



**Full Length Article**

## Does Biochar Amendment Influence Water Uptake Pattern of Winter Wheat (*Triticum aestivum*)? A Case Study in a Shallow Entisol

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### Abstract

Application of biochar in farmland to increase crop yield is globally used in agriculture. However, the benefits of biochar applications for agricultural drought-resistant in wheat farmland have not yet been sufficiently studied. To close this research gap, water isotopes (<sup>2</sup>H and <sup>18</sup>O) were used to explore the effect of biochar application in 0–20 cm soil on water use pattern of winter wheat (*Triticum aestivum* L.) at a shallow Entisol in the Southwest China. The results showed that isotopes of soil water (SW) with biochar addition were more enriched than the values of plots without biochar. Correspondingly, the stem water for winter wheat with biochar was also more enriched than the ones without biochar. Biochar significantly decreased the soil bulk density and increased SW content of the uppermost 0.1 m during wintering and filing stages. Winter wheat water uptake pattern correlated with its root distribution. Winter wheat absorbed more water (0.7–4.3%) from 0–0.2 m soil layer under biochar-amended treatment than the control experiment, but the differences were not significantly at the heading, filling and ripening stages. The mechanism was explained by that the amendment of biochar significantly improved the plant available water capacity because of increased storage pores (0.68–30 μm). This is due to the biochar which was a porous media itself improves soil structure by forming aggregates and increasing pore connectivity and quantity in the soils. Therefore, biochar application showed to be beneficial method in agricultural water management for alleviating the effect of drought on crop growth. © 2020 Friends Science Publishers

**Keywords:** Water use strategy; Stable isotopes; Soil water content; Biochar amendment; Lithological soil

### Introduction

Knowledge on water use of wheat is important to understand the response of crops (*e.g.*, crop frailer or death) to variable water conditions induced by human activities and climate change (Li *et al.* 2019). In addition, the ecological plasticity and variation of the water absorption depth of crops is critical information to understand the water flux within the soil-plant-atmosphere continuum in farmlands (Asbjornsen *et al.* 2008). Measurement of natural abundance of water isotopes (<sup>2</sup>H and <sup>18</sup>O) in the stem of plants and in the potential water bodies (soil water (SW), groundwater, stream water) has been verified to be a powerful tool to determine the source of water absorbed by different plants (Dawson and Ehleringer 1991; Ehleringer and Dawson 1992; Brooks *et al.* 2010; Dawson and Simonin 2011; Rothfuss and Javaux 2017). However, many experts argued that the understanding of root system function in vadose zone is still incomplete (Brunel *et al.* 1995;

Midwood *et al.* 1998). Albuquerque *et al.* (2013) found that biochar-amended soil management can increase wheat production due to an increase of available phosphorous (P). Blackwell *et al.* (2010) reported that application of biochar may increase crop yield. The positive effects are due to result from improved soil nutrient and water use. However, the results from Farrell *et al.* (2014) argued that no significant effect of biochar on the wheat yield. Basso *et al.* (2013) suggested that biochar amendment increases the water holding capacity and might increase available water content for plant use as the increase of the gravity-drained water content and bulk density for a sandy loam soil. Brockhoff *et al.* (2010) found that biochar may improve water content, reduce water use trough due to retaining more water and decrease in hydraulic conductivity. Laird *et al.* (2010) showed that biochar may substantially improve the agricultural soils quality in terms of greater water retention, gravity drained and larger specific surface areas.

In China, most agricultural farmland endures high

pressure to raise huge number of the population. Southwest China like Yangtze River basin (*e.g.*, Sichuan province and Chongqing Municipality) has the largest population density which means the farmland here has the highest pressure across China. At this region, a lithologic soil, called purple soil, develops from purple shale and occupies more than 300,000 km<sup>2</sup> at the hilly area of the Yangtze River region. Such shallow and rocky soils are typically susceptible to drought and erosion (Querejeta *et al.* 2016). This soil is classified as Entisol which is erodible and suffering from serious degradation (Zhao *et al.* 2013a). Due to its limited water holding capability, crop yield at these shallow soils is susceptible to the subtropical climate with seasonal dry (He *et al.* 2009).

Especially for such poor soils, biochar is proposed as a viable soil amendment, because of its potential to improve soil quality, increase crop yield and the soil's carbon sink for atmospheric CO<sub>2</sub> permanently (Nzanza *et al.* 2012; Hardie *et al.* 2014). Biochar is a fine-grained residue of biomass after pyrolysis processes in an oxygen-limited environment (Jeffery *et al.* 2011). Applying biochar to soils is considered to impact the soil physical quality in terms of water holding capacity, soil bulk density, total porosity, pore size, plant available water capacity (PAWC) (Atkinson *et al.* 2010). These changes are often referred to as key factors in explaining increased crop yields (Sohi *et al.* 2009). However, Verheijen *et al.* (2010) found that PAWC remains unchanged although total porosity is improved after biochar application. Contrasting results of biochar effect on PAWC may indicate that the role of biochar in affecting the crop water use strategy is still unclear. Moreover, the knowledge about biochar application on the water uptake of crops (*e.g.* wheat) is still scarce, despite its high relevance for water management in agriculture.

Although the studies of biochar amendment on soil is increasing (Sarma *et al.* 2018; El-Naggar *et al.* 2019), little has been done on its effect on crop water use in shallow soil, which is essential for water management in overburdened agricultural systems (Phillips *et al.* 2020). In addition, the biochar effects are soil type dependent and still unknown for drought-prone soils like the Entisols in Southwest China (Liu *et al.* 2016). To address this research gap, we conducted a study using a stable isotope approach in a hilly region in the Southwest of China where severe soil erosion occurs. We hypothesized that the biochar amendment can improve the water use strategy of crop in Entisol. The objectives of our study were (1) to determine the effects of biochar application on the water uptake of winter wheat and (2) to explore the mechanisms by which biochar amendment influences water use patterns of winter wheat.

