Development of Novel Random Network Theory-Based Approaches to Identify Network Interactions Among Nitrifying Bacteria

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Project Goals: Interactions among various microbial populations in a community play critical roles in determining the functioning of an ecosystem. However, little is known about such network interactions. Also, nitrification is an important step in the nitrogen cycle, and two groups of microorganisms, ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) are thought to play essential roles in this microbe-mediated process. Thus the ultimate goal of this project is to develop a novel molecular ecological network approach and analysis pipeline to understand the genetic diversity and interaction of soil AOA and AOB in a grassland ecosystem. Three specific objectives have been pursued: (i) To develop a molecular ecological network approach and analysis pipeline; (ii) To determine the genetic diversity of soil AOA and AOB populations by pyrosequening of bacterial and archaea *amoA* genes; and (iii) To examine interactions of soil AOA and AOB populations by molecular ecological network analysis of *amoA* pyrosequening data.

Development of functional molecular ecological network approach and pipeline. Theoretically, the interactions among various microbial populations in a community play critical roles in determining the functioning of an ecosystem; however, these relationships remain unclear due to the lack of appropriate experimental data and computational analytic tools. To address such challenges, a mathematical approach for identifying molecular ecological networks (MENs) from high-throughput metagenomics sequencing data has been developed. Two major steps are involved: (i) construction of network based on the Random Matrix Theory; and (ii) network characterization using various mathematical approaches. Compared to other network reconstruction methods, the RMT-based approach is remarkable in that the networks are automatically defined and robust to noise, thus providing excellent solutions to several common issues associated with high-throughput metagenomics data. The MEN topological analyses have integrated the most recent findings in social and biological network analyses, such as general network structures (small-world, scale-free, modularity and hierarchy structures) and module-based eigengene analysis, for dissecting community organizations at the whole and module levels. Furthermore, we associated module characteristics with environmental traits to understand the importance of network interactions in determining community functions. To facilitate application by the scientific community, all of these methods and statistical tools have been integrated into a comprehensive Molecular Ecological Network Analysis Pipeline (MENAP), which is open-accessible through the internet.

Pyrosequencing analysis of bacterial and archaeal *amoA* genes. We have designed universal primers for amplifying archaeal or bacterial *amoA* genes (ammonia monoxygenase subunit A). Both bacterial *amoA* and archaeal *amoA* genes were amplified from soil samples in a grassland experiment site, BioCON (CO_2 concentrations, nitrogen fertilization and plant species), followed by 454 pyrosequencing. A total of 1.2M reads were obtained. After preprocessing, about 651K reads remained with 140K for bacteria

and 511K for archaea. A total of 1911 OTUs for bacteria and 7922 OTUs for archaea were obtained at a similarity cutoff 0.95. Ammonium oxidizing bacteria (AOB) were dominated by species in *Nitosospiram*, *Nitrosovibrio* and *Nitrosomonas* genera (beta-proteobacteria) and Ammonium oxidizing archaea (AOA) were mostly from uncultured species in the *Crenarchaeota* phylum. Further analysis showed that plant diversity and nitrogen fertilization had clear impacts on the community structures of the nitrifying communities, while elevated CO_2 did not have significant effects.

To understand the interactions of AOA and AOB populations in a community, the novel RMT-based network analysis were performed based on the sequencing data. Networks with expected characteristics were obtained. Further topological analysis of AOA and AOB interactions is in progress.