

Global Climate Change-Induced Mutualism Breakdown Among Legumes and their Soil Symbionts

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I. Abstract

Mutualistic interactions within ecosystems help to increase species survival, growth, fitness, ecosystem services, and are key drivers of ecosystems success and function. While many studies have focused on the separate importance of mycorrhizal and rhizobial interactions in various systems, we show the importance of both of these types of interactions in a changing climate, depicted by an increasingly earlier snowmelt, and warmer, drier summer conditions. By using plots which advance snowmelt by two weeks, we show how the root-mutualist structure of legumes (*Lathyrus lanszwertii* var. *leucanthus* and *Vicia americana*) changes with early snowmelt and dry soil conditions. These results show how increasingly earlier snowmelt across the southern Rocky Mountains affects the ability of these legumes to form mutualisms with soil microbial species. Changes in how many and which partners legumes associate with will alter the amount of nitrogen in the plant and local ecosystem and potentially change the quantity and quality of pollinator rewards.

II. Introduction

Mutualisms are defined as interactions in which both species benefit, leading to an increased fitness and higher likelihood of survival of both partners. Mutualistic relationships are found in all ecosystems and (1) provide ecosystem services, like pollinators pollinating angiosperms, allowing for increased plant and insect fitness (Kearns et al., 1998); (2) enable the survival of species, an example being the Pied fantail birds which eat ticks off the Muntjac Indian Deer, thus decreasing the deer's risk for disease while providing a food source for the bird (Silmi, 2013); (3) promote growth, like mycorrhizal fungi, which increase plant-root surface area, allowing for greater amounts of nutrients and water to get to the plant (Finlay 2008); (4) increase the fitness of organisms, like gut endosymbionts that aid in animal digestion (Gibson et al., 2010); and (5) support global nutrient cycling, such as rhizobial bacteria which live in their symbiotic legume hosts and convert inert nitrogen into a usable form (Scheublin et al., 2006).

The benefits of mutualistic relationships are apparent in all systems, especially in instances of limited time and space for species interactions, such as areas with compressed growing seasons or islands. Isolated locations and short growing seasons illuminate the reliance of organisms on one another when the extinction of one species can lead to the extinction of a co-dependent species (Traveset, 1999). Mutualisms have

also shaped Earth's biodiversity and history by enabling the diversification of life, permitting regulated growth, behavior, and development (Douglas, 2014; Margulis, 1970; Pirozynski and Malloch, 1975; Bronstein, 2015), as shown in the evolution of eukaryotic cells and in the movement of plants from water to land (Bronstein, 2015).

Mycorrhizae and rhizobia are two plant-root mutualists that very commonly create mutualistic symbioses with legumes. Mycorrhizae and rhizobia serve as key symbionts for the make-up of the plant community, plant structure, and are vital to plant nutrition (Scheublin et al., 2006). Both mycorrhizal and rhizobial symbiotic mutualists can be found in and on the roots of legumes. Mycorrhizae are fungi which creates an increased surface area of the roots by binding inside of and on the root and allowing the plant to access more water and soil nutrients in exchange for plant sugars (Finlay, 2008). Rhizobia are bacteria which live in specialized plant organs called nodules. Rhizobia fix atmospheric nitrogen into a biologically usable form in exchange for plant sugars (Denison et al., 2011). When both are present in legume roots, mycorrhizae and rhizobia synergistically increase the plants fitness (Afkhami et al., 2016).

Rhizobia and mycorrhizae effectively work together to fix nitrogen and gather water and soil nutrients (respectively) that the plant requires. While rhizobia and mycorrhizae are able to interact with the plant in beneficial ways without the presence of the other, in circumstances of dry areas or during times of drought, the presence of both mycorrhizae and rhizobia allow for more nitrogen to be fixed because of present added water (Scheublin et al., 2006).

Climate Change:

The threat of global climate change on ecosystems and species involved in mutualistic interactions is a severe threat to interactions worldwide; the Earth is currently facing an estimated temperature increase of 0.2°C per decade, or 0.4°C to 0.6°C in high elevations, such as those present at the Rocky Mountain Biological Laboratory (RMBL), in Gothic, Colorado. The result of the temperature increase from anthropogenic climate change is not only affecting the severity of natural events, like greater variation in precipitation patterns and more extreme weather patterns globally (Root et al., 2005; Parry et al., 2007; Allan and Soden, 2008), but has lead to an unclear result for the fate of mutualisms (Rafferty, et al., 2015).

