

2015

# The Potential Effect of Mycorrhizal Fungi on Phosphate Levels in the Soil



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Environmental Science Summer  
Research Experience (E.S.S.R.E.)

7/24/2015

## Abstract

Mycorrhizal fungi are types of fungus that form a symbiotic relationship with plants and help plants increase their phosphorus and nitrogen intake. For its own benefit, the fungus receives the plant's carbon to help with its development. The 2015 Microclimate Biota Survey showed that the fungus levels in Microclimate 4 were around 20% lower than in the other three sites and over the past fifteen years, while the phosphorus level in site four have been rapidly increasing. Therefore, we hypothesized that as Microclimate 4's phosphorus levels increased its Mycorrhizal fungi levels decreased. We took soil samples from areas near two types of trees, two types of plants, and by the water with no plant life present, as a negative control. We then tested the soil samples for phosphorus levels and Mycorrhizal fungi levels. Our results were mixed. Around half of the results showed that as the phosphorus levels increased, and the Mycorrhizal fungi levels decreased a statistically significant amount. The other half showed no significant statistical relationship. In conclusion, our results proved our hypothesis incorrect. The Mycorrhizal fungi did not cause a significant change, either increase or decrease, in phosphorus. Further research could be conducted to test for all the varieties of fungi to observe any possible change in phosphorus and nitrogen levels that could have been caused by them.

# Introduction

Mycorrhizal fungi have a symbiotic relationship with the majority of terrestrial plants which enables these plants to boost their intake of soil phosphorus in the form of phosphate ( $\text{PO}_4^{3-}$ ), a vital nutrient for the healthy development of plants. When phosphate levels in the soil are low, mycorrhizal fungi, which are connected to a plant's roots, help convert the unusable phosphorus in rocks and sediment into phosphate ( $\text{PO}_4^{3-}$ ) which the plants can then absorb through their roots. Phosphorus improves the root growth of plants which enables them to develop larger and fuller leaf canopies (Namuth-Covert, 2015). Mycorrhizae, in turn, benefit from the increased sugar production from the host plant's photosynthesis (Puplett, 2014), using sugars donated by the plant to fulfill their own metabolic and carbon needs. (Ingham, 2015).



Figure 1 (Microclimate 4)

The symbiotic relationship between plants and fungi helps regulate phosphate levels in the soil, but, unfortunately, if the soil is disturbed or compacted or if certain chemicals are used in given area, the mycorrhizal fungi can be damaged and may disappear entirely from that soil (Puplett, 2014). Moreover, it can take decades for these critical microbes to repopulate, and, in the meantime, because there are fewer mycorrhizae, plants have a difficult time consuming enough of the nutrients they need from the soil, which has the potential to lead to a buildup of these nutrients – such as phosphorus – in the ground.

The data from the E.S.S.R.E. 2015 biota survey showed that the phosphorus levels (ppm) in E.S.S.R.E. Microclimate 4 (120.83ppm) were much higher than those in other three microclimates of the program (E.S.S.R.E. 2015a), and, a longitudinal analysis of Microclimate 4 (N 39° 21.470, W 0 76° 38.334) shows that the phosphorus levels in this site have been increasing steadily over the past fifteen years (E.S.S.R.E. 2015b). Microclimate 4 is home to many small plants and shrubs (see Figure 1), but there are fewer large trees in this location than the other three E.S.S.R.E. microclimates (see Figure 2). Large trees tend to house more mycorrhizae than other plants. Therefore, we hypothesized that the fungi population has fallen steadily over the past fifteen years ago and that it is this reduction in the density of mycorrhizae that has caused the long term increase in soil phosphorus levels.

