SIMULATING THE IMPACTS OF CLIMATE CHANGE ON UH MĀNOA LETTUCE (*LACTUCA SATIVA* L.) GROWTH BY MODIFYING AIR TEMPERATURE, SOIL WATER AVAILABILITY, AND ATMOSPHERIC CO_2 CONCENTRATION

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I certify that I have read this thesis and that, in my opinion, it is satisfactory in scope and quality as a thesis for the degree of Bachelor of Science in Global Environmental Science.

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For my family, who have never waned in their support of everything I do.
For my mentor Dr. Camilo Mora, who has shown me the power and beauty of science.
For Kira Webster, for her invaluable mentorship and advice
For Kyle McDowell, for his amazing technical guidance and support
For Lentina Villa, Heather Saito, Michael Guidry, Brian Glazer, and the rest of the SOEST/GES team, who have guided me through my journey at UH Mānoa
Finally, for the *ʻāina* I call home and that inspired my research. The land and the people are one.

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ABSTRACT

Plant species are adapted to survive under specific ranges of air temperature, soil water availability, and atmospheric CO_2 concentration. Climate change-induced shifts in these environmental conditions have the potential to significantly affect nearly all terrestrial plants. Many studies have explored the impacts of changing one or two of the conditions listed above, but few have examined the combined effects of all three. To study the interactive effects of the three environmental conditions, 350 UH Mānoa lettuce (*Lactuca sativa* L.) plants were grown in indoor growth chambers. Within the chambers, plants were grown under ambient (~430-520 ppm) or elevated (700 ppm) atmospheric CO_2 concentration, 60-100% of maximum soil water content, and 20-36 °C air temperature for 21 days. At the end of this period, the leaves of each plant were removed, dried, and weighed. Percent plant mortality and leaf nitrogen content percentage were also measured. Across all combinations of temperature and water availability, elevated CO_2 concentrations resulted in increased leaf mass production and decreased percent mortality. Percent mortality increased and leaf mass production decreased under high temperatures and both high and low water availability. The combination of environmental conditions that produced the largest amount of biomass was 700 ppm CO_2, 80% of soil water capacity, and 24 °C while the treatment that produced the least amount was ambient CO_2, 60% of soil water capacity, and 36 °C. Nitrogen content percentage increased under high temperatures and high water availability. These results suggest that although increased atmospheric CO_2 levels have the potential to promote lettuce growth, lettuce yield is still likely to decrease in many regions due to the negative effects of high temperatures, drought, and flooding.

Keywords: Lettuce, heat stress, water stress, CO_2 enrichment

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1.0 INTRODUCTION

1.1 BACKGROUND

The terrestrial biosphere and human civilization are fundamentally dependent on plants. As the dominant primary producers in nearly all non-aquatic environments, plants provide the biomass and energy that terrestrial consumers and decomposers need to function. Through photosynthesis, plants provide approximately 50% of the global oxygen production every year (NOAA, 2023) and terrestrial forests and soils represent the second largest non-lithospheric carbon stock (Janowiak et al., 2017). The roots of grasses and trees mitigate erosion on both slopes and the coast (Zuazo & Pleguezuelo, 2009) and improve water capture and quality in many regions (Ernst et al., 2004; Perkins et al., 2004). From a human perspective, the wild and cultivated plants of the world provide people with food, building materials, consumer goods, medicine, clothing, and cultural enrichment. Plant based agriculture directly provides approximately 82% of the global calorie supply (FAO, 2020), takes up half of the world's habitable land area through plant farming and plant-based animal agriculture (FAO, 2020), and produces a total value of well over four trillion US dollars a year (FAO, 2020). Global forest products are worth approximately 244 billion US dollars a year (FAO, 2020) and approximately 90% of the nearly one million single-family houses built in the United States in 2021 were wood framed (USCB, 2021). Without healthy, stable plant communities, terrestrial biomes and by extension human societies cannot exist.

1.2 PLANTS AND CLIMATE CHANGE

Due to the overwhelming global importance of plants, any threats to plant life posed by climate change could have severe global consequences. All organisms are evolved to survive within certain ranges of environmental conditions and changes to these conditions can negatively impact the ability of an organism to survive within an environment (Raza et al., 2019). Plants require water, atmospheric CO_2, and a favorable temperature regime to survive, all of which can be significantly altered by the effects of climate change (Raza et al., 2019). This study explores the individual and collaborative impacts of all three of these conditions on the growth and survival of plants.

1.2.1 IMPACTS OF HIGH TEMPERATURES

High temperatures present a threat to a number of plant functions, including photosynthesis, the production of biomass and reactive oxygen species (ROS), and reproduction. During extreme heat events, plants are known to experience damage to chloroplast protein complexes, chlorophyll, and grana stacks, all of which are vital to the normal functioning of photosynthesis (Bita & Gerats, 2013; Feller & Vaseva, 2014; Hasanuzzaman et al., 2013; Song et al., 2014). This can be compounded by heat-induced alterations in the activity of vital enzymes such as Rubisco and chlorophyllase (Bita & Gerats, 2013; Feller & Vaseva, 2014). Due largely to this decrease in photosynthesis, many plant species have been observed to exhibit reduced rates of growth and biomass production under high heat conditions (Bita & Gerats, 2013). This reduction can lead to decreased yield in important food crops such as wheat, rice, and maize (Hasanuzzaman et al., 2013). Heat-induced damage to enzymes and chlorophyll can also lead to the accumulation of dangerous ROS, such as superoxide, hydrogen peroxide, and hydroxyl radicals (Bita & Gerats, 2013; Feller & Vaseva, 2014; Hasanuzzaman et al., 2013). The presence of these molecules inside cells can lead to the irreversible oxidation of proteins, DNA, and fatty acids (Nafees et al., 2019). During periods of flowering and seeding, high temperatures can lead to poor seed production or sterility due to disturbances in reproductive organ development, the fertilization process, and embryo growth (Bita & Gerats, 2013; Hasanuzzaman et al., 2013; Lamaoui et al., 2018). These disturbances are reflected in the seedlings of heat-impacted parent plants, which experience higher levels of mortality and lower growth rates (Hasanuzzaman et al., 2013). All of these factors contribute to reduced plant success, which can lead to rippling effects on ecosystems and human communities.

1.2.2 IMPACTS OF LIMITED WATER AVAILABILITY

Water stress due to drought can lead to many of the same impacts on plants as extreme heat, but not always for the same reasons. Leaf stomata often close under drought conditions to reduce evapotranspiration, but this also reduces CO_2 uptake and, by extension, the amount of CO_2 that is available for photosynthesis (Kaur & Asthir, 2017; Lawlor, 2002; Osakabe et al., 2014). Low water availability also limits photosynthesis through chloroplast disfiguration, pigment synthesis reduction, disruptions to the thylakoid electron transport chain and Calvin cycle and, similarly to heat stress, a general reduction in the effectiveness of photosynthetic enzymes (Kaur & Asthir, 2017; Lawlor, 2002; Osakabe et al., 2014). Many of these limitations can be partly attributed to the actions of ROS, which can also be created due to water stress-induced NADPH accumulation (Feller & Vaseva, 2014; Kaur & Asthir, 2017). As well as reducing carbon assimilation through impaired photosynthesis, water deprivation can limit plant growth by preventing water flow to new cells and inhibiting mitosis (Kaur & Asthir, 2017; Seleiman et al., 2021). This can lead to reductions in root and shoot development, leaf size, crop yield, and reproductive success (Feller & Vaseva, 2014; Kaur & Asthir, 2017; Seleiman et al., 2021). Extended exposure to drought conditions has the potential to cause widespread leaf surface area reduction, leaf loss, air bubble formation in xylem tissue, a complete cessation of photosynthesis, significant metabolism disruption, and potentially complete plant death (Feller & Vaseva, 2014; Lawlor, 2002; Kaur & Asthir, 2017).

1.2.3 IMPACTS OF ELEVATED CO_2 CONCENTRATIONS

Increased atmospheric CO_2 concentration has the potential to mitigate some of the negative effects of water and temperature stress, but only to an extent. Exposure to CO_2-enriched air has been found to stimulate increased photosynthesis in a wide variety of plant species, which promotes higher levels of growth and biomass production (Cernusak et al., 2019). This increase in primary production and carbon assimilation has the potential to slightly reduce the rate of rising CO_2 concentrations, although it is unlikely to be significant enough to provide any substantial mitigation of climate change (Amthor, 1995; Cernusak et al., 2019). Additionally, increased CO_2 concentrations have been found to improve plant water use efficiency (WUE), which refers to the amount of water that is lost through evaporation for every unit of CO_2 assimilated (Amthor, 1995; Cernusak et al., 2019; Lamaoui et al., 2018). Increased WUE could allow plants to survive regional drying trends and exhibit higher levels of primary production under low water conditions (Amthor, 1995; Cernusak et al., 2019). However, while improved WUE and photosynthetic capacity are clearly visible under ideal laboratory conditions, these improvements become less significant when other environmental factors such as competing species, nutrient limitation, and the soil microbiome are introduced (Ackerly & Bazzaz, 1995; Díaz et al., 1993; Parmesan & Hanley, 2015). Additionally, the effects of increased CO_2 have been found to decrease over time as plants gradually acclimate to higher concentrations (Amthor, 1995; Parmesan & Hanley, 2015; Urban, 2003).

1.2.4 COMBINED IMPACTS

The reason why it is important to study temperature, water availability, and CO_2 concentration simultaneously is because these three factors can have interacting impacts on the health of a plant. A clear example of this can be seen in the case of stomatal conductance. The stomatal conductance of a plant is a measure of the exchange of gases like CO_2 and water vapor through the stomata and is primarily controlled by the degree to which leaf stomata are opened or closed (Damour et al., 2010). Increased atmospheric CO_2 concentrations allow plants to obtain appropriate amounts of CO_2 with lower stomatal conductance and water loss, which is why increased CO_2 levels tend to improve plant WUE (Amthor, 1995; Cernusak et al., 2019; Lamaoui et al., 2018; Xu et al., 2013). Alternatively, stomatal closure due to severe drought conditions could partially negate any stimulatory effects of increased CO_2 (Kaur & Asthir, 2017; Xu et al., 2013). The problem of stomatal conductance can be complicated further by temperature extremes, which can promote stomatal closure or opening regardless of atmospheric CO_2 concentrations or water availability (Feller & Vaseva, 2014; Urban et al., 2017). The impacts on photosynthesis and metabolism caused by any of the three can also play a role in the control of stomata and other plant functions (Lamaoui et al., 2018). Complex biological reactions such as those that control stomatal conductance can be found in most species and can play an important role in the survival of an organism (Lamaoui et al., 2018). However, many studies into the potential impacts of climate change do not take these complexities into consideration (Lamaoui et al., 2018).

1.3 LETTUCE AS A MODEL SPECIES

Lettuce was selected as a model organism to explore the impacts of climate change on plant growth because of its global economic importance, small size, fast growth rate, and sensitivity to changing environmental conditions. Lettuce is widely grown on both industrial and small farms around the world and is a relatively inexpensive and nutritious food source (Shatilov et al., 2019). Additionally, over 26 million tons of lettuce is grown a year (Shatilov et al., 2019) and the total value of the 2020 global crop of lettuce and the related species chicory was approximately three billion US dollars (FAO, 2020). Lettuce is useful as a model organism for studying climate change because it is a relatively small, fast-growing plant that has been shown to be particularly sensitive to changes in temperature and water availability (Holmes et al., 2019; Jiménez-Arias et al., 2019). Any findings collected on lettuce could potentially be extended to other members of the Asteraceae family, which includes sunflowers, safflowers, chicory, and artichokes among at least 32,000 other species. However, it should be noted that different plant species can respond to climate change in very different ways and the response of lettuce to climate variation should not be expected to be exactly mirrored in other species (Parmesan & Hanley, 2015).

