Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed for the year and note the personnel involved. It should also note any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. A complete statement of expenses should be included. In the event of major changes within the budget, supporting notes should be included. The report should capture a complete summary of activity for the final year and an overview of the entire project.

Project Title: Canola frequency effects on nutrient turnover and root-microbe interactions

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Bobbi Helgason	Agriculture and Agri-Food Canada Saskatoon RDC	**Co-Principal Investigator**
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Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Breanne Tidemann	AAFC Lacombe RDC	**Co-Investigator**
Steven Siciliano	University of Saskatchewan	**Co-Investigator**
Melissa Arcand	University of Saskatchewan	**Co-Investigator**
Jennifer Town	AAFC Canada Saskatoon RDC	**Co-Investigator**

Project Start Date: April 8, 2018 **Project Completion Date:** Sept 30, 2021

Reporting Period: Feb 15, 2021 to Sept 30, 2021

CARP Project Number: 2018.14

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition, a Final Extension Report is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for

consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as set out in the award letter. The Final Extension Report is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: __Sept 30, 2021___

_____ Ahead of Schedule _____ On Schedule _____ Behind Schedule __x___ Completed

Comments:

According to the planned project guidelines, the field sample data collection for the 2019 field season has been completed. In addition, all data for the 2018 and 2019 field seasons has been generated and all samples have been archived with a tracking database included. Due to COVID-19 related work interruptions, a 6 month no-cost extension was granted for this work. Complete analysis and interpretation of data for both field seasons has now been completed by September 30, 2021.

2. Abstract/Summary - Maximum of one page. This must include project objectives, results, and conclusions for use on the Funders' websites.

Crop rotation comprises an important facet of sustainable, healthy agroecosystems aiding in disease suppression, nutrient cycling and risk mitigation. However, on-farm cropping strategies are based on a complex suite of biological, economic and social factors which determine planting decisions from year to year. Both the total acreage and frequency of canola on individual farms have increased significantly in recent years and in some cases, this practice has resulted in negative impacts on disease pressures, balanced nutrient cycling and soil chemistry. Agronomic impacts of canola frequency for both glufosinate-resistant Liberty Link (LL) and glyphosate-resistant RoundUp Ready (RR) canola grown in continuous, canola-wheat and canola-pea-barley were examined by simultaneously measuring nutrient fluxes, changes in microbial community structures in the soil, rhizosphere and roots as well as the secretion of root exudates.

The effects of crop rotation strategy on soil nutrient fluxes varied significantly depending on the site and growing season, with the most consistent and significant effects found at Swift Current. Changes in root exudate profiles under monocropped vs. rotation management were also site dependent, with significant changes found at Lacombe and Swift Current, but not Scott.

The effect of crop rotation on the fungal community was greater compared to the bacterial community, with several fungal indicator species associated with different rotatioin strategies found consistently across sites and growing seasons. This included the association of *Olpidium brassicae*, *Alternaria* spp. Pleosporales, Stachybotryaceae and Sordariomycetes with monocropped canola and *Penicillum stolkiae*, *Volutella ciliae*, *Lachnum* sp., *Humicola nigrens* and *Diaporthe columnaris* with the canola-pea-barley rotation.

By generating complementary data about how nutrient cycling as well as soil and plant-root associated bacteria and fungi differ with varied crop rotation, we were able to identify positive and negative implications of canola frequency and refine agronomic recommendations for canola production. This research has provided new knowledge of the impacts of short rotation canola on soil properties and the microorganisms that associate with canola roots, ultimately affecting crop productivity and profitability.

3. Introduction – Brief project background, rationale and objectives.

Canola (*Brassica napus*) is an important crop for Canadian producers; both the total acreage and frequency of canola on individual farms have increased significantly in recent years. As a result, continuous or 2-year canola in rotation has become increasingly common. While the importance of crop rotation for reducing pathogen host-crop incidence is accepted as important, less is known about the indirect impacts of canola frequency on the broader soil and plant microbiome and how this subsequently affects soil nutrient cycling and uptake. It is important to demonstrate the potential direct and indirect benefits of using canola in diverse rotations to support best management recommendations that are convincing to producers.

Canola is known to have diverse and abundant root and rhizosphere microbiomes (Dunfield and Germida, 2001). This microbiome has important roles in plant nutrient uptake, resistance to pests and other stresses and for maintaining soil fertility. Because canola has a characteristic biochemical composition (e.g. glucosinolates) that may have allelopathic functions (Asaduzzaman et al. 2017), it is expected to have strong short term impacts on soil microbial community structure and function. Whether these effects are temporary (Mocai et al. 2015) or longer lasting may depend on the frequency of canola cropping as the influence of potentially persistent allelopathic compounds on soil biota is unknown. *Brassica* spp. do not associate with arbuscular mycorrhizal fungi (AMF), ubiquitous symbiotic soil fungi that have important roles in water and nutrient (especially P) acquisition by many other plants. Non-host crops decrease the AMF inoculum potential in the soil and mycorrhizal colonization of subsequent host crops (e.g. cereals) and may decrease translocation of fresh plant C to soil microbes (Kaiser et al. 2015). Because canola is not an AMF host crop, it may have other means of acquiring P such as direct excretion of P solubilizing organic acids as reflected in the root exudates. Root exudates aid in plant nutrient acquisition and also support increased abundance and activity of soil microorganisms; exudates are thought to be a primary mechanism through which plants manipulate their microbiomes (Steinauer et al. 2016). Together, these characteristics can act as soil microbiome disruptors, with greater potential benefits and detriments when canola is grown with high frequency.

Diverse crop rotations are an important component of healthy, resilient production systems (Dias et al. 2014). Compared to monoculture, diverse rotations have higher levels of nutrient cycling, and appear to have broader soil microbial metabolic capabilities perhaps owing to a greater diversity of residue input types (McDaniel et al. 2014; Souza et al. 2015). These attributes are important for maintaining soil productivity when disturbance events occur (e.g. extreme weather, new pest pressures). A long term experiment comparing monculture wheat to a diverse 4 yr rotation that includes canola and field pea at Swift Current, SK has shown that rotation leads to higher soil enzyme activity, changes in bacterial community structure, changes in soil organic matter composition and ultimately, improved soil fertility (Town et al. *in press*). This work has also demonstrated a stronger influence of canola on soil microbial community structure and their functions, compared to wheat and field pea. There is evidence that the decomposition of canola residues changes the dynamics of nitrogen cycling processes (Farrell et al. 2014). The extent to which these differences are realized through changes in microbial nutrient turnover dynamics (indirect mechanisms) or are more directly related to plant nutrient uptake is unknown.

