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Effects of Simulated Climate Change on Native Southeastern Grassland Vegetation Using Open Top Chambers

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**Effects of Simulated Climate Change on Native Southeastern Grassland Vegetation Using
Open Top Chambers**

A Thesis Submitted to the
Graduate Faculty of Jacksonville State University
in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
with a Major in Biology

By
Makaila Leigh Carpenter

Jacksonville, Alabama

August 2, 2024

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Makaila Leigh Carpenter

August 2, 2024

Abstract

The southeastern United States is a critical biodiversity hotspot once covered in prairie grassland ecosystems and now facing significant threats due to urbanization, habitat destruction, over-exploitation, biological invasions, pollution, and climate change. This study analyzes the response of Alabama's native prairie ecosystem to climate change. We used open top chambers (OTCs) to simulate climate change-induced warming. We assessed the response of ecosystem function and structure to higher temperatures (1-3°C). The indicators of ecosystem function included phenology, leaf surface area (LSA), specific leaf area (SLA), and aboveground and below net primary productivity (ANPP and BNPP respectively). Ecosystem structure was evaluated based on species diversity and composition. Our results showed ecosystem function in the OTC was significantly lower based on LSA, SLA, and leaf biomass. Although not statistically significant, ANPP and BNPP were also higher in control plots. We documented altered phenology in several species including early flowering and increase in the length of the growing season. We also documented decreased species diversity in the OTCs and the inability of some species to establish under the warmer conditions. Our results exemplify the negative effects of climate change on native prairie vegetation. Reduced ecosystem function has cascading impacts and will threaten food resources for other species, especially at higher trophic levels, thus putting at risk our already vulnerable prairie ecosystems. Moreover, a decline in healthy plants in an ecosystem will also alter ecosystem services like carbon sequestration, soil stabilization, and water cycle regulation. Changes in ecosystem services and biodiversity will also directly affect our food security.

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Thank you to Dr. Tenzing Ingty for being such a great mentor. Dr. Ingty guided me through my project with kindness and always helped me believe I was capable of hard work, including this thesis! I would also like to thank Dr. Triplett and Dr. Burns for being such supportive committee members and guiding me along with their ideas and valuable feedback. I would also like to thank Jacksonville State University's Biology Department, without which I would not have gained the knowledge, experiences, and incredible friends that I have today. A huge thank you to the BY104 Alabamazon labs, BY332 Ecology labs, and especially Claudia Williams for their help collecting much of the data that made this project possible. I want to thank my closest friends and biggest support systems, Maryellyn Hawbaker and Himani Patel, both of whom lifted me up when I was down and cheered me on, always. I want to thank my dad, Rowland Gidley, for his know-how, and ability to use a drill, skill saw, and copious amounts of silicone. Without my dad, the OTCs would not be standing today (or ever!). I want to thank my mom, Gloria Gidley. She pushes me to work hard, encourages me not to give up, and she is always there for me when I need her. Thank you, mom, for the determination I know I got from you, the kindness that you show the world, and for your constant love and encouragement. Lastly, my biggest thank you to my husband, Alex Meeker, for his constant support and ability to make me laugh when I feel down. Alex believed in me when I did not believe in myself and he motivated me throughout every step in this process, reminding me of how smart and capable he knew I was. I am beyond thankful for all the support from my loved ones. Thank you all.

Makaila Carpenter

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Introduction

Biodiversity and Threats of the Southeastern Grasslands

The southeastern United States is an area of high biodiversity, and is part of one of the 36 Global Biodiversity Hotspots, namely the North American Coastal Plain (NACP) (Noss et al., 2015). Alabama is a key state in the NACP and is considered one of the most biodiverse states in the country (Stein, 2002). The project is focused on Alabama's little-known and often misunderstood grasslands. Grasslands existed in much of southeastern United States in a pre-Euro-American landscape although these areas can hardly be recognized as grasslands today due to the thick trees and excessive groundcover essentially turning these areas to forests (Noss, 2013). In addition to urbanization and its associated changes, climate change is a major threat to Alabama's native prairie ecosystems.

The loss of the southeastern grasslands continues to threaten a concerning number of species, making up nearly one-third of species listed on the Endangered Species Act (Noss et al., 2021). The Southern Appalachians and Ridge and Valley Province, including central Alabama, are considered the hottest spots of endemism in the Southern United States, and among the hottest in the world for both plant and animal species (Noss, 2013), underlining the significance of conserving these species. Grassland ecosystems provide countless provisioning, cultural, supporting, and regulating services (Petermann, 2021). These include important processes like climate regulation through the sequestration of soil carbon, providing habitats for numerous native pollinators, and even simple services like providing aesthetic value, all of which justify the conservation of these grasslands as crucial to the well-being of the environment and society (Petermann, 2021).

Climate Change and the Southeastern Region

Climate scientists agree that temperatures in the Southeastern United States have increased since the industrial era, by about 1.1°C since 1970, and continue to remain in a distinct warmer climate than previously seen, with temperatures projected to increase 2.2 to 4.4°C by the end of the century (Belesky & Malinowski, 2016). Biodiversity across the globe is responding to climate change in three primary ways: change in phenology, a shift in range distribution, and extinctions. Phenology refers to the timing of plant life-cycle events, such as leaf bud bursting, flowering, and fruiting. The timing of these events, known as phenophases, are largely triggered by temperature (Ingty et al. 2023, Hart et al. 2014, Miller Rushing and Primack 2008). Studies have shown that spring phenology has advanced significantly along with delayed fall phenology across the world (Piao et al. 2019). Temperatures have been shown to speed up plant development, causing early anthesis and potentially leading to missed opportunities for pollination (Badeck et al., 2004). Plant species are also moving to higher, cooler elevations or higher latitudes due to climate change (Pecl et al., 2017). In some cases, moving to higher elevations can lead to plant success in a new location, but more often, plants can essentially trap themselves in unsuitable conditions leading to their extinction (Pecl et al., 2017). These changes in phenology will negatively impact pollination, pollinators, biodiversity, productivity, and even food security (Memmot et al., 2007; Klein et al., 2007, Cardinale et al., 2012, Bellard et al., 2012, Leadley et al., 2010, Lobell et al., 2011; Myers et al., 2014).

The response of vegetation to climate change has been studied using numerous methods ranging from long term recordings including historical records kept by scientists, amateur naturalists, and herbaria records (Hart et al. 2014, Miller-Rushing and Primack 2008, Primack et al. 2004), remotely sensed imagery (Ingty et al 2023), and temperature or carbon dioxide

manipulation experiments (Hollister and Webber 2000, Dorji et al. 2020, Wolkovich et al. 2012). Passive warming experiments (temperature manipulation) have been used to simulate climate change and its impacts on plant traits that define the structure and function of an ecosystem (Dorji et al 2020, Kudo 2016). This approach has been especially revealing to study the response of plant traits such as phenology, plant growth and vegetation cover (Hollister and Webber 2000, Dorji et al 2020, Willis et al 2010, Kudo 2016).

Passive Warming Experiments Using Open Top Chambers (OTCs)

Passive warming using open top chambers (OTC) has been shown to be an effective way to simulate climate change and measure the effects on vegetation (Hollister and Webber 2000, Suzuki and Kudo 1999, Kudo 2016). The OTC is a passive warming device, that is based on the experimental apparatus designed by the International Tundra Experiment (ITEX) (Hollister et al., 2023). The OTC warms ambient air temperatures by about 1-3°C which falls within the range of predictions from global climate models (Intergovernmental Panel on Climate Change, 2023). Studies simulating climate change via OTCs have recorded changes in productivity, diversity, growth rates and altered phenology (Cowles et al 2018, Hollister and Webber 2000).

OTCs were used in this project to understand the response of southeastern grasslands to simulated climate change induced warming at an ecosystem-level. This is especially significant since this is the first time passive warming experiments have been used to study grasslands communities of the Southeast. We used a multispecies/ecosystem-level approach that has proven to be most successful when looking at conservation policy (Noss et al., 2021).

We used our project site to test two questions regarding the response of native prairie grassland ecosystems to climate change.

- 1) What is the response of prairie ecosystem function to simulated climate change induced warming?

We used phenology, leaf surface area, biomass, specific leaf surface (a function of leaf surface area and leaf biomass), aboveground net primary productivity, and belowground net primary productivity as indicators of ecosystem function.

Our hypotheses and expected results for ecosystem function are as follows:

Alternative Hypothesis: Warmer conditions because of climate change will reduce ecosystem function and alter plant phenology.

Prediction: Vegetation in the OTC will show significantly lower values of NPP and SLA than the control group. Spring phenology will advance in the OTCs.

- 2) What is the response of prairie ecosystem structure to simulated climate change induced warming?

We used vegetation species richness and composition as an indicator of ecosystem structure.

Our hypotheses and expected results for ecosystem function are as follows:

Alternative Hypothesis: Climate change induced warming will reduce plant community structure.

Prediction: Vegetation in the OTC will show less species diversity (richness and evenness than the control group).

Community outreach was also an important aspect of this project. The project site is visible from the road, and through signs, social media, and word of mouth, we were able to foster some community participation. Our experimental plots were and will continue to be used for Course-based Undergraduate Research Experience (CURE) labs at JSU at the Introductory Biology level and the 300-level courses (Ecology). We have also received interest from other

educational institutions (particularly Oxford High School) who are using the CURE labs as a model for their dual enrollment courses.

In the long run, we foresee this project site expanding and even serving as a model for other institutions and communities to replicate and foster conservation of native prairie grasslands and encourage awareness and healthy discourses on the perils of climate change and our native prairies.

Methods

Study Site

Our project site was at Jacksonville State University in Jacksonville, Alabama (approximately located at 33.82359, -85.76115). Two plots of 15 X 5 feet each were prepared for our research. Each plot was first tilled in April of 2023 and covered with tarpaulin for a month to clear out any vegetation present. Thirteen native southeastern prairie species were then planted on June 16, 2023 (table 1). These species were chosen because they represent the major biodiversity of southeastern prairies.

