Research Article

Hibiscus cannabinus Biochar Enhances Crop Health and Soil Biochemical Functioning in Barley under Drought Stress

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Abstract

Drought stress is a critical constraint to crop health, productivity, and soil functional stability in arid and semi-arid agroecosystems. This study evaluated the potential of *Hibiscus cannabinus*-derived biochar to enhance physiological resilience and soil biochemical functioning in barley (*Hordeum vulgare* L., cv. 'TJ-70') under water deficit conditions imposed at tillering and grain-filling stages. A controlled pot experiment was conducted with biochar incorporated at 30 and 40 g kg^{-1} soil prior to sowing. Water deficit markedly impaired crop health by reducing biomass accumulation, chlorophyll content, photosynthetic rate, and grain yield, while elevating oxidative stress and suppressing soil enzymatic activities and microbial-associated biochemical indicators. Biochar amendment significantly alleviated drought-induced damage by improving photosynthetic performance, chlorophyll retention, and antioxidant defense systems, including superoxide dismutase, catalase, and peroxidase activities, thereby minimizing oxidative injury. In parallel, biochar application enhanced soil biochemical attributes, reflecting improved microbial functionality and soil health under moisture-limited conditions. The higher application rate (40 g kg^{-1}) consistently produced greater improvements in both plant physiological performance and soil biochemical stability. Overall, these findings demonstrate that *H. cannabinus*-derived biochar effectively enhances crop health by integrating plant stress tolerance with soil functional restoration, highlighting its potential as a sustainable management strategy for drought-prone cropping systems.

Keywords: *Hibiscus cannabinus*-biochar, soil microbial activity, soil traits, soil biofilm, barley, water stress

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1. Introduction

Barley, scientifically known as *Hordeum vulgare*, is a highly vigorous crop and the most crucial staple crop globally [1]. Furthermore, it assumes a crucial function in the economic dynamics of the agricultural industry [2]. Drought stress is a major abiotic stress that causes a substantial decrease in barley yield by adversely affecting seed germination, growth of seedlings, and physiological processes of crops [3]. The increased demand for water, coupled with diminished availability, intensifies drought susceptibility in some areas. Drought-induced stress constitutes a significant barrier to attaining sustainable barley output [4]. An increase in atmospheric carbon dioxide results in climate change, modifications in precipitation patterns, and seasonal drought stress [5]. The increasing severity of drought in response to shifting climatic events necessitates the use of appropriate crop production management strategies [6]. Drought stress can hinder the root development of barley plants during the initial growth stages. Insufficient hydraulic conductivity in the roots and leaves might impair the physiological and metabolic functions of the crop [7]. Food insecurity and global warming are compelling farmers to adopt strategies to increase agricultural productivity in the face of restricted water resources [8]. This work is exceedingly difficult due to persistent climate change. Water stress not only hinders nutrient absorption in plants but also interferes with fertilizer application, as many fertilizers become volatile and unavailable to plants in arid conditions. The processes of planting and crop management, which include all agricultural operations, might affect drought stress [9]. Efficient soil management, entailing the effective use of resources to sustain soil productivity, is essential in modern agriculture [10]. The widespread use of chemical fertilizers and insecticides in agriculture results in soil fertility reduction, adversely affecting the soil's physicochemical properties [11]. Microbial activities and the decomposition of different inorganic substances are detrimental as a result of the present use of pesticides [12]. The introduction of organic material for soil management is essential to maintain soil fertility [10]. Assessing soil quality in water-stressed conditions can identify critical regions for sustainable productivity and soil health, essential for increased crop yields [13]. Soil productivity pertains to the optimal conditions for plant development, characterized by an appropriate growth medium and nutrients [14].

The addition of organic supplements has been suggested to enhance soil properties, thus boosting productivity and mitigating the negative impacts of drought [15]. Recently, biochar, a carbonaceous product produced from the incineration of carbon-based resources, has garnered significant interest as an amendment to the soil and a protector under drought [16]. The biochar application in the soil was found to improve soil organic carbon levels, moisture content, and porosity. Furthermore, it enhances the cation exchange capacity and improves the retention of polyvalent cations. Kabir et al. [17] documented the impact of biochar application on improving soil texture stability and aggregate stability. In instances of low soil moisture, aggregate stability can significantly improve soil water retention [18]. Nevertheless, the water retention volume of soils altered with biochar may vary depending on soil type, feedstock type, and biochar addition rate, and the procedures used to produce the biochar [19].

Application of biochar is well recognized for enhancing soil characteristics in semiarid regions with restricted water resources [20]. The purpose of this work was to scrutinize the influence of *Hibiscus cannabinus*-biochar addition on chemical traits of soil and barley growth, as well as physiological and oxidative enzyme levels in drought-stressed environments. Published research has not analyzed the invasive species Hibiscus cannabinus-biochar for mitigating drought stress in barley. We utilized this biochar to enhance barley growth under drought conditions, aiming to provide insights for the future enhancement of this biomass biochar. We hypothesized that the addition of *Hibiscus cannabinus*-biochar to the soil would improve soil properties and barley growth due to improvement in biochemical and physiological parameters under drought conditions.

2. Materials and Methods

2.1. Study Site and Experimental Design

The study was performed at Islamia University of Bahawalpur, IUB, Punjab, Pakistan. The trial utilized the barley seed variety "TJ-70" from NIAB, Pakistan. Eight barley seeds were cultivated in each pot, each filled with 4 kg of soil. Before the experiment, analysis of the soil found the presence of 0.80% organic matter, 80 mg kg^{-1} available potassium, 30 mg kg^{-1} available phosphorus, EC 229 (µS/cm), TSS (0.49%), and 23%, 39%, and 38% clay, silt, and sand levels, respectively. The average humidity and temperature recorded throughout the experiment are provided in Table 1.

Table 1. Average humidity and temperature levels during the barley-growing phase.

Month	Temperature, °C	Humidity level
November	19	59
December	15	69
January	12	72
February	14	67
March	20	60
April	30	49

Biochar was applied as a soil supplement just 10 days before planting barley seeds. Biochar was made from *Hibiscus cannabinus* biomass (known as non-wood biomass). The experiment utilized biochar produced by the pyrolysis process at 550 °C. The applications of biochar varied in dose based on the treatments in pots: BC_0 (no biochar), BC_1 (30 g kg^{-1}), and BC_2 (40 g kg^{-1}). To induce water stress, irrigation was withheld at both the tillering phase (WS1) and grain-filling phase (WS_2) of barley growth. Fully irrigated (WS_0) plants were designated as the control group. Irrigation/watering was administered at crucial growth phases, and 4-inch containers were filled with water throughout irrigation. Properties of the *Hibiscus cannabinus*-biochar utilized in the trial are provided in Table 2. Totally, other components remained consistent with the recommended dose. A complete randomized (CRD) design with 4 replications was applied to settle the planting pots.

Table 2. Characteristics of the applied biochar.

Biochar traits	Values
pH	9.11
Ash level (%)	3.29
CEC (cmol$_c$ kg^{-1})	35.19
EC (µS cm^{-1})	941
Total carbon (%)	70.1
Total Nitrogen (%)	0.70
SSA (m^2 g^{-1})	113.6
Water soluble C (%)	149
Water soluble N (%)	89.54
$Cr_2O_7^{2-}$ oxidizable organic carbon (%)	4.61
Carbonate level (%)	12.31

2.2. Plant Harvesting and Quantification of Biomass

Upon reaching physiological maturity, which occurs after 125 days of germination, the plant was collected and divided into roots, branches, and spikes. After washing with distilled water, the plant components were meticulously rinsed with tap water. Initially, the root samples were rinsed with 1% hydrochloric acid solution. Each sample was divided into several components, including height of plant, length of spike, number of spikelets per spike, per spike grain numbers, 1000-grain weight, and per plant grain yield. The samples were dried in an oven at 70 °C until grasped a consistent weight. Dry biomass of each component was then measured individually, crushed, and kept for further analysis.

2.3. Determination of Gas Exchange Parameters and Photosynthetic Pigments

After 70 days of sowing, chlorophyll levels and gas exchange-related parameters were assessed by removing one plant from each treatment. The chlorophyll level was quantified with a spectrophotometer (Shimadzu UV-1800) at wavelengths of 470 nm, 647 nm, and 664.5 nm once samples were extracted with 85% (v/v) acetone (CH_3COCH_3) in the absence of light at 4 °C for 24 hours, followed by centrifugation at 4000×g for 15 minutes [21]. Stomatal conductance (Gs), water use efficiency (WUE), transpiration rate (Tr), and photosynthetic rate (Pn) were measured with a non-dispersive infrared gas analyzer (LI-6800). These variables were recorded during the period from 10:30 - 11:30 a.m. while barley plants were totally functional.

2.4. Quantification of H_2O_2, Malondialdehyde, and Electrolyte Leakage Levels

After 70 days of planting, electrolyte leakage in shoots was measured through uprooting one plant from every treatment. Samples were sliced into small pieces and positioned vertically in tubes containing 10 mL of DI water. The solution's initial EC was measured after a 2-hour incubation period at 30 °C. The samples were subjected to another autoclaving process for 25 minutes at 120 °C. The solution's final EC was measured after cooling the sample to 27 °C. Then, the electrolyte leakage was calculated with the equation suggested by Cuesta et al. [22].

$$EC = (EC1/EC2) \times 100 \qquad (1)$$

The malondialdehyde concentration quantification was conducted with an approach outlined by Mendes et al. [23]. To a 0.5-gram fresh sample, 5 mL of a 0.1% solution of thiobarbituric acid (TBA) was mixed. After centrifuging the aliquot for ten minutes at 12,000×g at 4 °C, the supernatant was obtained. After adding 4 mL of a 20% trichloroacetic acid solution (including 0.5% thiobarbituric acid) to 1 mL of supernatant, the mixture was immersed in boiling water at 95 °C for 30 minutes. The solution was then rapidly cooled in an ice bath and centrifuged at 10,000×g for 10 minutes. Samples were measured for absorbance at 532 nm, and non-specific absorbance at 600nm was subtracted. MDA levels were then determined with an extinction coefficient of 155 mM^{-1} cm^{-1}.

To quantify hydrogen peroxide, a 3 mL phosphate buffer solution (50 mM) was mixed with 50 mg of leaf samples to create a uniform mixture. The mixture was then centrifuged at 6000×g for 25 minutes at 4 °C.

Subsequently, the extracted solution was combined with 1 mL of 0.1% TiO (SO_4) in 20% (v/v) sulfuric acid. The resulting mixture was subjected to centrifugation at 6000×g for 20 minutes at a consistent temperature of 4 °C. Supernatant absorption was quantified at a wavelength of 410 nm. An extinction value of 0.28 μmol^{-1} cm^{-1} was employed to measure the amount of hydrogen peroxide.