A better understanding of the impacts of *Brassica napus* on soil biodiversity, the root microbiome and nutrient cycling processes is needed to help optimize canola productivity and to ensure the prolonged sustainability of farming systems that include canola as a cornerstone crop. This work will simultaneously measure nutrient fluxes and changes in microbial community structures in the soil, rhizosphere and in canola roots.

Characterization of root exudates will help to explain how nutrient cycling and microbial communities might be influenced by plant resource availability and needs. Agricultural management practices such as crop rotation impose important changes on soil characteristics that accumulate over time. Beginning with differences in crop nutrient demands and eventually leaving behind crop residues of varied quality, both the microbial decomposer community and eventually soil fertility are affected. By working in this model long-term rotation experiment, we were be able to measure important functional responses of the soil and plant microbiome to different canola frequencies over the past decade. This information will be used identify positive and negative implications of canola frequency to refine the development of agronomic recommendations for canola production used by extension specialists and producers.

4. Methods – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be cited and the reason(s) for the change should be specified.

Field study description and sample collection:

This CARP project provides functional information about nutrient cycling and plant root-microbe interactions of canola grown in long term rotations at different locations in Saskatchewan and Alberta. Established in 2008, this 12 year field experiment was established at 5 sites in Saskatchewan and Alberta. It examines frequency of both glufosinate-resistant Liberty Link (LL) and glyphosate-resistant RoundUp Ready (RR) canola in continuous, 2 and 3 year rotations (continuous canola, canola-wheat and canola-pea-barley) (see Harker et al. 2015 for further details).

We targeted Swift Current, Scott and Lacombe study sites which encompass the Brown, Dark Brown and Black soil zones to capture regional differences in climate and soil type (additional sites at Melfort, SK and Lethbridge, AB). Sampling efforts focused on canola. Specifically, we sampled both LL and RR canola in the canola phase of all 3 rotations in both 2018 and 2019 which represent the 11^{th} and 12^{th} years of the long-term experiment (Tidemann, Helgason).

Total number of samples collected: 3 sites x 6 treatments x 4 reps x 2 years = 144.

Specific objectives were to:

1) Measure nutrient fluxes (PRS probes) during a 6 week period of the growing season (Arcand and Helgason).
2) Perform targeted high throughput amplicon sequencing to characterize bacterial and fungal communities at canola flowering (Helgason).
3) Characterize functional plant-microbe interactions through root exudate profiling (Siciliano).
4) Archive samples for future use to build on the new knowledge built in the current proposed research (Helgason).

Plant Root Simulator (PRS) probes were installed and exchanged twice after 14 and 28 d, for a total of 3 sets over a 6-week period. Probes were rinsed and sent to Western Ag Innovations for analysis (Complete package including macro and micronutrients).

At peak canola flowering, we collected 3-4 plants from each plot using a hand trowel. Plants and accompanying soil were transported in cold storage immediately to the Helgason lab for processing the following day. Canola plants were removed from the sampling bag, shaken rigorously 5 times to remove any loosely adhering soil and the soil that remained adhered to the roots was considered rhizosphere soil.

Roots and adhering soil were weighed, placed in an Erlenmeyer flask with 100mL of 0.05M NaCl and shaken for 15 min. The roots were then removed from the flask and rinsed with sterile water. In year 2, the solution containing the rhizosphere soil was transferred to two 50mL centrifuge tubes and centrifuged at 5000rpm for 15 min. at 4°C. The supernatant was transferred to clean 50mL falcon tubes and frozen at -20°C until analysis.

Root exudate analysis (year 2 only):

The supernatant containing the root exudates suspended in 0.05M NaCl was filtered, measured for pH, purified and concentrated using a Strata TM-X-AW33 Polymeric Weak Anion solid phase extraction column and analyzed using ion chromatography on a Dionex ICS-2000. Organic acids including formate, malate and succinate were quantified based on known standards.

Root, rhizosphere and bulk soil microbiome characterization:

Rhizosphere soil were sub-sampled into two 1.5mL Eppendorf tubes and frozen at -80°C for further analysis. Roots were transferred to a sterile petri plate and cut into ~0.5cm segments using a sterile scalpel, then stored in two 1.5mL Eppendorf tubes at -80°C until DNA extraction. DNA bulk soil and rhizosphere samples were extracted from 200mg samples of soil using the Qiagen PowerSoil DNA extraction kit.DNA from roots was extracted from 250mg of minced root tissue using the Qiagen Power Plant DNA extraction kit. Products from the amplification of gene targets for bacteria (16S rRNA, V3-V4) and fungi (ITS1) were sequenced using Illumina chemistry. Sequencing data was used for amplicon sequence variant (ASV) calling with DADA2, and taxonomic profiling using the SILVA (bacteria) and UNITE (fungi) databases. The resulting frequency classification data was used to determine changes in alpha and beta diversity of the microbiome in response to rotation strategy.

5. Results – Present and discuss project results, including data, graphs, models, maps, design and technology development.

Project Activities related to objectives and deliverables:

1) **Objective: Measure nutrient fluxes (Plant Root Simulator probes) during a 6 week period of the growing season (Arcand/Helgason).**
   Deliverable: Data set of nutrient fluxes and root exudates.

