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MPSG FINAL EXTENSION REPORT

PROJECT TITLE: Frequency of soybean in rotation and persistence of rhizobia in Manitoba soils

PROJECT START DATE: 1 April 2017 **PROJECT END DATE: 31 March 2019**

DATE SUBMITTED: 14 February 2020

PART 1: PRINCIPAL RESEARCHER

PRINCIPAL

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PART 2: EXECUTIVE SUMMARY

Outline the project objectives, a summary of the activities and results, and their relevancy to pulse and soybean farmers.

The proposed objectives for this project were 1) to quanitfy the population of rhizobia that overwinter in the soil in Manitoba, 2) to compare the effect of the frequency of soybean in a four year crop rotation on populations of rhizobia that overwinter in the soil in Manitoba 3) to evaluate the microbal community and determine the functional catagories bacteria that are present, how they overwinter, and how are they influenced by frequency of soybean in rotation. 4) Evaluate the impact the fequency of soybean in a rotation has on the rhizobial/microbial population. In this project we were able to qunatify how Bradyrhizobium japonicum populations overwinter and how their populations change when soybean are not the planted crop, as well we determined the entire bacterial population of three sites that had four distinct crop rotations when they were all planted with soybean in their final test year.

The availability of nutreints to plants and the overall soil health is greatly determined by the microbes present. At present, very little is known about how crop rotations affect microbial communities as well as Rhizobium populations. The information from this study helps to provide a baseline of data for what may be happening. These data can help provide information for best practices of having soybean in rotation with other crops.

PART 3: EXPERIMENT DESCRIPTION & RESULTS

Concisely describe the experimental methods and results to date. You may include up to 3 graphs/tables/pictures in the Appendix.

Overwintering of B. japonicum and the effect of different crop rotations (objectives 1 and 2)

Samples were taken from three field sites (Carman, Kelburn and Melita). Each site had each of 4 rotations. The soil samples were collected and both extracted for their DNA content as well as archived as frozen samples at -80°C. The DNA that was extracted was used for qPCR analysis using two sets of primers. The J16S primers we had developed which could detect Bradyrhizobia, as well as a pair of primers to nodZ; a gene that is specific for Bradyrhizbia that can nodulate soybean.

The results of the analysis showed that both sets of primers could be used to show a decline in the B. japonicum population with time. The decline of the population is best described as an exponential decline (Figure 1). The decline after one winter were in found to be non-significant in the Canola-Soybean rotations, the corn-soybean rotations, and the diversify rotation that had soybean planted in rotation with wheat-canola-corn. The decline in the continuous soybean rotation was significant, however the overall population of B. japonicum was also higher in the continuous soybean rotation. Our data supports the hypothesis that in Manitoba, there is not a significant decline in the B. japonicum population after a single winter. The difference in the slope of the exponential declines that are seen with the nodZ primer probably reflect either the field history (with respect to previous soybean crops prior to the study), or the soil composition and other microbes found at the sites. We note that the steepest declines occurred at the Melita site. This site had never previously had a crop of soybean. The Carmen and Kelburn sites both had histories of soybean cropping. It is safe to conclude that if soybean crops were grown and inoculated within the last 2 years, inoculation is probably not necessary and may have limited benefits.

Microbial Community Analysis

The DNA that was extracted from the sites was sent for 16S community analysis. Briefly, whereas the majority of 16S ribosome is invariable and completely necessary for life, regions of variability exist and are used to determine the taxa that are present in the soil. One such region, termed the V3-V4 variable region is used to determine the taxa that are present in the soil.

Sequencing of amplicons derived from the samples of all three sites yielded a total of 8,294,238 assembled raw contigs. After filtering out low quality and chimeric sequences, there were 2,137,591 high quality sequences with an average length of 292 base pairs. These sequences were clustered into 12,9230 OTUs based on 97% similarity. The average number of sequences per sample was 29,215 (range: 13,465 – 64,033). All samples were sequenced to a sufficient depth and could be used for further analysis.

