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**Post-wildfire plant regeneration in arid ecosystems:  
Overcoming biotic and abiotic soil limitations**



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## Abstract

The threat of fire is a large concern in dryland, alpine shrubland ecosystems in Hawai'i. This unique ecosystem is dominated by māmane (*Sophora chrysophylla*), a dominant dry forest leguminous tree species, which is the exclusive food source for the Palila (*Loxioides bailleui*), an endemic and federally endangered Hawaiian bird. Curiously, in this ecosystem it is common to see many intact māmane seeds on the ground, but very few māmane seedlings. We focused on fertilization and *Rhizobium* inoculum to test potential restoration techniques in burned and unburned areas to increase the success of current habitat management and restoration efforts for Department of Defense (DoD) managed lands. The results suggested burned and unburned were similar in response to treatments. Growth was only moderately affected by fertilization or *Rhizobium* addition, but fertilization inhibited germination. This study pointed to soil properties (e.g., water-holding capacity, pH, soil microbial community composition) being a potentially important and unknown factor that may account for the low germination success, and we suggest further research and management to enhance germination is essential to the recovery of māmane forests.

## Introduction

Wildfire is a large and growing threat to native dryland ecosystems around the world. Increased wildfire severity and frequency is a global problem and Hawai'i is no exception. Wildfire incidence has been documented to increase more than four-fold in Hawai'i in recent decades (Trauernicht et al. 2015). In Hawai'i and the Pacific, forests and shrublands have been transformed into savanna and grasslands due to the invasion of fire-prone exotic grass and shrub species that have brought novel fire regimes to the region (Ellsworth et al. 2014). In addition, the threat of fire is predicted to increase in the future due to climate change leading to increased droughts, which is expected to increase fire occurrence at higher elevations in Hawai'i (Trauernicht 2019). Such large-scale transformation of the landscape destroys vegetation and alters soil properties, leading to reduced habitat quality for many species. The threat of fire is a large concern in dryland, alpine shrubland ecosystems in Hawai'i. This unique ecosystem is dominated by māmane (*Sophora chrysophylla*), a dominant dry forest leguminous tree species. This tree is the exclusive food source for the Palila (*Loxioides bailleui*), an endemic and federally endangered Hawaiian bird with a diet restricted to māmane's seeds, flowers, and caterpillars (Hess et al. 2014). In 2016, the population count was estimated at 1,934 birds, which is a 58% decline since 1998 (Camp et al. 2016). Non-native ungulate grazers have severely degraded and reduced the māmane woodland habitat. The present Palila population is restricted to the subalpine woodland on Mauna Kea on Hawai'i Island, at approximately 5% of its historical distribution (Hess et al. 2014).

An increase in māmane abundance and cover is vital to the recovery of the Palila, and the overall health of the ecosystem. However, māmane growth is slow (Francisco et al. 2015) and

regeneration is limited, especially after fire (Loh et al. 2009). Restoration attempts have focused on outplanting (Hess et al. 2014) and broadcasted seed, but little is known about how to encourage māmane regrowth post-fire. Curiously, in this ecosystem it is common to see many intact māmane seeds on the ground, but very few māmane seedlings (Figure 1). The observation suggests that there is: 1) a missing environmental factor necessary for seedling germination and growth or 2) environmental factors such as predation (Scowcroft and Conrad 1988) or disease may lead to seedling mortality. Because fire can burn off nutrients or alter soil microbial communities, we developed an experimental approach to test if amendments of these resources may benefit māmane germination and growth.

In previous research in the subalpine ecosystem, soil nutrients were found to be altered by fire. While total soil nitrogen (N) increased after fire (Questad et al. 2018), our current research indicates a severe soil phosphorus (P) loss following fire in this system (Cordell et al., unpub data funded by the DoD Strategic Environmental Research and Development Program and the DoD Environmental Security Technology Certification Program). Moreover, there is a strong consensus from studies in tropical, temperate and boreal conditions that long-term forest decline is related to P limitation (Wardle et al. 2004). However, the generality of P limitation in comparison to other factors is poorly understood. Some have suggested that mycorrhizal and nitrogen-fixing bacteria associations may also be highly disrupted in these systems following fire (Busse and DeBano 2005), thus potentially impacting māmane regeneration.

Better methods to enhance natural regeneration of critical habitats and key species are essential to ensure the sustainability of the māmane shrubland habitat. Intrigued by the number of ungerminated seeds in māmane subalpine areas, we conducted an experiment to test potential limitations of māmane seed germination and seedling growth, in a recently burned area in comparison to an unburned control site. We focused on fertilization and *Rhizobium* inoculum to help reveal the constraints for māmane regeneration and test potential restoration techniques that will help overcome these constraints and increase the success of current habitat management and restoration efforts for DoD managed lands. We hypothesized that māmane germination and growth is decreased by burning (H1), is increased by amendments of fertilizer (H2A) and *Rhizobium* N-fixing bacteria (H2B), and that there is a significant synergism between fertilization and *Rhizobium* (H3).

### **Military Mission Benefits**

Approximately 3% of Pōhakuloa Training Area (PTA) is within the Palila critical habitat designation (<https://ecos.fws.gov/ecp/report/table/critical-habitat.html>). Post-fire vegetation management fulfills two important DoD missions: 1) maintaining quality training lands, and 2) environmental stewardship of land resources, particularly in regard to protected species. Developing and testing effective procedures to improve post-fire regeneration of subalpine

forests in Hawai'i will provide critical tools needed to adapt to increasing aridity from climate change and will augment māmane habitat, which is a limiting factor for the endangered Palila bird. In addition, māmane forest cover has a number of benefits for military readiness and operations. First, plant cover stabilizes the soil and reduces dust and erosion; dust is a major management concern at PTA. Second, forest cover helps break the grass-fire cycle. In these arid ecosystems, fires generally lead to loss of tree cover and increase in invasive grass cover, that later fuels additional fires. Māmane is less flammability than grasses, and its shade also helps decrease grass cover.

## **Methods**

### *Study Site*

The research site is located in māmane dry woodland at Pōhakuloa Training Area (PTA) and within the Palila critical habitat designation. The study has a nested design, comparing a burned and unburned area (Figure 2). The burned area, centered at 19.734792 °N, -155.504697 °W, was in a 1,300 ha parcel that experienced a high-intensity anthropogenically-set wildfire in August 2010. The Unburned area was adjacent to the Burned area and has not burned in recent history, although we cannot rule out past burns over the sites history, given that charcoal has been documented at PTA (Kinney et al. 2015). The unburned area is centered at 19.731533° N, -155.507600° W and is 470 m from the burned site. Soils of both areas are Keekee ashy loamy sand, on 0 to 6% slopes (U.S. Department of Agriculture 2016) that appears to be quite hydrophobic.

