

The Direct and Interactive Effects of Warming and Species Interaction on Plant Functional Traits

Karin Rand

PIs: Aimée Classen and Kenna Rewcastle

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ABSTRACT:

Global climate change is a threat that continues to affect nearly every ecosystem on the planet through warmer temperatures, changes in precipitation patterns, and many other manifestations of a warmer world. Warming directly and indirectly affects plants and ecosystem functioning. In the natural world, both the direct and indirect effects of warming impact plant communities and the ecosystem functions simultaneously. Here, we conducted an experiment to determine how the direct and indirect effects of warming impacts plant functional traits in an alpine ecosystem. We leveraged an existing warming experiment in the Upper Gunnison Valley Basin in Colorado, USA. The ongoing 6-year study elevates plot temperatures using open-top warming chambers and manipulates species composition by removing the dominant plant species at a high and low elevation to evaluate the direct and indirect effects of warming on ecosystem structure and function. We sampled plant species from within the plots to answer a series of interrelated questions focused on how shifts in warming and species interactions alters plant functional traits: (1) How does warming directly alter plant functional traits? (2) How does removing the dominant species alter plant functional traits by shifting species interactions? (3) Will warming and species removal interact synergistically to alter plant traits? We hypothesized that: (1) Warming will shift the individual and community-wide traits to be more conservative by exacerbating water-stress in a moisture-limited environment. (2) Removing the dominant species will shift the plant community to be more acquisitive as the remaining plants are released from competitive pressure. (3) Warming and species removal will have counteractive effects on plant functional traits, possibly neutralizing the simulated effects of climate change on plant communities when both treatments occur simultaneously. Our results confirmed that warming and species removal have opposing effects on community shifts, though not in the direction that was expected. By comparing trait values within a species across the warming and removal treatments, we did not see overall shifts in most traits. The shifts in communities are therefore driven by shifts in the relative abundance of species as climate change selects for plant species that are best adapted to warmer environments in the absence of the dominant species, creating winners and losers in the plant community. From our results, it is evident that direct and indirect effects of climate change counteract each other rather than amplify each other. Functional traits of plants remain an effective lens through which we can observe the pathways through which climate change drives shifts in ecosystem structure and function.

INTRODUCTION

Global climate change is a threat that continues to affect nearly every ecosystem on the planet through warmer temperatures, changes in precipitation patterns, and many other manifestations of a warmer world. As humans continue to add more carbon to the atmosphere through fossil fuel combustion, scientists in recent decades have begun establishing warming experiments in the field to understand both how ecosystems will respond to climate change and if a warmer climate will trigger feedbacks that either release more carbon to the atmosphere through greater decomposition or store more carbon in soils with greater primary productivity. Many of these experiments have found that warming results in both shifts in ecosystem function. Others have also documented, through the use of warming experiments and observational experiments conducted over time as the climate has warmed, that shifts in climate alter the climate and habitat for local species. These local climate shifts alter the communities that can exist in a certain environment by changing the available niche-space. This shift in community composition can scale to affect numerous ecosystem functions. In alpine ecosystems, the effects of warming are exacerbated due to the organisms that are adapted to very unique and narrow ecosystem niches. Although there is evidence of overall shifts in the distributions of individual species with climate change (Pauli, Gottfried, and Grabherr, 1996), there is very little known about what is driving these community level shifts and how these novel communities will affect ecosystem function. The function of individual organisms in the face of warmer growing conditions, especially less mobile plants, may be the key to discerning how communities are both affected by and responding to climate change.

Warming directly affects plants in their natural environment. Direct warming of plants has been done across a variety of ecosystems with temperature manipulations that increase the average temperature by 2-4°C. Ecologists often measure the response of plant functional traits to natural temperature variation (i.e. across elevation gradients) or experimental temperature manipulation to understand how warming impacts plant physiology, resource allocation, and growth strategies (Wright et al. 2004). In tropical environments, a meta-analysis showed there was an average of 12.5% increase in biomass of terrestrial plants in warmed environments (Lin, Xia, & Wan, 2010). And yet the amount of biomass accumulations varied between different functional types of plants. A similar study conducted in a Colorado sub alpine environment saw

both an increase in different species biomass with warming under varying ecosystem moisture as well as enhanced seeding with warming (Hart and Shaw, 1995). In a tundra environment there is evidence of phenological shifts in budding and flowering to earlier dates in warmed environments (Arft et al. 1999). This variation in plant response to warming across different biomes indicates that while responses to warming are clear, the strength and direction of these responses likely vary across biomes and local climatic conditions.

Warming indirectly affects ecosystems as individual plant species respond variably to new climatic conditions, altering overall species composition and species interactions. As alpine environments are warmed, conditions change to be more or less favorable for certain plant species. Although plants have very limited mobility individually, the distribution of a species can shift over time to track their ideal thermal niches across temperature gradients. For example, with warming, species are expected to shift up slope as temperatures at higher elevations become warmer and more habitable to species from down-slope. Evidence of this shift has been recorded in the Swiss alps as the species richness of vascular plants increased on 70% of the study summits since between 1895-1953 and 1992-3 (Pauli, Gottfried, and Grabherr, 1996). This upward shift in species has accelerated in the past 50 years as we see species enrichment from 2007-16 is five times as high as the enrichment rates between 1957-66 (Steinbauer et al. 2018). As species distributions shift with changing climate, novel species interactions will occur as species that are able to remain despite the change in climatic species encounter species that have moved up-slope. In a high alpine environment where conditions are more stressful, positive interactions dominate between species (Callaway et. al., 2002), but a more competitive dynamic may be introduced as high alpine ecosystems warm. Both the occurrence of new species interactions and the shifts in abundances of existing species within a local community will undoubtedly affect community level dynamics and scale to impact the structure and function of ecosystems as a whole.

In the natural world, both the direct and indirect effects of warming impact plant communities and the ecosystem functions that they support simultaneously. As warming is directly affecting individual plants, their response in terms of trait shifts, productivity, and shifts in relative abundance drive indirect impacts of warming on plant species interactions at a

community scale, and in turn, ecosystem function. The function of plant communities can change due to shifts in the relative abundance of individual species with unique traits or due to shifts in trait expression within a species in response to new growing conditions. Therefore, changes in plant functional traits may be one of the many drivers by which climate change alters ecosystem function. In a tropical montane forest, plant functional traits were seen to have a more direct effect on the carbon stock of the ecosystem in comparison to the small indirect effect that the environment had (Bu et al 2019). The indirect effect of the environment on the amount of above ground carbon was mediated through the direct effect of the environment on the plant traits which in turn had a direct effect on the amount of aboveground carbon. These results indicate that shifts in plant functional traits, both at the species and community level, may mediate the impact of warming on ecosystems.

Here, we conducted an experiment to determine how the direct and indirect effects of warming impact plant functional traits in an alpine ecosystem. We leveraged an existing warming experiment—Warming and species Removal in Mountains (WaRM)—that occurs across an elevational gradient in the Upper Gunnison Valley Basin in Colorado, USA (Figure 1). The ongoing 6-year WaRM study elevates plot temperatures using open-top warming chambers and manipulates species composition by removing the dominant plant species at a high and low elevation to evaluate the direct and indirect effects of warming on ecosystem structure and function. We sampled plant species from within the WaRM plots to answer a series of interrelated questions focused on how shifts in warming and species interactions alters plant functional traits: (1) How does warming directly alter plant functional traits? (2) How does removing the dominant species alter plant functional traits by shifting species interactions? (3) Will warming and species removal interact synergistically to alter plant traits? We hypothesized that: (1) Warming will shift the individual and community-wide traits to be more conservative by exacerbating water-stress in a moisture-limited environment. (2) Removing the dominant species will shift the plant community to be more acquisitive as the remaining plants are released from competitive pressure. (3) Warming and species removal will have counteractive effects on plant functional traits, possibly neutralizing the simulated effects of climate change on plant communities when both treatments occur simultaneously. My project will provide some of the first data on how experimental warming and dynamic species interactions interact to influence

plant traits in alpine ecosystems, informing our predictions as to the structure and function of mountain ecosystems with climate change.

METHODS

To explore this series of questions we worked within a single existing site within the WaRM network. This site is located in the Almont Triangle in Almont, Colorado, USA. The site has 32, 2 m x 2 m plots organized across a low sloping north-northwest facing slope. There are eight replicates of four different treatments: ambient-control (AC), warmed-control (WC), ambient-removal (AR), and warming-removal (WR). The AC plot has no external manipulation for temperature or species removal and acts as a control for the experiment. The WC plots are warmed by open top warming chambers which raise the air temperature by $\sim 2^{\circ}\text{C}$. We removed the dominant species, a hybrid of *Wyethia amplexicaulis* and *Wyethia arizonica*, by clipping individuals at the ground level in AR plots. The WR plots received both the warming and *Wyethia* removal treatments. We maintained the removal treatments throughout the growing season. We placed warming chambers on warmed plots soil temperatures rise above freezing in the spring and remove chambers when the surface soils begin freezing again in late September. We collect all data at the peak of the growing season when plant biomass is highest in the plots. We collected data for this study during the summer of 2019, with plant specimens collected during the last week of July at peak biomass. Our site is fenced to prevent cattle from grazing within the plots.

On June 25th, 2019, we conducted plant community surveys by recording the percent cover of each plant species in a 70 cm radius circle in the center of each plot. We used this data to identify nine species that made up over 75% of the plant cover across all plots: *Achillea millefolium* (ACHMIL), *Alopecurus pratensis* (ALOPRA), *Delphinium nuttallianum* (DELNUT), *Festuca thurberi* (FESTHU), *Poa pratensis* (POAPRA), *Potentilla pulcherrima* (POTPUL), *Senecio* sp. (SENE1A), *Taraxacum officinale* (TAROFF), and *Wyethia* sp. (WYESP). For a single plot, we identified the presence or absence of each species. When present, we chose a random individual near the center of the plot and measured plant height in centimeters from the soil surface to the tallest non-reproductive part of that individual. We then harvested that individual making sure to include at least three leaves in the sample. If more plant

material was necessary, we also harvested the closest individual of that species. We then used a caliper to measure and record the thickness of the selected leaf sample in micrometers. We then stored the harvested sample in a sealed plastic bag alongside a paper towel soaked in deionized water which was stored in a cooler during the remainder of the field work. We repeated this in each plot to collect an individual from each of the nine focal species when present.

