The Effects of Elevated CO2 on the Growth and Water Conservation Potentials of Medicago Sativa L. And Chloris Gayana K.

Asma Khan Ahmadani

Follow this and additional works at: https://scholarworks.uaeu.ac.ae/all_theses

Part of the Environmental Sciences Commons

Recommended Citation
https://scholarworks.uaeu.ac.ae/all_theses/434

This Thesis is brought to you for free and open access by the Electronic Theses and Dissertations at Scholarworks@UAEU. It has been accepted for inclusion in Theses by an authorized administrator of Scholarworks@UAEU. For more information, please contact fadl.musa@uaeu.ac.ae.
THE EFFECTS OF ELEVATED CO₂ ON THE GROWTH AND WATER CONSERVATION POTENTIALS OF *MEDICAGO SATIVA* L. AND *CHLORIS GAYANA* K.

Asma Khan Ahmadani

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Sciences

Under the direction of Supervisor: Dr. Taoufik Ksiksi and Co-Supervisor: Dr. Khaled El-Tarabily

May 2014
DECLARATION OF ORIGINAL WORK

I, Asma Khan Ahmadani, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of the thesis titled "The effects of elevated CO$_2$ on the growth and water conservation potentials of Medicago sativa L. and Chloris gayana K.'", hereby solemnly declare that this thesis is an original work done and prepared by me under the guidance of Dr. Taoufik Ksiksi, and Dr. Khaled El-Tarabily, in the College of Science at UAEU. This work has not been previously formed as the basis for the award of any degree, diploma or similar title at this or any other university. The materials borrowed from the other sources and included in my thesis have been properly acknowledged.

Student's Signature.................................................. Date ..............................

II
Copyright © 2014 by Asma Khan Ahmadani

All Rights Reserved
Thesis Examining Committee:

1) Advisor (Committee Chair): Dr Taoufik Ksiki
   Department of Biology
   College of Science
   Signature: [Signature]
   Date: 28/5/2014

2) Member: Dr Khaled Tarably
   Department of Biology
   College of Science
   Signature: [Signature]
   Date: 28/5/2014

3) Member: Dr Shyam Kurup
   Department of Arid Land Agriculture
   College of Food and Agriculture
   Signature: [Signature]
   Date: 24/5/2014

4) Member (External Examiner): Dr. Makram Belhaj Fraj
   Division of Research and Innovation
   Institution: International Center For Biosaline Agriculture
   Signature: [Signature]
   Date: May 27, 2014
This thesis is accepted by:

Master's Program Coordinator: Dr. Taoufik S. Ksiksi

Signature: _______________________________ Date: 9/6/2014

Dean, College of Science: Prof. Peter Walter Werner

Signature: _______________________________ Date: 6/22/2014

Copy _____ of _____
ABSTRACT

Atmospheric carbon dioxide (CO₂) concentrations have been increasing for many decades. The impact of such greenhouse gas has been reported to affect ecosystems at all levels. Elevated atmospheric CO₂ can affect the growth of plants and their response to water intake. But, different plants show different degrees of response depending on whether they have C₃ or C₄ photosynthetic pathway. The aim of this study is to evaluate the effect of CO₂ enrichment on the growth and microbial activity of alfalfa (Medicago sativa) and Rhodes grass (Chloris gayana) and also its effect on the water use efficiency (WUE) of these plants. Both plant species were exposed to ambient CO₂ (ACO₂; ~500 ppm), enriched CO₂ (ECO₂; ~1000 ppm) under regular watering regime or under water stress. Soil samples were tested for microbial activity using Fluorescein diacetate (FDA) hydrolysis technique, shoot and root lengths were measured periodically. WUE was calculated using fresh and dry weight of the plants, and numbers of leaves were counted. Microbial activity under elevated CO₂ was 41% higher in Rhodes grass at water stress, than in ambient CO₂. Also, by inducing water stress on Rhodes grass, the WUE increased significantly by 4.6 times i.e. 21.5% than its control counterparts. Moreover, WUE of alfalfa in the treatment (elevated CO₂ + water stressed) group was 25.7% (3.8 times) greater than alfalfa grown at control (ambient CO₂ + normal water). Fresh weight biomass of Rhodes grass under treatment group showed no significant difference compared to the control group, whereas the biomass of alfalfa was significantly higher in both fresh (4%) and dry matter (11.3%) under treatment group, compared to the control group. Height of alfalfa grown under CO₂ enrichment was 30% lesser at water stress, than compared to
the ones grown at normal water conditions. Shoot/root ratio of Rhodes grass grown under elevated CO₂ was 28.8% lower than the ones grown at ambient CO₂ concentrations, whereas shoot/root ratio of alfalfa grown under CO₂ enrichment was 22.9% higher than the ones grown at ambient CO₂ concentrations. Number of leaves remained the same for both Rhodes grass and alfalfa under every treatment.

Keywords: CO₂, microbial activity, water use efficiency, global warming, C₃ plants, C₄ plants.
ACKNOWLEDGEMENTS

Firstly, I would like to thank God, for providing me this opportunity for enrolling into graduate studies program, and blessing me with the ability and skills to do so.

I would like to express my special appreciation and thanks to my supervisor, Dr. Taoufik Ksiksi, who has been a tremendous mentor for me. I would like to thank him for encouraging my research and for allowing me to grow as a research scientist. His advice on both research as well as on my career have been priceless. And also, I would like to express much appreciation to Dr. Khaled El-Tarabily for his time and efforts in reviewing my thesis, his corrections as well as his guidance throughout my thesis as well. Moreover, I would like to thank the chair and all members of Biology Department at UAEU for assisting me all over my studies and research.

To all my friends, for their constant encouragement and advises during good as well as in tough times. You friends really boosted my confidence. And finally, I would like to acknowledge that all this would not have been possible without the much needed guidance and support from my family members. A very special thank you, to my parents, to my brothers Tariq and Siraj, and to my sister Shama, for all the sacrifices you have made for on my behalf. It is your endless help and prayers that sustained me this far. Thank you everyone for the faith bestowed in me that I could do it. I hope I continue making you proud.
"I believe in innovation and that the way you get innovation is you fund research and you learn the basic facts." -Bill Gates

This thesis is dedicated to my beloved parents.
# TABLE OF CONTENTS

DECLARATION OF ORIGINAL WORK .......................................................... II
SIGNATURE PAGE ................................................................................... IV
ABSTRACT ............................................................................................... VI
ACKNOWLEDGEMENTS ......................................................................... VIII
DEDICATION ............................................................................................ IX
LIST OF TABLES ..................................................................................... XIV
LIST OF FIGURES .................................................................................. XVI

CHAPTER I: INTRODUCTION .................................................................. 2
1.1 Thesis motivation ............................................................................... 2
1.2 Thesis objectives ............................................................................... 4

CHAPTER II: LITERATURE REVIEW .................................................. 6
2.1 Carbon dioxide as a greenhouse gas and its historic trend ................. 6
2.2 Sources of elevation of atmospheric CO₂ .......................................... 7
2.3 Rise in CO₂ in the Arabian Gulf ...................................................... 8
2.4 Potential impacts of rising CO₂ levels .............................................. 9
2.5 Plant responses to very high CO₂ ................................................... 12
2.6 Reduced photosynthesis and growth at high CO₂ ......................... 13
2.7 C₃ and C₄ plants .............................................................................. 14
2.8 Effects of elevated CO₂ on C₃ and C₄ (Theoretical) ......................... 17
2.9 Effects of elevated CO₂ on C₃ and C₄ (Experimental) .................... 19
2.10 Soil microbial effect ...................................................................... 21
2.11 Alfalfa (*Medicago sativa*) ............................................................ 25
2.12 Effects of elevated CO₂ on alfalfa .................................................. 26
2.13 Rhodes grass (Chloris gayana) ......................................................... 27
2.14 Effects of elevated CO₂ on Rhodes grass .......................................... 28
2.15 Water stress ...................................................................................... 29

CHAPTER III: MATERIALS AND METHODS .................................................... 36

3.1 Location of the study ......................................................................... 36
3.2 Growth chambers ............................................................................... 36
3.3 Experimental plan ............................................................................. 36
3.4 Data collection .................................................................................. 37
  3.4.1 Testing soil microbial activity ......................................................... 37
  3.4.2 Weight of the shoots ..................................................................... 38
  3.4.3 Weight of the roots ....................................................................... 38
  3.4.4 Above ground shoot growth .......................................................... 39
  3.4.5 Below ground root growth ............................................................. 39
  3.4.6 Shoot/root ratio ............................................................................ 39
  3.4.7 Number of leaves .......................................................................... 39
3.5 Statistical analysis ............................................................................ 39

CHAPTER IV: RESULTS .................................................................................. 43

4.1 Effect of CO₂ enrichment on microbial activity on water stressed and non water stressed plants ................................................................. 43
  4.1.1 Microbial activity in Rhodes grass ................................................. 43
  4.1.2 Microbial activity in alfalfa ............................................................ 47
4.2 Effect of CO₂ enrichment on morphology of the plants ......................... 49
4.2.1 Fresh weight: Water use efficiency (WUE) ............................................. 49
4.2.2 Fresh weight: Biomass ............................................................................. 51
4.2.3 Dry weight: Water use efficiency (WUE) .............................................. 53
4.2.4 Dry weight: Biomass ............................................................................ 55
4.2.5 Height percentage .................................................................................. 57
4.2.6 Shoot/root ratio ...................................................................................... 73
4.2.7 Number of leaves ................................................................................... 81

CHAPTER V: DISCUSSION ............................................................................ 90

5.1 Effect of CO₂ enrichment on microbial activity on water stressed and non-
water-stressed plants. .................................................................................... 90
  5.1.1 Microbial activity in Rhodes grass ......................................................... 90
  5.1.2 Microbial activity in alfalfa .................................................................. 91
5.2 Effect of CO₂ enrichment and water stress on morphology of the plants ...... 92
  5.2.1 Fresh and dry weight: WUE in Rhodes grass ......................................... 92
  5.2.2 Fresh and dry weight: WUE in alfalfa .................................................... 94
  5.2.3 Fresh and dry weight: biomass of Rhodes grass .................................... 95
  5.2.4 Fresh and dry weight: biomass of alfalfa ............................................. 96
  5.2.5 Height of Rhodes grass ........................................................................ 97
  5.2.6 Height of alfalfa .................................................................................. 98
  5.2.7 Shoot/root ratio in Rhodes grass ............................................................ 99
  5.2.8 Shoot/root ratio in alfalfa .................................................................... 100
  5.2.9 Leaf percentage in Rhodes grass ........................................................... 101
  5.2.10 Leaf production in alfalfa ................................................................... 102
CHAPTER VI: CONCLUSION .................................................................................. 104
REFERENCES ..................................................................................................... 108
LIST OF TABLES

Table 1: ANOVA results for microbial activity of Rhodes grass grown with or without CO₂ enrichment under ambient water conditions. ......................................... 44

Table 2: ANOVA results for the microbial activity of Rhodes grass grown with and without CO₂ enrichment under water-stress conditions. ................................. 46

Table 3: T-test results for the microbial activity of Rhodes grass grown with and without CO₂ enrichment under water-stress conditions. ..................................... 46

Table 4: ANOVA results for plant height of Rhodes grass grown under no water stress conditions, with or without CO₂ enrichment. .................................................. 58

Table 5: ANOVA results for plant height of Rhodes grass grown under water stress conditions, with or without CO₂ enrichment. .................................................. 60

Table 6: ANOVA results for root length of Rhodes grass grown under ambient water conditions, with or without CO₂ enrichment. .................................................. 62

Table 7: ANOVA results for root length of Rhodes grass grown under water-stress conditions, with or without CO₂ enrichment. .................................................. 64

Table 8: ANOVA results of plant height of alfalfa grown under ambient water conditions, with or without CO₂ enrichment. ...................................................... 66

Table 9: ANOVA results of plant height of alfalfa grown under water stress conditions, with or without CO₂ enrichment. ...................................................... 68

Table 10: T-test results of Plant height of alfalfa grown under water stress conditions, with or without CO₂ enrichment.............................................................. 68

Table 11: ANOVA results of root length of alfalfa grown under ambient water conditions, with or without CO₂ enrichment. ...................................................... 70

Table 12: ANOVA results of root length of alfalfa grown under water stress conditions, with or without CO₂ enrichment. ...................................................... 72

Table 13: ANOVA results for shoot/root ratio of Rhodes grass grown at ambient water conditions, with or without CO₂ treatment. ............................................... 74
Table 14: T-test results for Shoot/root ratio of Rhodes grass grown at ambient water conditions, with or without CO₂ treatment

Table 15: ANOVA results Shoot/root ratio of Rhodes grass grown at water stress conditions, with or without CO₂ treatment

Table 16: ANOVA results of Shoot/root ratio of alfalfa grown at ambient water conditions, with or without CO₂ treatment

Table 17: T-test results of Shoot/root ratio of alfalfa grown at ambient water conditions, with or without CO₂ treatment

Table 18: ANOVA results for shoot/root ratio of alfalfa grown at water stress conditions, with or without CO₂ treatment

Table 19: ANOVA results for Leaf (%) of Rhodes grass grown at ambient water conditions, with or without CO₂ treatment

Table 20: ANOVA results of Leaf (%) of Rhodes grass grown at water stress conditions, with or without CO₂ treatment

Table 21: ANOVA results of leaf (%) of alfalfa grown at ambient water conditions, with or without CO₂ treatment

Table 22: ANOVA results for leaf (%) of alfalfa grown at water stress conditions, with or without CO₂ treatment
LIST OF FIGURES

Figure 1: Sources of CO₂ emissions in the GCC, International Environmental Agency (IEA) report, 2007 (Arman et al., 2013) .................................................. 8

Figure 2: Microbial activity of Rhodes grass grown with or without CO₂ enrichment under ambient water conditions .......................................................... 43

Figure 3: Microbial activity of Rhodes grass grown with or without CO₂ enrichment under water-stress conditions. * indicates the significant difference between the treatments at P < 0.005 ....................................................... 45

Figure 4: Microbial activity of alfalfa grown with or without CO₂ enrichment under ambient water conditions ................................................................. 47

Figure 5: Microbial activity of alfalfa grown with or without CO₂ enrichment under water-stress conditions ................................................................. 48

Figure 6: WUE of Rhodes grass grown at different CO₂ and water stress combinations ................................................................. 49

Figure 7: WUE of alfalfa grown at different CO₂ and water stress combinations ................................................................. 50

Figure 8: Biomass of Rhodes grass grown at different CO₂ and water stress combinations ................................................................. 51

Figure 9: Biomass of alfalfa grown at different CO₂ and water stress combinations ................................................................. 52

Figure 10: Water use efficiency (WUE) of Rhodes grass grown at different CO₂ and water stress combinations ................................................................. 53

Figure 11: Same as above WUE of alfalfa grown at different CO₂ and water stress combinations ................................................................. 54

Figure 12: Biomass of Rhodes grass grown at different CO₂ and water stress combinations ................................................................. 55

Figure 13: Biomass of alfalfa grown at different CO₂ and water stress combinations ................................................................. 56

Figure 14: Plant height of Rhodes grass grown under ambient water conditions, with or without CO₂ enrichment ................................................................. 57
CHAPTER I

INTRODUCTION
CHAPTER 1: INTRODUCTION

1.1 Thesis motivation

Greenhouse gases (GHG) in more than essential quantities in the atmosphere have been referred to as pollutants (US EPA, 2012). Among various GHG that exist, carbon dioxide (CO₂) has been the most studied gas, whose concentrations are known to be rising in the atmosphere over the years. Whether the sources are natural or manmade, the bottom line is that the levels in the atmosphere are higher than they have ever been as these numbers are increasing (Sanz-Sáez, Erice, Aguirreolea, Irigoyen, & Sánchez-Díaz, 2012). Dry and arid environments such as those found in the United Arab Emirates (UAE), do not escape the dilemma. Discovery of oil has led to the country's economic development, as well as being a cause for heavy carbon emissions by the industrial sector that contribute to the overall global problem (Arman & Murad, 2012). Besides rising CO₂ levels, UAE has also seen change in the country's climate over the span of 100 years. Country's highest average temperature in July between the years 1900-1930 was lower than the average which was noticed between the year 1990-2009 in July with temperatures being 32.3°C and 34.4°C respectively. Also, the rainfall has decreased over the same period of time; highest amount of rainfall (19.6 mm) noticed in February in the year 1900-1930, compared to 16.4 mm rainfall in February of 1990-2009 (World Bank, 2009). Climate change has exacerbated the water shortage problems in the country. High fresh water demand in the country especially by the agricultural sector and fewer rainfalls to replenish the withdrawal have led to declining of the water table in the UAE (Ebraheem, Sherif, Mulla, Akram, & Shetty, 2012; Falah et al., 2012). Ground water exploitation have
made the country dependent on desalination plants for their supply of potable fresh water (Alsalmi, Elkadi, & Leao, 2013). These measures might suppress the problem temporarily but the cause still exists. There is a need for water conservation by all the sectors of the country to reduce the rate at which UAE is running into water scarcity.

