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Bacterial Microbiomes Associated with the Rhizosphere, Root Interior, and Aboveground Plant Organs of Wheat and Canola at Different Growth Stages

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ABSTRACT

Beneficial bacteria associated with agricultural crops may potentially increase crop productivity and health. However, during various plant developmental processes, shifts in the diversity and function of bacterial communities often occur. This study investigated the diversity of bacterial communities associated with the rhizosphere, roots, and aboveground plant organs of wheat and canola at stem elongation, flowering, and ripening stages. The growth-chamber experiment consisted of wheat and canola grown in Orthic Brown Chernozem Calcic Kastanozem and Orthic Black Calcic Chernozem soils from agricultural fields in Saskatchewan, Canada. Rhizosphere bacterial communities of wheat and canola were mainly influenced by soil characteristics, whereas a specific root endophytic community was associated with each crop species. These results suggest that each crop may select distinct root bacterial endophytes from the rhizosphere. Bacteria associated with aboveground plant organs exhibited high variability among crop species and soils. The most abundant bacterial genera associated with the

rhizosphere of the crops included *Gemmatimonas*, *Solirubrobacter*, and *Nocardioides*, as well as unclassified genera of families *Commamonadaceae*, *Chitinophagaceae*, and *Sphingomonadaceae*. Other genera (e.g., *Stenotrophomonas*, *Streptomyces*, and *Variovorax*) were predominant in wheat roots, whereas species of *Lentzea* and *Pantoea* were the most abundant root endophytes detected in canola. Bacterial communities associated with aboveground organs consisted mostly of species of *Corynebacterium* and *Pseudomonas*, and unclassified members of the family *Enterobacteriaceae*. This study also revealed that plant growth stages can modulate the diversity of rhizosphere and endophytic bacteria. The influence of plant growth stages on the bacterial microbiome associated with wheat and canola was crop and organ specific.

Keywords: 16S rRNA high-throughput sequencing, crop microbiome, endophytic bacteria, plant growth stages, rhizosphere bacteria

The microbiome associated with plants includes all microorganisms inhabiting external surfaces and internal plant organs. Due to their relevance to plant growth and development, the microbiome is sometimes considered the plant's second genome (Turner et al. 2013). Among these microorganisms, bacteria are important in promoting plant growth and health, or improving the sustainability of crop production (Berg et al. 2014). For example, crop-associated bacteria have the potential to stimulate plant growth by several mechanisms, including (i) production of plant growth regulators, (ii) amelioration of abiotic stress, (iii) biological nitrogen fixation, and

(iv) phosphorus mobilization (Ali et al. 2014; Compant et al. 2010). In addition, bacteria may share habitats similar to those of phytopathogens, thus making it possible to limit the spread of potential pathogens (Reinhold-Hurek and Hurek 2011).

Bacterial communities colonize plant tissues mainly using two pathways, including horizontally (i.e., entering the plant tissues from the environment) or vertically (i.e., transmitted from the parent plant to its offspring through seed) (Bright and Bulgheresi 2010). Bacterial colonization of crops begins at seed germination, in which seed exudates attract bacteria from soil into the spermosphere (Nelson 2004). Additionally, during further crop growth, plant root exudates also are important in shaping the microbiome in the rhizosphere, the rhizoplane, and, subsequently, the root interior. After entering roots, bacterial endophytes may translocate into the xylem vessels and eventually colonize the aboveground plant tissues (Kandel et al. 2017). Bacteria may also colonize aboveground surfaces and penetrate plant organs such as stem, leaves, flowers, and fruit. Potential sources of bacteria for aboveground plant colonization include the atmosphere, rain, or pollinators (Frank et al. 2017). In addition,

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vertical transmission of bacteria through seed has been described as the main pathway for the colonization of most obligate endophytes, which depend strictly on the host plant for their growth and survival (Hardoim et al. 2008). Several studies have reported the diversity of bacteria colonizing aboveground tissues such as stems, leaves, and seed (Coombs and Franco 2003; Hardoim et al. 2012; Sessitsch et al. 2002).

Previous studies by el Zahar Haichar et al. (2008), Germida et al. (1998), Lay et al. (2018), and Ofek-Lalzar et al. (2014) reported that bacterial communities associated with crops are strongly influenced by plant species or cultivar. For example, the selection of rhizosphere microbiota from the bulk soil is modulated by the release of root exudates by the crops (Bulgarelli et al. 2013). Bacterial penetration into the root interior is also influenced by the morphology of the root system because bacterial colonization occurs mainly by accessing root tips or epidermis discontinuities (e.g., lateral root emergence sites or wounds) (Gaiero et al. 2013). Bacterial multiplication within internal plant tissues also involves the recognition of plant signals that may induce cellular processes necessary for the establishment of endophytes inside the host crop (Hardoim et al. 2008). Aerial vegetative organs are also colonized by bacterial endophytes adapted to specific plant niches in the aboveground plant organs (Hallmann 2001). Among plant characteristics, the physiological processes occurring during plant growth may influence the bacterial microbiome of crops. Previous studies report that rhizosphere bacterial communities associated with *Arabidopsis*, *Medicago*, maize, pea, wheat, canola, and sugar beet change during plant developmental stages (Baudoin et al. 2002; Dunfield and Germida 2003; Houlden et al. 2008; Micallef et al. 2009; Mougel et al. 2006). However, studies analyzing the influence of plant development stages on the bacterial microbiome associated with both below- and aboveground plant compartments are scarce.

