Final Report

Identification

Program Name: Collaborative Research and Development Grant
Due Date: 2021-05-31

Mohamed M. Hijri
Dép. des sciences biologiques
Université de Montréal
COMPLEXE DES SCIENCES
CP 6128 SUCC CENTRE-VILLE
MONTREAL QC H3C3J7
Telephone: 514-3432120
Extension:
E-mail Address: mohamed.hijri@umontreal.ca

Project Title: Enhancing the beneficial root microbiome in canola

File Number: CRDPJ 500507 - 2016
Co-Applicant: Marc MGJ. St-Arnaud, Recherche, Jardin botanique de Montréal
Collaborator: Yantai Y. Gan, Swift-Current Research and Development Centre, Agriculture and Agri-Food Canada
Collaborator: Luke D. Bainard, Swift-Current Research and Development Centre, Agriculture and Agri-Food Canada
Collaborator: Chantal C. Hamel, Land Resources and Environment, Agriculture and Agri-Food Canada
Supporting Organization: Canola Council of Canada
Saskatchewan Pulse Growers

Final Report

Canada

Identification

Public Summary of Outcomes and Benefits to Canada

This project aimed to assess the consistency and variability in the composition of the canola core rhizospheric and root microbiota, and determine the crop rotation systems best favouring the establishment of a beneficial root microbiome in canola. The main outcomes of this project demonstrated that biodiverse agricultural systems improve agricultural productivity, and climate management. The challenge in employing microbiome technologies for improving agricultural systems is the pre-requisite of understanding how soil microbial communities are structured. We tested two parameters of this process; the role of soil history in environmental filtering, and the selection pressure created by host plants. We described the impact of five Brassicaceae plants and their soil history on the structure of bacterial communities in their rhizosphere and roots. We found that soil history was significant in structuring the bacterial communities when soil chemistry was highly significant under possible drought conditions. Second, that the Brassicaceae host plants were consistently significant in structuring the bacterial communities.
The outcomes contributed to better understand canola microbiota which provided important information to improve canola yields and reducing greenhouse gaz emission by identifying the best rotation system that could potentially save nitrogen fertilizer uses.

Progress Towards Objectives/Milestones

To what extent were the objectives of the grant achieved? Rate your answer on a scale from 1 to 7.

Not at all			Somewhat			To a great extent
○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	● 7

Final Report

- **Description of the overall 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.
1 –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.

2 – 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:

(i) To describe the canola root microbiome as influenced by different rotation systems and time, on a Brown and a Black chernozem soil.
(ii) 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.
(iii) To identify the root microbiome taxonomic profiles and taxa related to efficient N use by canola crops.
(iv) To evaluate the potential of canola root microbiome to provide canola with tolerance to abiotic stress and pathogen pressure.
(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.

- **Description of the progress made towards these objectives as a result of the project**

  - ***Specific objective (i): To describe the canola root microbiome as influenced by different rotation systems and time, on a Brown and a Black chernozem soil.***

Lay et al. (2018)[2] 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

[2] Lay, C.-Y.., T.H. Bell, C. Hamel, K.N Harker, R. Mohr, C.W. Greer, E. Yergeau and M. St-Arnaud, M. (2018). Canola root-associated microbiomes in the Canadian Prairies. *Frontiers in Microbiology. 9:1188.*

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 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.

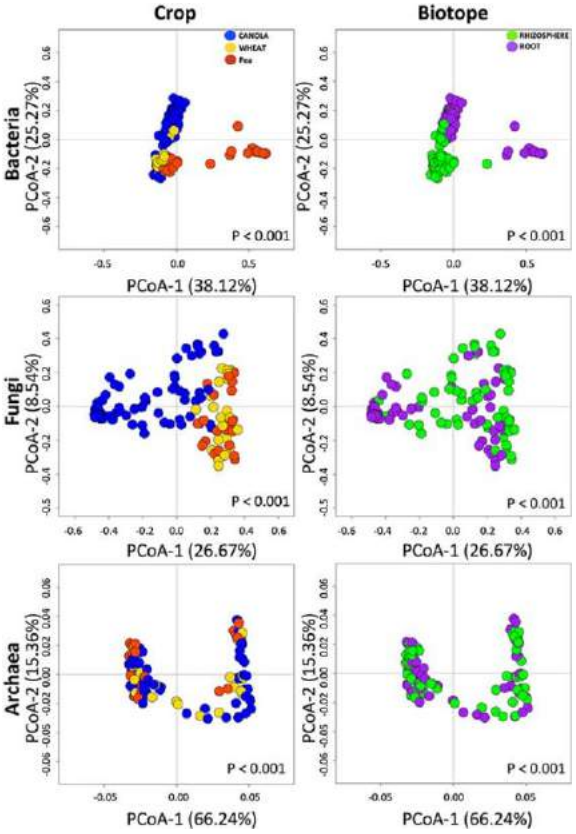

Figure 1: Principal coordinates analyses of the bacterial, fungal, and archaeal operational taxonomic units (OTUs), showing the grouping based on crops and biotopes. The percentages represent the variance explained by each axis.

