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Research article

Drought stress and *Acacia seyal* biochar effects on sorghum gas exchange and yield: A greenhouse experiment

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Abstract

Drought is the controlling abiotic stress factor affecting crop production in dryland environments and exposes millions of people to food insecurity in Africa and Asia. Although sorghum is drought tolerant, it is not sufficiently known if biochar can reduce drought-related losses in yields in clay soils for this particular crop. The stomatal morphology and gas exchange responses were investigated of a sorghum cultivar, ‘Wad Ahmed’ (widely grown throughout Sudan and South Sudan), to drought stress and *Acacia seyal* biochar application in a greenhouse pot experiment. The experiment was set up in a split-plot, randomized block design with two experimental factors: drought stress (60%, 40%, 20% of field capacity) and biochar (no biochar and 10 Mg/ha). The potting soil was clay textured with 5% carbon content. There were eight replicate pots of each treatment which were arranged randomly in six blocks giving a total of 48 pots. The experiment lasted 153 d from sowing, with 127 d of drought treatment. The results showed that while drought stress had a significant ($p < 0.05$) effect on gas exchange, water use efficiency, biomass and grain yield, biochar had no significant effect, and neither drought stress nor biochar had a significant effect on stomatal size and density. It may be that high doses of biochar are required to benefit crops grown under drought stress, particularly when the soils have initially high soil organic carbon content, with more time needed for any effect to become evident.

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is an important global multi-purpose crop that is used for food security, animal feed and energy (biofuel). It is grown on an estimated 501,000 km^2 (3% of the global cultivated area), but its cultivation is largely limited to arid and semiarid regions (Leff et al., 2004). Sorghum is believed to have originated in Africa, and, largely due to its resistance to drought (Brauer and Baumhardt, 2016), it is the second most important cereal crop in the continent after maize and the only affordable cheap source of food for many rural populations (Edmonds et al. 2009). Although there is potential to increase sorghum production globally, yields particularly in Sub-Saharan Africa are very variable depending on rainfall, soil fertility and agronomic management (Mulcahy et al., 2013; Deng et al., 2017).

Drought stress is the most limiting factor for crop production in arid and semiarid regions (Batista et al., 2019). Plant response to drought stress depends upon the intensity and duration of the drought, as well

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as the development stage of the plant (Pinheiro and Chaves, 2011). In the short term, the stomata close, the rate of transpiration decreases in an attempt to limit water loss and increase water use efficiency (WUE), and the rate of photosynthesis becomes reduced, whereas in the long term, the reduction in photosynthesis results in reduced growth and grain yields (Assefa et al., 2010). Tolerance to drought varies among species due to the adaptation of drought avoidance and drought tolerance mechanisms (Tari et al., 2013). In sorghum, leaf water potential, net photosynthesis and stomatal conductance have been shown to be significantly reduced by water stress (Pinheiro and Chaves, 2011). Ludlow et al. (1990) reported an ability of sorghum to control stomatal opening in a way that allowed photosynthesis at low water potentials. Sorghum can also avoid dehydration by producing a more extensive root system enabling it to extract moisture from deeper soil layers and by tolerating dehydration through osmotic regulation (Blum and Sullivan, 1986; Ludlow et al., 1990). Sorghum's ability to enhance tillering can compensate for drought stress damage to the main stem (Tari et al., 2013). Plants can also adapt to drought stress by increasing stomatal density and decreasing the size of the stomata (Xu and Zhou, 2008).

In recent years, the use of biochar has been promoted as a soil amendment especially in tropics because of its ability to retain nutrients in the soil and to improve soil water holding capacity (Omondi et al., 2016; Aller et al., 2017; Jeffery et al., 2017). Biochar has been reported to also improve some ecophysiological traits and yields of plants grown under drought stress (Major et al., 2010; Mulcahy et al., 2013), even when the amount of available water would be unaffected by biochar (Kammann et al., 2011).

Despite the importance of sorghum in Sub-Saharan Africa, only a handful of studies have been published on the potential use of biochar to alleviate drought stress and so improve the growth and grain yield**.** Laghari et al. (2015) reported biochar improved sorghum grain yields grown in desert sand (Kubuqi Desert, China) and attributed it to improved soil moisture retention. However, Deng et al. (2017) observed no increase in sorghum biomass and grain yield due to biochar addition (10 Mg/ha) from their study carried out in South Sudan even though the plant available water capacity (AWC) of the soil had been increased by 30%.

