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Soil water content and photosynthetic capacity of spring wheat as affected by soil application of nitrogen-enriched biochar in a semiarid environment

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Abstract

A field trial was conducted to determine the effect of nitrogen-enriched biochar on soil water content, plant's photosynthetic parameters, and grain yield of spring wheat at the Dingxi Experimental Station during the 2014 and 2015 cropping seasons. Results showed that biochar applied with nitrogen fertilizer at a rate of 50 kg ha^{-1} of N (BN_{50}) increased soil water content in the 0–30 cm depth range by approximately 40, 32, and 53% on average at anthesis, milking, and maturity, respectively, compared with zero-amendment (CN_0). Stomatal conductance and net photosynthetic rate after the BN_{50} treatment increased by approximately 40 to 50% compared to CN_0. Soil water content and photosynthetic traits also increased in other treatments using straw plus nitrogen fertilizer, but to lesser extent than that of BN_{50}. Grain yields were highest (1905 and 2133 kg ha^{-1} in 2014 and 2015, respectively) under BN_{50}. From this, biochar appears to have a potential for its use with N-fertilizer as a cost-effective amendment for crop production in semiarid environments.

Additional key words: biochar; chemical fertilizer; crop productivity; crop residues; gas exchange.

Introduction

Soil fertility is a fundamental factor underlying high productivity of intensively managed farming systems (Watson *et al*. 2002). Therefore, careful management of soil fertility is required for long-term agricultural sustainability (Biswas *et al*. 2014). The Loess Plateau is a dryland area of agricultural importance because of its contribution towards food security and employment for more than 30 million people (Zhao *et al*. 2012). The region is regarded as the cradle of agricultural production in China and is primarily used for cropping, but it is also severely affected by soil erosion and high evaporative losses, which therefore restrict productivity (Yin and Yin 2010). Progressive loss of soil organic matter, associated with traditional methods of soil cultivation (Sun *et al.* 2008), often accelerates soil erosion processes, the decline of soil fertility, and loss of soil organic C (Lal 2004, Wang *et al.* 2013). This process also progressively reduces the resilience of the soil and its water-holding capacity (Sun *et al.* 2008). Several studies (*e.g.*, Larney and Angers, 2012, Talgre *et al.* 2012) have shown that land application of organic materials is an effective means to restore soil organic C levels and overall soil fertility, as well as improving soil structural conditions and water-holding capacity.

In low-rainfall environments, such as the Loess Plateau (≈ 390 mm per year), the ability to develop and implement innovative soil management and water conservation practices plays an important role in maintaining, or where possible improving the productive capacity of soils and enhancing the resilience of the

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Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; ET – total evapotranspiration; g_s – stomatal conductance; LA – leaf area; L_S – stomatal limitation; P_N – net photosynthetic rate; RH – relative humidity; SN_{50} – nitrogen fertilizer; VPD – vapour pressure deficit; WUE – water-use efficiency; WUE_g – grain water-use efficiency; Ψ_w – leaf water potential.
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agro-ecosystem. Stubble retention and subsequent incorporation into the soil has gained interest amongst farmers at the Western Loess Plateau. This is practiced widely as a measure to mitigate impacts on soil, and also for water conservation purposes (Huang *et al*. 2008). However, the use of crop residues as a soil amendment is somehow not feasible in that region because they are also used as animal feed and as a source of fuel for domestic purposes (Lal 2007). Research (*e.g*., Yamato *et al.* 2006, Rondon *et al.* 2007, Spokas *et al.* 2012) has also shown that soil incorporation of biochar may be a cost-effective and technically feasible strategy to simultaneously enhance soil fertility and crop productivity in semiarid environments.

Photosynthesis is a sensitive physiological process, which can be used to assess rapid changes in plants' metabolic activity and growth in response to environmental conditions. Photosynthetic efficiency in crops is dependent on soil water availability and nutrient supply (Mengel and Kirkby 1987). Water stress due to limited soil water availability reduces stomatal conductance and net photosynthesis (Larcher 2003), which therefore reduces the rate of plant growth (Reynolds *et al.* 2000). Plants grown in arid and semiarid regions often suffer from periods of soil and atmospheric water deficits, which compromises yield potential (Ali *et al.* 1999). At present, only a reduced number of studies appears to be dealing with the effect of biochar on soil water content, photosynthetic traits, and crop productivity, with some exceptions (*e.g*., Case *et al.* 2012, Basso *et al*. 2013, Baronti *et al.* 2014). However, these studies have been conducted under conditions, which do not necessarily resemble those of the Western Loess Plateau of China. Furthermore, studies on gas exchange between the leaf and the atmosphere in response to nitrogen nutrition have been reported for a variety of soil and climatic conditions (Wang *et al*. 2012), but a paucity of experimental information appears to be for the semiarid conditions typical of northwestern China.

Much of the earlier work in this space was based on studies conducted under controlled environmental conditions in the laboratory or glasshouse, which may not be representative of field conditions (Tezara *et al.* 1999, Parry *et al.* 2002). Therefore, the objectives of the work reported in this article were to: (*1*) evaluate the effects of biochar, straw, and chemical fertilizer application on soil water content, grain yield, and water-use efficiency of spring wheat grown in field conditions, and (*2*) determine the effects of the above mentioned soil amendments on leaf water potential and photosynthetic rates. Experimental data derived from this work may be used to develop suitable crop models that may assist the establishment of practical guidelines and best management practices for soil amendments, such as biochar, used in combination with mineral fertilizers in northwestern China.

Materials and methods

Site description: The study was conducted at the Dingxi Experimental Station (35°28′N, 104°44′E, elevation of 1,971 m a. s. l.) at Gansu Agricultural University, which is located in Gansu Province, northwestern China. The work was undertaken under field conditions during the 2014 and 2015 cropping seasons. The research station is located in the semiarid Western Loess Plateau, which has relatively steep hills and active gullies. The aeolian soil in that region is locally known as Huangmian (Chinese Soil Taxonomy Cooperative Research Group, 1995), which equates to a Calcaric Cambisol in the FAO (1990) soil classification, and has a sandy loam (≥ 50% sand) texture. This soil has moderately low fertility and slightly alkaline pH (≈ 8.3), ≤ 7.65 g(soil organic carbon) kg^{-1}, and ≤ 13 mg(Olsen–P) kg^{-1}, and is the dominant soil type in the district, used primarily for cropping (Zhu *et al.* 1983). Long-term annual rainfall at Dingxi averages 391 mm, with about 54% received between July and September. Daily maximum temperatures can rise to 38ºC in July, while minimum temperature can drop to –22ºC in January. Long-term climatic records show that annual cumulative temperature > 10ºC is approximately 2240ºC and annual radiation is 5930 MJ m^2 with about 2480 h of sunshine per year. In summer, the climate is warm, sunny, and relatively moist. Potato (*Solanum tuberosum* L.) was the crop grown at the site prior to the experiment. Season rainfall recorded at the site during the course of the experiment was 164 mm in 2014 and 252 mm in 2015 (Fig. 1).