- ***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 N_2 fixation (N_2 reduction to NH_4^+), nitrification (oxidation of NH_4^+ to NO, NO_2^- and NO_3^-) and denitrification (reduction of NO_3^- to N_2O^-, NO and N_2). Biological fixation of atmospheric N_2 is a fundamental step in soil N-cycling. This process is catalysed by the enzyme nitrogenase encoded by the *nifH* gene of prokaryotes. Biological N_2 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 (NH_4^+ oxidation to NH_2^+) and the genes coding for ammonia monooxygenase A-subunits, *amoA*, are used as molecular markers to detect ammonia-oxidizing 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 NO_2^- to NO_3^-. Nitrate (NO_3^-) is the substrate for denitrification, the main nitrous oxide (N_2O) emissions process. Nitrate (NO_3^-) leaches easily and causes eutrophication of surface water bodies. It is also the substrate for the process of denitrification (NO_3 reduction to NO_2, to NO, to N_2O, to N_2), which is a source of the greenhouse gas (GHG) N_2O. The reduction of NO_2^- 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 cd_1-containing nitrite reductase (NirS) encoded by the gene *nirS* (Yang *et al*., **2018**). A range of microorganisms can oxidize NO to the GHG N_2O in soil. Nitrification and denitrification lead to N losses causing serious environmental problems. Nitrous oxide from 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 N_2O to the innocuous gas N_2 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. 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)[2] 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).

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).