2.0 METHODS

2.1 MORA LAB GROWTH CHAMBERS

This experiment utilized the Mora Lab Intelligent Plant Growing System (IPS) growth chambers (see Figures 1 and 2), which are located in the UH Mānoa Department of Geography and Environment. Each of the ten chambers consists of an approximately 2 m by 1 m by 1 m metal frame enclosed by reflective insulating material and each can contain up to 40 two-liter plant pots in five rows that are suspended approximately 0.2 m above the bottom of the chamber. Lighting within the chambers is provided by 8 Unifun grow lights located approximately 0.5 m above the top of the pots. The air temperature, soil moisture, and CO_2 availability of each chamber can be individually controlled, allowing researchers to study the effects of changing climate conditions on plant growth. The mechanisms that control each of these parameters are described in the following subsections.

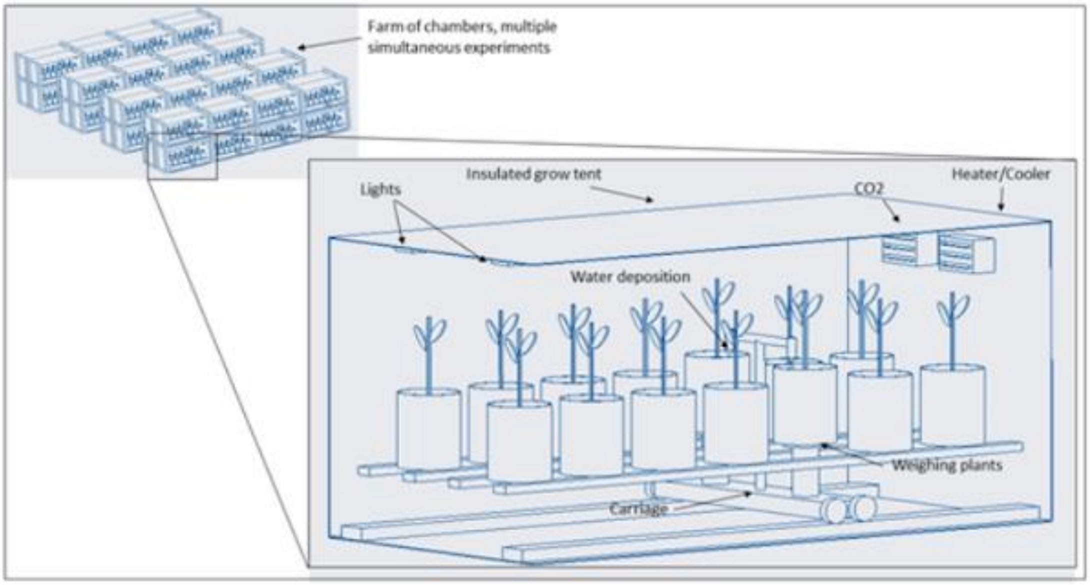


Figure 1: Simplified diagram of one of the Mora Lab Intelligent Plant Growing System (IPS) growth chambers, with key features labeled (Webster, 2021)

Figure 2: Picture of one of the Mora Lab Intelligent Plant Growing System (IPS) growth chambers with door open (Photo by author)

2.1.1 TEMPERATURE REGULATION

The temperatures of the chambers are regulated by custom-made IPS temperature controllers (Figure 3) (McDowell et al., 2021). Designed by Camilo Mora, Kyle McDowell, and Yang Zhong, each of these devices controls a small heater and air conditioner contained within the chambers as well as the day-night lighting cycle. When temperatures inside the chamber are below the set temperature - as determined by a built-in PT100 temperature probe - the controller turns on the heater for short bursts at regular intervals until temperatures reach the desired level. When temperatures are too high, the controller turns the air conditioner on steadily until chamber temperatures match the set temperature. To ensure a consistent temperature throughout the chamber, two fans provide constant air circulation.

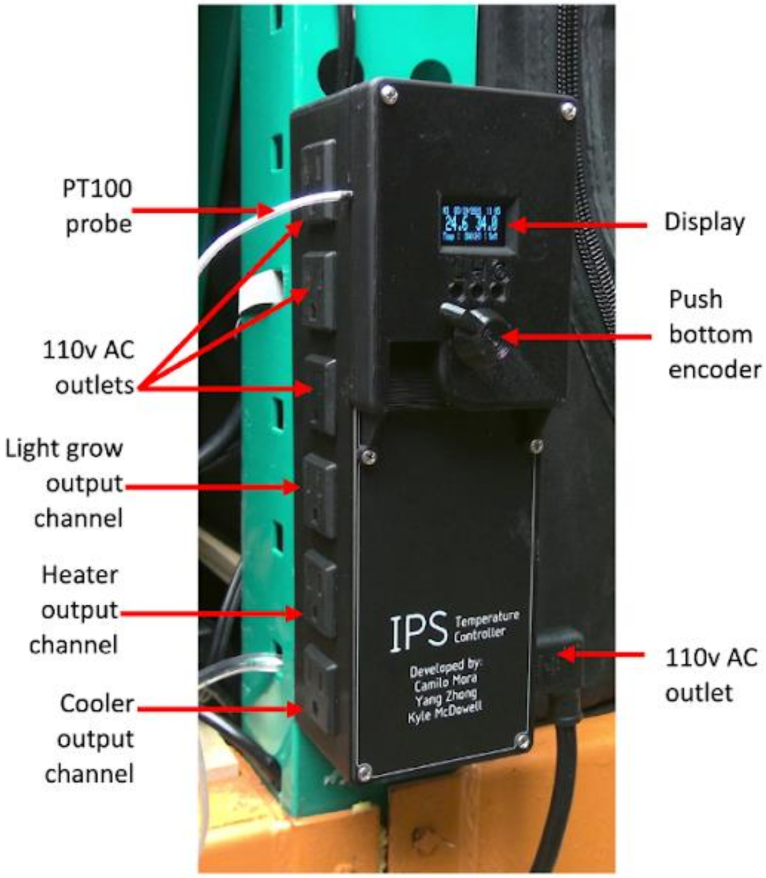


Figure 3: Picture of the IPS Temperature Controller, with key features labeled (McDowell et al., 2021)

2.1.2 WATERING GANTRY

The amount of water provided to the plants and thus the soil moisture within each pot was based on the field capacity of the growth medium. The field capacity of a soil is the maximum amount of water it can contain without the effects of gravity causing any of the water to drain downwards (Cassel & Nielsen, 1986). For this experiment, the field capacity of the media used - coarse vermiculite - was determined by saturating a known weight of media in a pot with drainage holes in the bottom, covering the top of the pot to prevent evaporation, and re-weighing the pot after 3 days. The initial and final weights were used to determine the weight of water that could be contained by a given volume of media. By determining the field capacity, the amount of water provided could be expressed as a fraction of the maximum weight of water the medium could contain.

The quantity of water added to each pot was controlled by the IPS robotic watering gantry, developed by Camilo Mora and a team of UH Mānoa mechanical and electrical engineers (Takara et al., 2021). This machine consists of a motorized rolling carriage that contains five load cells that control five water spigots, one for each row of pots. When the machine is switched on, it rolls parallel to the rows of pots until it detects the presence of a magnet, one of which is placed at the front of each column of pots. Upon stopping, the machine rises upwards and the five load cells determine the weight of each pot in the column. If the weight of a pot is below the programmed level, the spigot releases water into the pot until it reaches the desired weight. This process is repeated until every column has been weighed and watered, at which point the gantry returns to the starting point. For a simplified diagram of the operation of the machine, see Figure 4.

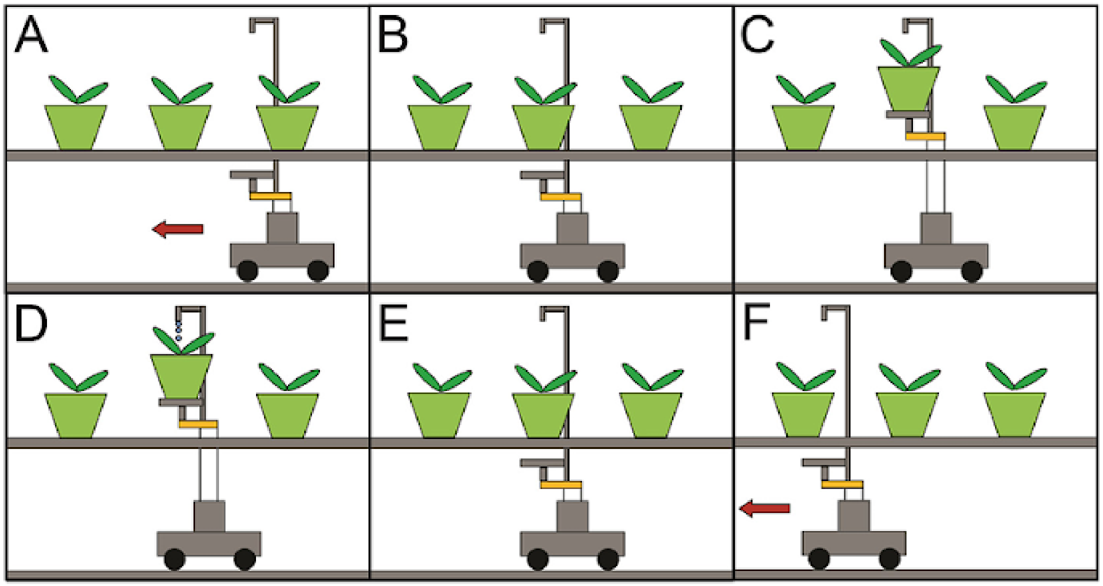


Figure 4: Simplified diagram depicting the watering gantry in action. Steps A-B show the gantry moving leftwards until it senses the magnet next to the column of pots shown in the middle. Steps C-D show the gantry weighing and then watering the pots. Steps E-F show the gantry lowering again and heading on to the next column. (Takara et al., 2021)

2.1.3. CO_2 REGULATION

Elevated atmospheric CO_2 concentrations were simulated by the addition of pure CO_2 gas from an external gas canister. The addition was controlled by a custom CO_2 regulator, again designed by Camilo Mora, Kyle McDowell, and Yang Zhong (see Figure 5). These devices constantly measure the CO_2 concentrations inside each chamber and add a short burst of gas whenever concentrations drop below the desired level. In the chambers without elevated CO_2 levels, a corner of the door of the chamber was left slightly open to allow the air in the chamber to interact with the external atmosphere. Because the ambient CO_2 concentration was influenced by the number of individuals in the laboratory building, levels in the ambient chambers ranged from 430-520 ppm daily.

