

146. Metatranscriptomes to explore the effects of nitrogen enrichment on forest soil microbial communities

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Project Goals: A diverse community of bacteria and fungi mediate plant litter decay in forests, within which they regulate the cycling and storage of C and N in soil. Anthropogenic perturbation of nitrogen balance alters the rate of forest litter decomposition and potentially influences overall soil community metabolism. However, the microbial mechanisms mediating a reduction in decay are largely unknown. In this study we are using environmental metatranscriptome techniques to explore the collective metabolism of natural microbial communities in forest floor material and soils exposed to experimental nitrogen enrichment. This poster focuses on the development of metatranscriptome methods that capture both eukaryotic (polyA) and prokaryotic mRNA and emphasizes the comparative analysis of carbohydrate active enzymes (CAZymes) within datasets.

Prior studies using soil metatranscriptomes suffered from low sequencing coverage or incomplete community representation (eukaryotic only). We developed a metatranscriptome approach using high-throughput Illumina sequencing that significantly enriched for mRNA in forest floor and soil RNA pools, and that included both eukaryote (primarily fungal) and bacterial transcripts. Our analyses included functional annotation of sequencing reads to PFAM, CAZymes, KEGG, FOAM, and Gene Ontology databases, allowing us to thoroughly explore the genomic basis of biogeochemical pathways mediated by soil fungi and bacteria. Metatranscriptome data was collected from three distinct biomes in North America: (1) soil from a pine-dominated temperate forest (North Carolina, USA), (2) decomposing leaf litter from a maple-dominated hardwood forest (Michigan, USA), and (3) a Cyanobacteria-dominated biological soil crust from a semi-arid grassland (Utah, USA). The work presented here focuses primarily on the forest biomes. Metatranscriptome expression profiles were correlated with additional biotic and abiotic measurements from the same samples including fungal and bacterial taxonomic abundance (rDNA), total bacterial and fungal abundance (qPCR), and soil chemistry. Results show that our mRNA isolation methods sufficiently deplete the rRNA fraction of total RNA and that subsequent metatranscriptome sequencing captures eukaryotic, bacterial, archaeal, and viral transcripts. KEGG analysis indicates we have covered the core metabolic pathways expected in microbial systems although the largest shifts in community metabolism among treatments are exhibited by the CAZyme analysis. In maple leaf litter, nitrogen enrichment suppresses a large suite of eukaryotic, primarily fungal, CAZymes while enriching unique sets of CAZymes at each site. In the pine forest, community expression was strongly differentiated by soil horizon and less so by nitrogen enrichment. Comparative analyses across all metatranscriptome datasets show distinct partitioning by biome and soil horizon correlating with broad-scale taxonomic shifts in the total microbial community.

Kuske CR, CN Hesse, JF Challacombe, D Cullen, JR Herr, RC Mueller, A Tsang, R Vilgalys (2015) Prospects and challenges for fungal metatranscriptomics of complex communities. *Fungal Ecol* doi:10.1016/j.funeco.2014.12.005

Funding statement: The information in this poster was supported by the U.S. Department of Energy Biological System Science Division, through a Science Focus Area Grant (2014LANLF260).