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Biochar

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Sustainable woody biochar application for improving net ecosystem carbon budget, yield and soil properties in red pepper cropping systems: a two-year field study

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Abstract

Woody biochar (WB) is a low carbon (C)-emitting organic resource that can be used to increase C sequestration by effectively storing C in the soil. However, there is a lack of research on optimal WB application levels for improving the annual net ecosystem C budget (NECB) in red pepper cropping systems. In this study, WB was applied annually at 0, 2.5, 5, and 10 Mg d.w ha^{-1} for 2 years in a red pepper cropping field. The annual NECB, including C input and output, red pepper biomass productivity, and soil properties, was investigated. The total C input from fertiliser and WB application over the two years was the highest at WB10, followed by WB5 > WB2.5 > WB0. In both years, CO_2 emissions increased with increasing WB application levels, whereas CH_4 emissions exhibited negative fluxes across all treatments, with a negligible impact on C output. WB application increased the cumulative fruit productivity and total biomass productivity. The optimal and sustainable WB application levels for improving the annual NECB were estimated to be 7.3–11.4 Mg d.w ha^{-1} when removing the whole biomass after harvest and 1.8–6.7 Mg d.w ha^{-1} when returning it to the soil. Increasing WB application levels significantly improved soil physicochemical properties, such as bulk density and soil organic carbon (SOC) content. Optimising the WB application level may improve organic matter management by enhancing NECB, red pepper productivity, and soil properties. Our results offer guidance for sustainable optimisation of WB application in red pepper cropping systems.

Highlights

- WB application improved annual NECB, overall biomass productivity, and soil properties in the pepper cropping system.
- To improve NECB, the optimal WB application levels were estimated to be 1.8–6.7 Mg d.w ha^{-1} when returning it to the soil.
- The optimal WB application levels were estimated to be 7.3–11.4 Mg d.w ha^{-1} when removing the whole biomass.
- WB application could be a sustainable strategy to mitigate climate change by improving the annual NECB.

Keywords Carbon budget, *Capsicum annuum*, CO_2, NECB, Organic amendment

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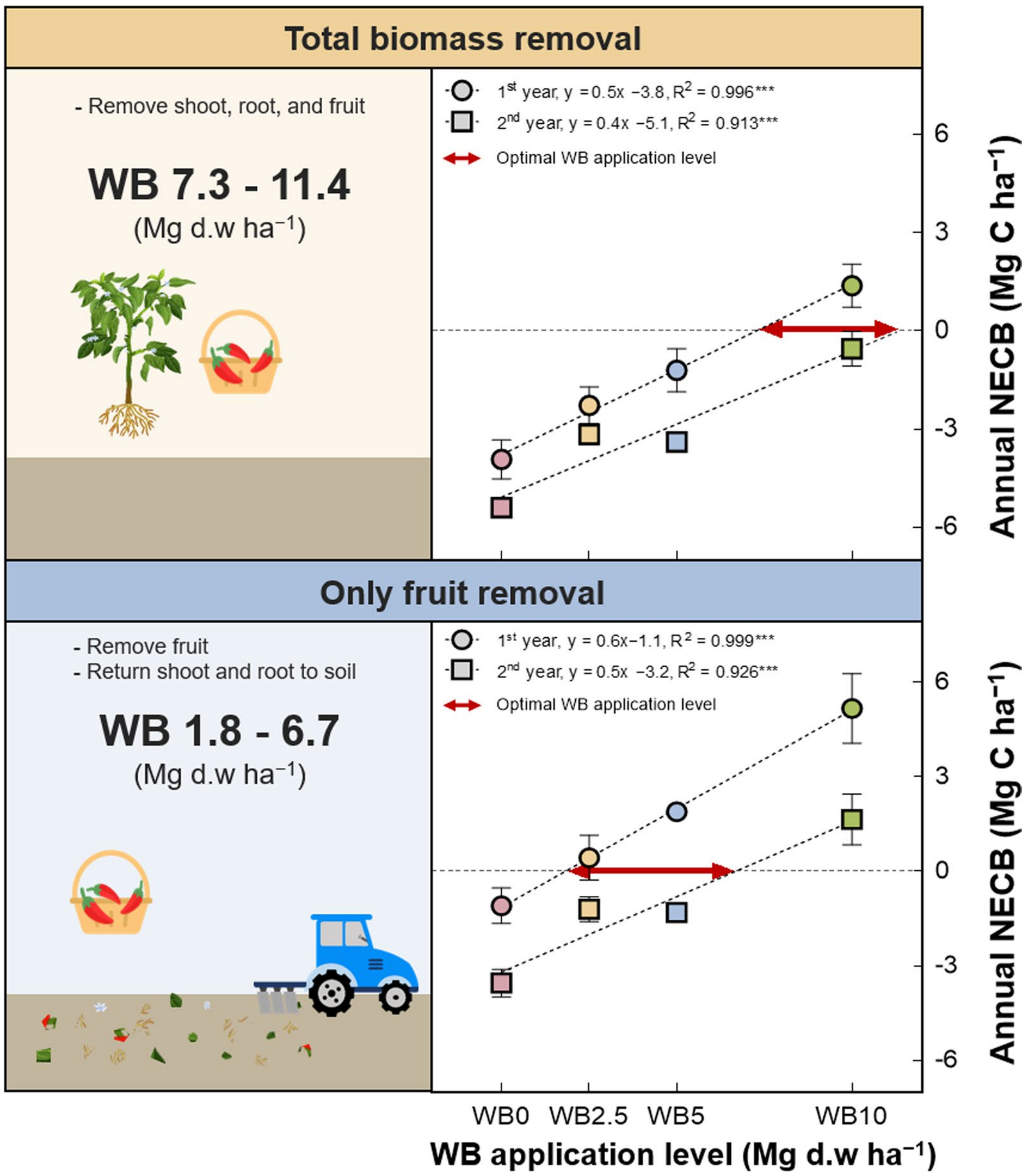
Graphical Abstract



1 Introduction

Climate change, caused by increased greenhouse gas (GHG) emissions, is a global challenge that negatively affects agricultural production and food security. Therefore, the development of sustainable agricultural management strategies is urgently needed to mitigate GHG emissions and enhance soil carbon (C) sequestration. Recently, the amendment of agricultural soils with biochar has been widely accepted as an effective way to enhance soil C sequestration and has gained significant attention worldwide (Bo et al. 2023; Neogi et al. 2022). Moreover, biochar application can significantly increase nutrient availability (Ding et al. 2016), improve soil quality (He et al. 2021), mitigate GHG emissions (Canatoy et al. 2022; Thomson et al. 2012; Zhang et al. 2024), and increase socioeconomic value (Inyang et al. 2016). Thus, biochar amendment can be recognised as a promising soil management strategy for mitigating global climate change while maintaining sustainable agricultural systems (Woolf et al. 2010).

Biochar is a stable form of a C-rich material produced by the pyrolysis of organic matter under low O_2 (oxygen) conditions (Schmidt and Noack 2000; Lehmann 2007). Woody biochar (WB) in particular has strong potential for enhancing soil C sequestration, exhibiting relatively low H/C ratios and high lignin contents as a proxy for organic matter stability (Al-Wabel et al. 2017). In addition, WB is more degradation-resistant than materials such as herbaceous feedstocks, effectively increasing C retention in soils (Luo et al. 2023). A previous study showed in an incubation experiment that wood biochar application had more potential to increase soil C storage than other organic resources, including compost and red pepper residue, because of its higher C input and lower degradation rate and Q_{10} value (Lee et al. 2023). The incorporation of WB into soil could play a crucial role in effectively sequestering C and lowering atmospheric CO_2 levels in agricultural environments.

