

<https://doi.org/10.17221/121/2023-PSE>

## Biochar addition enhances annual carbon stocks and ecosystem carbon sink intensity in saline soils of the Hetao Irrigation District, Inner Mongolia

RUXIN ZHANG<sup>1</sup>, ZHONGYI QU<sup>2,3\*</sup>, WEI YANG<sup>1,3</sup>, LIPING WANG<sup>1,3</sup>, DONGLIANG ZHANG<sup>1</sup>, LU LIU<sup>4</sup>, JUNJIE LI<sup>1</sup>, ZHIMIN ZHANG<sup>5</sup>

<sup>1</sup>College of Water Conservancy and Civil Engineering, Inner Mongolia Agricultural University, Hohhot, P.R. China

<sup>2</sup>College of Energy and Environment, Inner Mongolia University of Science and Technology, Baotou, P.R. China

<sup>3</sup>Autonomous Region Collaborative Innovation Center for Integrated Management of Water Resources and Water Environment in the Inner Mongolia Reaches of the Yellow River, Hohhot, P.R. China

<sup>4</sup>Northern Construction Management Department, China Three Gorges Renewables (Group) Co., Ltd., Beijing, P.R. China

<sup>5</sup>Inner Mongolia Hetao Irrigation District Water Development Center Yichang Branch Center Yihe Canal Water Supply Station, Bayan Nur, P.R. China

\*Corresponding author: [quzhongyi@imau.edu.cn](mailto:quzhongyi@imau.edu.cn)

**Citation:** Zhang R.X., Qu Z.Y., Yang W., Wang L.P., Zhang D.L., Liu L., Li J.J., Zhang Z.M. (2024): Biochar addition enhances annual carbon stocks and ecosystem carbon sink intensity in saline soils of the Hetao Irrigation District, Inner Mongolia. Plant Soil Environ., 70: 263–275.

**Abstract:** Biochar has demonstrated potential for stabilising high yields and sequestering carbon in dryland farmland, but it is unclear whether biochar affects the carbon sequestration capacity and carbon balance of annual farmland ecosystems. For this purpose, we conducted a plot control trial in salinised farmland in 2019–2021, where we set three treatments, control, and two biochar rates, 0 (CK), 15 (B15), and 30 t/ha (B30). The results showed that biochar application decreased soil organic carbon stocks in the early part of the experiment (first freeze and freeze period); these increased in the later part, and overall, the biochar treatments increased soil organic carbon storage by 3–6% compared with the control. Compared with the control (CK), biochar inhibited the total soil respiration rate and microbial respiration rate significantly ( $P < 0.05$ ) during the crop growing period compared with the freeze-thaw period. After two years of freeze-thaw cycling, biochar application increased sunflower plant carbon sequestration and net primary productivity and suppressed total soil microbial respiration, thereby increasing net ecosystem productivity. Therefore, the application of biochar is conducive to carbon sequestration in farmland ecosystems and presents a carbon sink effect, thus being a good choice for improving the soil carbon pool and reducing emissions in the northern dry zone.

**Keywords:** food production; straw biochar; agroecosystem; salinisation; biomass; permafrost

The IPCC (2022) report shows that atmospheric CO<sub>2</sub> and CH<sub>4</sub> concentrations have risen to their highest levels in the last eight decades and that sustained

emissions reductions are urgently needed. Agriculture is a significant source of greenhouse gas emissions globally, accounting for 34% of total anthropogenic

Supported by the National Natural Science Foundation, China, Projects No. 52279037 and 52109056, and by the Basic Research Operating Expenses-Multi-Disciplinary Research Program, Project No. BR22-15-03.

© The authors. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

emissions (Crippa et al. 2021). A large contribution comes from food production processes, such as CH<sub>4</sub> emissions caused by rice cultivation, soil N<sub>2</sub>O emissions caused by nitrogen fertiliser application, and CO<sub>2</sub> emissions caused by the processing and transportation of fertilisers and pesticides in agricultural production. Soil carbon sequestration can increase terrestrial carbon stocks and reduce atmospheric CO<sub>2</sub> concentrations; studies have shown that agroecosystems have the potential to increase soil carbon stocks and thus reduce greenhouse gas emissions (Li et al. 2021). Agroecosystems are complex systems that are strongly regulated and controlled by humans, and their carbon cycles are sensitive to the responses of different field management practices. Biomass conversion into biochar *via* pyrolysis and its subsequent addition to soil are considered important management practices for carbon sequestration (Nan et al. 2022, Feng et al. 2023).

Handling crop straw has become a serious obstacle to efficiently utilising straw resources. Biochar, as a carbon-based material made from straw, not only contributes to the sustainable development of agriculture but also reduces carbon emissions from agricultural ecosystems, making it an important strategy for increasing sinks and reducing emissions in agriculture (Gao et al. 2022, Li et al. 2022). Biochar applied to agricultural soils enhances soil carbon sequestration by stimulating plant growth and increasing the amount of plant (e.g., apoplastic and root) residues entering the soil (Lehmann et al. 2021). Factors such as cracking temperature, raw materials, land-use practices, and soil pH influence the effect of biochar on soil organic carbon (SOC) mineralisation. The mineralisation response of SOC in dryland soils to adding low amounts of biochar is strong (Cui et al. 2017) and can increase soil surface organic carbon sequestration (Gross et al. 2022). The CO<sub>2</sub> release from biochar applied to neutral and alkaline soils was 1.5–3.5 times lower than that of acidic soils (Sheng et al. 2016). In addition, some researchers have gradually considered the influence of environmental factors on the properties of biochar and biochar-soil mixtures. Freeze-thaw cycles reduce the hydraulic conductivity of biochar-soil mixtures, greatly affecting soil freeze-thaw deformation properties and contributing to soil carbon emissions by influencing soil hydrothermal conditions (Gao et al. 2020). Despite growing evidence of the benefits of biochar in enhancing soil carbon sequestration, how biochar addition over time affects soil carbon dynamics remains unclear.

Previous studies on the effects of biochar on soil carbon sequestration and carbon balance have focused on ecosystems with non-saline soils, such as grasslands, acidic farmlands, wetlands, and paddy fields. Globally, saline soils are increasing at a rate of  $1.0 \times 10^6$ – $1.5 \times 10^6$  ha per year. Soil salinisation poses a serious threat to agricultural production worldwide, and its impacts are increasing. The seasonal permafrost area in China accounts for approximately 53.5% of the national surface area (Xu 2010). The presence of freeze-thaw cycles in seasonal permafrost zones will intensify soil salinisation, affect soil structure and quality, and change soil carbon sequestration and emission reduction effects (Zuo et al. 2022). To address the above problems, this study selected the Hetao Irrigation District of Inner Mongolia as a typical seasonal permafrost area characterised by a high degree of soil salinisation, wide distribution area, and strong freezing and thawing. Changing patterns in annual carbon stocks and ecosystem carbon sink intensity in salinised soil in response to biochar were evaluated *via* a two-year field plot experiment. The main objectives were to analyse (1) the characteristics of soil respiration and microbial respiration changes in annual farmland ecosystems under different biochar treatments; (2) the characteristics of annual organic carbon changes in soils under different biochar treatments, and (3) carbon sequestration by plants and carbon sink potentials in salinised farmland ecosystems under different biochar treatments. The results of this study can provide theoretical parameters for carbon sinks and greenhouse gas emission reductions in saline areas, which can be used for accurate assessment of soil carbon emission potentials, model simulations, farmland management and climate change.