Canola and wheat production are important agronomical activities and food exports in Canada, accounting for 19 and 26 million metric tons, respectively, in 2020 (Statistics Canada 2021). Despite their relevance in Canadian agriculture, research about the microbiomes of canola and wheat is still scarce. Our recent investigation (Cordero et al. 2020) indicated that field-grown canola, wheat, field pea, and lentil collected at the flowering stage supported distinct root endophytic bacterial communities, suggesting that crop species strongly influence the selection of root bacterial microbiomes. Furthermore, we found that rhizosphere communities exhibited distinct profiles among crops and sampling locations. These results raised questions about how soil characteristics, plant growth stage, and plant compartments influenced bacterial microbiomes. In the present study, we assessed the diversity of bacteria colonizing the rhizosphere, roots, shoots, and seed of wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) plants at stem elongation, flowering, and ripening stages and grown on soils collected at Central Butte and Melfort, Saskatchewan, Canada. Our hypothesis was that plant compartment, plant growth stages, and soil characteristics affect the bacterial microbiomes of wheat and canola. The study was performed under controlled plant growth conditions, excluding effects of environmental conditions and agronomical practices existing between sampling locations.

MATERIALS AND METHODS

Experimental design. Orthic Brown Chernozem Calcic Kastanozem (hereafter referred to as brown) and Orthic Black Calcic Chernozem (hereafter referred to as black) soils were collected from wheat fields at Central Butte (50°43'N, 106°25'W) and Melfort (52°49'N, 104°36'W), Saskatchewan, Canada, respectively, which differed mainly in soil texture, organic matter content, and pH. These sites were adjacent to our previous field study (Cordero et al. 2020).

Bulk soil was air dried and sieved (<2 mm) and properties were determined by ALS Environmental Laboratory (Saskatoon, Saskatchewan, Canada). The brown soil was characterized as a sandy loam with pH = 7.6; organic matter (%) = 3.1; and available nutrients N-NO₃⁻ = 5.5, S-SO₄²⁻ = 6.6, P-PO₄³⁻ = 28.6, and K⁺ = 584 mg·g⁻¹ of soil; whereas the black soil was a silty clay loam with pH = 6.1; organic matter (%) = 14.6; and available nutrients N-NO₃⁻ = 15.9, S-SO₄²⁻ = 7.1, P-PO₄³⁻ = 10.7 and K⁺ = 624 mg·g⁻¹ of soil. Ten wheat (CDC Waskada) or canola (Invigor L150) seeds were seeded in 2.5-liter pots (*n* = 4) using 1.5 kg of soil, allowed to germinate, thinned to two plants, and grown in a growth chamber with a cycle of 16 h at 25°C (day, 1,500 μmol·m⁻²) and 8 h at 15°C (night). For each crop and soil, three individual sets of pots were prepared to be harvested independently at stem elongation, flowering, and ripening stages following a universal plant growth stage scale (Lancashire et al. 1991). Pots were arranged in a fully randomized block design with four replicates for a total of 48 pots. Plants were watered with autoclaved distilled water at 50% moisture-holding capacity throughout the experiment and rotated daily. To ensure an adequate fertilization regime, 1:5 Hoagland's nutrient solution was added to each pots on alternate weeks until plants reached the flowering stage.

Sampling of rhizosphere soil, root, stem, leaf, and seed. In total, 24 wheat and 24 canola plants were harvested from black and brown soils at stem elongation, flowering, and ripening. At each sampling time, a set of canola and wheat plants grown at brown and black soils were harvested (*n* = 16). After collecting plants, the bulk soil was manually removed from roots and the aboveground plant organs were separated from the roots using aseptic techniques. Plant roots (two roots/pot) with adhering soil were placed in a 500-ml Erlenmeyer flask containing 200 ml of sterile phosphate-buffered saline (PBS) and placed on a rotary shaker (150 rpm) at 22°C for 25 min. The resulting soil slurry was transferred to 50-ml Falcon tubes and centrifuged (2,000 × *g* for 5 min). The supernatant containing PBS buffer was discarded and the rhizosphere soil was stored at -80°C for DNA extraction (Dunfield and Germida 2003). Roots, stems, or leaves were washed with sterile tap water to eliminate soil and particles from the plant surface. Next, washed plant material (from a single plant) was surface disinfested using a 300-ml Erlenmeyer flask containing 100 ml NaClO (1.05% vol vol⁻¹) in autoclaved PBS. The flasks were placed on a rotary shaker (150 rpm) at 22°C for 15 min (Siciliano and Germida 1999). Next, the surface-disinfested plant material was cut into 0.5-cm portions using a sterile scalpel and stored at -80°C until processed for molecular analyses. Furthermore, surface disinfection of collected seed was accomplished by submerging the seed in 65% (vol vol⁻¹) ethanol for 3 min, followed by submersion in NaClO (1.2% vol vol⁻¹) for 5 min (Vincent 1970). After the disinfection process, plant material was rinsed 10 times in sterile tap water and efficacy of surface sterilization was checked by spreading 0.1 ml of the last washes onto 1/10th strength tryptone soy solidified with 1.5% agar (Siciliano and Germida 1999). Rhizosphere, root, and stem were collected at each growth stage. Leaves were collected only at stem elongation and flowering, whereas seed were collected only at ripening. In total, 96 wheat and 96 canola samples were obtained from the rhizosphere, root, stem, leaf, and seed.