2.5. Quantification of Antioxidant Enzymes

APX, CAT, POD, and SOD activities were measured using spectrophotometric instruments. For this purpose, shoot samples were pulverized in liquid nitrogen with a pestle and mortar. Standardizing the samples with a 0.05 M phosphate buffer at a pH of 7.8, the solution was filtered and centrifuged for 15 minutes at 12,000×g at 4 °C. The resulting supernatant was gathered and used for analysis. Peroxidase (SOD) and peroxidase (POD) activity were measured using the Pan et al. [24] technique.

The activity of catalase (CAT) was evaluated using the Ge et al. [25] technique. In summary, a 3 mL test mixture was prepared by adding 100 µL of enzyme extract, 100 µL of 250 mM hydrogen peroxide, and 2.8 mL of 50 mM phosphate buffer with 2 mM of CA at a pH of 7. Absorbance reduction at 240 nm was quantified to determine catalase activity. A reaction mixture containing 100 µL of enzyme extract, 100 µL of ascorbate (7.5 mM), 100 µL of Hydrogen peroxide (300 mM), and 2.7 mL of 25 mM tribasic potassium phosphate buffer with 2 mM CA was used to determine the activity of APX. Ascorbate oxidation activity was quantified by detecting at 290 nm ($\varepsilon = 2.8\ mM^{-1}cm^{-1}$), following the Ge et al. [25] method.

2.6. Soil Analysis

Samples of soil were gathered from pots at various stages of barley growth and after that preserved in plastic bags before their transportation to the lab. Supplementary containers were already prepared to gather soil samples from each phase of growth that underwent identical treatments and growing conditions. Phonological sampling was conducted at two distinct periods of barley growth: the tillering stage and the grain-filling stage. To accomplish this, many containers were set up for soil samples during the tillering and grain-filling stages, while maintaining equal conditions. Once the soil samples were moved to the laboratory, they were sifted through a sieve with a mesh size of less than 1.5 mm and then examined for soil moisture level. Once sieved, the soil was kept at a temperature of 4 °C.

The values of SOC, Bray P, and exchangeable potassium were calculated using established techniques as proposed by Sparks et al. [26]. Measurement of the soil pH level was conducted in a suspension of water and soil. The determination of total nitrogen was conducted through steam distillation, as documented via Mulvaney [27]. The quantification of carbon (DOC) and dissolved organic nitrogen (DON) was conducted following the methodology introduced by Smolander and Kitunen [28]. The nitrogen mineralization (NMIN) rate was determined by analyzing the soil both before and after a 10-day incubation period at a temperature of 30 °C. N, C, and P levels in the soil microbial biomass were quantified via fumigation extraction techniques as stated by Vance et al. [29]. Soil respiration (SR) was measured following the method described by Cuesta et al. [22]. The soil enzyme activities, including acid phosphatase (AcP), β-glucosidase (βG), dehydrogenase (DH), and urease (UR), were measured using the Tabatabai method [30].

2.7. Statistical Analysis

An analysis of variance was performed on data with Fisher's test with fixed factors biochar treatment (unamended, 30 g kg^{-1}, and 40 g kg^{-1}) and moisture treatment (continually irrigated, water stress during the tillering stage, and grain-filling phase). The Least Significant Difference (LSD) test was employed to identify significant differences in the interaction between amendment and moisture treatment at 95% confidence level ($p \geq 0.05$) (Steel et al., 1997).

3. Results

3.1. Barley Growth and Yield Parameters

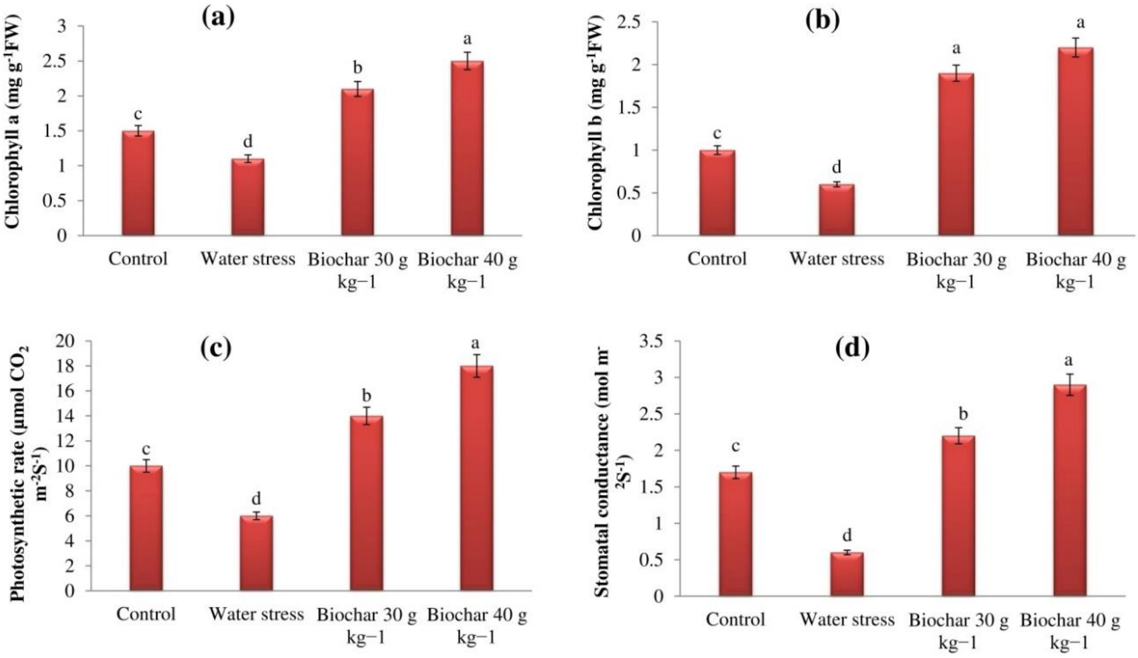
The drought stress had a significant impact on the barley growth indices and yield parameters. Additionally, it was detected that the addition of *Hibiscus cannabinus*-biochar had a notable effect in improving the barley growth as well as yield, as shown in Table 3. The best results were observed when biochar was applied at a dose of 40 g kg^{-1}, whereas the lower levels were obtained when no biochar was applied. The study revealed that the application of drought during the tillering stage resulted in the lowest spikelet numbers, spike length, and plant height. Conversely, the drought stress application during the grain-filling stage led to the lowest grain numbers, 1000-grain weight, as well as yield of grain. Similar patterns were observed in the results when the *Hibiscus cannabinus*-biochar was used in conjunction with drought stress.

Table 3. Evaluation of the impact of *Hibiscus cannabinus*-biochar on growth parameters and yield attributes of barley in conditions of drought-induced stress.

Application	Height of plant (cm)	Length of spike (cm)	Spikelet No. per-spike	Grains No. per-spike	1000-grain weight (g)	Grain yield per-plant (g)
WS_0	99 a (±0.889)	11.1 a (±0.139)	25.12 a (±0.119)	47 a (±0.349)	36 a (±0.360)	1.50 a (±0.008)
WS_1	88 c (±0.844)	8.77 c (±0.069)	23.07 b (±0.090)	39 b (±0.680)	33 b (±0.360)	0.89 b (±0.016)
WS_2	94 b (±0.399)	9.21 b (±0.159)	20.39 c (±0.040)	38 c (±0.909)	30 c (±0.160)	0.49 c (±0.030)
BC_0	89 c (±0.699)	10.09 c (±0.051)	22.31 c (±0.011)	38 c (±0.501)	31 c (±0.369)	1.19 c (±0.014)
BC_1	94 b (±0.398)	11.19 b (±0.090)	24.21 b (±0.020)	44 b (±0.909)	34 b (±0.401)	1.29 b (±0.020)
BC_2	98 a (±0.409)	12.11 a (±0.059)	26.39 a (±0.021)	48 a (±0.580)	37 a (±0.129)	1.50 a (±0.009)
WS_0BC_0	95.87 a (±1.041)	11.30 c (±0.069)	23.29 c (±0.039)	44 c (±0.399)	35 c (±0.140)	1.19 c (±0.010)
WS_0BC_1	97.41 b (±1.180)	12.19 b (±0.059)	25.30 b (±0.060)	47 b (±0.339)	37 b (±0.080)	1.29 c (±0.020)
WS_0BC_2	98.98 a (±0.932)	13.31 a (±0.260)	27.50 a (±0.089)	49 a (±0.380)	39 a (±0.109)	1.50 a (±0.010)
WS_1BC_0	85.40 h (±0.579)	8.19 h (±0.090)	20.10 f (±0.059)	40 f (±0.398)	29 f (±0.207)	0.90 f (±0.003)
WS_1BC_1	89.45 g (±1.159)	9.13 g (±0.180)	21.49 e (±0.129)	39 e (±0.180)	32 d (±0.030)	1.01 e (±0.008)
WS_1BC_2	89.61 f (±0.260)	10.14 f (±0.069)	22.39 d (±0.038)	40 d (±0.110)	34 e (±0.189)	1.09 d (±0.009)
WS_2BC_0	92.99 e (±0.388)	10.10 f (±0.049)	17.29 i (±0.029)	29 i (±0.359)	25 i (±0.090)	0.60 i (±0.006)
WS_2BC_1	94.32 d (±0.659)	10.99 d (±0.009)	18.19 h (±0.028)	34 h (±0.089)	27 h (±0.110)	0.70 h (±0.017)
WS_2BC_2	95.12 c (±0.630)	11.19 c (±0.169)	19.18 a (±0.008)	35 f (±0.409)	29 g (±0.079)	0.79 g (±0.010)

3.2. Analysis of Chlorophyll Levels and Gas-Exchange Characteristics

Quantification of chlorophyll a and b levels in barley showed a significant decline under drought stress (Figure 1). Both chlorophyll a and b showed a maximum reduction of around 40% in the presence of drought stress compared to the control group. The chlorophyll levels were significantly increased by biochar addition in comparison with the drought and control groups. 40 g kg^{-1} biochar treatment had the highest chlorophyll a and chlorophyll b levels, measuring around 81% and 72%, respectively, in comparison to the control. Water-induced stress reduced the values of WUE, Tr, Gs, and Pn in comparison to the control (Figure 1). The gas exchange-related parameters were significantly influenced by the biochar application in comparison to the comparable treatments without biochar. The highest increase in WUE, Tr, Gs, and Pn was recorded in 40 g kg^{-1} biochar, with values of approximately 55%, 89%, 49%, and 57% greater than the control samples, respectively.