   - Plant Root Simulator (PRS) probes from Western Ag were used to measure nutrient fluxes for NO_3, NH_4, P, K, S, Ca, Mg, Mn, Fe, Pb, Cu, Zn, Al, B and Cd. Results for Pb, Cu, and Cd were below the limit of detection for these assays and were excluded from further analysis

   Activity:

   - The effect of crop rotation on soil nutrient fluxes was variable depending on the field site and sampling year. Principal component analysis (PCA) of the PRS data showed that at Swift Current, rotation strategy was associated with significant differences in overall soil nutrient fluxes (PERMANOVA, α=0.05) before and during peak flowering in both the 2018 and 2019 growing seasons (Table 1). PERMANOVA is a multivariate statistical analysis where the p-value indicates a statistically significant effect and the R^2 value explains the proportion of the variation in the multivariate dataset that is explained by the factor (e.g. "before" flowering at Swift Current = 21%). Both Scott and Lacombe only showed significant differences in the overall nutrient fluxes at single time points in 2019; before flowering at Scott and during peak flowering at Lacombe (Table 1). Cultivar selection was not associated with significant differences in complete soil nutrient flux profiles at any site.

   - In terms of soil nutrient fluxes for specific macro- and micronutrients, Swift Current saw an increase in K fluxes from continuous canola in both growing seasons, and an increase in soil S in 2018. NO_3 fluxes were higher in the canola-pea-barley rotation in 2018 however in 2019, they were higher in the monocropped canola. Both Mg and Ca were significantly lower in the canola-wheat rotation compared to both monocropped canola and canola-pea-barley in 2018 at the early and peak flowering time points.

In 2019 at Scott, rotation was associated with significant differences in P and Fe while at Lacombe, significant differences in NO_3 and K were associated with rotation (Figure 1).

- At Swift Current, cultivar selection was associated with differences in P fluxes in the soil, with average soil P higher in plots planted with InVigor® L241C compared to Roundup Ready® 75-42 at peak flowering in 2018, and peak and late flowering in 2019. There were no significant differences between cultivars for specific nutrients at either Scott or Lacombe (Figure 2).

2) **Objective: Perform targeted high throughput amplicon sequencing to characterize bacterial and fungal communities at canola flowering (Helgason/Dumonceaux/Town).**
Deliverable: Sample collection and processing. DNA extracts from soil, rhizosphere and canola roots.

- Aliquots of all field sample soil, rhizosphere and root samples as well as extracted total genomic DNA have been archived at -80°C to be made available for further analysis if required.

Deliverable: Data set of bacterial and fungal abundance and community structure.

- All amplicon sequencing-based profiles for the bacterial (16S) and fungal (ITS) communities in the root, rhizosphere and bulk soil from all sites has been completed for both the 2018 and 2019 field season. This data will be deposited in the Short Read Archive (SRA) repository at GenBank (National Center for Biotechnology Information).

Activity:

- Analysis of the overall structure of the microbiome in soil, rhizosphere and root samples (beta diversity) revealed that the effects of crop rotation on bacterial community composition were variable depending on the study site while fungal community composition was significantly affected across all sites and sample compartments (PERMANOVA, α=0.05). PERMANOVA results also indicated that the effect of crop rotation on the fungal community was greater compared to the bacterial community. Cultivar selection was only significantly correlated to changes in the fungal community in the root samples at Lacombe (Table 2 & Table 3).

- Indicator species analysis identifies ASV sequences that are significantly associated with rotation strategy. The analysis consists of two metrics: first, the probability that the sequence is found in samples from a specific rotation strategy (positive predictive value); and second, that all samples from that rotation group contain the sequence (sensitivity). Significant values are scored at >0.75 (α=0.05). For each site, indicator ASVs that were identified in at least one sample type (root, rhiszosphere or bulk soil) in both 2018 and 2019 were retained. At Lacombe, there were several fungal ASV sequences that were very strongly associated with rotation strategy. Three ASVs classified as Olpidiales and most closely related to *Olpidium brassicae* were significantly associated with monocropped canola, with one sequence in particular, *Olpidium brassicae* f509, determined to be very abundant in the soil, rhizosphere and root libraries. An ASV identical to sequences from *Alternaria japonica* was also significantly associated with monocropped canola, and this fungus has been previously linked to leaf spot disease (Figure 3). Samples from both the Scott and Swift Current sites had several fungal ASV sequences that were significantly associated with either monocropping or the canola-pea-barley rotation. One sequence classified as *Penicillium stolkiae* was significantly associated with soil and rhizosphere samples from the canola-pea-barley rotation at both sites in both 2018 and 2019 (Figure 4 & Figure 5). *Penicillium* spp. have been previously associated with P solubilization in soil, and *Penicillium bilaiae* is currently marketed as the growth-promoting inoculant Jumpstart™ (Monsanto BioAg).

- Analysis of the fungal community profiles identified two closely-related *Olpidium brassicae* sequences that were hyper-abundant in root samples at all three sampling locations (Swift Current, Scott, and

Lacombe). In particular, samples from Swift Current and Scott were dominated by only one of these microorganisms, while the site at Lacombe revealed the presence of both of these microorganisms, with abundances that varied according both to rotation and to canola cultivar. These results were validated using real-time qPCR assays that were able to differentiate and quantify each of the ASVs independently in the Lacombe samples (Figure 6). While *O. brassicae* is not known to be a pathogen of canola, the related fungus *O. virulentis* is a known vector of plant pathogenic viruses (Maccarone LD Plant Dis. 2013;97(6):700-7). At present, it is unknown how these observations may relate to pathogenicity, but it is clear that we can discriminate between these microorganisms using the ASV approach for data analysis and that their abundances are dramatically different at the Lacombe site according to rotational strategy. It is important to note that traditional analysis methods using clustering at 97% identity would have grouped these two organisms into a single OTU and that this pattern would thereby have been completely missed.

3) **Objective: Characterize functional plant-microbe interactions through root exudate profiling (Helgason/Siciliano).**

Deliverable: Data set of nutrient fluxes and root exudates

- The washing procedure used to separate the rhizosphere soil from the root tissue was corrected for the 2019 field season so the root exudate data is available for the 2019 samples.

Activity:

- Principal component analysis (PCA) of the root exudate data showed that at Lacombe, rotation strategy was associated with significant differences in overall root exudate composition (PERMANOVA, α=0.05), while there were no significant differences found at either Swift Current or Scott (Table 4).

