

Alberta Bio Project B140390

Final Non-Confidential Report

Project title:

Intelligent NanoFertilizers - The dynamics of soil bacterial genomics associated with root exudates and N uptake by wheat and canola

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Executive Summary

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Little is known about the composition and dynamics of soil microbial accessions in the rhizosphere of N-fertilized and N-unfertilized crops. We characterized bacterial genomics after extracting and purifying soil DNA from rhizosphere soil samples collected during the growing season of canola (*Brassica napus*) and wheat (*Triticum aestivum*). We used a next generation sequencing technique to amplify the 16S DNA V3/V4 region by PCR. The quality-filtered reads obtained from soil alone, fertilized and unfertilized canola and wheat samples revealed complex and distinct bacterial diversity in these rhizosphere samples. The average number of operational taxonomic units (OTUs) at the order level decreased as follows: Canola (208,104 reads) > wheat (27,187 reads) > soil alone (26,557 reads), indicating a greater bacterial diversity under canola than under wheat. Among a total of 18 phyla, Proteobacteria, Actinobacteria, and Acidobacteria represented > 92% of all reads in the fertilized and unfertilized wheat planted soil. In canola rhizosphere, the latter three phyla plus Firmicutes represented > 93% of all reads in fertilized and unfertilized plants. At the order level, Xanthomonadales, Rhodospirales, Rhizobiales, Propionibacteriales, Myxococcales and Micrococcales represented > 70 % of all orders in the wheat soil samples, and Actinomycetales, Bacillales, Clostridiales, Rhizobiales, Solirubrobacterales, and Sphingomonadales represented 76 % of all orders under canola. Throughout the growing season, two uncultured species of Acidobacteria presented the highest OTUs (~2,300 readings) in soil alone and wheat. In comparison, *Shingomonas astaxanthinifaciens* DSM 22298 (13,000 readings), *Gaiella occulta* (~11,000 readings), *Gemmatimonas aurantiaca* T-27 (~8,000 readings) were the most abundant rhizosphere species during canola growth. The number of OTUs and species for two model species of *Nitrosomonas* and *Nitrobacter* were greater under canola than under wheat. Only *Nitrobacter vulgaris* was detected in wheat rhizosphere with a low reading (<30).

The addition of fertilizer-N to canola and wheat increased, decreased or did not change the temporal changes in the number of bacterial OTUs. At the Phylum level and under canola, the addition of N-fertilizer decreased the number of OTUs of Chloroflexi, Cyanobacteria and Firmicutes; increased that of Nitrospirae; and did not affect those of Acidobacteria, Actinobacteria, Bacteroides Dictyoglomi, Gemmatimonadete and Proteobacteria. Under wheat, the addition of fertilizer-N to wheat increased the number of OTUs for Acidobacteria, Actinobacteria, Elusimicrobia, Firmicutes, and Nitrospirae, and did not affect those of Bacteroidetes, Chloroflexi, Cyanobacteria, Fibrobacteres, Gammatomonadetes, Planctomycetes and Proteobacteria. We found that the dynamics of N uptake by canola was closely associated ($r^2=0.92$, $p<0.01$) with three specific soil bacterial accessions during the crop's growing season. No temporal association existed between the rate of N uptake and the number of soil bacterial OTUs in the rhizosphere of wheat.

Keywords: soil bacteria, genomics, rhizosphere, wheat, canola, nitrogen-fertilizer.

Introduction and Project Overview

Introduction

Much of the fertilizer-nitrogen (N) applied to crops is lost before plants take it up. Most of the soil- and fertilizer-N losses occur via volatilization, N₂O emissions, leaching or in run-off. At the farm level, a variety of soil-crop management strategies are used to minimize such losses (i.e., 4Rs, <http://fertilizercanada.ca>). An ideal approach to prevent N losses in crop production is to develop a

technology in which the fertilizer release is synchronized with the demand by the plant, dependent upon its stage and rate of growth. This concept has been explored since 2008 with financial support from Alberta Innovates Bio Solutions (AARI, project 2007A205R). Achieving this synchronization process is based on the central hypothesis established to develop INF: “in soil solutions, where N content and supply is limited for crop growth, plant roots emit specific chemical signals to stimulate soil microbial N mineralization for subsequent N uptake by crops”. Some of the basic scientific and technological challenges to develop novel INF products for enhancing NUE of crops are among others: a) Identifying the chemical composition of signals in root exudates that associate with crop N uptake; b) Detecting the identified chemical signals after preparation of the specific biosensors to be incorporated into polymer films of INF to help synchronize fertilizer release according to crop demand; c) Understanding the interactions between chemical signals, crop N uptake, and the dynamics of soil N cycling controlled by rhizosphere soil microorganisms. This project focused on increasing the understanding biotic components and their interaction as described in last c).

We expect that technology outputs developed from this project and subsequent research, commercialization and large-scale on-farm use of INF will help enhance nitrogen use efficiency (NUE) by crops upwards of 85 %, thus significantly reducing N losses from soil applied fertilizers. The main contribution of INF is to ensure the security of food production and the sustainability of agriculture in Canada and the world.

Project Goals

The main goal of the project was to understand the temporal dynamics of rhizosphere soil bacterial populations during the growing season of canola and wheat. Specific objectives were to determine: 1) the temporal changes of the soil bacterial genomics in the rhizosphere of wheat (*Triticum aestivum*) and canola (*Brassica napus*); and 2) identify the bacterial populations (genus and species) associated with soil N cycling processes and crop N uptake.

Materials and Methods

Growing canola and wheat in the greenhouse

Canola (*Brassica napus*) and wheat (*Triticum aestivum*) were grown in the greenhouse under controlled conditions of temperature, light and soil moisture from August 26th to November 5th, 2014. Prior to establishing the greenhouse experiment, a bulk sample (1 ton of Manotick soil series, loamy sand in texture) was collected from the 0-20 cm depth from a cultivated field located at the Central Experimental Farm in Ottawa. The bulk soil was sieved and placed into plastic pots. Soil pots were incubated with no plants (soil alone), and also planted to canola and wheat. Crops were unfertilized (0 kg N/ha) and fertilized with urea (equivalent to adding 100 kg N/ha). Phosphorus, K and micronutrients were added as a Hoagland solution as required by both crops at seeding time. Planted and unplanted soil pots were sampled destructively once a week from seeding to harvest. Samples of soil and plant materials (root and shoots) were collected, processed and stored under proper temperature for further chemical, biochemical and molecular analysis. For example, collected soil and plant samples were stored at -80 °C for extracting and characterizing 16S DNA and RNA.

