Reconciling phylogeny and function during plant litter decomposition by high-throughput functional metagenomics

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Project goals: The overall aim of this project is to link functional traits that influence carbon cycling with individual microbial taxa in order to build predictive trait-based models of ecosystem responses to global change. The functional traits are connected to microbial taxa with DNA sequencing, novel functional assays, experimental manipulations and mathematical models that are used to investigate the distribution of extra-cellular enzyme genes and functions involved in plant litter decomposition across taxonomic groups of microbes. The resulting trait-based frameworks are then used to model how altered water and nitrogen availability as well as plant species composition impact plant litter decomposition rates in annual grassland.

This presentation focuses on one aspect of the discovery and identification of functional traits involved in plant litter decomposition, the functional screening of metagenomic libraries. A total of twenty-four metagenomic fosmid libraries have been constructed from high molecular weight DNA isolated from decomposing plant litter collected over one year period from a global change experiment undergoing rainfall and nitrogen manipulations. The libraries encompass over 20 Gb of metagenomic DNA and cover a range of microbial taxonomic groups including Actinobacteria, Bacteroidetes and different classes of Proteobacteria as well as Fungi.

The 24 libraries are currently being screened for activities involved in cellulose, hemicellulose, chitin, lignin and starch degradation as well as peptide breakdown and mineralization of organic phosphate using novel functional assays. These automated high throughput assays are based on colorimetric and fluorescent substrates and have been optimized to allow multiplexed screening of both endolytic and exolytic hydrolysis activities required during different stages of decomposition. Several cellulose, hemicellulose, chitin and starch degrading as well as phosphatase and proteinase producing clones have been identified.

Clones expressing the targeted activities are sequenced with the Illumina sequencing platform and the identified hydrolytic genes are characterized for enzyme kinetics and given phylogenetic context for incorporation into trait-based microbial decomposition models.

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