In the lab we measured three additional functional traits for each harvested individual; stomatal density, leaf dry matter content (LDMC), and specific leaf area (SLA). We processed field samples in the lab within 24 hours of field harvesting. To measure stomatal density, a proxy for water stress experienced by that individual, we first painted clear nail polish on a swatch of the underside of the leaf for species that predominantly present stomates on a single side of the leaf, or in the case of the grasses, on both the top and bottom of the blade. We then peeled this dried print off the leaf with clear tape and secured it to a labeled microscope slide. Nail polish impressions, once mounted on a microscope slide, can be kept indefinitely for measurement. We then counted the stomata filling a graticule within the microscope lens field of view of known area on 40 x magnification using a compound microscope. We counted stomata in 10 different fields of view on each swatch. This was done for all species except ACHMIL and POTPUL due to the small size of the leaflets and the hairiness of the leaves, respectively, causing an inability to create a print of their stomata. We averaged the number of stomata counted across the 10 fields of view and divided this by the 0.0625mm² area of the graticule. We measured LDMC to determine the water capacity of each sample, another trait related to plants' physiological responses to water stress. We first set a leaf with attached petiole in a 2 mL floral tube of deionized water for ~12 hours in a refrigerator, allowing the leaf to absorb water until the water holding capacity of the leaf is reached before removing the petiole and measuring the tissue wet mass. We then dried this sample for at least 48 hours at 60 °C and measured the resulting dry mass. We calculated LDMC by dividing the mass of the water absorbed by the plant (fresh tissue mass – dry tissue mass) by the dry mass of the sample, with units of g H₂O g⁻¹ dry leaf tissue. We also measured SLA as an indication of the plants' allocation of resources to photosynthetic area, with higher SLA indicating a more resource acquisitive growth strategy and lower SLA indicating more resource conservative strategy. We first scanned a sample of leaves at 300 dpi resolution and used ImageJ software to deduce the area of the fresh leaf tissue in each image in

cm². For TAROFF, SENE1A, and WYESP, we were able to use a standard single hole punch to take a subsample of known area from the fresh leaf tissue due to the large leaf size. We then dried these hole-punch leaf subsamples for 48 hours at 60 °C and divided the area by the dry mass to calculate SLA in cm² g⁻¹ dry leaf tissue. Finally, we set aside a sample of dry leaf matter to measure C:N at a later date.

To understand the direct and indirect effects of climate change on plant functional traits at the community level, we calculated community weighted means (CWM) for LDMC, SLA, stomatal density, and leaf thickness. We calculated the CWM by first multiplying the trait value measured for a specific species in each plot by the relative abundance (percent cover) of that species within each plot. The sum of the abundance weighted trait values for each of the nine focal species in a plot was divided by the total percent cover of all nine focal species in that plot. We calculated CWM for each of the above mentioned four functional traits in each plot and then analyzed these data given the plot treatment to investigate community-scale trait differences driven by each of the climate change treatments. Then, to understand the degree to which treatment effects on plant functional traits varied across species, we conducted an ANOVA on a linear mixed model where variation in each plant functional trait (LDMC, SLA, stomatal density, plant height, leaf thickness) was explained by the climate change treatments, plant species, and the interaction between treatment and species. Finally, to investigate how trait plasticity (intraspecific trait variation) and shifts in relative abundance attributed to a single species contributes to overall community functional trait shifts, we conducted another ANOVA where a single species' trait value or relative abundance varied among the four climate change treatments. We conducted all analyses in R Studio (R version 3.4.1), and figures were created in the ggplot2 package.

RESULTS

At the community level, we saw significant changes in SLA and LDMC when considering how community-weighted trait means responded to dominant species removal and warming treatments. There was a significant effect of the climate change treatments on community-weighted SLA ($p = 0.005$, $R^2 = 0.294$) (Figure 2) with a slight decrease in SLA with removal, a significant increase in SLA with warming, and only a small increase in SLA with

removal and warming when compared to the controlled treatment. A similar pattern was seen for the response of community-weighted LDMC to the climate change treatments ($p < 0.001$, $R^2 = 0.475$) (Figure 3). LDMC decreased with removal, increased significantly with warming, and returned to control values with an interaction of treatments. We did not detect a significant difference in stomatal density or leaf thickness across treatments. We excluded plant height from community-weighted mean analyses as it is not a true trait constrained entirely by genetics and is confounded by many other variables.

To determine how the traits of each species responded individually to the climate change treatments, we analyzed the variation in trait values for each species across the climate change treatments separately. We saw a significant effect of the climate change treatments on SLA for the unweighted community (Figure 6), meaning that across all species and without considering the relative abundance of those species, the climate change treatments were correlated with significant variation in SLA ($p < 0.005$). We did not detect a significant effect of the interaction of species identity and climate change treatment, indicating that the treatments affected intraspecific SLA similarly across all species ($p = 0.966$). We found a highly significant effect of species on SLA, demonstrating that species occupy distinct niches according to resource allocation on the conservative to acquisitive spectrum. Overall, our model that correlates SLA with climate change treatment and species identity explained 49.9% of the variation in SLA. Although there was a significant difference between species for all traits, there was no significant difference for any trait across treatment for plant height, leaf thickness, LDMC, or stomatal density. To determine if there was a specific species driving the community shifts, we ran a series of ANOVAs to analyze the relationship between each separate species and the climate change treatments for all five leaf functional traits. The SLA of ACHMIL was the only significant result that was produced ($p = 0.006$, $R^2 = 0.322$) (Table 1). This comparison showed that there was a significant increase in SLA with warming, a pattern that closely matches the relationship between the climate change treatments and community-weighted SLA (Figure 11). However, because ACHMIL makes up a very small portion of the plant community, we suspect that the intraspecific variation in ACHMIL alone is insufficient to explain the driver of shifts in community-weighted SLA with warming and species removal.

Due to lack of significant intraspecific changes in traits in response to the climate change treatments, we looked at shifts in relative abundance of each individual species as this can also drive shifts in community-weighted trait changes. To further decipher the shift in traits at the community scale, we conducted an ANOVA to analyze changes in the relative abundance (percent cover) of each species across the warming and removal treatments. There were significant shifts in percent cover of ACHMIL, ALOPRA, SENE1A, and WYESP (see Table 1 for summary statistics). Each species that showed a significant shift in percent cover responded differently to the climate change treatments as some increased with warming while some decreased (Figure 12).

DISCUSSION

Our results confirmed that warming and species removal have opposing effects on community shifts, though not in the direction that was expected. Almont Triangle, in an average year, is typically a water-limited environment, characterized by a dry-steppe plant community. We therefore hypothesized that warming would increase plant stress in this already dry environment and result in physiological shifts across most species so that individuals in warmed plots exhibit more conservative traits. At lower elevations, plant communities have more available resources and therefore the dominant species interaction that shapes this community is competition (Callaway et al, 2002). This resulted in the hypothesis that with the removal of the dominant species, and therefore the removal of dominant competitor, the remaining species would express more acquisitive traits to take advantage of decreased competition for resources. Our results showed that with warming there was an increase in SLA within a community. An increase in SLA indicates that plants are allocating more resources to increase the surface area of their leaves and maximize photosynthetic capacity, leading to greater productivity. This shows that plants are not being more conservative as expected, but are rather shifting to more acquisitive growth strategies. This trend may be due to the extremely wet year. When water no longer limits a typically dry environment, all ecological processes, both above and belowground, are maximized to make the most of the conditions and to increase productivity. Removal of the dominant species resulted in a much more stressful environment than expected and therefore caused the remaining plants to express more conservative traits. The removal also counteracted the effects of the warming treatment. This shows that the typically water limited environment

may result in a facilitative community, in contrast to the theories that predict a competitive environment at low elevations, such that the loss of one species can limit the fitness of the rest of the community. The water limited ecosystems may also be more represented in certain traits such as LDMC. We saw a significant increase in LDMC with warming showing that communities are trying to maximize their ability to hold water. This shows that LDMC may be a less plastic trait and despite the wet year, exhibits a response to the legacy of a typically dry ecosystem. The opposing responses of these traits inspire further questions about what is driving the community shifts and how the direct and indirect impacts of climate change will affect plant functional traits in an average, dry year.

The shifting of an entire community can be driven by a disproportionately large shift in the functional traits of a particularly abundant species or by seemingly small shifts in the traits of all species in the community. We hypothesized that within a species there is enough intraspecific variation or trait plasticity so that individuals equipped with a favorable trait given environmental conditions or with the flexibility to shift traits in response to changing conditions will prosper in a given season. By comparing trait values within a species across the warming and removal treatments, we did not see overall shifts in most traits. Although there was variation in trait breadth, there was no indication that our climate change treatments affected trait breadth at all. This leads to the conclusion that these traits are less plastic than we believed, at least in the six years of climate change treatments in this experiment, and that individual plants are physiologically locked into a specific growth strategy. The shifts in communities are therefore driven by shifts in the relative abundance of species as climate change selects for plant species that are best adapted to warmer environments in the absence of the dominant species, creating winners and losers in the plant community. The results of a meta-analysis of warming experiments in tundra ecosystems showed that the percent cover of certain plant types such as shrubs do very well with warming while others such as lichens are in decline along with the species richness of the entire ecosystems (Walker et al. 2006). To support this idea, we saw a significant shift in the percent cover between climate change treatments for four of the plant species measured. In a study in a tundra ecosystem over the course of 30 years, there was insignificant intraspecific changes in plant functional traits and yet community-weighted means showed significant shifts with rising temperatures (Bjorkman et al. 2018). This shows that shifts

in the relative abundance of individual species likely drives overall changes in the function of the plant community as a whole.

From our results, it is evident that direct and indirect effects of climate change counteract each other rather than amplify each other. Each effect of climate change, both direct abiotic change and shifts in function driven indirectly through plant community response to climate change, is similar in magnitude and yet opposite in the direction of the effect, and not in the ways we predicted. Additionally, species-specific traits not only varied in the direction of their response to our climate change treatments but also the breadth of the trait, i.e. intraspecific variation. The highly variable response of plant functional traits to the climate change treatments was the most prevalent trend in the data across all species and traits. This variation may also stem from the incredibly wet year, highlighting the differences in the ability of individual species to capitalize on favorable conditions in a single growing season. It is likely that in a dry year the results would have been much different because the abnormal influx of moisture during this growing season likely confounded variation driven by treatment effects because the wet year alone drove additional variability in leaf traits. Although species are uniquely impacted by climate change and there is great variability in the response of plant functional traits to climate change, these changes can scale up quickly across communities to result in larger shifts in ecosystem function. As climate change selects for winner and losers within a community, the remaining winners will define the plant community structure and function, resulting in functional shifts at the ecosystem level. With plants being the foundation of many ecosystems, physiological traits specific to a species are what determine how that species will be affected by climate change and therefore mediate how the effects of climate change scale from species to ecosystems. Functional trait of plants remains an effective lens through which we can observe the pathways through which climate change drives shifts in ecosystem structure and function.