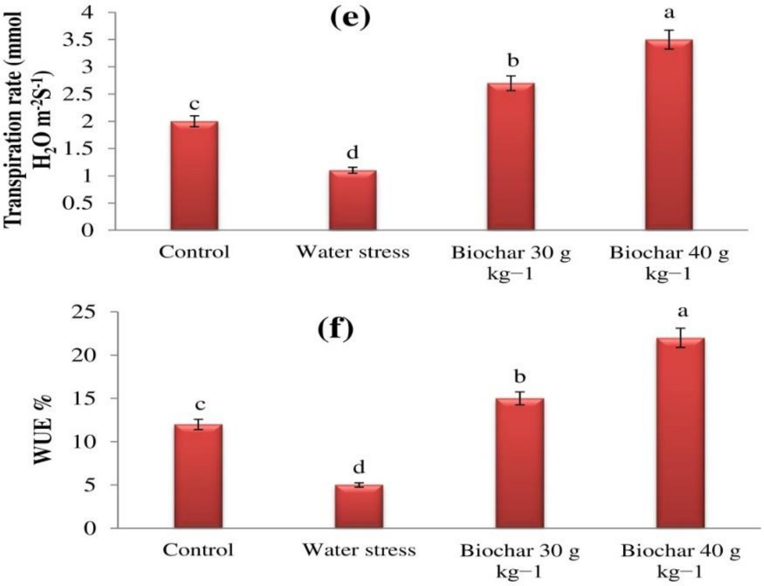
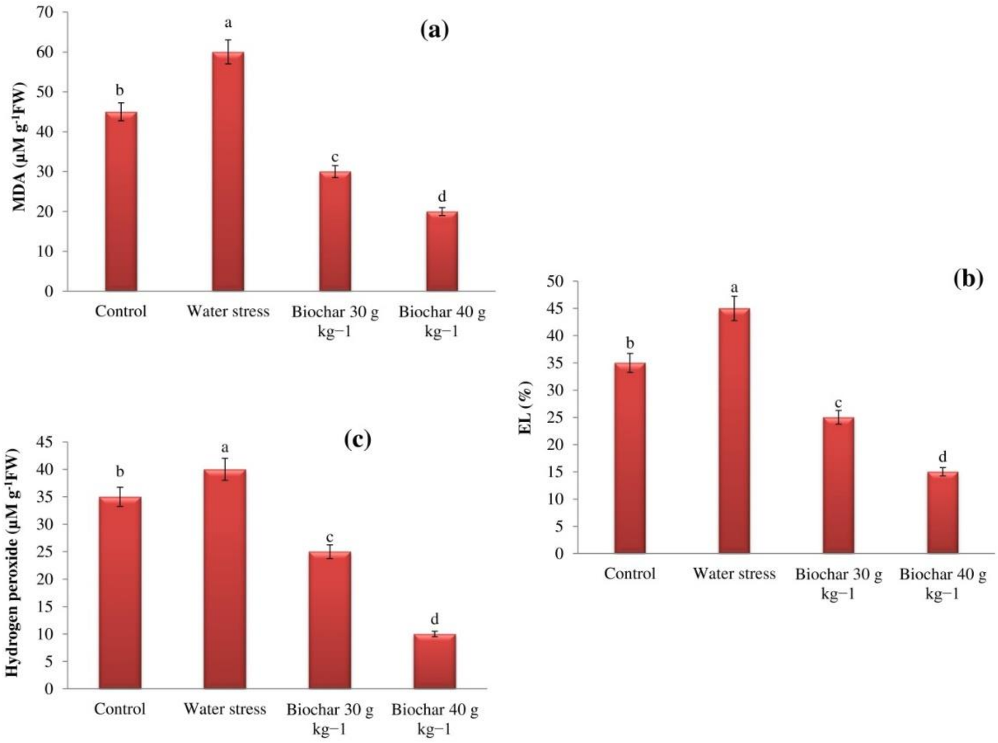


Figure 1. Evaluation of chlorophyll levels and gas exchange characteristics in barley cultivated under water stress, then subjected to escalating amounts of biochar. Values are means ± standard deviation (n = 4). Varying lowercase letters indicate a statistically significant difference among biochar treatments when the p-value is < 0.05.

3.3. Assessment of Oxidative Stress and Antioxidant Enzymes

Figure 2 depicts the data for oxidative stress, such as H_2O_2, EL, and MDA, as well as antioxidant enzymes, including CAT, POD, and SOD, in leaves. Drought stress resulted in elevated levels of H_2O_2, EL, and MDA in leaves compared to controls. Treatment with drought stress resulted in the highest increase in H_2O_2, EL, and MDA levels, with around 24%, 19%, and 29%, respectively to control. The biochar addition markedly decreased levels of H_2O_2, EL, and MDA compared to the similar drought application without biochar (Figure 2). The lowest levels of H_2O_2, EL, and MDA were detected in the 40 g kg^{-1} biochar. Reduced CAT and SOD activities and increased POD activity were observed during drought compared to the control. Application of biochar reduced POD activity and increased CAT and SOD activities in comparison to the drought application without biochar (Figure 2).



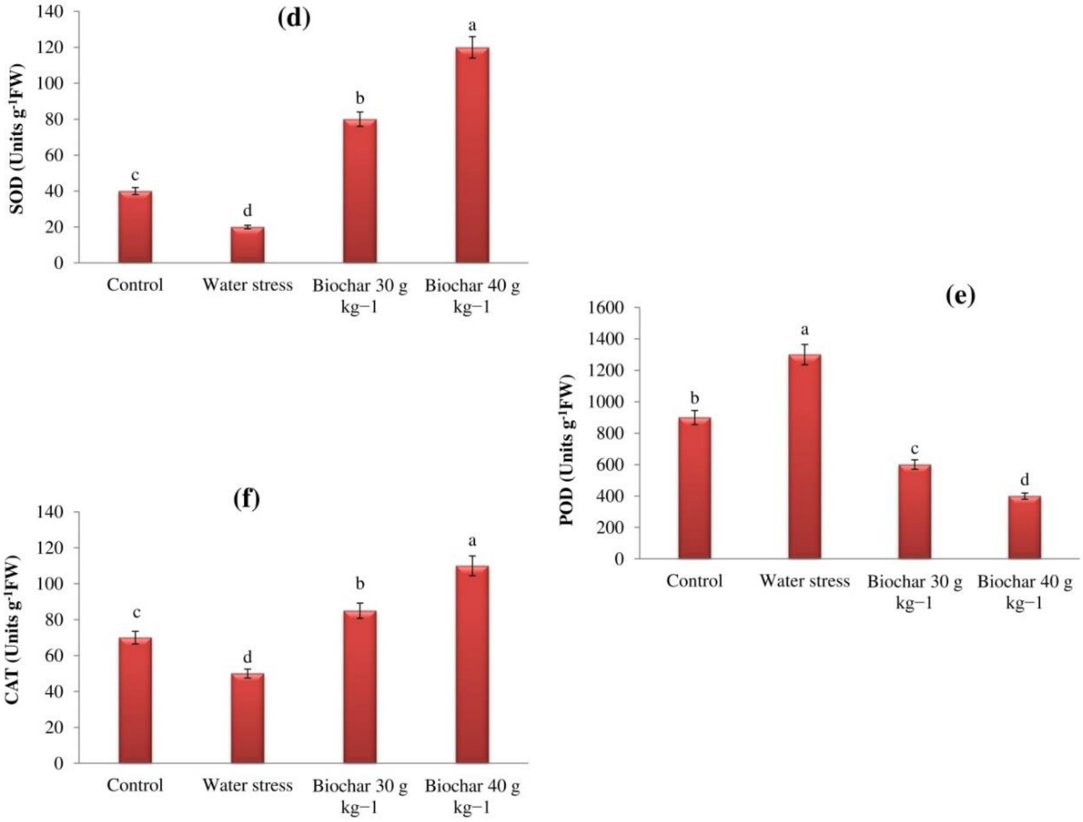


Figure 2. Evaluation of H_2O_2, EL, and MDA levels and antioxidant enzymes in barley cultivated under water stress, and then subjected to escalating amounts of biochar. Values are means ± standard deviation (n = 4). Varying lowercase letters indicate a statistically significant difference among biochar treatments when the p-value is < 0.05.

3.4. Soil Analysis

The soil pH level, total nitrogen, exchangeable potassium, as well as Bray phosphorus, recorded their maximum values in the soil under full irrigation with an elevated biochar treatment. Conversely, these values were lower when drought stress was applied at the grain-filling stage without biochar, as shown in Table 4. Under varying soil moisture circumstances (drought or full irrigation), higher doses of biochar resulted in high levels of various soil characteristics, whereas the lowest levels were observed in untreated soil. In general, both without and with biochar addition, these metrics were highest in the soil of plants that were completely irrigated but were lowest during the grain-filling phase under drought stress. No significant difference was observed in Bray phosphorus in biochar-added soil under drought during both the tillering and grain-filling stage.

Table 4. Impact of *Hibiscus cannabinus*-biochar on soil pH value, mineral nitrogen, phosphorus, and exchangeable potassium levels under water stress.

Treatments	pH of soil	Mineral nitrogen (mg kg^{-1})	Bray phosphorus	Exchangeable potassium
WS_0	7.10 a (±0.020)	84.60 a (±0.090)	7.79 a (±0.014)	169.49 a (±0.120)
WS_1	6.79 b (±0.090)	79.20 b (±0.050)	7.39 b (±0.017)	149.23 b (±0.069)
WS_2	6.80 c (±0.070)	75.50 c (±0.016)	6.89 c (±0.030)	139.34 c (±0.079)
BC_0	6.69 c (±0.059)	67.79 c (±0.040)	6.39 c (±0.210)	110.80 c (±6.321)
BC_1	6.90 b (±0.069)	85.49 b (±0.090)	7.19 b (±0.399)	180.99 b (±7.503)
BC_2	7.06 a (±0.080)	89.90 a (±0.050)	8.60 a (±0.130)	189. 21 a (±6.118)
WS_0BC_0	6.79 d (±0.012)	69.09 f (±0.088)	6.79 e (±0.015)	119.28 g (±0.088)
WS_0BC_1	7.02 b (±0.004)	90.29 b (±0.149)	7.79 c (±0.015)	197.00 b (±0.140)
WS_0BC_2	7.19 a (±0.020)	93.20 a (±0.079)	8.77 a (±0.015)	213.11 a (±0.131)
WS_1BC_0	6.69 e (±0.015)	66.23 g (±0.088)	6.39 f (±0.019)	99.63 h (±0.071)
WS_1BC_1	6.79 d (±0.008)	86.20 d (±0.089)	7.37 d (±0.007)	169. 20 e (±0.089)
WS_1BC_2	7.00 f (±0.011)	89.21 b (±0.099)	8.33 b (±0.019)	197.32 c (±0.059)
WS_2BC_0	6.59 e (±0.007)	64.09 h (±0.059)	6.09 f (±0.049)	98.31 i (±0.071)
WS_2BC_1	6.79 d (±0.006)	79.27 e (±0.058)	6.39 e (±0.015)	150.20 f (±0.086)
WS_2BC_2	6.89 c (±0.010)	87.16 c (±0.120)	8.39 b (±0.019)	179.45 d (±0.054)

Soil respiration, nitrogen mineralization, dissolved organic carbon, dissolved organic nitrogen, and soil organic carbon were greatest in the soil under full irrigation and the higher rates of biochar amendment. Contrariwise, these values were lowest in the absence of biochar addition at the grain-filling stage under drought stress, except for soil organic carbon, which was lowest under drought environments at the tillering phase at a biochar dose of 30 g kg^{-1} (as shown in Table 5). Both without and with biochar addition, these characteristics were highest in soil of completely watered plants and lowest at the grain-filling stage under drought stress, except for soil respiration, which exhibited no difference between the two water-stress conditions at a biochar dose of 30 g kg^{-1} and the low biochar level. No significant difference in soil respiration was observed between the two water-stress conditions when the biochar amount was reduced.