Experimental design: A complete randomized plot experiment with four treatments and three replicates per treatment was established in 2014. The treatments were as follows: CN_0 – control (zero-amendment), CN_{50} – 50 kg(N) ha^{-1}, BN_{50} – 15 t(biochar) ha^{-1} + 50 kg(N) ha^{-1}, and nitrogen fertilizer (SN_{50}) – 4.5 t(straw) ha^{-1} + 50 kg(N) ha^{-1}, respectively. Nitrogen fertilizer was applied in the form of urea (46% N). The text table below provides a full description of the treatments used in the study. The biochar was evenly spread by hand on the soil surface in March 2014, and subsequently incorporated into the soil using a rotary tillage implement to a depth of 10 cm. The biochar material was produced from maize straw through a slow pyrolysis process at a temperature of 500ºC, and it was acquired from a local supplier. Table 1S (*supplement available online*) shows the chemical composition of the biochar and straw used in the experiment. In straw-amended plots, the plant material from the previous crop was weighted and returned to the original plots immediately after threshing and spread evenly on the soil surface. The N-fertilizer (urea) was applied immediately before crop establishment. All the treatments received a

Treatment	Nutrient source	Detailed description
CN_0	N_0	Control (zero-amendment).
CN_{50}	50 kg(N) ha^{-1}	50 kg N ha^{-1} applied in the form of urea (460 g kg^{-1}) in 2014 and in 2015
BN_{50}	15 t ha^{-1} biochar+50 kg(N) ha^{-1}	a single biochar application at a rate of 15 t ha^{-1} in 2014; and 50 kg(N) ha^{-1} applied in 2014 and in 2015
SN_{50}	4.5 t ha^{-1} straw+50 kg(N) ha^{-1}	4.5 t ha^{-1} straw applied in 2014 and in 2015; and 50 kg(N) ha^{-1} applied in 2014 and in 2015

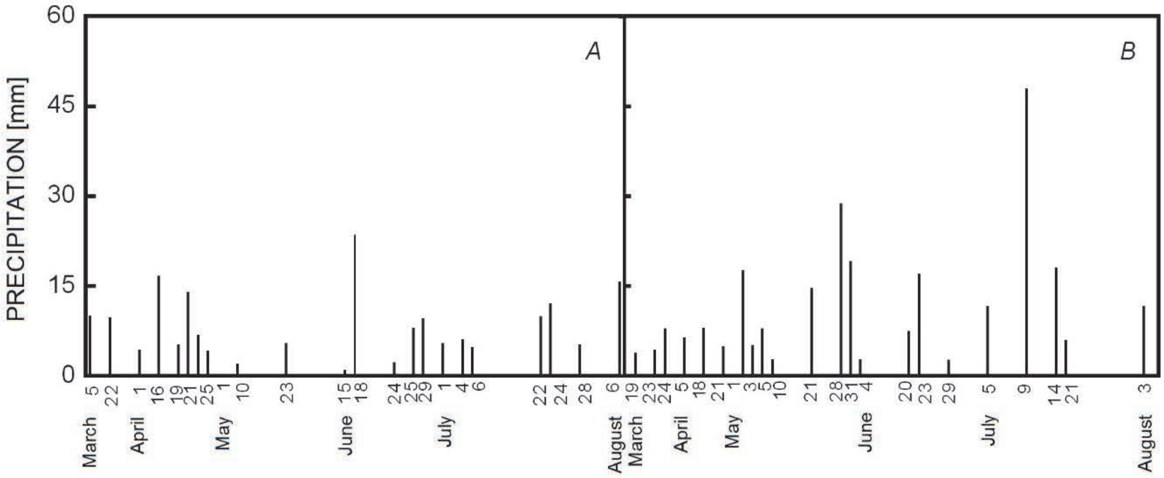

Fig.1. Daily rainfall records for the 2014 (*A*) and 2015 (*B*) cropping season.

blanket application of phosphorus at a rate of 46 kg(P) ha^{-1} as calcium superphosphate (6.1% P). Spring wheat (*Triticum aestivum* L.) was sown in the middle of March at a rate of 187.5 kg(seed) ha^{-1} with a row spacing of 20 cm, and harvested between late July and early August. This experimental setup had a total of twelve plots of 18 m^2 (plot dimensions: 3 m × 6 m).

Soil and plant measurements: Soil water content, leaf area (LA), chlorophyll (Chl) content, leaf water potential (Ψ_w), stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E), intercellular CO_2 concentration (C_i), ambient CO_2 concentration (C_a), relative humidity (RH), and vapour pressure deficit (VPD) were simultaneously measured at the following (critical) crop growth stages: anthesis, milking, and physiological maturity, respectively, based on Zadoks *et al.* (1974). Table 2*S* (*supplement available online*) gives a detailed description of growth and development stages of spring wheat based on the above mentioned scale.

Soil water content: Soil water content (%) was measured four times during the crop cycle, as follows: sowing, anthesis, milking, and maturity stages, respectively, and at nine depth intervals, as follows: 0–5, 5–10, 10–30, 30–50, 50–80, 80–110, 110–140, 140–170, and 170–200 cm, respectively. The soil water content in the 0–5 cm and 5–10 cm depth intervals was measured using the oven-drying method described in Jia *et al.* (2012). Gravimetric water content (0–5 and 5–10 cm) was multiplied by soil bulk density to obtain the volumetric water content. *Trime–Pico IPH* (*Precise Soil Moisture Measurement*, *IMKO Micromodultechnik GmbH*, Ettlingen, Germany) was used to measure volumetric soil water content in 10–200 cm depths. Soil water storage was extrapolated from the volumetric soil water content by multiplying it by the layer depth.

Leaf area, chlorophyll (Chl) content, and leaf water potential: Leaf area (LA) was determined using Eq. 1, which is described in Zhao *et al*. (2013):

$$LA = L_l \times L_w \times 0.78 \qquad (1)$$

where LA is leaf area, L_l is leaf length, L_w is leaf width, and 0.78 is a constant. Values of LA reported herein represent the mean value (n = 4) recorded at anthesis and milking. Chl content of fully developed leaves was assessed at anthesis and milking using a portable Chl meter (*SPAD Model 502*, *Minolta Camera Co*., Osaka, Japan). Measurements were performed between 09:00 h and 12:00 h on ten fully expanded leaves per plot. Measurements of water potential (Ψ_w) were carried out with a pressure chamber (*model WP4C*, *Decagon*, USA) on the first fully expanded leaf and near the leaves used for measurements of the photosynthetic parameters described below. Water potential was measured during the 2015 cropping season at anthesis and milking stages, respectively, and between 06:00 and 09:00 h to minimize effects of evaporative losses on Ψ_w readings. Water potential was measured on three leaves per plot.

