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MATERNAL EFFECTS ON GROWTH PARAMETERS OF CHLORIS GAYANA UNDER CO2 ENRICHMENT

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Cover: Rhodes grass grown under enrichment CO_2 condition

(Photo: By Ahmed Adnan Mohammed Mashli)

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Declaration of Original Work

I, Ahmed Adnan Mohammed Mashli, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Maternal Effects on Growth Parameters of Chloris gayana under* CO_2 *Enrichment Conditions*", hereby, solemnly declare that this is the original research work done by me under the supervision of Prof. Taoufik Ksiksi, in the College of Science at UAEU. This work has not previously formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Rhodes grass, *Chloris gayana*, is commonly used forage plant for grazers, livestock, and cattle. It significantly improves soil fertility and its overall structure by preventing nematode flourishment in soil. It is also used as a cover crop to upgrade soil health by reducing soil erosion and increasing water availability. The present study assessed the effects of CO_2 on the growth, development and maternal effects of *C. gayana*. The experimental setup was made in such a way that the potential impacts of CO_2 can be measured on the eco-physiological growth of *C. gayana* by studying successive generations. Generation 1 was grown by sowing the seeds of *C. gayana* in soil pots and generation 2 was propagated by cutting and regenerating the branches with their roots from generation 1 plants. Growth parameters including plant height, number of leaves, shoot/root ratio, chlorophyll content, stomatal conductance, and photosynthetic rate were measured at regular intervals in both generations. Significant differences in the growth parameters were observed in both generation 1 and 2 plants when plants were grown under enriched and ambient CO_2 conditions. The varying pattern of phenotypes in the generation 2 plants can be justified based on the Bet-hedging maternal effects. We hypothesize that the Rhodes grass parent plants when allowed to grow under ambient and enriched CO_2 conditions influenced the offspring plants by producing a variety of phenotypes to sustain maternal fitness. The present study can be helpful in understanding the *C. gayana* growth conditions and the vertical transfer of maternal effects from one generation to the other.

Keywords: Rhodes grass, CO_2 enrichment, Maternal effects, Plant assessment, *Chloris gayana*

Title and Abstract (in Arabic)

تأثير الأمهات على عوامل النمو لنبات كلوريس جايانا تحت تأثير ثاني أكسيد الكربون المرتفع

الملخص

يستخدم عشب رودس، كلوريس جايانا، بشكل كبير لعلف للرعي والماشية والقطعان حيث انه يحسن بشكل كبير خصوبة التربة وتركيبها عن طريق منع زيادة الديدان الخيطية في التربة. كما أنه يستخدم كمحصول كغطاء نباتي لتحسين صحة التربة عن طريق الحد من تآكل التربة وزيادة توافر المياه. قيمت الدراسة الحالية آثار ثاني أوكسيد الكربون على نمو والتطور التأثيرات الأمية على كلوريس جايانا. تم إجراء الإعداد للتجربة بطريقة يمكن من خلالها قياس التأثيرات المحتملة لثاني أكسيد الكربون على النمو الفسيولوجي البيئي لنبات كلوريس جايانا من خلال دراسة الأجيال المتعاقبة. نما الجيل 1 عن طريق زرع بذور كلوريس جايانا في أواني وتم زراعة الجيل 2 عن طريق قطع الجيل الأول واخذ فروع مع جذورها من نباتات الجيل الأول. تم قياس عوامل النمو بما في ذلك ارتفاع النبات، وعدد الأوراق، ونسبة المجموع الجذع / الجذر، ومحتوى الكلوروفيل ، والتوصيل الثغري، ومعدل التمثيل الضوئي على فترات منتظمة في كلا الجيلين.. لوحظت اختلافات كبيرة في معايير النمو في كل من نباتات الجيل 1 و2 عندما نمت النباتات في ظل ظروف محيطة بثاني أكسيد الكربون العادية والمرتفعة. يمكن تبرير النمط المتغير للأنماط الظاهرية في نباتات الجيل الثاني بناءً على تأثيرات التحوط ضد الرهان على الأمهات. نحن نفترض أن نباتات كلوريس جايانا الأم عند السماح لها بالنمو في ظل الظروف العادية والمرتفعة بثاني أكسيد الكربون قد أثرت على النسللالا خلال إنتاج مجموعة متنوعة من الأنماط الظاهرية للحفاظ على لياقة الأم. يمكن أن تكون الدراسة الحالية مفيدة في فهم ظروف نمو كلوريس جايانا والانتقال الرأسي لتأثيرات الأمهات من جيل إلى آخر.

مفاهيم البحث الرئيسية: عشب رودس، إثراء ثاني أكسيد الكربون، تأثيرات الأمهات، تقييم النبات، كلوريس جايانا

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I finally want to express my gratitude to anyone who supported me and assisted me to complete this project.

Dedication

To my beloved parents, wife and family

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List of Abbreviations

CO_2	Carbon dioxide
CFCs	Chlorofluorocarbon
IPCC	Intergovernmental panel on climate change

Chapter 1: Introduction

1.1 Climate change, the causes and impacts

Climate is the prevailing weather condition in a specific area for a long duration. The earth's climate is a network of natural resources, which together contribute in developing a specific pattern of environmental conditions. The sun, ocean, landscapes, rainfall, snow, wind, forests, savannas, deserts, wildlife, and human activities, everything is ultimately considered as a factor of our climate (Urry, 2015).

The climate of the earth is constantly getting changed from the day of its origin and contributing in the evolution of living beings. However, the acute climate changes occurring for the last few decades are discordant in permanent ways of interpreting the place of human in nature (Berrang-Ford et al., 2011). Climate change defines as the inevitable change on earth including local, regional, and global levels, and the potential impacts of these changes. More specifically, this term is used to explain the modifications in earth's atmosphere due to human activities in the pre-industrial time (Thomas, 2010). For instance, the fossil fuel burning and deforestation has resulted in the rapid emission of gases and carbon dioxide concentration.

An alternative term is global warming, refers to the slow but uneven temperature rise, which is the most prominent measure of global climate change and directly impact the ecosystem, wildlife, and human survival (Ahmed, 2020). About 97% of the published data claimed the human interference, since 20th century, to be the major cause of the global warming. When heat energy is applied to any closed system, temperature changes occur, since all the entities of global climate system are interconnected with each other, addition of heat, therefore, results in an increase of temperature of the whole system (Dow & Downing, 2016). As temperature and evaporation rate are directly proportional to each other, the rising temperature as a whole lead to more evaporation of water from oceans followed by an increased condensation or cloud formation which eventually result in the intense storms like typhoons/hurricanes and cause deforestation. Moreover, the global warming is causing the formation of polar ice cap, melting of Antarctic glaciers which are raising sea levels to an abnormal limit, changing patterns of wind leading to monsoons in

Asian countries and unpredictable drought and aridity (Bein et al., 2020; Benjamin et al., 2017).

The three school of thoughts about the global warming are:

(1) The opponents believe that no temperature rise is occurring specifically, but a routine cycle of events is going on

(2) The environmentalists believe that both temperature rise and climate change are interconnected and definitely occurring continuously but in a natural way of cyclic events without any human involvement

(3) The scientists and researchers consider that global warming and climate change is inevitably occurring due to human activities and adversely revolutionizing the earth's atmosphere (Goldberg et al., 2019; Mehnert, 2016).

Scientific evaluation by the Intergovernmental bodies on climate change policies have provided a vivid illustration of the climate change as a global issue, but a dispute of opinions exists between the impersonal and embedded experiences. A modified research strategy is therefore needed to frame out the importance of inevitable climate changes among the human population and realization of potential adaptations for species survival. Certainly, the challenge of survival is not new to the living beings on earth as each life form has been continuously adapting and evolving itself for a long time and developed the stress management strategies and modified physiology to cope up the changing climate (Arnold, 2019). The changing climate on earth is overwhelming and resulted in a non-uniform temperature increase, harmful gas emissions, deforestations, vulnerability towards genetic diseases, depleted resources, and geographical variations. The carbon dioxide level has increased from 280 ppm (pre-industrial time) to about 415 ppm and a safe level of 350ppm to fix the global warming issue by 2100 is the need of hour (Hoornweg & Pope, 2020).

1.2 Greenhouse gases

The greenhouse gases constitute all those gases which have the capability of absorbing and radiating the net heat/infrared radiation from the earth's surface. The most prominent ones are carbon dioxide, methane and evaporating water molecule, while few other gases such as nitrous oxide, chlorofluorocarbon (CFCs), surface level ozone also radiate infrared radiations and named as greenhouse gases (Kweku et al., 2017; Tian et al., 2016).

Besides contributing to atmosphere, the greenhouse gases impart an intense effect on the net energy of earth's system. Over the wide range of timescale, greenhouse gases have diversified considerably and lead to the substantial changes in earth's history (Jeffry et al., 2021). The concentrations of these gases are generally higher in warmer geographical areas than that of the colder regions. The factors such as sinks and ocean sources, vegetation, soil properties, deforestation, wetland, desertification, and rock formation/deformation had influenced the concentration of greenhouse gases over the period of thousands of years (Maucieri et al., 2017). Furthermore, human activities, after industrial revolution, like burning of fossils/petroleum are the primary culprits of the increasing the carbon dioxide, methane and CFCs in atmosphere (Shurpali et al., 2019).

1.2.1 Impacts of greenhouse gases on the earth-atmosphere system

Each greenhouse gas possesses specific chemical and physical properties, which define its radiative force, the potential for absorbing and radiating back the energy. According to Intergovernmental panel on climate change (IPCC), the radiative force accounts for the influence of greenhouse gas or other climatic factor on the radiant energy touching the earth's surface (Kweku et al., 2017).

An analysis performed by Loeb et al. (2009) declared the average value of global outgoing radiation to be 238.5Wm^{-2}. Keeping into consideration the principle of Stefan-Boltzmann law one can measure the emission temperature of earth atmospheric system. It is declared that the atmosphere has increased the earth's temperature as earth can be warmer by 33°C without the existence of atmosphere (Von Schuckmann et al., 2016). Hence, the satellite observation convinced the researchers about the greenhouse gas effect of atmosphere (Manabe, 2019).

The downward flow of the longwave heat energy at earth's surface is directly proportional to the greenhouse components such as water vapors and carbon dioxide, such as the vapor pressure of air at the oceans increases with the increasing temperature (Knutti, 2019). It is hypothesized that relative humidity of the earth's atmosphere does not change systematically due to this increased evaporation rate from wet surfaces of earth. The global warming does not only affect the rate of condensation/evaporation but also the geographical division of water transport system (Stouffer & Manabe, 2017).

1.2.2 Carbon dioxide

In general, the exchanges of energy, chemicals and momentum between the atmosphere or land surfaces cause the climate change (Kweku et al., 2017). Furthermore, atmospheric circulation, radiative transfer and turbulent oceanic circulations all are together involved in executing the climatic changes. Although the processes are naturally occurring but the extent of their magnitude depends on the human interferences as they involve the greenhouse gas emission and eventually lead to rapid changes in global climate (Voigt et al., 2017).

Carbon dioxide (CO_2) considered the most significant of all the greenhouse gases, which enter the atmosphere primarily through the combustion of natural resources such as coal, petroleum or gas and secondarily through agricultural or industrial activities. In other words, the rate of CO_2 emission is directly proportional to the human consumption rate of natural resources (Ritchie & Roser, 2020). Therefore, its concentration increases with the increase in human population in a given area, which result in trapping of radiation arising from earth's surface and eventually a drastic increase in temperature (Shurpali et al., 2019).

The investigation of Carbon in the context of surface warming at 60°N declared that increasing concentration of carbon dioxide is responsible for this interannual temperature variations. In order to understand the questions of increasing CO_2 and its influences on global climate, one has to address several processes influenced by its concentration, such as plant/crop cultivation, carbonate system in oceans, decomposition, combustion etc. All these processes together called as carbon cycling and human interferences changed the natural carbon cycle to a greater extent (Zhou & Wang, 2016).

However, a reliable assessment of human interference and critical investigation about carbon sinks are needed to evaluate the potential reduction of global carbon cycling. For instance, the enhanced ratio of greenhouse gases could affect the exchange rate of CO_2

with ocean and vegetation but such reactions still are the topics of dispute among environmentalists (Ritchie & Roser, 2020).

1.3 CO_2 enrichment and plant growth

Elevated rates of carbon dioxide significantly caught attention of researchers in 2013-14 period when a drastic increase of this most important greenhouse gas, 400 µmol mol^{-1}, was recorded in Hawaii over the past six million years (Zhou & Wang, 2016). It is estimated that atmospheric CO_2 will raise up to 940 µmol mol^{-1} near the end of twenty first century (IPCC, 2013). Since CO_2 is a limiting factor for a sustainable plant physiology, an increase in its availability is correlated with an increased plant metabolism especially, stomatal conductance, leaf surface area, photosynthetic rate and plant growth. To get a bright representation of this CO_2 enrichment and its effects on plant growth, each plant growth parameter will be discussed in detail (Willey, 2018).

1.3.1 Effect of CO_2 enrichment on stomatal conductance, size and density

The stomata are the pores in plant leaf epidermis, which control the gaseous exchange and considered to be the pivotal doors between incoming atmospheric gases into the plant and releasing water vapors from the plant. Their size, density and conductance potential, rate of transfer of CO^2 and other ions, water or gases, significantly influence the plant physiology (Sack & Buckley, 2016). The research analysis depicts that net photosynthesis increases by 40% with an increase of 69% in CO_2 concentration, followed by a uniform, not universal, 17-20% reduction of stomatal conductance (Batke et al., 2020), which helps in ameliorating drought stress with an improved water-consumption efficiency of plant.

Although it is proposed that an increased environmental CO_2 result in an increased carbon fixation and plant growth, but at the same time the reduced stomatal conductivity reduce the carbon fixation and stimulate water up taking efficiency of the plant. The correlation of stomata and CO_2 enrichment has been neglected for years and most of the data showed no distinct relation of stomatal conductance with elevated levels of greenhouse gases. Several factors affect the true findings, like choice of plant for experiment, time frame, and other photosynthesis constraints etc.(Gago et al., 2016).

In free environmental CO_2 enrichment studies (FACE) conducted on temperate plants, the rise in CO_2 level downregulate the stomatal conductance (Giammarchi et al., 2017; Gimeno et al., 2016; Pastore et al., 2019) with the accumulation of some end/by-products like nitrogenous compounds (Ainsworth & Rogers, 2007). Another FACE study revealed a decreased stomatal opening for water conductance by 22-25%.

Digging deeper into the mechanisms, the stomatal opening and closure is under the control of calcium ions level, turgor pressure on guard cells, hormonal signals and other environmental elements like water, CO_2 concentration, temperature, aridity and light (Ikawa et al., 2019; Pastore et al., 2019; Perez-Martin et al., 2009). Moreover, a plant type (genus/specie) further defines the stomatal aperture and its density (Seibert et al., 2021; Sekhar et al., 2020). The calcium ions frequency changed with the change in atmospheric carbon dioxide concentrations, which eventually impact the stomatal opening/closure. In the herbarium leaves, a 67% decrease in stomatal conductance was reported due to elevated level of CO_2 up to 340 μmol mol^{-1} under controlled conditions (Woodward et al., 2002).

Very few studies reported an unchanged stomatal conductance (Herrick et al., 2004; Tricker et al., 2005) and some of the reports declared that the non-vascular plants like hornworts and sporophyte moss were non-responsive to the CO_2 enrichment in the context of stomata (Rakocevic et al., 2018). However, one cannot ignore the external experimental factors, such as study duration, plant type, lab facilities, and biotic/abiotic stress (Seibert et al., 2021; Tricker et al., 2005; Woodward et al., 2002). Therefore, a proper care and background knowledge is necessary while using stomatal conductance as an indicator of plant response towards CO_2 elevation. Because the inhibited guard cells due to high CO_2 level stimulate the gene expression and evolutionary adaptations in plants.

1.3.2 CO_2 enrichment and photosynthetic rates

The plants were originally adapted to the preindustrial levels of CO_2, but over the passage of time with the human involvements in natural phenomena and occurrence of abrupt irreversible climatic changes; the increased ratio of atmospheric CO_2 stimulated the physiological changes in land plants especially their rates of photosynthesis and developmental steps. It has been reported that both long-term and short-term response of plant to elevated CO_2 involved the noticeable changes in photosynthesis but the changes are very complicated and one needs to differentiate between both types of responses before hypothesizing any fact (Jiang et al., 2010; Kirschbaum, 2010).

1.3.2.1 Short-term plant/photosynthesis responses towards CO_2 enrichment

The plants in short-term exposure are allowed to withstand the CO_2 enrichment for a very short duration like seconds, minutes to few hours. The CO_2 diffused through the stomatal pores into the intercellular spaces, cytosol, followed by passing through the plasmalemma, chloroplast cover and eventually into the stroma (Allen et al., 2020). This diffusion of CO_2 exerts a definite pressure in intercellular spaces, which causes the closure of stomatal pore (Xu & Zhou, 2008). The exact mechanism of CO_2 diffusion and stomatal closure is still unknown but it is evident that this flow of events must have a correlation with actin-based chloroplast movement (Evans et al., 2004).

Generally, the chloroplast of an efficiently photosynthesizing leaf avoids to get attached with neighboring cell membranes, but instead attach itself with the membranes exposed to air spaces (Kim et al., 2001). It is assumed that such relocation movement in chloroplast indirectly regulates the relative CO_2 pressure in stroma. The Rubisco (ribulose-1,5- bisphosphate (RuBP) catalyzes two competitive reactions in photosynthesis; (1) Carbon dioxide fixation and (2) the 2-phosphoglycolate production. The ratio or partial pressure of oxygen to carbon dioxide monitors the balance of these two reactions (Andersson & Backlund, 2008). With an increase in atmospheric CO_2 concentration, there will be an increased influx of CO_2 into the chloroplast, followed by an increased carbon fixation or carboxylation, which eventually lead to a decreased oxygenation due to an imbalance carbon-oxygen ratio. However, according to Rubisco kinetics, the rate of photosynthesis reported to be increased by 60% when the carboxylation rate got doubled (Makino et al., 2000).

1.3.2.2 Long-term plant/photosynthesis response towards CO_2 enrichment

Majority of the studies in past had declared an enhancement of photosynthesis after the stimulation of an enhanced CO_2 level, but the long-term effects of the increased CO_2 level when critically investigated, a reduced photosynthetic rate was found after a long-term exposure to CO_2. This refers to the hypothesis that a prolonged disclosure to abnormally high CO_2 results in a changed biochemical, morphological and physiological factors, which suppress the initially provoked photosynthesis (DOMEC et al., 2009). Furthermore, keeping into consideration the plant species, some plants show a definite down-regulated photosynthesis when exposed to CO_2 for a long duration such as weeks, months or years. An underlying fact is that the increased supply of CO_2 leads to the accumulation of photosynthetic product, the carbohydrate, and eventually the sink capacity reached to its limit. Hence, a general feedback inhibition mechanism is assumed present between accumulation of glucose/sucrose and capacity of plant sink, which causes the repression of photosynthesis after a certain deposition level of carbohydrate, is achieved. For instance, a definite sucrose accumulation in the plant leaf inhibit the sucrose synthesis as a feedback-inhibition strategy (Foyer et al., 1990; Krapp & Stitt, 1995).

However, such feedback mechanisms result in the synthesis of long-chain carbohydrates, like starch, which start to get deposited along with other metabolites without pausing or inhibiting elevated process of photosynthesis (Fünfgeld et al., 2021; Kleine et al., 2021). Interestingly, another feedback process gets started without limiting the photosynthesis, which lead to phloem overloading, its transport and then unloading at the sites of developmental plant tissues (Fünfgeld et al., 2021). In maize, sugarcane, and sorghum, physiological responses to increased CO_2, such as transpiration rate, stomatal conductance decreases, and photosynthetic enzymes, are expressed differently. Changes in gene regulation affecting carbohydrate metabolism and plant growth are proportional to the length of stress exposure and experimental facilities. C4 plants have benefits over the other plants to survive under high CO_2 habitats (Rudov et al., 2020).

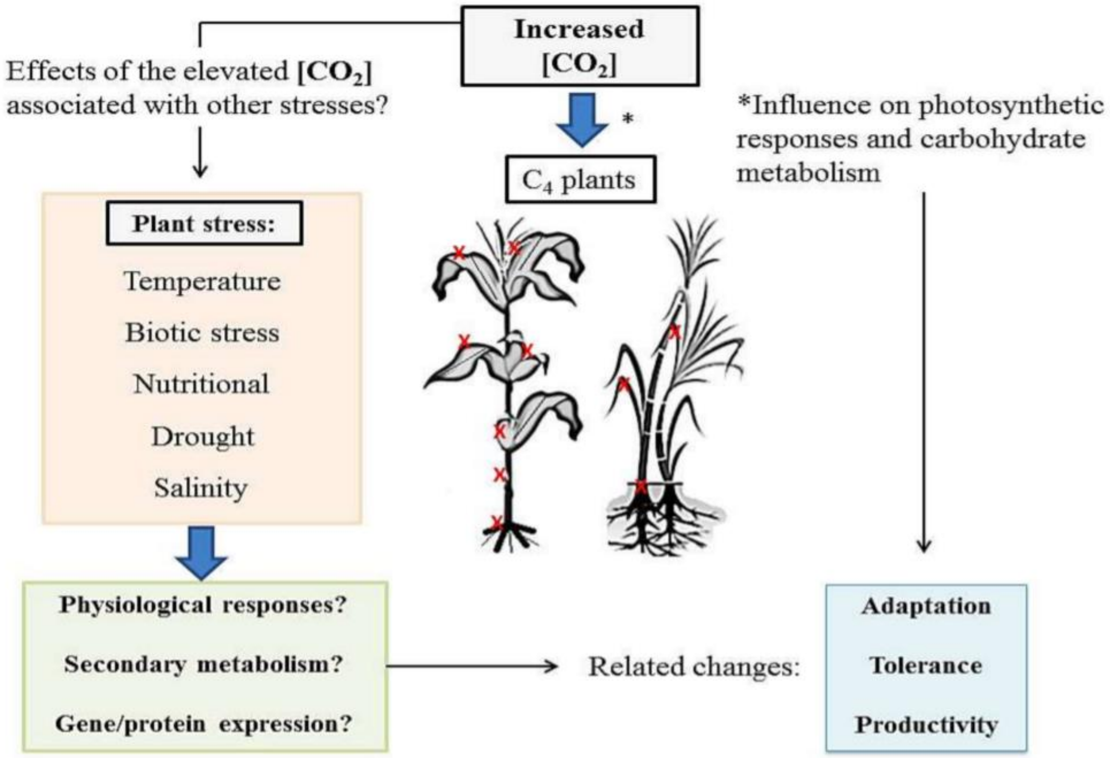


Figure 1: Influence of increased atmospheric CO_2 concentration and other environmental factors on general metabolism and physiology of C4 plants (Silva et al., 2020).