The present pot experiment was carried out to determine the effect of drought stress, biochar addition and their interaction on gas exchange, morphological and growth responses of the sorghum cultivar 'Wad Ahmed'. The specific objectives were to determine the effect of drought stress and biochar on: 1) stomatal conductance, transpiration, photosynthesis, leaf temperature and photosynthetic WUE; 2) stomatal size and density; and 3) biomass and grain yield of sorghum.

Materials and Methods

Experimental design and treatments

A pot experiment was carried out under controlled greenhouse conditions during May–December 2011 at the Viikki campus, Faculty of Agriculture and Forestry, University of Helsinki, Finland (60°13'40"N, 25°01'03"E, 13 m above sea level) The experiment was a split-plot, randomized block design with two experimental factors. The main plot factor was drought stress with three levels of soil moisture content: 60% of field capacity (well-watered), 40% (medium drought) and 20% (severe drought). The field capacity of the potting soil (see below) was determined prior to the experiment by filling two pots with soil and gently watering until drainage started and then allowing the pots to stand for 4 hr, after which their weights were recorded. The pots were subsequently dried at 80°C for 24 hr before reweighing. The difference in weight between the dried and watered pots was taken as the water content of the soil when at field capacity and the amounts of water for each of the three drought stress treatments calculated (600 mL for well-watered, 400 mL for medium drought, and 200 mL for severe drought). The subplot factor was biochar with two levels: no biochar and biochar addition (equivalent to 10 Mg/ha, a dose commonly used for biochar applications). There were eight replicate pots of each of the six treatments, with a pot of each treatment being randomly placed within a block of pots, giving a total of 48 pots.

Growth conditions

The experiment was carried out in a greenhouse room with an area of 18.6 m^2. Eight 400 W halogen lamps (E40; GE; Budapest, Hungary) were mounted 150 cm above the blocks. Automated black curtains fitted on the greenhouse walls and roof were used to regulate the photoperiod to 12 hr each of light and darkness. The maximum and minimum temperatures were 27.6°C and 17.2°C, respectively, and relative humidity was 60%. These conditions were used to mimic the conditions in South Sudan where a field experiment had been carried out (Deng et al., 2017).

The potting soil was made by mixing 8 kg clay soil (53% clay; 5% organic carbon) taken from an agricultural field in southern Finland with 1 kg of fine sand (0.2 mm) and 1 kg of coarse sand (0.5–1.2 mm). The clay soil was air-dried and sieved through a 1 cm sieve before it was mixed with the sand and used to fill 10 L plastic pots (22 cm height, 28 cm upper diameter and 21 cm bottom diameter) with five small holes at the bottom to allow for the drainage of excess water. This mixture was made to resemble the soil texture conditions of the field experiment carried out in South Sudan described in Deng et al. (2017). After filling the pots, 200 g agricultural lime and 0.9 g nitrogen fertilizer (NH_4NO_3) were added to each pot and thoroughly mixed in. For the biochar treatment, biochar from *Acacia seyal* produced in traditional mound kilns in Sudan was added at 6.9 g/kg soil (equivalent to 10 Mg/ha) to half (24) of the pots (chosen at random) and thoroughly mixed into the soil. More details about biochar production and properties are reported in Deng et al. (2017). Eight seeds of sorghum ('Wad Ahmed' cultivar from Sudan) per pot were sown at a depth of 2.5 cm on 30 June 2011. Then, all 48 pots were covered with perlite to minimize direct evaporation and arranged in eight blocks on the ground in the greenhouse. An additional 32 buffer pots were placed around the experiment pots to minimize edge effects (Fig. 1).

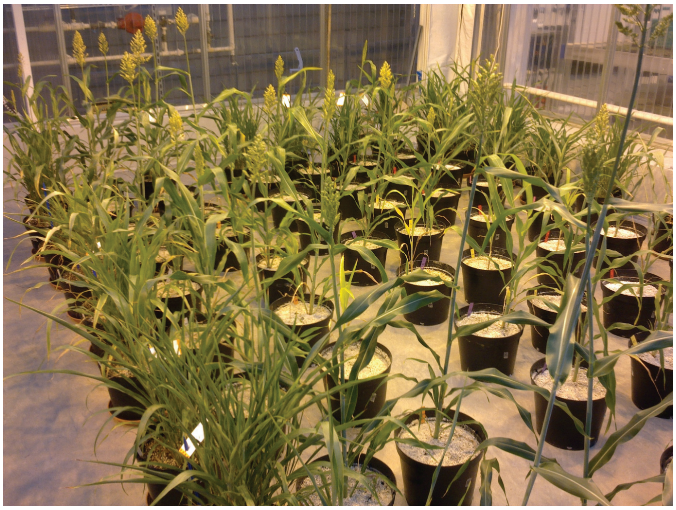

Fig. 1 Photograph showing sorghum pots in greenhouse experiment. (Photo taken by Bolajoko Bada, 2/11/2011)