The overall composition of the microbial community (microbiome) were similar. With a makeup dominated of Proteobacteria, Actinobacteria, Acidobacter (Figure 2). Worth noting at least 21% of the taxa that could be detected could not be identified as being present in the current data bases. This is not unique, but highlights a gap in our understanding of soil microbiology. At this level, the most striking difference was found within the Acidobacteria. Whereas the Kelburn and Melita sites were predominated by Gp6, in Carman this was completely supplanted by Gp1. This is also corroborated by Principle Coordinate Analysis (PCoA) analysis which shows the samples from Carmon are further separated from the other two sites. All the site samples clustered together using PCoA. Overlaying physical site data, the best explanation for this would be the measured soil pH which was more acidic in Carman. The analysis also showed that the three sites had a core-microbiome that consisted of 393 taxonomic units (Figure 3).

PART 4: RELEVANCE TO FARMERS AND FUTURE RESEARCH

Describe how the project results can be captured to benefit pulse and soybean farmers (production recommendations, innovation items, marketing plans, commercialization of technology etc). Identify any future research opportunities.

The project results represent the first in depth analysis of microbial communities associated with Manitoba fields. Although the data is robust, it does represent only a single field season. The project evolved over the time it was written and approved, to its completion. Initially we had anticipated only describing the microbial community of a single field. Since the price of sequencing has dropped, and become more accessible, we were able to analyze all three fields and have been able to carry out a more in-depth analysis. We have identified a number of trends that we see over a growing season, with respect to some types of bacteria either appearing to be selected for, or against. In addition, we can see that there is a correlation with respect to the microbial communities, and the previous crops.

With respect to the survival of populations of Bradyrhizbium, we can now demonstrate that there is a population of non-symbiotic Bradyrhizobia that are part of the natural soil microbiome. These, also respond to the presence of soybean and increase over a field season. We have also shown that over winter, the population of symbiotic B. japonicum does not appreciably decrease. There is a decline of B. japonicum that is observed with time since last inoculation. The rate of the decline may be a related to the history of soybean being grown in previous years. We have clearly shown that the decline on fields that have not been previously inoculated (Melita in this study), do have steep declines when comparted to areas where soybean has been already grown (Carman and Kelburn). The decline in these areas is such that if soybean was grown every other year, the necessity for inoculation may not be so great.

PART 5: COMMUNICATION

List extension meetings, papers produced, conference presentations made, project materials developed.

Results from this project has been presented by Patricia Ordonez (MSc) student at the 2018 Canadian Society of Microbiologists Meeting that was held in Winnipeg, the 24th North American Symbiotic Nitrogen Fixation that was held in Winnipeg in 2018, as well as she participated in the SoyLab nodulation/inoculation workshop that took place in Brandon and Dauphin in March 2018. The project has provided a baseline of microbial community data that is being used for Lawley's continued soybean rotation studies. In addition, the computer scripts for the analysis pipeline was developed so thatbacterial community analysis can be carried on in an ongoing manner.

The results of this study are going to be combined with some of Yvonne Lawley's field data and will be published in the upcoming year.