## MATERIAL AND METHODS

**Experimental materials.** The study site was located in Wu Yuan County, Bayan Nur City, Inner Mongolia Autonomous Region (40°46'30"–41°16'45"N, 107°35'70"–108°37'50"E). The study site is located in the Hetao irrigation area of Inner Mongolia, bordered by the Yellow River in the south and the Yinshan Mountains in the north. It belongs to a typical salinised irrigation area, with salinised soils with low organic carbon content, low microbial activity, and a slow rate of organic carbon mineralisation. Thus, it has a higher potential carbon sequestration capacity than other terrestrial ecosystems. Most

<https://doi.org/10.17221/121/2023-PSE>

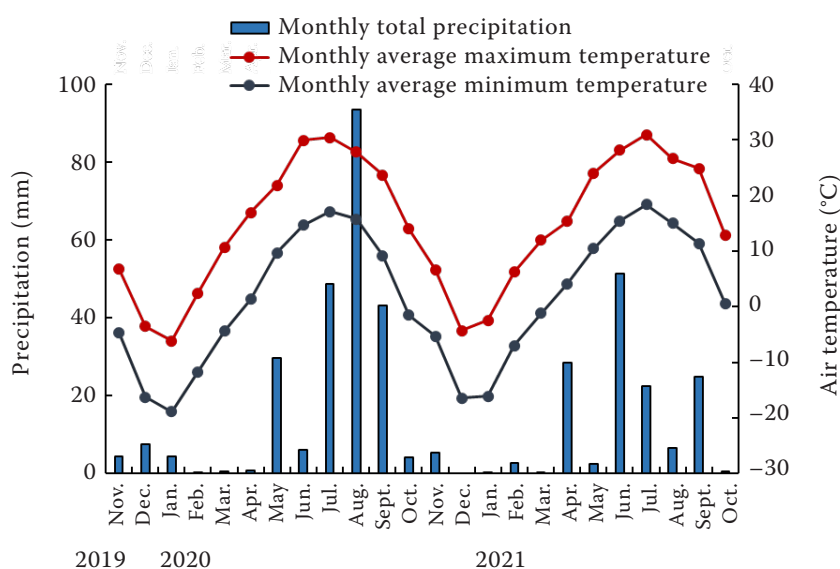


Figure 1. Maximum and minimum temperatures and rainfall during the study period, recorded in Xiaolancai Village

of the cultivated land is dominated by Solonchaks, with sunflower and maize planted in large areas, and rice, pasture, and sorghum planted in small areas. The site has a typical temperate continental dry climate, and considerable soil salt aggregation in winter and spring. According to local weather records, the average multiyear temperature was 6.1–7.6 °C, rainfall was 177 mm, average frost-free duration was 130 days, total radiation was 6 418 MJ/m<sup>2</sup>, annual potential evaporation was 1 993–2 373 mm, and sunshine hours was 3 231 h in the study area. The summer crop growing period from May to September accounts for approximately 75% of the total annual rainfall. The cumulative rainfall at the study site from November 2019 to April 2020 (first-year freeze-thaw period) was 18.1 mm, from June to September 2020 (first-year crop-growing period) was 191.6 mm, from November 2020 to April 2021 (second-year freeze-thaw period) was 45.4 mm, and from June to September 2021 (second-year crop-growing period) was 105.5 mm. The average minimum ground tem-

perature and depth of permafrost were –12.3 °C and 100–130 cm, respectively. The soil in the test area enters the first freeze in early November, and the freeze layer completely melts around mid-April of the following year, lasting for approximately 180 days. Changes in rainfall and temperature are shown in Figure 1.

Corn-straw biochar from Liaoning Jinhefu Agricultural Development Co., Ltd. was used as the test biochar. Biochar was prepared *via* pyrolysis at 360 °C under anaerobic conditions. The physical and chemical properties of the soil (0–20 cm) and biochar are listed in Table 1.

**Experimental design.** The experiment was conducted from 2019 to 2021 using three biochar levels, 0 (CK), 15 (B15), and 30 t/ha (B30), with three replications of each treatment for a total of nine plots arranged in completely randomised groups. A 2 m wide protection row surrounded the plots. The experimental plots were 160 m<sup>2</sup> (40 × 4 m) in size, and biochar was evenly spread on the experimental

Table 1. Basic properties of the test soil and biochar

Parameter	Soil texture	pH	EC (mS/m)	CEC (cmol <sub>+</sub> /kg)	Salt content (g/kg)	Available phosphorus	Available potassium	Carbon content	Nitrogen content	Particle size mass fraction (%)		
						(mg/kg)		(g/kg)		sand silt clay		
Soil	silty loam	8.9	1.9	7.6	3.6	7.5	351.5	8.4		16.7	79.0	4.3
Biochar		8.6				307.5	786.5	364.1	7.6			

EC – electrical conductivity; CEC – cation exchange capacity

plots onto October 15, 2019, and evenly mixed with the 20 cm soil layer using a rototiller, after which no additional biochar was applied.

The field trial was irrigated with 225 mm in spring on April 25, 2020, and April 20, 2021, using surface irrigation. Before sowing, the field was manually tilled to a depth of approximately 30 cm and fertilised with 450 kg/ha diamine phosphate (N mass fraction 18% and P mass fraction 46%) and 337.5 kg/ha compound fertiliser (N mass fraction 15%, P mass fraction 15%, and K mass fraction 15%) as substrates. The test crop was the sunflower cultivar 902, which is commonly grown by local farmers. Planting was performed using mulching and hand-sowing at 2 rows of crops per mulch for a total of 18 mulches and 36 rows. The spacing between the rows on the membrane was 40 cm, and the spacing between the rows between membranes was 100 cm. The planting density was the same in nine plots at 27 000 plants/ha. Other management practices were consistent with local production practices. The additional N rate was 210 kg/ha at the bud stage, and the irrigation rate was 90 mm. The specific division of the test period is shown in Figure 2.

**Soil respiration measurement.** The soil respiration dynamics of different treatments were measured from November 2019 to September 2021 from 09:00 to 12:00, approximately every 20 days and the measurement time was appropriately adjusted when rainfall was encountered (Wang et al. 2021). Detailed information regarding the determination of soil respiration using an ultra-portable greenhouse gas analyser can be found in earlier publications (Li et al. 2021, Zhang et al. 2022). The CO<sub>2</sub> emission rate (μmol/(m<sup>2</sup>/s)) was measured using the dynamic confined gas chamber analyser (UGGA) method, and the soil respiration rate on the day of measurement was used as the CO<sub>2</sub> emission rate,  $R_s$ . The meas-

uring instrument model was a US PS-3000 (Beijing LICA United Technology Limited, Beijing, China) fully automatic portable respiration system with an SC-11 respiration chamber. To reduce disturbance to the soil surface, the burial position of the polyvinyl chloride (PVC) ring (191 mm inner diameter, 200 mm outer diameter, and 10 cm length) was kept constant throughout the test cycle. The chamfered end of the PVC ring was pressed approximately 5 cm into the soil, and approximately 5 cm was left exposed to remove the debris inside the ring (Figure 3).

A 20 cm diameter PVC was placed in each test plot into a bare area with no plant growth (Zhou et al. 2007). The bare area was a circular area of 35 cm in diameter, and the visible roots were removed before pipe placement to measure the soil microbial respiration rate  $R_m$ . The PVC pipe measuring the soil microbial respiration rate was 50 cm high and embedded 45 cm into the soil. Holes were drilled 5 cm from the mouth of the pipe and every 10 cm from top to bottom along the pipe wall to facilitate the exchange of soil, water, and nutrients inside and outside the PVC pipe and to exclude roots from entering (Zhou et al. 2007, Chen et al. 2016). Live plants were periodically removed from the PVC pipes during the experiment.