Photosynthetic parameters: Diurnal variation of g_s, P_N, E, C_i, C_a, RH, and VPD were measured on cloudless days under natural light. The photosynthetic parameters were measured at anthesis and milking on the middle portions of a fully developed leaf, which had full exposure to sunlight. Three representative plants per plot from the six inner rows were chosen for the measurement. Subsequently, one leaf per plant was chosen to conduct the measurement over a period of 1.5 min in which three readings were recorded. Measurements were conducted at regular intervals of two hours between 08:00 h and 06:00 h using a portable gas-exchange fluorescent system (*GFS–3000*, *Heinz Walz GmbH*., Eichenring, Germany). Stomata limitation (L_S) was calculated using Eq. 2 described in Yin *et al*. (2006):

$$L_s = 1 - \frac{C_i}{C_a} \quad (2)$$

where L_S is stomata limitation, C_i is intercellular CO_2 concentration, and C_a is ambient CO_2 concentration. The conditions in the gas exchange device were set as follows: flow rate of air through the chamber was 750 µmol s^{-1}, CO_2 absorbance 393.3 ppm, H_2O absorbance 14,598 ppm, area of 4 cm^2, and temperature of 24.74°C, respectively.

Grain yield and water-use efficiency: The entire area of the plot was harvested manually using sickles at 5 cm above ground. The edges (0.5 m) of the plot were trimmed and discarded. Grain yield was determined on a dry mass basis after oven-drying the plant material at 105°C for 45 min and then to constant mass at 85°C. Grain water-use efficiency (WUE_g) was determined using Eq. 3 described in Wang *et al*. (2013):

$$WUE_g = \frac{Y}{ET} \quad (3)$$

where WUE_g is grain water-use efficiency, Y is grain yield (kg ha^{-1}), and ET is total evapotranspiration over the entire growing season (mm). Evapotranspiration (ET) was estimated using Eq. 4:

$$ET = P - \Delta W \quad (4)$$

where ET is total evapotranspiration, P is total precipitation for the growing season, and ΔW is the difference between soil water storage at sowing and harvest, respectively. All parameters are expressed in mm. Previous studies conducted at the study site reported no significant runoff or drainage during the growing season (Huang *et al*. 2008). Water-use efficiency (WUE) at the leaf level was calculated using Eq. 5, described in Polley (2002), as follows:

$$WUE = \frac{P_N}{E} \quad (5)$$

where WUE is water-use efficiency at the leaf level, P_N is net photosynthetic rate, and E is transpiration rate, respectively.

Statistical analyses: Statistical analyses were undertaken using the statistical package *SPSS 22.0* (*IBM Corporation*, Chicago, IL, USA), and evaluated by one-way analysis of variance (*ANOVA*) at a probability level of 5% ($p<0.05$). Mean separation was obtained by *Duncan*'s multiple range test. Data were analyzed on a per-year basis and pooled for bivariate correlation analysis (two-tailed) using *Pearson*'s correlation coefficients.

Results

Soil water content measured at anthesis, milking, and maturity stages increased with increasing soil depth, which was observed for all treatments (Fig. 2). Significant differences in soil water content were only observed in some depth intervals, as shown in Fig. 2 and Tables 3S, 4S. The BN_{50} treatment showed consistently higher soil water contents compared to other treatments both at depth and at all stages of crop development (Fig. 2; Tables 3S,4S). These observations were fairly consistent both years. Differences in soil water content between treatments were larger in the 0–30 cm depth interval. Significant differences in soil water content between treatments were also observed at anthesis, milking, and maturity in both years (Fig. 2; Tables 3S,4S). In the 0–30 cm soil depth interval, BN_{50} and SN_{50} increased soil water content by 45 and 25% (anthesis), 44 and 28% (milking), and 60 and 46% (maturity), respectively, compared to CN_0 in 2014 (Fig. 2*A–C*; Table 3S, *supplement available online*). Similar observations were recorded for these two treatments in 2015 (anthesis: 37 and 21%, milking: 21 and 17%, and maturity: 46 and 41% for BN_{50} and SN_{50}, respectively) although values were marginally lower compared to the previous year, and despite of relatively lower amount of rainfall recorded during the crop season (Fig. 2*D–F*; Table 4S, *supplement available online*). This effect may be attributed to relatively higher yields, and therefore water use by crop, in the second compared to the first year. Soil water content in the 30–110 cm depth interval exhibited significant differences between treatments at anthesis and maturity (Fig. 2; Tables 3S, 4S). Soil water content within that depth range decreased in the order: $BN_{50} > SN_{50} > CN_{50} > CN_0$. In 2014, the BN_{50} treatment showed increases in soil water content in the 30–110 cm depth range of approximately 15% at anthesis and 16% at maturity compared with the CN_0 treatment (Fig. 2*A–C*; Table 3S), and by about 18% at anthesis and 14% at maturity in 2015 (Fig. 2*D–F*; Table 4S). The BN_{50} and SN_{50} treatments improved soil water content in the 0–200 cm depth range at anthesis, milking, and maturity in both years compared with CN_0, but the effect of BN_{50} was consistently higher (Fig. 2, Tables 3S, 4S).

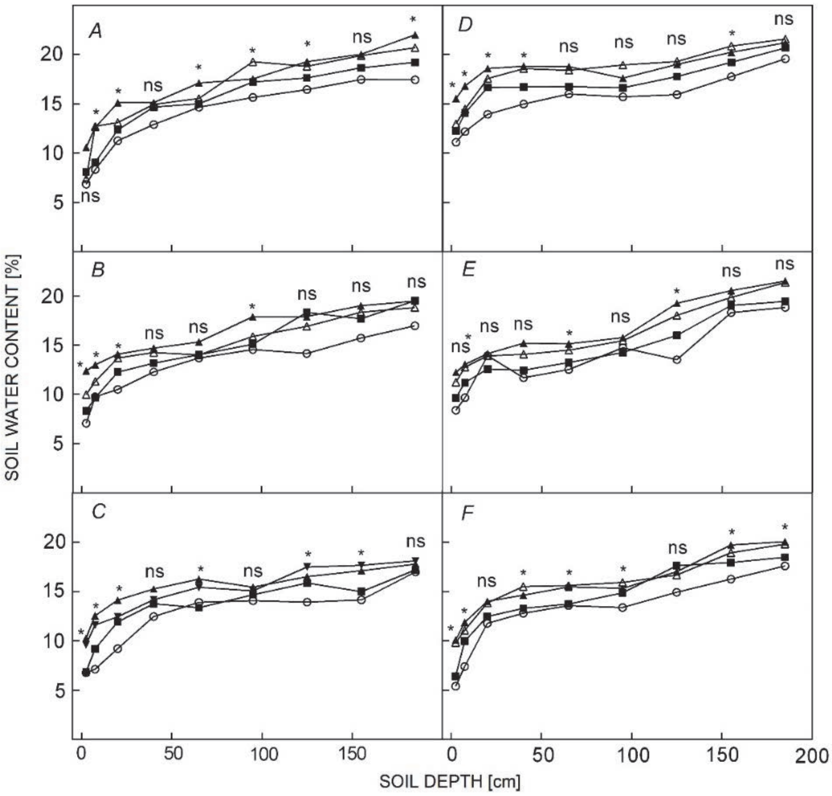

Fig. 2. Soil water content at the 0–200 cm depth range recorded at anthesis, milking and maturity in 2014 (*A–C*), and 2015 (*D–F*), respectively. Symbols are: (○) CN_0; (■) CN_{50}; (▲) BN_{50}; (Δ) SN_{50}. Mean values ± SE (n = 3), and means comparison based on *Duncan*'s multiple range test (p<0.05). Significance (p<0.05) is indicated with an asterisk.