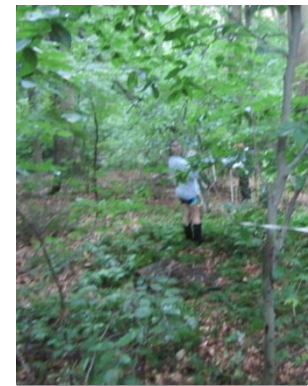


Figure 2  
(Microclimate 1)

## Method

In the ESSRE Microclimate 4 (N 39.35733; W 076.63840), 15 soil samples each 15 ½ cm deep with a diameter of 2 cm were extracted simultaneously in sets of 3 based on the plant type living at each location. 3 samples were extracted from the roots of an *Acer rubrum* found in quadrant 3; 3 samples were extracted from the roots of an *Ulmus rubra* found in quadrant 2; 3

samples were extracted from where *Leersia virginica* grows in quadrant 4; and 3 samples were extracted from where *Boehmeria cylindrical* grows in quadrant 1. 3 samples, used as a negative control, were extracted from an area without plants located between quadrants 2 and 3. All of the soil samples were collected on the morning of July 16, 2015.

All 15 samples were tested for phosphorus levels (ppm) using a LaMotte Test Kit Combination Soil, Model STH-14 (Code 5010-01) kit. Simultaneously, all 15 soil samples were serially diluted using sterile water to the  $10^{-3}$  degree. 100  $\mu$ L aliquots of each dilution for each of the 15 samples was plated onto its own individual 3M Petrifilm™ Yeast and Mold Count Plate and allowed to grow for 72 hours to determine the density of Mycorrhizal fungi in the soil samples. Two more complete sets of 15 soil samples were simultaneously extracted from these locations the mornings of July 17 and July 20, 2015 respectively and were tested for both phosphorus levels and mycorrhizae density.

## Results

Figure 1A

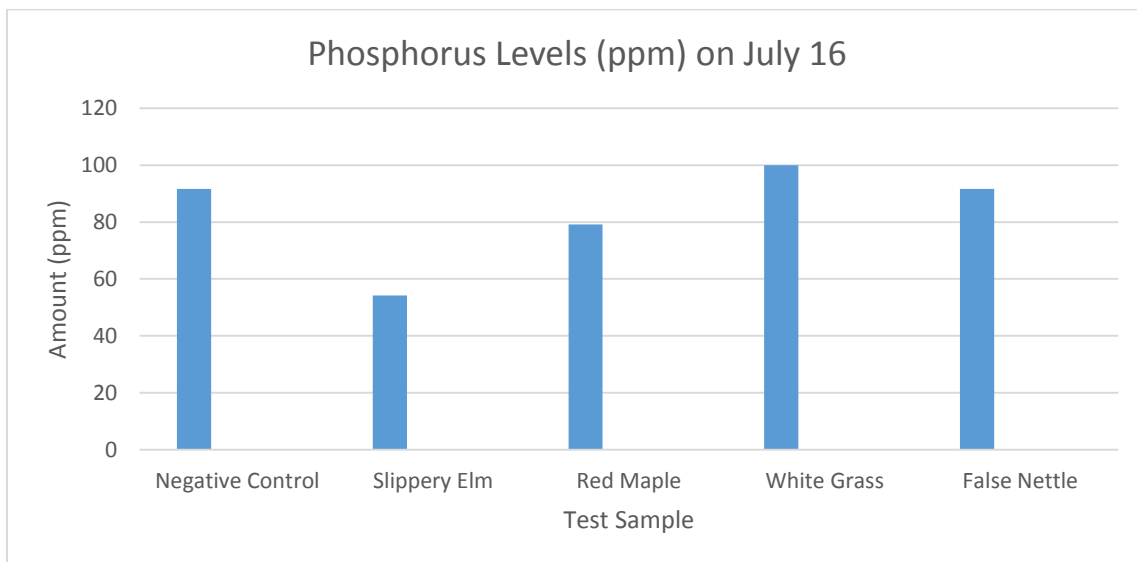


Figure 1A shows the relationship between the level of soil phosphorus and the location from which the sample was collected on July 16.

