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EFFECTS OF BIOCHAR AMENDMENT, CO_2 ELEVATION AND DROUGHT ON LEAF GAS EXCHANGE, BIOMASS PRODUCTION AND WATER USE EFFICIENCY IN MAIZE

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Abstract

The effects of biochar amendment, CO_2 concentration ([CO_2]), drought and their interactions on the growth and physiology of maize were investigated. Maize was grown in pots with soil treated with (2%, w/w) or without biochar in one greenhouse chamber at a [CO_2] of 400 mol l^{-1}, and in a second chamber at a [CO_2] of 800 mol l^{-1}. At 19 days after planting, the plants were subjected to one of two watering regimes for one week, each plant either being well-watered or being drought-stressed by withholding irrigation. Before starting drought treatment, biochar amendment increased root dry biomass (RDM) and the root to shoot ratio (RSR), while CO_2 elevation increased leaf area (LA) and specific leaf area (SLA) but decreased the chlorophyll content index (CCI) and stomatal conductance (g_s). After drought, elevated [CO_2] increased RDM, RSR, LA, SLA and photosynthetic rate (A_n), but decreased root water potential (Ψ_r). A clear tendency of increasing water use efficiency (WUE) was noticed in maize grown under elevated [CO_2] (P =0.080). Biochar amendment reduced evapotranspiration (ET). Drought decreased shoot dry biomass, total dry biomass, LA, A_n, g_s, Ψ_r and ET, but increased intrinsic WUE. It was concluded that elevated [CO_2] enhanced maize growth and WUE, and biochar amendment tended to ameliorate some negative effects of drought stress on both growth and physiology under both ambient and elevated [CO_2].

Keywords: Climate change, Drought, Photosynthetic rate (A_n), Stomatal conductance (gs), Root water potential (Ψ_r), Water use efficiency (WUE).

Introduction

Anthropogenically induced global climate change is likely to result in a high frequency of extreme drought in many regions (Burke *et al*., 2006). It is therefore necessary to understand plant reaction to water deficiency as well as [CO_2] elevation under future climate scenarios. The increase of anthropogenic atmospheric CO_2 concentration ([CO_2]) has direct effects on photosynthesis, crop growth and productivity (Schapendonk *et al*., 2000; Jaggard *et al*., 2010). Many researchers report that increased [CO_2] could lead to greater water use efficiency (WUE) in crops (Magliulo *et al*., 2003; Leakey *et al*., 2006; Fleisher *et al*., 2008; Jaggard *et al*., 2010). Several mechanisms may be involved in the effect. First, the net CO_2 assimilation rate (A_n), growth and yield could be stimulated directly by elevated [CO_2] as a result of the decrease in photorespiration and acceleration of the rubisco carboxylation rate. Furthermore, stomatal conductance (g_s) could be decreased, leading to a decrease in the transpiration rate, which may reduce plant water use and thus possibly ameliorate the negative effect of water stress.

A large body of research indicates that both rising [CO_2] and greater water deficiency could result in significant increases in intrinsic water use efficiency (WUE_i, A_n/g_s) and plant water use efficiency (WUE_p, plant biomass increment/accumulated plant water use) (Kumar *et al*., 2014; Pazzagli *et al*., 2016). On the other hand, biochar amendments could possibly enhance soil water holding capacity (Karhu *et al*., 2011; Streubel *et al*., 2011; Basso *et al*., 2013; Akhtar *et al*., 2014). Pore size distribution could be altered by the application of biochar, resulting in enhanced soil water retention (Sukartono *et al*., 2012). This implies that biochar amendment could help soil retain more water from both rainfall and irrigation, which will reduce the external water demand without sacrificing crop production (Jeffery *et al*., 2011). Studies have also shown that increasing soil water holding capacity by the amendment of biochar can help to improve water use efficiency in crop production (Akhtar *et al*., 2014).

Recently, integrated approaches have been developed to improve crop productivity under drought stress or other abiotic stress conditions. The use of biochar could be an effective part of such an approach to maintain crop yield under drought in a future climate with elevated [CO_2]. This study aimed to examine biochar effects on maize growth and physiology under drought and elevated [CO_2]. It was hypothesized that biochar addition could increase WUE under elevated [CO_2] and drought-stressed conditions.