Table 1. List of native prairie species planted in plots.

Family	Scientific Name	Common Name
Asclepiadaceae (Milkweed)	<i>Asclepias verticillata</i> L.	Whorled Milkweed
Asteraceae (Aster)	<i>Anaphalis margaritacea</i> (L.) Benth. & Hook.f.	Pearly Everlasting
Asteraceae (Aster)	<i>Coreopsis palmata</i> Nutt.	Prairie Coreopsis
Asteraceae (Aster)	<i>Rudbeckia hirta</i> L.	Black-Eyed Susan
Asteraceae (Aster)	<i>Symphyotrichum oblongifolium</i> (Nutt.) G.L.Nesom	Aromatic Aster
Fabaceae (Legume)	<i>Baptisia tinctoria</i> (L.) R.Br.	Small Yellow Wild Indigo
Fabaceae (Legume)	<i>Dalea purpurea</i> Vent.	Purple Prairie Clover
Lamiaceae (Mint)	<i>Monarda punctata</i> L.	Spotted Bee Balm
Poaceae (Grass)	<i>Panicum virgatum</i> L.	Switch Grass

Poaceae (Grass)	<i>Schizachyrium scoparium</i> (Michx.) Nash	Little Bluestem
Scrophulariaceae (Figwort)	<i>Agalinis tenuifolia</i> (Vahl)Raf.	Slender False Foxglove
Verbenaceae (Verbena)	<i>Verbena stricta</i> Vent.	Hoary Vervain
Violaceae (Violet)	<i>Viola sororia f. priceana</i> House	Common Blue Violet, Bi-Color

Open Top Chambers

Four clear plexiglass sheets each 0.093 inches thick were used with sides cut at an angle of 65° (Fig 1) to construct one OTC. We constructed four OTCs in total. The sides were secured together using Gorilla Glue and reinforced with zip ties at each corner. On June 26th, 2023, we placed two OTCs in each plot for a total of four OTCs. Parts of the plot outside the OTC were sampled as the control. Each OTC had a height of 28cm covering 0.66m² of ground area. The top opening of OTC was 0.026 m².

Ambient air temperature was measured inside OTC and control. Measurements were taken at 1-hour intervals during the experimental period by automatic recording thermometers (HOBO Pendant MX Data Logger).

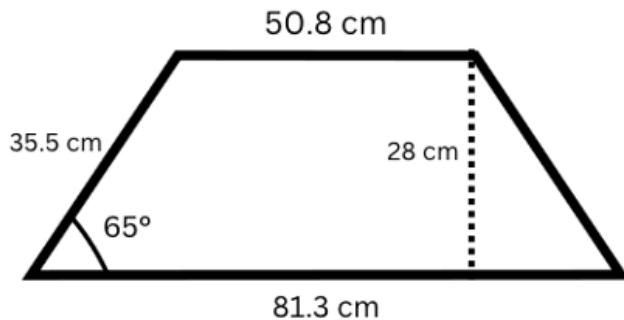


Figure 1: Model of OTC with dimensions in details

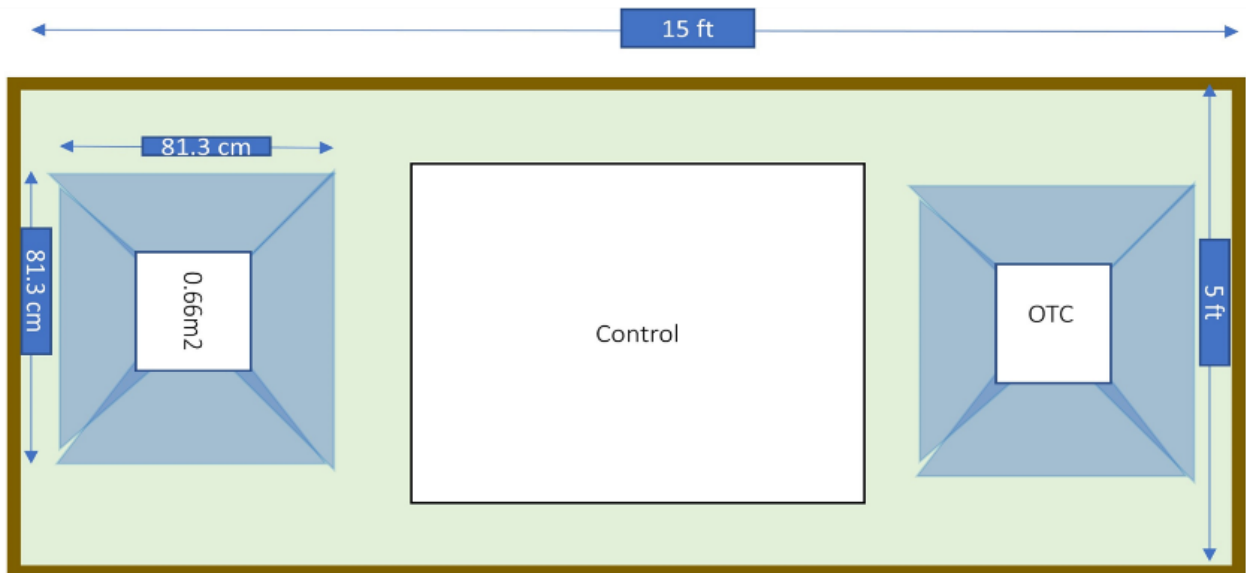


Figure 2: Model of a plot of the study site. On our site, we had this setup duplicated for a total of 4 treatment areas and 2 control areas.

Ecosystem Structure

Plant species diversity was documented at monthly intervals from October 2023 to April 2024 to determine ecosystem structure inside OTC and control. Data was always collected by the same individual to maintain consistency.

Ecosystem Function

Phenology, net primary productivity –both above and below ground (ANPP and BNPP respectively), leaf surface area (LSA), leaf biomass, and specific leaf area (SLA) were documented to measure change in ecosystem function. Additional data was collected by undergraduate students in the Introductory Biology (BY 104) labs for LSA and SLA and the Ecology (BY 332) course for net primary productivity. Only data that was cross checked by graduate teaching assistants were used in this study.

Phenology:

During the experimental period, phenology was recorded weekly by noting the species present inside and outside of the OTCs, particularly which phenophase the plant was presenting.

For the study, we identified and recorded two major phenophases: leafing and flowering. Data was recorded once a week from each plot for both OTC and control from August 2023 to June 2024.

Net Primary productivity:

ANPP was collected at peak standing crop by clipping an area of 15 X 15 cm in the month of November. Each sample was then oven dried at 60°C for 48 hours and the dry weight was measured using a precision scale.

BNPP was collected by using a soil core to dig below the vegetation and collect six inches deep of soil. Different-sized sifters were used to separate dirt, twigs, rocks, and other debris from the roots in our below-ground samples. Once isolated, the roots were oven dried at 60°C for 48 hours and the dry weight was measured using a precision scale. The dry weight for ANPP and BNPP were standardized to per unit area (cm^2) area by dividing by their respective sampling area- 225 cm^2 for ANPP and 3.14 cm^2 for BNPP.

Leaf Surface Area:

Leaves were collected at petiole length from both OTC and control. After collecting, leaves were placed on graph paper and photographed from straight above. Photographs were uploaded into ImageJ software to accurately measure leaf surface area for all leaves.

Specific Leaf Area:

Specific leaf area is a function of leaf surface area and biomass. Leaf surface area was collected and calculated as detailed above. Leaf biomass was calculated by drying the leaves at 60°C for 48 hours and the dry weight was measured on a precision scale. SLA was calculated by using the formula below.

$$SLA = \frac{\text{leaf surface area (mm}^2\text{)}}{\text{leaf dry weight (mg)}}$$

Statistical Analysis

Statistical analysis and graphical representation were conducted using *vegan* and *ggplot2* packages in R-Studio. Normality of the data was checked using the Shapiro Wilks test. When the data was normally distributed the parametric Students T test was used to test for significance ($p < 0.05$) of differences and the Wilcoxon Rank Sum test was used for non-parametric data. To explore the response of species composition to warming a triangular similarity matrix was constructed using Bray Curtis similarity coefficients based on cover data. The similarity matrix was displayed in an ordination plot using non-metric multidimensional scaling (NMDS). The stress function was used to assess how well the calculated sample relationships are represented in the two-dimensional plot. A lower value (< 0.2 commonly accepted) represents a more accurate ordination.

The Analysis of similarity (ANOSIM) was used to check the degree of dissimilarity of species across treatments. ANOSIM based on the Bray Curtis similarity matrix was used to check significance levels using a permutation test with 999 simulations. R values close to 1 suggest sites within a group are similar to each other and dissimilar to sites in other groups. While an R value close to 0 suggests an even distribution of high and low ranks within and between groups.

Results

Open Top Chamber:

Temperature data was collected over 130 days (10/27/2023 to 3/6/2024) in both OTC and control. The mean daily temperature in OTC was 10.06°C and control was 9.35°C. The mean temperature inside the OTC was 0.71°C higher than temperatures recorded in the control plots (Fig 2).