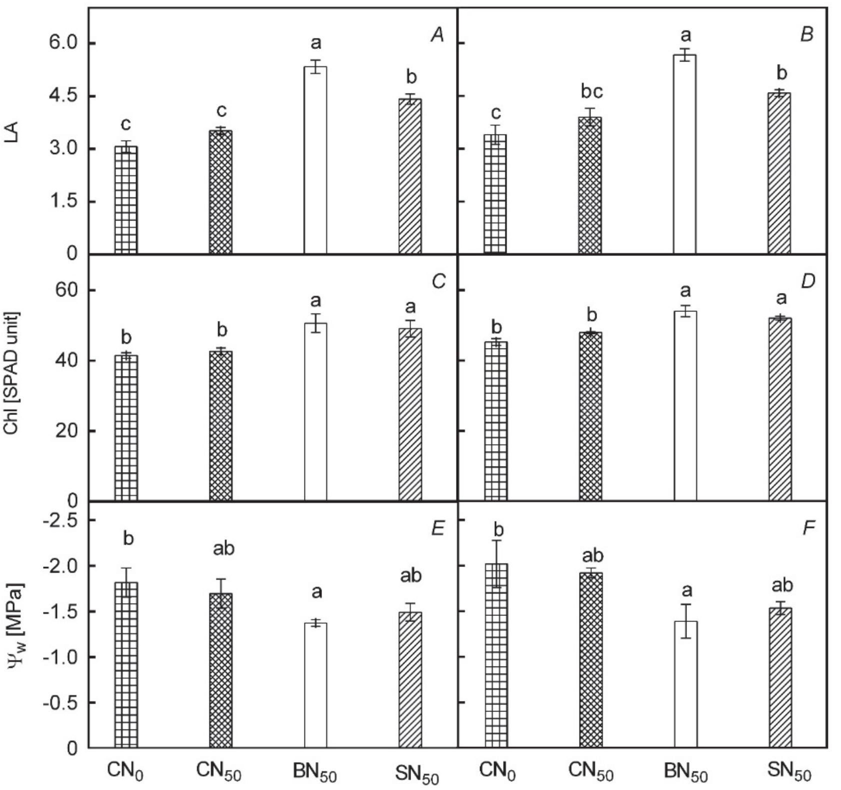

Fig. 3. Leaf area (LA, *A*: 2014, *B*: 2015), chlorophyll content (Chl, *C*: 2014, *D*: 2015) and leaf water potential (Ψ_w) recorded at anthesis (*E*) and milking (*F*), respectively. *Different letters* denote statistically different values at p<0.05. Error bars represent the SE. Mean values ± SE (n = 3), and means comparison based on *Duncan*'s multiple range test (p<0.05).

Leaf area, Chl content and leaf water potential: Overall, there were significant differences between treatments in LA, Chl content, and Ψ_w, respectively, which were observed both years (Fig. 3; Table 5S, *supplement available online*). Application of BN_{50} increased LA by 74 and 52% in 2014 and by 67 and 45%

in 2015 compared with CN_0 and CN_{50}, respectively (Fig. 3*A*–*B*). The SN_{50} treatment increased LA by 44 and 26% in 2014 compared with CN_0 and CN_{50} (Fig. 3*A*), and by 35% in 2015, respectively (Fig. 3*B*). The BN_{50} treatment improved Chl content in both years (*i.e.*, by 22 and 19% in 2014, and by 19 and 13% in 2015) compared with CN_0 and CN_{50}, respectively (Fig. 3*C*–*D*). SN_{50} improved Chl content both in 2014 (by 18 and 15%) and 2015 (by 15 and 9%) compared with CN_0 and CN_{50}, respectively. Water potential was highest at anthesis (–1.37 MPa) and milking (–1.39 MPa) phases in the BN_{50} treatment whereas the lowest Ψ_w values were recorded at the same phases (–1.82 and –2.02 MPa), respectively, in control plots (CN_0) (Fig. 3*E*–*F*).

Diurnal dynamics of photosynthetic parameters: Results derived from measurements of photosynthetic parameters are summarized in Table 1. In both years, the treatments showed similar peak times and daily patterns in the photosynthetic traits at the two critical crop stages (anthesis and milking) investigated. Therefore, the values reported correspond to the mean of both growth stages for each year.

Table 1. Stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E), intercellular CO_2 concentration (C_i), stomatal limitation (L_S) and water-use efficiency (WUE) as affected by treatment. Mean values ± SE (n = 3), and means comparison based on *Duncan*'s multiple range test (p<0.05). *Different letters* within columns denote significance at p<0.05.

Treatment	g_s [mmol m^{-2} s^{-1}]	P_N [mmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	C_i [mmol m^{-2} s^{-1}]	L_S	WUE [μmol(CO_2) mmol^{-1}(H_2O)]
2014						
CN_0	96.49 ± 5.25^b	3.73 ± 0.12^b	2.16 ± 0.19^b	323.81 ± 14.10^a	0.44 ± 0.46^a	1.73 ± 0.21^a
CN_{50}	105.85 ± 5.61^b	3.95 ± 0.19^b	2.18 ± 0.08^b	321.48 ± 4.88^a	0.39 ± 0.005^a	1.81 ± 0.18^a
BN_{50}	146.80 ± 6.72^a	5.58 ± 0.14^a	2.85 ± 0.21^a	296.89 ± 4.78^a	0.29 ± 0.005^b	1.96 ± 0.15^a
SN_{50}	139.88 ± 8.10^a	5.29 ± 0.10^a	2.82 ± 0.12^a	309.76 ± 23.35^a	0.31 ± 0.02^b	1.88 ± 0.04^a
2015						
CN_0	124.57 ± 2.98^b	4.03 ± 0.15^b	2.63 ± 0.20^b	335.26 ± 7.34^a	0.23 ± 0.01^a	1.53 ± 0.13^a
CN_{50}	131.10 ± 2.42^b	4.44 ± 0.26^b	2.72 ± 0.43^b	322.02 ± 7.02^a	0.22 ± 0.033^{ab}	1.63 ± 0.11^a
BN_{50}	174.25 ± 2.91^a	6.10 ± 0.05^a	3.39 ± 0.13^a	312.28 ± 8.26^a	0.15 ± 0.024^b	1.80 ± 0.06^a
SN_{50}	168.57 ± 6.13^a	5.83 ± 0.46^a	3.37 ± 0.06^a	316.28 ± 4.00^a	0.18 ± 0.028^{ab}	1.73 ± 0.16^a