Numerous studies have been conducted to decipher the impacts of biochar amendment on mitigating GHG emission, enhancing crop production and C sequestration in agricultural soils (Al-Wabel et al. 2017; Bo et al. 2023; Luo et al. 2023). In general, incorporating biochar can directly and indirectly influence soil physicochemical properties and microbial activities, which in turn affect soil organic carbon (SOC) mineralisation (Bo et al. 2023). In particular, the addition of biochar significantly increased SOC mineralisation, accelerating the decomposition of soil organic matter by creating optimal environmental conditions. This is mainly associated with improved soil physical properties (Baiamonte et al. 2019), increased labile C sources (Wang et al. 2016) and enhanced soil nutrient availability and fertility (Ding et al. 2016). However, several reports have demonstrated that biochar incorporation significantly decreases SOC mineralisation rates by reducing microbial accessibility to organic substrates (Yang et al. 2016), increasing encapsulation and adsorption protection (Bo et al. 2023) and facilitating the development of biochar-mineral-organic aggregates to be stabilised in soils (Han et al. 2020). Thus, the influence of biochar application on overall soil C dynamics in cropping systems remains unclear.

In cropping systems, C inputs are regulated by biomass productivity, which is mainly influenced by soil conditions and management practices. Biochar addition can directly add C to soils and enhance crop yield and biomass productivity, including crop residues and roots, by improving soil properties and indirectly increasing C input. However, the addition of fresh organic biomass such as root, litter, and fruit residues can also increase soil microbial respiration and activity, accelerating C loss from increased SOM decomposition rates during the cropping seasons. Furthermore, the remaining biochar can also be degraded in soils after crop harvesting during the fallow season because of its ageing process, affecting the overall C dynamics in the cropping systems. Therefore, a comprehensive evaluation of the response to biochar application is required by considering overall C balances over the entire cropping period, which can simultaneously investigate C inputs and outputs, narrowing a significant research gap in sustainable agricultural practices.

Red peppers (*Capsicum annuum* L.) are a major crop worldwide. In Korea, red peppers were cultivated on approximately 27,129 ha in 2023, accounting for 13% (205,829 ha) of the total open-field fruit and vegetable crop cultivation area (MAFRA 2023). As a thermophilic vegetable, red pepper is typically cultivated in (sub)tropical or temperate regions, including China, India, and Ethiopia, for a long duration under either greenhouse or open-field conditions, which could be vulnerable to accelerating SOC decomposition during cultivation. With the rapid expansion in vegetable cultivation areas worldwide, substantial amounts of residues are generated during vegetable production (Pavi et al. 2017). In red pepper cropping systems, incorporating pepper residues into soil is widely recognized a sustainable way to maintain SOM, supply available nutrients to the plant, and reduce agricultural waste. However, little information is available on how residue incorporation affects C balance by either stimulating C accumulation as positive feedback or accelerating C decomposition as negative feedback in red pepper cropping systems. Therefore, developing an effective management strategy for organic matter in red pepper cultivation fields is necessary for sustainable vegetable production (Singh et al. 2012), especially for enhancing the soil C balance.

The net ecosystem C budget (NECB) is a precise tool for estimating the soil C balance between C sequestration and mineralisation loss (Chapin et al. 2006; Smith et al. 2010) that provides a scientific basis for the C budget at a crop seasonal cropping scale under field conditions (Lai et al. 2017; Tang et al. 2021). However, little information is available about the response of NECB to vegetable cultivation (Jia et al. 2012), especially following biochar addition. NECB assessment has been applied to rice and maize cropping systems, with a focus primarily on organic amendments such as compost and biochar in paddy soils and agricultural practices such as plastic mulching systems during cultivation (Canatoy et al. 2022, 2023; Lee et al. 2021a, b). Notably, there is limited information available on the NECB for vegetable crops, including red pepper, under field cropping conditions, which is probably due to labour-intensive cultivation, such as frequent harvesting (Dessie et al. 2019). Therefore, a systematic evaluation of NECB is necessary to determine the optimal application level of WB to enhance C sequestration and mitigate GHG emissions without excessive inputs in red pepper cropping systems. Recently, a few studies have recommended optimal biochar application levels of approximately 10 and 20 Mg ha^{-1} in saline–alkali soils and vegetable rotation systems, respectively (Zhang et al. 2022, 2024). These studies suggest appropriate levels based on enhancing C storage without significant loss of crop yield by measuring gas emissions and SOC changes. However, these measurements may be too difficult to quantitatively estimate the overall C dynamics, including C fixation, emission, and translocation, which may lead to over- or under-estimation of the optimal level of biochar application across the entire cropping boundary. Therefore, the optimal application levels of WB should be estimated based on the C balance for better agricultural production and sustainability. In this study, we hypothesized that WB application would improve annual NECB by balancing C inputs and outputs, with optimal rates varying based on residue management.

The objectives of this study were to evaluate the overall impact of WB application on NECB, crop biomass productivity, and soil properties in two different harvesting scenarios (whole biomass removal or only fruit removal) and to suggest optimal biochar application levels to maintain NECB at a positive value in the cropping boundary as a novel approach. Moreover, the main contributing factors that significantly influenced the C balance were evaluated based on the NECB method over the cropping period, including the cultivation and non-cultivation seasons. In this study, we conducted a field experiment with different application levels of WB under two types of residue incorporation scenarios over two successive years for suggesting a better sustainable soil management.

2 Materials and methods

2.1 Experimental design, WB application level, and crop cultivation

This experiment was conducted at the Suncheon University Research Farm in Suncheon, Jeollanam-do, South Korea (35°00′03.2″N, 127°30′30.1″E), which has a typical monsoon climate, a mean annual temperature of 14.8 ℃, and annual precipitation of 1467 mm based on the last 30 years from 1991 to 2020 (KMA 2023). The meteorological characteristics throughout the cultivation period are consistent with those shown in Fig. S1. The soil was classified as *Deogcheon* series, a loamy skeletal, mixed, and mesic family of Typic Udifluvents. The main chemical properties of the soil before the experiment included a slightly acidic pH (5.77, 1:5 with H_2O) and high total C (31.0 g kg^{-1}) (Table 1).

Table 1 Chemical properties of soils before the experiment

Parameters	Value
pH (H_2O, 1:5)	5.77 ± 0.01
Electrical conductivity (dS m^{-1})	0.62 ± 0.01
Total C (g kg^{-1})	31.0 ± 0.82
Total N (g kg^{-1})	2.83 ± 0.13
C/N ratio	11.0 ± 0.71
Available P_2O_5 (mg kg^{-1})	555 ± 20.8
Exchangeable cations (cmol$_c$ kg^{-1})	
Ca^{2+}	6.15 ± 0.41
Mg^{2+}	1.62 ± 0.07
K^+	0.77 ± 0.03
Soil texture	*Silt Loam*

Table 2 Chemical properties of WB used in this study

Parameters	1st year	2nd year
pH (H_2O, 1:5)	7.4 ± 0.03	7.8 ± 0.02
Electrical conductivity (dS m^{-1})	3.5 ± 0.09	2.9 ± 0.07
Total contents (g kg^{-1})		
C	566 ± 0.75	546 ± 7.75
N	10.8 ± 0.31	7.97 ± 0.68
P_2O_5	3.49 ± 0.09	0.36 ± 0.03
K_2O	3.74 ± 0.03	7.52 ± 1.91
Aromatic ratio		
C/N	52.5 ± 1.52	68.9 ± 6.56
O/C	0.61 ± 0.01	0.70 ± 0.02
H/C	0.095 ± 0.002	0.104 ± 0.001
Water content (%, wt wt^{-1})	39.2 ± 2.15	50.8 ± 0.28