## Materials and Methods

### Study area

The study was conducted at the Yanting Agro-ecological Experimental Purple Soil Station of the Chinese Academy of

Sciences, located in the purple soil hilly area of southwest China (31°88' N and 105°28' E). This region is characterized by a moderate subtropical monsoon climate with an annual mean temperature of 17.3°C and a mean annual precipitation of 826 mm year<sup>-1</sup> from 1981 to 2006. The investigated soil is classified as loamy Entisol that has an average pH of 8.3, a bulk density of 1.33 g cm<sup>3</sup>, organic matter content of 8.75 g kg<sup>-1</sup>, total N content of 0.62 g kg<sup>-1</sup>, alkali-hydrolyzed N content of 42.29 mg kg<sup>-1</sup>, and a saturated hydraulic conductivity of about 10<sup>-2</sup> to 10<sup>-1</sup> mm min<sup>-1</sup> with a low PAWC (Zhao *et al.* 2013a; Wang *et al.* 2015).

### Sampling and measurements

Field experiments were conducted on six sloping farmland plots, three plots with biochar application (named BK) and three control plots without biochar application (named CK). Each plot has an area of 100 m<sup>2</sup> (20 m long and 5 m wide) and a thin soil profile (0.4 m thick on average). Winter wheat (*Triticum aestivum* L.) was sown on November 1, 2015 with a density of four plants per square meter and was harvested on May 10, 2016. Prior to our experiment, corn and wheat were planted for two years and there was no significant difference of the biomass and crop yields among the six plots. We assumed that the soil basic properties of the plots were not significantly different.

A total of 16 t ha<sup>-1</sup> of biochar was added on three 100 m<sup>2</sup> plots. The biochar application rate was equivalent to 6.0 g kg<sup>-1</sup> soil within 0.2 m depth. The soil bulk density was 1.35 g cm<sup>3</sup>. To avoid biochar dust losses, the application was conducted on a day with small rain in May 2014. Biochar which produced from pyrolysis of crop straws was spread on the plots soil. The basic properties of biochar were provided by Liu *et al.* (2016). Our sampling was carried out 1.5 years after biochar application.

Water samples including precipitation, SW, and stem water were collected for plant water uptake pattern analysis. During the wheat growing season, every rainfall event was sampled with a glass funnel (0.2 m in diameter) connected to a high-density polyethylene bottle. A table tennis ball was placed in the funnel to reduce evaporation. Both soil and plant stem samples were collected in six growth periods of winter wheat. Three replicates of stem water samples were collected for each treatment. The first internode of each wheat stem was collected at 8 A.M. for plant water sampling during in the six growing periods of wintering (on December 11, 2015), greening (on January 9, 2016), jointing (on February 4, 2016), heading (on March 16, 2016), filing (on April 17, 2016); and ripening stages (on May 2, 2016). Soil samples were taken at 0–0.1, 0.1–0.2, 0.2–0.3, and 0.3–0.4 m depths with duplicates using a hand-operated auger. The soil and wheat stem samples were taken in parallel. SW content was measured by oven drying at 105°C for 8 h. Immediately after soils and wheat stems were sampled, they were stored in airtight glass vials wrapped in Parafilm and were placed in a refrigerator at -4°C for isotope analysis.

Water from bulk soil and xylem was extracted from their respective matrix using the cryogenic vacuum distillation method described by Ehleringer and Osmond (1989) with an extraction time of 4–5 h at 100°C and -1 MPa, as suggested by Araguás-Araguás *et al.* (1995). All samples were weighted before and after water extraction, as well as after an additional oven drying (48 h at 105°C) to assess the extraction efficiency. Only samples that reached a water recovery higher than 98% were used for further isotope analysis (Araguás-Araguás *et al.* 1995; Orłowski *et al.* 2016). Deuterium and oxygen isotope analysis were carried out with an L2120-i analyzer (Picarro, U.S.A.). A micro-pyrolysis module (Ao214) and ChemCorrect post processing software were employed to remove the interference of organic material and correct the results. The isotope ratios ( $^2\text{H}/\text{H}$  and  $^{18}\text{O}/^{16}\text{O}$ ) are expressed in the delta notation as per mil (‰) with  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  being defined relative to the Vienna-Standard Mean Ocean Water (V-SMOW):

$$\delta^{18}\text{O} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \text{‰ OR } \delta^2\text{H} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \text{‰}$$

Where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the  $^2\text{H}/\text{H}$  or  $^{18}\text{O}/^{16}\text{O}$  ratios of the sample and the V-SMOW, respectively. The analytical precision for each sample was 0.2 ‰ for  $\delta^{18}\text{O}$  and 0.5 ‰ for  $\delta^2\text{H}$ .

Pits of one square meter were dug to a depth of 0.4 m at each plot to assess the wheat root density. Wheat roots were sampled at depth intervals of 0.1 m. The root samples were taken on the same day as plant and soil sampling. Samples were placed on sieves, suspended and washed until they were free of soil. Afterwards, the roots were dried at 75°C for 48 h to a constant weight.

Leaf water potential ( $\Psi_L$ ) was measured with a pressure chamber (WP4, Decagon, U.S.A.) on three randomly selected top leaves of wheat at 12 A.M. on the day of plant sampling. A paired samples t-test was used to test if the difference of  $\Psi_L$  with and without biochar application were different from zero.

Soil water retention curve (SWRC) were derived by measuring SW content along 12 different pressure heads for each plot based on the methods of Corneli *et al.* (2005). The pressure heads ranging from -1 to -100 cm was analyzed in a sand box, while a Pressure Membrane (Soilmoisture Equipment Corp., CA, USA) were applied for the range of -340 to -15300 cm. When the soil reached equilibrium at -15300 cm, soil samples were dried in an oven with 105°C for about 24 h. Then, the soil bulk density and gravimetric SW content were measured. The fitting of the volumetric SW content versus pressure head were conducted by a bi-exponential model of Dexter *et al.* (2008):

$$\theta = \theta_r + \theta_{\text{txt}} e^{-\frac{h}{h_{\text{txt}}}} + \theta_{\text{str}} e^{-\frac{h}{h_{\text{str}}}}$$

Where  $\theta_r$  represent the residual volumetric water content ( $\text{m}^3 \text{m}^{-3}$ ),  $\theta_{\text{txt}}$  and  $\theta_{\text{str}}$  are the matrix pore and structural pore

water contents ( $\text{m}^3 \text{m}^{-3}$ ), respectively;  $h$  represents the pressure head (cm); and  $h_{\text{txt}}$  and  $h_{\text{str}}$  are matrix pore and structural pore pressure head, respectively. Soil physical parameters including PAWC were derived from the SWRCs. The saturation of SW  $S$  was calculated as follow:

$$S = \frac{\theta - \theta_r}{\theta_s - \theta_r}$$

Where  $\theta_s$  is the saturation water content ( $\text{m}^3 \text{m}^{-3}$ ). The  $\theta_s$  and  $\theta_r$  represent the corresponding soil volume water content under the pressure of 0 and 15300 cm, respectively.

