectively. n.s., not significant.
Figure captions:

**Fig. 1.** Location of agricultural field sites sampled in Saskatchewan during 2013 and 2014 (A), sampling points (B) and sampling procedure (C). Map Source (Malbarry 2013).

**Fig. 2.** Venn diagram representing bacterial OTUs associated with the rhizosphere (RZ) and root interior (EN) of canola, wheat, pea and lentil at flowering, grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan. Percentage of read counts, indicated within parenthesis, designates the proportion of sequence reads associated with the bacterial OTUs that were detected in both rhizo-compartments, in relation to the total number of sequence reads in the rhizosphere (RZs) and/or root interior (ENs). Different letters indicate significant differences between crop species at $\alpha=0.05$ using Tukey’s post hoc test.

**Fig. 3.** Principal coordinate analysis of rhizosphere bacterial communities based on Bray–Curtis dissimilarity between crops and locations (N=96).

**Fig. 4.** Principal coordinate analysis of root endophytic bacterial communities based on Bray–Curtis dissimilarity between crops and locations (N=96).

**Fig. 5.** Hierarchical clustering (Bray–Curtis) of bacterial genera (abundant >1.5%) associated with the rhizosphere of canola, wheat, pea and lentil at flowering, grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan (N=96).

**Fig. 6.** Hierarchical clustering (Bray–Curtis) of bacterial endophyte genera (abundant>0.5%) associated with the roots of canola, wheat, pea and lentil at flowering, grown at Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan (N=96).

**Fig. 7.** Redundancy analysis (RDA) based on the relative abundance of bacterial genera and phyla and soil properties (pH, organic matter, texture and available N, P, S and K) of rhizosphere
samples associated with crops at flowering, grown in Central Butte, Stewart Valley, Saskatoon and Melfort, Saskatchewan (N=96).

**Fig. S1.** Relative abundance of bacterial phyla from the rhizosphere of canola, wheat, pea and lentil at flowering, grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan (N=96).

**Fig. S2.** Relative abundance of bacterial endophyte phyla from the roots of canola, wheat pea and lentil at flowering, collected at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan (N=96).

**Fig. S3.** Relative abundance of *Rhizobium* in the rhizosphere and root interior of canola, wheat, pea and lentil at flowering, grown at Central Butte (CB), Stewart Valley (SV), Saskatoon (SN) and Melfort (MF), Saskatchewan (N=96).
Melfort
Saskatoon
Central Butte
Stewart Valley

Excavating 15 cm depth (4-6 plants)

182x119mm (300 x 300 DPI)
canola – 9073\textsuperscript{a} OTUs

wheat – 8748\textsuperscript{b} OTUs

pea – 7617\textsuperscript{b} OTUs

lentil – 6922\textsuperscript{b} OTUs

182x49mm (300 x 300 DPI)