- At Swift Current, tartarate was significantly higher in the root exudates from the canola-pea-barley rotation canola while formate was significantly higher in continuous canola. At Lacombe, tartarate, formate and oxalate were all significantly higher in continuous canola compared to either the canola-wheat or canola- rotations (Figure 7).

4) Objective: Archive samples for future use to build on the new knowledge built in the current proposed research (Helgason/Dumonceaux).
Deliverable: A set of characterized, catalogued soil samples to be used for future research projects.

- Aliquots of all field sample DNA extracts and soil samples have been archived at -80°C to be made available for further analysis if required.

Activity:

- A new ultra low temperature freezer was procured to house the samples, and a database of sample IDs was prepared to catalog the samples for future analysis as required.

Summary:

- The effects of crop rotation strategy on soil nutrient fluxes varied significantly depending on the site and growing season, with the most consistent and significant effects found at Swift Current.
- The effect of crop rotation on the fungal community was greater compared to the bacterial community, while cultivar was significantly correlated to changes in fungal community in the root samples at a single sampling location (Lacombe). While changes in the microbiota in response to rotation strategy were variable depending on the field site, there were some findings that were consistent across both growing

seasons and at multiple sites. This included the identification of bacterial and fungal indicator sequences with abundances that are strongly associated with crop rotation strategy: *Olpidium brassicae*, *Alternaria* spp. Pleosporales, Stachybotryaceae and Sordariomycetes (significantly associated with monocropped canola); *Penicillum stolkiae*, *Volutella ciliae, Lachnum* sp., *Humicola nigrens* and *Diaporthe columnaris* (canola-pea-barley).

- The fungus *Olpidium brassicae* was found to be ubiquitous and highly abundant in the roots and rhizosphere of canola under all conditions at all sites. *O. brassicae* strain 'd3f1' was the most abundant across all sites while strain 'f509', found exclusively at Lacombe, was significantly higher in the monocropped canola in both growing seasons. Given the lack of literature examining the role of *Olpidium* spp. in canola, further research characterizing its impact on health and resiliency warrants further examination.
- Significant effects of crop rotation strategy on root exudate profiles were found only at the Lacombe site. Interestingly, there were consistently significant effects of crop rotation on bacterial and fungal communities in the root, rhizosphere and bulk soil at this site in 2019.

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

The effects of long term canola frequency on nutrient fluxes during the critical period of flowering were transient and site-dependent. We speculate that the addition of rotation–specific fertilizer each year based on soil test helped to ensure adequate nutrient availability for crop growth. It was more likely soil and environmental factors like precipitation that led to the differences in nutrient fluxes that were observed. This finding indicates that soil-test based fertilizer management may be an important tool for ensuring adequate nutrient supply for short rotations.

It was surprising that although canola frequency effects on bacterial communities in the soil, rhizosphere and roots were observed, there was not a consistent impact at the whole community level. At Swift Current, shifts in bacterial community structure were observed in all but one instance, indicating that crop rotation may be most impactful for shifting bacterial community composition at this location. Fungal community structure on the other hand, was consistently affected by crop rotation at all sites in the root, rhizosphere and bulk soil. This indicates that increased frequency of canola in rotation is fundamentally changing the composition of fungi in all of these environments with likely impacts for nutrient cycling and crop performance. The stronger impacts of crop rotation on the fungal community may be related to the fact that canola is a non-mycorrhizal crop and thus with increasing canola frequency there will be a selective pressure (lack of a host crop) on these important symbionts. Although we didn't examine arbuscular mycorrhizal fungi (AMF) directly, shifts in AMF abundance and/or diversity may have had cascading effects on the broader fungal community.

Digging deeper in the changes in key species within the bacterial and fungal communities, we saw that there were impacts of crop rotation on the abundance of individual organisms, particularly fungi. These differences in the prominence of individual fungi and bacteria were greatest in the soil and rhizosphere indicating that the primary impact on function was more likely related to nutrient cycling and/or decomposition than to disease.

Continuously cropping with canola promoted the dominance of *Olpidium brassicae*, a poorly understood colonizer of canola roots. This fungus was present in the roots at all sites and was increasingly dominant in the fungal root community in short rotation canola. Because of the similarity in life cycle and root colonization to

clubroot, further investigation is warranted to determine whether this dominant root fungus plays a role in the disease profile of canola roots. For example, it is possible that *O. brassicae* might exclude other root pathogens by "competitive exclusion" of the root niche, that is by dominating the environment and preventing colonization by more virulent pathogens. The ecology and physiology of the relationship between *O. brassicae* and canola is an important avenue for future research.

7. Extension and communication activities: (e.g. extension meetings, papers produced, conference presentations made, photos).

Dr. Town gave an interview to Carolyn King of Top Crop Manager (*to be published fall, 2021*).

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

9. Literature Cited.

Dias et al. 2014. J. Sci. Food Agric. Doi:10.1002/jsfa.6565.
Dunfield and Germida. 2001. FEMS Microbiol. Ecol. 38:1-9.
Farrell et al. 2014. ADF Project No. 20100190 Final Report
Harker et al. 2015. Can. J Plant Sci. 95:9-20.
McDaniel et al. 2014. Soil Biol. Biochem. 2014:243-254.
Mocai et al.2015. Indust. Crops and Prod. 79-90.
Souza et al. 2015 Appl. Soil Ecol. 86:106-112.
Steinauer et al. 2016. Ecol and Evol. Doi:10.1002/ece3.2454.
Town et al. 2021. Applied Soil Ecology. (*in press*)

10. Other Administrative Aspects: personnel involved; equipment bought; project materials developed

Personnel: Dr. Jennifer Town (postdoctoral associate); Christine Hammond (Biologist; transferred to project upon maternity leave of Dr. Town); Sam Horovatin (casual computer scientist hired to assist with data analysis in final year of project).