With equation (3),  $\theta \sim \log h$  was converted to  $S \sim \log h$ . The cubic spline function was used to fit the curve of  $S \sim \log h$  (Kastanek and Nielsen 2001), with which the differential curve of  $dS(\log h)/d(\log h) \sim \log h$  was obtained. Based on the capillary equation, the relationship of pore diameter  $D$  ( $\mu\text{m}$ ) and  $h$  (cm) is expressed as:

$$D = \frac{3000}{h}$$

With which,  $dS(\log h)/d(\log h) \sim \log h$  was converted to  $dS(\log D)/d(\log D) \sim \log D$  to represent characteristics of pore size distribution (Kutílek and Nielsen 1994).

### Analysis method

A Bayesian model approach was used to determine the sources of water uptake by winter wheat with and without biochar amendment. To this end, we applied the SIAR (stable-isotope analysis in R) Bayesian mixing model statistical package (Parnell *et al.* 2010). As maximum rooting depth was 0.4 m and groundwater depth was far below 0.4 m, SW can be considered as the sole water source to plant growth. Samples were collected when no precipitation occurred at least four days prior to sampling to eliminate rainfall interference. Three potential sources of stem water were classified when running the Bayesian model: (1) SW at 0–0.1 m; (2) SW at 0.1–0.2 m; and (3) SW at 0.2–0.4 m. The trophic enrichment factor and concentration dependence of the original model were set to 0. The model was run with 50000 iterations and a source water's likelihood of a source water's contribution to stem water (*i.e.*, the mean of the posterior distribution of the MCMC simulation) to stem water was obtained to calculate the average values of stem and SW isotope composition. Water proportion data were subjected to one-way ANOVA followed by post hoc Fisher's least significant difference test to detect significant differences ( $P < 0.05$ ) between the water proportions from different soil layers. Statistical analyses were conducted using the R (R Core Team 2017).

**Results**

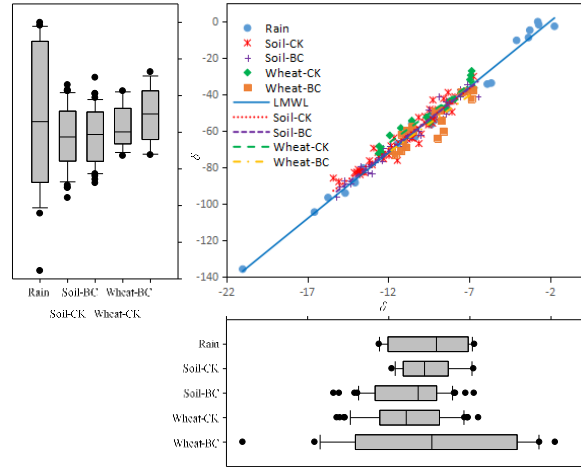
**Isotopic composition of various water samples**

Fig. 1 shows the precipitation, wheat water, and SW samples in dual isotope space ( $\delta^2\text{H}$  vs.  $\delta^{18}\text{O}$ ) from November 1, 2015 to May 30, 2016. Precipitation stable isotope compositions were highly variable. The  $\delta^2\text{H}$  ranged from -115.26 to 10.56‰, with a weighted mean value of -72.12‰; the  $\delta^{18}\text{O}$  ranged from -16.11 to 2.01‰, with a weighted mean value of -11.96‰. The local meteoric water line (LMWL), derived from the regression line through the precipitation samples, was  $\delta^2\text{H} = 7.34\delta^{18}\text{O} + 15.41\text{‰}$  ( $R^2 = 0.97$ ).

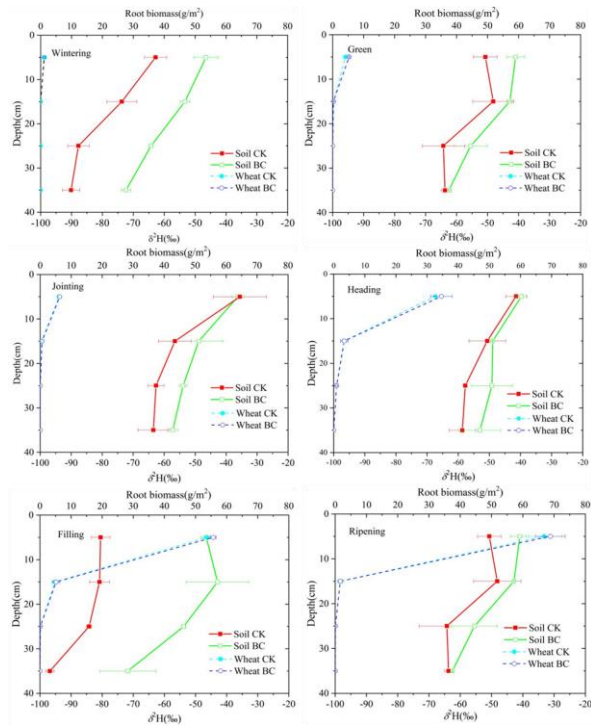
At the plots without biochar application (referred to as CK), SW  $\delta^2\text{H}$  ranged from -95.84 to -34.17‰ with a mean value ( $\pm$  SD) of  $-62.81 \pm 16.43\text{‰}$  and SW  $\delta^{18}\text{O}$  ranged from -15.40 to -6.74‰ with a mean value of  $-10.80 \pm 2.34\text{‰}$ . At the plots with biochar (referred to as BC), SW  $\delta^2\text{H}$  ranged from -87.76 to -30.15‰, with a mean value of  $-61.68 \pm 15.37\text{‰}$ ; SW  $\delta^{18}\text{O}$  ranged from -15.20 to -6.48‰, with a mean value of  $-10.74 \pm 3.60\text{‰}$ . The variation in the amplitude of the isotopic ratios (IRs) was smaller in the SW than in precipitation. The IRs of SW were more enriched in heavy isotopes at the BC plots than the CK plots. This indicated that SW evaporation was higher at the BC plots than the CK plots, although the differences in IRs values were not statistically significant. In addition, IRs of SW at the BC plots were more damped and had a smaller range than the IRs at the CK plots. The linear regressions in dual isotope space for the SW samples were  $\delta^2\text{H} = 6.90\delta^{18}\text{O} + 11.74\text{‰}$  ( $R^2 = 0.96$ ) for the CK plots and  $\delta^2\text{H} = 6.65\delta^{18}\text{O} + 9.75\text{‰}$  ( $R^2 = 0.93$ ) for the BC plots, respectively. The slope of these two regression lines were not significantly different, and they were slightly lower than that of the LMWL ( $P > 0.05$  using SMA estimation).