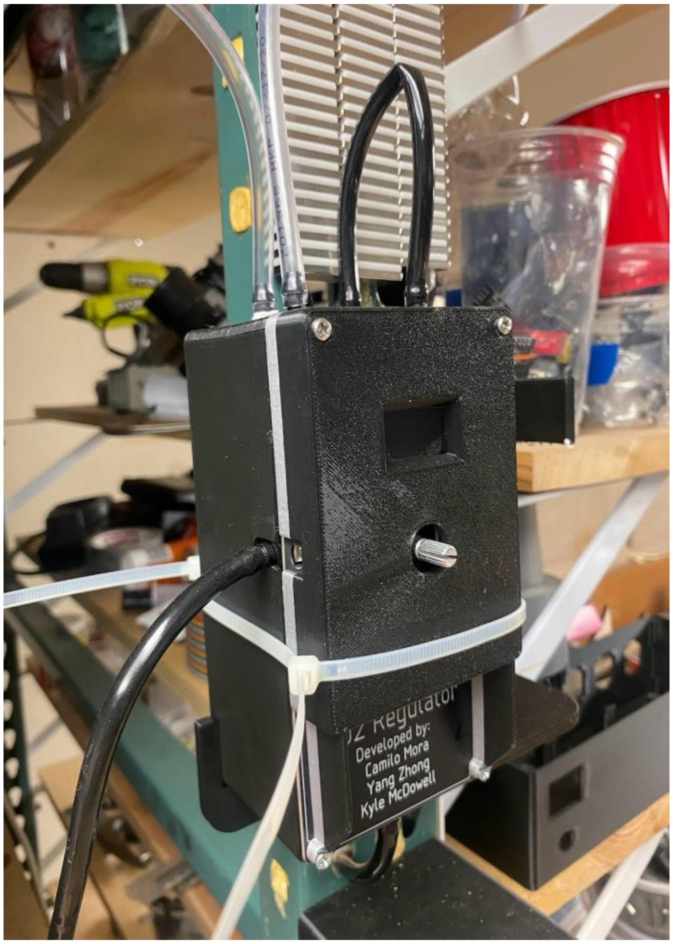


Figure 5: Picture of the Mora Lab CO_2 regulator

2.2 LETTUCE GROWTH PROCEDURES

For this experiment, UH Mānoa lettuce (*Lactuca sativa*) plants were grown under a range of potential climate change conditions. Lettuce seeds - purchased from the UH Mānoa CTAHR seed project - were sprouted in rock wool cubes set in a shallow tray of water under a 12-hour day-night cycle at approximately 20 ^{o}C. After 10 days, both the seedlings and the intact rock wool cubes were transplanted to 2 liter pots of coarse vermiculite in the growth chambers. All plants were approximately the same size and age upon planting. Before planting, 10 grams of Island Supreme 16-16-16 controlled release NPK fertilizer (Pacific Agricultural Sales & Services, Kapolei, HI) was mixed in with the top three centimeters of vermiculite in each pot. After allowing the seedlings to acclimate to the pots for one week under a 12-hour day-night cycle, approximately 20 ^{o}C, and the range of soil moisture levels that would be used in the experiment, the chambers were closed and the temperature and CO_2 conditions in each were set. Of the ten chambers, half were left at ambient CO_2 concentration, which ranged from 430-520 ppm daily. CO_2 concentrations in the other five chambers were elevated to 700 ppm, meant to represent global CO_2 concentrations in 2100 under moderate emissions (SSP2-4.5) (IPCC, 2021). Within each group of five chambers, the daytime temperatures of the chambers were set at 4 degree intervals from 20 °C to 36 °C. At night, temperatures inside each chamber were decreased by five degrees. Within each chamber, each row of pots was given a different degree of soil moisture, ranging from 100% of field capacity to 60% of field capacity. Daily watering and a 12-hour day-night cycle were maintained for the duration of the experiment. Once these conditions were established, the lettuce plants were allowed to grow for 21 days before harvesting.


Figure 6: Lettuce growing in the chambers

2.3 DATA COLLECTION

The three variables that were recorded in this experiment were plant mortality, leaf dry biomass, and percent leaf nitrogen content. Throughout the experiment, plant mortality percentage across all treatments was recorded regularly by visually examining coloration. Plants that no longer exhibited any traces of green coloration were recorded as deceased. After 21 days, each plant was cut at the base and all aboveground biomass was collected, dried for 24 hours at 70 °C in a Fisher Scientific Isotemp Oven (Pittsburgh, PA), and weighed with a Ohaus Adventurer scientific scale (Parsippany, NJ). Following this process, three samples from different plants within each experimental group (each unique combination of temperature, water availability, and CO_2 treatments) were sent to the UH Mānoa Agricultural Research center to be tested for percent nitrogen content, which can be used as a proxy for percent protein content. For experimental groups that had less than 3 living plants remaining by the end of the experiment, all remaining plants were tested. After data collection was complete, the information we collected on mortality, dry leaf biomass, and protein content was processed via regression analysis in the R studio statistical and visualization program.

Figure 7: Harvested lettuce samples

Figure 8: Lettuce samples before and after drying for 24 hours at 70 ^{o}C

3.0 RESULTS

3.1 STATISTICAL ANALYSES

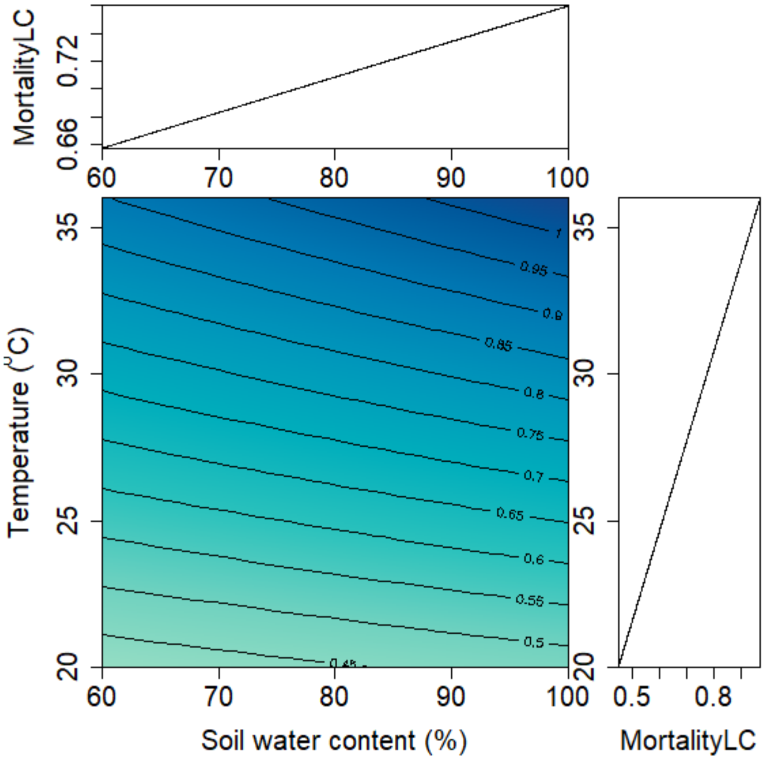
Regression analysis found that, although most predictor variables used in this experiment were not statistically significant, the regression models fit the data in all six models in Table 1. The model comparing the effects of water availability and temperature on dry leaf biomass under elevated CO_2 concentrations was the only model in which the predictor variables had p-values under 0.05. In all other models, the p-values for the t-statistics of the predictor variables ranged from 0.05 to 1, indicating a lack of statistical significance for individual variables. However, the p-values for the F-statistics of all six regression models were less than 0.05, indicating that all six models were generally accurate representations of the expected values of the response variables.

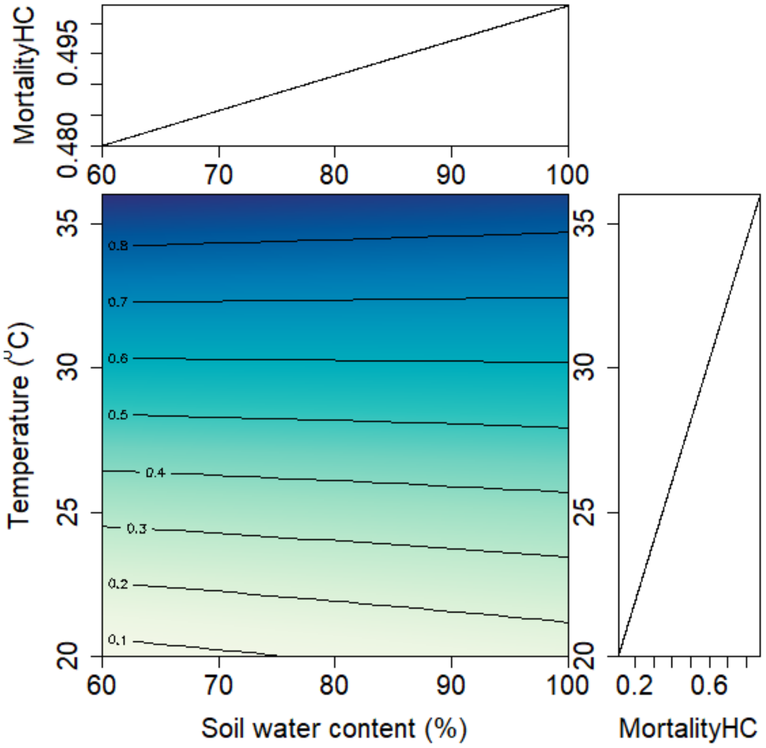
Table 1: The 6 models and associated figures used in the experiment.

Model	Associated Figure
Mortality vs. temperature and water availability under ambient CO_2 concentration	Figure 9
Mortality vs. temperature and water availability under elevated CO_2 concentration	Figure 10
Dry leaf biomass vs. temperature and water availability under ambient CO_2 concentration	Figure 16
Dry leaf biomass vs. temperature and water availability under elevated CO_2 concentration	Figure 17
Percent nitrogen content vs. temperature and water availability under ambient CO_2 concentration	Figure 22
Percent nitrogen content vs. temperature and water availability under elevated CO_2 concentration	Figure 23

3.2 MORTALITY

The effects of the environmental conditions studied in this experiment on plant survival are compiled in the two figures below. Mortality was defined as the lack of any green coloration in the leaves of the plant.


MortalityLC
0.66
0.72
60
70
80
90
100
35
30
25
20
Temperature (°C)
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
Soil water content (%)
0.5
0.8
MortalityLC


MortalityHC
0.480
0.495
60
70
80
90
100
35
30
25
20
Temperature (°C)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Soil water content (%)
0.2
0.6
MortalityHC

Figures 9 & 10: The central figures show the impacts of temperature and water availability on lettuce mortality under ambient (LC) and elevated (HC) CO_2 concentrations. Darker colors represent higher mortality rates. See contour lines for specific mortality rates, which range from 0 for complete mortality to 1 for complete survival. The smaller graphs to the right of and above the main figures depict the effects of temperature and water content respectively on mortality.

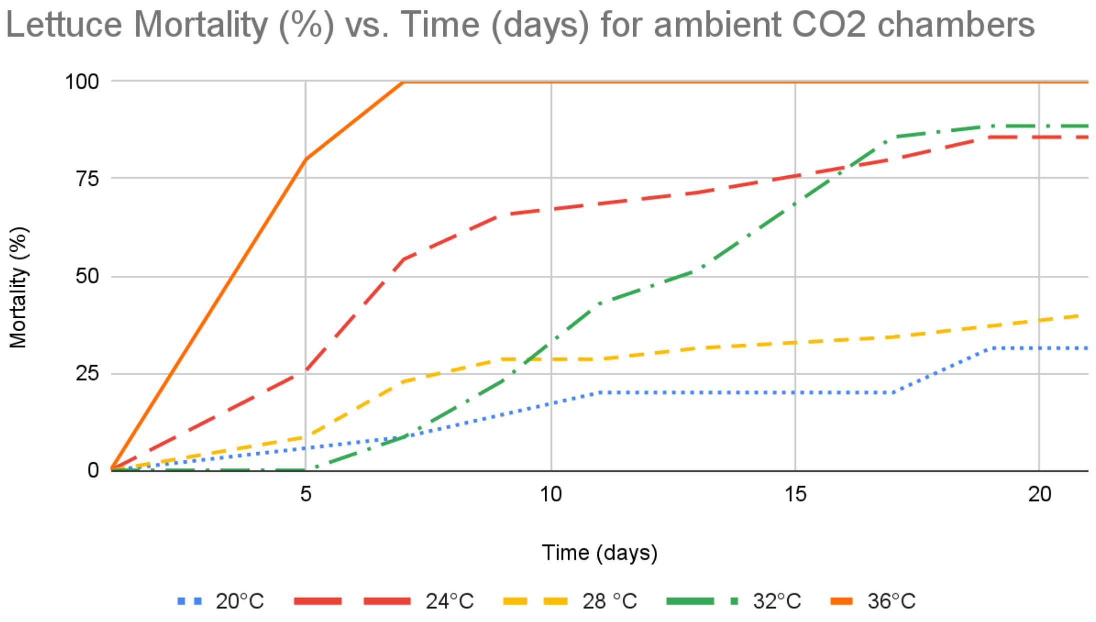
As seen in Figure 9, the highest survival rates were found under low temperatures and low water availability. Mortality increases at a relatively linear rate as both temperature and water availability increase. In Figure 10, mortality also increases at a linear rate with increasing temperatures, but soil water availability has a very limited impact on mortality. Between the two figures, it should be noted that overall mortality was lower under elevated CO_2 concentrations than under ambient conditions.

