PROJECT DETAILS

- **Title**: Enhancing the beneficial root microbiome in canola
- **Funders:** Alberta Canola, Manitoba Canola Growers, SaskCanola and Natural Sciences and Engineering Research Council of Canada (NSERC)
- **Research program:** Canola Agronomic Research Program (CARP)
- **Principal investigator:** Chantal Hamel
- **Collaborators/additional investigators:** Mohamed Hijri, Yantai Gan, Luke Bainard, Marc St. Arnaud, Chih-Ying Lay
- **Year completed:** 2019

Final report

Objectives

The project had two principal objectives, which are fundamental for unraveling the canola roots microbiota, for identification of the core microbiome in soil under crop rotation systems, as well as for the improvement of fertilizer efficiency for canola production:

- We assessed the consistency and variability in the composition of the canola core root microbiome. We validated a list of the reliable microbial taxa, i.e. the list of those taxa that are always present and abundant in canola core root microbiome.
- We determined the crop rotation systems best favoring the establishment of a beneficial root microbiome in canola and in other rotation crops.

Using two field experiments, one in Swift Current and the other in Indian Head, we reached these goals by addressing a series of five specific objectives, which were:

- To describe the canola root microbiome as influenced by different rotation systems and time, on a Brown and a Black chernozem soil.
- To identify the rotation systems with best efficiency of N cycling in the canola rhizosphere by quantifying the expression of genes involved in the processes of biological N2-fixation, nitrification, and denitrification in canola rhizosphere.
- To identify the root microbiome taxonomic profiles and taxa related to efficient N use by canola crops.
- To evaluate the potential of canola root microbiome to provide canola with tolerance to abiotic stress and pathogen pressure.
- To correlate the changes in microbial compositions with plant performance and rotational practices in order to improve understanding of the interactions between the microbial community and the plants.

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Lay et al. (2018) determined whether canola has a core root microbiome (defined here as a set of microbes that are consistently selected in the root environment), and whether this is distinct from the core microbiomes of other crops that are commonly grown in the Canadian Prairies, pea, and wheat as a complex rotation system.

We also assessed whether selected agronomic treatments can modify the canola microbiome, and whether this was associated to enhanced yield. We used a field experiment with a randomized complete block design, which was repeated at three locations across the canola-growing zone of Canada. Roots and rhizosphere soil were harvested at the flowering stage of canola. We separately isolated total extractable DNA from plant roots and from adjacent rhizosphere soil, and constructed MiSeq amplicon libraries for each of 60 samples, targeting bacterial, and archaeal 16S rRNA genes and the fungal ITS region. We determined that the microbiome of the roots and rhizosphere of canola was consistently different from those of wheat and pea (Fig. 1). These microbiomes comprise several putative plant-growth promoting rhizobacteria, including:

Amycolatopsis sp.
Serratia proteamaculans
Pedobacter sp.
Arthrobacter sp.
Stenotrophomonas sp.
Fusarium merismoides
Fusicolla sp.

The presence of this core microbiome correlated positively with canola yield. Crop species had a significant influence on bacterial and fungal assemblages, especially within the roots, while higher nutrient input or seeding density did not significantly alter the global composition of bacterial, fungal, or archaeal assemblages associated with canola roots. However, the relative abundance of *Olpidium brassicae*, a known pathogen of members of the *Brassicaceae*, was significantly reduced in the roots of canola planted at higher seeding density. Our results suggest that seeding density and plant nutrition management modified the abundance of other bacterial and fungal taxa forming the core microbiomes of canola that are expected to impact crop growth. This

