Evaluating the Effect of Carbon Dioxide on the Rate of Transfer in Mycorrhizal Networks.

Grant Proposal

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Author Note

I would like to acknowledge and thank Dr. Kevin Crowthers for mentoring and supporting me throughout my project, and the Massachusetts Academy of Math and Science for providing me with equipment and lab space for me to conduct my experiment.

Abstract (RQ) or Executive Summary (Eng)

Mycorrhizal networks are symbiotic relationships between mycorrhizae and plants; the fungi can receive and send molecules from the plants it forms the relationship with. The fungi are able to create relationships with multiple plants, allowing molecules to be transferred between plants. The relationship has been hypothesized to be based on carbon: fungi vary molecules transferred based on the amount of carbon received. However, the hypothesis has been debated, with multiple differing sources of evidence. The amount of CO2 in the atmosphere has increased over twenty percent by 2022, largely due to human pollution. Plants use CO2 in a process called photosynthesis in which plants isolate carbon, which could be used in the network. Mycorrhizal networks are essential to plant survival; Therefore, understanding how the increase of CO2 affects it is vital to predicting the effects of pollution on ecosystems. An experiment was conducted on how the amount of carbon dioxide a plant is exposed to affects the rate at which the network's transfer molecules changes. The percentage of CO2 plants exposed to was varied, with the mycorrhizal fungi exposed to nutrients, and the amount of transfer being measured by the growth of the plant's roots. The results are expected to show that as carbon dioxide increases, the length of plant's roots increase, showing a positive relationship between CO2 and the rate of transfer within mycorrhizal networks, providing evidence that as global warming increases, the relationship between the plants and mycorrhizae increases, and the plants receive molecules that benefit its survival.

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Mycorrhizal networks are symbiotic relationships between mycorrhizae fungi and plants. In this relationship, the fungus within the soil can receive and send molecules from the plants it forms the relationship with, which is extremely beneficial to plants, as they can receive the nutrients that they are lacking (Gorzelak et al., 2015). The relationship between mycorrhizal fungi and plants has been hypothesized to be based on carbon. Plants give the fungi carbon molecules, and the fungi send helpful molecules to the plants. Fungi survive off breaking dead matter into carbon, and plants rely on nitrogen, phosphorus, and other nutrients in the soil. The hypothesis of the carbon relationship between plants and fungi has been long debated, with multiple studies providing evidence for and against the relationship (Fellbaum et al., 2014).

The biggest way plants receive carbon is from CO2 (Bassham & Lambers, 2024). However, the amount of carbon dioxide in the atmosphere has increased by over twenty percent within the years 1979 and 2022, largely due to the use of fossil fuels, as well as numerous other human activities that lead to the production of carbon dioxide, which hascontinued to be used and exploited since the industrial revolution (*Atmospheric carbon dioxide*, 2022). Plants use carbon dioxide to create sugars needed for growth, in a process called photosynthesis. Plants can take in carbon dioxide and isolate the carbon to form sugars it will consume for energy (Bassham & Lambers, 2024). As the amount of carbon dioxide increases within the atmosphere, plants will have greater opportunities to absorb carbon dioxide and therefore come into possession of a significantly greater amount of carbon.

How is Mycorrhizal networks affected? If the symbiotic relationship between plants and Mycorrhizal networks was correctly hypothesized to be based around carbon, an increase of carbon dioxide would allocate plants more carbon, strengthening the mycorrhizal relationship.

Since Global warming has been expanding rapidly, it is important to understand how such an environmentally impactful effect is impacting the plants around us, especially mycorrhizal networks.

I am conducting an experiment on how carbon dioxide exposure will affect the rate of molecule transfer through a mycorrhizal network by varying the percentage of carbon dioxide both plants are exposed to and measuring the change in each plant's root length. As the amount of carbon dioxide increases, the change in the root lengths will increase, showing a positive relationship between carbon dioxide and the rate of transfer within mycorrhizal networks, effectively showing the effects global warming has on mycorrhizal networks, furthering our understanding of how ecosystems have been affected by climate change.

Section II: Specific Aims

Specific Aim 1: determining the existence of mycorrhizal networks.

Specific Aim 2: Showing how the change in CO2 has affected mycorrhizal networks and predicting rates of transfer in the future.

Section III: Project Goals and Methodology

Relevance/Significance

This project is now more relevant than ever before as carbon dioxide continues to increase, showing no evidence that it will slow down as it reaches historical highs. It is vital to understand all parts of the ecosystem to fully understand the effects of greenhouse gases on our planet.

Moreover, mycology, the study of fungi, is a critically understudied field due to a multitude of reasons; difficulty studying fungi in a laboratory, difficulty in growing and culturing, as well as a lack of interest (Ralls, 2024). The lack of research in mycology is rather disappointing, especially due to the relevance that fungi can have over an entire ecosystem.

