Understanding the effect of abiotic and biotic stresses and below-ground microbial diversity on sustainable woody biomass cultivated on marginal land

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Abstract: A well recognized benefit of biomass energy from forest trees is the ability to cultivate on marginal lands not suitable for food or other agricultural crops. Long studied for woody plant biomass, the tree *Populus* can be sustainably harvested without replanting in coppice style cultivation. Despite a long history of use in the paper and pulp industry, details of *Populus* biomass accumulation for biofuel or ethanol in field settings are minimal, especially on marginal lands where incidents of biotic and abiotic stress are common. Pre-harvest treatments with the ethylene blocking agent 1-methylcyclopropene (1-MCP) may reduce stress and total lignin content of biomass tissues in *Populus.* We have set up a two-factor completely randomized design consisting of planting space, 1-MCP treatment, and presence or absence of the nitrogen fixing legume, Black Locust (*Robinia pseudoacacia*). In 2009 and 2010, both field measurements and a genomics based gene expression strategy, including above and below ground biological diversity associated with these trees, were taken to assess the growth of these treatments on the accumulation of woody biomass. The goal of this study is to understand the system biology of *Populus* plantations on woody biomass and to determine methods farmers can maximize biomass yields for ethanol production.

Project Overview

We have established a series of hybrid *Populus* biomass plots to determine the best growth considerations with regard to the maximization of biomass. In addition to planting regimes, the presence of a nitrogen fixing legume, (*Robinia pseudoacacia*), and the below-ground microbial composition of the research plots is being investigated. The role of a ethylene blocking agent (1-methylcyclopropene; 1-MCP) is being investigated for drought protection and reduced lignin synthesis.

Year One Goals

1. Establish field plot with Poplar hybrids at three spacing treatments and apply ethylene blocker 1-MCP to specified plots.
2. Conduct suite of biological measurements to assess the growth characteristics of year one.
3. Sample soil at various seasonal times for metagenomic analysis.

Year Two Goals

1. Interplant fast growing nitrogen fixing legume tree (Black Locust) in designated field plots.
2. Apply 1-MCP to treatment plots, sample soil, and conduct measurements to assess growth characteristics.
3. Measure biomass components from each treatment plot.

Year 3 to 5 Goals

1. Continue field measurements and soil sampling, +/-1-MCP, treatments.
2. Assess biomass accumulation from each treatment plot at rotation.
3. Study expression of cell wall genes during stress-induced secondary growth in poplar using both microarray and chemical analysis

- **Despite a long history of growing *Populus* in the paper and pulp industry, details of biomass accumulation in field settings are minimal, especially on marginal lands.**
- **Pre-harvest treatments with the ethylene blocking agent 1-methylcyclopropene (MCP; Choi & Huber 2009), may reduce stress and total lignin content of biomass tissues in *Populus* (Carroll & Somerville 2009, Hendriks & Zeeman 2009).**
- **We set up a two-factor completely randomized design consisting of planting space (1X5, 3X5, and 5X5 foot spacing), 1-MCP treatment (half treated at 1,000 nl/L application), and presence or absence of the nitrogen fixing legume, Black Locust (*Robinia pseudoacacia*) treatments (Kumar et al 2009). Poplar trees were planted as unrooted dowels and were clonally propagated from identical parent material (Neumann *et al* 2009). These trees were planted on a fallow field that had been mowed and reflected marginal crop production land.**
- **A large array of field measurements were taken during the field seasons of 2009 and 2010. The measurements included tree height (Figures 1, 2,& 3), total number of leaves per tree, stem diameter (Figure 4), leaf weight, leaf area removed by insect herbivory (Figure 5), and average number of leaves per tree (Figure 6).**
- **Soil samples were collected for a metagenomic study of fungi (mycorrhizal and non-mycorrhizal) and bacteria associated with the low-input conversion of a marginal agricultural land site into a poplar biomass plantation. Metagenomic data reflects control plots of 10 and 30 year old poplar trees.**
- **This study reflects preliminary data of currently ongoing research plots. No data regarding the affect of 1-MCP or *Robinia* on growth was observed in the first year of growth.**