Table 5. Impact of *Hibiscus cannabinus*-biochar on soil respiration, nitrogen mineralization, dissolved organic N, soil organic carbon, and dissolved organic carbon in conditions of drought stress.

Treatments	SOC (g kg^{-1})	DOC (µg g^{-1})	DON (µg g^{-1})	Mineralization of nitrogen	Soil respiration
WS_0	1.29 a (±0.020)	139.75 a (±0.079)	22.12 a (±0.089)	78.77 a (±0.099)	24.17 a (±0.020)
WS_1	1.26 b (±0.0021)	136.52 b (±0.0080)	20.07 b (±0.080)	66.76 b (±0.080)	23.12 b (±0.219)
WS_2	1.24 c (±0.0007)	131.21 c (±0.130)	16.39 c (±0.080)	49.52 c (±0.089)	22.19 c (±0.006)
BC_0	0.89 c (±0.0060)	127.07 c (±1.214)	17.31 c (±1.801)	52.76 c (±5.591)	21.99 c (±0.401)
BC_1	1.50 b (±0.0181)	134.19 b (±2.175)	20.21 b (±1.806)	66.76 b (±7.989)	23.11 b (±0.843)
BC_2	1.49 a (±0.0180)	149.12 a (±3.680)	22.39 a (±1.811)	77.54 a (±7.997)	24.98 a (±0.711)
WS_0BC_0	98 d (±0.0013)	134.30 f (±0.059)	20.29 d (±0.079)	66.30 c (±0.119)	23.09 b (±0.021)
WS_0BC_1	1.49 b (±0.0020)	139.19 d (±0.089)	23.30 b (±0.078)	79.76 b (±0.089)	25.30 ab (±0.022)
WS_0BC_2	1.55 a (±0.0019)	160.31 a (±0.094)	25.50 a (±0.120)	88.89 a (±0.080)	26.20 a (±0.029)
WS_1BC_0	0.97 d (±0.0014)	129.19 f (±0.067)	18.10 f (±0.059)	49.76 e (±0.112)	22.29 cd (±0.009)
WS_1BC_1	1.51 d (±0.0025)	136.13 e (±0.099)	21.49 c (±0.070)	66.43 c (±0.069)	22.88 c (±0.711)
WS_1BC_2	1.51 ab (±0.0024)	149.14 b (±0.071)	23.39 b (±0.110)	79.09 b (±0.059)	25.12 b (±0.004)
WS_2BC_0	0.97 e (±0.0007)	130.10 g (±0.130)	14.29 h (±0.070)	46.21 f (±0.119)	21.21 e (±0.006)
WS_2BC_1	150 c (±0.0008)	133.99 f (±0.070)	17.19 g (±0.079)	49.43 e (±0.079)	22.15 d (±0.007)
WS_2BC_2	1.51 b (±0.0010)	147.19 c (±0.190)	19.18 a (±0.079)	59.19 d (±0.099)	23.41 c (±0.004)

Values in brackets are the standard error of the mean (n = 4).

Under full watering with the maximum biochar treatment (40g kg^{-1}), the soil exhibited the highest levels of microbial biomass C (MBC), microbial biomass N (MBN), and microbial biomass P (MBP). Conversely, when drought stress was introduced at grain-filling without biochar application, these values were the lowest (Figures 3 and 4). Across various soil moisture circumstances (drought or full irrigation), the soil indices exhibited the highest values when the biochar level was at its maximum, but the lowest values in untreated soil. In general, both without and with biochar addition, these metrics were highest in the soil of plants that were completely irrigated, but were lowest during the grain-filling stage under water stress.

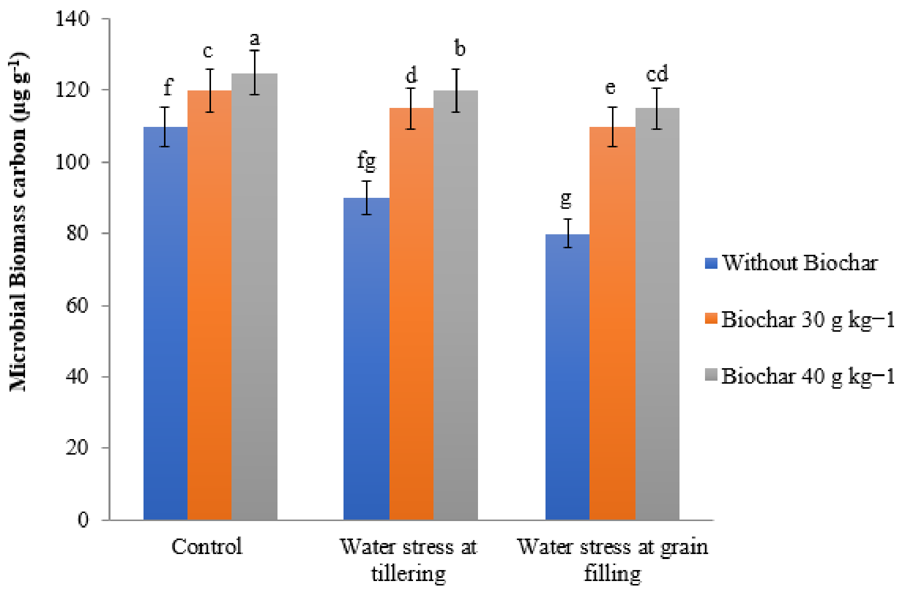


Figure 3. Impact of *Hibiscus cannabinus*-biochar on soil microbial biomass carbon under water stress conditions.

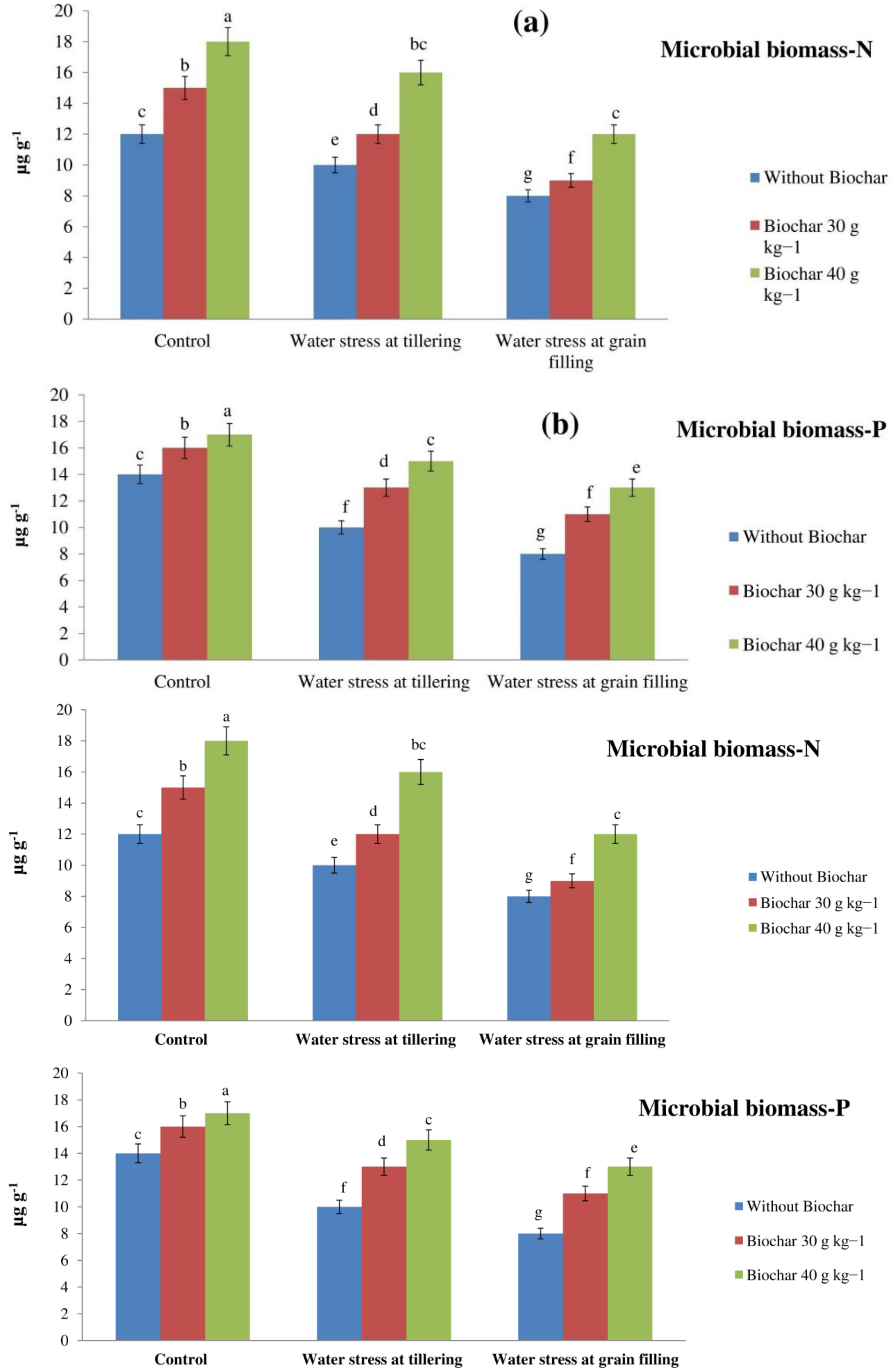


Figure 4. Impact of *Hibiscus cannabinus-biochar on microbial biomass* phosphorus and microbial biomass nitrogen and of the soil under water-stress conditions.