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CITATIONS:

- Arft, A. M., Walker, M. D., Gurevitch, J., Alatalo, J. M., Bret-Harte, M. S., Dale, M., . . . Wookey, P. A. (1999). RESPONSES OF TUNDRA PLANTS TO EXPERIMENTAL WARMING: META-ANALYSIS OF THE INTERNATIONAL TUNDRA EXPERIMENT. *Ecological Monographs*, 69(4), 491-511. doi:10.1890/0012-9615(1999)069[0491:rotpte]2.0.co;2
- Bu, W., Huang, J., Xu, H., Zang, R., Ding, Y., Li, Y., . . . Zhang, C. (2019). Plant Functional Traits Are the Mediators in Regulating Effects of Abiotic Site Conditions on Aboveground Carbon Stock-Evidence From a 30 ha Tropical Forest Plot. *Frontiers in Plant Science*, 9(1958). doi:10.3389/fpls.2018.01958
- Bjorkman, A. D., Myers-Smith, I. H., Elmendorf, S. C., Normand, S., Rüger, N., Beck, P. S. A., . . . Weiher, E. (2018). Plant functional trait change across a warming tundra biome. *Nature*, 562(7725), 57-62. doi:10.1038/s41586-018-0563-7
- Callaway, R. M., Brooker, R. W., Choler, P., Kikvidze, Z., Lortie, C. J., Michalet, R., . . . Cook, B. J. (2002). Positive interactions among alpine plants increase with stress. *Nature*, 417(6891), 844-848. doi:10.1038/nature00812
- Harte, J., & Shaw, R. (1995). Shifting Dominance Within a Montane Vegetation Community: Results of a Climate-Warming Experiment. *Science*, 267, 876-880. doi:10.1126/science.267.5199.876
- Lin, D., Xia, J., & Wan, S. (2010). Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. *New Phytologist*, 188(1), 187-198. doi:10.1111/j.1469-8137.2010.03347.x
- Pauli, H., Gottfried, M., & Grabherr, G. (1996). Effects of climate change on mountain ecosystems -- Upward shifting of alpine plants. *World Resource Review*, Medium: X; Size: pp. 382-390.
- Steinbauer, M. J., Grytnes, J.-A., Jurasinski, G., Kulonen, A., Lenoir, J., Pauli, H., . . . Wipf, S. (2018). Accelerated increase in plant species richness on mountain summits is linked to warming. *Nature*, 556(7700), 231-234. doi:10.1038/s41586-018-0005-6
- Walker, M. D., Wahren, C. H., Hollister, R. D., Henry, G. H. R., Ahlquist, L. E., Alatalo, J. M., . . . Wookey, P. A. (2006). Plant community responses to experimental warming across the tundra biome. *Proceedings of the National Academy of Sciences*, 103(5), 1342-1346. doi:10.1073/pnas.0503198103
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., . . . Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821-827. doi:10.1038/nature02403

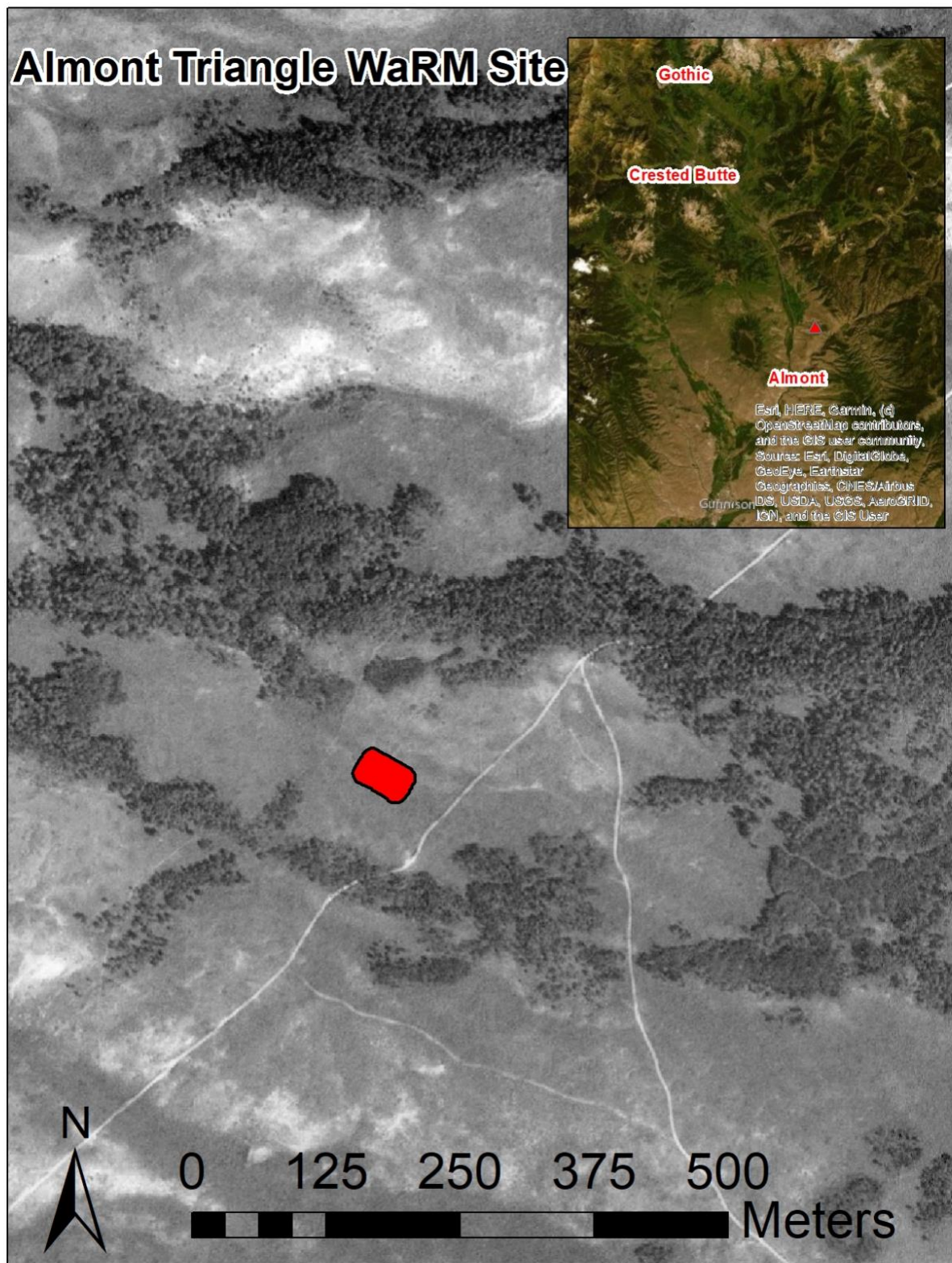


Figure 1: Site map of the Almont Triangle WaRM site in the East River Valley.

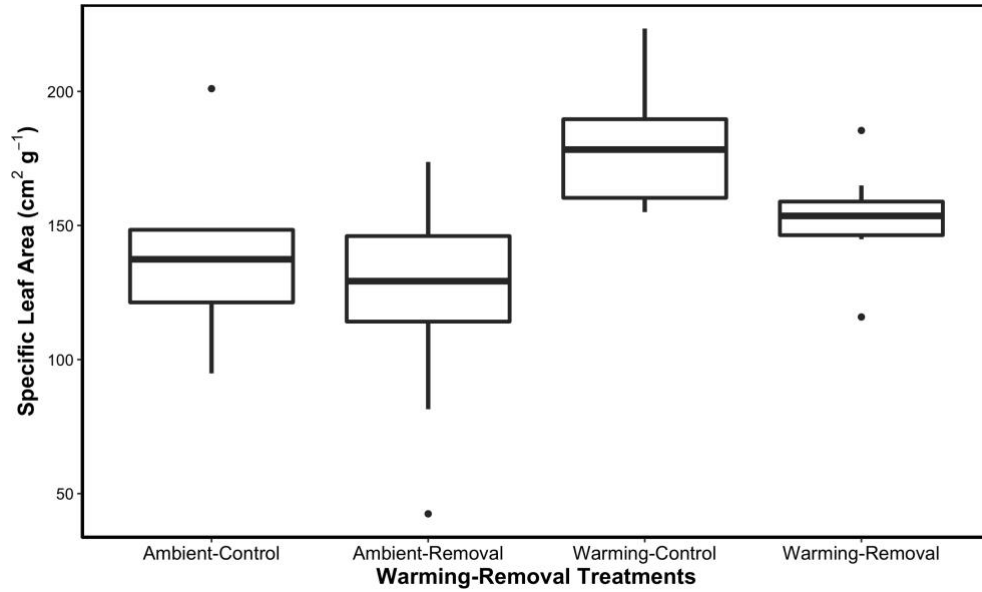


Figure 2: Plot showing the distribution of community weighted mean values for Specific Leaf Area (cm²/g) by treatment. There is a statistically significant difference in the mean community SLA between treatments ($p=0.005$). There is a significant difference in SLA between the Ambient-Control and Warming-Control treatment ($p=0.049$) as well as between the Warming-Control and Ambient-Removal treatments ($p=0.003$). The model explains 29.4% of the variation in the data ($R^2=0.294$).

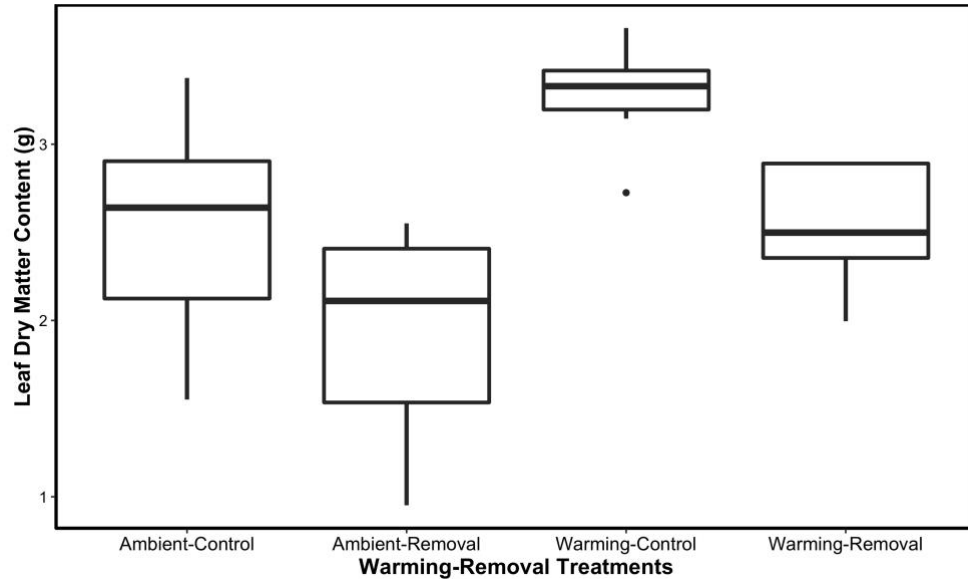


Figure 3: Plot showing the distribution of community weighted mean values for Leaf Dry Matter Content by treatment. There is a statistically significant difference in the mean community LDMC between treatments ($p=9.385e-05$). There is a significant difference in LDMC between the Warming-Control and Ambient-Control treatments ($p=0.024$), between the Warming-Control and Ambient-Removal treatments ($p<0.001$), and between the Warming-Control and Warming-Removal treatments (0.026). The model explains 47.51% of the variation in the data ($R^2=0.4751$).