To enhance establishment of the plants, all the pots received 600 mL water at 3 d intervals starting from the time of sowing until the emergence of the fifth leaf. The seedlings were thinned on 13 July 2011 to one viable seedling per pot. The drought stress treatments were imposed on 27 July 2011 (27 d after sowing) when the plants were at the vegetative stage and the watering given at 3 d intervals until the end of the experiment on 30 November 2011 (127 d after start of drought treatment) when the sorghum grain was ready for harvesting.

Gas exchange and stomatal morphology measurements

Rates of photosynthesis (P_n, measured in micromoles CO_2 per square meter per second) and transpiration (E, measured in millimoles H_2O per square meter per second) and leaf temperature (measured in degrees Celsius) were measured on four occasions during the experiment (20 July 2011, 3 August 2011, 12 October 201, 29 November 2011) using an LI-6400 portable open gas exchange system (LI-COR Inc.; Lincoln NE, USA). The first set of measurements (20 July 2011) were made 7 d prior to the start of the drought treatment. A photosynthesis photon flux density of 1,000 µmol $^/$m^2/s was used. A supplementary injecting CO_2 cartridge was connected to the system and the CO_2 flow rate was set at 400 µmol/mol. Stomatal conductance ($g_{s,}$ measured in millimoles H_2O per square meter per second) was measured using the same LI-6400 system, but only on the last two dates. All measurements were made from the youngest fully expanded leaf on each plant at 30 s intervals and carried out across the experiment between 0900 hours and 1300 hours. The gas exchange values were calculated automatically using the LI-6400 system software. Photosynthetic WUE at each of the four gas exchange measurement dates was calculated by dividing the photosynthesis rate by the transpiration rate.

Stomatal length (SL), width (SW) and density (SD) were determined from a single, fully expanded leaf taken from each plant in five of the eight blocks. The measurements were made using the nail varnish method (Voleníková and Tichá, 2001). Imprints of both the adaxial (upper) and abaxial (lower) epidermis of each sample leaf surface were made from the broader part of the leaf avoiding the midribs. SL and SW were measured using a Laborlux S binocular microscope (Ernst Leitz GmbH; Wetzlar, Germany) at ×40 magnification. The stomatal area (SA) was calculated as the product of average SL and SW values. The number of stomata were counted from three fields of view on each of the adaxial and abaxial imprints and SD was calculated for each surface as the average stomatal count divided by the area of the field of view.

Biomass and yield measurements

The plants were harvested on 30 November 2011 (153 d after sowing) when most of the plants had reached maturity and produced seeds. Plant height, number of leaves per plant, panicle length, number of seeds and their weights were recorded at harvest. Plant height and panicle length were both measured in centimeters using a scaled ruler. Aboveground biomass was determined by cutting the plants at a height of 3–5 cm above the pots. Root biomass was determined by separating the roots from the soil and gently washing with water. The biomass samples were put in paper bags and oven dried at 80°C for 24 hr using a Memmert dryer (Memmert 800; Schwabach, Germany). All weighing was done using a Mettler balance (PJ3000; Mettler-Toledo; Columbus, OH, USA).

Statistical analysis

The gas exchange, stomatal morphology, biomass and yield measurements were described using the mean and coefficient of variation (%) and presented by drought stress and biochar treatments. To test for differences between the drought stress and biochar treatments univariate analysis of variance was used. Drought stress and biochar treatments were considered as fixed effect factors. Comparisons of the drought stress treatments were conducted using Tukey's honestly significant difference test and biochar treatments using Student's t test. The statistical analyses were conducted using the SPSS software (version 23.0; SPSS Corp.; Chicago, IL, USA).

Results

Effects of drought stress and biochar on stomatal morphology

The stomatal size and density of the abaxial and adaxial leaf surfaces of the sorghum plants are summarized in Table 1. Neither the drought stress nor biochar treatments had a significant effect on any of the stomatal morphological traits (Table 2).

