PUNCS: Towards Predictive Understanding of Nitrogen Cycling in Soils

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Project Goals: In anoxic environments, nitrate is largely consumed by dissimilatory nitrate reduction to ammonium (DNRA) and nitrate reduction to N_2O and N_2 (denitrification). DNRA oxidizes more C per mole of nitrate than denitrification and generates a cation (NH_4^+), which is retained in most soils, indicating that these processes have profoundly different impacts on N retention and greenhouse gas (CO_2, N_2O) emissions. Microbes capable of DNRA or denitrification coexist but the regulatory controls for these competing processes are unknown, and their relative contributions cannot be predicted faithfully with current models. This paucity of information limits the development of more accurate, predictive models of N-flux including the effects of N-retention on plant growth and biomass yield for bioenergy production, as well as greenhouse gas (e.g., N_2O) emissions.

To elucidate the environmental factors controlling N-turnover in soils, the project team will conduct physiological studies, stable isotope probing (SIP) experiments, (meta)genomics and (meta)transcriptomics analyses, along with high resolution imaging (NanoSIMS) on systems of increasing complexity (i.e., pure cultures to mesocosms established with distinct soil types). We will quantitatively monitor genes and transcripts related to N-turnover in response to changes in pH, temperature, soil moisture, C- and N-content and assess the emitted gases using a mass balance approach based on (geo)chemical and stable isotope measurements. By comparing the gene-centric and genomic (*who is there?*) to the transcriptomic, SIP, and NanoSIMS (*who is how active?*) datasets gathered from different treatments, ***system-level insights into the pathway controls and the functional redundancy within microbial communities controlling N-flux in soils will be obtained***.

One project task determines the pathway controls for nitrate depletion in anoxic soil environments; denitrification versus DNRA. Previous observations suggested that the carbon to nitrogen (C:N) ratio controls the fate of nitrate to gaseous products (N_2O, N_2) or NH_4^+. *Shewanella loihica*, a unique bacterium with both complete denitrification and DNRA pathways, is used to explore the environmental factors (e.g., C:N ratio, type of C available, pH, temperature) that determine which N-oxide reduction pathway predominates. The results obtained to date suggest that the C:N ratio is one of several factors that influence the fate of nitrate. DNRA appears to be favored over denitrification under conditions of low nitrate:nitrite ratios and high C:N ratios. Further, nitrate reduction to ammonium predominated in mineral medium augmented with amino acids. Gene expression studies are being used to monitor the expression of DNRA and denitrification genes under these different growth conditions.

Another project goal is to better understand the diversity of *nosZ* genes involved in N_2O reduction to N_2. Genome analysis of the nitrite-to-NH_4^+-reducing, non-denitrifying bacterium *Anaeromyxobacter dehalogenans* strain 2CP-C revealed the presence of a complete *nosZ* gene cluster. Subsequent physiological studies corroborated that this organism uses N_2O as a growth-supporting electron acceptor. The denitrifier- and *Anaeromyxobacter*-types of *nosZ* genes share sequence similarity; however, the primers used for environmental surveys of denitrifier *nosZ* have failed to detect the *Anaeromyxobacter*-type *nosZ*. PCR primers specifically targeting the *Anaeromyxobacter*-type *nosZ* determined its distribution in different soil ecosystems, suggesting that an important, yet unrecognized N_2O sink exists. Recent isolation and sequencing efforts revealed an unexpected diversity of non-denitrifying N_2O reducers and *nosZ* genes in soil ecosystems suggesting that current N_2O emission models are missing a possible significant N_2O sink.

To elucidate the relative abundance of genes implicated in nitrate, nitrite and N_2O transformation in soils, we have embarked on surveys of existing metagenome datasets. The datasets incorporate millions of short-read sequences (e.g., shorter than 400 bp), obtained using either the Illumina or the Roche 454 sequencers. Therefore, the first objective of our work is to develop the bioinformatics pipeline that will allow us to reliably identify and align short fragments of the target genes recovered in the available metagenomes. Subsequently, we will evaluate in-silico the specificity of available primers for chosen target genes and design new primers for microbial groups that are not encompassed by the primers currently available. These approaches will enable the identification and enumeration of keystone microbial groups that respond to different incubation conditions and perturbations in mesocosm systems. We have also validated and applied Illumina Hi-Seq 2000 sequencing on soil samples, and have established the bioinformatics approaches to assemble such high-volume data (>50Gb per sample) and allow comparisons of different communities based on metagenomic datasets. We will report on recent developments including preliminary results from metagenome sequencing efforts.

This research is supported by DOE Genomic Science Grant DE-FG02-11ER65267.