Figure 1B

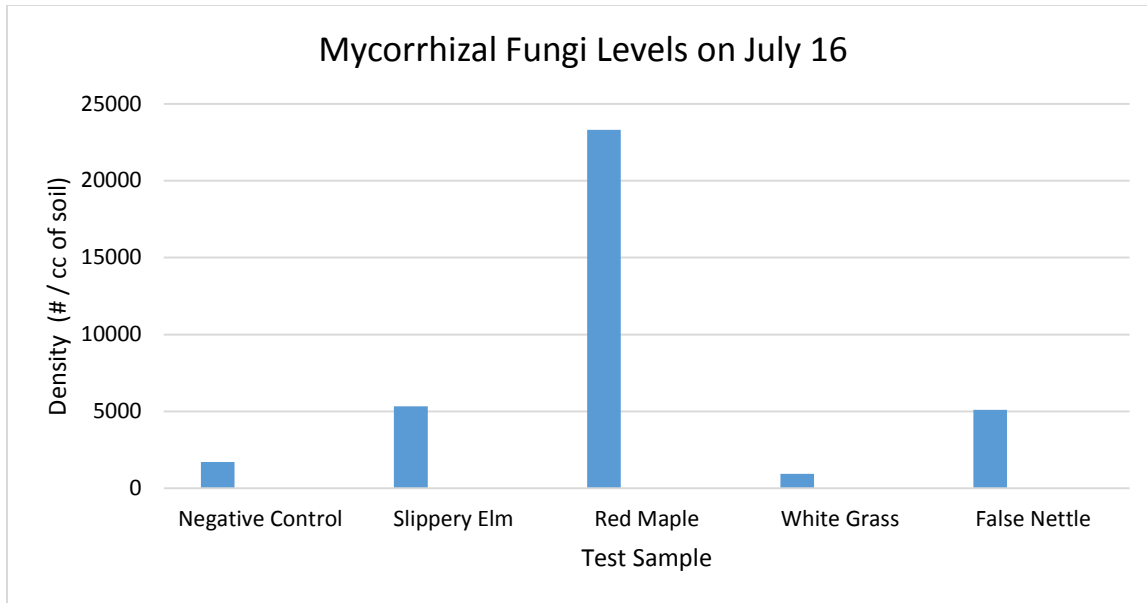


Figure 1B shows the relationship between the density of the mycorrhizal fungi in the soil and the location from which the sample was collected on July 16.