The discovery of mycorrhizal networks completely changes our understanding of how forests and other habitats interact. Whereas before, it was considered that plants worked by themselves, now we understand that plants often rely on other plants, as well as fungi, to survive, changing theories of evolution between plants, as well as their history.

The implantation of mycorrhizal fungi into farming has provided benefits to their growth. Plants can react to stimuli and absorb nutrients that they would be unable to even identify on their own, improving the livelihood of the plants, as well as improving the crop themselves (郭涛, 刁向银, 2013).

Mycorrhizal networks are relevant in nature that not including or considering them in environmental studies decreases the value of that study. Currently, scientists have identified 50,000 fungal species that form mycorrhizal associations with 250,000 plant species (van der Heijden et al., 2015), showing how widespread they are.

Therefore, by excluding mycorrhizal fungi from studies about the effects of increasing carbon dioxide, science fails to make predictions accurately to nature itself as a large part of ecosystems are ignored (*Atmospheric carbon dioxide*, 2022).

Hence, why it is so important to understand how the rate of transfer is affected by changes in the amount of carbon dioxide. The change in transfer rate may show how ecosystems can react to changes in carbon dioxide. If plants have increased amounts of transfer, they may change the whole function and communication of the ecosystem. With this study, I hope to prove how the rate of change in mycorrhizal networks change as CO_2 exposure changes, to show the effects of global warming on the networks.

Innovation

Currently, their is a lot of debate on the effects CO_2 has on the rate of transfer in mycorrhizal networks because of the conflicting evidence provided across studies providing differing evidence on how this relationship functions. This experiment will help elevate debate by providing evidence on how differing levels of CO_2 affect nutrient transfer to plants (Fellbaum et al., 2014).

Methodology

For this project, A chamber out of a 2-inch diameter PVC pipe was created (figure 2) inspired by the 2014 study conducted by Carl Fellbaum et al. A 50-micron mesh was placed directly in-between the pipe, creating two sides of the pipe to ensures that the plant receiving the transferring molecules will not accidentally pick it up from the soil or the roots of the plant, meaning it must be through the mycorrhizal fungi. I then sealed the bottom of the pipe with a 2-inch diameter PVC pipe cap.

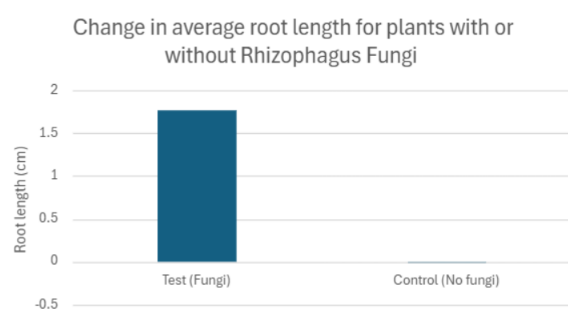
A plant was placed on one side of the mesh, and then nutrient capsules containing nitrogen and phosphorous on the other side. The testing chambers had soil containing 500 spores per gram of soil of a Rhizaphagus fungi mix. The control contained just the soil. Plants were then placed at 640 ppm of CO2 (ambient level), 900-1300 ppm, and 1500-1900 ppm, with testing plants and control plants in both. The average root length of the plants before the experiment and after two days of the experiment was taken, with greater increase in root length showing more nutrient transfer.

Specific Aim #1: Determining the existence of mycorrhizal networks.

Justification and Feasibility. I hope with my study I will be able to create a model of a mycorrhizal network that shows that the transfer is from the fungi, and not from other causes. I will use a control of without fungi spores to prove that the connection between the roots and the fungi is driving the transfer of molecules and not an outside force to show not only the effect of carbon dioxide, but the existence of the network between the plants, as well as its ability to transfer molecules.

Summary of Preliminary Data

Graph 1 shows the growth of roots before and after the experiment. The control has no fungi, while the test has a mix of Rhizophagus fungi. Each plant was placed in a growth chamber separated in half by a 50-micron mesh. On one side of the chamber the plant was placed; on the other side,



Graph 1: The change in the average root length of *Chamaedorea Elegan* plants germinated with a mix of Rhizophagus fungi (test) or without (control) after two days of growing.

nutrient capsules containing nitrogen and phosphorus were placed. The roots were measured before being planted, and again after two days. After the two days, the test plants on average showed significant root length growth, while the control showed no growth at all, proving that the mycorrhizal relationship must exist as the plants with the mycorrhizae had more root growth due to the network passing the nutrients allocated for it to grow.

Expected Outcomes. The overall outcome of this aim is to show that for all test cases, the plants with mycorrhizae have greater root growth than the plants without. This knowledge will be used to show that the relationship between the plants and the mycorrhizae is present and responsible for any root growth change in the project.

Potential Pitfalls and Alternative Strategies. A potential failure is no significant evidence found showing an existence of a mycorrhizal relationship. This could happen due to the environmental conditions being unsuitable for fungi germination. The solution would be to germinate the fungi separately from the plant in 40 degrees C and then forming the connection with the plant to ensure that the fungi is able to germinate.