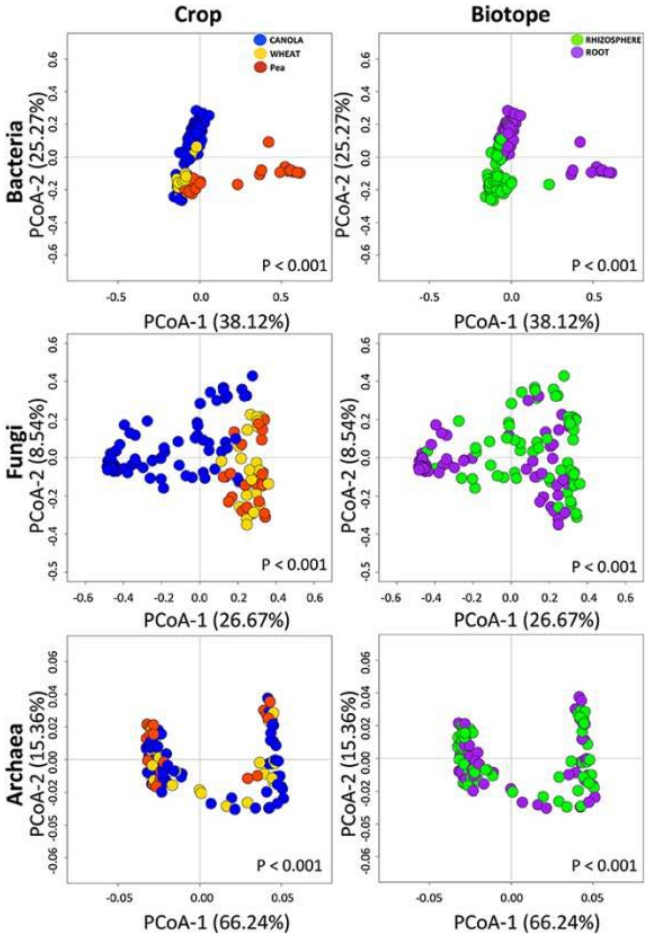

Figure 1: Principal coordinates analyses of the bacterial, fungal, and archaeal operational taxonomic units (OTUs), showing the grouping based on crops and biotopes. The percentages represent the variance explained by each axis.

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work helps us to understand the microbial assemblages associated with canola grown under common agronomic practices and indicates microorganisms that can potentially benefit or reduce the yield of canola.

o ***Specific objectives (ii) and (iii): To identify the rotation systems with best efficiency of N cycling in the canola rhizosphere by quantifying the expression of genes involved in the processes of biological N2-fixation, nitrification, and denitrification in canola rhizosphere; and to identify the root microbiome taxonomic profiles and taxa related to efficient N use by canola crops.***

Nitrogen cycles process in the air and in the soil through eight key inorganic nitrogen species of different oxidation states. Three major biological processes involved in N cycling are N2 fixation (N2 reduction to NH4+), nitrification (oxidation of NH4+ to NO, NO2− and NO3−) and denitrification (reduction of NO3− to N2O−, NO and N2). Biological fixation of atmospheric N2 is a fundamental step in soil N-cycling. This process is catalysed by the enzyme nitrogenase encoded by the *nifH* gene of prokaryotes. Biological N2 fixation generates ammonia, which can be oxidized by ammonia monooxygenase to produce nitrate in the nitrification process. Ammonia monooxygenase mediates the first stage of nitrification (NH4+ oxidation to NH2+) and the genes coding for ammonia monooxygenase A-subunits, *amoA*, are used as molecular markers to detect ammoniaoxidizing bacteria (AOB) and archaea in many environments. The nitrite oxidoreductase gene, *nxrA*, codes for the enzyme carrying out a subsequent process in nitrification, i.e. the oxidation of NO2− to NO3−. Nitrate (NO3−) is the substrate for denitrification, the main nitrous oxide (N2O) emissions process. Nitrate (NO3−) leaches easily and causes eutrophication of surface water bodies. It is also the substrate for the process of denitrification (NO3 reduction to NO2, to NO, to N2O, to N2), which is a source of the greenhouse gas (GHG) N2O. The reduction of NO2− to nitric oxide (NO) by nitrite reductase (Nir) is a crucial step in denitrification. The enzyme Nir has two forms: the copper-containing nitrite reductase (NirK) encoded by the gene *nirK* and the cytochrome cd1-containing nitrite reductase (NirS) encoded by the gene *nirS* (Yang *et al*., **2018**). A range of microorganisms can oxidize NO to the GHG N2O in soil. Nitrification and denitrification lead to N losses causing serious environmental problems. Nitrous oxide from

