Impact of Agricultural Practices on Nitrogen Cycle Genes and Nitrous Oxide Emissions from Midwestern Soils

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Project Goals:
The goals of this project are to fill existing knowledge gaps in our understanding of N-flux and associated C-turnover in soils and sediments. Novel information about the diversity, distribution, abundance and gene expression contributing to N-transformation is required to link desirable (i.e., N-retention) and undesirable (i.e., N-loss, such as N_2O emissions) activities with measurable microbial parameters. Linking molecular- and organismal-level information with environmental factors that control N- and C-turnover can predict the impact of land management practices on greenhouse gas emissions. Such integrated approaches generate novel information on multiple scales of resolution and contribute to system-level understanding of key nutrient cycles in soils. In the present work, we analyzed the response of microbial communities to agricultural practices (e.g., addition of N-species) in two agricultural sites, an important soil ecosystem for bioenergy crop production in Midwest US.

Abstract:
Assessing the impact of fertilizer overuse on microbial soil communities is important for a better understanding of the cycling of nitrogen and carbon and the emissions of potent greenhouse gasses. We analyzed short-read metagenomes obtained from two agricultural sites with contrasting soil textures (sandy versus silty-loam) during four seasons in 2012 at two depths: surface (0-5cm) and deep (20-30 cm). The predicted protein-coding sequences recovered in the 16 metagenomes, based on the SEED and Gene ontology databases, revealed a clear separation between surface and deep samples. For instance, genes related to light-dependent stress, DNA repair and nutrient uptake were more abundant (> 2-fold) in the surface samples in both soils (p-adjusted < 0.05). Distinct archaeal populations and nitrogen metabolism genes were characteristic of the deep samples. To overcome the limitations of fixed e-values cut-offs for annotation of short-read metagenomes and to reduce false positive matches, we developed a novel computational approach, called ROCker, that employs the receiver operating characteristic (ROC) curve to minimize the false discovery rate (FDR) based on how simulated shotgun metagenomic reads of known composition map onto well-curated reference protein sequences. ROCker typically showed 60-fold lower false positive rates compared to the common practice of using fixed e-values and hidden Markov models. Application of the ROCker approach to the time series metagenomes showed that most N cycling genes (e.g., *nosZ*, *amoA* and *nirK,* among others) varied in abundance over the course of the year. For instance, we found a remarkably high abundance of metagenomic reads related to the Clade II *nosZ* (reduction of N_2O to N_2) sequences, accounting for approximately 90% of the total *nosZ* reads found in both soil layers. Approximately 12% of the *nosZ* reads were taxonomically assigned to the *Anaeromyxobacter* genus, indicating their potential relevance in N_2O reduction. In addition, six *amoA* (ammonia

oxidation) genes, each encoded by distinct archaeal and bacterial populations, became abundant in the deep sandy samples when seasonal nitrogen fertilization was applied. Population binning allowed the recovery of several draft genomes for novel (at least at the species level) ammonia-oxidizing archaea, bacteria and nitrite oxidizing bacteria. The most abundant populations (ranging from 10 to 35X coverage), related to the *Thaumarchaeota* and *Nitrospira* phyla, were observed to sharply increase in abundance upon N fertilizer application. The activity and abundance of these populations were more closely examined by combining metatranscriptomic, metagenomic and metaproteomics approaches in ^{15}N-labeled ammonia incubations under controlled laboratory settings. This analysis allowed the quantification of the activity of each population, and assessed the strength of each feature (gene, transcript or protein) in explaining ammonia oxidation and N_2O emission rates. Collectively, our study identified key microbial populations and genes responding to seasonal and human-induced perturbations (e.g., fertilization) and controlling the fate of nitrogen in agricultural soils. It also advanced the molecular toolbox for studying N cycling in soils, and is applicable to other important environmental processes.

References:

1. Robert A. Sanford, Darlene D. Wagner, Qingzhong Wu, Joanne C. Chee-Sanford, Sara H. Thomas, Claribel Cruz-García, Gina Rodríguez, Arturo Massol-Deyá, Kishore K. Krishnani, Kirsti M. Ritalahti, Silke Nissen, Konstantinos T. Konstantinidis, and Frank E. Löffler. 2012. **Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils.** Proc. Natl. Acad. Sci. U. S. A. **109**: 19709–14.

2. Orellana, L. H., L. M. Rodriguez-R, S. Higgins, J. C. Chee-Sanford, R. A. Sanford, K. M. Ritalahti, F. E. Löffler, and K. T. Konstantinidis. 2014. **Detecting nitrous oxide reductase (*nosZ*) genes in soil metagenomes: method development and implications for the nitrogen cycle**. mBio **5**: e01193-14.

3. Orellana, L. H., L. M. Rodriguez-R and K. T. Konstantinidis. **ROCker: a pipeline for accurate detection and quantification of target genes in short-read metagenomic datasets**. *Under review.*

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