At the CK plots, wheat water  $\delta^2\text{H}$  ranged from -82.85 to -22.28‰, with a mean value ( $\pm$ SD) of  $-52.50 \pm 15.71\text{‰}$  and stem  $\delta^{18}\text{O}$  ranged from -15.58 to -6.78‰, with a mean value of  $-10.99 \pm 3.39\text{‰}$ . At the BC plots,  $\delta^2\text{H}$  of wheat water ranged from -72.39 to -14.12‰, with a mean value of  $-47.53 \pm 19.95\text{‰}$  and wheat water  $\delta^{18}\text{O}$  ranged from -14.92 to -5.86‰, with a mean value of  $-10.03 \pm 2.83\text{‰}$ . There were no significant ( $P > 0.05$ ) differences between the mean IRs of plant water at the BC and CK plots. The linear regression in dual isotope space for the wheat water at the CK was  $\delta^2\text{H} = 6.44\delta^{18}\text{O} + 10.49\text{‰}$  ( $R^2 = 0.95$ ). The linear regression for wheat stem water samples at the BC plots was  $\delta^2\text{H} = 6.24\delta^{18}\text{O} + 3.12\text{‰}$  ( $R^2 = 0.82$ ). Thus, the slopes were lower than that of the precipitation and SW at the CK and BC, respectively ( $P > 0.05$  using SMA estimation).

Fig. 2 shows SW  $\delta^2\text{H}$  values over depth at the six stages. SW  $\delta^2\text{H}$  usually decreased with depth at both BC and CK plots. SW  $\delta^2\text{H}$  values were more similar between BC and CK plots at lower SW condition. Moreover, SW  $\delta^2\text{H}$  was generally more enriched in the BC than the CK plots.

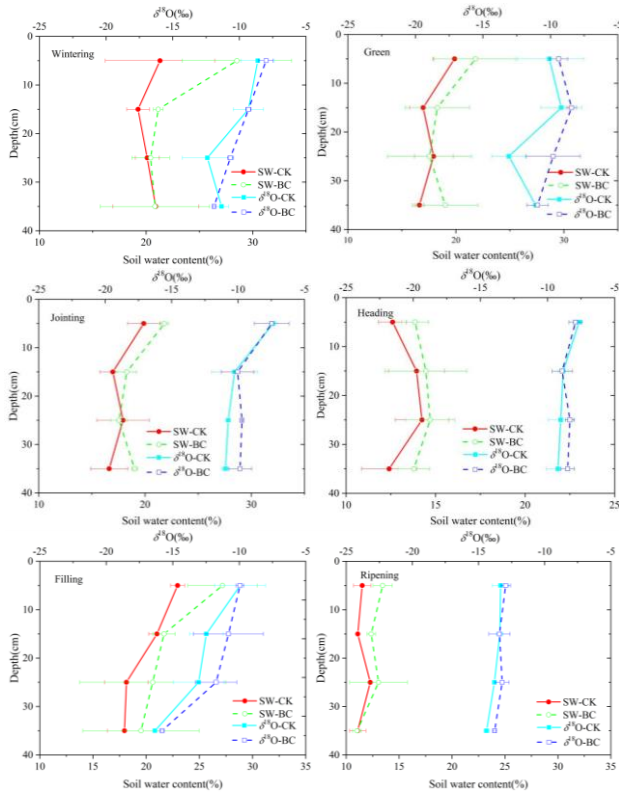


**Fig. 1:** Dual isotopes plot stable isotopes of water from rainwater (N=22), wheat water of the BC plots (N=18), wheat water of the CK plots (N=18), bulk soil water of the BC plots (N=48), bulk soil water of the CK plots (N=48), (LMWL:  $\delta^2\text{H} = 7.34\delta^{18}\text{O} + 15.41$ ,  $R^2 = 0.97$ ; Linear regression for soil water at the BC plots:  $\delta^2\text{H} = 6.65\delta^{18}\text{O} + 9.75$ ; for soil water at CK:  $\delta^2\text{H} = 6.90\delta^{18}\text{O} + 11.74$ ; for wheat water at BC:  $\delta^2\text{H} = 6.24\delta^{18}\text{O} + 3.12$ ; and for wheat water at CK:  $\delta^2\text{H} = 6.44\delta^{18}\text{O} + 10.49$ ). Boxplots show 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> percentiles, while data extremes are shown by black points



**Fig. 2:** Root biomass (upper axis) and  $\delta^2\text{H}$  values (lower axis) across soil profiles

Fig. 3 compares the SW content and the  $\delta^{18}\text{O}$  values between the BC and CK plots. At the winterring and filing stages, SW content of 0–0.1 m was significantly ( $P < 0.05$ ) higher at the BC than the CK plots. For other stages and depths,



**Fig. 3:** Soil water content (lower axis) and  $\delta^{18}\text{O}$  values (upper axis) across soil profiles in different growing period of wheat (error bars represent SD)

