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Research Article

Impact of Elevated CO_2 and Drought Stress on Maize Plant Growth

Khadija Ummer[1], Abdul Qadeer[1], Tahseen Afzal[1], Ahmad Mujtaba[1], Tanvir Shahzad[2], Muhammad Sanaullah[1,*]

[1]Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan
[2]Department of Environmental Sciences & Engineering, Government College University Faisalabad, Pakistan
*Corresponding author's e-mails: sanasial@gmail.com; msanaullah@uaf.edu.pk

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Abstract

Due to anthropogenic activities, there is an increasing trend of carbon dioxide (CO2) and other greenhouse gases (GHGs) emissions that contribute to climate change. These climatic changes may lead to drought stress due to uncertain rainfall. Plant responses to these climatic indicators (elevated CO2 and drought stress) are of immense concern because the extent of these changes is predicted to increase in near future. The present study aimed to elucidate the impact of elevated CO2 and drought stress on maize plant growth, and soil biochemical health parameters. Plants were grown at 80% water holding capacity (WHC, optimum moisture conditions) for two weeks and then half plants were exposed to drought stress by maintaining 30% WHC for the next three weeks. Two levels of CO2 enrichment were applied i) ambient CO2 – 400 ppm and ii) elevated CO2 – 500 ppm for both moisture levels. The results of the present study showed that drought stress reduced plant growth parameters. Drought stress also affected the root architecture by disturbing the root system. Elevated CO2 minimized the negative impact of drought on plant growth parameters by enhancing photosynthetic activities. At elevated CO2, the maximum increase in microbial biomass carbon (462.29 mg C kg-1), the activity of chitinase (152.48 nM g-1 h-1), acid phosphatase (463.44 nM g-1 h-1), and leucine aminopeptidase (5227.8 nM g-1 h-1) was recorded under the presence of drought conditions. The study showed that plant growth decreased, enzymatic activities were affected, and nutrient uptake was minimized under drought stress. Elevated CO2 minimized the negative impacts of drought, on plants' growth parameters (13%) and had a strong influence on the microbial biomass carbon and affected the enzymatic activities. Hence, elevated CO2 could be beneficial to minimize the negative impact of drought.

Keywords: Elevated CO2; Climate change; Drought stress; Microbial biomass carbon; Enzyme activities

1. Introduction

CO_2 is the most important gas in the scenario of climate change. The effects of this change are shifting weather patterns, temperature increases sea-level rise, disturbances in agricultural practices, and the shortage of good-quality water (Kumar et al., 2018). Global warming is caused due to the continuous rise of CO_2 that traps the outgoing radiations in the troposphere. The amount of carbon dioxide has been steadily increasing since the industrial revolution. It has gone up from 280 parts per million (ppm) to 400 ppm. Predictions warn that land-use change, mining, deforestation, and burning of fossil fuel are contributing to enhanced atmospheric CO_2 levels. However, the use of fossil fuels for mechanization, agrochemical, transport, and fertilizer production are also indirect emitters of CO_2. According to predictions, the concentration of CO_2 is expected to surpass 550 ppm by the end of this century (Nölte et al., 2023).

The consequential rise in CO_2 has several negative and positive effects on plants. Elevated CO_2 can have a significant impact on plants, leading to a decrease in the concentration of vital nutrients and essential micro and macro elements (Naz et al., 2023). Following are the positive impacts of elevated CO_2 on plants: Photosynthesis is enhanced by elevated CO_2 causing increased synthesis of carbohydrates, and biomass accumulation that changes plant nitrogen or carbon metabolism (Ainsworth and Long, 2021). Respiration is a crucial factor in determining crop yield and plant carbon balance (Li et al., 2021). Due to these impacts, it is very essential to predict plant mechanisms to elevated CO_2 because the concentration of CO_2 is rising rapidly.