Analysis of bacterial communities using 16S ribosomal RNA high-throughput sequencing. Total genomic DNA was extracted from rhizosphere soil and plant material using soil and plant DNA extraction kits, respectively (MO BIO Laboratories, Inc.). The DNA extractions were conducted following the manufacturer's protocols. The DNA yield was quantified using a Qubit DNA HS Assay Kit (Thermo Fisher Scientific). DNA visualization was performed after running DNA electrophoresis in 1% agarose gels stained with the SYBR safe DNA gel stain (Invitrogen) by comparison with a DNA mass ladder (Invitrogen) using a Bio-Rad Gel Doc XR System

(Bio-Rad Laboratories, Mississauga, ON, Canada). The DNA samples were submitted for high-throughput sequencing to the Génome Québec Innovation Centre, McGill University, using Illumina MiSeq PE250 technology. The PCR was conducted using the primers 520F (5'-AGCAGCCGCGGTAAT-3') and 799R2 (5'-CAGGGTATCTAATCCTGTT-3') that amplified the V4 region of the 16S ribosomal RNA (rRNA) gene (Edwards et al. 2007). Sample libraries were prepared according to the MiSeq reagent kit preparation guide (Illumina, San Diego, CA, U.S.A.), and the sequencing protocol from Caporaso et al. (2010).

Bioinformatics and statistical analyses. The DNA sequences derived from rhizosphere and plant endophytic bacteria using high-throughput Illumina technology were analyzed using Mothur v.1.34.3 (Kozich et al. 2013). The standard operating procedure included the combination of forward and reverse reads and removal of sequence errors and chimeras. Taxonomic classification was performed with naive Bayesian classifier using the SILVA database (Silva.seed_v128). During sequence processing, 16S rRNA sequences identified as archaea, plant plastid, and mitochondria were detected. For both crops, the removed chloroplast sequences accounted for <0.01% in the rhizosphere, 0.02% in the root, 1.5% in the stem, 3.3% in the leaf, and 1.2% in the seed. The removed mitochondrial sequences accounted for 0.05% in the rhizosphere, 17% in the root, 87% in the stem, 84% in the leaf, and 95% in the seed (Supplementary Fig. S1). These sequences were removed from the dataset before further microbiome analysis. Reads displaying at least 97% identity were clustered into operational taxonomic units (OTU). Relative abundance of individual 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. 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 visualize the bacterial community structure of rhizosphere, root interior, and aboveground organs. The influence of crop, soil, plant compartment, and development stages 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 of the most abundant genera (relative abundance higher than 0.5%) and the calculation of Simpson's reciprocal (1/D) diversity and Chao 1 richness were conducted using the VEGAN package (version 2.0-7) in R, version 2.15.2 (R Core Team 2021). Differential abundance analyses was used to detect OTUs that showed significantly differential relative abundance between plant compartments, crop species, soil types, and plant growth stages. Differential abundance analyses were conducted using the DESeq2 package in R, version 4.05 (R Core Team 2021) with the Wald statistical test (Love et al. 2014). Log₂ fold-changes and adjusted *P* values were reported for each comparison. The top 10 OTUs with adjusted *P* values < 0.05 were considered to be significantly different in a differential comparison. The richness and diversity indexes were subjected to analysis of variance and Tukey post hoc test using SAS software, version 9.6 (SAS Institute Inc., Cary, NC, U.S.A.). The sequence data can be accessed in NCBI under Genome Project ID 510722 (accession PRJNA510722).

RESULTS

Community structure and diversity of bacterial communities in plant compartments. The 16S rRNA gene sequencing of all samples from wheat and canola rhizosphere and plant organs at stem elongation, flowering and ripening stages generated a total of 3,769,710 high-quality reads. Sequence processing identified 9,857 bacterial OTUs at 97% similarity across all samples, corresponding to 8,453

OTUs and 8,984 OTUs in wheat and canola, respectively (Table 1). The rhizocompartments (rhizosphere and roots) in both crops exhibited the highest number of OTUs, followed by stems and leaves, with seed exhibiting the lowest values (Table 1). Venn diagrams representing the OTU distribution revealed that 7,580 OTUs were shared between wheat and canola, which represent more than 80% of the bacterial OTUs of both crops (Fig. 1). Plants grown in black and brown soils shared 2,850 and 3,270 OTUs in wheat and canola, respectively, which represent more than 40% of the bacterial microbiome of each crop. The rhizosphere, the root interior, and the above-ground plant organs shared 359 and 449 OTUs in wheat and canola, respectively. In both crops, the number of OTUs shared between the rhizosphere and aboveground plant organs was higher than OTUs shared between the root interior and aboveground plant organs (Fig. 1). In addition, the number of OTUs shared between the rhizosphere and root interior was higher in comparison with the number of OTUs shared among other plant compartments.

