Denitrification revisited: Contributions of chemodenitrifiers and fungi to soil denitrification

Jenny Onley,1 Steven A. Higgins,1 Luis Orellana,2 Konstantinos Konstantinidis,2 Joanne Chee-Sanford,3 Robert A. Sanford,4 and Frank E. Löffler1,5* (frank.loeffler@utk.edu)

1University of Tennessee, Knoxville, TN; 2Georgia Institute of Technology, Atlanta, Georgia; 3US Department of Agriculture-Agricultural Research Service, Urbana, IL; 4University of Illinois, Urbana, IL; 5Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN

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 loss, such as N2O emissions) 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 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 show that coupled biotic-abiotic processes contribute to denitrification, and we demonstrate the utility of new p450nor-targeted PCR primers to assess the fungal contribution to N2O formation in soils.

Abstract:
To meet the needs of a growing human population, the use of N-based fertilizers has increased substantially in the last 50 years. Denitrification of nitrate is a major pathway for nitrate turnover and is associated with soil N loss and nitrous oxide (N2O) emissions (Sanford et al., 2012). The assessment of denitrification potential has been largely based on the enumeration of bacterial nirK and nirS genes, which encode nitrite reductases (nitrite à→ nitric oxide, NO). NO is subsequently enzymatically reduced to N2O and dinitrogen. An alternate pathway for nitrite reduction is chemodenitrification, the abiotic reduction of nitrite to N2O mediated by ferrous iron. Anaeromyxobacter dehalogenans, a common and metabolically versatile soil bacterium, utilizes nitrate and ferric iron as electron acceptors. Consistent with gene content (i.e., the presence of nrfA encoding the ammonia-forming cytochrome c nitrite reductase), the organism reduces nitrate to ammonium via nitrite. Copies of the key denitrification genes nirK and nirS are absent on the genome, but an atypical Clade II nos operon encoding a functional nitrous oxide reductase (NosZ) is present (Sanford et al., 2012). Interestingly, ammonium was not the major product of nitrate reduction in axenic cultures amended with ferric iron, and nitrate was predominantly reduced to N2O in an abiotic reaction with ferrous iron. The N2O formed was subsequently reduced by the activity of the Clade II NosZ. Even though A. dehalogenans lacks nirS or nirK, the organism contributes to complete denitrification through a combination of direct enzymatic and coupled biotic-abiotic reactions. The prevalence of ferric iron minerals and iron-reducing bacteria in soils suggest that chemodenitrifiers have relevant contributions to N turnover and N2O flux.
Fungi are key contributors to carbon (C) cycling in soils and their role in C turnover is well established; however, the broader roles of fungi for soil N turnover remain largely unexplored. Recent evidence suggested a significant role for dominant soil fungi in denitrification and associated N₂O production (Chen et al., 2014). Fungi are distinguished from their denitrifying bacterial counterparts due to the formation of N₂O as the major end product of denitrification, and mounting evidence suggests that fungal denitrification could be a major source of N₂O in soils. Fungi possess a unique gene, *p450nor*, encoding an approximately 44 kDa cytochrome P450 protein that catalyzes the reduction of NO to N₂O by direct electron transfer from NADH or NADPH (Shoun et al., 2012). Cultivation-based approaches to assess the fungal contribution to N₂O production in soils are highly biased, and culture-independent tools for detecting fungal denitrifiers are desirable. Therefore, we designed novel PCR primer sets targeting the fungal *p450nor* gene. Amplification of *p450nor* from DNA of 37 denitrifying fungal isolates validated the approach and application to agricultural soil yielded 23 unique *p450nor* amplicons (Higgins et al., 2016). Phylogenetic analysis demonstrated monophyly and provided insights into the taxonomic diversity of denitrifying fungi in the soils studied. Interestingly, *p450nor* genes were not detected in metagenomes generated from the same agricultural soil samples, emphasizing the value of the novel PCR-based approach for assessing potential fungal contributions. Collectively, our studies demonstrate that N cycling (and associated C turnover) cannot be predicted based on gene content (e.g., *nirS, nirK*) alone, and chemodenitrifiers may be major contributors to N₂O flux in soils. The new *p450nor*-targeted primer set complements the molecular toolbox for studying N₂O formation in soils, and has broad utility for assessing fungal denitrification activity.

References:

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