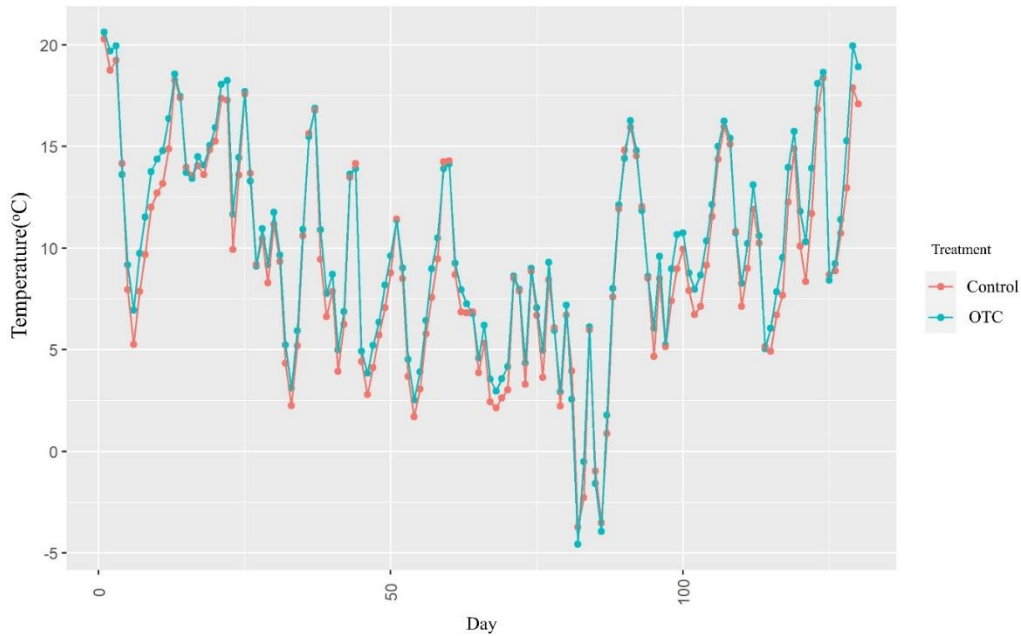


Figure 3: Daily average temperature recordings in OTC and control. Data recorded by HOBO Pendant MX Data Logger daily over the course of the experiment.

Ecosystem Function:

Leaf Surface Area

250 leaves were sampled (125 from OTC and 125 from control). The mean LSA for the OTC leaves was 569.25cm² and mean LSA for control was 796.26cm². Leaves in the control plots had significantly ($p < 0.05$) higher surface area than leaves in the OTC with the mean LSA in the control being 39.6% higher (Fig 3, table 2). Normality of data was checked using the

Shapiro Wilk's Test and consequently significance of difference was tested using the Wilcoxon signed-rank test.

Specific Leaf Area

We sampled 30 leaves to measure SLA, which included measuring both LSA and individual leaf biomass. The mean LSA for leaves in OTC was 886.96cm² and 1406.41cm² in the control. Control leaves were 58.6% higher than the OTC. The mean biomass was 35.01mg in OTC and 83.62mg in control. Control leaves were 138.8% higher than the OTC leaves. (Fig 3, table 2)

We found that the SLA in the OTC plots were 99.85% higher than the control plots. The Wilcoxon test showed that the SLA in OTC was significantly higher than control with a P-value of 6.71e-08. (Fig 3, table 2)

Aboveground and Belowground Net Primary Productivity

Wilcoxon tests were run to compare OTC and control plot values for ANPP and BNPP. ANPP was found to be higher in the OTCs by 15.40%; where the mean value in the OTCs was 20.31mg/cm² and the mean value in the control was 17.60mg/cm². This difference was not considered significant ($p= 0.8287$) (Fig 3). BNPP was higher in the control plots by 3.12%; with a mean dry weight of 273.72mg/cm² and 282.30mg/cm² in OTC and control respectively. Again, this difference was not considered significant with a P-value of 0.9025. (Fig 3)

Net Primary Productivity was also compared between aboveground and belowground. The mean value of ANPP was 18.96mg/cm² and the mean value of BNPP was 278.03mg/cm². BNPP was significantly greater than ANPP with a P value of 3.824e-05. (Fig 3).

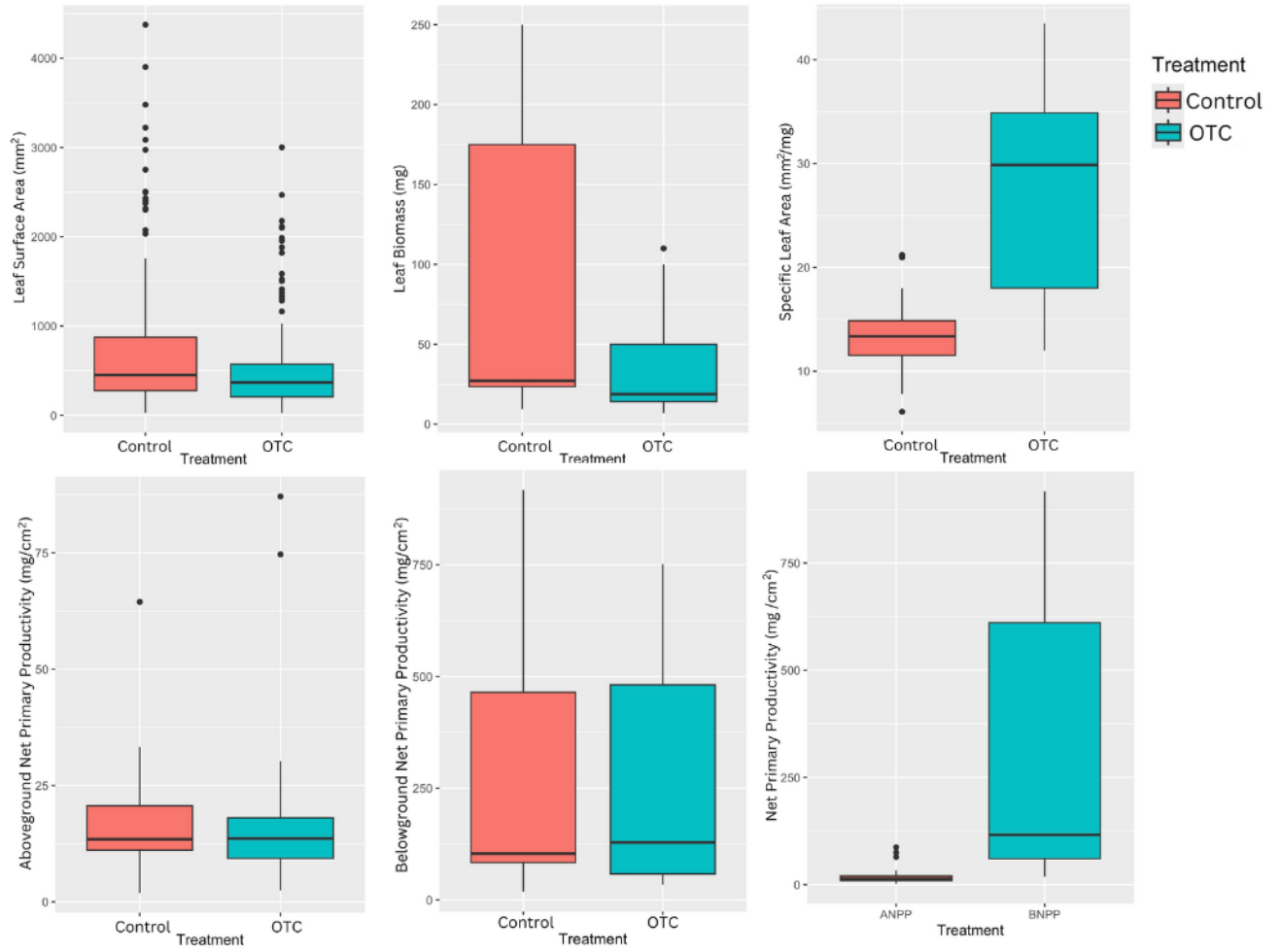


Figure 4: Boxplots of OTC vs Control for LSA, Biomass, SLA, ANPP, BNPP

Table 2: Ecosystem function traits under simulated climate change (OTC) treatments and in control plots. P values represent significance ($p < 0.05$) of difference using the Wilcoxon signed rank non-parametric test

PLOT	LSA (mm ²)	Leaf Biomass (mg)	SLA (mg/mm ²)	ANPP (mg)	BNPP (mg)
All (mean)	696.26	59.31	19.91	18.96	278.03
OTC (mean)	569.26	35.01	26.54	20.31	273.76
Control (mean)	796.26	83.62	13.28	17.60	282.30
Difference (Control- OTC)	227.01	48.61	-13.26	-2.71	8.54
P-value (two tailed)	0.03	0.006	1.34E-07	0.83	0.90
P-value (one tailed)	0.02	0.003	6.71E-08	0.32	0.47

Phenology

In summer 2024 we noted two species flowering in the plot namely *Rudbeckia hirta* (Black Eyed Susan) and *Monarda punctata* (Spotted Beebalm) (Fig 4,5,6). We noted early flowering of *Rudbeckia hirta* in the OTC when compared to the control plots. The first flowering individuals of *Rudbeckia hirta* in the OTC were documented 35 days before the same species flowered in the control (Fig 6). *Monarda punctata* flowered only on the control and not in the OTC (Fig 6).

2023 phenology data was limited since this was the first year that the plots were established. Nevertheless, several species were documented flowering in the control plots that did not flower or grow in the OTCs; these included-, *Euphorbia maculata* (Spotted Spurge),

Senna tora, *Cyperaceae sp.*, and *Asclepias verticillata*. Notably, *Rudbeckia hirta* showed delayed flowering in the fall in the OTC than the control (October 16 versus August 18).

Leafing phenophase showed similar results with several species absent in the OTC that were found in the control. This included *Chamaesyce maculata*, *Senna tora*, and *Dalea purpurea*.