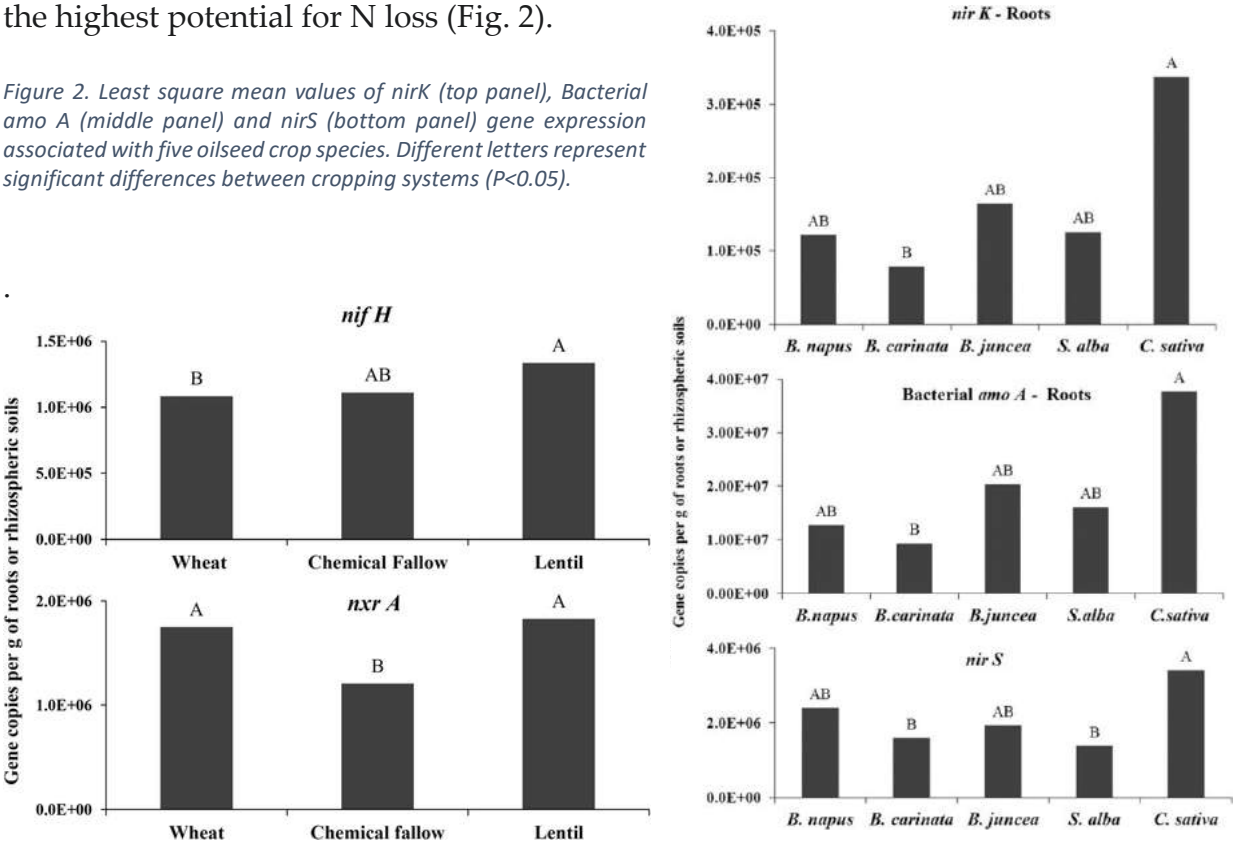

Figure 3. Figure 3. Least square mean values of nifH (top panel) and nxrA (bottom panel) gene expression, after a preceding wheat, fallow, or lentil crop. Different letters represent significant differences between cropping systems (Tukey's HSD test, P < 0.05).

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 N_2 fixation, decrease N fertilization application and reduce the agricultural

[2] Wang L, Gan Y, Bainard L, Hamel C, St-Arnaud M, Hijri M (2020) Expression of N-cycling genes of root microbiomes provides insights for sustaining oilseed crop production. Environmental Microbiology 22 (11), 4545-4556.

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 N_2 fixation and retention and minimize N loss in productive cropping systems.

- ***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.

- ***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

[3] Masse J, Hamel H, Bainard, LD, Gan Y, Yergeau É, Greer CW, Hijri M, & St-Arnaud M (2021) Does canola negatively affect the root and rhizospheric arbuscular mycorrhizal fungal microbiome of successive crops in canola-pulse-cereal rotations? In Preparation

prairies, Floc'H et al. (2020)[4] 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; and (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). 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.

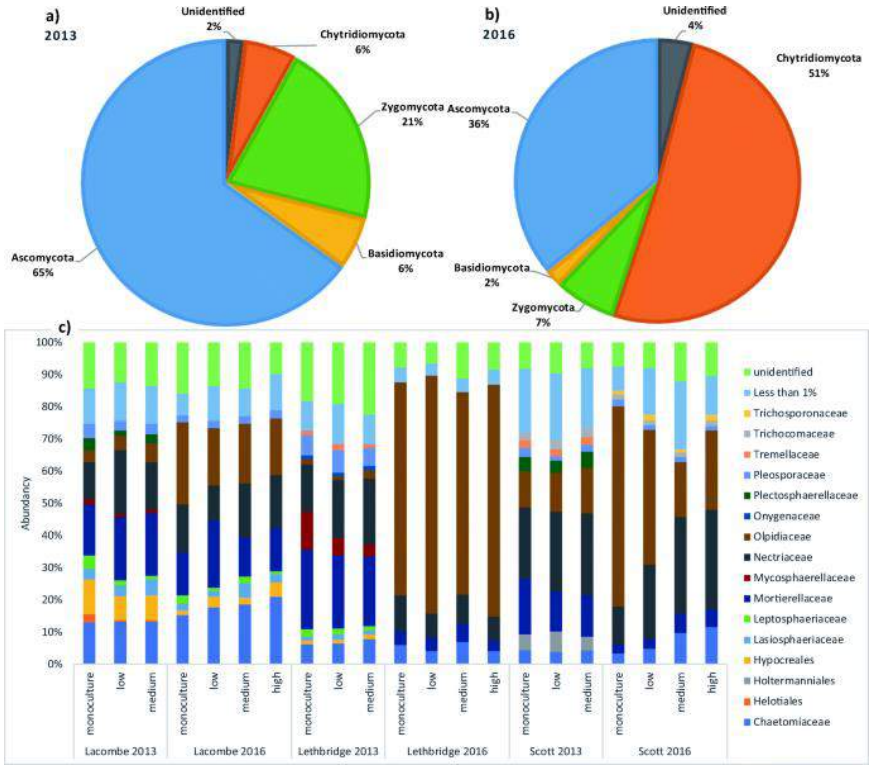

Figure 4. Variation in taxonomic profiles is characterized by an increase in the abundance of the Olpidiaeae in the phylum Chytridiomycota in 2016. Fungal families also varied with site, crop diversification level, and year (c).

[4] Floc'H JB., Hamel C., Harker N., St-Arnaud M., (2020). Fungal communities of the canola rhizosphere: keystone species and substancial between-year variation of the rhizosphere microbiome. Microbial Egology 80 (4): 762-777.

- **A justification for any deviations from the original objectives**

Not applicable as we have achieved all objectives of the project.

- **A description of the 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 N_2 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.