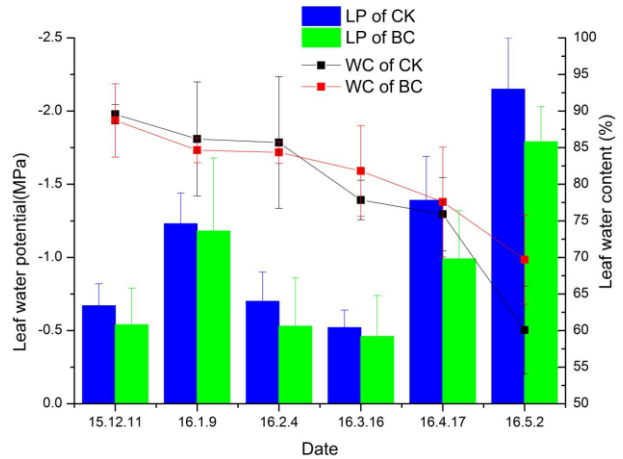
although SW content at the BC plots was always higher than the CK plots, the differences were not significant ( $P > 0.05$ ). The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  showed similar trends over depth.

### Soil physical properties and leaf water potential

Table 1 shows the comparison of soil basic properties with and without biochar amendment at two different days. Bulk density was lower and total porosity was higher at the BC plots than at the CK plots. These differences were significant ( $P < 0.05$ ) at 0–0.1 m depth but not for depths below 0.1 m. Xiao *et al.* (2020) also reported that biochar use decreased bulk density from 1.47 (CK) to 1.34  $\text{g}\cdot\text{cm}^{-3}$ . SW content at BC plots was significantly higher than at CK plots for the 0–0.1 m depth in March ( $P < 0.05$ ).

Fig. 2 further shows root biomass of winter wheat over depth. At the wintering and green stages, rooting depth was limited to the upper 0.2 m. At jointing and ripening stages, roots reached down to 0.3 and 0.4 m, respectively. However, more than 90% of the roots were located at 0–0.1 m thorough out the study period. Root biomass was generally higher at the BC than the CK plots but the differences were only significant for the topsoil (0–0.1 m).

The  $\Psi_L$  of winter wheat grown at the CK was generally lower than at the BC plots and differences were most pronounced in April and May (Fig. 4). The lowest



**Fig. 4:** Leaf water potential (LP) and leaf water content (WC) of wheat leaf at the BC and CK plots (error bars represent SD)

water potential occurred during the ripening stage. In addition, water content of wheat leaf was higher at the BC than at the CK plots during the last three stages, but there were little differences during the first three stages.

Fig. 5 shows the average pore size distributions of the 0–0.1 and 0.1–0.2 m soil layers at the CK and BC plots. The pore size distribution of the Entisol shows a bimodal form. Two peaks were found at the pores with diameter of 1 and 50  $\mu\text{m}$ . It seems that the pore size distribution of 0.1–0.2 m soil layer was not affected much by biochar application. At 0–0.1 m layer, however, biochar amendment resulted in a larger equivalent pore (1.95  $\mu\text{m}$ ) and peak value for the first peak. However, it did not change the size of equivalent pore but decreased peak value for the second peak. Therefore, biochar application increased the quantity of pores with diameter from 0.68  $\mu\text{m}$  to 39  $\mu\text{m}$ , but decreased the quantity of pores with diameter from 39  $\mu\text{m}$  to 1490  $\mu\text{m}$  for the upper 0.1 m of this Entisol. Consequently, biochar application increased meso-porosity and decreased macro-porosity of the topsoil (0–0.1 m layer).

Table 2 shows the comparison of some soil physical properties in the upper 0.2 m soil between the CK and BC plots. The application of biochar did not significantly change the particle size, residual water content, saturated water content, field capacity, and permanent wilting water content. PAWC increased by biochar application from 4.4 to 6.0% at 0–0.1 m and from 5.0 to 6.9 at 0.1–0.2 m soil depth, demonstrating an increase in soil's capability of keep high PAWC. Phillips *et al.* (2020) found that biochar amendment showed a significant increase in saturated water content of 0.05–1.7% per  $\text{Mg}\cdot\text{ha}^{-1}$  and decreases in wilting point by 0.09–0.8% per  $\text{Mg}\cdot\text{ha}^{-1}$  in four soil textures. Safaei Khorram *et al.* (2020) also reported that water holding capacity increased 25.00–28.00% after the first year of biochar amendment. These results were comparable to our data with application amount.



**Table 1:** Soil basic properties of soil profiles at the BC and CK plots for two campaigns. Shown are mean values ( $\pm$ SD) of three replicates