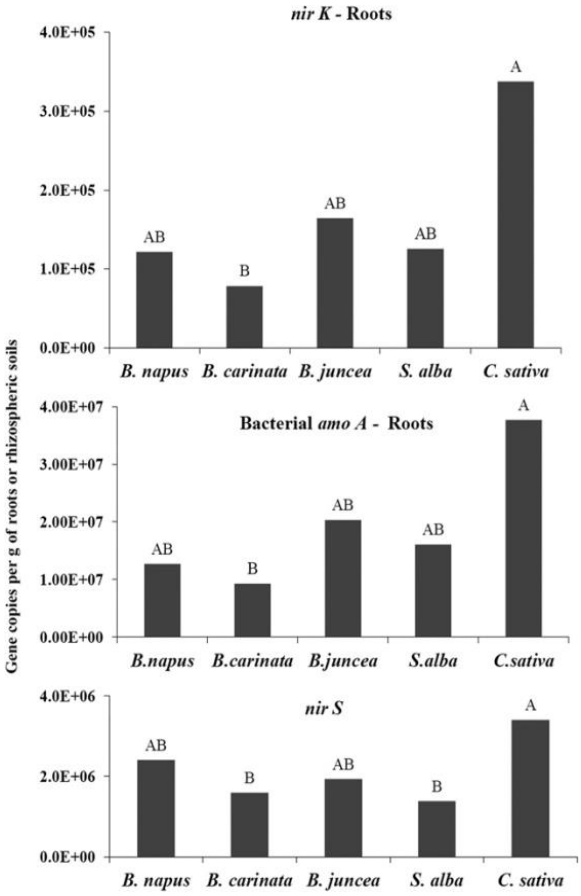

Figure 2. Least square mean values of nirK (top panel), Bacterial amo A (middle panel) and nirS (bottom panel) gene expression associated with five oilseed crop species. Different letters represent significant differences between cropping systems (P<0.05).

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denitrification contributes about 6% of current anthropogenic GHG emissions and ranks third among anthropogenic emission of GHGs. More than a third of all nitrous oxide emissions are due to agriculture. However, the subsequent reduction of N2O to the innocuous gas N2 mitigates the negative impact of denitrification on air quality. Nitrous oxide reductase (Nos), the enzymes carrying out this reaction, is encoded by the gene *nosZ* . Furthermore, many previous studies have reported that in agricultural cropping systems, N-cycling functional genes are highly correlated with N transformation processes, since various agricultural cropping systems change plant–soil micro-ecosystem environment. For example, plant-induced changes to the soil environment stimulate N-cycling gene transcripts to produce active N transformation enzymes to act on the process of N transformation directly. Therefore, N-cycling gene expression patterns could be used as a proxy to assess *in situ* N-cycling transformations.

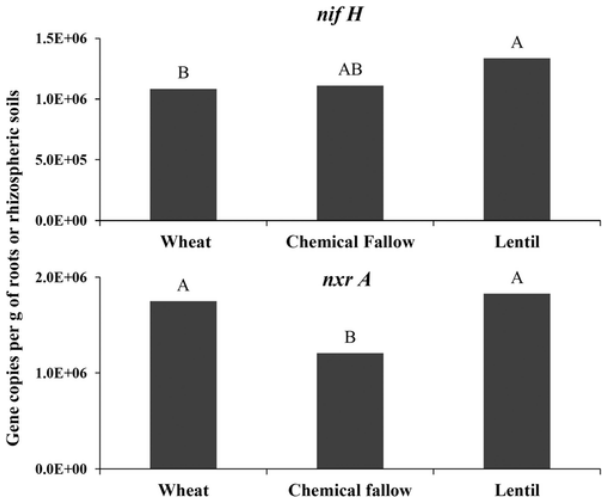

Figure 3. Least square mean values of nifH (top panel) and nxrA (bottom panel) gene expression, after a preceding wheat, fallow, or lentil crop. Different letters represent significant differences between cropping systems (Tukey's HSD test, P < 0.05).

The abundance of the microbial N-cycling genes is different in soil and roots and the abundance of these genes is affected directly and/or indirectly by the identity of the crop plant.

Wang et al. (2020) assessed N-cycling gene expression patterns in the root and rhizosphere microbiomes of five oilseed crops as influenced by three 2-year crop rotations. The first phase consisted of fallow, lentil or wheat, and the second phase consisted of one of five oilseed crops. Expression of bacterial *amoA*, *nirK* and *nirS* genes showed that the microbiome of Ethiopian mustard had the lowest and that of camelina the highest potential for N loss (Fig. 2).

These results provide new insights that have the potential to improve crop production while reducing the environmental footprint of agriculture. Among the five oilseed plants tested in our study, *B. carinata* showed the best performance with the highest yield and lowest impact on potential greenhouse gas emissions. *Camelina sativa* exhibited the opposite trend, with lower yield and higher denitrification potential. Our results also demonstrated that the preceding crop is an important factor to consider in crop production systems. Lentil, as a preceding crop for oilseed production, could help to increase N2 fixation, decrease N fertilization application and reduce the agricultural footprint on the environment. Wheat as a previous crop for oilseed production was a poor performer for sustaining oilseed production and could result in nitrogen loss and potentially higher greenhouse gas emissions than a lentil. Overall, our findings highlight that diversified pulse-oilseed cropping sequences are highly desirable on the semiarid northern Great Plains of North America to achieve high N2 fixation and retention and minimize N loss in productive cropping systems.