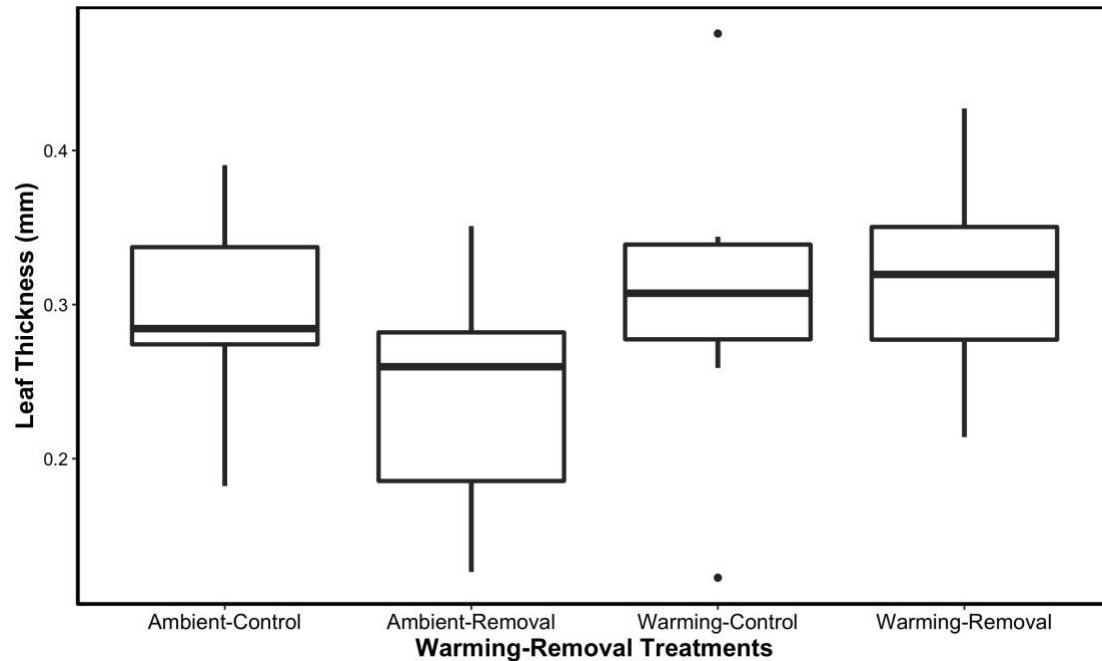


Figure 4: Plot showing the distribution of community weighted mean values for Leaf Thickness (mm) by treatment. There is no statistically significant difference in the mean community thickness between treatments ($p = 0.245$). The model explains 4.3% of the variation in the data ($R^2 = 0.043$).

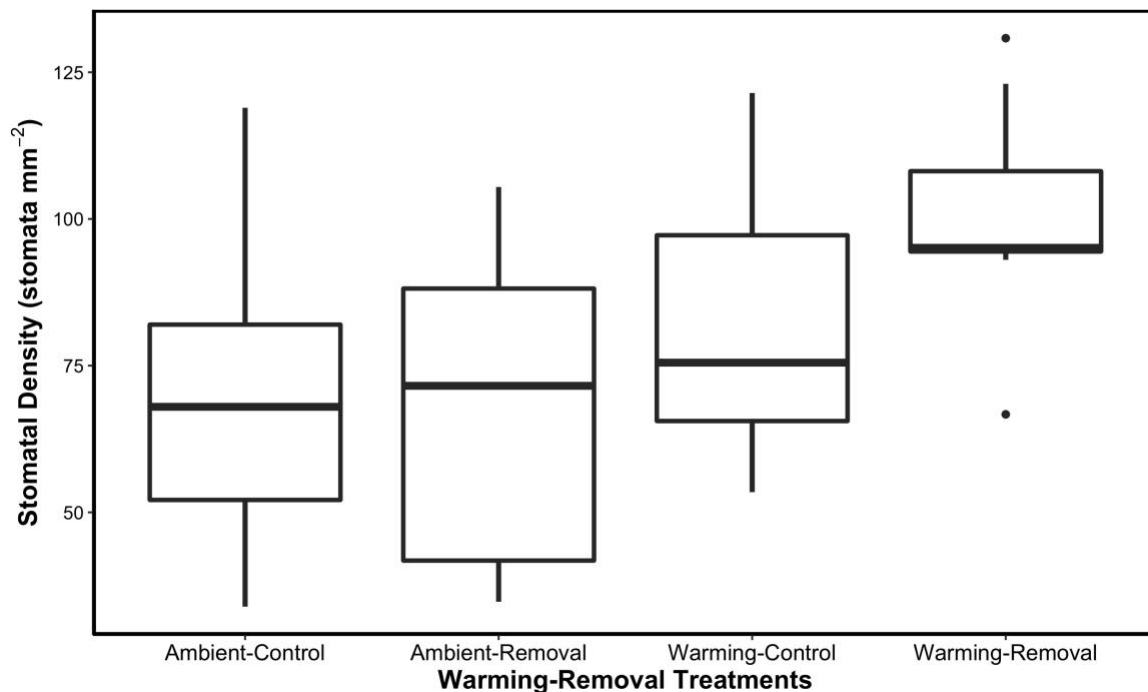


Figure 5: Plot showing the distribution of community weighted mean values for Stomatal Density (stomata/mm²) by treatment. There is no statistically significant difference in the mean community SLA between treatments ($p = 0.056$). The model explains 15.1% of the variation in the data ($R^2 = 0.151$).

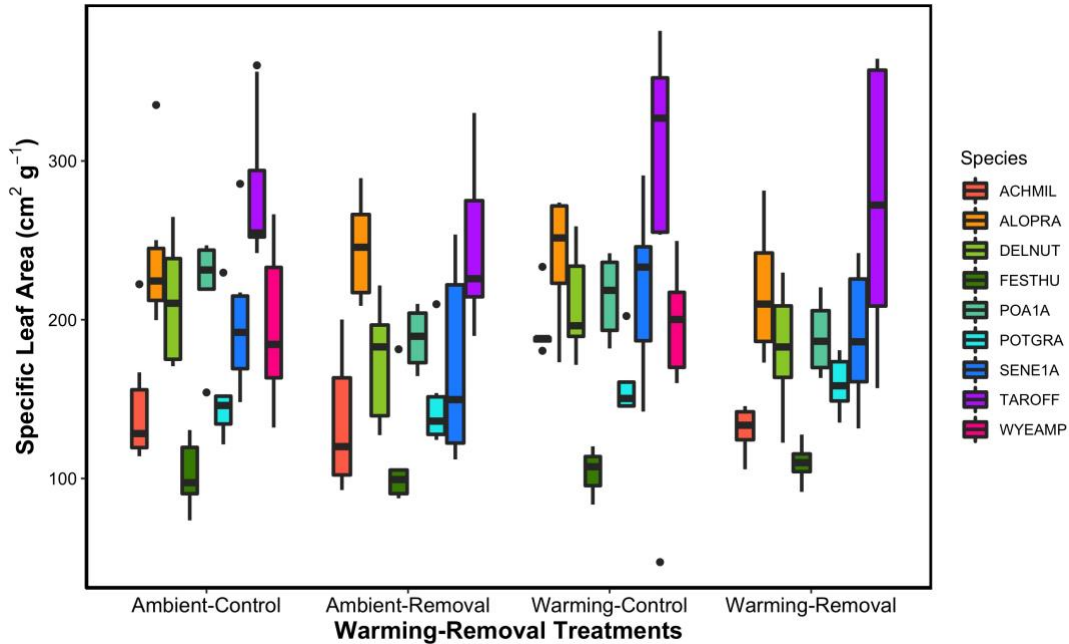


Figure 6: Plot showing the distribution of Specific Leaf Area (cm^2/g) for each species grouped by treatment. SLA varies significantly between different species ($p = <0.001$) and between treatments ($p = 0.005$). There is no significant variation in species specific SLA across treatments ($p = 0.966$). The model explains 49.9% of the variability in the data ($R^2 = 0.499$).

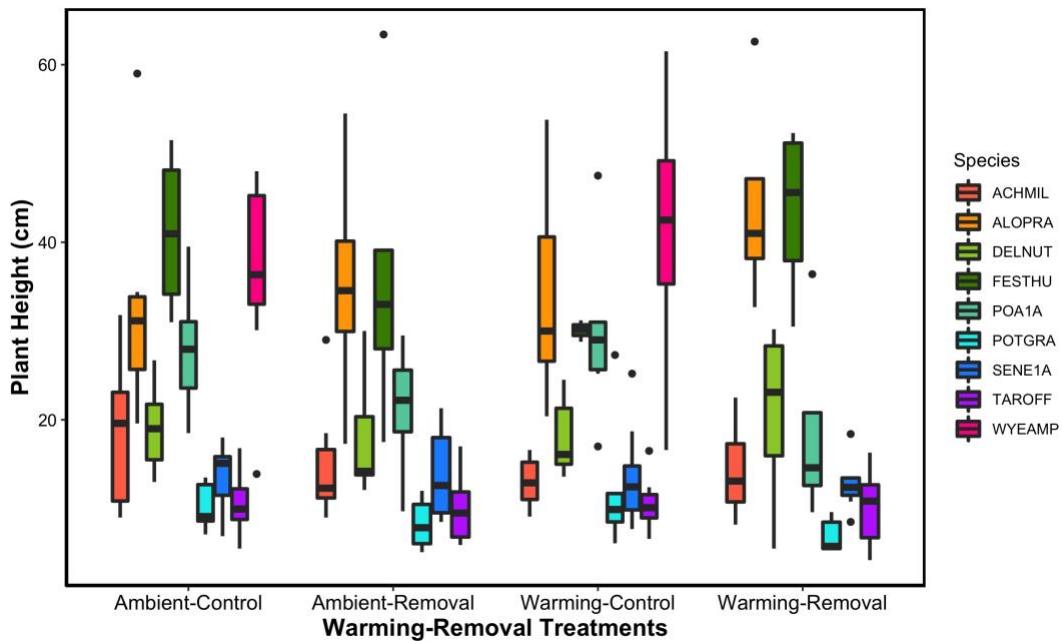


Figure 7: Plot showing the distribution of plant height (cm) for each species grouped by treatment. Plant height varies significantly between different species ($p = <0.001$). There is no significant variation of plant height with treatment ($p = 0.773$) or significant variation of species specific height across treatments ($p = 0.413$). The model explains 63.7% of the variability in the data ($R^2 = 0.637$).

