## 66. Denitrification in the Absence of Bacterial Nitrite Reductases

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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., N2O emission) activities with measurable microbial parameters. Correlating 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 (N2O, CO2) 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 design tools to assess the contribution of fungi to N2O production in soils, and demonstrate that bacteria without the nitrite reductase genes nirS or nirK contribute to denitrification.

Abstract: The overuse of synthetic, nitrogen-based fertilizers stimulates microbial N- turnover, effectively accelerating the global N-cycle. Bacterial denitrification is generally considered the major pathway for nitrate turnover under anoxic conditions although recent findings suggest that respiratory ammonification can predominate under certain geochemical conditions (1). Monitoring nirS or nirK genes, which encode the nitrite reductases, is often employed to assess denitrification potential. Interestingly, Anaeromyxobacter dehalogenans, a Deltaproteobacterium lacking nirS or nirK, reduced nitrite to dinitrogen gas in medium amended with ferric iron and acetate as electron donor. Detailed studies revealed that ferrous iron, the product of ferric iron reduction, chemically reduced nitrite to N2O (i.e., chemodenitrification). Anaeromyxobacter dehalogenans strains possess a functional atypical nosZ gene conferring the ability to reduce N2O to dinitrogen (2), and the cultures reduced N2O to dinitrogen. Despite the absence of key denitrification genes, A. dehalogenans contributed to the conversion of nitrite to dinitrogen in the presence of ferric iron. Ferric iron minerals are common to soils and sediments suggesting that coupled abiotic-biotic processes (i.e., chemodenitrification followed by enzymatic N2O reduction) contribute to N-cycling and affect N2O flux. These findings also demonstrate that the role of a microorganism in N- cycling cannot be predicted based on gene content alone, and ferric iron reducers lacking nitrite reductase genes can contribute to denitrification.

Fungal activity is another source of N2O in soils; however, the involvement of fungi in soil geochemical transformations is poorly understood. Filamentous Ascomycetes and Basidiomycetes are key contributors to the degradation of plant-derived organic matter (e.g., lignin, cellulose), and affect soil carbon flux. Further, members of these taxa have been implicated in nitrate/nitrite reduction to N2O (3). Though relevant contributions of fungi to C- and N-turnover in soils have been recognized, molecular tools to selectively target fungal taxa and their functional genes involved in geochemical cycling are lacking. To address these shortcomings, more than 200 fungal isolates capable of nitrate or nitrite reduction were obtained from two distinct soil sites in Illinois. Degenerate PCR primers amplifying an approximately 650-bp fragment of the fungal p450nor gene, which is responsible for N2O production, were designed. The primer set amplified p450nor genes from many denitrifying fungal isolates, and amplicons were also obtained from soil DNA extracts. Sequence analysis confirmed specific amplification of p450nor gene fragments. This PCR tool is now being applied to assess the dynamics of fungal p450nor genes over spatial and temporal scales at the two study sites. To assess the diversity and dynamics of soil fungi from

the two different soil types, automated ribosomal intergenic spacer analysis (ARISA) was conducted on samples collected over defined spatial and temporal scales (e.g., with depth and across seasons). The average number of ARISA fragment sizes representing unique operational taxonomic units (OTUs) was higher in the well- drained sandy soil (n=95) than in the clay-containing silt loam (n=76). Overall fungal communities were significantly distinct across soil depths at any time of year, but assemblages shifted seasonally with depth. qPCR assays targeting the ribosomal intergenic spacer regions of 15 fungal morphotypes were designed and are being applied in concert with the p450nor primers to assess fungal population dynamics in terms of abundance and denitrification potential. This study provides new tools to assess soil denitrification and associated C-turnover in the absence of nirS or nirK in soil ecosystems.

References:

1. Yoon, S., C. Cruz-García, R. Sanford, K. M. Ritalahti, and F. E. Löffler. 2014. Denitrification versus ammonification: environmental controls of two competing respiratory nitrate/nitrite reduction pathways in Shewanella loihica. ISME J. doi: 10.1038/ismej.2014.201.
2. 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
3. E. Löffler. Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. Proc. Natl. Acad. Sci. U. S. A. 109: 19709-19714.
4. Shoun H, Fushinobu S, Jiang L, Kim S-W, Wakagi T. 2012. Fungal denitrification and nitric oxide reductase cytochrome P450nor. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 367:1186-94.

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