Figure 2A

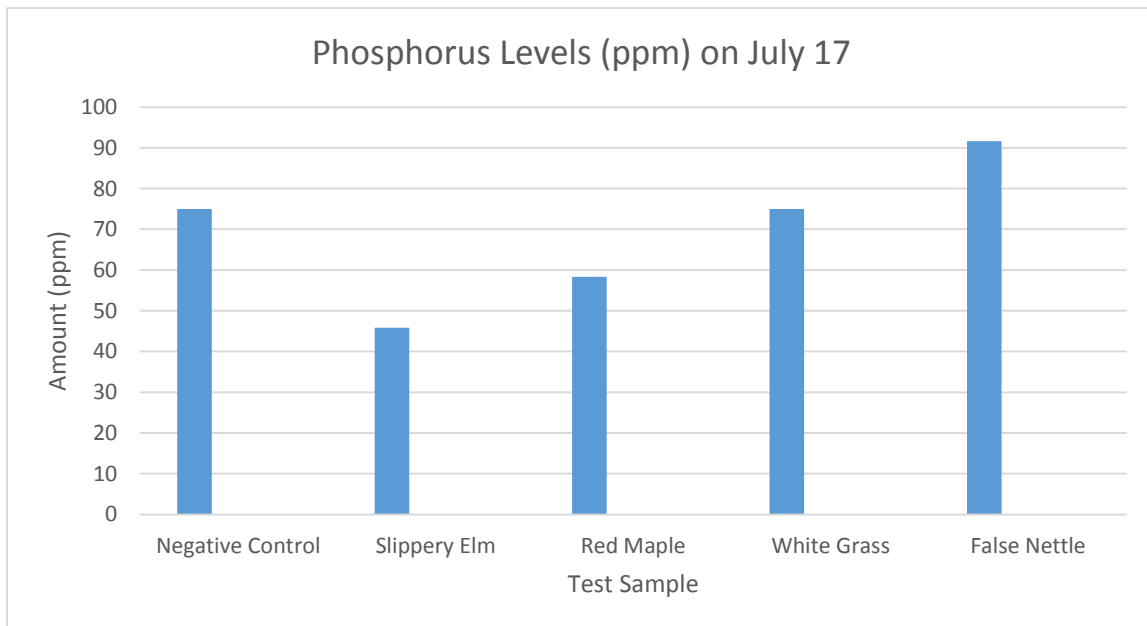


Figure 2A shows the relationship between the level of soil phosphorus and the location from which the sample was collected on July 17.

Figure 2B

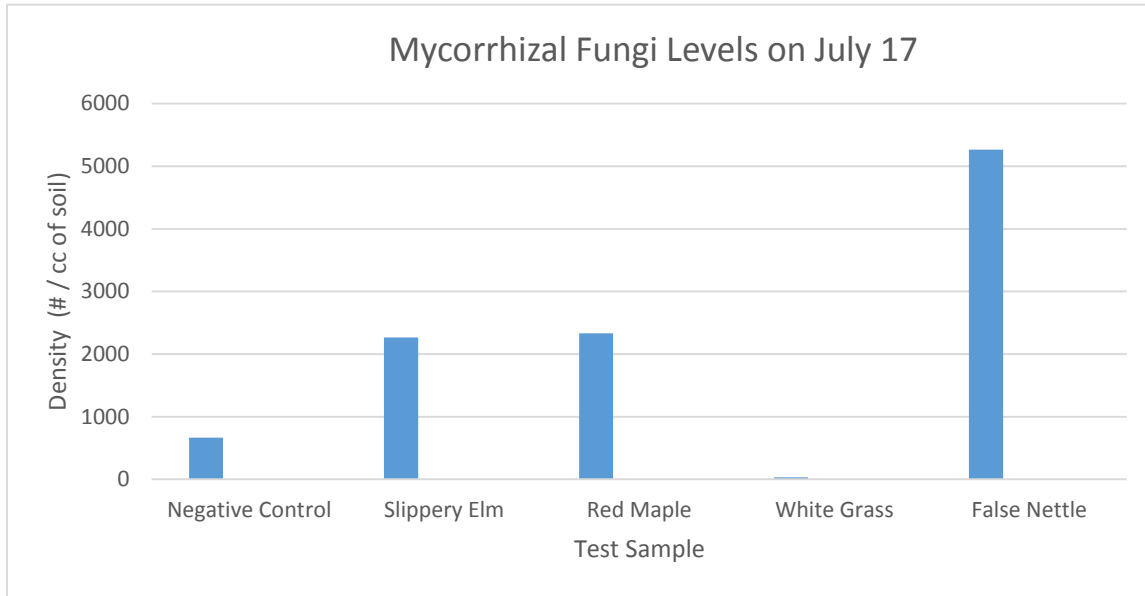


Figure 2B shows the relationship between the density of the mycorrhizal fungi in the soil and the location from which the sample was collected on July 17.