Specific Aim #2: Show how the change in CO_2 has affected mycorrhizal networks and predicting rates of transfer in the future.

Justification and Feasibility. Currently, there are very few studies on how the changing amount of carbon dioxide has affected or will continue to affect the rate of transfer for plants connected between mycorrhizal networks. I hope to identify whether the amount of Carbon dioxide has a positive relationship to the rate of transfer. The 2014 study conducted

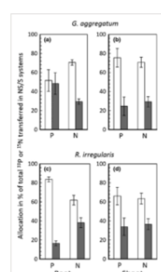
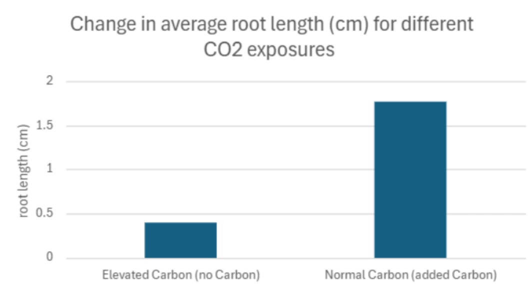


Figure 1. Results of the experiment conducted by Fellbaum et la. Allocation of the total 33P or 15N taken up by the common mycorrhizal network (CMN) of Glomus aggregatum (a, b) and Rhizophagus irregularis (c, d) and transferred to the roots (a, c) or shoots (b, d) of nonshaded (open bars) and shaded (closed bars) Medicago truncatula plants in NS/S systems (calculated as a percentage based on the nitrogen (N) or phosphate (P) content in shaded (S) and nonshaded (NS) plants). Shown is the average of $n = 5 \pm$ SE.

by Carl Fellbaum et al. varied the amount of carbon plants intake by shading plants, reducing the amount of photosynthesis. They found that plant growth was reduced when plants were shaded, showing that carbon increases nutrient transfer in mycorrhizal networks. Therefore, as CO2 is increased, there should also be an increase in root growth.

Summary of Preliminary Data

Graph 2 shows the growth of roots before and after the experiment. The Normal Carbon and Elevated Carbon have a mix of Rhizophagus fungi. Each plant was placed in a growth chamber separated in half by a 50-micron mesh. On one side of the chamber the plant was placed; on the other side, nutrient capsules containing nitrogen and phosphorus were placed. The Normal Carbon was placed in 640 ppm of CO2, while the Elevated Carbon was placed in a range of 900 ppm to 1300 ppm. The roots were measured before being planted, and again after two days. After the two days, the Elevated Carbon on average showed significantly less root growth than the Normal Carbon, showing that CO2 has a negative relationship with molecule transfer in mycorrhizal networks, as the Elevated Carbon passed less nutrients than the normal Carbon, leading to less root growth, which could show that this network does not follow the excepted relationship with carbon, providing evidence that CO2 actually reduces the rate of transfer.



Graph 1: The change in the average root length of *Chamaedorea Elegan* plants germinated with a mix of Rhizophagus fungi exposed to 640 ppm of CO2 (Normal Carbon) or a range of 900-1300 ppm of CO2 (Elevated Carbon)

Expected Outcomes. The overall outcome of this aim is to show that CO2 influences the rate of nutrient transfer in mycorrhizal networks. This knowledge will be used to show the effects that increasing CO2 within Earth's atmosphere has been impacting the mycorrhizal networks within ecosystems.

Potential Pitfalls and Alternative Strategies. The change in CO_2 could potentially affect the growth of the fungi outside of mycorrhizal nutrient transfer, which in turn would inaccurately portray the effects of the CO_2 on mycorrhizal nutrient transfer. If this were to happen, the methodology of this experiment would be changed by putting fluorescein, a flouriest molecule, in the soil instead of the nutrients, and measuring the increase of light.

Section III: Resources/Equipment

For this project, a CO_2 tank would be needed for the exposure of CO_2 to the mycorrhizal network. A CO_2 regulator is also required so the amount of CO_2 can be precisely changed, and the effects of nutrient transfer evaluated over multiple CO_2 levels.

Section V: Ethical Considerations

The fungi used, Rhizophagus, is a non-toxic, non-pathogenic, microorganism, therefore exhibiting low risks. However, usage could result in potential cross-contamination with other organisms, potential allergic reactions, and potential introduction in non-native environments. To combat , the following safety procedures were used- Safety goggles, gloves, proper disinfectant of all equipment and surfaces, hoods to reduce spore exposure - as well as proper disposal of any cultures + spores - 20% bleach solution - to prevent any of these potential dangers from happening.

Section VI: Timeline

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Section VII: Appendix

1.

Figure 2. Chamber create out of PVC pipe. The dotted lines represent the 50-micron mesh.

Section VIII: References

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