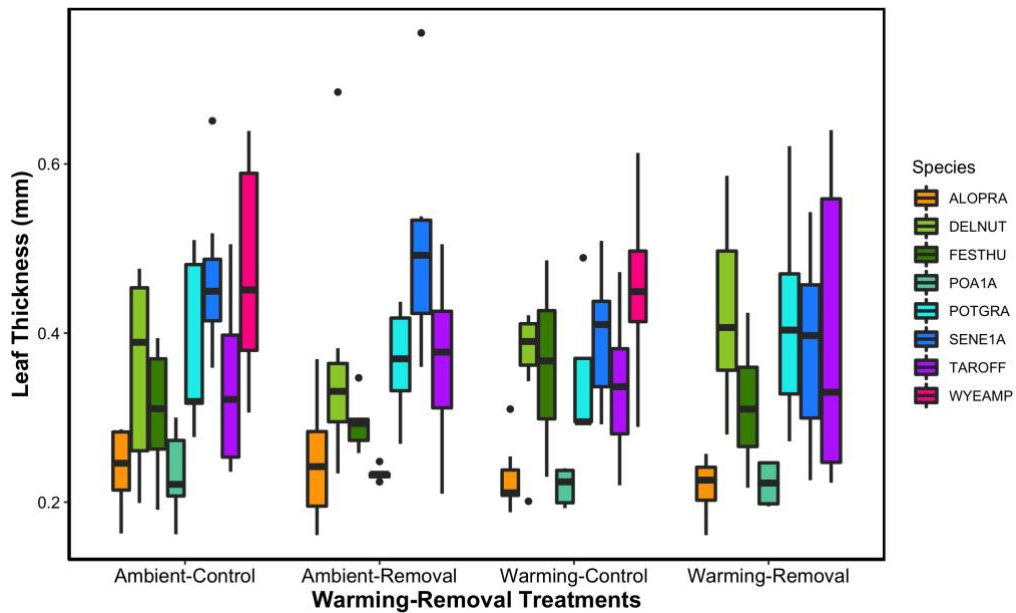


Figure 8: Plot showing the distribution of Leaf Thickness (mm) for each species grouped by treatment. Thickness varies significantly between different species ($p = <0.001$). There is no significant variation of thickness with treatment ($p = 0.472$) or significant variation of species specific thickness across treatments ($p = 0.855$). The model explains 36.66% of the variability in the data ($R^2 = 0.367$).

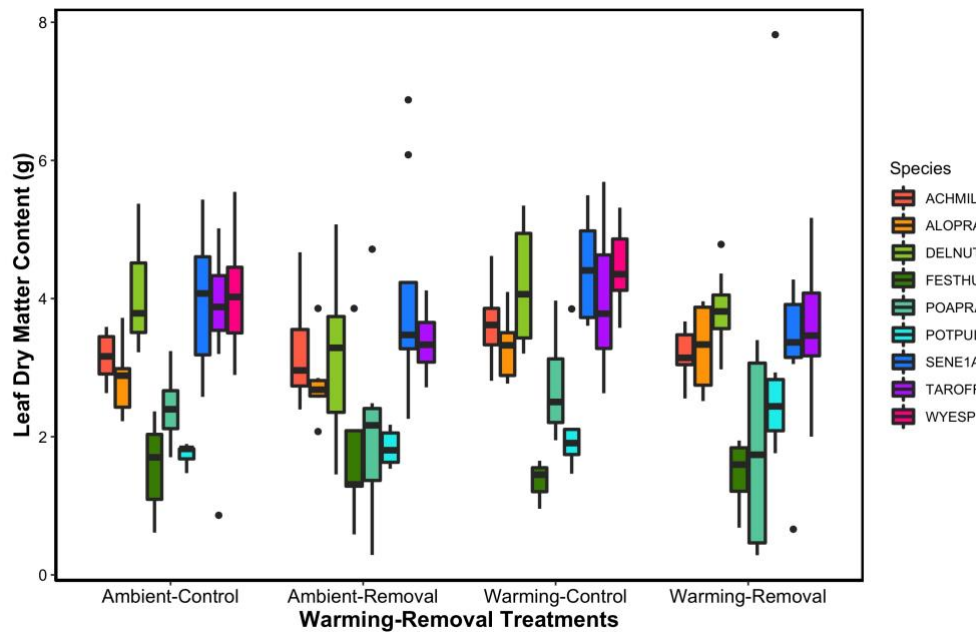


Figure 9: Plot showing the distribution of Leaf Dry Matter Content (g) for each species grouped by treatment. LDMC varies significantly between different species ($p = <0.001$). There is no significant variation of LDMC with treatment ($p = 0.0185$) or significant variation of species specific LDMC across treatments ($p = 0.426$). The model explains 37.8% of the variability in the data ($R^2 = 0.378$).

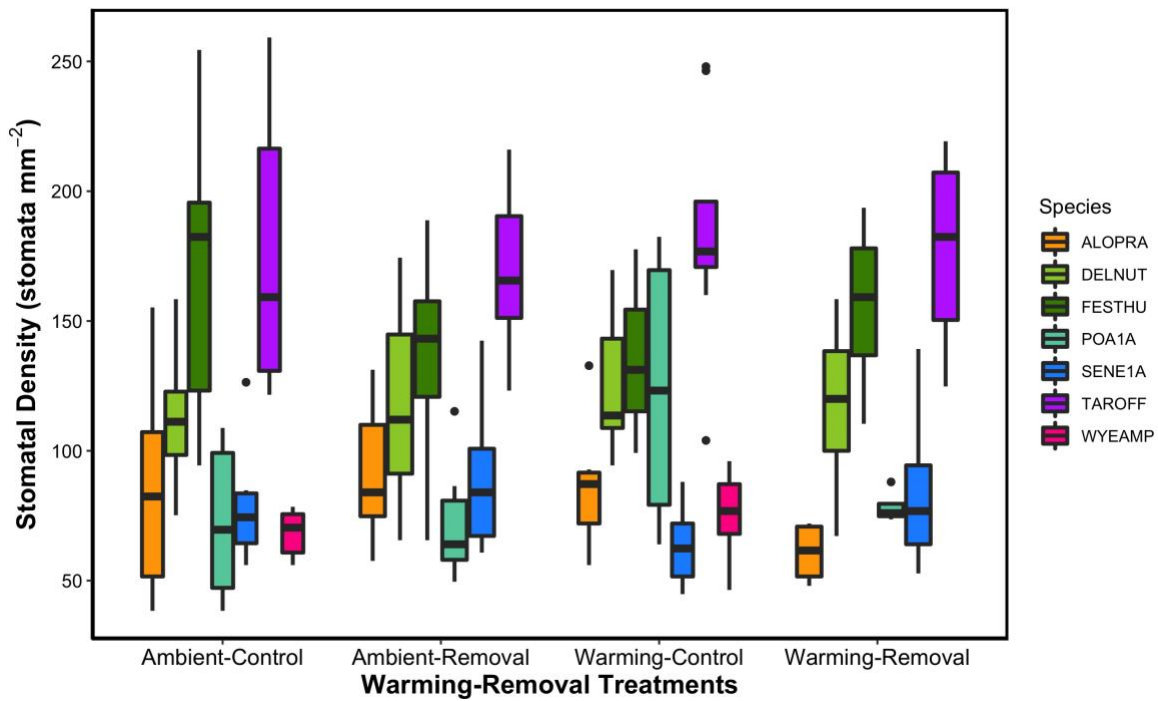


Figure 10: Plot showing the distribution of Stomatal Density (stomata/mm²) for each species grouped by treatment. SD varies significantly between different species ($p < 0.001$). There is no significant variation of SD with treatment ($p = 0.838$) or significant variation of species specific SD across treatments ($p = 0.422$). The model explains 54.2% of the variability in the data ($R^2 = 0.542$).

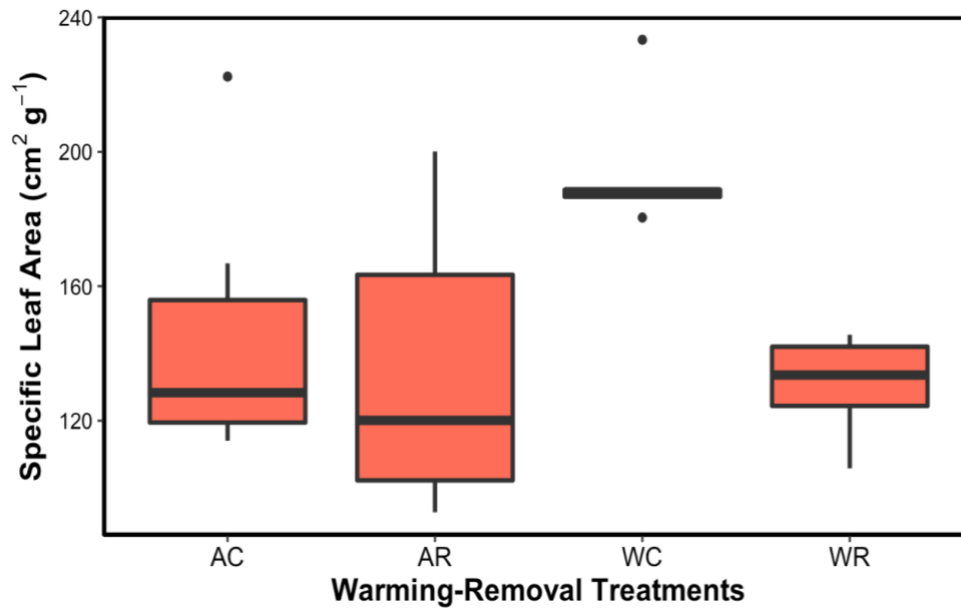


Figure 11: Plot showing the distribution of Specific Leaf Area (cm²/g) for ACHMIL between treatment. There is a significant effect of treatment on species ($p = 0.006$). The model explains 32.2% of the variation in the data ($R^2 = 0.322$).