Materials and Methods

Plant material and treatments: During the period between April and May, 2016, maize plants were grown in two greenhouse chambers at the experimental farm of the University of Copenhagen, Denmark. Uniform maize seedlings were selected and transferred to plastic pots with 0.320 kg of sandy loam soil, which had been sieved through a 2-mm mesh. The pot water holding capacity was 32.0% (v/v). Air temperature was maintained at 22±2°C in the day, 16±2°C at night and 60% relative humidity. The photoperiod was 15 h per day, and the photosynthetic active radiation was above 500 µmol m^{-2} s^{-1} by sunlight and meta-halide lamps. Maize plants were randomly assigned to 8 treatments, each with 8-12 replicates. In one chamber, the plants were grown with ambient [CO_2] (400 µmol l^{-1}) and in the other chamber plants were grown under elevated

$[CO_2]$ (800 µmol l^{-1}). The $[CO_2]$ in the chambers was calibrated using a CO_2 Transmitter Series GMT220 (Vaisala Group, Helsinki, Finland).

In each of the chambers, half of the plants were grown with the addition of biochar at the rate of 2% by weight (B2) and half without biochar as controls (B0). The biochar used, from the UK Biochar Research Centre, was of soft wood pellets and had been produced at 550°C. After crushing into fine powder, biochar and soil were mixed thoroughly to fill the pot. The basic properties of the biochar are shown in Table 1. For the first 18 days after planting, all plants were well-watered to 95% of pot holding capacity. From day 19 onwards, the maize was subjected to two watering treatments: well-watered (W) control group, with 95% of water holding capacity maintained per day by irrigating the plants; and drought-stressed (D) group, with the plants receiving no irrigation water until 30% of pot holding capacity was reached. The pots in the two chambers were randomly positioned on benches with 8-12 replications of each treatment. Two harvests of four random replicates of each treatment were conducted: one was taken 19 days after planting, just before the watering treatments started (H1), and the other was taken 26 days after planting (H2).

Table 1. Physicochemical properties of biochar used in the present study

Attributes	Biochar
Moisture, wt% (a.r.)	1.52
C_{tot}, wt% (d.b.)	85.52
H, wt% (d.b.)	2.77
O (by difference), wt% (d.b.)	10.36
Total ash, wt% (d.b.)	1.25
Total N, wt% (d.b.)	<0.10
Mineral N, mg/kg (d.b.)	<3
Total P, wt% (d.b.)	0.06
Total K, wt% (d.b.)	0.25
Volatile Matter, wt% (d.b.)	14.20
Total Surface Area, m^2/g (d.b.)	26.40
pH	7.91
EC, dS/m	0.09

Measurements: Four physiological indices were measured at each harvest time. A portable LiCor-6400 photosynthetic system was used to determine leaf A_n and g_s when upper leaves were fully expanded during the period between 11:00 and 14:00 h. Measurements were performed on one leaf per plant at 25°C chamber temperature and 1000 µmol m^{-2} s^{-1} photon flux density, and at a $[CO_2]$ of 400 µmol l^{-1} for ambient $[CO_2]$ and 800 µmol l^{-1} for elevated $[CO_2]$ treatments, respectively. A portable CCM-200 chlorophyll content meter (Opti-Science, Tyngsboro, MA, USA) was used to measure the chlorophyll content index (CCI) of the leaf, and a LI-3100C area meter (Li- Cor Lin- coln, NE, USA) was used to measure leaf area (LA).

The pot weight was recorded every day to measure the accumulative evapotranspiration (ET). Shoot was cut at a point 2–3 cm from the stem base, and a Scholander-type chamber from Soil Moisture Equipment Corp., USA, was used to pressurize roots of the uprooted plants at each harvest time to determine root water potential (Ψ_r). At each harvest, root, stem and leaf samples were collected. Roots and soil were separated carefully by rinsing gently with water. The shoots (stems and leaves) and roots were dried in an oven at 70 °C for two days, in order to measure the shoot dry biomass (SDM) and root dry biomass (RDM).

Plant water use efficiency (WUE_p, g l^{-1}) was calculated as:

$$WUE_p = \frac{plant\ total\ biomass\ increment}{plant\ water\ consumption} \qquad [1]$$

Intrinsic water use efficiency (WUE_i, mmol CO_2 mol^{-1} H_2O) was calculated as:

$$WUE_i = \frac{A_n}{g_s} \qquad [2]$$

where A_n is photosynthetic rate (µmol m^{-2} s^{-1}), and g_s is stomatal conductance (mmol m^{-2} s^{-1}).