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o ***Specific objectives (iv) To evaluate the potential of canola root microbiome to provide canola with tolerance to abiotic stress and pathogen pressure.***

Masse et al. (In Preparation)3 tested the impact the canola-cereals-pea rotation systems with different crop intensities, 1) on crop productivity, 2) on arbuscular mycorrhizal (AM) fungal diversity and community structure in the roots and in the rhizosphere of each crop, and 3) pinpointed relationships between specific AM fungal microbiome members and crop productivity. We used three rotation systems (intensifying canola, cereals or pulse over four years) and tested them in a complete random block design. The root and rhizosphere microbiomes were sampled for each of the rotation phases at two growing stages. DNA was extracted and sequenced using an Illumina MiSeq sequencer.

Increasing the frequency of canola in a 4-year rotation did not reduce the productivity of the other crops in the rotation nor did it translate into reduced biodiversity of AM fungi in the roots or in the rhizosphere of those crops, except for canola itself. Conversely, crop and cropping system did modify the AM fungal community structure in both roots and rhizospheric environments of the plants with positive or negative correlations with crop productivity. These results support the hypothesis that a simple modification of the cropping system could be used to manipulate root or endophytic microbiomes to improve crop productivity without increasing the amount of input in crop production.

o ***Specific objective (v) To correlate the changes in microbial compositions with plant performance and rotational practices in order to improve understanding of the interactions between the microbial community and the plants.***

Rhizosphere microbes influence one another, forming extremely complex webs of interactions that may determine plant success. Identifying the key factors that structure the fungal microbiome of the plant rhizosphere is a necessary step in optimizing plant production. In a long-term field experiment conducted at three locations in the Canadian prairies, Floc'H et al. (2020) tested the following hypotheses:

(1) diversification of cropping systems influences the fungal microbiome of the canola (*Brassica napus*) rhizosphere

(2) the canola rhizosphere has a core fungal microbiome, i.e., a set of fungi always associated with canola

(3) some taxa within the rhizosphere microbiome of canola are highly interrelated and fit the description of hub taxa. Our results show that crop diversification has a significant effect on the structure of the rhizosphere fungal community but not on fungal diversity. We also discovered and described a canola core microbiome made up of one zero-radius operational taxonomic unit (ZOTU), cf. *Olpidium brassicae*, and an eco-microbiome found only in 2013 consisting of 47 ZOTUs (Fig. 4).

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Using network analysis, we identified four hub taxa in 2013: ZOTU14 (*Acremonium* sp.), ZOTU28 (*Sordariomycetes* sp.), ZOTU45 (*Mortierella* sp.) and ZOTU179 (cf. *Ganoderma applanatum*), and one hub taxon, ZOTU17 (cf. *Mortierella gamsii*) in 2016. None of these most interacting taxa belonged to the core microbiome or eco-microbiome for each year of sampling. This temporal variability puts into question the idea of a plant core fungal microbiome and its stability. Our results provide a basis for the development of ecological engineering strategies for the improvement of canola production systems in Canada.