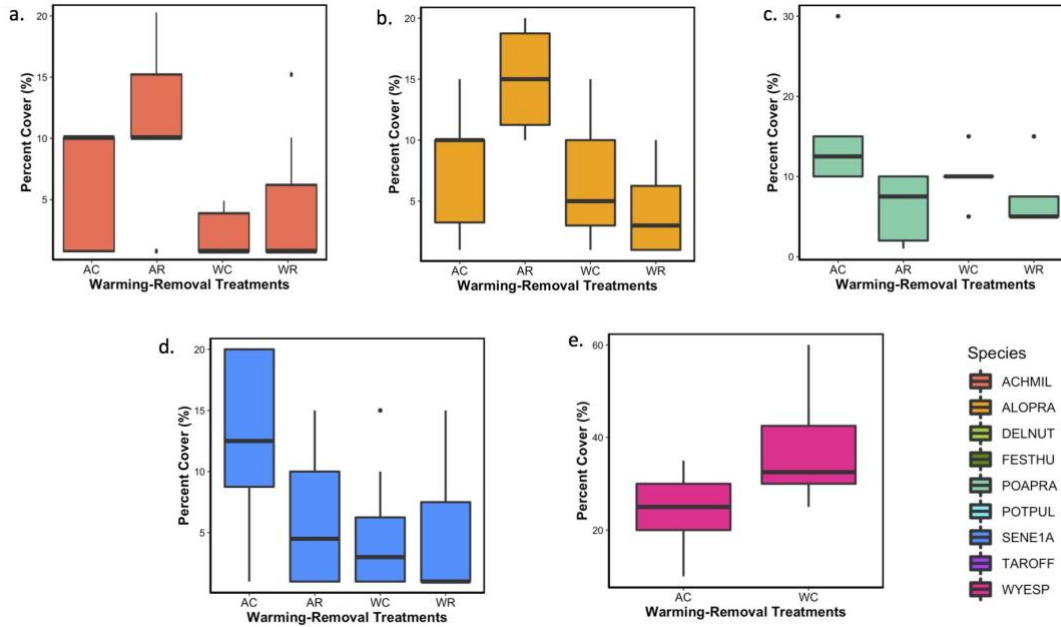


Figure 12: Plot showing the distribution of percent cover of ACHMIL ($p = 0.013$), ALOPRA ($p = 0.013$), POAPRA ($p = 0.057$), SENE1A ($p = 0.04128$), and WYESP ($p = 0.02079$) between treatment. Percent cover varies significantly between treatment all species.

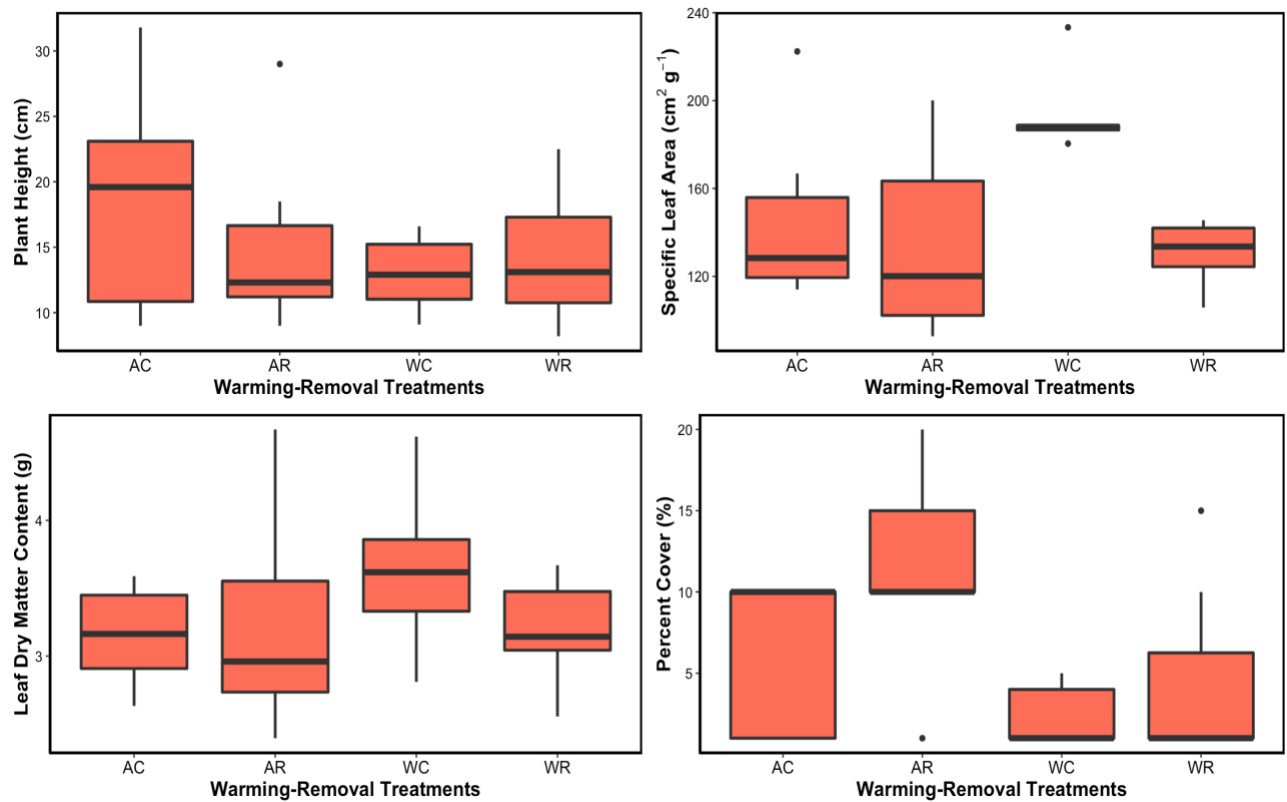


Figure 13: Plot showing the distribution of each trait across treatment for ACHMIL. Refer to Table 1 for statistics.

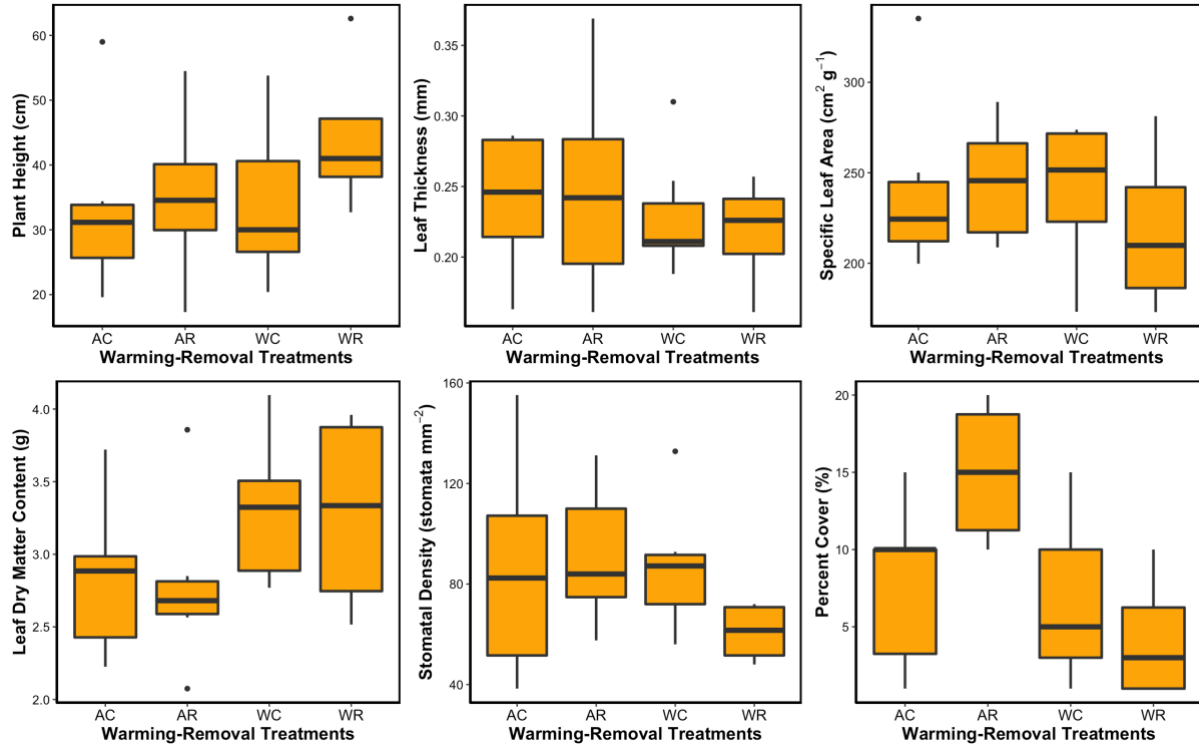


Figure 14: Plot showing the distribution of each trait across treatment for ALOPRA. Refer to Table 1 for statistics.