Figure 3A

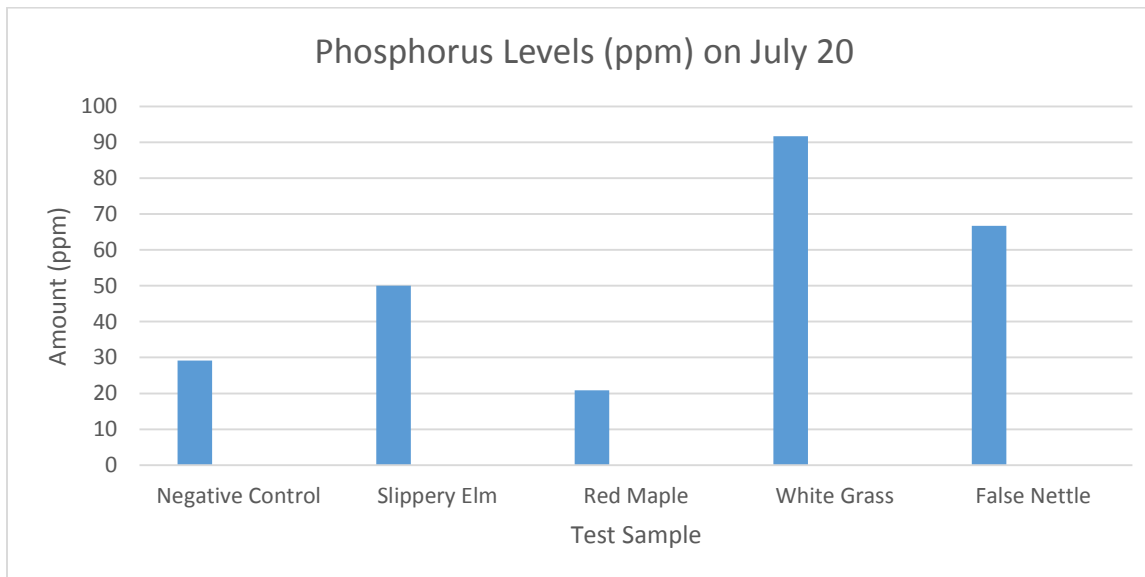


Figure 3A shows the relationship between the level of soil phosphorus and the location from which the sample was collected on July 20.

Figure 3B

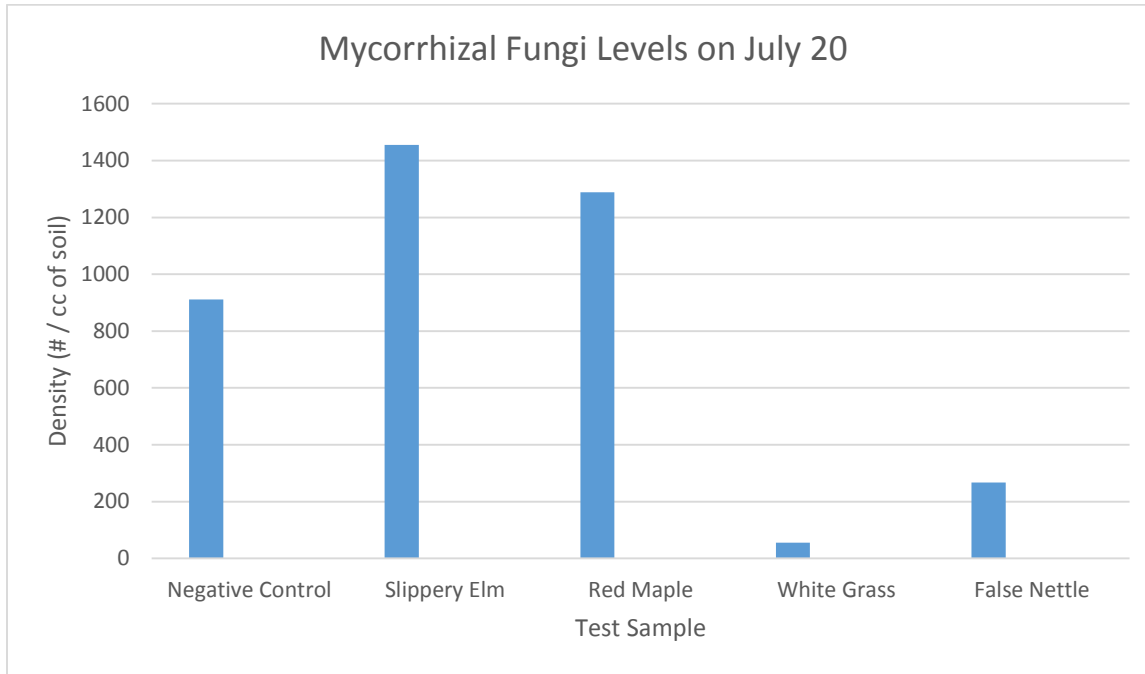


Figure 3B shows the relationship between the density of the mycorrhizal fungi in the soil and the location from which the sample was collected on July 20.