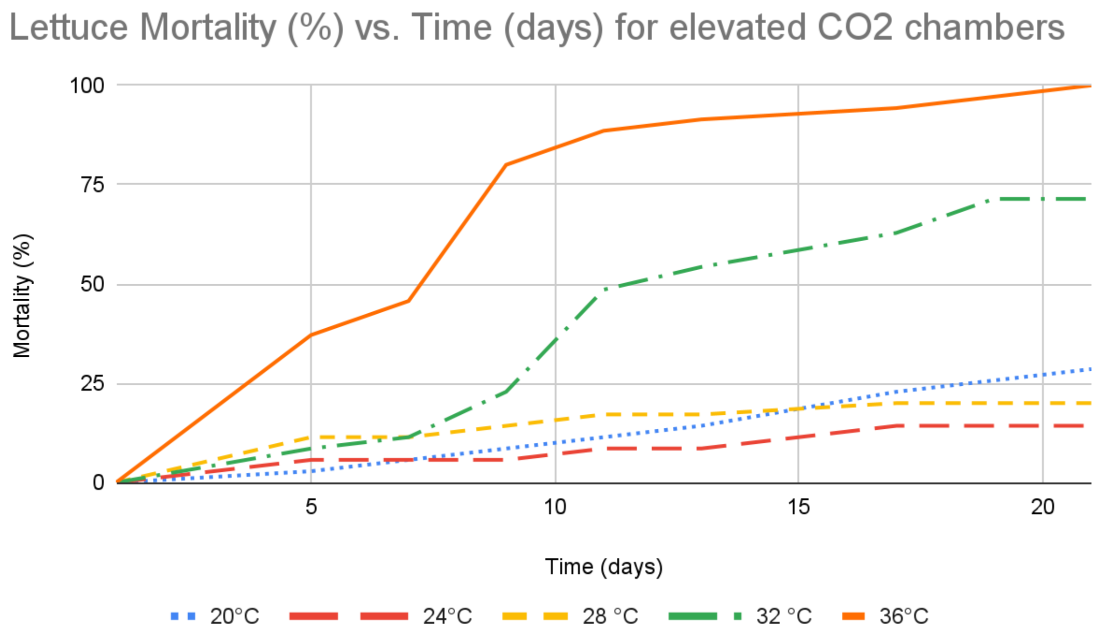
3.2.1 RELATIONSHIP WITH TEMPERATURE

High mortality rates occurred across most chambers used in this experiment, with mortality generally increasing with daytime temperature. However, although Figures 9 and 10 imply a linear relationship between temperature and mortality, neither the ambient nor elevated CO_2 chambers experienced a consistent increase in mortality with increasing temperatures. For the ambient CO_2 chambers, both the 32 °C chamber and the 24 °C experienced 89% mortality, yet the 28 °C chamber had 43% mortality. The lowest mortality rate under ambient CO_2 was 34% in the 20 °C chamber. For the elevated CO_2 chambers, the highest mortality rate (100%) occurred in the 36 °C chamber while the lowest rate (14%) occurred in the 24 °C chamber. The mortality rate of the 20 °C chamber (34%) was higher than in both the 24 °C and 28 °C chambers. Both ambient and elevated chambers set at 36 °C experienced 100% mortality, although it took the elevated chamber 14 days longer to reach 100%. See Table 1 and Figures 11 and 12 for a complete comparison of temperature and mortality at the end of the experiment and over time.

Table 2: Percent mortality of lettuce plants at ambient or elevated CO_2 concentrations under different air temperature regimes

Daytime temperature (°C)	Final percent mortality at ambient CO_2 (%)	Final percent mortality at elevated CO_2 (%)	Increase between elevated and ambient CO_2 (%)
36	100	100	0
32	89	74	125
28	43	23	35
24	89	14	650
20	34	34	0





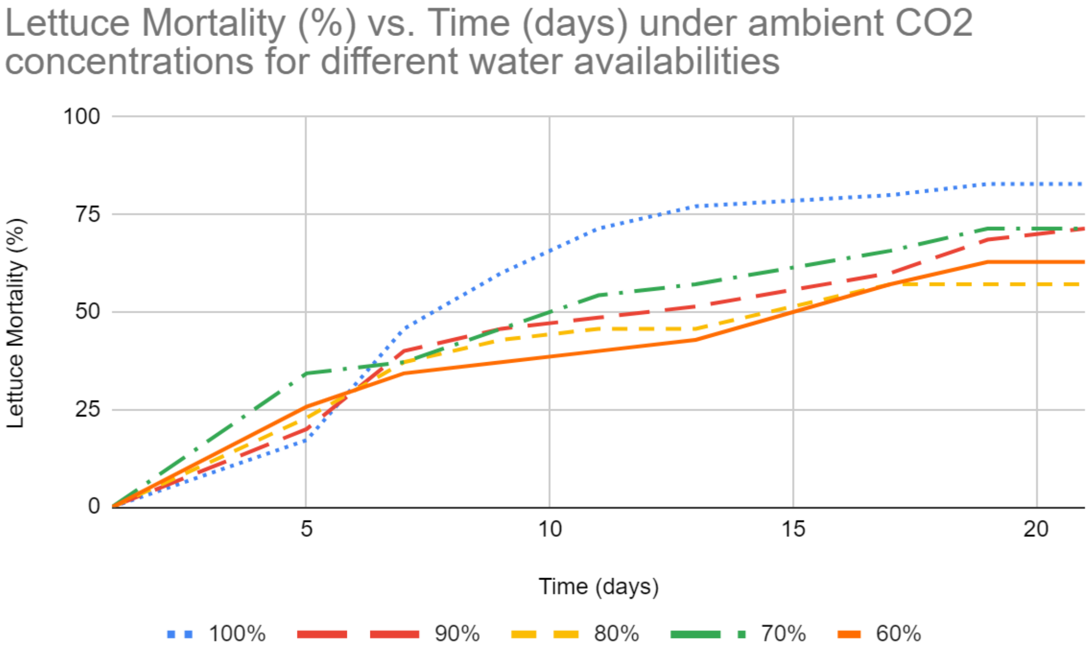
Figures 11 & 12: Line graphs showing percent mortality over time (days) for chambers set at different temperatures. Data for ambient CO_2 chambers is on the upper graph and data for elevated CO_2 chambers is on the lower graph.

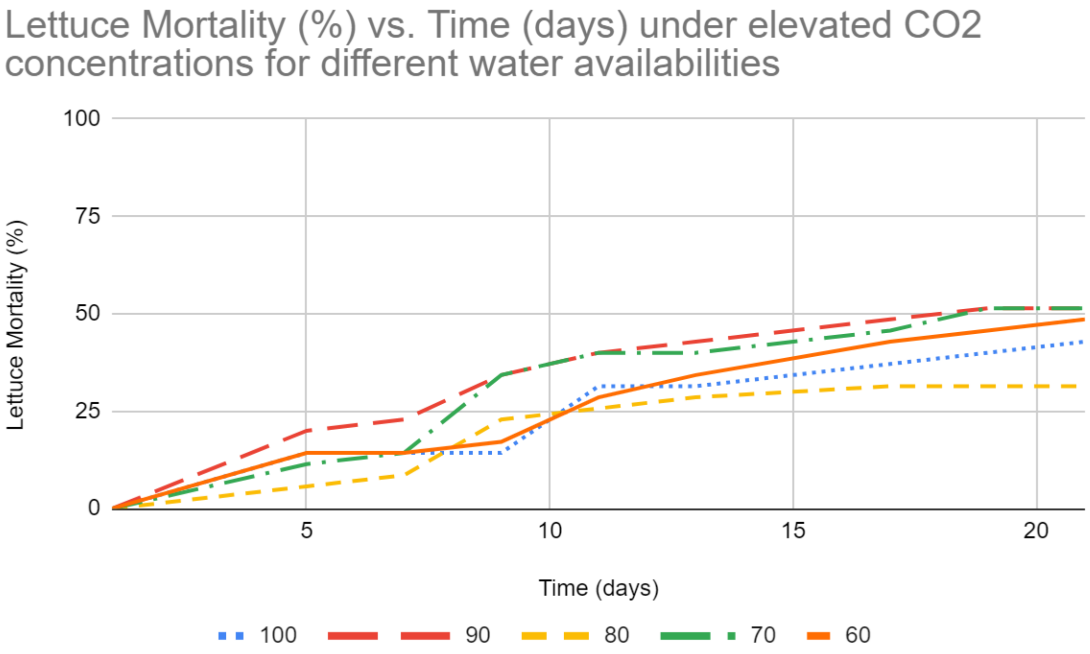
3.2.2 RELATIONSHIP WITH SOIL WATER CONTENT

Soil water content had a very small effect on lettuce mortality in the elevated CO_2 chambers, with a slight increase (<2%) in mortality as water availability increased from 60% to 100%. This effect was only somewhat more pronounced in the ambient CO_2 chambers, where plants experienced a moderate (18%) increase in mortality as water availability increased. See Table 2 and Figures 13 and 14 for a comparison of mortality over time for plants grown under different soil water content levels.

Table 3: Percent mortality of lettuce plants at ambient or elevated CO_2 concentrations under different water availability regimes.

Soil water availability	Final percent mortality at ambient CO2 (%)	Final percent mortality at elevated CO2 (%)	Increase between elevated and ambient CO2 (%)
100%	83	43	93
90%	71	51	39
80%	57	31	84
70%	71	51	39
60%	63	49	29





Figures 13 & 14: Line graphs showing percent mortality over time (days) for chambers set at different levels of soil water availability. Data for ambient CO_2 chambers is on the upper graph and data for elevated CO_2 chambers is on the lower graph.

3.2.3 RELATIONSHIP WITH CO_2 CONCENTRATION

Chambers with elevated CO_2 generally experienced lower mortality rates than those with ambient CO_2 concentrations, with an average mortality rate of 45% compared to the 69% average mortality found in ambient chambers. However, the numerical relationship between the mortality rates of elevated and ambient chambers varied widely, as described in section 3.2.1 and Table 1. See Figure 15 for a comparison of mortality and CO_2 concentration over time.