The estimation of total soil respiration and total soil microbial respiration (t/ha of CO<sub>2</sub>) for different treatments was calculated using the following equations (Li et al. 2018):

$$C_S = \sum_{i=fist}^{i=last-1} \left[ \frac{D_i + D_{i+1}}{2} \times (N_{i+1} - N_i - 1) + D_{last} \right] \quad (1)$$

$$D_i = R_s \times 3600 \times 24 \times 44 \times 10^{-6} \quad (2)$$

where:  $C_S$  – total amount of soil CO<sub>2</sub> emission (t/ha);  $D_i$  – CO<sub>2</sub> emission rate on the day of measurement (g/m<sup>2</sup>);  $R_s$  – CO<sub>2</sub> emission rate on the day of measurement (μmol/

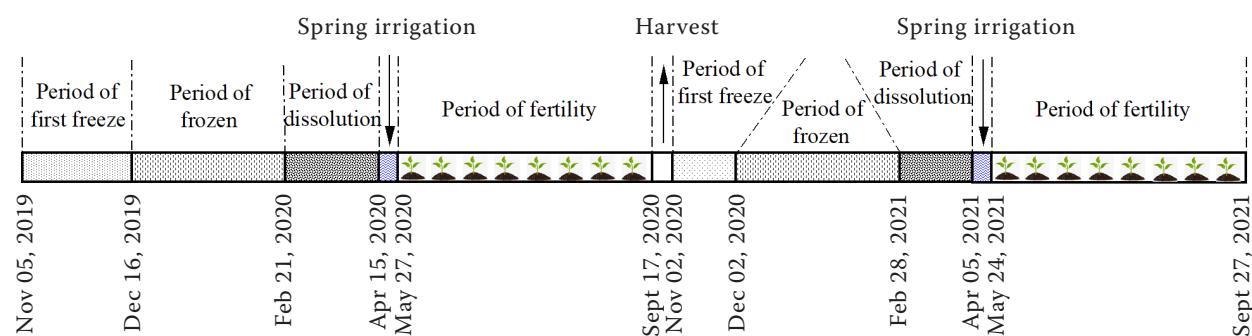


Figure 2. Phases of the test period and their corresponding dates

<https://doi.org/10.17221/121/2023-PSE>

(m<sup>2</sup>/s)), 44 is the molar mass of CO<sub>2</sub> (g/mol), 3 600, 24, and 10<sup>-6</sup> are conversion factors (24 and 3 600 are conversion factors between days and seconds; 10<sup>-6</sup> is conversion factors between μmol and mol);  $N_{i+1}$  and  $N_i$  – interval between two adjacent measurements (d), respectively.

**Soil organic carbon measurement.** Soil samples were collected from different treatments during the 2019–2021 experimental cycle at a depth of 40 cm in 20 cm levels with three replicates for each treatment. Soil samples were air-dried after removing roots and stones and soil organic carbon content (g/kg) was determined using an external heating method using potassium dichromate (De et al. 1978).

The calculation of SOC stocks using an iso-mass approach uses the following equations (Ellert and Janzen 1999):

$$M_{\text{soil}} = 10^4 \times \rho_b \times T \quad (3)$$

$$M_{\text{element}} = 10^{-3} \times M_{\text{soil}} \times M_{\text{SOC}} \quad (4)$$

where:  $M_{\text{element}}$  – SOC stocks (t/ha);  $M_{\text{soil}}$  – soil mass per unit area (t/ha);  $\rho_b$  – bulk density of each soil layer (t/m<sup>3</sup>);  $T$  – depth of the soil layer (m);  $M_{\text{SOC}}$  – SOC content of each soil layer (kg/t), and 10<sup>-3</sup> and 10<sup>4</sup> are the conversion factors.

**Estimation of carbon balance in agricultural ecosystems.** After the sunflower seeds were harvested, the aboveground biomass (straw or stubble) of sunflowers was measured in three 1 m<sup>2</sup> plots selected from different treatments, and the straw and stubble were dried at 60 °C for 72 h. After harvest, four holes 8 cm in diameter and 60 cm in depth (two on the

plant rows and two between the rows) were punched into each treatment plot, and the root biomass of sunflowers in the 0–60 cm soil layer was measured. Roots were placed into nylon bags and rinsed with tap water, after which the roots were dried at 80 °C until constant weight and root dryness were determined. Root biomass was calculated according to planting density. The carbon content of leaves stems, and roots passing through a 0.25 mm sieve was determined using the potassium dichromate-sulfuric acid oxidation method (Bao 2000).

Approximately five sunflower plants were planted per square meter. The net C plant production (NPP) was calculated by Cao et al. (2003):

$$\text{NPP} = 5(m_l\omega_l + m_q\omega_q + m_r\omega_r) \quad (5)$$

where: NPP – net C plant production (g/m<sup>2</sup>);  $m_p$ ,  $m_q$ , and  $m_r$  – sunflower leaf, stem, and root dry matter masses (g/plant), respectively;  $\omega_p$ ,  $\omega_q$ , and  $\omega_r$  – sunflower leaf, stem, and root carbon contents (%), respectively.

Net C ecosystem productivity (NEP) was calculated by Gao et al. (2017) and Wang et al. (2021):

$$\text{NEP} = \text{NPP} - C_s \quad (6)$$

where: NEP – net C ecosystem productivity (g/m<sup>2</sup>);  $C_s$  – total carbon emissions from microbial respiration (g/m<sup>2</sup>). When the NEP > 0, the farm ecosystem is a sink for CO<sub>2</sub> absorption; otherwise, it is a source of CO<sub>2</sub> emissions.

**Statistical analysis.** All values were expressed as the means ± standard error. The normal distribution and homogeneous variance tests were performed

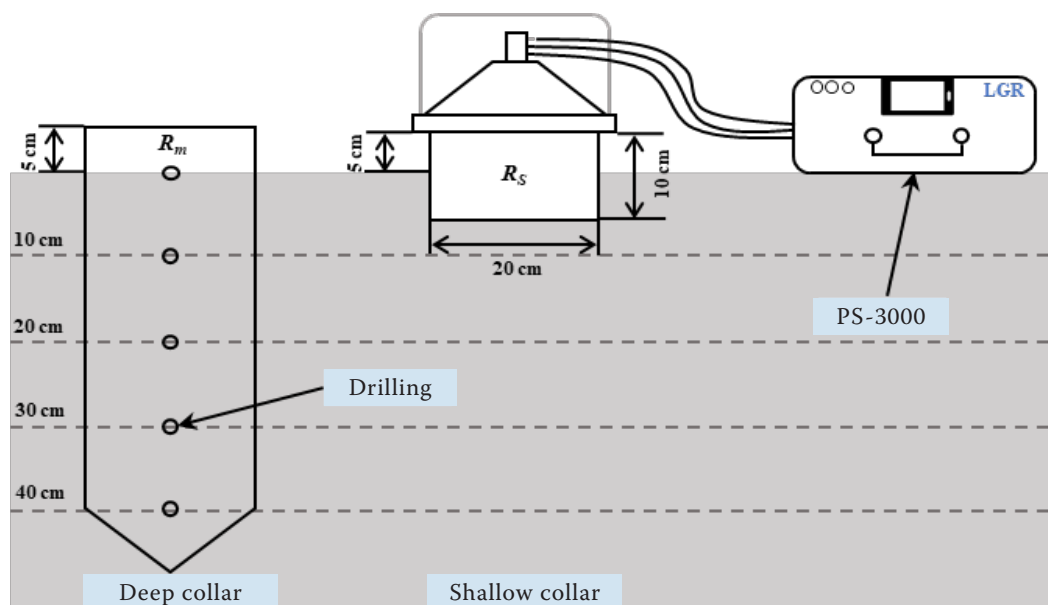


Figure 3. Gas extraction device arrangement.  $R_m$  – soil microbial respiration rate;  $R_s$  – soil respiration rate



before subjecting the results to analysis of variance (ANOVA). One-way ANOVA was conducted using SPSS (v22.0, IBM, Chicago, USA) to compare the effects of biochar addition on SOC, soil respiration, dry matter mass, carbon content, and carbon sequestration of plant organ indicators. The significance of the observed differences was tested using the least significant difference (*LSD*) test at a level of 0.05. Finally, plots were created using Microsoft Excel 2021 (Redmond, USA).