Problems Encountered

Identify the problems encountered during the research project. (Select all that apply.)

- ☐ Technical or scientific problems
- ☐ Problems with direction of research or findings
- ☐ Equipment and facilities
- ☐ Staffing issues (e.g., availability of students, staff leaving project)
- ☐ Funding problems
- ☐ Partners withdrew from project
- ☐ Partners interaction issues
- ☑ No problems were encountered
- ☐ Other (specify)

Problems Encountered

If problems were identified, briefly describe them and the steps taken to resolve each one.

We have asked for a no-extension cost to allow PhD students to achieve their projects. Covid-19 pandemic slowed down the project as for all other research activities.

Research Team

Entry 1 of 4

Consent obtained: ● Yes ○ No

Name: Marc

Role: Co-Applicant

If role is "Other", specify:

Contribution

Co-supervision of graduate students; involved in conceptualization of experiments; Revision of manuscript; Involved in project management.

Entry 2 of 4

Consent obtained: ● Yes ○ No

Name: Luke

Role: Collaborator

If role is "Other", specify:

Contribution

Experiment setup; Sampling; Revising manuscripts

Entry 3 of 4

Consent obtained: ● Yes ○ No

Name: Yantai

Role: Collaborator

If role is "Other", specify:

Contribution

Supervision of field experiments

Research Team

Entry 4 of 4

Consent obtained: ● Yes ○ No

Name: Chantale

Role: Collaborator

If role is "Other", specify:

Contribution

Co-supervision of graduate students; involved in conceptualization of experiments; Revision of manuscript;

Training of Highly Qualified Personnel (HQP)

What types of interactions did the HQP have with the partners during the project? (Select all that apply.)

- [x] HQP presented research results to the partners
- [x] HQP discussed the project directly with partners to obtain input
- [ ] Partners jointly supervised thesis projects of HQP
- [ ] HQP worked regularly in the partner's facilities
- [ ] HQP did not interact with the partners
- [ ] Other (specify)

Entry 1 of 11

Name:	Éloïse
Type:	Undergraduate Student
If type is "Other", specify:	
Start Date yyyy/mm:	2020/05
End Date yyyy/mm:	2020/08
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	0
Total person-months:	3
To the best of your knowledge trainee is:	Continuing Academic Training
If "Employed by Other", specify:	

Entry 2 of 11

Name:	Marilou
Type:	Undergraduate Student
If type is "Other", specify:	
Start Date yyyy/mm:	2020/05
End Date yyyy/mm:	2020/08
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	0
Total person-months:	3
To the best of your knowledge trainee is:	Continuing Academic Training
If "Employed by Other", specify:	

Training of Highly Qualified Personnel (HQP)

Entry 3 of 11

Name:	Thomas
Type:	Undergraduate Student
If type is "Other", specify:	
Start Date yyyy/mm:	2020/05
End Date yyyy/mm:	2020/08
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	0
Total person-months:	3
To the best of your knowledge trainee is:	Continuing Academic Training
If "Employed by Other", specify:	

Entry 4 of 11

Name:	Emmy
Type:	Undergraduate Student
If type is "Other", specify:	
Start Date yyyy/mm:	2019/05
End Date yyyy/mm:	2019/08
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	100
Total person-months:	3
To the best of your knowledge trainee is:	Continuing Academic Training
If "Employed by Other", specify:	

Entry 5 of 11

Name:	Raphaella
Type:	Undergraduate Student
If type is "Other", specify:	
Start Date yyyy/mm:	2017/05
End Date yyyy/mm:	2017/08
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	0
Total person-months:	3
To the best of your knowledge trainee is:	Do not know
If "Employed by Other", specify:	

Training of Highly Qualified Personnel (HQP)

Entry 6 of 11

Name:	Florence
Type:	Undergraduate Student
If type is "Other", specify:	
Start Date yyyy/mm:	2021/05
End Date yyyy/mm:	2021/08
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	0
Total person-months:	3
To the best of your knowledge trainee is:	Continuing Academic Training
If "Employed by Other", specify:	

Entry 7 of 11

Name:	Andrew
Type:	Doctoral Student
If type is "Other", specify:	
Start Date yyyy/mm:	2017/09
End Date yyyy/mm:	2022/04
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	100
Total person-months:	55
To the best of your knowledge trainee is:	Continuing Academic Training
If "Employed by Other", specify:	

Entry 8 of 11

Name:	Jean-Baptiste
Type:	Doctoral Student
If type is "Other", specify:	
Start Date yyyy/mm:	2018/09
End Date yyyy/mm:	2022/03
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	100
Total person-months:	42
To the best of your knowledge trainee is:	Continuing Academic Training
If "Employed by Other", specify:	