Literature Cited

- Carroll A & C Somerville (2009) *Annual Review of Plant Biology* 60: 165-182.
- Choi ST & DJ Huber (2009) *Postharvest Biology and Technology* 52: 62-70
- Kumar P, DM Barrett, MJ Delwiche, & P Stroeve (2009) *Industrial Engineering and Chemistry Research* 48: 3713-3729.
- Hendriks ATWM & G Zeeman (2009) *Bioresource Technology* 100: 10-18.
- Neumann, K.-H., A. Kumar, and J. Imani. 2009.Springer-Verlag,New York, 333 pp.

Acknowledgements

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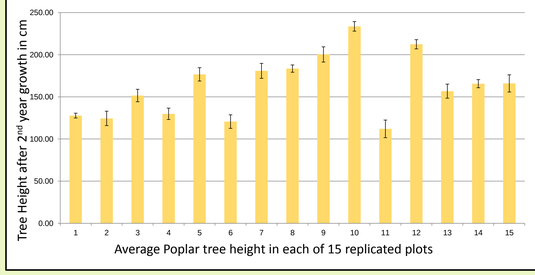
Above-Ground Measures of Poplar Growth

Plot Height Variation In Clonal Plant Material

- **After two years of growth, average tree height and standard error varied and was not completely correlated with spacing regime or plant growth characters measured in our plots (below).**



- **These results suggest that numerous environmental factors, such as above ground stresses and below ground nutrient availability, are influencing the above ground biomass accumulation.**

Variation In Plant Growth Characters in 1 ft, 3 ft, and 5 ft spacing regimes

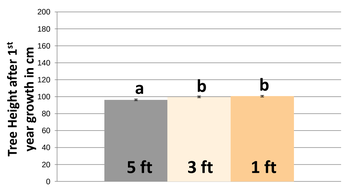


Figure 2 - Average Poplar tree height in replicated plots for 5 ft, 3 ft, and 1 ft plant spacing regimes in 2009

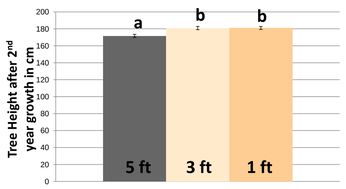


Figure 3 - Average Poplar tree height in replicated plots for 5 ft, 3 ft, and 1 ft plant spacing regimes in 2010

- **We measured growth characteristics from 3647 clonal Poplar trees planted in five replicates of 1, 3, and 5 foot spacing plots in both the 2009 and 2010 growing seasons.**
- **Poplars planted at both 3 and 5 foot spacing had a greater height in both the 2009 and 2010 growing seasons.**
- **Stem diameter (Figure 4), Leaf Area Removed (Figure 5), and number of leaves per tree (Figure 6) were measured on row 3 of each replicated plot. Leaf area and leaf weight were measured but are not shown.**

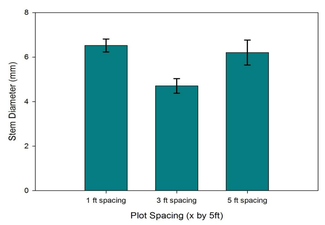


Figure 4 – Average stem diameter at plant spacing regimes.

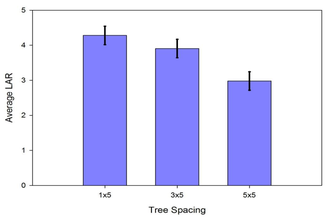


Figure 5 – Average LAR (leaf area removed) from herbivory at three plant spacing regimes.

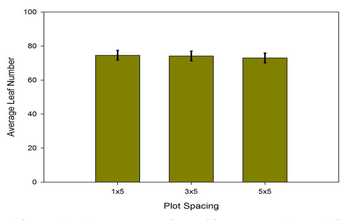
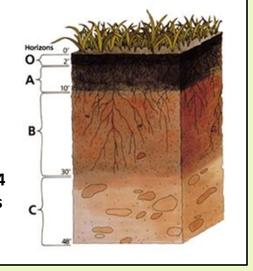


Figure 6 – Average number of leaves per tree at three plant spacing regimes.

