The Effects of AMF on Nitrogen-Rich Soil and Normal Soil in a Normal Environment on *clitoria ternatea* (Butterfly Pea Plants)

Sai Deeksha Munimadireddy[1], Neha Anadure[2], Gia Ginjupalli[3] and Bharat Poudyal[#]

[1]Irvington High School, USA
[2]Mountain House High School, USA
[3]Mission San Jose High School, USA
[#]Advisor

ABSTRACT

Nitrogen is an essential component of chlorophyll, which is important in the process of photosynthesis. In addition, Arbuscular Mycorrhizal Fungi (AMF) have a symbiotic relationship with plants and improve nutrient absorption and resistance to abiotic stressors. This fungal mycelium colonizes the roots of plants and specifically improves nutrient absorption by regulating the transfer of phosphorus and nitrogen in the soil. To understand the impact AMF and nitrogen have on the growth of pea plants, research was conducted. The hypothesis states that the sugar snap pea plants will grow best with the introduction of AMF and nitrogen-rich soil into the growth environment. For this experiment, 4 planters with an equal amount of soil but differing amounts of AMF and nitrogen fertilizer were used.

Introduction

Plants take up different types of mineral nutrients and chemicals from the soil during their growth process. Primary nutrients of plants are Nitrogen (N), phosphorus (P), and potassium (K) (Plant Nutrients in Soil, n.d.). Nitrogen, specifically, is an essential component of chlorophyll, which is important in the process of photosynthesis (Ikarashi N., 2020). Nitrogen is also a major component of amino acids in plants, as well as a key part of the energy molecule ATP (adenosine triphosphate), and of nucleic acids, specifically DNA (deoxyribonucleic acid). Nitrogen in soil comes in three forms: ammonium ions (NH_4^+), nitrate ions (NO_3^-), and organic nitrogen compounds, all of which are extremely important to balance the soil for essential plant growth (Hoorman, 2016).

Another important component of soil are different types of fungi ("Nitrogen in Soil", n.d.). They exist in the soil to mostly break down organic matter and are more common in slightly acidic or low pH soils ranging from 5.5 to 6.5 (Hoorman, 2016). Varieties of fungi, saprophytes, and mycorrhizae are very beneficial to plant growth. A type of mycorrhizae, called arbuscular mycorrhizal fungi (AMF) are proven to have multiple growth inducing qualities. AMFs develop a symbiotic relationship with its host plant's roots, where AMF receives essential nutrients from the plants and provides minerals to the plant in return. This fungus specifically improves nutrient absorption by regulating the transfer of minerals such as nitrogen, phosphorus, potassium, calcium, zinc, and sulfur (Khaliq A., & Perveen S., 2022).

For this experiment, the effects of AMF on nitrogen-rich soil and normal soil will be observed on butterfly pea plants (*clitoria ternatea*). The hypothesis was that the butterfly pea plants will grow best with nitrogen fertilizer and AMF mixed into the soil.

This research goes further than the lab. This can be applied to real-life situations, especially farmers striving to optimize their crop yield. To grow an optimal amount of crop to sustain a business, cultivators are always looking for new methods and what combinations of fertilizer or mycorrhizae to avoid. During this experiment, different combinations will be tested, which can be applied to real-world farms and gardens to increase yield.

However, because this experiment is confined to a lab greenhouse, it does not confirm the effects of AMF and nitrogen on a more large-scale level. Ultimately, this experiment will deepen understanding on the effects of different substances on crops, which will be beneficial to any cultivator or gardener.

Materials & Methods

Germinating the Seeds

Thirty two butterfly pea seeds were soaked in water for about 24 hours, and then transferred into wet paper towels. Eight seeds each were placed in each paper towel. The paper towels were folded and each was placed into a ziploc to germinate the seeds. They were left there for about two days, and the seeds began to sprout. After that, they were transferred into the soil.

Preparing the Planters

1/16 teaspoon of raw nitrogen fertilizer was mixed into one gallon of DI water. Soil was added two-thirds of the way into each planter. 1.5 teaspoons of AMF were added to two of the planters. In the remaining two planters, one was filled with AMF, and the other one without AMF, ¼ gallon of nitrogen fertilizer was mixed into each. After preparation, there was one planter with just soil, one with AMF, one with nitrogen fertilizer, and one with both AMF and nitrogen fertilizer as the control treatments.

