Inter-Kingdom Networks of Canola Microbiome Reveal Bradyrhizobium as Keystone Species and Underline the Importance of Bulk Soil in Microbial Studies to Enhance Canola Production.

Jean-Baptiste Floc'h
Université de Montréal and Jardin Botanique de Montréal

Chantal Hamel
Université de Montréal and Jardin Botanique de Montréal

Mario Laterriere
uebec Research and Development Centre

Breanne Tidemann
Agriculture and Agri-Food Canada

Marc St-Arnaud
Université de Montréal and Jardin Botanique de Montréal

Mohamed Hijri (mohamed.hijri@umontreal.ca)
Université de Montréal: Universite de Montreal  https://orcid.org/0000-0001-6112-8372

Research Article

Keywords: sequencing, plant microbiota, soil, rhizosphere, roots

DOI: https://doi.org/10.21203/rs.3.rs-700628/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

The subterranean microbiota of plants are of great importance for plant growth and health, as root-associated microbes can perform crucial ecological functions. As the microbial environment of roots is extremely diverse, identifying keystone microorganisms in plant roots, rhizosphere and bulk soil is a necessary step towards understanding the network of influence within the microbial community associated with roots and enhancing its beneficial elements. To target these hot spots of microbial interaction, we used inter-kingdom network analysis on the canola growth phase of a long-term cropping system diversification experiment conducted at four locations in the Canadian prairies. Our aims were: to verify whether bacterial and fungal communities of canola roots, rhizosphere and bulk soil are related and influenced by diversification of the crop rotation system; to determine whether there are common or specific core fungi and bacteria in the roots, rhizosphere, and bulk soil under canola grown in different environments and with different levels of cropping system diversification; and to identify hub taxa at the inter-kingdom level that could play an important ecological role in the microbiota of canola. Our results showed that fungi were influenced by crop diversification but not by bacteria. We found no core microbiota in canola roots but identified three core fungi in the rhizosphere, one core mycobiota in the bulk soil and one core bacteria shared by the rhizosphere and bulk soil. We identified two bacterial and one fungal hub taxa in the inter-kingdom networks of the canola rhizosphere, and one bacterial and two fungal hub taxa in the bulk soil. Among these inter-kingdom hub taxa, *Bradyrhizobium sp.* and *Mortierella sp.* are particularly influential on the microbial community and the plant. To our knowledge, this is the first inter-kingdom network analysis utilized to identify hot spots of interaction in canola microbial communities.

Introduction

Plant subterranean microbiota have often been described as a “black box” [1–4]—a term referring to an inherent complexity of inner workings that makes a system difficult to grasp in its entirety. We can define plant subterranean microbiota as composed of different biotopes, notably: the root interior, the rhizosphere, and the bulk soil. The root and rhizosphere microbiota are highly influenced by the plant. Rhizodeposits recruit root symbionts and shape the microbial community of the rhizosphere [5–7]. Microbial communities shaped by the plant in the rhizosphere and root interior can protect it against pathogens and enhance its growth and production [6, 8–11]. Bulk soil microbiota, while important to plant health, are less influenced by the plant due to their distance from the roots. However, the bulk soil microbiota are the inoculum from which the plant recruits its rhizosphere microbiota, and there is a direct link between bulk soil microbial composition and rhizosphere composition, as a substantial portion of the microbiota of these two biotopes interact [12–14]. In addition, the microbes in the bulk soil can produce volatile compounds that influence plant health and development [13, 15–17].

Due to their proximity, the biotopes of the plant subterranean microbiota should influence each other, and their microbes should interact. Microbial co-occurrence network analysis is a tool increasingly used to apprehend the complexity of microbial dynamics in plant and soil ecosystems [18–23]. Analysis of these networks helps identify microbial taxa of ecological interest, particularly those linked to other members of the microbial community: the hub taxa [24–28]. Hub taxa can modulate, via their shared interactions, the composition and diversity of the plant microbiota, affecting agronomic production, plant growth and productivity [25, 29, 30].
Network analysis at the inter-kingdom level can reveal different microbial dynamics that are complementary. Fungal and bacterial communities interact with each other, and, considered as a whole, can be used to identify hub taxa at a higher level of ecological complexity than previously reported [26, 31].

In this study, our targeted plant is canola (B. napus), a highly valuable crop grown by producers across the Canadian Prairies [32, 33]. Most studies about canola microbial ecology have targeted the root interior and the rhizosphere [27, 28, 34–37]. Little is known about the ecology of canola bulk soil microbiota or their overlap with root and rhizosphere microbiota. Within these three biotopes, aside from the hub taxa, other microbes may be ecologically important. Floc'h et al., (2020a) and (2020b) reported several fungi and bacteria in the canola rhizosphere that were always present despite variations in crop rotation and environmental conditions. These organisms, also reported in canola roots by Lay et al., (2018a) were identified as core fungi and core bacteria. According to Vandenkoornhuyse et al., (2015), the ever-present taxa associated with a given plant form its core microbiome and have a preferential interaction with their host.

We aimed to identify hub taxa and universal components of the core microbiota in canola roots, rhizosphere, and bulk soil. We used a gradient of crop diversification levels to create variability in these biotopes. Building on Floc'h et al., (2020a) and (2020b) who identified hub taxa in the fungal and bacterial fractions of the canola rhizosphere microbiota, respectively, we aimed to consider the canola subterranean microbiome as a whole in order to identify core fungi, core bacteria, and several inter-kingdom hub taxa that could be of ecological importance for canola health and production.

**Material And Methods**

**Experimental Design and sampling**

Our study was conducted in 2018 using a subset of plots from a long-term experiment initiated in 2008. The experiment, which was replicated at four locations in the Canadian Prairies, tested the effects of diversification in canola-based cropping systems, with all rotation phases present each year. Our study used the canola phases of three crop rotation systems. The canola grown was the glufosinate-tolerant variety L241C. The three crop diversification treatments used in this study were: (1) monoculture of canola, (2) wheat-canola, and (3) pea-barley-canola (Table 1). These treatments were applied in a randomized complete block design with four blocks at each of four experiment sites.
Table 1
Selected crop diversification treatments from a long-term experiment established in 2008 at three different sites in the Canadian prairies [39]. The rotation phases examined in this study in 2018 are underlined.

| Cropping systems | Diversification level 2008–2018 |
|------------------|-------------------------------|
| Monoculture      | C1-C-C-C-C-C-C-C-C-C-C-C   |
| Low              | C-W-C-W-C-W-C-W-C-W-C-W-C   |
| Medium           | P-B-C-P-B-C-P-B-C-P-B-C-P-B-C |

1 C. canola; W. wheat; P. pea; B. barley.

The four experiment sites were located in three pedoclimatic zones of the canola-producing regions of Canada. Two sites were in Alberta: one in Lacombe (lat. 52.5°N, long. 113.7°W) and the other in Lethbridge (lat. 49.7°N, long. 112.8°W), and two were in Saskatchewan, in Melfort (lat. 52.8°N, long. 104.6°W) and Swift Current (lat. 50.3°N, long. 107.7°W). Crops were grown according to best management practices, as described in Harker et al., (2015). With the exception of the Lethbridge site, the growing season at all sites was characterized by more frequent rain in July just before sampling (Figure S4).

Root, rhizosphere, and bulk soil samples were collected at the end of the canola flowering period, at full flower after 50% of flowers on the raceme had opened. This occurred in the fourth week of July 2018. Three to four plants within each plot were randomly selected and uprooted with a shovel. The shoots were removed, and roots were placed in plastic bags and brought to the laboratory on ice in a cooler. About 5 g of rhizosphere soil per plot was collected by gently brushing the roots. The brushed roots were then gently washed with sterilized distilled water. The bulk soil was sampled with a 2 cm diameter soil probe at a 7 cm depth, exactly in between two seed rows. The samples were kept at 4°C before being shipped on ice to the laboratory in Québec City, Québec, where they were preserved at -80°C until DNA extraction.

More details on site description, experimental design and sampling methods are provided in Floc’h et al. (2020a).