1.3.3 Effect of CO_2 enrichment on leaf surface area and chlorophyll content

A proper understanding of crop responses towards climatic changes is necessary for developing new varieties to combat the issues of global hunger and the plants with desirable traits (Bourgault et al., 2016; Tausz-Posch et al., 2015). Along with several other significant effects of CO_2 enrichment on plant system, a primary effect is reported on leaf physiology and chlorophyll content. Though increase in CO_2 in atmosphere escalates the photosynthetic rate in crops but it does not associate with the increase in biomass or crop yield enhancement (Jin et al., 2018).

Chen et al. (2014) reported that the capacity of source in the Takanari variety rice (*Oryza sativa* L.) leaves under elevated CO_2 was higher than that of the Koshihikari variety. These results were in accordance with the previously reported data by Chen et al., (2014) and confirm the high-yield of Takanari rice crop (Ueda et al., 2016). This benefit of Takanari rice over Koshihikari rice was observed when plants from both groups were exposed to elevated CO_2 through FACE experiment.

In another study by Saldanha et al. (2014), the effects of forced aeration and elevated CO_2 exposure (about 360 to 720 µmol mol^{-1}) were evaluated in *Pfaffia glomerata* plants under lab conditions. *P. glomerata* is an endangered medicinal plant. *P. glomerata* nodal parts were found to have a significant difference in growth parameters like relative water loss from leaves, stomatal density, photosynthetic pigments and leaf anatomy under the two different exposures. The CO_2 enrichment with a lack of sucrose accumulation resulted in an increased photosynthetic component and a reduced stomatal density/conductance along. All the growth parameters reported to be increased followed by a lower relative water loss in a CO_2-enriched environment, with or without sucrose accumulation. Such studies supported the evidence that a photoautotrophic or photomixotrophic condition under CO_2-enrichment could be appropriate for large-scale propagation of *P. glomerata* (Saldanha et al., 2014).

Ksiksi et al. (2018) conducted another experimental study to assess the CO_2-enrichment effects on maize and alfalfa plants. Drought stress along with excessive CO_2 supply was evaluated in maize (*Zea mays L.*), native to hot climate of the United Arab Emirates (UAE), and alfalfa (*Medicago sativa L.*) under greenhouse setup three different sets of conditions (1) 1000 ppm CO_2, (2) 700 ppm CO_2, and (3) routine greenhouse CO_2 (435 ppm) were studied in experimental and control group plants. Similarly, three treatments of water stress (200 ml per week, 400 ml per week and 600 ml per week) were provided to each setup. The results showed that total chlorophyll content in all of the water stress treatments was either same or increased as compared to that of control plants. However, under CO_2 enrichment, the stomatal lengths of maize plants found to be increased with lower water stress but decreased with the increase in water stress. In alfalfa plants, no significant change in total chlorophyll content was noticed under CO_2 elevation (Ksiksi et al., 2018).

1.3.4 Effect of CO_2 enrichment on plant morphological parameters

Plants carry out photosynthesis to remove/fix CO_2 from the atmosphere and give rise to organic compounds along with oxygen release during this process. As CO_2 ratio increases, it is obvious that photosynthesis also increases. However, at the same time the stomatal conductance also reduces and leads to the lower transpiration rate. The transpiration is in correlation with the evaporative cooling effect and hence eventually the cooling effect due to evaporation or water loss becomes lower (Ksiksi et al., 2018).

The plant morphological parameters like branching, yield, biomass, monopodial or sympodial branching, growth patterns and root/shoot elongation are collectively affected by the balance of atmospheric gases especially CO_2. Besides biomass, the carbon and nitrogen metabolism of a plant is monitored by its exposure to CO_2 concentration, any changes in this concentration result in a change of chemical composition of plant parts, such as vegetables, fruits, roots and even the phloem sap (Azoulay-Shemer et al., 2015).

Keeping into consideration, the changes in a plant’s physiology and morphology due to CO_2 enrichment, a concern raised for the inheritance of traits from a parent plant to the offspring; whether an excessive exposure of a plant to the elevated level of greenhouse gases is influencing the next successive generations or not? (Van Etten & Brunet, 2013) The long-term effects of global warming are flowing through plant generations or not or could eventually these factors revolutionize the global ecosystem or not. In order to understand these aspects, one should understand the concept of maternal effects, asexual reproduction, and asexual propagation using maternal parts (Cici, 2017).

1.4 C3 vs C4 responses to CO_2 enrichment

The theory and experimental data prove that C_4 plant species (which produce four-carbon molecule as their primary product of carbon fixation) are less benefited from elevated carbon dioxide (CO_2) levels as compared to the C_3 species (which produce three-carbon molecule as their basic product). The distinct reason for the contradictory responses in both species is their different photosynthetic physiologies, which undergo different behaviors under enriched CO_2 levels (Reich et al., 2018).

The direct effects of CO_2 enrichment on C3 and C4 plants have been evaluated in several studies. Chen et al., in (1996) conducted a study to estimate the interaction between the atmosphere and the biochemical mechanisms of C_3 and C_4 photosynthetic pathways. The experimental plants were *Pascopyrum smithii* (C_3) and *Bouteloua gracilis* (C_4) which were tested for successive two-years under controlled levels atmospheric CO_2, precipitation and temperature in the growth chamber. The data collected from both experimental plants showed that elevated CO_2 resulted in an enhanced biomass production. However, the shoot production in both species found to be varied under different combinations CO_2 levels and temperature/precipitation.

Another study conducted by Navie et al. (2005) declared that the C3 weeds possess a competitive benefit over the C4 grasses under the higher atmospheric CO_2 levels (from 360 (ambient) to 480 (enriched) ppmv). The enhanced growth of C3 specie, *Pascopyrum hysterophorus*, especially the plant height, above-ground biomass, stem base diameter, capitula and seed production, under elevated CO_2 confirmed the potential of this weed to become more aggressive when atmospheric CO_2 levels will be raised in future. Furthermore, C3 plants under enriched CO_2 showed more physiological development than those grown at the ambient CO_2 level. On the other hand, the growth of *Cenchrus ciliaris*, the C4 pasture species, except for a few differences in its growth was not significantly affected by CO_2 enrichment (Navie *et al.,* 2005).

However, Reich et al. (2018) proposed a reversal of the conventional theory by conducting a long-term 20-year CO_2 enrichment study on the grassland plots with different types of plants. It was found that during the first thirteen years of experiment, the biomass of C3 plants increased with an increased atmospheric CO_2 and no significant effect was observed in C_4 plants. Later on, over the next eight years, this growth pattern reversed i.e., Biomass started to increase in C_4 plants but not in C_3 plants. Further studies also confirmed these findings (Habermann et al., 2019; Li et al., 2019; Fay et al., 2021) and raised questions against the conventional C_3-C_4 *e*CO_2 prototype. It can be seen that even the best-supported short-term studies on plant physiology might not predict long-term results to explain the global change.

1.5 Maternal effects on plants

Over the last few decades, several studies are carried out by quantitative geneticists to investigate the maternal effects on the phenotype of a plant. The genotype and environmental factors do not solely determine the phenotype of the plant, but the maternal effects also contribute to a greater extent (Mousseau & Fox, 1998). The maternal effects are defined as the primary influence of the female parent plant on the genotypic or phenotypic characteristics of the offspring. The maternal effect is considered as a process or phenomenon, which flows from one generation to the other, and hence provide a coordination between ecological and evolutionary mechanisms. These effects play a significant role in developing the diversity, evolution, population dynamics, niche construction, response towards natural selection, plasticity of phenotype and eventually the overall plant ecosystem (Roach & Wulff, 1987).

Originally, maternal effects were thought as bothersome means of variation for quantitative studies, but now evolutionary ecologists and environmentalists declared it as one of the most significant impacts on next generation phenotype. For instance, experimental findings have suggested that mother plants can resemble the offspring's phenotype towards local environmental changes and serve as the effective source of developing resistance in them against biotic/abiotic stresses (Fox et al., 1997; Mousseau et al., 2009). Likewise, these maternal effects could degrade the offspring's' fitness by transferring few inappropriate traits and it might lead to the evolutionary imbalance (Johnsen et al., 2005).

1.5.1 Classifications of maternal effects

The maternal effects are categorized into four major types based on the alternative and outcome-based approaches (Donohue, 1999). These are selfish, bet-hedging, transmissive and anticipatory types. The demographic and evolutionary consequences will be clearer by understanding these classes thoroughly.

1.5.1.1 Selfish maternal effects

The reducing effects from mother plant, which cause decreased fitness of the offspring, are categorized as selfish effects. While a decrease in the standard of the environment could cause the mother plant to increase the quality of their offspring's traits, few variations in the maternal conditions could result in a decreased performance/quality of her newly produced offspring (Marshall & Uller, 2007; Paul, 2016; Russell & Lummaa, 2009). The choice on mothers can result in the effects that decrease the offspring's performance when: a) the effect is costly to the mother, b) mothers have a potential of reproducing for multiple times, like varying selection procedures, c) mother got better conditions for reproducing, or d) The strategies that decrease the lethal maternal effects are inefficiently selected due to some hindrance (Marshall & Uller, 2007).

The first condition is quite true because few changes such as offspring size, phenotype and maternal care are induced by mother like an investment. Therefore, a mother would suffer if she increases the investment in offspring's performance, which reduce her current or future fertility (Russell & Lummaa, 2009). Hence, the choices exist for mothers to get the benefits of long-term fitness by lowering or upgrading their input for offspring. Since the mother would prefer to establish her own survival by compromising the performance of her offspring either by terminating the parental care or affecting the plant phenotype. In any case, the mother would be able to accomplish the higher performance by trading the quantity verses quality in the context of offspring, i.e. making fewer offspring (Marshall & Uller, 2007).

1.5.1.2 Bet-hedging maternal effects

The maternal effects where the offspring with a variety of phenotypes are produced in order to sustain the maternal fitness are termed as Bet-hedging effects (Philippi, 1993). A certain combination of the environmental settings is more appropriate for a certain type of phenotype than another and hence any variation that specific phenotype can result in the dwindling offspring's fitness. When mother give rise to the offspring with a variety of phenotypic traits, some of those offspring are potentially misfit or less suitable than others (Kudo, 2006).

Under uncertain heterogeneity of temporal/spatial environmental and selective pressures, mother plant potentially produces a broad range of phenotypes in her offspring to boost-up her geometric mean fitness throughout the breeding events (Olofsson et al., 2009; Starrfelt & Kokko, 2012). For instance, under any inevitable circumstances, when mothers are unaware of the proposed competitive survival conditions for their offspring, they produce a number of offspring constituting varying sizes and morphological features instead of producing a single offspring (Marshall & Uller, 2007; Marshall et al., 2008).

The phenomenon of such production is named as diversified bet hedging. Alternatively, conservative bet-hedging is another condition, where mothers give rise to high quality offspring's who are capable of survival regardless of complications/ challenges in their environment (Marshall & Uller, 2007). However, few studies have declared the conservative bet-hedging to be specifically occurred only under very precise conditions/ circumstances, while diversified bet-hedging is more common to occur under any condition (Einum & Fleming, 2004).

1.5.1.3 Transmissive maternal effects

The transmissive maternal effects cause a reduction in both offspring and material's fitness. Such effects benefit none of the mother or the offspring and hence usually not classify as proper maternal effects but satisfy a condition of no give no take (Marshall & Uller, 2007). For instance, a mother exposed to the toxic agents will produce the offspring having no or a very minimum potential of survival. Similarly, the transmission of pathogens from mother to offspring also comes under this category where neither of them is getting any advantage (Bernardo, 1996; Wiklund & Sundelin, 2001). The exact principle behind such maternal effects is still under study but they are named as transmissive because the mother affects the offspring phenotype by transmitting the traits, which she has attained due to certain environmental changes. The researchers hypothesized that such effects occur due to the co-evolving pathogens with their hosts (Einum & Fleming, 2004).

1.5.1.4 Anticipatory maternal effects

This effect involves the adjustment by maternal parents to adapt the offspring phenotypically according to the local conditions in order to achieve maximum offspring fitness (Marshall et al., 2008; Itonaga 2011). This kind of maternal approach can be seen in seed beetles, where mother while laying on thick seed coat tend to produce large-sized egg and therefore, push the offspring towards the extra resources by boring through the thicker coat, which eventually result in an enhanced fitness of both offspring and mother (Rollinson & Hutchings, 2011). The term anticipate is used for the mothers who possess the capability of influencing or anticipating the offspring environment and give rise to an adaptive offspring with the suitable phenotype under temporal or spatial heterogeneity (Marshall et al., 2008).

1.6 Asexual propagation in plants

The asexual process of plant reproduction in which a parent plant part, such as stem, leaf or root, is cut out and allow to regenerate itself or evolve into a new plant called offspring. The offspring created by asexual reproduction are genetically identical to their parent plant (Hartmann & Kester, 1963). The process of removing a plant part for propagation is called cutting and the parental stem, leaf or branch is cut for this purpose. This is quiet cost-sufficient and rapid method of propagating the plant to give rise a large number of genetically identical/uniform plants (Melnyk, 2017).

Different plant tissues/organs are selected for different plants, such us woody branch cutting is used as a mean of asexual propagation in several plants such as fig, bougainvillea, rose, pomegranate, and grapes. Likewise, plants with fleshy leaves, such as Alvera, Chrysanthemum, Pilea, Iresine, Dahlia, and Petunia are allowed to reproduce by leaf section cuttings. Within the notches of *Bryophyllum* or begonia rex (leaf margins or veins respectively), there exist the vegetative buds, when a plant part bearing that vegetative bud is taken out and placed in a rooting medium, supplemented with specific hormones, growth regulators and other growth promoting factors, a new healthy plant is developed. Therefore, the plant species, phenotype, strength, and available conditions etc. decide which method to be used for its asexual reproduction (Berhe & Negash, 1998; Nanda & Melnyk, 2018; Senthilkumar et al., 2014).

1.6.1 Asexual propagation by cutting plant part

The stem cutting method allows the vegetative propagation of several plants and give rise to the uniform offspring under controlled conditions. Generally, a healthy non-flowering stem part from a mother parent is removed and shifted to a root promoting growth medium (Banik, 1984; Schmid & Dolt, 1994). The medium has a specific ratio of cytokines and auxins to support the roots development. Under controlled environmental conditions, the rooting established and plantlets are acclimatized to further develop into a completely new plant. These plants are then shifted to the field or greenhouse for further growth and productivity (Azad & Matin, 2015).

There are four categories of branch propagation based on the plant's age or maturity. These are, (i) Hardwood cutting (ii) Semi-hardwood cutting (iii) Softwood cutting and (iv) Herbaceous cutting. The softwood and herbaceous cutting are used for plants like coleus, *Alternanthera*, duranta, *Clerodendrum*, *Chrysanthemum*, Pilea, Iresine, Petunia, marigold etc. One-two months old soft shoots or stems are selected for cutting and processed for plant development. In contrast, hardwood or a mature tree branch is used for propagation in woody deciduous plants (Dhillon et al., 2011; Islam et al., 2011).

1.6.2 Asexual propagation using maternal branch

The one-year-old branch from a healthy maternal plant is used for this propagation. The long woody, isolated branch is cut into 10-13cm sections. Each section bear 4-6 vegetative buds with complete removal of leaves, thorns and other small shoots to control the water loss through transpiration (Marte, 1990). The cut is made at the base, just beneath the node of the cutting in a slanting angle followed by a straight cut distant from the upper bud. The angle of a cut is very significant to identify the position, geotropism and phototropism of the propagating plant (Husen & Pal, 2007). The slanting cut at the lower portion is meant for providing a maximum area of cutting-medium contact so that rooting can be induced rapidly under the effect of growth hormones already supplemented to the rooting medium (Husen & Pal, 2007).

On the other hand, the straight cut near the top portion of the cutting is wrapped by the waxy material, which is meant for reducing the chances of water loss due to transpiration. The cambium in the wood gives rise to the callus tissue, a mass of undifferentiated cells, which differentiate into different plant organs once the growth established. Each cutting is allowed to submerge in growth promoting medium, packed within a nursery bed or polythene bag under standard planting conditions in the greenhouse. The most commonly used practice is to cultivate the stem cutting of conifers, gymnosperms and some oaks in the monsoon season. However, deciduous plants are best propagated from November to February (Bonga, 2016; Husen, 2004).

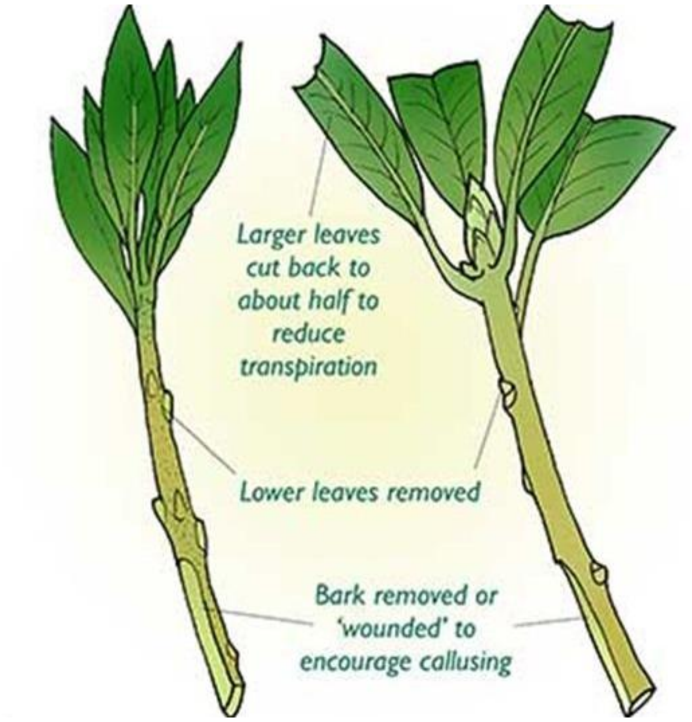


Figure 2: Maternal branch cutting for asexual propagation. The slanting cut at lower portion is made to promote callus formation while the lower leaves, thorns are removed to avoid transpiration water loss (Bonga, 2016)

The stem cutting propagation is significant because it is independent of meiosis and the energy required for making gametes. No specific sex organs are required to clone a plant through this method as simple mitotic division takes place in cambium and meristematic shoot or root to develop the whole new plant. Furthermore, the positive features of maternal plants are transferred exactly to the newly grown plant and this way it provides a basis for creating the multiple clones of the same healthy plant (Husen, 2004).

1.7 *Chloris gayana*

The fodder crops are the backbone of the agriculture and significantly affect a country's economy due to their utilization by livestock. The *Chloris gayana*, generally called the Rhodes grass (Figure 3), is one of the most important among the tropical and subtropical fodder grasses. It is used in hay and pasture for feeding the livestock in dairy farms or household cattle. This grass has the potential to withstand the dry and arid weather and produce high yield when it is fully developed. *C. gayana* is an annually grown grass with a morphology of leafy green grassy structures of about 2m length, which can be vary in different habitats. The nodes and internodes division is present on the stem with tufted culms which are either creepy or erected (Hussain et al., 2021).

Figure 3: Rhodes grass (*C. gayana*) (Image Source: Animal feed resources information system)

Usually, the rooting occurs from the nodes and the roots along with crawling on the ground they penetrate deep into the soil up to 5 m downward. The leaf is linear in shape and consists of flat or bended glassy blade like structures, which can be 15-55 cm in length and 15-20 mm in width. When the grass is young, its inflorescence is pale green to brown or yellow in color, while it turns to dark brown when grass is fully matured. Its fruit is grooved longitudinally (Allah & Bello, 2019; Hussain et al., 2021).

1.7.1 Utilization of C. gayana

Rhodes grass is commonly used forage plant for grazers, livestock and cattle. It is not appropriate for silage formation but can be cut and used for hay or pasture making. A number of cultivars have been developed through selection breeding methods, some of them like prostrate cultivars are used by grazers, while others (erect ones) are the best choice for hay production (Hussain et al., 2021; Mazimpaka et al., 2017). Besides, *C. gayana* is also significantly important for improving the soil fertility and its overall structure by preventing the nematode flourishment in soil. The nematodes are the soil-dwelling worms, which severely damage the growth of roots, stem and other plant parts.

They inhibit the plant growth by attacking the root system by piercing holes in the cell walls.

The Rhodes grass is one of the best remedies to get rid of these culprits and hence enhance the soil structure. Moreover, it is also used as a cover crop to upgrade the soil health by reducing the soil erosion and increasing the water availability. This phenomenon protects the field ground from disease causing pests and promote biodiversity (Abu-Alrub et al., 2018; Gupta & Arora, 2015). A three-month of covering period is readily provided by the *C. gayana* among the sowing of crops. Researchers have reported its significant role in lowering ground temperature during hot weather, improving water-holding potential and infiltration. Possessing the ability to creep and penetrate the soil, *C. gayana* helps maintaining the soil stabilization, especially in the soil affected by mining. Experimental studies have declared the successful soil rejuvenation in Australian soil which was destructed due to mining (Fox et al., 2004).