Table 1 Mean (% coefficient of variation in parentheses) stomatal length, width, area and density on lower (abaxial) and upper (adaxial) leaf surfaces of sorghum plants measured at the end of the experiment, where neither drought nor biochar treatments had a significant ($p < 0.05$) effect on any of the stomatal characteristics (see Table 2)

Stomatal	Leaf	Severe drought						Medium drought						Well-watered					
characteristic	surface	No biochar		Biochar		Mean		No biochar		Biochar		Mean		No biochar		Biochar		Mean	
Length (μm)	Abaxial	12.0	(23)	12.3	(11)	12.2	(17)	12.8	(17)	10.3	21	11.6	(21)	12.2	(13)	12.6	11	12.4	(12)
	Adaxial	10.8	(16)	10.8	(8)	10.8	(12)	11.5	(12)	12.8	7	12.2	(10)	11.5	(27)	13.2	15	12.3	(21)
Width (μm)	Abaxial	9.7	(17)	8.8	(24)	9.2	(20)	9.4	(12)	8.0	22	8.7	(18)	8.7	(22)	9.3	19	9.0	(19)
	Adaxial	7.5	(22)	9.1	(9)	8.3	(18)	7.8	(18)	8.9	18	8.4	(18)	7.5	(10)	8.5	13	8.0	(13)
Area (μm²)	Abaxial	117	(28)	109	(32)	112.8	(28)	121	(22)	80	15	100.5	(29)	106	(24)	118	28	112.1	(25)
	Adaxial	81	(30)	98	(12)	89.6	(23)	91	(26)	115	22	102.9	(25)	86	(25)	112	20	98.7	(25)
Density (number/mm²)	Abaxial	101	(11)	104	(19)	102.5	(15)	100	(9)	119	32	109.4	(26)	125	(13)	114	38	119.3	(26)
	Adaxial	87	(21)	98	(17)	92.3	(19)	91	(25)	98	18	94.7	(21)	85	(35)	99	24	92.1	(29)

Table 2 Results of univariate analysis of variance of stomatal length, width, area and density on the abaxial and adaxial leaf surfaces by the main treatments (drought stress and biochar) and their interaction, where neither treatment had a significant ($p < 0.05$) effect on any of the stomatal characteristics

Stomatal		Drought		Biochar		Drought x Biochar	
characteristic		*F* value	*p* value	*F* value	*p* value	*F* value	*p* value
Length	Abaxial	0.484	0.622	0.794	0.382	1.641	0.215
	Adaxial	2.230	0.129	2.118	0.159	0.554	0.582
Width	Abaxial	0.223	0.802	0.929	0.345	0.898	0.421
	Adaxial	1.401	0.266	0.022	0.882	0.912	0.415
Area	Abaxial	0.590	0.562	1.432	0.243	2.159	0.137
	Adaxial	0.056	0.945	1.573	0.222	1.074	0.357
Density	Abaxial	1.014	0.378	0.119	0.734	0.812	0.456
	Adaxial	0.043	0.958	1.765	0.196	0.053	0.949

Effects of drought stress and biochar on gas exchange

The biochar treatment had no significant effect on any of the gas exchange traits at any of the drought stress levels (Fig. 2, Tables 3–4). Stomatal conductance under the severe drought treatment was significantly less (half) than under both the medium drought and well-watered treatments, but the difference between the latter treatments was not significant (Fig. 2A, Tables 3–4). The same pattern was also shown for both the photosynthetic (Fig. 2B) and transpiration rates (Fig. 2C). Photosynthetic WUE increased with the level of drought stress, but only that of the severe drought and well-watered treatments significantly differed from each other (Fig. 2D). Compared to the well-watered treatment, the reduction in stomatal conductance in the severe drought treatment was 56%. For photosynthesis, the reduction was 18% and for transpiration 31%. There was a 32% increase in the photosynthetic WUE of the severe drought-stressed plants compared to the well-watered treatment. There was no significant difference in leaf temperatures between any of the drought stress treatments (Fig. 2E).

Effects of drought stress and biochar on biomass and yield

While the drought stress treatments had a significant effect on biomass and yield parameters with the exception of panicle biomass, the biochar treatment had no significant effect (Tables 5–6). Generally, the effects of the drought stress treatment on the biomass and grain yield were consistent, with values decreasing with increasing level of drought stress. The greatest reduction was associated with the number and weight of seeds produced per plant. Compared to the well-watered treatment, the severe drought treatment resulted in 92% and 95% reductions in the number and weight of seeds produced per plant, respectively.