Before crop cultivation, six treatments were established in a completely randomised block design with three replicates, each with a size of 1.6 m^2. In this study, WB was incorporated every year as a basal fertiliser for two years under different application levels (0 as a control and 2.5, 5, and 10 Mg ha^{-1} based on dry weight) under the recommended quantities for the cultivation of red pepper (*Capsicum annuum* L., N-P_2O_5-K_2O = 190-112-149 kg ha^{-1}) (NIAST 2019). Biochar was prepared from commercial products pyrolysed from coniferous wood at 400 ℃ for 1 h. Their chemical properties are summarised in Table 2. An evaluation based on their O/C and H/C ratios ($O/C \leq 0.4$ and $H/C \leq 0.7$) indicated typical biochar properties (Rodrigues et al. 2023).

Red peppers were transplanted on 3 May 2022 and 21 April 2023 at a spacing of 35 cm. During the red pepper cropping season, 103 kg N ha^{-1} and 91 kg K ha^{-1} were applied at the time of transplanting, and the first (43.5 kg N ha^{-1}, 29 kg K ha^{-1}) and second top dressing (43.5 kg N ha^{-1}, 29 kg K ha^{-1}) were applied approximately 30 days after transplanting (DAT) and 60 DAT, respectively. In contrast, P fertiliser was applied as a basal fertiliser. Fruits were harvested over two weeks, starting approximately 80 days after transplanting and continuing for four harvests, with red peppers larger than 8 cm collected. The final harvest, reflecting the total fruit biomass, was conducted by harvesting red peppers larger than 2 cm on 3 September 2022 and 25 August 2023.

2.2 Measurement of gaseous C losses

To investigate gaseous C losses (CO_2 and CH_4) during red pepper cropping, gas samples were collected using the static closed-chamber method (Rolston 1986). Opaque cylindrical chambers (12 cm diameter × 30 cm height) were installed with three replicates in each treatment plot. Gas samples were collected for 30 min between 10 a.m. and 11 a.m. using a 60 mL syringe at 0-, 15-, and 30-min intervals (Lee et al. 2025). During gas sampling, the chamber temperature was monitored using a thermometer to determine its potential influence on GHG emissions. The collected gas samples were analysed using a gas chromatograph (GC8890, Agilent, USA) equipped with a flame ionisation detector (FID) for CH_4 measurements. CO_2 was reduced to CH_4 using hydrogen in a nickel catalytic converter and detected using FID. The carrier gas was argon (Tr) containing 5% CH_4 at a flow rate of 40 mL min^{-1}. The temperature of the FID detector was maintained at 250 ℃, and the oven and column were operated at 60 ℃.

GHG emission rates were calculated using the following formula (Rolston 1986):

$$\mathrm{CO_2\ and\ CH_4,\ emission\ rate\ (kg\ ha^{-1}\ day^{-1})} = \rho \times \left(\frac{V}{A}\right) \times \left(\frac{\Delta c}{\Delta t}\right) \times \left(\frac{273}{T}\right)$$

where ρ (mg cm^{-3}) is the gas density of CO_2 and CH_4 under standard conditions; V (m^3) and A (m^2) are the volume and surface area of the chamber used, respectively; $\Delta c/\Delta t$ is the difference in gas concentration (mg m^{-3} h^{-1}) inside the headspace before and after chamber closing; and T (K) is the absolute temperature during the gas sampling period inside the chamber.

The total greenhouse gas emissions generated during each experimental year were evaluated using the following equation (Singh 1999):

$$\mathrm{Total\ CO_2\ and\ CH_4\ flux\ (Mg\ ha^{-1})} = \sum_{i}^{n} (F_i \times D_i)$$

where n is the total number of gas samples, F_i is the mean daily flux (kg ha^{-1} day^{-1}) of CO_2 and CH_4 on the ith sampling date, and D_i is the number of days during the ith sampling period.

2.3 Crop productivity analysis

To evaluate the productivity of red peppers, biomass was harvested and separated into shoot, root, and fruit biomass, following the standard method of NIAST (2000). The harvested biomass was oven-dried at 70 °C for 72 h, and the dry weight was measured. The fruit yield was calculated as the sum of the dry weights of red peppers harvested four times annually. The total C and N contents of the dried biomass were measured using an elemental analyser (EA2400II, PERKIN ELMER, USA).

2.4 Estimation of NECB

2.4.1 C input

To estimate the NECB for different WB application levels, the C input was estimated using the following equation:

$$\mathrm{Fertiliser\ (Mg\ C\ ha^{-1})} = \mathrm{Urea\ application\ rate\ (Mg\ ha^{-1})} \times \mathrm{Urea\ C\ content\ (20\%)}$$

$$\mathrm{WB\ (Mg\ C\ ha^{-1})} = \mathrm{WB\ application\ level\ (Mg\ d.w\ ha^{-1})} \times \mathrm{WB\ total\ C\ content\ (kg\ Mg^{-1})}/1000$$

$$\mathrm{NPP\ (Mg\ C\ ha^{-1})} = \mathrm{NPP_{shoot}} + \mathrm{NPP_{root}} + \mathrm{NPP_{fruit}} + \mathrm{NPP_{litter}} + \mathrm{NPP_{rhizodeposits}}$$

where fertiliser is the C input due to urea; WB is the C input due to WB; NPP is the net primary production of red pepper; and NPP_{shoot}, NPP_{root}, NPP_{fruit}, NPP_{litter}, and $NPP_{rhizodeposits}$ are the C contents of shoots, roots, fruits, litter, and rhizodeposits of red pepper, respectively. The NPP of the litter and that of the rhizodeposits were calculated using an allometric relationship. Litter was assumed to be 5% of the total (shoot, root, and fruit) biomass productivity (Smith and Falloon 2004), and rhizodeposits were assumed to be 7% of the total biomass productivity (Kuzyakov and Domanski 2000).

2.4.2 C output

Respired C loss refers to C output from soil respiration (CO_2 and CH_4). Harvested C removal is the C uptake of red pepper biomass, including shoots, roots, and fruits, which is removed at the harvesting stage.

$$\begin{aligned}&\text{Respiration C loss (Mg C ha}^{-1})\\&=\text{Total CO}_2\text{ flux (Mg ha}^{-1})\times 12/44\\&\quad+\text{Total CH}_4\text{ flux (Mg ha}^{-1})\times 12/16\end{aligned}$$

$$\begin{aligned}&\text{Harvested C removal (Mg C ha}^{-1})\\&=\text{NPP}_{\text{shoot}}+\text{NPP}_{\text{root}}+\text{NPP}_{\text{fruit}}\end{aligned}$$

where harvested C removal refers to the scenario in which the red pepper biomass is not returned to the soil. On the other hand, only fruit removal refers to the scenario in which only the fruit is removed by harvesting, and the shoots and roots are returned to the soil.