Soil bacterial genomics

Extraction and purification of soil 16S DNA

Initially, we tested two different genomic DNA extraction protocols in 4 soil samples using the University of Alberta protocol (Foght et al., 2004; Norgene kit protocol), and decided to use the Univ. of Alberta protocol as it extracted greater amount of DNA. We conducted four sets of 36 tagged 16S, for a total of 108 PCR amplifications, targeting the V2 region of the 16S bacterial gene. The PCR amplicons were run on a 1.5 % agarose gel, the amplified 250 bp fragment from each sample was then extracted using a Qiagen gel extraction kit. The purified DNA fragments were sent to Genome Quebec Innovation Center (GQIC), Montreal, Quebec, for sequencing using a HiSeq Illumina platform through paired end sequencing procedure. The initial amplicons produced failed the quality control (QC) protocol established by GQIC. As a result, Genomic DNA was further purified using an AMPure (Agilent) bead protocol in the lab of Dr. Vidmar. The latter purified genomic DNA approach was successfully used as a template for PCR amplification of the 16S V3/V4 region in rhizosphere soil samples obtained from the canola and wheat growth studies. The protocol involved the PCRed (450bp) for the 16S V3/V4 region (347F GGAGGCAGCAGTRRGAAT, and 803R CTACCRGGGTATCTAATCC). High throughput Illumina Paired End 250 bp runs were done with 18-tagged samples per run. A total number of 70 soil samples run for canola and 72 soil samples from wheat experiments. The software CLC bio was used to do quality control and initial statistical analysis of the unique sequence reads. A list of unique reads for the soil samples obtained from the N-fertilized and unfertilized crop treatments was compiled (6,778,634 unique reads for canola and 27,233,968 for wheat). For statistical analysis, data having a mean read of > 1 (in canola) or > 2 (in wheat) under both fertilizer treatments over all the sampling times were selected as subsets for statistical analysis. There were a total of 34,152 unique reads for canola, and 155,815 for wheat. Multivariate analysis (i.e., Principal component analysis, PCA) helped analyze and interpret the experimental data. ANOVA and t-tests were used to test for significant effects of N-fertilizer addition and phenological growth stage on the operational taxonomic units (OTUs).

Results

1. Influence of Canola and Wheat on Soil Bacteria

Tables 1, 2 and 3 show the distribution of the average number of OTUs in the untreated soil, and in the N-fertilized and -unfertilized canola and wheat rhizosphere. The analysis of 34,152 quality-filtered reads obtained from canola and 155,815 reads obtained for wheat fertilized and unfertilized samples revealed complex and distinct bacterial diversity in these rhizosphere samples. Table 3 shows that the average number of operational taxonomic units (OTUs) at the order level decreased as follows: canola (208,104 reads) > wheat (27,187 reads) > soil alone (26,557 reads), indicating a greater bacterial diversity under canola than under wheat. Canola and wheat rhizospheres shared 11 common soil bacterial phyla. The canola soil rhizosphere samples, with 1,010 soil bacterial operational taxonomic units (OTUs) versus 723 OTUs under wheat, exhibited the highest diversity as indicated by the diversity of classes, orders and species. Among a total of 18 phyla, Proteobacteria, Actinobacteria, and Acidobacteria represented > 92% of all reads in the fertilized and unfertilized wheat planted soil. In canola rhizosphere, the latter three

phyla plus Firmicutes represented > 93% of all reads in fertilized and unfertilized plants. At the order level, Xanthomonadales, Rhodospirales, Rhizobiales, Propionibacteriales, Myxococcales and Micrococcales represented > 70 % of all orders in the wheat soil samples, and Actinomycetales, Bacillales, Clostridiales, Rhizobiales, Solirubrobacterales, and Sphingomonadales represented 76 % of all orders under canola. A couple of uncultured species of Acidobacteria presented the highest OTUs (22,806 readings) in wheat. *Shingomonas astaxanthinifaciens* DSM 22298 (13,000 readings), *Gaiella occulta* (~11,000 readings), *Gemmatimonas aurantiaca* T-27 (~8,000 readings) were the most abundant species in canola (data not shown).

2. Soil Bacterial Genomics in Canola Rhizosphere

2.1. Effects of N-fertilizer addition

Figure 1 shows results of the PCA analysis conducted for the 16S DNA sequence data between the N-fertilized and unfertilized canola crop. The collected experimental data, that was first normalized and then log transformed, found 3,923 unique sequences that were significantly different ($p < 0.05$) between the N fertilized and the unfertilized canola treatment. These 16S DNA sequences were identified using NCBI's 16S Blast dataset, clustered, and re-examined to determine the change in read numbers over the different sampling dates (as reported in Milestone 2 report). Our statistical analysis of the transformed data shows two separated data sets, and a t-test was done on it to determine the unique sequences that showed a significant statistical difference between fertilizer conditions ($p < 0.05$).

We found significant sequence differences in bacteria accessions in the soil-canola receiving fertilizer-N and the soil-canola not receiving N. Two PCA groups or clusters (containing 23 unique sequences) showed the largest increase in number of reads in the soil samples receiving fertilizer-N with a log change difference between 0.94 and 0.41 in the transformed data. On the other hand, 141 unique sequences showed a large increase in reads in the unfertilized soil samples, representing a difference between 0.4 to 0.24 log in the transformed data (Figure 1). Cluster 5, showed that specific bacterial species numbers repressed by the presence of N, versus cluster6 bacterial species numbers induced by the presence of N-fertilizer.

Operational taxon units (OTUs) were identified from the sequences showing significant different readings. Rarefaction data analysis indicated that there were enough counts for each OTU in each sample confirming that the sampling scheme (i.e., by fertilizer treatment and phenology) was sufficient for soil samples taken from the experimental set-up (Figure 2).

Figure 3A and B show the phylum, class and order established for the average counts over the different times of soil sampling under each fertilizer treatment during canola growth. Overall, slight changes were observed in bacterial type profiles between the N-fertilized and not fertilized canola treatments. Overall, and considering all sampling dates, the average number of OTUs in canola rhizosphere soil tended to decrease in the following order: unfertilized soil planted to wheat (222,401) > fertilized soil planted to wheat (203,522). According to two-tailed paired t-tests, these differences were

not statistically significant.

Further, t-tests of the experimental data showed that the addition of fertilizer-N resulted in a significant increases, decreases or no effects on the number of OTUs at the Phylum, Class and Order level. For example, addition of N-fertilizer decrease the number of OTUs established for Chloroflexi ($p=0.036$), Cyanobacteria ($p=0.046$), and Firmicutes ($p<0.002$). In comparison, addition of fertilizer-N significantly increased the number of OTUs for Nitrospirae ($p<0.001$), but only tended to increase the number of OTUs for Gemmatimonadetes and Bacteroidetes (Tables 1, 2 and 3).

2.2. Soil bacterial genomics and phenology

We used the statistically significant data set obtained from the genomic analysis to examine the temporal variability of OTUs during the growing season of canola. Figure 4A shows that the rates of N uptake by canola shoots (leaves and stems) presented two maxima, at 29 (7 to 8 leaves, stem elongation begins) and 42 days after seeding (70 % of final stem length, 40 % flower buds open in main raceme), and a minimum at day 35 (flower buds free level with youngest leaves, buds closed). Uptake of N by canola was negligible and not measurable beyond day 49 of crop growth. Because of the cost of sample analysis, we focused on characterizing bacterial genomics in soil samples taken from 21 to 63 days after seeding. Interestingly, the log number of the assigned 16S DNA bacterial OTUs also presented two maxima and a minimum peak. Figure 4B shows that the dynamics of the rate of N uptake in canola was associated with the average OTU numbers in two out of 14 phylum, two out of 37 classes, and two out of 72 orders. Examples of OTU species presenting the temporal dynamics similar to that of N uptake rates in canola were *Sphingomonas* sp. within the taxonomic hierarchy of Proteobacteria - Sphingobacteriia – Sphingomonadales. Similar temporal associations with N uptake rates were observed with other bacteria involving *Lysobacter* and *Luteimonas* sp. within the hierarchy Bacteroidetes – Gammaproteobacteria - Xanthomonadales (Figure 4B). *Sphingomonas* are chemoheterotrophic, gram negative, rod shaped, and strictly aerobic bacteria residing in various environments including soil-root microcosms.