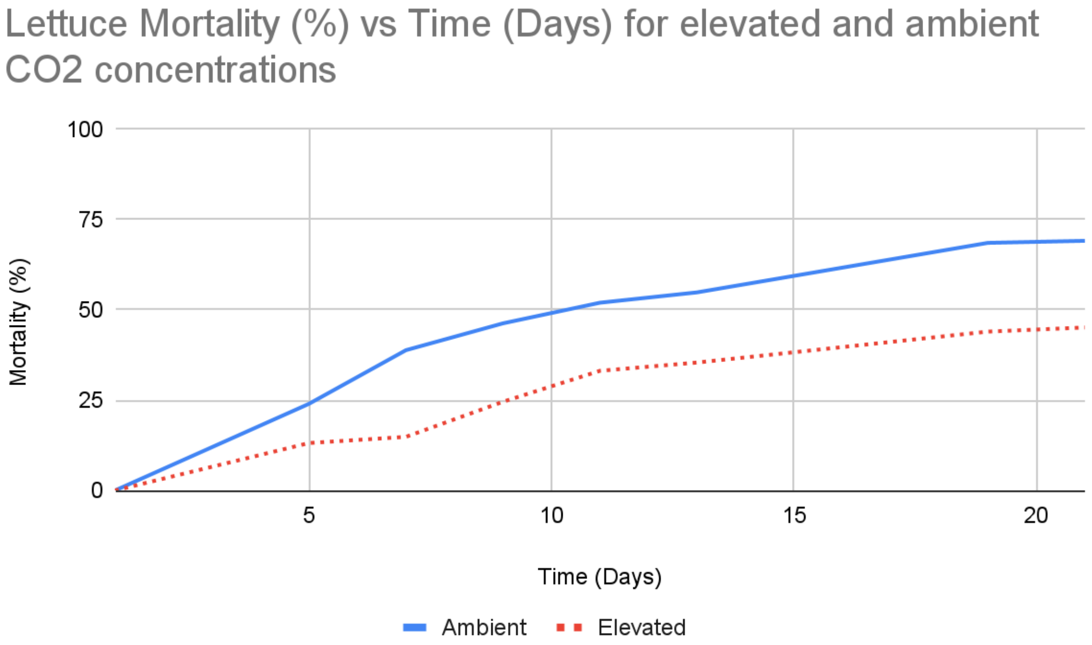
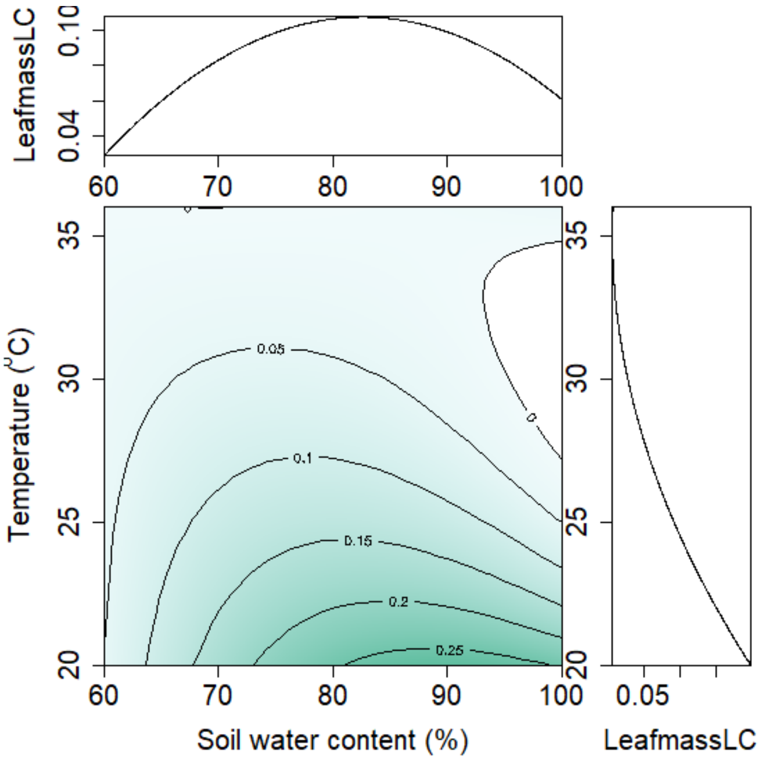
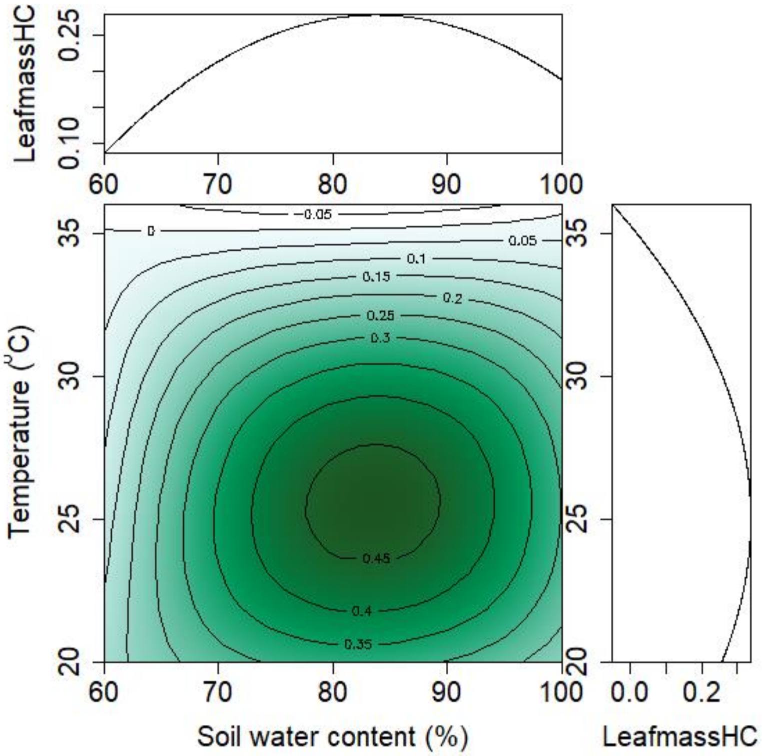


Figure 15: Line graphs showing average percent mortality over time (days) for chambers set at ambient and elevated CO_2 concentrations.

3.3 LEAF MASS

The effects of the environmental conditions studied in this experiment on lettuce leaf dry biomass are compiled in the two figures below.


LeafmassLC
0.04
0.10
60
70
80
90
100
Temperature (°C)
20
25
30
35
0.05
0.1
0.15
0.2
0.25
0
Soil water content (%)
0.05
LeafmassLC


LeafmassHC
0.10
0.25
60
70
80
90
100
Temperature (°C)
20
25
30
35
-0.05
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
Soil water content (%)
0.0
0.2
LeafmassHC

Figures 16 & 17: The central figures show the impacts of temperature and water availability on lettuce dry lettuce leaf biomass under ambient (LC) and elevated (HC) CO_2 concentrations. Darker colors represent higher leaf mass. Values on the contour lines are given in grams. It should be noted that dead plants were included in the leaf mass data and listed as having zero mass, meaning that the masses indicated by the contour lines are lower than they would be otherwise. The smaller graphs to the right of and above the main figure depict the effects of temperature and water content respectively on leaf mass.

In Figure 16, leaf mass was highest at low temperatures and medium-high water availability. In Figure 17, leaf mass was highest at medium temperature and water availability. In both figures, both temperature and water availability had significant impacts on leaf biomass production, with temperature having a larger impact than water availability. Between the two figures, leaf mass was generally much higher under elevated than ambient CO_2 concentrations.

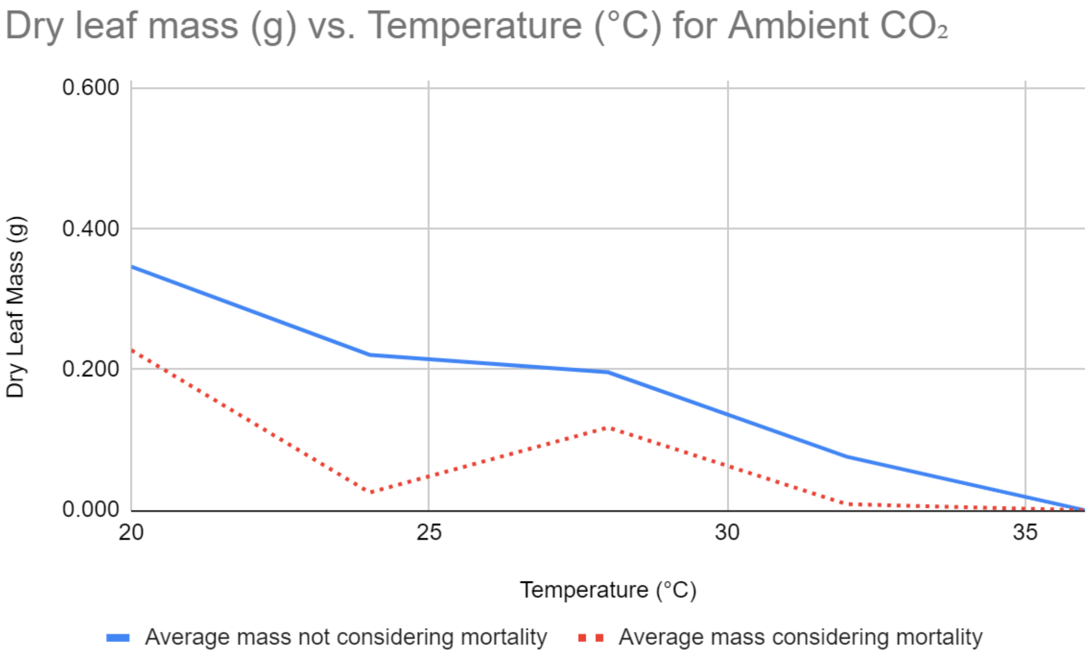
3.3.1 RELATIONSHIP WITH TEMPERATURE

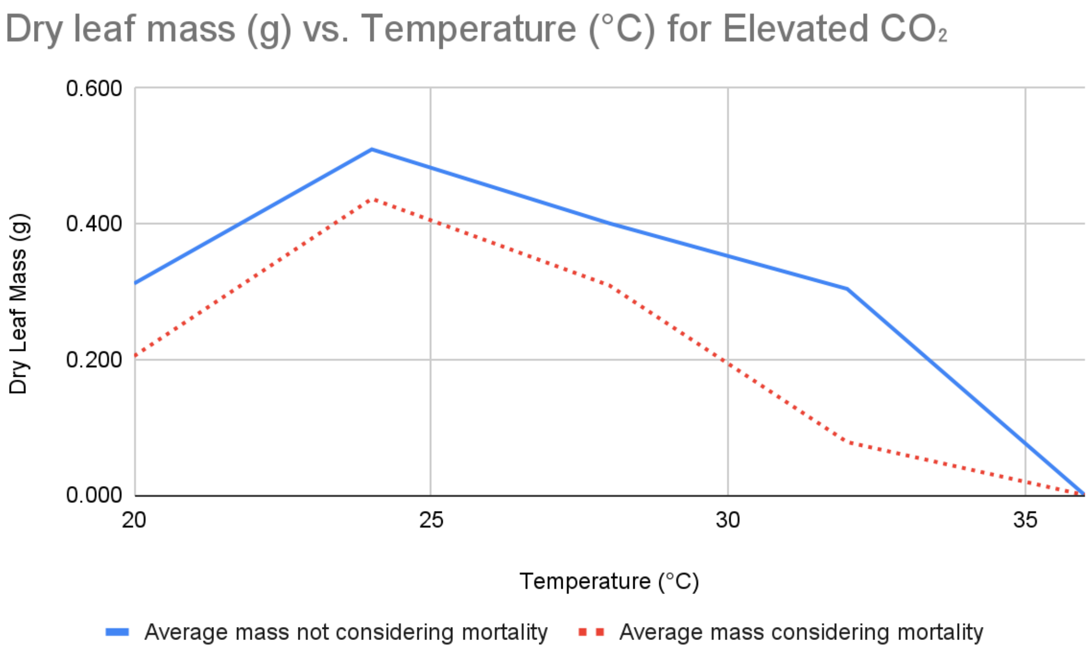
In ambient chambers, when considering the average dry lettuce leaf biomass of surviving plants grown under different temperatures, mass decreased with increasing temperature. The highest average leaf mass of plants grown in ambient chambers was 0.346 g in the 20 °C chamber, a value that consistently decreased with increasing temperature until reaching 0 g in the 36 °C chamber. When including dead plants - which are considered to have a leaf mass of zero - in the data, leaf mass still declined with increasing temperature, but with less consistency. Most notably, there is a large dip in leaf mass at 24 °C due to high mortality in the 24 °C chamber, even though the average mass of living 24 °C plants was the second highest of all plants grown in ambient chambers.