The plant faces diverse abiotic or biotic stresses in different environmental conditions that affect plant growth and production. Physiological, morphological, and molecular responses of plants can alter due to drought stress and climate change. Drought stress reduces turgor pressure, reduce cell enlargement, and growth of cells (Pamungkas and Farid, 2022). Therefore, it affects the yield of the plant due to physiological, and biochemical damage. Under drought stress, plants attain a variety of molecular and physiological approaches for tolerance (Kumar et al., 2023). Extreme drought stress has caused significant disruptions to agricultural production in a vast region of the world (Al-Salman et al., 2023). Hence, it is very important to know the impact of drought on maize. Investigating the impact of elevated

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CO_2 and drought stress on maize plant growth is important because maize is a staple crop and plays a vital role in global food production. The present study aimed to elucidate the basic mechanisms of plants under elevated CO_2 and drought so that production measures could be better in response to climate change.

2. Materials and Methods

2.1 Collection of soil samples

For the incubation study, topsoil (0-15 cm) was collected from the research field of the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad Pakistan (31.4391° N, 73.0700° E). The environmental conditions of the region are semi-arid having a mean annual temperature of about 31.8 °C, mean annual rainfall of 22.8 mm, and relative humidity ranging from 19 to 48%. After removing visible plant roots and debris, the soil was dried, ground, and finally sieved (2 mm) for analysis of its basic physical and chemical properties (Bouyoucos, 1927). The textural class of soil was loam containing sand (43%), silt (37.6%), and clay (20.4%). The pH of the saturated soil paste and the electrical conductivity of the extract of soil were 7.9 and 1.41 dS m^{-1}, respectively. The water-holding capacity of the soil (WHC) was 19.50%. The soil organic carbon (SOC), total soil N content and C:N ratio were 0.57%, 0.054% and 10.56, respectively. The soil extractable P was 12.9 mg kg-1 and soil exchangeable K was 104.3 mg kg-1 soil.

2.2 Experimental design and growth conditions

To study the interactive effects of elevated CO_2 and drought on maize plant growth, a two-factorial experiment was conducted. In this study, we utilized a hybrid variety of maize (P1429) that was cultivated in a laboratory on moist filter paper in a nursery. In this study, we planted the plants in pots, maintaining 3 plants per pot with 400 g of soil.. Before transplanting the seeds, we applied fertilizer doses to the pots. A total of 16 pots were used for growing maize. During the initial 2 weeks of plant growth, we maintained an optimum water level of 80% of the water holding capacity (WHC) for all the plants. After 15 days of plant growth, we adjusted the soil moisture to two different levels: optimum conditions (80% WHC) and drought conditions (30% WHC). Then, elevated CO_2 (500 ppm) treatment was applied for three hours. The detailed procedure for CO_2 treatment is given in previous studies (Sanaullah et al., 2012). After 1 week of elevated CO_2 treatment, plants were harvested, and plant growth parameters were recorded.

2.3. Microbial biomass carbon

Two soil subsamples each of 5 g were taken from the same replicate one for fumigation with the help of chloroform and the other for non-fumigation. Fumigated as well as non-fumigated samples were dissolved in 20 mL of 0.5 M (K_2SO_4) solution and after shaking extracted in a plastic bottle. Then, 4 mL of that extract was titrated with 0.0337 M ferrous ammonium sulfate solution in the presence of 5 ml (H_2SO_4), 1 mL of ($K_2Cr_2O_7$), and 2-3 drops of phenanthroline indicator. The difference between fumigated and non-fumigated subsamples is soil microbial biomass carbon. The conversion factor of microbial C flush into microbial biomass C is 0.45 (Vance et al., 1987).

2.4 Enzymatic activities

Extracellular enzyme activities from fresh soil samples were determined by using fluorogenically labeled substrates technique (Pritsch et al., 2004; Sanaullah et al., 2011). The three methylumbelliferone (MUF)-based fluorogenic enzyme substrates used were MUF-β-D-glucopyranoside for β-glucosidase enzyme, MUF-N-acetyl-β-D-glucosaminide dehydrate for chitinase enzyme, and MUF-phosphate monoester for acid phosphomonoesterase. The L-Leucine-7-amino-4-methylcoumarin (AMC) substrate was used to determine the L-Leucine aminopeptidase activity.

