Development of High Throughput Primer Design and Quantification for Nitrogen Cycle Genes in Bioenergy Crop Soils

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Project Goals: To improve understanding of how plant-soil-microbe interactions influence nitrogen cycling in bioenergy crop soils, this project aims to relate microbial functional gene diversity and abundance to rates of gross nitrogen cycling and gaseous nitrogen fluxes in bioenergy crop soil and to develop cost-effective, scalable tools to capture the phylogenetically diverse nitrogen cycle genes in environmental samples.

The development of molecular biological tools in combination with advanced sequencing technologies (e.g., 16S rRNA amplicon sequencing) has enabled high throughput characterization of community composition and structure. However, these approaches are often restricted to characterizing microbial community structure and cannot reliably provide information on the functional potential of genes. Metagenomic sequencing can be an improved approach to investigating diverse functional genes in environmental samples. However, these functional groups often comprise only a small fraction of the environmental DNA, resulting in high costs and low sequencing coverage. Another method to characterize functional genes leverages PCR-based methods. For PCR-based methods to target functional genes, the reliability of primer sets is a prerequisite, as the primer sets ultimately determine what is amplified in the environmental samples. Unfortunately, conventional PCR primers are known to detect a limited range of the diverse genes involved in nitrogen cycle. Further, the majority of currently available primers have been designed mainly for isolated strains instead of environmental biodiversity, and there is a lack of cost-effective and scalable platforms to cover the high diversity of target genes. As high throughput qPCR has become applicable for environmental samples, we can now assay hundreds of primer sets and genes in a single run. Thus, we have developed a pipeline to perform high throughput primer design based on abundant nitrogen cycle genes based on their abundance in over 1,900 existing soil metagenome samples. Through the pipeline, >400 novel primer sets were designed targeting denitrification (*nap*A, *nar*G, *nir*K, *nir*S, *nor*B, *nos*Z) and nitrification (*amo*A-AOA/AOB) genes. We have optimized BioMark HD, a high throughput qPCR system, for studying nitrogen cycling Miscanthus soil samples from the DOE CABBI LAMPS site.

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