13. Nutrient Cycling for Biomass: ChIP Sequencing of Transcriptional Factors to Infer Gene Regulatory Networks for Carbon and Nitrogen Sequestration During Poplar x *Laccaria* Ectomycorrhizal Interactions

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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:
RNA sequencing (RNA-Seq) and ChIP sequencing (ChIP-Seq) generate comprehensive transcript abundance data along with increased resolution on transcriptional regulation. However, these next-generation approaches are only beginning to be used to understand the mechanisms underlying increased plant biomass during beneficial plant-microbial interactions. In our study, we use these approaches to explore carbon and nitrogen sequestration by employing a laboratory-based *Populus tremuloides* X *Laccaria bicolor* ectomycorrhizal system. Based on past RNA-Seq analyses of this system, 16 transcription factors (TFs) were identified that are important in mycorrhiza-specific carbon and nitrogen flux metabolism. We performed ChIP-Seq analyses using antibodies generated against these TFs, including several MADS-Box TFs known to be involved in root and shoot development as well as a commercially available RNA- polymerase II antibody as an experimental control. 961 loci were identified as the potential targets of these MADS-Box regulators. In addition, the CC[A/T]$_6$GG motif, termed the CArG- box, is one known binding site for the MADS domain, and this motif was over-represented in the promoter sequences of the identified target genes, thus validating the ChIP-Seq analysis pipeline in *P. tremuloides*. Moreover, some of the identified target genes are involved in key biochemical and metabolic pathways associated with cellulose and lignin biosynthesis during growth and development. Thus, it appears that the ChIP-Seq approach will work well to enhance our understanding of biomass production in forest trees. Such data will be linked with transcriptome data to build gene regulatory networks and develop system-scale models that are predictive of the molecular mechanisms that control plant carbon management and allocation.