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Triple impact of biochar, no-tillage and cover crop: Enhancing soil carbon and climate resilience in soybean farming

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ABSTRACT

With the dissemination of conservation agriculture, no-tillage (NT) and cover crops have been widely adopted globally. However, their effects on greenhouse gas (GHG) emissions are controversial. Biochar is posited to mitigate climate change by increasing carbon (C) sequestration and decreasing GHG emissions in soil. To investigate the comprehensive effect of NT, cover crop, and biochar on soil organic C (SOC) sequestration and net global warming potential (GWP), a split-split-plot experiment was conducted in the experimental field at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan. The experiment involved various combinations of two tillage methods, NT and moldboard plowing (MP), two cover crop treatments, fallow (FA) and rye (RY), and two biochar treatments, biochar application (WB) and no biochar application (NB). The NT and RY treatments demonstrated a trend of increasing N_2O emission, while WB tended to reduce the N_2O emission in NT plots. Compared with the MP-FA-NB treatment, the NT-RY-WB treatment increased SOC stock (0–30 cm) by 23.2% in 2020 and 30.2% in 2021, indicating that this combination promoted C sequestration. Due to the heightened SOC stock, the net carbon dioxide (CO_2) retention effectively compensated for the GWP arising from non-CO_2 emissions. Consequently, the combination of NT, RY, and WB positively contributed to a decreased net GWP in the soybean field ($-1\ 231$ and $-2\ 767$ kg CO_2 equivalent ha^{-1} year^{-1} in 2020 and 2021, respectively). These findings highlight the considerable potential of the combination of NT, RY, and WB for SOC sequestration and net GWP decrease, positioning it as an environmentally beneficial agricultural system for mitigating climate change during long-term food production in Asia.

Key Words: biochar application, carbon sequestration, climate change, conservation tillage, global warming potential, greenhouse gas emission, rye, soil organic C

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INTRODUCTION

Agricultural production is one of the main anthropogenic sources of greenhouse gas (GHG) emissions (Shukla *et al.*, 2019). With a growing global population comes a greater demand for agricultural production, which is likely to result in increased agroecosystem-based GHG emissions. Therefore, low-carbon (C) sustainable agricultural practices are essential to mitigating the warming trend of the climate system.

For the agricultural sector, reducing GHG emissions and increasing C sequestration effectively alleviates climate change (Wollenberg *et al.*, 2016; Nazir *et al.*, 2024). Some researchers have reported that no-tillage (NT) has a positive effect on soil organic C (SOC) accumulation in top layers, significantly increasing C sequestration (Hou *et al.*, 2012; Higashi *et al.*, 2014). No-tillage improves soil structure and provides a more stable physical environment for soil aggregation by reducing soil disturbance, resulting in enhanced SOC content in the surface layer (Andruschkewitsch *et al.*, 2013; Liu *et al.*, 2014). While considering the benefit of increased SOC sequestration, the effect of NT on GHG emissions should also be considered (Abdalla *et al.*, 2016; Huang *et al.*, 2018). Some studies reported that NT increased methane (CH_4) uptake and decreased nitrous oxide (N_2O) emission (Liu *et al.*, 2006; Alskaf *et al.*, 2021). However, another long-term experiment conducted in northern France indicated that NT tended to emit more carbon dioxide (CO_2) and N_2O than conventional tillage (Oorts *et al.*, 2007). These inconsistent results reveal that the effect of NT on GHG

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emission is complex and changeable. For instance, the effect of NT on N_2O is time-dependent and climate-dependent (Van Kessel *et al.*, 2013).

Based on the meta-analysis on a global scale, researchers found that crop productivity under sole application of NT showed a clear decline compared with conventional tillage (Pittelkow *et al.*, 2015; Xiao *et al.*, 2019). However, the negative effect of NT on crop yield can be weakened or even reversed when NT is combined with the two other conservation agriculture principles of straw mulching and crop rotation (Pittelkow *et al.*, 2015). As a management practice with a long application history, cover crop is widely used in agriculture alongside the global spread of conservation agriculture, which is fully in line with the conservation agriculture principles of straw mulching and crop rotation (Groff, 2015). After termination, the cover crop serves as green manure, thereby increasing the C input to soil and soil fertility (Hubbard *et al.*, 2013; Paustian *et al.*, 2016). A previous study found that rye as a cover crop combined with NT could maintain considerable productivity of soybean in Po Valley, Italy (Fiorini *et al.*, 2020). Although the effects of cover crops on SOC stock improvement vary based on climate, soil texture, and cover crop species, cover crops have the potential to increase SOC stock in general (Jian *et al.*, 2020). For instance, many studies found rye to be an effective cover crop to increase SOC stock in the surface soil layer (Higashi *et al.*, 2014; Gong *et al.*, 2021; Ardenti *et al.*, 2023). Cover crop, through its effect on key soil properties, can also influence GHG emissions (Mitchell *et al.*, 2013) by increasing the mineral C and nitrogen (N) sources for soil microbial activities and changing soil microbial community structure (Abalos *et al.*, 2014; Singh and Kumar, 2021). Notably, cover crop residue can increase N_2O emissions (Brozyna *et al.*, 2013; Thomas *et al.*, 2017).