Planting the Seeds

Eight holes were poked into each planter, 1 inch deep and about 2 inches apart. One seed was placed in each hole and covered up with soil. The planters without nitrogen fertilizer were watered with ¼ gallon of water after the seeds were planted.

Results

During this experiment, the plants were divided into four groups and placed under different conditions. Each planter was watered weekly with a quarter of a gallon (approximately 945 mL). The height of the plants was measured, and the number of leaves was counted.

Table 1. Number of Leaves and Height of Each Plant Under Nitrogen Fertilizer Condition

Nitrogen Only; No change in salinity

	1	2	3	4	5	6	7	8
Height	8.5	17 cm	9 cm	7 cm	6 cm	7.5 cm	13 cm	n/a
Leaves	6	15	5	8	7	10	13	n/a

Table 2. Number of leaves and height of each plant under AMF

AMF Only; No change in salinity

	1	2	3	4	5	6	7	8
Height	12 cm	10 cm	7.5 cm	8.5 cm	13 cm	9.5 cm	4.5 cm	6.5 cm
Leaves	12	11	10	11	11	13	8	8

Table 3. Number of leaves and height of each plant under nitrogen fertilizer and AMF

AMF + Nitrogen; No change in salinity

	1	2	3	4	5	6	7	8
Height	14 cm	4 cm	4.5 cm	2.5 cm	n/a	14 cm	6 cm	n/a
Leaves	9	3	2	2	n/a	11	7	n/a

Table 4. Number of Leaves and Height of Each Plant Under Control Condition (No Added Substances)

Control; No change in salinity

	1	2	3	4	5	6	7	8
Height	4 cm	6 cm	3 cm	6.5 cm	10.5 cm	n/a	1 cm	n/a
Leaves	7	5	3	5	9	n/a	2	n/a