The data presented in Table 6 indicate that the soil β-glucosidase (βG), acid phosphatase (AcP), urease (UR), and dehydrogenase (DH) concentrations were highest in the absence of water stress, followed by drought at the grain-filling phase. Conversely, the lowest levels were observed when water stress was employed during the grain-filling stage. The biochar incorporation at a dose of 40 g kg^{-1} yielded the maximum soil enzymatic activity, followed by the biochar addition at a level of 30 g kg^{-1}. The treatment without biochar showed the lowest enzyme activities. A significant interaction was observed between the administration of *Hibiscus cannabinus*-biochar and water-stress treatments.

Table 6. Impact of Hibiscus cannabinus-biochar on soil β-glucosidase (βG), acid phosphatase (AcP), urease (UR), and dehydrogenase (DH) concentrations in conditions of drought stress.

Applications	DH (nmol TPF g^{-1} soil h^{-1})	UR (µmol NH_3-N g^{-1} h^{-1})	AcP (µmol *p*-Nitrophenol g^{-1} h^{-1})	βG (µmol *p*-Nitrophenol g^{-1} h^{-1})
WS_0	69 a (±0.799)	16.29 a (±0.280)	27.12 a (±0.250)	7.70 a (±0.059)
WS_1	34 c (±1.120)	10.28 c (±0.119)	18.07 c (±0.169)	3.39 c (±0.089)
WS_2	66 b (±0.897)	13.29 b (±0.110)	22.39 b (±0.139)	5.49 b (±0.070)
BC_0	66 c (±1.432)	17.09 c (±0.019)	25.31 c (±0.269)	6.76 c (±0.319)
BC_1	77 b (±1.112)	18.19 b (±0.090)	29.21 b (±0.240)	7.76 b (±0.240)
BC_2	83 a (±0.872)	22.12 a (±0.030)	33.39 a (±0.502)	8.54 a (±0.089)
WS_0BC_0	64 c (±0.872)	14.30 c (±0.089)	23.29 c (±0.109)	7.30 c (±0.091)
WS_0BC_1	69 b (±1.112)	17.19 b (±0.130)	28.30 b (±0.499)	7.76 b (±0.200)
WS_0BC_2	76 a (±0.873)	19.29 a (±0.260)	32.50 a (±0.229)	8.89 a (±0.407)
WS_1BC_0	29 h (±1.431)	5.39 g (±0.260)	15.10 g (±0.529)	3.76 h (±0.140)
WS_1BC_1	36 g (±0.699)	7.13 f (±0.070)	17.49 g (±0.470)	4.43 g (±0.050)
WS_1BC_2	40 g (±0.801)	9.13 e (±0.060)	18.39 f (±0.699)	4.09 f (±0.059)
WS_2BC_0	59 f (±0.439)	10.10 e (±0.099)	18.29 f (±0.601)	4.21 e (±0.070)
WS_2BC_1	66 e (±0.399)	12.99 d (±0.100)	20.19 e (±0.501)	5.43 d (±0.129)
WS_2BC_2	69 d (±0.857)	13.19 c (±0.169)	22.18d (±0.811)	5.87 d (±0.120)

4. Discussion

Current research shows that water-induced stress during the tillering stage and the grain-filling stage significantly reduced the soil pH level, while soil pH remained higher in continually watered soils. Similar findings were documented by Katohar et al. [31], Ashfaq et al. [32] observed a significant reduction in soil pH when exposed to drought stress, ascribed to insufficient phosphate solubilization. The parameters associated with the growth and yield of the barley plants are interconnected. An increased 1000-grain weight results in a greater grain yield per plant [33]. Seleiman et al. [34] found that drought stress, observed at any stage of growth, significantly impairs barley growth and production due to the key role of water in the development and growth of plants. Drought stress leads to reduced availability of nutrients from the soil and decreased synthesis of photosynthetic components in crops, resulting in reduced growth of plants. The biochar addition in the soil enhanced barley growth and plant biomass, except in the case of drought, when biochar was not effective. Enhancing the quantity of biochar incorporation showed an improvement in barley plant growth under conditions of water scarcity [35]. Furthermore, multiple studies have revealed the enhancement of growth-related parameters with biochar in the presence of water deficit conditions [36]. The positive impact of biochar on barley yield and biomass in stressed environments was attributed to the enhancement of soil physicochemical characteristics in response to drought stress [37].

Drought stress resulted in a reduction in chlorophyll levels and gas exchange characteristics. The reduction in chlorophyll content is attributable to chloroplast damage induced by reactive oxygen species. Drought stress induces the generation of reactive oxygen species (ROS), including O_2^- and H_2O_2, resulting in the degradation of chlorophyll [38]. Conversely, the application of biochar enhanced barley plant chlorophyll levels and gas exchange characteristics more than control and drought treatments (Figure 1). Several reasons are rooted in its distinctive characteristics and interaction with plants and soil. Biochar improves nutrient accessibility in the soil, facilitating the uptake of vital elements including nitrogen, potassium, and phosphate. These nutrients are essential for synthesizing chlorophyll, the primary photosynthetic pigment in crops [39]. Studies have shown that the use of biochar under drought stress leads to an increase in chlorophyll levels, Gs, and Pn in the leaves of different plants, including wheat, barley, maize, and potato [40]. Similarly, biochar application enhanced photosynthesis in crops experiencing drought stress [41]. Biochar serves as an effective technique for increasing photosynthesis in crops experiencing drought stress by promoting water retention, nutrient accessibility, soil structure, and overall plant vitality. It alleviates the detrimental impacts of drought, guaranteeing that plants possess the necessary resources to sustain effective photosynthesis, resulting in improved agricultural yields even in water-scarce conditions [42]. The enhancement of photosynthesis observed with biochar addition under drought stress may be attributed to the ultrastructural alteration of barley seedlings. This is because the stress induces significant damage to the chloroplasts [43].

Oxidative stress affects various cellular processes in crops by promoting lipid peroxidation and inducing structural damage to nucleic acids [44]. The generation of reactive oxygen species (ROS) in crops is counteracted by important antioxidant enzymes found in crops, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Sodium oxidase (SOD) transforms $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2), catalase (CAT) directly converts hydrogen peroxide to water and O_2, and peroxidase (POD) catalyzes the dissociation of hydrogen peroxide. Therefore, plants undergo ROS detoxification in this manner, and a proper balance is necessary between the generation and breakdown of ROS for crop functioning [45]. Nevertheless, in stressed conditions, plants are incapable of eliminating the reactive oxygen species, and so, oxidative stress increases in crops [46]. In this work, drought was found to elevate the levels of H_2O_2, MDA, and EL and suppress the POD activity in barley seedling leaves, while reducing activities of CAT and SOD compared to the control (Figure 2). Drought stress frequently results in an imbalance between reactive oxygen species (ROS) generation and the plant's capacity to neutralize them via antioxidant enzymes such as peroxidase (POD), hence diminishing its ability to detoxify hydrogen peroxide (H_2O_2) and other ROS [47]. The observed elevation in oxidative stress and decrease in CAT and SOD activity under drought stress have significantly impaired the plant's antioxidative system. Choudhury et al. (2022) revealed that drought stress leads to an elevation of APX, POD, and CAT activities while causing a decrease in SOD activity in Poaceae plants. The research findings indicate that drought stress leads to a rise in MDA levels and activities in POD and CAT in maize leaves under drought stress compared to the control [47]. Drought-induced oxidative stress increased MDA levels in Castor and Indian mustard, with the particular reaction varying bestowing to the species and development phase duration [48]. Ishfaq et al. [49] observed that drought stress induces a dose-additive rise in the CAT, POD, SOD, $O_2^{\bullet-}$, H_2O_2, and MDA activities in both the shoot and root of the Poaceae family compared to the control group. The application of Hibiscus cannabinus-biochar to barley plants under water-stress conditions can lead to improved physiological responses and antioxidant enzymatic activity. The biochar treatment can enhance the plant's antioxidant defense system, increasing the activity of enzymes such as superoxide dismutase, catalase, and peroxidase, which help mitigate oxidative stress caused by water stress. Additionally, kenaf-biochar can improve plant water status, reducing the negative impacts of drought on plant growth and physiology. The treatment can also modulate physiological responses, such as increasing stomatal conductance, photosynthetic rate, and chlorophyll content, ultimately leading to improved plant growth and resilience under water-stress conditions. By enhancing antioxidant enzymatic activity and physiological responses, kenaf-biochar can help barley plants better cope with water stress.

Biochar is a highly efficient soil supplement that enhances soil characteristics and effectively sustains soil production over a long period [50]. The application of biochar as a soil supplement has a beneficial impact on the plant's growth and development [51]. As biochar can elevate soil organic carbon (SOC) and soil organic matter (SOM) levels, while also augmenting the activity of soil microbes that facilitate nutrient availability [52]. In conditions of low soil water content, including drought stress, the buildup of OM can lead to a decrease in soil pH by releasing H^+ ions [53]. Furthermore, increased polarity of deposited cations caused by soil drying can induce a decrease in the soil pH values [54]. Furthermore, Chanthini et al. [55] revealed that a reduction in soil pH under drought stress may suggest that moisture increases the relationship between phosphatase activities in soil.

Nevertheless, biochar addition improved water-induced reductions in soil pH levels, primarily because of its alkaline nature and buffering capacity, along with its ability to influence soil properties and microbial communities [56]. The increase in soil pH resulting from biochar addition has been ascribed to the release of intrinsic alkaline compounds [19], as well as potentially elevated levels of calcium carbonate equivalent and calcium carbonate [57]. Su et al. [58] stated that biochar alkalinity is a critical determinant of its liming ability. The biochar addition impact on soil pH shows a positive correlation with biochar dose application under various soil water treatments in the current study.

Exposure to drought stress greatly reduced the nutrient levels (total nitrogen, mineral nitrogen, bray phosphorus, and exchangeable potassium) in soil, as compared to soils that were consistently irrigated, due to several interconnected physical, chemical, and biological processes such as (a) reduced nutrient mineralization, (b) impaired nutrient solubility and mobility (c) soil Drying and nutrient leaching (d) soil compaction and root activity (e) reduced cation exchange capacity that occur in the soil under water-limited conditions [59]. Under drought stress, decreased soil water level impedes microbial activity, leading to diminished breakdown of organic waste, according to Doroski et al. [60]. Phosphorus pool and soil respiration were reduced in soil with a lower water level (-1.700 MPa) compared to soil with an optimal water level (-0.078 MPa). Dry soil has reduced microbial activity and phosphorus availability in comparison to soils that are consistently irrigated [61]. The presence of higher nutrient levels in soil continuously watered soils may indicate the influence of soil microorganisms on the breakdown of SOM, leading to nutrient mineralization [62].

