Denitrification revisited: Contributions of chemodenitrifiers and fungi to soil denitrification

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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 loss, such as N_2O 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 N_2O 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 (N_2O) 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 → nitric oxide, NO). NO is subsequently enzymatically reduced to N_2O and dinitrogen. An alternate pathway for nitrite reduction is chemodenitrification, the abiotic reduction of nitrite to N_2O 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 N_2O in an abiotic reaction with ferrous iron. The N_2O 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 N_2O 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 N_2O production (Chen *et al.*, 2014). Fungi are distinguished from their denitrifying bacterial counterparts due to the formation of N_2O as the major end product of denitrification, and mounting evidence suggests that fungal denitrification could be a major source of N_2O in soils. Fungi possess a unique gene, *p450nor*, encoding an approximately 44 kDa cytochrome P450 protein that catalyzes the reduction of NO to N_2O by direct electron transfer from NADH or NADPH (Shoun *et al.*, 2012). Cultivation-based approaches to assess the fungal contribution to N_2O 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 N_2O flux in soils. The new *p450nor*-targeted primer set complements the molecular toolbox for studying N_2O formation in soils, and has broad utility for assessing fungal denitrification activity.

References:

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