Figure 4

The Relationship Between Phosphorus and Mycorrhizal Fungi Levels

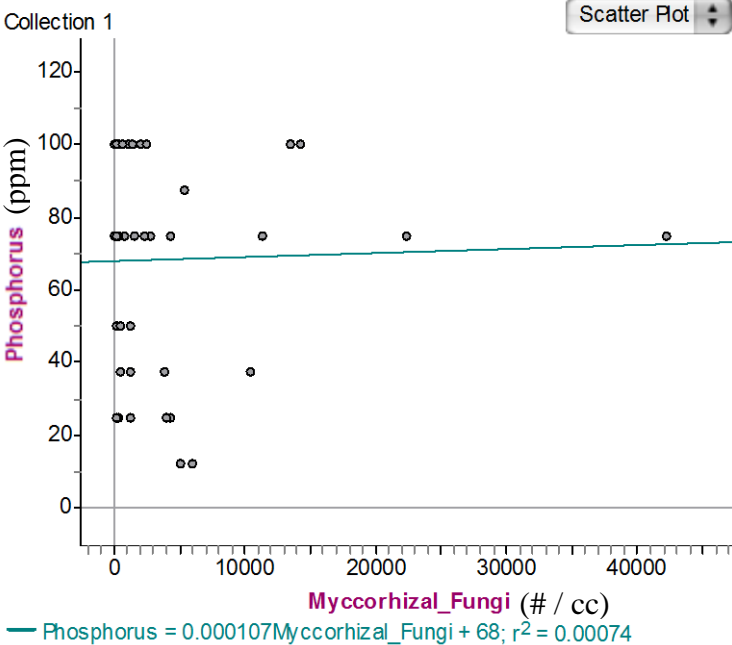




Figure 5

	16-Jul		17-Jul		20-Jul	
	Phosphorus Averages "p" values	Fungus Averages "p" values	Phosphorus Averages "p" values	Fungus Averages "p" values	Phosphorus Averages "p" values	Fungus Averages "p" values
Negative control – slippery elm	0.058	0.306	0.234	0.252	0.283	0.692
Negative Control – red maple	0.274	0.179	0.493	0.274	0.438	0.615
Negative Control – white grass	0.423	0.502	1	0.214	0.007	0.159
Negative Control – false nettle	1	0.505	0.387	0.413	0.228	0.234

Slippery elm – white grass	0.053	0.238	0.234	0.159	0.082	0.35
Slippery elm – false nettle	0.058	0.965	0.073	0.575	0.567	0.413
Red maple – white grass	0.038	0.17	0.493	0.178	0.004	0.162
Red maple – false nettle	0.274	0.224	0.173	0.584	0.163	0.209
Slippery elm – red maple	0.139	0.967	0.607	0.967		0.906

p value < .20

Table 1: p-values for all Phosphorus and Fungi Data

## Discussion

As graph 1A shows, there was a statistically significant difference in the amount of phosphorus found in the negative control and the *Acer rubrum*, the first tree, on July 16 ( $p = 0.058$ ), but as graph 1B indicates, there was not a significant difference between the Mycorrhizal fungi levels between the negative control and the *Acer rubrum* on July 16 ( $p = 0.306$ ). Therefore, because there is no correspondence in significance between these data sets, this July 16 data does not affirm our hypothesis.

