72. Cultivation and PCR-based Approaches Elucidate the Functional Diversity of Soil Fungal Populations Contributing to Nitrogen Cycling in Soils

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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 are desirable to interpret field-scale observations, and 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 molecular approaches to study the diversity of fungi contributing to nitrous oxide production within two field sites in Illinois. The current study addresses the need for a better understanding of the contributions of fungal populations to N-turnover and associated C-flux in soils. Specifically, molecular tools were designed and applied to assess the fungal diversity and the abundance of fungal p450nor genes implicated in fungal denitrification and N_2O production.

The kingdom Fungi is estimated to comprise anywhere from 700,000 to 1,500,000 species. Of the known diversity, a majority is housed within the phyla Ascomycota and Basidiomycota. These taxa are distinguished by their activities in soils, such as nutrient transport and retention, engaging in beneficial symbiotic associations with plants, and enhancing overall soil health. Although recent efforts suggest that fungi play relevant roles for nutrient turnover in soils, their involvement in soil geochemical transformations is poorly understood. Anthropogenic activities (i.e., large-scale agriculture and industrialization) over the past two centuries have affected soil microbial activity, and in turn have led to increased greenhouse gas emissions from soil. Filamentous Ascomycetes and Basidiomycetes are key contributors to the degradation of plant- derived organic matter (e.g., lignin, cellulose), and affect soil carbon flux (1, 2). Moreover, members of these taxa have been implicated in denitrification, the conversion of nitrate/nitrite to gaseous products (N_2O, N_2) (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, 214 fungal isolates capable of using nitrate or nitrite as the sole nitrogen source in liquid medium were obtained from two physicochemically distinct soil sites in Illinois. The majority (70%) of the isolates were denitrifiers and produced N_2O from nitrite. Phenotypic characterization distinguished 15 morphotypes represented by isolates from both field sites. For functional characterization of denitrifying isolates, degenerate PCR primers amplifying an approximately 650-bp fragment of the fungal *p450nor* gene, which is responsible for N_2O production, were designed. The primer set amplified the *p450nor* gene from many denitrifying fungal isolates, and to date 13 *p450nor* genes were cloned and sequenced. 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 within depth. The distinction between fungal communities across spatiotemporal scales was more prominent in sand than silt loam. The ribosomal intergenic spacer regions from the 15 distinct fungal morphotypes were cloned and sequenced. This sequence information will guide the design of qPCR assays to target fungal populations and their dynamics in terms of abundance and activity. In concert with soil metadata analysis (e.g., soil moisture, pH, temperature), these efforts will quantify the fungal contribution to N- and C-turnover in agricultural soil ecosystems.

References:

1. **Rousk J, Brookes PC, Bååth E**. 2009. Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization. Appl. Environ. Microbiol. **75** :1589–1596.
2. **Fontaine S, Henault C, Aamor A, Bdioui N, Bloor JMG, Maire V, Mary B, Revaillot S, Maron PA**. 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol. Biochem. **43**:86–96.
3. **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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