**DNA extraction and PCR amplification**

DNA extraction was conducted as described in Floc’h et al. (2020a) with a QIAGEN DNeasy extraction kit. We constructed amplicon libraries for fungal ITS sequences by using target-specific PCR primers attached to Illumina overhang sequences for Nextera library preparation. The primer pairs were ACACGTACGACATGGTCTACACTTGGTCAATTAGAGGAATGAA (ITS1F-Illu) and TACGGTAGCAGACAAGCTTTGCCGTCTGCTCTTCTATCATCGAT (5.8A2R-Illu). Each 25-µL PCR reaction consisted of 0.10 µL of forward and reverse primers, with 19.6 µL H2O, 2.5 µL 25 mM MgCl2, 12.5 µL KAPA HiFi Hotstart ReadyMix (Kapa Biosystem, Cape Town, South Africa) and 1 µL of sample DNA. The reaction conditions were as follows: 95°C for 5 min, 25 cycles of 94°C for 45 sec, 52°C for 60 sec, and 72°C for 30 sec with a final extension at 72°C for 7 min. PCR products were verified by electrophoresis on 1% agarose gels. Dual Nextera
indices were then attached to PCR products based on the protocol “16S Metagenomic Sequencing Library Preparation” provided by Illumina (part no. 15044223 rev. B). The final purified product was quantified by Qubit Fluorometric Quantitation (Thermofisher Scientific, Waltham, MA). Libraries were pooled in equimolar amounts before sequencing in rapid paired-ends 250 bp (PE250) mode on an Illumina MiSeq system, using the 500-cycle MiSeq reagent kit v.2 in accordance with the manufacturer’s recommendations.

Amplicon libraries for bacterial 16S rRNA gene sequences were constructed by using target-specific PCR primers attached to Illumina overhang sequences for Nextera library preparation. The primer pairs were GTGCCAGCMGCCGCGGTAA (515F-Illu) and GGACTACHVGGGTWTCTAAT (806R-Illu). This primer set was selected because it is used by the Earth Microbiota Project (http://www.earthmicrobiota.org/emp-standard-protocols/16s/). Two PCR reactions were performed to prepare the amplicon library. In the first PCR reaction, the V4 hypervariable region of prokaryotic 16S RNA genes was amplified using the previously described primers (515F and 806R). The PCR reaction was performed in a 25-µL reaction mixture containing 1 µL of template DNA, 1 × PCR-buffer (Qiagen, Germantown, MD, USA), 1.8 mM MgCl₂, 1.25 µL of 5% dimethylsulfoxide (DMSO), 0.2 mM dNTP, 0.5 U Taq DNA polymerase (Roche, Branford, CT, USA), and 0.6 µM of each primer. The 5’ ends of the forward and reverse primers were tagged with CS1 (ACACTGACGACATGGTTCTACA) and CS2 (TACGGTAGCAGAGACTTGGTCT), respectively, which were used as anchors for the second PCR reaction. The conditions to amplify the prokaryotic 16S rRNA fragments consisted of an initial denaturation at 94°C for 2 min, 33 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and elongation at 72°C for 30 s, followed by a final elongation at 72°C for 7 min.

The second PCR reaction was used to add barcodes to each sample and the Illumina sequencing adapters. This PCR reaction was performed in a 20-µl reaction mixture, containing 1× PCR-buffer (Qiagen, Germantown, MD, USA), 1.8 mM MgCl₂, 1 µL of 5% DMSO, 0.2 mM dNTP, 0.5 U Taq DNA polymerase (Roche, Branford, CT, USA), 2 µM of Nextera XT index primers (Illumina Inc., San Diego, CA, USA), and 1 µL of 1/150 dilution of the first PCR products. The PCR conditions were as follows: initial denaturation at 95°C for 10 min, 15 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s followed by a final elongation at 72°C for 3 min. After the second amplification, PCR products were quantified using a Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, Canada) and a Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (D-Mark, Canada). The amplicon library was purified using calibrated AMPure XP beads (Agencourt Bioscience, Beverly, MA), and the average size and quantity of each library were assessed on the LabChip GX (Perkin Elmer, Waltham, MA) instrument. The library was then sequenced on Illumina MiSeq using the paired-end 250 protocol at the Genome Québec Innovation Centre of McGill University (Montréal, Canada).

**ASV determination and bioinformatic pipeline**

Bioinformatics were used in QIIME2 environment version 2021.4 [40]. The bioinformatic pipeline used for the processing of our ITS and 16S rRNA gene sequences was DADA2 v1.18.0 [41]. First, we used Cutadapt 3.4 to remove the primer part of the ITS and 16S rRNA gene sequences with “minimum-length” at 50 and “p-error-rate” at 0.1. Then, we excluded the sequences with less than 220 bp with the command “—p-trunc-len”, as the base quality of the sequences tended to diminish below that threshold in our data. Next, the amplicon sequence variant (ASV) table was calculated, and chimeras eliminated, resulting in a sequence length ranging from 250 to 253 nucleotides. ASVs were then identified using the naïve Bayesian classifier method on the databases SILVA and RDP, and the identities of ASVs of interest were verified manually using BLAST on the NCBI nt
With the taxonomic resolution of the ITS and 16S RNA gene, it is generally not possible to identify a bacterium or fungus at the species level. Thus, the identifications at species level presented here must be considered with caution despite the fact that they perfectly match (100% similarity and coverage) the NCBI reference sequences.

The MiSeq sequencing data generated as part of this work are publicly available on Zenodo (https://doi.org/10.5281/zenodo.5028181).

**Data processing and statistical analyses**

To assess the variation induced in the canola rhizosphere by crop diversification systems, the datasets were filtered from their rare ASVs using QIIME2 with “--p-min-frequency” set to 17 and “--p-min-samples” set to 1.

The effect of crop diversification on bacterial and fungal community structure was assessed by permutational multivariate analysis of variance (PERMANOVA) [42], considering 16 blocks (four blocks per each of the four sites), using the function “adonis” of the vegan package v2.5.7 [43] in R v4.1.0, and the set of relative abundance data.

We then aimed to identify the universal fungal and bacterial components of the core microbiota and hub taxa in each biotope of the canola subterranean microbiome. We defined the core microbiota of a biotope as the set of microbial ASVs that were present in the microbiota of a particular biotope at all sites and plots. To assess the interactions among bacterial and fungal taxa in the microbiota, we created a co-occurrence inter-kingdom network using the package Spiec-Easi v1.1.0 in R 4.1.0 [21]. The analysis incorporated the roots, rhizosphere, and bulk soil fungal and bacterial community. The input data consisted of the raw abundance matrixes of the ITS and 16S ASVs. We first filtered the datasets to remove ASVs with a frequency lower than 20%. The Spiec-Easi run was conducted with the algorithm “mb” with the lambda min ratio set at $10^{-2}$ and 50 repetitions. We then imported the networks into Cytoscape 3.8.2 [44] for plotting and used the “organic” layout to draw the networks. Edges were defined as co-occurrences or mutual exclusion based on the positive or negative values of inverse covariance linking the nodes. Betweenness centrality, defined as the fraction of the shortest path between all other nodes in the network containing the given node, and degree score, highlighted central nodes and provided information about network architecture. A score of betweenness centrality and degree of connectivity greater than 95% of the network taxa suggested participation in multipartite interactions in the community and allowed us to flag the highly connected taxa as hub taxa.

**Results**

**Bioinformatic yield and taxonomic profile of the microbiota of canola roots, rhizosphere, and bulk soil**

Sequencing yielded 10,118,613 ITS and 10,273,048 16S reads. Our bio-informatic pipeline retained 6,934,809 (68.03%) non-chimeric ITS-reads and 7,157,985 (68.21%) non-chimeric 16S reads after filtering, trimming, denoising and merging. These reads were respectively assigned to 2140 ITS amplicon sequence variants (ASV) and 9814 16S ASV. Rarefaction curves indicated that read abundances reached saturation for all samples (Figure S1).
Fungal ASV in bulk soil belong mainly to three families: Chaetomiaceae (~ 16%), Mortierellaceae (~ 13%) and Nectriaceae (~ 11%) (Fig. 1D). In the rhizosphere, the most dominant fungal families were Olpidiaceae (~ 24%), Chaetomiaceae (~ 12%), Mortierellaceae (~ 10%) and Nectriaceae (~ 10%) (Fig. 1C). In the roots, the most dominant fungal families were Olpidiaceae (~ 54%) and Glomeraceae (11%) (Fig. 1B). Unknown Ascomycota were abundant in the bulk soil (~ 20%), rhizosphere soil (~ 13%), and roots (~ 14%) (Fig. 1B).