2.4.3 NECB

To estimate NECB under the different WB application levels, NECB was estimated to use the following equation (Smith et al. 2010; Canatoy et al. 2023):

$$\begin{aligned}&\text{NECB (Mg C ha}^{-1})\\&=\text{Total C input (fertiliser + WB + NPP)}\\&\quad-\text{Total C output (respiration C loss}\\&\quad+\text{harvested C removal)}\end{aligned}$$

2.5 Evaluation of soil physicochemical properties

To assess the physical properties of the soil, soil bulk density was determined using the gravimetric method (Blake and Hartge 1986). Soil samples with a total volume of 100 cm^3 were collected using a core sampler after the red pepper harvest. These samples were oven-dried at 105 °C for 48 h, and the dry weight was measured to calculate the bulk density (Lee et al. 2021a, b). To evaluate the soil's chemical properties, soil samples were collected from the surface layer (0–15 cm) of the plots using an auger sampler. The collected samples were air-dried, sieved through a 2-mm sieve, and used for chemical analysis before and after harvest. The soil texture was determined using the pipette method. Soil pH and electrical conductivity (EC) were measured with a 1:5 water extraction using a pH meter (Orion Star A211, Thermo Scientific, Indonesia) and an EC meter (Orion Star A212, Thermo Scientific, Indonesia), respectively. The total C and N contents in the soil were measured using an elemental analyser (EA2400II, PERKIN ELMER, USA). Available phosphorus was extracted using the Lancaster method and quantified by colorimetric determination using a discrete analyser (AQ400, SEAL Analytical, USA). The ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) as inorganic nitrogen in soil were determined following extraction with 2 M KCl. NH_4^+-N was measured using the salicylate method (Kempers and Zweers 1986), and NO_3^--N was quantified using the cadmium reduction method (Dorich and Nelson 1984), both measured with a discrete analyser (AQ400, SEAL Analytical, USA). Exchangeable cations were determined by extracting the soil with 1N NH_4OAc, followed by filtration and analysis using atomic absorption spectrometry (AAS, Shimadzu, Japan). The cation exchange capacity (CEC) was measured using the ammonium saturation method by saturating the soil with 1N NH_4OAc solution, removing excess ammonium with ethanol, displacing exchangeable ammonium ions with 1 M KCl, and quantifying them using a discrete analyser (AQ400, SEAL Analytical, USA).

2.6 Statistical analysis

Statistical analyses were conducted using SAS software (version 9.4; SAS Institute Inc., 1995). One-way and Two-way analyses were used to determine the differences between factors, including the WB application level and red pepper cropping system year. Post hoc analysis was conducted using Tukey's honest significant difference test at a 5% significance level ($p < 0.05$) when significant differences among treatments were observed.

3 Results

3.1 Meteorological characteristics

The meteorological characteristics of the region, including the seasonal and annual mean air temperature (MAT) and seasonal total precipitation (STP), were monitored over the entire cultivation season (Fig. S1). The seasonal MAT and STP were much higher during the red pepper cultivation period, with ranges of 21.6–22.8 °C and 555–1394 mm, respectively, than during the non-cultivation period, with ranges of 8.3–9.5 °C and 485–906 mm, respectively. Unexpectedly, the total annual precipitation (2300 mm) in the second year was more than twice that in the first year (1040 mm). The annual MATs in the two years were almost the same. However, the temperature was higher during the cultivation season in the first year (22.8 °C) and during the non-cultivation period in the second year (9.5 °C).

3.2 WB application on C input

Irrespective of the year of investigation, WB application significantly increased C input with proportionally increas the application levels (Table 3 and 4). Net primary production (NPP), including shoots, fruits, litter, roots, and rhizodeposits, was significantly affected by

Table 3 Red pepper biomass productivity under the different WB application levels for two years

Year (A)	Treatment (B)	Biomass productivity (Mg d.w ha^{-1})		Fruit productivity (Mg d.w ha^{-1})					Total productivity (Mg d.w ha^{-1})
		Shoot	Root	1st	2nd	3rd	4th	Sum	
1st year	NPK + WB0	6.61[1]^{b}	0.36^{a}	1.44^{a}	0.75^{a}	1.45^{a}	2.31^{a}	5.94^{b}	12.9^{b}
	NPK + WB2.5	6.38^{b}	0.34^{a}	2.11^{a}	1.01^{a}	1.28^{a}	2.21^{a}	6.61^{b}	13.3^{b}
	NPK + WB5	6.85^{b}	0.43^{a}	1.69^{a}	0.77^{a}	1.49^{a}	2.66^{ab}	6.61^{b}	13.9^{b}
	NPK + WB10	8.93^{a}	0.46^{a}	2.00^{a}	1.00^{a}	1.79^{a}	3.09^{b}	7.88^{a}	17.3^{a}
2nd year	NPK + WB0	4.51^{a}	0.22^{a}	1.11^{a}	0.64^{a}	0.60^{b}	1.71^{a}	4.12^{b}	8.86^{b}
	NPK + WB2.5	4.69^{a}	0.24^{a}	1.51^{a}	0.49^{a}	0.73^{b}	1.83^{a}	4.56^{ab}	9.49^{ab}
	NPK + WB5	4.97^{a}	0.29^{a}	1.46^{a}	0.43^{a}	0.71^{b}	2.17^{a}	4.77^{ab}	10.0^{ab}
	NPK + WB10	5.20^{a}	0.30^{a}	1.46^{a}	0.48^{a}	1.07^{a}	1.99^{a}	5.00^{a}	10.5^{a}
Statistical analysis									
A[2]		[3]***	***	**	***	***	***	***	***
B		**	*	*	Ns	**	*	***	***
A × B		Ns	Ns	Ns	Ns	Ns	Ns	Ns	*

[1] Different letters indicate significant differences between the treatments (at $p < 0.05$, Tukey's test). The same letter means there are no significant differences between the treatments. [2] A and B denote year and treatment in 2 way ANOVA analysis. [3] ns denotes not significant, and *, **, and *** denote significant differences at levels of $p < 0.05$, *0.01*, and *0.001* respectively

WB application levels and investigation year. Shoot and fruit biomass constituted the main C inputs during the entire cultivation period, accounting for 86–87% of the total NPP. Fertiliser C input (mainly urea) had a negligible effect on C input over the investigation period.

3.3 WB application on C output (CO_2 and CH_4 as respired losses)

Respired C losses (CO_2 and CH_4 emission rates) were investigated over the cropping season, including the red pepper cultivation and non-cultivation seasons for two years (Fig. 1). Irrespective of the biochar application level, the patterns of CO_2 and CH_4 loss were similar in both years. CO_2 emissions were generally low at the initial stage of red pepper cultivation and gradually increased with crop growth until approximately mid-August of each year. Thereafter, the CO_2 emission rates declined sharply to their lowest levels during winter. However, the CH_4 emission rates were negligible, showing negative values throughout the cultivation seasons, irrespective of the application level. During the entire investigation period, the cultivation period was the main contributor to respired C loss, showing approximately 60–79% compared to the non-cultivation season (22–41%) during both years (Table 4). Overall, respired C losses over the cultivation period increased proportionally with increasing biochar application levels. In addition, the respired C loss during the cultivation period was significantly affected by biochar application, but there was no significant impact on the investigation year. However, the respired C loss during the non-cultivation season was significantly affected only by the investigation year and not by application levels. Interestingly, the value was almost 2 times higher in the second year (2.46 Mg C ha^{-1} on average) than in the first year (1.02 Mg C ha^{-1} on average). The C loss from crop harvesting, including shoot, root, and fruit biomass, gradually increased with increasing biochar application levels, showing significant differences between the NPK + WB0 and NPK + WB10 treatments. The sum of C outputs gradually increased with increasing biochar application levels.