Noteworthy, further examination of the temporal variability of rates of N uptake (y) showed a close association with the log of OTUs for the species *Sphingomonas jaspi* ($r=0.76$), *Lysobacter arseniciresistens* ZS79 ($r=0.89$) and *Luteimonas vadosa* ($r=0.79$). The temporal changes in the rates of N uptake, however, was best described as a function of the log number of three specific OTUs during the growing season of canola. The model was $y = -2.24 + 1.675 X_1 + 3.867 X_2 - 5.928 X_3$; ($r^2=0.91$), where y = rate of N uptake (kg N/ha/d); X_1 = log number of *Sphingomonas jaspi*; X_2 = log number of *Lysobacter arseniciresistens* ZS79, and X_3 = log number of *Luteimonas vadosa*.

3. Soil Bacterial Genomics in Wheat Rhizosphere

3.1 Effect of fertilizer addition

Overall, and considering all treatments and sampling dates, the average number of OTUs in wheat

rhizosphere soil tended to decrease in the following order: unfertilized soil planted to wheat (32,177) > soil alone (29,420) > fertilized soil planted to wheat (26,822). According to two-tailed paired t-tests, these differences were, however, not statistically significant. Figure 5A shows the results from Principal Components Analysis (PCA) conducted to test for differences in the soil 16S DNA sequences between the treatments of soil alone, and N-fertilized and unfertilized soil planted to wheat. The PCA of normalized and log transformed sequence data associated with the three treatments showed clear overlapping and no clear separation. These results indicate that a number of soil bacterial accessions were not significantly influenced by the imposed soil and soil-plant treatments. Of all the sequence data, only a handful of outliers were found (green dots, circled in orange, and data circled in blue and purple). Further PCA analysis was only conducted between the fertilized and unfertilized wheat planted soil. Figure 5B shows the results from the PCA analysis conducted after removing data obtained from the soil-alone treatment. Such analysis resulted in a clear separation of two clusters representing differences in a number of bacterial accessions found in the unfertilized soil-plant and the N-fertilized soil-plant treatments (Figure 5B). In this case, PCA analysis of transformed data shows that the addition of fertilizer-N to the soil-wheat system significantly influenced soil bacterial accessions by decreasing its total average number of OTUs.

Further, analysis of variance (ANOVA) was conducted using the unique sequences that showed a significant statistical difference ($p < 0.05$) (statistical analysis data not shown). The 722 unique accessions or OTUs were used to establish PCA clusters to test for increases or decreases of reads over the type of treatment and time of sampling. A total of nine PCA clusters or groups were created, with cluster 4, 5 and 7 showing increased reads in the N-fertilized soil-wheat over the soil-alone or unfertilized soil-wheat treatment, whereas the other 6 clusters showed other pattern effects (i.e., no change or decreases). Clusters 4, 5, and 7 contained 44 accessions with the following genus': *Arthrobacter*, *Mesorhizobium*, *Massilia*, *Sphingomonas*, *Nitrospira*, *Terrabacter*, *Nonomuraea*, *Knoellia*, *Janibacter*, *Streptomyces*, *Devosia*, *Pseudomonas*, *Kineosporia*, *Phenylobacterium*, uncultured *Oxalobacteraceae*, *Nocardioides*, *Phycococcus*, *Quadrisphaera*, *Lapillicoccus*, *Agromyces*, *Leifsonia*, *Marmoricola*, *Lysobacter*, *Mycobacterium*, *Noviherbaspirillum*, *Rhodanobacter*, *Caulobacter*, *Microvirga*, *Pseudaminobacter*, *Nitrospira*, uncultured *Planococcaceae*, and uncultured *Rhodobacteraceae*. These 44 accessions showed an increased number of OTUs in the fertilized soil-wheat system relative to the unfertilized soil-wheat system, or the soil-alone treatment, indicating that the addition of fertilizer-N stimulated the number of specific soil bacterial accessions. All the 44 bacterial accessions found in wheat rhizosphere were similar to those found in the canola experiment. Noteworthy, *Nitrobacter* and *Nitrosomonas* are important species regulating soil mineral-N fluxes and the supply of inorganic-N to crops. *Nitrobacter vulgaris* was the only species of the genus detected in the three treatments with an average reading of 36 OTUs. The presence of wheat plants and especially the addition of fertilizer-N increased the OTU number to 53. On the other hand, various species of *Nitrosomonas* were present in soil alone and in wheat planted soil (average reading of ~ 950 OTUs), and their OTU number was slightly higher under wheat than in soil alone; but the addition of fertilizer-N reduced the OUT number by 30 %.

An analysis conducted after removing data obtained from the soil-alone treatment, showed that three of nine clusters increased the number of OTUs in the fertilized soil-wheat treatment. Noteworthy, 97 of the OTUs in the wheat planted soil comprised 65 genus of all the OTUs found under canola.

3.2. Soil bacterial genomics and phenology

We have recently received the analysis for the total content of N in wheat plants. Figure 5 shows a preliminary analysis of the total N content data, and indicates that the rate of N uptake by unfertilized wheat shoots (leaves + stems) increased during the first 5 weeks of growth with a maximum value measured at day 28 (7 leaves unfolded, 3 tillers), and a minimum at day 42 after seeding (6 tillers, 2nd node detected in main shoot, appearance of flag leaf). Thereafter, the N uptake rate increased slowly until day 56. There was little or no N uptake by unfertilized wheat measured beyond day 63 and 70 (anthesis complete, early grain filling). Noteworthy, the dynamics of the log number of bacterial OTUs in the rhizosphere of unfertilized and fertilized wheat did not follow the dynamics of N uptake rates during the growing season. For example and in the unfertilized wheat, the log number of OTUs was minimum at day 35 with a maximum number observed at day 42 after seeding (Figure 4). In addition, a careful examination of all the genomics data for wheat rhizosphere showed that most assigned species under the different orders, classes and phylum showed similar temporal trend as that shown in Figure 5. Consequently and in relation to the rate of N uptake, the dynamics of bacterial genomics under wheat was different than under canola. The reasons for the latter differences in OTUs during the growing season of wheat and canola are unknown, these differences in the dynamics of OTUs between wheat and canola, however, may be associated with the type and amount of compounds exuded during the growing season, the presence of endomycorrhizae in wheat roots, or some other unknown root-microbial interaction factors. Noteworthy, the bacterial diversity in the rhizosphere of wheat was not affected by the presence of wheat plants or the addition of fertilizer-N.

In summary, The addition of fertilizer-N to canola and wheat increased, decreased or did not change the temporal changes in the number of bacterial OTUs. At the Phylum level and under canola, the addition of N-fertilizer decreased the number of OTUs of Chloroflexi, Cyanobacteria and Firmicutes; increased that of Nitrospirae; and did not affect those of Acidobacteria, Actinobacteria, Bacteroides Dictyoglomi, Gemmatimonadetes and Proteobacteria. Under wheat, the addition of fertilizer-N to wheat increased the number of OTUs for Acidobacteria, Actinobacteria, Elusimicrobia, Firmicutes, and Nitrospirae, and did not affect those of Bacteroidetes, Chloroflexi, Cyanobacteria, Fibrobacteres, Gammatomonadetes, Planctomycetes and Proteobacteria. We found that the dynamics of N uptake by canola was closely associated ($r^2=0.92$, $p<0.01$) with three specific soil bacterial accessions during the crop's growing season. Preliminary data evaluation indicates no clear temporal association between the rate of N uptake and the number of soil bacterial accessions in the rhizosphere during the growing season of wheat.