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

		Swift Current			Scott			Lacombe		
		pseudo-F	R^2	p-value	*pseudo-F*	R^2	p-value	*pseudo-F*	R^2	p-value
2018	Before	**2.56**	**0.20**	**0.004**	0.74	0.06	0.76	1.10	0.10	0.32
	Peak	**2.81**	**0.21**	**0.005**	0.81	0.08	0.635	1.08	0.10	0.369
	After	0.73	0.06	0.755	0.82	0.07	0.643	0.85	0.07	0.609
2019	Before	**1.74**	**0.21**	**0.05**	**1.91**	**0.15**	**0.027**	1.16	0.10	0.299
	Peak	**1.66**	**0.14**	**0.05**	0.77	0.07	0.735	**2.30**	**0.19**	**0.008**
	After	1.26	0.11	0.227	1.05	0.09	0.397	1.30	0.11	0.195

Table 1. PERMANOVA Principal Component Analysis (PCA) of the complete soil nutrient profile at each site as measured by the Plant Root Simulator (PRS) probes. The pseudo-F statistic reflects the effect size and p-value≤0.05 indicates a significant correlation between crop rotation strategy and differences in overall soil nutrient composition.

16S Bray-Curtis PERMANOVA			Swift Current		Scott		Lacombe	
			pseudo-F	p-value	*pseudo*-F	p-value	*pseudo*-F	p-value
Soil	2018	Rotation	1.195	0.158	**2.860**	**0.001**	0.864	0.587
		Cultivar	0.818	0.798	1.095	0.271	0.712	0.835
		Rotation:Cultivar	0.615	0.998	0.716	0.905	0.541	0.996
	2019	Rotation	**1.908**	**0.020**	**1.847**	**0.051**	**2.813**	**0.001**
		Cultivar	0.757	0.757	0.798	0.674	1.299	0.107
		Rotation:Cultivar	0.990	0.416	0.766	0.743	0.751	0.884
Rhizosphere	2018	Rotation	**1.489**	**0.033**	1.218	0.190	**2.583**	**0.018**
		Cultivar	0.670	0.974	0.955	0.481	1.355	0.195
		Rotation:Cultivar	1.048	0.388	0.847	0.709	1.416	0.133
	2019	Rotation	**2.624**	**0.01**	1.050	0.361	**2.048**	**0.001**
		Cultivar	1.013	0.406	0.992	0.439	1.044	0.378
		Rotation:Cultivar	0.803	0.662	0.681	0.920	1.145	0.209
Rt	2018	Rotation	**1.488**	**0.042**	**2.290**	**0.001**	1.382	0.087
		Cultivar	1.068	0.320	1.223	0.141	1.006	0.496
		Rotation:Cultivar	0.992	0.487	0.795	0.800	0.986	0.470
	2019	Rotation	**2.160**	**0.017**	1.241	0.140	**2.032**	**0.002**
		Cultivar	0.916	0.523	1.052	0.385	**2.092**	**0.002**
		Rotation:Cultivar	0.723	0.784	0.856	0.689	1.071	0.321

Table 2. PERMANOVA analysis of the Bray-Curtis dissimilarity matrix for the bacterial communities from 2018 and 2019 field samples at each site. The pseudo-F statistic reflects the effect size and p-value≤0.05 indicates a significant correlation between the variable and differences in bacterial community composition.

ITS Bray-Curtis PERMANOVA			Swift Current		Scott		Lacombe	
			pseudo-F	p-value	*pseudo*-F	p-value	*pseudo*-F	p-value
Soil	2018	Rotation	**2.236**	**0.001**	**2.571**	**0.008**	**10.337**	**0.002**
		Cultivar	1.022	0.407	1.621	0.091	1.134	0.295
		Rotation:Cultivar	1.091	0.318	1.181	0.247	0.542	0.557
	2019	Rotation	**2.794**	**0.031**	**5.330**	**0.001**	**6.266**	**0.001**
		Cultivar	0.848	0.449	0.536	0.906	1.060	0.317
		Rotation:Cultivar	0.753	0.555	0.738	0.641	0.666	0.740
Rhizosphere	2018	Rotation	**6.522**	**0.001**	**2.692**	**0.043**	**7.965**	**0.001**
		Cultivar	1.351	0.201	1.060	0.329	1.770	0.154
		Rotation:Cultivar	0.891	0.442	0.408	0.895	1.656	0.155
	2019	Rotation	**2.710**	**0.057**	**3.239**	**0.022**	**11.515**	**0.001**
		Cultivar	0.709	0.531	0.355	0.906	1.334	0.242
		Rotation:Cultivar	0.446	0.750	0.334	0.938	2.533	0.071
Rt	2018	Rotation	**7.011**	**0.004**	**5.302**	**0.006**	**18.121**	**0.001**
		Cultivar	**3.972**	**0.017**	0.922	0.312	1.196	0.320
		Rotation:Cultivar	2.102	0.130	0.752	0.421	3.270	0.103
	2019	Rotation	**5.424**	**0.013**	**2.237**	**0.053**	**18.068**	**0.001**
		Cultivar	0.368	0.759	0.658	0.718	1.144	0.287
		Rotation:Cultivar	0.399	0.712	0.745	0.613	-0.159	1.000

Table 3. PERMANOVA analysis of the Bray-Curtis dissimilarity matrix for the fungal communities from 2018 and 2019 field samples at each site. The pseudo-F statistic reflects the effect size and p-value≤0.05 indicates a significant correlation between the variable and differences in fungal community composition.

		pseudo-F	R^2	p-value
Rotation 2019	Swift	0.17	0.02	0.999
	Scott	0.87	0.09	0.539
	Lacombe	**5.02**	**0.32**	**0.002**

Table 4. PERMANOVA of Principal Component Analysis (PCA) for 2019 root exudate profiles. The pseudo-F statistic reflects the effect size and p-value≤0.05 indicates a significant correlation between rotation and differences in root exudate composition.