### *Germination Experiment in the Greenhouse*

Initially, a germination experiment was conducted in the field, but due to heavy seed predation from non-native game birds at the unburned site, we were unable to see the effect of the treatments. In order to exclude the birds from the experiment, we repeated the experiment in a completely enclosed greenhouse at the Institute of Pacific Islands Forestry (IPIF) in Hilo, HI using soil from the 40 research study plots in PTA. Soil from each of the 40 plots filled 10, 4 inch square pots, each pot had 10 seeds total. All 4000 māmane seeds used in the germination experiment were scarified with a nail clipper and soaked in distilled water overnight before planting. Guard N was applied to the soaked seeds at 9.30 grams per 2000 seeds. Nutricote type 180 slow release fertilizer was applied at the sensitive crop rate of 7.5 grams per pot.

The four treatments were applied to the pots in the greenhouse in the same manner and level of replication as they were in the growth experiment in the field. Germination was recorded 2 times per week for a total of 6 weeks, ending after germination had stopped. An additional treatment was implemented in the greenhouse germination experiment using commercially available sterile potting media (that was non-fertilized and non-*Rhizobium* inoculated) and no fertilizer or *Rhizobium* inoculate was added to this treatment of 10 pots. The pH of soil collected

from the treatment sites and of the sterile potting media used in the greenhouse germination experiment was determined with a pH meter using a 1:1 slurry of soil and distilled water (Robertson et al. 1999).

### *Experimental Layout*

At each of the burned and unburned area there are ten 5 x 5 m enclosures built to exclude large ungulates (Figure 3). These 5 x 5 m enclosures were further split in half (plots). The plots were one of four treatments (see Application of treatments section), and there was a total of 5 replicate plots per treatment per site.

Māmane seedlings used in the experimental treatments were started in the greenhouse at IPIF and were brought to PTA to be hardened on site, where they were watered 2-3x/week during hardening and placed under the shade of surrounding vegetation. After hardening on site for five weeks, the plants were planted into 23 cm diameter pots using the soil from the associated plot area at the site. One plant was planted into each pot and 10 pots were placed into each treatment plot area. The pots were buried in the plot treatment area with approximately 2 inches remaining of the pot above the soil surface to mimic a seedling's natural environment in the ground while providing a physical barrier around each plant that would prohibit the movement of added nutrients and inoculum between treatments (see application of treatments below). Roots did not extend beyond the pot for any plant during the duration of the experiment. Leaf litter from the site was used to mulch the surface of the soil in each pot to reduce evaporative water loss in this extremely dry site. Plants that died within the first month following planting were replaced with new potted plants. Most mortality occurred due to drought stress.

### *Application of Treatments*

The experiment utilized 4 treatments: control, fertilization, *Rhizobium* inoculum, and fertilization and *Rhizobium* in combination. Treatments were randomly assigned to plot areas and were initiated on July 14, 2017, one day after planting. The control treatment had no additions. The fertilization treatment was a surface application of Nutricote 13-11-11 Type 180 (slow release over 180 days) applied at a "sensitive crop rate" of 78g/ft<sup>2</sup> or 20g/pot. The *Rhizobium* treatment was applied at the rate of 2.0 x 10<sup>5</sup> g CFU/seedling by mixing 0.2 grams of Guard N with 600mL non-chlorinated water. Each plant in a *Rhizobium* treatment received 3 mL of the mixture once per week for 2 weeks. *Rhizobium* treatments were applied to a small hole made with the end of a Sharpie to the depth of 3 cm and following the application the hole was filled. The Fertilization x *Rhizobium* treatment had both treatments applied. To avoid cross-contamination, several steps were taken. Fertilization was applied to plots that were randomly chosen to receive that treatment over a 1 day period. *Rhizobium* was applied to plots that were randomly selected to receive that treatment on a separate day from the application of the fertilizer treatment to prevent cross contamination of the *Rhizobium* treatment. In addition,

70% isopropyl alcohol was sprayed on hands and tools between visiting *Rhizobium* treatment plants and non-*Rhizobium* treatment plants. If any plants died during the first month after planting, a new seedling was replanted; in these cases, additional fertilizer was not added because it was left in the soil from the first application but *Rhizobium* treatments were repeated.

#### *Plant Maintenance and Data Collection*

Due to the intense drought pressure of this site, plants were watered to saturate the pot 2-3 times per week during the duration of the experiment. Data were collected on survival, basal diameter, plant height, and plant height x width x length (an indicator of plant volume) at four periods during the experiment (8 weeks, 12 weeks, 17 weeks, and 19 weeks) (Figure 4). The experiment was intended to be continued to 24 weeks, but a frost event occurred on November 21, 2017 at week 19 that resulted in mortality of 98% of the plants. At that point, plants were harvested (above- and belowground material) immediately following the frost event and all soil material was carefully removed from the roots (Figure 5). Plant material was dried at 70 °C to constant mass to determine aboveground and belowground dry biomass. Determination of survival and growth was made based on the last measurement at 17 weeks before the frost hit.

#### *Nutrient Analysis of Plant Tissue*

All biomass was collected at the end of the growth experiment. Above and below ground biomass was separated. Belowground biomass was rinsed of soil. All samples were placed in the drying oven at 70 degrees C. All biomass samples of the 10 plants per plot were pooled and two composite samples were collected per plot, one with leaf tissue only, the other with a mix of all tissues from the plant (leaf, stem, and root biomass). All samples were analyzed for carbon ©, N, <sup>15</sup>N, and <sup>15</sup>C. Laboratory analyses were performed by the University of Hawai'i at Hilo Analytical Lab.

#### *Statistical Analysis*

Data were analyzed using R Studio version 1.0.136 (R Core Team 2013). For the field experiment, response variables analyzed were proportion surviving, and relative growth rates of height, basal diameter, and volume. Relative growth rates were calculated as  $\ln(\text{final}/\text{initial}/\text{time})$ . For germination we analyzed the mean number of seeds germinating. For all data, we calculated a mean for each plot and compared the plots ( $n = 5$  per treatment 4 treatments x 2 sites).

