110. Effect of Nitrogen and Phosphorus Limitation on the Growth and Morphology of Engineered Microbial-Plant Communities.

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Project Goals: The Argonne "Environment Sensing and Response" Scientific Focus Area (SFA) program seeks to identify the molecular basis of cellular transport and sensory pathways that mediate the response of terrestrial ecosystems to environmental nutrients. The mechanistic links between and within ecosystems comprised of plants, fungi, and soil bacteria involved in the production of biomass for fuel are currently very poorly defined. The effects of nutrient availability, closely linked to climate, on those mechanistic links, are also inadequately understood. This program will address this knowledge gap by mapping transport and sensor proteins to specific environmental compounds to define their function and biological roles and establish a series of defined connections between the environment and the cell. The knowledge will facilitate the development of system-level models predictive of cellular response to changes in environmental conditions.

Plant microbial communities play an important role in terrestrial ecosystem carbon (C) storage. These communities are typically comprised of symbiotic fungi and diverse species of bacteria that are modulators of plant response to the environment and change carbon budgeting in forest ecosystems. Carbon allocation (biomass) and partitioning (biomass quality) in plants are affected by nutrient availability in soils and are influence by the microbial community. Changes in plants C allocation are elicited by nutrient availability. To understand the molecular mechanism underlying these plant microbe interactions, we examined the molecular and phenotypic changes of aspen seedlings that were inoculated with mycorrhizal fungi and / or mycorrhizal helper bacteria, evaluated under either nitrogen or phosphorous nutrient stress conditions.

To determine the colonization-induced changes in plant signaling or metabolism that alter P / N use efficiency, aspen seedlings were inoculated with mycorrhizal fungi and / or mycorrhizal helper bacteria. The plant transcriptome was sequenced from the plant root samples colonized with and without the microbial community for five weeks in controlled conditions. Expression profiles of the plant microbial community demonstrated 2482 genes were significantly differentially regulated in response to bacterial colonization (Pseudomonas fluorescens SBW25) which contributes 6% of total number of genes. Plant transporters and transcription factors were highly up-regulated during bacterial colonization, whereas enzyme related genes were down-regulated during the interaction. Over 3000 plants genes were significantly differentially regulated due to mycorrhizal colonization by Laccaria bicolor.
We also examined the effect of nitrogen and phosphorous nutrient stress on Aspen phenotype after

microbial colonization. Aspen trees were subjected to low levels of nitrogen (2.94 mM, 1.47 mM, 0.98 mM and 0.49 mM) and phosphorus (0.236 mM, 0.1 mM, 0.075 mM and 0.041 mM) along with optimum nitrogen (14.7 mM) and phosphorus levels (1.25 mM). Experimental setup included appropriate controls consisting of the one- and two-component combinations of P. fluorescens Pf-01 and Laccaria with the aspen trees. Phenotypic data was collected that included fresh and dry weights, leaf number, and stem length along with root tissue scans for analysis of root branching.

Phenotypic analysis indicated there was a decrease in plant biomass with reducing levels of nitrogen, while significant increases in plant biomass were observed when colonized by either Laccaria alone or Laccaria in combination with P. fluorescens strain Pf-01. However, increase in biomass was most significant at optimum nutrient levels, 56% increase in biomass with Laccaria alone and 74% with combination of Laccaria and Pf-01. At 2.94 mM nitrogen there was a 10% increase in plant biomass with Laccaria alone and no effect on biomass when in combination with Pf-01. Below 2.94 mM nitrogen there was no significant increase in biomass; however, in each of these nitrogen-limiting conditions there was a slight increase in biomass when Laccaria was in combination with Pf-01. It is evident from the study that Laccaria alone or in combination with Pf-01 is not mobilizing nitrogen from growth medium with very low nitrogen levels (below 2.94 mM).

Under phosphorus limitation there was significant increases in plant biomass when colonized by either Laccaria alone or Laccaria in combination with Pf-01. An increase in biomass was most significant at 1.25 mM phosphorus (optimum level), 30% increase in biomass with Laccaria alone and 80% with combination of Laccaria and Pf-01. Also, at 0.236 mM and 0.1 mM phosphorus, there was around 10-20% increase in biomass with Laccaria alone or in combination with Pf-01. At lower phosphorus levels (0.236 mM, 0.1 mM and 0.075 mM) Pf-01 alone had negative impact on plant health, i.e. decrease in biomass. Overall, it appears that the Laccaria treatment alone or in combination with Pf-01 was alleviating phosphorus stress.

This knowledge will facilitate the development of more accurate systems-level models predictive of root response to environmental conditions or changes and potential for ecological niche enhancement by soil symbioses. The elucidation of function, regulation, and system response will support the Genomic Sciences Program goal of achieving a genome-based, dynamic systems-level understanding of organism and community function.

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