Briefly, 0.5 g fresh soil was dispersed in 50 mL sterile distilled water on a reciprocating shaker at 320 rpm for 30 minutes. Then 50 µL of soil suspension was pipetted into a 96-well microplate containing 50 µL of buffer solutions, such as 2-(N-morpholino) ethanesulfonic acid (MES) salt for MUF enzymes (β-Glucosidase, Chitinase and Acid Phosphatase) and Tris (hydroxymethyl) aminomethane (TRIZMA)-Base and TRIZMA-HCl salts for AMC substrates enzyme (Leucine-Aminopeptidase). After that, 100 µL of respective substrates of each enzyme were added, making the final concentration of 200 µmol g^{-1} soil in each well. After pipetting all these, the soil suspensions were incubated with fluorogenic substrates for 2 hours at room temperature. The fluorescence at an excitation/emission wavelength of 360/460 nm was measured using a multilabel microplate reader (Synergy, Biotek, USA). The enzyme activities were expressed as MUF or AMC released in nmol g^{-1} hr^{-1}.

2.5. Statistical Analysis

The significant difference among treatments was tested by using two-way ANOVA following Tukey,s honest significance test at a 5% probability level, depending on the distribution of data, and its normality tests were done by using Statistics version 8.1 (Steel and Torrie, 1960).

3. Results

3.1. Plant growth parameters

Under drought stress, plant growth parameters such as plant height, root and shoot dry weights, root length, and chlorophyll SPAD values were significantly decreased compared with optimum moisture conditions (Table 1). However, with the treatment of elevated CO_2, the negative impact of drought stress was minimized, especially for plant height, root length, and root dry weight (Table 1).

Table 1. Impact of elevated CO_2 and drought stress on plant growth parameters. Data represented as mean ± SE (n=4). Letters with the values indicate significant differences between treatments according to LSD test ($p < 0.05$).

	Treatments	Plant height (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	SPAD Value
Ambient CO_2	Optimum moisture	51± 1.32 ^{a}	3.09±0.06 ^{a}	16 ±0.4 ^{a}	0.84± 0.03 ^{b}	39.53± 0.79 ^{a}
	Drought stress	36 ± 0.88 ^{d}	1.47±0.06 ^{c}	13±0.25 ^{c}	0.57± 0.01 ^{d}	33.13± 0.49 ^{c}
Elevated CO_2	Optimum moisture	48± 1.22 ^{ab}	2.74±0.03 ^{b}	17±0.11 ^{a}	0.99± 0.01 ^{a}	37.55± 0.84 ^{ab}
	Drought stress	41 ± 0.96 ^{c}	1.57±0.06 ^{c}	15± 0.30 ^{b}	0.69± 0.02 ^{c}	35.43± 0.64 ^{bc}

Table 2. Impact of elevated CO_2 and drought stress on root architecture. Data represented as mean ± SE (n=4). Letters with the values indicate significant differences between treatments according to LSD test ($p < 0.05$).

	Treatments	Primary root length (cm)	Primary root diameter (cm)	Primary root area (cm)	Lateral root length (cm)	Lateral root diameter (cm)	Lateral root area (cm)
Ambient CO_2	Optimum moisture	13.21 ± 0.04 ^{a}	0.05±0.001 ^{a}	0.40± 0.004 ^{a}	10.18 ± 0.02 ^{a}	0.05±0.002 ^{ab}	0.28±0.001 ^{b}
	Drought stress	12.44± 0.04 ^{c}	0.04± 0.002 ^{c}	0.35± 0.003 ^{c}	9.74± 0.002 ^{c}	0.03±0.001 ^{c}	0.27± 0.001 ^{d}
Elevated CO_2	Optimum moisture	13.10± 0.04 ^{a}	0.06±0.002 ^{a}	0.41± 0.002 ^{a}	10.05± 0.03 ^{b}	0.06± 0.002 ^{a}	0.29±0 .001 ^{a}
	Drought stress	12.8 ± 0.04 ^{b}	0.05±0.001 ^{bc}	0.38±0.002 ^{b}	9.76± 0.02 ^{c}	0.05± 0.001 ^{b}	0.28± 0.001 ^{c}