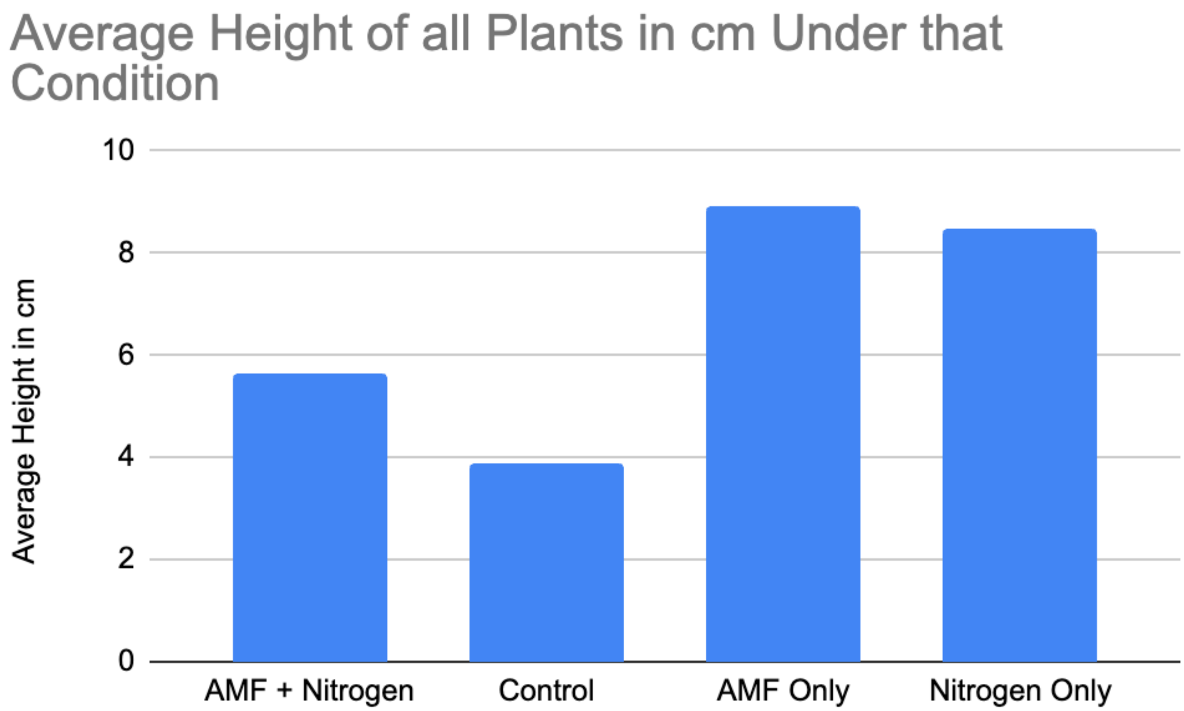

Figure 1. Mean height of all the plants under each condition

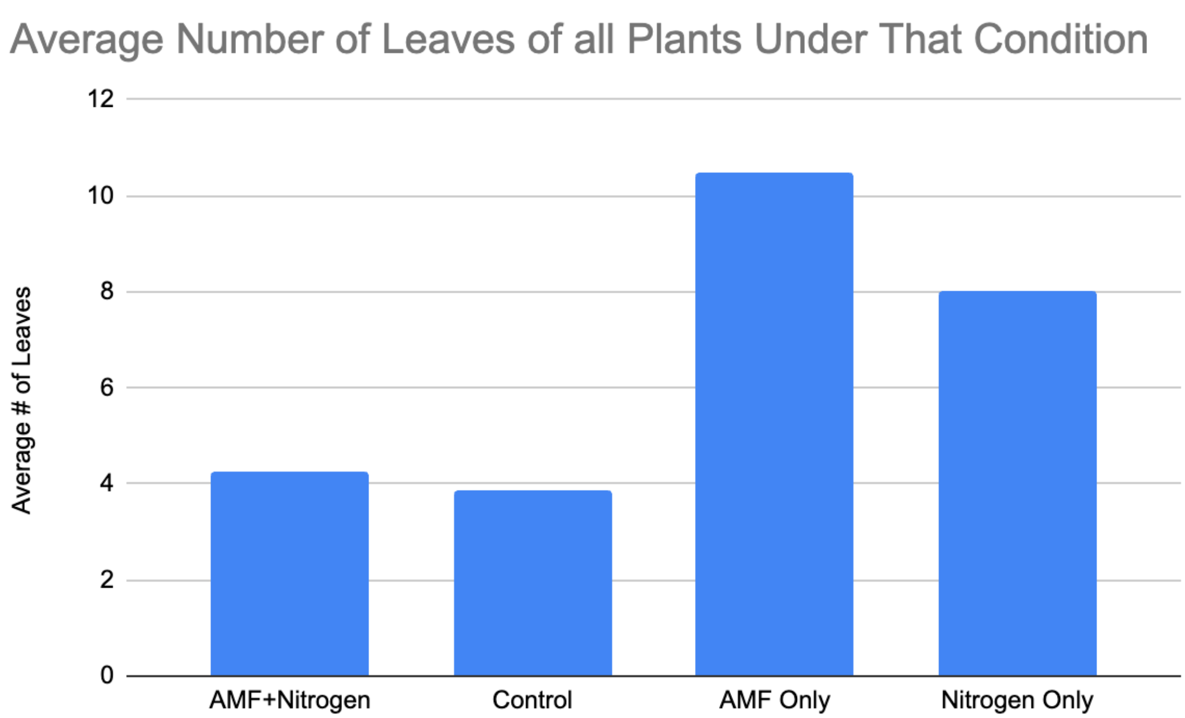

Figure 2. Mean number of leaves for all plants under each condition

Based on the data collected from our experiment, the plants that grew with only arbuscular mycorrhizal fungi (AMF) exhibited the most favorable growth outcomes. Specifically, under the condition with just AMF, the plants had an average of approximately 10 leaves and a mean height of 9 centimeters. This suggests that AMF alone significantly enhanced plant growth compared to the other conditions tested.

Discussion

Amount of Water

During the course of the experiment, we encountered an issue with water absorption in the soil. The soil failed to properly absorb the water, leading to a thin layer of water pooling beneath the soil surface. Despite efforts to reduce the amount of water used, the problem persisted, indicating that overwatering was not the cause. This issue likely impacted plant growth and the absorption of nitrogen fertilizer, potentially skewing the results of the experiment.