## RESULTS

**Characteristics of SOC changes in annual farmland ecosystems.** The effects of mixed biochar soils on SOC content and storage in the tillage layer at different periods are shown in Figures 4A–D and 4E–H, respectively. After two years of freezing and thawing, the biochar treatments increased SOC content by 6–13% and organic carbon storage by 3–6% compared with the control. Compared with the CK and B15 treatments, the response of the B30 treatment to SOC content in the 0–20 cm soil layer was more significant ( $P < 0.05$ ). However, the effect of biochar on SOC stock exhibited a fluctuating trend during the two-year experiment (Figure 4I, J), with a negative effect on SOC stock in the early part of the experiment (first freeze and freeze period), followed by a positive effect on SOC stock. More prolonged *in situ* field monitoring is required to understand this phenomenon further.

**Characteristics of soil respiration and soil microbial respiration changes in annual farmland ecosystems.** Horizontal fluxes in the test area showed a strong seasonal emission pattern, and the pattern of soil microbial respiration changes in each treatment in each period was consistent with that of total soil respiration (Figure 5). The total soil respiration rate (mean  $\pm$  standard deviation) was the highest during the crop growth period ( $20.57 \pm 5.76 \text{ g/m}^2$ ), lowest during the freezing period ( $0.88 \pm 0.06 \text{ g/m}^2$ ), and began to increase again during the thawing period ( $8.09 \pm 0.59 \text{ g/m}^2$ ) (Figure 5A, B). In conventional tillage, the crop straw is completely removed, no root respiration takes place during the freeze-thaw period, and the microbial respiration rate is equal to the total soil respiration rate; therefore, in the following sections, we analyse the characteristics of microbial respiration rate changes during the freeze-thaw period by using the total soil respiration rate as an example.

The mean total soil respiration rate and microbial respiration rate were significantly lower ( $P < 0.05$ ) in the biochar treatment than in the control treatment in both growing seasons but exhibited differences in the three phases of the soil freezing and thawing period. One-way ANOVA showed that in the pre-existing phase of biochar application (first freeze in 2019–2020), biochar treatment increased the mean total soil respiration rate, but the effect was not significant (B15:  $P = 0.277$ ; B30:  $P = 0.167$ ). The inhibitory effect of biochar addition on the total soil respiration rate and microbial respiration rate gradually increased with the increase in biochar application time, in which the B30 treatment reached a significant difference ( $P < 0.05$ ) from the control treatment during the remaining period, except for the freezing period in 2020–2021 in which there was no significant difference between treatments.

**Characteristics of changes in dry-matter mass, carbon content, and carbon sequestration of plant organs in an annual agroecosystem.** During the two-year growing season, the higher the amount of biochar applied and the longer the period after application, the higher the dry-matter mass, carbon content, and carbon fixation of each organ (Figure 6). Compared with no biochar application (CK), the B30 treatment significantly ( $P < 0.05$ ) increased the aboveground dry-matter mass of all sunflower organs by 53.04, 26.13, and 16.58% for roots, stems, and leaves, respectively, in 2020–2021 (Figure 6A), and by 36.47% for the whole plant. Biochar treatments did not significantly differ ( $P > 0.05$ ) for sunflower stems in 2019–2020 and sunflower leaves in 2020–2021, except for the B30 treatment, which significantly increased sunflower carbon content ( $P < 0.05$ ) (Figure 6B). From the results of carbon sequestration in each sunflower plant organ (Figure 6C), all treatments exhibited significant differences ( $P < 0.05$ ) in 2019–2020, and only the B30 treatment showed a significant difference ( $P < 0.05$ ) from the CK treatment in 2020–2021, which indicated that the carbon sequestration capacity of the B15 treatment on each sunflower organ slowed over time.

**Carbon balance analysis of farmland ecosystems under different treatments on an annual basis.** The net C ecosystem productivity of farmland under different biochar treatments is shown in Table 2. The experimental results showed that applying biochar increased plant carbon sequestration and net C plant production and suppressed total soil microbial respiration, thus improving net C ecosystem productivity.

<https://doi.org/10.17221/121/2023-PSE>

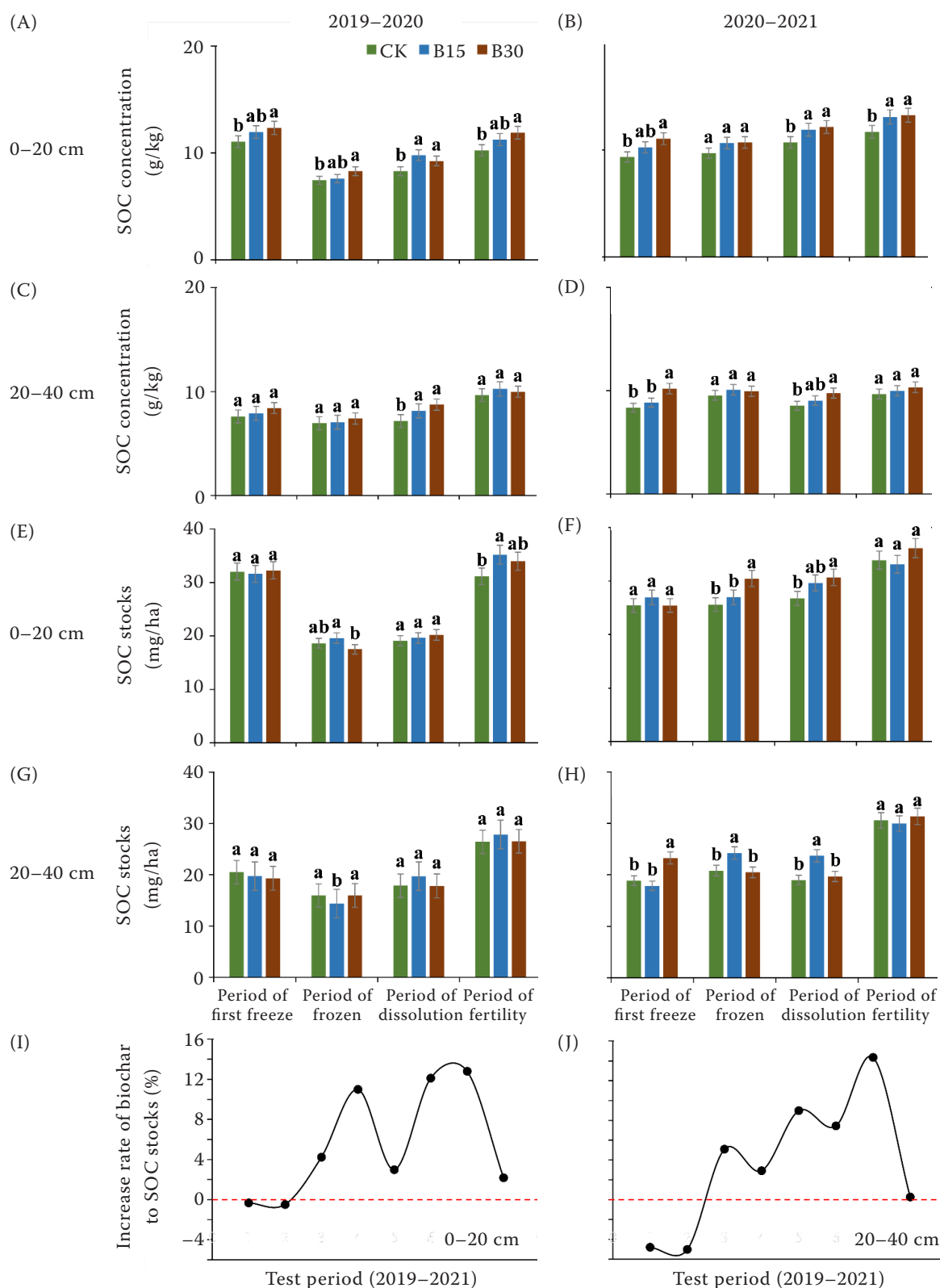


Figure 4. Change the pattern of soil organic carbon (SOC) content and organic carbon stock of each treatment during the experimental period. CK – blank control; B15 and B30 are treatments of biochar rates 15 t/ha and 30 t/ha. (A–D) shows SOC content; (E–H) shows SOC stock; (I) and (J) show average increase rates of SOC stocks with biochar treatment (B15 and B30) compared with blank treatment (CK). Different lowercase letters represent significant differences between the treatments at  $P < 0.05$