Statistical analysis: The effects of biochar application, $[CO_2]$ elevation, watering regime, and the interaction between these three variables were analyzed by three-way ANOVA. Data were analyzed with SPSS 22.0 and presented as the means of four replicates ± S.E. (standard error of the mean); difference between treatments was checked at the $p \leq 0.05$ level of significance. Regression analyses were also performed among some variables, at the $p \leq 0.05$ level of significance.

Results

Leaf gas exchange and intrinsic water use efficiency: Photosynthetic rate (A_n) was affected significantly by $[CO_2]$ and watering regimes, being 8% higher for maize grown under elevated $[CO_2]$ than those with ambient $[CO_2]$, and 13% higher with well-watered (W) treatment than under drought-stressed (D) conditions, respectively ($p<0.001$; Fig. 1a; Table 3). Before starting the water treatment, g_s was significantly decreased by 37% under elevated $[CO_2]$ (Table 2). At H2, g_s was only affected by watering treatment, being 64% higher under W compared to plants grown under D ($P< 0.001$; Fig. 1b; Table 3). Furthermore, we found a significant positive correlation between A_n and g_s across all treatments (Fig. 2). WUE_i at H2, calculated as A_n/g_s, was only affected by watering treatment, being 30% lower under W than under D ($p<0.001$, Fig. 1c; Table 3).

Root and shoot biomass: Before starting the watering treatment (i.e., at H1), plants grown under elevated $[CO_2]$ had increased RDM, SDM, root to shoot ratio (RSR) and LA (by 10%, 2%, 12% and 43%, respectively) compared to plants grown under ambient $[CO_2]$; however, a significant increase was found only for LA ($p<0.001$; Table 2). RDM and RSR were significantly increased by biochar addition (Table 2; $p<0.01$ and $p<0.05$, respectively). However, plants responded differently between the two chambers, with RDM under ambient $[CO_2]$ increased by 13% with the application of biochar and that under elevated $[CO_2]$ increased by 38%.

Consequently, a larger increase in RSR with biochar application was found with elevated [CO_2] than with ambient [CO_2] (42% vs. 12%). Specific leaf area (SLA, leaf area/leaf dry weight) was significantly affected by [CO_2], being 51% higher under elevated [CO_2] compared to the treatment under ambient [CO_2]. Effects of elevated [CO_2] and biochar application on RDM and total dry biomass (TDM) were not significant at H1 (Table 2).

At the second harvest (H2), [CO_2] elevation resulted in greater RDM and SDM compared to the treatments at ambient [CO_2] (Fig. 3a; Fig. 3b; Table 3). Elevated [CO_2] enhanced RSR by 19% compared to RSR under ambient [CO_2] (Fig. 3c; Table 3).

Evapotranspiration and plant water use efficiency: Accumulative evapotranspiration (ET) after imposition of water treatment was significantly affected by biochar and watering treatment ($p<0.001$; Fig. 4a; Table 3). ET with biochar addition was 21% lower than without biochar, and ET in the treatments under ambient [CO_2] was 5% lower than those under elevated [CO_2]. Compared to plants grown under drought-stressed conditions, the TDM of plants grown under well-watered regimes was 18% higher at H2 ($p<0.05$; Fig. 4b; Table 3). Plant water use efficiency (WUE_p), calculated based on biomass increment and accumulative ET from H1 to H2, was 35% higher with biochar amendment compared to the biochar control; however, the effect was not statistically significant (P = 0.104, Fig. 4c; Table 3). Also, CO_2 elevation had a clear tendency to increase WUE_p, although the change was statistically insignificant (P = 0.080, Fig. 4c; Table 3).

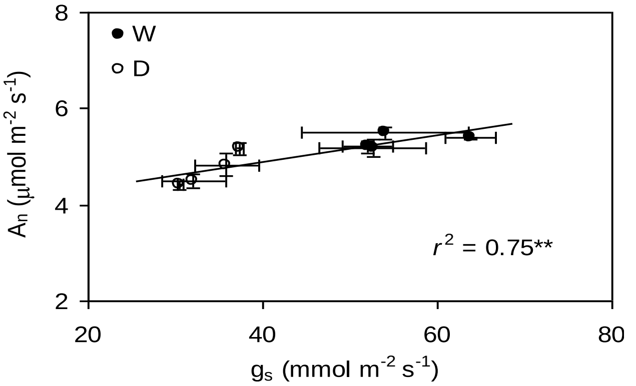

Fig. 2. Relationship between photosynthetic rate (A_n) and stomatal conductance (g_s) of maize leaves affected by biochar, CO_2 concentration and watering treatment (drought-stressed, D; well-watered, W). ** indicates the significance of the regression line at p<0.01. Error bars indicate S.E. (n=4).