3.4 WB application on annual NECB

To evaluate the effect of WB application on soil C stock changes, annual NECB was calculated during the red pepper cultivation and non-cultivation periods for two years (Table 4). Regardless of the investigation year, the annual NECB gradually increased with increasing application levels, mostly showing negative values except for NPK + WB10 in the first investigation year. WB application was effective in significantly improving the annual NECB in both investigation years, which was significantly affected by the investigation year, application level, and the interaction between these factors. To maintain positive values of the annual NECB for the entire red pepper cropping system in the field, the optimal application level of WB was estimated using first-order regression equations based on two different scenarios: the red pepper residue was totally removed or incorporated (only fruit removal) after crop harvesting (Fig. 2). The results suggested that the optimal application levels of WB based on the annual NECB at two common

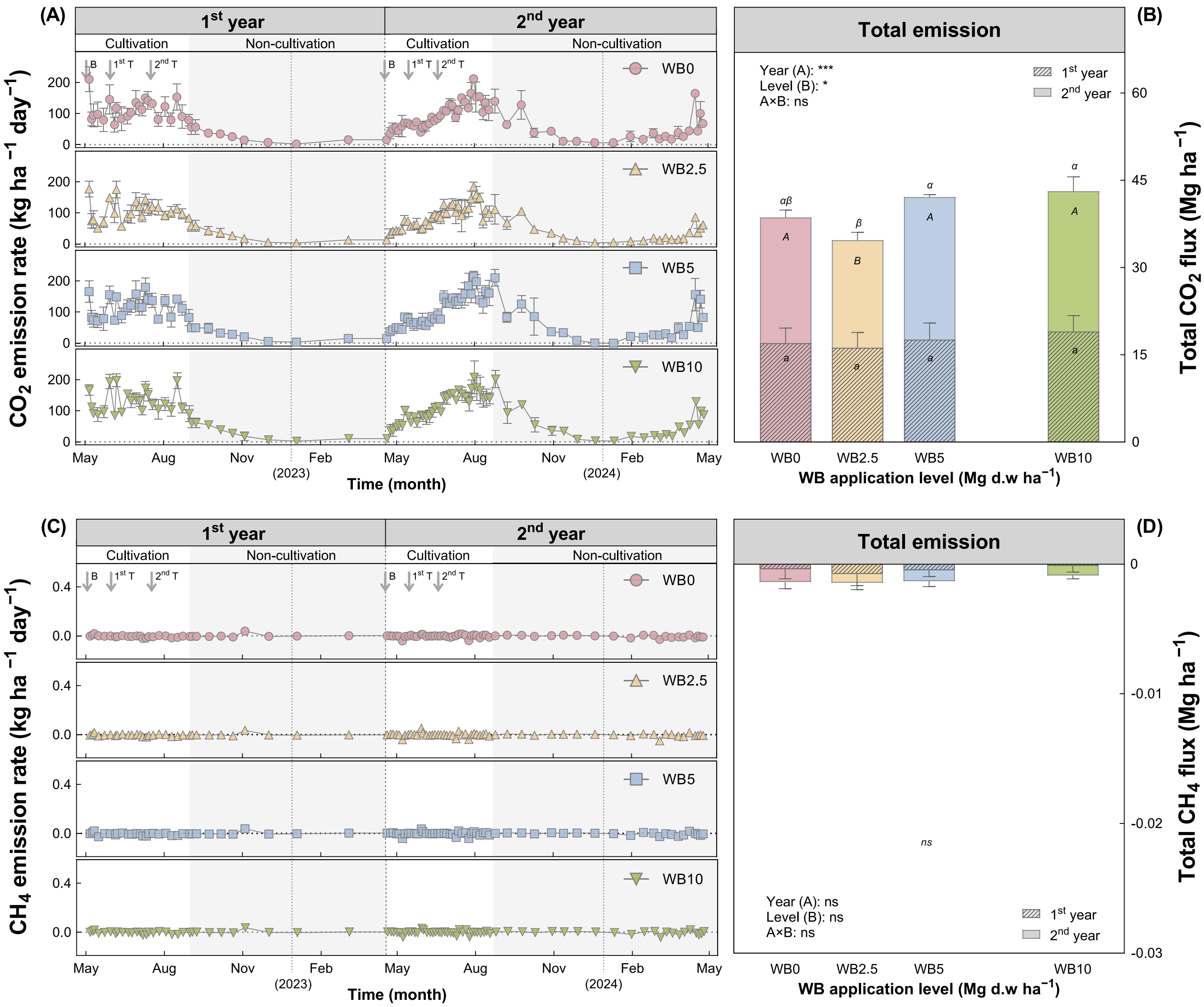


Fig. 1 Changes in CO_2 and CH_4 emission rates under the different WB application levels during the two years (**A**, **C**) and total flux (**B**, **D**). Vertical bars represent standard deviations (*n* = *3*). The different letters indicate significant differences between treatments ($p < 0.05$, Tukey's test). 'ns' means no significant differences among treatments. B, 1st T and 2nd T indicate the timings of basal fertilizer, first top dressing, and second top dressing, respectively

scenarios for residue management practice were 7.3–11.4 Mg d.w ha^{-1} (mean value of two years: 9.4 Mg d.w ha^{-1}) and 1.8–6.7 Mg d.w ha^{-1} (mean value of two years: 4.3 Mg d.w ha^{-1}) with whole biomass removal and with residue incorporation, respectively (Figs. 2A and B). The optimal application level of WB was much lower in the fruit removal scenario than in the total biomass removal scenario, showing a 55% reduction in the application level, suggesting that red pepper residue incorporation into the cropland could be a better option for improving annual NECB as well as saving the amount of biochar application, even though the residue could be easily decomposed by potentially increasing C losses from the red pepper cropping boundary. The annual NECB was generally lowered in the second year than in the first year, mainly because of decreased shoot biomass as an NPP input in the second year (Table 4).

3.5 WB application on red pepper fruit yield and biomass production

Red pepper fruit yield was significantly affected by the WB application level and year of investigation (Table 3). WB application significantly increased red pepper fruit yield, particularly in the NPK + WB10 treatments in both years, but showed no significant increase at other application levels. Biochar application increased overall fruit productivity by up to 18% in the first year and 16%

Table 4 Changes of NECB components in red pepper cropping system under different WB application levels