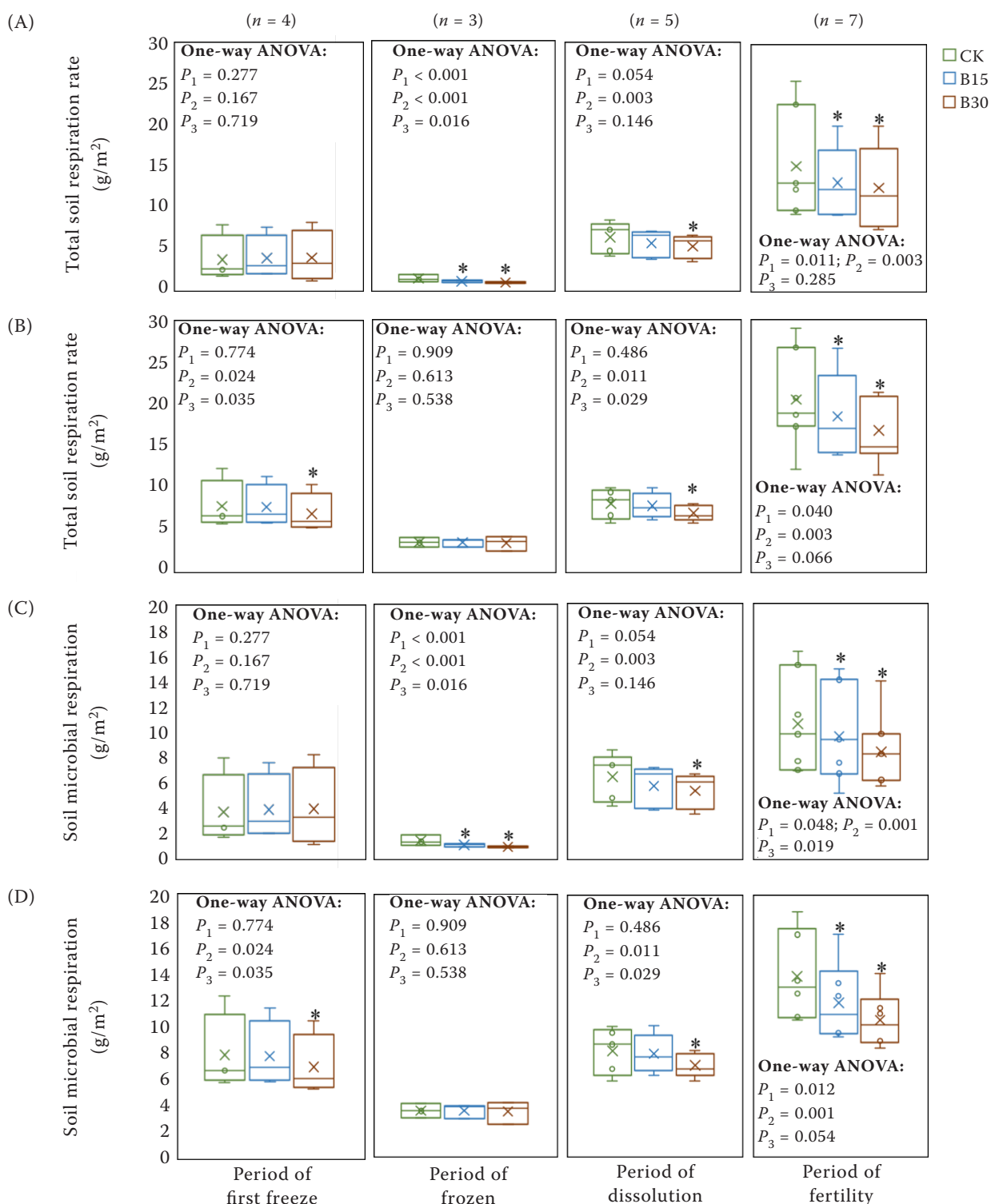


Figure 5. Changes in soil respiration and soil microbial respiration (g/m<sup>2</sup> of C) for each treatment during the experimental period. CK – blank control; B15 and B30 are treatments of biochar rates 15 t/ha and 30 t/ha. (A) and (B) denote the total soil respiration rate during the experimental periods 2019–2020 and 2020–2021, respectively; (C) and (D) denote the soil microbial respiration rate during the experimental periods 2019–2020 and 2020–2021, respectively.  $P_1$  – variability between B15 and CK treatments at the  $P < 0.05$  level;  $P_2$  – variability between B30 and CK treatments;  $P_3$  – differences between B15 and B30 treatments at the  $P < 0.05$  level. Asterisks indicate significant differences between biochar treatments (B15 and B30) and CK at the  $P < 0.05$  level.



<https://doi.org/10.17221/121/2023-PSE>

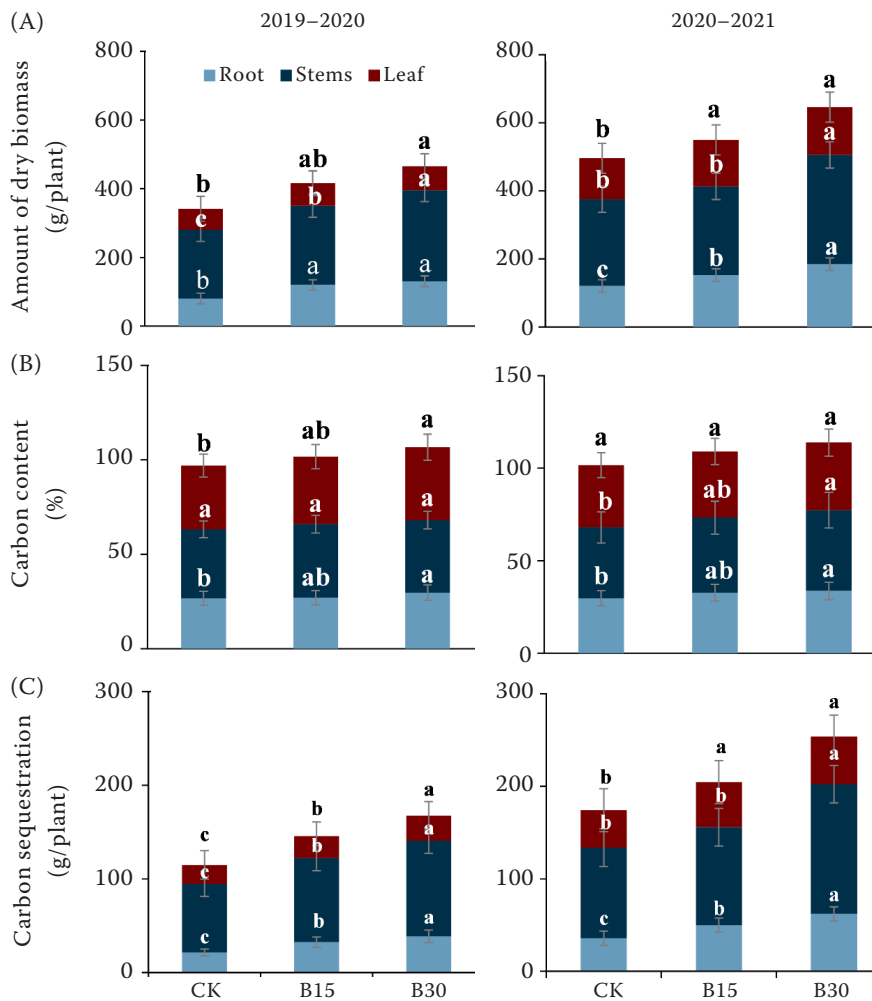


Figure 6. Changes in dry biomass, carbon content, and carbon sequestration of sunflower organs under different treatments after harvest. CK – blank control; B15 and B30 are treatments of biochar rates 15 t/ha and 30 t/ha. (A) shows the dry-matter mass of each sunflower organ; (B) shows the carbon content of each sunflower organ; and (C) shows the carbon sequestration in each sunflower organ. Different lowercase letters represent significant differences between treatments at  $P < 0.05$