3.2. Root architecture

Like other plant growth parameters, drought stress negatively affected root architecture. There was a significant decrease in both primary (12.44±0.04 cm) and lateral root length (9.74±0.002 cm), diameter, and area under drought stress (Table 2). With the treatment of elevated CO_2, root architecture parameters such as primary root length (12.8±0.04 cm) and primary root area (0.38±0.002 cm) as well as lateral root diameter (0.05±0.001 cm) and area (0.28±0.001 cm) were improved compared with drought stress alone at ambient CO_2 (Table 2).

3.3. Nutrient uptake by plant

At optimum moisture conditions, there was no significant impact of elevated CO_2 on NPK uptake by both plant root and shoot (Fig.1). Under drought stress conditions, there was a significant decrease in nutrient (NPK) uptake at both plant root and shoot level. Exposure of plants to elevated CO_2 helped in the improvement of N and K uptake by shoot compared with ambient CO_2 conditions (Fig. 1A and 1E). While P uptake in the shoot was further decreased (0.04 ± 0.01%) under drought stress due to elevated CO2 treatment (Fig. 1C). In the case of NPK uptake by roots, there was no

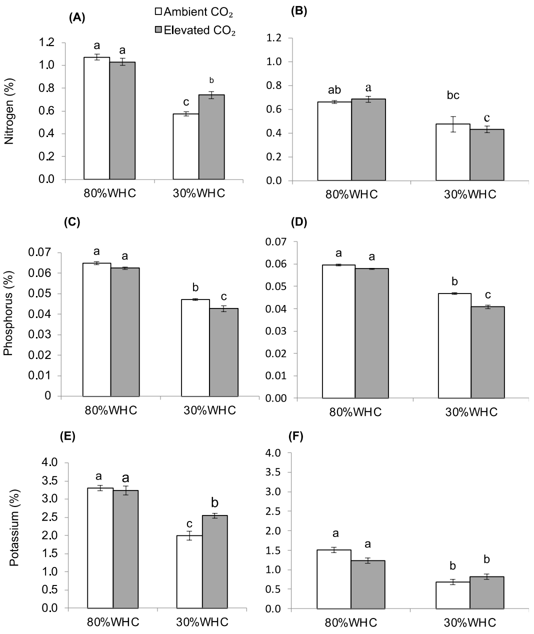

Fig. 1. Impact of elevated CO_2 and drought stress on nitrogen contents in (A) shoot and (B) root, phosphorous contents in (C) shoot and (D) root and potassium contents in (E) shoot and (F) root. Data represented as mean ± SE (n=4). Letters above the bars indicate significant differences between treatments according to LSD test ($p < 0.05$).

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significant impact of elevated CO_2 treatment on nutrient uptake, except for P uptake in the root which was significantly decreased with elevated CO_2 treatment (Fig. 1D)

3.4. Soil Microbial biomass and enzyme activities

At ambient CO_2, there was no significant impact of drought stress on microbial biomass carbon (364 ±15.31 mg C kg^{-1} soil) (Fig. 2A) as well as on extracellular enzyme activities, except for β-glucosidase and acid phosphatase activities which increased under drought stress (Fig. 3).