Biochar enhanced nutrient levels (mineral nitrogen, total nitrogen, Bray phosphorus, and exchangeable potassium) in both drought-stressed and continuously irrigated soils compared to untreated soils. Biochar is an effective amendment that boosts soil nutrient levels, improving plant growth and resilience in both drought-stressed and continuously irrigated soils by increasing nutrient availability and preventing nutrient losses [63]. Comparable findings were noted in the study conducted by Luo et al. [64]. Bankole et al. [65] also confirmed that the use of biochar enhanced the retention of nitrogen and SOM in the soil, leading to enhanced soil WUE. The increased soil nutrient availability caused by biochar under consistent drought and watering-imposed treatments may be attributed to either the release of existing nutrients from char or the accelerated breakdown of soil organic matter, known as "soil priming" [66]. Furthermore, Priya et al. [67] revealed that biochar addition to the soil increased nutrient availability and improved soil fertility. Significant influence on the nutrient levels released into amended soil, attributed to the biochar production temperature as well as feedstock [63]. The application of Hibiscus cannabinus-biochar can positively impact microbial activity and nutrient cycling in soil. The biochar provides a habitat for beneficial microorganisms, increasing microbial abundance and diversity, which can enhance nutrient cycling and availability. Kenaf-biochar's porous structure and high surface area can also adsorb and retain nutrients, reducing leaching and making them available to microorganisms and plants. This can lead to improved soil fertility, structure, and overall ecosystem health. Additionally, the biochar can stimulate microbial processes, such as nitrogen fixation, decomposition, and solubilization of minerals, further enhancing nutrient cycling and availability for plant uptake. By promoting beneficial microbial activity and nutrient cycling, kenaf biochar can contribute to a more sustainable and resilient soil ecosystem

Drought stress reduces the size of macroaggregates in the soil organic carbon pool, which subsequently impacts soil stability [68]. Biological carbon metabolism is extremely responsive to soil moisture levels [69]. Dissolved organic carbon (DOC), soil organic carbon (SOC), and dissolved organic nitrogen (DON) are interconnected with soil moisture availability and are all diminished under drought stress. Conversely, Nazir et al. [70] concluded that drought stress led to a rise in dissolved organic carbon (DOC) generation from marshland. Bai et al. [71] found that biochar contains carbon, so its incorporation into the soil also improves the soil's organic carbon and DOC. Furthermore, biochar increases organic matter levels in soil and promotes microbial activity, therefore enhancing the dissolved organic nitrogen levels. Biochar facilitates habitats for microorganisms owing to its porous structure and by encouraging the development of stable soil micro-aggregates. Biochar functions as a source of carbon and nutrients. Biochar modifies the physical and chemical characteristics of soil, establishing optimal conditions for microbial diversity [72]. The influence of soil moisture availability on nitrogen mineralization was documented by Naorem [68]. These conditions lead to a surge in nitrogen loss because of increased synchronicity between nitrogen generated via mineralization and its uptake by plants. Any soil additives capable of augmenting soil OM have the potential to alleviate nitrogen loss. By enhancing SOM and reducing nitrogen loss, the use of biochar also promotes nitrogen mineralization in soil [72].

Therefore, soil respiration is crucial for the long-term development of plants, microbes, and soil fauna. This parameter quantifies the speed at which SOM breaks down and the activities of microbes [73]. In this work, soil respiration was reduced by 7.11% and 11.01% during the tillering and grain-filling stages, respectively, under drought stress. However, after biochar was utilized and then exposed to drought stress at similar stages, much greater soil respiration was observed. The findings of Raj et al. [74] demonstrated that reduced soil moisture leads to a decline in soil microbial activity. Therefore, a higher number of microbes and roots of plants allocate energy towards the synthesis of protective chemicals, which in turn reduce soil respiration.

The soil microbial biomass is crucial in the process of organic matter decomposition. Enhanced soil fertility and increased soil nutrient availability are facilitated by higher microbial biomass. Furthermore, it acts as an association between soil nutrient sources and sinks [75]. Prasad et al. [76] documented that drought stress induces osmotic stress, leading to microbial mortality and a decrease in microbial biomass. A significant reduction in organic matter decomposition was observed under drought stress. Numerous studies have documented that biochar addition has the potential to increase microbial activities, nutrient levels, and organic matter in soil. Thus, this leads to an enhancement in soil microbial biomass, hence promoting plant growth and soil fertility.

Research findings indicate that drought stress leads to a significant reduction in soil enzymatic activities. Furthermore, Chen et al. [77] observed a decline in enzymatic activity in soil due to a climatic gradient under conditions of limited drought stress. Insufficient soil water availability reduces soil microbial processes responsible for recycling different soil nutrients. Various levels of soil nutrient availability and plant growth requirements at various phases of barley growth of barley result in distinct impacts of water stress on soil enzymatic activity. A reduction in enzymatic activity is found when there is nitrogen availability during drought stress, as described by Muhammad et al. [78]. Consistent with previous studies, our results indicate that dehydrogenase and phosphate activities, β-glucosidase, and urease are positively associated with soil water availability [79]. The biochar addition also enhances organic carbon content, which can greatly benefit the improvement of soil microflora [80]. Microbial processes in soil also improve the organic matter and nutrient content of soil. Several studies have also documented that biochar addition in soil leads to an increase in organic matter, which in turn improves soil enzymatic activity and yields positive effects on plants [81]. Babur et al. [82] presented that biochar addition as a soil conditioner enhances the levels of soil organic matter, which improves soil quality and health.

5. Conclusion

The current study results indicated that the application of biochar soil effectively enhanced barley growth and yield while alleviating the impact of drought stress on the plants. Under conditions of drought stress, biochar addition reduced oxidative stress and increased the activity of antioxidant enzymes. In comparison to control and drought treatments, the chlorophyll levels and gas exchange characteristics were enhanced. The biochar addition was observed to have a significant impact on the soil biochemical characteristics. The majority of organic matter exhibited a significant increase with biochar application. The biochar application under drought-stress environments has the potential to enhance both plant growth and soil chemical characteristics. Under drought stress, biochar addition at a dose of 40 g kg^{-1} of soil is more beneficial compared to a rate of 30 g kg^{-1}. Therefore, it is advisable for farmers and researchers exploring crops to employ biochar, particularly under drought stress, to enhance plant growth and as an alternative technique for enhancing nutrient availability for plant growth.

Author Contributions

Conceptualization, G.M., and M.U.; methodology, software, validation, R.I.; formal analysis, G.M.; investigation, G.M. and M.U.; resources, G.M; data curation, writing-original draft preparation, R.I.; writing-review, editing, G.M., and R.I.; All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors stated that they had no interest that might be perceived as posing a conflict or bias.

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References

[1] Kaur A, Purewal SS, Phimolsiripol Y, Punia Bangar S. Unraveling the hidden potential of barley (Hordeum vulgare): an important review. Plants. 2024 Aug 30;13(17):2421. https://doi.org/10.3390/plants13172421

[2] H Pangaribuan D, Widagdo S, Muhammad Hariri A, Siregar S. The effect of rice straw mulch and cow urine on growth, yield, quality on sweet corn and pest population density. Asian Journal of Agriculture and Biology. 2022(Online). https://doi.org/10.35495/ajab.2021.03.123

[3] Visioni A, Basile B, Amri A, Sanchez-Garcia M, Corrado G. Advancing the conservation and utilization of barley genetic resources: insights into germplasm management and breeding for sustainable agriculture. Plants. 2023 Sep 6;12(18):3186. https://doi.org/10.3390/plants12183186

[4] Zewodu A, Mohammed W, Shiferaw E. Analysis of genetic diversity and population structure of some Ethiopian barley (Hordeum vulgare L.) accessions using SSR markers. Plos one. 2024 Jun 25;19(6):e0305945. https://doi.org/10.1371/journal.pone.0305945

[5] He M, Cui J, Yi Y, Chen HW, Zhang Q, Li L, Huang L, Hong S. Vegetation increases global climate vulnerability risk by shifting climate zones in response to rising atmospheric CO2. Science of The Total Environment. 2024 Nov 1;949:174810. https://doi.org/10.1016/j.scitotenv.2024.174810

[6] Fatemi R, Yarnia M, Mohammadi S, Vand EK, Mirashkari B. AJAB. Asian J Agric & Biol. 2023(2). https://doi.org/10.35495/ajab.2022.071

[7] Sallam A, Alqudah AM, Dawood MF, Baenziger PS, Börner A. Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. International journal of molecular sciences. 2019 Jun 27;20(13):3137. https://doi.org/10.3390/ijms20133137

[8] Wahab A, Abdi G, Saleem MH, Ali B, Ullah S, Shah W, Mumtaz S, Yasin G, Muresan CC, Marc RA. Plants' physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: A comprehensive review. Plants. 2022 Jun 21;11(13):1620. https://doi.org/10.3390/plants11131620

[9] Fu Y, Li P, Mounkaila Hamani AK, Wan S, Gao Y, Wang X. Effects of single and combined drought and salinity stress on the root morphological characteristics and root hydraulic conductivity of different winter wheat varieties. Plants. 2023 Jul 19;12(14):2694. https://doi.org/10.3390/plants12142694

[10] Kiryushin VI. The management of soil fertility and productivity of agrocenoses in adaptive-landscape farming systems. Eurasian Soil Science. 2019 Sep;52(9):1137-45. https://doi.org/10.1134/s1064229319070068

[11] Garai S, Mondal M, Nayak J, Sarkar S, Banerjee H, Brahmachari K, Hossain A. Input use efficiency for improving soil fertility and productivity. In Input use efficiency for food and environmental security 2022 Jan 11 (pp. 305-333). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-16-5199-1_10

[12] Vasu D, Tiwary P, Chandran P, Singh SK. Soil quality for sustainable agriculture. In Nutrient dynamics for sustainable crop production 2019 Sep 7 (pp. 41-66). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-8660-2_2

[13] Mohanty LK, Singh NK, Raj P, Prakash A, Tiwari AK, Singh V, Sachan P. Nurturing crops, enhancing soil health, and sustaining agricultural prosperity worldwide through agronomy. Journal of Experimental Agriculture International. 2024 Feb 3;46(2):46-67. https://doi.org/10.9734/jeai/2024/v46i22308

[14] Murtaza G, Ahmed Z, Usman M, Tariq W, Ullah Z, Shareef M, Iqbal H, Waqas M, Tariq A, Wu Y, Zhang Z. Biochar induced modifications in soil properties and its impacts on crop growth and production. Journal of plant nutrition. 2021 May 7;44(11):1677-91. https://doi.org/10.1080/01904167.2021.1871746

