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Article

Analyzing the effect of mycorrhizal fungi on plant communication of nutrients

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SUMMARY

The goal of our experiment was to determine if phosphate transfer occurred between plants, using mycorrhizal fungi. We hypothesized that a "communication" network existed between plants when the mycorrhizal fungi were present and that there would be no transfer of nutrients if the mycorrhizal fungi were not present. This study extended the analysis of Suzanne Simard who studied Paper Birch, Douglas Fir, and Western Red Cedar in Canada. She was able to confirm that there is a massive underground communication network where different plants cooperate with resources using mycorrhizal fungi. The purpose of our experiment was to see if plants with nutrients would transfer their excess levels of phosphate to the plant that had a limited amount of nutrients. Overall, no definitive conclusion could be drawn from the treatments conducted. The measurements greatly deviated from the hypothesized results and demonstrated a more complicated relationship than originally thought. Further study will need to be conducted to determine if there are further conditions that must be met, such as a minimum amount of phosphate concentration or a minimum differential between the resources (in terms of this study, phosphate concentration) of the two plants, for this transfer to occur. The results of this study, if replicated and shown to be conclusive at long distances, could be used to aid forest management.

INTRODUCTION

In ecosystems, several abiotic and biotic factors interact with one another to form complex, interrelated relationships. One such symbiotic association between trees and fungi was discovered to have established an underground "communication network" to share resources (1). As Suzanne Simard suggested, the communication network in forests parallels our neural networks in that there are components that are both interconnected and interdependent (2). With this in mind, our experiment mimicked this communication network apparent in forests, but with small plants instead. By placing two plants in a close vicinity and creating a channel of "communication" with mycorrhizal fungi, we sought to investigate whether the plant with excess phosphate levels would transfer its nutrients to the neighboring plant. While doing so, we also sought to evaluate whether the plant-to-plant system, with a differential in nutrient concentration, truly initiated the phosphate transfer mechanism as opposed to a natural occurrence prompting the soil to reach equilibrium in terms of phosphate levels (3).

The fungi aided in this "communication" between plants by utilizing their roots. The fungi attached to the roots and utilized them to relay signals and nutrients from one plant to the other. In exchange, the fungi received sugar to consume from the plants (4). This relationship is symbiotic in that all parties involved benefit.

A previous study looked into the effect mycorrhizal fungi have on the lifespan and growth of plants (5). Similar studies provide a tentative understanding of the existence of a mycorrhizal association between plant roots and fungi and how fungi facilitate nutrient extraction from the ground (6). In this sense, there are no studies that investigate how fungi aid in moving nutrients from one plant to another plant in its vicinity. Dr. Simard performed a study where she observed the effect that mycorrhizal fungi had on nutrient transfer between trees (1). Yet, like the other pre-existing studies, Dr. Simard's study did not investigate smaller plants such as the annual and perennial plants that we chose to explore, and the effect of the fungi on nutrient transfer.

The goal of our study was to determine if plants with nutrients would transfer the excess levels of phosphate to the plant that had a limited amount of nutrients when the fungi were present (7). For all treatments, the measured phosphate level for each plant was technically the phosphate level of the surrounding soil. An additional consideration was evaluated by this study which was whether phosphate transfer between the plant-to-plant system and mycorrhizal fungi was unilateral and maintained this direction even after extended time periods, or when the differential between the phosphate concentration in the plants changed.

We set up two experiments to study the relationship between mycorrhizal fungi and plants by investigating the transfer of phosphate using different levels of phosphate in the soil. Multiple treatments were included, which involved a two-plant system, one fertilized and the other unfertilized. Some plants were "connected" with a visible line of concentrated mycorrhizal fungi **(Table 1).** The initial study was conducted in a back room, which limited the amount of light and photosynthesis. This treatment resulted in a positive correlation between the fungi and the transfer of phosphate between the two plants. Despite the promising results, this study had a relatively small data set that limited extended evaluation of how the phosphate transfer between plants persisted over time. This limitation prompted the second iteration of the experiment which was completed in a greenhouse over an extended period of time. Our second experiment involved numerous controls in which single plants were exposed to the fungi and the phosphate levels were observed. Other treatments were replications of the