Below-Ground Measures of Microbial Diversity

- **To understand below ground microbial diversity in Poplar monoculture, we sampled from 10 and 30 year old hybrid Poplar plantations as both a proof of principle and experimental control. We sampled from the O and A horizon of the two Poplar types.**
- **We also have sampled seasonally from each of our plots to determine microbial diversity and patchiness on a plot level scale. The analysis of this data is pending.**
- **Total DNA was extracted from soil and sequenced directly with 454 pyrosequencing techniques. We also used both 16S and ITS primers to select for the Bacterial and Archaea (16S) and Eukaryotic (ITS) microflora.**



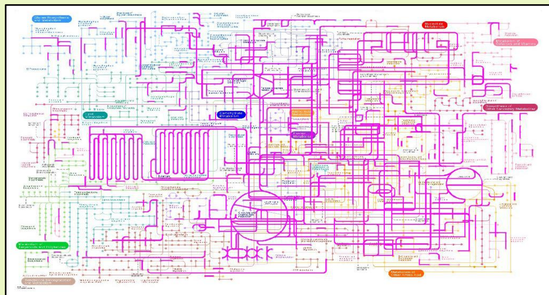


Figure 7 – KEGG metabolism network map of the total genes present in both the 10 year and 30 year poplar plantations. These genes were represented by the total DNA extracted from soil and were not separated by any taxonomic level. Both poplar plantations are identical hybrid genotypes planted on adjacent plots.

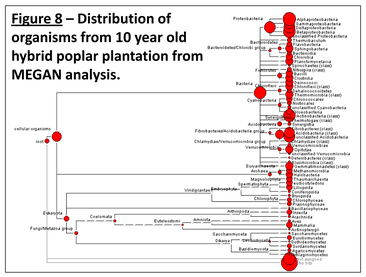


Figure 8 – Distribution of organisms from 10 year old hybrid poplar plantation from MEGAN analysis.

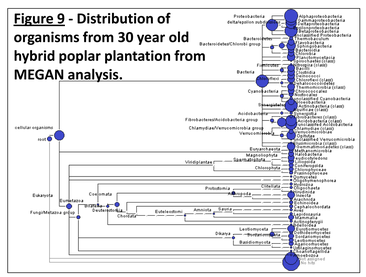


Figure 9 - Distribution of organisms from 30 year old hybrid poplar plantation from MEGAN analysis.

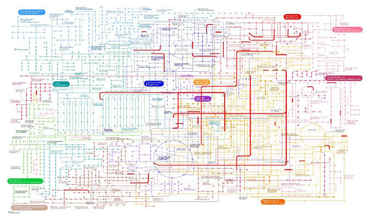


Figure 10 – Genes in KEGG pathway represented only in the 10 year old hybrid poplar plantation.

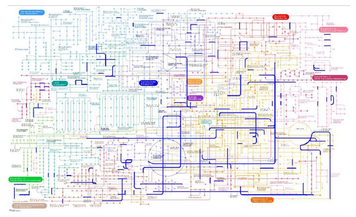


Figure 11 – Genes in KEGG pathway represented only in the 30 year old hybrid poplar plantation.

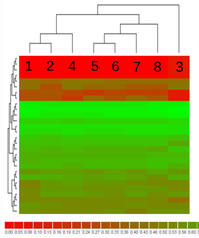


Figure 12 – Neighbor joining gene analysis from 8 samples from Poplar plantations. Samples 1 to 4 represent 10 year old plantation. Samples 5 to 8 represent 30 year old plantation. Genes in reference (red, less abundant; green, more abundant) to average soil genes in MG-RAST database. Sample from both plots are generally homologous

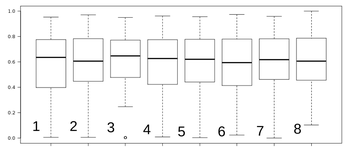


Figure 13 –Functional representation of gene diversity from total genes for 4 samples taken from each Poplar plantation. Shows sample homology irrespective of plot age.