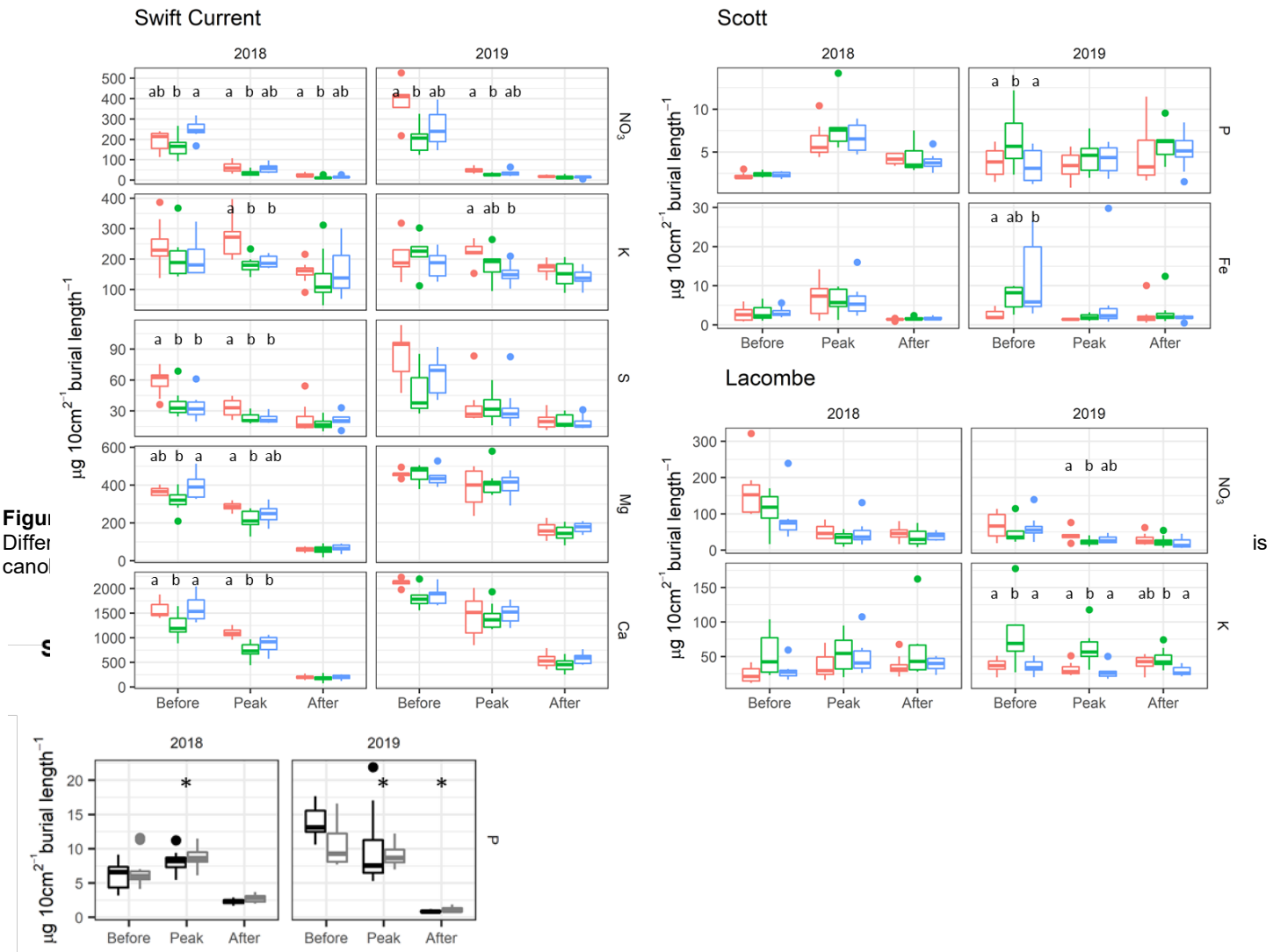


Figure 2: Soil P concentration was significantly associated with cultivar selection at Swift Current. Time points with a significant difference (p≤0.05) between cultivars are indicated with an asterisk (*).

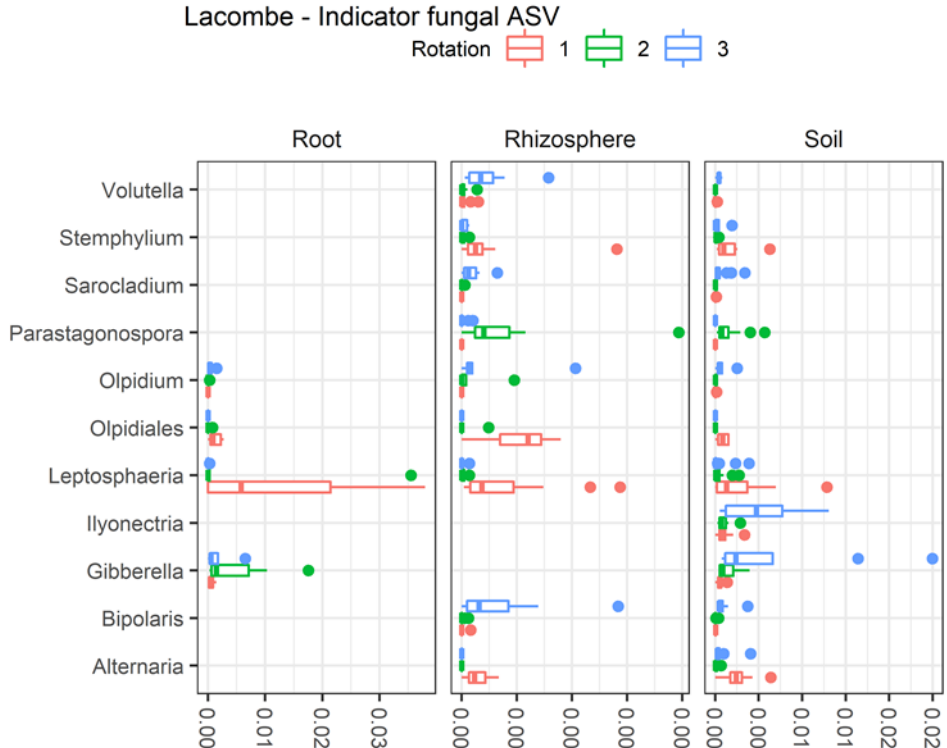
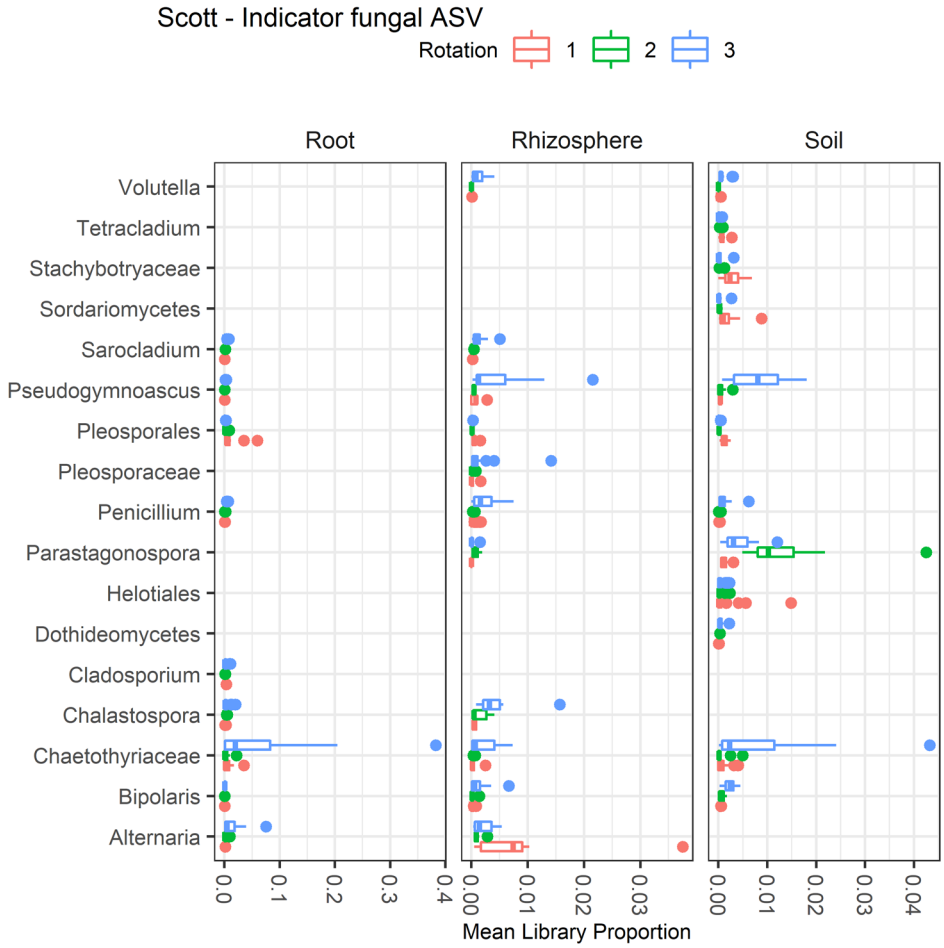