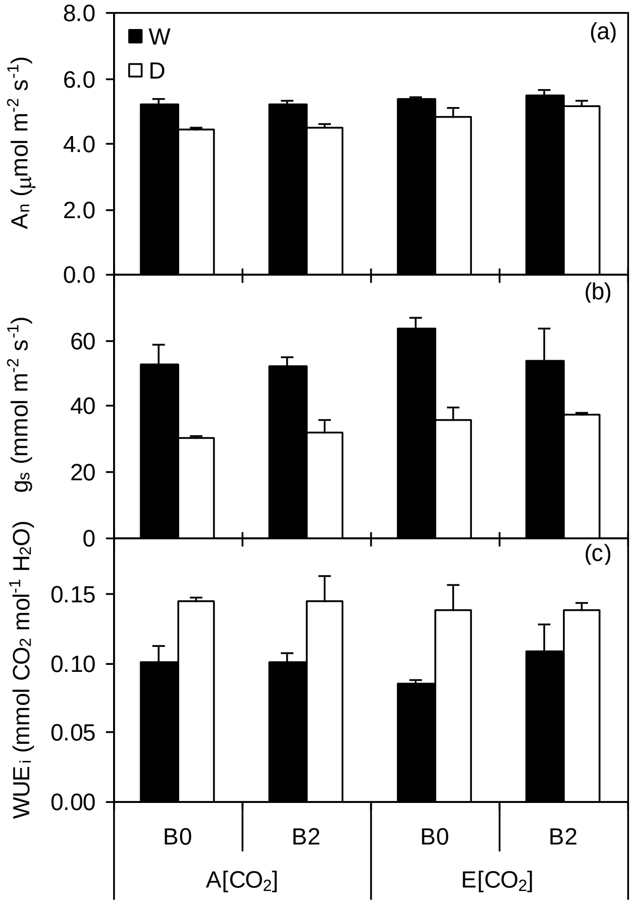

Fig. 1. Photosynthetic rate (A_n), stomatal conductance (g_s), and intrinsic water use efficiency (WUE_i) of maize subjected to different biochar, CO_2 concentration and watering treatments, 26 days after planting (drought stressed, D; well-watered, W; ambient, A; elevated, E; B0 and B2 indicate biochar application at a rate of 0% and 2% by weight in soil, respectively). Error bars indicate S.E. (n=4).