Conclusions

Our results from the characterization of the soil bacterial genomics in the rhizosphere of canola and wheat during their growing season indicate the following:

- 1) Greater bacterial diversity was observed in canola than in wheat rhizosphere throughout the growing season of both crops. The total average number of operational taxonomic units (OTUs) decreased as follows: canola >> wheat = soil alone.
- 2) Overall and at the level of Phylum, class and order, the addition of fertilizer-N decreased a large number of OTUs but this trend was only consistent for some accession numbers. Thus, the addition of fertilizer-N to canola increased or decreased the number of OTUs of some taxa. For example, at the Phylum level, it increased Nitrospirae; decreased that of Chloroflexi, Cyanobacteria and Firmicutes; and did not affect the accession number for Acidobacteria, Actinobacteria, Bacteroides Dictyoglomi, Gemmatimonadete and Proteobacteria.
- 3) The addition of fertilizer-N to wheat at the Phylum level increased the number of OTUs for Acidobacteria, Actinobacteria, Elusimicrobia, Firmicutes, and Nitrospirae, and did not affect those of Bacteroidetes, Chloroflexi, Cyanobacteria, Fibrobacteres, Gammatomonadetes, Planctomycetes and Proteobacteria.
- 4) The rate of N uptake was closely associated with the dynamics of three specific soil bacterial accessions in canola rhizosphere during the crop's growing season. There was a low association between the rate of N uptake and the number of bacterial accessions in wheat rhizosphere.

Scientific Achievements

1. *Published Book Chapters*

Monreal, C.M. 2015. The chemistry of labile organic matter in soil solution: I. A model for metabolites of chemical signaling pathways associated with plant-microbial interactions. *In* Z. He and F. Wu, eds. Labile organic matter – chemical compositions, function, and significance in soil and the environment. Soil Science Society of America, Madison, WI. doi:10.2136/sssaspecpub62.2014.0073

Monreal, C.M. and M. Schnitzer. 2015. Labile organic matter in soil solution: II. Separation and identification of metabolites from plant-microbial communication in soil solutions of wheat rhizospheres. *In* Z. He and F. Wu, eds. Labile organic matter - compositions, function, and significance in soil and the environment. Soil Sci. Soc. of America, Madison, WI. DOI: 10.2136/sssaspecpub62.2014.0074.

Mastronardi, E., Tsae, P., Zhang, X., **Monreal, C.** and M.C. DeRosa. 2015. Strategic role of nanotechnology in fertilizers: Potential and limitations. In: Nanotechnologies in Food and Agriculture. Rai, M., Ribeiro, C., Mattoso, L., Duran, N. Eds., pages 250-268. Springer, New York. Chapter 2, p 347. DOI 10.1007/978-3-319-14024-7.

2. *Peer Reviewed Journal Articles*

Monreal, C.M., DeRosa, M., Mallubhotla, S.C., Bindraban, P.S. and C. Dimkpa. 2015. Nanotechnologies for increasing the crop use efficiency of fertilizer-micronutrients. *Biol. Fertil.*

Soils. DOI: 10.1007/s00374-015-1073-5.

Heinrichs, R., **Monreal, C.M.**, Santos, E.T., Soares Filho, C.V., Rebonatti, M.D., Teixeira N.M. and A. Moreira. 2016. Phosphorus sources and rates associated with nitrogen fertilization in Mombasa grass yield. Commun. Soil Sci. & Plant Analys. DOI: 10.1080/00103624.2016.1141923

Zhang, X., Chabot, D., Sultan, Y., **Monreal, C.** and M. DeRosa. 2013. Aptamer-based targeted delivery microcapsules by layer-by-layer assembly. Appl. Mater. Interfaces 5: 5500-5507.

a. Other (manuscripts under preparation)

-1) Vidmar, J., Zhang, J., and C. M. Monreal. 2016. Evaluation of soil bacterial genomics aspects in wheat rhizosphere. (Possible submission to PLOS ONE Biology or BMC Plant Biology).

-2) Vidmar, J., Zhang, J., and C. M. Monreal. 2016. Assessment soil bacterial genomics population in canola rhizosphere. (Possible submission to PLOS ONE Biology or BMC Plant Biology).

Greenhouse Gas Impacts

- b. Expected GHG benefits projected over a ten-year period, including direct impacts from implementation of the project and future impacts based on market adoption.

Environment Canada (2013) reported that the annual N₂O emissions from fertilizer-N are 8.0 kt for Alberta and 29.6 kt for all of Canada. The latter Greenhouse Gas (GHG) emissions are equivalent to 2.48 Mt CO₂e in Alberta, and 9.18 Mt in Canada. Thus, and assuming a NUE of 85 % for Intelligent NanoFertilizers (INF), the successful development, commercialization and adoption of novel INF, at the farm level, have the potential to reduce GHG emissions by at least 2.1 Mt CO₂e in Alberta and 7.8 Mt CO₂e in Canada. Thus far, work conducted under this project contributes to basic scientific knowledge on the genomics of soil bacterial population and their interactions with crop N uptake. This incremental new knowledge supports in part a rigorous approach to develop a new and efficient fertilizer technology for reducing greenhouse gas emissions from agriculture. Information can be used in collaboration with other scientific teams interested in associating greenhouse gas emissions with the dynamics of bacterial genomics in crop rhizospheres.

Overall Conclusions

Next Steps:

- Discussion of the next steps for the technology/process/innovation, including potential follow-up projects.
1. Scientific information from this study has helped to identify soil bacterial taxa that are associated with N uptake by canola and wheat. Further data analysis will help determine the soil bacterial taxa associated with soil N mineralization and immobilization.
 2. The acquired knowledge will help understand and establish the basis for manipulating the synchrony of fertilizer-N release according to crop demand for increasing N use efficiency by crops.

3. The acquired knowledge will help establish the basis for understanding key biotic components and mechanisms influencing the synchrony of fertilizer-N release according to crop demand for increasing N use efficiency by crops.
4. This information will provide a scientific rigorous basis for the development and deployment of a novel technology such as Intelligent NanoFertilizer (INF) for delivering urea to wheat and canola.
5. The bacterial genomics information is central to establishing a **bacterial species-enzyme-gene-plant transporter model** system to research key components of communication signaling networks between crop roots and active soil microorganisms.
6. We hope that future funding efforts will contribute to establishing the above model to better understand the regulation of the soil N and P cycles in crop rhizosphere. This model system approach will help examine bacterial species, genes, and key transporters controlling the mineralization, immobilization, supply and uptake of N and P by crops. In the future, this information will contribute to elucidate chemical signals and molecular components in roots, root exudates, soil solutions and soil microbial cells that are involved in N and P cycling under various conditions of nutrient availability in the rhizosphere.

- Long-term plan for commercialization of the project technology/learnings.

