71. Detecting Nitrous Oxide Reductase (nosZ) Genes in Soil Metagenomes: Method Development and Implications for the Nitrogen Cycle

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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 expression of genes contributing to N-transformation is required to link desirable (i.e., N-retention) and undesirable (i.e., N_2O emission) 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 (N_2O, CO_2) emissions. Such integrated approaches generate novel information at multiple scales of resolution and contribute to system-level understanding of key nutrient cycles in soils. In the present work, we developed and applied bioinformatic approaches to study the abundance and diversity of the nitrous oxide reductase gene, currently known as the only gene directly involved in the reduction of N_2O to an innocuous form, N_2.

Abstract: The anthropogenic fixation of N_2, by means of the Haber-Bosch process, has led to the overuse of synthetic, nitrogen-based fertilizers in agriculture. As a consequence of the increased nitrogen content in soils, the atmospheric N_2O concentration increased nearly 20% relative to preindustrial era levels. Microbial processes including ammonium oxidation, dissimilatory nitrate reduction to ammonium, and primarily denitrification contribute to N_2O emissions. The key enzyme for mitigating N_2O emissions is the nitrous oxide reductase (NosZ), which catalyzes N_2O reduction to N_2 and is generally attributed to denitrifying microorganisms. We have recently described a novel group of "atypical" functional NosZ encoded on the genomes of denitrifiers and incomplete denitrifiers, most of which were missed in previous PCR-based surveys (Sanford et al., PNAS 2012). We analyzed the abundance and diversity of both types of *nosZ* genes in whole-genome shotgun metagenomes obtained from sandy and silty-loam soils in Illinois that typify the Midwest US corn belt, frequently used in bioenergy production. We tested different algorithms and defined appropriate cut-offs for detecting typical and atypical *nosZ* fragments based on *in silico* generated (mock) metagenomes. Based on the determined cut-offs, more than 71 distinct reference representatives (obtained from clustered sequences at 95% amino acid identity), encoding typical and atypical NosZ, were detected in both soil types. Remarkably, more than 70% of the total *nosZ*-encoding reads in both soils were classified as atypical. About 12% of the total *nosZ* reads were taxonomically assigned to the *Anaeromyxobacter* genus, indicating the potential relevance of these organisms for N_2O reduction. Further analyses indicated that atypical *nosZ* genes outnumbered typical counterparts in most publicly available soil metagenomes from various important soil ecosystems in North America, underscoring the ecological importance of atypical *nosZ* in soils. Our work provides a bioinformatic strategy to detect target genes in data-rich short-read metagenomes and has implications for better understanding N-cycling in soils.

References:

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***Funding Statement**: This research was supported by the US Department of Energy, Office of Biological and Environmental Research, Genomic Science Program, Award DE-SC0006662.*