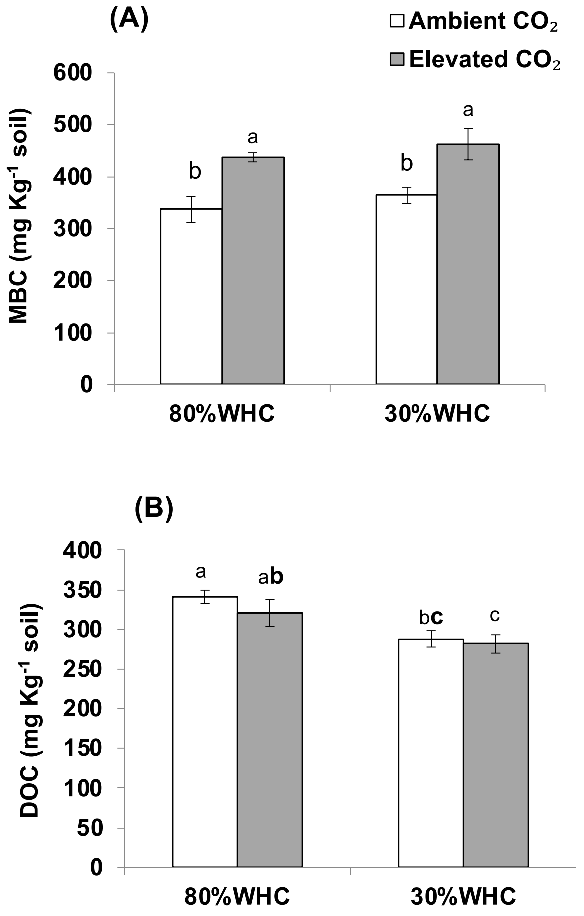

Fig. 2. Impact of elevated CO_2 and drought stress on (A) microbial biomass C and (B) dissolved organic C in soil. Data represented as mean ±SE (n=4). Letters above the bars indicate significant differences between treatments according to LSD test ($p < 0.05$).

While with elevated CO_2 treatment, MBC was significantly higher for both moisture levels (Fig. 2A). With elevated CO_2, extracellular enzyme activities were not changed for both moisture levels, except for β-glucosidase activity which was significantly decreased with elevated CO_2 (Fig. 3). The DOC contents decreased with drought stress (287 ± 9.99 mg C kg^{-1} soil) but there was no significant impact of elevated CO_2 on DOC (287 ±9.99 mg C kg^{-1} soil) (Fig. 2B).

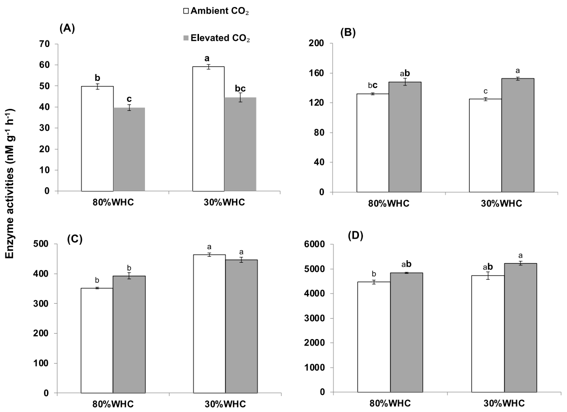

Fig. 3. Impact of elevated CO_2 and drought stress on (A) β-glucosidase, (B) Chitinase, (C) Acid phosphatase, and (D) Leucine aminopeptidase. Data represented as mean ± SE (n=4). Letters above the bars indicate significant differences between treatments according to LSD test ($p < 0.05$).

4. Discussion

The maximum increase in plant height was recorded under optimum moisture conditions because photosynthesis and nutrient uptake reach their maximum efficiency when plants are exposed to optimum moisture conditions (Abaza et al., 2023). Whereas drought stress caused a significant reduction in plant height. The plant's biological processes, such as cell division and mitosis, can have an impact on its growth, potentially causing it to slow down. Elevated CO_2 has been found to have a profound impact on plant growth, particularly in terms of height. This is attributed to the increased development of chloroplasts and mesophyll cells, which play a crucial role in photosynthesis and overall plant productivity.