Training of Highly Qualified Personnel (HQP)

Entry 9 of 11

Name:	Jacynthe
Type:	Postdoctoral Fellows
If type is "Other", specify:	
Start Date yyyy/mm:	2017/04
End Date yyyy/mm:	2019/12
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	25
Total person-months:	32
To the best of your knowledge trainee is:	Employed by Government
If "Employed by Other", specify:	

Entry 10 of 11

Name:	Li
Type:	Postdoctoral Fellows
If type is "Other", specify:	
Start Date yyyy/mm:	2017/07
End Date yyyy/mm:	2019/12
Percentage (%) of time this individual spent on this project:	100
Percentage (%) of salary from this grant (NSERC and industry contribution):	100
Total person-months:	29
To the best of your knowledge trainee is:	Employed in Academia / Faculty
If "Employed by Other", specify:	

Entry 11 of 11

Name:	Chih-Ying
Type:	Postdoctoral Fellows
If type is "Other", specify:	
Start Date yyyy/mm:	2017/05
End Date yyyy/mm:	2018/12
Percentage (%) of time this individual spent on this project:	50
Percentage (%) of salary from this grant (NSERC and industry contribution):	0
Total person-months:	10
To the best of your knowledge trainee is:	Employed by Other (specify)
If "Employed by Other", specify:	Researcher R&D Biofertilizer's company

Dissemination of Research Results

Refereed Journal Articles Submitted :	2
Refereed Journal Articles Accepted or Published:	11
Conference Presentations/ Posters:	30
Other (Technical Reports, Non-Refereed Articles, etc.):	10
How many of the publications, conference presentations, etc. identified above were co-authored with a non-academic partner?	11

Research Contributions of the project

1. Articles in preparation for submission

Jacynthe Masse, Chantal Hamel, Luke D. Bainard, Yantai Gan, Étienne Yergeau, Charles W. Greer, Mohamed Hijri and Marc St-Arnaud. Does canola negatively affect the root and rhizospheric arbuscular mycorrhizal fungal microbiome of successive crops in canola-pulse-cereal rotations? In Preparation

Lay C-Y, L Wang, J Tremblay, TH Bell, C Hamel, KN Harker, R Mohr, M Hijri, M St-Arnaud. Rhizosphere metatranscriptomes under different canola field management practices and relationships to canola yield. In Preparation

2. Refereed Journal Articles, Accepted or Published

Lay, C.-Y.., T.H. Bell, C. Hamel, K.N Harker, R. Mohr, C.W. Greer, E. Yergeau and M. St-Arnaud, M. (2018). Canola root-associated microbiomes in the Canadian Prairies. *Frontiers in Microbiology. 9:1188.*

Lay, C.-Y., C. Hamel, & M. St-Arnaud (2018). Taxonomy and pathogenicity of *Olpidium brassicae* and its allied species. *Fungal Biology, 122*(9), 837-846.

Niu, Y., L.D. Bainard, W.E. May, Z. Hossain, C. Hamel and Y. Gan (2018). Intensified pulse rotations buildup pea rhizosphere pathogens in cereal and pulse based cropping systems. *Frontiers in Microbiology, 9*: 1909. doi:10.3389/fmicb.2018.01909

Li Y., Laterrière M., Lay C.-Y., Klabi R., **Masse J**., St-Arnaud M., Yergeau É., Lupwayi N.Z., Gan Y., Hamel C. (2021) Effects of arbuscular mycorrhizal fungi inoculation and crop sequence on root-associated microbiome, crop productivity and nutrient uptake in wheat-based and flax-based cropping systems. Applied Soil Ecology. 168.

Floc'H JB., Hamel C., Harker N., St-Arnaud M., (2020). Fungal communities of the canola rhizosphere: keystone species and substancial between-year variation of the rhizosphere microbiome. Microbial Egology 80 (4): 762-777.

Floc'H JB., Hamel C., Lupwayi N., Harker N., Hijri M., St-Arnaud M., (2020). Bacterial communities of the canola rhizosphere: Network analysis reveal a core bacterium shaping microbial interactions. Frontiers in Microbiology 11: 1587. 10.3389/fmicb.2020.01587

Kamrun N., Floc'H JB., Goyer C., Zebarth BJ., Whitney S. (2020) "Diversity of soil bacterial communities is influenced by spatial location and time but not potato cultivar." Phytobiomes Journal 4: 225. 10.1094/PBIOMES-01-20-0002-R

Berthouly-Salazar C., Mariac C., Couderc M., Pouzadoux J., Floc'h JB., Vigouroux Y., (2016). Genotyping-by-Sequencing SNP Identification for Crops without a Reference

Genome: Using Transcriptome Based Mapping as an Alternative Strategy. Frontiers in Plant Science 7: 777. 10.3389/fpls.2016.00777

Wang L, Gan Y, Bainard L, Hamel C, St-Arnaud M, Hijri M (2020) Expression of N-cycling genes of root microbiomes provides insights for sustaining oilseed crop production. Environmental Microbiology 22 (11), 4545-4556.