The results of P_N, RH, and VPD are presented in Fig. 1S (*supplement available online*). RH showed relatively high levels at 08:00 h, then decreased sharply, and remained relatively low until around midday, and then reached the minimum at about 16:00 h (Fig. 1S). As expected, VPD increased steadily from 08:00 h and reached the maximum at 16:00 h (Fig. 1S). Diurnal variation of g_s, P_N, E, and L_S showed similar patterns throughout the day, and observations were fairly consistent in both years (Fig. 4). The g_s, P_N, and E increased steadily from 08:00 to 10:00 h, reaching the maximum value at around 12:00 h. Subsequently, they all decreased progressively to reach the minimum at about 16:00 h (Fig. 4). The variability of these parameters was generally similar across years and treatments, and followed the changes observed in diurnal variation of RH and VPD (Fig. 1S). Water loss by transpiration was compensated at dusk as indicated by the bimodal curves of the photosynthetic traits. Such a response was observed in all measurements. Intercellular CO_2 concentration (C_i) was relatively high at 08:00 h and 16:00 h, but it reached a constant value from about 10:00 h to 14:00 h. Regardless of the treatment, L_S was consistently high around midday, and relatively lower in the morning and evening, respectively (Fig. 4*I,J*).

Maximum and minimum g_s, P_N, E, and L_S values were dependent on the treatment and occurred at specific times during the day, as shown in Fig. 4 and Tables 6S–10S (*supplements available online*), respectively. Significant effects of treatments on g_s and P_N were observed on three occasions in 2014 and on two occasions in 2015, as shown in Fig. 4 and Tables 6S, 7S. For example, diurnal g_s and P_N were the highest in the BN_{50} treatment, and the lowest in the control (CN_0). In 2014, BN_{50} showed that at 12:00 h and 14:00 h, maximum g_s and P_N values were 194.34 and 126.71 mmol m^{-2} s^{-1}, respectively. These values were higher than those observed for CN_0 (*i.e.*, g_s = 127.25, and P_N = 87.22 mmol m^{-2} s^{-1}). Significant treatment effects on E and L_S were also observed on four and two occasions in 2014 and 2015, respectively (Fig. 4, Tables 8S, 10S).

Application of BN_{50} and SN_{50} increased diurnal g_s, P_N, and E significantly compared with both CN_0 and CN_{50} (Tables 2, 6S, 7S, 8S). The BN_{50} and SN_{50} application improved g_s, P_N, and E in both years, but the effect of BN_{50} was relatively higher. The CN_0 treatment exhibited higher L_S than BN_{50} (Tables 2, 10S). Generally, g_s, P_N, and E values were higher in 2015 than that in 2014, and treatments with high g_s, P_N, and E had lower C_i and L_S, respectively.

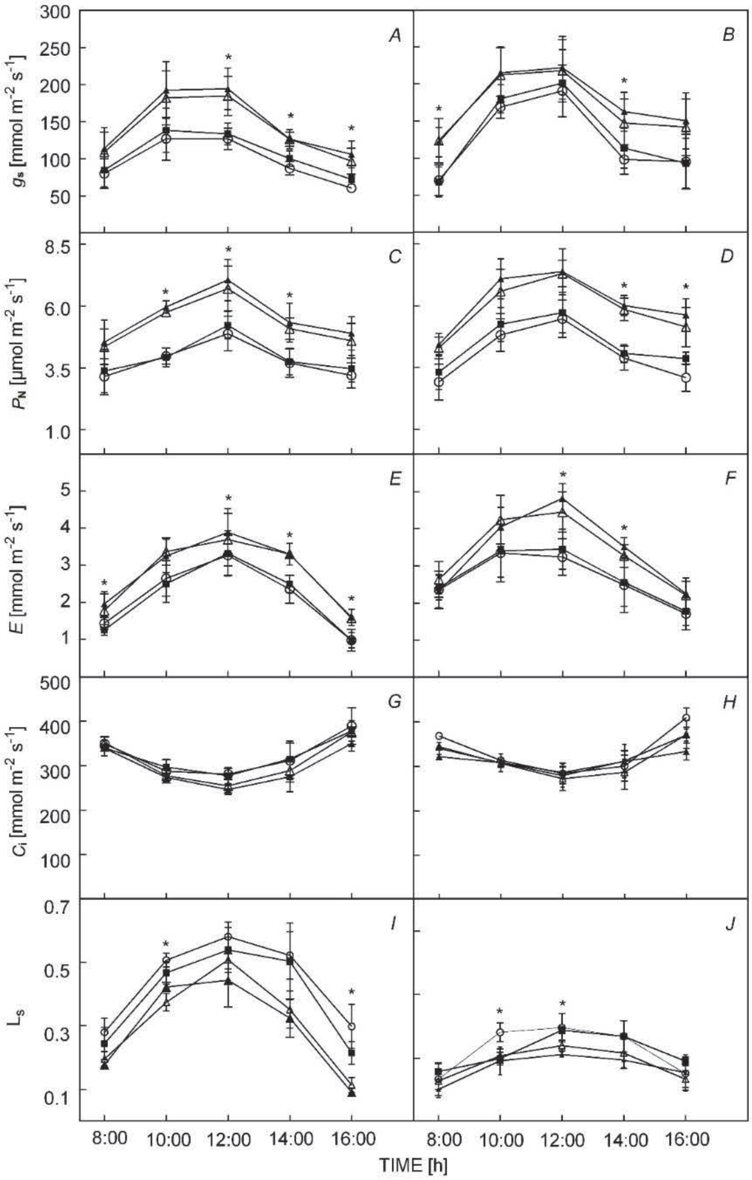

Fig. 4. Diurnal variation in stomatal conductance (g_s, 2014: *A*, 2015: *B*), net photosynthetic rate (P_N, 2014: *C*, 2015: *D*), transpiration rate (*E*, 2014: *E*, 2015: *F*), intercellular CO_2 concentration (C_i, 2014: *G*, 2015: *H*), and stomatal limitation (L_S, 2014: *I*, 2015: *J*). Error bars represent the SE. Mean values ± SE (n = 3), and means comparison based on *Duncan*'s multiple range test ($p<0.05$). Significance ($p<0.05$) is denoted with asterisk.

Table 2. Grain yield, evapotranspiration (ET), and grain water-use efficiency (WUE_g) as affected by treatment. Mean values ± SE (n = 3) and means comparison based on *Duncan*'s multiple range test ($p<0.05$). *Different letters* within columns denote significance at $p<0.05$.