For legumes, increased droughts and a lower overall soil moisture has the potential to severely affect the ability to continue fixing nitrogen. With the mutualists mycorrhizae and rhizobia obtaining sugars from the plant in exchange for increased water and nitrogen, the legume decides which mutualists to continue trading sugars with in times of stress. In most cases of changing climate conditions, water is the most limiting growth factor, causing legumes to force rhizobia out in order to maintain mycorrhizal interactions. Legumes abandoning rhizobial interactions can cause the mutualism to break down completely over evolutionary time, making it so the nitrogen fixing interactions are no longer possible (Kiers, et. al, 2010; Sachs, et. al, 2006).

Phenology:

Phenology refers to the study of the natural cycle of the biological events of organisms. Climate change has shifted the timing of events such as migration, germination, and reproduction to, on average, much earlier than the historical timing. The results of the shifted timing are often seen with species-specific manner (Parmesan and Yohe, 2003; Root et al., 2003; Parmesan 2007; CaraDonna et al., 2014).

Some of the most extensive plant phenology has been measured from the Rocky Mountain Biological Laboratory, where researchers have been looking at flowering phenology since the 1970s. Recent studies focusing on the interaction of mutualistic relationships show there is a non-symmetrical interaction between species in a changing climate (Sutherst et al., 2007; Tylianakis et al., 2008; Hegland et al., 2009; Kiers et al., 2010), meaning flowers would not alter at the same rates as others. Some mismatches have been noticed to lead to a partial or complete loss of temporal or spatial overlap among partners (Rafferty et al., 2015), meaning the mismatches could harm mutualistic relationships (Bascompte, 2019). Plant phenology has been studied at RMBL since 1974 in the same plots, finding that over the past 40 years there had been an advanced increase in temperature and snowmelt dates, with flowering increasing over 1.5 days per decade (Kearns et al., 1998). From increasing rates of phenological shift, results are predicted to continue impacting the attraction of mutualists to plants, increasing the likelihood of phenological mismatches (Elzinga et al., 2007).

Independent phenological shifts among mutualists can also cause phenological mismatches where there is a partial or complete loss of temporal overlap among interacting species relative to historical conditions, which may lead to affected interaction strength, duration, and outcome within partners (Yang and Rudolf, 2010; Rafferty et al., 2015). This was shown in a study out of RMBL, with *Lathyrus lanszwertii* plants and *Hoplitis fulgida* bees having a broad overlap in phenology in 2008, and only slight overlap in 2009, due to advanced flowering phenology in 2009 compared to 2008, without a corresponding advance in the phenology of the bees (Forrest, 2017).

Another example of phenological mismatch was shown in the affects of early/late snowmelt on *Claytonia lanceolata*, with alterations most negatively affecting the flowers reproduction by reduction in pollination, bringing to light the possible result climate change will have on mutualisms (Gezon et al., 2016).

Importance:

RMBL is an excellent system to answer questions about mutualisms and climate change because of the noticeable effects of climate change already altering the seasonal weather patterns. Since the 1980s, precipitation in June has significantly decreased; the snowmelt shifting to earlier dates, and monsoon season historically occurring in early July pushed back even later with less rainfall (Lambert, 2017). With the effects of climate change already clear at RMBL, the possible effects this could have on legume root symbionts to become mismatched with or later abandoned by their legume host could put germination success, phenology, and interactions with other mutualists at risk (Lau and Lennon, 2012; Wagner et al., 2014).