We used the nlme package (Pinheiro et al. 2019), and analyzed each response variable as a two-factor nested ANOVA. In the field experiment, the plots that contained the 10 pots with māmane seedlings were nested within an amendment treatment, and those treatments were nested within the large enclosure (either in the Burned or the Unburned area). The plots nested within treatments were considered a random factor, and the ANOVA allowed us to test for the

effect of management type (Burned vs. Unburned), amendment treatment, and their interaction. Tukey's multiple comparisons were used as post-hoc tests.

In the greenhouse experiment we used a one-way ANOVA followed by Tukey's post hoc tests. We compared the nine treatments with one-way ANOVA because we added an additional treatment that was not part of the nested setup in the field. Soil pH in the greenhouse experiment was tested among the Burned soil, Unburned Soil, and sterile potting media using a one-way ANOVA followed by a Tukey's test.

## Results

### *Germination*

The greenhouse germination experiment showed that there was a significant difference among treatments ( $F_{8, 492} = 41.84$ ,  $p < 0.001$ ). The greenhouse treatment in which the māmane seeds were germinated in sterile potting media had by far the greatest number of germinants (Figure 6). Among the pots with soil used from the field site, the highest germination was from the Unburned control, suggesting that something about the soil at the field site may make it less suitable for germination. The pH differed significantly among the soils used for germination ( $F_{2, 11} = 13.95$ ,  $p = 0.002$ ), with the sterile potting media having the lowest pH ( $6.71 \pm 0.01$ ,  $n = 2$ ), followed by the Unburned site ( $7.2 \pm 0.06$ ,  $n = 5$ ) and the Burned site ( $7.6 \pm 0.13$ ,  $n = 5$ ), which were not significantly different than each other.

In the Unburned soil, the control had greater germination than any of the other treatments, and the addition of fertilizer and *Rhizobium* appears to suppress germination. In contrast, for the Burned soils, *Rhizobium* addition increased germination. In both soils, the combination of *Rhizobium* x Fertilizer led to the lowest germination rates. Thus, it appears that the treatments generally inhibit germination, but the effects of that suppression are greater at the Unburned Site.

### *Growth and Survival in the Field*

Survival of plants was not significantly different among sites ( $F_{1, 32} = 3.16$ ,  $p = 0.0847$ ), treatments ( $F_{3, 32} = 1.84$ ,  $p = 0.16$ ) or the interaction between the two ( $F_{3, 32} = 1.82$ ,  $p = 0.1639$ ). The average survival by the end of the experiment (before the frost) was  $54.5 \pm 3.7\%$  standard error (SE) ( $n = 40$  plots) across all treatments and sites.

Although the experiment did not affect survival, it had a number of influences on the different growth parameters. For basal diameter, there were no significant differences among burned vs. unburned areas ( $F_{1, 32} = 1.27$ ,  $p = 0.2688$ ) but there was a significant difference among treatments ( $F_{3, 32} = 4.38$ ,  $p = 0.0108$ ) and a significant interaction ( $F_{3, 32} = 3.28$ ,  $p = 0.0333$ ). Basal diameter growth was highest under the fertilizer treatments at both sites (Figure 7). At the unburned site, Fertilizer x *Rhizobium* also increased basal diameter relative to the control and

at the burned site, led to a small increase over the control (Figure 7). Taken together, it appears that fertilizer and *Rhizobium* were moderately effective at increasing diameter growth. We focus on basal diameter because it was an easy trait to measure and was not usually affected by herbivory.

For the other growth variables, the influence of site and treatment varied, but was generally more influenced by fertilizer than by *Rhizobium*. Trees were significantly taller at the burned site ( $F_{1, 29} = 4.55$ ,  $p = 0.0416$ ) and also after fertilization ( $F_{3, 29} = 5.76$ ,  $p = 0.0032$ ). There was also a significant interaction ( $F_{3, 29} = 3.18$ ,  $p = 0.0387$ ); however, the controls were not significantly different from the treatments at either site (Figure 8). For plant volume, the burned site had a higher volume than the unburned site ( $F_{1, 30} = 4.49$ ,  $p = 0.0424$ ), but treatment ( $F_{3, 30} = 2.57$ ,  $p = 0.0730$ ) and the interaction ( $F_{3, 30} = 2.54$ ,  $p = 0.0753$ ) were not significant (Figure 9). Total tree biomass was not significantly different by site ( $F_{1, 31} = 2.42$ ,  $p = 0.1297$ ), but was influenced by treatment ( $F_{3, 31} = 7.45$ ,  $p = 0.0007$ ). There was also a significant interaction ( $F_{3, 31} = 9.41$ ,  $p = 0.0001$ ), with the treatments with fertilizer having larger plants (Figure 10). The ratio of root to shoot was not significant for site, treatment, or their interaction.

#### *Nutrient Concentrations*

Leaf %N was significantly influenced by treatment ( $F_{3, 31} = 13.73$ ) but was not different among sites ( $F_{1, 31} = 1.81$ ,  $p = 0.189$ ) or the interaction ( $F_{3, 31} = 1.43$ ). The addition of fertilizer led to higher foliar nitrogen concentrations (Figure 11). In addition, the treatment ( $F_{3, 31} = 26.5$ ,  $p < 0.0001$ ) and interaction ( $F_{3, 31} = 4.07$ ,  $p = 0.0151$ ) varied in  $^{15}\text{N}$  values while the sites did not ( $F_{1, 31} = 0.33$ ,  $p = 0.57$ ). The strongest result is that fertilized plants had more negative  $^{15}\text{N}$  values, suggesting that less N in the plant tissue was coming from atmospheric sources (Figure 12). These changes were also reflected in the  $^{13}\text{C}$  values (Figure 13), which is a proxy for integrated water-use efficiency (WUE). In this case, there was greater WUE at burned site ( $F_{1, 31} = 15.2$ ,  $p < 0.0001$ ) and with fertilization ( $F_{3, 31} = 32.4$ ,  $p < 0.0001$ ) and the interaction was significant ( $F_{3, 31} = 8.8$ ,  $p < 0.0001$ ).