The most abundant bacterial higher taxa in the bulk soil were: Actinobacteria (~ 18%), Thermoleophilia (~ 15%) and Alphaproteobacteria (11%). In the rhizosphere, Actinobacteria (~ 24%), Gammaproteobacteria (~ 16%) and Thermoleophilia (~ 14%) were the dominant bacterial higher taxa and in the roots, Gammaproteobacteria (~ 60%), Bacili (~ 19%) and Actinobacteria (13%) were the most abundant (Fig. 2).

Principal coordinate analysis (PCoA) showed a site effect on both ITS and 16S communities (Figure S2 and S3). PERMANOVA thus conducted by site reported a significant effect of cropping system diversification on fungal communities in certain sites and microbiota compartments, but no significant effect of diversification on bacterial communities was detected in any compartment of the subterranean microbiota of canola (Table 2). Fungal communities were significantly affected by crop diversification in canola roots only in Lethbridge; in the rhizosphere at Melfort and Lacombe; and in the bulk soil at Lacombe and Lethbridge.
Table 2
Effects of crop diversification on the structure of Bacterial 16S and Fungal ITS communities in the canola subterranean microbiome, according to PERMANOVA (α = 0.05, n = 12) for each site.

| ITS communities       | 16S communities |                  |                  |                  |                  |                  |
|-----------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                       | Root             | Rhizosphere      | Bulk soil        | Root             | Rhizosphere      | Bulk soil        |
| Scott                 |                  |                  |                  |                  |                  |                  |
| Factor                | Df, Degree of Freedom | P                | P                | P                | P                | P                |
| Cropping system       | 2                | 0.48             | 0.332            | 0.282            | 0.814            | 0.51             | 0.138            |
| Residual              | 9                |                  |                  |                  |                  |                  |                  |
| Total                 | 11               |                  |                  |                  |                  |                  |                  |
| Melfort               |                  |                  |                  |                  |                  |                  |
| Cropping system       | 2                | 0.444            | 0.044*           | 0.494            | 0.193            | 0.418            | 0.156            |
| Residual              | 9                |                  |                  |                  |                  |                  |                  |
| Total                 | 11               |                  |                  |                  |                  |                  |                  |
| Lacombe               |                  |                  |                  |                  |                  |                  |
| Cropping system       | 2                | 0.535            | 0.006**          | 0.035*           | 0.313            | 0.724            | 0.647            |
| Residual              | 9                |                  |                  |                  |                  |                  |                  |
| Total                 | 11               |                  |                  |                  |                  |                  |                  |
| Lethbridge            |                  |                  |                  |                  |                  |                  |
| Cropping system       | 2                | 0.002**          | 0.206            | 0.001***         | 0.252            | 0.354            | 0.586            |
| Residual              | 9                |                  |                  |                  |                  |                  |                  |
| Total                 | 11               |                  |                  |                  |                  |                  |                  |

1DF, Degree of Freedom

“*” = p < 0.05. “**” = p < 0.01 and “***” = p < 0.001, Level of significance.

Shared microbiota and Core-microbiota of canola roots, rhizosphere, and bulk soil.

The canola roots, rhizosphere, and bulk soil biotopes shared 318 fungal ASV. These ASV represent 59%, 24% and 21% of the fungal communities of the roots, rhizosphere, and bulk soil. Thirty-three fungal ASV representing respectively 5.6% and 2.5% of the root and rhizosphere fungal communities were shared in these ecospheres, but absent in bulk soil. Forty-two fungal ASV found only in the bulk soil and roots made up 7.2% and 2.7% of these fungal communities, respectively. Five hundred and fifty-two fungal ASV representing 42% and 36% of the rhizosphere and bulk soil fungal communities, respectively, were absent in the roots (Fig. 3).
The 2405 bacterial ASV that were shared between the three compartments of the canola subterranean microbiota represented 69.4%, 29.9% and 29.2% of the bacterial communities of the roots, rhizosphere, and bulk soil, respectively. The 453 ASV shared exclusively between canola roots and rhizosphere soil represented 13% and 5.6% of the root and rhizosphere bacterial communities. The 268 ASV shared only between bulk soil and roots represented 7.7% and 3.2% of the roots and bulk soil bacterial communities, respectively. There were 4353 ASV shared exclusively between the rhizosphere and bulk soil. These shared ASV represented 54.2% and 53% of the rhizosphere and bulk soil bacterial communities, respectively (Fig. 4).

We were unable to detect a core microbiota in roots. However, three fungal amplicon sequence variants (FASV) were always present in the rhizosphere of canola, at all sites and regardless of crop rotation specifications. This fungal core microbiota was composed of FASV1 (Trichocladium sp.), FASV4 (Fusarium sp.) and FASV7 (Cryptococcus sp.). Only one fungal ASV, FASV2 (Fusarium sp.), was present in the bulk soil sample of all plots. Only one bacterial amplicon sequence variant (BASV) was present in the canola rhizosphere sample of all plots. This BASV, BASV46 (Marmoricola sp.), was also the only BASV present in the bulk soil sample of all plots (Table 3).

| Core fungi in canola rhizosphere | Core fungi in bulk soil | Core bacterium in both rhizosphere and bulk soil |
|---------------------------------|------------------------|-----------------------------------------------|
| ASV                             | Identity               | Confidence score     |
| FASV1                           | Trichocladium sp.      | 100                 |
| FASV4                           | Fusarium sp.           | 100                 |
| FASV7                           | Cryptococcus sp.       | 100                 |
| FASV2                           | Fusarium sp.           | 100                 |

Inter-kingdom network analysis of the subterranean canola microbiota.

Network analysis detected no potential inter-kingdom interaction in canola roots; thus, no network was drawn.

In the rhizosphere, the inter-kingdom network of putative interactions involved 77 ASV sharing 120 edges and included 33 mutual exclusions and 87 co-occurrences (Fig. 5a). This network, formed with the bacterial and fungal abundance datasets, revealed 3 hub taxa (Table 4), namely BASV45 (Bradyrhizobium sp.), BASV134 (Pseudonocardia sp.) and FASV21 (Mortierella sp.). BASV45 (Bradyrhizobium sp.) had a betweenness centrality score of 0.25. It was connected to 7 BASV and 3 FASV (Fig. 5b), including the hub taxon FASV21 (Mortierella sp.). FASV21 was connected to 6 BASV and 2 FASV (Fig. 5D) and had a betweenness centrality score of 0.25. The third inter-kingdom hub taxon, BASV134 (Pseudonocardia sp.), was highly connected with
FASVs (Fig. 5c), with 4 connections to FASV and 4 connections to BASV. BASV134 had a betweenness centrality score of 0.11.
Table 4
Interaction cohorts of inter-kingdom hub-taxa in canola subterranean microbiome. The taxa in bold are inter-kingdom hub-taxa.