Figure 3: Indicator species analysis for the Lacombe site showed several fungal ASV sequences that were significantly associated with monocropping in both 2018 and 2019. Of note, the highly abundant sequence classified as O*lpidium brassicae* f509 was strongly associated with mono-cropping in the soil, rhizosphere and root samples. There were no bacterial ASV sequences that were significantly associated with rotation consistently in 2018 and 2019.



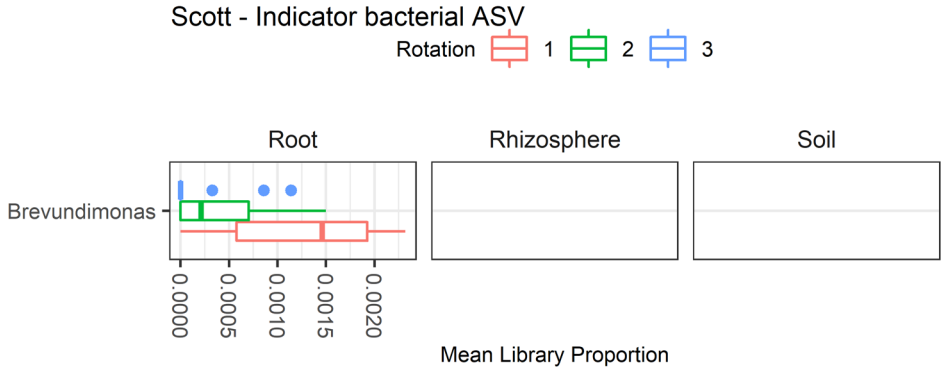


Figure 4: Indicator species analysis for the Scott site identified two ASVs classified as *Penicillium stolkiae* and *Chaetothyriaceae* that were significantly associated with multiple sample compartments from the canola-pea-barley rotation. Similar to the Lacombe site, an ASV 100% identical to the sequence for *Alternaria alternata*, a causal agent of leaf spot in canola, was significantly associated with monocropped canola in the root and rhizosphere samples.

Figure 5: Indicator species analysis for the Swift Current site also identified the same ASV sequence classified as *Penicillium stolkiae* as the Scott site as being significantly associated with the soil and rhizosphere from the canola-pea-barley rotation. Sequences classified as *Fusarium* sp. were significantly associated with the canola-pea-barley rotation in the root, rhizosphere and soil.