Floc'H JB., Hamel C., Laterriere M., Tidemann B., St-Arnaud M., Hijri M., (2021) Inter-kingdom networks of canola microbiome reveals *Bradyrhizobium* as keystone species and underline the importance of bulk soil in microbial studies to enhance canola production. Microbial Ecology, DOI: 10.1007/s00248-021-01905-6

Yang T., Lupwayi N., St-Arnaud M., Siddique K., Bainard L.B. 2021. Anthropogenic drivers of soil microbial communities and impacts on soil biological functions in agroecosystems. Global Ecology & Conservation 27, e01521.

3. Conferences Presentations and Posters

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PHOTOS COURTESY OF MARC ST-ARNAUD

ENHANCING THE BENEFICIAL ROOT MICROBIOME IN CANOLA

Improving crop performance of canola in the Canadian Prairies.

by Donna Fleury

Increasingly, research is showing that canola root-associated microbiomes can impact crop growth and nutrient uptake. Some of the soil micro-organisms are beneficial to plants that protect them against pathogens, mitigate the impact of abiotic stress, improve plant nutrition or stimulate plant growth by phytohormones. Gaining a better understanding of the influence these organisms have on each other and the complex webs of interactions that can be enhanced to optimize crop production is important for canola and other crops in Western Canada.

Researchers Marc St-Arnaud and Mohamed Hijri at the University of Montreal, and collaborators with Agriculture and Agri-Food Canada in Western Canada and Quebec are studying the root and rhizosphere microbiomes of canola. Through various projects, researchers are trying to better understand the core root microbiome of canola, and whether or not it is distinct from other crops such as wheat and pea. They are also trying to determine agronomic practices and crop rotations that favour the establishment of a beneficial root microbiome in canola-based rotations to optimize the efficiency of plant production. Field experiments have been conducted at several locations in Western Canada, while most of the DNA, molecular genetics and other lab analysis are conducted at the University of Montreal.

"Results from recent projects have shown that the microbiome associated with crops is very different between key species," St-Arnaud explains. "Crop diversification also has a significant impact on the microbiome." In one recently completed project, graduate student Jean-Baptiste Floc'h, compared the results of field

ABOVE: **Andrew Blakney, PhD student, and post-doc Jacynthe Masse preparing DNA for sequencing the canola microbiome to identify the beneficial microbes.**

Post-doctoral researcher Wang Li extracting RNA from canola roots to analyze the microbial genes involved in canola growth.

experiments conducted in 2013 and 2016 at three locations in Western Canada: Lacombe, Alta., Lethbridge, Alta., and Scott, Sask. The study was based on the canola phase of an existing long-term five cropping system field experiment, including one of two types of canola (Roundup Ready and Liberty Link), and compared continuous canola to very diversified rotations. The results showed that crop diversification has significant impact on the structure of rhizosphere fungal communities. "We also discovered and described a canola core microbiome, which included a very diverse number of microbes that have a positive correlation to canola yield." Floc'h will be continuing research into the canola microbiome through a PhD program started in the fall of 2018.

"One of our priorities is to find a way to increase the proportion of beneficial organisms, through agronomic practices such as seeding density, fertilizer rates or other treatments, and their effect on the microbiome population. "We are also studying the effect of crop rotation and intensity on the beneficial or detrimental organisms in the microbiome," says St-Arnaud. "As part of this study, we are also trying to look at all of the microbes associated with the canola rhizosphere, not only to identify which organisms are there but also which ones are the most active and what they are doing in the soil. The beneficial nature of the micro-organisms is being determined based on identity and abundance of important functional genes, as well as on crop performance. One other factor that has shown to have a significant influence on the combination of micro-organisms in the soil is the effect of rainfall and temperature. We have found big differences between a rainy year and a dry year, particularly in areas like Swift Current where rainfall can be variable. Therefore, understanding the microbiome population and proportion of beneficial organisms under variable field conditions is also a priority."