In wheat and canola, the Chao 1 richness estimator and Simpson's 1/D values were influenced (*P* < 0.0001) by soils and plant compartment, as well as the interaction soil-compartment (Supplementary

TABLE 1 Number of bacterial sequences and operational taxonomic units (OTUs) in wheat and canola grown in brown and black soils from Saskatchewan ^a				
Crop, soil	Compartment	Samples	Sequences	OTUs
Wheat				
Brown	Rhizosphere	12	474,997	5,906
	Root	12	190,596	533
	Stem	12	22,664	298
	Leaf	8	24,307	182
	Seed	4	4,966	114
Black	Rhizosphere	12	429,340	5,032
	Root	12	285,028	819
	Stem	12	13,322	291
	Leaf	8	4,521	234
	Seed	4	482	101
Total	–	96	1,450,223	8,453
Canola				
Brown	Rhizosphere	12	480,725	6,096
	Root	12	610,845	1,431
	Stem	12	34,383	320
	Leaf	8	65,132	172
	Seed	4	1,754	103
Black	Rhizosphere	12	461,826	5,107
	Root	12	549,861	1,328
	Stem	12	73,477	470
	Leaf	8	39,125	161
	Seed	4	2,359	85
Total	–	96	2,319,487	8,984
Grand total	–	192	3,769,710	9,857

^a In total, 24 wheat and 24 canola plants were harvested from black and brown soils at stem elongation, flowering, and ripening (*n* = 4). Rhizosphere, roots, and stems were collected at each growth stage. Leaves were collected only at stem elongation and flowering, whereas seed were collected only at ripening.

Table S1). In both crops, the highest richness was detected in the rhizosphere, followed by root, whereas no significant differences were detected between stem, leaf, and seed (Supplementary Tables S2 and S3). In addition, the rhizosphere of both crops exhibited a higher bacterial richness in plants grown in the brown soil when compared with black soil. However, no differences were observed when comparing bacterial richness in the roots, stems, leaves, and seed of plants grown on different soils. The bacterial diversity (Simpson's index) in both crops grown in brown or black soils was higher in the rhizosphere, whereas no significant differences were detected between root, stem, leaf, and seed (Supplementary Tables S2 and S3).

Plant growth stages, as well as soil-stage and compartment-stage interactions, influenced ($P < 0.05$) bacterial Chao 1 richness and Simpson 1/D diversity in wheat (Supplementary Table S1). The rhizosphere and roots of wheat plants grown in black soils exhibited higher richness and diversity at the flowering stage (Supplementary Table S2). A similar trend was noted for the bacterial richness in the roots and stems of wheat plants grown in brown soils. In canola, only bacterial richness was affected ($P < 0.05$) by plant growth stages and the compartment-stage interaction, whereas diversity was not influenced (Supplementary Table S1). The stems of canola plants grown in brown and black soils exhibited higher richness at stem elongation and flowering stage, respectively (Supplementary Table S3). The bacterial richness was also higher at flowering in the roots and rhizosphere of wheat grown in brown and black soils, respectively (Supplementary Table S3).

PCoA revealed distinct patterns among the rhizosphere, root, and aboveground bacterial communities associated with the crops, with the first two axes explaining 33% of the total variation of the bacterial communities (Supplementary Fig. S2). Bacterial patterns of the rhizosphere and roots exhibited individual clusters in the ordination. However, bacterial profiles associated with the stem, leaf, and seed greatly overlapped. PERMANOVA determined that bacterial community structure differed between the plant compartments ($P \leq 0.001$) (Supplementary Table S4). PCoA of bacteria associated with the

rhizocompartments indicated that rhizosphere bacterial communities of plants grown on black and brown soils separated along axis 1 (Fig. 2). Root bacterial communities of canola and wheat plants greatly separated along axis 1, whereas wheat communities also separated along axis 2, corresponding with each soil type (Fig. 2). Although clustering was observed based on plant species and soils, no clusters were observed among plant growth stages in the rhizocompartments (Fig. 2; Table 2). PERMANOVA confirmed that root endophytic communities were influenced by soil or crop species as well as the crop-soil interaction, whereas rhizosphere communities were only influenced by soil type (Table 2).

The PCoA of the bacteria associated with the aboveground plant organs revealed that bacterial patterns in the stem exhibited a cluster of samples at ripening stage, whereas no clustering was detected at stem elongation or maturity (Fig. 2). PERMANOVA confirmed that endophytic bacterial communities associated with the stem were highly influenced ($P < 0.001$) by plant growth stage, and crop and plant species also affected ($P < 0.01$) bacterial stem profiles (Table 2). Bacterial communities associated with the stem exhibited no clustering among samples in response to soils, crop species, or plant growth stages (Fig. 2). However, PERMANOVA indicated that crops species and plant growth stages influenced ($P < 0.01$) bacterial profiles (Table 2). Seed bacterial communities were not influenced by soils or crops (Fig. 2; Table 2).