Treatment	Grain yield [kg ha^{-1}]		ET [mm]		WUE_g [kg ha^{-1} mm^{-1}]	
	2014	2015	2014	2015	2014	2015
CN_0	1,305.17 ± 55.68^{b}	1,500.00 ± 83.88^{c}	205.98 ± 20.85^{a}	218.72 ± 22.79^{a}	6.41 ± 0.35^{b}	7.15 ± 0.73^{c}
CN_{50}	1,537.67 ± 162.87^{ab}	1,896.89 ± 44.44^{bc}	202.87 ± 5.21^{a}	217.92 ± 7.65^{a}	7.56 ± 0.72^{ab}	8.22 ± 0.10^{bc}
BN_{50}	1,905.17 ± 210.51^{a}	2,133.33 ± 138.77^{a}	194.71 ± 12.01^{a}	200.24 ± 8.36^{a}	9.73 ± 0.52^{a}	10.69 ± 0.74^{a}
SN_{50}	1,852.33 ± 202.08^{ab}	1,944.44 ± 40.06^{ab}	201.18 ± 20.12^{a}	203.59 ± 2.70^{a}	9.34 ± 1.09^{a}	9.55 ± 0.11^{ab}

Grain yeld and water-use efficiency: Overall, there were significant treatment effects on grain yield and WUE_g, which were observed in both years, as shown in Table 2. BN_{50} produced the highest grain yields both in 2014 (1,905 kg ha^{-1}) and 2015 (2,133 kg ha^{-1}). On average, this was approximately 45% higher than that of CN_0 and 13% higher than that of CN_{50}, respectively. There were no treatment effects on ET ($p>0.05$). On average, WUE_g was ≈ 50% and ≈ 30% higher in BN_{50} than that in CN_0 and CN_{50}, respectively.

Table 3. Correlation coefficients between soil water content (SWC), leaf area (LA), leaf water potential (Ψ_w), stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E), intercellular CO_2 concentration (C_i), grain yield (GY), water-use efficiency (WUE), and grain water-use efficiency (WUE_g). Not significant (ns), significant (*) at $p<0.05$, and significant (**) at $p<0.01$.

	LA	Ψ_w	g_s	P_N	E	C_i	GY	WUE	WUE_g
SWC	0.951*	0.981*	0.967*	0.969*	0.933ns	0.987*	1.000**	0.997**	0.992**
LA		0.970*	0.933ns	0.939ns	0.895ns	–0.988*	0.947ns	0.928ns	0.966*
Ψ_w			0.992**	0.994**	0.973*	–0.986*	0.976*	0.962*	0.997**
g_s				1.000**	0.993**	–0.959*	0.959*	0.948ns	0.988*
P_N					0.992**	–0.962*	0.961*	0.949ns	0.989*
E						–0.921ns	0.923ns	0.910ns	0.963*
C_i							–0.986*	–0.975*	–0.990**
GY								0.998**	0.989**
WUE									0.979*

Correlation analyses: The *Pearson*'s correlation coefficient is presented in Table 3. LA showed a significant (positive) correlation with soil water content ($r^2 = 0.951$, $p<0.05$) and Ψ_w ($r^2 = 0.970$, $p<0.05$). Significant correlations were also observed between soil water content and Ψ_w, g_s, P_N, and C_i ($r^2 = 0.95$, $p<0.05$). Highly significant correlations were observed between soil water content and grain yield ($r^2 = 1.000$, $p<0.01$), WUE_g ($r^2 = 0.992$, $p<0.01$), and WUE ($r^2 = 0.997$, $p<0.01$). A significant linear relationship was found between Ψ_w and g_s ($r^2 = 0.992$, $p<0.01$) and also P_N ($r^2 = 0.994$, $p<0.01$). Significant correlations were also observed between g_s, P_N, and grain yield.

Discussion

Soil water content, particularly in the 0–30 cm depth interval is important for crop production in the Western Loess Plateau. Studies (*e.g.*, Huang *et al.* 2012) have shown that about 60–70% of root biomass of wheat crops grown in northwest China is found within this depth. Such a distribution of root biomass enables the crop to be responsive to rainfall events in relatively dry environments, and therefore it is responsible for > 80% of the water uptake by the crop during the season (Ali *et al.* 1999; Jamieson and Ewert 1999). Therefore, increasing soil water retention at this rooting depth should also increase uptake of water and nutrients by crops. In the present study, the use of biochar combined with N-fertilizer was shown to increase soil water availability, particularly in the top soil (0–30 cm depth range). This observation was consistent across all three stages of crop development and years. Application of biochar has also been reported to increase volumetric water content in soil (*e.g.*, Novak *et al.* 2012), improve soil water retention (*e.g.*, Glaser *et al.* 2002), and increase water infiltration and plant available nutrients (*e.g.*, Major *et al.* 2009, Slavich *et al.* 2012). Stubble retention is also mentioned in several studies (*e.g.*, Huang *et al.* 2008) to have improved water holding capacity in soils of the Western Loess Plateau. Application of biochar could therefore be used as a reliable technique to store rainfall in soil and increase rainfall-use efficiency in dryland areas. Increased soil water holding capacity and plant available water that follows biochar addition (Brockhoff *et al.* 2010, Kammann *et al.* 2011) is explained by increased total porosity in soil and specific surface area (Verheijen *et al.* 2010, Slavich *et al.* 2012). Increased soil water content observed within this study after addition of biochar to soil significantly increased Ψ_w and photosynthetic traits. These findings suggest that biochar applied with N fertilizer at the rates used in this study, have potential to increase the resilience of spring wheat crops grown under semiarid conditions. Thus, this practice offers promise as a means to ameliorate plant water stress and improve crop performance in those environments.

Diurnal variation of g_s, P_N, and E showed similar trends in all treatments. Photosynthetic traits exhibited relatively low levels in the morning and late afternoon, but higher levels around midday, which occurred in response to the diurnal variation of photosynthetic RH and VPD, and water availability to the plant. Several studies (*e.g.*, Flexas *et al.* 2004; Cramer *et al.* 2008; Han and Zhao 2010) argued that relative humidity, vapor pressure deficit, and water availability are the main environmental factors influencing photosynthetic CO_2 uptake and transpiration in wheat. Stomatal conductance directly controls photosynthetic activity and transpiration. This study confirmed the above statement given that the patterns of P_N and E mirrored that of g_s. C_i is also dependent on g_s and the ability of mesophyll cells to assimilate intracellular CO_2. High C_i observed at 08:00 h and 16:00 h may be explained by low g_s and the constant low values recorded between 10:00 h and 12:00 h, which may be associated with high g_s, allowing depletion of C_a

in the plant canopy. The trend of photosynthetic traits during the day may be due to changes in radiation intensity and temperature during the day, and therefore metabolic activity, and importantly plant response to water status leading to stomatal closure/ opening which drives photosynthesis.