| Cohort of hub-taxa BASV45 inter-kingdom - Rhizosphere | Cohort of hub-taxa BASV69 inter-kingdom - Soil |
|--------------------------------------------------------|------------------------------------------------|
| ASV          | Identity               | Confidence score | ASV          | Identity               | Confidence score |
| FASV8        | Chaetomium mareoticum  | 100              | FASV1       | Humicola nigrescens    | 100              |
| FASV21       | Mortierella sp.        | 99               | FASV5       | Ascomycota sp.         | 89               |
| FASV23       | Trichoderma pubescens  | 99               | FASV14      | Ascomycota sp.         | 75               |
| BASV43       | Sphingomonas sp.       | 99.99            | FASV25      | Alternaria metachromatica | 100              |
| BASV60       | Bacillus sp.           | 99.89            | FASV101     | Sordariomycetes sp.    | 100              |
| BASV77       | Micromonosporaceae sp. | 72.41            | BASV27      | Rubrobacter sp.        | 99.99            |
| BASV43       | Jatrophihabitans sp.   | 92.99            | BASV47      | Chloroflexi KD4-96     | 72.03            |
| BASV134      | Pseudonocardia sp.     | 90.75            | BASV52      | Skermanella sp.        | 99.99            |
| BASV181      | Ilumatobacter          | 98.24            | BASV61      | Agromyces sp.          | 85.99            |
| BASV200      | Chitinophagaceae sp.   | 99.99            | BASV109     | Vicinamibacteraceae sp. | 94.91            |

| Cohort of hub-taxa BASV134 inter-kingdom - Rhizosphere | Cohort of hub-taxa FASV8 inter-kingdom - Soil |
|--------------------------------------------------------|------------------------------------------------|
| ASV          | Identity               | Confidence score | ASV          | Identity               | Confidence score |
| FASV7        | Cryptococcus fuscescens| 100              | FASV7       | Cryptococcus fuscescens| 100              |
| FASV9        | Mortierella hyalina    | 98               | FASV9       | Mortierella hyalina    | 98               |
| FASV109      | Microascales sp.       | 98               | FASV11      | Nectriaceae sp.        | 99               |
| FASV221      | Ilyonectria sp.        | 100              | FASV63      | Dendryphion sp.        | 100              |
| BASV43       | Sphingomonas sp.       | 99.99            | FASV104     | Mortierella sp.        | 99               |
| BASV45       | Xanthobacteraceae sp.  | 99.99            | FASV151     | Exophiala equina       | 100              |
| BASV84       | Jatrophihabitans sp.   | 92.99            | FASV160     | Tetracladium sp.       | 98               |
| BASV107      | Gemmatimonadaceae sp.  | 72.1             | BASV43      | Sphingomonas sp.       | 99.99            |

Legends of the figures
In the bulk soil, the inter-kingdom co-occurrence network had the highest complexity, with 96 ASV and 149 edges (Fig. 6a). All three modules of the network were organized around 3 inter-kingdom hub taxa (Table 4). One of these, FASV8 (*Corynascella inaequalis*), shared 7 interactions with FASV and 3 with BASV (Fig. 6c) and had a betweenness centrality score of 0.24. The other two inter-kingdom hub taxa were FASV114 (*Mortierella sp.*) and BASV69 (*Bacterium sp.*). FASV114 shared connections with 5 FASV and 8 BASV (Fig. 6d) and had a betweenness centrality score of 0.29. BASV69 shared connections with 5 FASV and 5 BASV (Fig. 6b) and had a betweenness centrality score of 0.25.

**Discussion**
Our results support the existence of stable core bacterial and fungal components of the canola rhizosphere and in the bulk soil of canola-producing fields. Inter-kingdom network analysis revealed hub taxa in rhizosphere and bulk soil, but no significant potential interaction was found in canola roots. Hub taxa BASV45 (*Bradyrhizobium sp.*), BASV134 (*Pseudonocardia sp.*), and FASV21 (*Mortierella sp.*) in the rhizosphere and bulk soil hub taxa FASV8 (*Corynascella sp.*), FASV114 (*Mortierella sp.*) and BASV69 (*Bacterium sp.*) are strongly suspected to act as structuring factors in canola microbial communities and could exclude pathogens or enhance plant health.

### Canola subterranean microbiota compartmentation and response to crop diversification

Crop diversification is known to increase canola yield by preventing the accumulation of pests and pathogens in soil [27, 28, 39, 45]. We have previously shown the insignificance of the crop diversification effect on the bacterial communities of the canola rhizosphere in the Canadian prairies [28]. In this paper, our results confirm that bacterial communities are insensitive to diversification of cropping systems not only in the canola rhizosphere, but also in its roots and bulk soil (Table 2). This could be explained by the fact that bacteria are more influenced by soil physical properties and weather conditions than by crop rotations [46].

A contrario, the composition of the fungal communities in canola roots, rhizosphere and bulk soil appeared to be sensitive to cropping systems diversification. The sensitivity of the rhizosphere fungal community was previously reported [27, 36]. Geographic location and soil physical properties appear to affect fungal community composition as the crop rotation effect was site-dependant (Table 2).

Crop rotation systems are known to influence microbial community structure in a wide range of crops [47–49]. Crop rotations can be used to modulate biological N-fixation and to modify soil structure, with feedback on microbial communities [50–52]. Changing the fungal microbiota of canola with crop rotation systems could thus be useful for suppressing pathogens or enhancing canola nutrient uptake. The shaping of canola microbial communities must also take into account the environmental conditions, as fungal communities are subject to substantial geographical variations [53–55].

The dominance of Olpidiomyctota in the taxonomic profiles of our fungal communities was similar to that previously reported in canola [27, 28, 36, 56]. The dominance of Olpidiomyctota in the fungal microbiota of the roots and rhizosphere is mainly due to the abundance of *Olpidium brassicae* [27, 57]. This particularity of a single dominant species spread across the roots and rhizosphere is usually linked to situations of pathogen infestations [58–60] or symbiosis [61] in other crops. The predominance of *O. brassicae* was reported to have little influence on canola yield [27, 57].

In the soil, compartments like roots, rhizosphere and bulk soil are known to show a concentration gradient of plant chemical compounds that attract microbes [5, 7, 62]. Thus, the colonization of these three different niches share certain similarities in terms of the composition of their microbial communities. Cordero et al. (2019) reported 77% similarity between bacterial communities in the roots and rhizosphere of canola. In our case, canola subterranean microbiota were similar in terms of the proportion of bacterial ASV shared between the different compartments, with a significant percentage of shared community between the bulk soil and
rhizosphere (Figs. 3 and 4). The proportion of bacterial species shared between the root interior and rhizosphere of canola (82.4%) was similar to the proportion reported by Cordero et al., (2019). To the best of our knowledge, this is the first report of the percentage of mycobiota shared between canola roots, rhizosphere, and bulk soil communities. We found the same trend in fungi as in bacteria. The fact that a significant part (30 ~ 60%) of the microbial communities are shared between these ecological niches could reflect their physical proximity. The fact that between the three biotopes, bacteria are shared more than fungi could be explained by the presence of filamentous fungi that allow bacteria to migrate following their hyphae, thus allowing a wide range of bacteria to navigate between the compartments.

*Canola core-microbes in roots, rhizosphere, and bulk soil.*

The canola root interior was devoid of any core fungi or bacteria. This lack of detection could be attributed to the fact that canola roots produce glucosinolates that are toxic to microbial life and lead to limited root colonization by fungi and bacteria [32, 36, 64]. *Olpidium brassicae*, an obligatory endophyte previously reported as a core fungi in canola rhizosphere in the Canadian prairies [27, 36], was present and dominant in canola roots (Fig. 1A) but not enough to be flagged as a core fungus according to our threshold of 99%. This difference between our results and results previously published can be explained by the difference in bioinformatics methods used. In this study, DADA2 allowed us to form ASVs (amplicon sequence variants) that are much more discriminating than the 97% identity threshold used in USEARCH to form OTUs. We can thus identify different genetic variants of the same species as different ASVs in DADA2 (Callahan et al., 2016). This permits more precise inference of the structure and ecology of microbial communities. *Olpidium brassicae* shows significant genotypic variation and similarity with other pathogenic species known to affect canola, such as *O. virulentus* [57]. Its significant genetic variability and abundance in the roots and rhizosphere of canola suggest that *O. brassicae* should be the target of population genetics studies in the near future.

In the rhizosphere, three fungi and one BASV were detected as core microbes: FASV1 (*Trichocladium sp.*), FASV4 (*Fusarium sp.*), FASV7 (*Cryptococcus sp.*), and BASV46 (*Marmoricola sp.*). These three fungi were previously reported by [27] to be part of the canola rhizosphere fungal core microbiota. Paired with the observations reported in the previous article, the findings of the present study illustrate the stability over time of the concept of core microbiota in the canola rhizosphere, reinforcing the need for long term studies with recurrent samplings.

In canola bulk soil, a core fungus and core bacterium were found: FASV2 (*Fusarium sp.*) and BASV46 (*Marmoricola sp.*), respectively. The latter was also present as a core bacterium in the canola rhizosphere. Regarding the former, fusaria are well known commensalists and pathogenic fungi, widely abundant in agricultural soils [65–67]. As no core microbiota has ever been identified in canola bulk soil, these results should be taken with caution. Core fungi and bacterium are subject to variation in presence and abundance over time and depending on weather conditions [27, 28].