Taxonomic analysis of bacterial communities in the plant compartments. Taxonomic classification of the bacterial OTUs from potted-soil-grown wheat and canola revealed 13 phyla. Overall, nine bacterial phyla were observed concomitantly in wheat and canola grown in brown and black soils (Supplementary Fig. S3). *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, and *Firmicutes* were the dominant phyla in both wheat and canola plants.

Differential abundance analysis revealed the top 10 bacterial OTUs that exhibited higher significant differences ($P < 0.05$) in relative abundance among plant compartments, crop species, and soil types. However, OTUs did not exhibit significant differences among plant

Fig. 1. Venn diagram representing the bacterial operational taxonomic units associated with the rhizosphere (RZ), root (RT), and aboveground plant organs (AG) of wheat and canola grown in an Orthic Brown Chernozem Calcic Kastanozem and Orthic Black Calcic Chernozem soils from Central Butte and Melfort, Saskatchewan, respectively. Data presented were obtained from plants harvested at stem elongation, flowering, and ripening.

growth stages (Supplementary Table S5). Relative abundance of the genera *Cellvibrio* and *Vasilyevaea* was higher in the rhizosphere, whereas *Cupriavidus* and *Chitinophaga* were predominant in the roots (Supplementary Fig. S4). The genera *Paenibacillus* and *Arthrobacter* were the most abundant bacterial taxa in the stems, whereas leaf and seed exhibited a low abundance of the detected differential OTUs. Differential analysis between crop species revealed that the genera *Moraxella* and *Methylophilus* were predominant in canola whereas, in wheat, *Stenotrophomonas*, *Asticcacaulis*, and *Sphingobium* were the most abundant genera (Supplementary Fig. S4). Most of

bacterial taxa detected as differential OTUs among soils were highly abundant in plants grown on brown soil; however, *Amycolatopsis* and *Porphyrobacter* were predominant in plants grown black soils (Supplementary Fig. S4).

The most abundant bacterial genera associated with the rhizosphere and root interior of wheat were grouped into four main clusters: rhizosphere of wheat grown in brown soil (cluster A) and in black soil (cluster B), and root interior of wheat grown in black soil (cluster C) and in brown soil (cluster D) (Supplementary Fig. S5). The most prevalent genera in the rhizosphere of wheat grown in brown soil

Fig. 2. Principal coordinate (PC) analysis based on Bray-Curtis dissimilarity between bacterial communities associated with wheat and canola grown in brown and black soils from Saskatchewan.

(cluster A) were *Lysobacter* (3%), *Sphingomonas* (3%), and unclassified genera of the families *Burkholderiales* (4%), *Comamonadaceae* (4%), *Chitinophagaceae* (4%), *Rhizobiales* (4%), and *Sphingomonadaceae* (3%). In addition, the rhizosphere of wheat grown in black soil (cluster B) was dominated by *Arthrobacter* (4%), *Gemmatimonas* (3%), *Nocardiodes* (4%), *Solirubrobacter* (5%), and unclassified genera of the families *Actinomycetales* (3%), *Geodermatophilaceae* (4%), *Rhizobiales* (5%), and *Solirubrobacterales* (7%). In contrast, the root interior of wheat grown in black soil (cluster C) exhibited a high abundance of *Chitinophaga* (7%), *Lysobacter* (4%), *Streptomyces* (10%), *Variovorax* (4%), and unclassified genera of the families *Enterobacteriaceae* (33%), and *Oxalobacteraceae* (4%). However, the bacterial communities associated the root interior of wheat grown in brown soil (cluster D) mainly consisted of *Variovorax* (10%), *Devosia* (3%), and unclassified genera of the families *Comamonadaceae* (3%), *Enterobacteriaceae* (3%), and *Pseudonocardiaceae* (6%). These findings suggest that rhizosphere communities of wheat and canola were influenced by soil characteristics.

The most abundant bacterial genera associated with the rhizosphere and root interior of canola were grouped in three main clusters: root interior of canola grown in brown and black soils (cluster A), and rhizosphere of canola grown in brown soil (cluster B) and in black soil (cluster C) (Supplementary Fig. S6). Root samples (cluster A) were characterized by the prevalence of the genera *Streptomyces* (3%), *Variovorax* (3%), and unclassified genera of the families *Enterobacteriaceae* (38%) and *Pseudonocardiaceae* (11%). Additionally, the dominant genera in the rhizosphere of canola grown in brown soil (cluster B) were *Sphingomonas* (3%) and unclassified genera of the families *Burkholderiales* (7%), *Comamonadaceae* (4%), *Chitinophagaceae* (4%), *Enterobacteriaceae* (3%), *Rhizobiales* (3%), and *Sphingomonadaceae* (3%). In contrast, *Gemmatimonas* (5%), *Solirubrobacter* (4%), *Lysobacter* (4%), *Variovorax* (4%), *Sphingomonas* (3%), *Nocardiodes* (3%), and unclassified genera of the families *Geodermatophilaceae* (3%), *Rhizobiales* (3%), *Solirubrobacterales* (5%), and *Xanthomonadaceae* (5%) were dominant in the rhizosphere of canola grown in black soil (cluster C). These results suggest during endophytic colonization, each crop may select distinct bacterial communities.