Biochar is mainly produced from organic waste (*e.g.*, straw, sludge, and stool) through high-temperature pyrolysis (Leng *et al.*, 2019) and used as a soil amendment owing to its unique physicochemical properties (Steiner *et al.*, 2007; Bhattacharyya *et al.*, 2024). Biochar improves soil physicochemical properties by directly enhancing soil aggregate stability (Sharma, 2024), thus reducing SOC loss (Situ *et al.*, 2022; Yang *et al.*, 2022). Furthermore, by stimulating and altering soil microbial community, biochar application significantly affects SOC stock and GHG emissions (Farrell *et al.*, 2013; Liu *et al.*, 2016). Compared with conventional management practices, biochar application effectively mitigates N_2O emissions (Case *et al.*, 2012; Mukherjee *et al.*, 2014), notably in paddy and sandy soils (Borchard *et al.*, 2019). Meanwhile, the effect of biochar on CH_4 emission is variable and affected by soil type. Many studies have reported that biochar application could reduce CH_4 emission in paddy fields (He *et al.*, 2020; Sriphirom *et al.*, 2022). Conversely, biochar was found to increase CH_4 emission in neutral or alkaline dryland (Jeffery *et al.*, 2016; Shakoor *et al.*, 2021a).

No-tillage and cover crops are utilized synergistically in conservation agricultural practices as a combined beneficial management practice. Studies published on a long-term NT-cover crop system demonstrated that such a combination significantly improved soil health and crop yield (Mitchell *et al.*, 2017; Nouri *et al.*, 2019; Wulanningtyas *et al.*, 2021). Although the NT-cover crop system has been an effective climate-smart agriculture practice, the GHG emissions induced by such a system should be considered (Gong *et al.*, 2021). Biochar, due to its positive effect on GHG emission reduction (Case *et al.*, 2012; Mukherjee *et al.*, 2014), can be used to promote C sequestration to strengthen the effect on mitigating climate change (Wang *et al.*, 2022). Several published studies have analyzed the interaction of tillage and biochar in GHG emission (Lyu *et al.*, 2022; Zhang *et al.*, 2023). However, there is still a lack of studies on the effects of the triple application of NT, cover crop, and biochar on GHG emissions and C sequestration.

Therefore, this study employed randomized combinations of diverse tillage practices, cover crop applications, and biochar management as distinct treatments and compared their effects on crop productivity, GHG emission, and SOC content. This study aimed to evaluate the potential of NT, cover crop, and biochar combined management practice for strengthening C sequestration and reducing net global warming potential (NGWP), thereby exploring the most suitable practice for organic soybean production. We hypothesized that biochar application would reduce CH_4 and N_2O emissions in the NT-cover crop system, thereby decreasing the NGWP in organic soybean production. Additionally, the combined application of NT, cover crop, and biochar will enhance C sequestration, effectively alleviating climate change.

MATERIALS AND METHODS

Experimental site

This study was conducted at the Center for International Field Agriculture Research and Education (36°2′ N, 140°12′ E), Ibaraki University, Japan. The experimental field had been executing NT-cover crop system studies for almost 19 years since 2002. Across the entire experiment period in this research (June 2020 to June 2022), the mean air temperature was 15.4 °C, and the total precipitation was 2 543 mm (Japan Meteorological Agency, 2022). Based on the soil classification in the World Reference Base for Soil Resources, the soil type at this site is a typical Andosol. Specifically, sandy loam occupies the upper surface, and clay content increases gradually with depth. Selected properties of the soil (0–30 cm) in this field are shown in Table I (Wulanningtyas *et al.*, 2021).