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Treatment Group	Fertilized Daisy	Unfertilized Marigold	Unfertilized Mint	Organic Mycorrhizal Fungi
#1	+	+	-	+
#2	+	-	+	+
#3	+	+	-	-
#4	+	-	+	-
#5	+	+	-	+
#6	+	-	+	+
#7	+	+	-	-
#8	+	-	+	-
#9	-	-	-	-
#10	+	-	-	-
#11	+	-	-	+

Table 1. Treatment Groups. Eleven treatment groups were prepared and observed in the experiment. Each treatment had a different purpose and therefore included different combinations of variables. For instance, some included mycorrhizal fungi while others did not. Some treatment groups explored the transfer of phosphate between a fertilized daisy and an unfertilized mint while others explored the transfer of phosphate between a fertilized daisy and an unfertilized marigold.

initial treatments but were performed over a greater range of days to evaluate how the transfer of phosphate, through the hypothesized "communication network," persisted.

In both of our experiments specifically, the treatment subjects were marigolds, African daisies, and mints. These plants were chosen because they are relatively hardy plants and are not expected to die due to unexpected factors. While there are several studies involving small plants, such as annuals and perennials, there is no research on the interaction between these species and their relationship with mycorrhizal fungi. Thus, this study set out to determine if smaller plants, such as annuals and perennials, had the same interaction with mycorrhizal fungi that the trees did in Dr. Simard's study (1).

RESULTS

To study the relationship between mycorrhizal fungi and plants we investigated the transfer of phosphate between different combinations of fertilized and unfertilized plants over four days as well as three weeks, in four-day increments. Combinations of plants in the treatment groups included a daisy and marigold or a daisy and mint that were placed three feet apart from one another. The daisy in each treatment was given an excess of phosphate with fertilizer and the other plant was given basic soil. The original phosphate level was measured for all plants and soil-to-soil control. The mycorrhizal fungi were placed around the roots of the plants and a channel of mycorrhizal fungi was made connecting the two plants with enough concentration that a visible line of mycorrhizal fungi could be seen. We then took the measurements and compared the soil around the plant looking for changes in the level of phosphate. Some treatments did not involve mycorrhizal fungi to understand the baseline and whether or not other factors affected the transfer of nutrients.

In treatments #1 and #2, the daisy was given phosphate while either the mint or marigold, depending on the treatment, was deprived. Based on the data and analysis of both treatments, the phosphate level in the daisy decreased and that of the marigold or mint increased **(Figure 1A-B)**. This change in phosphate level was expected given the fact that mycorrhizal fungi were present in both treatments. In treatments #3 and #4, mycorrhizal fungi were not present. For treatment #3, the phosphate level in the daisy remained unchanged, while that of the marigold increased from 25 ppm to 30 ppm **(Figure 1C)**. For treatment #4, the phosphate level in the daisy increased from 45 ppm to 50 ppm, and that of the marigold increased from 25 ppm to 30 ppm **(Figure 1D).** Both treatments #3 and #4 showed a constant reading of ±5 ppm. Although there were small changes in phosphate level, there were only two treatments and one of each arrangement, whereby confidence in these trends could not be ascertained.

In treatments #5 and #6, the average phosphate level over three trials in the daisy experienced periods of increase and decrease **(Figure 2A-B).** The average phosphate level in the marigold or mint, depending on the treatment, over three trials also, experienced periods of increase and decrease. Although the first three measurements followed the hypothesized trend where the daisy would lose phosphate while the marigold or mint would gain phosphate, results after day eight were seemingly arbitrary. This suggests that there may be other factors that come into play over the extended time period, which could have skewed the results.

For treatment #7, the daisy was given the initial nutrients while the marigold was deprived. The average phosphate level in the daisy, over three trials, experienced periods of increase and decrease **(Figure 2C).** The average phosphate level in the marigold or mint also experienced periods of increase and decrease over three trials. Even though no mycorrhizal fungi were present, a similar trend was observed: the daisy lost phosphate and the marigold gained phosphate up until the third measurement where the levels then started returning to their original levels.