As graph 1A shows, there was a statistically significant difference in the amount of phosphorus found in *Ulmus rubra*, the second tree, and the *Leersia virginica*, the first plant, on July 16 ( $p = 0.038$ ), and as graph 1B indicates, there was also a significant difference between the Mycorrhizal fungi levels between the *Ulmus rubra* and the *Leersia virginica* on July 16 ( $p = 0.17$ ). Therefore, because there is a correspondence in significance between these data sets and the expected correlation between fungi and phosphorus is present (see graph 1A and 1B), this July 16 data does affirm our hypothesis.

As graph 2A shows, there was a statistically significant difference in the amount of phosphorus found in the *Ulmus rubra*, the second tree, and the *Boehmeria cylindrical*, the second plant, on July 17 ( $p = 0.173$ ), and as graph 2B indicates, there also was a significant difference between the Mycorrhizal fungi levels between the *Ulmus rubra*, the second tree, and the *Boehmeria cylindrical*, the second plant, on July 17 ( $p = 0.178$ ). The *Boehmeria cylindrical*'s Mycorrhizal fungi levels were higher when they should have been lower in correlation to the phosphorus levels. Therefore, because there is an incorrect correlation in significance between these data sets, this July 17 data disproves our hypothesis.

Graph 2A shows a significant difference between the phosphorus levels of the *Acer rubrum*, the first tree, and the *Leersia virginica*, the first plant ( $p = 0.234$ ). Graph 2B indicates a statistically significant difference in the amount of Mycorrhizal fungi found by the *Acer rubrum*, the first tree and the *Leersia virginica*, the first plant ( $p = 0.159$ ) on July 17. Therefore since there is a correspondence in significance between these data sets and the expected correlation between fungi and phosphorus is present (see graph 2A and 2B), this July 17 data does affirm our hypothesis.

As graph 3A shows, there was a statistically significant difference in the amount of phosphorus found in the *Ulmus rubra*, the second tree and the *Leersia virginica*, the first plant, on July 20 ( $p = 0.004$ ), and as graph 3B indicates, there was not a significant difference between the Mycorrhizal fungi levels between the *Ulmus rubra*, the second tree, and the *Leersia virginica*, the first plant, on July 20 ( $p = 0.162$ ). Therefore, because there is a correspondence in significance between these data sets and the expected correlation between fungi and phosphorus is present (see graph 3A and 3B), this July 20 data does affirm our hypothesis.

As graph 3A shows, there was a statistically significant difference in the amount of phosphorus found in the *Ulmus rubra*, the second tree, and the *Boehmeria cylindrical*, the second plant, on July 20 ( $p = 0.163$ ), and as graph 3B indicates, there was a significant difference between the Mycorrhizal fungi levels between the *Ulmus rubra*, the second tree, and the

*Boehmeria cylindrical*, the second plant, on July 20 ( $p = 0.209$ ). Therefore, because there is a correspondence in significance between these data sets and the expected correlation between fungi and phosphorus is present (see graph 3A and 3B), this July 20 data does affirm our hypothesis.

However, while these individual sets of daily data would seem collectively to support our hypothesis strongly, an analysis of the collective data (see Figure 4) shows that there was no actual correlation between fungal density and phosphorus levels in the soils studied ( $r^2 = 0.00074$ ). Therefore, our hypothesis was disproven.

Interestingly, another group studying the effect of sugar maples on nitrogen levels in the soil in Microclimate 4 found that the nitrogen cycle has once again stabilized in this location (Miller, Rubin, Walsh, 2015c). We believe this stabilization may also be contributing to the stability we observed in our own data. In the future, we would test for all of the varieties of fungi along with phosphorous and nitrogen levels in the soils located in ESSRE Microclimate 4.

## Acknowledgements

We thank the following people and organizations for their contributions: Dr. Holliday Cross Heine, The Jennings Family, Human Capital Development, Inc. for funding our research. We would also like to thank Jean Brune and Roland Park Country School for the use of the RPCS backwoods. Finally we would like to thank David Brock, Annie Blalock, Kendall McCoach and Emma Wilson for reviewing this paper.

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