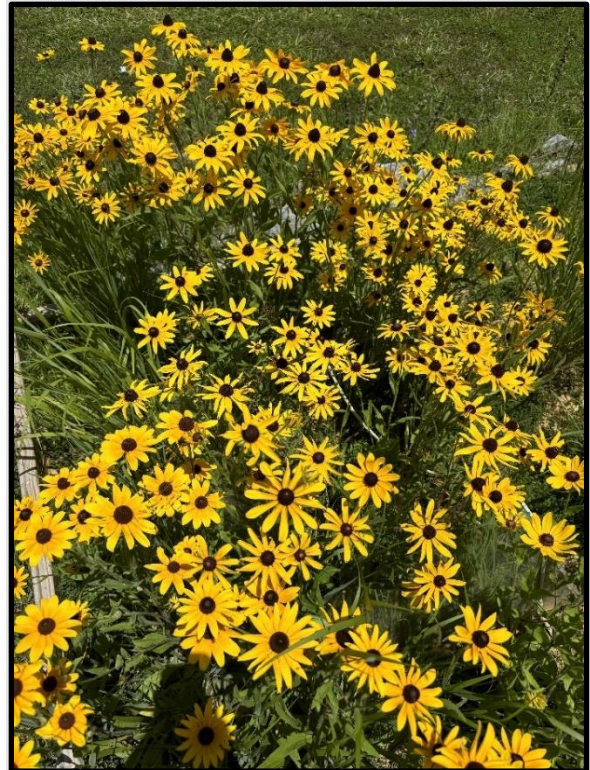


Fig 5: Left: *Monarda punctata* (Spotted Beebalm) flowering outside the passive heating chambers (OTC). No individuals of the species were found in the OTC.

Fig 6: Right: *Rudbeckia hirta* (Black Eyed Susan) flowering in the OTC and control plots.

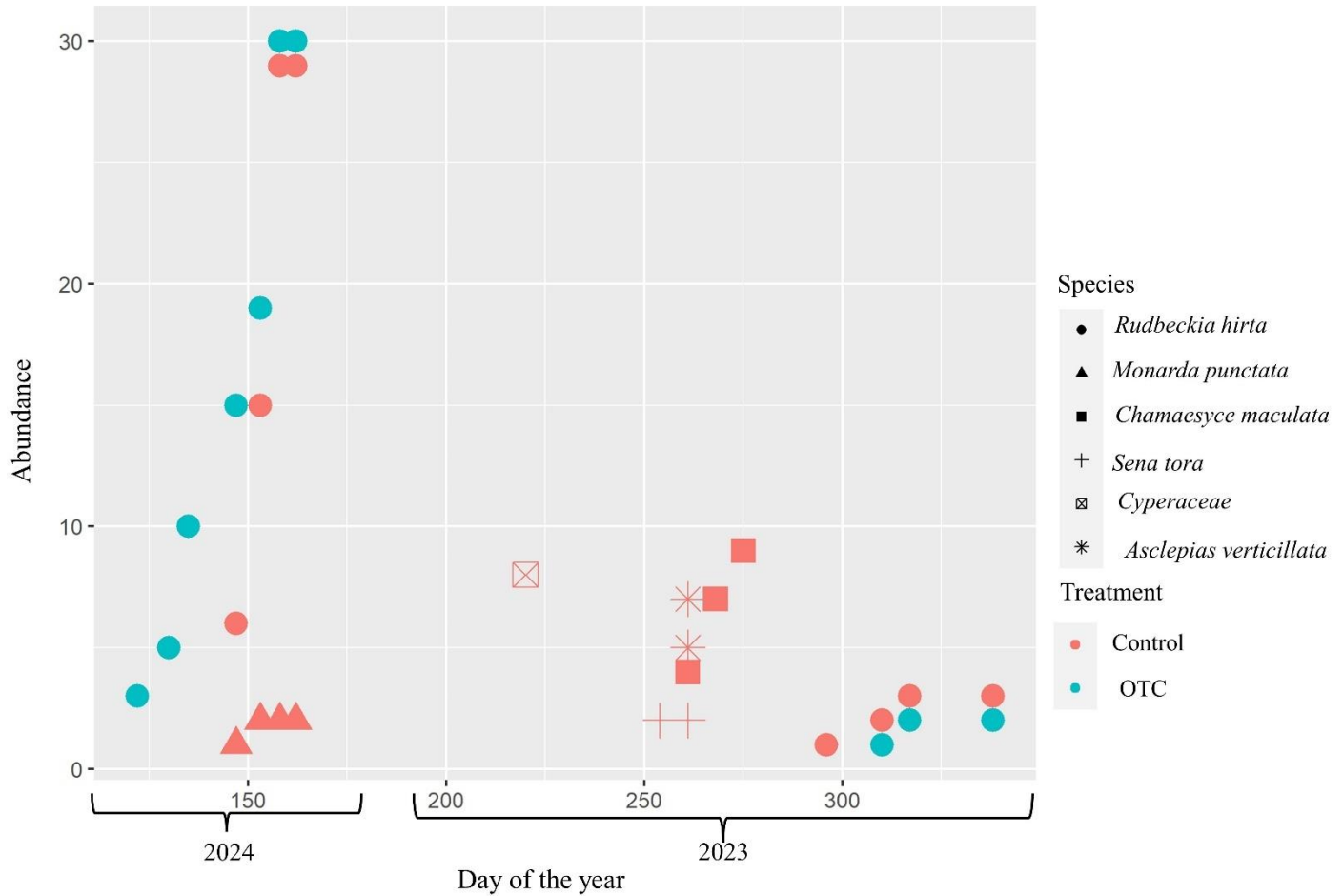


Figure 7: Flowering phenology of 6 species over a one-year period in both OTC and control (Fall 2023 to Summer 2024).

Ecosystem Structure:

Species Richness and composition

24 species were sampled in the 8 plots between April 2023 and April 2024. This included 19 species in the control plots 18 species in the OTC. 13 species were found in both OTC and control, 5 exclusively in the OTC and 6 in control only. The species richness was significantly higher in the control than the OTC (table 3). Although not significant the control plots showed higher diversity (both Shannon and Simpsons) than the OTC. The mean Shannon index was 1.67 and 1.85 in the OTC and control respectively (Fig 7, table 3). The mean Simpsons diversity

index was 0.77 in the OTC and 0.8 in the control (Fig 7, table 3). Beta diversity a measure of species turn over in each quadrat was higher in OTC than control plots. Higher beta diversity suggests a more heterogenous species composition from one quadrat to the next.

The results of an ANOSIM (Analysis of similarity) did not show a significant difference ($p=0.6$) in species composition in the OTC and control plots (Fig 7).

Table 3: Diversity indices of plant species found in the OTC and control plots.

** represent statistically significant ($p<0.05$) differences.

DIVERSITY INDEX	OTC	CONTROL	Difference (Control-OTC)
Mean species richness	7.75	11.5	3.75**
Mean Shannon index	1.67	1.85	0.18
Mean Simpson diversity index	0.77	0.81	0.04
Beta diversity	1.32	0.67	-0.65

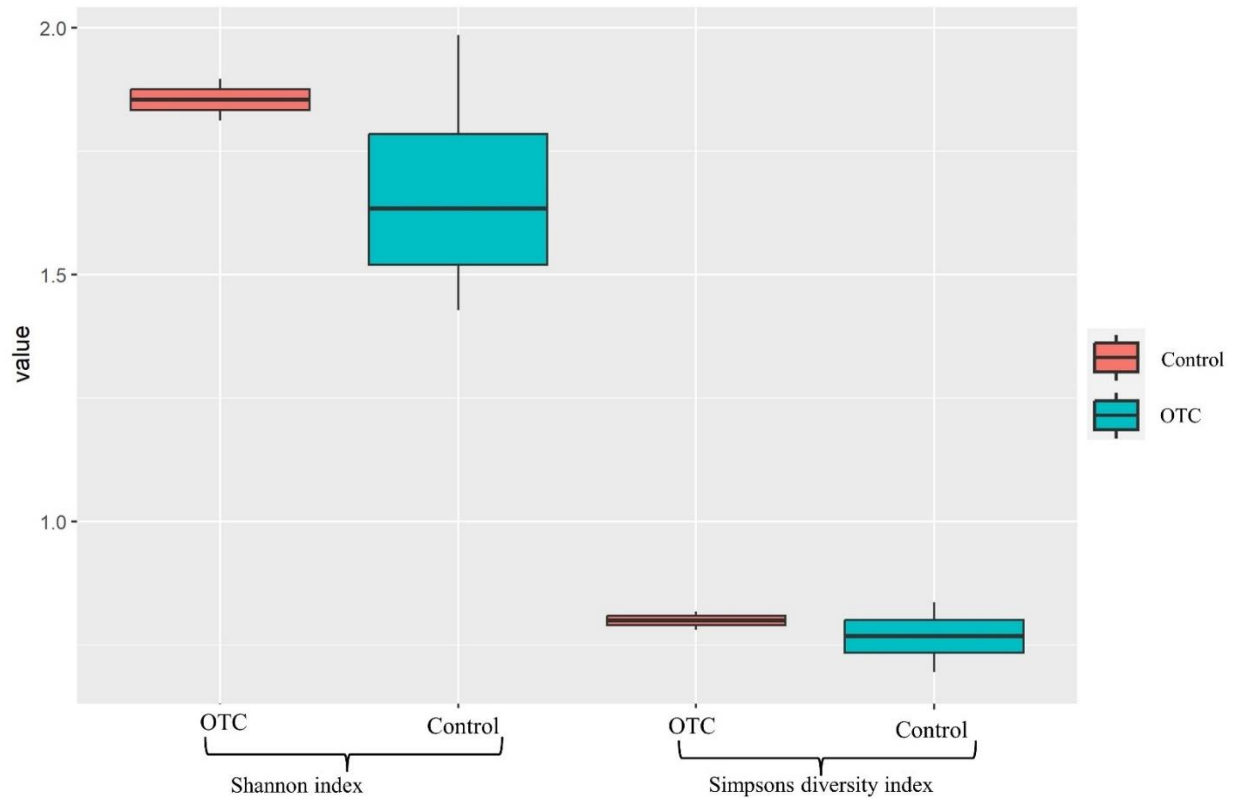


Fig 8: Boxplots representing diversity indices in OTC and control plots.