Year (A)	Treatment (B)	C input (Mg C d.w ha^{-1})								C output (Mg C d.w ha^{-1})				Annual NECB (Mg C ha^{-1})
		Fertiliser	Biochar	Net primary production (NPP)					Sum	Respired C loss period		Harvest removal[4]	Sum	
				Shoot	Fruit	Litter	Root	Rhizo-deposit		Cultivation	Non-cultivation			
1st year	NPK+WB0	0.04	0[1]^{d}	2.68^{b}	2.62^{b}	0.27^{b}	0.14^{a}	0.38^{b}	6.14^{d}	3.59^{a}	1.04^{a}	5.45^{b}	10.1^{b}	−3.93^{d}
	NPK+WB2.5	0.04	1.41^{c}	2.57^{b}	2.91^{b}	0.28^{b}	0.14^{a}	0.39^{b}	7.75^{c}	3.42^{a}	0.99^{a}	5.62^{b}	10.0^{b}	−2.28^{c}
	NPK+WB5	0.04	2.83^{b}	2.93^{ab}	2.92^{b}	0.30^{b}	0.17^{a}	0.42^{b}	9.61^{b}	3.74^{a}	1.06^{a}	6.02^{b}	10.8^{b}	−1.21^{b}
	NPK+WB10	0.04	5.66^{a}	3.61^{a}	3.44^{a}	0.36^{a}	0.18^{a}	0.51^{a}	13.8^{a}	4.19^{a}	0.99^{a}	7.24^{a}	12.4^{a}	1.38^{a}
2nd year	NPK+WB0	0.04	0^{d}	1.78^{a}	1.86^{a}	0.19^{b}	0.09^{a}	0.26^{b}	4.21^{d}	3.37^{b}	2.53^{a}	3.72^{b}	9.62^{b}	−5.41^{d}
	NPK+WB2.5	0.04	1.37^{c}	1.86^{a}	2.04^{a}	0.20^{ab}	0.10^{a}	0.28^{ab}	5.89^{c}	3.11^{b}	1.95^{b}	4.00^{ab}	9.06^{b}	−3.17^{b}
	NPK+WB5	0.04	2.73^{b}	1.96^{a}	2.14^{a}	0.21^{ab}	0.12^{a}	0.30^{ab}	7.50^{b}	3.87^{a}	2.80^{a}	4.22^{ab}	10.9^{a}	−3.40^{c}
	NPK+WB10	0.04	5.46^{a}	2.06^{a}	2.22^{a}	0.22^{a}	0.13^{a}	0.31^{a}	10.4^{a}	4.03^{a}	2.55^{a}	4.40^{a}	11.0^{a}	−0.55^{a}
Statistical analysis														
A[2]		–	[3]***	***	***	***	***	***	***	Ns	***	***	***	***
B		–	***	**	***	***	*	***	***	*	Ns	***	***	***
A × B		–	***	Ns	Ns	*	Ns	*	*	Ns	Ns	*	*	***

[1] Different letters indicate significant differences between the treatments (at $p < 0.05$, Tukey's test). The same letter means there are no significant differences between the treatments. [2] A and B denote year and treatment in 2 way ANOVA analysis. [3] ns denotes not significant, and *, **, and *** denote significant differences at levels of $p < 0.05$, *0.01*, and *0.001* respectively. [4] Harvest removal as C output was calculated by the sum of C from the shoot, fruit and root biomass

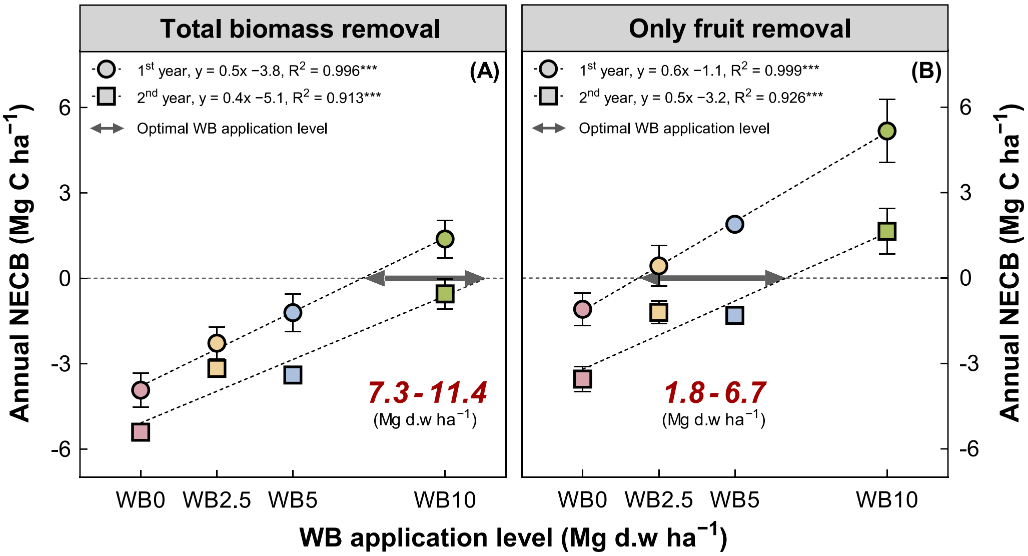


Fig. 2 Estimation of optimal application levels of WB to improve NECB under the two different harvesting scenarios in red pepper cropping systems (**A**: total biomass removal, **B**: only fruit removal)

increases in the second year compared to the control (4.12 and 5.94 Mg d.w ha^{-1}, respectively). Shoot biomass productivity generally increased with increasing application levels but only showed a significant increase for the NPK + WB10 treatment in the first year. However, root biomass did not differ significantly among treatments, regardless of the year of investigation. Total biomass production, including that of shoots, roots, and fruits, was significantly affected by the application level and investigation year. Biochar incorporation increased total red pepper biomass productivity by 19–34% over the control (8.9–12.9 Mg d.w ha^{-1}), resulting in significant differences for the NPK + WB10 treatment in both years. Overall, red pepper biomass decreased by approximately 32% in the second year compared to that in the first year, likely due to unfavourable weather conditions, such as frequent and excessive precipitation events (Fig. S1).

3.6 WB application on soil properties

WB application significantly altered the soil physicochemical properties, mainly influencing soil C-related properties, including bulk density, pH, total C, C/N ratio, exchangeable K^{+}, and cation exchange capacity after whole-crop harvesting (Table 5 and Fig. S2). Bulk density, pH, and total C were significantly affected by the biochar application levels. However, the bulk density, pH, C/N, exchangeable K^{+}, and cation exchange capacity were also significantly influenced by the year of investigation. For example, biochar application was effective in decreasing the bulk density as a proxy for soil physical properties compared to the control. With increasing application levels, the bulk density gradually decreased, showing that WB application had a significant impact in the second year. Soil pH was improved by biochar application with increasing levels because of the comparatively higher pH of the WB than that of the soil used in this study (Tables 1 and 2). Moreover, soil C content significantly increased with increasing the application levels, showing a 15–20% increase compared to the control (29.6–31.1 g kg^{-1}). The overall C/N ratio of the soil also increased with increasing application levels, but there was no significant impact on the total N content. Other properties such as electrical conductivity, available P_2O_5, extractable Ns (NH_4^{+}-N and NO_3^{-}-N), exchangeable Ca^{2+} and Mg^{2+} were not significantly affected by WB application or the year of investigation.

4 Discussion

WB application significantly affected soil C input and output, as well as red pepper biomass and fruit production, which led to a significant increase in annual NECB in red pepper cropping systems. Moreover, biochar application significantly improved overall soil physicochemical properties, such as bulk density and SOC content with increasing application levels. Our results provide the first evidence of the optimal levels of WB application under two different scenarios to improve annual NECB for a more sustainable agricultural cropping system.

