# Temperate Tree Response to Microbial Inoculation During Restoration: Soil and Leaf Nutrient Status



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#### **OBJECTIVE**

The objective of my summer project was to determine the effects of mycorrhizal inoculum on the soil nutrient status and plant growth response during the early-establishment phase of a restoration project. These findings will contribute to a larger experimental question of whether tree establishment is impacted by differences in the mycorrhizal inoculum used in restoration.

#### BACKGROUND

- Plant communities are dependent upon organisms within the soil microbiome, including mycorrhizal fungi, to obtain many of the nutrients necessary for survival.
- Disturbed / man-made sites often have different microbial community compositions than those of native forests. Frequently, commercial mycorrhizal inoculums are used in forestry and restoration to supplement the existing microbial communities and aid in tree establishment. However, research suggests that inoculums collected from local forest sites may be better adapted to facilitate colonization and tree growth than commercial brands.
- Acacia Reservation, operated by the Cleveland Metroparks, is a 155-acre nature preserve located in Lyndhurst, OH. The site was managed as a golf course for nearly 100 years before being converted into an area for forest restoration and public recreation.
- microbial communities in Acacia have been Soil significantly impacted by the former golf course, which may impact the success of tree restoration initiatives. The use of mycorrhizal inoculum may be beneficial to these restoration-planning efforts.



Figure 1. Aerial map of the Acacia study site, on which 435 saplings were planted.



Figure 2. The field site at Acacia Reservation. Photo taken in June, 2017, approximately 1 year after original tree planting.

## **STUDY DESIGN AND METHODS**

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• The experimental site was established at Acacia Reservation in 2016. 435 saplings were planted, composing of 3 different tree species, sourced from 3 populations, into 3 soil inoculation treatments.

• Species: Liriodendron tulipifera (Tulip Tree), Prunus serotina (Black Cherry), and Quercus rubra (Red Oak)

**Populations:** West Virginia, Missouri, and Indiana

**Inoculant Treatments:** Control (water), commercially produced inoculum (MycoGrow Soluble), and forest soil transfer (three replicates).



Figure 3. Collecting leaves from a Tulip Poplar for nutrient and specific leaf area determination.

Soil was collected from a subsample of 90 trees, dried, and used for nutrient analysis

• Ammonium: Determination of  $NH_4$  by the phenate method for ammonia / nitrogen.

**Carbon / Nitrogen:** C/N ratio determination by the dry combustion method

**Phosphorus:** P determination by the modified ascorbic acid method



**Figure 4:** Example of soil NH4 determination using a color standard spectrum and spectrophotometer.

• Leaves were also collected from the subsample and will be analyzed for nutrient content and specific leaf area

## Carbon / Nitrogen

Phosphorus

Specific Leaf Area : Ratio of leaf area / dry weight of three leaves, collected from each tree.

### RESULTS

Data on soil NH4 and CN, as well as leaf Specific Leaf Area have been collected thus far and analyzed using linear mixed-effect models and ANOVA



Mycorrhizal Inoculant Type

Figure 5. Soil C/N ratio was significantly affected by mycorrhizal inoculant type. (p = 0.0024) Forest soils have a higher CN ratio than commercial and control. . The control group was not significantly different from the commercial inoculant.



Figure 6. Soil ammonium was significantly affected by mycorrhizal inoculant type. (p = 0.0323) Forest soils have higher NH<sub>4</sub> content than commercial soil. The control group was not significantly different from either forest or commercial inoculant.



Figure 7. Strip chart illustrating the leaf area differences between each species type. There were no significant differences in specific leaf area between any of the 3 treatments (p > 0.05)



## **CONCEPTUAL MODEL**



Figure 8. Conceptual model of these experimental results. It is predicted that the forest inoculated trees are exhibiting a higher CN ratio and NH<sub>4</sub> content because the belowground microbial communities are more taxonomically and functionally diverse than the commercial inoculant or control. These communities, being more active, are liberating inorganic N in the soil by converting it to  $NH_4$ , which is more accessible to trees.

### Conclusions

- Elevated ammonium and C/N ratio in forest inoculated soils is indicative of a more active and productive microbial community, possibly having higher species diversity and a higher diversity of fungal types
- As trees form relationships with mycorrhizal fungi, they are likely to be benefitting from microbial communities which free up accessible nutrient resources  $(NH_4)$ ; promoting early establishment growth.
- Forest inoculants, being locally adapted and potentially supporting more productive microbial communities, may be a more effective soil inoculant in restoration projects when compared to the commercial brand, which did not differ from the control.

## **Future Work**

- The remainder of this summer will be spent collecting data for the determination of soil and leaf Phosphorus content and leaf Carbon / Nitrogen content. All samples have been collected and processed for long-term storage.
- Predictions: •
- The forest inoculate treatment will have elevated soil phosphorus, elevated leaf nitrogen and phosphorus, and higher fungal diversity than the control or commercial treatment.

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