#### **Discussion**

Māmane has a hard seed and is extremely abundant on the ground. This is common through māmane's range on the island of Hawai'i. This species is highly desired for restoration as it is critical for the survival of the endangered Palila bird. Natural resource managers have tested numerous protocols and have developed a successful strategy for facilitating the germination and growth of seedlings in the greenhouse. This involves a scarification technique to break dormancy followed by inoculation with *Rhizobium* to promote growth and survival. Of late, managers have also had success using a broadcast seeding approach with scarified seeds. It is important to note that all of the outplanting sites to date are found on younger substrates and have higher values of mean annual precipitation than this site on the PTA installation. Given

that a portion of PTA is considered critical habitat for Palila, we attempted to utilize known methods described above to promote māmane regeneration as the tree is abundant but is comprised of mature trees with little to no seedlings or saplings. In a previous study (Cordell et al., unpub data funded by the DoD Strategic Environmental Research and Development Program and the DoD Environmental Security Technology Certification Program), we tested the expected limiting factors to māmane regeneration which included watering, fencing to reduce impacts of ungulate grazing, and fertilization and seed scarification to promote germination. Much to our surprise we were unable to induce germination across all of our treatments. It was therefore determined that germination was the bottleneck at these sites. In this study, we removed impacts of ungulate grazing as before, but rather than promoting germination of seeds in the seed bank we added scarified seeds to the soil to test for germination and implemented fertilizer and *Rhizobium* treatments to look at survival and growth. In addition, these treatments were conducted on burned and unburned landscapes to address the larger issue of the impacts of increased anthropogenic fire in this region.

We hypothesized that māmane germination and growth is decreased by burning (H1), is increased by amendments of fertilizer (H2A) and *Rhizobium* N-fixing bacteria (H2B), and that there is a significant synergism between fertilization and *Rhizobium* (H3). In general, the measurements relating to growth were not as expected. The Burned site had increases in growth, therefore not supporting H1. In addition, *Rhizobium* addition usually did not enhance growth (H2B not supported), while fertilizer addition did (H2A supported) and in combination a synergistic effect was not noticeable (H3). Germination results were more complicated, but suggest that fertilization and soil properties may have an inhibitory effect, due to the fact that germination was greater in potting soil than at soil from the field site.

### *Germination*

Seed germination began 17 days after sowing in the field. Within 21 days we began to notice seedling predation by introduced game birds. We attempted to scare away the birds but unfortunately lost all germinants to predation. This situation was likely exacerbated because the experiment occurred during fledging season when the caloric need of birds is at its highest. We opted to repeat the experiment in the greenhouse using field collected soils. In addition we used a sterile potting media to determine background rates of germination. Germination rates ranged from 5-80% with strong patterns across the treatments. The most intriguing results were that controls (Burned and Unburned) had higher germination than the treatments and that germination in sterile potting media was the highest (80%). This result implies that something about the biotic or abiotic characteristics of the site or soil negatively impacts māmane regeneration. In reviewing the literature there are many examples of this including seed antagonism by soil microorganisms (Long et al. 2015), hormone exudates from roots and decomposing leaves (Zhao et al. 2011), and soil pH, moisture content, and nutrient availability (Pakeman et al. 2012). Further, the fertilizer and *Rhizobium* treatments had a more negative impact on germination in the Unburned sites relative to the Burned sites indicating that fire

may remove exogenous inhibitors or denature residual chemicals in the soil (Christensen and Muller 1975). These data indicate that older māmane substrates such as those found at PTA have a negative impact on māmane germination thus providing insights as to why regeneration of māmane is so challenging at this site relative to other areas where māmane regeneration can occur once limiting factors are addressed. Zhao et al. (2011) found a similar result across a forest successional gradient in China where young soils were relatively free of the phytohormone abscisic acid (ABA) (a growth inhibitor) whereas older forests showed increases in ABA with impacts on seed germination of certain species. It would be interesting to follow up these experiments with additional treatments to better understand these relationships. Our results suggest that the soil in these field sites may have a microbial community composition that is inhibiting seed germination. We hypothesize that native soils have a significantly lower germination rate than commercial soils due to soil pathogens, or due to water retention differences between commercial and site soils.

### *Growth and Survival*

The overarching effects of our treatments on māmane growth and survival were: 1) there were no treatment effects on survival; 2) basal diameter growth was highest in the fertilizer treatments; 3) fertilizer plus *Rhizobium* also increased basal diameter growth and that these effects were more pronounced in the burned site; and 4) trees were taller at the burned site but there was no impact of the treatments on height. These results surprised us in that natural resource managers have had great success using *Rhizobium* bacteria to enhance the growth and survival of māmane in other areas. The lack of a treatment effect on survival was expected. This species is drought adapted with conservative growth traits geared for survival. The fact that fertilizer had a greater impact on basal diameter than fertilizer plus *Rhizobium* is interesting and one possibility is that the commercial *Rhizobium* inoculum (Guard N) is not compatible with this species. Guard N was used in our experiment as this is the product that the largest commercial grower of māmane in Hawai'i uses for inoculating all of their māmane plants. It is also possible that the combination of fertilizer and *Rhizobium* inoculum may not be effective in water stressed ecosystems due to a lack of the energetics necessary for N-fixation (Mrema et al. 1997). We suggest that this might be the case because the  $^{15}\text{N}$  values indicate that there is little N-fixation going on in these trees. If atmosphere is the source of N, then  $^{15}\text{N}$  values should be around zero; however, there was no change in  $^{15}\text{N}$  values between the control and the *Rhizobium* treatment, indicating that the bacterial addition is not affecting fixation rates. It is also interesting that in general the Burned site had a more positive response to the treatments than the Unburned site indicating that biotic characteristics such as available nutrients and soil water-holding capacity may be enhanced – at least in the short term from disturbance.

### *Applications*

Despite many surprises in the data we feel confident that we have improved our understanding of the limiting factors associated with māmane regeneration in the PTA landscape. We have learned that burning does not seem to negatively affect māmane growth, while nutrient

availability seems to be a limiting factor, but not *Rhizobium*. While we did not study water availability, it is clear that these plants are water stressed and nitrogen fixation rates at this site may be very low. The key to understanding the low amount of māmane regeneration at these sites appears to be related to the soil characteristics and further studies on the water-holding capacity and microbial communities of the soil may provide guidance to better understand if some special soil properties are limiting māmane generation at this site, while if protected from ungulates, it appears to regenerate well across many other sites in the subalpine habitat.

While this study worked on a species endemic to Hawai'i, the approach of testing factors that might limit recruitment is one that is transferable to other military installations. The process of systematically testing potential limiting resources with amendments is one that can be done for any species facing recruitment limitations. Of particular interest to others may be the use of *Rhizobium*, as projects may add the bacteria as a matter of course without testing if they are actually beneficial. Finally, many military installations deal with wildfire, and as our study shows, it is important to consider that fire may not always have negative effects on growth regeneration of dominant species.