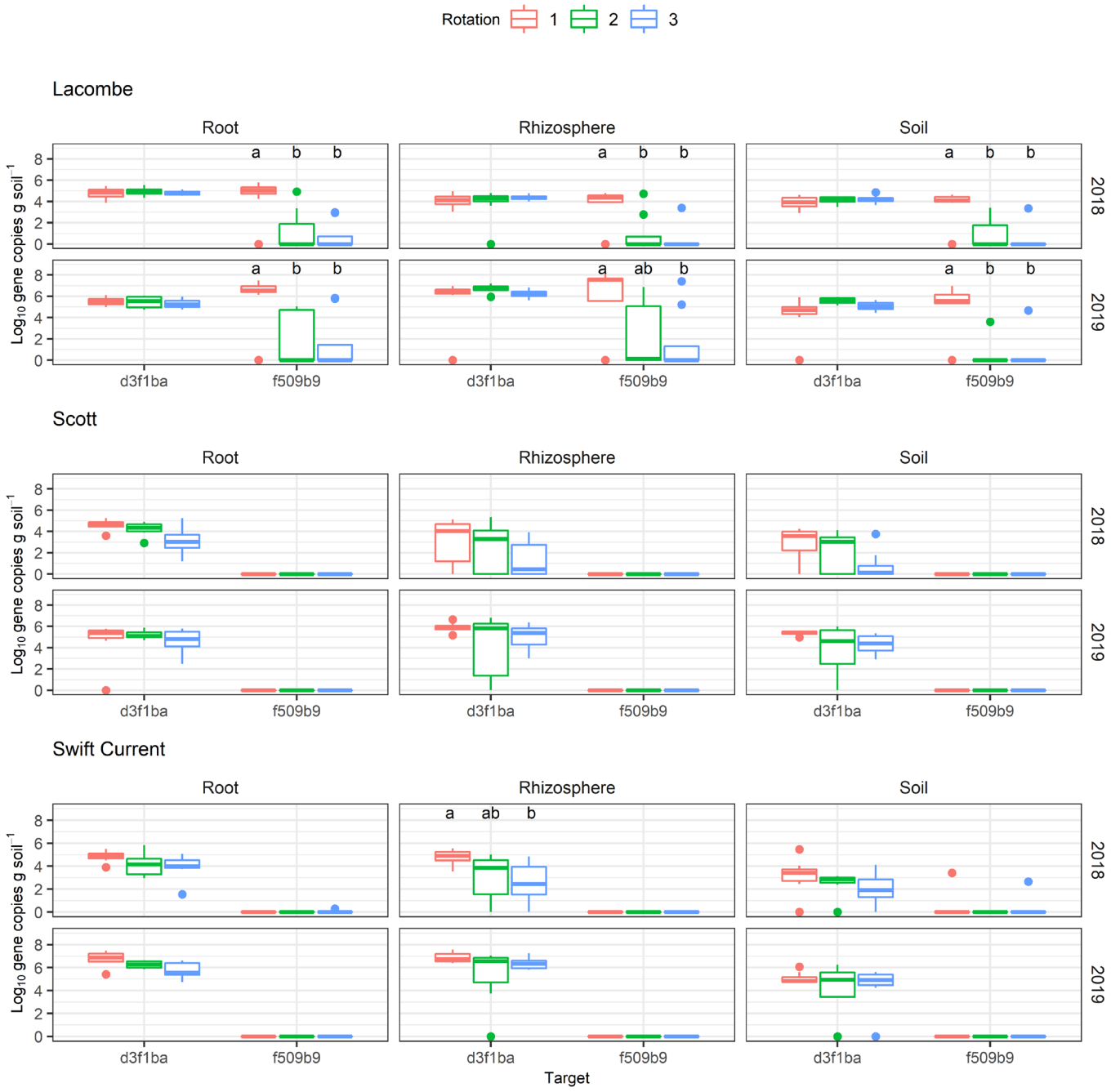


Figure 6. Abundances of two closely related ASV in the root, rhizosphere and soil from Swift Current, Scott and Lacombe quantified using qPCR. These ASV were very closely related to one another, and both shared very high sequence identity with the fungus *Olpidium brassicae*. While samples from Swift Current and Scott were dominated by only one of these organisms, samples from Lacombe had detectable levels of both of these ASV. At Lacombe the relative abundance of the two ASV was significantly different between crop rotation strategies in both 2018 and 2019.

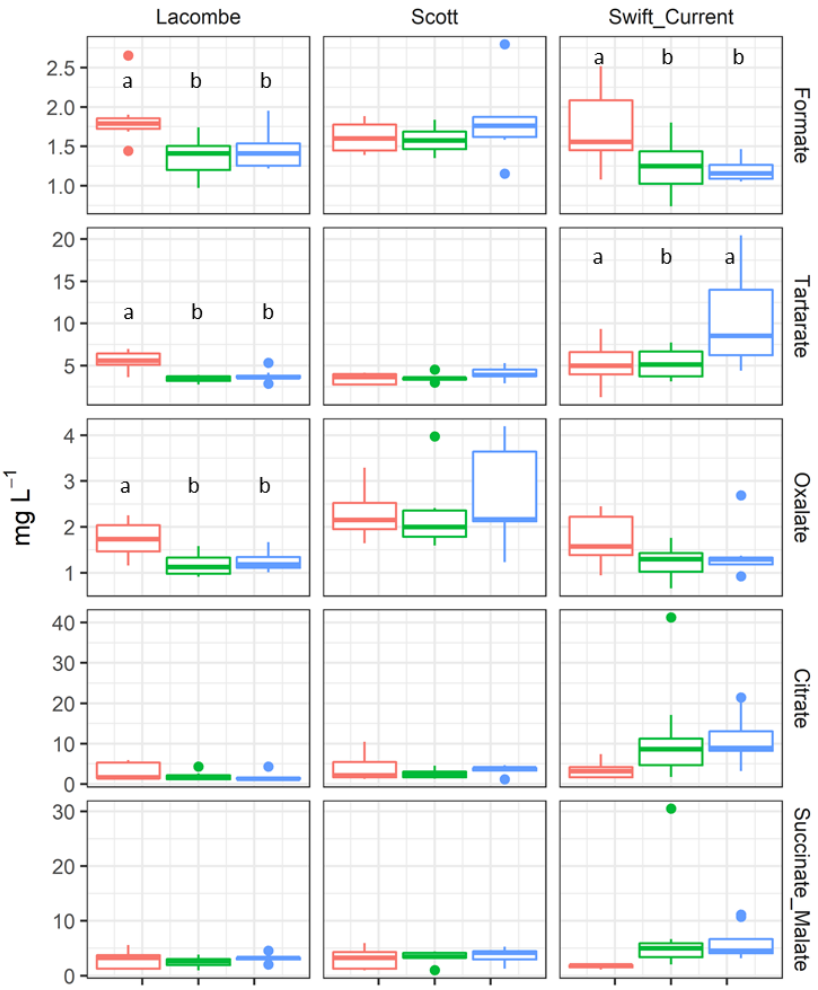


Figure 7. Quantification of organic acids in the root exudates of canola root samples.

12. Financial (to be provided to each Funding Agency (at the addresses indicated in 11.2)

a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding.
b. Explanation of variances from budget which are greater than 10%.
c. An invoice for each Funding Agency

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