For treatment #8, the daisy was given the initial nutrients while the mint was deprived. The average phosphate level over three trials in the daisy experienced periods of increase and decrease **(Figure 2D).** The net change in phosphate level was 0%. The average phosphate level in the mint, over three trials, also observed periods of increase and decrease. The net phosphate level of the mint increased by 31.28%. While the phosphate level for the mint fluctuated around the observed trendline, the phosphate level in the daisy remained fairly constant which was expected without mycorrhizal fungi. In regard to the control groups, the average phosphate level for the soil in treatment #9 decreased from 20 ppm to 10 ppm **(Figure 3).** With a range of an average from 5 ppm to 30 ppm, that left a ±16 ppm error from the average. Treatment #10 (control #2) included one daisy with phosphate at one end of the 3-foot box and only soil on the other end, with no mycorrhizal fungi present. The phosphate level in the daisy decreased from 50 ppm to 30 ppm **(Figure 4).** Although there were some arbitrary fluctuations in phosphate levels, the overall trends were fairly horizontal with a slope of less than negative one. Lastly, in treatment #11 (control #3), the phosphate level in the soil increased from 11.7 ppm to 15 ppm with the presence of mycorrhizal fungi and added fertilizer **(Figure 5)**. There were some random fluctuations in measurement over a fairly constant trendline.

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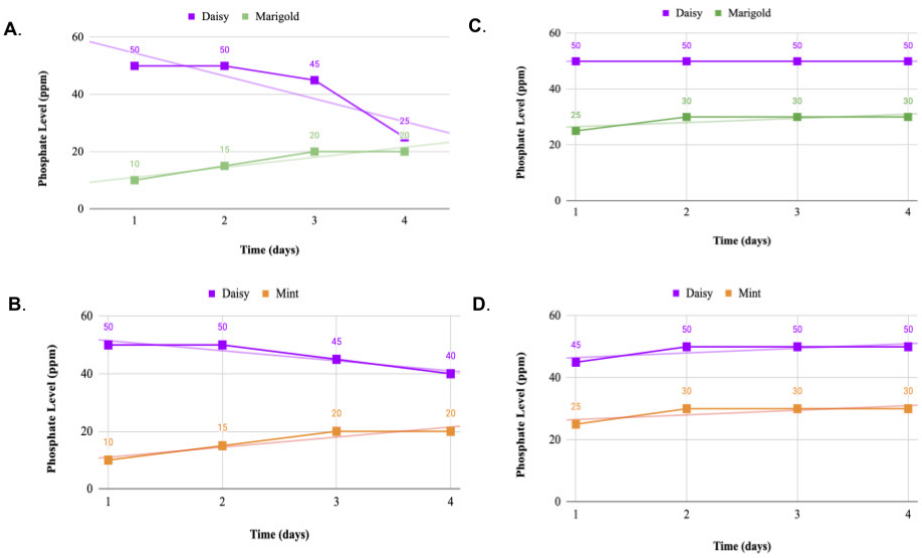

Figure 1. Changes in Phosphate Levels for Fertilized-Unfertilized Plant System. Over four days, the phosphate levels in various two-plant systems were measured every day. **A)** Fertilized Daisy and Unfertilized Marigold with Mycorrhizal Fungi, **B)** Fertilized Daisy and Unfertilized Mint with Mycorrhizal Fungi, **C)** Fertilized Daisy and Unfertilized Marigold without Mycorrhizal Fungi, and **D)** Fertilized Daisy and Unfertilized Mint without Mycorrhizal Fungi. A visible line of concentrated mycorrhizal fungi connected the two plants in both treatments #1 **(A)** and #2 **(B). A, B)** Changes in phosphate level suggest that the daisy was transferring its nutrients. **C, D)** Minimal phosphate transfer that occurred in treatments lacking mycorrhizal fungi could be from uneven distribution of phosphate in the soil or an indication that the soil was over-diluted during the measurement.

