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**June 18, 2013**

**MELNHE**

**Bartlett Experimental Forest**

**The effect of nitrogen on mycorrhizal colonization associated with Populus *grandidentata*.**

Hypothesis:

As nitrogen availability increases, a decline in mycorrhizal abundance is anticipated as the biomass and activity of mycorrhiza reduces.

Introduction:

Atmospheric nitrogen deposition has fallen from the air onto forest ecosystems, which causes fluctuations in nutrient availability in soils. The symbiotic fungi, mycorrhiza, are vital to tree nutrient uptake, since tree roots are not able to uptake enough on their own. For the 85-90% of land plants that form them, mycorrhizal represents the crucial link between the root systems and soil and by creating resistances against parasites and other stress factors (Smith and Read 1997). However, studies have shown that ecosystems have been found to decline in abundances and diversity in response to nitrogen deposition, with possible damaging effects on plant uptake of soil resources. In low nutrient areas, plants generally invest more carbon in mycorrhizal fungi (Mosse & Phillips, 1971) But if nutrient availability rises (fertilization), plants allocate carbohydrates elsewhere in the plant and a decline in mycorrhizal abundance is expected with fertilization (Read, 1991) The purpose of the proposed research is to understand the impact of nitrogen deposition on the two guilds of mycorrhizal fungi associated with big-tooth aspen (Populus *grandidentata)*. Under normal conditions, aspens are able to colonize due to mutualistic relationships with mycorrhizal fungi (Cripps 1996, 2001). In addition, aspen has been reported to transpire within phosphate mining dumps (Harris and Jurgensen, 1977). Aspen can associate with over 60 different species of fungi, but only a portion occurs on acidic soils (Cripps, 2001; Cripps and Miller, 1993). This research will investigate the resistance of the mycorrhizal networks at a guild level to nitrogen disturbances.

Methods and Materials:

 Mycorrhizal abundance will be measured by directly collecting Populus grandidentata roots. Both AM and EM mycorrhizal colonization will be quantified on each root with a microscope and staining techniques.

Field Methods:

 The collection of the roots will each come from the C4 site nitrogen plots. However, since a control plot for P. *grandidentata* was not found, a set of P. *grandidentata* trees outside of the nitrogen plots will be used for constant variability. Field sampling procedures include: selecting representative sites, sampling by horizon, and designating and sampling a subhorizon if root mass and morphology change. A total of 20 roots will be examined under the following experiment. A soil probe can be used to collect root samples from the tree for analysis and evaluation of mycorrhization or manually extracting the roots from the soil can be of use also.

Each root will be placed within zip lock bags and labeled for the specific plot to compare amounts of nitrogen abundances. Three soil cores (subsamples) will be collected from different sides of the tree (e.g., North, South, etc.). Fragments of finer roots that can be found closer to the soil surface that can be unearthed manually should be added to the core. At least 10% of each tree will be root sampled from the organic soil horizon. After gathering the samples, each root will be placed within the corresponding labeled zip lock bag. Once placed the roots are placed in zip lock bags, the samples are then placed in a freezer until later use.

Lab Methods:

After removing the roots from the freezer, the roots are then separated from the soil. Roots are removed from the soil by hand rinsing the soil from the roots by sieving followed by drying. The soil must be isolated for effective separation of the roots and plant excess from the soil sample. Tap water, rather than distilled water, will be used to help avoid dispersion problems. Report root biomass in lbs/ac or kg/ha, over a given depth, and as a “root bulk density” g/cc of oven dry root biomass in the soil of a particular horizon

Structures produced by AM fungi are invisible in fresh roots because internal structures are obscured by the natural pigments and cell contents within roots, while whole EM roots can often be identified by direct observation with a dissecting microscope (Gardner 1975). A compound microscope will be used for AM mycorrhizae structures within roots.

Fungal structures in plant tissues will be observed by the use of differential stains which bond to fungal hyphae without much background staining of the cleared plant material. Stains such as, Chlorazol black E (CBE) or ink in vinegar are used to stain mycorrhizal structures can differentiate the fungi from the root (Brundrett et al. 1984). For complete microscopic examinations, roots can be stained with Chlorazol black E (CBE) in a lactoglycerol solution (Brundrett et al. 1984). The prime stain concentration will depend on the dye source and microscope procedure used. Roots are stained by heating for several hours at 90° C, or 15 minutes in an autoclave using a liquids cycle at 121° C, or by leaving them in staining solution at room temperature for one or more days. (Brundrett et al. 1984). The staining solution may be reused several times is filtered through folded cheesecloth or fine nylon screen after each use (to remove root fragments), until it becomes translucent. A more recently developed staining method uses ink and vinegar (Vierheilig et al. 1998, 2005). This staining solution consists of a 5% ink diluted in vinegar (5% acetic acid). Staining with black writing inks (Shaeffer Jet Black; Cross Black; Pelikan Black) and some blue inks (Pelikan Blue).

Materials/ Budget:

 Lab:

* Dissecting microscope
* Compound microscope
* Microscope slides
* Cover slips
* Quarter liter of Differential staining dyes
* 1 bottle of 3% Hydrogen peroxide
* A liter of Glycerol

Timeline

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| Week | Task |
| 1 | Collecting roots from each represented plot following storing of roots. |
| 2 | Root separation from soil (sieving)  |
| 3 | Staining of roots |
| 4 | Data and analysis  |

Cited Sources:

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Gardner JH, Malajczuk N. 1988. Recolonisation of rehabilitated bauxite mine sites in Western Australia by mycorrhizal fungi. Forest Ecology and Management 24: 27-42

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