Plants require CO₂ for carrying out photosynthesis (Nzewi, 2011). This means, rising global CO₂ levels can prove to be beneficial for the growth of plants and may act as a fertilizer (Gielen & Ceulemans, 2001). Plants often respond to high CO₂ by increasing their photosynthetic rates (Drake, González-Meler, & Long, 1997; Ziska, Hogan, Smith, & Drake, 1991) Many studies done on plants under elevated CO₂ conditions have shown positive results, with better growth, and more allocation of resources to shoots and roots (Baker & Allen Jr., 1994; Kimball, 1983). Therefore, it is most likely that plants grow better at higher CO₂ levels. Not only are the photosynthesis and growth rates positively affected, scientists have noticed remarkable decline in transpiration rates when CO₂ levels are increased. This is mainly due to the fact that at elevated CO₂ levels, stomatal conductance is reduced, and this over all improves the water use efficiency of the plant (Drake et al., 1997). With transpiration rates declining, plant requires lesser water to produce equal or more biomass, which is also known as water use efficiency. As studied by Wong (1979), water use efficiency increased in cotton and maize plants under elevated CO₂ mainly due to reduced transpiration rates and by increase in assimilation. Studies still continue till date, to find out the relation between CO₂ elevation and water use efficiency of the plant.
By understanding this concept, the global increase in atmospheric CO₂ levels could actually prove to be beneficial in helping the farmers conserve more water. To help with UAE's water scarcity issues, and to help farmers produce more while using up less water, the current study was undertaken. This study may help establish potential benefits or otherwise, of using CO₂ elevation in greenhouses, to support plant growth and to aid water conservation in general.

1.2 Thesis objectives

Specific objectives of this thesis were:

- To compare alfalfa (*Medicago sativa L.*) growth under both high CO₂ and ambient CO₂ concentrations.
- To compare Rhodes grass (*Chloris gayana K.*) growth under high CO₂ and ambient CO₂ concentrations.
- To evaluate the overall growth of alfalfa (as a C₃ species) and Rhodes grass (as a C₄ species) under high CO₂ concentrations.
- To compare microbial activity for both plant species under high CO₂ and ambient CO₂ concentration.
- To assess water conservation potentials of both plant species under enriched CO₂ concentrations.
CHAPTER II

LITERATURE REVIEW
CHAPTER II: LITERATURE REVIEW

2.1 Carbon dioxide as a greenhouse gas and its historic trend

Carbon dioxide (CO\textsubscript{2}) is one of the main greenhouse gases and is increasing in the atmosphere due to natural and human activities (Sanz-Sáez et al., 2012; US EPA, 2012). It is of significant concern in the world, because of its global warming potential which is leading to a global climate change. It is estimated that concentration of atmospheric CO\textsubscript{2} are increasing all over the world at a rate of 1% or more every year (Scarascia-Mugnozza, Karnosky, Ceulemans, & Innes, 2001). During the pre-industrial times the atmospheric CO\textsubscript{2} concentration was estimated to be around 280 ppm (Hofmann, Butler, & Tans, 2009) and that number has considerably increased by about 30%, since the beginning of the industrial revolution (Hofmann et al., 2009; Scarascia-Mugnozza et al., 2001) to 379 ppm in 2005 (Change, 2007). An increase in trend has been noticed in CO\textsubscript{2} levels of the atmosphere, and currently it is about 390 ppm, which is expected to reach 500 ppm or higher this century (Procter, 2013). In other studies this rate of increase in levels has also been reported as average of 1.72 ppm per year in the Southern hemisphere (Metzl, 2009) or 0.5% per year (Hugate & Koch, 2003). In a text book written by Miller Jr & Spoolman (2011) called ‘Living in the Environment’, Figure 19-5 illustrates the trend of CO\textsubscript{2} between the years 1880-2009. In 1880, the concentration was only around 290 ppm in the atmosphere which gradually rose to around 320 ppm in the year 1960. After that a steep elevation was noticed from 1960 to 2009, with CO\textsubscript{2} rising up to 385 ppm in 2009 (Miller Jr & Spoolman, 2011). Many sources of these increases in CO\textsubscript{2} concentration are known and well established.
2.2 Sources of elevation of atmospheric CO$_2$

The natural carbon cycle is responsible for about 93% of the CO$_2$ in the atmosphere, the remaining could be associated with human activities (Miller Jr & Spoolman, 2011). The current increase in CO$_2$ concentrations can mainly be linked to human activities such as fossil fuel burning, deforestation, cement manufacturing, etc (Hungate & Koch, 2003; Sanz-Sáez et al., 2012). However, human activity that emits largest amounts of CO$_2$ is the combustion of fossil fuels (coal, natural gas, and oil) for electrical energy and transportation (US EPA, 2012). It was also predicted that the normal passenger cars would dominate the world CO$_2$ production, road passenger transport within the decade 2000 to 2010, and that the contribution of cars to total CO$_2$ emissions would be 95% of total CO$_2$ emissions from road passenger transport (Paravantis & Georgakellos, 2007). Industries also play a significant role in emitting CO$_2$ into the atmosphere. In the USA the overall industrial processes were accountable for about 14% of total USA CO$_2$ emissions and 20% of total USA greenhouse gas emissions in 2010 (US EPA, 2012). Burning fossil fuel in general, seems to be the key factor responsible for major CO$_2$ emissions. Besides human activity, there are also two important biological processes that strongly and positively influence the atmospheric CO$_2$ concentration, by reducing its levels in the atmosphere. The first important process is photosynthesis by plants and the second are the algae (marine and terrestrial) both of which convert the atmospheric CO$_2$ to organic carbon for their own use (Hungate & Koch, 2003). Thus, clearing the CO$_2$-absorbing forests and grasslands can cause changes in its concentration in the atmosphere (Miller Jr & Spoolman, 2011).
2.3 Rise in CO$_2$ in the Arabian Gulf

The Gulf Cooperation Council (GCC) consisting of Bahrain, Kuwait, Oman, Qatar, Saudi Arabia and the UAE formed in 1981, ranks among the top 25 countries of CO$_2$ emissions per capita (Arman & Murad, 2012). This is due to the fact that the average CO$_2$ emissions of the GCC countries in the last 50 years (1960-2009) are about 22.8 metric tons per capita (World Bank, 2007). Therefore, it is evident from this record that the average GCC countries' CO$_2$ emissions in the last 50 years have been about 6 times higher than the world average which is 4.1 metric tons per capita. Figure (1) shows sources of CO$_2$ emissions in the GCC (Arman, Basarir, & Ibrahim, 2013).

![Sources of CO$_2$ Emission](image)

Figure 1: Sources of CO$_2$ emissions in the GCC. International Environmental Agency (IEA) report, 2007 (Arman et al., 2013).

Considering UAE specifically, which after the discovery of oil, took a huge leap towards urbanization and economical growth since 1971 (Kazim, 2007). In one decade (1997-2007) the primary energy production of UAE increased by 55.8% (BP, 2009) and CO$_2$ emissions during this time (1997-2006) increased about the range of
33%-35% (Radhi, 2010). This means to say that as the portion of total energy increased, the production of CO₂ emitted also increased (Radhi, 2010). The resulting CO₂ records were alarming. In the last 48 years, CO₂ emissions of the UAE averaged about 33.6 metric tons per capita, which was about 8 times higher than the world average at 4.1 metric tons per capita (World Bank, 2007). In the year 2000, UAE topped second after Qatar, by producing 41.8 metric tons per capita of CO₂ that year which is more than 10 times the global average (World Bank, 2007). Although the rate has gone down fairly low (19.9) metric tons per capita in the year 2010, it is still much higher than the world average (World Bank, 2007). Rise in global CO₂ concentrations is very likely to have profound effects on the environment and its climate.

2.4 Potential impacts of rising CO₂ levels

"Climate is determined by the average weather conditions of the earth or a particular area, especially temperature and precipitation, over a long period of time, ranging from decades, centuries to thousands of years" (Miller Jr & Spoolman, 2011). Some of the major concerns of rising CO₂ levels are changes in global climate and differences in the usual global precipitation patterns as the current CO₂ concentration doubles (Baker & Allen Jr., 1994). Also the doubling of the present CO₂ concentration forecasting a maximum temperature-increase of 0.01-0.03 °C (Florides & Christodoulides, 2009) and as predicted by The Intergovernmental Panel on Climate Change (IPCC) the temperatures will rise about 1.4-5.8° C by the middle of this century (Houghton et al., 2001). The rise in temperatures will cause the basins to dry out in the Mediterranean Regions (Houghton et al., 2001). There is a strong co-
relation between temperature and CO₂ elevations. These changes in temperatures and climatic variations arise as CO₂ is being added to the atmosphere faster than it is removed by the natural carbon cycle (Miller Jr & Spoolman, 2011). These can contribute to severe droughts in some areas, or excessive flooding in other areas, melting of snow and ice caps, melting of permafrost, rising sea levels, extreme weather, threat to biodiversity, human health problems such as heat exhaustion, agricultural decline leading to the reduction of food supplies in some areas, while causing water shortages in other areas (Miller Jr & Spoolman, 2011).

However, the rising CO₂ concentrations may also have a positive side. In contrast to other greenhouse gases, CO₂ is a plant fertilizer rather than a pollutant (Gielen & Ceulemans, 2001). This may cause a difference in growth patterns in plants as plants use CO₂, water and sunlight at appropriate temperatures to carry out photosynthesis. “Photosynthesis is a complex anabolic process that takes place in cells of green plants” (Nzewi, 2011). “Radiant energy from sun is used to combine CO₂ and water to produce oxygen and carbohydrates and other nutrient molecules” (Miller Jr & Spoolman, 2011). It is a way of plants to reduce atmospheric CO₂ to produce organic compounds (Ehleringer & Cerling, 2002). Therefore, significant impact of increasing atmospheric CO₂ on plants is expected. Lots of studies have been done to test its effects on plants. Kimball (1983), extracted information from 70 reports published during the past 64 years, and found that plants grown in growth chambers/green houses respond better to enriched CO₂ compared to the outdoor plants because nutrient levels in indoor plants are higher than agricultural lands. Firstly, it can be assumed that the photosynthetic activity and its output will greatly
increase, if there is an increase in concentration of atmospheric CO$_2$ from 330 ppm to 1000 ppm as studied by Wittwer (1979). This is because most crops have higher photosynthetic rates when CO$_2$ concentrations are maintained at high levels (Drake et al., 1997; Reicosky, 1989; Ziska et al., 1991) and usually result in increased biomass, and seed yield (Baker & Allen Jr., 1994) over short term exposures. Secondly, plants also respond to rising atmospheric CO$_2$ by reducing stomatal conductance, transpiration, and improving water use efficiency (Drake et al., 1997), resulting in lower transpiration rates than in plants grown at ambient CO$_2$ levels. This response increases the overall resource use efficiency of the plant (Drake et al., 1997). Thirdly, at the same time, increase in photosynthesis rates and light-use efficiency can also be noticed in plants (Baker & Allen Jr., 1994; Drake et al., 1997). Moreover, if higher CO$_2$ concentrations are coupled with elevated temperatures, then further increase in plant biomass, enhance leaf surface area (Bala et al., 2006) and photosynthesis can be noticed, which leads to say that higher CO$_2$ concentrations may only produce higher plant output when temperatures are elevated (Aranjuelo, Irigoyen, Perez, Martinez-Carrasco, & Sanchez-Diaz, 2006). Finally, in order to take up extra water and nutrients to promote growth, plants may also respond by increasing the root biomass (Schroeder, 2011). Excess photosynthetic product may be stored below the ground in roots and other structures, as a response to increasing atmospheric carbon (Schroeder, 2011). Different plant species respond differently and these responses are also strongly influenced by other environmental factors including temperature, light level, and the availability of water and nutrients (Baker & Allen Jr., 1994). However, the effect of CO$_2$ on plant growth can be limited by biotic factors (bacteria, viruses,
fungi, etc.) together with abiotic factors such as: water availability, atmospheric humidity, temperature, solar radiation, wind speed, nutrient limitation, etc (Aranjuelo et al., 2006).

To sum up, increasing concentration of CO₂ in the atmosphere should result in a general increase in the net primary productivity of most cultivated species and forest species, assuming no counterproductive climatic changes occur (Rosenberg, 1981).

2.5 Plant responses to very high CO₂

So far the literature reviewed, only looked upon the aspect of CO₂ concentration doubling in the next 100 years. If this trend continues, the concentration might even increase three fold or more in the next 300 years. Some researchers have been carried out experiments on plants using very high CO₂ concentration and reported their effects. When researching on the effects of eight CO₂ concentration, ranging from 348 to 1791 ppm, on Raphanus sativus (common radish) a C₃ plant, the authors observed an increase in biomass (38% above ground and 55% below ground) at higher concentrations of CO₂ (700ppm – 1791 ppm) (Schubert & Jahren, 2011). Similar results were noticed by Japanese researchers, who studied the response of Cymbidium plantlets (orchid), grown at 0 ppm, 3000 ppm and 10,000 ppm, which also showed increases in shoot and root dry weight, higher percentage at 10,000 ppm when compared to 3000 ppm, at both high and low light regimes (Norikane, Takamura, Morokuma, & Tanaka, 2010). In another study, Cuphea viscosissima seedlings were exposed to very high CO₂ elevation that resulted in greatest seedlings fresh weight, leaf number, root number, and seedling length when supplemented with
10,000 μmol mol⁻¹ CO₂ increasing 607%, 184%, 784%, and 175%, respectively, when compared to seedlings grown without CO₂ enrichment (Tisserat, Vaughn, & Berhow, 2008). Mint and thyme also exhibit more biomass at very high CO₂ concentration as compared to ambient CO₂. When grown at ambient O₂ concentration, increase in shoot fresh weight of mint was 3.1% and thyme was 5.8% when grown at 10,000 ppm CO₂, when compared to thyme and mint grown at ambient O₂ and CO₂ (350 ppm) concentration (Tisserat, Vaughn, & Silman, 2002). However, this might not be the case all the time.

**2.6 Reduced photosynthesis and growth at high CO₂**

Rising atmospheric CO₂ may increase potential net leaf photosynthesis under short-term exposure, but this response decreases under long-term exposure because plants acclimate to elevated CO₂ concentrations through a process known as down regulation (Aranjuelo et al., 2006; Erice, Irigoyen, Pérez, Martínez-Carrasco, & Sánchez-Diaz, 2006a). A long term study revealed no major changes in the relative photosynthetic or respiratory responses of the leaves of the sour orange trees to atmospheric CO₂ enrichment over the 4 years of the experiment (Idso & Kimball, 1992). This may be due to the observed downward regulation of photosynthesis and growth at high CO₂ levels to be due to experimental protocols that result in restricted root growth (Idso & Kimball, 1992). Other authors have concluded that during elevated CO₂ concentrations, plants exhibit a reduced photosynthetic activity at a given, partial pressure of CO₂, and it is believed that this may be a response to accumulation of abundance of carbohydrates stored in the leaves (Kramer, 1981; Sage, 1994). This effect was seen when two tomatoes species were exposed to 330
and 900 microlitres per litre, for 10 weeks. Carbon exchange rates were significantly higher in CO₂ enriched plants for the first few weeks of treatment but thereafter decreased as tomato plants acclimated to high atmospheric CO₂, and no significant accumulation of starch and sugar was seen in the seedlings (Yelle, Beeson, Trudel, & Gosselin, 1989). Another reason for acclimation could be reduced stomata on leaves in response to long term increase in CO₂ concentrations (Wagner et al., 1996). When exposed to long-term elevated CO₂ five species of C₃ plants showed different responses: (a) the initial slope of the CO₂ response was unaffected, but the photosynthetic rate at high CO₂ increased (b) the initial slope decreased but the CO₂-saturated rate of photosynthesis was little affected (c) both the initial slope and the CO₂-saturated rate of photosynthesis decreased (Sage, Sharkey, & Seemann, 1989).

These results indicate that during growth in CO₂-enriched air, leaf RuBisCO content remains in excess of CO₂, of that required to support the observed photosynthetic rates so therefore, no further increase in photosynthesis will be noticed (Sage et al., 1989).

However, it is insufficient to generalize responses without appreciating the differences among the plants themselves. Plants can be classified according the pathway they choose to fix the atmospheric CO₂, hence, affecting their response to the elevated atmospheric CO₂ concentrations.

2.7 C₃ and C₄ plants

There are three main types of plants species, the C₃, C₄ and CAM plant species (Ward, 2009). They differ from each other by the type of photosynthetic
pathway used by each. However, the current thesis dealt only with C₃ and C₄ photosynthetic pathways.

### 2.7.1 C₃ Plants

The most common and very frequently used pathway used by plants is the C₃ pathway for photosynthetic activities (Ward, 2009). It is called so, because the initial product of this pathway is a C₃ molecule. This type of photosynthesis occurs in two phases: the ‘Light Dependent Reaction’ and the ‘Dark Reaction’ also known as the ‘Calvin Cycle’ (Taiz & Zeiger, 2003). The ATP’s and NADPH’s produced in the initial light dependent reaction are carried to the Calvin cycle to further complete the process (Taiz & Zeiger, 2003).

In the process of Calvin cycle, a molecule of CO₂ gets attached to the 5 carbon sugar called RuBP (ribulose 1,5-biphosphate), producing two 3 carbon molecules of sugar called phosphoglyceroate (PGA) as its photosynthetic product (Ward, 2009).