Discussion

Given the importance of sorghum as a major food staple throughout much of the developing world and the anticipated increases in drought stress in dryland environments related to climate change, it is important to investigate the response of sorghum to drought stress and whether the use of biochar can help offset any negative effects. Sorghum, as a crop of dry environments, has been reported to preserve its growth and production through adopting dehydration avoidance or escape mechanisms (Blum, 2005). These mechanisms involve the regulation of stomatal opening and closure to minimize water loss, to maintain leaf water potential and to sustain cellular hydration. The current study looked specifically at the stomatal conductance and gas exchange responses of sorghum to drought stress and not cellular hydration mechanisms.

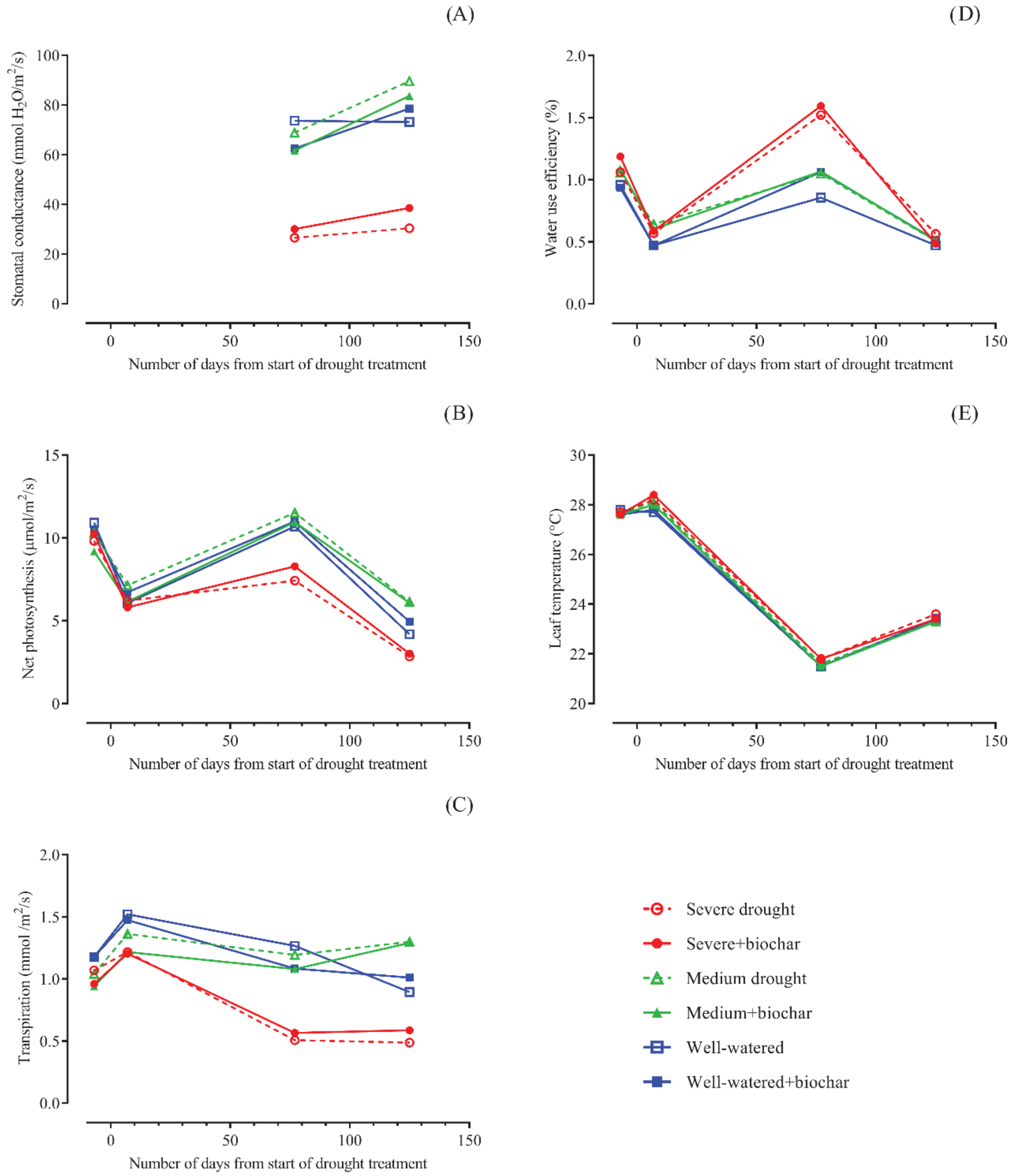

Fig. 2 Physiological traits and leaf temperature by drought and biochar treatments as mean values of measurements made on four (only two for stomatal conductance) occasions during the experiment, the first occasion being 7 d before the start of the drought treatment