We were able to identify hub taxa at the intra- and inter-kingdom levels that are known as inter-kingdom hub taxa (Table 4). Each of these could be of importance for canola production and manipulation of canola subterranean microbial communities.
BASV45 (*Bradyrhizobium sp.*) is a hub taxa that has been linked to other inter-kingdom hub taxa of the canola rhizosphere. *Bradyrhizobium* is a nitrogen-fixing bacterial genus known to nodulate Fabaceae such as soybeans (*Glycine max*), cowpeas (*Vigna unguiculata*), Bambara groundnuts (*Vigna subterranea*) and chickpeas (*Cicer arietinum*) [68–70]. Furthermore, these bacteria demonstrate other ecological functions as plant growth promoting rhizobacteria (PGPR) through hormone secretions and antagonism in non-legume plants [10, 71, 72, 73]. Reported *Bradyrhizobium*-induced nodular structures on canola roots. Thus, our detection of BASV45 (*Bradyrhizobium sp.*) as an inter-kingdom hub-taxon in the canola rhizosphere highlights this taxon as a potential PGPR for canola production, and as an agent for community manipulation in canola microbial networks. Indeed, high connectivity microbes are potentially beneficial for the plant, particularly in the rhizosphere [30, 74]. Given how this taxon interacts with other plant species, ASV45 could be an important actor in the canola microbiome.

In the plant rhizosphere, microbes compete for space. One of the mechanisms used to compete with other microbes is the production of anti-microbial compounds [75–77]. Such is the case for the second most connected inter-kingdom hub taxon, BASV134 (*Pseudonocardia sp.*). This genus of actinobacteria is known as an important producer of antibiotics associated with leaf cutter ants [78–80]. This genus can be encountered in a broad range of environments from marine ecosystems to rhizosphere soil [81–83]. BASV134 was negatively connected to FASV221 (*Ilyonectria sp.*), a fungus known to cause root rot in a wide spectrum of hosts, including olive trees, panax ginseng, strawberries and avocados [84–87]. The fact that our network analysis revealed negative connectivity between these two ASVs could indicate a suppressive effect of BASV134 on FASV221 through antibiosis. It could also indicate that these two species target different exclusive ecological niches and, thus, are not frequently associated. Either way, the fact that BASV45 (*Bradyrhizobium sp.*) is negatively linked to BASV134 (Fig. 5C) suggests that *Bradyrhizobium sp.* may have biocontrol abilities in the canola rhizosphere.

The third cross-kingdom hub taxon in the canola rhizosphere was a fungus of the genus *Mortierella FASV21*. *Mortierella* is often reported as a plant growth promoting fungi (PGPF) enhancing plant phosphate nutrition [88–91]. In the canola rhizosphere, *Mortierella* was previously reported as a dominant genus of fungi [27, 36, 92]. FASV21, which was negatively linked to BASV45 (*Bradyrhizobium sp.*), and FASV151 (*Exophiala sp.*), is also a potentially beneficial organism for canola production. FASV151 (*Exophiala sp.*) was reported by [27] to be strongly positively linked to canola yield in the Canadian Prairies. *Exophiala* is a genus of fungi belonging to the dark septate endophytes (DSE) group, which hosts a broad range of plant growth promoting fungi [93, 94]. This mutual exclusion between potentially mutualistic organisms could be explained by these microbes competing for similar ecological niches and may feed on similar rhizodeposits [95–97].

Bulk soil is an important part of plant microbiota, but very few studies of canola-related microbiota have taken bulk soil into consideration [45, 98–100]. Despite the fact that bulk soil is important to plant health and rhizosphere microbiota, most studies of canola-related microbiota have been restricted to root and rhizosphere microbiota. Bulk soil microbial communities emit volatile compounds that can enhance plant growth, protect against pathogens and even influence plant root architecture [13, 15–17]. These microbial volatile compounds also have an influence on the structure of rhizosphere microbial communities [14, 101]. Compared to the rhizosphere microbial network, the bulk soil network showed a higher overall connectivity and a higher number of potential interactions between fungi and bacteria (Fig. 6). The bulk soil network had three modules, each
centered on a hub taxon. In canola bulk soil, we were able to identify three inter-kingdom hub taxa, two fungi and one bacterium: FASV8 (Corynascella sp.), FASV114 (Mortierella sp.) and BASV69 (Bacterium sp.). None of these taxa were linked with each other. This finding, coupled with the modularity of the networks, suggests a strong ecological differentiation between the canola bulk soil hub taxa.

FASV114 (Mortierella sp.) was the most connected hub taxon in the network, with a dominance of mutual exclusions. It was negatively linked with Pseudonocardia and with FASV129 (Davidiella sp.). The latter genus, the teleomorph form of Cladosporidium, is known to count among its ranks numerous plant pathogens [102, 103]. As we discussed previously, Mortierella can act as a PGPF. Its negative links with potential pathogens reinforce the need to investigate the impact this genus could have on canola production and health, as it is present as hub taxa in both the bulk soil and rhizosphere soil.

The potential ecological role of the two other hub taxa, FASV8 (Corynascella sp.) and BASV69 (unknown bacterium sp.), is rather difficult to attribute, as FASV8 (Corynascella sp.) is only reported in donkey dung from Iraq [104] and BASV69 is unknown. FASV8 was positively linked with DSE FASV151 (Exophiala sp.), which is known to be of interest for canola cropping. The position of these two hub taxa in bulk soil suggests their potential importance as microbes in canola production and thus the need for subsequent studies defining their ecological functions.

The fungal microbiota in canola roots, rhizosphere and bulk soil respond to cropping system diversification, but show different responses depending on geography and weather conditions. The microbiota in canola roots, rhizosphere and bulk soil demonstrate ecological interconnectivity and recruitment, as a significant part of the microbial communities of these biomes is shared. This first inter-kingdom network analysis of canola rhizosphere and bulk soil microbiota allowed identification of two particular microbes of interest for canola production: Bradyrhizobium sp. and Mortierella sp. The latter is a hub taxon in canola rhizosphere and roots and is linked to Exophila sp., a taxon previously described as associated with canola and positively correlated with high canola yield. The hub taxa matching Bradyrhizobium and Mortierella at the genus level could be of potential interest for bioengineering of canola subterranean microbiota and enhancing canola production.

Declarations

Acknowledgements

We are grateful to the technical staff of the AAFC research centers in Lacombe, Lethbridge, Melfort and Swift Current for carrying out the sampling and providing useful advice.

Funding: This study was supported by funds the Natural Sciences and Engineering Research Council of Canada CRD grant, the Canola Council of Canada, the Agricultural Bioproducts Innovation Program (ABIP), the Prairie Canola Agronomic Research Program (PCARP), the Agriculture and Agri-Food Canada Canola Cluster Initiative, the Alberta Canola Producers Commission, the Saskatchewan Canola Development Commission, the Manitoba Canola Growers Association, and the Western Grains Research Foundation.

Conflicts of interest/Competing interests: authors do not declare any access.
**Availability of data and material:** The MiSeq sequencing data generated as part of this work are publicly available on Zenodo at the following link: https://doi.org/10.5281/zenodo.5028181