When dealing with dry conditions, such as drought, mycorrhizal interactions are important; mycorrhizal fungi increase the ability to take in water by increasing root surface area. Legumes allocating photosynthate to mycorrhizal fungi to increase the water for plant growth. Legumes allocate sugars to mycorrhizal expansion instead of rhizobial interactions which may lower the amount of nitrogen present in the plant. Allocating resources to mycorrhizae rather than rhizobia forces the bacteria into a dormant or free-living state in the soil (Blagodatskaya & Kuzyakov, 2013; Classen et al., 2015). Rhizobia are not able to fix nitrogen unless in the nodule. The abandonment of rhizobia leads to a short-term breakdown of the mutualisms (Zahran, 1999; Comerford et al., 2013). Over evolutionary time while one partner is dormant and the other is not, this mutualism can break down via the loss of a segment of rhizobial DNA important for legume interactions, leading to an alteration of the mutualisms fate; rhizobia cannot interact with legumes thereafter (Sachs & Simms 2006; Rafferty et al., 2015). Rhizobia evolving away from legume interactions is not only negative to the health of the legume and the surrounding soil, but also for pollinators who get their nutritional nitrogen needs from the nectar and pollen of legumes. If climate change creates conditions that do not permit rhizobia to be present in legumes because of the increasing need for water, lots of mutualistic interactions with legumes will be lost (Finlay, 2008).

Predictions:

Using legumes *Lathyrus lanszwertii* var. *leucanthus*, *Vicia americana*, and rhizobial bacteria as a study system to understand how drought will affect this crucial mutualism (rhizobia fix nitrogen which is a very limiting resource), we predict a decrease of rhizobia-filled nodules in areas of low soil moisture (early snowmelt plots), with an increase of mycorrhizae. The prediction for less rhizobial colonization in areas of low soil moisture comes from two mechanisms: 1) phenological mismatches via early snowmelt and 2) mutualism abandonment in dry summer conditions. We predict plots with higher soil moisture (control plots) to have relatively high number of rhizobial nodules because the more standard snowmelt allowed for the plants to not be as water stressed as the early snowmelt plots, lessening the requirement for plants to have as many mycorrhizae in the roots, and more energy to spend on rhizobial nodules. We hypothesize that if conditions are dry enough, legumes will favor hosting mycorrhizae over rhizobia to obtain more water.

Dry Summer Conditions:

If premature snowmelt is an important determinant of mycorrhizae-legume interactions, I predict to observe a greater proportion of mycorrhizal interactions under dry summer conditions than under wet summer conditions. If true, increasing mycorrhizal interactions will cause an increased amount of water and nutrients taken up by the roots, lessening available sugar for the plant to use for rhizobia; lessening the amount of nitrogen available to the legume or in surrounding soil. With reduction of nitrogen and nutrients in nectar, this could further alter the phenology of legumes from pollinators not visiting the legumes as often because nitrogen/nutrient reduction.

Wet Summer Conditions:

If early snowmelt is an important determinant of rhizobia-legume interactions, we expected to observe a greater number of nodules present under short-summer conditions than under long-summer conditions. Wetter summer conditions mean a shorter amount of time for legume mutualistic interactions to be affected by dry conditions. When conditions are such that legumes can allocate more sugar to rhizobial interactions, nitrogen content is increased in the plant and soil. High nitrogen in nectar and pollen leads to an increased visitation and health of pollinators, which is in turn beneficial for the legumes.

III. Methods

To investigate how legume mutualistic interactions change with early snowmelt conditions, we used Dr. Berry Brosi's two-week early snowmelt plots to determine alterations in legumes root-mutualists with reduced available groundwater. These meadow sites around the East River Valley in Gothic, Colorado, vary in elevation; all plots contain a control and a two-week early snowmelt plot. These plots were ideal locations for measuring early snowmelt because of their similar plant community composition and varying elevation. The plots were 10 x 14 meters each, with the manipulated plot covered with shade cloth prior to complete snowmelt, causing the plot to melt out two weeks before the control plot. Everything else within the plots remained the same, only varying in the timing of snowmelt.

Field Sampling Methods:

An iButton temperature gauge was buried the first day of collection 3" below the ground at each site to record temperature every fifteen minutes, until obtained at the end of the summer. Soil moisture was taken at all sites weekly, using a volumetric soil moisture probe with 1.5" probes at eight points around the perimeter of site (four around the corners, four halfway between each corner). This length of probes was chosen because of study species roots only reaching 2 inches in the soil, taking an accurate reading for the moisture the legumes had available for use. Moisture collection points were statistically analyzed to find the overall trend of soil moisture throughout the summer, and comparing the control and early snowmelt plots to see if the available water was significantly different in the plots.