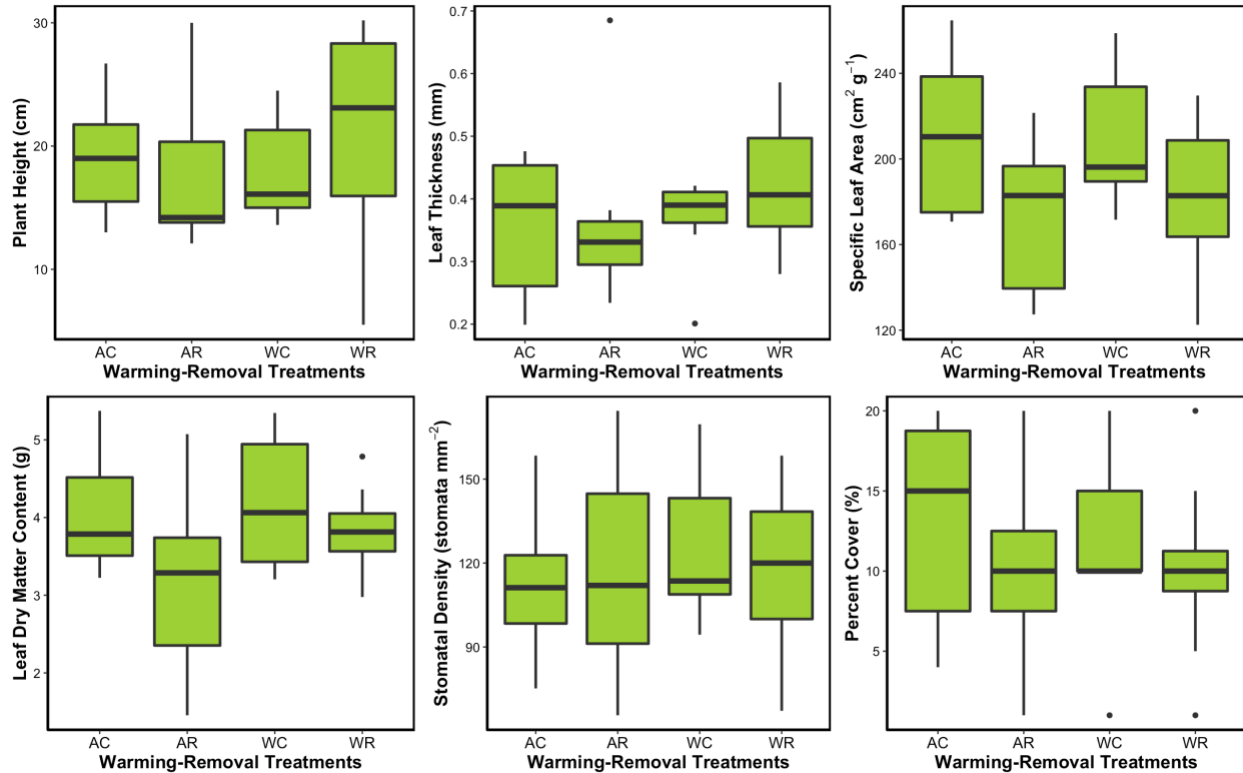


Figure 15: Plot showing the distribution of each trait across treatment for DELNUT. Refer to Table 1 for statistics.

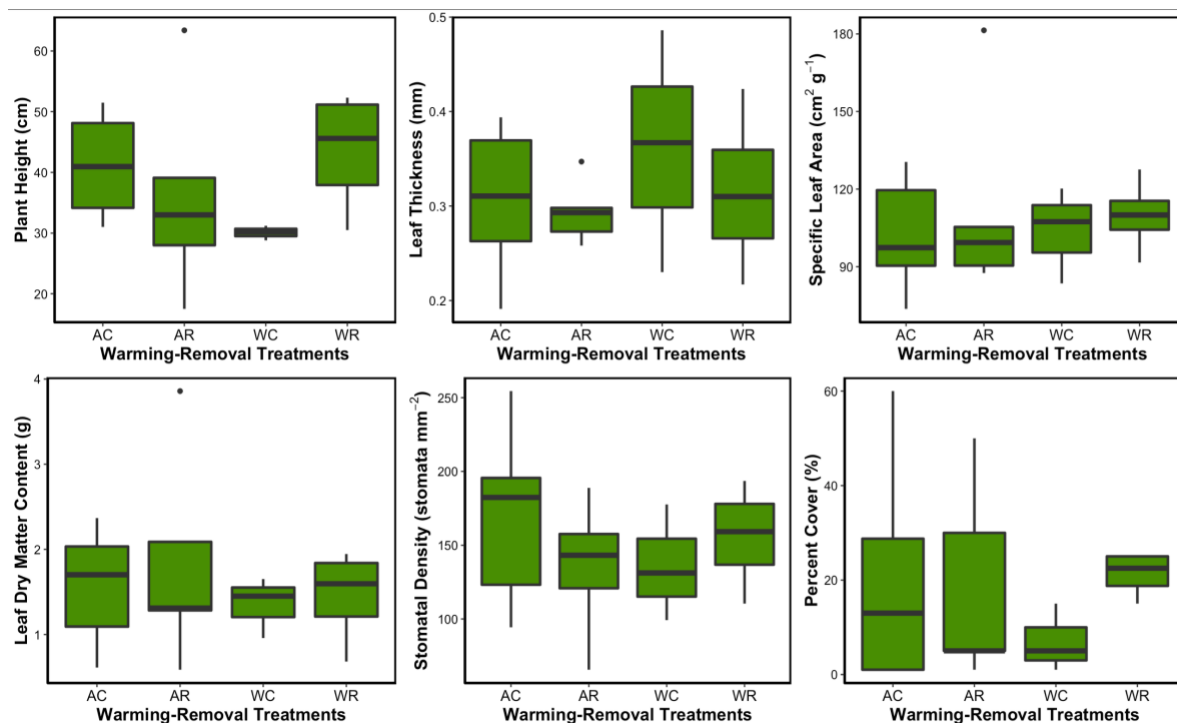


Figure 16: Plot showing the distribution of each trait across treatment for FESTHU. Refer to Table 1 for statistics.

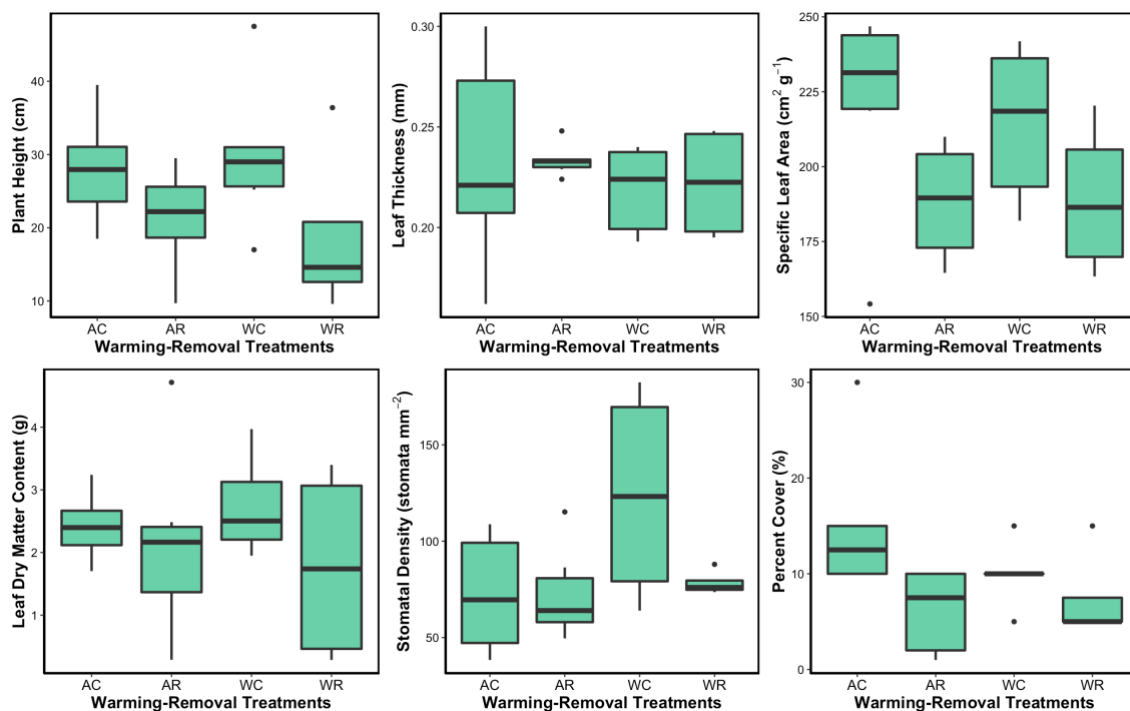


Figure 17: Plot showing the distribution of each trait across treatment for POAPRA. Refer to Table 1 for statistics.

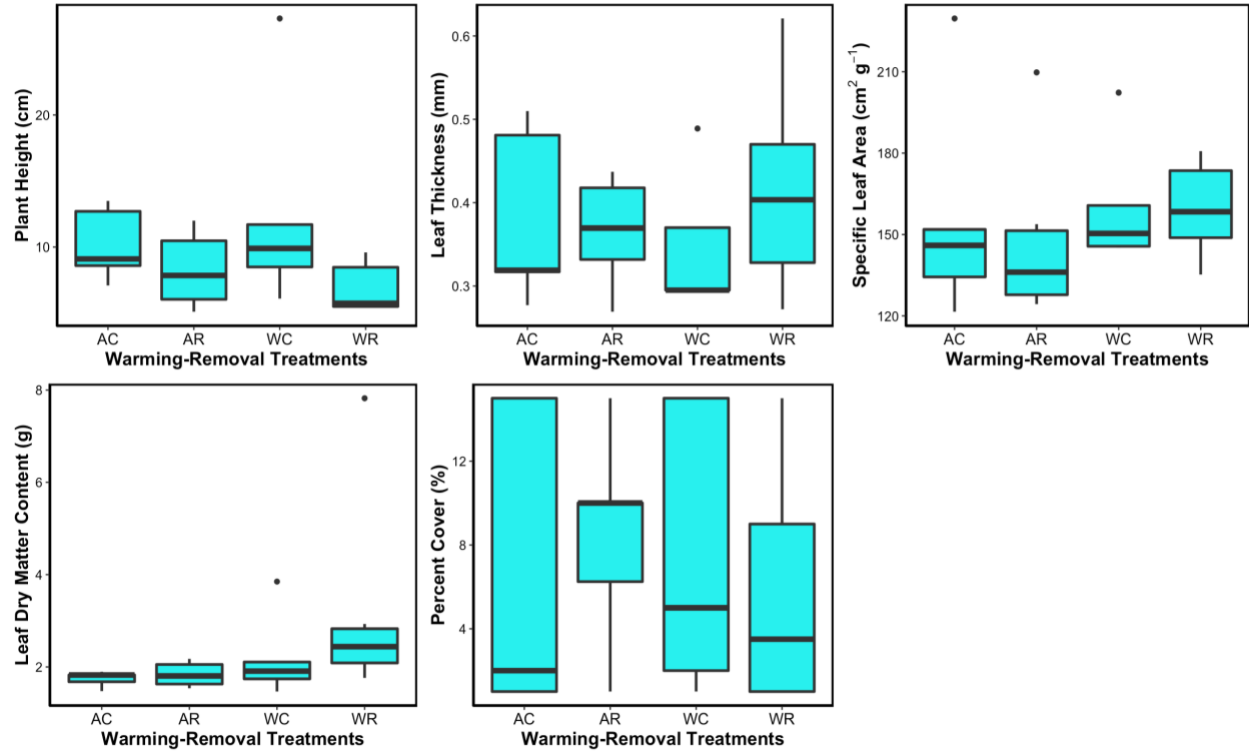


Figure 18: Plot showing the distribution of each trait across treatment for POTPUL. Refer to Table 1 for statistics.