**Code availability:** not applicable

**References**

1. Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. MEC Mol Ecol 10:1855–1871
2. Cortois R, De Deyn GB (2012) The curse of the black box. Plant Soil 350:27–33. https://doi.org/10.1007/s11104-011-0963-z
3. Pickles BJ, Pither J (2014) Still scratching the surface: how much of the ‘black box’ of soil ectomycorrhizal communities remains in the dark? New Phytol 201:1101–1105. https://doi.org/10.1111/nph.12616
4. Fernandez CW, Kennedy PG (2015) Moving beyond the black-box: fungal traits, community structure, and carbon sequestration in forest soils. New Phytol 205:1378–1380
5. Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-Secreted Malic Acid Recruits Beneficial Soil Bacteria. Plant Physiol 148:1547–1556. https://doi.org/10.1104/pp.108.127613
6. Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001
7. Patéis JS, Singh A, Singh HB, Sarma BK (2015) Plant genotype, microbial recruitment and nutritional security. Front Plant Sci 6:. https://doi.org/10.3389/fpls.2015.00608
8. Bais HP, Weir TL, Perry LG et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159
9. Chaparro JM, Badri DV, Vivanco JM (2013) Rhizosphere microbiome assemblage is affected by plant development. ISME J. https://doi.org/10.1038/ismej.2013.196
10. Pageni BB, Lupwayi NZ, Akter Z et al (2014) Plant growth-promoting and phytopathogen-antagonistic properties of bacterial endophytes from potato (Solanum tuberosum L.) cropping systems. Can J Plant Sci 94:835–844. https://doi.org/10.4141/cjps2013-356
11. Latz E, Eisenhauer N, Rall BC et al (2016) Unravelling Linkages between Plant Community Composition and the Pathogen-Suppressive Potential of Soils. Sci Rep 6:23584. https://doi.org/10.1038/srep23584
12. Bhattacharyya D, Lee YH (2016) The bacterial community in the rhizosphere of Kimchi cabbage restructured by volatile compounds emitted from rhizobacterium Proteus vulgaris JBL202. Appl Soil Ecol 105:48–56. https://doi.org/10.1016/j.apsoil.2016.03.020
13. Schenkel D, Maciá-Vicente JG, Bissell A, Splivallo R (2018) Fungi Indirectly Affect Plant Root Architecture by Modulating Soil Volatile Organic Compounds. Front Microbiol 9:. https://doi.org/10.3389/fmicb.2018.01847
14. Raza W, Wang J, Jouset A et al (2020) Bacterial community richness shifts the balance between volatile organic compound-mediated microbe–pathogen and microbe–plant interactions. Proc R Soc B Biol Sci
15. Ryu C-M, Farag MA, Hu C-H et al (2003) Bacterial volatiles promote growth in Arabidopsis. Proc Natl Acad Sci 100:4927–4932. https://doi.org/10.1073/pnas.0730845100

16. Cordovez V, Schop S, Hordijk K et al (2018) Priming of Plant Growth Promotion by Volatiles of Root-Associated Microbacterium spp. Appl Environ Microbiol 84:. https://doi.org/10.1128/AEM.01865-18

17. Garbeva P, Weisskopf L (2020) Airborne medicine: bacterial volatiles and their influence on plant health. New Phytol 226:32–43. https://doi.org/10.1111/nph.16282

18. Aderhold A, Husmeier D, Lennon JJ et al (2012) Hierarchical Bayesian models in ecology: Reconstructing species interaction networks from non-homogeneous species abundance data. Ecol Inform 11:55–64. https://doi.org/10.1016/j.ecoinf.2012.05.002

19. Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10:538–550. https://doi.org/10.1038/nrmicro2832

20. Aires T, Moalic Y, Serrao EA, Arnaud-Haond S (2015) Hologenome theory supported by cooccurrence networks of species-specific bacterial communities in siphonous algae (Caulerpa). FEMS Microbiol Ecol 91:fiv067. https://doi.org/10.1093/femsec/fiv067

21. Kurtz ZD, Müller CL, Miraldi ER et al (2015) Sparse and Compositionally Robust Inference of Microbial Ecological Networks. PLOS Comput Biol 11:e1004226. https://doi.org/10.1371/journal.pcbi.1004226

22. Alshawaqfeh M, Serpedin E, Younes AB (2017) Inferring microbial interaction networks from metagenomic data using SgLV-EKF algorithm. BMC Genom 18:228. https://doi.org/10.1186/s12864-017-3605-x

23. Nahar K, Floc'h J-B, Goyer C et al (2020) Diversity of Soil Bacterial Community Is Influenced by Spatial Location and Time but Not Potato Cultivar. Phytobiomes J PBIOMES-01-20-0. https://doi.org/10.1094/PBIOMES-01-20-0002-R

24. Benedek Z, Jordán F, Báldi A (2007) Topological keystone species complexes in ecological interaction networks. Community Ecol 8:1–7. https://doi.org/10.1556/ComEc.8.2007.1.1

25. Berry D, Widder S (2014) Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Front Microbiol 5:. https://doi.org/10.3389/fmicb.2014.00219

26. Banerjee S, Kirkby CA, Schmutter D et al (2016) Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biol Biochem 97:188–198. https://doi.org/10.1016/j.soilbio.2016.03.017

27. Floc'h J-B, Hamel C, Harker KN, St-Arnaud M (2020) Fungal Communities of the Canola Rhizosphere: Keystone Species and Substantial Between-Year Variation of the Rhizosphere Microbiome. Microb Ecol. https://doi.org/10.1007/s00248-019-01475-8

28. Floc'h J-B, Hamel C, Lupwayi N et al (2020) Bacterial Communities of the Canola Rhizosphere: Network Analysis Reveals a Core Bacterium Shaping Microbial Interactions. Front Microbiol 11:. https://doi.org/10.3389/fmicb.2020.01587

29. Agler MT, Ruhe J, Kroll S et al (2016) Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. PLOS Biol 14:e1002352. https://doi.org/10.1371/journal.pbio.1002352

30. van der Heijden MGA, Hartmann M (2016) Networking in the Plant Microbiome. PLOS Biol 14:e1002378. https://doi.org/10.1371/journal.pbio.1002378
31. Vannini Candida C, Andrea S, Alessandra et al (2016) An interdomain network: the endobacterium of a mycorrhizal fungus promotes antioxidative responses in both fungal and plant hosts. New Phytol 211:265–275. https://doi.org/10.1111/nph.13895
32. Smith EG, Kutcher HR, Brandt SA et al (2013) The profitability of short-duration canola and pea rotations in western Canada. Can J Plant Sci 93:933–940. https://doi.org/10.4141/cjps2013-021
33. Rempel CB, Hutton SN, Jurke CJ (2014) Clubroot and the importance of canola in Canada. Can J Plant Pathol 36:19–26. https://doi.org/10.1080/07060661.2013.864336
34. Dunfield KE, Germida JJ (2003) Seasonal Changes in the Rhizosphere Microbial Communities Associated with Field-Grown Genetically Modified Canola (Brassica napus). Appl Environ Microbiol 69:7310–7318. https://doi.org/10.1128/AEM.69.12.7310-7318.2003
35. Farina R, Beneduzi A, Ambrosini A et al (2012) Diversity of plant growth-promoting rhizobacteria communities associated with the stages of canola growth. Appl Soil Ecol 55:44–52. https://doi.org/10.1016/j.apsoil.2011.12.011
36. Lay C-Y, Bell TH, Hamel C et al (2018) Canola Root–Associated Microbiomes in the Canadian Prairies. Front Microbiol 9:1188. https://doi.org/10.3389/fmicb.2018.01188
37. Taye ZM, Helgason BL, Bell JK et al (2020) Core and Differentially Abundant Bacterial Taxa in the Rhizosphere of Field Grown Brassica napus Genotypes: Implications for Canola Breeding. Front Microbiol 10:. https://doi.org/10.3389/fmicb.2019.03007
38. Vandenkoornhuyse P, Quaiser A, Duhamel M et al (2015) The importance of the microbiome of the plant holobiont. New Phytol 206:1196–1206. https://doi.org/10.1111/nph.13312
39. Harker KN, O’Donovan JT, Turkington TK et al (2015) Canola rotation frequency impacts canola yield and associated pest species. Can J Plant Sci 95:9–20. https://doi.org/10.4141/cjps-2014-289
40. Bolyen E, Rideout JR, Dillon MR et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37:852–857. https://doi.org/10.1038/s41587-019-0209-9
41. Callahan BJ, McMurdie PJ, Rosen MJ et al (2016) DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869
42. Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x
43. Oksanen J, Blanchet F, Kindt R et al (2013) Vegan: Community Ecology Package
44. Smoot ME, Ono K, Ruscheinski J et al (2011) Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics 27:431–432. https://doi.org/10.1093/bioinformatics/btq675
45. Hilton S, Bennett AJ, Keane G et al (2013) Impact of Shortened Crop Rotation of Oilseed Rape on Soil and Rhizosphere Microbial Diversity in Relation to Yield Decline. PLoS ONE 8:e59859. https://doi.org/10.1371/journal.pone.0059859
46. Leitner ZR, Daigh ALM, DeJong-Hughes J (2021) Temporal fluctuations of microbial communities within the crop growing season. Geoderma 391:114951. https://doi.org/10.1016/j.geoderma.2021.114951
47. Xuan DT, Guong VT, Rosling A et al (2012) Different crop rotation systems as drivers of change in soil bacterial community structure and yield of rice, Oryza sativa. Biol Fertil Soils 48:217–225. https://doi.org/10.1007/s00374-011-0618-5
48. Venter ZS, Jacobs K, Hawkins H-J (2016) The impact of crop rotation on soil microbial diversity: A meta-analysis. Pedobiologia 59:215–223. https://doi.org/10.1016/j.pedobi.2016.04.001

