Multiomic Insights into the Activity and Dynamics of Soil Nitrifier Communities in Midwestern Agricultural 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 can be used to better predict the impact of land management practices on N- and C-turnover. 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-fertilizer) in two distinct agricultural soils, 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 C and N in soils. However, integrating field metadata (e.g., temperature, moisture, and oxygen) with the activity and dynamics of microbial communities in order to provide a systems-level understanding of nutrient cycling remains challenging, especially for soils. To this end, we analyzed short-read metagenomes obtained from two agricultural sites with contrasting soil textures (sandy versus silt loam) during four seasons in 2012 at two depths: surface (0-5cm) and deep (20-30 cm). Distinct archaeal populations and N 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 under-studied 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. Population binning allowed the recovery of 69 draft genomes, including novel nitrifier archaea and bacteria. Six bins encoding *amoA* represented five new members of the *Thaumarchaeota* phylum and

three nitrifier populations represented a new bacterial genus, most related to the commamox *Nitrospirae.* These nitrifier populations, especially the archaeal ones, were observed to sharply increase in abundance upon N fertilizer application in the 20-30 cm soil layer in sandy soils, suggesting that they were responsible for a large part of (the fertilized) ammonia oxidation. In addition, time-series metatranscriptomic analyses of N-amended soil mesocosms (simulating a fertilization event) showed a high correspondence between *amoA* transcript abundance and ammonium disappearance ($r^2 \sim 0.96$), and that bacterial nitrifiers were comparatively more responsive than their archaeal counterparts. Collectively, our study identified measurable biomarkers and novel microbial populations controlling the fate of fertilizer N and N_2O emissions in agricultural soils, revealed key differences in the responding populations from field and incubated soils, and advanced the molecular toolbox for studying *in-situ* processes.

References:

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