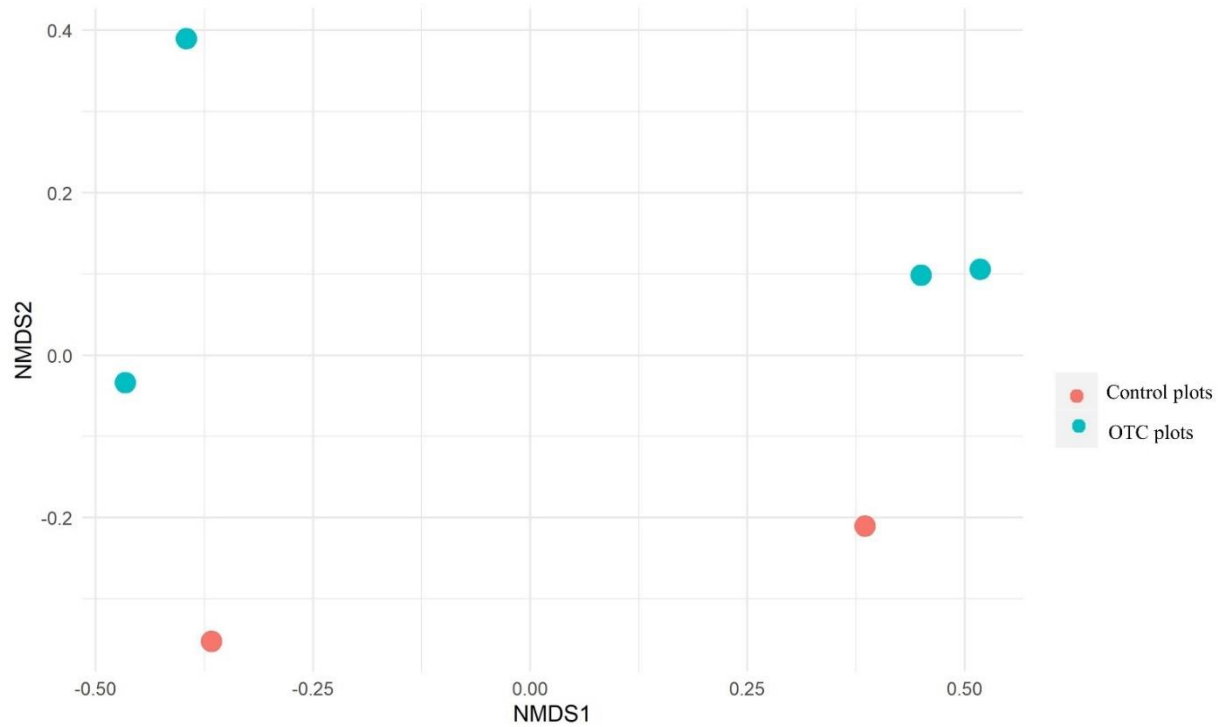


Figure 9: Results of a non-metric Multidimensional scaling (NMDS) multivariate analysis. The similarity matrix was displayed in an ordination plot using the Bray Curtis index.

Discussion

Phenology

In our study we had limited phenology data, but our results suggest three important findings. First, some species flowered earlier in response to simulated climate change driven warming in the OTCs. Second, some species failed to establish in the OTCs, suggesting an inability to thrive in the warmer climate. Third, as shown by early spring and delayed fall flowering of *Rudbeckia hirta* in the OTCs plant species showed early spring and delayed fall phenology indicating an increase in the length of growing season. This clearly suggests that while some plants will benefit from warmer temperatures leading to longer growing seasons (like *Rudbeckia hirta*) numerous other plants will not be able to survive in elevated temperatures.

Our results are consistent with multiple studies that show early flowering in warmer temperatures (Beaubien & Hamann, 2011; Craufurd & Wheeler, 2009; Miller-Rushing & Primack, 2008). Historical records have already shown that common plant species and crops are developing faster and flowering earlier in the year than they have historically (Beaubien & Hamann, 2011; Craufurd & Wheeler, 2009; Miller-Rushing & Primack, 2008). Along with these historical records, researchers have worked to track phenology with ground observations, remote sensing, and analysis of atmospheric CO₂ signal (Badeck et al., 2004; Ingty et al., 2023). Ground observations showed a correlation between warmer environments and the advancement of spring bud burst and flowering dates; Normalized Difference Vegetation Index (NDVI), a measure of vegetation phenology using remote sensing, also showed a trend for advanced green-up in spring (Badeck et al., 2004). Analysis of remotely sensed data in the Himalaya revealed that spring start of season was advanced by 21.3 days over a 17-year study period, while dates for maturity and senescence were all delayed; supporting the trend of advanced spring phenology and delayed fall phenology due to climate change (Ingty et al., 2023).

Other studies have used OTCs to measure the changes in phenology associated with climate change. In a study using OTCs on alpine shrubs in China, all species used showed earlier flowering and a longer flowering stage in the OTCs when compared with the control plots (Xu et al., 2009). The same results were found with tundra plants, noting a 3% longer growing season in passive warming when compared to control (Collins et al., 2021). Our results suggesting higher temperatures tend to lead to quicker development, early flowering, and a longer length of growing season are consistent with numerous other studies (Beaubien & Hamann, 2011; Craufurd & Wheeler, 2009; Miller-Rushing & Primack, 2008, Ingty et al., 2023).

Leaf Surface Area

In this study, we found that leaf surface area reduced significantly in response to climate change driven simulated warming. Leaves experiencing higher temperatures in the OTC are adapting by reducing their surface area, resulting in less solar radiation being absorbed. Our results align with multiple studies that show reduced leaf surface area in higher temperatures (Milthorpe, 1959; Pilau & Angelocci, 2015). A study done on the leaves of cucumber plants showed that the leaf surface area remained a consistent size until temperatures reached 30°C and then significantly decreased in size (Milthorpe, 1959). Another study focused on orange trees showed the same results: as temperatures rose, leaf surface area reduced (Pilau & Angelocci, 2015). These studies, along with our results, show a direct correlation between higher temperatures and reduced leaf surface area suggesting that leaves are shrinking their surface area in an adaptive measure to avoid excess solar radiation.

Specific Leaf Area

Our results support the idea that SLA (a function of LSA and leaf biomass) increases in the OTCs suggesting that leaves are adapting by becoming thinner in response to warmer temperatures. Thicker leaves will tend to absorb more solar radiation than thinner leaves. Leaves in the OTCs are thinning out to save energy and absorb less solar radiation. This is especially interesting since we found that LSA showed a significant decrease in the OTCs suggesting that leaf biomass too must compensate for this loss by decreasing by a significant amount,

Our results align with multiple studies that show similar results. A study completed in 2009 with alpine shrubs in China observed significant differences in SLA between three out of four species growing in the OTC when compared with the control vegetation; it was found that higher temperatures led to an increase in SLA (Xu et al., 2009). In another study in which OTCs

were used, there were significant differences observed in SLA between the OTC and control vegetation wherein OTC SLA was higher when compared to control vegetation; however, only one out of the two species used in this study showed significant results (Zhen-Feng et al., 2008).

SLA was introduced as a concept to help analyze whole plant growth and is often used to understand the relative thickness in leaves (Gunn et al., 1999). Understanding the relationship between SLA and leaf thickness gives insights into plant adaptation strategies to changes in their environment and implications for leaf function. Leaves with high SLA tend to be thinner because they invest more resources into their photosynthetic tissues, which contributes to a larger surface area for gathering light and exchanging gases (Wright et al., 2004). On the contrary, lower SLA is associated with thicker leaves, caused by the leaves allocating more resources to the conservation of water which reduces the proportion of leaf mass devoted to photosynthetic tissues (Wright et al., 2004). Using Open Top Chambers (OTCs) to simulate climate change on a small scale is one way that researchers have studied SLA and its response to temperature increases. We would like to continue this study to further our understanding of the relationship between climate change and SLA. It would also be beneficial to see more studies on this topic specifically.

Aboveground and Belowground Net Primary Productivity

Our study reveals two important results regarding net primary productivity (NPP). First, that BNPP reduced in response to warming while ANPP increased in warmer temperatures. Second, we found that BNPP is significantly higher than ANPP both in the OTC and in the control plots. This clearly indicates that more energy is being allocated below ground, where the plants are forming their roots, and less energy is spent in above ground structures. It is important

to note that this project took place during the first year after planting. It is likely that the vegetation is using more energy to produce strong below ground structures and roots instead of vulnerable above ground structures, and the differences between ANPP and BNPP could shift in the future as the vegetation matures.

ANPP and BNPP are both important in quantifying the amount of carbon stored in living organisms and understanding the role of the ecosystem in carbon sequestration (Gayathri et al., 2021). Changes in both ANPP and BNPP are extremely sensitive to variables, including disturbance, anthropogenic impacts, topography, and climate. It is well established that elevated CO₂ and warming can directly influence and alter ANPP and BNPP (Field et al., 1995; Garbutt et al., 1990; Kardol et al., 2010). Higher biomass often indicates greater productivity and nutrient cycling within an ecosystem (Gayathri et al., 2021; Parresol, 2002).

Our results align with several studies done thus far analyzing ANPP that tend to support the idea that an increase in temperature will also increase ANPP (Wang et al., 2017; Poudel et al., 2011; Garbutt et al., 1990). Our study found that ANPP increased in the OTCs compared to the control plots, although this difference was not significant.

Our results also align with studies analyzing BNPP. A study found that with a 2°C increase, BNPP is affected negatively (reduced by 41%) in grasslands (Li et al., 2018), while another study found that while BNPP was generally sensitive to climate change, though there was not a significant change of BNPP (Xu et al., 2016). Our study found that BNPP decreased with higher temperatures in the OTC; however, these results were not significant. We suggest that our study site continue to be used to study both ANPP and BNPP. More studies are needed to better understand the relationship between climate change and net primary productivity.

Ecosystem Structure:

Species Richness and composition:

This was the first year the plots were established so we did not expect to find any major results regarding diversity and composition. However, we did find some interesting preliminary indicators. The species richness was significantly higher in Control than OTC plots and although not statistically significant, all species diversity indices were higher in the control. This indicates the negative impacts of climate change driven warming trends on biodiversity. This is further exemplified by the absence of six species in the OTC found only in the control. This includes the purple prairie clover (*Dalea purpurea*), a species of immense ecological significance to prairie ecosystems. In addition to its nitrogen fixing properties *Dalea purpurea* is an important species for numerous native insect species including the charismatic Southern Dogface butterflies (*Zerene cesonia*) (Fenner et al., 2018).