In elevated chambers, the average dry lettuce leaf biomass of surviving plants was highest in the 24 °C chamber (0.510 g) and decreased with increasing temperature until reaching zero at 36 °C. The average leaf mass of surviving plants grown at 20 °C (0.312 g) was approximately equal to the average leaf mass of surviving plants grown at 20 °C (0.304 g). This trend is generally mirrored when including dead plants in the calculations, although there is a dip in the data at 32 °C due to high mortality in the 32 °C chamber. When comparing chambers with elevated CO_2 concentrations with ambient chambers set at the same temperature, almost all elevated chambers have larger average leaf masses, regardless of whether dead plants are included or not. The only exceptions to this trend are the two 20 °C chambers, as the average leaf mass of the ambient 20 °C chamber is slightly higher than that of the elevated 20 °C chamber.

Table 4: Average dry lettuce leaf biomass from elevated and ambient CO_2 chambers set at different temperatures. When included in the average, dead plants are given a leaf mass of 0 g.

	Ambient CO_2		Elevated CO_2	
Temperature (°C)	Average dry lettuce leaf biomass (g)	Average dry lettuce leaf biomass (g), including dead plants	Average dry lettuce leaf biomass (g)	Average dry lettuce leaf biomass (g), including dead plants
20 °C	0.346	0.228	0.312	0.205
24 °C	0.221	0.025	0.510	0.437
28 °C	0.196	0.118	0.401	0.309
32 °C	0.076	0.009	0.304	0.078
36 °C	0.000	0.000	0.000	0.000





Figures 18 & 19: Relationship between dry lettuce leaf biomass and temperature under ambient (top) and elevated (bottom) CO_2 concentrations. In both figures, the solid line represents the average dry lettuce leaf biomass of plants in chambers set at different temperatures (20 °C, 24°C, etc.) while the dotted line represents the same data but with dead plants included in the calculation. Dead plants are considered to have zero leaf mass.

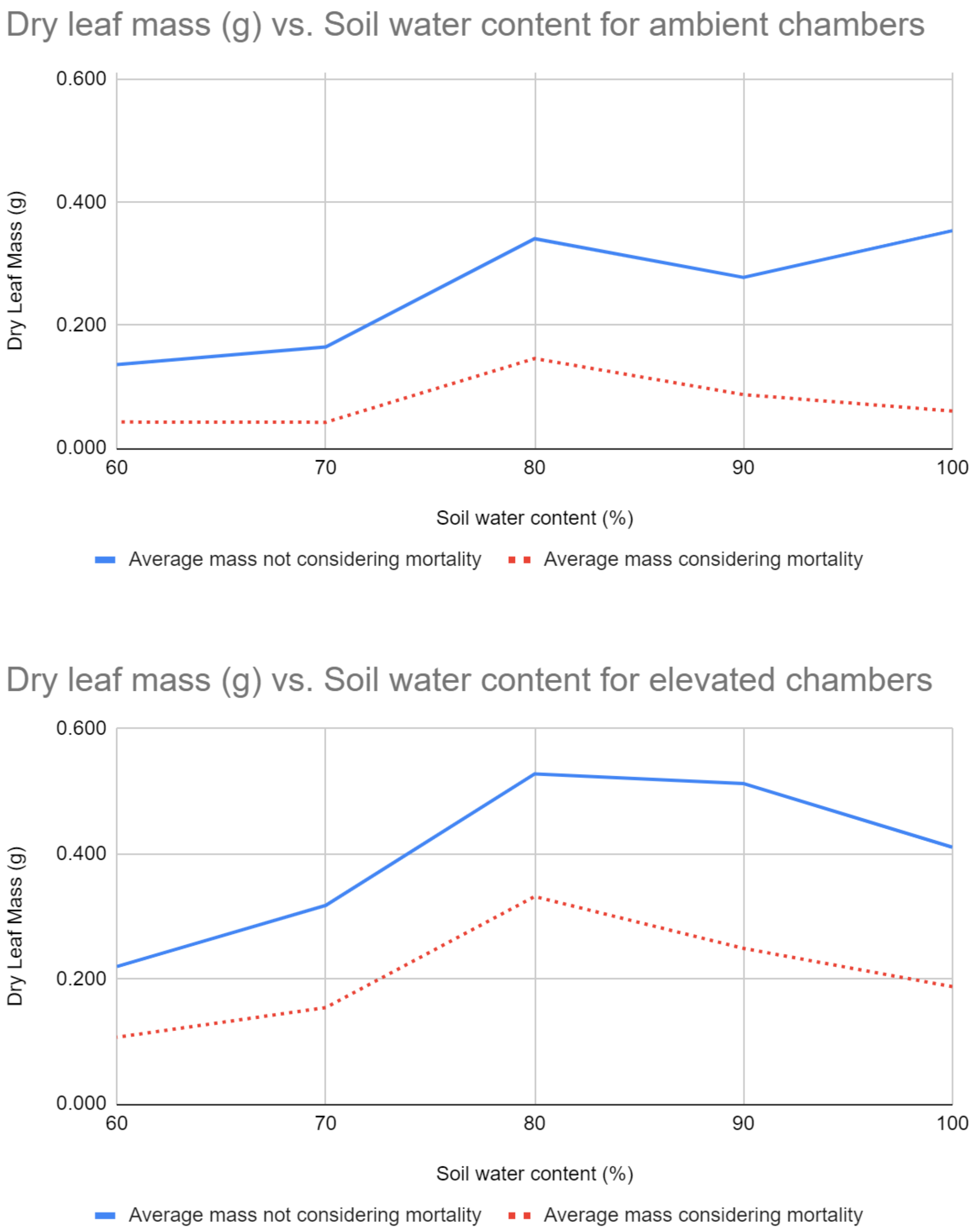
3.3.2 RELATIONSHIP WITH SOIL WATER CONTENT

In chambers with ambient CO_2 concentrations, dry leaf biomass generally increased with increasing soil water content, with the highest average biomass (0.354 g) occurring at 100% field capacity and the lowest (0.136 g) occurring at 60% field capacity. However, when the high levels of mortality under 100% field capacity are taken into consideration by including zero values for dead plants, the highest average biomass (0.146 g) occurred at 80% field capacity. Lettuce grown in chambers with elevated CO_2

followed a similar pattern, with the highest dry biomass occurring at 80% field capacity regardless of whether mortality is considered in the calculation or not. The highest average dry biomass of living plants in the elevated chambers was 0.528 g and the highest average dry biomass when considering mortality was 0.332 g, both of which occurred at 80% field capacity. It should be noted that in both ambient and elevated chambers and whether or not mortality was included, plants grown at 60% field capacity always had a lower average dry biomass than plants grown at 100% field capacity.

Table 5: Average dry lettuce leaf biomass of elevated and ambient CO_2 chambers set at different soil water content levels. When included in the average, dead plants are given a leaf mass of 0 g.

	Ambient CO_2		Elevated CO_2	
Soil water content (%)	Average dry lettuce leaf biomass (g)	Average dry lettuce leaf biomass (g), including dead plants	Average dry lettuce leaf biomass (g)	Average dry lettuce leaf biomass (g), including dead plants
100	0.354	0.061	0.411	0.188
90	0.278	0.087	0.512	0.249
80	0.341	0.146	0.528	0.332
70	0.165	0.042	0.318	0.154
60	0.136	0.043	0.220	0.107



Figures 20 & 21: Relationship between dry lettuce leaf biomass and water availability under ambient (top) and elevated (bottom) CO_2 concentrations. In both figures, the solid line represents the average dry lettuce leaf biomass of plants in chambers set at different soil water percentages (100%, 90%, etc.) while the dotted line represents the same data but with dead plants included in the calculation. Dead plants are considered to have zero leaf mass.

3.3.3 RELATIONSHIP WITH CO_2 CONCENTRATION

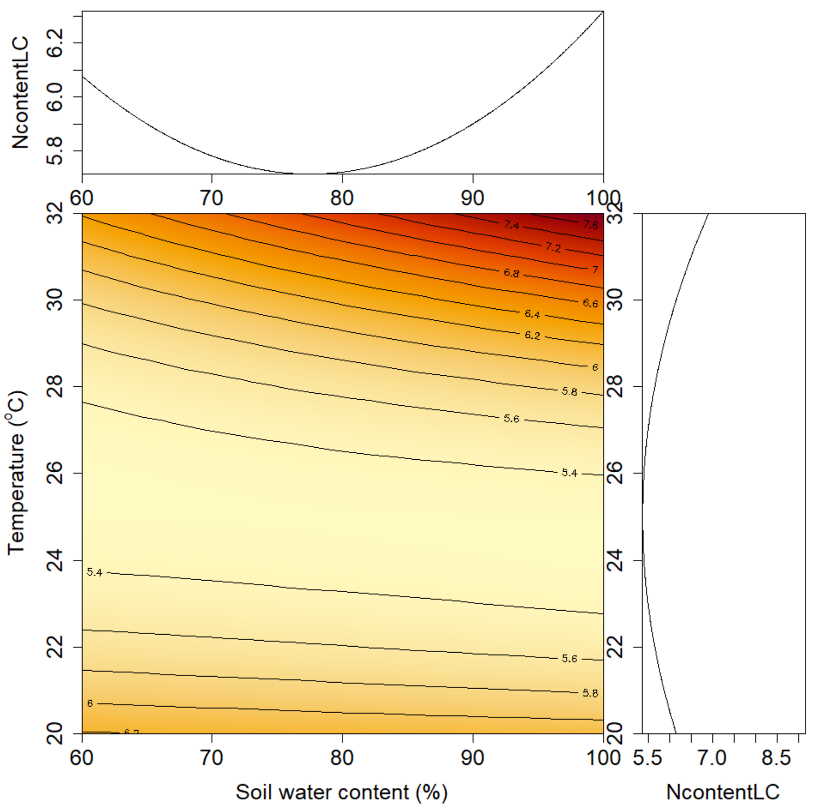
Under elevated CO_2 concentrations, lettuce plants that survived until the end of the experiment had an average dry leaf biomass that was approximately 59% greater than that of plants grown under ambient concentrations. This difference became more prominent when mortality was considered, as the average dry leaf biomass of elevated CO_2 plants was over 171% greater than that of ambient plants when dead plants were included as zero values.

Table 6: Total and average dry lettuce leaf biomass of elevated and ambient CO_2 chambers. When included in the average, dead plants are given a leaf mass of 0 g. Total dry lettuce leaf biomass refers to the sum of the dry lettuce leaf biomass of all surviving plants grown under ambient or elevated CO_2 concentrations.