APPENDIX

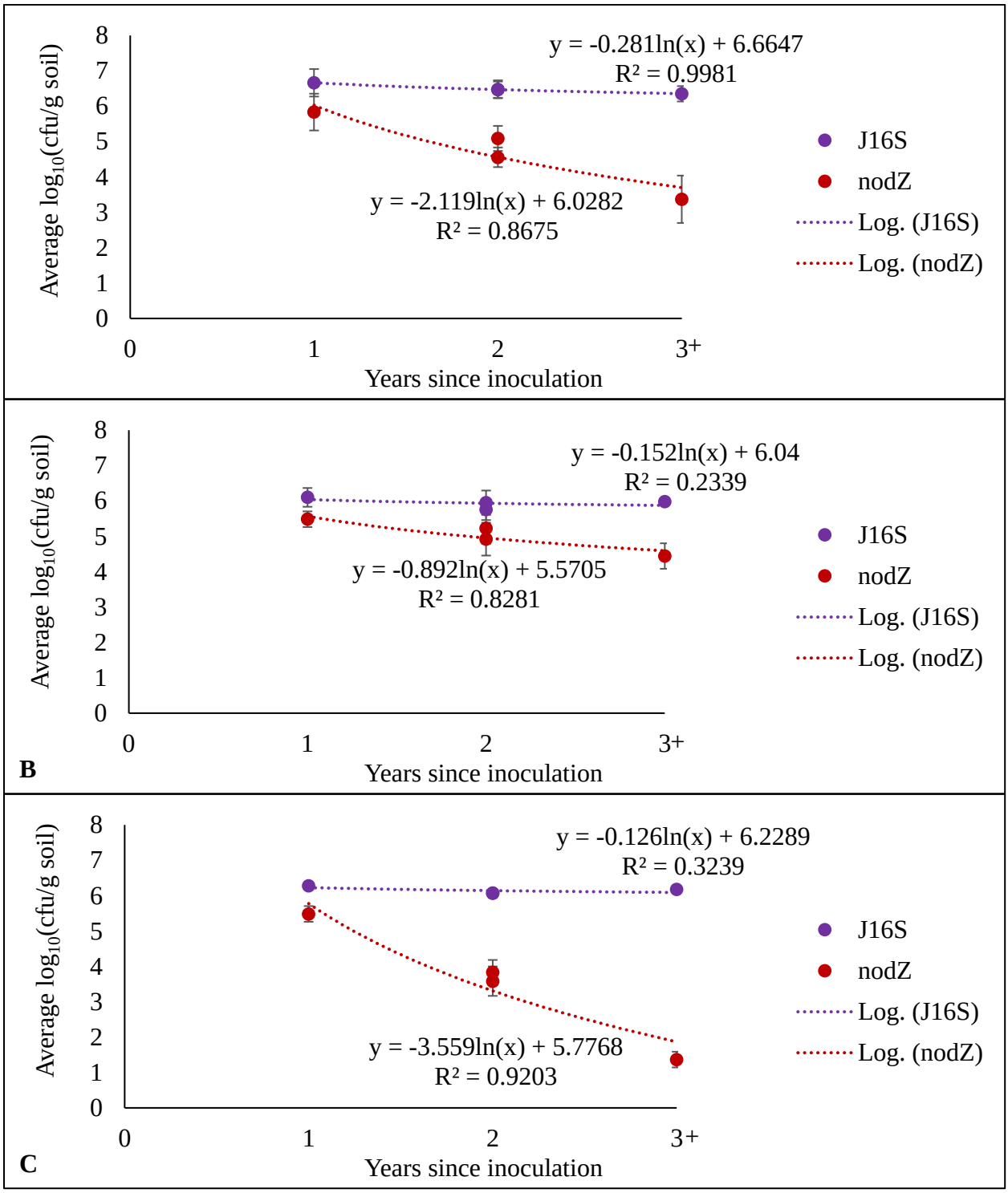
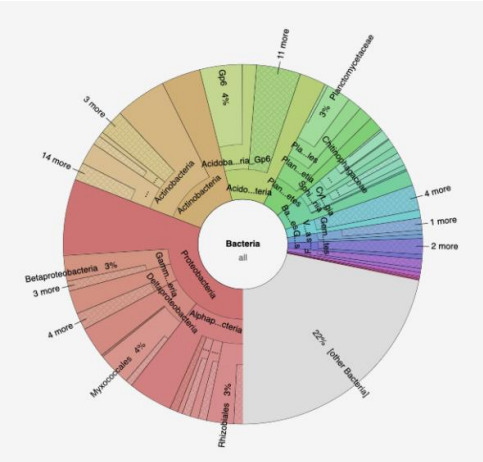


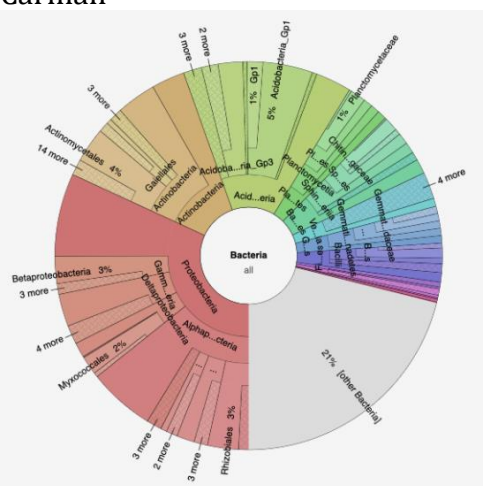
Figure 1. Quantification of *Bradyrhizobium* (J16S) and *B. japonicum* (nodZ) over years since last inoculation. Data was collected from before planting (BP) soil samples. Manitoba locations are as follows: **A)** Carman, **B)** Kelburn, **C)** Melita.