Non-confidential scientific findings of our research will be published in the peer reviewed scientific literature. The bacterial genomics knowledge will provide credible basis for the successful long-term commercialization of a new generation of highly efficient INF for delivering macro- and micronutrients in agriculture. Noteworthy, the path to commercialization of INF involves negotiations first with a Canadian fertilizer company. Alternatively, a commercialization path may also involve negotiations with foreign fertilizer manufacturing companies interested in the smart fertilizer technology for crops.

- Commercialization-related actions to be undertaken within two years of project completion.

Commercialization-related actions to produce and use an Intelligent Nanofertilizer for urea will be undertaken once the advanced prototype is successfully tested in greenhouse and field studies. If successful, steps will be taken to register a patent for the potential commercialization of an advanced prototype of the INF product to be developed under AIBS Project #2013F014R.

Communications plan:

- c. Plans for communicating information about the project, project findings and results or the underlying technology with third parties.

1) In close collaboration with CCEMC, the collected scientific information will be communicated to farm producer organizations, fertilizer industry, Canadian citizens and academic communities.

2) Present R&D results at national and international conferences, symposia, university lectures, workshops, and field demonstration days organized by government, fertilizer industry and/or farmer producer organizations.

3) Other dissemination mechanisms may include websites (i.e., CCEMC, Carleton University),

newsletters, and releasing information through interviews with newspapers, TV and radio.

4) At least, two scientific articles will be published from this project (two draft articles on bacterial genomics are being drafted). Other scientific knowledge may be also published in book chapters and in open access peer reviewed scientific journals.

5) This project trained a highly qualified personnel (two Post-doctoral fellow), two technicians and a summer student.

- d. What non-confidential communication tools are intended to be employed to enhance consistent and effective communication with respect to the project?

Communication tools and reported information to be discussed and agreed with CCEMC before its release. Information obtained from this research project is considered non-confidential, thus, there are no special strategies planned to separate the diffusion of confidential and non-confidential information.

Table 1. At the Phylum level, the average number of operational taxonomic units (OTUs) for bacteria: in soil alone, and in unfertilized and N-fertilized canola and wheat rhizosphere.

Phylum	Soil alone	Canola		Wheat	
		No fertilizer-N	Fertilizer-N	No fertilizer-N	Fertilizer-N
Acidobacteria	7,178	12,213	11,137	7493	4737
Actinobacteria	8,023	83,142	74,248	7756*	8072*
Bacteroidetes	367	475		489	487
Chloroflexi	115	2,857*	2,335*		96
Cyanobacteria	321	353*	296*		5
Dictyoglomi	ND	174	152	ND	ND
Elusimicrobia	226	ND	ND	270*	92*
Fibrobacteres	94	ND	ND	179	40
Firmicutes	336	33,542*	26,291*	783*	608*
Gemmatimonadetes	708	7,787	8,001	623	394
Nitrospirae	47	1,370*	1,796*	54*	97*
Planctomycetes	40	ND	ND	49	16
Proteobacteria	12,206	76,213	74,019	14,309	12,185
Total	29,343	218,125	198,763	32,105	26,756

*Within each crop, values followed by an asterisk are significantly different at $p < 0.05$ according to a paired, two tailed t-test. T-tests were conducted on log-transformed data.

Table 2. At the Class level, the average number of operational taxonomic units (OTUs) for bacteria: in soil alone, and in unfertilized and N-fertilized canola and wheat rhizosphere.

Class	Soil alone	Canola		Wheat	
		No fertilizer-N	Fertilizer-N	No fertilizer-N	Fertilizer-N
Acidimicrobidae	17	6,163	5,528	24*	11*
Acidobacteriales	6,682	5,922	5,134	7,020	4,481
Actinobacteridae	7,765	54,586	50,832	7,487*	7,899*
Alphaproteobacteria	5,065	53,675	52,889	5,457	5,452
Bacilli	266	20,042*	15,591*	298*	514*
Betaproteobacteria	2,302	7,127	8,159	2,431	2,203
Clostridia	69	13,142*	10,445*	465*	92*
Coriobacteria	3	ND	ND	44	2
Cytophagia	57	ND	ND	60	88
Dehalococcidia	ND	2,111*	1,541*	ND	ND
Deltaproteobacteria	1,817	11,629*	9,141*	3,297	1,131
Elusimicrobia	226	ND	ND	270	92
Fibrobacteria	94	ND	ND	179	40
Flavobacteria	1	ND	ND	7	12
Gammaproteobacteria	3,019	3,778	3,823	3,120	3,394
Gemmatomonadales	708	7,787	8,001	623	394
Holophagae	491	797	744	468	255
Negativicutes	ND	263	194	ND	ND
Nitrospirales	47	1,370*	1,796*	54*	97*
Rubrobacteridae	ND	21,124*	16,895*	ND	ND
Solibacteres	ND	5,493	5,260	ND	ND
Sphingobacteriia	303	405	420	317	320
Thermomicrobia	157	ND	ND	136	130
Total	29,089	215,414	196,391	31,758	26,608

*Within each crop, values followed by an asterisk are significantly different at $p < 0.05$ according to a paired, two tailed t-test. T-tests were conducted on log-transformed data.

Table 3. At the Order level, the average number of operational taxonomic units (OTUs) for bacteria in soil alone, and in unfertilized and N-fertilized canola and wheat rhizosphere.

Order	Soil alone	Canola		Wheat	
		No fertilizer-N	Fertilizer-N	No fertilizer-N	Fertilizer-N
Acholeplasmatales	ND	68*	29*	ND	ND
Acidimicrobiales	17	6,163	5,528	24	11
Acidobacteriaceae	31	4,755*	4,121*	19	112
Actinomycetales	ND	54,586	50,832	ND	ND
Alteromonadales	ND	5	2	ND	ND
Anaerolineales	ND	210	161	ND	ND
Ardenticatenales	1	127	118	0.2	1
Bacillales	265	20,042*	15,591*	298	511
Bacteroidales	ND	ND	ND	298	511
Bdellovibrionales	11	823	86	29	79
Blastocatella	ND	672	665	ND	ND
Burkholderiales	500	4,252	4,626	604	972
Caldilineales	ND	51	47	ND	ND
Campylobacterales	ND	7	5	ND	ND
Caulobacterales	108	756	689	115	393
Chlorobiales	64	ND	ND	57	30
Chromatiales	ND	211*	165*	ND	ND
Chroococcales	ND	41	56	ND	ND
Clostridiales	68	9,235*	6,962*	464	91
Coriobacteriales	3	587	507	44	2
Corynebacteriales	74	ND	ND	94	116
Cytophagales	57	48	50	60	88
Dehalococcoidales	ND	221	152	ND	ND
Dehalogenimonas	ND	1,891*	1,389*	ND	ND
Desulfobacterales	1	2,553*	1,906*	12	ND
Desulfocarbo	ND	18	15	ND	ND
Desulfovibrionales	ND	860	724	ND	ND
Desulfurellales	ND	32	23	ND	ND
Desulfuromonadales	96	3,157	2,513	1,427	100
Dictyoglomaceae	ND	174	146	ND	ND
Dissulfuribacter	ND	3	1	ND	ND
Entomoplasmatales	ND	3	2	ND	ND
Enterobacteriales	ND	ND	ND	1	8
Erysipelotrichales	ND	41	28	ND	ND
Fibrobacteraceae	94	7	3	179	40
Flavobacteriales	1	22	19	7	12
Gaiellales	119	10,157	12,509	108	108
Geminicoccus	ND	473	406	ND	ND
Gemmatimonadaceae	676	7,787	8,001	581	381
Gloeobacterales	ND	4	1	ND	ND
Halanaerobiales	ND	6	3	ND	ND
Holophagales	26	797	744	6	ND