In another project conducted in 2014 using a similar long-term cropping system at three locations in Western Canada (Lacombe and Beaverlodge, Alta., and Brandon, Man.), researchers compared the canola root-associated microbiome with those of wheat and pea grown alongside canola in the same fields. They also compared the effect of selected agronomic treatments, including two canola seeding rates (recommended rate and 150 per cent of the recommended rate) and two fertilizer rates (recommended rate and 150 per cent of the recommended rate) on the canola microbiome. The effect of crop and treatment on the diversity of bacterial, fungal, and archaeal assemblages associated with the roots and rhizosphere soil was assessed. The results showed that canola has a core microbiome distinct from those of wheat and pea and that the root and rhizosphere microbiomes significantly responded to the agronomic treatments. Researchers also found treatment-specific changes in the relationship between bacterial and fungal microbiome members.

"Along with crop rotation, we also found that different crop cultivars can be an important factor influencing the microbiome," Hijri adds. "Farmers should be aware that some cultivars respond better than others in terms of biodiversity of the microbiome, and will want to consider that in cultivar selection. As well, unlike most other crops, canola is one of the rare plants that cannot form mycorrhiza and the symbiotic association between the plant roots and soil fungi. Therefore, the impact of more intensive canola rotations may also reduce the proportion of other important beneficial fungi more than first considered."

Other research underway is a focus on studying the microbial

Intellectual Property Protection

Filing of patent applications:	Not applicable
Registration of copyright for computer software or databases:	Not applicable
Registration of copyright for educational materials:	Not applicable
Registration of industrial designs:	Not applicable
Filing for protection of trademarks:	Not applicable
Registration of integrated circuit topographies:	Not applicable
Filing of applications for plant breeders' rights:	Not applicable
Execution of non-disclosure or confidentiality agreements:	Not applicable
Other (specify):	Not applicable

Collaboration with the Partners

How was this research project initiated?

- [x] The university researcher approached the partners
- [ ] The partners approached the university researcher
- [ ] The government partner approached the university
- [x] There was a previous collaboration with the partners
- [ ] This is a new collaboration
- [ ] Other (specify)

Did this project arise from a grant funded by the NSERC Strategic Workshops Program? ● Yes ○ No

Did this project arise from a grant funded by the Interaction and/or Engage Program? ○ Yes ● No

Collaboration with the Partners

Briefly describe the process.

Three collaborators at Agriculture Canada Swift Current have been received funds from Canola Council of Canada and Saskatchewan Pulse Growers.
Our team applied in a Canola Council of Canada's competition which was successful. After securing Canola Council of Canada funds, we applied for NSERC-CRD grant as a matching fund.

Collaboration with the Partners

To what extent were the partners involved in the project? Rate your answer on a scale from 1 to 7.

Not at all			Somewhat			To a great extent
○ 1	○ 2	○ 3	○ 4	○ 5	● 6	○ 7

In what way were the partners directly involved in the project? (Select all that apply.)

- [x] Partners were available for consultation
- [ ] Partners provided facilities
- [ ] Partners provided training
- [ ] Partners co-supervised students' theses
- [ ] Partners received training from university personnel
- [ ] Personnel from the partner organization received training from the university
- [x] Partners discussed the project regularly with the university team

  Average number of meetings per year: 2
- [ ] Partners were involved in the research
- [x] Other (specify)

  Partners were involved in the outreach

Collaboration with the Partners

Describe the partners' involvement and comment on the collaboration.

Canola Council if Canada greatly helped for media coverage and outreach for canola growers.

Future Plans

What links are you maintaining with the partners? (Select all that apply.)

- [x] Collaborating with the partners on the same research
- [x] Collaborating with the partners on other research
- [ ] Collaborating with other partners on the same research
- [ ] Continuing the research without partners
- [ ] No contact with the partners currently and none planned
- [ ] No contact with the partners currently but future collaboration planned

Future Plans

Describe any follow-up or related work that will be undertaken as a result of this project, who will be involved in this work (including partners) and how it will be funded.

During this project, we generated a huge amount of next-generation sequencing that we will continu to put our effort for bioinformatics and biostatistics analyses.

Future Plans

Describe any additional links that the partners will maintain with the university.

Our team gained expertise n canola's and pulse's microbiota and we will be happy to provide technical support to Canola Council of Canada and Saskatchewan Pulse Growers through consultations.