Once legumes began emerging from the soil with leaves large enough to collect (at least 3 leafettes), samples were collected weekly, from three marked locations in each plot, along with a (120 gram) soil sample. To obtain consistent data about mycorrhizal and rhizobial relationships within individual legumes, individuals were identified and marked within three locations in each plot - these legume species are clonal; plants within a one-meter radius of one another come from the same individual. This permitted for the same individuals to be taken week to week, allowing us to detect the change in mycorrhizal and rhizobial interactions over time. Additionally, we recorded the phenological stage of each plant each week to account for phenology between the plots. From the three identified points in each plot, small *Lathyrus* and *Vicia* samples were collected from each site. At

least 3" of root matter was collected and stored in bags labeled with the date, site, plot, and individual number, allowing for identification for analysis. Once roots were collected, holes were refilled to allow further growth of the plants. Once back at the lab, individuals were stored in a refrigerator at 2°C. Testing for nitrogen composition and presence of mutualist rhizobia in the soil will be obtained by analyzing the 120 gram samples collected from around clusters of legume from each plot weekly. Soil samples were stored in the freezer in the lab at 0°C.

Measuring Rhizobia and Mycorrhizae:

Nodule quantification was done in the lab post-collection. For every individual, nodules were marked in a spreadsheet along with information including site, plot, date, and time, to compare trends among sites and plots. In addition to quantifying nodule counts, the Rafferty lab will assess the type of nitrogen legumes are acquiring by using N15 isotopic signatures. Leaves from field collection were removed from the stem and weighed before and after dehydration in ovens set to 60°C, for a minimum of three days. After dehydration, weight was recorded and analyzed by subtracting previous weight of leaves to the dehydrated weight, calculating the amount of water retained in leaves, an indication of soil water availability.

To measure fungal colonization of mycorrhizae, legume roots were washed and snipped into 2 cm sections (lateral roots preferred because of the smaller size and shorter time to become translucent to observe mycorrhizae), placed into labeled test tubes containing 10% potassium hydroxide (KOH). After clearing, roots were transferred into 1% HCl to acidify then 0.01% Trypan Blue for staining. Note: taproots and lateral roots took a much longer than the expected 48-hours to become translucent, causing mycorrhizal interactions to not be accounted for in time for this study.

IV. Results

Soil moisture data gathered throughout the growing season showed the inverted bell shaped curve, as hypothesized, with soil gradually becoming drier throughout sites until the rain began in late July/early August (Figure 1).

Soil moisture in June compared to July was significantly higher (Figures 2 and 3; $p < 2.2e-16$; F-statistic: 219 on 3 and 201 DF) from the snowmelt having completed in June, leaving the ground to dry in July. The difference in soil moisture between the control and manipulated plots in June was also statistically significant (Figure 2; $p < 4.333e-07$; F-statistic: 73.44 on 2 and 11 DF), with the control plot having much higher soil moisture than the manipulated plot.

When investigating nodules on the roots, June versus July showed a significant difference in rhizobia occurrences, and rhizobial findings were occurring at the same time as peaks in the soil moisture (Figures 4 and 5; $p < 0.001388$). Comparing the control and manipulated plots in June also showed a significant difference in rhizobial occurrences, in that there were more rhizobia in the control plots than early snowmelt plots (Figure 4;

$p < 9.213 \times 10^{-5}$). July was not found to have significant differences in rhizobial occurrences (Figure 5; $p < 9.213 \times 10^{-5}$).

V. Discussion

I hypothesized that premature snowmelt is an important determinant of mycorrhizae/rhizobial-legume interactions, with increasingly earlier snowmelt leading to a higher likelihood of phenological mismatch between legumes and mutualists, in turn, resulting in mutualism abandonment in dry summer conditions. From this, I predicted that dry summer conditions would lessen the available sugar for the plant to use for rhizobia, and in turn would allocate more photosynthate for mycorrhizal interactions, lowering the overall nitrogen in the soil from reduced rhizobial interactions.

Our results showed the overall soil moisture for the summer (Figure 1) created an inverted bell shape, with the soil moisture being highest in the beginning of the summer as the snow began to melt, decreasing as conditions became drier, with soil moisture lowering in turn. In the end of July/beginning of August when the rain began, soil moisture rose, but then decreased again from lack of continuing rain.