	Total dry lettuce leaf biomass (g)	Average dry lettuce leaf biomass (g)	Average dry lettuce leaf biomass (g), including dead plants
Ambient CO_2	13.278	0.255	0.076
Elevated CO_2	36.032	0.405	0.206

3.4 NITROGEN CONTENT

The effects of the environmental conditions studied in this experiment on lettuce leaf nitrogen content are compiled in the two figures below.


NcontentLC
5.8
6.0
6.2
60
70
80
90
100
Temperature (°C)
20
22
24
26
28
30
32
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
5.8
5.6
5.4
5.4
5.6
5.8
6
6.2
Soil water content (%)
5.5
7.0
8.5
NcontentLC

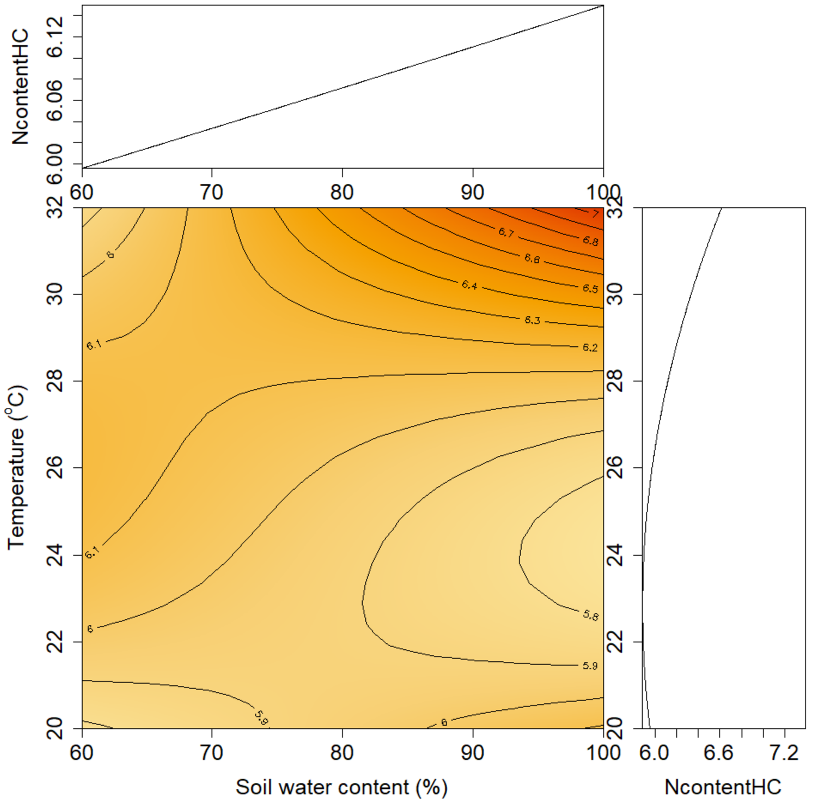

NcontentHC
6.00
6.06
6.12
60
70
80
90
100
Temperature (°C)
20
22
24
26
28
30
32
7
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6
6.1
6.1
6
5.8
5.9
5.9
6
Soil water content (%)
6.0
6.6
7.2
NcontentHC

Figure 22 & 23: The central figures show the impacts of temperature and water availability on dried lettuce leaf nitrogen content under ambient (LC) and elevated (HC) CO_2 concentrations. Darker colors represent higher nitrogen content. Values on the contour lines are given as percentages. The only plants included in the data are those that underwent lab testing, which did not include plants that died before the end of the experiment. The smaller graphs to the right of and above the main figure depict the effects of temperature and water content respectively on leaf nitrogen content. These figures do not include nitrogen data from chambers set at 36 °C, as no protein samples were available from said chambers.

In Figure 22, the highest leaf nitrogen content was found at high temperature and water availability. This decreased under medium temperatures before slightly increasing again at the lowest temperature. In general, temperature had a more important effect on nitrogen content than water availability. In Figure 23, the highest nitrogen levels were again found at high temperatures and water availabilities and nitrogen content decreased with both. Temperature generally had a more important effect than water availability. Between the two figures, there is little average difference in nitrogen content between ambient and elevated CO_2 concentrations, with elevated chambers leading to slightly higher nitrogen content.

3.4.1 RELATIONSHIP WITH TEMPERATURE

Regarding temperature, the highest average percent nitrogen found in dry leaf samples occurred in the 32 °C chambers for both ambient and elevated CO_2. These values - 6.92 % and 6.76 % respectively - were also the two highest average nitrogen content values found in any of the chambers. In the ambient chambers, N content decreased with decreasing temperature until increasing again at 20 °C. The lowest average N content of all chambers (5.44 %) was found at 20 °C and ambient CO_2. The elevated chambers expressed a less definite relationship between temperature and N content, with a decrease in N content when temperatures decreased from 32 °C to 28 °C but few changes in N content between the other three chambers.

Table 7: Average percent nitrogen content of dry lettuce leaf biomass grown under different temperatures and under ambient and elevated CO_2.

Temperature (°C)	Average Nitrogen Content Under Ambient CO_2 (%)	Average Nitrogen Content Under Elevated CO_2 (%)
20	6.14	5.89
24	5.44	6.10
28	5.63	5.92
32	6.92	6.76

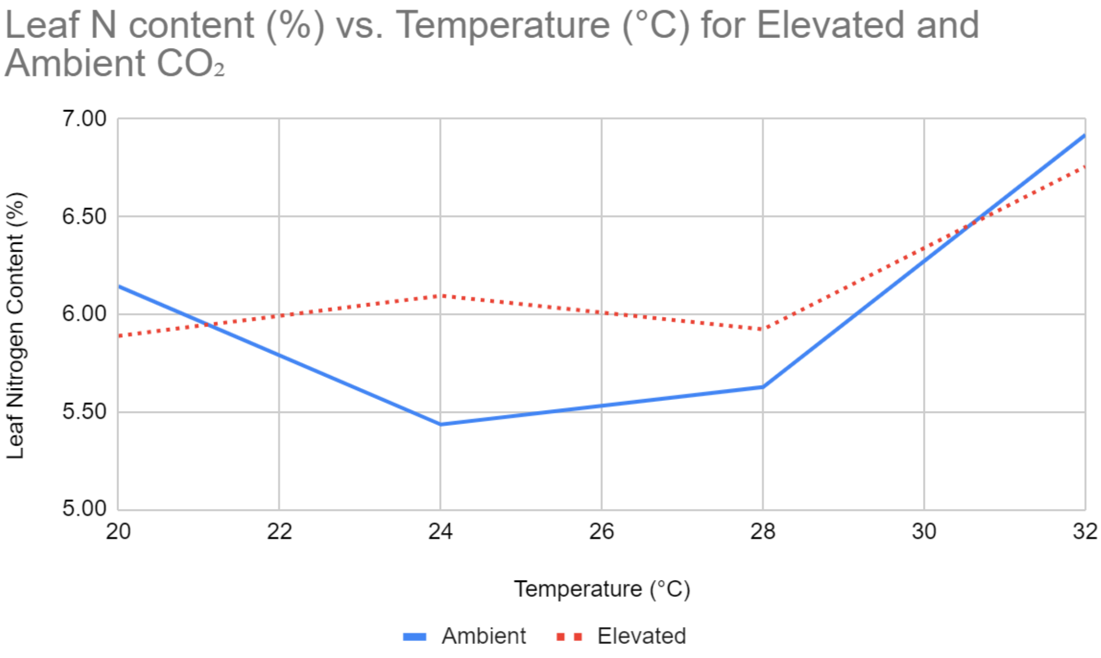


Figure 24: Relationship between dry leaf nitrogen content and temperature under ambient and elevated CO_2 concentrations. The solid line represents the average nitrogen content of dry leaf samples grown under ambient CO_2 levels while the dotted line represents the same data for plants grown under elevated CO_2 levels.

3.4.2 RELATIONSHIP WITH WATER AVAILABILITY

Within the elevated chambers, there was a very small increase in N content with increasing soil water content. The highest average N content found in elevated chambers (6.16%) was at 100% field capacity and the lowest (6.01%) was found at 60% field capacity. There was less of a trend within ambient chambers, as the highest value (6.27%) was found at 90% field capacity and the smallest (5.53%) was found at 80%.

Table 8: Average percent nitrogen content of dry lettuce leaf biomass grown under different levels of water availability and under ambient and elevated CO_2.

Soil Water Content (%)	Average N Content (%) Under Ambient CO_2	Average N Content (%) Under Elevated CO_2
100	6.098	6.158
90	6.286	6.071
80	5.531	6.123
70	5.670	5.992
60	6.196	6.008

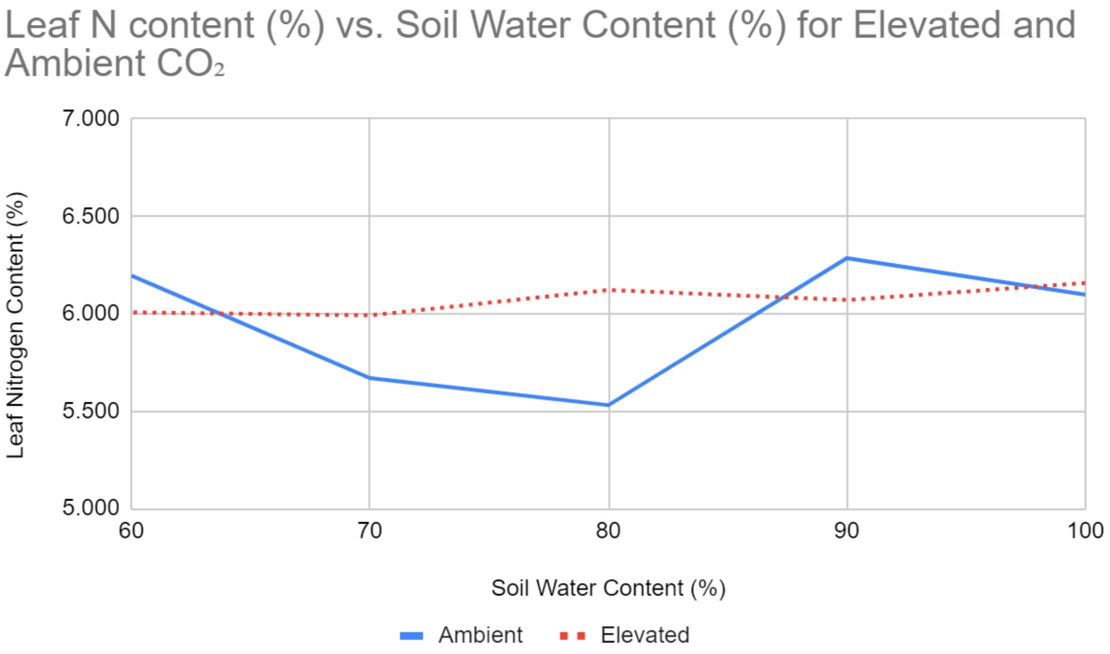


Figure 25: Relationship between dry leaf nitrogen content and water availability under ambient and elevated CO_2 concentrations. The solid line represents the average nitrogen content of dry leaf samples grown under ambient CO_2 levels while the dotted line represents the same data for plants grown under elevated CO_2 levels.

3.4.3 RELATIONSHIP WITH CO_2

CO_2 concentration had little effect on average lettuce leaf nitrogen content. On average, plants grown under ambient CO_2 concentration had a dry leaf biomass that was 5.93% nitrogen and plants grown under elevated concentrations had an average dry leaf biomass that was 6.08% nitrogen. The only point where CO_2 concentration significantly altered nitrogen content was at high temperatures and water availability, where plants grown under elevated CO_2 concentration had approximately 6.8% nitrogen content versus the 7.6% nitrogen content found in plants grown under ambient CO_2 conditions.