[15] Murtaza G, Ahmed Z, Dai DQ, Iqbal R, Bawazeer S, Usman M, Rizwan M, Iqbal J, Akram MI, Althubiani AS, Tariq A. A review of mechanism and adsorption capacities of biochar-based engineered composites for removing aquatic pollutants from contaminated water. Frontiers in Environmental Science. 2022 Nov 14;10:1035865. https://doi.org/10.3389/fenvs.2022.1035865

[16] Murtaza G, Ahmed Z, Eldin SM, Ali B, Bawazeer S, Usman M, Iqbal R, Neupane D, Ullah A, Khan A, Hassan MU. Biochar-Soil-Plant interactions: A cross talk for sustainable agriculture under changing climate. Frontiers in Environmental Science. 2023 Feb 14;11:1059449. https://doi.org/10.3389/fenvs.2023.1059449

[17] Kabir E, Kim KH, Kwon EE. Biochar as a tool for the improvement of soil and environment. Frontiers in Environmental Science. 2023 Dec 18;11:1324533. https://doi.org/10.3389/fenvs.2023.1324533

[18] Nepal J, Ahmad W, Munsif F, Khan A, Zou Z. Advances and prospects of biochar in improving soil fertility, biochemical quality, and environmental applications. Frontiers in Environmental Science. 2023 Feb 16;11:1114752. https://doi.org/10.3389/fenvs.2023.1114752
[19] Murtaza G, Ahmed Z, Usman M, Iqbal R, Zulfiqar F, Tariq A, Ditta A. Physicochemical properties and performance of non-woody derived biochars for the sustainable removal of aquatic pollutants: A systematic review. Chemosphere. 2024 Jul 1;359:142368. https://doi.org/10.1016/j.chemosphere.2024.142368
[20] Osei AF, Jin XL, Abdullah WZ, Sidique SN. Silicon improves strawberry plants nutrient uptake and epicuticular wax formation in a rhizosphere cooling system. Asian J. Agric. Biol. 2023 Jan (2): 2022060. https://doi.org/10.35495/ajab.2022.060
[21] Hamani AK, Wang G, Soothar MK, Shen X, Gao Y, Qiu R, Mehmood F. Responses of leaf gas exchange attributes, photosynthetic pigments and antioxidant enzymes in NaCl-stressed cotton (Gossypium hirsutum L.) seedlings to exogenous glycine betaine and salicylic acid. BMC Plant Biology. 2020 Sep 21;20(1):434. https://doi.org/10.1186/s12870-020-02624-9
[22] Cuesta B, Vega J, Villar-Salvador P, Rey-Benayas JM. Root growth dynamics of Aleppo pine (Pinus halepensis Mill.) seedlings in relation to shoot elongation, plant size and tissue nitrogen concentration. Trees. 2010 Oct;24(5):899-908. https://doi.org/10.1007/s00468-010-0459-0
[23] Mendes R, Cardoso C, Pestana C. Measurement of malondialdehyde in fish: A comparison study between HPLC methods and the traditional spectrophotometric test. Food Chemistry. 2009 Feb 15;112(4):1038-45. https://doi.org/10.1016/j.foodchem.2008.06.052
[24] Pan Y, Wu LJ, Yu ZL. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (Glycyrrhiza uralensis Fisch). Plant Growth Regulation. 2006 Jul;49(2):157-65. https://doi.org/10.1007/s10725-006-9101-y
[25] GE TD, SUI FG, BAI LP, LU YY, Zhou GS. Effects of water stress on the protective enzyme activities and lipid peroxidation in roots and leaves of summer maize. Agricultural Sciences in China. 2006 Apr;5(4):291-8. https://doi.org/10.1016/s1671-2927(06)60052-7
[26] Sparks DL, Page AL, Helmke PA, Loeppert RH, editors. Methods of soil analysis, part 3: Chemical methods. John Wiley & Sons; 2020 Jan 22.
[27] Mulvaney RL. Nitrogen—inorganic forms. Methods of soil analysis: Part 3 Chemical methods. 1996 Jan 1;5:1123-84. https://doi.org/10.2136/sssabookser5.3.c38
[28] Smolander A, Kitunen V. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. Soil Biology and Biochemistry. 2002 May 1;34(5):651-60. https://doi.org/10.1016/s0038-0717(01)00227-9
[29] Vance ED, Brookes PC, Jenkinson DS. An extraction method for measuring soil microbial biomass C. Soil biology and Biochemistry. 1987 Jan 1;19(6):703-7. https://doi.org/10.1016/0038-0717(87)90052-6
[30] Tabatabai MA. Soil enzymes. Methods of soil analysis: Part 2 Microbiological and biochemical properties. 1994 Jan 1;5:775-833. https://doi.org/10.2136/sssabookser5.2.c37
[31] Katohar I, Soothar RK, Chandio FA, Talpur MA, Soomro SA, Singha A, Bin L, Mirjat MU. Drought priming and subsequent irrigation water regimes enhanced grain yield and water productivity of wheat crop. Water. 2023 Oct 23;15(20):3704. https://doi.org/10.3390/w15203704
[32] Ashfaq W, Brodie G, Fuentes S, Pang A, Gupta D. Silicon improves root system and canopy physiology in wheat under drought stress. Plant and Soil. 2024 Sep;502(1):279-96. https://doi.org/10.1007/s11104-023-06202-4
[33] Matías J, Rodríguez MJ, Carrillo-Vico A, Casals J, Fondevilla S, Haros CM, Pedroche J, Aparicio N, Fernández-García N, Aguiló-Aguayo I, Soler-Rivas C. From 'farm to fork': Exploring the potential of nutrient-rich and stress-resilient emergent crops for sustainable and healthy food in the Mediterranean region in the face of climate change challenges. Plants. 2024 Jul 11;13(14):1914. https://doi.org/10.3390/plants13141914
[34] Seleiman MF, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH, Battaglia ML. Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants. 2021 Jan 28;10(2):259. https://doi.org/10.3390/plants10020259
[35] Martínez-Gómez Á, Poveda J, Escobar C. Overview of the use of biochar from main cereals to stimulate plant growth. Frontiers in Plant Science. 2022 Aug 2;13:912264. https://doi.org/10.3389/fpls.2022.912264

[36] Kondrlova E, Horak J, Igaz D. Effect of biochar and nutrient amendment on vegetative growth of spring barley ('Hordeum vulgare'L. var. Malz). Australian journal of crop science. 2018 Feb 1;12(2):178-84. https://doi.org/10.21475/ajcs.18.12.02.pne476
[37] Kumar A, Bhattacharya T, Shaikh WA, Roy A. Sustainable soil management under drought stress through biochar application: Immobilizing arsenic, ameliorating soil quality, and augmenting plant growth. Environmental Research. 2024 Oct 15;259:119531. https://doi.org/10.1016/j.envres.2024.119531
[38] Rao MJ, Zheng B. The role of polyphenols in abiotic stress tolerance and their antioxidant properties to scavenge reactive oxygen species and free radicals. Antioxidants. 2025 Jan 10;14(1):74. https://doi.org/10.3390/antiox14010074
[39] Murtaza G, Rizwan M, Usman M, Hyder S, Akram MI, Deeb M, Alkahtani J, AlMunqedhi BM, Hendy AS, Ali MR, Iqbal R. Biochar enhances the growth and physiological characteristics of Medicago sativa, Amaranthus caudatus and Zea mays in saline soils. BMC Plant Biology. 2024 Apr 22;24(1):304. https://doi.org/10.1186/s12870-024-04957-1
[40] Mahmoud AW, Samy MM, Sany H, Eid RR, Rashad HM, Abdeldaym EA. Nanopotassium, nanosilicon, and biochar applications improve potato salt tolerance by modulating photosynthesis, water status, and biochemical constituents. Sustainability. 2022 Jan 10;14(2):723. https://doi.org/10.3390/su14020723
[41] Wu Y, Wang X, Zhang L, Zheng Y, Liu X, Zhang Y. The critical role of biochar to mitigate the adverse impacts of drought and salinity stress in plants. Frontiers in Plant Science. 2023 May 8;14:1163451. https://doi.org/10.3389/fpls.2023.1163451
[42] Naeem MB, Jahan S, Rashid A, Shah AA, Raja V, El-Sheikh MA. Improving maize yield and drought tolerance in field conditions through activated Biochar application. Scientific Reports. 2024 Oct 23;14(1):25000. https://doi.org/10.1038/s41598-024-76082-w
[43] Ali AB, Elshaikh NA. Performance of biochar under diminish water stress in plants. Communications in Soil Science and Plant Analysis. 2022 Jan 2;53(1):1-6. https://doi.org/10.1080/00103624.2021.1984508
[44] Rezayian M, Niknam V, Ebrahimzadeh H. Oxidative damage and antioxidative system in algae. Toxicology reports. 2019 Jan 1;6:1309-13. https://doi.org/10.1016/j.toxrep.2019.10.001
[45] Biswas MS, Mano JI. Lipid peroxide-derived reactive carbonyl species as mediators of oxidative stress and signaling. Frontiers in plant science. 2021 Oct 28;12:720867. https://doi.org/10.3389/fpls.2021.720867
[46] Tabassum, Jeena AS, Pandey D. Metal induced genotoxicity and oxidative stress in plants, assessment methods, and role of various factors in genotoxicity regulation. In Induced genotoxicity and oxidative stress in plants 2021 Aug 4 (pp. 133-149). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-16-2074-4_5
[47] Zeeshan M, Wang X, Salam A, Wu H, Li S, Zhu S, Chang J, Chen X, Zhang Z, Zhang P. Selenium nanoparticles boost the drought stress response of soybean by enhancing pigment accumulation, oxidative stress management and ultrastructural integrity. Agronomy. 2024 Jun 26;14(7):1372. https://doi.org/10.3390/agronomy14071372
[48] Nalina M, Saroja S, Chakravarthi M, Rajkumar R, Radhakrishnan B, Chandrashekara KN. Water deficit-induced oxidative stress and differential response in antioxidant enzymes of tolerant and susceptible tea cultivars under field condition. Acta Physiologiae Plantarum. 2021 Jan;43(1):10. https://doi.org/10.1007/s11738-020-03174-1
[49] Ishfaq N, Waraich EA, Ahmad M, Hussain S, Zulfiqar U, Din KU, Haider A, Yong JW, Askri SM, Ali HM. Mitigating drought-induced oxidative stress in wheat (Triticum aestivum L.) through foliar application of sulfhydryl thiourea. Scientific Reports. 2024 Jul 10;14(1):15985. https://doi.org/10.1038/s41598-024-66506-y
[50] Ojuederie OB, Olanrewaju OS, Babalola OO. Plant growth promoting rhizobacterial mitigation of drought stress in crop plants: implications for sustainable agriculture. Agronomy. 2019 Nov 4;9(11):712. https://doi.org/10.3390/agronomy9110712
[51] Zhang K, Khan Z, Khan MN, Luo T, Luo L, Bi J, Hu L. The application of biochar improves the nutrient supply efficiency of organic fertilizer, sustains soil quality and promotes sustainable crop production. Food and Energy Security. 2024 Jan;13(1):e520. https://doi.org/10.1002/fes3.520