Similarly, in Hawaii, it is a common cultivation practice of farmers to use this grass as clipping to provoke soil mulching as well as prevention from erosion due to wind or aridity. Moreover, being a source of rich organic content, it is used as a convenient seed bed for some crops like cabbage, onions, eggplant and other horticultural plants (Brima, 2007). A report in 2007 from Queensland, Australia revealed that the *C. gayana* produced the seeds richly and rapidly in its rainforest that a smooth fertile habitat was created for the flourishment of the native flora (Nichols et al., 2007). Another study declared the *C. gayana* as the competitor of summer-weeds and therefore, assist in preventing their development (Döndü & TANSI, 2020).

1.7.2 Concerns about using the C. gayana

Originally, the Rhodes grass was grown in African subcontinent, from where it spread to the other continents, such as Asia, Australia and America. In the early years of 20th century, it was introduced to Australia after world war, through the returning soldiers, and later became the most widely spread grass of the subtropical regions, such as Western Australia (Moore et al., 2014). Looking into its habitat, this grass tends to flourish in the widespread woodlands or grasslands especially during spring-summer season, favorably at 16-25°C temperature and 5-6 pH. It also grows at river banks, along the roads, and unattended sites/plots.

Rhodes grass can resist extreme weather conditions for more than six months due to the deep penetrating roots. It possesses the potential to withstand the waterlogging, hottest weather (50°C), wind, pH, aridity, poor soil, etc. Hence, it is widely used as a preferable choice of covering crops throughout the world. However, few limitations are there, as it needs proper sunlight to grow, it cannot flourish well in shady areas. Moreover, it is resistant to several acidic or basic chemicals and metals, but not to Manganese and Magnesium (Allah & Bello, 2019; Faji et al., 2021). Soil salinity is a growing problem of all the agricultural countries, especially, Asia and Africa. This issue is so alarming among the environmentalists and food authorities. To combat the potential risk of food scarcity, researchers are trying hard to transform the arid saline soil into the fertile land either through leaching methods or by upgrading the drainage procedures (Qin et al., 2012).

Bioremediation is also one of the best strategies to overcome this issue and the introduction of fodder grasses in all such salt-affected areas. Because they do not only have the potential to reduce salinity by developing a mulching layer, but also strengthen up the rooting system of plants by preventing the upward flow of water table in soil. *C. gayana* is one of the best choices among these grasses which tends to give rise biomass and improve water availability (Gelaye et al., 2019). The fodder grasses are accounted for their nutritive values, yield, effect on grazer's health and livestock performance and the biochemical properties. Research studies have indicated that *C. gayana* is a valuable source of biomass for livestock feeding and soil improver as well.

However, few concerns associated with its cultivation are there (De Luca et al., 2001; Granzin & Dryden, 2003; Lukashe et al., 2020; Milford & Minson, 1968) such as, the Rhodes grass is not resistant to the acidic or infertile soil, although it can survive extreme weather conditions, but when water is available, it tends to consume significantly high amount of water to grow. This eventually could lead to water scarcity. Another important point to be considered is its limitation to grow only in sunny areas and cannot establish in a shady place. Under specific climatic conditions, it needs highly fertile soil to grow appropriately. Although it provides a nutritionally valuable biomass for livestock, the peak season for such enriched yield is however, very short and rapid (Abu-Alrub *et al.,* 2018).

Furthermore, the cultivation of this grass requires a field preparation, which is a laborious job. The field preparation involves repeated ploughing for creating suitable seedbed and immediate sowing (Arshad et al.*,* 2016). Grass cutting is needed after six months of the seed plantation and then every two months to sustain the quality of biomass. The digestibility rates vary with the growth period of grass. For instance, the young fresh grass constitutes the digestibility rate of 60-65% in grazing cattle and this rate decreases down as the grass gets old/mature (Tefera et al.*,* 2015). It flowers lately and hence makes the harvesting of seeds tedious. Hand picking is possible in smaller areas but at larger sector seeds need a proper clean-up process before storage. It is adapted to a wide range of weather conditions, but it can occasionally tolerate the frost or extreme cold temperature (Abebe et al., 2015; Manalil et al.*,* 2020).

1.7.3 Gulf countries and cultivating C. gayana

Gulf countries are located at the bank of Arabian Gulf and are well known for their financial stability worldwide. The majority of the land in these countries is deficient of fresh water, lakes, biodiversity and forests. There are deserts with sand dunes and many aridity issues. Using the modern biotechnology methods, a number of areas have been provided with vegetation, forests and successful development of green belt, but still the water availability is not up to the mark (Arshad et al., 2016). A decade ago, the UAE stopped the farmers to rely on Rhodes grass as the cover crop.

The fact behind this decision was the research studies reports, which declared the *C. gayana* a culprit of consuming significantly high amount of water as compared to the other grasses. The statistical evidence revealed that this grass consumed around 50% of the one and half billion cubic meters’ irrigation water. Hence, the government banned the production of Rhodes grass and all other such water-efficient crops to maintain the water availability at an appropriate level. Around 1.25 million tons of hay and pasture was imported from Australia alternatively, to cope up the loss in local crop system (Belgacem, 2016; “End to Subsidy for Farmers’ Rhodes Grass,” 2010).

The alternative grasses like Jet grass (family *Poaceae*) have grown for livestock feeding. Jet grass is also valuable nutritionally and consume less water than *C. gayana*. Other substitutes are some salinity resistant grasses like sporobolus, wheat, saltbrush, and barley, which allow direct consumption by cattle rather than processing and plantation. Although there is a shortage of available seeds in seed banks for replacing the Rhodes grass with other, but the stakeholders are confident that this strategy will save water up to 40-50%. Especially during the hottest seasons. The ecologists are planning to develop the database of local flora and fauna to protect the biodiversity through various conservation methods (Singh et al., 2014).

In UAE, experimental studies were conducted to assess the recycled wastewater effect on soil physiochemical properties and consumption rate of water in different fodder crops, such as Alfalfa, Buffelgrass, and Rhodes grasses. All the grasses were allowed to grow in random block under controlled cultivation conditions. The highest biomass was found in Buffelgrass and lowest in Alfalfa. Soil properties were found to be improved and showed that processed wastewater can be significantly used for irrigation (Mazahrih et al., 2018). Since gulf countries are deprived of the fresh water due to desert land, their primary source of water is sea desalination. The governmental bodies are developing new strategies to upgrade the desalination system to meet up the irrigation and domestic water needs (Hussain et al., 2021).

The objectives of this study are as following:

- To examine the direct impact of CO_2 on *C. gayana* growth parameters.
- To determine the maternal effects on growth parameters of *C. gayana* offspring.
- To identify specific traits in Eco- physiological growth of offspring because of maternal effects.

Chapter 2: Methods and Materials

2.1 Experiment setup

The whole study was performed in research lab facility at F3 building, United Arab Emirates University, Al Ain city. It was a one-year research work, which was performed in two successive parts. The first part involved the growth and assessment of "Generation 1" plants and it was done from 26th February to 27th August, 2017.The second part of the study consists a propagating and evaluating of "Generation 2" plants and it was conducted from 27th August to 1st January, 2018.

The experimental setup was made in such a way that the potential impacts of CO_2 can be measured on eco-physiological growth of *C. gayana* by studying successive generations. The following schematic illustration (Figure 4) shows the experimental setup. The first generation of *C. gayana* was grown under standard agricultural practices in two separate experimental groups. The one group was provided excessive CO_2, while the other group was grown under ambient supply of CO_2. Keeping all other cultivation parameters like temperature, moisture content and light, constant, the growth of these two groups was assessed under varying concentrations of CO_2. The growth parameters were measured for each group of *C. gayana* plants and a successive generation was replanted to further investigate the CO_2 effects. The similar pattern of CO_2 supply was followed in both groups of second generation, i.e. (1) CO_2 enriched and (2) CO_2 ambient supply.

2.1.1 Pot preparation and seed germination

The sixty (60) pots were prepared by filling the EcoBalt soil composite. The soil mixture was made fertile by mixing peat moss, clay, and sand in equal proportions along with the minute quantity of antifungal solution. The soil was autoclaved appropriately prior to filling up in pots and moistened with adequate water. The *C. gayana* seeds, originated from Zimbabwe, were sown in the pots. Sixty seeds were used, one in each pot. The soil was supplemented with (Adfert NPK 20-20-20) Fertilizer to promote the smooth growth of the seedlings.

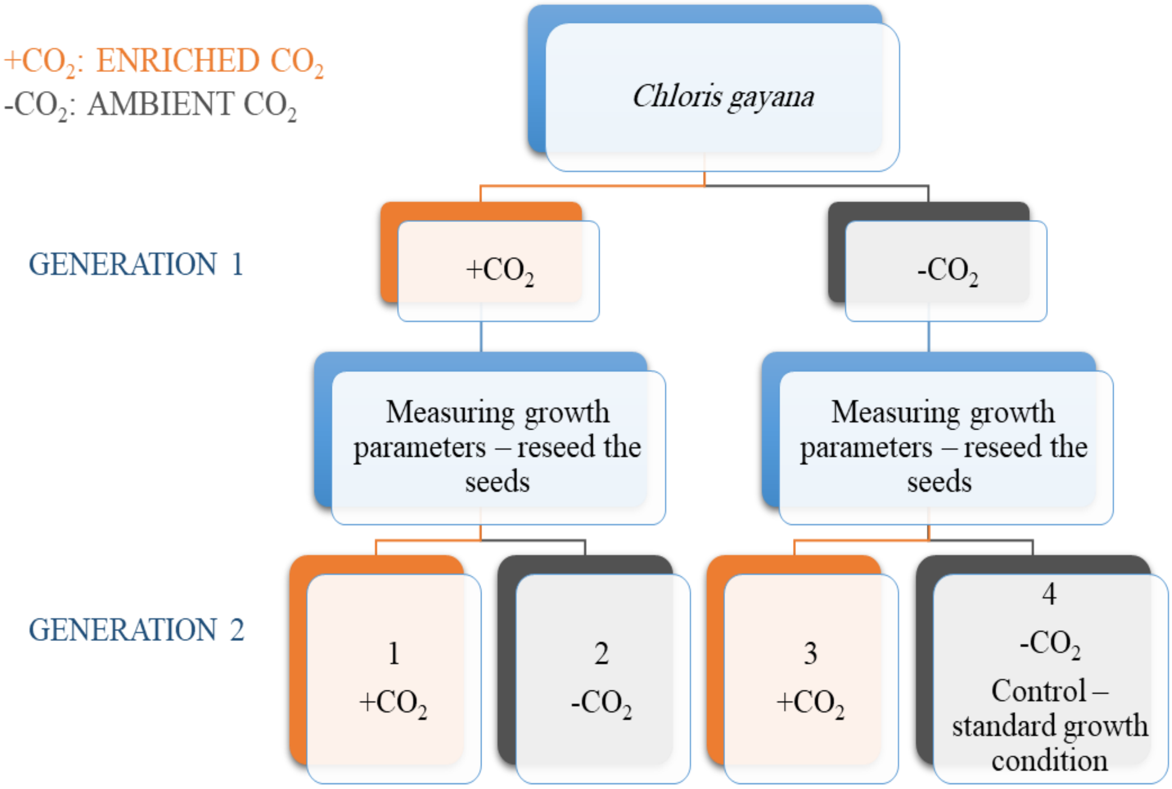


Figure 4**:** The schematic diagram of experimental setup

2.2 Growing Generation 1 plants

The pots with sowed seeds were placed in two separate growth chambers, (BINDER Growth Chamber KBW#720), under controlled lab conditions. Each growth chamber with equal number of thirty pots was provided with different supplies of CO_2 through a CO_2 regulator, an Autopilot Digital Environmental Controller and a CO_2 cylinder. CO_2 enrichment condition was kept at around 1000 ppm. While the other growth chamber was maintained for an ambient CO_2 supply which was around 500 ppm. The seeds were allowed to germinate under both conditions for up to 3 days until seedling emergence. These plants were named as “Generation 1”.

2.2.1 Assessment of growth parameters in Generation 1 plants

The *C. gayana* plants were allowed to grow under the above-mentioned conditions and the growth parameters were measured at regular intervals. Plant height, number of leaves, shoot/root ratio, chlorophyll content, stomatal conductance and photosynthetic rate were measured in the Generation 1 plants. The collected data was organized in excel sheets and prepared for statistical analysis.

2.2.2 Evaluation of growth parameters in Generation 1 plants

Random individual plants were selected from each growth chamber (CO_2 enriched and CO_2 ambient) and assesses for plant height, number of leaves and shoot/root ratio at regular intervals. The plant height assessment was done from first week to 13th week of plant growth. For this purpose, the selected plant was taken and undergone the length measurement using standard meter scales. The plant shoot/root ratio was measured after second month of growth and assessment was done at regular intervals up to fourth months of plant growth. To measure shoot/root ratio, the selected plant was removed, cleaned and oven dried to remove the moisture content i.e., dry shoot weight, dry root weight, shoot length, and root length. Likewise, the leaf number and length was also calculated in the randomly selected plants. The data of leaf measurements was taken from week 1 to week 17 through manual leaf count per plant and using the meter rod. Data from both plant groups was compared statistically to determine the potential effect of CO_2 on *C. gayana*.

Stomatal conductance, photosynthetic rate and chlorophyll content were also measured. Since a proper leaf surface area is required to assess the physiological parameters, hence the measurements were done from week 7 to week 13 of plant growth. The stomatal conductance was calculated through SC-1 leaf Porometer (Decagon Devices), the photosynthetic rate was measured using miniPPM (EARS), and the Chlorophyll content was estimated by Hansatech system (model CL-01) which displayed in the range 0 – 2000 units. Data from both plant groups was compared statistically to determine the potential effect of CO_2 on *C. gayana* physiological growth.

2.3. Propagation of Generation 2 through replantation

The generation 2 of the *C. gayana* plants was initiated by replanting the plant parts of the best grown Generation 1 plants. For this purpose, the soil pots were prepared by filling the fertile soil compost and branches with their roots of Generation 1 plants were removed and replanted in the newly prepared soil pots. For each group of Generation 1 plants, two successive groups of plants were propagated, i.e., the pots with replantation were divided into two groups of CO_2 enriched and CO_2 ambient supply under controlled growth conditions in (BINDER Growth Chamber, KBW#720). The planted branches of Generation 1 were allowed to give rise the Generation 2 plants under controlled supplies of CO_2.

2.3.1 Assessment of growth parameters in Generation 2 plants

The *C. gayana* plants were allowed to grow under the above-mentioned controlled conditions and the growth parameters were measured at regular intervals. Plant height, number of leaves, shoot/root ratio, chlorophyll content, stomatal conductance and photosynthetic rate were measured in Generation 2 plants. The collected data was organized in excel sheets and prepared for statistical analysis.

Random individual plants were selected from each growth chamber and assesses for plant height, number of leaves and shoot/root ratio. Data from both plant groups (CO_2 enriched and CO_2 ambient) were compared statistically to determine the potential effect of CO_2 on *C. gayana* phenotype for generation 2.

Stomatal conductance, photosynthetic rate and chlorophyll content were measured at regular intervals using the same equipment and methodology as done for Generation 1 plants. Data from both plant groups (CO_2 enriched and CO_2 ambient) was compared statistically to determine the potential effect of CO_2 on *C. gayana* physiology in generation 2.

2.4 Experimental Design and Statistical Analyses

2.4.1 Analyses performed

The Randomized Completely Block Design was used for the experiment in this research. The analyses of the quantitative data collected during the experiment was done by using Jamovi 1.6.23 software. Two-way analysis of variance (ANOVA) was performed towards the data to compare the effect of ambient and enriched CO_2 treatment on growth parameters of Rhodes grass. During the analyses, CO_2 treatment and date kept as a fixed factors and their effect and interactions were evaluated on dependent variables, which includes; plant height, number of leaves, shoot/root ratio, chlorophyll content, stomatal conductance and photosynthetic rate. The Post hoc comparison was conducted on significant results. The Line graphs were used to represent the comparison of means between different groups.

2.4.2 Treatments Tested

In first part of the experiment, which was for generation 1, Rhodes grass was grown under two CO_2 treatments (ambient and enriched). While, in second part of the experiment which was for generation 2, Rhodes grass was divided into four groups which are ambient CO_2 treatment and parent comes from ambient CO_2 (AA), ambient CO_2 treatment and parent comes from enriched CO_2 (AE), enriched CO_2 treatment and parent comes from ambient CO_2 (EA) and enriched CO_2 treatment and parent comes from enriched CO_2 (EE).

Chapter 3: Results

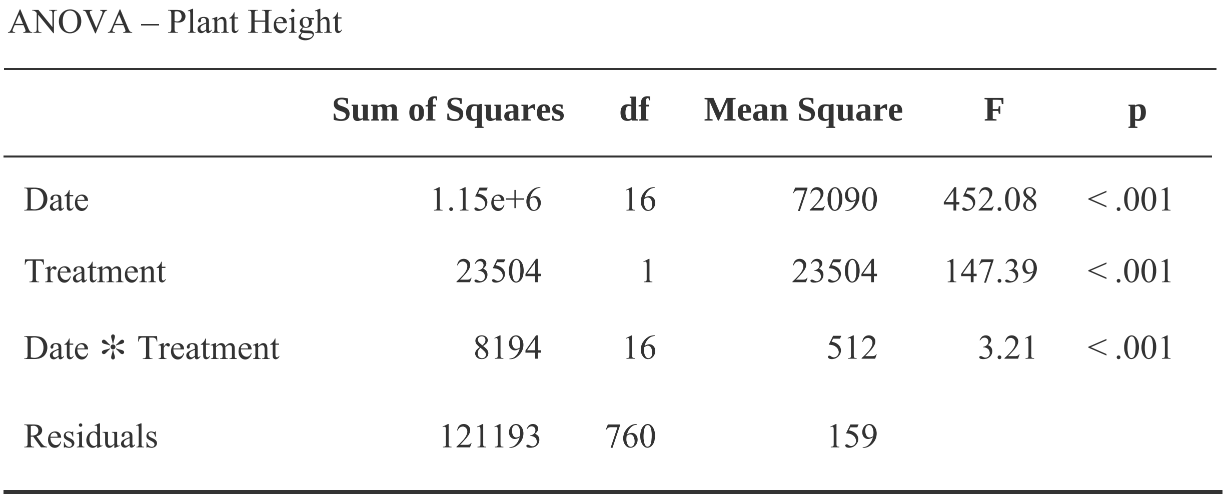
3.1 Growth pattern assessment - Generation 1 plants

3.1.1 Plant height

Plant height was measured in cm from week 1 to 17 and an increase in plant height was measured over the 17 weeks in both ambient and enriched environments. As shown in (Table 1), there was a significant difference ($p< 0.05$) observed in effect of Date, Treatment and interaction of Date* Treatment.

The average plant height under enriched CO_2 was higher than ambient (Figure 5). There was a slight decrease in plants average height under enriched and ambient CO_2 in week 15 and week 14 respectively. The maximum plants average height recorded in enriched and ambient plant groups was in week 17, which was 124 cm and 118 cm respectively. Similarly, the least average plants height was 7.00 cm in the ambient plant during week 1 of growth and 18.0 cm in the enriched plant group in same week.

Table 1: ANOVA results for plant height of Rhodes grass grown under ambient and enriched CO_2



ANOVA – Plant Height

	Sum of Squares	df	Mean Square	F	p
Date	1.15e+6	16	72090	452.08	< .001
Treatment	23504	1	23504	147.39	< .001
Date ✻ Treatment	8194	16	512	3.21	< .001
Residuals	121193	760	159		

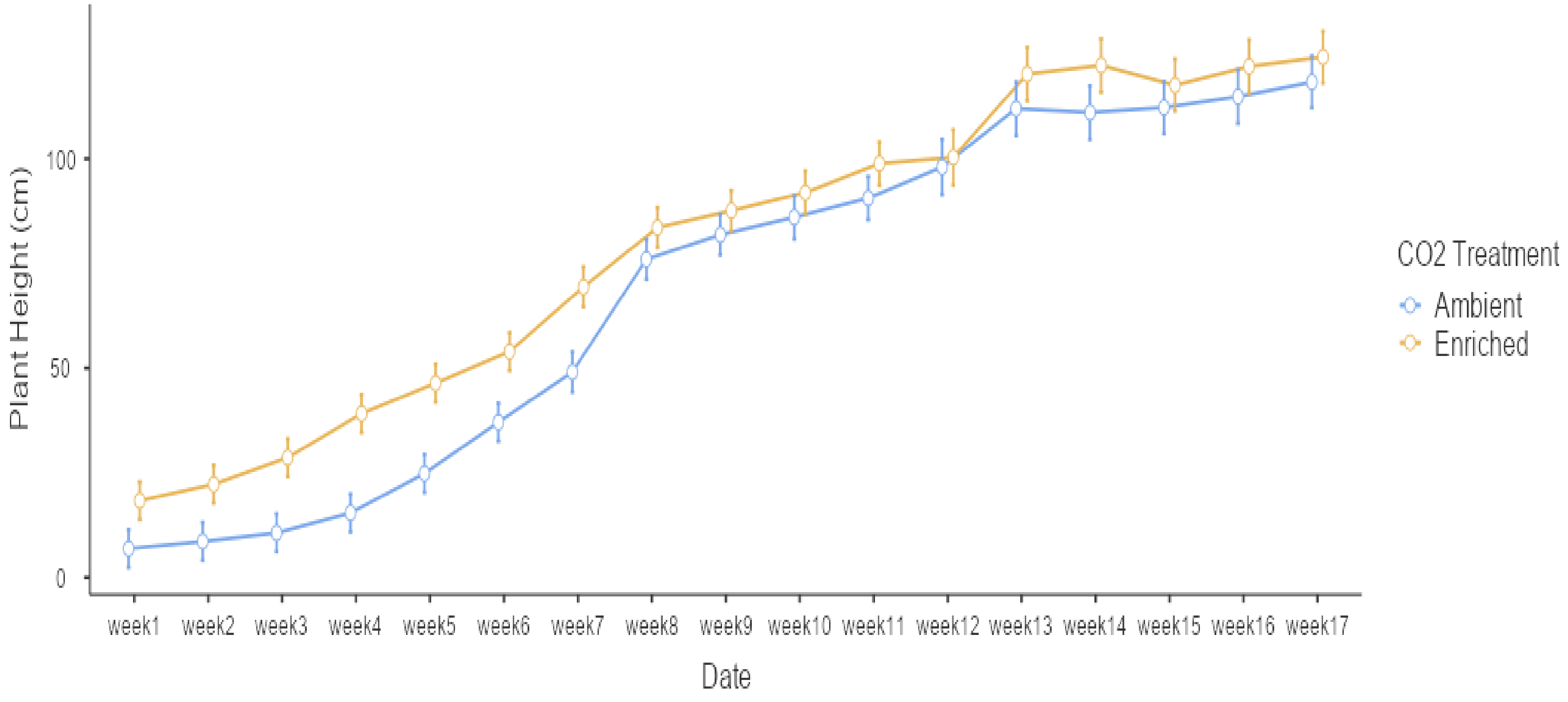


Figure 5: Comparison of plant height of Rhodes grass grown under ambient and enriched CO_2

3.1.2 Number of leaves

The number of leaves was counted from week 1 to 17. There was a significant difference ($p < 0.05$) in effect of Date, Treatment and Date*Treatment interaction (Table 2). As shown in (Figure 6), the number of leaves increased over the week in plants grown under ambient and enriched CO_2 but there was a slight drop in leaf number under the ambient condition in week 15 and under the enriched environment in week 14. The highest mean of leaf number was recorded in plants under enriched CO_2 with 48 leaves in week 13. In addition, the maximum mean of leaf number in ambient CO_2 condition was in week 14 with 34 leaves. While the minimum mean of leaf number was recorded in week 1 for both conditions.