Melita



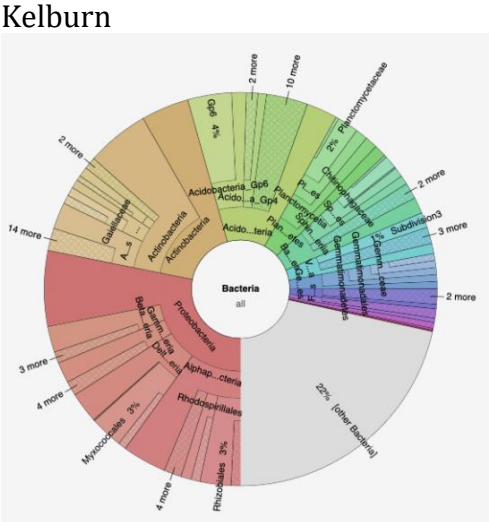
Proteobacteria: 31%
Actinobacteria: 15%
Acidobacteria: 11% (Gp6 4%)
Planctomycetes: 6%
Bacteroidetes: 6%
Verrumicrobiota: 3%
Firmicutes: 2%
Chloroflexi: 1%
Armatimonadetes: 0.4%
Other Bacteria: 22%

Carman



Proteobacteria: 32%
Actinobacteria: 13%
Acidobacteria: 14% (Gp1 5%)
Planctomycetes: 4%
Bacteroidetes: 6%
Verrumicrobiota: 3%
Firmicutes: 3%
Gemmatimonadetes: 2%)
Chloroflexi: 0.6%
Armatimonadetes: 0.7%
Other Bacteria: 21%

Kelburn



Proteobacteria: 28%
Actinobacteria: 18%
Acidobacteria: 12% (Gp6 4%)
Planctomycetes: 5%
Bacteroidetes: 6%
Verrumicrobiota: 3%
Firmicutes: 2%
Gemmatimonadetes: 3%
Chloroflexi: 1%
Armatimonadetes: 0.5%
Other Bacteria: 22%

Figure 2. Krona graphs showing the distribution and abundance of bacteria determined by 16S community analysis. For clarity, major groups and abundances are listed at right.

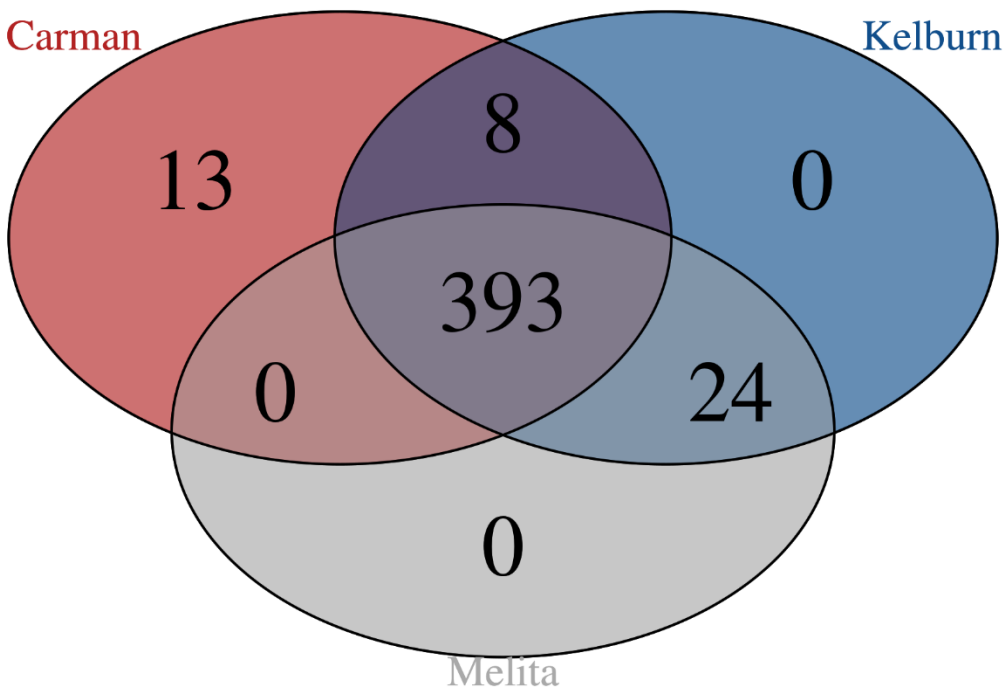


Figure 3. Venn diagram depicting the core microbiome (OTU level) between the three sites locations