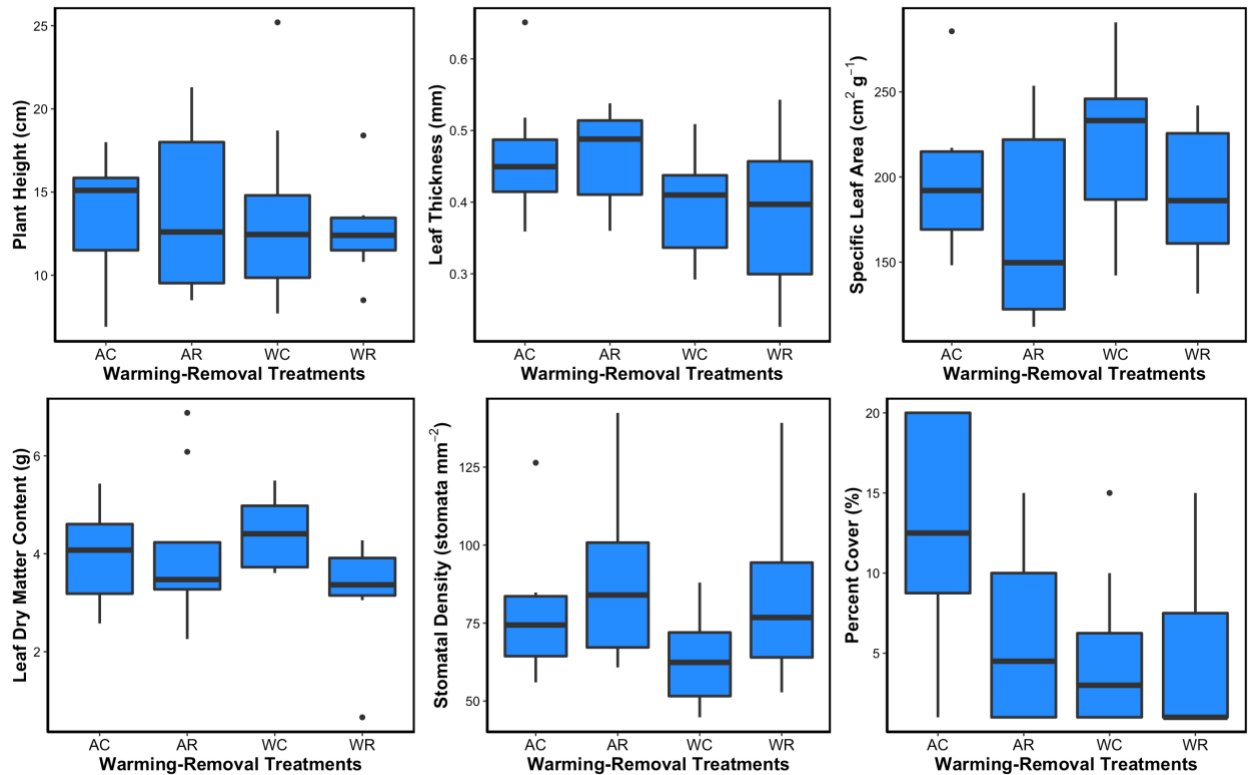


Figure 19: Plot showing the distribution of each trait across treatment for SENE1A. Refer to Table 1 for statistics.

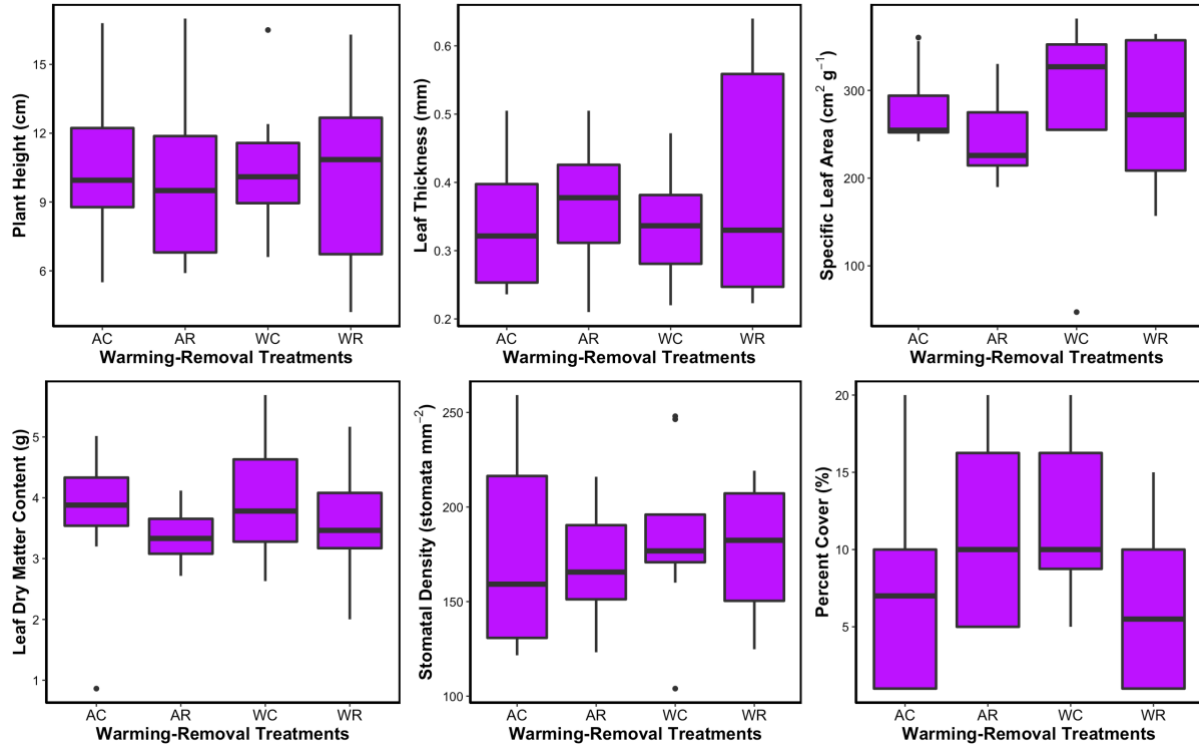


Figure 20: Plot showing the distribution of each trait across treatment for TAROFF. Refer to Table 1 for statistics.

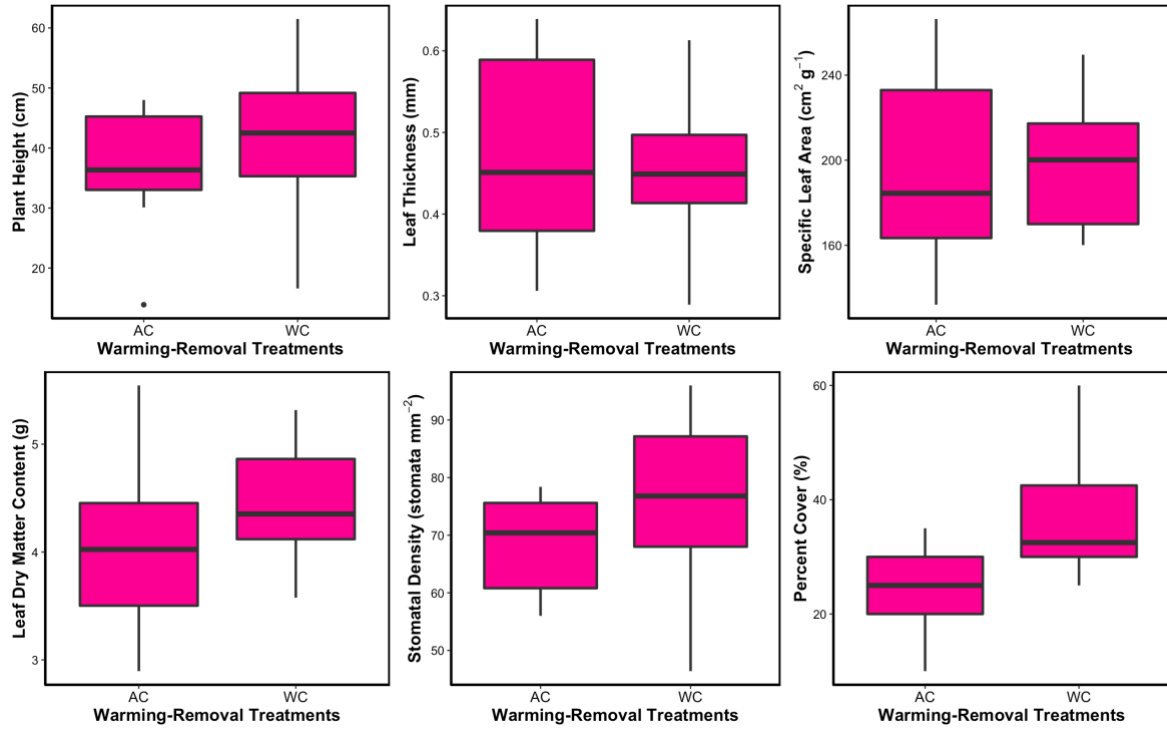


Figure 20: Plot showing the distribution of each trait across treatment for WYESP. Refer to Table 1 for statistics.

Table 1: Table showing the R^2 and p value results from ANOVAs comparing intraspecific variation in traits across treatments.

Species	Height (cm)		Leaf Thickness (mm)		SLA (cm ² /g)		LDMC (g water/ g dry mass)		SD (stomata/mm ²)		Percent Cover (%)	
	R ²	p	R ²	p	R ²	p	R ²	p	R ²	p	R ²	p
ACHMIL	-0.008	0.443			0.322	0.006**	0.002	0.401			0.276	0.013*
ALOPRA	-0.041	0.558	-0.118	0.854	-0.094	0.776	0.044	0.293	-0.020	0.478	0.333	0.013*
DELNUT	-0.070	0.747	-0.050	0.638	0.104	0.134	0.082	0.174	-0.104	0.906	-0.077	0.786
FESTHU	-0.008	0.440	-0.107	0.719	-0.171	0.913	-0.161	0.851	-0.104	0.690	-0.117	0.750
POAPRA	0.098	0.190	-0.112	0.827	0.140	0.131	-0.061	0.623	0.223	0.057	0.223	0.057
POTPUL	0.085	0.213	-0.081	0.704	-0.127	0.886	0.080	0.222			-0.126	0.882
SENE1A	-0.101	0.970	0.111	0.105	0.046	0.240	0.093	0.140	0.057	0.211	0.177	0.041*
TAROFF	-0.106	0.998	-0.058	0.733	-0.060	0.740	-0.054	0.707	-0.055	0.714	0.067	0.182
WYESP	-0.012	0.382	-0.065	0.713	-0.070	0.895	0.023	0.263	0.028	0.252	0.278	0.021*