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## List of Abbreviations

C: Carbon

DoD: Department of Defense

H: Hypothesis (e.g., H1, H2A, H2B, H3)

HawCC: Hawai'i Community College

IPIF: Institute of Pacific Islands Forestry

N: Nitrogen

P: Phosphorous

PTA: Pōhakuloa Training Area

SE: Standard error

USDA: United States Department of Agriculture

WUE: water-use efficiency



Figure 1. Māmāne seeds litter the ground under a mature māmāne tree at the Unburned site but young māmāne are uncommon.



Figure 2: View of Mauna Loa and exclosures at the Unburned site. Fenced exclosures exclude feral ungulates such as goats and sheep.

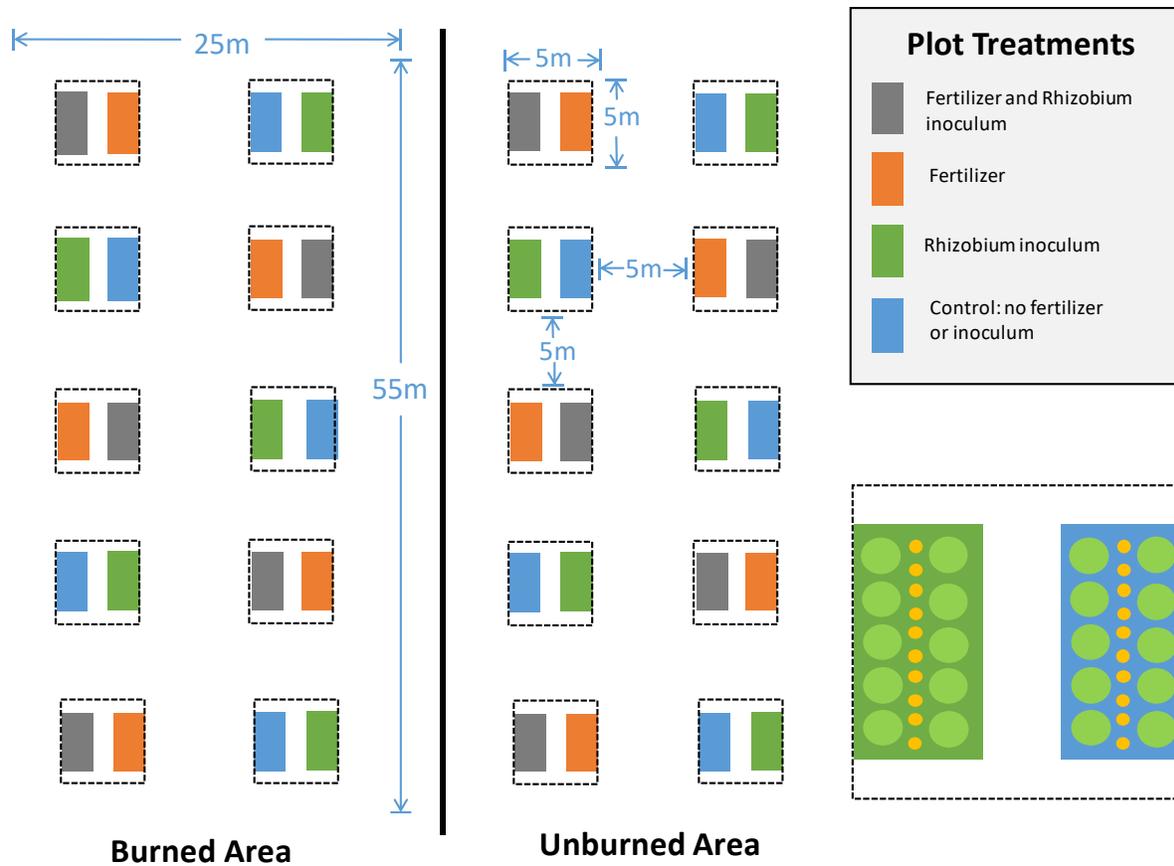


Figure 3. At both the Burned and Unburned area there are ten 5 x 5 m fenced plots (Figure 1). These 5 x 5 m plots were further split in half (plots). The plots were one of four treatments (see Application of treatments section), and there was a total of 5 replicate plots per treatment per site. Treatments were randomly assigned to plots in the experiment.



Figure 4. Basal diameter, height, and volume were all recorded in the field, on living plants one time per month after establishment.



Figure 5. Plot 12 after the frost event. This plot was at the Unburned site and received fertilizer and *Rhizobium* treatments.

## Greenhouse Germination

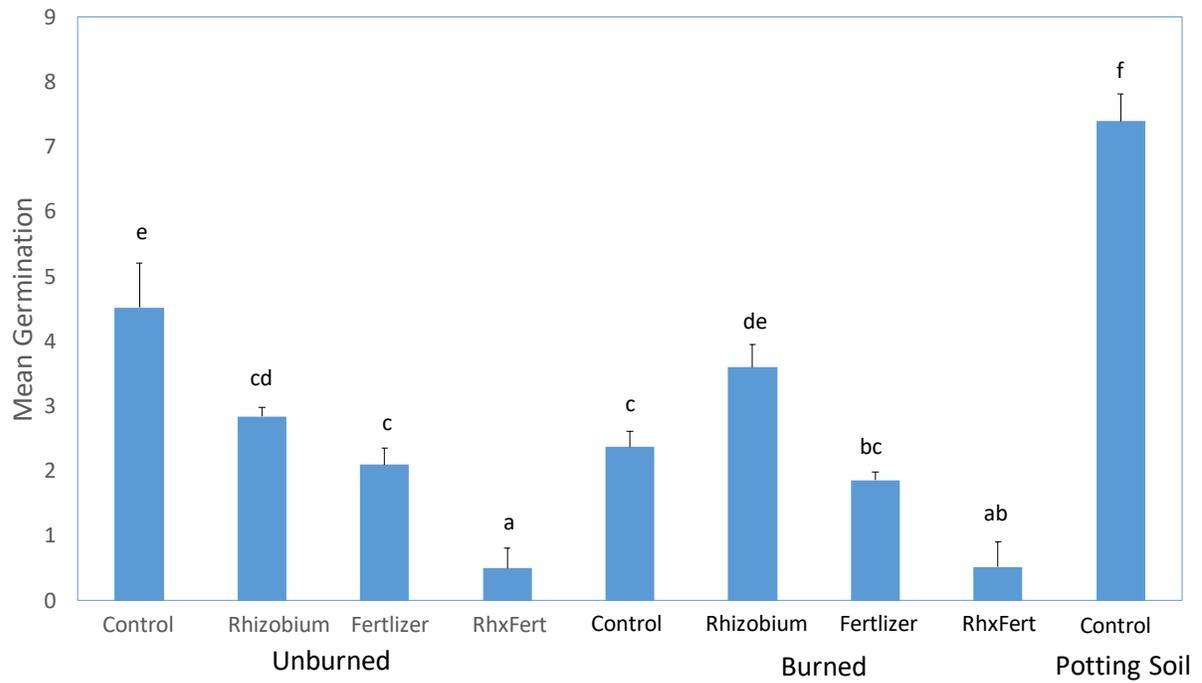


Figure 6. The greenhouse germination experiment in which soil from both sites was used and treatments were applied. Data show mean number and SE of germinants per pot per treatment. Different letters above SE bars indicate statistical significance.