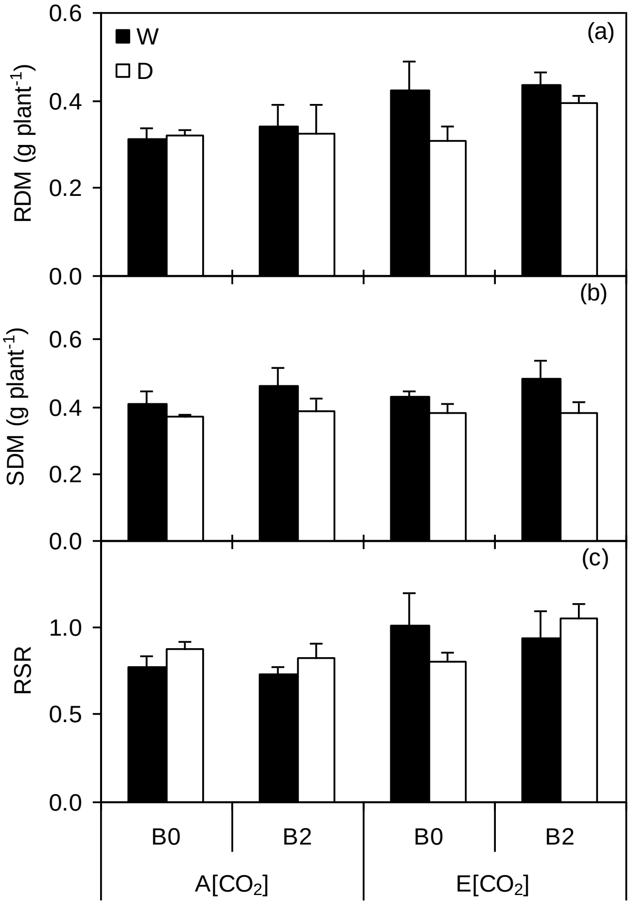

Fig. 3. Root dry weight (RDW), shoot dry weight (SDW) and root to shoot ratio (RSR) of maize subjected to different biochar, CO_2 concentration and watering treatments (drought stressed, D; well-watered, W; ambient, A; elevated, E; B0 and B2 indicate biochar application at a rate of 0% and 2% by weight in soil, respectively). Error bars indicate S.E. (n=4).

Table 2. Effects of CO_2 concentration ([CO_2]), biochar (B) and their interactions ([CO_2] × B) on shoot dry mass (SDM), root dry mass (RDM), root to shoot ratio (RSR), total dry mass (TDM), leaf area (LA), specific leaf area (SLA), chlorophyll content index (CCI), root water potential (Ψ_r), and stomatal conductance (g_s) of maize plants, 18 days after planting. A[CO_2] indicate ambient CO_2, E[CO_2] indicate elevated CO_2, B0 indicate treatments without biochar and B2 indicate treatments with 2% of biaochar.

Treatment		SDM (g)	RDM (g)	RSR	TDM (g)	LA (cm^2)	SLA (cm^2 g^{-1})	SPAD	Ψ_r (MPa)	g_s (mmol $m^{-2}s^{-1}$)
A[CO_2]	B0	0.29	0.20	0.69	0.49	45.59	237.87	8.98	-0.43	65.75
	B2	0.29	0.22	0.77	0.51	47.76	234.94	9.53	-0.39	78.93
E[CO_2]	B0	0.29	0.19	0.68	0.48	66.34	336.66	7.21	-0.47	40.85
	B2	0.30	0.27	0.96	0.55	66.77	374.97	7.40	-0.42	50.65
ANOVA										
[CO_2]		ns	ns	ns	ns	***	**	**	ns	**
B		ns	**	*	ns	ns	ns	ns	ns	ns
[CO_2] × B		ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **, *** denote significance level at $p<0.05$, $p<0.01$ and $p<0.001$, respectively, from two-way analysis of variance (ANOVA); ns denotes no significance

Table 3. Output of three-way analysis of variance (ANOVA) for the effects of CO_2 concentration ([CO_2]), biochar (B), watering treatment (WT) and their interactions ([CO_2] × B, [CO_2] × WT, B × WT and [CO_2] × B × WT) on shoot dry mass (SDM), root dry mass (RDM), root to shoot ratio (RSR), total dry mass (TDM), leaf area (LA), specific leaf are (SLA), photosynthetic rate (A_n), stomatal conductance (g_s), intrinsic water use efficiency (WUE_i), root water potential (Ψ_r), chlorophyll content index (CCI), evapotranspiration (ET) and plant water use efficiency (WUE_p) of maize plants, 26 days after planting.

Factors	SDM	RDM	RSR	TDM	LA	SLA	A_n	g_s	WUE_i	Ψ_r	CCI	ET	WUE_p
[CO_2]	ns	*	*	ns	**	***	***	ns	ns	***	ns	ns	ns
B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	ns
WT	**	ns	ns	*	**	ns	***	***	***	***	ns	***	ns
[CO_2]×B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
[CO_2]×WT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B×WT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
[CO_2]×B×WT	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **, *** indicate significance level at $p<0.05$, $p<0.01$ and $p<0.001$, respectively; ns denotes no significance