The distribution of the bacterial genera associated with the aboveground plant organs in both crops was highly variable among soils and growth stages; thus, no clusters were detected in response to these factors (Supplementary Figs. S7 and S8). Overall, the relative abundance of unclassified genera of the family *Enterobacteriaceae* was high in the aboveground plant samples and accounted for up to 99 and 97% of bacterial communities in wheat and canola, respectively (Supplementary Figs. S7 and S8). Similarly, wheat and canola

aboveground plant organs also exhibited a high abundance of genera *Corynebacterium* and *Pseudomonas* that accounted for up to 40 and 37% of the bacterial profile, respectively. In addition, *Tumebacillus* (2%) and unclassified genera of the family *Planococcaceae* (2%) were also detected in wheat aboveground plant organs (Supplementary Fig. S7). Conversely, *Acinetobacter* (2%), *Staphylococcus* (2%), and unclassified genera of the order *Actinomycetales* (2%) were also detected in canola plants (Supplementary Fig. S8).

Influence of plant development stages on bacterial microbiome.

Bacterial communities associated with the rhizosphere, roots, and stems of canola and wheat were analyzed for their bacterial profiles at different plant growth stages (Figs. 3 and 4). The leaves and seed of both crops were excluded from this analysis due to their absence during the plant development stages assessed. In this study, the predominance of the most abundant bacterial families at stem elongation, flowering, and ripening was influenced by crop species and plant compartments. For example, the bacterial profile in the rhizosphere exhibited an even distribution of the relative abundance of most families among growth stages in both crops. However, the family *Enterobacteriaceae* was higher in the rhizosphere of canola at ripening when compared with stem elongation and flowering (Figs. 3 and 4).

The analyzes of roots indicated that the relative abundance of bacterial families in canola exhibited an even distribution among various plant growth stages (Fig. 4). In contrast, wheat roots exhibited a more variable bacterial distribution among plant growth stages. For example, at stem elongation stage, families *Bacillaceae*, *Geodermatophilaceae*, *Gemmatimonadaceae*, *Planococcaceae*, and *Solirubrobacteraceae* were highly abundant in wheat roots, whereas the family *Oxalobacteraceae* was predominant at flowering stage. Additionally, at ripening, a high abundance of the families *Mycobacteriaceae*, *Pseudonocardiaceae*, *Streptomycetaceae*, *Enterobacteriaceae*, *Comamonadaceae*, *Hyphomicrobiaceae*, *Rhizobiaceae*, and *Bradyrhizobiaceae* was detected (Fig. 3).

The distribution of bacterial families varied greatly among plant growth stages of wheat and canola stems (Figs. 3 and 4). For example, in wheat, most of bacterial families were scarce or absent at stem elongation. In contrast, the families *Geodermatophilaceae*, *Pseudonocardiaceae*, *Solirubrobacteraceae*, *Streptomycetaceae*, and *Enterobacteriaceae* were dominant in wheat stems at flowering. At ripening, the families *Chitinophagaceae*, *Sphingobacteriaceae*, *Alicyclobacillaceae*, *Bacillaceae*, *Planococcaceae*, *Nocardioidaceae*, and *Bradyrhizobiaceae* were also prevalent in wheat stems (Fig. 3). Conversely, in canola stems, the relative abundance of most families was higher at stem elongation and flowering stages, except that families *Chitinophagaceae* and *Sphingobacteriaceae* were highly prevalent at ripening (Fig. 4).

TABLE 2
Effect of crop species, soil type, and plant growth stage on bacterial operational taxonomic unit community composition assessed with permutational multivariate analysis of variance^a

Variable	dF	Rhizosphere		Root		Stem		Leaf		Seed	
		F	P	F	P	F	P	F	P	F	P
Crop	1	1.7	0.15 ns	9.7	0.0002***	1.7	0.001**	2.2	0.003*	1.2	0.19 ns
Soil	1	56.5	0.0002***	4.2	0.0002***	1.5	0.008*	1.0	0.47 ns	1.4	0.07 ns
Stage	2	0.8	0.62 ns	1.3	0.07 ns	1.8	0.0002***	1.8	0.01*	–	–
Crop × soil	1	2.9	0.05 ns	2.9	0.0006***	0.8	0.89 ns	1.1	0.25 ns	1.4	0.07 ns
Crop × stage	2	1.8	0.10 ns	1.3	0.12 ns	1.4	0.006**	0.9	0.61 ns	–	–
Soil × stage	2	0.6	0.77 ns	1.1	0.21 ns	1.0	0.45 ns	1.2	0.17 ns	–	–

^a Asterisks *, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively; ns = not significant.