TABLE I

Selected properties[a)] of the soil (0–30 cm) in the experimental field at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan (Wulanningtyas *et al*., 2021)

Property	Unit	Value
pH		6.5
SOC	g kg^{-1}	38
TN	g kg^{-1}	4.5
AP	mg kg^{-1}	63
CEC	cmol kg^{-1}	32
Exchangeable cation		
K^+	mg kg^{-1}	220
Ca^{2+}	mg kg^{-1}	1 300
Mg^{2+}	mg kg^{-1}	125

[a)] SOC = soil organic C; TN = total N; AP = available P; CEC = cation exchange capacity.

Experimental design and agronomic management

The field experiment from June 2020 to June 2022 was a split-split-plot design and set in a completely randomized block with four replications. The main factor was tillage method: NT and moldboard plowing (MP). The first split factor was cover crop: fallow (FA) and rye (*Secale cereal*) (RY). Within the principal split plot, a second split factor was represented by the application (WB) or no application (NB) of biochar. Each main plot had an area of 72 m^2 (6 m × 12 m), while the sub-plot and sub-sub-plot were sized at 36 m^2 (6 m × 6 m) and 18 m^2 (3 m × 6 m), respectively (Fig. S1, see Supplementary Material for Fig. S1).

In this field, winter cover crops were planted as a rotation with soybean in the RY plots since 2008. Cover crops were sown manually and covered with a thin layer of soil to overwinter in November. The seeding density of RY was 100 kg ha^{-1} each year. At the same time, weeds were allowed to grow naturally without manual intervention in the FA plots. Subsequently, the weeds within FA and cover crops within RY were terminated and crushed by a flail mower on the soil surface in the next May. The residues of weeds and cover crops were left on the surface in the NT plots, whereas in the MP plots, residues were buried in the soil by summer tillage after cover crop termination as green manure. Under MP, the soil was plowed to a depth of around 30 cm. The biochar used in the study was produced from rice husk at a pyrolysis temperature of 350–400 °C. Based on previous research (Oladele, 2019), biochar was broadcast in plots of the WB treatment at a rate of 8 Mg ha^{-1} year^{-1} before summer tillage since 2020. Until late June, all plots were intentionally kept fallow to facilitate land restoration. Soybean (cv. *Sachiyutaka*) was sown in early July at a seed rate of 60 kg ha^{-1} by an NT sowing device (MJSE 18-6, Mitsubishi, Japan) with a row spacing of 0.3 m. No fertilizers, herbicides, or pesticides were used throughout soybean cultivation. During the soybean planting period, weed control was conducted by human labor and backpack mowers. Owing to precipitation meeting the water requirement, no extra manual irrigation was carried out. After the harvest of soybeans in late October, we conducted the autumn tillage for cover crop sowing in early November, and its arrangement was the same as for summer tillage. Unlike for cover crops, the residue of stems and pods after soybean threshing was not returned to the field as green manure.

Measurements of crop biomass and yield

Cover crop biomass in RY and weed biomass in FA were sampled using a 0.25-m^2 (0.5 m × 0.5 m) quadrat in the center of each plot in May during the 2-year experiment. In November of each year, soybean biomass samples were taken from the center of each plot using a 0.6-m^2 (1 m × 0.6 m) quadrat. The biomass of both the cover crop and soybean was quantified through the measurement of oven-dried subsamples, subjected to a temperature of 60 °C for a duration of 72 h. Soybean yield was determined from grain weight threshed from the soybean biomass subsample.

Soil sampling and analysis

In October 2020 and October 2021, soil samples were collected for measurement of SOC content using a soil sampling cylinder with a diameter of 5 cm and a length of 30 cm. A singular soil core sample was extracted from each plot and measured for soil bulk density (BD). Soil samples for SOC measurement in each layer were air-dried for 7 d. Then, the samples were ground through a 2-mm mesh sieve. After grinding, any undesirable plant residues and biochar were manually removed using tweezers. Finally, the samples were loaded into small bottles and dried for 72 h at 105 °C, and SOC content was quantified with a C/N analyzer (JM3000, J-science Lab, Japan). Based on the equivalent soil mass method (Ellert and Bettany, 1995), SOC stock was calculated based on SOC content, soil BD, and soil depth.