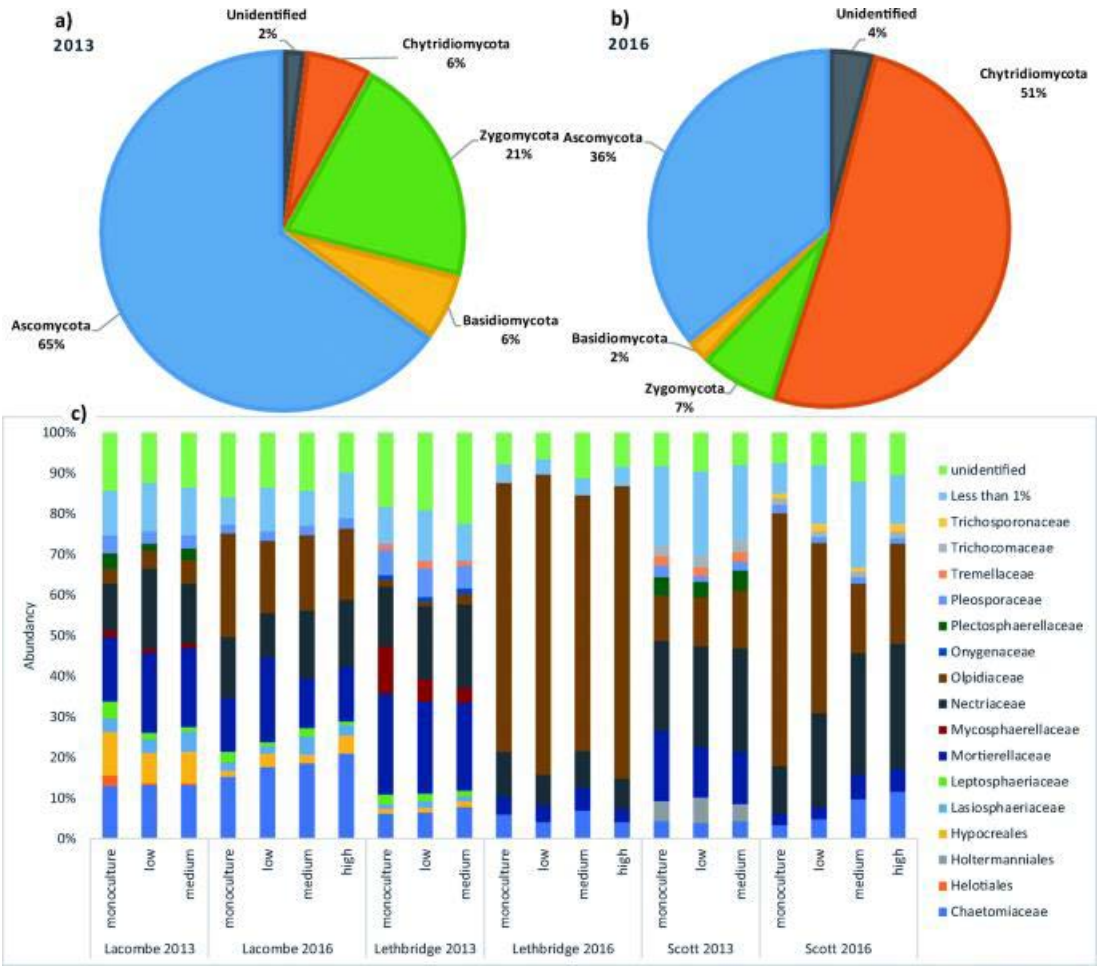

Figure 4. Variation in taxonomic profiles is characterized by an increase in the abundance of the Olpidiaeae in the phylum Chytridiomycota in 2016. Fungal families also varied with site, crop diversification level, and year (c).

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Scientific and/or engineering significance of the results achieved

We documented canola microbiomes for three communities of microorganisms, namely, bacteria, fungi, and archaea. We found that canola microbiomes were distinguished between the two biotopes (roots and rhizosphere) and were significantly different from those of the reference crops (wheat and pea). We highlighted the potential PGPR among those microorganisms by correlating the core microbiome members in the Canadian Prairies with canola yield. Taxa related to *Amycolatopsis* sp., *S.proteamaculans,Pedobacter* sp., *Arthrobacter* sp., *Stenotrophomonas* sp., *F. merismoides*, and *Fusicolla* sp. are potentially beneficial to canola due to their status as members of the core or eco microbiome and their positive correlation with canola yield. Fertilization and seeding rates seem to influence certain taxa forming the core and eco microbiomes of canola based on the relative abundances profiles, notably the parasite *O. brassicae* which was less abundant at the higher seeding rate. Certain archaeal taxa showed some specificity to crops and treatments. Furthermore, the putative interactions between the members of bacterial and fungal core microbiomes were weaker with higher fertilization and seeding than the recommended treatments in canola rhizospheres. Our study provides information about the canola root microbiome that is fundamental for the design of microbiome management strategies for improving canola yield and health.

A preceding rotation phase of lentil significantly increased the expression of *nifH* gene by 23% compared with wheat (Fig. 3) and improved *nxrA* gene expression by 51% with chemical fallow in the following oilseed crops respectively. Lentil substantially increased biological N2 fixation and reduced denitrification in the following oilseed crops. Our results also revealed that most N-cycling gene transcripts are more abundant in the microbiomes associated with roots than with the rhizosphere. The outcome of our investigation brings a new level of understanding on how crop diversification and rotation sequences are related to N-cycling in annual cropping systems.

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