Lower beta diversity in the control suggests a more homogenous species composition from one quadrat to the next in the control plots. This indicates similar species being found in the different quadrats in the control plots. The higher beta diversity in the OTC indicate that species found in one quadrat in the OTC was different than the next. This may be because species are taking longer to establish themselves in the warmer conditions and it will be interesting to see how beta diversity changes over the next few years after the community stabilizes and most species establish themselves. The NMDS ordination plot and ANOSIM analysis did not show any clear results, this was primarily because of the low sample size, since we did not have many species in 2023 and winter of 2024 when the bulk of sampling effort was put.

Conclusion

Our study revealed results that are consistent with multiple studies analyzing climate change and its effects on vegetation and biodiversity. When ambient temperatures are increased, we saw altered phenology, significantly reduced leaf surface area, significantly lower specific leaf area, and lower belowground net primary productivity. Each of these changes to vegetation can affect the overall health of plants and cause unexpected damage that affects the environment and even humans.

It has been well established that climate change is affecting phenology and causing advanced flowering (Beaubien & Hamann, 2011; Craufurd & Wheeler, 2009; Miller-Rushing & Primack, 2008). Changes in phenology, particularly the timing of plant blooming, have significant impacts on humans, affecting biodiversity and food supply (Kjølhl, 2011). In a study done to simulate the effects of rising temperatures on a plant pollinator network, it was found that shifts in phenology reduced the resources for 17 to 50% of pollinator species (Memmot et al., 2007). A temporal mismatch can be detrimental for plants, pollinators, and consequently humans. These mismatches cause poor pollination that reduce crop yield thus affecting our food supply (Memmot et al., 2007; Klein et al., 2007). Thirty five percent of global food production is dependent upon animal pollination; this includes fruit, vegetable, and seed production from eighty-seven of the world's leading food crops (Klein et al., 2007). Altered plant phenology would result in less pollination events, less resources for pollinators and decreasing agricultural yield, threatening food security.

It is already well known that climate change has negative effects on biodiversity (Cardinale et al., 2012, Bellard et al., 2012, Leadley et al., 2010). Bellard et al's (2012) seminal study examined multiple models attempting to understand the future of biodiversity with rising

temperatures across the globe; while all modeling methods have weaknesses and can vary, they all found the same results: alarming consequences for biodiversity. Keeping a diverse community is important because these communities are more productive and functionally more efficient (Cardinale et al., 2012).

Changes in primary productivity (biomass), specifically reduced biomass, can lead to habitat loss and food insecurity for animals in the surrounding ecosystem. Reduced primary productivity will threaten food resources for species, especially at higher trophic levels thus putting at risk our already vulnerable prairie ecosystems. Moreover, a decline in healthy plants in an ecosystem will also alter ecosystem services like carbon sequestration, soil stabilization, and water cycle regulation (Cardinale et al., 2012).

Along with changes in ecosystem services and biodiversity, climate change will also directly affect our food security. Several studies show that increased CO₂ and increased temperatures have negative effects on food nutrition and supply (Lobell et al., 2011; Myers et al., 2014). Plants with lower leaf surface area and net primary productivity function less efficiently, producing a lower crop yield which can directly impact food supply (Lobell et al., 2011; Myers et al., 2014). Research models show that the global maize and wheat production declined by 3.8% and 5.5% respectively when temperatures were increased (Lobell et al., 2011). Not only is the supply affected; the quality of our food will be reduced as well. Lower production efficiency will reduce the amount of nutrients in our food (Myers et al., 2014). Researchers found a direct decrease in concentrations of zinc and iron in C3 grains and legumes grown at the elevated CO₂ concentration that is predicted for the middle of this century, reflective of our changing climate (Myers et al., 2014).

Changes in climate can lead to decreases in biodiversity, reduced ecosystem services, and increased food insecurity. Understanding these changes at an ecosystem level- such as southeastern prairie grasses- is essential in learning how we can better address these concerns for future generations.

Reflections

Upon the completion of this thesis project, there are a few things that I would do differently. First, while I do believe that our LSA results were accurate, I would have preferred to use a scanner instead of phone photos to run ImageJ for LSA. Using a scanner would've made this portion of the project easier and marginally more accurate. I also would have liked to pay more attention to genetic variations and other differences within the vegetation that was chosen. Genetics were beyond the scope of this project, although I think that would have been an interesting exploration. I hope this will be explored in the future with the established plots. Lastly, I would have loved to have had time to include other variables that were measured and studied by students in the Ecology 332 course, such as stomata count and pubescence and their changes due to passive warming.

Overall, I am proud of the work that was done for this project. I believe this project will be a good start for future graduate students to carry on and dig deeper in their research concerning climate change induced warming and its effects on southeastern native prairie grasslands using our plots with OTCs.

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Appendix A: Tables

Table 1: List of native prairie species planted in plots

Family	Scientific Name	Common Name
Asclepiadaceae (Milkweed)	<i>Asclepias verticillata</i> L.	Whorled Milkweed
Asteraceae (Aster)	<i>Anaphalis margaritacea</i> (L.) Benth. & Hook.f.	Pearly Everlasting
Asteraceae (Aster)	<i>Coreopsis palmata</i> Nutt.	Prairie Coreopsis
Asteraceae (Aster)	<i>Rudbeckia hirta</i> L.	Black-Eyed Susan
Asteraceae (Aster)	<i>Symphyotrichum</i> <i>oblongifolium</i> (Nutt.) G.L.Nesom	Aromatic Aster
Fabaceae (Legume)	<i>Baptisia tinctoria</i> (L.) R.Br.	Small Yellow Wild Indigo
Fabaceae (Legume)	<i>Dalea purpurea</i> Vent.	Purple Prairie Clover
Lamiaceae (Mint)	<i>Monarda punctata</i> L.	Spotted Bee Balm
Poaceae (Grass)	<i>Panicum virgatum</i> L.	Switch Grass
Poaceae (Grass)	<i>Schizachyrium scoparium</i> (Michx.) Nash	Little Bluestem
Scrophulariaceae (Figwort)	<i>Agalinis tenuifolia</i> (Vahl)Raf.	Slender False Foxglove
Verbenaceae (Verbena)	<i>Verbena stricta</i> Vent.	Hoary Vervain
Violaceae (Violet)	<i>Viola sororia</i> f. <i>priceana</i> House	Common Blue Violet, Bi-Color

Table 2: Ecosystem function traits under simulated climate change (OTC) treatments and in control plots. P values represent significance ($p < 0.05$) of difference using the Wilcoxon signed rank non-parametric test

PLOT	LSA (mm²)	Leaf Biomass (mg)	SLA (mg/mm²)	ANPP (mg)	BNPP (mg)
All (mean)	696.2552	59.31451	19.908025	18.956645	278.0297
OTC (mean)	569.252	35.00968	26.53723	20.31047	273.7622
Control (mean)	796.2584	83.61935	13.27882	17.60282	282.2972
Difference	227.0064	48.60967	-13.25841	-2.70765	8.535
P-value (two tailed)	0.03313	0.006715	1.34E-07	0.8287	0.9025
P-value (one tailed)	0.01656	0.003358	6.71E-08	0.3207	0.4706

Table 3: Diversity indices of plant species found in the OTC and control plots.

** represent statistically significant ($p < 0.05$) differences.

DIVERSITY INDEX	OTC	CONTROL	Difference (Control-OTC)
Mean species richness	7.75	11.5	3.75**
Mean Shannon index	1.67	1.85	0.18
Mean Simpson diversity index	0.77	0.81	0.04
Beta diversity	1.32	0.67	-0.65

Appendix B: Figures

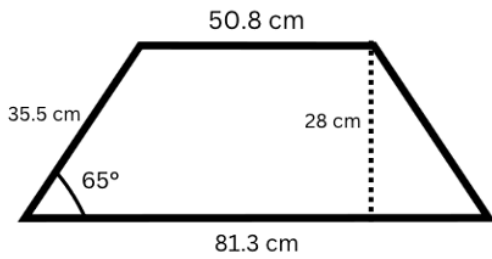


Figure 1: Model of OTC with dimensions in details

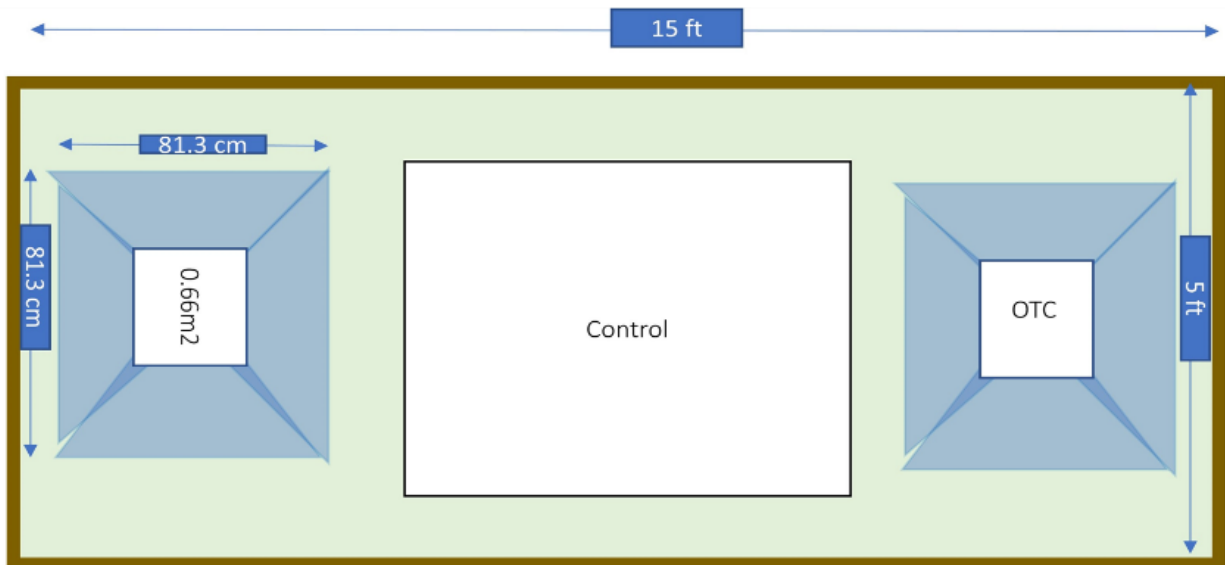


Figure 2: Model of a plot of the study site. On our site, we had this setup duplicated for a total of 4 treatment areas and 2 control areas.