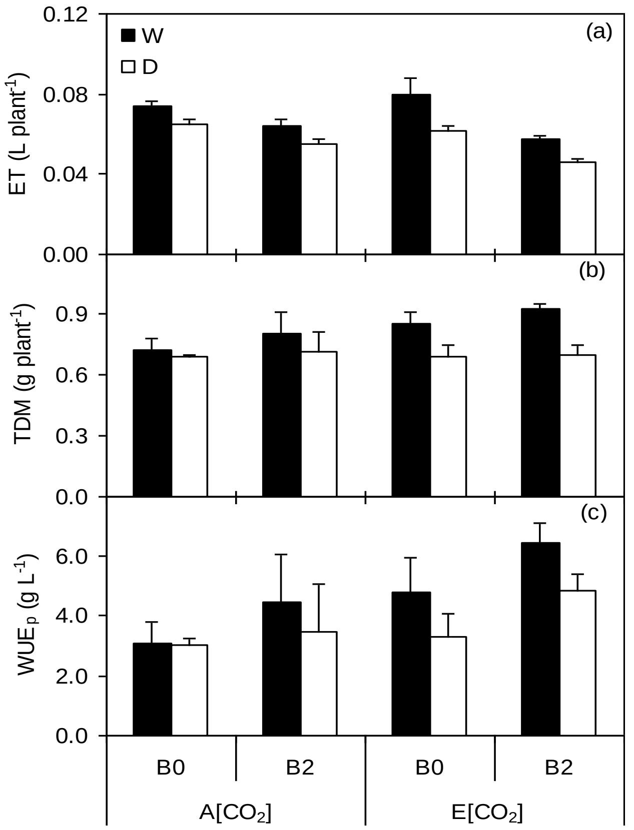

Fig. 4. Evapotranspiration (ET), total dry mass (TDM) and plant water use efficiency (WUE_p) of maize subjected to different biochar, CO_2 concentration and watering treatments (drought stressed, D; well-watered, W; ambient, A; elevated, E; B0 and B2 indicate biochar application at a rate of 0% and 2% by weight in soil, respectively). Error bars indicate S.E. (n=4).

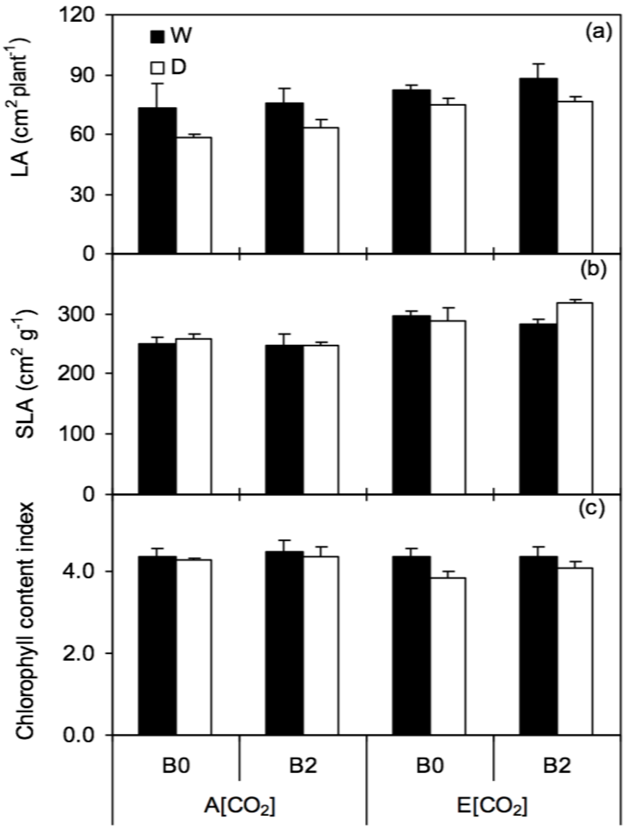

Fig. 5. Leaf area (LA), specific leaf area (SLA) and leaf chlorophyll content index of maize subjected to different biochar, CO_2 concentration and watering treatments (drought stressed, D; well-watered, W; ambient, A; elevated, E; B0 and B2 indicate biochar application at a rate of 0% and 2% by weight in soil, respectively). Error bars indicate S.E. (n=4).

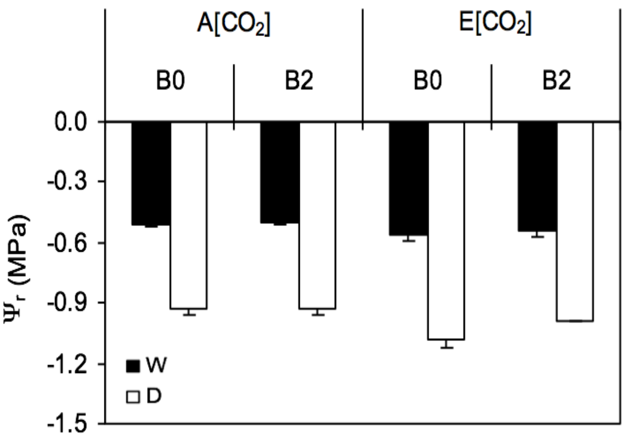

Fig. 6. Root water potential (Ψ_r) of maize subjected to different biochar, CO_2 concentration and watering treatments (drought stressed, D; well-watered, W; ambient, A; elevated, E; B0 and B2 indicate biochar application at a rate of 0% and 2% by weight in soil, respectively). Error bars indicate S.E. (n=4).