Soil moisture percentages between the control and manipulated plots was an important check for significance in changes in rhizobial and mycorrhizal interactions. In June, soil moisture between the two plots was statistically different (Figure 2; $p < 4.333 \times 10^{-7}$), showing that inducing two-week early snowmelt dried the soil more quickly than natural snowmelt timing. Global climate change is causing increasingly earlier snowmelt timing, and our results show that this could affect plant and soil community survival and fitness; dry soils affect germination success, plant growth, and survival (Kiers, et. al, 2010; Sachs, et. al, 2006)

Soil becoming drier quicker in the early snowmelt plot than in the control plot allowed for representation of what climate change conditions could have on rhizobial mutualisms, and was shown by analyzing rhizobial interactions in June between the two plots. Results showed that in the control plot (where soil moisture was significantly higher), rhizobial interactions were more likely to occur than in the manipulated plot (with lower soil moisture) (Figure 4, $p < 9.213 \times 10^{-5}$). These results show that if climate change were to continue to increase the date of snowmelt, legumes could begin to lose the rhizobial interactions sooner in the season than would with historical snowmelt conditions. The abandonment of rhizobia from the legume nodules in dry conditions could have severe consequences for the fate of this mutualism (Sachs & Simms 2006).

In July, soil moisture was significantly lower across control and manipulated plots than soil moisture recorded in June (Figures 2 and 3; $p < 2.2 \times 10^{-16}$). This may be because monsoon season was extremely delayed this year compared to past years, allowing all soils to dry to very low soil moisture levels regardless of snowmelt timing. Dry conditions also caused soil moisture between the two plots in July to not show significant difference (Figure 3, $p < 4.333 \times 10^{-7}$). Rhizobial interactions between control and manipulated plots were also not statistically significant (Figure 5; $p < 0.1721$), for there were not many observations of rhizobial interactions in either plot. Observing fewer

rhizobial interactions in times of drought/dry soil conditions demonstrates legumes ability and preference to host and allocate more photosynthate to mycorrhizal interactions than to rhizobial interactions when water is limited.

Soil moisture was shown to be statistically greater in June than in July (Figure 2 and 3; $p < 2.2 \times 10^{-16}$), displaying the drying conditions of the soil. Nodule counts between the two months also displayed rhizobial interactions becoming much less common in July, the driest month of the study (Figure 3 and 4; $p < 0.001388$). The effects of low soil moisture on rhizobial interactions within such a short time span exhibits the ability of legumes to abandon rhizobia in dry conditions.

Soil moisture and rhizobial interactions, shown in Figure 6, also helps to graphically display rhizobial interactions with legumes as soil moisture fluctuates. When soil moisture increases, rhizobial interactions are then more likely to be present on roots. This supports the idea that legumes prefer to allocate photosynthate to rhizobia when water is less limited, and in times of dry conditions, rhizobial interactions are expelled and likely exchanged for mycorrhizal interactions

Short Term and Long Term Effects:

Decreased soil moisture caused by early snowmelt could be causing phenological mismatch among legumes and rhizobia (Figure 4). When rhizobia and legumes become mismatched early in the season, this could affect legume germination success, germination phenology, and plant growth (preliminary results from Andrea Keeler from a common garden study at UC Riverside).

The long-term effect of snow melting out increasingly earlier could lead to mutualism abandonment of rhizobia and legumes. Over evolutionary time, this could cause rhizobia to discard their ability to interact with legumes via natural selection. If the manipulated plot conditions from this study were to become the natural climatic conditions in the future, population sizes of *Lathyrus* and *Vicia* would be severely affected from such reduced rhizobial interactions. Legumes would result in stunted growth from decreased nitrogen content and could recruit fewer pollinators via decreased pollen and nectar nitrogen, both causing a decrease in legume population sizes. This would also affect the survival of other plants surrounding legumes as well, because of the increase in soil nitrogen from rhizobium-associating legumes.

This year, snow melting later in the season than historical snowmelt timing has created a later blooming for legumes *Lathyrus* and *Vicia* than was predicted. In turn, this shifted the root collection timing to one week later than planned because of plants not being large enough to collect from without harming growth of legumes. This resulted in less rhizobia and mycorrhizae information to be collected in the beginning of summer, when soil moisture was highest and rhizobia were likely more present. Not having data from the times of highest soil moisture could have caused inaccurate data about how many rhizobial and mycorrhizal interactions were present in the beginning of the summer.