DISCUSSION

After extending the work of previous studies by ecologist Suzanne Simard, no definitive conclusion could be drawn from the treatments conducted in our study. Not only did the measurements demonstrate a much more complicated relationship than originally thought, but they were also much more variable than initially suspected.

Two experiments were conducted to determine if plants in a close vicinity would transfer their excess nutrients to one another, via the symbiotic fungal association. The preliminary experiment involved four treatments, conducted over four days. The secondary experiment included four replications of the original treatments and three control groups, for which phosphate levels were measured every four days over approximately three weeks. With the control groups, some of the confounding variables were observed. In addition, the control groups, specifically treatment #11, provided a basis for understanding whether the plant-to-plant system, connected through the "channel" of mycorrhizal fungi, truly initiated the transfer mechanism by "communicating" a need when one plant had excess phosphate levels in comparison to the other.

Based on the results of treatment #1, the net change in phosphate levels for the daisy and marigold over four days supported our hypothesis **(Figure 1A)**. Similarly, the results for treatment #2, with the 10 ppm decrease in the phosphate level for the daisy, and 10 ppm increase in phosphate level for the mint, suggested that fungi could be facilitating nutrient transfer through the hypothesized "communication" network **(Figure 1B)**. For treatment #3, the phosphate level in the daisy remained unchanged, while that of the marigold increased by 20%**.** For treatment #4, the phosphate level in the daisy increased by 11% and that of the marigold increased by 20%. Both treatments #3 and #4 exhibited results that slightly deviated from the hypothesized trend, as net transfer occurred even though mycorrhizal fungi were not present **(Figure 1C-D).** Even then, the minimal transfer that occurred over a relatively constant trendline was expected as other confounding variables had not been considered. Nevertheless, the results for treatment #1 and treatment #2 suggested that the fungi, to some degree, could be facilitating phosphate transfer as the demonstrated trendline is consistent with the hypothesized results (**Figure 1A-B).**

To overcome some of the limitations involved in the first set of treatments, such as the short study period that limited evaluation of how the hypothesized fungal association persisted, another round of treatments was completed. The results of the preliminary treatments strongly suggested that the fungi were facilitating nutrient transfer between the plant-to-plant system. With this knowledge, the replicated treatments were conducted over an extended time period and allowed us to further assess whether the transfer of phosphate was unilateral or bidirectional and maintained these directions over time. However, in an attempt to create a second iteration of the experiment, there was limited access to AquaChek treatment strips which are required to ensure uniform measurements and minimal bias. As a result, the time period under which the treatments were conducted had to be adjusted. Phosphate levels were intended to be measured daily, over one to two weeks. However, given the circumstances, phosphate measurements were limited to being taken every four days, over three weeks.

When evaluating the measurements taken every four days over three weeks, the hypothesized trend was observed in treatment #5 until day eight. For treatment #5, the daisy experienced a period of decrease in phosphate level, while the marigold experienced a period of increase in phosphate

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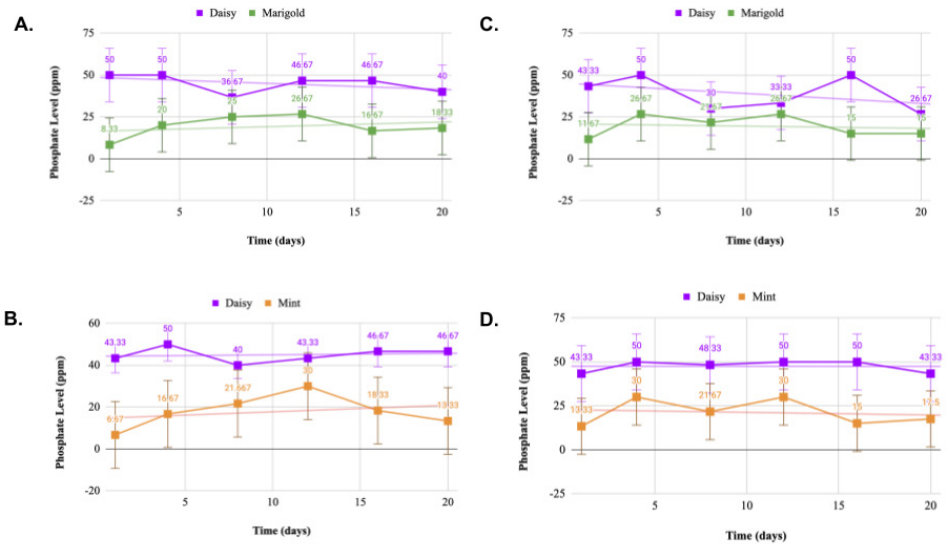