Table 5 Physicochemical properties of soils under the different WB application levels at the whole red pepper harvesting stage in first and second year

Year (A)	Treatment (B)	Bulk density	pH	Electrical conductivity	Total contents (g kg^{-1})		C/N ratio	Available P_2O_5	NH_4^+-N	NO_3^--N	Exchangeable cations (cmol$_c$ kg^{-1})			Cation exchange capacity
		(g cm^{-3})	(1:5)	(dS m^{-1})	C	N		(mg kg^{-1})			Ca^{2+}	Mg^{2+}	K^+	(cmol$_c$ kg^{-1})
1st year	NPK + WB0	1.08$^{1)a}$	6.49^{b}	1.30^{a}	29.6^{b}	2.29^{a}	13.1^{b}	490.5^{a}	1.17^{b}	38.4^{a}	9.24^{a}	4.67^{a}	1.01^{a}	25.5^{a}
	NPK + WB2.5	1.07^{a}	6.51^{ab}	1.07^{a}	31.6^{ab}	2.16^{a}	14.7^{ab}	511.7^{a}	1.30^{b}	61.0^{a}	7.73^{a}	3.85^{a}	0.81^{b}	25.6^{a}
	NPK + WB5	1.04^{a}	6.62^{ab}	0.72^{a}	32.7^{ab}	2.23^{a}	14.6^{ab}	509.3^{a}	1.91^{a}	57.4^{a}	7.00^{a}	2.89^{a}	0.77^{b}	26.1^{a}
	NPK + WB10	1.02^{a}	7.27^{a}	0.66^{a}	35.6^{a}	2.17^{a}	16.6^{a}	510.4^{a}	1.71^{ab}	39.3^{a}	7.69^{a}	3.47^{a}	0.85^{ab}	25.1^{a}
2nd year	NPK + WB0	1.07^{a}	6.19^{a}	0.60^{a}	31.1^{c}	2.03^{a}	16.0^{a}	458.6^{b}	2.02^{a}	29.8^{a}	9.11^{a}	4.73^{a}	0.74^{a}	22.2^{a}
	NPK + WB2.5	1.01^{b}	6.28^{a}	0.78^{a}	33.0^{bc}	2.00^{a}	16.7^{a}	452.0^{b}	1.01^{b}	42.9^{a}	9.02^{a}	4.56^{a}	0.72^{a}	22.1^{a}
	NPK + WB5	0.96^{b}	6.52^{a}	0.72^{a}	34.1^{ab}	2.00^{a}	17.1^{a}	543.2^{a}	1.65^{ab}	41.1^{a}	7.45^{a}	3.83^{a}	0.67^{a}	21.4^{a}
	NPK + WB10	0.96^{b}	6.55^{a}	0.77^{a}	35.8^{a}	2.17^{a}	16.6^{a}	509.2^{a}	1.69^{ab}	30.9^{a}	8.17^{a}	3.95^{a}	0.66^{a}	21.9^{a}
Statistical analysis														
A$^{2)}$		$^{3)}$*	*	Ns	Ns	Ns	*	Ns	Ns	Ns	Ns	Ns	**	***
B		*	*	Ns	***	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
A × B		Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	*

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[1] Different letters indicate significant differences between the treatments (at $p < 0.05$, Tukey's test). The same letter means there are no significant differences between the treatments. [2] A and B denote year and treatment in 2 way ANOVA analysis. [3] ns denotes not significant, and *, **, and *** denote significant differences at levels of $p < 0.05$, *0.01*, and *0.001* respectively

4.1 Optimal WB applications on annual NECB during the whole red pepper cropping periods

The NECB has been widely employed to evaluate the overall impact of organic resources on soil C input and output in agricultural ecosystems (Smith et al. 2010; Xiao et al. 2024), providing a comprehensive understanding of the changes in the C budget for better soil C management strategies. Our study demonstrated that the control treatment exhibited the lowest (negative) NECB values in both years, indicating soil C depletion in the long-term management of agriculture, possibly leading to deterioration of soil quality and productivity (Haque et al. 2015). However, WB application significantly increased the annual NECB over the red pepper cropping period compared to the control. This result implies that biochar incorporation has a strong potential to increase C input, accounting for 30–37% of the total, effectively enhancing soil C accumulation owing to its degradation stability (Kuzyakov et al. 2014). Despite its exceptional stability, WB application gradually increased soil C output with increasing the application levels during the whole cultivation period by potentially stimulating microbial respiration by up to ca. 12% compared to the control; however, there were no statistical differences among all treatments (Fig. 1). This result indicates that certain fractions of the added biochar may be vulnerable to organic matter decomposition in soil environments throughout the cropping period owing to facilitated ageing and priming effects (Bo et al. 2023), although WB is considered a stable material for enhancing soil C stocks. Therefore, it is evident that WB can effectively improve the annual NECB in pepper cropping systems. However, increasing the application level may also lead to higher C output (Fig. 1). A previous study argued that the optimal application levels of biochar in agricultural ecosystems are still debatable, with the recommended levels varying widely (Farhangi-Abriz et al. 2021). Consequently, the optimal level of WB appropriate for maintaining optimal C levels as a positive NECB value for sustainable red pepper cultivation remains uncertain.

Numerous studies have been conducted using biochar at levels ranging from <1 to 100 Mg ha^{-1} in arable soils (Major et al. 2010), mainly depending on the soil conditions and incorporation method (Al-Wabel et al. 2017). Most such studies have focused on achieving the highest crop yields (Arif et al. 2016), lowest greenhouse gas emissions (Hu et al. 2024), and soil organic C sequestration (Zhang et al. 2022). This result indicates that determining the appropriate levels of biochar may be nearly impossible, as it is challenging to account for all ecological changes and parameters, including C inputs (NPP, organic amendment, and fertiliser) and outputs (respiration and harvesting crops based on whole cropping periods). In addition, many studies have been conducted on arable lands, mainly focusing on rice (Canatoy et al. 2022), maize (Guo et al. 2024), and other crop productions, such as sunflowers (Hu et al. 2024), but little information is available for red pepper cropping systems, highlighting the need to narrow the gaps in current knowledge. Consistent with what was mentioned earlier, Al-Wabel et al. (2017) addressed the optimal application levels of biochar to enhance agricultural benefits, which have not been evaluated thus far under specific conditions, including the red pepper cropping system. In this study, we propose an appropriate WB application to determine the optimal levels under two different scenarios to improve the annual NECB and maintain it at a positive value for a more sustainable cropping system in agriculture.

Red pepper residues, mainly stems and roots, are typically managed under two different scenarios in red pepper fields (Lee et al. 2017). For example, the residues are mostly removed from fields to facilitate successive crop production (Moreno-Cornejo et al. 2017), to use as solid biofuel feedstock (Maia and de Morais 2016) or as additional material for composting (Yasin et al. 2023). Alternatively, whole red pepper residues were directly incorporated into croplands after their final harvest to improve soil physicochemical and biological properties, providing soil organic C sources and additional nutrients (Moreno-Cornejo et al. 2017). Considering the two different residue management scenarios, the annual NECB was evaluated for two years under field conditions over the red pepper cultivation period, including the cultivation and non-cultivation seasons, to estimate the optimal levels of WB (Fig. 2). Our results suggest that the optimal WB application level for maintaining positive annual NECB in the red pepper cropping system is in the range of 7.3 to 11.4 Mg d.w ha^{-1} (mean value: 9.4 Mg d.w ha^{-1}) when removing the whole of the residue after the final harvest and in the range of 1.8 to 6.7 Mg d.w ha^{-1} (mean value: 4.3 Mg d.w ha^{-1}) when returning it to the soil. Interestingly, incorporation of the whole residues after the final harvest into the soil is regarded as an appropriate way to improve NECB in red pepper cropping lands by reducing C output from the harvest removal (approximately 3.7 to 7.2 Mg C ha^{-1}), even though the fresh residue may increase respiration rates, facilitating C loss from the soil. In terms of NECB, the incorporation of crop residues after the final harvest (ranging from -1.11 to 1.60 Mg C ha^{-1} on average of all treatments in the first and second year, respectively) enhanced the C budget in this study, showing an increase of approximately 160% over the non-amended conditions (ranging from -1.51 to -3.13 Mg C ha^{-1} on average), where complete residue removal was assumed (Table 4). In this study, all annual NECB showed negative values except for the NPK + WB10 treatment in the first year. However, the annual NECB in the 2nd year was much lower than that in the first year, and the benefits of improving the NECB on annual WB application gradually diminished over the year, mainly because of respiration loss, particularly during the non-cultivation period, as well as decreased NPP during the cultivation season. Temperature is one of the most important factors influencing microbial activity and the decomposition rate of soil organic matter (Kim et al. 2013), primarily affecting annual NECB in cropping systems. Under unfavourable climate conditions, such as excessive precipitation events, which are closely related to reduced sunshine hours, the overall decomposition of organic matter may decrease owing to reduced microbial activity, thereby lowering C output during the non-cultivation seasons in the second year. For example, pepper biomass decreased by approximately 32% in the second year compared to the first (Table 3), possibly due to unfavorable weather conditions (Fig. S1), which may slightly diminish the benefits of NECB in this study. At the same time, crop growth is significantly affected by climate conditions (Hatfield et al. 2011), which mainly lower biomass productivity, leading to a significant decline in the overall NPP during cropping periods. This indicates that soil C stocks may decline in the long term under more vulnerable climatic conditions. Therefore, optimal conditions over the long-term should be further investigated under a range of soil conditions and biochar application methods (Al-Wabel et al. 2017), while the application of WB could be beneficial for maintaining annual NECB in red pepper cropping systems.