Knowledge and Technology Transfer

Research results transferred to the partners

- [x] Through informal discussions
- [x] Through reports provided to the partners
- [ ] As a result of the partners participating in the research
- [ ] Through formal publications
- [ ] Through patents
- [ ] Through licencing arrangements
- [ ] The research results have not been transferred to the partner
- [ ] Other (specify)

Research results being used and/or will be used by the partners

As a stimulus for future R&D:	Potential to be used
To enhance the skills and knowledge of personnel in the partner's organization:	Potential to be used
To improve an existing product:	Have been used
To improve an existing process:	Have been used
To improve an existing service:	Potential to be used
To develop a new product:	No potential to be used
To develop a new process:	No potential to be used
To develop a new service:	No potential to be used
To contribute to a policy, regulation or standard:	Potential to be used

Other (specify):

Knowledge and Technology Transfer

Briefly describe these outcomes

Our results demonstrated clearly that some rotation consequences can save Nitrogen fertilization use while others can have negative effect on greenhouse gaz emission through denitrification.
We also documented that monoculture of canola doesn't have a negative effect of mycorrhizal fungal communities as previously bileived.

Knowledge and Technology Transfer

Describe any environmental or social benefit that resulted or could result in the future from this research

Nitrogen fertilization contributes greenhouse gaz emission through denitrification. We documented that lentil followed by canola is the best agricultural practice that reduces denitrification process which also translates to reduce nitrogen fertilizer's uses and save input costs for farmers.

Impact on Researcher

Impact the project had on your teaching

- [x] Creation of new courses
- [x] New content for existing courses
- [ ] Use of real world examples in courses
- [ ] Guest lectures from partners
- [ ] New equipment/material
- [ ] Project has had no impact on my teaching
- [ ] Other (specify)

Impact the project had on your research

- [ ] Influenced the direction to more industrially relevant topics
- [x] Opened up new opportunities for research beyond the original objectives
- [ ] The project has had no impact on my research
- [ ] Other (specify)

Contributions from Other Sources

Partners	Total Cash		Total In-Kind	
Company Name	**Committed**	**Received**	**Committed**	**Received**
Canola Council of Canada	200,000	200,000	0	0
	Have you had previous research collaborations with this partner? Yes - In the same and other research area			
Saskatchewan Pusle Growers	0	0	173,820	173,820
	Have you had previous research collaborations with this partner? Yes - In the same and other research area			
	0	0	0	0
	Have you had previous research collaborations with this partner?			
	0	0	0	0
	Have you had previous research collaborations with this partner?			
	0	0	0	0
	Have you had previous research collaborations with this partner?			
	0	0	0	0
	Have you had previous research collaborations with this partner?			
	0	0	0	0
	Have you had previous research collaborations with this partner?			

Contributions from Other Sources

Other Sources	Total Cash		Total In-Kind	
		Received		Received
		0		0
		0		0
		0		0
		0		0
		0		0
		0		0
		0		0
Total (partners and other sources)	**$200,000**	**$200,000**	**$173,820**	**$173,820**

Contributions from Other Sources

Variation described between commitment and actual cash and in-kind contributions

The in-kind contribution of Saskatchewan Pulse Growers supported indirectly
costs of research related to filed experiments (setup, inputs, fellow up, physics-chemical analyses, sampling, shipping and harvest).

Financial Information

Consolidated balance remaining at the end of the project:

Budget Items	Total Budget	Total Actual Expenditure	Percent Variation
1) Salaries and benefits			
PhD students	33,000	87,999	167
Master's students	0	13,765	999
Undergraduate students	0	2,700	999
Postdoctoral fellows	288,981	59,951	-79
Technical/professional assistants	22,500	43,094	92
In-Kind Contribution	83,820	83,820	0
2) Equipment or facility			
Purchase or rental	6,204	9,355	51
Operation and maintenance costs	0	0	0
User fees	0	0	0
Sequencing	43,189	12,052	-72
3) Materials and supplies			
Materials and supplies	95,604	110,658	16
Soil/Plant analyses	9,600	0	-999
	0	0	0
4) Travel			
Conferences	8,000	11,010	38
Field work	2,000	0	-999
Project related travel	2,300	0	-999
In-King Field work	90,000	90,000	0
5) Dissemination			
Publication costs	3,500	0	-999
	0	0	0
6) Technology transfer activities			
Field trials	0	0	0
Prototypes	0	0	0
Meetings	3,000	0	-999
7) Others (specify)			
Overhead Cost	0	26,073	999
	0	0	0
Total	691,698	550,477	-20

Financial Information

Explanation for the variation of each budget item

Salaries and benefits
Jacynthe Masse received a postdoc scholarship from Quebec Government which explain the difference of the amount of fund planned for postdoc salary.
However, we fully supported two PhD students for a period of 4 years which resulted an increase of expenses. Jean-Baptiste Floc'h was supported as Master Student at the first year, and then continued as PhD student.
We underestimated the cost of technical support.

Equipment or facility
We used other funds to partially support sequencing services resulting in saving funds for this category and used it for other category.

Dissemination
We used other funds for publication expenses.

Technology transfer activities
Our meeting were done via visioconférences. We didn't spend funds for this category.