"The C₃ photosynthetic pathway uses light energy to reduce CO₂ to carbohydrates and other organic compounds and is a central component of biological metabolism." (Vogan, 2010). It is a process that occurs during the day time (diurnal), has a high photorespiration due to a very low CO₂ to O₂ ratio and about 1/3rd of the efficiency when compared to C₄ pathway (Ward, 2009). This process is catalyzed by an enzyme called RuBisCo, that has the ability to fix both CO₂ and O₂; and in the cycle where O₂ gets fixed, it may not be so beneficial to the plant (Taiz & Zeiger, 2003). A major problem with the C₃ cycle is that the enzyme RuBisCo catalyzes two competing reactions: carboxylation and oxygenation (Portis Jr & Parry, 2007). The oxygenation reaction directs the flow of carbon through the photo respiratory pathway, and this
can result in losses of between 25% and 30% of the carbon fixed (Valeria & Santiago, 2011). The cycle in which oxygen is fixed instead of CO₂ produces no end product, but uses up much of the ATP and NADPH, lowering the efficiency of the overall cycle. This process is called oxygenase reaction or photorespiration (Taiz & Zeiger, 2003). Environmental variables such as high temperature and drought can result in an increase in the oxygenase reaction (Valeria & Santiago, 2011). Both light dependent and Calvin cycle reactions occur in the mesophyll cells of the leaf (Ehleringer & Cerling, 2002).

2.7.2 C₄ Plants

However, to avoid the wastages occurring from photorespiration, plants have evolved over the years to be able to selectively utilize only CO₂ without the interference of O₂ by a process called C₄ photosynthesis pathway (Ehleringer & Cerling, 2002). C₄ pathway also diurnal in nature, is a slightly more complex and more efficient pathway compared to the C₃, and as the name says, produces a four carbon (C₄) sugar as its photosynthetic product (Ehleringer & Cerling, 2002). However, this pathway is frequent in plants (commonly grasses) that belong to the arid environment (Ward, 2009) and, this pathway helps them be more efficient in low CO₂, dry and high temperature environment (Ehleringer & Cerling, 2002; Ward, 2009). C₄ plants usually out-compete C₃ plants in hot, dry weather due to the absence of photorespiration (Nzewi, 2011). The CO₂ molecule is converted to oxaloacetate (4C) compound initially, and later into other sugars by RuBP carboxylase (RuBisCo), a process which occurs at high CO₂/O₂ ratio thus reducing the photorespiration and minimizing the plant’s water requirements (Ward, 2009). Therefore, The C₄
photosynthesis is an adaptation of the C₃ pathway that overcomes the limitation of the photorespiration, improving photosynthetic efficiency and minimizing the water loss in hot, dry environments (Edwards & Walker, 1983). In C₄ plants, the photorespiration is suppressed by elevating the CO₂ concentration at the site of RuBisCo though suppressing the oxygenase activity of the enzyme (Valeria & Santiago, 2011). This is achieved by a biochemical CO₂ pump and relies on a spatial separation of the CO₂ fixation and assimilation (Valeria & Santiago, 2011).

The initial step happens inside the mesophyll cells, where C₄ acid sugar (oxaloacetate) is produced, and later transferred to the bundle sheath cells, where it gets converted back to the three carbon compound (pyruvate) (Ehleringer & Cerling, 2002). This process releases CO₂ into the bundle sheath cell which is essentially the whole point, as this process selectively only brings CO₂ into the cells, allowing the RuBisCo enzyme to further fix it by Calvin cycle to produce carbohydrates without the interference of O₂ (Ehleringer & Cerling, 2002).

In short, C₃ plants use Calvin cycle to fix carbon obtained from atmospheric CO₂ and produce a 3-carbon molecule as the product of photosynthesis, whereas, C₄ group of plants are believed to be more efficient than the C₃ plants because they first produce a 4-carbon molecule, before the 3-carbon molecule (Nzewi, 2011).

2.8 Effects of elevated CO₂ on C₃ and C₄ (Theoretical)

Growth at elevated CO₂ levels has only two direct effects on plant physiology, these are direct effect on the primary carboxylating enzyme in C₃ photosynthesis, and the second direct effect of elevated CO₂ concentrations on C₃ plants is to reduce stomatal conductance (Leakey et al., 2012). This decrease in stomatal conductance of
individual leaves at elevated CO$_2$ concentrations can integrate across the whole plant
to reduce water use and soil moisture depletion, improve water use efficiency while
increasing canopy temperatures (Leakey et al., 2012). About 85% of plants belong to
C$_3$ category, e.g. oaks, barley, rice, peanuts, cotton, tobacco, soya beans and most
trees (Nzewi, 2011).

Scheme summarizing the main effects of growth at elevated CO$_2$ on C$_3$ plants
is shown in the picture by Leakey et al. (2012), where it is illustrated that C$_3$ plants
grown under elevated CO$_2$ are more likely to have fewer transpiration and
photorespiration rates than compared to their ambient CO$_2$ plant counterparts, and
that it would result in increased photosynthetic rate, higher crop yield, improved soil
moisture content, and overall, increased net primary productivity.

Because, C$_4$ synthesis evolved due to the decrease in atmospheric CO$_2$
previously in history, it is very likely that this type of photosynthesis may perish as
result of increasing CO$_2$ levels (Ehleringer & Cerling, 2002). C$_4$ photosynthesis
compensates for photosynthetic limitations imposed by low atmospheric CO$_2$.
C$_4$ plants concentrate CO$_2$ into the bundle sheath (BS) cells where RuBisCo is
localized (Levin, 2013). This leads to more efficient photosynthesis in warm climates
and thus facilitates domination of open landscapes of low-to-mid latitude (Levin,
2013). C$_4$ photosynthesis also allows exotic C$_4$ grasses to become aggressive weeds.
Where humans facilitate grass establishment, invasive C$_4$ grasses can initiate
autocatalytic grass-fire cycles that destroy tropical forests (Levin, 2013). Although
exotic C$_4$ grasses are expanding, much of the Earth's native C$_4$ diversity is being
degraded due to overexploitation of their habitat for agricultural purposes (Levin, 2013).

C₄ plants make up less than 1% of known plant species (Washio, 2013) but are disproportionately important since they include a number of the world’s major crops and weeds and account for 18% of the global productivity (Ehleringer, Cerling, & Helliker, 1997). The C₄ species dominate both tropical and temperate Grasslands (Archibald, 1995). C₄ grasslands also contribute ~25–30% of global terrestrial productivity (Gillon & Yakir, 2001). Elevated CO₂ doesn’t usually elevate photosynthesis in C₄ plants, this is a feature that distinguishes them from C₃ plants (Leakey et al., 2012). C₄ plants are photosynthetically more efficient than C₃ plants, hence need only about half as much water as C₃ plants for photosynthesis, but only in dry night conditions (Nzewi, 2011). Elevated CO₂ like in C₃ plants, also reduces stomatal conductance in C₄ plants so in places where droughts are frequent, C₄ plants may score well (Leakey et al., 2012). In general, C₄ plants also have a high optimum temperature range; while C₃ plants have a lower optimum temperature range (Angelo, 2012). The C₄ cycle is also helpful to the plants growing in dense tropical forests, where there is poor supply of CO₂ because they have an internal supply of CO₂, which helps them survive in poor CO₂ conditions (Parry, Canziani, Palutikof, van der Linden, & Hanson, 2007). The C₃ and C₄ plant species may differ in their response to elevated CO₂ experimentally as well.

2.9 Effects of elevated CO₂ on C₃ and C₄ (Experimental)

Wand, Midgley, Jones, & Curtis (1999), did a large scale literature review on articles published between 1980 and 1997 to analyze the responses of C₃ and C₄
species towards elevated CO\textsubscript{2}. These authors determined that both species showed positive responses in doubled CO\textsubscript{2} concentrations, by both increasing their photosynthetic rates to 33\% and 25\% respectively; above ground biomasses 44\% and 33\% respectively (Wand et al., 1999). Similar results can also be noticed by experiments done by other researchers. Some authors have estimated that plants having C\textsubscript{3} photosynthetic pathway, may have an increased growth as a result to increasing atmospheric CO\textsubscript{2} (Conroy, Smillie, Küppers, Bevege, & Barlow, 1986; Schroeder, 2011). Cotton plants grown at elevated CO\textsubscript{2} conditions in a study showed significantly heavier roots and stem weight and also increased the dry weight of cotton reproductive structures (Robana, 1996). Also, in the research by Ziska et al. (1991) increased photosynthesis activity, and water use efficiency was noticed in all five C\textsubscript{3} plant species when the concentration of CO\textsubscript{2} was doubled, as compared to the ambient conditions. Additionally, four out of five C\textsubscript{3} species tested, showed significant increase in total plant dry weight (Ziska et al., 1991). However, no significant increases in either photosynthesis or total plant dry weight were noted for the C\textsubscript{4} grasses used in the study (Ziska et al., 1991). Not all C\textsubscript{4} plant species respond to the elevated CO\textsubscript{2} the same way. In a comparative study done by Kellogg, Farnsworth, Russo, & Bazzaz (1999) it was concluded that out of the nine species of C\textsubscript{4} plants studied, only two species which were also the members of the Aristidoideae were significantly larger in height along with a higher biomass in elevated CO\textsubscript{2}; whereas the response of other species varied considerably. Similarly, Chloris gayana (C\textsubscript{4}) plant species may demonstrate significantly higher plant heights when grown under the high CO\textsubscript{2} concentration, but, may over all have a lower number of leaves
and shoot/root ratio than those grown under the ambient CO₂ level (Ksiksi & Youssef, 2010). Two perennial grass species (C₃ and C₄) respond differently to the elevated CO₂. The net primary production of a C₃ species increased to 36% on doubling the CO₂ levels, whereas in a C₄ plant, the increase was just 29% (Chen, Hunt, & Morgan, 1996). Effect of additional temperature elevation on these species increased the primary net production to 43% and 24% respectively (Chen et al., 1996). All the above studies have been done on high value crops but the results should be applicable to the three-fourths of the world agriculture represented by the C₃ crops and possibly to the remaining C₄ crops as well, so keeping these limitations in mind, the analysis showed that yields probably will overall increase by 33% (with a 99.9% confidence interval from 24 to 43%) with a doubling of atmospheric CO₂ concentration (Kimball, 1983).

In conclusion, in C₃ species increased growth under elevated CO₂ is primarily due to decreased photorespiration (photosynthetic fixation of O₂ rather than CO₂), while in C₄ species, increased growth under elevated CO₂ is primarily due to decreased stomatal conductance and transpiration, which decreases soil water use (Kassem, Joshi, Sigler, Heckathorn, & Wang, 2008; Rosenberg, 1981). However, not just the plant height, biomass, and the structure of plant roots, but also the biotic and microbial environment of the soil is also altered by increased atmospheric CO₂ concentration (Sadowsky & Schortemeyer, 1997).

2.10 Soil microbial effect

Microorganisms defined as a collection of many thousands bacteria, protozoa, fungi and microalgae (Miller Jr & Spoolman, 2011), are present everywhere. They
are unseen by naked eyes, and are very abundant in the soil as well (Van Der Heijden, Bardgett, & Van Straalen, 2008). The role of microorganisms in soil is of particular importance. They either directly affect the plants and their root systems, or they are free living microorganisms that alter the nutrient supply of the soil (Van Der Heijden et al., 2008). The direct effect can be understood by the presence of nitrogen fixing bacteria in the soil. They fix the nitrogen present in the atmosphere and convert it to ammonium which can be utilized by the plants; which would otherwise be a limiting nutrient (Sprent & Gardens, 2001). This symbiotic relationship can be commonly seen in nodulated roots of the legume plants, and where the microbes and plants are interdependent on each other (Graham & Vance, 2003). The indirect effect can be noticed by witnessing the effect of free microbes in the soil. They play an important role of decomposition of organic and inorganic matter and recycling the minerals and nutrients back to the soil making it simple forms that can be easily utilized by the plants (Van Der Heijden et al., 2008). These processes mentioned above increase the soil fertility. However, these microbes may affect the plants negatively as well. Some might compete with the plants for nutrients and make it less available for the plants to grow especially in the colder regions (Nordin, Schmidt, & Shaver, 2004).

There can be many factors which can affect the soil microbial content. There could be a persistent change in the soil microbial population and their numbers depending on the drought of that soil and the levels of CO₂ in that place, affecting their relationship with plants (Adam, Owensby, & Ham, 2000). Other reasons may also include; chemical factors such as: pH, oxygen and cation exchange capacity; physical factors such as: soil (texture, pores, structure, moisture, aggregates fauna),
temperature and microbial interactions (Pikuta, Hoover, & Tang, 2007). However, in this thesis soil moisture and CO₂ levels were considered, because all the other factors have been kept constant.

According to many studies done on the soil, the soil sample from the pot of enriched CO₂ (475 ppm) tested by Procter (2013) showed higher microbial biomass as compared to the ambient CO₂ soil (300 ppm). Similarly, elevated atmospheric CO₂ also increased numbers of heterotrophic bacteria in C₃ grass species *Lolium perenne* (Marilley, Hartwig, & Aragno, 1999). In some studies, on the other hand, no significant difference in microbial biomass was detected despite significant increases in the CO₂. As noticed by Kampichler, Kandeler, Bardgett, Jones, & Thompson (1998) there were negligible effects of CO₂ concentration on microbial activity of the soil. The reason it is unlikely that increasing atmospheric CO₂ will have any direct influence on the rhizosphere microbial biomass is because, the CO₂ concentration on the soil is already 10–50 times higher than that in the atmosphere (Sadowsky & Schortemeyer, 1997). However, it is more likely that effect on the microbial biomass maybe be indirect, by increasing root growth and rhizodeposition rates, and decreasing soil water shortage (Sadowsky & Schortemeyer, 1997). Compart, Van Der Heijden, & Sessitsch (2010), reviewed the results of 135 studies investigating the effects of climate change factors on beneficial microorganisms and their interaction with host plants. They found out that, in most cases, plant-associated microorganisms had a beneficial effect on plants under elevated CO₂ (Compant et al., 2010). The majority of studies showed that elevated CO₂ had a positive influence on the abundance of arbuscular and ectomycorrhizal fungi, whereas the effects on plant
growth-promoting bacteria and endophytic fungi were more variable (Compant et al., 2010). Soil microbial biomass was increased by 48% and also their enzymatic activity was improved by the influence of nine years of enriched CO$_2$ (600 ppm) on the function and structural diversity of soil microorganisms in a grassland ecosystem under free air CO$_2$ enrichment (Drissner, Blum, Tscherko, & Kandeler, 2007). In another study, Ronn, Ekelund, & Christensen (2003) grew wheat at enriched atmospheric carbon (ambient +320 ppm) in open top chambers and investigated its effects on bacteria and protozoa of the soil microbial mass (Ronn et al., 2003). It was found that there was no effect on the number of bacteria at elevated CO$_2$ (Lesaulnier et al., 2008; Ronn et al., 2003), but the number of bacterivorous protozoa was higher, which they claim was due to increased root biomass, rather than the effect of rhizodeposition (Ronn et al., 2003). There is also an increase in the response of heterotrophic decomposers and ectomycorrhizal fungi observed at elevated atmospheric CO$_2$ on plants (Alberton, Kuyper, & Gorissen, 2005; Lesaulnier et al., 2008). In the six years of experimental CO$_2$ doubling reduced soil carbon in a scrub-oak ecosystem by Carney, Hungate, Drake, & Meginigal (2007), soils exposed to elevated CO$_2$ had higher relative abundances of fungi and higher activities of a soil carbon-degrading enzyme, which led to more rapid rates of soil organic matter degradation than soils exposed to ambient CO$_2$.

However, in the study of Insam et al. (1999), elevated CO$_2$ was found to have no significant impact on microbial community composition, even after nearly 18 months of atmospheric CO$_2$ enrichment, on artificial tropical ecosystems, each
composed of 77 plants representing seven C3 species, but it did increase the soil humic substances (organic residues of decaying matter) by 30%.

As noticed, different plant species respond differently to atmospheric CO2 enrichment and alfalfa (*Medicago sativa*), being a C3 species might also respond differently.

### 2.11 Alfalfa (*Medicago sativa*)

Alfalfa (*Medicago sativa*) is a valuable (Safarnejad, Collin, Bruce, & McNeilly, 1996), temperate forage crop plant forming a symbiosis with soil nitrogen fixing bacteria which is frequently exposed to elevated temperature, low water availability and relative humidity, nitrogen poor soils etc., in Mediterranean environments (Aranjuelo, Irigoyen, Pérez, Martinez-Carrasco, & Sanchez-Diaz, 2005). The soil used for growing alfalfa should be deep and well drained, and the plant can be expected to grow in less ideal soils as well (Lacefield, Henning, Rasnake, & Collins, 1997). Alfalfa, is a perennial plant, with plantations done mostly done during winters (Cafa, 2004), and is of particular value because of its salt tolerating properties, and is often cultivated in salt affected areas that have high temperatures and few precipitation (Safarnejad et al., 1996). It has an extensive root system sometimes stretching more than 15 m (Cafa, 2004), which enables it to obtain water and nutrients from a large volume of soil (Lacefield et al., 1997). Alfalfa has been grown as a forage crop since the beginning of recorded history and can now be found almost anywhere in the world (Lacefield et al., 1997). This plant is capable of self fertilization by the help of nitrogen fixing bacteria. Fixed nitrogen leads to a higher protein concentration in its various plant parts which in turn enhances our diet.
and can also be recycled into the environment as a form of fertilizer (e.g., green manure) (Bouton, 1996). Alfalfa also has many uses. One of its main uses is as a hay and silage crop, but when dried, ground, and pellet, alfalfa is marketed as a dehydrated feed for many animals (Conrad & Klopfenstein, 1988). The other uses of this plant may include: direct grazing by animals, protein supplements in dehydrated form for bovines and rodents, as sprouts in human diet, production of industrial enzymes, paper pulp production, grain and oil seed uses, and ornament use (Conrad & Klopfenstein, 1988).