Table 3 Mean (% coefficient of variation in parentheses) physiological traits and leaf temperature of sorghum plants measured four times (stomatal conductance only twice) during the experiment by drought and biochar treatments, where biochar treatment had no significant ($p < 0.05$) effect on any of the characteristics (see Table 4)

Physiological trait	Severe drought							Medium drought							Well-watered						
	No biochar		Biochar		Mean			No biochar		Biochar		Mean			No biochar		Biochar		Mean		
Stomatal conductance (mmol $H_2O/m^{-2}/s$)	28.5	(34)	34.3	(56)	31.4	(48)	A	79.2	(42)	72.7	(48)	76.0	(44)	B	73.4	(61)	70.6	(57)	72.0	(58)	B
Net photosynthesis (µmol $CO_2/m^{-2}/s$)	6.6	(49)	6.8	(52)	6.7	(50)	A	8.8	(37)	8.1	(41)	8.4	(39)	B	8.0	(45)	8.3	(42)	8.1	(44)	B
Transpiration (mmol $H_2O/m^{-2}/s$)	0.8	(55)	0.8	(59)	0.8	(57)	A	1.2	(39)	1.1	(48)	1.2	(43)	B	1.2	(41)	1.2	(36)	1.2	(38)	B
Water use efficiency (%)	9.3	(54)	9.6	(59)	9.5	(56)	A	8.1	(47)	8.2	(49)	8.2	(48)	AB	6.9	(43)	7.5	(44)	7.2	(43)	B
Leaf temperature (°C)	25.3	(11)	25.3	(12)	25.3	(11)	A	25.2	(12)	25.1	(12)	25.1	(12)	A	25.1	(11)	25.1	(11)	25.1	(11)	A

A,B = different uppercase letters indicate significant ($p < 0.05$) differences between drought stress treatments (Tukey honestly significant difference).

Table 4 Results of univariate analysis of variance of physiological traits and leaf temperature of sorghum plants by the main treatments (drought stress and biochar) and their interaction, where significant ($p < 0.05$) differences are indicated in bold

Physiological trait	Drought treatment		Biochar treatment		Water x Biochar	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
Stomatal Conductance	18.353	**0.000**	0.032	0.859	0.303	0.739
Net photosynthesis	4.691	**0.010**	0.004	0.949	0.442	0.644
Transpiration	12.258	**0.000**	0.286	0.594	0.183	0.833
WUE	4.702	**0.010**	0.258	0.612	0.063	0.939
Leaf temperature	0.107	0.898	0.004	0.948	0.002	0.998

Table 5 Mean (% coefficient of variation in parentheses) growth, biomass (dry weight) and yield of the sorghum plants measured at the end of the experiment presented by drought stress treatment, where biochar treatment had no significant effect on any of the characteristics (see Table 6)

Plant characteristic	Severe drought				Medium drought				Well-watered			
	No biochar	Biochar	Mean		No biochar	Biochar	Mean		No biochar	Biochar	Mean	
Plant height (cm)	66.3 (40)	65.0 (21)	65.6 (31)	A	98.3 (20)	98.0 (17)	98.1 (18)	B	107.6 (17)	107.8 (19)	107.7 (18)	C
Panicle length (cm)	7.6 (91)	4.9 (52)	6.3 (84)	A	21.9 (14)	21.1 (47)	21.5 (33)	B	33.4 (35)	30.0 (52)	31.7 (42)	C
Number leaves/plant	7 (26)	6 (14)	6 (21)	A	7 (30)	7 (26)	7 (28)	AB	8 (13)	8 (17)	8 (15)	B
Number seeds/plant	49 (105)	42 (77)	46 (92)	A	336 (46)	366 (54)	351 (49)	B	636 (28)	545 (55)	590 (41)	C
Root biomass (g)	10.3 (56)	12.7 (52)	11.5 (53)	A	16.5 (44)	16.4 (56)	16.4 (48)	AB	22.8 (22)	16.9 (54)	19.9 (39)	B
Panicle biomass (g)	1.5 (46)	2.0 (47)	1.8 (49)	A	1.7 (56)	2.3 (45)	2.0 (51)	A	2.2 (41)	1.9 (81)	2.0 (60)	A
Shoot biomass (g)	46.4 (21)	47.0 (16)	46.7 (18)	A	72.8 (33)	77.3 (32)	75.1 (31)	B	122.3 (16)	101.6 (37)	112.0 (27)	C
Total biomass (g)	58.2 (26)	61.7 (20)	59.9 (22)	A	91.0 (34)	96.0 (35)	93.5 (33)	B	147.3 (16)	120.4 (38)	133.8 (28)	C
Grain yield (g/plant)	0.8 (94)	0.8 (79)	0.8 (84)	A	8.5 (53)	9.1 (53)	8.8 (51)	B	17.7 (30)	14.0 (53)	15.9 (41)	C