Reduced soil water content causes loss of leaf turgor and reduction of stomatal aperture limits photosynthetic CO_2 uptake, internal conductance, and assimilation ability (Cramer *et al.* 2008). Diurnal variation in photosynthetic parameters within our experiment demonstrated that improved soil water content led to increases in g_s, P_N, and E. Moreover, reduced soil water content led to increased L_s, and this may be due to plant stress (Rosales-Serna *et al.* 2004). BN_{50} exhibited higher values of g_s, P_N, and E than the other treatments, indicating the potential of biochar plus N-fertilizer to increase crop's photosynthetic capacity. Increased soil water content improved plant water status and leaf Ψ_w. Water potential was a strong indicator of the trends in the photosynthetic traits. The fact that the Ψ_w of biochar applied with N-fertilizer was less negative than the controls provided evidence of improved water status. This is also supported by the increase observed in photosynthetic rates as soil water deficit tends to reduce g_s and P_N when Ψ_w decreases below a critical level (Baronti *et al.* 2014). From the operational perspective, our results suggest that biochar application could increase the resilience of spring wheat to water deficits that may occur or be induced at critical phases of crop development due to high water demand. Thus, promoting biochar as a soil water conservation and climate change mitigation strategy may be encouraged. Treatments with relatively higher soil water contents, such as BN_{50}, showed therefore greater LA, Ψ_w, g_s, and P_N with lower L_S compared with other treatments. This finding confirms that stomatal closure is an important factor controlling photosynthetic activity. Flexas *et al.* (2006) found that stomatal closure occurs as a protective mechanism against xylem cavitation caused by water stress. Differences in photosynthetic capacity of spring wheat within our study were attributed to variations in the amount of soil water content and the associated (detrimental) effect on LA, Chl, and Ψ_w. Soil water content and Ψ_w accounted for more than 95% of the variation in g_s and P_N.

Grain yield and WUE_g were significantly improved in BN_{50} compare to CN_0, which was observed in both years. Grain yield observed with biochar addition was achieved under low ET, and considered to be high relative to median grain yields (1,400 kg ha^{-1}) typically achieved in the Western Loess Plateau (Yeboah *et al.* 2016). Improved water-use efficiency is needed in environments, where water is the main limiting factor to crop production, and this research demonstrated that such improved efficiency might be achieved when N-fertilizer is used together with biochar. In fact, more than 95% of the variability in the grain yield was explained by WUE. Joint application of nitrogen fertilizer and biochar were also reported in previous studies (*e.g.*, Solaiman *et al.* 2010), which showed significant increases in the grain yield of wheat crops, and therefore agree with our experimental results. This also implies that biochar applied to soil has potential to increase the use efficiency of N-fertilizer because of concurrent improvement in rainfall-use efficiency. Despite this, other studies (*e.g.*, Jeffery *et al.* 2011) showed neutral effect on the grain yield when biochar was applied with N-fertilizer. Grain yield correlated with soil water content and g_s; these two factors explained more than 90% of the variation in grain yield. Parry *et al.* (2011) reported that increased grain yield of wheat was associated with changes in photosynthetic traits, such as g_s and P_N, which also agrees with our observations.

Conclusions: Application of biochar plus N-fertiliser to spring wheat at the rates used in this study increased soil water content, particularly within the 0–30 cm depth interval, to significantly greater extent than the other treatments tested.

Increased soil water retention had a beneficial effect on leaf water potential and photosynthetic activity, which translated into the higher grain yield and water-use efficiency when biochar was applied to soil in combination with N-fertilizer. Improved soil water availability at critical stages of crop development (anthesis and milking) reduced water stress, which therefore contributed to formation of grain yield.

The results reported in this study were consistent with positive correlations observed between photosynthetic traits, leaf water potential, and soil water content. This confirmed that the photosynthetic capacity of the crop is sensitive to changes in available soil water, and that small water stresses can result in significant impacts on grain yield. This set of results offers new insights into beneficial use of biochar as a soil conditioner, particularly when applied with nitrogen fertilizer. From this, it seems to be potential for further development of management practices involving use of biochar in crop production under semiarid environments.

References

Ali M., Jensen C.R., Mogensen V.O., Bahrun A.: Drought adaptation of field grown wheat in relation to soil physical conditions. – Plant Soil **208**: 149-159, 1999.

Baronti S., Vaccari F.P., Miglietta F. *et al.*: Impact of biochar application on plant water relations in *Vitis Vinifera* (L.). – Eur. J. Agron. **53**: 38-44, 2014.

Basso A.S., Miguez F.E., Laird D.A. *et al.*: Assessing potential of biochar for increasing water–holding capacity of sandy soils. – GCB Bioenergy **5**: 132-143, 2013.

Biswas S., Ali M.N., Goswami R. *et al*.: Soil health sustainability and organic farming: A review. – J. Food Agric. Environ. **12**: 237-243, 2014.

Brockhoff S.R., Christians N.E., Killorn R.J. *et al*.: Physical and mineral-nutrition properties of sand-based turfgrass root zones amended with biochar. – Agron. J. **102**: 1627-1631, 2010.

Case S.D., McNamara N. P., Reay D.S., Whitaker J.: The effect of biochar addition on N_2O and CO_2 emissions from sandy loam soil. The role of soil aeration. – Soil Biol. Biochem. **51**: 125-134, 2012.

Chinese Soil Taxonomy Cooperative Research Group.: [Chinese Soil Taxonomy (Revised Proposal).] Pp. 137-147. Inst. Soil Sci., Chinese Agricult. Sci. Technol.Press, Academic Sinica, Beijing 1995. [In Chinese]

Cramer M. D., Hoffmann V., Verboom G.A.: Nutrient availability moderates transpiration in Ehrharta calycina. – New Phytol. **179**: 1048-1057, 2008.

FAO.: Soil Map of the World: Revised Legend. World Soil Resources Report 60. Pp. 7. FAO, Rome 1990.

Flexas J., Bota J., Galmés J., *et al*.: Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. – Physiol. Plantarum **127**: 343-352, 2006.

Flexas J., Bota J., Loreto F. *et al*.: Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. – Plant Biol. **6**: 269-279, 2004.

Glaser B., Lehmann J., Zech W.: Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal. A review. – Biol. Fert. Soils **35**: 219-230, 2002.

Han G., Zhao Z.: [Light response characteristics of photosynthesis of four xerophilous shrubs under different soil moistures.] – Acta Ecol. Sin. **30**: 4019-4026, 2010.

Huang G.B., Chai Q., Feng F.X., Yu A.Z.: Effects of different tillage systems on soil properties, root growth, grain yield, and water use efficiency of winter wheat (*Triticum aestivum* L.) in arid Northwest China. – J. Integr. Agr. **11**: 1286–1296, 2012.