2.12 Effects of elevated CO2 on alfalfa

Exposing alfalfa sprouts to high concentrations of CO2 for 80 minutes in full sun showed increased photosynthetic activity by doubling the photosynthetic activity as the concentrations of CO2 were increased to two folds, till up to 2500 ppm concentration of CO2 (Kramer, 1981). CO2 enrichment also increased aboveground leaf and stem mass, but not that of below ground components such as root and nodule, at the end of one month of normal vegetative growth (Erice, Irigoyen, Pérez, Martinez-Carrasco, & Sánchez-Diaz, 2006b). However regardless of water availability, the effect of elevated CO2 on plant development of nodulated alfalfa plants grown was temperature dependent and no effect of CO2 elevation was observed, when applied at ambient temperature (Aranjuelo et al., 2006). Also that alfalfa is a forage crop that shows photosynthetic acclimation during vegetative normal growth (Aranjuelo et al., 2005). The lack of fresh water availability is also a current global concern and any plant that is more water efficient can be beneficial in the long run. CO2 enrichment on plants might seem promising in that aspect.
According to few studies, alfalfa, dry weight (biomass) responses to atmospheric CO$_2$ enrichment at 300 ppm, around 37 % (Erice, Irigoyen, Sánchez-Díaz, Avice, & Ourry, 2007; Sanz-Sáez et al., 2012) but it increased to 64% when the concentration was tripled (900 ppm) (Zebian & Reekie, 1998).

2.13 Rhodes grass (*Chloris gayana*)

*Chloris gayana* is a species of grass known by the common name Rhodes grass. It is one of the major tropical forage grasses, originating from Africa, but widely grown and naturalized throughout the tropics and sub-tropics (Ponsens, Hanson, Schellberg, & Moeseler, 2010). Rhodes grass is a grazing tolerant grass, which is mainly the reason for it being useful for pasturing of animals, which makes it preferable choice for silage, hay and fodder (Humphreys, 1980). Similar to alfalfa, Rhodes grass is good as a rotational crop in both tropical and sub-tropical areas (Humphreys, 1980). It is said to make excellent hay, which possesses a high nutritive value (particularly proteins and fiber (Abate, Kayongo-Male, & Karue, 1981), while its aroma and palatability rendering it very acceptable to stock (Boonman, 1993). It is one of the summer growing grasses and belongs to the C$_4$ grass species that is moderately tolerant to drought (Valeria & Santiago, 2011). Rhodes grass is one of the main sub-tropical grasses used in agriculture and is widely grown in Africa, Australia, Japan, South America and under irrigation in the Middle East for both forage and soil conservation purposes (Moore, Sanford, & Wiley, 2006). It is readily established from seed and the seed germinates quickly (1-7 days) depending on temperature (Moore et al., 2006). Rhodes grass displays good seedling vigor and often achieves full groundcover within three months of sowing (Moore et al., 2006).
Rhodes grass has a high shoot/root ratio and a weak primary root system, so plants rely on developing a strong secondary root system and are easily pulled out by stock during the establishment period (Moore et al., 2006). Rhodes grass prefers high temperatures with maximum growth at 30°C/25°C (day/night temperature) under controlled conditions (Moore et al., 2006). Growth is reduced greatly below 18°C/13°C and there is negligible growth when the average daily temperature is below 8°C (Moore et al., 2006). It is a saline tolerant plant (Tomar, Minhas, Sharma, & Gupta, 2003) as it has a number of mechanisms to deal with salinity including the ability to excrete sodium from salt glands on the leaves, accumulate salt in plant tissues and actively exclude salt from the roots (Moore et al., 2006). Rhodes grass is also tolerant to alkalinity, drought, frost, high pH, low pH, salt, as well as other various soil stresses (Duke, 1983).

As mentioned earlier, Rhodes grass is suitable to tolerate fairly long and dry climates, and is adapted to tropical and subtropical summer-rainfall areas with a small amount of rainfall around 7.5–12.5 dm per year, however, it does not grow well in areas that exceed 18 dm rainfall (Duke, 1978, 1983). Seeds produced by Rhodes grass are usually 3.3–4.4 million per kilogram of the grass (Reed, 1976).

2.14 Effects of elevated CO₂ on Rhodes grass

In a research done by Ksiksi & Youssef (2010), it was found that Chloris gayana (C₄) plant species may demonstrate significantly higher plant heights when grown under the high CO₂ concentration, but, may overall have a lower number of leaves and shoot/root ratio than those grown under the ambient CO₂ level.
In UAE, Rhodes grass is considered one of the most important grass used for animal feeds (SCAD, 2012). The statistics showed that, Rhodes grass accounted of 94 per cent of the total field crops in 2011, with alfalfa taking up 4%, while barley, dry corn and other crops were grown on the remaining 2% of the total area cultivated with field crops (SCAD, 2012). However, it is also the most water consuming crop in the field of agriculture, considering the fact that UAE heavily relies on desalination plants to obtain fresh water for irrigation (Al-Qusaili, 2010). In the year 2010, Abu Dhabi Food Control Authority decided to ban the planting of Rhodes grass in Abu Dhabi, as it was using up the land’s 60% of total water used for irrigation (Al-Qusaili, 2010). The government had also reduced the subsidy for the farmers going this crop, for the same purpose (Al-Mazroui, 2010). It would be very useful to know if elevation of atmospheric CO2 could positively reduce the need to irrigate the plant to the normal extent. This could be tested by inducing water stress on the plants.

2.15 Water Stress

The environment all over the world has constantly been subjected to various amounts of stresses ever since life on this planet began. Stresses have caused the living species to either to perish from the face of earth or to adapt and evolve to suit the new environmental conditions. Examples of few such stresses could be: drought, salinity, extreme temperatures, chemical toxicity, oxidative stress, etc. (Lisar, Motafakkerazad, & Rahm, 2012). As mentioned earlier, if the world green house gases continue to rise, a rise in global temperatures from around 1-6°C can be expected this century (Houghton et al., 2001) and this temperature can have a weighty impact on the world’s climate, particularly on the world’s precipitation (Held & Soden,
2000). Huntington (2006), in his study stated that as the climate warms, there may be an intensification of water cycle and this could result in frequent, and severe storms, flood and droughts in an irregular pattern. In this research the effects on plants due to only drought stress will be considered. Since the beginning of 1900, there have been an increase in overall drought patterns and also precipitation increment have been seen in different parts of the world (Dai, Trenberth, & Karl, 1998). According to couple of studies, the drought is expected "to increase in frequency and severity in the future as a result of climate change, mainly as a consequence of decreases in regional precipitation but also because of increasing evaporation driven by global warming." (Dai, 2011; Sheffield & Wood, 2008). Drought can be defined as "a condition of moisture deficit sufficient to have an adverse effect on vegetation, animals, and man over a sizeable area" (Warwick, 1975) or as a climatic excursion involving a shortage of precipitation sufficient to adversely affect crop production or range production (Rosenberg, 1980).

The term drought and water stress have been used interchangeably for periods in which a plant observes low water availability than it would generally require for normal growth in many literatures, or periods when the demand of water is more than the availability (UN, 2007). It may occur when less water reaches the roots, the transpiration rate is very high, and/or, high soil salinity (Lisar et al., 2012). Every life on this planet depends on water (Hsiao, 1973), and especially non-woody plants contain about 80-90% of water in their structures and require it as a medium for transporting metabolites and nutrients (Lisar et al., 2012). Hence, the plant may have a significant negative impact during the periods of water unavailability. Severe and
prolonged droughts now affect 30% of the earth’s land, and it is predicted that regions
that have avoided drought so far, like USA, might see persistent droughts in the next
20–50 years (Dai, 2011). There could be many reasons for a land to experience
drought which could be natural or human influenced (Haile, 1988). General
atmospheric circulation could be the main cause which might cause fluctuations in
rainfall, creating prolonged periods with less precipitation, leading to a drought
(Haile, 1988). Over cultivation, over grazing, over population and deforestation
maybe be few of the human influences that may cause drought in an area (Haile,
1988).

To cope with drought stress, plants exhibit various drought tolerating and
drought avoiding strategies such as: drought escaping, drought evading, drought
enduring, and drought resisting (Ward, 2009). Within these strategies plants respond
by many different methods which include: reduced transpiration and stomatal closure,
reduced CO₂ assimilation in light due to stomatal closure, changes in respiration,
slower above ground growth rate in response to mild stress, enhanced root
proliferation etc (Hsiao, 1973).

The term water use efficiency is used to express the ratio of transpiration to
carbon assimilation (Leakey et al., 2012). Which means that low stomatal
conductance leading to low transpiration rate, coupled with increased carbon
assimilation at increased CO₂ increased water use efficiency in plants (Leakey et al.,
2012). Periods of low water availability are expected to increase in Mediterranean
ecosystems (Aranjuelo et al., 2005). While working on the pine trees in controlled
growth chambers Wertin, McGuire, & Teskey (2010) found that the plant
productivity (net assimilation rate) in enriched CO\textsubscript{2} and low water treatment, to be higher or equal to that in the high water treatment. CO\textsubscript{2} enrichment on plants also improved the water efficiency in under drought conditions by 19.8\% in winter wheat (C\textsubscript{4}) (Qiao et al., 2010); 80\% increase in water use efficiency in sour orange trees (C\textsubscript{3}) (Leavitt et al., 2003); and, 20-40\% less water supplied to enriched CO\textsubscript{2} corn (C\textsubscript{4}) to achieve similar growth as compared to ambient CO\textsubscript{2} (Chun, Wang, Timlin, Fleisher, & Reddy, 2011). Similar results were also found by Bunce (1995), who reported that canopy conductance to water vapor on an average was 23\% less at high CO\textsubscript{2} than at low CO\textsubscript{2} concentrations in the orchard grass plots (C\textsubscript{3} grass), and 14\% less in the alfalfa plots (C\textsubscript{3}) and also that leaf conductance was reduced in the high CO\textsubscript{2} environment to 77-86\% as compared to the values found at ambient CO\textsubscript{2} conditions. When compared with ambient CO\textsubscript{2} conditions, high CO\textsubscript{2} increased water use efficiency in wheat (C\textsubscript{4}) by 20\% under well-watered conditions but water use efficiency increased by 42\% in response to high CO\textsubscript{2} under drought conditions (Manderscheid & Weigel, 2007). In addition, in a canopy of cotton plants grown at Beltsville, Maryland, USA, the soil water use rates of plants grown under elevated CO\textsubscript{2} were significantly lower than those grown under ambient levels for both water-stressed and well-watered plants (Ephrath, Timlin, Reddy, & Baker, 2011), however plants grown at 350 ppm CO\textsubscript{2} concentration depleted more water from the deepest part of the profile (0.35-0.85 m) than did the plants from the elevated CO\textsubscript{2} treatment (Ephrath et al., 2011). Moreover, few more studies prove similar results. Barley pots used 18\% more water at ambient CO\textsubscript{2} than compared to elevated CO\textsubscript{2} (700 umol l\textsuperscript{-1}) (Robredo et al., 2007), similarly peanuts grown in a greenhouse at elevated CO\textsubscript{2}
extracted much less water from the soil by the end of growing season (Clifford et al., 1995). Soya beans showed 10% less transpiration rates (Jones, Jones, & Allen Jr., 1985) and lower transpiration rates was also seen for rice (Drake & Leadley, 1991) at elevated CO₂ levels. The reduction in transpiration rate also leads to higher water contents in the soil compared to plants grown under ambient CO₂ levels (Adam et al., 2000; Ephrath et al., 2011).

Alfalfa is generally a Mediterranean plant constantly being exposed to high temperatures and low water availability (Aranjuelo et al., 2006), alfalfa is much suited to drought tolerance which is the physiological adaptions that enable a plant to continue functioning in spite of the water deficits (Jones, 1992). Drought does also have effects on soil microbial relationship. Adam et al., (2000); De Luis, Irigoyen, & Sánchez-Diaz (1999); Sgherri, Quartacci, Menconi, Raschi, & Navari-Izzo (1998) concluded that elevated CO₂ enhances growth of water stressed plants by stimulating carbon fixation, preferentially increasing the availability of photosynthates to below-ground production (roots and nodules) without improving water status. This means that elevated CO₂ enhances the ability to produce more biomass in N₂-fixing alfalfa under given soil water stress, improving drought tolerance (De Luis et al., 1999; Sgherri et al., 1998). Rhodes grass is widely grown in drought-prone regions and credited with reasonable drought resistance (Ponsens et al., 2010). In their study, Ponsens et al. (2010) found that the drought resistance abilities in Rhodes grass mainly depends on the phenotypic diversity of the plant. In a study done by 10 researchers, on effects of elevated CO₂ on soil water content, they found that their enriched CO₂ treatment (600 ppm) increased the annual soil water content by 17.3%
compared to productivity of native mixed-grass prairie (C₄ grass species) at 385 ppm (Morgan et al., 2011). They demonstrated that the water conservation effects of elevated CO₂ can completely cancel the desiccating effects of moderately warmer temperatures (Morgan et al., 2011). Similar effects were seen by authors who grew C₄ grass *Panicum coloratum*, at 360 ppm and 1000 ppm by withholding water from half these plants after 3 weeks (Seneweera, Ghannoum, & Conroy, 2001). They noticed, that after water restriction, the plants at ambient CO₂ fared more poorly than compared to the ones at elevated CO₂ (Seneweera et al., 2001). Clearly, there is enough information to support that elevated CO₂ does increase the water use efficiency of plants.
CHAPTER III
MATERIALS & METHODS
CHAPTER III: MATERIALS AND METHODS

3.1 Location of the study

The experiment was carried out in United Arab Emirates (UAE) University campus in Al-Ain city (N 24.19, E 55.62) within the time period of March 2013 to November 2013. The experimental procedure was used as the study design.

3.2 Growth chambers

Research was conducted in two plant growth chambers (Binder, Model: 720, KBW E5.1). One growth chamber had enriched/elevated CO₂ conditions (ECO₂ ~ 1000 ppm) whereas the second growth chamber was used as a control with ambient CO₂ conditions (ACO₂ ~ 500 ppm). All other parameters such as temperature, light, humidity etc., within both growth chambers were kept close to identical. Source of CO₂ were 20-kg CO₂ canisters. The levels of CO₂ were monitored on a daily basis, using a CO₂ monitor and controller (TONGDY td.) and (Extech Desktop Indoor Air Quality CO₂ Datalogger C0210).

3.3 Experimental plan

Two species of plants; alfalfa (Medicago sativa) and Rhodes grass (Chloris gayana), were grown and tested in two different CO₂ concentrations and water levels. Although the difference in Nitrogen fixing ability is well understood, and it is also known that there is difference in their photosynthetic pathway, but it was believed that species will represent C₃ and C₄ general plant ecophysiological characteristics. Total of 96 plastic growing pots (13.8 cm diameter; 13 cm height) were used in the experiments, which were divided into two groups, alfalfa and Rhodes grass. Forty-eight plastic growing pots were assigned for each species. Within each category, the
pots were further divided into ‘ambient’ (ACO2) and ‘elevated’ CO2 concentrations (ECO2); each with twenty-four pots. Within ACO2 concentrations, 12 of the 24 pots were exposed to water stress i.e. were given restricted amount of water (50 ml, once per week), while the other 12 pots were watered normally (50 ml, twice per week). Quantity of water was fixed to 50 ml per pot, by observations during the initial trial phase of the experiment, where 50 ml of water was noticed to be sufficient for the soil to be evenly moist. The same method was used for the pots under ECO2 concentrations. All the other parameters (temperature, humidity, moisture, light intensity), were kept constant for all the 96 pots. The growing medium used was (Potgrond H) soil substrate mixture and normal tap water was used for irrigation purpose.

3.4 Data collection

Soil microbial activity, above ground growth (shoots and leaves), below ground growth (roots), and were measured destructively on a regular basis.

3.4.1 Testing soil microbial activity

The total microbial activity of the freshly sampled sediments was observed by measuring the rate of hydrolysis of Fluorescein diacetate FDA (Sigma Chemical Co., St Louis, Miss, USA) by the soil microbes. Around 2 g of the each soil sample was placed separately in 100 ml Erlenmeyer flasks. Next, 20 ml of 60 mM potassium phosphate buffer was added to each flask. The reaction was initiated by adding 0.2 ml (200 µl) of FDA stock solution. All the flasks were shaken on a rotary shaker at 25 °C for 20 minutes. Finally, the reaction was stopped by adding 20 ml of acetone to each flask. All the samples were then filtered on a muslin cloth first and then through a
filter paper. Filtrate was collected in glass tubes with screw caps to prevent any evaporation. Fluorescein concentration in the solution was measured using spectrophotometer (Perkin Elmer precisely, Lambda 25, UV/VIS spectrometer) by reading the optical density at 490 nm. To prepare the 60 mM potassium phosphate buffer and FDA stock solution the method of (Inbar et al., 1991) was employed.