For this study, mycorrhizae were scheduled to have been processed in time for this paper, but from roots being too large to process in a condensed time span, information about mycorrhizal interactions are not available at this time. When mycorrhizae are processed, more information will be available for analysis about legumes altering mutualistic interactions between rhizobia and mycorrhizae in times of drought, and the number present in various conditions.

VI. Conclusions

The importance of this study brings to light the impact that climate change and premature snowmelt will likely have the mutualistic rhizobial and mycorrhizal relationships hosted by legumes, altering the plants ability to gain nitrogen from rhizobia, in turn which could affect productivity, nectar quality, seed production, and environmental nitrogen inputs. With soil moisture drastically lowering throughout the summer and monsoon rains coming later in the season, rhizobial interactions with legumes were seen to decrease significantly as the season wore on, in turn likely lowering the nitrogen in the soil. Even in control plots, illustrating more historical snow melt conditions, rhizobial interactions decreased throughout the dry summer, but even more significantly in two-week early snowmelt plots.

If global warming continues to accelerate the phenology of legumes and induce droughts, legumes will be more water deprived and the entire ecosystem will suffer from reduced nitrogen. Early snowmelt reducing the effectiveness of these plants and their mutualists, there will not only be an impact on the plants themselves, but interactions with other species, like pollinators, as well as nearby plants that rely on the nitrogen that comes from the rhizobia after legume senescence.

As seen in with the manipulated plots in this study, legumes in areas with lower soil moisture are less likely to host rhizobia which alters the phenology of legumes even further. If climate change and global warming conditions prove to mimic the results of this study, rhizobial and legume interactions could lead to a long-term break-down between mutualists, with legumes completely phasing out of the ability to fix atmospheric nitrogen in legume nodules.

Legumes, the only plants able to interact with rhizobia to fix nitrogen, are essential for ecosystem health. If legumes were to lose this interaction because of climate change forcing rhizobia to phase out from phenological mismatch or mutualism abandonment, there would be great implications for not only legumes fitness, but also for other plant species that benefitted from legume nitrogen-fixing abilities, and pollinators who obtain nitrogen from legumes.

VII. Acknowledgements:

I want to thank my mentor Andrea Keeler for allowing me to be part of her project this summer, as well as the immense support, patience, and assistance in teaching me so much about mutualisms, legumes, and how to conduct field work - this would not have been possible without her! I would also like to thank Dr. Nicole Rafferty for allowing me to assist in her lab based out of the University of California, Riverside, as well as Jimmy Lee for reviewing my paper, the Rocky Mountain Biological Laboratory, for the site, supplies, and funding, as well as my professor, John Gutrich, for pushing me to apply to RMBL. This summer and this project would not have been possible without every one of these people.

VIII. Figures

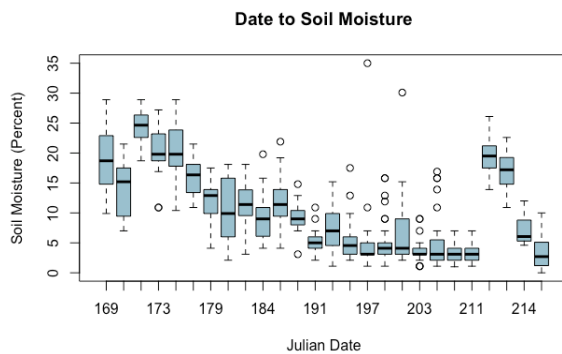


Figure 1: Soil moisture data collected throughout the summer months at all sites. Soil moisture beginning highest in the beginning of the season when snowmelt had just occurred and lowering over the time in late June to July, then raising again when the monsoon season started ($p < 4.333e-07$).

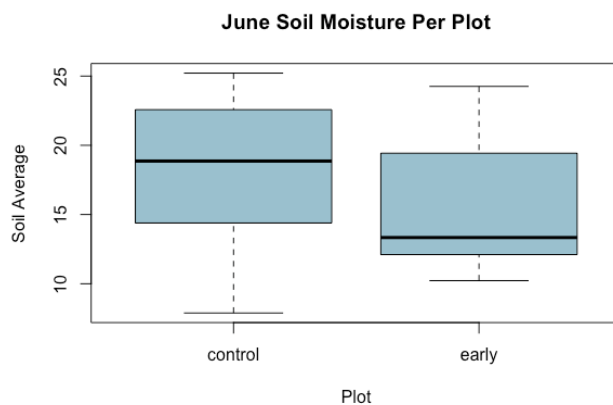


Figure 2: A significant difference ($p < 4.333e-07$) in soil moisture between the control and manipulated plots in the month of June. This illustrates how the shade cloth inducing the

early snowmelt did portray the result of climate change conditions altering the phenology of legumes.