Huang G.B., Zhang R.Z., Li G.D. *et al*.: Productivity and sustainability of a spring wheat-field pea rotation in a semi-arid environment under conventional and conservation tillage systems. – Field Crop. Res. **107**: 43-55, 2008.

Jamieson P. D., Ewert F.: The role of roots in controlling soil water extraction during drought: an analysis by simulation. – Field Crop. Res. **60**: 267-280, 1999.

Jeffery S., Verheijen F.G.A., van der Velde M., Bastos A.C.: A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. – Agr. Ecosyst. Environ. **144**: 175-187, 2011.

Jia D., Dai X., He M.: Polymerization of glutenin dying grain development and quality expression in winter wheat in response to irrigation levels. – Crop Sci. **52**: 1816-1827, 2012.

Kammann C., Linsel S., Gößling J.W. *et al*.: Influence of biochar on drought tolerance of *Chenopodium quinoa* Wild and on soil-plant relations. – Plant Soil **345**: 195-210, 2011.

Lal R.: Constraints to adopting no–till farming in developing countries. – Soil Till. Res. **94**: 1-3, 2007.

Lal R.: Soil carbon sequestration to mitigate climate change. – Geoderma **123**: 1-22, 2004

Larcher W.: Physiological Plant Ecology Ecophysiology and Stress: Physiology of Functional Groups (4^{th} ed.). Pp. 514., Springer Verlag, Berlin – Heidelberg 2003.

Larneyn F.J., Angers D.A.: The role of organic amendments in soil reclamation: A review. – Can. J. Soil Sci. **92**: 19-38, 2012.

Major J., Steiner C., Downie A. *et al*.: Biochar effects on nutrient leaching. – In Lehmann J., Joseph S. (ed.): Biochar for Environmental Management: Science and technology. Pp. 271-288. Earthscan, London 2009.

Mengel K., Kirkby E. A.: Principles of Plant Nutrition (4^{th} ed.). Pp. 687. International Potash Institute, Bern 1987.

Novak J.M., Busscher W.J., Watts D.W. *et al*.: Biochars impact on soil-moisture storage in an ultisol and two aridisols. – Soil Sci. **177**: 310-320, 2012.

Parry M.A.J., Reynolds M., Salvucci M.E. *et al*.: Raising yield potential of wheat II. Increasing photosynthetic capacity and efficiency. – J. Exp. Bot. **62**: 453-467, 2011.

Parry M.A.J., Andralojc P.J., Khan S.L. *et al*.: Rubisco activity. Effects of drought stress. – Ann. Bot.-London **89**: 833-839, 2002.

Polley H.W.: Implications of atmospheric and climatic change for crop yield and WUEg. – Crop Sci. **42**: 131-140, 2002.

Reynolds M.P., van Ginkel M., Ribaut J.M.: Avenues for genetic modification of radiation use efficiency in wheat. – J. Exp. Bot. **51**: 459-473, 2000.

Rondon M .A., Lehmann J., Ramirez J. *et al*.: Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with biochar additions. – Biol. Fert. Soils **43**: 699-708, 2007.

Rosales-Serna R., Kohashi-Shibata J., Acosta-Gallegos J.A. *et al*.: Biomass distribution, maturity acceleration and yield in drought-stressed common bean cultivars. – Field Crop. Res. **85**: 203-211, 2004.

Slavich P.G., Sinclair K., Morris S.G. *et al*.: Contrasting effects of manure and green waste biochars on the proportions of an acidic ferralsol and productivity of a subtropical pasture. – Plant Soil **366**: 1-15, 2012.

Solaiman Z.M., Blackwell P., Abbott L.K. *et al*.: Direct and residual effect of biochar application on mycorrhizal root colonisation, growth and nutrition of wheat. – Soil Res. **48**: 546-554, 2010.

Spokas K.A., Cantrell K.B., Novak J.M. *et al*.: Biochar: A synthesis of its agronomic impact beyond carbon sequestration. – J. Environ. Qual. **41**: 973-989, 2012.

Sun X., Liu Q., Wang D.J. *et al*.: [Effect of long-term application of straw on soil fertility.] – Chin. J. Ecolog. Agricult. **16**: 587-592, 2008. [In Chinese]

Talgre L., Lauringson E., Roostalu H. *et al*.: Green manure as a nutrient source for succeeding crops. – Plant Soil Environ. **58**: 275-281, 2012.

Tezara W., Mitchell V., Driscoll S.: Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. – Nature **401**: 914-917, 1999.

Verheijen F., Jeffery S., Bastos A.C. *et al*.: Biochar Application to Soils – A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. EUR 24099 EN. Pp. 166. Office for the Official Publications of the European Communities, Luxembourg 2010.

Wang D., Yu Z.W., White P. J.: The effect of supplemental irrigation after jointing on leaf senescence and grain–filling in wheat. – Field Crop. Res. **151**: 35-44, 2013.

Wang M., Shi S., Lin F. *et al*.: Effects of soil water and nitrogen on growth and photosynthetic response of manchurian ash (*Fraxinus mandshurica*) seedlings in northeastern China. – PLoS ONE **7**: e30754, 2012.

Watson C.A., Atkinson D., Gosling P. *et al.*: Managing soil fertility in organic farming systems. – Soil Use Manage. –**18**: 239-247, 2002.

Yamato M., Okimori Y., Wibowo I.F. *et al.*: Effects of the application of charred bark of *Acacia mangium* on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. – Soil Sci. Plant Nutr. **52**: 489-495, 2006.

Yeboah S., Zhang R., Cai L. *et al.*: Tillage effect on soil organic carbon, microbial biomass carbon and crop yield in spring wheat – field pea rotation. – Plant Soil Environ. **62**: 279-285, 2016.

Yin C.Y., Berninger F., Li C.Y.: Photosynthetic responses of *Populus przewalski* subjected to drought stress. – Photosynthetica **44**: 62-68, 2006.

Yin R., Yin G.: China's primary programs of terrestrial eco-system restoration: Initiation, implementation, and challenges. – Environ. Manage. **45**: 429-441, 2010.

Zadoks J.C., Chang T.T., Konzak C.F.: A decimal code for the growth stages of cereals. – Weed Res. **14**: 415-421, 1974.

Zhao F., Yang, X.Y., Strahler, A. H. *et al.*: A comparison of foliage profiles in the Sierra National Forest obtained with a full-waveform under-canopy EVI lidar system with the foliage profiles obtained with an airborne full-waveform LVIS lidar system. – Remote Sens. Environ. **136**: 330-341, 2013.

Zhao X.Z., Li F.M., Mo F. *et al.*: Integrated conservation solutions for the endangered Loess Plateau of Northwest China Special Issue. – Pak. J. Bot. **44**: 77-83, 2012.

Zhu X., Li Y., Peng X. *et al.*: Soils of the loess region in China. – Geoderma **29**: 237-255, 1983.