Figure 2. Phosphate Levels in a Fertilized-Unfertilized Plant System. Over three weeks, the phosphate levels in various two-plant systems were measured every four days. **A)** Fertilized Daisy and Unfertilized Marigold, **B)** Fertilized Daisy and Unfertilized Mint, **C)** Fertilized Daisy and Unfertilized Marigold without Mycorrhizal Fungi, and **D)** Fertilized Daisy and Unfertilized Mint without Mycorrhizal Fungi. Measured phosphate levels were averaged over the three trials conducted. In treatments where it was present, mycorrhizal fungi were placed in the roots and a visible line of concentrated mycorrhizal fungi connected the two plants. **A, B)** Over the first three days, the two-plant systems exhibit a relevant trend in phosphate transfer. **A, B)** After the first three measurements, the trend became chaotic in the gains and losses of phosphate. **C, D)** In these cases, the plants nearly restore their initial phosphate levels. A ±16 ppm error from the average was measured from Treatment #9.

level **(Figure 2A).** Despite these promising results, the measurements after day eight greatly deviated from our hypothesized trend. After day eight, the daisy in treatment #5 experienced periods of increase in phosphate level while the marigold experienced periods of decrease in phosphate level. For treatment #6, the change in the phosphate levels for the daisy and the mint was relatively consistent with the hypothesized trend, but only until day eight **(Figure 2B)**. After day eight, the data demonstrated each plant following a trend opposite to that hypothesized, where each plant's phosphate levels began returning to their initial levels **(Figure 2B)**.

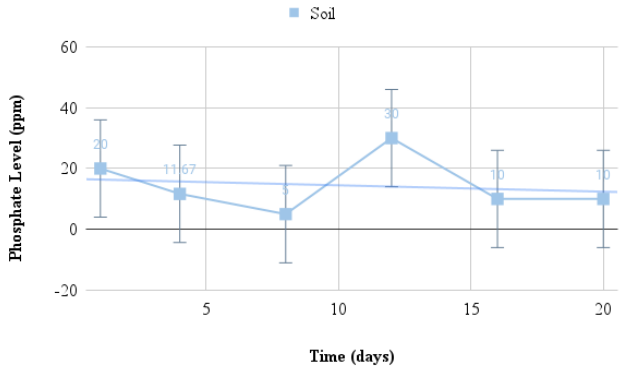

Figure 3. Background Phosphate Levels in Topsoil. No plants or mycorrhizal fungi were present. Over three weeks, the phosphate levels in the topsoil, represented by the blue line, were measured every four days. Measured phosphate levels were averaged over the three trials conducted. There was a clear fluctuation in the readings even though no changes were made. With a range of an average from 5 ppm to 30 ppm, a ±16 ppm error from the average was measured, which meant that a very strong trend must be present to rule out random fluctuations of the phosphate in the soil. Error bars for treatment #5 - #11 were set to the ±16 ppm error, calculated from this treatment, to account for the deviation from the average of 14.4 ppm.

For treatment #5 and treatment #6, the first three measurements for each seemed to be consistent with that of the hypothesized trend, where the plant with excess nutrients would transfer its phosphate to the neighboring plant, via the fungal association. Even in the preliminary treatments, such as treatment #1 and treatment #2, the observed trend supported our hypothesis. We speculated that the trends that were most consistent with our hypothesis were those observed over the shorter time periods since there may have been other factors that came into play over the extended time period, which skewed the results overtime: in our case, after day eight for the treatments in the second experiment.