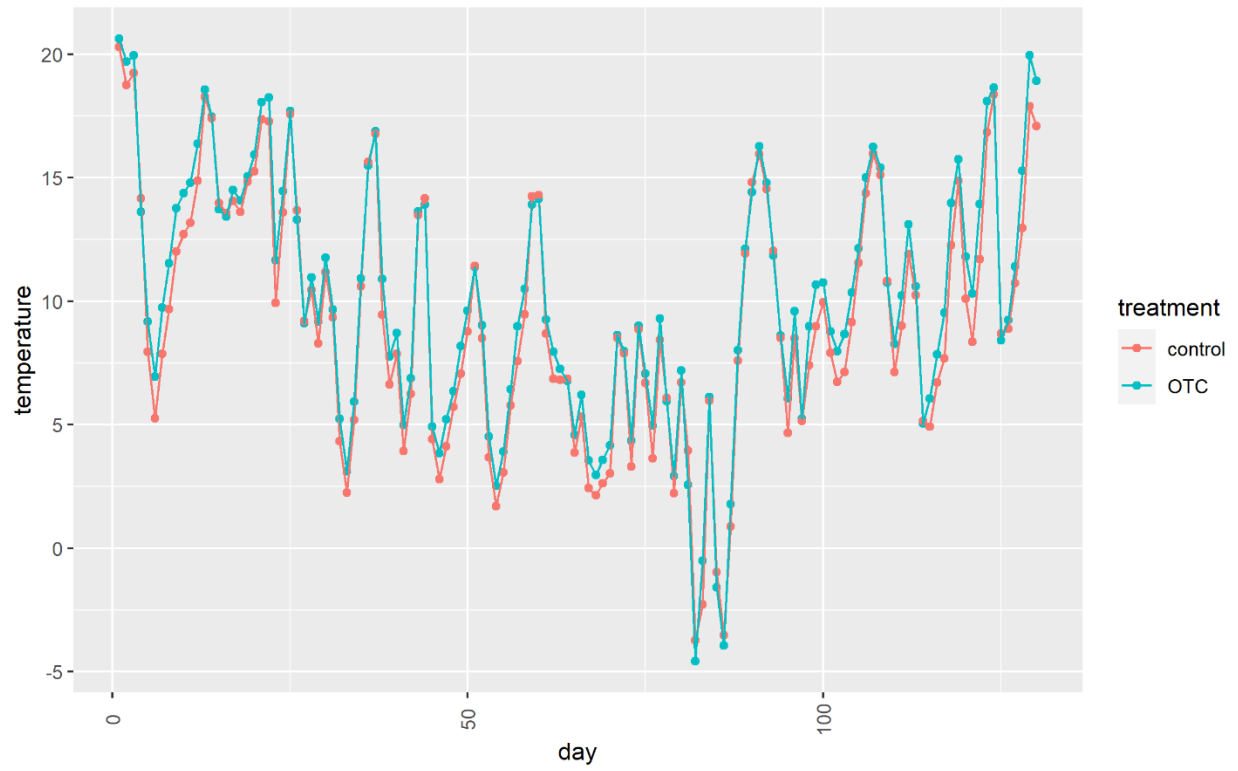


Figure 3: Daily average temperature recordings in OTC and control.

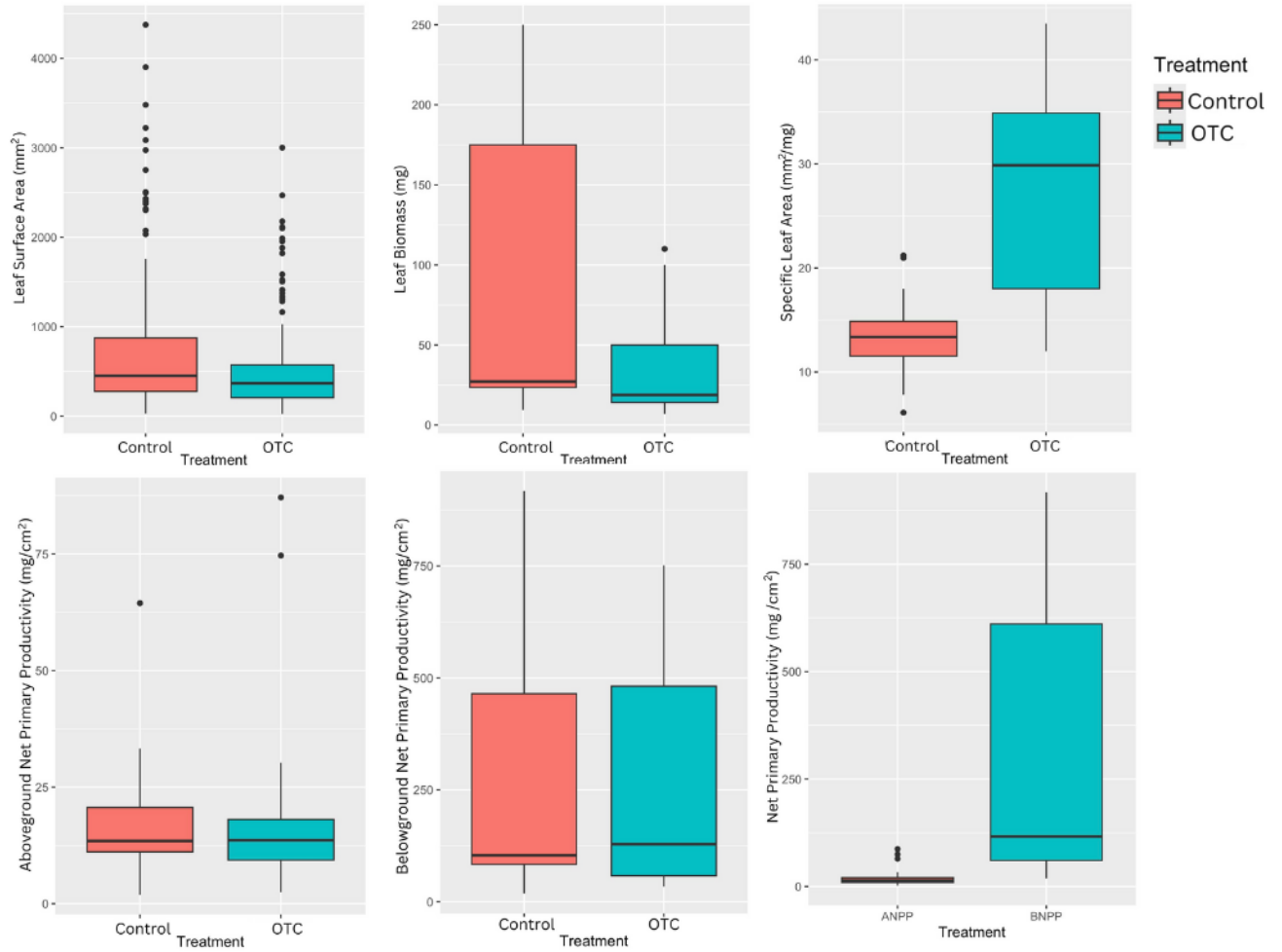


Figure 4: Boxplots of OTC vs Control for LSA, Biomass, SLA, ANPP, BNPP



Fig 5: Left: *Monarda punctata* (Spotted Beebalm) flowering outside the passive heating chambers (OTC). No individuals of the species were found in the OTC.

Fig 6: Right: *Rudbeckia hirta* (Black Eyed Susan) flowering in the OTC and control plots.