Severe drought stress may decrease the biosynthesis of abscisic acid in roots and result in limited root growth (Muhammad Aslam et al., 2022). Elevated CO_2 caused structural changes in the root system function which affects the rhizospheric soil, and increases root exudates this, in turn, enhances carbon sequestration, ultimately leading to increased root dry weight.

Photosynthetic activities are enhanced under optimum moisture conditions therefore maximum chlorophyll contents are recorded under optimum moisture conditions (Vennam et al., 2023). These organic molecules are then broken down, releasing electrons that enter the electron transport chain. As a result, energy is released and used to create carbohydrates (Singh et al., 2022).

Root systems provide structural support to the plant and help for nutrient uptake from the soil. Results revealed that the root architecture system is better under optimum water conditions because the length of root, and germination of roots is maximum under optimum moisture conditions (Khaeim et al., 2022). Significant reduction was recorded in primary and lateral root length, primary root, and lateral root diameter, due to drought stress. Elevated CO_2 improved the primary and lateral root length, primary root and lateral root area, and diameter by increasing the sugar allocation to roots (Prescott et al., 2020).

Droughts can have severe consequences in storing carbon due to the decreasing activity of the hydrolytic enzymatic, it disturbs the cleavage of bonds in molecules thus decomposition is inhibited due to the altered inhibitory effect of phenolic compounds, and the redox reaction is altered. Carbon transferring enzyme activities like β-glucosidase are correlated with CO_2 enrichment. The maximum β-glucosidase activity was recorded when treated with ambient CO_2 drought stress Highly significant association between β-glucosidase and soil labile carbon fractions was noticed (Moreno et al., 2022). Under drought plants, roots release mucilage that increases the activity of β-glucosidase. β-glucosidase activity decreased when treated with elevated CO_2 under optimum moisture conditions because enzymatic activities are closely associated with the degradation of labile substrates and it decreases at elevated CO_2 (Ullah et al., 2023).

The activity of acid phosphatase increased under drought stress. In the presence of drought acid phosphatase plays a very important role in sustaining adverse environmental conditions associated with low phosphorus levels. Depending on the persistence of stresses plants respond to phosphorus deficiency by adopting multiple pathways (biochemical, and physiological morphological), changes to resist change in environmental conditions (Khan et al., 2023). Drought stress has increased the acid phosphatase function, and membrane permeability, and increased plant tolerance to drought stress by enhancing the activity of protective enzymes.

By increasing CO_2 chitinase activity is increased because decomposition is increased, and soil organic matter is increased. The maximum activity of leucine aminopeptidase is recorded under drought conditions. Leucine aminopeptidase is an enzyme involved in the degradation of proteins, which is also involved in the N cycle, and has a better response to drought in contrast to enzymes involved in the C cycle (Bogati and Walczak, 2022). Our result revealed that drought stress negatively affected plant growth and soil biological health but applying elevated CO_2 can reduce the adverse effects of drought on plant and soil ecological parameters. Further research hence is needed to explore this phenomenon in actual field conditions.

5. Conclusion

Drought stress can have detrimental effects on plant growth and soil biological health. However, our study highlights the promising potential of elevated CO_2 in mitigating these negative impacts and reducing the adverse effects of drought on both plants and soil ecological parameters. Our result concluded that drought stress negatively affected plant growth and soil biological health but applying elevated CO_2 can reduce the adverse effects of drought on plant and soil ecological parameters. These results emphasize the importance of further research to delve deeper into this phenomenon, particularly in real-world field conditions. By exploring this topic in more diverse we can gain a more comprehensive understanding of the benefits and implications of elevated CO_2 in combating the challenges posed by drought stress. This knowledge will undoubtedly contribute to the development of effective strategies for sustainable agriculture and ecosystem management.

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Author Contributions

K. Umar and **M. Sanaullah**: Conceptualization, Methodology, Writing- Original draft preparation. **A. Qadeer, T. Afzal and A. Mujtaba**: Data curation, Writing- Original draft preparation. **M. Sanaullah**: Supervision. **T. Shazad**: Writing- Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors have no conflict of interest.

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