Table 2: ANOVA results for number of leaves of Rhodes grass grown under ambient and enriched CO_2

ANOVA – Number of Leaves

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Date	146435	16	9152.2	99.03	< .001
Treatment	10467	1	10466.6	113.25	< .001
Date ✻ Treatment	3505	16	219.1	2.37	0.002
Residuals	70240	760	92.4		

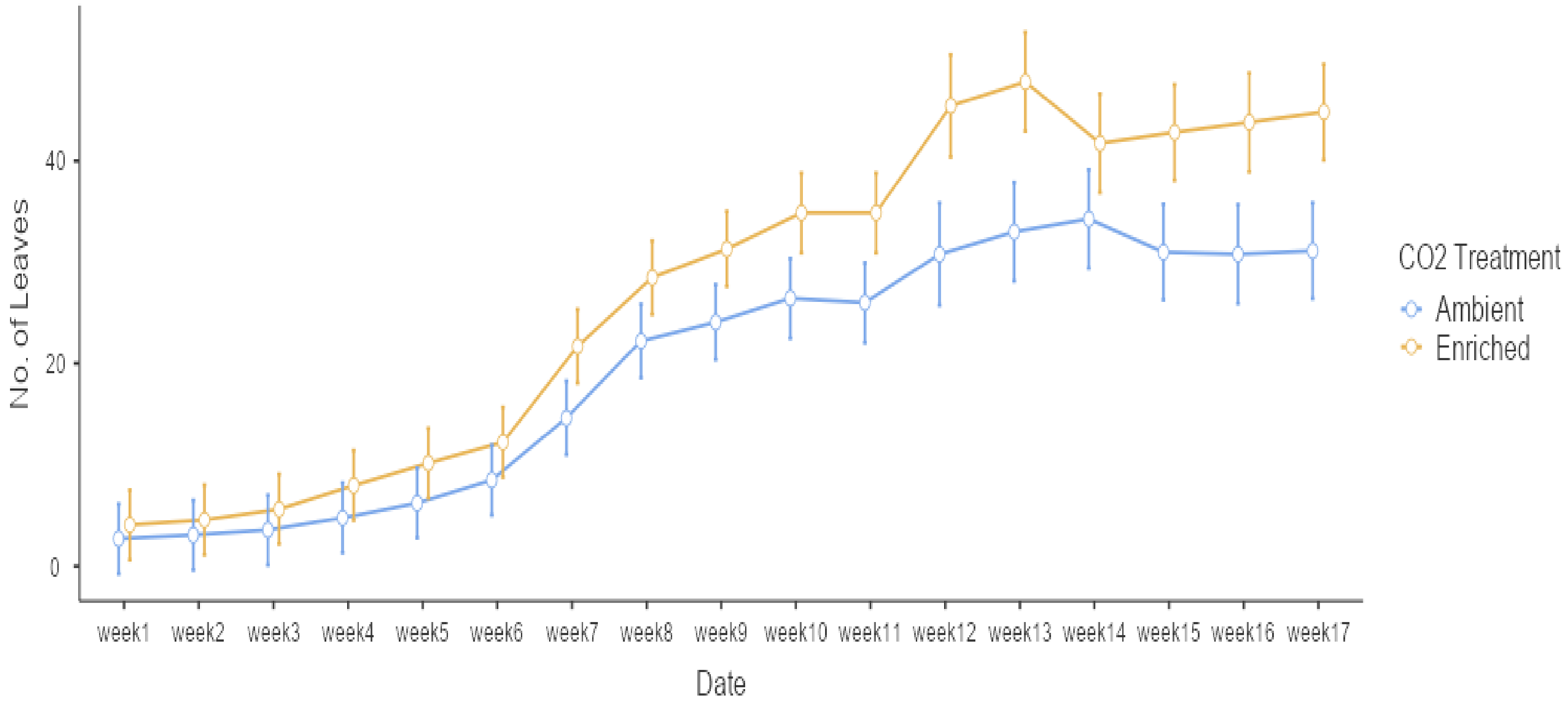
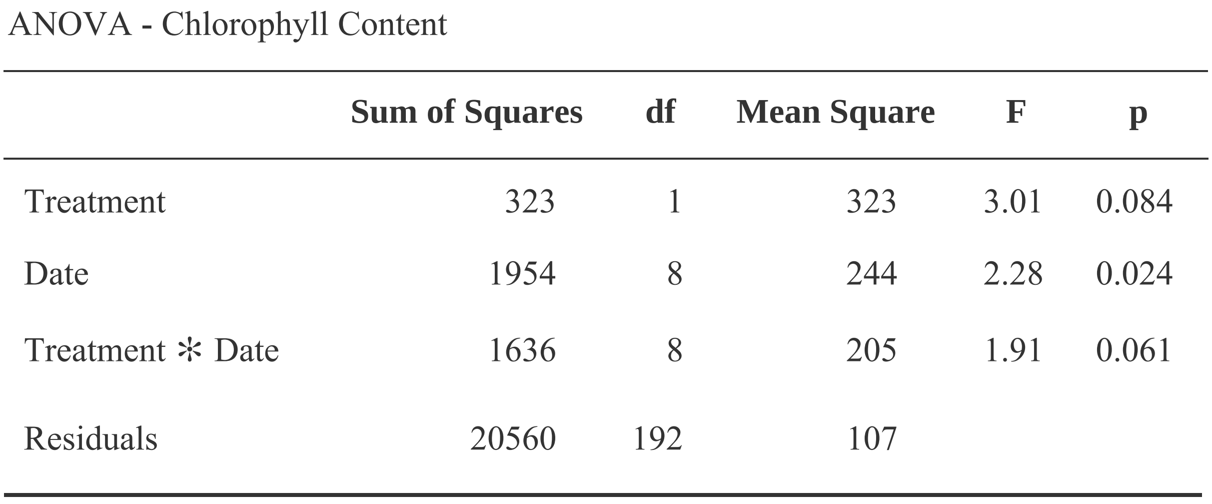


Figure 6: Comparison of number of leaves of Rhodes grass grown under ambient and enriched CO_2

3.1.3 Chlorophyll content

The photosynthetic pigment, chlorophyll was measured in all the plant groups from week 8 to week 17. The comparison among all plant groups showed a significant difference in the effect of Date ($p < 0.05$) (Table 3). In general, the chlorophyll content in the plants grown under enriched CO_2 conditions was greater than or equal to plants grown under ambient CO_2 (Figure 7). The Rhodes grass grown under enriched CO_2 conditions reached the highest chlorophyll content in week 11 with a mean of 26 units, while week 8 and 10 had the lowest chlorophyll content with a mean of 11 units. The Rhodes grass grown under ambient CO_2 conditions had the greatest chlorophyll content in week 17 with a mean of 21 units and had the lowest chlorophyll content in week 12 with a mean of 9 units.

Table 3: ANOVA results for chlorophyll content of Rhodes grass grown under ambient and enriched CO_2



ANOVA - Chlorophyll Content

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Treatment	323	1	323	3.01	0.084
Date	1954	8	244	2.28	0.024
Treatment ✻ Date	1636	8	205	1.91	0.061
Residuals	20560	192	107		

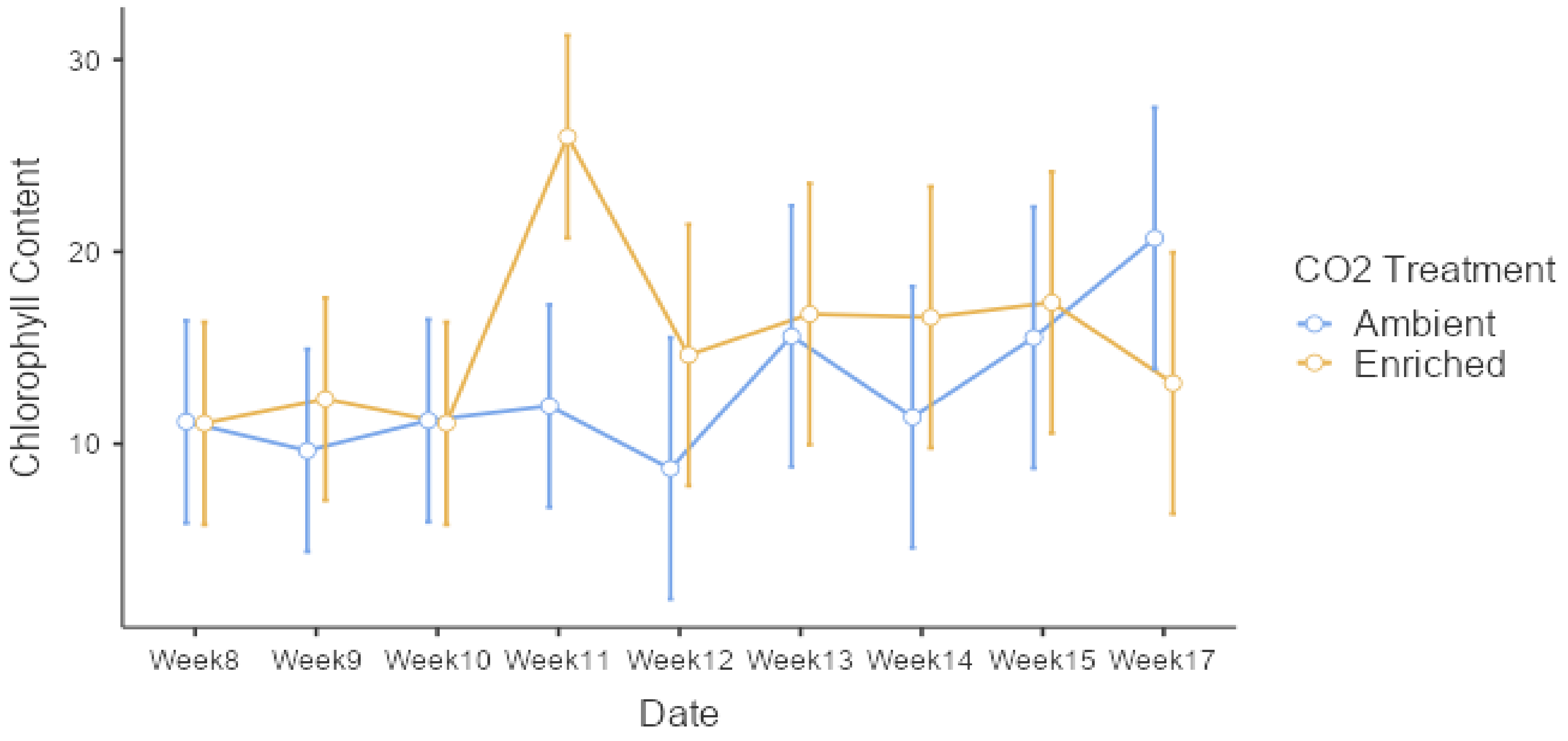


Figure 7: Comparison of chlorophyll content of Rhodes grass grown under ambient and enriched CO_2

3.1.4 Stomatal conductance

The stomatal conductance of plants was measured from week 5 to week 13. There was a significant effect of Date ($p< 0.05$) on the stomatal conductance of the plants (Table 4). Week 11 with a mean of 119.1 mmol/m^{2}s was significantly higher than week 15 with a mean of 74.6 mmol/m^{2}s and week 17 with a mean of 65.5 mmol/m^{2}s. However, the mean in week 14 and week 15, which were 76.5 and 74.6 mmol/m^{2}s, were significantly lower than week 9 with a mean of 122.8 mmol/m^{2}s. Also, week 17 with a mean of 65.5 mmol/m^{2}s was significantly lower than the mean in week 8 and week 9, which were 102.4 and 122.8 mmol/m^{2}s.

Rhodes grass grown under ambient and enriched CO_2 had fluctuating stomatal conductance (Figure 8). For example, an increase of stomatal conductance in enriched CO_2 condition was noticed from week 8 to week 9. However, in week 10 a sharp drop in stomatal conductance with a mean of 84 mmol/m^{2}s, followed by a sharp rise in week 11 with a mean of 128 mmol/m^{2}s, which is considered the maximum stomatal conductance. From week 11 to 15, a decline in stomatal conductance until reached the minimum, which was a mean of 63 mmol/m^{2}s in week 15. For the plants grown under ambient CO_2 condition, the maximum stomatal conductance was in week 9 with a mean of 120 mmol/m^{2}s and the lowest stomatal conductance with a mean of 58.4 mmol/m^{2}s in week 17.

Table 4: ANOVA results for stomatal conductance of Rhodes grass grown under ambient and enriched CO_2

ANOVA - Stomatal conductance (mmol/m^{2}s)

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Treatment	2619	1	2619	2.00	0.159
Date	64633	8	8079	6.17	< .001
Treatment ✻ Date	13612	8	1702	1.30	0.246
Residuals	235616	180	1309		

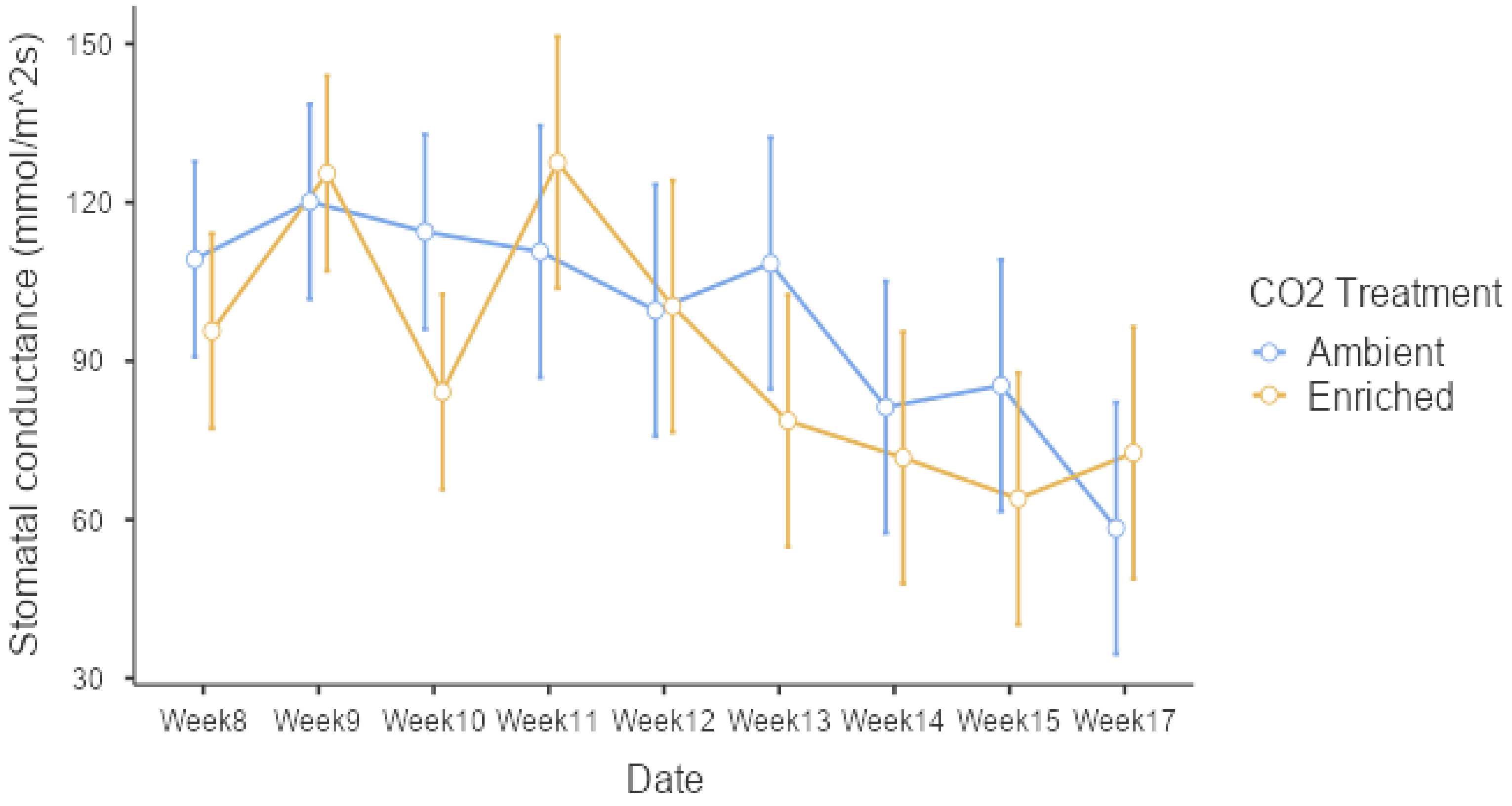


Figure 8: Comparison of stomatal conductance of Rhodes grass grown under ambient and enriched CO_2

3.1.5 Photosynthetic rate

The photosynthetic rate of plants was measured from week 8 to week 17. A significant difference ($p < 0.05$) in the effect of Date was observed on the photosynthetic rate of Rhodes grass (Table 5). The mean of photosynthetic rate in week 17 was 72.9 % and it was significantly lower than in the mean of photosynthetic rate week 8, 9, 11, 13, 14, and 15, which were 74.1%, 74.2%, 74.7, 75% and 74.6 respectively. While, the mean of photosynthetic rate in week 13 was 75% and it was significantly higher than the mean of photosynthetic rate in week 10 and week 12 which were 73.8% and 73.7%. The mean photosynthetic rate ranged between 72 to 75% in Rhodes grass grown under ambient and enriched CO_2 (Figure 9). The highest recorded mean of photosynthetic rate was 75.3 % in the enriched condition plants in week 13. The lowest mean of photosynthetic rate was 73 % in plants grown under ambient and enriched CO_2 in week 17.