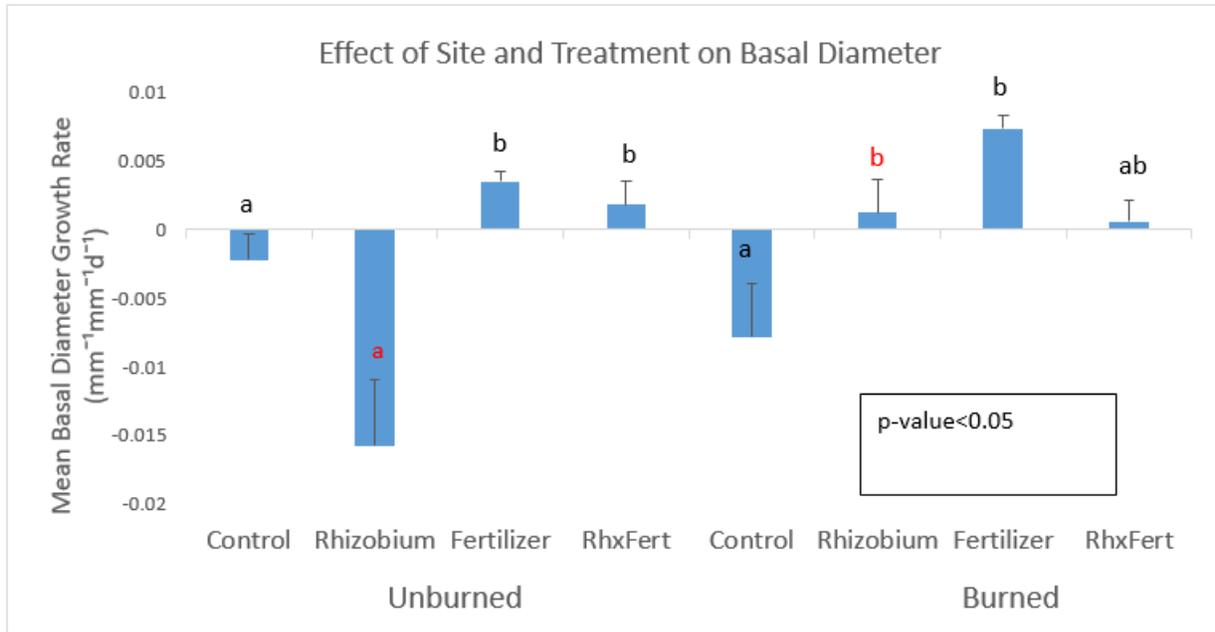


Figure 7. Data show mean basal diameter and SE of plants in the field per pot per treatment at the two different sites. Different letters above SE bars indicate statistical significance.

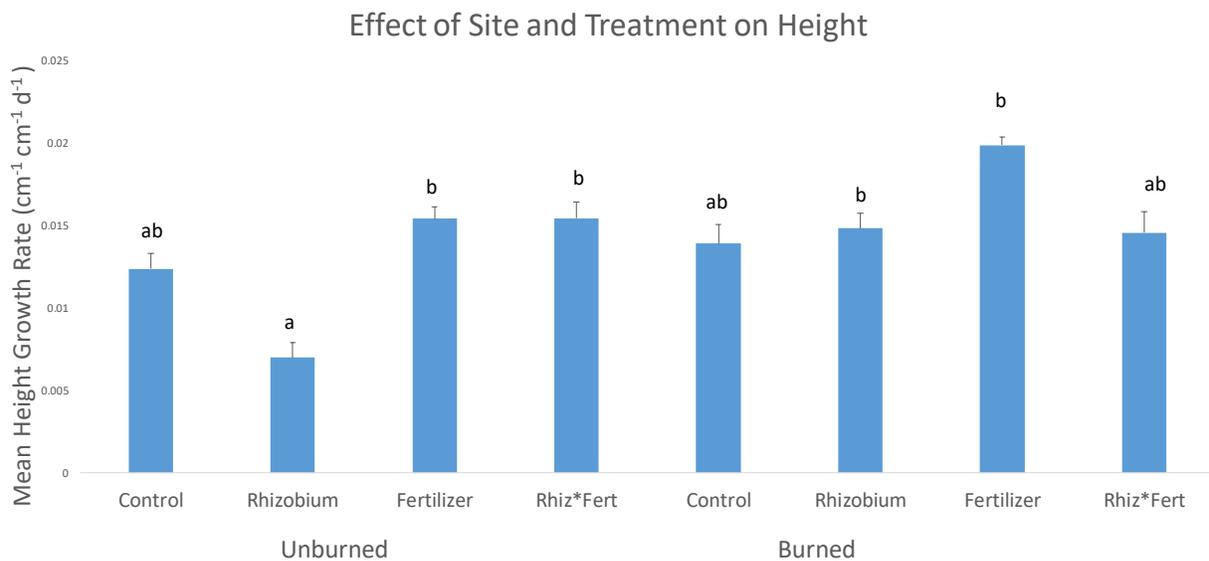


Figure 8. Data show mean height and SE of plants in the field per pot per treatment at the two different sites. Different letters above SE bars indicate statistical significance.