One interesting outcome of the experiment came from the results for treatment #5 and treatment #6 whose measurements suggested that there was some degree of fluctuation in measured phosphate levels as there were periods of increase and decrease in phosphate levels that occurred over the study period **(Figure 2A-B).** While these fluctuations in phosphate levels suggested that phosphate transfers from daisy to marigold/mint and from marigold/mint to daisy (not only unilaterally) through a relatively low-affinity transport system, there was not enough data to support the hypothesized idea regarding a certain threshold of phosphate concentration that a plant must maintain depending on external conditions. For example, a minimum availability of phosphate for its own survival or a difference in levels between the two plants warrants a redistribution of resources. In other words, when one plant transfers some of its phosphate nutrients through the fungal association, it

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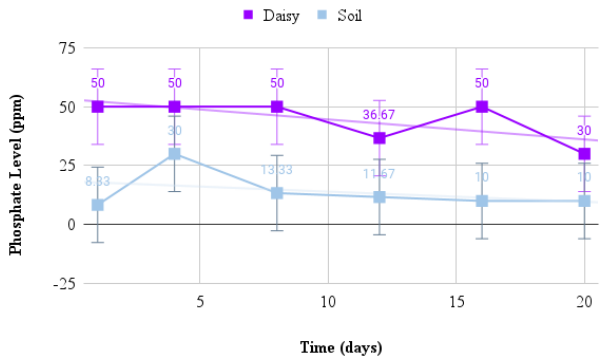

Figure 4. Phosphate Levels in a Fertilized Daisy without Mycorrhizal Fungi. No Mycorrhizal fungi were present. Over three weeks, the phosphate levels in the daisy and the topsoil were measured every four days. Measured phosphate levels were averaged over the three trials conducted. Arbitrary fluctuations were observed in the measurement, but overall, both trends were fairly horizontal with a slope of less than a negative one, as demonstrated by the line of best fit.

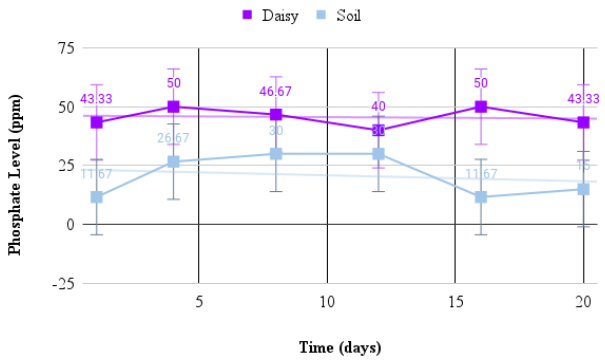

Figure 5. Phosphate Levels in a Fertilized Daisy with Mycorrhizal Fungi Averaged Phosphate Levels vs. Days. Over three weeks, the phosphate levels in the daisy and the topsoil were measured every four days. Mycorrhizal fungi were present in this treatment. Measured phosphate levels were averaged over the three trials conducted. There were some random fluctuations in the phosphate levels over a fairly constant trendline.

may reach a point where it requires a certain concentration of phosphate to sustain, facilitating phosphate transfer back to itself. This raised the question of the extent to which the plant with greater phosphate concentration will utilize the fungal communication network to transfer phosphate to the plant with less concentration. However, further study will need to be conducted to verify if there is a certain nutrient threshold that is required for each plant or a specific differential that warrants redistribution.

As for treatment #7 and treatment #8, the net phosphate level should have remained fairly constant according to the proposed hypothesis. The results for treatment #8 strongly supported the hypothesis that minimal phosphate transfer would occur between the plants in proximity when fungi were absent **(Figure 2D).** However, the fluctuation in phosphate levels for treatment #7 suggested that other, confounding variables may have been involved (**Figure 2C).**

As for control #2 (treatment 10), it was apparent that the phosphate levels for both the daisy and the soil fluctuated around a fairly constant trend line **(Figure 4).** Treatment #11 demonstrated some arbitrary fluctuations over a fairly constant trendline, suggesting that phosphate levels remained relatively constant for both the plant and the soil **(Figure 5).** This trend was expected since we hypothesized that the mycorrhizal fungi would only facilitate phosphate transfer when a need was "communicated" through the plant-to-plant system rather than the transfer mechanism occurring as an automatic process of two neighboring plants trying to reach equilibrium, in terms of phosphate concentration.