3.4.2 Weight of the shoots

3.4.2.1 Fresh weight

First the fresh weight of the sample plant shoots was recorded. Each plant was taken separately, and weighed in a container. The weight of the empty container was subtracted from the total weight measured.

3.4.2.2 Dry weight

The samples after they were weighed for the fresh weight were kept in the hot oven (JSR Oven J$O F -240) along with the containers for 48 hours at 70°C to dry out the moisture in the sample shoots and weighed again for the dry weight.

3.4.3 Weight of the roots

3.4.3.1 Fresh weight

Roots were first separated by cutting from the plant shoots and then washed in clean tap water to remove any excess soil attached to them. Then they were placed on paper towels to remove excess moisture. Next, they were weighed on a weighing balance in the containers and the weight of the empty container was then deducted from the total weight.

3.4.3.2 Dry weight
Same method was used to dry the roots as well, by placing them in the oven for 48 hours at 70°C to dry out the moisture in the sample roots and weighed again for the dry weight.

3.4.4 Above ground shoot growth

The whole plant was carefully pulled out by the root from the pots. Plant was then placed on to white sheets of paper and the height of the shoot was marked. Then using an ordinary ruler/measuring tape for tall plants, distances between the two marks were measured giving the approximate height of the plant in centimeters.

3.4.5 Below ground root growth

The roots that were extracted from the soil along with the plant were first placed on the white sheet of paper and the length of the root was marked on the paper, which was then measured using a ruler and recorded to approximate centimeters.

3.4.6 Shoot/root ratio

Shoot/root ratio was calculated using the obtained shoot height and dividing it by root length from the same sample plant to give the ratio.

3.4.7 Number of leaves

The leaves were counted manually in each separate sample plant and recorded on the data sheet.

3.5 Statistical analysis

SPSS (IBM SPSS Statistics Version 21.0) was used to perform analysis of variance (GLM-ANOVA), t-tests and descriptive statistics to analyze the effect of each variable in the study. Raw data was converted into percentages, ratios, cumulative results etc, depending whatever was suitable for the treatment used.
Shoot/root ratio was calculated by dividing the shoot length in centimeters by root length in centimeters. Biomass of the plant was calculated by adding up the weight of shoot and weight of root of the same plant. Water use efficiency (WUE) of the plant was calculated by dividing cumulative amounts of water given to a plant in milliliters divided by the biomass of the same plant. Results were expressed in ml/g units. Height was calculated as percentage of growth, by dividing each sample height, to the initial height at day 1 of the experiment, multiplied by 100. Results were expressed in percentages. Leaves were also expressed in percentages, by dividing number of leaves of each sample, by the number of leaves of that sample at day 1 of the experiment, multiplied by 100. Results were expressed in percentages.

Two-way analysis of variance (ANOVA) analysis was done to compare the main effects (ambient and enriched CO2) on the growth of plant observed in this study. CO2 treatment and month were kept as fixed factors, and their interactions were assessed on the dependent variables such as; microbial activity, plant height, root length, number of leaves, and shoot/root ratio. Graphs, descriptive data and post hoc results were expressed by splitting the files species (Rhodes grass or alfalfa), and their water treatment conditions. Finally, T-test was done on the same raw data to assess the significant differences between the results of both CO2 treatments on each variable.

One way analysis of variance (ANOVA) was done for plant biomass and water use efficiency, to understand the effects and comparisons of the two variables under each treatment. Independent T-test was done, afterwards to assess the
significant differences between the results of both CO₂ treatments on each variable. Graphs were manually made in Excel using the means from the ANOVA results.

If the variability in the data was large, and there was a large range of standard deviations, outliers were removed from the raw data. It was ensured that equal number of outliers were removed from each treatment if any.
CHAPTER IV
RESULTS
4.1 Effect of CO₂ enrichment on microbial activity on water stressed and non-water stressed plants

4.1.1 Microbial activity in Rhodes grass

Rhodes grass

There were significant effects of month (P < 0.005) on the microbial activity of Rhodes grass grown under ambient water conditions (Table 1). Microbial activity in the month of October was significantly different (P < 0.01) compared to other months. Over all, Rhodes grass grown under enriched CO₂ conditions had a higher microbial activity compared to the ones grown under ambient CO₂ conditions (1.17 and 0.84, respectively as over all means). Figure (2) illustrates the trend of microbial activity under different CO₂ treatment in each month.

![Figure 2: Microbial activity of Rhodes grass grown with or without CO₂ enrichment under ambient water conditions.](image-url)
Table 1: ANOVA results for microbial activity of Rhodes grass grown with or without CO₂ enrichment under ambient water conditions.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>9.033</td>
<td>7</td>
<td>1.290</td>
<td>4.026</td>
<td>.024</td>
</tr>
<tr>
<td>Intercept</td>
<td>10.771</td>
<td>1</td>
<td>10.771</td>
<td>33.607</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>2.84</td>
<td>1</td>
<td>.284</td>
<td>.886</td>
<td>.369</td>
</tr>
<tr>
<td>Month</td>
<td>8.035</td>
<td>3</td>
<td>2.678</td>
<td>8.357</td>
<td>.004</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>499</td>
<td>3</td>
<td>.166</td>
<td>.519</td>
<td>.679</td>
</tr>
<tr>
<td>Error</td>
<td>3.205</td>
<td>10</td>
<td>.321</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30.441</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>12.238</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* R Squared = .738 (Adjusted R Squared = .555)
Rhodes (Water stress)

The analysis of variance revealed significant strong interaction between CO\textsubscript{2} and month at P < 0.001 (Table 2). As shown in Figure (3), in the plants grown under enriched CO\textsubscript{2} conditions, there was a fairly constant microbial activity for the first three months, with sharp increase in the microbial activity thereafter, reaching the maximum in the month of October. Lowest activity was noticed in the month of May. On the other hand, plants grown at ambient CO\textsubscript{2} conditions had a short increase in activity in the month of May, which slightly declined and reached to its lowest in September. The microbial activity then increased to its maximum level in October. In general, the overall activity in the plants grown at enriched CO\textsubscript{2} conditions was significantly higher (P < 0.005; Table 3) than in plants grown at Ambient CO\textsubscript{2} conditions, (0.90 and 0.53, respectively as overall means).

![Figure 3: Microbial activity of Rhodes grass grown with or without CO\textsubscript{2} enrichment under water-stress conditions. * indicates the significant difference between the treatments at P < 0.005.](image-url)
Table 2: ANOVA results for the microbial activity of Rhodes grass grown with and without CO2 enrichment under water-stress conditions.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>5.430</td>
<td>7</td>
<td>7.776</td>
<td>13.968</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>7.109</td>
<td>1</td>
<td>7.109</td>
<td>128.008</td>
<td>.000</td>
</tr>
<tr>
<td>CO2</td>
<td>.641</td>
<td>1</td>
<td>.641</td>
<td>11.545</td>
<td>.007</td>
</tr>
<tr>
<td>Month</td>
<td>3.099</td>
<td>3</td>
<td>1.033</td>
<td>18.602</td>
<td>.000</td>
</tr>
<tr>
<td>CO2 * Month</td>
<td>2.824</td>
<td>3</td>
<td>.941</td>
<td>16.952</td>
<td>.000</td>
</tr>
<tr>
<td>Error</td>
<td>.555</td>
<td>10</td>
<td>.056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14.787</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>5.985</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .907 (Adjusted R Squared = .842)

Table 3: T-test results for the microbial activity of Rhodes grass grown with and without CO2 enrichment under water-stress conditions.

<table>
<thead>
<tr>
<th>Levene's Test for Equality of Variances</th>
<th>T-test for Equality of Means</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>Sig.</td>
<td>t</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>11.193</td>
<td>.004</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>1203</td>
<td>1.203</td>
</tr>
</tbody>
</table>

46
### 4.1.2 Microbial activity in alfalfa

Alfalfa (Non water stress)

No significant difference was observed between the two treatments on microbial activity of alfalfa grown under ambient water conditions. As shown in Figure (4), both treatments showed a similar trend of microbial activity. There was a slight rise in microbial activity in May, which declines till September, followed by a sharp rise in the activity in the month of October.

![Figure 4: Microbial activity of alfalfa grown with or without CO₂ enrichment under ambient water conditions.](image-url)
No significant difference was observed between the two treatments on microbial activity of alfalfa grown under water-stress conditions. As shown in Figure (5), for the plants grown under enriched CO$_2$ conditions, there was a gradual increase in microbial activity for the first three months, followed by a sharp increase reaching its maximum in the month of October. Lowest activity was noticed in April. For the plants grown under ambient CO$_2$ conditions, there was initial rise in activity in May, after which the activity declined till September, followed by a sharp rise in the month of October. Over all, alfalfa grown under enriched CO$_2$ conditions had a higher microbial activity compared to the ones grown under ambient CO$_2$ conditions (0.88 and 0.76, respectively as over all means).

**Figure 5:** Microbial activity of alfalfa grown with or without CO$_2$ enrichment under water-stress conditions.
4.2 Effect of CO\textsubscript{2} enrichment on morphology of the plants

4.2.1 Fresh weight: Water use efficiency (WUE)

Analysis of variance results followed by T-test results showed significant effects of water stress on WUE of Rhodes grass (P < 0.05). As shown in Figure (6), when water stress was induced onto Rhodes grass, the Rhodes grass achieved its maximum WUE potential, as compared to its counterpart (control) grown under ambient CO\textsubscript{2} and water conditions. However, when Rhodes grass was exposed to both, water stress and CO\textsubscript{2} elevations, there was no significant difference noticed, but overall average WUE was higher than its control counterpart (132.70 ml/g, 538.59 ml/g) respectively. Lowest WUE (538.59 ml/g) was noticed in control (green: ambient CO\textsubscript{2} and water conditions).

![Rhodes WUE (Fresh)](image)

**Figure 6:** WUE of Rhodes grass grown at different CO\textsubscript{2} and water stress combinations.
Analysis of variance results followed by T-test results showed significant combined effects of elevated CO$_2$ and water stress on WUE of alfalfa (P < 0.05). As shown in Figure (7), when elevated CO$_2$ combined with water stress was induced onto alfalfa, the alfalfa achieved its maximum WUE potential, as compared to its counterpart (control) grown under ambient CO$_2$ and water conditions. Although not significant, the highest average WUE was noticed when alfalfa was induced to water stress only (1540.43 ml/g). Lowest WUE (6838.53 ml/g) was noticed in control (green; ambient CO$_2$ and water conditions).

![Alfalfa WUE (Fresh)](image)

**Figure 7:** WUE of alfalfa grown at different CO$_2$ and water stress combinations.
4.2.2 Fresh weight: Biomass

Analysis of variance results followed by T-test results showed significant individual effects of CO2 elevations (P = 0.005) and water stress (P < 0.01) on the biomass of Rhodes grass. As shown in Figure (8), when only CO2 was elevated keeping water the same, it significantly lowered the plant mean biomass. Only inducing water stress lowered the biomass of the plant significantly. However, when both CO2 enrichment and water stress treatments were induced in a combination onto Rhodes grass, the mean biomass of the two groups were not significantly different. Over-all, highest biomass was noticed in plants exposed to elevated CO2 conditions and water stress (19.13 g) and lowest biomass was noticed in plants exposed to CO2 elevation only (10.38 g).

![Rhodes Biomass (Fresh)](image)

*Figure 8: Biomass of Rhodes grass grown at different CO2 and water stress combinations.*
Analysis of variance results followed by T-test results showed significant combined effects of elevated CO\textsubscript{2} and water stress on biomass of alfalfa ($P < 0.05$). As shown in Figure (9), when elevated CO\textsubscript{2} combined with water stress was induced onto alfalfa, alfalfa achieved significantly higher biomass as compared to its control counterpart (green; ambient CO\textsubscript{2} and water conditions). Effects of individual treatments such as exposure to elevated CO\textsubscript{2} and exposure to water-stress alone had no significant differences compared to the control. However, not significant, highest average biomass was noticed when alfalfa was induced to water stress only (2.81 g) and lowest WUE (0.90 g) was noticed in control.

![Alfalfa Biomass (Fresh)](image)

**Figure 9:** Biomass of alfalfa grown at different CO\textsubscript{2} and water stress combinations.
4.2.3 Dry weight: Water use efficiency (WUE)

Analysis of variance results followed by T-test results showed significant effects of water stress on WUE of Rhodes grass (P < 0.05). As shown in Figure (10), when water stress was induced onto Rhodes grass, Rhodes grass achieved its maximum WUE potential, as compared to its counterpart (control) grown under ambient CO₂ and water conditions. However, when Rhodes grass was exposed to both, water stress and CO₂ elevations, there was no significant difference noticed, but overall average WUE was higher than its control counterpart (3993.44 ml/g, 547.44 ml/g) respectively. Lowest WUE (547.44 ml/g) was noticed in control (green; ambient CO₂ and water conditions).

![Rhodes WUE (Dry)](image)

**Figure 10:** Water use efficiency (WUE) of Rhodes grass grown at different CO₂ and water stress combinations.
No significant effect of any treatment was noticed for WUE of alfalfa calculated using dry weight. Generally the overall means of alfalfa grown under water stress are lower compared to the ones grown at normal water conditions (Figure 11). Highest mean WUE was noticed in the control group (29014.33 ml/g), and the lowest was noticed in water stressed group (6492.63 ml/g).

![Alfalfa WUE (Dry)](image)

**Figure 11**: Same as above WUE of alfalfa grown at different CO₂ and water stress combinations.
4.2.4 Dry weight: Biomass

Analysis of variance results followed by T-test results showed significant individual effects of CO₂ elevations (P < 0.05) and water stress (P < 0.05) on biomass of Rhodes grass. As shown in Figure (12), when only CO₂ was elevated keeping water the same, it significantly lowered the mean biomass of the plant. On the other hand, by only inducing water stress, biomass of the plant increased significantly. But when both CO₂ enrichment and water stress treatments were induced in a combination onto Rhodes grass, mean biomass of the two groups were not significantly different. Over-all, highest biomass was noticed in plants exposed only to water stress (3.38 g) and lowest biomass was noticed in plants exposed to CO₂ elevation only (1.16 g).

![Rhodes Biomass (Dry)](image)

**Figure 12:** Biomass of Rhodes grass grown at different CO₂ and water stress combinations.
Analysis of variance results followed by T-test results showed significant combined effects of elevated CO$_2$ and water stress on biomass of alfalfa ($P < 0.05$). As shown in Figure (13), when elevated CO$_2$ combined with water stress was induced onto alfalfa, alfalfa achieved significantly higher biomass as compared to its control counterpart (green; ambient CO$_2$ and water conditions). Effects of individual treatments such as exposure to elevated CO$_2$ and exposure to water-stress alone had no significant differences compared to the control. Although not significant, highest average biomass was noticed when alfalfa was induced to water stress only (0.66 g) and lowest WUE (0.19 g) was noticed in control.

**Figure 13:** Biomass of alfalfa grown at different CO$_2$ and water stress combinations.
4.2.5 Height percentage

Rhodes grass: Shoot (Non water stress)

Analysis of variance showed significant interactions of CO$_2$ × month at $P < 0.001$ (Table 4) on the height of plants grown at ambient water conditions. As shown in Figure (14), plants grown in enriched CO$_2$ treatment had higher height in September as compared to the ones grown without CO$_2$ enrichment. However in October, heights of plants grown under ambient CO$_2$ conditions exceeded the heights of plants grown under CO$_2$ enrichment and attained their maximum height in that month. Overall, the average height percentages (%) of plants grown at ambient CO$_2$ conditions (2029%) were higher than the average height percentages (%) of plants grown under enriched CO$_2$ treatment (1508%).

![Figure 14: Plant height of Rhodes grass grown under ambient water conditions, with or without CO$_2$ enrichment.](image-url)
Table 4: ANOVA results for plant height of Rhodes grass grown under no water stress conditions, with or without CO₂ enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>385636941.843</td>
<td>7</td>
<td>55090991.692</td>
<td>185.648</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>268350733.183</td>
<td>1</td>
<td>268350733.183</td>
<td>904.299</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>483901.673</td>
<td>1</td>
<td>483901.673</td>
<td>1.631</td>
<td>.210</td>
</tr>
<tr>
<td>Month</td>
<td>360924667.747</td>
<td>3</td>
<td>120308222.582</td>
<td>405.420</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>9134907.870</td>
<td>3</td>
<td>3044969.290</td>
<td>10.261</td>
<td>.000</td>
</tr>
<tr>
<td>Error</td>
<td>10386246.142</td>
<td>35</td>
<td>296749.890</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>531559236.111</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>396023187.984</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .974 (Adjusted R Squared = .969)
Rhodes grass; Shoot (Water stress)

There was a significant interaction of month on the height (%) of the plants ($P < 0.001$; Table 5), grown at both enriched and ambient CO$_2$ conditions under water stress. The trend in Figure (15) showed a higher plant height of plants grown at ambient CO$_2$ conditions at each month, as compared to enriched CO$_2$ conditions. The trend of plants grown at both CO$_2$ treatments are almost the same, indicating that under water stress conditions, plants grown at CO$_2$ enrichment, grow equally as well as the ones grown at ambient CO$_2$ environment. However, the average mean of plants grown under ambient CO$_2$ treatment is still higher (2342.0%) than the ones grown under enriched CO$_2$ treatment (1840.9%).