Table 5: ANOVA results for photosynthetic rate of Rhodes grass grown under ambient and enriched CO_2

ANOVA - Photosynthetic Rate [%]

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Treatment	0.0157	1	0.0157	0.0154	0.901
Date	60.6369	8	7.5796	7.4222	< .001
Treatment ✻ Date	8.7271	8	1.0909	1.0682	0.387
Residuals	196.0724	192	1.0212		

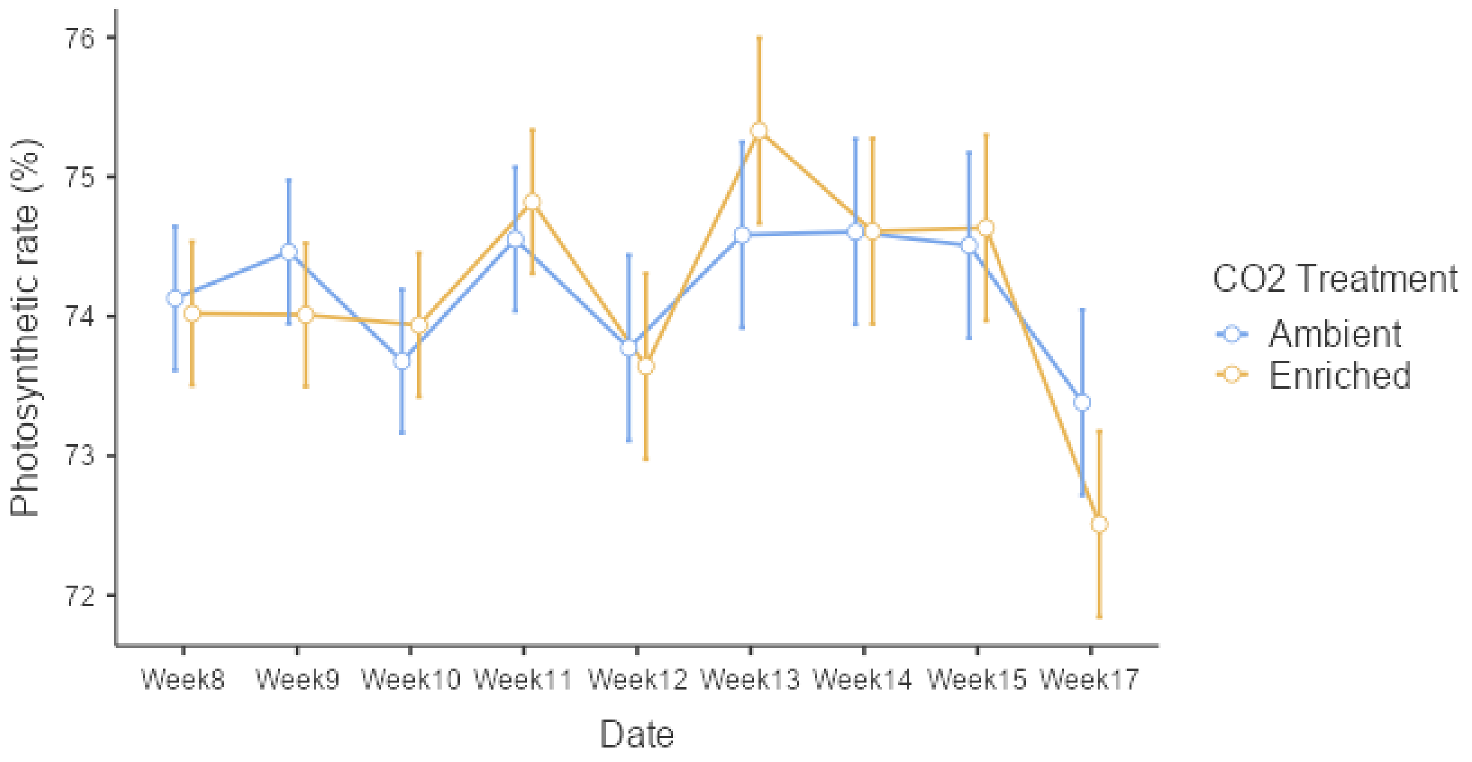


Figure 9: Comparison of photosynthetic rate of Rhodes grass grown under ambient and enriched CO_2

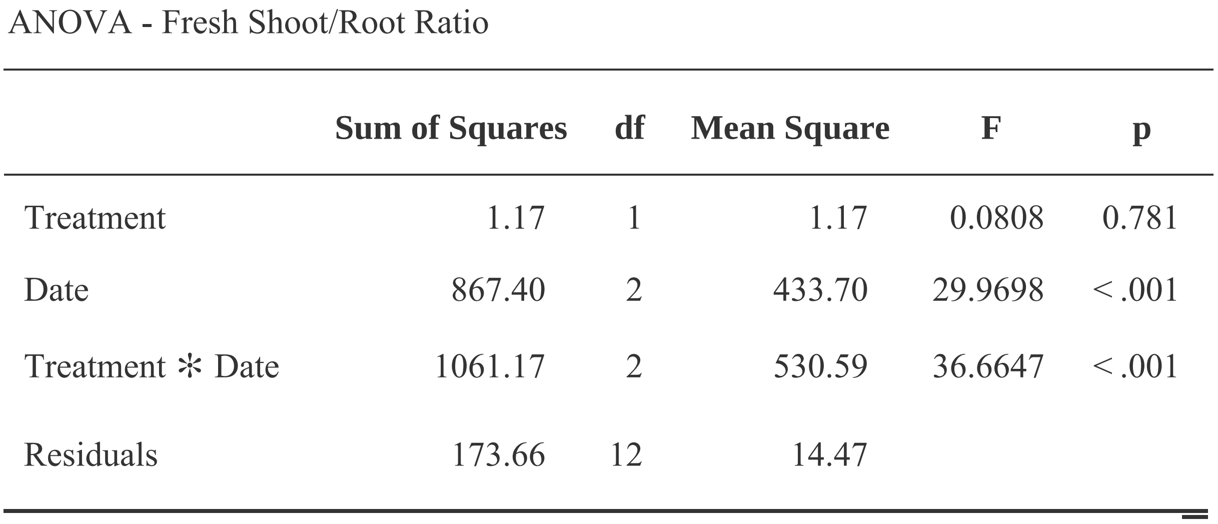
3.1.6 Shoot/Root ratio

3.1.6.1 Fresh Shoot/Root ratio

Fresh Shoot/Root ratio was measured from month 2 to 4 and a significant difference ($p < 0.05$) was noticed in the effect of Date and Treatment*Date interaction (Table 6). For Date effect, the month 3 and 4 with a mean of 34.1 and 26 were significantly higher than month 2 with a mean of 17.1 but month 4 with a mean of 26 was significantly lower than month 3, which had a mean of 34.1. For Treatment*Date interaction, it has been found that plants grown under ambient CO_2 in month 2 with a mean of 14.9 was significantly lower than plants grown under ambient CO_2 in month 3 and 4 which had a mean of 25.5 and 36. Also, the mean of plants grown under ambient CO_2 in month 3 with a mean of 25.5 was significantly lower than plants grown under ambient CO_2 in month 4 and enriched CO_2 in month 3 which their mean were 36 and 42.6. However, plants grown under ambient CO_2 in month 4 with a mean of 36 was significantly higher than plants grown under enriched CO_2 in month 2 and 4 with a mean of 19.2 and 16.1. Besides, Rhodes grass grown under enriched CO_2 in month 3 had a mean of 42.6 and it was significantly higher than plants grown under enriched CO_2 in month 2 and 4 with a mean of 19.2 and 16.1.

Generally, the mean of fresh Shoot/Root ratio of Rhodes grass under enriched CO_2 was higher than ambient CO_2 (Figure 10) except in month 4, which dropped to reach the lowest ratio with a mean of 16.1. While, the mean of Fresh Shoot/Root ratio of plants under ambient CO_2 gradually increased by a mean of 10 in each month until reached the highest mean of ratio in month 4, which was 36.

Table 6: ANOVA results for fresh Shoot/Root ratio of Rhodes grass grown under ambient and enriched CO_2



ANOVA - Fresh Shoot/Root Ratio

	Sum of Squares	df	Mean Square	F	p
Treatment	1.17	1	1.17	0.0808	0.781
Date	867.40	2	433.70	29.9698	< .001
Treatment ✻ Date	1061.17	2	530.59	36.6647	< .001
Residuals	173.66	12	14.47		

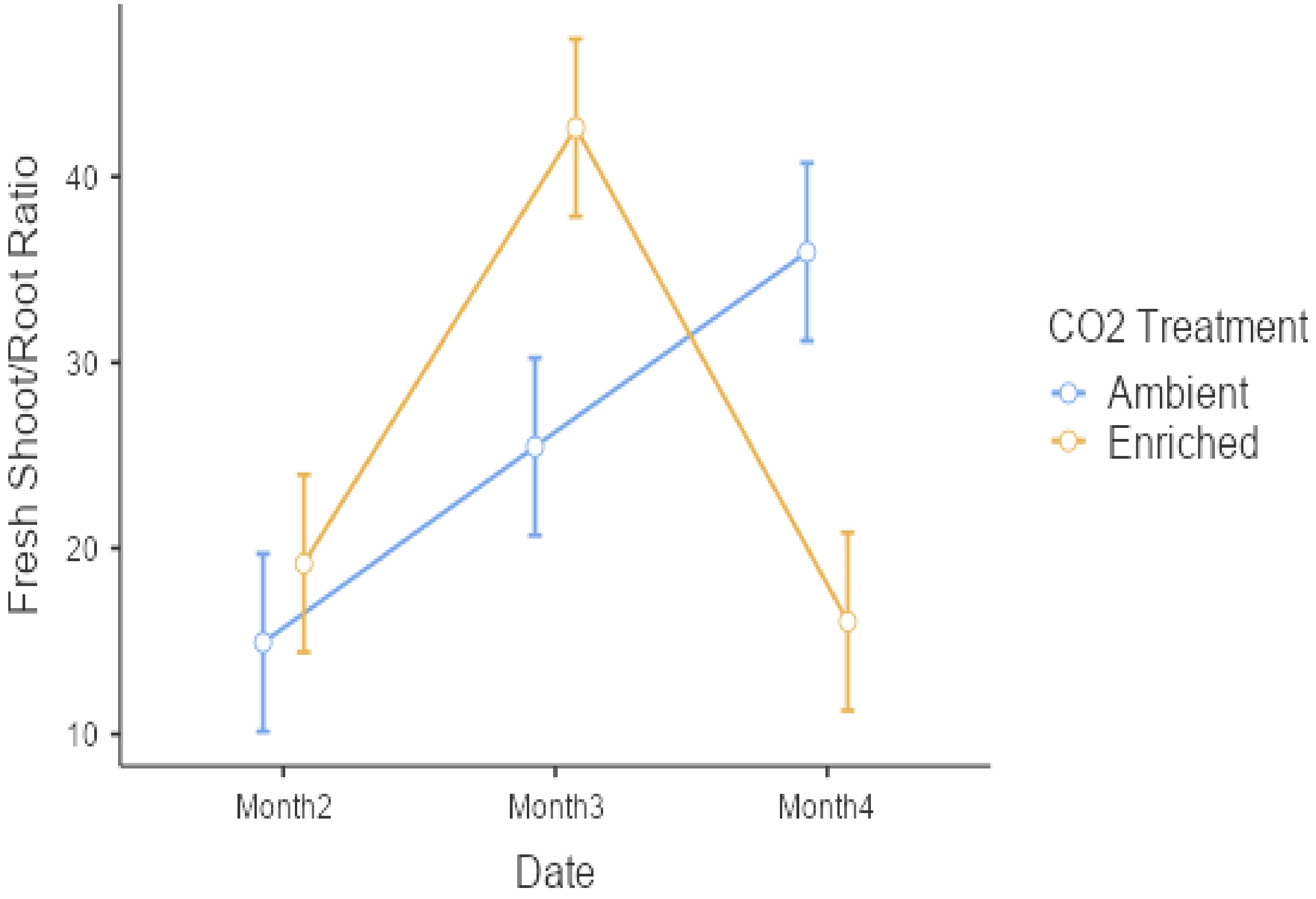


Figure 10: Comparison of fresh Shoot/Root ratio of Rhodes grass grown under ambient and enriched CO_2

3.1.6.2 Dry Shoot/Root ratio

Dry Shoot/Root ratio was measured from month 2 to month 4. There was a significant effect of Date ($p< 0.05$) on dry Shoot/Root ratio as result shown in (Table 7). Month 4 with a mean of 17.26 was significantly higher than month 3 with a mean of 12.97. Overall, the Rhodes grass grown under ambient CO_2 conditions had a higher mean of Dry Shoot/Root ratio compared to the ones grown under enriched CO_2 conditions (Figure 11). In both conditions, the mean dry Shoot/Root ratio increased gradually until reached 19 in the ambient condition and 15 in the enriched condition considered the highest.

Table 7: ANOVA results for dry Shoot/Root ratio of Rhodes grass grown under ambient and enriched CO_2

ANOVA - Dry Shoot/Root Ratio

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Treatment	24.20	1	24.20	1.140	0.307
Date	264.95	2	132.47	6.239	0.014
Treatment ✻ Date	5.00	2	2.50	0.118	0.890

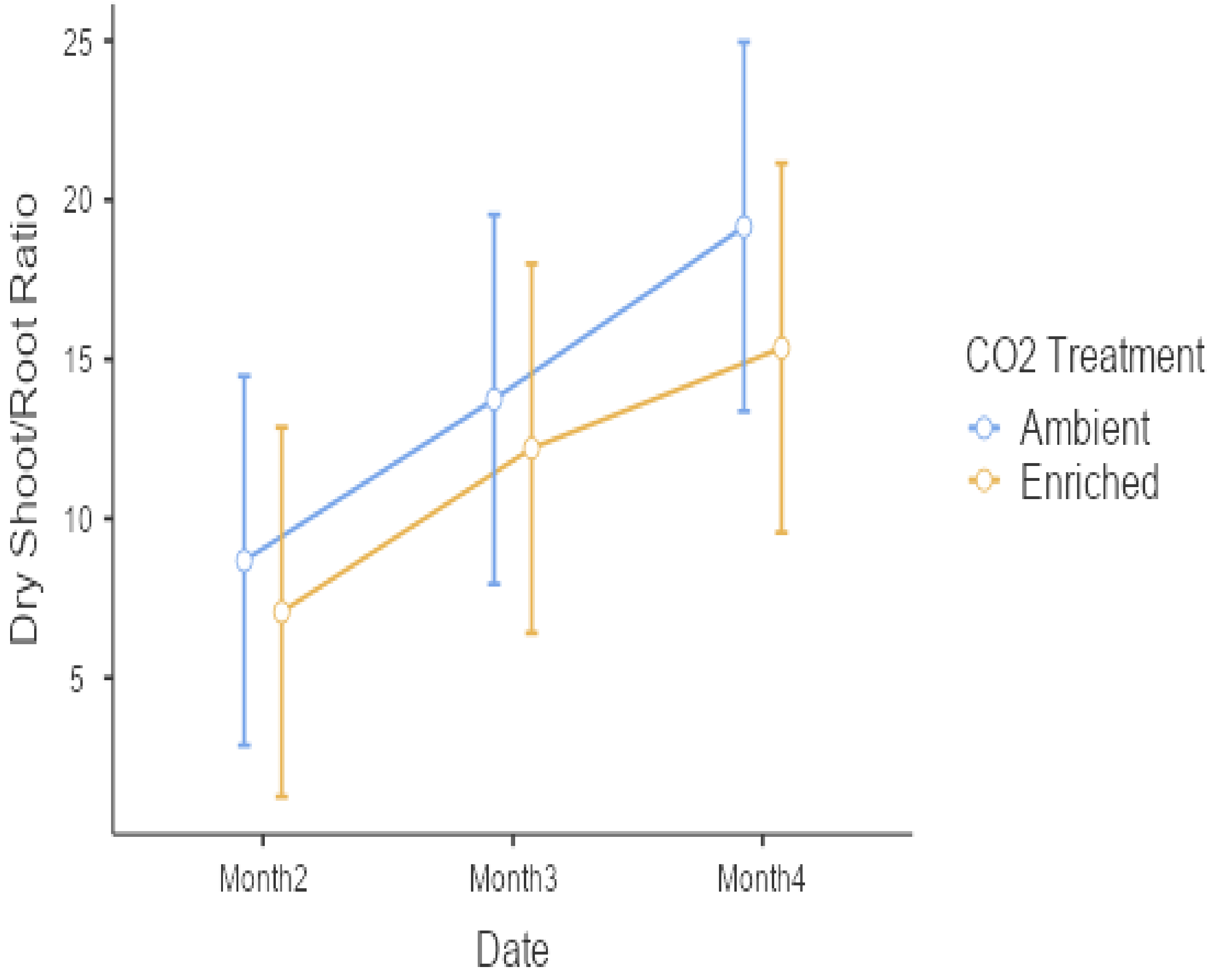


Figure 11: Comparison of dry Shoot/Root ratio of Rhodes grass grown under ambient and enriched CO_2

3.2 Growth pattern assessment – Generation 2 plants

3.2.1 Plants height

The plant height was measured in cm from week 1 to 13. There was a significant difference ($p< 0.05$) showed in the effect of Date and Treatment*Parent (TxP) among plant groups. For TxP, it has been found that (AE) group with a mean of 65.1 cm was significantly lower than (EA) group with a mean of 82.2 cm. In addition, the mean of (EA) group was 82.2 cm and it was significantly lower than (EE) group, which was 82.6 cm. There was an increase in plant height in all experimental groups during all weeks (Figure 12). The highest mean of plant height was 116 cm in week 13 for (EE) group and the least mean of plant height was 20 cm in week 1 for (AE) group.

Table 8: ANOVA results for plants height of Rhodes grass for four plant groups grown under ambient and enriched CO_2

ANOVA – Plants Height

	Sum of Squares	df	Mean Square	F	p
TxP	19163	3	6388	9.349	< .001
Date	353849	12	29487	43.158	< .001
TxP ✻ Date	7073	36	196	0.288	1.000
Residuals	304043	445	683		

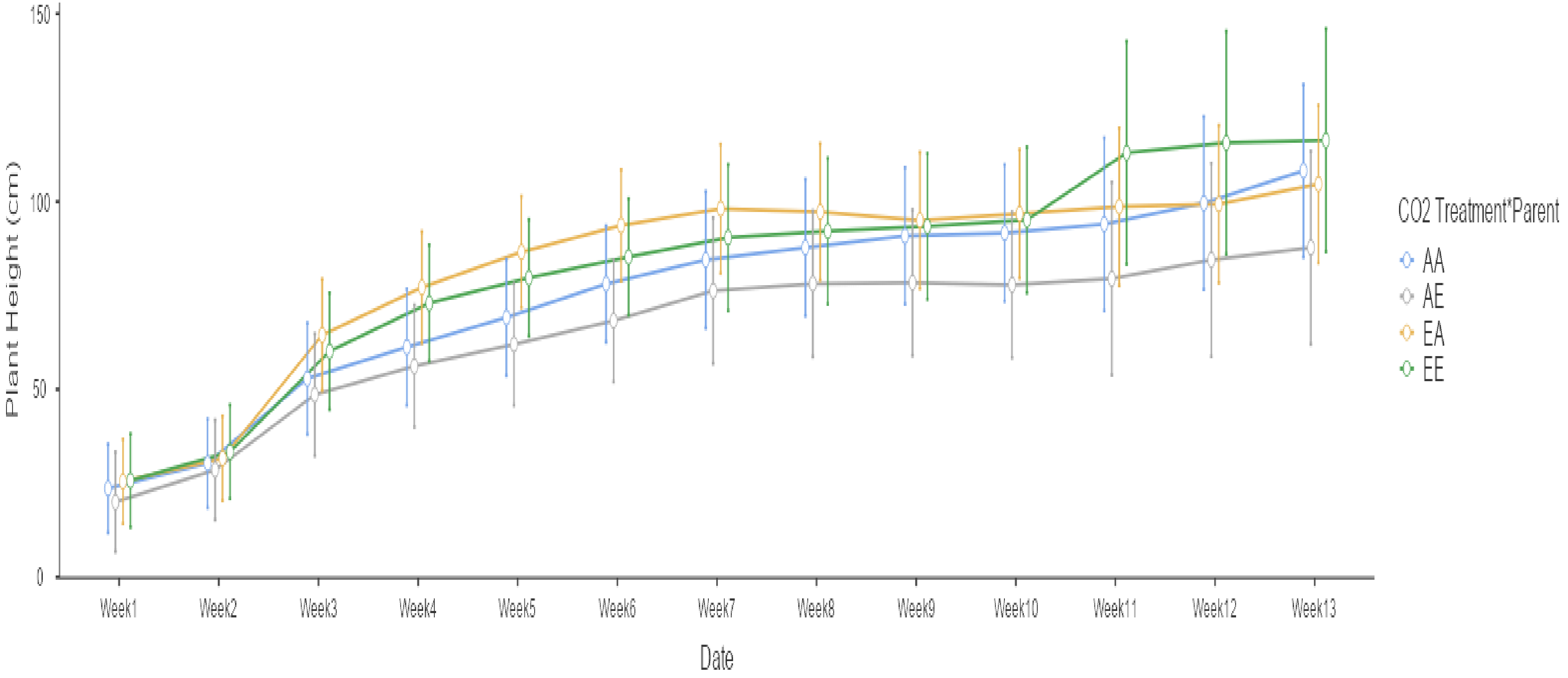


Figure 12: Comparison of plant heights in four experimental groups

3.2.2 Number of leaves

The number of leaves was counted from week 1 to 13. After analyzing the data, a significant effect of Date ($p< 0.05$) was noticed (Table 4). Week 1 and 2 with a mean of 5.89 and 6.76 leaves were significantly lower than week 7, 8, 9, 10, 11, 12 and 13 with a mean of 25.89, 27.88, 30.01, 31.83, 38.60, 40.76 and 47.63 leaves respectively. Moreover, week 4 and 5 with a mean of 16.24 and 18.25 leaves were significantly lower than week 11,12, and 13 with a mean of 38.60, 40.76 and 47.63 leaves. The mean of week 6 was 20.11 and it was significantly lower than week 12 and 13 with a mean of 40.76 and 47.63 leaves. Week 7 with a mean of 25.89 leaves was significantly lower than week 13 with a mean of 47.63 leaves.

As shown in (Figure 13), the number of leaves increased generally over the experimental period and reach the maximum in week 13. The highest leaf count with a mean of 56 leaves was in (EE) group in week 13. The lowest leaf count with a mean of 5 leaves was in (EA) group in week 1.