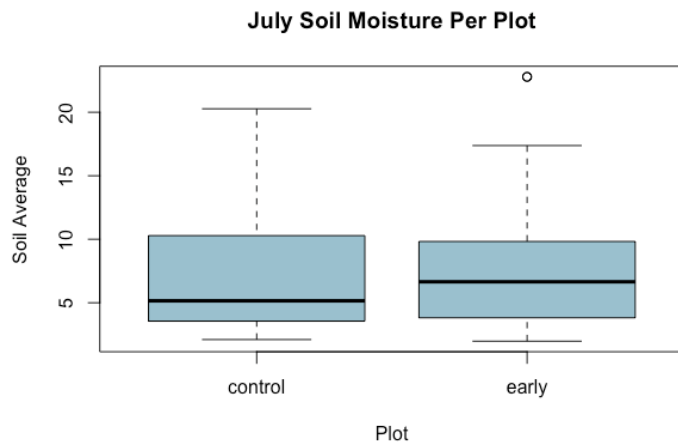


Figure 3: An insignificant difference of soil moisture between the control and manipulated plots in the month of July. This shows how as dry summer conditions continued, the soil all dried to approximately the same soil moisture percentage from the lack of rain leveling the soil moisture average.

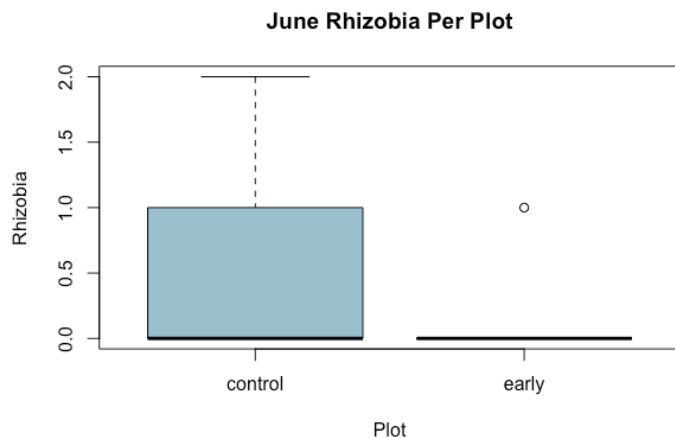


Figure 4: A significant difference ($p < 9.213 \times 10^{-5}$) of rhizobia present between the control and manipulated plots in June. The importance of this is to display how in more moist soil conditions, such as the control plot, legumes are more able to host rhizobial interactions than drier soil conditions.

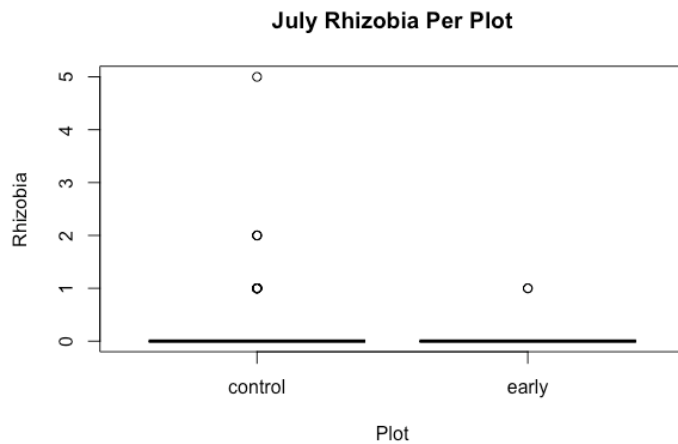


Figure 5: An insignificant difference ($p < 0.1721$) of rhizobia present between control and manipulated plots in July. The importance of this is to display how during dry conditions in July, when the soil moisture in both sites was very low, legumes were not able to host as many rhizobial interactions than drier soils.