Leaf area and leaf chlorophyll content index: Before starting water treatment (H1), LA and SLA were 43% and 51% greater, respectively, in the treatments with elevated [CO_2] than those with ambient [CO_2] (Table 2). At H2, LA was significantly affected by [CO_2] and watering treatment, being 19% and 18% greater in plants grown under conditions of elevated [CO_2] and well-watered treatments, respectively, than those grown at ambient [CO_2] and under drought stress ($P< 0.01$; Fig. 5a; Table 3). SLA was significantly affected by [CO_2] at H2, being higher in the treatments with elevated [CO_2] than those at ambient [CO_2] ($p<0.001$; Fig. 5b; Table 3).

The chlorophyll content index (CCI) was significantly affected by [CO_2] at H1, being 21% lower in elevated [CO_2] than ambient [CO_2] ($p<0.01$; Table 2); however, there was no obvious difference at H2 (Table 3).

Root water potential: The root water potential (Ψ_r) of plants with biochar was less negative compared to plants without biochar addition at H1, but the influence was not significant (Table 2). There was no significant effect of biochar on Ψ_r at H2. However, at H2, Ψ_r was significantly affected by [CO_2] and watering treatment ($p<0.001$; Fig. 6; Table 3), being lower (more negative) in the treatments with elevated [CO_2] than those at ambient [CO_2], and higher (less negative) under well-watered conditions than under drought stress.

Discussion

Leaf gas exchange: It is well known that elevated [CO_2] often leads to a significant decrease in g_s in various plant species (Ghannoum *et al*., 2009; Meng *et al*., 2014). Consistent with this, at H1 before starting the watering treatment, elevated [CO_2] significantly decreased plant g_s compared to ambient [CO_2]. However, at H2 the effect of [CO_2] on g_s was not significant. Most previous studies have also reported a significant increase of A_n with increased [CO_2] (Wall *et al*., 2001; Leakey *et al*., 2006; Wang *et al*., 2013; Pazzagli *et al*., 2016). In agreement with this, in the present study A_n was increased by 8% in the treatments at elevated [CO_2] than those under ambient [CO_2] at H2.

Drought often decreases g_s in different plant species (Liu *et al*., 2008; Pazzagli *et al*., 2016), while biochar addition can lead to higher g_s in tomato grown with less irrigation (Akhtar *et al*., 2014). The latter authors proposed that biochar amendment may increase soil water holding capacity which could help the plants to sustain a better water status and hence a greater g_s. However, such an effect of biochar on g_s was not evident in the present study, which could have been due to the small pots used in the experiment, or to the g_s of C4 maize plants being less sensitive to soil water deficit compared to C3 tomato plants.

In the present study, WUE_i was significantly higher in plants with drought stress than in those under well-watered conditions at H2, which agrees well with earlier findings that reduced irrigation increases WUE_i compared to full irrigation (Liu *et al*., 2005; Wang *et al*., 2012). Previous studies have also found increases in WUE_i under elevated [CO_2] (Yelle *et al*., 1989; Pazzagli *et al*., 2016), as a consequence of either an enhanced A_n or a lowered g_s or both. In our study, however, significant differences were not observed in WUE_i under two [CO_2] treatments; even though A_n was greater in plants grown under elevated [CO_2], the slightly higher g_s of the plants in elevated [CO_2] could have offset the positive effect of A_n on WUEi.

Biomass production: Earlier studies have indicated that the RSR under either well-watered or drought-stressed conditions is not affected by [CO_2] enrichment, and that LA per plant also does not respond to [CO_2] elevation (Samarakoon *et al*., 1996; Kim *et al*., 2006). In contrast, in the present study the results indicated that root biomass was higher in plants grown under elevated [CO_2], especially under biochar treatment. Quilliam *et al*., (2013) found that shoot biomass of plants grown under non-biochar amended soil was not significantly different compared to biochar amended soil, but the RSR was decreased slightly with biochar treatment. In line with this, here the shoot biomass was not affected by biochar application, and RSR was decreased slightly with biochar treatment at H2 under ambient [CO_2].