[52] Thapa RB, Coupal RH, Dangi MB, Stahl PD. An assessment of plant growth and soil properties using coal char and biochar as a soil amendment. Agronomy. 2024 Feb 1;14(2):320. https://doi.org/10.3390/agronomy14020320

[53] Pratt C, Kingston K, Laycock B, Levett I, Pratt S. Geo-agriculture: reviewing opportunities through which the geosphere can help address emerging crop production challenges. Agronomy. 2020 Jul 6;10(7):971. https://doi.org/10.3390/agronomy10070971

[54] Suddarth SR, Ferreira JF, Cavalcante LF, Fraga VS, Anderson RG, Halvorson JJ, Bezerra FT, Medeiros SA, Costa CR, Dias NS. Can humic substances improve soil fertility under salt stress and drought conditions?. Journal of Environmental Quality. 2019 Nov;48(6):1605-13. https://doi.org/10.2134/jeq2019.02.0071

[55] Chanthini KM, Pavithra GS, Murugan P, Malarvizhi P, Deva-Andrews A, Ramasubramanian R, Thulasi-Raman N, Malafaia G, Senthil-Nathan S, Prockow J. Management of excessive soil H+ ion induced toxicities by application of organic seaweed amendment enhances photosynthesis and resource use efficiencies in rice (Oryza sativa). Environmental research. 2024 Apr 15;247:118179. https://doi.org/10.1016/j.envres.2024.118179

[56] Dong Y, Zhang X, Wang X, Xie C, Liu J, Cheng Y, Yue Y, You X, Li Y. Modified biochar affects CO2 and N2O emissions from coastal saline soil by altering soil pH and elemental stoichiometry. Science of the Total Environment. 2024 Dec 1;954:176283. https://doi.org/10.1016/j.scitotenv.2024.176283

[57] Wang X, Ding J, Han L, Tan J, Ge X, Nan Q. Biochar addition reduces salinity in salt-affected soils with no impact on soil pH: A meta-analysis. Geoderma. 2024 Mar 1;443:116845. https://doi.org/10.1016/j.geoderma.2024.116845

[58] Su Z, Liu X, Wang Z, Wang J. Biochar effects on salt-affected soil properties and plant productivity: A global meta-analysis. Journal of Environmental Management. 2024 Aug 1;366:121653. https://doi.org/10.1016/j.jenvman.2024.121653

[59] de Faria Melo CC, Amaral DS, de Mello Prado R. The role of mineral nutrients in plant growth under drought stress. In Sustainable Agriculture Under Drought Stress 2025 Jan 1 (pp. 195-207). Academic Press. https://doi.org/10.1016/b978-0-443-23956-4.00013-2

[60] Doroski DA, Bradford MA, Duguid MC, Hallett RA, Pregitzer CC, Ashton MS. Diverging conditions of current and potential future urban forest patches. Ecosphere. 2022 Mar;13(3):e4001. https://doi.org/10.1002/ecs2.4001

[61] Siebielec S, Siebielec G, Klimkowicz-Pawlas A, Gałązka A, Grządziel J, Stuczyński T. Impact of water stress on microbial community and activity in sandy and loamy soils. Agronomy. 2020 Sep 19;10(9):1429. https://doi.org/10.3390/agronomy10091429

[62] Prudent M, Dequiedt S, Sorin C, Girodet S, Nowak V, Duc G, Salon C, Maron PA. The diversity of soil microbial communities matters when legumes face drought. Plant, Cell & Environment. 2020 Apr;43(4):1023-35. https://doi.org/10.1111/pce.13712

[63] Cao X, Zhang J, Yu Y, Ma Q, Kong Y, Pan W, Wu L, Jin Q. Alternate wetting–drying enhances soil nitrogen availability by altering organic nitrogen partitioning in rice-microbe system. Geoderma. 2022 Oct 15;424:115993. https://doi.org/10.1016/j.geoderma.2022.115993

[64] Luo X, Chen W, Liu Q, Wang X, Miao J, Liu L, Zheng H, Liu R, Li F. Corn straw biochar addition elevated phosphorus availability in a coastal salt-affected soil under the conditions of different halophyte litter input and moisture contents. Science of The Total Environment. 2024 Jan 15;908:168355. https://doi.org/10.1016/j.scitotenv.2023.168355

[65] Bankole GO, Aghorunse AC, Azeez JO. Nitrogen, phosphorus and sulphur mineralization kinetic as affected by biochar, manure and ash amendments—An incubation study. Arabian Journal of Geosciences. 2024 May;17(5):148. https://doi.org/10.1007/s12517-024-11942-3

[66] Aboagye DA, Adjadeh WT, Nartey EK, Asuming-Brempong S. Co-application of biochar compost and inorganic nitrogen fertilizer affects the growth and nitrogen uptake by lowland rice in northern Ghana. Nitrogen. 2022 Jul 6;3(3):414-25. https://doi.org/10.3390/nitrogen3030027

[67] Priya S, Somenahally AC, Obayomi O, Gentry TJ, Sarker TC, Brady JA, Adams CB. Exploring the application of signaling compounds and soil amendments to modulate plant–microbe interactions for improved plant salinity tolerance. Plant and Soil. 2024 Aug;501(1):267-88. https://doi.org/10.1007/s11104-024-06512-1

[68] Naorem A, Jayaraman S, Dang YP, Dalal RC, Sinha NK, Rao CS, Patra AK. Soil constraints in an arid environment—challenges, prospects, and implications. Agronomy. 2023 Jan 11;13(1):220. https://doi.org/10.3390/agronomy13010220
[69] Thotakuri G, Angidi S, Athelly A. Soil carbon pool as influenced by soil microbial activity—an overview. American Journal of Climate Change. 2024 Jun 17;13(02):175-93. https://doi.org/10.4236/ajcc.2024.132010
[70] Nazir MJ, Li G, Nazir MM, Zulfiqar F, Siddique KH, Iqbal B, Du D. Harnessing soil carbon sequestration to address climate change challenges in agriculture. Soil and Tillage Research. 2024 Mar 1;237:105959. https://doi.org/10.1016/j.still.2023.105959
[71] Bai J, Huang Y, Bai Y, Chen D, Haider S, Song J, Moreira BR, Ren G, Yang G, Feng Y, Wang X. Impact of straw-biochar amendments on microbial activity and soil carbon dynamics in wheat-maize system. Soil and Tillage Research. 2024 Dec 1;244:106284. https://doi.org/10.1016/j.still.2024.106284
[72] Xiao Y, Wang M, Song B, Zhang X, Zhuang W. The effect of moisture on nitrogen acquisition strategies of ephemeral desert plants in north-western China are regulated by temperature. Environmental and Experimental Botany. 2024 Jun 1;222:105219. https://doi.org/10.1016/j.envexpbot.2023.105219
[73] Datt N, Singh D. Enzymes in relation to soil biological properties and sustainability. In Sustainable management of soil and environment 2019 Oct 3 (pp. 383-406). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-8832-3_11
[74] Raj A, Jhariya MK, Yadav DK, Banerjee A, Meena RS. Soil for sustainable environment and ecosystems management. In Sustainable agriculture, forest and environmental management 2019 May 29 (pp. 189-221). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-6830-1_6
[75] Dhaliwal SS, Dubey SK, Kumar D, Toor AS, Walia SS, Randhawa MK, Kaur G, Brar SK, Khambalkar PA, Shivey YS. Enhanced organic carbon triggers transformations of macronutrients, micronutrients, and secondary plant nutrients and their dynamics in the soil under different cropping Systems-A review. Journal of Soil Science and Plant Nutrition. 2024 Sep;24(3):5272-92. https://doi.org/10.1007/s42729-024-01907-6
[76] Prasad S, Malav LC, Choudhary J, Kannojiya S, Kundu M, Kumar S, Yadav AN. Soil microbiomes for healthy nutrient recycling. In Current trends in microbial biotechnology for sustainable agriculture 2020 Nov 11 (pp. 1-21). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-15-6949-4_1
[77] Chen Q, Cao X, Nie X, Li Y, Liang T, Ci L. Alleviation role of functional carbon nanodots for tomato growth and soil environment under drought stress. Journal of hazardous materials. 2022 Feb 5;423:127260. https://doi.org/10.1016/j.jhazmat.2021.127260
[78] Muhammad I, Lv JZ, Yang L, Ahmad S, Farooq S, Zeeshan M, Zhou XB. Low irrigation water minimizes the nitrate nitrogen losses without compromising the soil fertility, enzymatic activities and maize growth. BMC Plant Biology. 2022 Apr 1;22(1):159. https://doi.org/10.1186/s12870-022-03548-2
[79] Baloch H, Sabir IA, Leghari SK, Saddiq MS, Alam P, Khan S, Fatima EM, Sajid M, Raza MH, Hussain MA, Ayoub M. AJAB. Asian J Agric & Biol. 2024(4). https://doi.org/10.35495/ajab.2023.328
[80] Woźniak MM, Siebielec S, Siebielec G, Bojarszczuk J, Gałązka A, Urbaniak M. Microbially modified effect of exogenous organic matter on soil chemical and biological indices and plant responses. Journal of Soils and Sediments. 2024 Jan;24(1):70-85. https://doi.org/10.1007/s11368-023-03632-6
[81] Hidayat F, Pane RD, Sapalina F, Listia E, Koga T, Winarna, Lubis ME, Oshiro M, Sakai K, Utami SN, Tashiro Y. Long-term application of organic matter improves soil properties and plant growth-promoting bacteria in soil communities of oil palm plantation. Soil Science and Plant Nutrition. 2024 Nov 1;70(5-6):393-405. https://doi.org/10.1080/00380768.2024.2380881
[82] Babur E, Dindaroğlu T, Roy R, Seleiman MF, Ozlu E, Battaglia ML, Uslu ÖS. Relationship between organic matter and microbial biomass in different vegetation types. In Microbial syntrophy-mediated eco-enterprising 2022 Jan 1 (pp. 225-245). Academic Press. https://doi.org/10.1016/b978-0-323-99900-7.00005-5