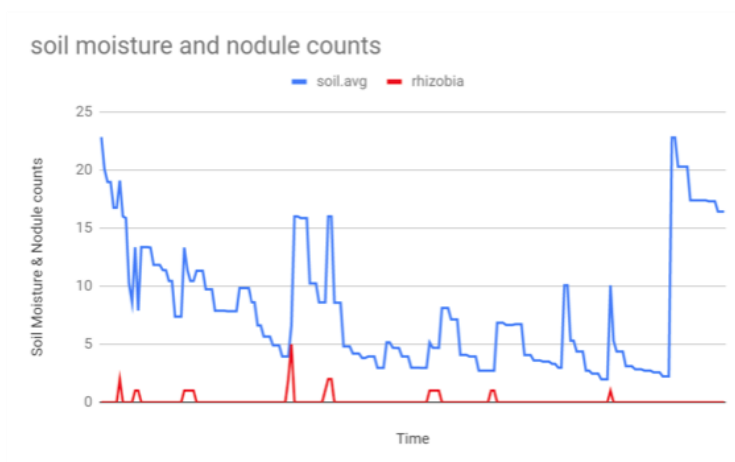


Figure 6: The frequency of rhizobial interactions with the soil moisture percentage. This illustrates how when there is a higher soil moisture, one or more rhizobial interactions becomes more common.

Work Cited:

- Afkhami, M.E., Strauss, S.Y. 2016. Native fungal endophytes suppress an exotic dominant and increase plant diversity over small and large spatial scales. *Ecological Society of America*. 97(5).
- Bascompte, J. García, M.B., Ortega, R., Rezende, E.L., Pinonon, S. 2019. Mutualistic interactions reshuffle the effects of climate change on plants across the tree of life. *Science Advances*. 5(5).
- Denison, R.F., Kiers, T.E. 2011. Life histories of symbiotic rhizobia and mycorrhizal fungi. *Current Biology*. 21(18)
- Douglas, A.E. 2014. Symbiosis as a general principle in eukaryotic evolution. *Cold Springs Harbor Perspectives in Biology*. 6(2)
- Finlay, R. D. 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium, *Journal of Experimental Botany*. 59, 5
- Gezon ZJ, Inouye DW, Irwin RE 2016. Phenological change in a spring ephemeral: implications for pollination and plant fitness. *Global Change Biology*. 22(5)
- Gibson, C.M., Hunter, M.S. 2010. Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. *Ecology Letters*. 13(2)
- Kearns, C.A., Inouye, D.W., Waser N.M. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*. 29(1)
- Toby Kiers, E., Palmer, T. M., Ives, A. R., Bruno, J. F., & Bronstein, J. L. (2010). Mutualisms in a changing world: an evolutionary perspective. *Ecology letters*, 13(12), 1459-1474.
- Lambert, A. M., Miller-Rushing, A. J., Inouye, D. W., 2017. Changes in snowmelt date and summer precipitation affect the flowering phenology of *Erythronium grandiflorum*. *American Journal of Botany*. 97(9).
- Lau, J.A., Lennon, J.T. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. *Proceedings of the National Academy of Sciences of the United States of America*. 109(35)
- Nelson AS, Pratt RT, Pratt JD, Smith RA, Symanski CT, Prenot C, Mooney KA 2019. Progressive sensitivity of trophic levels to warming underlies an elevational gradient in ant-aphid mutualism strength. *Oikos Journal*. 128(4)
- Sachs, J. L., & Simms, E. L. (2006). Pathways to mutualism breakdown. *Trends in ecology & evolution*, 21(10), 585-592.
- Scheublin, TR., Van Der Heijden, MGA. 2006. Arbuscular mycorrhizal fungi colonize non-fixing root nodules of several legume species. *New Phytologist Trust*. 172(4)

- Silmi, M. 2013. First evidence of mutualism between pied fantail (*Rhipidura javanica*) and bornean red muntjac (*Muntiacus muntjak*). *Journal of Indonesian Natural History*. 1(1)
- Traveset, A. 1999. The importance of mutualisms for biodiversity conservation in insular ecosystems. *Revista Chilena de Historia Natural*. 4(72)
- Wadgyman SM, Ogilvie JE, Inouye DW, Weis AE, Anderson JT 2017. Phenological responses to multiple environmental drivers under climate change: insights from a long-term observational study and a manipulative field experiment. *New Phytologist Trust*. 2(218)