Table 9: ANOVA results for number of leaves of Rhodes grass for four plant groups grown under ambient and enriched CO_2

ANOVA – Number of leaves

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
TxP	386	3	128.6	0.318	0.812
Date	65130	12	5427.5	13.444	< .001
TxP ✻ Date	1618	36	44.9	0.111	1.000
Residuals	179649	445	403.7		

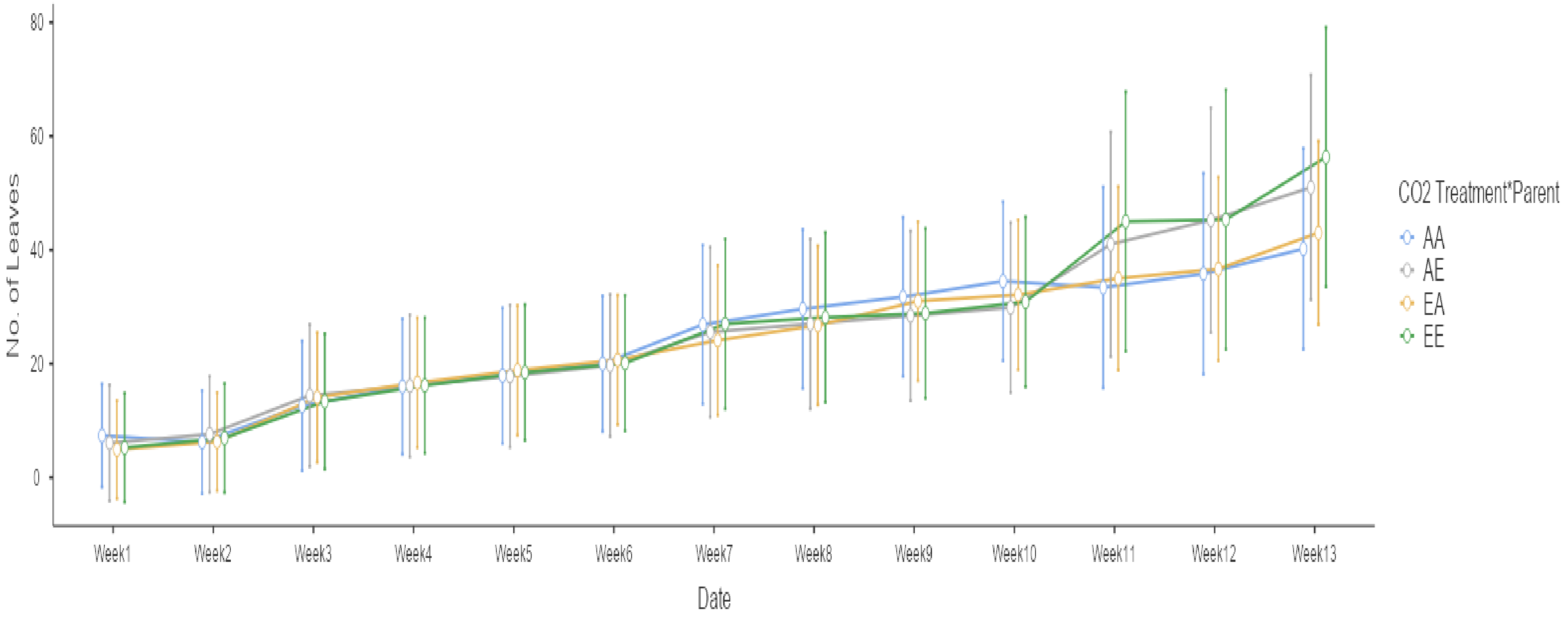


Figure 13: Comparison of number of leaves in four experimental groups

3.2.3 Chlorophyll content

The chlorophyll content was measured in all the plant groups from week 5 to week 13. No significant difference ($p< 0.05$) was showed among all plant groups (Table 10). There was a fluctuating trend in all experimental groups as shown in (Figure 14). The highest mean of chlorophyll content recorded was 22 units in (EE) group in week 9. Contrarily, the least mean of chlorophyll content was 8 units in (EA) group in week 13.

Table 10: ANOVA results for chlorophyll content of Rhodes grass for four plant groups grown under ambient and enriched CO_2

ANOVA - Chlorophyll content

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
TxP	330	3	110.1	2.50	0.060
Date	405	8	50.6	1.15	0.331
TxP ✻ Date	1582	24	65.9	1.50	0.067
Residuals	12695	288	44.1		

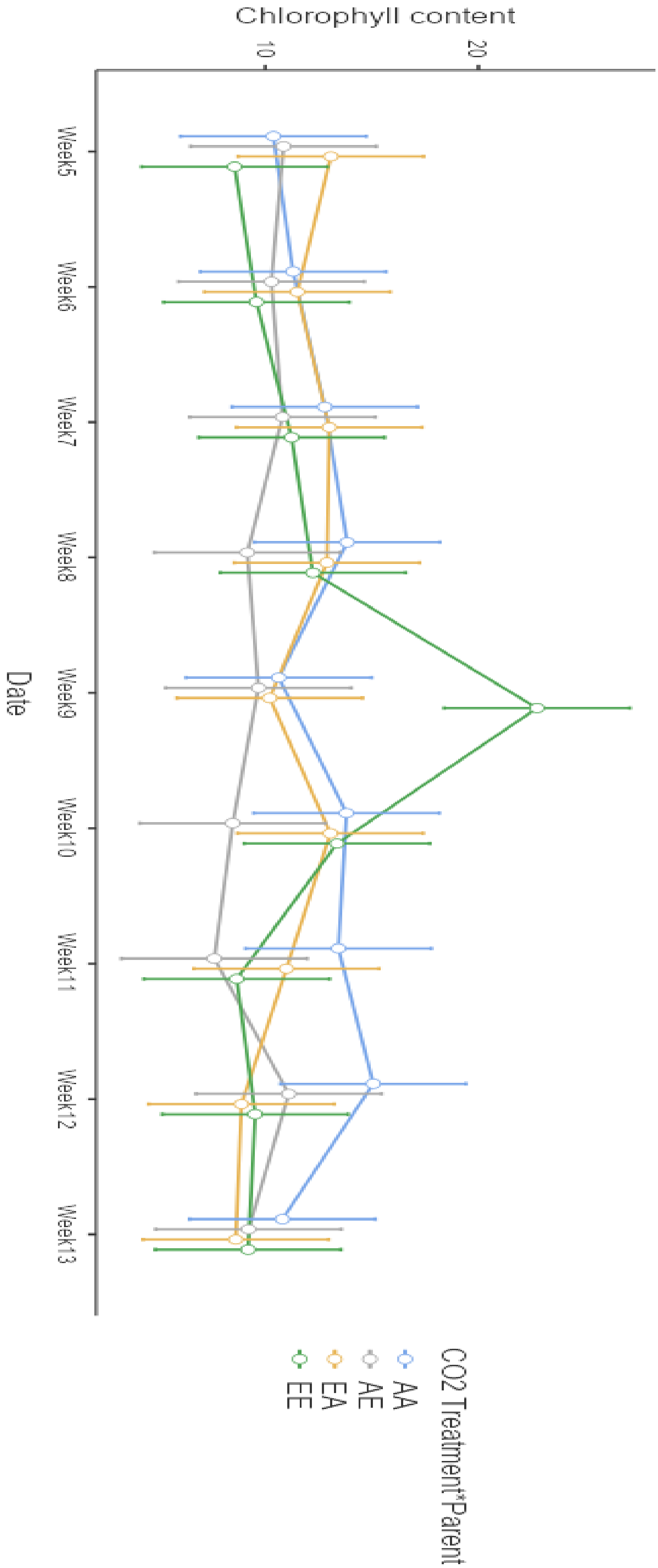


Figure 14: Comparison of chlorophyll content in four experimental groups

3.2.4 Stomatal conductance

The stomatal conductance of plants was measured from week 5 to week 13. A significant difference ($p < 0.05$) was observed in effect of Date (Table 11). Week 5, 6, and 7 with a mean of 141.1, 128 and 128.4 mmol/m^{2}s were significantly higher than week 9, 11, and 12 with a mean of 80.7, 80.7 and 71.9 mmol/m^{2}s respectively. A sharp dropped was observed in week 6 on the stomatal conductance of (AA) group which had a value of 227 mmol/m^{2}s in week 5 (considered the maximum value) and became 111 mmol/m^{2}s in week 6 (Figure 15). However, the least stomatal conductance of 65 mmol/m^{2}s was found in (AA) and (AE) groups during week 11and 13 respectively.

Table 11: ANOVA results for stomatal conductance of Rhodes grass for four plant groups grown under ambient and enriched CO_2

ANOVA - Stomatal Conductance (mmol/m^{2}s)

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
TxP	3220	3	1073	0.262	0.853
Date	186045	8	23256	5.679	< .001
TxP ✻ Date	131763	24	5490	1.341	0.136
Residuals	1.18e+6	288	4095		

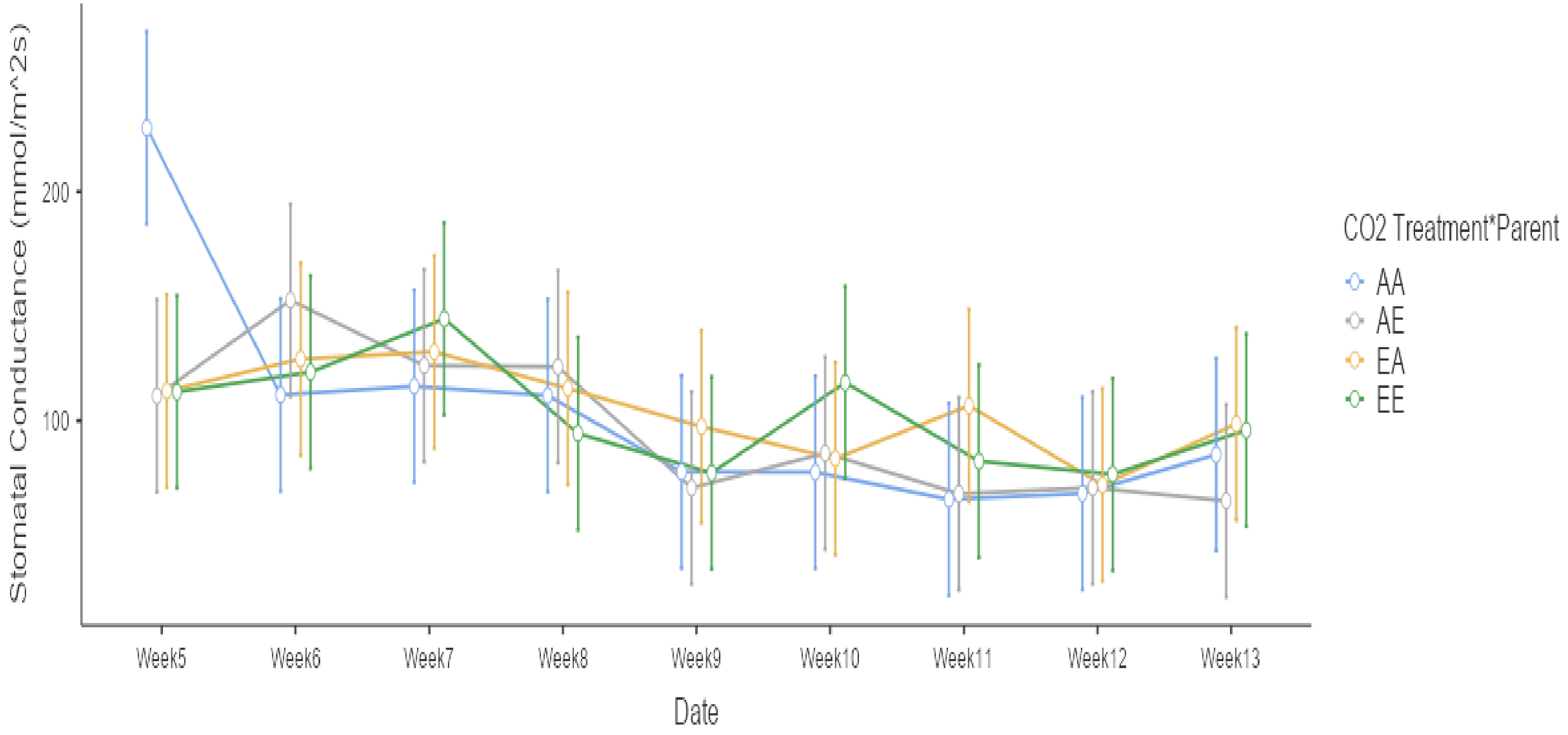


Figure 15: Comparison of stomatal conductance in four experimental groups

3.2.5 Photosynthetic rate

The measurement of the photosynthetic rate was conducted from week 5 to week 13. After analyzing the data, a significant difference ($p < 0.05$) was observed among all plant experimental groups (Table 12). For TxP, it has been found that (AE) group with a mean of 73.5% was significantly higher than (EA) group with a mean of 72.3%. However, (EA) group with a mean of 72.3% was significantly lower than (EE) group with a mean of 73.4%. As shown in (Figure16), plants whose parent comes from ambient CO_2 were lower in photosynthetic rate than the plants whose parent originally comes from enriched CO_2. However, in certain weeks, plants, which their parent comes from ambient CO2, became the highest, such as in week 11 for (EA) group and week 13 for (AA) group.

Table 12: ANOVA results for photosynthetic rate of Rhodes grass for four plant groups grown under ambient and enriched CO_2

ANOVA - Photosynthetic rate [%]

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
TxP	78.0	3	26.01	7.32	< .001
Date	117.0	8	14.62	4.11	< .001
TxP ✻ Date	170.7	24	7.11	2.00	0.004
Residuals	1023.8	288	3.55		

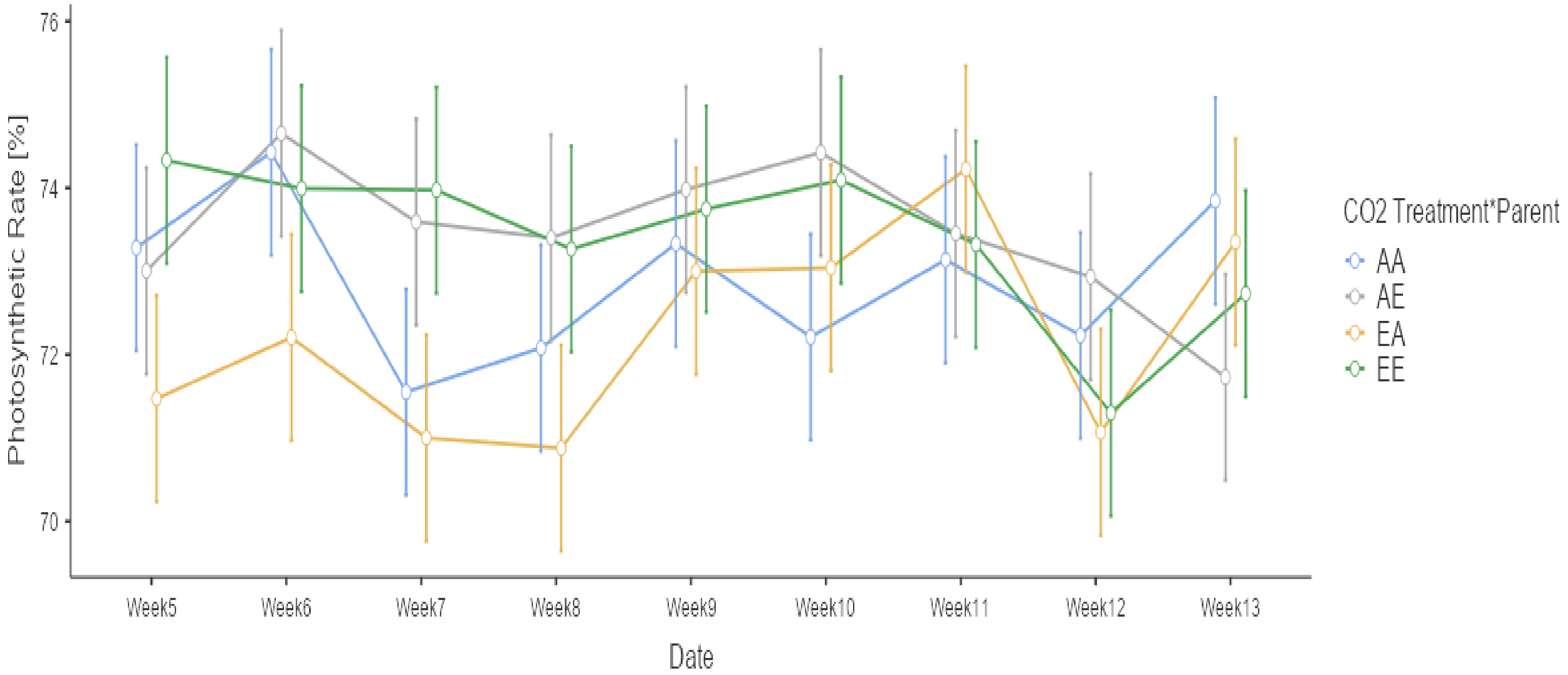


Figure 16: Comparison of photosynthetic rate in four experimental groups

3.2.6 Shoot/Root ratio

3.2.6.1 Fresh Shoot/Root ratio

Fresh Shoot/Root ratio was measured from month 2 to 4. A significant difference ($p< 0.05$) was observed in effect of Date (Table 13). The mean of month 2 was 6.99 and it was significantly lower than month 3 and 4 with a mean of 15.81 and 15.66. According to (Figure 17), all experimental groups increased in fresh Shoot/Root ratio in month 3 and 4 except group (AA) and (EA) were decreased in month 4. In month 4, the highest fresh Shoot/Root ratio with a mean of 18.5 was noticed in (EE) group. While in month 2 the lowest Fresh Shoot/Root ratio with a mean of 5.50 was for (AE) group.

Table 13: ANOVA results for fresh Shoot/Root ratio of Rhodes grass for four plant groups grown under ambient and enriched CO_2

ANOVA - Fresh Shoot/Root ratio

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
TxP	114.8	3	38.25	0.676	0.575
Date	612.5	2	306.25	5.415	0.011
TxP ✻ Date	36.1	6	6.02	0.107	0.995
Residuals	1357.3	24	56.55		

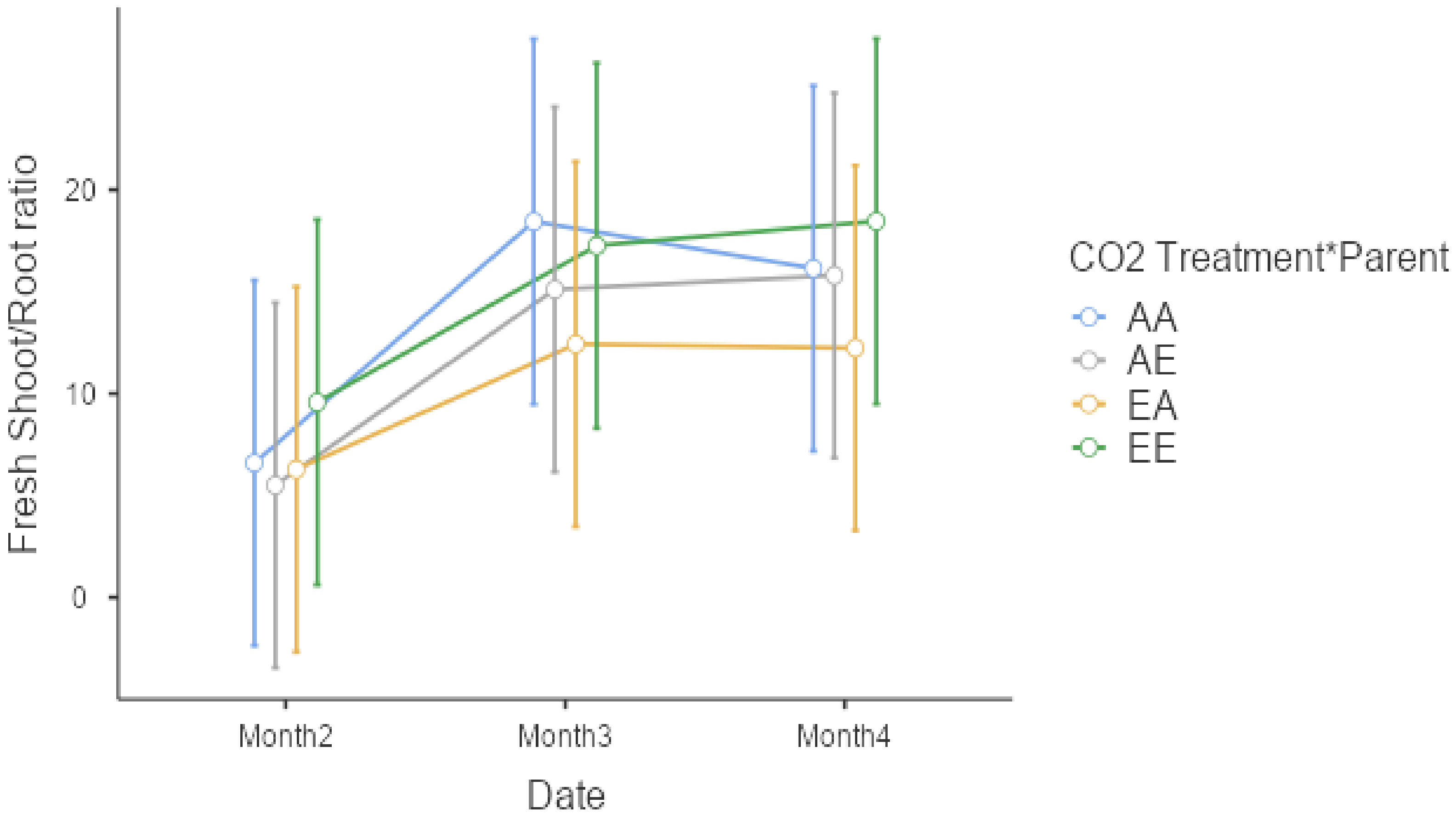


Figure 17: Comparison of fresh Shoot/Root ratio in four experimental groups

3.2.6.2 Dry Shoot/Root ratio

Dry Shoot/Root ratio was measured from month 2 to 4. There was a significant effect of Date ($p< 0.05$) detected on dry Shoot/Root ratio (Table 14). Month 2 with a mean of 4.89 was significantly lower than month 3 and 4 with a mean of 7.83 and 10.03. The plants grown under ambient CO_2 were constant increasing. However, plants grown under enriched CO_2 were in frequent changes (Figure 18). The highest dry Shoot/Root ratio with a mean of 11 was observed in group (EE) in month 4. While the lowest dry Shoot/Root ratio was in month 2 with a mean of 3.13 in group (EA).