Figure 15: Plant height of Rhodes grass grown under water stress conditions, with or without CO$_2$ enrichment.
Table 5: ANOVA results for plant height of Rhodes grass grown under water stress conditions, with or without CO₂ enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>452056128.858</td>
<td>7</td>
<td>64579446.980</td>
<td>100.051</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>449502346.748</td>
<td>1</td>
<td>449502346.748</td>
<td>696.400</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>1066733.635</td>
<td>1</td>
<td>1066733.635</td>
<td>1.653</td>
<td>.207</td>
</tr>
<tr>
<td>Month</td>
<td>443811752.244</td>
<td>3</td>
<td>147937250.748</td>
<td>229.195</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>916244.164</td>
<td>3</td>
<td>305414.721</td>
<td>.473</td>
<td>.703</td>
</tr>
<tr>
<td>Error</td>
<td>23882226.080</td>
<td>37</td>
<td>645465.570</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>673828750.000</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>475938354.938</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .950 (Adjusted R Squared = .940)
Rhodes grass; Root (Non water stress)

Significant interaction of month was noticed on the root length (%) of Rhodes grass grown under no water stress conditions at both with or without CO\textsubscript{2} enrichment (P < 0.001; Table 6). As shown in Figure (16), the trend shows a sharp increase in root length grown under enriched CO\textsubscript{2} treatment in September and October, as compared to moderate but consistent increase in the ambient CO\textsubscript{2} treatment. However, the overall mean of plants grown under ambient CO\textsubscript{2} conditions have a higher mean (725.2%), than the ones grown under enriched CO\textsubscript{2} treatment (694.8%).

Figure 16: Root length of Rhodes grass grown under ambient water conditions, with or without CO\textsubscript{2} enrichment.
Table 6: ANOVA results of root length of Rhodes grass grown under ambient water conditions, with or without CO₂ enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>53390345.180</td>
<td>7</td>
<td>7627192.169</td>
<td>35.656</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>39700973.375</td>
<td>1</td>
<td>39700973.375</td>
<td>185.594</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>665864.268</td>
<td>1</td>
<td>665864.268</td>
<td>3.113</td>
<td>.086</td>
</tr>
<tr>
<td>Month</td>
<td>52576079.545</td>
<td>3</td>
<td>17525359.848</td>
<td>81.928</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>847530.418</td>
<td>3</td>
<td>282510.139</td>
<td>1.321</td>
<td>.283</td>
</tr>
<tr>
<td>Error</td>
<td>7486941.001</td>
<td>35</td>
<td>213912.600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>82575537.190</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>60877286.181</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .877 (Adjusted R Squared = .852)
Rhodes grass; Root (Water stress)

Significant interaction of month were revealed by analysis of variance on the root length (%) of Rhodes grass grown under water stress conditions at both with or without CO₂ enrichment (P < 0.001; Table 7). As shown in Figure (17), the trend shows a sharp increase in root length grown under enriched CO₂ treatment in September and October, as compared to moderate increase in length under ambient CO₂ treatment. The overall mean of plants grown under enriched CO₂ treatment have a higher mean (859.5%), than the ones grown under ambient CO₂ condition (692.6%). This indicates that under water stress conditions, there is more investment in the root of the plant under enriched CO₂ treatment.

![Figure 17: Root length of Rhodes grass grown under water stress conditions, with or without CO₂ enrichment.](image-url)
Table 7: ANOVA results for root length of Rhodes grass grown under water stress conditions, with or without CO\textsubscript{2} enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>65884837.567\textsuperscript{a}</td>
<td>6</td>
<td>10980806.261</td>
<td>22.477</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>50459528.442</td>
<td>1</td>
<td>50459528.442</td>
<td>103.285</td>
<td>.000</td>
</tr>
<tr>
<td>CO\textsubscript{2}</td>
<td>1409070.988</td>
<td>1</td>
<td>1409070.988</td>
<td>2.884</td>
<td>.098</td>
</tr>
<tr>
<td>Month</td>
<td>64944260.932</td>
<td>3</td>
<td>21648086.977</td>
<td>44.311</td>
<td>.000</td>
</tr>
<tr>
<td>CO\textsubscript{2} * Month</td>
<td>2464315.561</td>
<td>2</td>
<td>1232157.781</td>
<td>2.522</td>
<td>.094</td>
</tr>
<tr>
<td>Error</td>
<td>17587597.567</td>
<td>36</td>
<td>488544.377</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>109500495.868</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>83472435.134</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} R Squared = .789 (Adjusted R Squared = .754)
Alfalfa; Shoot (Non water stress)

Analysis of variance revealed significant interaction of month on the height (%) of alfalfa plants grown under ambient water conditions at $P < 0.001$ (Table 8). As shown in Figure (18), there was a gradual and consistent increase in plant heights grown under enriched CO$_2$ treatment. On the other hand, plants grown without CO$_2$ enrichment, showed a gradual increase in the month of April and May but then an abrupt decrease in height (%) was noticed in September, followed by a steep increase in October. In general, the overall mean of alfalfa height % under both CO$_2$ treatments is nearly the same, (ambient: 120.6%; enriched: 122.2%) indicating that there is very little effect of CO$_2$ enrichment on plant height under CO$_2$ enrichment at normal water conditions.

![Figure 18](image.png)

**Figure 18**: Plant height of alfalfa grown under ambient water conditions, with or without CO$_2$ enrichment.
Table 8: ANOVA results of plant height of alfalfa grown under ambient water conditions, with or without CO$_2$ enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>37311.974*</td>
<td>7</td>
<td>5330.282</td>
<td>8.159</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>351663.727</td>
<td>1</td>
<td>351663.727</td>
<td>538.267</td>
<td>.000</td>
</tr>
<tr>
<td>CO2</td>
<td>1381.385</td>
<td>1</td>
<td>1381.385</td>
<td>2.114</td>
<td>.155</td>
</tr>
<tr>
<td>Month</td>
<td>34508.059</td>
<td>3</td>
<td>11502.686</td>
<td>17.606</td>
<td>.000</td>
</tr>
<tr>
<td>CO2 * Month</td>
<td>2956.342</td>
<td>3</td>
<td>985.447</td>
<td>1.508</td>
<td>.229</td>
</tr>
<tr>
<td>Error</td>
<td>23519.746</td>
<td>36</td>
<td>653.326</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>710171.650</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>60831.720</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .613 (Adjusted R Squared = .538)
Alfalfa; Shoot (Water stress)

Significant interaction of CO$_2$ x month revealed by analysis of variance on the height (%) of alfalfa plant under water stress conditions at P = 0.05, (Table 9). As shown in Figure (19), sharp increase in height (%) is noticed in alfalfa plants grown without CO$_2$ enrichment, in May and October, with heights only slightly declining in September. However, plants grown under CO$_2$ enrichment have shown only slight increase in plant height over the course of experiment. Overall, mean heights of plants grown without CO$_2$ enrichment are significantly different at P = 0.01 (Figure 10) as compared to plants grown with CO$_2$ enrichment with mean height percentages as 163.01% and 113.41% respectively. These results indicate that CO$_2$ enrichment suppresses plant height under water stress.

![Figure 19](image-url): Plant height of alfalfa grown under water stress conditions, with or without CO$_2$ enrichment. * indicates significant difference between the treatments at P = 0.01.
Table 9: ANOVA results of Plant height of alfalfa grown under water stress conditions, with or without CO₂ enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>1699908.717</td>
<td>7</td>
<td>24272.674</td>
<td>7.226</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>456878.736</td>
<td>1</td>
<td>456878.736</td>
<td>136.018</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>19988.132</td>
<td>1</td>
<td>19988.132</td>
<td>5.951</td>
<td>.019</td>
</tr>
<tr>
<td>Month</td>
<td>76341.971</td>
<td>3</td>
<td>25447.324</td>
<td>7.576</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>49929.395</td>
<td>3</td>
<td>16643.132</td>
<td>4.955</td>
<td>.005</td>
</tr>
<tr>
<td>Error</td>
<td>127640.022</td>
<td>38</td>
<td>3358.948</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1176288.286</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>297548.739</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .571 (Adjusted R Squared = .492)

Table 10: T-test results of Plant height of alfalfa grown under water stress conditions, with or without CO₂ enrichment.

<table>
<thead>
<tr>
<th>Levene's Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>7.268</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>2.151</td>
</tr>
</tbody>
</table>

68
Alfalfa; Root (Non water stress)

Analysis of variance show significant interaction of $CO_2 \times$ month at $P < 0.001$ on the root length (%) of alfalfa plant grown in without water stress, under both $CO_2$ treatments (Table 11). As shown in Figure (20), the root length (%) for plants grown under $CO_2$ enrichment increases moderately till September, after which there is a sharp rise in length in October. For the plants grown without $CO_2$ enrichment, the root length increase is slow and gradual over time, with slight reductions seen in September. Overall, the mean root length (%) grown under enriched $CO_2$ treatment is higher (120.0%) than the ones grown at ambient $CO_2$ conditions (115.5%).

![Graph showing root length of alfalfa grown under ambient water conditions, with or without CO2 enrichment.](image)

**Figure 20**: Root length of alfalfa grown under ambient water conditions, with or without $CO_2$ enrichment.
Table 11: ANOVA results of root length of alfalfa grown under ambient water conditions, with or without CO₂ enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>227222.934</td>
<td>7</td>
<td>32460.419</td>
<td>27.133</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>428806.110</td>
<td>1</td>
<td>428806.110</td>
<td>358.427</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>6040.533</td>
<td>1</td>
<td>6040.533</td>
<td>5.049</td>
<td>.031</td>
</tr>
<tr>
<td>Month</td>
<td>190471.488</td>
<td>3</td>
<td>63490.496</td>
<td>53.070</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>36527.888</td>
<td>3</td>
<td>12175.963</td>
<td>10.178</td>
<td>.000</td>
</tr>
<tr>
<td>Error</td>
<td>45461.488</td>
<td>38</td>
<td>1196.355</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>910903.591</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>272684.423</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .833 (Adjusted R Squared = .803)
Significant interaction of CO$_2$ × month was noticed for the roots grown under water stress at both CO$_2$ treatments ($P = 0.01$; Table 12). As shown in Figure (21), the curve of roots grown under enriched CO$_2$ treatment show a very steep rise in length in September but then also reduces sharply in October. On the other hand, the roots of plants grown under ambient CO$_2$ conditions show gradual but constant rise in root length (%). Overall, the average root length (%) is higher (131.0%) at ambient CO$_2$ conditions as compared to enriched CO$_2$ treatment (119.0%).

Figure 21: Root length of alfalfa grown under water stress conditions, with or without CO$_2$ enrichment.
Table 12: ANOVA results of root length of alfalfa grown under water stress conditions, with or without CO₂ enrichment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>210825.259</td>
<td>7</td>
<td>30117.894</td>
<td>18.596</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>610291.632</td>
<td>1</td>
<td>610291.632</td>
<td>376.819</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>4164.512</td>
<td>1</td>
<td>4164.512</td>
<td>2.571</td>
<td>.117</td>
</tr>
<tr>
<td>Month</td>
<td>181560.629</td>
<td>3</td>
<td>60520.210</td>
<td>37.368</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>20989.023</td>
<td>3</td>
<td>6996.341</td>
<td>4.320</td>
<td>.010</td>
</tr>
<tr>
<td>Error</td>
<td>63163.946</td>
<td>39</td>
<td>1619.588</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1010037.691</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>273989.203</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a, R Squared = .769 (Adjusted R Squared = .728)
4.2.6 Shoot/root ratio

Rhodes (Non water stress)

Significant effect of month ($P = 0.001$) was noticed on the shoot/root ratio of non-water stressed Rhodes grass at both CO$_2$ concentrations (Table 13). As shown in Figure (22), under ambient CO$_2$ conditions, the trend is not consistent. There is a steep rise in the ratio until May, after which the ratio declines in September, then again shoots up, reaching its maximum in October. Also under enriched CO$_2$ conditions, trend is not consistent. There is a gradual increase in the ratio from April until September reaching its highest peak, which then declines by almost 1 point in October. In general, the shoot/root ratio in the plants grown at ambient CO$_2$ conditions was significantly higher ($P = 0.001$; Table 14) than in plants grown at enriched CO$_2$ conditions, (2.63 and 1.87, respectively as overall means) under non-water stress conditions.

Figure 22: Shoot/root ratio of Rhodes grass grown at ambient water conditions, with or without CO$_2$ treatment.
Table 13: ANOVA results for shoot/root ratio of Rhodes grass grown at ambient water conditions, with or without CO₂ treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>41.785*</td>
<td>7</td>
<td>5.969</td>
<td>4.796</td>
<td>.001</td>
</tr>
<tr>
<td>Intercept</td>
<td>135.284</td>
<td>1</td>
<td>135.284</td>
<td>108.692</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>2.523</td>
<td>1</td>
<td>2.523</td>
<td>2.027</td>
<td>.163</td>
</tr>
<tr>
<td>Month</td>
<td>27.310</td>
<td>3</td>
<td>9.103</td>
<td>7.314</td>
<td>.001</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>7.088</td>
<td>3</td>
<td>2.363</td>
<td>1.898</td>
<td>.148</td>
</tr>
<tr>
<td>Error</td>
<td>43.563</td>
<td>35</td>
<td>1.245</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>304.964</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>85.348</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .490 (Adjusted R Squared = .388)

Table 14: T-test results for Shoot/root ratio of Rhodes grass grown at ambient water conditions, with or without CO₂ treatment.

<table>
<thead>
<tr>
<th>ShootRoot Ratio</th>
<th>Levene's Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>Equal variances</td>
<td>12.594</td>
<td>.001</td>
</tr>
<tr>
<td>Root assumed</td>
<td>1.793</td>
<td>.039</td>
</tr>
<tr>
<td>Equal variances</td>
<td>-1.826</td>
<td>.079</td>
</tr>
<tr>
<td>not assumed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

74
Rhodes (Water stress)

Analysis of variance revealed significant effect of month for shoot/root ratio for Rhodes grass grown under water-stress conditions at both CO₂ treatments (P < 0.001; Table 15). As shown in Figure (23), under ambient CO₂ conditions, there is a steep increase in ratio from April till May, after which the increase in ratio slows down, with a very gradual increase till October where it reaches the peak. On the other hand, there is a consistent, yet steep increase in the over-all ratio of Rhodes grass grown under enriched CO₂ conditions from April till September, after which there is a sudden sharp decrease in the ratio, and it declines in October. Over all, the average of shoot/root ratio under ambient CO₂ condition is higher (2.33) than the ones grown at enriched CO₂ treatment (2.00).

![Figure 23: Shoot/root ratio of Rhodes grass grown at water stress conditions, with or without CO₂ treatment.](image-url)
Table 15: ANOVA results Shoot/root ratio of Rhodes grass grown at water stress conditions, with or without CO$_2$ treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>30.143$^a$</td>
<td>6</td>
<td>5.024</td>
<td>6.010</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>164.034</td>
<td>1</td>
<td>164.034</td>
<td>196.224</td>
<td>.000</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>1.622</td>
<td>1</td>
<td>1.622</td>
<td>1.941</td>
<td>.172</td>
</tr>
<tr>
<td>Month</td>
<td>9.042</td>
<td>3</td>
<td>10.817</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>CO$_2$ * Month</td>
<td>1.727</td>
<td>2</td>
<td>0.863</td>
<td>1.033</td>
<td>.366</td>
</tr>
<tr>
<td>Error</td>
<td>30.094</td>
<td>36</td>
<td>.836</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>262.041</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>60.237</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .500 (Adjusted R Squared = .417)
Alfalfa (Non water stress)

Significant effect of month ($P = 0.053$) was noticed on the shoot/root ratio of non-water stressed alfalfa at both CO$_2$ concentrations (Table 16). Unlike Rhodes grass, the trend of alfalfa is negative, and gradually declines under both CO$_2$ treatments, suggesting that with time, the speed of root length out-grows the speed at which the shoots develop. As shown in Figure (24), under ambient CO$_2$ conditions, there is a steep decline in the ratio from April until September, after which there is a notable increase in the ratio, at the end, in the month of October. However, under enriched CO$_2$ treatment it is the opposite. Shoot/root ratio slightly increases in the beginning from April to May, and then steeply declines from May until October. In general, the shoot/root ratio in the plants grown at enriched CO$_2$ conditions was significantly higher ($P < 0.05$; Table 17) than in plants grown at ambient CO$_2$ conditions, (1.61 and 1.31, respectively as overall means) under non-water stress.

Figure 24: Shoot/root ratio of alfalfa grown at ambient water conditions, with or without CO$_2$ treatment.
Table 16: ANOVA results of Shoot/root ratio of alfalfa grown at ambient water conditions, with or without CO₂ treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>5.379*</td>
<td>7</td>
<td>.768</td>
<td>2.263</td>
<td>.051</td>
</tr>
<tr>
<td>Intercept</td>
<td>34.888</td>
<td>1</td>
<td>34.888</td>
<td>102.759</td>
<td>.000</td>
</tr>
<tr>
<td>CO2</td>
<td>.222</td>
<td>1</td>
<td>.222</td>
<td>.653</td>
<td>.424</td>
</tr>
<tr>
<td>Month</td>
<td>2.863</td>
<td>3</td>
<td>.954</td>
<td>2.811</td>
<td>.053</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>1.520</td>
<td>3</td>
<td>.507</td>
<td>1.493</td>
<td>.233</td>
</tr>
<tr>
<td>Error</td>
<td>12.223</td>
<td>36</td>
<td>.340</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>112.214</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>17.602</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 17: T-test results of Shoot/root ratio of alfalfa grown at ambient water conditions, with or without CO₂ treatment.