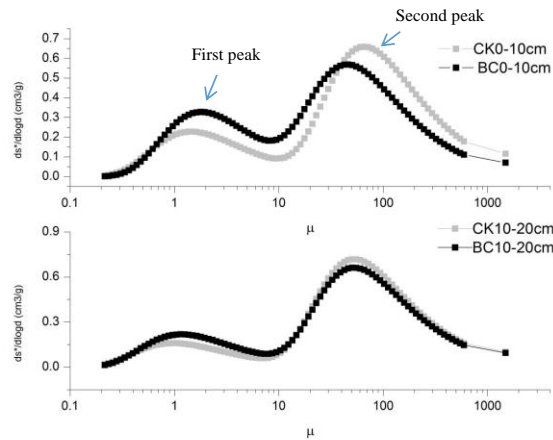
Date	Depth (cm)	Soil water content (%)		Soil bulk density ( $\text{g cm}^{-3}$ )		Total porosity (%)	
		CK	BC	CK	BC	CK	BC
12-17-2015	0-10	21.57 $\pm$ 0.06% <sup>a</sup>	32.14 $\pm$ 6.92 % <sup>b</sup>	1.43 $\pm$ 0.14 <sup>a</sup>	1.13 $\pm$ 0.04 <sup>b</sup>	46.10 $\pm$ 5.25% <sup>a</sup>	57.51 $\pm$ 1.34% <sup>b</sup>
	10-20	18.97 $\pm$ 0.52% <sup>a</sup>	21.08 $\pm$ 2.39% <sup>a</sup>	1.61 $\pm$ 0.04 <sup>a</sup>	1.52 $\pm$ 0.01 <sup>a</sup>	39.17 $\pm$ 1.48% <sup>a</sup>	42.57 $\pm$ 0.49% <sup>b</sup>
	20-30	17.10 $\pm$ 1.19% <sup>a</sup>	20.54 $\pm$ 2.42% <sup>a</sup>	1.66 $\pm$ 0.03 <sup>a</sup>	1.56 $\pm$ 0.02 <sup>a</sup>	37.36 $\pm$ 1.21% <sup>a</sup>	40.95 $\pm$ 0.68% <sup>a</sup>
	30-40	18.04 $\pm$ 0.52% <sup>a</sup>	22.18 $\pm$ 1.12% <sup>a</sup>	1.59 $\pm$ 0.03 <sup>a</sup>	1.54 $\pm$ 0.08 <sup>a</sup>	39.91 $\pm$ 1.14% <sup>a</sup>	41.79 $\pm$ 2.96% <sup>a</sup>
3-28-2016	0-10	19.16 $\pm$ 0.59% <sup>a</sup>	22.12 $\pm$ 0.04% <sup>a</sup>	1.36 $\pm$ 0.08 <sup>a</sup>	1.23 $\pm$ 0.09 <sup>b</sup>	48.64 $\pm$ 3.02% <sup>a</sup>	53.65 $\pm$ 3.48% <sup>b</sup>
	10-20	17.17 $\pm$ 0.95% <sup>a</sup>	19.68 $\pm$ 3.04% <sup>a</sup>	1.46 $\pm$ 0.11 <sup>a</sup>	1.49 $\pm$ 0.19 <sup>a</sup>	45.08 $\pm$ 4.05% <sup>a</sup>	43.95 $\pm$ 7.19% <sup>a</sup>
	20-30	18.44 $\pm$ 1.99% <sup>a</sup>	18.56 $\pm$ 3.76% <sup>a</sup>	1.56 $\pm$ 0.02 <sup>a</sup>	1.56 $\pm$ 0.09 <sup>a</sup>	41.04 $\pm$ 0.56% <sup>a</sup>	41.12 $\pm$ 3.52% <sup>a</sup>
	30-40	18.00 $\pm$ 1.00% <sup>a</sup>	19.11 $\pm$ 3.96% <sup>a</sup>	1.69 $\pm$ 0.00 <sup>a</sup>	1.59 $\pm$ 0.10 <sup>a</sup>	36.31 $\pm$ 0.16% <sup>a</sup>	40.10 $\pm$ 3.82% <sup>a</sup>

Different character indicates different at 0.05 level

**Table 2:** Comparison of soil properties of 0-0.2 m soil between the CK and BC plots. Shown are mean values ( $\pm$ SD) of three replicates

Soil properties	CK		BC	
	0-10 cm	10-20 cm	0-10 cm	10-20 cm
Clay (%)	20.8 $\pm$ 0.6 <sup>a</sup>	19.9 $\pm$ 0.3 <sup>a</sup>	18.3 $\pm$ 0.4 <sup>a</sup>	20.6 $\pm$ 3.2 <sup>a</sup>
Silt (%)	51.2 $\pm$ 4.0 <sup>a</sup>	57.1 $\pm$ 5.6 <sup>a</sup>	47.5 $\pm$ 2.2 <sup>a</sup>	52.8 $\pm$ 7.2 <sup>a</sup>
Residual water content (%)	19.1 $\pm$ 1.9 <sup>a</sup>	21.9 $\pm$ 6.7 <sup>a</sup>	19.3 $\pm$ 1.0 <sup>a</sup>	18.8 $\pm$ 6.0 <sup>a</sup>
Saturated water content (%)	60.1 $\pm$ 5.0 <sup>a</sup>	52.0 $\pm$ 6.0 <sup>a</sup>	59.7 $\pm$ 2.0 <sup>a</sup>	51.7 $\pm$ 2.0 <sup>a</sup>
Field water capacity (%)	25.5 $\pm$ 2.9 <sup>a</sup>	31.1 $\pm$ 1.1 <sup>a</sup>	26.5 $\pm$ 4.6 <sup>a</sup>	28.8 $\pm$ 1.7 <sup>a</sup>
Permanent wilting water content (%)	21.1 $\pm$ 4.6 <sup>a</sup>	26.1 $\pm$ 2.8 <sup>a</sup>	20.5 $\pm$ 7.0 <sup>a</sup>	21.9 $\pm$ 1.9 <sup>a</sup>
Plant available water capacity (%)	4.4 $\pm$ 0.7 <sup>a</sup>	5.0 $\pm$ 1.1 <sup>a</sup>	6.0 $\pm$ 0.4 <sup>a</sup>	6.9 $\pm$ 0.8 <sup>a</sup>

Different letters indicate significant difference between CK and BC at the same depth at 0.05 level

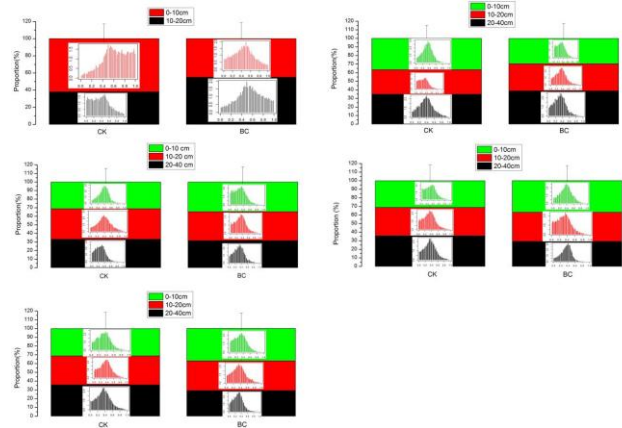


**Fig. 5:** The corresponding pore size distributions of soil cores of 0-10 cm (a) and 10-20 cm (b) in the plots with (BC) and without (CK) biochar amendment

**Water source of wheat water**

Fig. 6 shows the wheat water sources at the BC and CK plots in the growing season. At the wintering stage, only SW from 0–0.1 m depth was available for wheat roots. At the green stage, wheat water sources at the CK plots were 55.0  $\pm$  23.3% (mean  $\pm$  SD) from 0–0.1 m layer and 45.0  $\pm$  23.6% from 0.1–0.2 m layer; the corresponding values at the BC plots were 38.0  $\pm$  22.3 and 62.0  $\pm$  23.3%, respectively. This indicated that wheat at the CK plots acquired greater share of water from 0–0.1 m layer and wheat at the BC plots absorbed greater share of water from 0.1–0.2 m layer.