Relationship Between Nitrogen and AMF

Our original theory, which proposed that plants would develop most optimally when both nitrogen fertilizer and arbuscular mycorrhizal fungi (AMF) were applied, was not supported by the experiment's outcomes. The idea behind the hypothesis was that while nitrogen fertilizer and AMF both promote plant development on their own, applying them together would provide the best outcomes. In contrast to what was anticipated, plants grown in soil containing both nitrogen and AMF performed noticeably worse than plants produced in soil containing AMF alone.

According to theory, AMF affects nitrification, denitrification, and mineralization, among other processes that have a substantial impact on the intake of nitrogen. It is well known that AMF increases the potentials for nitrification and nitrogen mineralization, which should help plants absorb more nitrogen (Fang C., & Xie J., 2023). But given the subpar results seen in plants getting both nitrogen fertilizer and AMF, it is possible that these two treatments will not work well together. It is also possible that complicated interactions between the two treatments that were overlooked prevented the combination between AMF and nitrogen fertilizer in our experimental settings from enhancing growth as was expected.

Conclusion

The findings of this study also have significant ramifications for soil management and sustainable agriculture. The results underscore the need for more focused and accurate nutrient application by indicating that the use of nitrogen fertilizer in excess can have detrimental effects on plant growth and soil quality. Furthermore, the advantages of AMF for plant growth highlight how crucial it is to support healthy soil microbes in agricultural settings. Future studies should investigate the underlying processes of these relationships and devise methods for maximizing agricultural output with the least amount of negative environmental effects. In summary, this research offers significant understanding of the intricate interactions between soil microbes and plants, which might guide the development of more productive and sustainable farming methods.

Future Research

In today's time, the severity of clean soil to sustain agriculture is inadequate. The common instance of high salt levels in soil are concerning the health of crops' growth. In order for plants to survive and grow in soil with medium to high salinity, it is crucial that they maintain proper osmotic balance. Without this, the cells within the plants may dehydrate and eventually die. Altered soil salinity can lead to issues with ion toxicity, osmotic stress, nutrient deficiency, and oxidative stress, which can limit the uptake of water from the soil. Additionally, soil salinity reduces the phosphorus levels in the plant, because Ca ions precipitate with phosphate ions. Many salts are also essential nutrients in the soil, so high salt levels in the soil, or high salinity, can interfere with nutrient uptake (Shrivastava, P., & Kumar, R., 2015).

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References

Begum, N., & Qin, C. "Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance." *Northwest A&F University,* 18 September 2019, https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.01068/full

Eckert D., "Nitrogen in Soil." *Mosaic*, 2024 www.cropnutrition.com/nutrient-management/nitrogen/

Fang C., & Xie J., "Arbuscular mycorrhizal fungi drive soil nitrogen transformation under wheat varieties with different nitrogen utilization efficiencies." *Rhizosphere ScienceDirect Journal*, 20 September 2023, https://www.sciencedirect.com/science/article/abs/pii/S2452219823001143

Hoorman, J. J. "Role of Soil Fungus." *Ohio State University*, 7 June 2016, https://ohioline.osu.edu/factsheet/anr-37#

Ikarashi N., "Nitrogen as a major essential element of plants." *Niigata University, Faculty of Agriculture*, 2020, https://www.researchgate.net/profile/Takuji-Ohyama/publication/234135771_Nitrogen_as_a_major_essential_element_of_plants/links/0c96051ac0b614543a000000/Nitrogen-as-a-major-essential-element-of-plants.pdf

Khaliq A., & Perveen S., "Arbuscular Mycorrhizal Fungi Symbiosis to Enhance Plant–Soil Interaction" *Sustainability Journal*, 27 June 2022, https://www.mdpi.com/2071-1050/14/13/7840

"Plant Nutrients in Soil." *Department of Primary Industries*, www.dpi.nsw.gov.au/agriculture/soils/soil-testing-and-analysis/plant-nutrients

Shrivastava, P., & Kumar, R. (2015). "Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation." *Saudi Journal of Biological Sciences*, 22(2), 123–131 https://doi.org/10.1016/j.sjbs.2014.12.001