A,B,C = different uppercase letters indicate significant ($p < 0.05$) differences between drought stress treatments (Tukey honestly significant difference).

Table 6 Results of univariate analysis of variance of mean growth, biomass and yield of the sorghum plants by the main treatments (drought stress and biochar) and their interaction, where significant ($p < 0.05$) differences are indicated in bold

Plant characteristic	Drought treatment		Biochar treatment		Water × Biochar	
	F value	*p* value	*F* value	*p* value	*F* value	*p* value
Plant height	20.034	**0.000**	0.006	0.937	0.005	0.995
Panicle length	28.925	**0.000**	0.704	0.406	0.080	0.923
Number leaves/plant	4.011	**0.025**	0.307	0.583	0.026	0.975
Number seeds /plant	37.759	**0.000**	0.195	0.661	0.491	0.615
Root biomass	5.300	**0.009**	0.342	0.562	1.337	0.274
Panicle biomass	0.308	0.737	1.056	0.310	1.017	0.370
Shoot biomass	33.334	**0.000**	0.638	0.429	1.439	0.249
Total biomass	25.620	**0.000**	0.532	0.470	1.523	0.230
Grain yield/plant	43.019	**0.000**	0.655	0.423	1.026	0.367

In general, stomatal conductance is a function of stomatal density, aperture and stomatal size and controls both photosynthesis and transpiration (gas exchange) rates (Larcher, 1983). Earlier studies have shown that developing plants decrease stomatal size and increase stomatal density when subject to at least moderate drought stress (Muchow and Sinclair, 1989; Xu and Zhou, 2008). Hepworth et al. (2015) indicated that stomatal formation and density during leaf formation are regulated by a peptide signaling mechanism in epidermal cells. While stomatal size and density values of the sorghum plants in the current study were within the ranges reported by Turner and Begg (1973) and Muchow and Sinclair (1989), no significant differences were recorded in stomatal size or density related to drought stress (Table 1). Owing to the wide variation in stomatal size and density among sorghum genotypes (Muchow and Sinclair, 1989), it is expected that stomatal size and density within a plant may react differently to drought stress during the growth.

As there was no significant effect on stomatal morphology, the significant differences in P_n, E and WUE between the well-watered and the severe drought treatments must have been solely due to stomatal opening control (Chaves, 1991; Massacci et al., 1996). This was consistent with the findings of Fracasso et al. (2016), who reported that all gas exchange parameters (stomatal conductance, photosynthetic rate, transpiration) were significantly affected under water-limiting conditions for all tested sorghum genotypes. Although, the reduction in CO_2 assimilation in response to drought stress is attributed to stomatal limitations (Farooq et al., 2009; Fracasso et al., 2016), non-stomatal (biochemical) limitations are also expected in severe drought stress treatment (Ghannoum, 2009; Keshavarz Afshar et al., 2015).

It is well documented that exposure of plants to drought stress during vegetative and reproductive stages hinders growth, biomass and grain yields (de Oliveira Neto et al., 2014; Hmielowski, 2017). In the current study, drought stress clearly had a negative effect on sorghum biomass and grain yield in spite of increased WUE. Similar results have also been reported elsewhere. For example, in greenhouse experiments Manjarrez-Sandoval et al. (1989) found that drought stress caused a 20–30 % reduction in sorghum grain yield and de Oliveira Neto et al. (2014) reported up to 50% reduction in grain yield and panicle development under drought stress conditions. Beheshti and Behboodi Fard (2010) also demonstrated a reduction (up to 50%) in sorghum grain yield and panicle development under drought stress conditions in a field experiment. Similarly, Tsuji et al. (2003) reported significant reductions in the shoot dry mass and leaf area of three Sudanese sorghum cultivars (‘Gadambalia’, ‘Arous el Rimal’, ‘Tabat’) grown under drought stress from their field study carried out in Japan.