DISCUSSION

The current study investigated the effect of plant compartment and soil properties on the diversity of bacterial communities associated with the rhizosphere and plant interior of wheat and canola plants grown in different soils in a growth chamber. Although our study was focused on bacterial microbiomes associated with crops, it is important to note that fungal communities (mycobiomes) also represent a significant fraction of the crop microbes and play essential roles in sustainable agricultural ecosystems (Pagano et al. 2017). High-throughput sequencing analysis revealed that bacterial community structure of wheat and canola differed among the rhizosphere, root, and aboveground plant organs. The rhizosphere and roots of both

crops exhibited a higher number of OTUs compared with the aboveground plant organs. These findings support the idea that endophytic bacterial communities are a subset of the rhizosphere microbiome (Bulgarelli et al. 2013; Edwards et al. 2015; Germida et al. 1998). The lower diversity and species richness of the bacterial communities associated with wheat and canola aboveground plant organs may be related to the low number of endophytes that are able to colonize aerial plants organs. In fact, Hallmann (2001) reported that bacterial endophytes have to overcome several plant morphological barriers and physiological limitations to establish in the stems, leaves, and seed.

Our analysis of bacterial community structure associated with wheat and canola indicated that rhizosphere communities were mainly influenced by soil characteristics, as compared with the effect of host crop or plant growth stages. Similarly, Simonin et al. (2020) also reported that soil characteristics had the largest influence on microbial taxa richness and in community structure of wheat grown in African and European soils. In addition, Garbeva et al. (2004) reported that soil properties have the ability to influence the bacterial communities not only in the bulk soil but also in microbial communities in the plant's rhizosphere. Rhizosphere bacteria associated with wheat and canola were characterized by the dominance of bacterial genera with known beneficial effects on these crops. For example, the genus *Gemmatimonas* was predominant in the rhizosphere of wheat and canola grown in both brown and black soils in the growth chamber, confirming our recent findings for field-grown canola and wheat grown in soils from the same locations (Cordero et al. 2020). Similarly, DeBruyn et al. (2011) and Gkarmiri et al. (2017) reported *Gemmatimonas* to be widely distributed in agricultural systems. These authors also reported a high relative abundance of members of the phylum *Gemmatimonadetes* associated with plants grown in soils with pH near neutrality, somewhat similar to the pH of the brown and black soils that were used in the current study. The rhizosphere of canola and wheat grown in brown soil exhibited a high abundance of the genera *Solirubrobacter* and *Nocardioideis*, which have been previously detected in rhizosphere soils associated with wheat and canola (Donn et al. 2015; Monreal et al. 2018). Similar to the current investigation, Germida et al. (1998) and Germida and Siciliano (2001) also reported that *Arthrobacter* was detected in the rhizosphere of wheat, thus suggesting that *Arthrobacter* is among the most prevalent genera associated with the rhizosphere of wheat grown in Saskatchewan agricultural soils.

In the current study, root bacterial endophytes differed between wheat and canola, suggesting that each crop selected distinct bacterial communities. The selection of endophytic communities from the rhizosphere occurs during the root colonization as the host crop regulates the colonization and multiplication of specific bacterial taxa in the root interior. For example, Bulgarelli et al. (2013) reported that the release of root exudates in the rhizosphere has an important function for the selection of root microbiota by the crop. Several plant characteristics such as plant health, plant developmental stages, root morphology, and the presence of plant wounds also may affect the penetration of bacteria into the host plant roots (Gaiero et al. 2013; Garbeva et al. 2004). Furthermore, bacterial colonization within the plant tissues involve physiological traits of bacteria such as the production of lipopolysaccharides, the activity of flagella or pili, the secretion of cell-wall-degrading enzymes, and the degradation of plant-derived compounds (Compant et al. 2010). Boller and He (2009) also suggested that some bacterial endophytes may possess effective mechanisms to avoid detection by a plant's immune system and, thus, establish viable colonies inside the plant tissues. The root interior of wheat grown in potted soils in the current growth-chamber study exhibited a high abundance of *Stenotrophomonas*, *Streptomyces*, and *Variovorax*. This confirms our previous and other

Fig. 3. Relative abundance of bacterial families (>0.5% abundant) associated with rhizosphere, roots, and stems of wheat harvested at stem elongation, flowering, and ripening. Relative abundance of bacterial families was averaged from plants grown in brown and black soils in Saskatchewan.

Fig. 4. Relative abundance of bacterial families (>0.5% abundant) associated with rhizosphere, roots, and stems of canola harvested at stem elongation, flowering, and ripening. Relative abundance of bacterial families was averaged from plants grown in brown and black soils in Saskatchewan.

researchers' observations for field-grown crops (Cordero et al. 2020; Lay et al. 2018) in Canadian prairie soils. These bacterial genera are reported to exert beneficial effects on wheat (Bertrand et al. 2001; Coombs and Franco 2003; Coombs et al. 2004; Conn and Franco 2004; Dal Bello et al. 2002; Germida and Siciliano 2001; Germida et al. 1998; Majeed et al. 2015; Mouloud et al. 2015; Rascovan et al. 2016; Ryan et al. 2009; Salantur et al. 2006; Singh and Jha 2017; Yin et al. 2013).