Hydrogenispora	ND	44	29	ND	ND
Hydrogenophilales	1	1,027	1,207	2	4
Kineosporiales	379	ND	ND	403	480
Ktedonobacterales	ND	302	414	ND	1
Legionellales	545	127	64	713	488
Methylophilales	ND	45	46	ND	ND
Micavibrio	ND	42	17	ND	ND
Micrococcales	3,353	ND	ND	3,336	3,821
Micromonosporales	224	ND	ND	182	132
Myxococcales	1,709	6,542*	5,078*	1,825	951
Natranaerobiales	ND	25	13	ND	ND
Neisseriales	1	5	7	8	5
Nitrosomonadales	993	1,017*	1,539*	1,079	753
Nitrospiraceae	47	1,370*	1,796*	54	97
Oceanospirillales	ND	3	1	1	1
Oscillatoriales	ND	4	7	ND	ND
Propionibacteriales	1,994	ND	ND	1,790	1,806
Pseudomonadales	63	146	126	75	430
Pseudonocardiales	116	ND	ND	90	73
Rhizobiales	2,188	24,103	21,811	2,438	2,985
Rhodobacterales	283	249	231	295	363
Rhodocyclales	4	749	709	28	2.44
Rhodospirillales	2,110	6,039	5,264	2,172	1,281
Rickettsiales	13	76	46	100	70
Rivulariaceae	ND	306	230	ND	ND
Rubrobacterales	84	ND	ND	65	32
Selenomonadales	1	263	194	28	3
Solibacterales	ND	5,493	5,260	ND	ND
Solirubrobacterales	38	8,615*	6,738*	27	20
Sphaerobacterales	1	16	13	0	2
Sphingobacteriales	303	420	405	317	320
Sphingomonadales	363	21,936	24,425	330	354
Streptomycetales	503	ND	ND	555	534
Streptosporangiales	44	ND	ND	57	62
Synergistales	ND	21	9	ND	ND
Syntrophobacterales	ND	438*	335*	4	0
Thermoanaerobacterales	ND	3,876	3,467	2	1
Thermoanaerobaculum	ND	496	348	ND	ND
Thermodesulfobacteriaceae	ND	23	19	ND	ND
Thermogemmatisporales	ND	40	41	ND	ND
Thermoleophilales	ND	683	487	ND	ND
Thermolithobacterales	ND	11	5	ND	ND
Thiohalobacter	ND	37	33	ND	ND
Thiotrichales	4	ND	ND	8	8
Xanthomonadales	2,475	2,437*	2,818*	2,320	2,456
Total	21,240b	217,871a	202,057a	23,805b	22,091b

*Within each crop, values followed by the asterisk and same letters are significantly different at $p < 0.05$ according to a paired, two tailed t-test that were conducted using log-transformed data.

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Figure 3. Average bacterial profiles of canola rhizosphere showing phylum, class, and order for all identified operational taxonomic units (OTUs), only categories over 0.2% were graphed. A) No fertilizer-N added, B) fertilizer-N added.

Figure 4. The dynamics of N uptake with the OTU number of three bacterial species level during the growing season of canola. The temporal changes in the rates of N uptake in unfertilized canola was best described as a function of the log number of three specific OTUs during the growing season of canola. The model was $y = -2.24 + 1.675 X_1 + 3.867 X_2 - 5.928 X_3$; $r^2=0.91$), where y = rate of N uptake (kg N/ha/d); X_1 = log number of *Sphingomonas jaspi*; X_2 = log number of *Lysobacter arseniciresistens* ZS79, and X_3 = log number of *Luteimonas vadosa*.

Figure 5. The dynamics of N uptake by wheat with the log number of OTUs for an uncultured *Shingomonadales* sp. found in the crop's rhizosphere during the growing season.

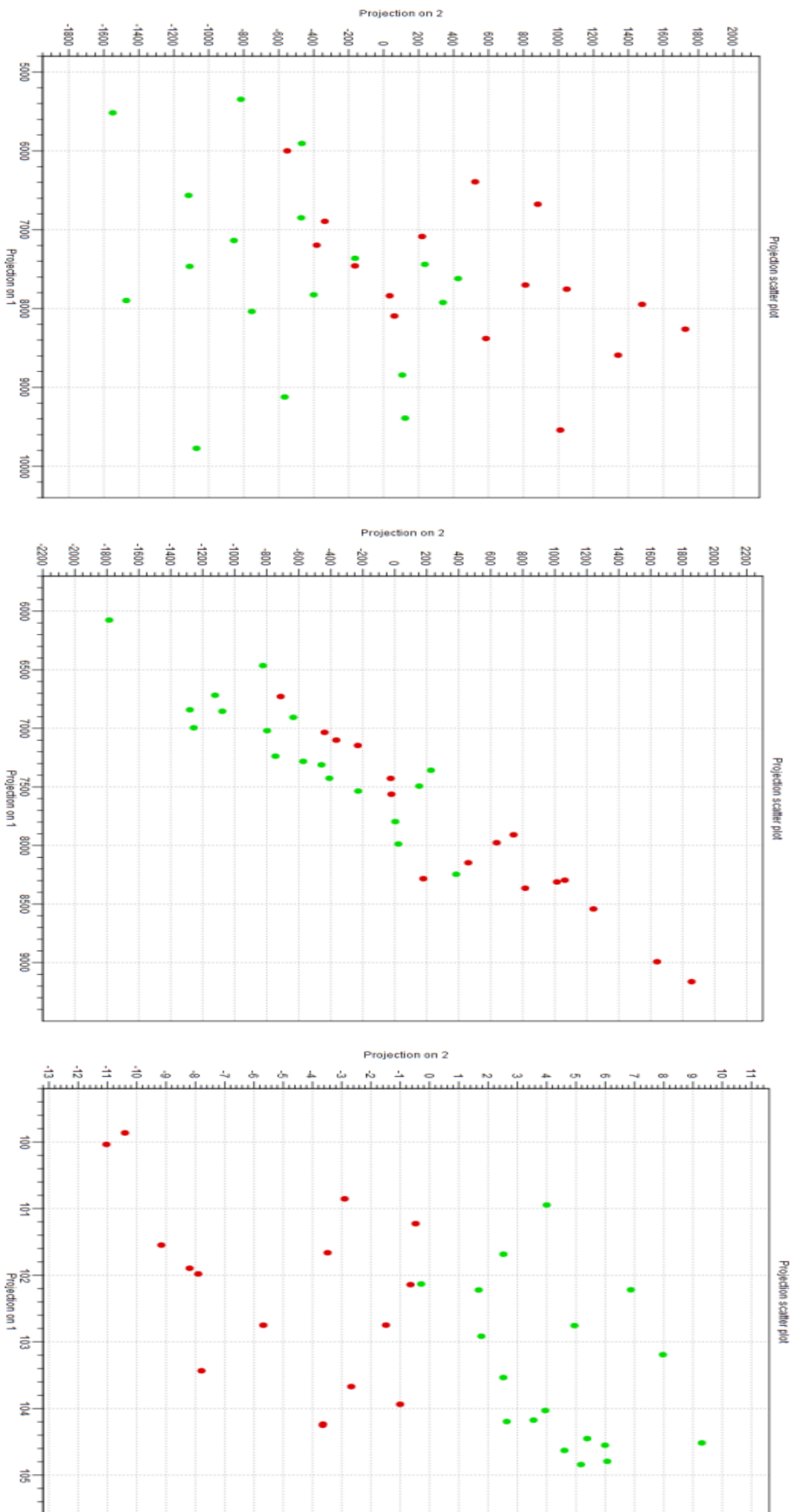


Figure 1.