Our experiment can be extended by repeating for more precise and accurate results with a more natural environment, taking into account any difference in plant growth/photosynthesis due to each treatment being conducted in a greenhouse vs. in an outside environment where solar radiation is not limited/filtered. In addition, the fluctuation of the phosphate readings based on the soil's dilution limited the study's accuracy. A potential bias in interpretation included the treatment strips used to measure the phosphate level as the measurement was made based on the color of the treatment strip, which is not a precise, numerical measurement. A viable alternative to treatment strips would be sodium bicarbonate testing.

The soil used for each treatment group contained food for phosphate-solubilizing microbes to be established. Soil microorganisms like these mediate phosphate uptake. Thus, treatment #3, treatment #4, and treatment #7 may have experienced net transfer, without mycorrhizal association, due to microbe abundance within the standard soil itself. The overall comprehensive was lacking to consider the role of naturally occurring soil microbes in facilitating phosphate uptake, which could have interfered with the communication network. To eliminate this bias, the soil should be sterilized or exposed to high temperatures to kill these preexisting microbes.

For comparison purposes, a control group with organic soil, fertilizer, mycorrhizal fungi, and no plants should be included as a way to evaluate whether the plant-to-plant system, with a differential in nutrient concentration, truly initiates the phosphate transfer mechanism. This treatment group would pose as a negative control as the variable that is perceived to initiate the phosphate transfer is removed. If a difference in phosphate levels were to be seen, then it could validate the presence of other confounding variables involved in the treatments. Another bias was discovered from the results of treatment #9 (control #1) which demonstrated that there was a variation in the phosphate level of the topsoil itself even though there was no added fertilizer, fungi, or plants **(Figure 3).** These results suggested that a more homogeneous soil is necessary to maintain a fairly consistent phosphate level throughout each treatment box before the phosphate transfer mechanism can be properly observed.

Withal, the experiment should be conducted over a short period of time with more precise measurements as our results suggest that other confounding factors come into play over longer periods of time. Since these factors were not controlled, the hypothesized communication network, through which plants with high phosphate levels transfer their excess nutrients to plants with lower phosphate levels, was seemingly temporary. However, this observation cannot be

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confirmed as the relationship between the plants and fungi seems to be more complex than initially suspected.

The results of our study, if replicated without the precision, environmental, and systematic errors mentioned, and shown to be conclusive, can be used to aid plants located in less favorable environmental conditions by placing plants with excess phosphate in their close vicinity. If other macronutrients such as nitrogen and potassium exhibit similar transportation properties via mycorrhizal fungi as the phosphate seemingly does, future iterations of the experiment could be performed to have these other nutrients transported to plants lacking in them.

MATERIALS & METHODS

Experimental Treatments

This experiment involved a total of eleven treatments: eight experimental treatments and three control treatments. Select plants for each treatment were planted into a cardboard box, three feet apart, and were surrounded by gardenpro Organic non-fertilized topsoil. BioAdvanced Fertilizer was placed into the soil of the daisy. Using AquaChek High Range phosphate treatment strips, the soil of each plant was treated for its phosphate levels. The results were recorded and the strip color was compared to a scale provided on the bottle to tell the range of the phosphate level. One trial was completed for treatments 1-4, while three trials were conducted for treatments 5-8. For treatments 5-8, the measurement process was repeated every four days over approximately three weeks and the phosphate levels for each day of the three trials conducted were then averaged. In terms of the control groups, each was conducted for different purposes and therefore, had relatively distinct conditions. Three trials of each treatment were completed and involved the same methods of measurement and planting as the aforementioned treatments. For treatment #9 (control #1), no plants or mycorrhizal fungi were added. For treatment #10 (control #2), only one daisy was planted in the box and no mycorrhizal fungi were present. For treatment #11 (control #3), only one daisy was planted in the box and mycorrhizal fungi were present. Specific conditions that vary across treatments are further delineated in **Table 1**.

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