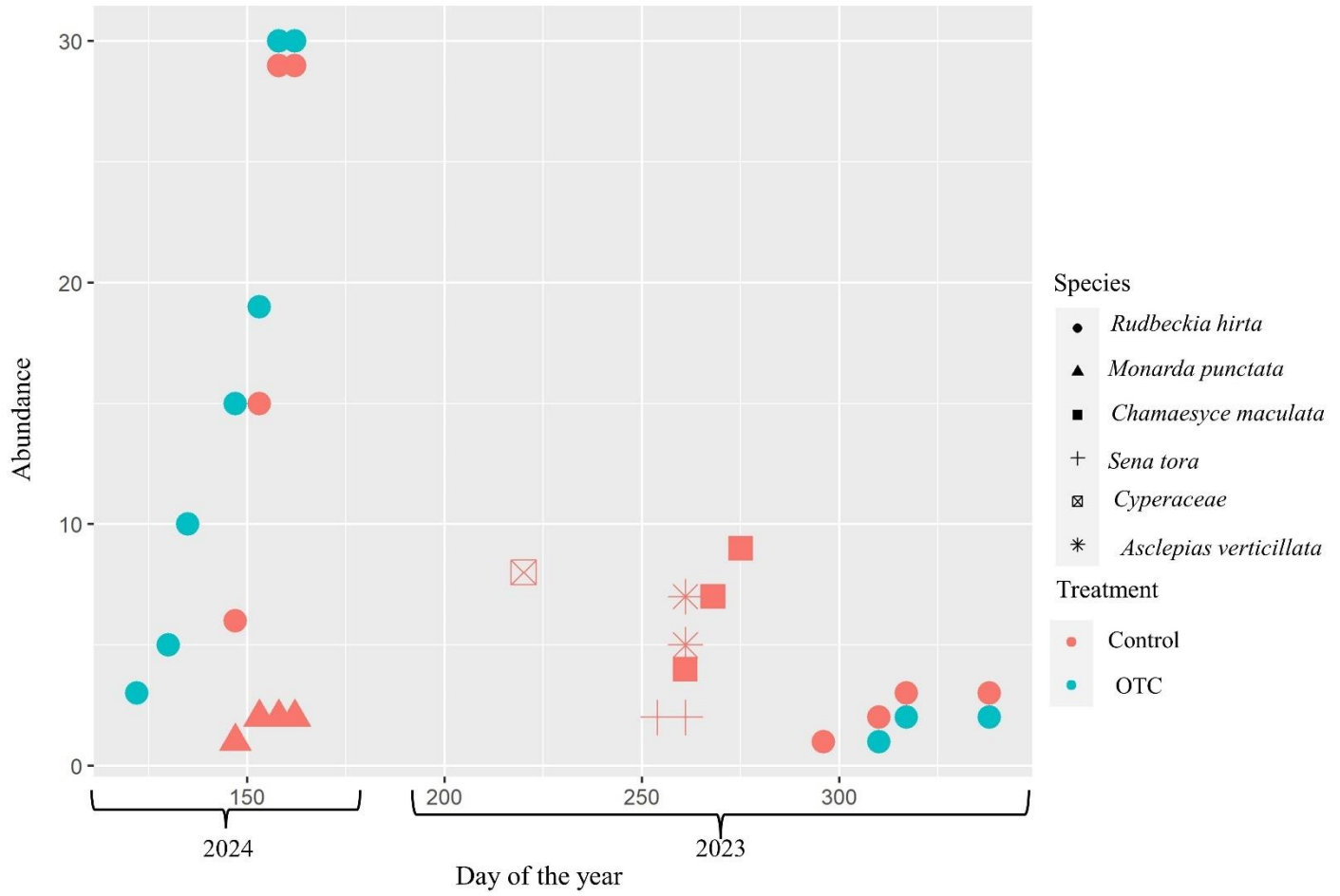


Figure 7: Flowering phenology of 6 species over a one-year period (Fall 2023 to Summer 2024).

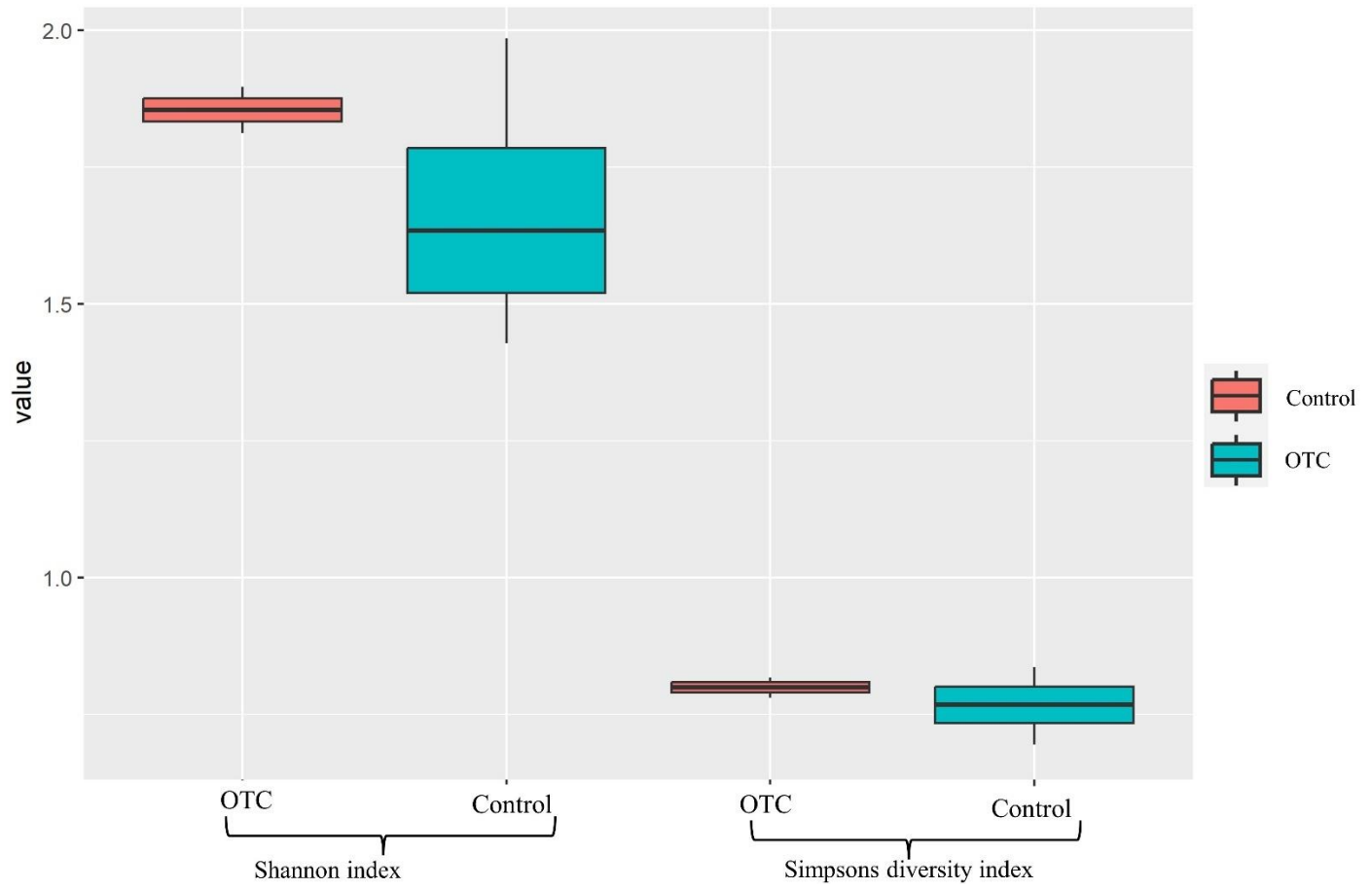


Fig 8: Boxplots representing diversity indices in OTC and control plots.

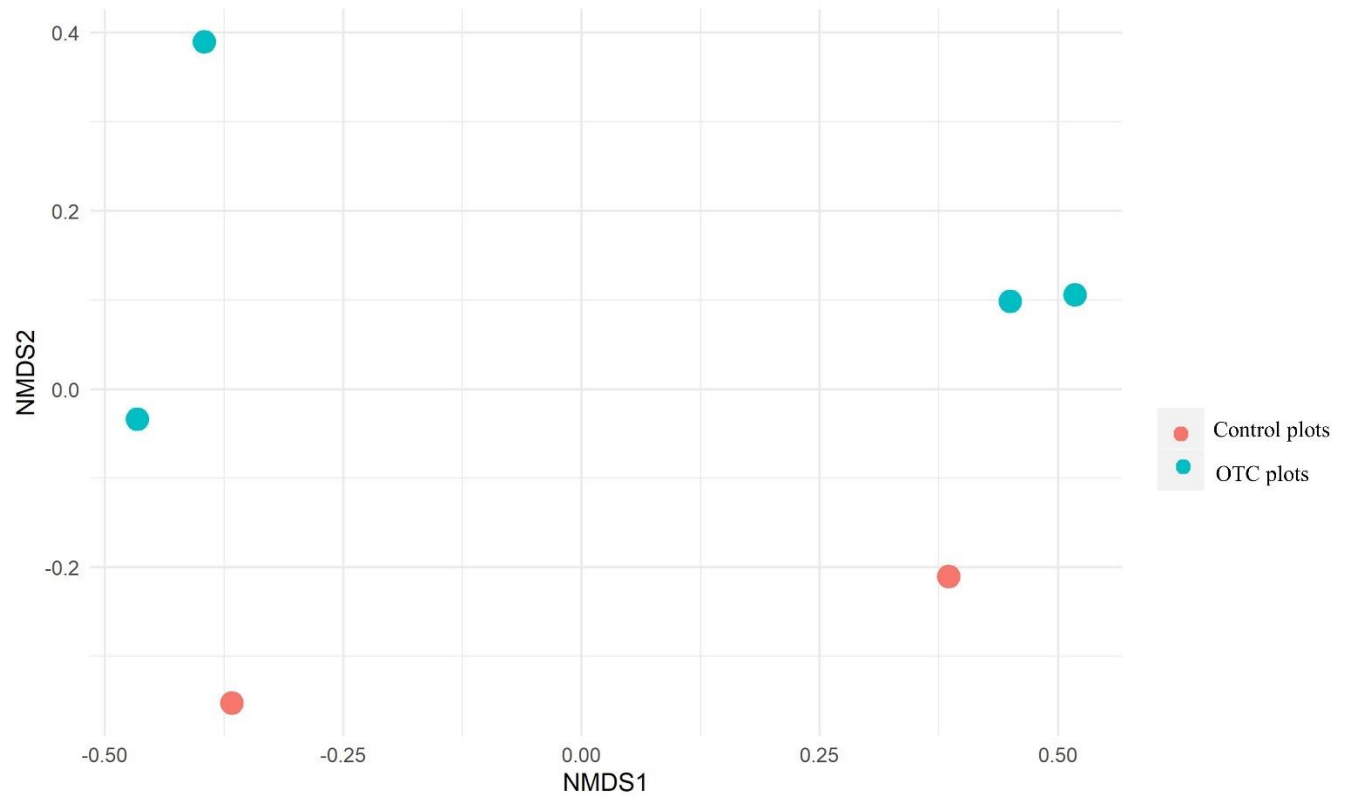


Figure 9: Results of a non-metric Multidimensional scaling (NMDS) multivariate analysis. The similarity matrix was displayed in an ordination plot using the Bray Curtis index.