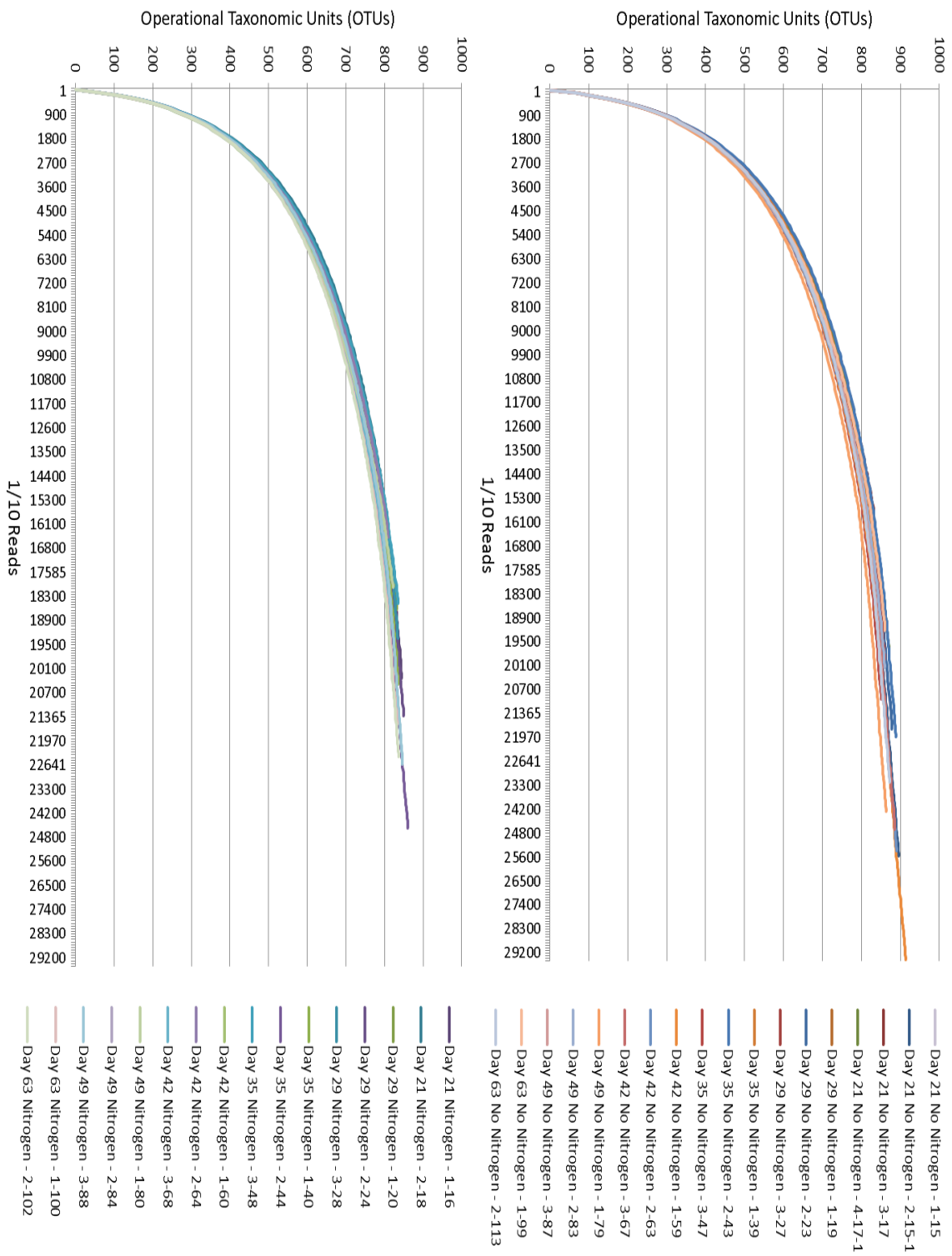


Figure 2.

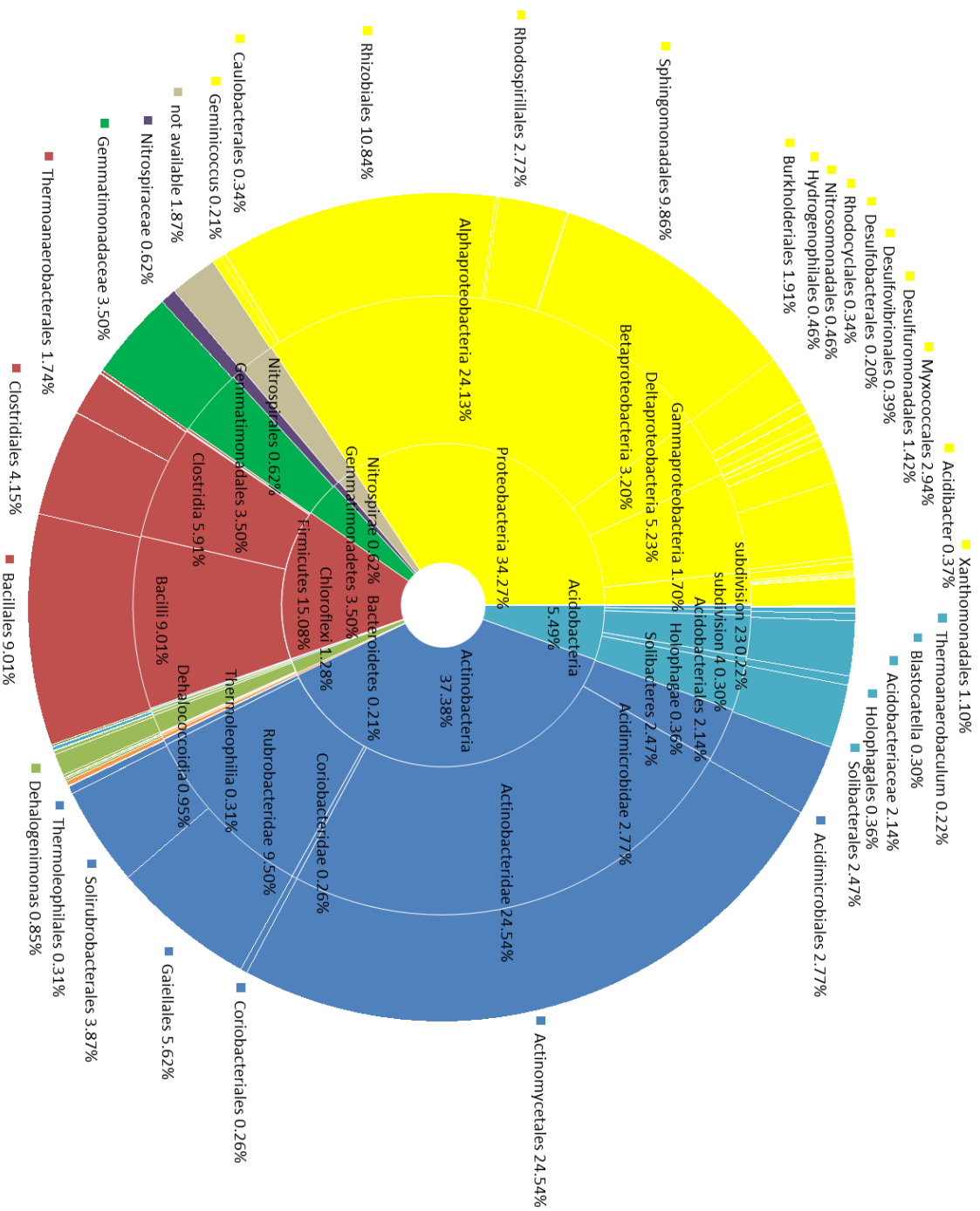


Figure 3A.

