Improved High-Throughput Quantification of Nitrogen Cycling Genes in Bioenergy Crop Soils

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Project Goals: We will characterize plant-microbe interactions associated with nitrogen cycling in miscanthus and corn soils. Denitrification genes will be compared between miscanthus and corn crops over a growing season, under different fertilization rates, and with various stand ages. Our results will help us to understand how plant, soil, and microbe interactions provide nutrients to varying feedstocks and how we may sustainably manage their productivity.

Metagenomic sequencing has helped us to understand the immense diversity of microbial strains that participate in nitrogen (N) cycling in soils. However, this diversity represents a challenge for identifying and measuring the key drivers of nitrogen cycling, especially as metagenome sequencing is not practical for large number of samples. Consequently, we still have a limited understanding on the abundance of the microorganisms carrying out these processes and related interactions between soil microbes and bioenergy crops. The development of high-throughput qPCR presents a novel opportunity to characterize nitrogen genes in complex soil environments but is limited by selection of probes for representative gene targets. Our objective was to develop genomic probes for characterizing denitrification in bioenergy crops. Among N cycle pathways, denitrification is an important process in agricultural soils because it removes fixed N that can otherwise be used for primary production and produces N_2O (one of the major global warming gases). Our results confirmed that currently available probes, designed based on well-characterized isolates, cannot cover diverse denitrification genes in our soils. Thus, we developed gene probes that can cover diverse denitrification genes present in agricultural soils and are also appropriate for high-throughput qPCR, to quantify denitrification genes in bioenergy crop soils. A novel primer-design tool, EcoFunPrimer, was used to redesign 384 novel primer sets targeting *nap*A, *nar*G, *nir*K, *nir*S, and *nos*Z genes based on the abundances of gene clusters enumerated in 1,950 publicly available soil metagenomes. These probes were initially tested against a subset of miscanthus soil samples from the CABBI Long-Term Assessment of Miscanthus Productivity and Sustainability (LAMPS) site. Our results show that new primers

can detect a significantly higher proportion of denitrification genes in soil systems and justifies using these probes for all 700 soil samples available from the LAMPS experiment. Further, we are expanding probe design to other N cycle genes.

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