The benefits of biochar on plant growth and grain yields in water-stressed environments are generally attributed to an increase in the water holding capacity of the soil (Kammann et al., 2011). The increase in the soil carbon content resulting from the dose of biochar used in the current study would theoretically increase the AWC of the soil, although probably by less than the 30% increase for soils of low soil organic carbon (SOC) content in South Sudan (Deng et al., 2017). The effect of biochar on soil water retention properties is dependent on soil and biochar properties (Tammeorg et al., 2017), but the initial SOC content of soils is perhaps the most important characteristic determining the extent of the effect. Probably much greater biochar addition rates than the 10 Mg/ha used in the current study would have been needed in order to have a significant effect on the AWC of the experimental soil with a SOC content of about 5%. Similarly, Keshavarz Afshar et al. (2015) found no significant effects on the AWC with a 2% biochar addition to soil having an initial C content of 4.1%. Therefore, as the amount of plant-available water was the same in both the biochar and non-biochar treatments in the current experiment, any effect of biochar would have to have been the result of direct changes in stomatal morphology or gas exchange.

Kammann et al. (2011) found that peanut hull biochar at rates of 100 Mg/ha and 200 Mg/ha increased the growth, leaf-N, drought tolerance and water-use efficiency of quinoa (*Chenopodium quinoa* Willd.) grown in sandy soil, even though the plants received the same amount of water and in amounts below field capacity. As possible explanations, the authors suggested biochar-related increases in K^+ ions and subsequent changes in osmotic activity and stimulated fine root growth. In a field-based study carried out in South Sudan using the same *A. seyal* biochar and dose as in the current study, the soil K^+ contents increased by 32% (Deng et al., 2017). The total K content of the same *A. seyal* biochar was 96 g/kg. Although the severe drought stress treatment in the current greenhouse study reduced root biomass by 42% compared to the well-watered treatment (Table 5), the biochar treatment had no effect on root biomass.

Another possible effect of biochar is related to its effects on phytohormonal signalling (Kammann and Graber, 2015). For example, Di Lonardo et al. (2013) explained improved root development of white poplar clones to the adsorption of growth limiting phytohormonal ethylene by the biochar that had been added to the soilless agar growth medium. However, the *A. seyal* biochar application at 10 Mg/ha had no significant effect on any of the measured stomatal morphological, gas exchange or on biomass and grain yields of the sorghum plants. Keshavarz Afshar et al. (2015) also found biochar (maple wood at doses of 1% and 2%) and its interactions with drought stress had no significant effect on the physiological traits (photosynthesis and transpiration rates, stomatal conductance) of milk thistle (*Silybum marianum* L. (Gaertn.) in their greenhouse study. Those authors attributed this lack of a response to the initially high C content of the soil paired with the low biochar dosage used and to the short period of the experiment which may not have been long enough to result in a significant impact on soil properties and thus on plant performance under drought stress.

The positive effects shown in the greenhouse experiment with *Chenopodium quinoa* by Kammann et al. (2011) were achieved with a sandy soil and biochar applications an order of magnitude greater than used in the current study. It may be that the ecophysiological effects of biochar on crops grown under drought stress require high doses and are limited to sandy soils, and only appear after the biochar has become fully integrated into the soil (Cornelissen et al., 2013; Mulcahy et al., 2013). More research is needed to explore whether biochar addition in field conditions can improve the water availability to crops in the longer term, as it could be expected that SOC contents would increase as a result of the negative priming effect of biochar on the mineralization of soil organic matter (Aller et al., 2017; Madari et al., 2017).

The current study showed that drought stress had clear effects on sorghum plant physiology and that these changes resulted in a significant reduction in growth and yield. However, the addition of 10 Mg/ha Acacia biochar to the soil had no significant effect on the stomatal morphology, gas exchange or yield of sorghum at any level of drought treatment. As demonstrated in other biochar studies, more time or higher doses may be required before beneficial changes in soil properties are induced, particularly in clay soils, which would yield agronomic benefits. Therefore, long-term studies are needed to properly evaluate the potential effects of biochar in improving soil moisture conditions and drought tolerance for crop production.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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