4.0 DISCUSSION

4.1 IMPLICATIONS OF CLIMATE CHANGE IMPACTS ON LETTUCE GROWTH AND MORTALITY

Due to the wide variety of environments lettuce is grown under and the differing ways that these environments could be impacted by climate change, it is difficult to make any concrete, overarching predictions as to how climate change could affect the global lettuce crop. With this being said, our research suggests that climate change has the potential to improve lettuce growth and survival due to the beneficial effects of elevated CO_2 concentrations. However, this improvement could be negated by the deleterious effects of high temperatures, drought, and/or flooding. The above conclusions are largely corroborated by similar studies, but because most research into the effects of climate change on lettuce considers only one or two environmental factors, discussion of the literature on the topic will be divided between the sections below.

4.1.1 CO_2 IMPACTS

Across all treatments, elevated CO_2 concentrations resulted in greater plant biomass and improved plant survival. This increase in productivity is likely due primarily to the photosynthesis promoting effects of increased CO_2 availability (Holley et al., 2022, Pérez-López et al., 2015). Our data also suggests that increased CO_2 concentrations improve lettuce thermal tolerance, as illustrated by Figures 16 and 17. As shown in these figures, while lettuce biomass increased with decreasing temperature under ambient CO_2 concentration, biomass was greatest at approximately 25 °C under elevated CO_2 concentration. This shift is different from the relationship between CO_2, water availability, and biomass, as the graph of biomass vs. water availability has a nearly identical shape in Figures 16 and 17. The similar relationship between water availability and biomass in both ambient and elevated treatments suggests that improved water use efficiency due to elevated CO_2 concentrations does not have a significant effect on lettuce grown under the levels of water availability used in this study.

Comparing our research to other studies in the literature shows that the relationship we found between CO_2 availability and biomass yield is supported by the findings of Holley et al. (2022), Pérez-López et al. (2015), Giri et al. (2016), and Frantz et al. (2004), all of which found that elevated CO_2 concentrations increased lettuce biomass production. Frantz et al. (2004) also found that artificially elevating CO_2 concentrations led to an increase in the optimal temperature for lettuce cultivar biomass growth from 25 °C to 30 °C. However, when comparing the growth rates of lettuce grown under a wide range of CO_2 concentrations, Holley et al. (2022) found that increasing CO_2 levels gave diminishing returns in biomass increases once concentrations surpassed 800 ppm.

4.1.2 TEMPERATURE IMPACTS

Within both the ambient CO_2 group and the elevated CO_2 group, temperature was the most important factor in determining lettuce biomass production and mortality. At least within the IPS chambers, it appears that the success of Mānoa lettuce declines rapidly with increasing temperatures and that the species cannot survive at temperatures exceeding 36 °C. This could be due to a number of factors, including damaged cellular apparatuses, impaired enzyme function, and the accumulation of reactive oxygen species (Bita & Gerats, 2013; Feller & Vaseva, 2014; Hasanuzzaman et al., 2013). Biomass and survival both increased at lower temperatures, with the optimum temperature for lettuce grown under elevated CO_2 around 25 °C and the optimum temperature under ambient CO_2 around 20 °C or lower. This is notable, as the Mānoa lettuce cultivar is ordinarily known to experience a decline in yield starting at approximately 24 °C (Hartmann et al., 2000).

The relationship between temperature and lettuce growth has been explored by past studies, but there is limited consensus as to the nature of this relationship. Studies such as Chen et al. (2020) and Sublett et al. (2018) have found that lettuce biomass increased as temperature increased from 20 °C to 24 °C and 25 °C to 33 °C, respectively. However, studies by Iqbal et al. (2022) and Wheeler et al. (1993) found that biomass decreased as temperatures increased from 20 °C to 30 °C and 14 °C to 21 °C, respectively. This discrepancy could be due to different lettuce cultivars having differing optimal temperatures, as demonstrated by studies by Smeets (1977), Choi et al. (2000), and Thakulla et al. (2021).

4.1.3 WATER AVAILABILITY IMPACTS

Although water availability did not have as dramatic of an impact as temperature, it still played an important role in lettuce growth and survival. Our findings from both elevated and ambient treatments suggest that the optimum level of substrate water content for Mānoa lettuce grown in coarse vermiculite is approximately 80% of the experimentally determined field capacity of the media. The decrease in lettuce productivity at lower water availability could be due to lower rates of water flow to new growth (Kaur & Asthir, 2017; Seleiman et al., 2021) or closed stomata reducing the amount of CO_2 available for photosynthesis (Kaur & Asthir, 2017; Lawlor, 2002;

Osakabe et al., 2014). At higher water availability, it is possible that waterlogging could lead to root oxygen deprivation, which has a detrimental effect on the uptake of water and nutrients in most plants (Sairam et al., 2008, Manik et al. 2019).

Other studies on the effect of water availability on lettuce have also found that both drought and waterlogging can have negative impacts on lettuce biomass production. When growing lettuce plants under waterlogging and drought stress, Cabillo (2019), while both water extremes had a negative effect on water growth, waterlogging had a larger impact than drought. Both Eichholz et al. (2014) and Trang et al. (2010) compared the effects of waterlogging and water restriction on different lettuce cultivars and found that, although both water extremes reduced plant growth, the degree of this impact depended on the cultivar being grown. Additionally, the soil microbial content of the growing media can modify the effects of both drought stress (Ruiz-Lozano et al. 2015, Duran et al. 2016) and waterlogging (Ruiz-Lozano et al. 2015, Irfan et al. 2010, Laanbroek 1990) on plant growth and survival.

4.2 IMPACTS OF CLIMATE CHANGE FACTORS ON NITROGEN CONTENT

Based on our findings, it appears that lettuce percent nitrogen content may increase when both temperature and water availability are at a high level. Our data suggests that this increase may be less prominent under elevated CO_2 concentrations, which could potentially lead to reduced nutritional quality in lettuce under climate change conditions. However, the small sample size of high temperature lettuce that was available for testing reduces the reliability of this prediction. Additionally, the negative impacts of increased temperature and water availability on plant survival and yield could negate any increase in nutritional value.

Our findings regarding the impacts of climate change on lettuce nitrogen content are partially supported by other studies in the literature, although few studies have examined the impacts of climate change on lettuce nitrogen content specifically. A review of 54 studies by Dong et al. (2018) found that, although the nitrogen content of most vegetables decreased when grown under elevated CO_2, the nitrogen content of leafy vegetables was generally unaffected by increased concentrations. Regarding temperature, some studies have concluded that lettuce protein content increases with warming conditions (Chen et al. 2020) while others have reported the opposite trend (Ouyang et al. 2020). It is possible that the higher levels of nitrogen under high temperatures and lower water levels could simply be due to the small size of surviving lettuce plants under these conditions, as although larger plants generally have greater total nutrient uptake, the increased amount of biomass in larger plants can reduce nutrient content per unit weight (Peet and Wolfe, 2000).

4.3 LIMITATIONS

Because our experiment took place in a laboratory, there are several areas in which our research conditions differed from the conditions lettuce would grow under in a farm setting. For one, the media used to grow our lettuce was vermiculite, a sterile, inorganic media that lacks the beneficial microbiome found in most natural soils. Vermiculite also has a high water capacity that allows it to hold a larger amount of water than many organic soils. Additionally, because the pots used in the IPS system lack drainage holes, there was no opportunity for excess water or nutrients to escape from each pot. This has the potential to promote the growth of harmful bacteria and increase waterlogging. Growing plants indoors also eliminates many of the pressures that plants face in outdoor farm settings, including insect pests, diseases, or weeds. Finally, the relatively limited range of factors examined in this experiment disregards several environmental conditions that can have major impacts on plants growing in the field, such as wind, fluctuating light levels, nutrient availability, and pollutant exposure.

Other factors that could have contributed to inaccuracy in our data include CO_2 fluctuations and laboratory malfunctions. As mentioned earlier, the ambient CO_2 concentrations in the lab fluctuated widely over the course of each day and depended largely on the number of people present in the building. On an ordinary day, this meant that CO_2 levels ranged from approximately 430 ppm to 520 ppm. When the lab was actively in use for long periods of time, CO_2 concentrations could reach 600 ppm or even higher. As a result, the average CO_2 concentration in the ambient chambers was well above the modern average of 420 ppm (NASA, 2023), meaning that the atmospheres of chambers with ambient CO_2 concentrations did not accurately mirror the modern atmosphere. Additionally, the 20 °C chambers - which were the most energy-intensive - experienced two power outages each over the course of the experiment. During these outages, the chambers warmed to room temperature (approximately 25 °C at night), lost all light if the outage occurred during the 12 hour “daytime”, and, in the case of the elevated chamber, returned to ambient CO_2 concentrations for the duration of the outage. Although only two of these events occurred during the experiment, power loss could have impacted lettuce growth rates.

4.4 POSSIBLE FUTURE RESEARCH

There are multiple possible options for future research into the effects of climate change on lettuce and other plants. Although this study covered the upper ends of the survivable spectrums of temperature and water availability well, future research could explore the lower limits as well. Examining the lower limit of water availability under ambient and elevated CO_2 is especially important, as elevated CO_2 should theoretically allow lettuce to survive under drier conditions due to improved water use efficiency. For similar reasons, future research could explore the responses of lettuce plants to different nutrient concentrations. Plant nutrient uptake can be influenced by temperature, CO_2 concentration, and water stress, so it is fully possible that climate change could change the way lettuce interacts with soil nutrients (Bassirirad, 2000, Alam, 1999). Nutrient uptake can also be influenced by the soil microbiome, another factor that could be incorporated in future studies (Ruiz-Lozano et al. 2015, Irfan et al. 2010). Finally, there is a great need for more research into the effects of climate change on other lettuce cultivars as well as other food crops. Climate change impacts are highly species- and cultivar-specific, so the results of one study on one lettuce cultivar are not enough to provide accurate insight into the effects of environmental change on the global food system (Parmesan & Hanley, 2015).

5.0 CONCLUSION

As demonstrated by this study, climate change has the potential to greatly impact plant growth - and by extension the global agricultural system - in varied and surprising ways. To investigate the impacts of climate change on one particular crop - lettuce - we grew Mānoa lettuce under a wide variety of environmental conditions and observed that 1) Lettuce growth and survival was benefited by increasing atmospheric CO_2 concentration, 2) This benefit was reduced or negated by the detrimental effects of high atmospheric temperatures and high or low water availability, and 3) At high temperatures, lettuce nitrogen content was lower at elevated CO_2 concentrations than ambient concentrations. From these pieces of information, it can be concluded that lettuce yield could increase due to the effects of climate change, but only if the area in which it is grown is not expected to experience significant temperature increases or long-term increases or decreases in rainfall. To prepare for this future, modern farmers could consider focusing lettuce farming efforts on regions that are expected to experience limited environmental change in the coming decades or growing different crops if migration is impossible. This study provides information that could potentially help to inform modern and near-future agricultural climate adaptation measures, but we only examined the ways in which a relatively small range of three environmental conditions affected three aspects of one cultivar of one species. It is vital that future studies explore the potential impacts of climate change on the many other plant and animal species that ecosystems and human societies depend on. To adequately prepare for a future where climate change may radically alter the way organisms grow and function, the scientific community must invest time and resources into understanding the ways in which the species of today may respond to the environment of tomorrow.

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