49. Fan P, Lai C, Yang J et al (2020) Crop rotation suppresses soil-borne Fusarium wilt of banana and alters microbial communities. Arch Agron Soil Sci 0:1–13. https://doi.org/10.1080/03650340.2020.1839058

50. Roberts BA, Fritschi FB, Horwath WR et al (2011) Comparisons of Soil Microbial Communities Influenced by Soil Texture, Nitrogen Fertility, and Rotations. Soil Sci 176:487–494. https://doi.org/10.1097/SS.0b013e31822769d3

51. Suzuki C, Takenaka M, Oka N et al (2012) A DGGE analysis shows that crop rotation systems influence the bacterial and fungal communities in soils. Soil Sci Plant Nutr 58:288–296. https://doi.org/10.1080/03807682.2012.694119

52. Soman C, Li D, Wander MM, Kent AD (2017) Long-term fertilizer and crop-rotation treatments differentially affect soil bacterial community structure. Plant Soil 413:145–159. https://doi.org/10.1007/s11104-016-3083-y

53. Jansa J, Erb A, Oberholzer H-R et al (2014) Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. Mol Ecol n/a-n/a. https://doi.org/10.1111/mec.12706

54. Barnes CJ, van der Gast CJ, Burns CA et al (2016) Temporally Variable Geographical Distance Effects Contribute to the Assembly of Root-Associated Fungal Communities. Front Microbiol 7:. https://doi.org/10.3389/fmicb.2016.00195

55. Coleman-Derr D, Desgarennes D, Fonseca-Garcia C et al (2016) Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. New Phytol 209:798–811. https://doi.org/10.1111/nph.13697

56. Gkarmiri K, Mahmood S, Ekblad A et al (2017) Identifying the Active Microbiome Associated with Roots and Rhizosphere Soil of Oilseed Rape. Appl Environ Microbiol 83:e01938–e01917, /aem/83/22/e01938-17.atom. https://doi.org/10.1128/AEM.01938-17

57. Lay C-Y, Hamel C, St-Arnaud M (2018) Taxonomy and pathogenicity of Olpidium brassicae and its allied species. Fungal Biol 122:837–846. https://doi.org/10.1016/j.funbio.2018.04.012

58. Bakker MG, Otto-Hanson L, Lange AJ et al (2013) Plant monocultures produce more antagonistic soil Streptomyces communities than high-diversity plant communities. Soil Biol Biochem 65:304–312. https://doi.org/10.1016/j.soilbio.2013.06.007

59. Shen Z, Penton CR, Lv N et al (2018) Banana Fusarium Wilt Disease Incidence Is Influenced by Shifts of Soil Microbial Communities Under Different Monoculture Spans. Microb Ecol 75:739–750. https://doi.org/10.1007/s00248-017-1052-5

60. Gao Y-H, Lu X-H, Guo R-J et al (2021) Responses of Soil Abiotic Properties and Microbial Community Structure to 25-Year Cucumber Monoculture in Commercial Greenhouses. Agriculture 11:341. https://doi.org/10.3390/agriculture11040341

61. Andrade DS, Murphy PJ, Giller KE (2002) Effects of liming and legume/cereal cropping on populations of indigenous rhizobia in an acid Brazilian Oxisol. Soil Biol Biochem 34:477–485. https://doi.org/10.1016/S0038-0717(01)00206-1
62. van der Heijden MG, Bruin S de, Luckerhoff L et al (2016) A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. ISME J 10:389–399. https://doi.org/10.1038/ismej.2015.120

63. Cordero J, Freitas JR de, Germida JJ (2019) Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. Can J Microbiol. https://doi.org/10.1139/cjm-2019-0330

64. Smith BJ, Kirkegaard JA, Howe GN (2004) Impacts of Brassica break-crops on soil biology and yield of following wheat crops. Aust J Agric Res 55:1. https://doi.org/10.1071/AR03104

65. Ioos R, Belhadj A, Menez M (2004) Occurrence and distribution of Microdochium nivale and Fusarium species isolated from barley, durum and soft wheat grains in France from 2000 to 2002. Mycopathologia 158:351–362. https://doi.org/10.1007/s11046-004-2228-3

66. Cha J-Y, Han S, Hong H-J et al (2016) Microbial and biochemical basis of a Fusarium wilt-suppressive soil. ISME J 10:119–129. https://doi.org/10.1038/ismej.2015.95

67. Klein E, Katan J, Gamliel A (2016) Soil suppressiveness by organic amendment to Fusarium disease in cucumber: effect on pathogen and host. Phytoparasitica 44:239–249. https://doi.org/10.1007/s12600-016-0512-7

68. Suzuki Y, Adhikari D, Itoh K, Suyama K (2014) Effects of temperature on competition and relative dominance of Bradyrhizobium japonicum and Bradyrhizobium elkanii in the process of soybean nodulation. Plant Soil 374:915–924

69. Valetti L, Iriarte L, Fabra A (2016) Effect of previous cropping of rapeseed (Brassica napus L.) on soybean (Glycine max) root mycorrhization, nodulation, and plant growth. Eur J Soil Biol 76:103–106. https://doi.org/10.1016/j.ejsobi.2016.08.005

70. Grönemeyer JL, Reinhold-Hurek B (2018) Diversity of Bradyrhizobia in Subsahara Africa: A Rich Resource. Front Microbiol 9:. https://doi.org/10.3389/fmicb.2018.02194

71. Hasegawa T, Kato Y, Okabe A et al (2019) Effect of Secondary Metabolites of Tomato (Solanum lycopersicum) on Chemotaxis of Ralstonia solanacearum, Pathogen of Bacterial Wilt Disease. J Agric Food Chem 67:1807–1813. https://doi.org/10.1021/acs.jafc.8b06245

72. Al-Mallah MK, Davey MR, Cocking EC (1990) Nodulation of Oilseed Rape (Brassica napus) by Rhizobia. J Exp Bot 41:1567–1572. https://doi.org/10.1093/jxb/41.12.1567

73. Trinick MJ, Hadobas PA (1995) Formation of nodular structures on the non-legumes Brassica napus, B. campestris, B. juncea and Arabidopsis thaliana with Bradyrhizobium and Rhizobium isolated from Parasponia spp. or legumes grown in tropical soils. Plant Soil 172:207–219. https://doi.org/10.1007/BF00011323

74. Rout ME (2014) The Plant Microbiome. In: Advances in Botanical Research. Elsevier, pp 279–309

75. Whyte AC, Gloer KB, Koster B, Malloch D (1997) New antifungal metabolites from the coprophilous fungus Cercophora sordarioides. Can J Chem 75

76. Bandani AR, Kambay BPS, Faull JL et al (2000) Production of efrapeptins by Tolypocladium species and evaluation of their insecticidal and antimicrobial properties. Mycol Res 104:537–544. https://doi.org/10.1017/S0953756299001859
77. de Felício R, Pavão GB, de Oliveira ALL et al (2015) Antibacterial, antifungal and cytotoxic activities exhibited by endophytic fungi from the Brazilian marine red alga Bostrychia tenella (Ceramiales). Rev Bras Farmacogn 25:641–650. https://doi.org/10.1016/j.bjp.2015.08.003

78. Moore JM, Bradshaw E, Seipke RF et al (2012) Chapter Eighteen - Use and Discovery of Chemical Elicitors That Stimulate Biosynthetic Gene Clusters in Streptomyces Bacteria. In: Hopwood DA (ed) Methods in Enzymology. Academic Press, pp 367–385

79. Seipke RF, Grüschow S, Goss RJM, Hutchings MI (2012) Chapter Three - Isolating Antifungals from Fungus-Growing Ant Symbionts Using a Genome-Guided Chemistry Approach. In: Hopwood DA (ed) Methods in Enzymology. Academic Press, pp 47–70