<table>
<thead>
<tr>
<th>Shoot/Root Ratio</th>
<th>Levene's Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>Equal variances</td>
<td>5.664</td>
<td>.022</td>
</tr>
<tr>
<td>assumed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equal variances</td>
<td>5.664</td>
<td>.022</td>
</tr>
<tr>
<td>not assumed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Alfalfa (Water stress)

Significant effect of month ($P < 0.05$) was noticed on the shoot/root ratio of water stressed alfalfa at both CO$_2$ concentrations (Table 18). As shown in Figure (25), the trend for both plants grown under enriched CO$_2$ as well as plants grown under ambient CO$_2$ conditions show a similar trend. The trend declines in ratio from April till September, after which the trend shows a sharp increase in the month of October for plants grown under both CO$_2$ treatments. However, the overall average ratio of alfalfa grown under ambient CO$_2$ treatment is higher (1.66) than those grown at enriched CO$_2$ conditions (1.42).

![Shoot/root ratio of alfalfa grown at water stress conditions, with or without CO$_2$ treatment.](image)

**Figure 25:** Shoot/root ratio of alfalfa grown at water stress conditions, with or without CO$_2$ treatment.
Table 18: ANOVA results for shoot/root ratio of alfalfa grown at water stress conditions, with or without CO2 treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>5.338°</td>
<td>7</td>
<td>.763</td>
<td>1.690</td>
<td>.141</td>
</tr>
<tr>
<td>Intercept</td>
<td>35.446</td>
<td>1</td>
<td>35.446</td>
<td>78.560</td>
<td>.000</td>
</tr>
<tr>
<td>CO2</td>
<td>.889</td>
<td>1</td>
<td>.889</td>
<td>1.969</td>
<td>.169</td>
</tr>
<tr>
<td>Month</td>
<td>4.070</td>
<td>3</td>
<td>1.357</td>
<td>3.007</td>
<td>.042</td>
</tr>
<tr>
<td>CO2 * Month</td>
<td>.792</td>
<td>3</td>
<td>.264</td>
<td>.585</td>
<td>.629</td>
</tr>
<tr>
<td>Error</td>
<td>17.146</td>
<td>38</td>
<td>.451</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>131.680</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>22.483</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .237 (Adjusted R Squared = .097)
4.2.7 Number of leaves

Rhodes Grass (Non water stress)

Analysis of variance revealed significant interaction of CO$_2$ × month for leaves grown under ambient water conditions at both CO$_2$ treatments (P < 0.05; Table 19). As shown in Figure (26), the steep rise in leaf (%) for Rhodes grass is noticed in September under enriched CO$_2$ treatment, after which the increase slows down, reaching to a maximum (%) in October. On the other hand, there is a gradual rise in leaf (%) in plants grown under ambient CO$_2$ conditions, followed by a steep rise in October, reaching its maximum. Over all, the average of leaf (%) under enriched CO$_2$ treatment is slightly higher (781.0%), than the ones grown at ambient CO$_2$ condition (771.4%).

![Graph showing leaf (%) of Rhodes grass grown at ambient water conditions, with or without CO$_2$ treatment.](image)

**Figure 26:** Leaf (%) of Rhodes grass grown at ambient water conditions, with or without CO$_2$ treatment.
Table 19: ANOVA results for Leaf (%) of Rhodes grass grown at ambient water conditions, with or without CO₂ treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>40816507.937*</td>
<td>7</td>
<td>5830929.705</td>
<td>26.792</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>35969853.362</td>
<td>1</td>
<td>35969853.362</td>
<td>165.274</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>1766648.427</td>
<td>1</td>
<td>1766648.427</td>
<td>8.117</td>
<td>.007</td>
</tr>
<tr>
<td>Month</td>
<td>38716499.219</td>
<td>3</td>
<td>12905499.740</td>
<td>59.298</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>2106026.211</td>
<td>3</td>
<td>702008.737</td>
<td>3.226</td>
<td>.035</td>
</tr>
<tr>
<td>Error</td>
<td>7399682.540</td>
<td>34</td>
<td>217637.722</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>73520000.000</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>48216190.476</td>
<td>41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .847 (Adjusted R Squared = .815)
Rhodes Grass (Water stress)

Significant interaction of CO$_2$ × month was noticed for leaves grown under water stress conditions at both CO$_2$ treatments ($P = 0.002$; Table 20). As shown in Figure (27), trend of leaf (%) under both CO$_2$ treatments start off, almost similar until May, after which there is a steep increase in leaf (%) under enriched CO$_2$ treatment till October, whereas, under ambient CO$_2$ conditions, there is a much steeper rise in leaf (%) till September, which slows down in October.

![Figure 27](image)

**Figure 27:** Leaf (%) of Rhodes grass grown at water stress conditions, with or without CO$_2$ treatment.
Table 20: ANOVA results of Leaf (%) of Rhodes grass grown at water stress conditions, with or without CO₂ treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>56713840.18²</td>
<td>7</td>
<td>8101977.170</td>
<td>83.253</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>65947714.457</td>
<td>1</td>
<td>65947714.457</td>
<td>677.655</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>94504.462</td>
<td>1</td>
<td>94504.462</td>
<td>.971</td>
<td>.331</td>
</tr>
<tr>
<td>Month</td>
<td>56340256.866</td>
<td>3</td>
<td>18780085.622</td>
<td>192.977</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>16859009.566</td>
<td>3</td>
<td>561969.855</td>
<td>5.775</td>
<td>.002</td>
</tr>
<tr>
<td>Error</td>
<td>3503432.540</td>
<td>36</td>
<td>97317.571</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>97680000.000</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>60217272.727</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .942 (Adjusted R Squared = .931)
Alfalfa (Non water stress)

Significant effect (interaction between 2 factors) of month \((P < 0.001)\) was noticed for leaf \((\%\) of alfalfa grown under ambient water conditions, at both \(CO_2\) treatments (Table 21). As shown in Figure (28), trend of leaf \((\%)\) under both \(CO_2\) treatments start off, almost similar until May, after which there is a steep increase in leaf \((\%)\) under enriched \(CO_2\) treatment till October reaching its maximum, whereas, under ambient \(CO_2\) conditions, the leaf percentage decreases in September, followed by a sharp increase in October almost reaching to the maximum value of leaf \((\%)\) grown at enriched \(CO_2\) treatment. Over all, the average of leaf \((\%)\) under enriched \(CO_2\) treatment is higher \((751.5\%)\), than the ones grown at ambient \(CO_2\) condition \((711.1\%)\).

![Graph showing leaf percentage of alfalfa under ambient and enriched CO2 conditions over months.](image)

**Figure 28:** Leaf \((\%)\) of alfalfa grown at ambient water conditions, with or without \(CO_2\) treatment.
Table 21: ANOVA results of leaf (%) of alfalfa grown at ambient water conditions, with or without CO$_2$ treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>35675788.616</td>
<td>7</td>
<td>5096541.231</td>
<td>14.759</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>25396931.257</td>
<td>1</td>
<td>25396931.257</td>
<td>73.545</td>
<td>.000</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>399130.784</td>
<td>1</td>
<td>399130.784</td>
<td>1.156</td>
<td>.290</td>
</tr>
<tr>
<td>Month</td>
<td>35059503.426</td>
<td>3</td>
<td>11686501.421</td>
<td>33.842</td>
<td>.000</td>
</tr>
<tr>
<td>CO$_2$ * Month</td>
<td>544984.863</td>
<td>3</td>
<td>181661.621</td>
<td>.526</td>
<td>.667</td>
</tr>
<tr>
<td>Error</td>
<td>12086330.247</td>
<td>35</td>
<td>345323.721</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>70788888.889</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>47762118.863</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .747 (Adjusted R Squared = .696)
Alfalfa (Water stress)

Significant as above of month (P < 0.001) was noticed for leaf (%) of alfalfa grown under water stress conditions, at both CO2 treatments (Table 22). As shown in Figure (29), trend of leaf (%) under both CO2 treatments start off almost similar until May, after which in enriched CO2 treatment, there is a slight increase in leaf (%) till September after which growth slows down by October with not much increase in leaf (%) as compared to the previous month. On the other hand, under ambient CO2 conditions, the leaf (%) moderately increases till September, followed by a sharp increase in October reaching to its maximum. Over all, the average of leaf (%) under ambient CO2 condition is higher (612.1%), than the ones grown at enriched CO2 treatment (1005.7%).

Figure 29: Leaf (%) of alfalfa grown at water stress conditions, with or without CO2 treatment.
Table 22: ANOVA results for leaf (%) of alfalfa grown at water stress conditions, with or without CO₂ treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>45848941.358</td>
<td>7</td>
<td>6549848.765</td>
<td>8.305</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>32822108.975</td>
<td>1</td>
<td>32822108.975</td>
<td>41.617</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂</td>
<td>893014.363</td>
<td>1</td>
<td>893014.363</td>
<td>1.132</td>
<td>.294</td>
</tr>
<tr>
<td>Month</td>
<td>34371422.638</td>
<td>3</td>
<td>11457140.879</td>
<td>14.527</td>
<td>.000</td>
</tr>
<tr>
<td>CO₂ * Month</td>
<td>5756247.652</td>
<td>3</td>
<td>1918749.217</td>
<td>2.433</td>
<td>.080</td>
</tr>
<tr>
<td>Error</td>
<td>29180836.420</td>
<td>37</td>
<td>788671.255</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>104797777.778</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>75029777.778</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a R Squared = .611 (Adjusted R Squared = .537)
CHAPTER V
DISCUSSION
CHAPTER V: DISCUSSION

5.1 Effect of CO₂ enrichment on microbial activity on water stressed and non-water-stressed plants.

5.1.1 Microbial activity in Rhodes grass

Depending on the kind of micro-organisms present and the type of stress the soil is exposed to, microbial activity of the plant's soil may vary according to the circumstances. In the present study, when plants exposed to water stress, microbial activity under enriched CO₂ conditions was significantly higher (41%) than compared to the ones in ambient CO₂ conditions. In general, as the plants aged, their mean microbial activity increased with time, with the maximum recorded activity in the final month.

As noticed in the results section, elevated CO₂ concentrations masked the effects of water stress on Rhodes grass. Considering this, it could be possible that under elevated CO₂ concentrations combined with water stress on Rhodes grass invested its photosynthates more towards the roots of the plant, hence the rise in microbial activity. This phenomena is supported by a study done by Sadowsky & Schortemeyer (1997) that it is more likely that effect on the microbial biomass maybe be indirect, by increasing root growth and rhizodeposition rates, and decreasing soil water shortage. In another study, it was noticed that soil microbial biomass increased by 48% in grassland with enriched CO₂ (Drissner et al., 2007). However, in some different investigations, no significant difference in microbial biomass or negligible differences were noticed despite significant increases in the CO₂. (Kampichler et al., 1998). In general, soil microbial activity might have a direct relation with the root...
biomass of the plant. The significant increase in the soil microbial activity in the month of October compared to the previous months (April, May and September) in soils cultivated with Rhodes grass in the present study can be explained on the basis of increase in the root mass with time. It is reported that plants with more root mass will harbor more microorganisms in their rhizosphere as the roots secrete root exudates in the rhizosphere area (Whipps, 2001).

5.1.2 Microbial activity in alfalfa

Roots of alfalfa are characterized by the presence of root nodules, serving as home for nitrogen fixing bacteria, which live symbiotically with the crop (Aranjuelo et al., 2005). This gives an idea of the kind of microorganisms present in the soils of alfalfa. The present study found no significant difference was noticed in the activity of microorganisms between both CO₂ and water treatments. Although not significant, but when exposed to water stress overall average microbial activity of alfalfa grown under enriched CO₂ conditions was higher than its counterpart.

This finding is similar to the study of Insam et al. (1999), who concluded that elevated CO₂ was found to have no significant impact on microbial community composition, even after nearly 18 months of atmospheric CO₂ enrichment, on artificial tropical ecosystems, each composed of 77 plants representing seven C₃ species. This may be due to the fact that the CO₂ concentration on the soil is already 10–50 times higher than that in the atmosphere. Therefore, it is unlikely increasing atmospheric CO₂ will have any direct influence on the rhizosphere microbial biomass (Sadowsky & Schortemeyer, 1997). It might suggest that nitrogen fixing bacteria are already adapted to increasing CO₂ stress, so no further changes happen to their
numbers. In general as most studies concluded, elevated CO$_2$ has relatively small, or insignificant effects on soil inorganic nitrogen pools and fluxes, indicating very minor or no effects on soil microbial activity of nitrogen fixing plants. The significant increase in the soil microbial activity in the month of October compared to the previous months (April, May and September) in soils cultivated with alfalfa in the present study can be explained on the basis of increase in the root biomass with time. It is reported that plants with more root biomass will carry more microbial flora in their rhizosphere as the roots secrete root exudates in the rhizosphere area (Whipps, 2001).

5.2 Effect of CO$_2$ enrichment and water stress on morphology of the plants.

5.2.1 Fresh and dry weight: WUE in Rhodes grass

WUE in this study has been calculated by measuring the total amount of water a plant requires to produce its total fresh weight at the point of sampling. This gave an estimate of how efficient the plant was in utilizing a most demanded water resource into its total productivity. Effects of different CO$_2$ concentrations, water treatments and combination of both treatments on WUE were recorded. The objective was to find out if any of the treatments were successful in rendering the plant more efficient in conserving water, while keeping its productivity the same, or even higher than under normal conditions. It was found that just by inducing water stress on Rhodes grass its efficiency increased significantly by 4.6 times i.e. 21.56% than its control counter parts.

No effect of CO$_2$ elevation was noticed on the WUE of the plant. This may be due to the fact that, Rhodes grass belongs to the C$_4$ grass family. C$_4$ photosynthetic
pathway is an adaptation that plants have acquired from C3 photosynthetic pathways over the years. The benefit is that C4 pathway gets rid of the inefficiencies occurring due to photorespiration, which minimize the need for any excess CO2 as the leaf is already efficient in acquiring all the CO2 it needs even under normal conditions, as there are no wastages of energy in the process (Valeria & Santiago, 2011). C4 pathway has evolved in a high CO2 environment which means they have greater rate of CO2 assimilation even at ambient conditions (Valeria & Santiago, 2011). With the plant being more efficient in obtaining CO2 from the atmosphere, it eliminated the need for stomata to be open for longer periods of time, which leads to lower transpiration rates and therefore, minimizing water loss to the environment (Valeria & Santiago, 2011). This explains why even under drought conditions, Rhodes grass was more efficient in utilizing lesser water as compared to its counterpart.

When comparing the findings obtained in the current study with other studies done in the same fields, opposite results were achieved by other researchers. It was found that when exposed to higher CO2 concentrations, transpiration rates and stomatal conductance decreased which lead the plant to be more water efficient (Allen Jr., Kakani, Vu, & Boote, 2011). Also in other studies it was also found that CO2 enrichment on plants improved the water efficiency in under drought conditions by 19.8% in (C4) winter wheat (Qiao et al., 2010), and 19% increase in WUE based on biomass of sorghum in elevated CO2 levels (Conley et al., 2001). Over all from the literature we can conclude that, C4 plant species are efficient in water use to begin with, having an addition CO2 enrichment in the plant’s atmosphere maybe be an added benefit in most cases.
5.2.2 Fresh and dry weight: WUE in alfalfa

Alfalfa belongs to the C₃ photosynthetic pathway species, which is expected to respond differently as compared to C₄ photosynthetic pathway species. Similarly, alfalfa crops used in the present study too responded differently. Combined effects of elevated CO₂ concentration and induced water-stress on the plant showed significantly greater WUE, compared to its control counterpart. WUE in the treatment group was 25.77% (3.8 times) greater than alfalfa grown at ambient CO₂ concentration and well watered conditions.

These findings can be related to the fact that C₃ photosynthetic pathway the most common, and the most inefficient of all pathways in utilizing the available CO₂. Efficiency of C₃ pathway is only 1/3rd of C₄ pathway due to occurrence of photorespiration (Ward, 2009) and loses 25% and 30% of the total carbon fixed (Valeria & Santiago, 2011). Therefore there is a need for the plant to keep stomata open for longer period of time to acquire required amounts of CO₂ which might possibly result in water losses due to transpiration. Effects of increasing atmospheric concentration can reduce the need for stomatal opening for longer periods of time thus reducing stomatal conductance. This decrease in stomatal conductance of individual leaves at elevated CO₂ concentration can render the whole plant to reduce water use and soil moisture depletion, improve WUE (Leakey et al., 2012).

