12. Nutrient Cycling for Biomass: Biochemical and Proteomic Profiling of a Nutrient Limited Poplar x *Laccaria* Ectomycorrhizal System

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Project Goals: This project addresses the need to develop system-scale models at the symbiotic interface between ectomycorrhizal fungi (such as *Laccaria bicolor*) and tree species (such as poplar) in response to environmental nutrient availability/biochemistry. A multiple "omics" approach is implemented that integrates next generation sequencing transcriptomics, proteomics, biochemical analyses and ChIP-Seq analyses to construct ectomycorrhizal regulatory networks and computational modeling approaches to predict how atmospheric carbon is sequestered as plant and/or subsurface fungal biomass.

Poster Abstract:

Ectomycorrhizal (ECM) symbiosis significantly affects carbon (C), nitrogen (N) and phosphorus (P) uptake in many plants, including commercially important Poplar trees. In order to understand the molecular mechanisms underlying this essential ecological phenomenon, our project employs a controlled laboratory *Populus tremuloides* X *Laccaria bicolor* model system. Biochemical analyses were performed to quantify key C, N and P metabolites within the leaves, stems and roots of ECM trees subjected to nutrient limiting conditions, and differential shifts in C and N allocation were identified that are indicative of the tree's ability to produce biomass. This data was correlated with the biochemical pathways associated with each metabolite as a means to identify important proteins that drive significant biochemical processes. Out of the key pathways, ~600 selected proteins will be analyzed by quantitative nanoLC-pseudoMRM-MS targeted proteomics. To determine the proteotypic peptides needed for such protein profiling, we developed an updated global protein database for *P. tremuloides* by assembling previously obtained transcriptomic data (780 million RNA-Seq short reads). Using global nanoLC-MS analyses, the newly predicted protein sequences were compared to trypsinized proteins extracted from *P. tremuloides*, resulting in a 22.4% increase in successful protein identifications over previously available databases. A major objective of this project is to integrate transcriptomic, proteomic and biochemical data in order to build system-scale models of the molecular mechanisms involved in improved nutrient uptake and carbon sequestration in ECM systems under nutrient limiting conditions. Such models will be the basis for the prediction of ecosystem responses to environmental nutrient availability.