Table 2. Carbon (C) balance analysis of different treatment systems

Period	Treatment	Net C plant production	Total soil microbial respiration	Net C ecosystem productivity
			(g/m <sup>2</sup> )	
First-year freeze-thaw period ( $n = 12$ )	CK	–	253.28 ± 13.25 <sup>a</sup>	–253.28 ± 12.66 <sup>a</sup>
	B15	–	249.60 ± 13.44 <sup>a</sup>	–249.60 ± 12.48 <sup>a</sup>
	B30	–	227.79 ± 11.23 <sup>b</sup>	–227.79 ± 11.39 <sup>a</sup>
First-year crop-growth period ( $n = 7$ )	CK	574.85 ± 28.74 <sup>c</sup>	402.16 ± 20.20 <sup>a</sup>	172.69 ± 8.63 <sup>c</sup>
	B15	728.08 ± 36.40 <sup>b</sup>	351.62 ± 18.56 <sup>a</sup>	376.46 ± 18.82 <sup>b</sup>
	B30	837.40 ± 41.87 <sup>a</sup>	313.26 ± 17.74 <sup>b</sup>	524.14 ± 26.21 <sup>a</sup>
Second-year freeze-thaw period ( $n = 12$ )	CK	–	112.75 ± 6.29 <sup>a</sup>	–112.75 ± 5.64 <sup>b</sup>
	B15	–	104.21 ± 5.41 <sup>ab</sup>	–104.21 ± 5.21 <sup>ab</sup>
	B30	–	93.19 ± 5.10 <sup>b</sup>	–93.19 ± 4.66 <sup>a</sup>
Second-year crop-growth period ( $n = 7$ )	CK	870.63 ± 45.53 <sup>c</sup>	364.88 ± 18.21 <sup>a</sup>	505.74 ± 25.29 <sup>c</sup>
	B15	1 022.78 ± 51.14 <sup>b</sup>	335.71 ± 17.23 <sup>b</sup>	687.07 ± 34.35 <sup>b</sup>
	B30	1 268.70 ± 63.44 <sup>a</sup>	284.59 ± 14.14 <sup>c</sup>	984.12 ± 49.21 <sup>a</sup>

CK – blank control; B15 and B30 are treatments of biochar rates 15 t/ha and 30 t/ha. Different lowercase letters represent significant differences between the treatments at  $P < 0.05$

The ecosystems in the region under different biochar treatments were carbon "sinks" for atmospheric CO<sub>2</sub> during the crop-growth period and carbon "sources" for atmospheric CO<sub>2</sub> during the freeze-thaw period. The net C ecosystem productivity of all treatments reached significant differences ( $P < 0.05$ ) during the crop-growth period but not during the freeze-thaw period in the second year ( $P > 0.05$ ). The farmland net C ecosystem productivity increased with biochar application, which implied that the carbon "sink" intensity gradually increased.

## DISCUSSION

**Effect of biochar application on SOC storage and soil respiration.** This study's SOC storage in the 0–40 cm soil layer increased after biochar application (Figure 4). Biochar is a carbon-rich material that can directly input exogenous organic matter into the soil (Matovic 2010). From the previous analysis, the SOC content in this study increased significantly with increasing biochar application owing to the high carbon content, complex aromatisation structure, and inherent chemical inertness of biochar. Biochar is considered an inert carbon pool with high chemical and microbial stability in the soil environment, and the magnitude of its effect depends on its amount and stability (Zwieten et al. 2010). After two years of freeze-thaw, biochar treatment (B15 and B30) increased SOC by 6–13% and SOC storage by 3–6% compared to the control. It indicates that straw biochar can be used as a better carbon sequestration material, which has a better carbon sequestration effect when prepared as biochar and returned to the soil and positively affects soil carbon sequestration. Consistent with the results of this study, Criscuoli et al. (2021) found a significant increase in soil carbon storage in the short term through a field trial compared with the original soil control. The present study further showed a highly consistent covariance between SOC storage and SOC, with a decreasing trend of soil carbon storage and SOC with increasing soil depth. This may be due to microorganisms' high abundance and activity in the top soil layer, which decomposes organic matter faster and accumulates relatively more SOC. In contrast, the lower soil layer has lower microbial activity and numbers, forming a relatively enriched SOC pool. However, it has also been shown that the microbial biomass of surface soil is significantly higher than that of deeper soil, and microorganisms produce more soil enzymes for

the decomposition of SOC; therefore, the organic carbon content of surface soil is lower than that of deeper layers (Wang et al. 2013). This may be because we studied the annual SOC stock change law, in which the soil microbial activity was lower during the freeze-thaw period. Furthermore, differences in other environmental factors, such as soil type, may also produce different results. In addition, even in replicated experiments, the lack of measurements at the beginning of the experiment may lead to some bias (Poeplau et al. 2016), e.g., when the initial SOC of the trial is low, the increase in SOC storage is instead greater (Poulton et al. 2018); it takes decades or even longer to validate the characterisation of significant changes in SOC storage (Smith et al. 2020). However, our understanding of the underlying mechanisms of soil carbon stocks over the long term is limited by the lack of relevant studies, particularly regarding the contributions of belowground biomass and soil microbes to soil carbon stocks.

The extent and direction of the effects of biochar additions on total soil CO<sub>2</sub> emissions remain controversial, despite numerous studies conducted over the past decade (Zimmerman et al. 2011, Wang et al. 2016). Our study showed that the effects of both biochar treatments on total soil respiration and microbial respiration did not reach a significant level ( $P > 0.05$ ) during the stabilised soil freezing period in the second year of the experiment, which could be attributed to the lower microbial abundance and activity under the low-temperature environment, which suppressed CO<sub>2</sub> emissions. As shown in Figure 5, the peak of soil carbon emissions in winter is during the melting period, which is similar to the results of Yang et al. (2014). Increasing soil moisture and temperature during thawing may stimulate soil microbial activity (Aanderud et al. 2013). In addition, repeated freeze-thaw cycles during thawing can rupture microbial cells or induce the release of readily decomposable organic matter, which may also lead to an increase in CO<sub>2</sub> emissions (Feng et al. 2007). In this study, biochar application significantly reduced the total soil respiration. Biochar has a large specific surface area and a certain content of minerals such as CaCO<sub>3</sub> and Fe(OH)<sub>3</sub>, which have a strong physical and chemical fixation effect on CO<sub>2</sub>, prompting biochar to form organic-inorganic complexes with soil and organic matter to generate more stable agglomerates; the organic carbon stored therein is not easily decomposed and used by external microorganisms, thus reducing soil CO<sub>2</sub> emissions

<https://doi.org/10.17221/121/2023-PSE>

(Huang et al. 2017). Alternatively, the addition of biochar not only promotes the formation of soil humus but also contributes to the formation of organic macromolecules such as carbohydrates, esters, and aromatic hydrocarbons that are difficult to use by microorganisms (Cross and Sohi 2011); this process reduces the microbial utilisation of organic carbon, thus reducing soil CO<sub>2</sub> emissions. Simultaneously, this study found significant differences in the effects of biochar at different periods after application to the soil. Different biochar application rates increased soil respiration in the early stage of soil application compared to the blank control, whereas the B15 and B30 treatments gradually inhibited soil respiration over time. The application of biochar improved the soil microenvironment and increased the content of readily decomposable organic matter in the soil, resulting in a sharp increase in soil carbon emissions in the short term; however, this excitation effect gradually disappeared with the mineralisation of readily decomposable organic carbon (Ventura et al. 2014). Therefore, this study needs to conduct field monitoring over a longer period to validate the above findings. Overall, any comparisons or conclusions should be made cautiously, considering that these syntheses tend to ignore biochar age's complex but important effects on soil CO<sub>2</sub> emissions.