4.2 WB applications on improving red pepper fruit yield and soil properties

WB application increased the overall fruit productivity in red pepper fields by 16–18% compared with the control (Table 3). Similarly, a recent meta-analysis by Singh et al. (2022) highlighted the positive effects of biochar amendments on crop productivity across various agricultural systems. These findings are similar to our results, which demonstrated an approximately 30% increase in crop yield under field cropping conditions compared to the control. Previous studies have shown that biochar has the potential to enhance agricultural productivity across diverse crops (Bo et al. 2023). Biochar applications can improve crop performance mainly for several reasons, including the direct supply of plant-available nutrients (Xu et al. 2013), neutralising soil pH, particularly under acidic conditions (Wang et al. 2014), and enhancing physicochemical properties such as soil CEC (Liang et al. 2006) and bulk density (Nelissen et al. 2015) in soils. In this study, WB incorporation significantly altered the overall soil physicochemical characteristics ($p < 0.05$, Table 5 and Fig. S2), which increases soil C accumulation and leads to better soil properties in agriculture (Bo et al. 2023; Zhang et al. 2022). Biochar applications can improve soil aeration and porosity, soil water and nutrient holding capacity, cation exchangeable capacity (CEC), nutrient availability, and microbial abundance and diversity (Al-Wabel et al. 2017; Ding et al. 2016; Singh et al. 2022), feasibly providing favourable soil environments for better crop growth. Our results showed that fruit yield was positively correlated with shoot ($p < 0.001$, $r = 0.971$) and root biomass ($p < 0.001$, $r = 0.945$), total C input ($p < 0.1$, $r = 0.753$), soil pH ($p < 0.05$, $r = 0.858$), and CEC ($p < 0.05$, $r = 0.835$) (Fig. S3). Soil organic matter plays an important role in enhancing soil physicochemical and biological properties (Bo et al. 2023), particularly by improving soil pH, CEC, nutrient-holding capacity, and microbial activity (Thangarajan et al. 2013). This result indicated that the enhancement of red pepper crop biomass by WB application could be related to increased soil C content and its related properties, potentially improving the overall soil properties and yields. In this study, biochar application clearly had a positive impact on fruit yield, increasing it by 18% compared with the control in the first year (Table 3). However, the positive effect on crop yield declined slightly in the second year, showing a 16% increase compared with the control. These results are similar to those of Arif et al. (2016), who found that biochar increased maize yields by 20% after the first year and 13% after the second year compared to the control, showing a positive impact on crop yield. A previous study addressed the negative effects of biochar on crop productivity, mainly due to the immobilisation of available nutrients, including N, and the presence of phytotoxic compounds (PAHs and metals), potentially inhibiting plant growth (Bruun et al. 2012). Unexpectedly, the total biomass productivity of red pepper was much lower in the second year than in the first year, suggesting negative effects on either continuous red pepper cropping or annual WB incorporation into the soils. In this study, we observed that the overall biomass productivity in the first year also decreased in the control, indicating that the decrease may be more closely related to continuous cultivation than to annual biochar amendments. Therefore, WB application could be an effective soil management strategy for increasing red pepper biomass and yield, but long-term experiments are required to investigate the possible negative impacts of successive applications on crop productivity and soil properties.

4.3 Limitations and perspectives

The limitations of this study include the lack of in situ gas measurements. In this study, the NECB was calculated as the difference in the C value between residue removal and incorporation. Ideally, the realistic C output from the field under the residue incorporations should have been measured, which could have provided additional insights to estimate annual NECB. This is because crop residue incorporation can stimulate microbial growth and activity by adding exogenous decomposable substances to soils, resulting in increased soil C loss due to increased enzyme activity (Kuzyakov and Domanski 2000). Despite these limitations, our study provides valuable insights into the optimal levels of WB application for NECB in red pepper cropping systems. Further studies are required to assess the annual NECB by monitoring C losses during the non-cultivation seasons after the incorporation of red pepper residue into the soil. Additionally, a detailed analysis of soil organic matter fractions could provide clearer evidence for understanding the C stabilisation mechanisms and microbial interactions in biochar-amended soils. From a practical perspective, it is also essential to determine the optimal incorporation strategies for WB, including application frequency, timing, and placement. Nevertheless, the effects of biochar on crop productivity remain inconsistent because of multiple influencing factors, such as variations in feedstock composition, pyrolysis conditions, soil properties, plant species, and experimental settings (Al-Wabel et al. 2017; Bo et al. 2023). These findings suggest that the effect of WB application on crop productivity can vary significantly, primarily depending on soil and crop characteristics, biochar properties, and field conditions. Despite these insights, this study has several limitations. The long-term effects of biochar application were not fully captured, and the external environmental factors that might have influenced the results were not entirely controlled. Moreover, variations in soil microbial communities and their functional roles in C dynamics have not been explicitly examined. Future research should address these aspects to provide a more comprehensive understanding of biochar applications for sustainable soil management and crop productivity in agricultural ecosystems.

5 Conclusions

This study revealed the optimal application level of WB based on the annual NECB and provided the first evidence of the usefulness of the annual NECB as a tool for evaluating the overall C budget in cropping systems. The optimal WB application ranges for improving the annual NECB were found to be 7.3–11.4 Mg d.w ha^{-1} (mean value: 9.4 Mg d.w ha^{-1}) when removing the whole biomass after harvest and 1.8–6.7 Mg d.w ha^{-1} (mean value: 4.3 Mg d.w ha^{-1}) when returning it to the soil. Increasing the WB application level improved the physicochemical properties of the soil, such as bulk density and SOC content, and significantly improved crop productivity. Optimising the WB application level could be a sustainable organic matter management strategy for improving the NECB, red pepper productivity, and soil quality in red pepper cropping systems. Our results provide useful information for effectively minimising excessive biochar application by suggesting optimal levels for maintaining the annual NECB and enhancing crop productivity and soil quality in agricultural environments. The proposed optimal levels of WB in the two residue management scenarios will contribute to the development of sustainable options for effectively improving the annual NECB, red pepper productivity, and soil properties in red pepper cropping systems in the future.

Supplementary Information

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Supplementary material 1.

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Data availability
Data will be made available on request.

Declarations

Competing interests
The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

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