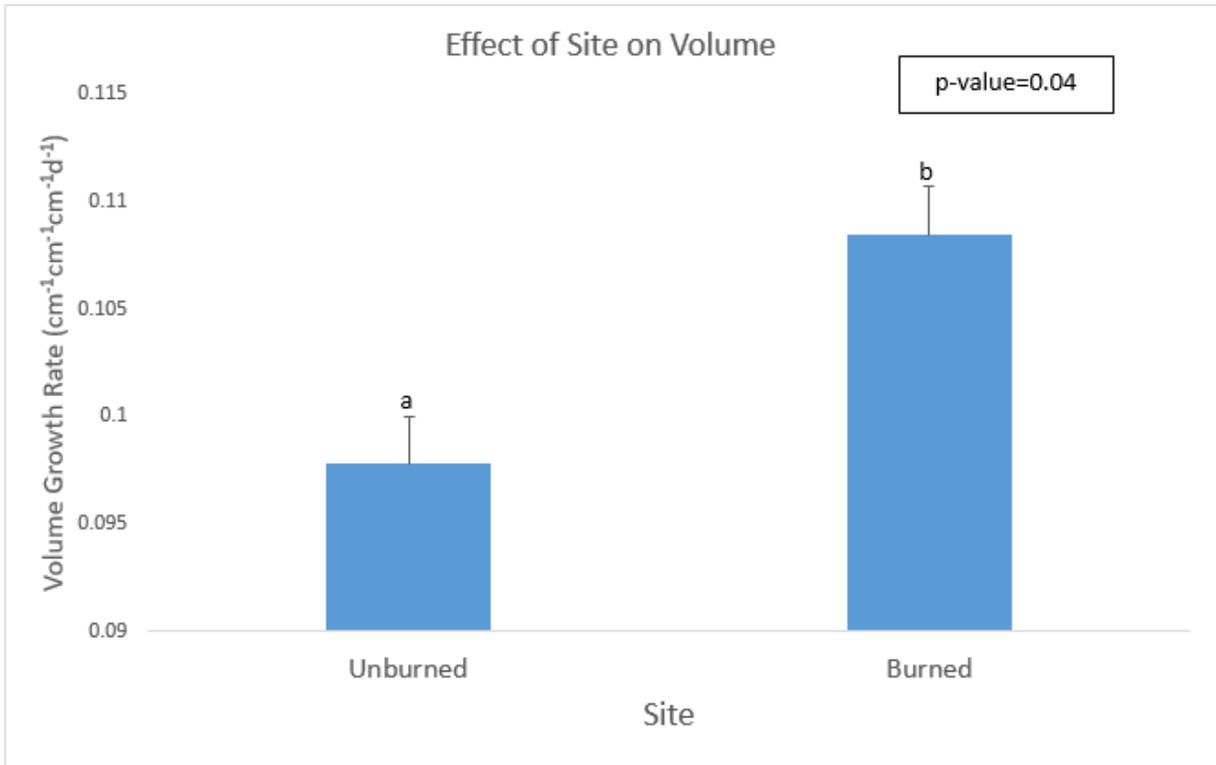


Figure 9. Data show mean plant volume (plant length x width x height) and SE per pot per treatment at the two different sites. Different letters above SE bars indicate statistical significance.

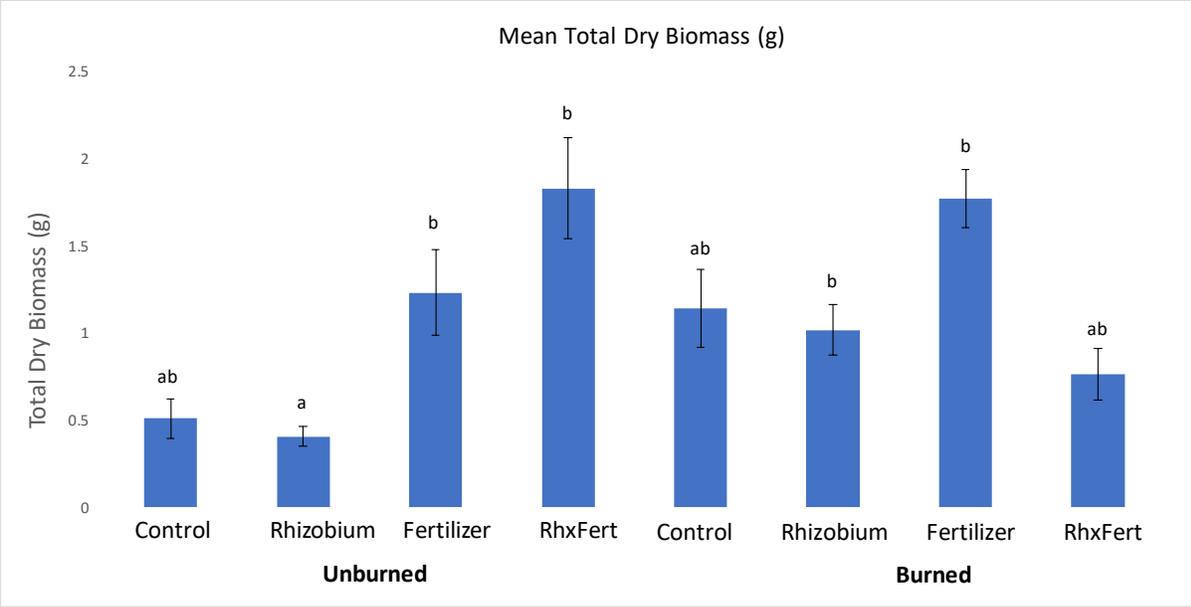


Figure 10. Data show mean total dry biomass and SE of plants in the field per pot per treatment at the two different sites. Different letters above SE bars indicate statistical significance.

