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~ Presents ~

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TABLE OF CONTENTS

Alburuj Rahman '15
College of Agriculture and Life Sciences
arr75@cornell.edu
Columbus, OH

Biological Sciences
Advisor: James Shapleigh
Department of Microbiology

Study of modular denitrification in microbial communities using sequence analysis

Denitrification, the reduction of nitrate to nitrogen gas, is one way bacteria carry out nitrate respiration, and happens in anoxic environments. The process of denitrification happens in steps requiring four separate enzymes referred to as reductases: (i.) nitrate reductase (Nar or Nap), (ii.) nitrite reductase (Nir), (iii.) nitric-oxide reductase (Nor), and (iv.) nitrous-oxide reductase (Nos). The accepted view of the denitrification process is that it is carried out by individual microbial organisms. A more recent hypothesis suggests that denitrification in environments happens through modularity. Modularity arises through the observation that each reductase can provide benefit in the absence of other reductases. Thus, presence of modularity is unclear in many environments. Consequently, denitrifying bacteria can be either partial or complete. Finding out whether denitrification in soil is carried out by partials working communally or by complete denitrifiers working independently is a goal for the project.

To determine if modularity is important in soil, DNA samples were collected from the soil of the Hubbard Brooke Experimental Forest, a site reserved for ecosystem studies. Soil was collected from three distinct locations of different pH, altitude with respect to a body of water, and soil properties. The DNA was sequenced using the Illumina sequencing platform. Trimmed and paired reads were imported into MG-RAST, a metagenomic database sequence analysis tool.

Efforts were made to validate the MG-RAST based taxonomic classifications and functional characteristics of the sequenced DNA by studying the collected DNA sequences using local NCBI blastx and blastn searches, and by aligning and comparing sequences for identity match between groups of classified organisms' genomes. We will be looking for relationships between samples to determine how different Hubbard Brooke properties may influence denitrification. We plan to identify the groups of proteins that may belong to one or more organism in Hubbard Brooke. G+C content and known properties of the Hubbard Brooke collection site were considered when determining bias or inaccuracies in the assigning specificity of sequences by blast. Based on the current data, the sequences that have been classified appear to be linked to or containing either Nir or Nor, but rarely both, supporting modularity. We will work on building more appropriate databases and validation tools to reasonably determination nitrate reductase distribution at Hubbard Brook.