Surprisingly, our results revealed that neither crop species nor soil characteristics influenced the composition of bacterial endophytes associated with wheat or canola aboveground plant organs. In contrast to the root bacterial communities, endophytic bacteria associated with stem, leaf, and seed of wheat and canola exhibited similar bacterial community profiles among crops. This observation may suggest that bacterial communities associated with aboveground plant organs were less influenced by crop genotype in comparison with the root-associated bacteria (Gdanetz and Trail 2017; Ofek-Lalzar et al. 2014; Peiffer et al. 2013). We also found that bacterial communities associated with wheat and canola were highly variable among stems, leaves, and seed. Hirano and Upper (2000) reported that microbial communities associated with the aboveground plant organs are influenced by several abiotic factors such as temperature, humidity, light irradiation, and access to nutrients, which are more fluctuating in the aboveground plant organs in comparison with the rhizosphere and root environments. However, our growth-chamber experiment environmental conditions were maintained constant, in contrast to field studies, where fluctuations in abiotic factors may occur. Therefore, a possible explanation for the diverse bacterial profiles detected in the aboveground organs is that, during colonization of aerial organs, bacteria have to overcome several morphological barriers as well as need to possess the physiological requirements to establish in different plant niches existing in stems, leaves, or seed (Compant et al. 2010). In addition, these bacterial endophytic communities colonizing aboveground organs may originate from different sources, including roots or phyllosphere (Frank et al. 2017).

As indicated by high-throughput sequencing analysis, the bacterial microbiome associated with wheat and canola at stem elongation, flowering, and ripening varied among plant growth stages. Earlier studies examining the influence of growth stages on the bacterial communities associated with the rhizosphere, roots, and leaves of wheat and canola also reported a shift on the bacterial communities among growth stages (Chen et al. 2019; Copeland et al. 2015; de Campos et al. 2013; Dunfield and Germida 2003; Farina et al. 2012; Gdanetz and Trail 2017; Smalla et al. 2001). Similar to the results reported by Donn et al. (2015) and Smalla et al. (2001), the diversity of rhizosphere bacteria associated with wheat and canola was higher at ripening and flowering stages, when compared with the stem elongation growth stage. Gdanetz and Trail (2017) reported that fluctuations in the diversity of rhizosphere bacteria at flowering and maturity could be explained by three possible factors, including (i) root surface increases high availability of habitats and resources in the rhizosphere, (ii) signaling between host plant and micro-organisms that colonized the rhizosphere at earlier stages, and (iii) availability of complex metabolites released by mature plant roots. Additionally, bacterial changes during plant growth may be associated with the activation of the plant defense system that can limit the spread of certain bacterial groups (Marag and Suman 2018). Our results revealed that the influence of growth stage on the bacterial microbiome was more noticeable in the stem compared with rhizosphere and roots compartments. Moreover, the influence of growth stage on bacterial richness and diversity was more significant in wheat compared with canola. These findings suggest that the influence of growth stage on the bacterial microbiome of wheat and canola was crop and organ specific. Taxonomic analysis of the most

abundant bacterial family in both crops revealed that bacterial distribution among growth stages was even in the rhizosphere and highly variable at the stem. However, the roots of wheat exhibited a high variation in bacterial family among stages whereas, in canola, the distribution was even. These changes in the relative abundance of bacterial families among growth stages suggest that crops may select specific bacterial taxa within different plant compartments at each plant growth stage. In fact, previous studies concluded that the presence of certain bacterial groups at specific growth stages was related to the different bacterial ecological strategies within the host crop. For example, at early plant stages, fast-growing and efficient carbon-degrading bacteria (r-strategist) may colonize such rapidly changing environments as the young growing roots (Chiarini et al. 1998). In contrast, at mature stages, bacterial communities in the roots are reported to be dominated by K-strategists (i.e., bacteria exhibiting low growth rates and high persistency, even under low nutrient availability) (Brimecombe et al. 2000; Chiarini et al. 1998). Similarly, a previous study by Marag and Suman (2018) reported that, in the aboveground organs, a declining of bacterial populations occurs at maturity stage, partially due to factors such as nutrient deficiency, water stress, and free radical formation.

In conclusion, this study provides information on the diversity of the bacterial microbiome associated with plant organs of wheat and canola grown in agricultural soils from Saskatchewan, Canada. Bacterial community structure in both crops exhibited distinct profiles between the rhizosphere, root, and aboveground plant organs. Rhizosphere bacteria associated with wheat and canola differed between plants grown in brown and black soils, suggesting that soil properties influenced the rhizosphere microbiome, whereas crop species influenced endophytic bacteria associated with the roots of the crops. In contrast, the aboveground plant organs exhibited a high variability among crops and soils, thus suggesting that additional factors, including physiological and morphological complexity and diverse bacterial colonization pathways of aboveground tissues, may have influenced the bacterial microbiome in stems, leaves, and seed. Additionally, plant growth stages also may influence the bacterial microbiome associated with the rhizosphere, root, and aboveground plant organs of wheat and canola. The influence of growth stages on the bacterial microbiome of wheat and canola was crop and organ specific. Overall, our results suggest that transmission and dynamics of bacteria in the rhizosphere, root, and aboveground plant organs of crops may be controlled by interactions between multiple factors. Bacterial communities associated with the rhizosphere are highly influenced by soil properties whereas root endophytes are mainly controlled by crop species. Aboveground communities are affected by both plant physiological process and habitat complexity within the plant tissues, which may influence the colonization and diversity of endophytic bacteria in stem, leaf, and seed.

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