**Effect of biochar application on dry matter accumulation and carbon sequestration in sunflower plants.** To achieve the ultimate goal of carbon sequestration and emission reduction in farmland, further enhancements to the carbon sequestration capacity of farmland and reductions in carbon emissions should be realised. This study showed that most of the sunflower carbon stocks were accumulated in the above-ground parts of the plant, with the highest carbon sequestration in the stem, which accounted for 51.8–63.7% of the total carbon sequestration in the plant under different biochar methods. In contrast, in the below-ground carbon sequestration in sunflowers, the limitation of the root biomass resulted in low carbon sequestration in the roots, which accounted for only 18.6–24.5% of the total carbon sequestration in the plant. The carbon cycle pathway in farmland is such that atmospheric CO<sub>2</sub> is absorbed and fixed by crops, intervened by biological and human activities, and finally returned to the atmosphere in the form of CO<sub>2</sub> and CH<sub>4</sub>. Therefore, the effective fixation of CO<sub>2</sub> in the air by plants in farmland ecosystems is key to maintaining the carbon balance of this ecosystem. In this study, biochar

application increased sunflower plants' dry matter accumulation and carbon fixation (Figure 6). The higher the amount of biochar applied and the longer the period after application, the higher the dry matter mass, carbon content, and carbon fixation of each sunflower organ. It is assumed that the promotional effect is because biochar can enhance the uptake of nitrogen, phosphorus, and potassium and reduce the leaching of nutrient ions such as ammonium and calcium. This may also be due to the large specific surface area and microporous structure of biochar. Biochar has a strong adsorption capacity and can improve the soil structure and water, fertiliser, air, and heat conditions (Tang et al. 2021), increase the water-holding and salt-leaching capacity of the soil, facilitate soil desalination in the root zone, create a good growth environment in the root zone, and promote the growth of each crop organ.

**Effects of biochar application on the carbon balance of farmland ecosystems.** Recently, the net C ecosystem productivity has been adopted by many researchers to evaluate the carbon balance of agricultural ecosystems, which, to a certain extent, compensates for the shortcomings of estimating soil carbon emission efficiency. Different ecosystems' carbon inputs and outputs vary yearly depending on climatic conditions and vegetative development (Tezza et al. 2019). Adding biochar plays an important role in controlling these systems' net soil carbon balance, bringing them closer to carbon neutrality. This study showed that all treatments exhibited net carbon uptake, and the application of biochar increased the net C plant production of the ecosystem owing to increased net ecosystem exchange compared to the control. The longer the application period, the greater the net C plant production (Table 2). The net C ecosystem productivity was also used to evaluate the carbon balance of salinised farmland ecosystems, and the net C ecosystem productivity of annual salinised farmland soils collectively was a carbon "sink" of atmospheric CO<sub>2</sub>; however, there were significant differences between different periods. The net C ecosystem productivity was positive in all treatments during the crop growth period, implying that the ecosystem carbon input was significantly higher than the carbon output during this period, and the farmland ecosystem was a carbon "sink" for atmospheric CO<sub>2</sub>. This is consistent with several studies that have concluded that agricultural soils function as a carbon "sink" during the crop growing period (Zhang et al. 2013), whereas soils during the

freeze-thaw period act as a "source" of atmospheric CO<sub>2</sub> emissions, probably because there is no plant growth during the freeze-thaw period in this study area. Generally, the influence of climatic conditions on the stability of biochar in soil is long-lasting. In addition, biochar contains a large amount of inert carbon, prolonging the time it affects the soil; studying the seasonal dynamics of the microbial load and carbon source metabolism requires an even longer monitoring time. Presently, the study of biochar's physical and chemical properties on its stability is relatively mature. Nevertheless, several uncertainties exist regarding the influence of the soil environment on biochar stability, and further research is still needed.

**Acknowledgement.** We thank the Bureau of Agriculture, Animal Husbandry and Science and Technology of Wuyuan County, Bayannur City, for providing the field for the experiment.

## REFERENCES

- Aanderud Z.T., Jones S.E., Schoolmaster D.R., Fierer N., Lennon J.T. (2013): Sensitivity of soil respiration and microbial communities to altered snowfall. *Soil Biology and Biochemistry*, 57: 217–227.
- Bao S.D. (2000): *Soil and Agricultural Chemistry Analysis*. 3<sup>rd</sup> Edition. Beijing, Agriculture Press.
- Cao M.K., Prince S.D., Li K.R., Tao B., Small J., Shao X.M. (2003): Response of terrestrial carbon uptake to climate interannual variability in China. *Global Change Biology*, 9: 536–546.
- Chen J., Luo Y.Q., Xia J.Y., Shi Z., Jiang L.F., Niu S.L., Zhou X.H., Cao J.J. (2016): Differential responses of ecosystem respiration components to experimental warming in a meadow grassland on the Tibetan Plateau. *Agricultural and Forest Meteorology*, 220: 21–29.
- Crippa M., Solazzo E., Guizzardi D., Monforti-Ferrario F., Tubiello F.N., Leip A. (2021): Food systems are responsible for a third of global anthropogenic GHG emissions. *Nature Food*, 2: 198–209.
- Criscuoli L., Ventura M., Wiedner K., Glaser B., Panzacchi P., Ceccon C., Loesch M., Raifer B., Tonon G. (2021): Stability of woodchips biochar and impact on soil carbon stocks: results from a two-year field experiment. *Forests*, 12: 1350.
- Cross A., Sohi S.P. (2011): The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biology and Biochemistry*, 43: 2127–2134.
- Cui Y.F., Meng J., Wang Q.X., Zhang W.M., Cheng X.Y., Chen W.F. (2017): Effects of straw and biochar addition on soil nitrogen, carbon, and super rice yield in cold waterlogged paddy soils of North China. *Journal of Integrative Agriculture*, 16: 1064–1074.
- De W. (1978): *Simulation of Assimilation, Respiration and Transpiration of Crops*. Simulation Monographs, Wageningen: Pudoc, 112.
- Ellert B.H., Janzen H.H. (1999): Short-term influence of tillage on CO<sub>2</sub> fluxes from a semi-arid soil on the Canadian Prairies. *Soil and Tillage Research*, 50: 21–32.
- Feng X.J., Nielsen L.L., Simpson M.J. (2007): Responses of soil organic matter and microorganisms to freeze-thaw cycles. *Soil Biology and Biochemistry*, 39: 2027–2037.
- Feng J., Yu D.L., Sinsabaugh R.L., Moorhead D.L., Andersen M.N., Smith P., Song Y., Li X.Q., Huang Q.Y., Liu Y.R., Chen J. (2023): Trade-offs in carbon-degrading enzyme activities limit long-term soil carbon sequestration with biochar addition. *Biological Reviews of the Cambridge Philosophical Society*, 98: 1184–1199.
- Gao X., Gu F.X., Hao W.P., Mei X.R., Li H.R., Gong D.Z., Mao L.L., Zhang Z.G. (2017): Carbon budget of a rainfed spring maize cropland with straw returning on the Loess Plateau, China. *Science of the Total Environment*, 586: 1193–1203.
- Gao X.L., Zhao N., Lu Y.H., Han X., Yang Z.P. (2022): Effects of supplementary irrigation on soil respiration of millet farmland in a semi-arid region in China. *Atmosphere*, 13: 1584.
- Gao Y., Li T.X., Fu Q., Li H., Liu D., Ji Y., Li Q.L., Cai Y.P. (2020): Biochar application for the improvement of water-soil environments and carbon emissions under freeze-thaw conditions: an *in-situ* field trial. *Science of the Total Environment*, 723: 138007.
- Gross C.D., Bork E.W., Carlyle C.N., Chang S.X. (2022): Biochar and its manure-based feedstock have divergent effects on soil organic carbon and greenhouse gas emissions in croplands. *Science of the Total Environment*, 806: 151337.
- Huang R., Lan M.L., Liu J., Gao M. (2017): Soil aggregate and organic carbon distribution at dry land soil and paddy soil: the role of different straws returning. *Environmental Science and Pollution Research International*, 24: 11356.
- Lehmann J., Cowie A., Masiello C.A., Kammann C., Woolf D., Amonette J.E., Cayuela M.L., Camps A.M., Whitman T. (2021): Biochar in climate change mitigation. *Nature Geoscience*, 14: 883–892.
- Li J., Tian D., Huang R., Xu G.X., Li J., Gao M., Wang Z.F. (2018): Effects of straw and biochar addition on soil carbon balance and ecological benefits in a rape-maize rotation planting system. *Environmental Science*, 39: 4338–4347.
- Li Y., Wang G.X., Bing H.J., Wang T., Huang K.W., Song C.L., Chen X.P., Hu Z.Y., Rui P.F., Song X.Y., Chang R.Y. (2021): Watershed scale patterns and controlling factors of ecosystem respiration and methane fluxes in a Tibetan alpine grassland. *Agricultural and Forest Meteorology*, 306: 108451.
- Li Y., Feng H., Chen J., Lu J.S., Wu W.J., Liu X.Z., Li C., Dong Q.G., Siddique K.H.M. (2022): Biochar incorporation increases winter wheat (*Triticum aestivum* L.) production with significantly improving soil enzyme activities at jointing stage. *Catena*, 211: 105979.
- Matovic D. (2010): Biochar as a viable carbon sequestration option: global and canadian perspective. *Energy*, 36: 2011–2016.
- Nan H.Y., Mašek O., Yang F., Xu X.Y., Qiu H., Cao X.D., Zhao L. (2022): Minerals: a missing role for enhanced biochar carbon