When compared to other studies, similar results were noticed. Bernacchi, Kimball, Quarles, Long, & Ort (2007), in their study found out that, when a canopy of soybean was exposed to elevated CO₂ concentrations, the stomatal conductance reduced and over all transpiration of the canopy had decreased. Also, in the research
by Ziska et al., (1991); increased photosynthesis activity, and WUE was noticed in all C₃ plant species he experimented with, when the concentration of CO₂ was doubled. On the other hand, some researchers found that, when CO₂ was elevated, the leaf surface area of the plant increased, which exposed more stomata to the air, thus cancelling the effect reduced stomatal conductance and showing no improvement in WUE (Morison & Gifford, 1984). Overall, maximum numbers of studies agree that significant improvements in C₃ plants are achieved in terms of WUE, when plants are exposed to high CO₂ concentrations.

### 5.2.3 Fresh and dry weight: biomass of Rhodes grass

Biomass of Rhodes grass is the total fresh or dry weight of the shoots and roots combined. When plants are exposed to different stresses, plants respond to stresses by either investing in the biomass or reducing it. In the present study, when Rhodes grass was subjected to elevated CO₂ and water stress alone in separate treatments, average biomass significantly decreased as a result of individual stresses by 38.5% and 0.02% respectively. However, when both stresses were induced together on the plants, the combined effect had no significant difference in the biomass (fresh and dry both) compared to the control counterparts. This suggests that, when both treatments were combined, Rhodes grass was able to grow and invest in biomass no different to its counterparts, while efficiently using up only 50% of the water used in control to produce the same, if not more.

Although, there is not much research demonstrating the net biomass production between treatment and control group, but there are ample of studies establishing the fact that a higher biomass production results at elevated CO₂
conditions. When Rhodes grass were exposed to high CO\textsubscript{2} treatment, 65% increase in the total dry matter was observed (Ksiki & Youssef, 2010). In a study carried out by Conley et al., (2001), they found that CO\textsubscript{2} elevation significantly increased biomass in plots of sorghum that were dry, and there was inconsistent or no effect of CO\textsubscript{2} elevation on biomass of the wet plots. Biomass of calcareous grass community also increased as a result of elevated CO\textsubscript{2} in Switzerland (Leadley, Niklaus, Stocker, & Körner, 1999). Also when a C\textsubscript{4} grass ‘Pensacola’ bahiagrass was exposed to elevated CO\textsubscript{2} the result was 15% increase in overall dry matter production (Newman, Sollenerberger, Boote, Allen, & Littell, 2001). On the other hand, when tested for effects of increased CO\textsubscript{2} concentration on savanna grasslands, native grasses showed no increase in biomass, but exotic species showed much greater biomass (Tooth & Leishman, 2014). Overall, these studies suggest that depending on the species of C\textsubscript{4} plant, combined effect of water-stress and CO\textsubscript{2} elevations can help conserve water, by producing similar or greater biomass results in general.

5.2.4 Fresh and dry weight: biomass of alfalfa

As a C\textsubscript{3} plant species, alfalfa too displays effects of different stresses on its biomass (fresh and dry). In the present study, the combined effect of CO\textsubscript{2} elevation and water stress significantly increased the overall biomass in both fresh (4%) and dry matter (11.34%) of the plant. Exposures to these stresses separately on plants had no significant effects on biomass otherwise. These results suggest that when exposed to water stress, plant opportunistically captures and uses elevated CO\textsubscript{2} to deposit it in various parts thus increasing the biomass, to combat the negative effects of reduced water availability.
Findings of this study can be compared with other studies done on similar interest. De Luis et al. (1999) noticed that when alfalfa were exposed to water stress, negative effects of water stress were counter balanced by increment of atmospheric CO₂. According to them, elevated CO₂ enhances growth of water stressed plants by improving carbon fixation, by investing the photosynthetic products more to below-ground production (roots and nodules), without much effect on the water status (De Luis et al., 1999). This means that elevated CO₂ enhances the ability to produce more biomass in nitrogen-fixing alfalfa under given soil water stress, improving drought tolerance (De Luis et al., 1999). In another study, plant dry weight was also increased substantially by high CO₂ in all species tested by Morison & Gifford (1984), with an average increase of 64% in dry mass. A study done in California revealed that increase in CO₂ concentrations, increased the overall biomass of the riparian plants, but however water-stressed reduced the biomass of the seedlings by 70-97%, which suggests that elevated CO₂ is unlikely to counteract the negative effects of increased aridity on riparian woody seedling recruitment (Perry, Shafroth, Blumenthal, Morgan, & LeCain, 2013). Carbon dioxide elevation combined with water stress can have a significant impact on biomass of plants.

5.2.5 Height of Rhodes grass

Plant height was calculated by measuring the height of shoot and the length of root separately using a normal ruler and converting it to percentage of growth from the initial reading. In this study it was found that the height percentages of shoot and root both grew significantly with each passing time, especially in the final month the height was at its highest in both all four treatments. Although not significant, overall
height percentages of shoot grown under ambient CO₂ was higher than those grown under elevated CO₂ conditions. Opposite is true for root, during the final two months, the average height percentage of roots in elevated CO₂ was higher than those at ambient CO₂ conditions. These results suggest that when exposed to higher CO₂ levels, Rhodes grass tends to invest more in the root growth compared to shoot.

When comparing the findings of this study, it was found that in a study carried out by Bazzaz (1990), it was mentioned that when CO₂ levels are elevated, there is generally more allocation to the roots of the plants, especially if the plant is under water stress. Moreover, even the shoot height is also expected to rise under higher CO₂ concentrations, as was the case in a study done by Ksiksi & Youssef (2010), where a clear trend of increased Rhodes grass height was noticed under CO₂ enrichment. Therefore, it is difficult to mention what the response will be, but it is obvious that more allocation goes towards the roots.

5.2.6 Height of alfalfa

Height percentages of shoot and root both grew significantly with each passing time, especially in the final two months; the height was at its highest in all four treatments for alfalfa too. Under normal water conditions, average shoot height percentages under both CO₂ treatments are almost the same, but however, when exposed to water stress, height percentage of alfalfa grown under CO₂ enrichment was significantly lower (30% lesser) than its counterpart. With the case of root length, over all in non-water stress plants, the mean root height percentage of alfalfa grown under CO₂ enrichment was higher than its counterpart. On the other hand plants grown under water stress conditions, the mean root height percentage of alfalfa grown
under CO₂ enrichment was lower than its counterpart. These results indicate that CO₂ enrichment suppresses plant shoot and root height percentages under water stress.

Findings of current study contradict the results noted by Bazzaz & Carlson (1984), where they found that three of the C₃ plant species that were exposed to high levels of CO₂ had a higher plant height in general. Also in the book written by Bazzaz (1990), it was mentioned that when CO₂ levels are elevated, there is generally more allocation to the roots of the plants, especially if the plant was under water stress. Likewise, many other studies have also shown that elevation of CO₂ in the atmosphere could lead to improved plant growth, height, more root allocations and overall improve yield of the plant (Deepak & Agrawal, 1999). But this might not necessarily be true, as in a study done by Wu, Ma, Li, & Wan (2014), introducing high levels of CO₂ on maize and alfalfa showed decreased height, leaf number, leaf area, and root length trends as the levels increased. This may have been due to the reduced photosynthetic rates and reduced dry matter accumulation in the plant. These results suggest that alfalfa plant might have a certain threshold to tolerate the rises in atmospheric CO₂. Any increment beyond the threshold might interfere with the growth and development of the plant.

5.2.7 Shoot/root ratio in Rhodes grass

Shoot/root ratio in this study was calculated to notice the growth pattern in the plants over the period of time. It gives an indication about allocation of resources of the plant during the course of time. As noticed in the present study, when grown at normal water conditions, shoot/root ratio was significantly higher (28.8%) in the grass grown under ambient CO₂ conditions as compared to their enriched CO₂
counterparts. On the other hand, when exposed to water stress, although not significant, but overall mean shoot/root ratio of Rhodes grass grown under ambient CO₂ conditions was higher than the ones grown at elevated CO₂ conditions. In general these results suggests that when exposed to higher CO₂ levels, Rhodes grass invests in creating longer roots thus improving the water availability.

When plants are exposed to elevated CO₂ concentrations, root growth dramatically increases (Rogers, Prior, Runion, & Mitchell, 1995). Also elevated CO₂ concentrations could potentially enhance the root growth speed, length and the biomass of the roots (Rogers, Runion, Prior, & Torbert, 1999), suggesting the possible reasons for lower shoot to root ratio in Rhodes grass at elevated CO₂ in the present study.

5.2.8 Shoot/root ratio in alfalfa

Unlike Rhodes grass, alfalfa showed a negative trend in shoot/root ratio, with decreasing shoot/root ratio with time, in all treatments. These results suggest that with time, the speed of root length out-grows the speed at which the shoots develop. In plants grown at normal water conditions, average shoot/root ratio in general in alfalfa grown under CO₂ enrichment was significantly higher (22.90%) than its counterpart. While on the other hand, plants exposed to water stress had no significant differences between the ratios of the two treatments. In general it can be said that, when exposed to high CO₂ concentrations and normally watered, shoots are longer than roots.

The findings of present study were compared with review paper done by Rogers et al. (1995), in which out of all the plant researches reviewed, almost 60% had increased root/shoot ratio, 3% had no effect and 37% had lower root/shoot ratio.
Possibly, alfalfa falls under the last category of plants that allocate more towards the shoots. CO\textsubscript{2} enrichment also increased aboveground leaf and stem mass, but not of belowground components such as root and nodule, at the end of one month of normal vegetative growth in a study done by Erice et al. (2006b), which further illustrated that alfalfa normally does not invest in root production under elevated CO\textsubscript{2} circumstances as much as it would under ambient CO\textsubscript{2} exposure. In general, the downward trend shows an overall investment in roots of alfalfa with passage of time under any environment, possibly due to the nodules that serve to store most photosynthate.

5.2.9 Leaf percentage in Rhodes grass

Leaves of Rhodes grass showed no significant differences between all four treatments suggesting that the number of leaves was not affected by any kind of stress. Similar results were noticed in a study done by Bazzaz & Carlson (1984), who found that when C\textsubscript{4} plant species were tested under three different CO\textsubscript{2} levels, leaf weight of those plants showed no response at water stress levels, nor did they decline at ambient water levels. However, on the other hand, Ksiksi & Youssef (2010), found that when Rhodes grass was grown under CO\textsubscript{2} enrichment, it had fewer number of leaves compared to its counterparts. Their finding was supported by the fact that upon more allocation towards the roots at higher CO\textsubscript{2} investments in the shoots and leaves is compromised significantly according to (El-Satnawi, Ksiksi, Makhadmeh, & Haddad, 2003).
5.2.10 Leaf production in alfalfa

Leaves of alfalfa also showed no significant differences between all four treatments suggesting that number of leaves is not affected by any kind of stress. Although not significant, alfalfa when exposed to water stress showed a higher mean leaf percentage under ambient CO$_2$ than under CO$_2$ enrichment ($P = 0.078$). As suggested by the results, under water stress conditions, alfalfa has fewer leaves when exposed to CO$_2$ enrichment.

Radoglou & Jarvis (1990), exposed four poplar clones to double the ambient CO$_2$ concentrations, and the number of leaves, total leaf area and total leaf dry weight positively increased with the increment in CO$_2$. Similar results were found by Sionit (1983), where increase in CO$_2$ produced positive results on specific leaf weight of soybean plant. As fewer studies are done on effects of CO$_2$ enrichment on leaf count, it may be believed that leaf numbers are not affected by CO$_2$ elevation. In conclusion in this study it can be said that under ambient CO$_2$ conditions, alfalfa focuses on producing more leaves, but under CO$_2$ enrichment, alfalfa invests on shoot elongation (length) more than its leaves.
CHAPTER VI
CONCLUSION
CHAPTER VI: CONCLUSION

Rhode grass is a C$_4$ grass species and is one of the most popular agricultural crops grown in the UAE, particularly for its value as a fodder species for animal feeds. This recently banned crop could be brought back into the market, if there are means to help conserve water. Alfalfa too is one of the plant species grown in UAE, second after Rhodes grass for its use as animal feeds, and could potentially use the benefits rising global CO$_2$ levels. One of the ideas was to use CO$_2$ enrichment alone or in combination with water stress treatment and monitor their effects on the plant.

The objectives of the current study were: i) to compare Rhodes grass (*Chloris gayana*), alfalfa (*Medicago sativa*) growth under both ‘high’ CO$_2$ and ‘ambient’ CO$_2$ concentrations ii) to evaluate the overall growth of alfalfa (as a C$_3$ species) and Rhodes grass (as a C$_4$ species) under high CO$_2$ concentrations iii) to compare microbial biomass for both plant species under high CO$_2$ and ambient CO$_2$ concentration iv) to assess water conservation potentials of both plant species under enriched CO$_2$ concentrations. In general, plants showed growth under all four treatments and survived the six month study period, without any sickening or death of any plant. Following detailed results were noticed after the six month study period.

Combination of elevated CO$_2$ and water stress resulted in significantly higher microbial activity in the Rhodes grass. Depending on the type of micro-organisms present, its causes would be more apparent. Exposure to elevated CO$_2$ in general caused the Rhodes grass height to shorten, and roots to elongate more than their counterparts. This result was further confirmed by a significantly smaller shoot/root ratio in Rhodes grass grown at enriched CO$_2$ compared to those grown at ambient
CO₂. Rhodes grass proved to be sensitive to both kinds of stresses, which meant that when exposed to individual stresses such as elevated CO₂ alone and water stress alone. Rhodes grass responded by significantly reducing its fresh weight biomass. However, when both treatments were used in combination, there was no difference in the biomass compared to control. WUE was the highest in water stressed plants under ambient CO₂. Stresses overall had no effect on the number of leaves of Rhodes grass. It can be said that using a combination of stresses can be beneficial in conserving water, without compromising the biomass or the number of leaves in general of the plant.

Alfalfa too had its own responses to stress exposure. CO₂ also had some influence on the microbial activity in the soil, particularly showing higher means under CO₂ elevation. Also in general, CO₂ elevation caused shorter shoots and roots means in alfalfa under both water stressed and normal water conditions. Shoot/root ratio further confirmed the finding, as shoot/root ratio in the control group was significantly higher than its counterpart at elevated CO₂ indicating that exposure to high CO₂ suppresses the plant growth in terms of height. A positive impact of combination treatment was noticed on the biomass (fresh and dry both) of the plant which was significantly higher in plants exposed to water-stress and elevated CO₂ combined. Combination treatment also proved to be beneficial in significantly enhancing the WUE of the crop compared to the control group. Leaves in general showed no positive response to any of the treatments, although the mean of leaf percentages in control group was higher than in enriched CO₂ treatments. Overall, a higher biomass, reduced shoot and root height, and fewer leaves indicate that at
elevated CO₂ concentrations, plant resource allocations in a way that results in possible increased leaf size and thickness, thicker stems and most likely bulkier and more nodulated roots.
REFERENCES
REFERENCES


Insam, H., Bååth, E., Berreck, M., Frostegård, Å., Gerzabek, M. H., Kraft, A., … Tschuggnall, G. (1999). Responses of the soil microbiota to elevated CO2 in


1ypv7xcC&oi=find&pg=PR5&dq=living+in+the+environment&ots=bk94nz5G2t&sig=XPKaMCfYd-RpTjmtqWjJPkINts

%20perennial%20pastures%20for%20wa.pdf


planting-2010-10-18-1.305519


الملخص العربي
أثبتت هذه الدراسة أيضاً أنه لم تظهر أي زيادة في المجموع الخضري لنباتات الروذس في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 1000 جزء في المليون مقارنة بنباتات الروذس في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 500 جزء في المليون. ولكن في حالة استخدام نباتات البرسم لملاحظة هذه الزيادة في الوزن النضج بنسبة 4% بالوزن النافذ بنسبة 11% في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 1000 جزء في المليون مقارنة بالمعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 500 جزء في المليون.

أثبتت الدراسة الحالية أيضاً أن طول نباتات البرسم في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 1000 جزء في المليون كان أقل بنسبة 30% في حالة الإجهاد المائي مقارنة بطول نباتات البرسم تحت الظروف الري التقليدية.

أثبتت هذه الدراسة أيضاً أن نسبة المجموع الخضري إلى المجموع الجذري في نباتات الروذس في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 1000 جزء في المليون كانت 29% أقل من نباتات الروذس عنها في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 500 جزء في المليون. ولكن نسبة المجموع الخضري إلى المجموع الجذري في نباتات البرسم في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 1000 جزء في المليون كانت 23% أعلى في نباتات البرسم عنها في المعاملة التي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 500 جزء في المليون.

أثبتت هذه الدراسة الحالية أيضاً أن عدد الأوراق في نباتات البرسم ونباتات الروذس لم يتأثر معناويًا بين جميع المعاملات والتي تم فيها اضافة غاز ثاني أكسيد الكربون بتركيز 1000 جزء في المليون وتركيز 500 جزء في المليون.
000 1 = 10 000 5 = 50 000 1 = 1 000 5 = 5 000 1 = 5 000 000 1 = 1 000 000 5 = 5 000 000 1 = 5 000 000
تأثير زيادة تركيز غاز ثاني أكسيد الكربون على نمو نباتات البرسيم و الحدائق والقدرة المثلية على استخدام الماء

رسالة مقدمة من:

اسماء حنان أحمدان

مقدمة إلى:

جامعة الإمارات العربية المتحدة

استكمالاً لمنطلقات الحصول على درجة الماجستير في مقدمة

Shrieen
2016.12.07
10:30:34
+04'00'

مايو- 2014

126