Figure 3B.

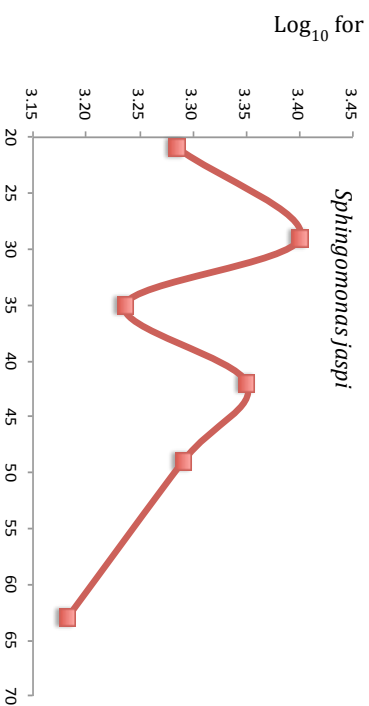
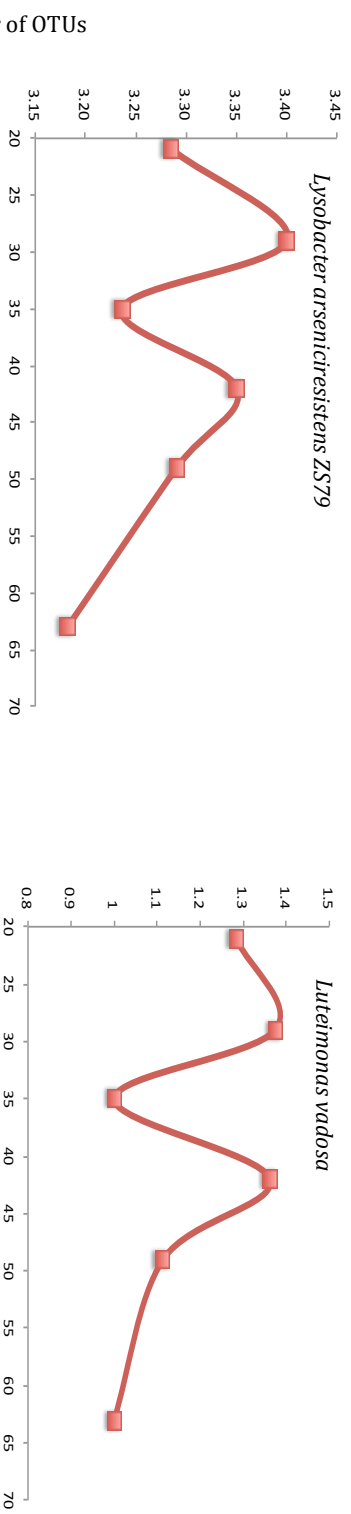
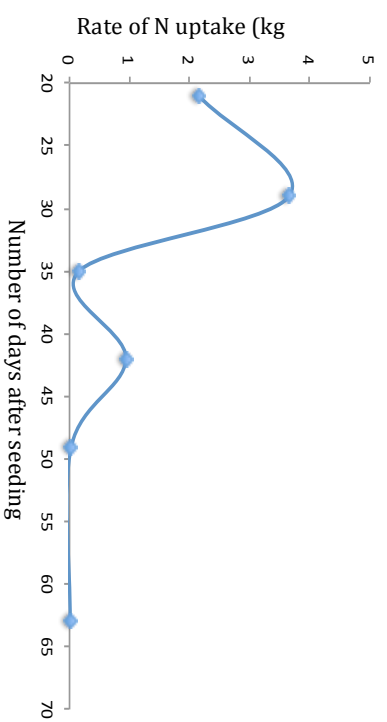


Figure 4.

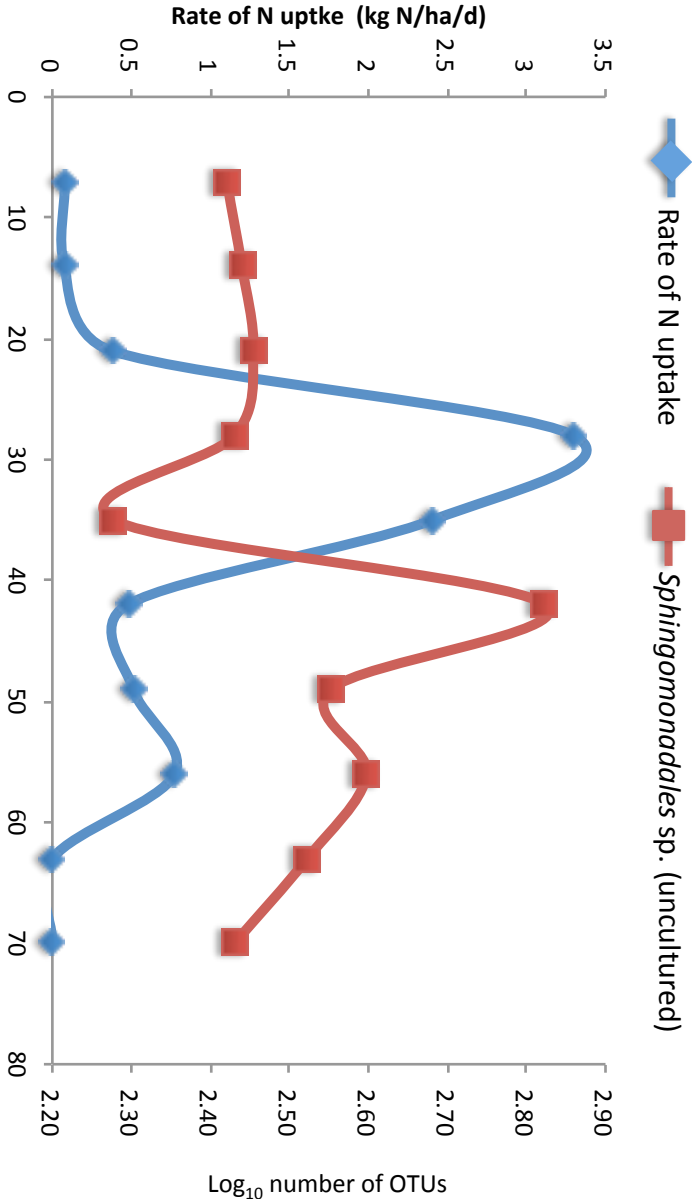


Figure 5.