At the jointing stage, wheat grown at the CK plots used 38.7  $\pm$  14.3, 31.7  $\pm$  15.1 and 29.5  $\pm$  14.2% water from



**Fig. 6:** Source water partitioning using Bayesian mixing model. Also shown are the respective probability density plots of each putative source water superimposed on plots of relative contribution to wheat water (error bars represent 1 SD)

0–0.1, 0.1–0.2, and 0.2–0.4 m layer, respectively. The corresponding values at the BC plots were 34.8  $\pm$  14.5, 28.7  $\pm$  17.1 and 36.5  $\pm$  15.1%, respectively.

At heading stage, wheat at the CK plots acquired 26.9  $\pm$  15.1, 39.6  $\pm$  18.9 and 33.4  $\pm$  15.6% water from 0–0.1, 0.1–0.2, and 0.2–0.4 m layer, respectively, while the corresponding values at BC plots were 32.4  $\pm$  17.1, 34.9  $\pm$  18.2, and 32.6  $\pm$  17.5%, respectively. Thus, wheat grown at BC plots used more water from 0–0.1 m than wheat at CK plots, but the differences were non-significant ( $P > 0.05$ ).

At the filing stage, wheat grown at the CK plot used 29.1  $\pm$  15.8, 34.0  $\pm$  17.6 and 36.9  $\pm$  17.5% water from the 0–0.1, 0.1–0.2, and 0.2–0.4 m layer, respectively, while

wheat grown at the BC plots acquired  $35.7 \pm 18.8$ ,  $33.1 \pm 18.1$  and  $31.1 \pm 18.6\%$  from 0–0.1, 0.1–0.2, and 0.2–0.4 m layer, respectively. Again, wheat at the BC plots used more water from 0–0.1 m than wheat at the CK plots, but the differences were not statistically significant ( $P > 0.05$ ).

At the ripening stage, wheat grown at CK the plot used  $33.3 \pm 18.4$ ,  $32.5 \pm 17.9$  and  $31.4 \pm 17.9\%$  water from 0–0.1, 0.1–0.2, and 0.2–0.4 m layer, respectively, while the wheat grown at the BC plots acquired  $36.8 \pm 19.1$ ,  $33.3 \pm 18.9$  and  $29.9 \pm 17.8\%$  water from 0–0.1, 0.1–0.2, and 0.2–0.4 m layer, respectively. At this stage, wheat grown at the BC plots again tended to use similar higher water proportions from shallow soil layers than wheat grown at the CK plots. At the last three stages, wheat acquire higher water proportion from 0–0.2 m depth in BC plots than the CK's which ranges from 0.7 to 4.3%.

## Discussion

The lower slopes of  $\delta^2\text{H}$  vs  $\delta^{18}\text{O}$  lines for SW compared with the LMWL indicated evaporation effects on SW. SW at the BC plots was more isotopically enriched compared to SW at CK plots. Because of the smaller atomic weight, deuterium is more likely to be evaporated than  $^{18}\text{O}$  (Craig *et al.* 1963). This disproportional enrichment of  $^{18}\text{O}$  relative to  $^2\text{H}$ , as a result of kinetic non-equilibrium fractionation, resulted in a moderately lower slope in  $\delta^2\text{H}$  vs.  $\delta^{18}\text{O}$  plots for SW compared to precipitation. The lower slope of the SW regression line at the BC plots compared to the CK plots showed that SW at the BC plots experienced stronger soil evaporation than the SW at the CK plots. In general, previous findings suggest that isotopic fractionation effects are more pronounced for soils with a higher fraction of small pores where immobile SW is stored (Barnes and Turner 1998; Brooks *et al.* 2010; Zhao *et al.* 2013b). The small pores have diameter less than  $0.2 \mu\text{m}$ , mesopores have a diameter of  $0.2\text{--}30 \mu\text{m}$ , and macropores have a diameter large than  $30 \mu\text{m}$ . This implied that the biochar amendment prolonged the SW retention time by increasing microporosity, which led to longer experience of evaporative enrichment and more pronounced isotopic fractionation for these waters. Thus, our stable isotope data support that biochar addition results in a higher water retention, as reported in other studies (Brockhoff *et al.* 2010; Laird *et al.* 2010; Karhu *et al.* 2011). This was mainly due to the increased mesopores quantity by biochar where a portion of immobile water was stored (Devereux *et al.* 2012). The increase of SW content by biochar application was more pronounced during higher soil wetness. Phillips *et al.* (2020) stated that biochar amendment resulted in an increase in saturation water content. This can explain the reason that the effects of biochar application on SW were pronounced for high soil wetness. Compared with the total porosity, water-filled porosity was more affected by biochar application due to the different effects of biochar on mesoporosity and macro-porosity (Fig. 6). However, drainage

and evaporation would exhaust water stored in the macropores and most part of meso-pores during the dry periods, left the water in the micropores which had little differences between the BC and CK plots.

## Wheat water source in a shallow Entisol

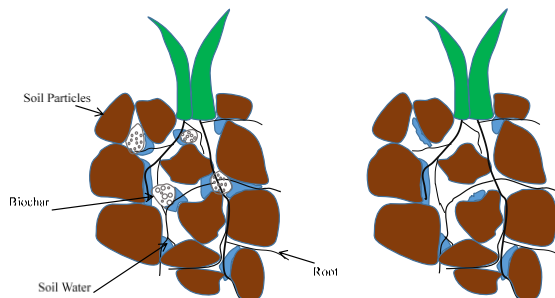
The wheat rooting depth was generally limited to the upper 0.4 m in this shallow soil. Following the growth of root, wheat gradually used deeper SW throughout the growing season. At the heading, filing and ripening stages, root depth reached 0.4 m depth. The results of Bayesian model analysis showed that wheat preferably took up water from 0–0.2 m compared to 0.2–0.4 m soil depths, which agrees with the rooting distribution that most roots were located in the upper 0.1 m (Fig. 2). Thus, wheat acquired most water from the topsoil, which provided enough water to supply the plants. Guan *et al.* (2015) also reported that winter wheat mainly acquired water from 0–0.2 m soil depth during the winter, green and jointing stages.