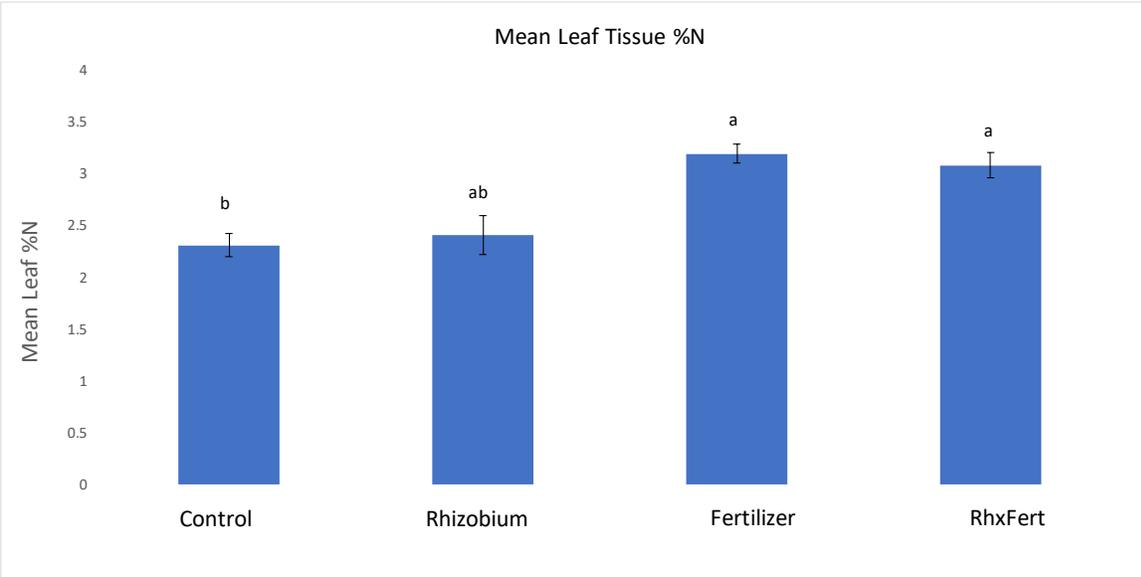


Figure 11. Data show mean leaf tissue percent nitrogen and SE of plants in the field per pot per treatment averaged across the two different sites. Different letters above SE bars indicate statistical significance.

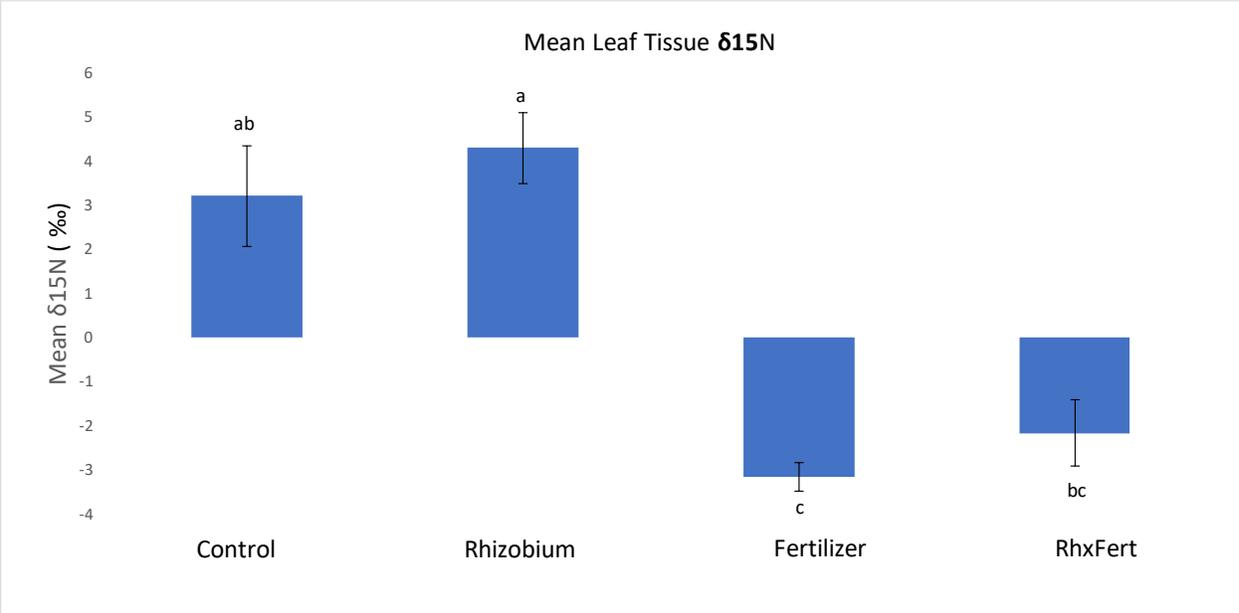


Figure 12. Data show mean leaf tissue  $^{15}\text{N}$  and SE of plants in the field per pot per treatment averaged across the two different sites. Different letters above SE bars indicate statistical significance.

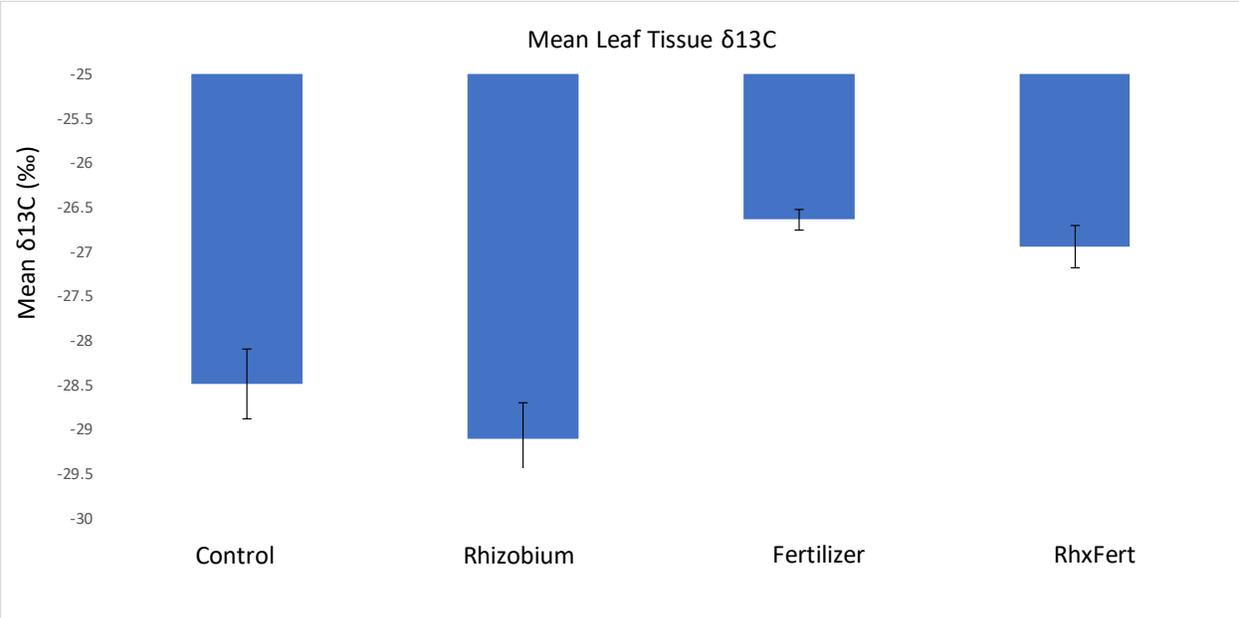


Figure 13. Data show mean leaf tissue  $^{13}\text{C}$  and SE of plants in the field per pot per treatment averaged across the two different sites. Different letters above SE bars indicate statistical significance.