Table 14: ANOVA results for dry Shoot/Root ratio of Rhodes grass for four plant groups grown under ambient and enriched CO_2

ANOVA - Dry Shoot/Root ratio

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
TxP	46.3	3	15.42	1.90	0.157
Date	159.4	2	79.72	9.81	< .001
TxP ✻ Date	77.6	6	12.94	1.59	0.193
Residuals	195.0	24	8.12		

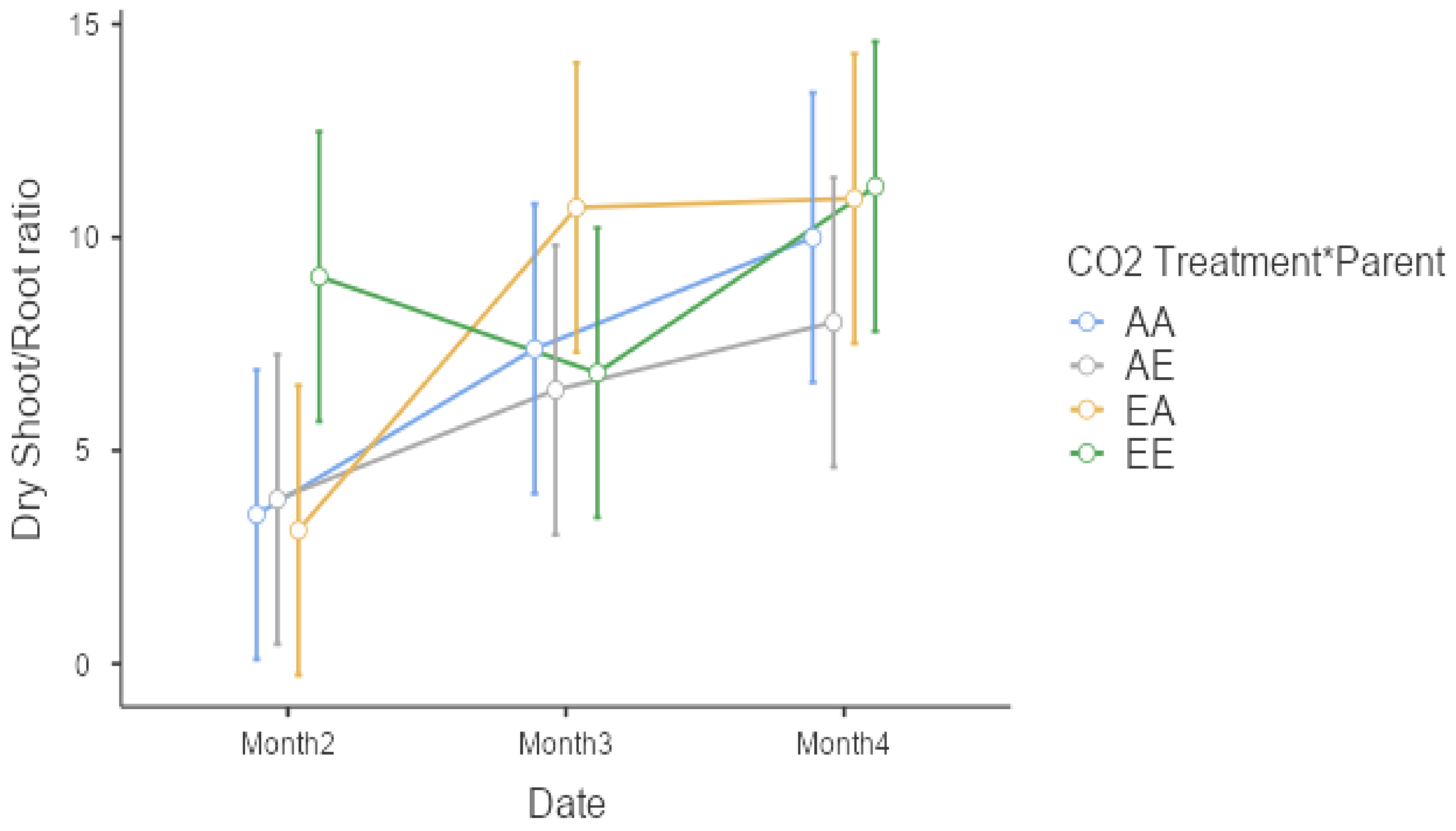


Figure 18: Comparison of dry Shoot/Root Ratio in four experimental groups

Chapter 4: Discussions

Rhodes grass, C. *gayana*, is a grass species native to Africa but also found in tropical and subtropical areas. In the context of sustainable agriculture, C. *gayana* can be efficiently beneficial to the farmers with the least resources, because they intercorporate the C. *gayana* with food crops. For instance, C. *gayana* can be sown under to maize crop without reducing maize grain output. Moreover, another practical implication of this grass is that it helps in weed control by out-competing the wild weeds. Furthermore, C. *gayana* is worth mentioning for its potential for managing soil erosion, especially on sloped fields as it gets easily mixed with legumes and alfalfa, and facilitates the improvement in soil nutrient levels. Hence, when it comes to resolving problems like weed growth, soil erosion, and soil quality, the C. *gayana* is considered one suitable option for farmers because it can resist extreme weather conditions for more than six months due to its deep penetrating roots.

Rhodes grass possesses the potential to withstand waterlogging, the hottest weather (50°C), wind, pH, aridity, poor soil, and salinity, etc. (Allah & Bello, 2019; Faji et al., 2021). Soil salinity is a growing problem in all agricultural countries, especially, in Asia and Africa (Qin et al., 2012). However, the Rhodes grass is not resistant to acidic or infertile soil, although it can survive extreme weather conditions. However, when water is available, it tends to consume significantly high amounts of water to grow. This eventually could lead to water scarcity (De Luca et al., 2001; Granzin & Dryden, 2003; Lukashe et al., 2020; Milford & Minson, 1968). Since 2011, the UAE banned Rhodes grass cultivation as the cover crop because; *C. gayana* was declared as a culprit for consuming significantly high amounts of water. Around 1.25 million tons of hay and pasture were imported from Australia alternatively, to cope up the loss in the local crop system (Belgacem, 2016; "End to Subsidy for Farmers' Rhodes Grass," 2010).

Keeping into consideration the significance of *C. gayana*, the present study was designed to investigate the effects of greenhouse gas CO_2 on maternal effects and growth parameters. The study is in accordance with the previously done research work by Ahmadani (2014) who checked out the effects of CO_2 enrichment on the growth parameters and water conservation capacity of *C. gayana*. Certainly, as the CO_2 ratio increases, photosynthesis also increases, but at the same time, the stomatal conductance also reduces and leads to a lower transpiration rate. Since CO_2 is a limiting factor for sustainable plant physiology, an increase in its availability is correlated with an increased plant metabolism especially, stomatal conductance, leaf surface area, photosynthetic rate, and plant growth. The studies revealed that C3 plants under enriched CO_2 showed more physiological development than those grown under the ambient CO_2 level. While, the C4 plants were not found to be significantly affected by the CO_2 enrichment (Navie et al., 2005; Reich et al., 2018; Willey, 2018).

To get a bright representation of CO_2 enrichment and its effects on the Rhodes grass. The experimental plan was set up in such a way that plants of *C. gayana* were allowed to grow as Generation 1 in two different growth chambers, i.e., CO_2 ambient chamber supplied with 500ppm CO_2 and CO_2 enriched chamber supplemented with 1000ppm CO_2 (as shown in Figure 4, chapter 2). The branches with their roots of Generation 1 plants were cut and germinated to develop the Generation 2 plants, which were also kept in the above-mentioned chambers of CO_2 supply. The data was collected at regular time intervals and organized in excel sheets, which were then curated statistically to plot the graphs. All the growth parameters were measured among plants grown in different CO_2 supplies.

In generation 1 plants, the growth parameters include plant height, number of leaves, shoot/root ratio, chlorophyll content, stomatal conductance and photosynthetic rate were measured at regular intervals. The plant height and number of leaves were higher in enriched CO_2 than in ambient CO_2 as shown in the results. These findings are similar to what was reported by Wand et al. (1999) which found that C4 species grown under enriched CO_2 conditions increased total biomass by 33%.

The chlorophyll content is the key factor of a plant's physiological processes. The chlorophyll was assessed in both plant groups from week 8 to week 17. In the experiment, chlorophyll content in plants grown under enriched CO_2 conditions was higher than in plants grown under ambient CO_2 in most weeks. The results are in accordance with a previously done study by Miri et al. (2012) which found that in increasing of CO_2 concentration, the rate of chlorophyll raised in millet, soybean, pigweed, and lambs quarter.

The dry shoot/root ratio of the plants under ambient conditions was higher than those under enriched conditions. The results are in accordance with the study done by Reich et al. (2018) who conducted a long-term 28-year CO_2 enrichment study on C3 and C4 plants. He found out that the Biomass started to increase in C_4 plants but not in C_3 plants under ambient CO_2 conditions. Further studies also confirmed these findings (Habermann et al., 2019; Li et al., 2019; Fay et al., 2021).

The growth parameters in generation 2 plants were evaluated in four groups. There was a significant difference in ($p< 0.05$) observed on plant height in effect of (TxP) among plant groups. Initially, the plant height of (AE) group plants was significantly lower than those in (EA) group. In addition, (EA) group was significantly lower than (EE) group. The results are quite relevant to a previous study where an increased level of CO_2 promoted the plant height as compared to those grown under ambient levels of CO_2 (Ksiksi and Youssef, 2010). Contrarily, few studies reported the reverse of these results and non-significant differences in plant heights were reported in different groups under CO_2 enrichment (Voelker et al., 2016; Klein et al., 2016).

A fluctuating trend was observed in all experimental groups for the chlorophyll measurement, but no significant difference was observed statistically. However, significant differences were calculated for the stomatal conductance and the photosynthetic rates in all four experimental groups. The results can be justified by the fact that chlorophyll is considered the basic machinery needed to carry out photosynthesis. Hence, its quantity might or might not be influenced by the maternal effects. However, photosynthetic rates and stomatal conductance are the physiological processes, which involve gene expression and several enzyme participations. Therefore, the maternal effects could impart effects on the metabolism or photosynthetic rates of a plant (Tanaka et al., 2006). The results of the current study are in accordance with the research study done by Ksiksi et al. (2018) which also reported elevated stomatal conductance under CO_2 enriched condition. Moreover, a research team in China also reported that CO_2 enrichment in plants is directly proportional to improved photosynthetic rates (Pan et al., 2019).

It is evident from the data that the plants from parents grown under ambient conditions showed a consistent growth pattern as compared to the plants from an enriched environment. The varying pattern of phenotypes in the generation 2 plants can be justified based on the bet-hedging maternal effects. We hypothesize that the Rhodes grass parent plants when allowed to grow under ambient and enriched CO_2 conditions influenced the offspring plants by producing a variety of phenotypes to sustain maternal fitness. When a mother gives rise to offspring with a variety of phenotypic traits, some of those offspring are potentially misfits or less suitable than others (Kudo, 2006).

The maternal environment has a prominent effect on the offspring's phenotype. The maternal effects represent a vertical transfer or transgenerational form of phenotypic traits transmitted without modifications in the DNA sequence to the offspring. The maternal effects influence plant development, disease resistance, and overall physiology throughout the subsequent development of the mature plant. The maternal environment influences trans-generationally passing from one generation to the other (Mousseau & Fox, 1998). However, the possible reason for non-significant differences in some of the parameters in the Generation 2 plants can be justified based on the plant's behavior of withstanding the abiotic stress.

Conclusively, this study leads to further investigation of maternal environment influences on *C. gayana* and other species by subjecting them to a long-term experimental study of 10-20 years, so that successive generations of plants can be compared and maternal effects patterns can be illustrated more clearly. Furthermore, the present study can be helpful in the successful deployment of *C. gayana* cultivation under varying environmental conditions of abiotic stress.

Chapter 5: Conclusion

The present study was carried out to evaluate the effects of the maternal influence of the Rhodes grass grown under two different environments; CO_2 enriched and ambient. The two successive generations of the Rhodes plants were studied in this regard and we hypothesize that the bet-hedging maternal approach was used by the parent plant to promote the offspring fitness under enriched and ambient CO_2 supply. The varying pattern of phenotypes in the generation 2 plants is the effect imparted by the generation 1 plant to sustain both the maternal and offspring fitness. The present study can be helpful in understanding the *C. gayana* growth under enriched and ambient CO_2 conditions and the vertical transfer of maternal effects from one generation to the other.

References

Abu-Alrub, I., Marcum, K. B., Kabir, N., Ahmed, A., & Al Hammadi, M. (2018). Productivity and nutritional value of four forage grass cultivars compared to Rhodes grass irrigated with saline water. *Australian Journal of Crop Science*, *12*(2), 203-209.

Abebe, Y., Tafere, M., Dagnew, S., Tolla, M., Gebre-Selassie, Y., Amane, A., & Molla, D. (2015). Best fit practice manual for Rhodes grass (Chloris gayana) production. *Capacity building for scaling up of evidence-based practices in agricultural production in Ethiopia. BDU-CASCAPE Working. Paper*, *10*.

Ahmed, M. (2020). Introduction to Modern Climate Change. Andrew E. Dessler: Cambridge University Press, 2011, 252 pp, ISBN-10: 0521173159. *The Science of the Total Environment*, *734*, 139397. https://doi.org/10.1016/j.scitotenv.2020.139397

Ahmadani, A. K. (2014). The Effects of Elevated CO_2 on the Growth and Water Conservation Potentials of Medicago Sativa L. And Chloris Gayana K.

Arshad, I., Ali, W., Khan, Z. A., & Bhayo, W. A. (2016). Effect of water stress on the growth and yield of Rhodes grass (Chloris gayana. L. Kunth.). *PSM Biol. Res*, *1*(2), 58-61.

Agarwala, V., Flannick, J., Sunyaev, S., Vinetta, A., & Altshuler, D. (2013). Abu-Alrub, I., Marcum, K. B., Kabir, N., Ahmed, A., & Al Hammadi, M. (2018). Productivity and nutritional value of four forage grass cultivars compared to Rhodes grass irrigated with saline water. *Australian Journal of Crop Science*, *12*(2), 203-209.

Ainsworth, E. A., & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, cell & environment*, *30*(3), 258-270.

Allah, Y. N., & Bello, A. (2019). The Potentials of Rhodes Grass (ChlorisGayanaKunth) as Drought Resistant Perennial Forage Grass in Nigeria. *Am J Biomed Sci & Res*, *6*(3).

Allen, L., Kimball, B., Bunce, J., Yoshimoto, M., Harazono, Y., Baker, J., Boote, K., & White, J. (2020). Fluctuations of CO_2 in Free-Air CO_2 Enrichment (FACE) depress plant photosynthesis, growth, and yield. *Agricultural and forest meteorology*, *284*, 107899.

Andersson, I., & Backlund, A. (2008). Structure and function of Rubisco. *Plant Physiology and Biochemistry*, *46*(3), 275-291.

Arnold, F. H. (2019). Innovation by evolution: bringing new chemistry to life (Nobel Lecture). *Angewandte Chemie International Edition*, *58*(41), 14420-14426.

Azad, M., & Matin, M. (2015). Effect of indole-3-butyric acid on clonal propagation of Swietenia macrophylla through branch cutting. *Journal of Botany*, *2015*.

Azoulay-Shemer, T., Palomares, A., Bagheri, A., Israelsson-Nordstrom, M., Engineer, C. B., Bargmann, B. O., Stephan, A. B., & Schroeder, J. I. (2015). Guard cell photosynthesis is critical for stomatal turgor production, yet does not directly mediate CO 2-and ABA-induced stomatal closing. *The Plant Journal*, *83*(4), 567-581.

Banik, R. L. (1984). Macropropagation of bamboos by pre-rooted and pre-rhizomed branch cutting. *Bano Biggyan Patrika*, *13*(1/2), 67-73.

Batke, S. P., Yiotis, C., Elliott-Kingston, C., Holohan, A., & McElwain, J. (2020). Plant responses to decadal scale increments in atmospheric CO_2 concentration: comparing two stomatal conductance sampling methods. *Planta*, *251*(2), 1-12.

Bein, T., Karagiannidis, C., & Quintel, M. (2020). Climate change, global warming, and intensive care. *Intensive care medicine*, *46*(3), 485-487.

Benjamin, D., Por, H.-H., & Budescu, D. (2017). Climate change versus global warming: Who is susceptible to the framing of climate change? *Environment and Behavior*, *49*(7), 745-770.

Belgacem, A. (2016). *United Arab Emirates and ICARDA Highlight of 20 years of Collaboration and Achievements*. International Center for Agricultural Research in the Dry Areas (ICARDA). https://repo.mel.cgiar.org/handle/20.500.11766/6064

Berhe, D., & Negash, L. (1998). Asexual propagation of Juniperus procera from Ethiopia: a contribution to the conservation of African pencil cedar. *Forest Ecology and Management*, *112*(1-2), 179-190.

Bernardo, J. (1996). Maternal effects in animal ecology. *American Zoologist*, *36*(2), 83-105.

Berrang-Ford, L., Ford, J. D., & Paterson, J. (2011). Are we adapting to climate change? *Global environmental change*, *21*(1), 25-33.

Bonga, J. (2016). Conifer clonal propagation in tree improvement programs. *Vegetative Propagation of Forest Trees; Park, Y.-S., Bonga, JM, Moon, H.-K., Eds*, 3-31.

Bourgault, M., Brand, J., Tausz, M., & Fitzgerald, G. J. (2016). Yield, growth and grain nitrogen response to elevated CO2 of five field pea (Pisum sativum L.) cultivars in a low rainfall environment. *Field Crops Research*, *196*, 1-9.

Brima, F. I. A. (2007). *Effect of Seed Rate and NPK Fertilization on Growth, Yield and Forage Quality of Rhodes Grass (Chloris gayana L. kunth.)* UOFK].

Chen, D. X., Hunt, H. W., & Morgan, J. A. (1996). Responses of a C3 and C4 perennial grass to CO2 enrichment and climate change: Comparison between model predictions and experimental data. *Ecological Modelling*, *87*(1-3), 11-27.

Chen, C. P., Sakai, H., Tokida, T., Usui, Y., Nakamura, H., & Hasegawa, T. (2014). Do the rich always become richer? Characterizing the leaf physiological response of the high-yielding rice cultivar Takanari to free-air CO_2 enrichment. *Plant and Cell Physiology*, *55*(2), 381-391.

Cici, S. (2017). Maternal environment modulates dormancy and germination in Vaccaria hispanica. *Agrotechnology*, *6*(154), 2.

Céccoli, G., Ramos, J. C., Ortega, L. I., Acosta, J. M., & Perreta, M. G. (2011). *Salinity induced anatomical and morphological changes in Chloris gayana Kunth roots*. Universidad Nacional de Cuyo.

De Luca, M., Seffino, L. G., Grunberg, K., Salgado, M., Corrdoba, A., Luna, C., Ortega, L., Rodríguez, A., Castagnaro, A., & Taleisnik, E. (2001). Physiological causes for decreased productivity under high salinity in Boma, a tetraploid Chloris gayana cultivar. *Australian Journal of Agricultural Research*, *52*(9), 903-910.

Dhillon, R., Hooda, M., Pundeer, J., Ahlawat, K., & Chopra, I. (2011). Effects of auxins and thiamine on the efficacy of techniques of clonal propagation in Jatropha curcas L. *Biomass and bioenergy*, *35*(4), 1502-1510.

DOMEC, J. C., Palmroth, S., Ward, E., Maier, C. A., Thérézien, M., & Oren, R. (2009). Acclimation of leaf hydraulic conductance and stomatal conductance of Pinus taeda (loblolly pine) to long-term growth in elevated CO_2 (free-air CO_2 enrichment) and N-fertilization. *Plant, cell & environment*, *32*(11), 1500-1512.

Döndü, P. S. F., & TANSI, V. (2020). PERENNIAL WARM SEASON GRASSES; CULTIVATION OF. *INNOVATIVE APPROACHES IN MEADOW-RANGELAND AND FORAGE CROPS*, 177.

Donohue, K. (1999). Seed dispersal as a maternally influenced character: mechanistic basis of maternal effects and selection on maternal characters in an annual plant. *The American Naturalist*, *154*(6), 674-689.

Dow, K., & Downing, T. E. (2016). *The atlas of climate change*. University of California Press.

Einum, S., & Fleming, I. A. (2004). Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evolutionary Ecology Research*, *6*(3), 443-455.

End to subsidy for farmers' Rhodes grass. (2010, September 28). *The National*. https://www.thenationalnews.com/uae/environment/end-to-subsidy-for-farmers-rhodes-grass-1.567671

Evans, J. R., Vogelmann, T. C., Williams, W. E., & Gorton, H. L. (2004). Chloroplast to leaf. In *Photosynthetic adaptation* (pp. 15-41). Springer.

Faji, M., Abebe, A., & Ahmed, K. (2021). FORAGE BIOMASS YIELD PERFORMANCE OF RHODES GRASS (Chloris gayana) ACCESSIONS IN BENISHNGUL-GUMUZ REGION OF WESTERN ETHIOPIA. *Journal of Global Ecology and Environment*, 35–41.

Fay, P. A., Hui, D., Jackson, R. B., Collins, H. P., Reichmann, L. G., Aspinwall, M. J., ... & Polley, H. W. (2021). Multiple constraints cause positive and negative feedbacks limiting grassland soil CO_2 efflux under CO_2 enrichment. *Proceedings of the National Academy of Sciences*, *118*(2), e2008284117.

Fox, C. W., Thakar, M. S., & Mousseau, T. A. (1997). Egg size plasticity in a seed beetle: an adaptive maternal effect. *The American Naturalist*, *149*(1), 149-163.

Fox, D., Bryan, R., & Price, A. (2004). The role of soil surface crusting in desertification and strategies to reduce crusting. *Environmental monitoring and assessment*, *99*(1), 149-159.

Foyer, C., Furbank, R., Harbinson, J., & Horton, P. (1990). The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. *Photosynthesis Research*, *25*(2), 83-100.

Fünfgeld, M. M., Wang, W., Ishihara, H., Arrivault, S., Feil, R., Smith, A. M., Stitt, M., Lunn, J., & Niittylä, T. (2021). The pathway of starch synthesis in Arabidopsis thaliana leaves. *bioRxiv*, https://doi.org/10.1101/2021.01.11.426159

Gago, J., Daloso, D. d. M., Figueroa, C. M., Flexas, J., Fernie, A. R., & Nikoloski, Z. (2016). Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: a multispecies meta-analysis approach. *Plant Physiology*, *171*(1), 265-279.

Gelaye, K. K., Zehetner, F., Loiskandl, W., & Klik, A. (2019). Comparison of growth of annual crops used for salinity bioremediation in the semi-arid irrigation area. *Plant, Soil and Environment*, *65*(4), 165-171.

Giammarchi, F., Cherubini, P., Pretzsch, H., & Tonon, G. (2017). The increase of atmospheric CO_2 affects growth potential and intrinsic water-use efficiency of Norway spruce forests: insights from a multi-stable isotope analysis in tree rings of two Alpine chronosequences. *Trees*, *31*(2), 503-515.