In addition, the 0–0.2 m SW played a more important role in winter wheat water use at the BC than at the CK plots. Water used by wheat at the BC plots was more isotopically enriched than at the CK plots. Evaristo *et al.* (2015) also showed that the isotopic composition of plant water is usually kinetically fractionated, which stems from the SWs, the potential water source, that are as well kinetically fractionated (Sprenger *et al.* 2016; Zhao *et al.* 2016). Although the biochar amendment significantly increased SW content at 0–0.1 m soil in wintering and filing stages, biochar amendment did not significantly increase the water use proportion from 0–0.1 m depth at any stages. In this shallow Entisol, wheat acquired water from the whole soil profiles to meet the transpiration requirement. As SW content usually decreased with soil depth, the use of deep SW weakened the effect of top SW in the wheat water use. There are no significant differences in water use strategy between the wheat at the BC and CK plots. This may partly explain why biochar application did not significantly promote crop yield (Tammeorg *et al.* 2014).

Biochar amendment effects on wheat water use strategy were only found at the heading, filing and ripening stages when the root reached its maximum depth. Only at these stages, wheat took up SW from the entire profiles to meet their transpiration needs. Compared with the CK plots, wheat at the BC plots acquired more water from the 0–0.1 m layer, but the differences were not statistically significant. In addition, leaf water potentials at the BC plots were always higher than that at the CK plots, but again there were no significant differences. Higher leaf water potential implied more water supply at the BC plots than the CK plots, especially during dry days. Baronti *et al.* (2014) also found that biochar increased available SW content and led to higher leaf water potential during droughts. They attributed these effects to an increased quantity of micropores and mesopores by biochar. In this research, the

total porosity of the soil increased after biochar application, but the pore size distribution shifted toward smaller pore size range. Phillips *et al.* (2020) showed that biochar amendment resulted in water increase was due to the decrease of wilting point. Theoretically, the water in pores with diameter of 0.2–30  $\mu\text{m}$  calculated by the SW characteristic curves is available for plant water use (Feiza *et al.* 2015). Biochar treatments increased the share of pores with diameters from 0.68  $\mu\text{m}$  to 38.86  $\mu\text{m}$ . This implied that the pores with diameter of 0.68–30  $\mu\text{m}$  were also increased for plant water use considering the fact that biochar application increased the water content. Andrenelli *et al.* (2016) also stated that application of biochar increased soil pores (30–0.2  $\mu\text{m}$ ) for the amended soils. Consequently, the biochar amendment increased PAWC and thus may improve this drought-prone soil. Jones *et al.* (2010) found that biochar addition decreased the share of macropores and increased the quantity of meso- and micro-pores, leading to an increase in available water-holding capacity with the application of biochar to sand. Nawaz *et al.* (2019) indicated that biochar application is effective for increase of water productivity of wheat under limited water conditions. They believed this benefit was due to the biochar triggered wheat plant antioxidant defense system and improved performance of gas exchange behavior. In addition, the root biomass at the BC plots was also higher than the CK plots which may be partly related to the introduced nutrients and water by the added biochar. Higher root biomass facilitated water uptake by winter wheat.

Biochar amendment affected the soil pore distribution and PAWC in two ways. First of all, biochar is a porous media with low bulk density of 0.30–0.43  $\text{g cm}^{-3}$  (Pastor-Villega *et al.* 2006). Low bulk density implies biochar can hold water in its own pores. These pores can reduce soil bulk density and increase the SW content directly. Other studies also found reduced soil bulk density and increased SW content from biochar addition (Major *et al.* 2010; Karhu *et al.* 2011; Vaccari *et al.* 2011; Hussain *et al.* 2017), confirming that this effect is present at a wide range of soil type and biochar concentrations. This was associated with increased biochar porosity, improved soil aggregation or increased store pores (Novak *et al.* 2009). Other factors such as the hydrophilic substances at the surface of biochar could have also increased PAWC (Fig. 7). Secondly, the interaction of biochar with the soil can also benefit to the water storage in soil interaction-effects of biochar. Soil with biochar was more complicated than a mixer of two different materials (Peake *et al.* 2014). The biochar surface could adsorb clay particles to increase the soil aggregate sizes. This is an important factor in improving the soil physical properties such as increasing macro-aggregate formation (Jien and Wang 2013; Xiao *et al.* 2020). The applied biochar media could increase pore quantity and connectivity as a binding agent (Safaei Khorram *et al.* 2020). These processes improve the soil structure for holding more SW (Liu *et al.* 2016).



**Fig. 7:** The sketch map of biochar amendment affecting the soil water distribution and soil water content

Consequently, the biochar amendment changes the pore size distribution, resulting in changes in wheat water use pattern that are highly relevant for water management of the agricultural fields.

## Conclusion

We analyzed the effects of biochar amendment on the water uptake pattern of winter wheat growing on a shallow Entisols in a sloping farmland. Our study showed that soil physical properties like bulk density and soil pore size distribution were improved when biochar was applied to the soil. Biochar amendment significantly increased the SW content at 0–0.1 m soil depth under wet conditions due to an increased pore volume. Wheat grown on soils with biochar amendment acquired more water from the 0–0.1 m soil layer during heading, filling and ripening stages than wheat without biochar influence, because of an improved soil structure and increased PAWC as a result of biochar amendment. However, wheat generally tends to use SW from the entire soil profile to meet the transpiration requirement.

The results of this study have important implications for agricultural water management since the application of biochar to Entisol could be used to mitigate drought stress by allowing soil to retain more water available to support crop production. However, further testing is required to validate this finding, including work over longer time scales.

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