80. Goldstein SL, Klassen JL (2020) Pseudonocardia Symbionts of Fungus-Growing Ants and the Evolution of Defensive Secondary Metabolism. Front Microbiol 11:. https://doi.org/10.3389/fmicb.2020.621041

81. Li J, Zhao G-Z, Huang H-Y et al (2010) Pseudonocardia rhizophila sp. nov., a novel actinomycete isolated from a rhizosphere soil. Antonie Van Leeuwenhoek 98:77–83. https://doi.org/10.1007/s10482-010-9431-7

82. Ye X, Anjum K, Song T et al (2016) A new curvularin glycoside and its cytotoxic and antibacterial analogues from marine actinomycete Pseudonocardia sp. HS7. Nat Prod Res 30:1156–1161. https://doi.org/10.1080/14786419.2015.1047775

83. Song J, Qiu S, Zhao J et al (2019) Pseudonocardia tritici sp. nov., a novel actinomycete isolated from rhizosphere soil of wheat (Triticum aestivum L.). Antonie Van Leeuwenhoek 112:765–773. https://doi.org/10.1007/s10482-018-01210-2

84. Dann EK, Cooke AW, Forsberg LL et al (2012) Pathogenicity studies in avocado with three nectriaceous fungi, Calonectria ilicicola, Gliocladiopsis sp. and Ilyonectria liriodendri. Plant Pathol 61:896–902. https://doi.org/10.1111/j.1365-3059.2011.02579.x

85. Úrbez-Torres JR, Peduto F, Gubler WD (2012) First Report of Ilyonectria macrodidyma Causing Root Rot of Olive Trees (Olea europaea) in California. Plant Dis 96:1378–1378. https://doi.org/10.1094/PDIS-04-12-0330-PDN

86. Guan YM, Ma YY, Jin Q et al (2020) Multi-Locus Phylogeny and Taxonomy of the Fungal Complex Associated With Rusty Root Rot of Panax ginseng in China. Front Microbiol 11:. https://doi.org/10.3389/fmicb.2020.618942

87. Erper I, Ozer G, Alkan M et al (2021) First report of Dactylonectria torresensis causing black root rot of strawberries in Kyrgyzstan. J Plant Pathol 103:379–380. https://doi.org/10.1007/s42161-020-00706-z

88. Zhang H, Wu X, Li G, Qin P (2011) Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (Mortierella sp.) and their effects on Kosteletzkya virginica growth and enzyme activities of rhizosphere and bulk soils at different salinities. Biol Fertil Soils 47:543. https://doi.org/10.1007/s00374-011-0563-3

89. Li F, Chen L, Redmile-Gordon M et al (2018) Mortierella elongata's roles in organic agriculture and crop growth promotion in a mineral soil. Land Degrad Dev 29:1642–1651. https://doi.org/10.1002/ldr.2965

90. Li F, Zhang S, Wang Y et al (2020) Rare fungus, Mortierella capitata, promotes crop growth by stimulating primary metabolisms related genes and reshaping rhizosphere bacterial community. Soil Biol Biochem 151:108017. https://doi.org/10.1016/j.soilbio.2020.108017
91. Ozimek E, Hanaka A (2021) Mortierella Species as the Plant Growth-Promoting Fungi Present in the Agricultural Soils. Agriculture 11:7. https://doi.org/10.3390/agriculture11010007

92. Monreal CM, Zhang J, Koziel S et al (2018) Bacterial community structure associated with the addition of nitrogen and the dynamics of soluble carbon in the rhizosphere of canola (Brassica napus) grown in a Podzol. Rhizosphere 5:16–25. https://doi.org/10.1016/j.rhsph.2017.11.004

93. Kauppinen M, Raveala K, Wäli PR, Ruotsalainen AL (2013) Contrasting preferences of arbuscular mycorrhizal and dark septate fungi colonizing boreal and subarctic Avenella flexuosa. Mycorrhiza 1–7. https://doi.org/10.1007/s00572-013-0526-7

94. Zhang Q, Gong M, Yuan J et al (2017) Dark Septate Endophyte Improves Drought Tolerance in Sorghum. Int J Agric Biol 19:53–60. https://doi.org/10.17957/IJAB/15.0241

95. Hibbing ME, Fuqua C, Parsek MR, Peterson SB (2010) Bacterial competition: surviving and thriving in the microbial jungle. Nat Rev Microbiol 8:15–25. https://doi.org/10.1038/nrmicro2259

96. Foster KR, Bell T (2012) Competition, Not Cooperation, Dominates Interactions among Culturable Microbial Species. Curr Biol 22:1845–1850. https://doi.org/10.1016/j.cub.2012.08.005

97. Ghoul M, Mitri S (2016) The Ecology and Evolution of Microbial Competition. Trends Microbiol 24:833–845. https://doi.org/10.1016/j.tim.2016.06.011

98. Lupwayi NZ, Hanson KG, Harker KN et al (2007) Soil microbial biomass, functional diversity and enzyme activity in glyphosate-resistant wheat–canola rotations under low-disturbance direct seeding and conventional tillage. Soil Biol Biochem 39:1418–1427. https://doi.org/10.1016/j.soilbio.2006.12.038

99. Lupwayi NZ, Harker KN, Dosdall LM et al (2009) Changes in functional structure of soil bacterial communities due to fungicide and insecticide applications in canola. Agric Ecosyst Environ 130:109–114. https://doi.org/10.1016/j.agee.2008.12.002

100. Rathore R, Dowling DN, Forristal PD et al (2017) Crop Establishment Practices Are a Driver of the Plant Microbiota in Winter Oilseed Rape (Brassica napus). Front Microbiol 8:1489. https://doi.org/10.3389/fmicb.2017.01489

101. Schenkel D, Deveau A, Niimi J et al (2019) Linking soil’s volatilome to microbes and plant roots highlights the importance of microbes as emitters of belowground volatile signals. Environ Microbiol 21:3313–3327. https://doi.org/10.1111/1462-2920.14599

102. Schubert K, Groenewald JZ, Braun U et al (2007) Biodiversity in the Cladosporium herbarum complex (Davidiellaceae, Capnodiales), with standardisation of methods for Cladosporium taxonomy and diagnostics. Stud Mycol 58:105–156. https://doi.org/10.3114/sim.2007.58.05

103. Bensch K, Braun U, Groenewald JZ, Crous PW (2012) The genus Cladosporium. Stud Mycol 72:1–401. https://doi.org/10.3114/sim0003

104. Guarro J, Al-Saadoon AH, Gené J, Abdullah SK (1997) Two new cleistothecial Ascomycetes from Iraq. Mycologia 89:955–961. https://doi.org/10.1080/00275514.1997.12026867

Figures
Figure 1

(A) Abundance of fungal families in all samples. (B) Relative abundance of main fungal families in canola roots, (C) rhizosphere and (D) bulk soil
Figure 2

(A) Abundance of Bacterial class in all samples. (B) Relative abundance of main bacterial families in canola roots, (C) rhizosphere and (D) bulk soil
Figure 3

Venn diagram of the ASV of the fungal community shared between root, rhizosphere and bulk soil, taking all sites in account.
**Figure 4**

Venn diagram of the ASV of the bacterial community shared between root, rhizosphere and bulk soil, taking all sites in account.
Figure 5

(A) Network of inter-kingdom interactions between the bacteria and fungi forming the microbiome of canola rhizosphere. Node shades indicate the degree of connectivity: ASVs with warm colors are more connected with the other members of the network than the cold colored ones. Green edges indicate positive relationships and red edges, negative relationships. (B) Sub-network centered around BASV45. (C) Sub-network centered around BASV134. (D) Sub-network centered around FASV21.
Figure 6

(A) Network of inter-kingdom interactions between the bacteria and fungi forming the microbiome of bulk soil in canola field. Node shades indicate the degree of connectivity: ASVs with warm colors are more connected with the other members of the network than the cold colored ones. Green edges indicate positive relationships and red edges, negative relationships. (B) Sub-network centered around BASV69. (C) Sub-network centered around FASV8. (D) Sub-network centered around FASV114.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.
• Supplementaryinformation.docx