<https://doi.org/10.17221/121/2023-PSE>

- sequestration from the thermal conversion of biomass to the application in soil. *Earth-Science Reviews*, 234: 104215.
- Poepplau C., Bolinder M.A., Katterer T. (2016): Towards an unbiased method for quantifying treatment effects on soil carbon in long-term experiments considering initial within-field variation. *Geoderma*, 267: 41–47.
- Poulton P., Johnston J., MacDonald A., White R., Powlson D. (2018): Major limitations to achieving "4 per 1 000" increases in soil organic carbon stock in temperate regions: evidence from long-term experiments at Rothamsted Research, United Kingdom. *Global Change Biology*, 24: 2563–2584.
- Sheng Y.Q., Zhan Y., Zhu L.Z. (2016): Reduced carbon sequestration potential of biochar in acidic soil. *Science of the Total Environment*, 572: 129–137.
- Smith P., Soussana J.F., Angers D., Schipper L., Chenu C., Rasse D.P., Batjes N.H., Van E.F., McNeill S., Kuhnert M., Arias-Navarro C., Olesen J.E., Chirinda N., Fornara D., Wollenberg E., Álvaro-Fuentes J., Sanz-Cobena A., Klumpp K. (2020): How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. *Global Change Biology*, 26: 219–241.
- Tang Y., Gao W.C., Cai K., Chen Y., Li C.B., Lee X.Q., Cheng H.G., Zhang Q.H., Cheng J.Z. (2021): Effects of biochar amendment on soil carbon dioxide emission and carbon budget in the karst region of southwest China. *Geoderma*, 385: 114895.
- Tezza L., Vendrame N., Pitacco A. (2019): Disentangling the carbon budget of a vineyard: the role of soil management. *Agriculture, Ecosystems and Environment*, 272: 52–62.
- IPCC (2022): The Intergovernmental Panel on Climate Change. *Climate Change 2022: Mitigation of Climate Change*. Switzerland, The Physical Science Basis.
- Ventura M., Zhang C., Baldi E., Fornasier F., Sorrenti G., Panzacchi P., Tonon G. (2014): Effect of biochar addition on soil respiration partitioning and root dynamics in an apple orchard. *European Journal of Soil Science*, 65: 12095.
- Wang D., Chen J., Felton A.J., Xia L.L., Zhang Y.F., Lou Y.Q., Cheng X.L., Cao J.J. (2021): Post-fire co-stimulation of gross primary production and ecosystem respiration in a meadow grassland on the Tibetan Plateau. *Agricultural and Forest Meteorology*, 303: 108388.
- Wang J., Dokohely M.E., Xiong Z., Kuzyakov Y. (2016): Contrasting effects of aged and fresh biochars on glucose-induced priming and microbial activities in paddy soil. *Journal of Soils and Sediments*, 16: 191–203.
- Wang X.F., Wang S.L., Zhang W.D. (2013): Effects of Chinese fir litter on soil organic carbon decomposition and microbial biomass carbon. *Chinese Journal of Applied Ecology*, 24: 2393–2398.
- Wang Y., Liu S.R., Luan J.W., Chen C., Cai C., Zhou F., Di Y.P., Gao X.M. (2021): Nitrogen addition exacerbates the negative effect of throughfall reduction on soil respiration in a bamboo forest. *Forests*, 12: 724.
- Xu X.Z. (2010): *Permafrost Physics*. Beijing, Science Press.
- Yang J.S., Zhou W.M., Liu J.S., Hu X.J. (2014): Dynamics of greenhouse gas formation in relation to freeze/thaw soil depth in a flooded peat marsh of Northeast China. *Soil Biology and Biochemistry*, 75: 202–210.
- Zhang J.F., Zhou J.G., Lambers H., Li Y.W., Li Y.X., Qin G.M., Wang F.M. (2022): Nitrogen and phosphorus addition exerted different influences on litter and soil carbon release in a tropical forest. *Science of the Total Environment*, 832: 155049.
- Zhang M.Y., Wang F.J., Chen F., Malemela M.P., Zhang H.L. (2013): Comparison of three tillage systems in the wheat-maize system on carbon sequestration in the North China Plain. *Journal of Cleaner Production*, 54: 101–107.
- Zimmerman A.R., Gao B., Ahn M.Y. (2011): Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology and Biochemistry*, 43: 1169–1179.
- Zhou X., Wan S.Q., Luo Y.Q. (2007): Source components and inter-annual variability of soil CO<sub>2</sub> efflux under experimental warming and clipping in a grassland ecosystem. *Global Change Biology*, 13: 761–775.
- Zuo Y.T., Meng F.X., Li T.X., Fu Q., Liu D., Hou R.J., Li Q.L., Li M. (2022): Effect of biochar application on freezing-thawing deformation of farmland soil during freeze-thaw cycling. *Geoderma*, 405: 115510.
- Zwieten L.V., Kimber S., Morris S., Chan K.Y., Downie A., Rust J., Joseph S., Cowie A. (2010): Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil*, 327: 235–246.

Received: March 20, 2023

Accepted: March 4, 2024

Published online: April 10, 2024