Gimeno, T. E., Crous, K. Y., Cooke, J., O'Grady, A. P., Ósvaldsson, A., Medlyn, B. E., & Ellsworth, D. S. (2016). Conserved stomatal behaviour under elevated CO_2 and varying water availability in a mature woodland. *Functional Ecology*, *30*(5), 700-709.

Goldberg, M. H., van der Linden, S., Maibach, E., & Leiserowitz, A. (2019). Discussing global warming leads to greater acceptance of climate science. *Proceedings of the National Academy of Sciences*, *116*(30), 14804-14805.

Granzin, B., & Dryden, G. M. (2003). Effects of alkalis, oxidants and urea on the nutritive value of rhodes grass (Chloris gayana cv. Callide). *Animal feed science and technology*, *103*(1-4), 113-122.

Gupta, R., & Arora, S. (2015). Agroforestry as alternate landuse system for sustaining rural livelihoods in Himalayan ecosystem. *Adv. Soil Water Resour. Manag. Food Livelihood Secur. Chang. Clim.*

Hartmann, H. T., & Kester, D. E. (1963). Plant propagation: Principles and practice. *Soil Science*, *95*(1), 89.

Habermann, E., San Martin, J. A. B., Contin, D. R., Bossan, V. P., Barboza, A., Braga, M. R., ... & Martinez, C. A. (2019). Increasing atmospheric CO_2 and canopy temperature induces anatomical and physiological changes in leaves of the C4 forage species Panicum maximum. *PloS one*, *14*(2), e0212506.

Herrick, J. D., Maherali, H., & Thomas, R. B. (2004). Reduced stomatal conductance in sweetgum (Liquidambar styraciflua) sustained over long-term CO_2 enrichment. *New Phytologist*, *162*(2), 387-396.

Hoornweg, D., & Pope, K. (2020). Cities: growing threats, growing opportunities. In *Handbook of Megacities and Megacity-Regions*. Edward Elgar Publishing.

Husen, A. (2004). Clonal propagation of Dalbergia sissoo Roxb. by softwood nodal cuttings: effects of genotypes, application of IBA and position of cuttings on shoots. *Silvae Genetica*, *53*(2), 50-54.

Husen, A., & Pal, M. (2007). Effect of branch position and auxin treatment on clonal propagation of Tectona grandis Linn. f. *New Forests*, *34*(3), 223-233.

Hussain, M. I., Akhtar, N., Qureshi, A. S., & Gallacher, D. (2021). Perennial Forage Grass Production on the Marginal Arabian Peninsula Land. In *Sustainable Agriculture Reviews 52* (pp. 279-308). Springer.

Itonaga, K. (2011). *Maternal effects on offspring fitness in Pseudemoia entrecasteauxii: selective advantages and physiological mechanisms* (Doctoral dissertation, University of Tasmania).

Ikawa, H., Sakai, H., Chen, C. P., Soong, T. H., Yonemura, S., Taniguchi, Y., Yoshimoto, M., Tokida, T., Zhang, G., & Kuwagata, T. (2019). High mesophyll conductance in the high-yielding rice cultivar Takanari quantified with the combined gas exchange and chlorophyll fluorescence measurements under free-air CO_2 enrichment. *Plant Production Science*, *22*(3), 395-406.

Islam, M., Bhuiyan, M., Hossain, M., & Hossain, M. (2011). Clonal propagation of Bambusa vulgaris by leafy branch cuttings. *Journal of Forestry Research*, *22*(3), 387-392.

Jeffry, L., Ong, M. Y., Nomanbhay, S., Mofijur, M., Mubashir, M., & Show, P. L. (2021). Greenhouse gases utilization: A review. *Fuel*, *301*, 121017.

Jiang, Z. J., Huang, X. P., & Zhang, J. P. (2010). Effects of CO_2 enrichment on photosynthesis, growth, and biochemical composition of seagrass Thalassia hemprichii (Ehrenb.) Aschers. *Journal of Integrative Plant Biology*, *52*(10), 904-913.

Jin, Z., Ainsworth, E. A., Leakey, A. D., & Lobell, D. B. (2018). Increasing drought and diminishing benefits of elevated carbon dioxide for soybean yields across the US Midwest. *Global Change Biology*, *24*(2), e522-e533.

Johnsen, Ø., Dæhlen, O. G., Østreng, G., & Skrøppa, T. (2005). Daylength and temperature during seed production interactively affect adaptive performance of Picea abies progenies. *New Phytologist*, *168*(3), 589-596.

Kim, H., Lieffering, M., Miura, S., Kobayashi, K., & Okada, M. (2001). Growth and nitrogen uptake of CO_2-enriched rice under field conditions. *New Phytologist*, 223-229.

Kirschbaum, M. (2010). Does Enhanced Photosynthesis Enhance Growth? Lessons Learned from CO_2 Enrichment Studies. *Plant Physiology*, *155*, 117–124. https://doi.org/10.1104/pp.110.166819

Kleine, T., Nägele, T., Neuhaus, H. E., Schmitz-Linneweber, C., Fernie, A. R., Geigenberger, P., Grimm, B., Kaufmann, K., Klipp, E., & Meurer, J. (2021).

Acclimation in plants–the Green Hub consortium. *The Plant Journal*, *106*(1), 23-40.

Klein, T., Bader, M. K. F., Leuzinger, S., Mildner, M., Schleppi, P., Siegwolf, R. T., & Körner, C. (2016). Growth and carbon relations of mature Picea abies trees under 5 years of free-air CO_2 enrichment. *Journal of Ecology*, *104*(6), 1720-1733.

Knutti, R. (2019). Closing the knowledge-action gap in climate change. *One Earth*, *1*(1), 21-23.

Krapp, A., & Stitt, M. (1995). An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. *Planta*, *195*(3), 313-323.

Ksiksi, T., & Youssef, T. (2010). Effects of CO_2 enrichment on growth partitioning of Chloris gayana in the arid environment of the UAE. *Grassland science*, *56*(3), 183-187.

Ksiksi, T. S., Ppoyil, S. B. T., & Palakkott, A. R. (2018). CO_2 enrichment affects eco-physiological growth of maize and alfalfa under different water stress regimes in the UAE. *Physiology and Molecular Biology of Plants*, *24*(2), 251-259.

Kudo, S.-i. (2006). Within-clutch egg-size variation in a subsocial bug: the positional effect hypothesis. *Canadian journal of zoology*, *84*(11), 1540-1544.

Kweku, D. W., Bismark, O., Maxwell, A., Desmond, K. A., Danso, K. B., Oti-Mensah, E. A., Quachie, A. T., & Adormaa, B. B. (2017). Greenhouse effect: Greenhouse gases and their impact on global warming. *Journal of Scientific research and reports*, 1-9.

Li, P., Li, B., Seneweera, S., Zong, Y., Li, F. Y., Han, Y., & Hao, X. (2019). Photosynthesis and yield response to elevated CO_2, C4 plant foxtail millet behaves similarly to C3 species. *Plant Science*, *285*, 239-247.

Loeb, Norman G., Bruce A. Wielicki, David R. Doelling, G. Louis Smith, Dennis F. Keyes, Seiji Kato, Natividad Manalo-Smith, and Takmeng Wong. 2009. "Toward Optimal Closure of the Earth's Top-of-Atmosphere Radiation Budget." *Journal of Climate* 22 (3): 748–66. https://doi.org/10.1175/2008JCLI2637.1.

Lukashe, N. S., Mnkeni, P. N. S., & Mupambwa, H. A. (2020). Growth and elemental uptake of Rhodes grass (Chloris gayana) grown in a mine waste-contaminated soil amended with fly ash-enriched vermicompost. *Environmental Science and Pollution Research*, *27*(16), 19461-19472.

Makino, A., Nakano, H., Mae, T., Shimada, T., & Yamamoto, N. (2000). Photosynthesis, plant growth and N allocation in transgenic rice plants with decreased Rubisco under CO_2 enrichment. *Journal of Experimental Botany*, *51*(suppl_1), 383-389.

Manalil, S., Mobli, A., & Chauhan, B. S. (2020). Competitiveness of windmill grass (Chloris truncata) and feathertop Rhodes grass (Chloris virgata) in mungbean (Vigna radiata). *Crop and Pasture Science*, *71*(10), 916-923.

Manabe, S. (2019). Role of greenhouse gas in climate change. *Tellus A: Dynamic Meteorology and Oceanography*, *71*(1), 1620078.

Marshall, D., & Uller, T. (2007). When is a maternal effect adaptive?. *Oikos*, *116*(12), 1957-1963.

Marshall, D. J., Bonduriansky, R., & Bussière, L. F. (2008). Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology*, *89*(9), 2506-2517.

Marte, R. J. (1990). *Pre-production, production and post-harvest handling of carambola*. IICA.

Maucieri, C., Barbera, A. C., Vymazal, J., & Borin, M. (2017). A review on the main affecting factors of greenhouse gases emission in constructed wetlands. *Agricultural and forest meteorology*, *236*, 175-193.

Mazahrih, N., Sayari, A., Al-Hamoodi, A., Nejatian, A., & Belgacem, A. (2018). Impacts of Irrigation with Reclaimed Wastewater on Forages Production, Nutrients, and Heavy Metals Content. *Journal of Agricultural Science*, *10*, 206. https://doi.org/10.5539/jas.v10n2p206

Mazimpaka, E., Mbuza, F., Michael, T., Gatari, E. N., Bukenya, E., & James, O.-A. (2017). Current status of cattle production system in Nyagatare District-Rwanda. *Tropical animal health and production*, *49*(8), 1645-1656.

Mehnert, A. (2016). *Climate change fictions: representations of global warming in American literature*. Springer.

Melnyk, C. W. (2017). Plant grafting: insights into tissue regeneration. *Regeneration*, *4*(1), 3-14.

Milford, R., & Minson, D. (1968). The digestibility and intake of six varieties of Rhodes grass (Chloris gayana). *Australian Journal of Experimental Agriculture*, *8*(33), 413-418.

Miri, Hamid Reza, Ahmad Rastegar, and Ali Reza Bagheri. 2012. “The Impact of Elevated CO_2 on Growth and Competitiveness of C3 and C4 Crops and Weeds.” *European Journal of Experimental Biology* 2 (4): 0–0.

Moore, G., Albertsen, T., Ramankutty, P., Nichols, P., Titterington, J., & Barrett-Lennard, P. (2014). Production and persistence of subtropical grasses in environments with Mediterranean climates. *Crop and Pasture Science*, *65*(8), 798-816.

Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in ecology & evolution*, *13*(10), 403-407.

Navie, S. C., McFadyen, R. E., Panetta, F. D., & Adkins, S. W. (2005). The effect of CO2 enrichment on the growth of a C3 weed (Parthenium hysterophorus L.) and its competitive interaction with a C4 grass (Cenchrus ciliaris L.). *Plant Protection Quarterly*, *20*(2), 61-66.

Nanda, A. K., & Melnyk, C. W. (2018). The role of plant hormones during grafting. *Journal of plant research*, *131*(1), 49-58.

Nichols, P., Loi, A., Nutt, B., Evans, P., Craig, A., Pengelly, B., Dear, B., Lloyd, D., Revell, C., & Nair, R. (2007). New annual and short-lived perennial pasture legumes for Australian agriculture—15 years of revolution. *Field Crops Research*, *104*(1-3), 10-23.

Olofsson, H., Ripa, J., & Jonzén, N. (2009). Bet-hedging as an evolutionary game: the trade-off between egg size and number. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1669), 2963-2969.

Pastore, M. A., Lee, T. D., Hobbie, S. E., & Reich, P. B. (2019). Strong photosynthetic acclimation and enhanced water-use efficiency in grassland functional groups persist over 21 years of CO_2 enrichment, independent of nitrogen supply. *Global Change Biology*, *25*(9), 3031-3044.

Pan, T., Ding, J., Qin, G., Wang, Y., Xi, L., Yang, J., ... & Zou, Z. (2019). Interaction of supplementary light and CO_2 enrichment improves growth, photosynthesis, yield, and quality of tomato in autumn through spring greenhouse production. *HortScience*, *54*(2), 246-252.

Paul, S. C. (2016). *The price of defence: Maternal effects in an aposematic ladybird*. https://ore.exeter.ac.uk/repository/handle/10871/25374

Perez-Martin, A., Flexas, J., Ribas-Carbó, M., Bota, J., Tomàs, M., Infante, J., & Diaz-Espejo, A. (2009). Interactive effects of soil water deficit and air vapour pressure deficit on mesophyll conductance to CO_2 in Vitis vinifera and Olea europaea. *Journal of Experimental Botany*, *60*(8), 2391-2405.

Philippi, T. (1993). Bet-hedging germination of desert annuals: variation among populations and maternal effects in Lepidium lasiocarpum. *The American Naturalist*, *142*(3), 488-507.

Qin, X., Tang, J., Li, D., & Zhang, Q. (2012). Effect of salinity on the bioremediation of petroleum hydrocarbons in a saline-alkaline soil. *Letters in applied microbiology*, *55*(3), 210-217.

Rakocevic, M., Ribeiro, R. V., Ribeiro Marchiori, P. E., Filizola, H. F., & Batista, E. R. (2018). Structural and functional changes in coffee trees after 4 years under free air CO2 enrichment. *Annals of botany*, *121*(5), 1065-1078.

Reich, P. B., Hobbie, S. E., Lee, T. D., & Pastore, M. A. (2018). Unexpected reversal of C3 versus C4 grass response to elevated CO_2 during a 20-year field experiment. *Science*, *360*(6386), 317-320.

Ritchie, H., Roser, M., & Rosado, P. (2020). CO_2 and Greenhouse Gas Emissions. *Our World in Data*. https://ourworldindata.org/co2-and-other-greenhouse-gas-emissions

Roach, D. A., & Wulff, R. D. (1987). Maternal effects in plants. *Annual review of ecology and systematics*, *18*(1), 209-235.

Rollinson, N., & Hutchings, J. A. (2011). Body size-specific maternal effects on the offspring environment shape juvenile phenotypes in Atlantic salmon. *Oecologia*, *166*(4), 889-898.

Rudov, A., Mashkour, M., Djamali, M., & Akhani, H. (2020). A review of C4 plants in southwest Asia: An ecological, geographical and taxonomical analysis of a region with high diversity of C4 eudicots. *Frontiers in plant science*, *11*, 1374.

Russell, A. F., & Lummaa, V. (2009). Maternal effects in cooperative breeders: from hymenopterans to humans. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1520), 1143-1167.

Sack, L., & Buckley, T. N. (2016). The developmental basis of stomatal density and flux. *Plant Physiology*, *171*(4), 2358-2363.

Saldanha, C. W., Otoni, C. G., Rocha, D. I., Cavatte, P. C., Detmann, K. d. S. C., Tanaka, F. A. O., Dias, L. L. C., DaMatta, F. M., & Otoni, W. C. (2014). CO 2-enriched atmosphere and supporting material impact the growth, morphophysiology and ultrastructure of in vitro Brazilian-ginseng [Pfaffia glomerata (Spreng.) Pedersen] plantlets. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *118*(1), 87-99.

Schmid, B., & Dolt, C. (1994). Effects of maternal and paternal environment and genotype on offspring phenotype in Solidago altissima L. *Evolution*, *48*(5), 1525-1549.

Seibert, R., Donath, T. W., Moser, G., Laser, H., Grünhage, L., Schmid, T., & Müller, C. (2021). Effects of long-term CO2 enrichment on forage quality of extensively managed temperate grassland. *Agriculture, Ecosystems & Environment*, *312*, 107347.

Sekhar, K. M., Kota, V. R., Reddy, T. P., Rao, K., & Reddy, A. R. (2020). Amelioration of plant responses to drought under elevated CO_2 by rejuvenating photosynthesis and nitrogen use efficiency: implications for future climate-resilient crops. *Photosynthesis Research*, 1-20.

Senthilkumar, S., Kumar, N., Soorianathasundaram, K., & Kumar, P. J. (2014). Aspects on asexual propagation in papaya (Carica papaya L.)-a review. *Agricultural Reviews*, *35*(4), 307-313.

Shurpali, N., Agarwal, A. K., & Srivastava, V. (2019). *Greenhouse gas emissions: Challenges, technologies and solutions*. Springer.

Silva, R. G. d., Alves, R. d. C., & Zingaretti, S. M. (2020). Increased [CO_2] Causes Changes in Physiological and Genetic Responses in C4 Crops: A Brief Review. *Plants*, *9*(11), 1567.

Singh, Y. P., Singh, G., & Sharma, D. K. (2014). Bio-amelioration of alkali soils through agroforestry systems in central Indo-Gangetic plains of India. *Journal of Forestry Research*, *25*(4), 887-896.

Starrfelt, J., & Kokko, H. (2012). Bet-hedging—a triple trade-off between means, variances and correlations. *Biological Reviews*, *87*(3), 742-755.

Stouffer, R. J., & Manabe, S. (2017). Assessing temperature pattern projections made in 1989. *Nature Climate Change*, *7*(3), 163-165.

Tanaka, A., & Tanaka, R. (2006). Chlorophyll metabolism. *Current opinion in plant biology*, *9*(3), 248-255.

Tausz-Posch, S., Dempsey, R. W., Seneweera, S., Norton, R. M., Fitzgerald, G., & Tausz, M. (2015). Does a freely tillering wheat cultivar benefit more from elevated CO_2 than a restricted tillering cultivar in a water-limited environment? *European Journal of Agronomy*, *64*, 21-28.

Tefera, G., Tegegne, F., Mekuriaw, Y., Melaku, S., & Tsunekawa, A. (2015). Effects of different forms of white lupin (Lupinus albus) grain supplementation on feed intake, digestibility, growth performance and carcass characteristics of Washera sheep fed Rhodes grass (Chloris gayana) hay-based diets. *Tropical Animal Health and Production*, *47*(8), 1581-1590.

Thomas, C. D. (2010). Climate, climate change and range boundaries. *Diversity and Distributions*, *16*(3), 488-495.

Tian, H., Lu, C., Ciais, P., Michalak, A. M., Canadell, J. G., Saikawa, E., Huntzinger, D. N., Gurney, K. R., Sitch, S., & Zhang, B. (2016). The terrestrial biosphere as a net source of greenhouse gases to the atmosphere. *Nature*, *531*(7593), 225-228.

Tricker, P. J., Trewin, H., Kull, O., Clarkson, G. J., Eensalu, E., Tallis, M. J., Colella, A., Doncaster, C. P., Sabatti, M., & Taylor, G. (2005). Stomatal conductance and not stomatal density determines the long-term reduction in leaf transpiration of poplar in elevated CO 2. *Oecologia*, *143*(4), 652-660.

Ueda, H., Shibuya, T., Sawaki, Y., Saitoh, M., Takai, K., & Maruyama, S. (2016). Reactions between komatiite and CO_2-rich seawater at 250 and 350 C, 500 bars: Implications for hydrogen generation in the Hadean seafloor hydrothermal system. *Progress in Earth and Planetary Science*, *3*(1), 1-14.

Urry, J. (2015). Climate change and society. In *Why the social sciences matter* (pp. 45-59). Springer.

Van Etten, M. L., & Brunet, J. (2013). The impact of global warming on floral traits that affect the selfing rate in a high-altitude plant. *International Journal of Plant Sciences*, *174*(8), 1099-1108.

Voelker, S. L., Brooks, J. R., Meinzer, F. C., Anderson, R., Bader, M. K. F., Battipaglia, G., ... & Wingate, L. (2016). A dynamic leaf gas-exchange strategy is conserved in woody plants under changing ambient CO_2: evidence from carbon isotope discrimination in paleo and CO_2 enrichment studies. *Global Change Biology*, *22*(2), 889-902.

Voigt, C., Lamprecht, R. E., Marushchak, M. E., Lind, S. E., Novakovskiy, A., Aurela, M., Martikainen, P. J., & Biasi, C. (2017). Warming of subarctic tundra increases emissions of all three important greenhouse gases–carbon dioxide, methane, and nitrous oxide. *Global Change Biology*, *23*(8), 3121-3138.

Von Schuckmann, K., Palmer, M., Trenberth, K. E., Cazenave, A., Chambers, D., Champollion, N., Hansen, J., Josey, S., Loeb, N., & Mathieu, P.-P. (2016). An imperative to monitor Earth's energy imbalance. *Nature Climate Change*, *6*(2), 138-144.

Wand, Stephanie J. E., GuY. F. Midgley, Michael H. Jones, and Peter S. Curtis. 1999. “Responses of Wild C4 and C3 Grass (Poaceae) Species to Elevated Atmospheric

CO_2 Concentration: A Meta-Analytic Test of Current Theories and Perceptions." *Global Change Biology* 5 (6): 723–41. https://doi.org/10.1046/j.1365-2486.1999.00265.x.

Wiklund, A.-K. E., & Sundelin, B. (2001). Impaired reproduction in the amphipods Monoporeia affinis and Pontoporeia femorata as a result of moderate hypoxia and increased temperature. *Marine Ecology Progress Series*, *222*, 131-141.

Willey, N. (2018). *Environmental plant physiology*. Garland Science.

Woodward, F., Lake, J., & Quick, W. (2002). Stomatal development and CO_2: ecological consequences. *New Phytologist*, 477-484.

Xu, Z., & Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, *59*(12), 3317-3325.

Zhou, P., & Wang, M. (2016). Carbon dioxide emissions allocation: A review. *Ecological economics*, *125*, 47-59.

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The present study assessed the effects of CO_2 on the growth, development and maternal effects of *Chloris gayana*. The experimental setup was made in such a way that the potential impacts of CO_2 can be measured on the eco-physiological growth of *C. gayana* by studying successive generations. Significant differences in the growth parameters were observed in both generation 1 and 2 plants when plants were grown under enriched and ambient CO_2 conditions. The present study can be helpful in understanding the *C. gayana* growth conditions and the vertical transfer of maternal effects from one generation to the other.

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