Evapotranspiration and plant water use efficiency: At the onset of the watering treatment, TDM was lower in plants at ambient [CO_2] than those growing under conditions of elevated [CO_2]; at the end of the watering treatment, the [CO_2] effect was greater in well-watered plants than in plants grown under drought stress (Fig. 4b). Consistent with this, earlier studies have also reported an increase in TDM with elevated [CO_2] and well-watered conditions (Driscoll *et al*., 2006; Meng *et al*., 2014). However, other authors have found the biomass of maize under elevated [CO_2] to be unaffected under full irrigation (Leakey *et al*., 2006; Maekelz *et al*., 2011). Erbs *et al*., (2015) postulate that a C_4 plant such as maize has no response to an increase in atmospheric [CO_2]unless there is prominent drought stress.

In the present study, ET was lower at elevated [CO_2] than at ambient [CO_2], which agrees with earlier findings by Leakey *et al*., (2006), who reported that the water use at leaf or canopy scales declines when plants are exposed to elevated [CO_2]. The ET of plants grown with 2% biochar addition was lower across all watering and [CO_2] treatments, and led to a higher WUE_p. Previous studies have also found an increase in WUE_p with biochar amendment in all irrigation treatments compared to the non-biochar controls (Akhtar *et al*., 2014). In our study, WUE_p was insignificantly higher (P = 0.080) in plants treated at elevated [CO_2] than ambient [CO_2], which agrees with the findings of Pazzagli *et al*., (2016). Wang *et al*., (2010) found that WUE_p increased with a reduction in irrigation. However, the present study found that the WUE_p of maize under drought stress was 28% lower than under well-watered conditions (although not statistically significant, P = 0.161), which was mostly attributed to the lowered TDM.

Leaf area and leaf chlorophyll content index: The prevailing view is that leaves with a high SLA are productive and function well in environments that are rich in resources, and leaves with a low SLA perform better in an environment lacking in resources where retention of captured resources is a higher priority (Wilson *et al*., 1999). Previous studies have reported a decline in SLA as [CO_2] increases (Yin *et al*., 2002); in contrast with this, in the present study elevated [CO_2] was shown to have a positive effect on SLA, which was most likely attributed to the greater LA. Olmo *et al*., (2014) reported that biochar addition results in a decrease in SLA, in contrast to this study where biochar was shown to have a positive effect on SLA, which was most probably due to it improving the soil moisture status under drought treatment.

Many studies have indicated that leaf chlorophyll content (i.e., CCI) decreases with elevated [CO_2] due to a decrease in leaf nitrogen concentration (Epron *et al*., 1996; Haque *et al*., 2006), while some other studies report that CCI does not differ between treatments of elevated and ambient [CO_2] (Reddy *et al*., 2005; Kim *et al*., 2006). In our study, CCI was only affected by [CO_2] at H1, and the value was lower in plants treated under elevated [CO_2], in good agreement with earlier findings (e.g., Li *et al*., 2016). However, at H2 there was no significant difference in CCI between [CO_2], biochar and watering treatments. Several studies have reported that biochar amendment has a negative effect on CCI compared to non-biochar controls (Asai *et al*., 2009; Akhtar *et al*., 2014; Akhtar *et al*., 2015a), while others have found that biochar addition significantly increases CCI (Akhtar *et al*., 2015b; Hafeez *et al*., 2017), indicating that the effect of biochar addition on CCI is inconsistent across different experiments.

Root water potential: As expected, drought stress decreased Ψ_r of maize plants (Fig. 6), and similar results have been reported in previous studies (e.g., Vivin *et al*., 1996). Earlier studies have reported that CO_2 elevation increases Ψ_r (Wullschleger *et al*., 2002; Pazzagli *et al*., 2016); however, in the present study, [CO_2] enrichment led to a decreased Ψ_r. The reason behind this remains unknown and merits further investigation.

Conclusion

Although there were no synergistic effects of CO_2 elevation, drought, and biochar amendment on maize growth and physiology observed during the short period of the early growing stage in the experiment, the results clearly indicate that [CO_2] elevation enhanced growth and water use efficiency of maize plants, while biochar addition tended to reduce plant water use thereby ameliorating the negative effect of drought stress on maize under both ambient and elevated [CO_2].

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