Monitoring and analysis of CH_4 and N_2O emissions

Gas (CH_4 and N_2O) samples were collected in the field weekly between 8:30 and 10:30 a.m. from June 2020 to May 2022 using the static closed chamber method (Gong *et al*., 2021). The components of the chamber included an anchor and a cylindrical lid (220 mm in diameter, 280 mm in height). The chamber anchor was inserted into the soil (5 cm depth) in the center of the plot, and aboveground weeds residing within the confines of the anchor were removed prior to installation. The cylindrical lid was installed to cover the anchor closely on each sampling day. A polypropylene syringe was employed to extract a 100-mL gas sample from the chamber and transfer it to a 1-L Tedlar bag (As One, Japan) at 0, 20, 40, and 60 min following lid closure. The concentrations of CH_4

and N_2O were measured within the sampling day using a GC-2014 gas chromatograph (Shimadzu, Japan) with a flame ionization detector (FID) for CH_4 and an electron capture detector (ECD) for N_2O. Linear interpolation of daily fluxes between gas sampling dates was used to quantify cumulative GHG emissions (Bayer *et al.*, 2016; Zhou *et al.*, 2017). The cumulative emissions from June 2020 to May 2022 were used to calculate the annual GHG emissions. During each gas collection, the soil volumetric water content (VWC, %) in each plot was measured by a soil moisture sensor (Campbell Scientific, Inc., USA) in the 0–15 cm layer. The water-filled pore space (WFPS, %) in the 0–15 cm layer was calculated as follows:

$$\text{WFPS} = \text{VWC}\left(1 - \frac{\text{BD}}{\text{PD}}\right) \quad (1)$$

where BD is the soil BD in the 0–15 cm layer (g cm^{-3}), and PD is the soil particle density in the 0–15 cm layer, which is 2.65 g cm^{-3}.

Calculation of NGWP and GHG intensity (GHGI)

In the agricultural system, NGWP depends on net CO_2 retention (NCDR) and CH_4 and N_2O emissions. To more efficiently evaluate the integrated climatic effect of CH_4 and N_2O emissions, the annual CH_4 and N_2O emissions were transformed into CO_2-equivalent global warming potential (GWP, kg CO_2 equivalent ha^{-1} $year^{-1}$):

$$\text{GWP} = 265\text{AE}_{\text{N}_2\text{O}} + 28\text{AE}_{\text{CH}_4} \quad (2)$$

where 265 and 28 are the GWP values for N_2O and CH_4 over a 100-year time horizon, respectively (Field *et al.*, 2014), $\text{AE}_{\text{N}_2\text{O}}$ is the annual N_2O emission (kg ha^{-1} $year^{-1}$), and AE_{CH_4} is the annual CH_4 emission (kg ha^{-1} $year^{-1}$). Then, NGWP (kg CO_2 equivalent ha^{-1} $year^{-1}$) was calculated as follows (Gong *et al.*, 2021):

$$\text{NGWP} = \text{GWP} - \text{NCDR} \quad (3)$$

where NCDR (kg ha^{-1} $year^{-1}$) can be calculated from the annual change in SOC stock (Gong *et al.*, 2021):

$$\text{NCDR} = \frac{\text{SOC}_{2020/2021} - \text{SOC}_{2008}}{n} \times 3.67 \quad (4)$$

where $\text{SOC}_{2020/2021}$ is the SOC stock in 2020 or 2021 (kg ha^{-1}), SOC_{2008} is the SOC stock in 2008 (kg ha^{-1}), which was taken from a previous study in this soybean field (Higashi *et al.*, 2014), n is the duration of the experimental period, which is 12 (for 2020) or 13 year (for 2021), and 3.67 is the conversion coefficient from C to CO_2.

The integrated effect of NT, cover crop, and biochar application on global warming and soybean productivity was evaluated using GHGI (kg CO_2 equivalent Mg^{-1} yield), which was quantified as follows (Nouri *et al.*, 2019; Gong *et al.*, 2021):

$$\text{GHGI} = \frac{\text{NGWP}}{\text{Soybean yield}} \quad (5)$$

Statistical analysis

Statistical analyses were performed using Statistix 8 and Hiplot (https://hiplot.com.cn). A split-split-plot model was used to evaluate the impacts and interactions of tillage (main factor), cover crop (first split factor), and biochar (second split factor) on cover crop biomass, soybean biomass, soybean yield, WFPS, soil BD, SOC stock, GHG daily emissions, GHG annual emissions, NCDR, NGWP, and GHGI. The mean values of various treatments were tested for statistical significance using the Tukey test at the $P < 0.05$ level. In addition, Pearson correlation analysis was performed using Hitplot to examine the relationships between the total inputs of C and N, SOC and soil total N (TN) contents, N_2O emission, and SOC stock.

RESULTS

Crop performance

The cover crop factor had a significant effect on the biomass of plants (*i.e.*, weeds in FA and rye in RY), which was 60.2% and 59.0% lower in 2020 and 2021, respectively, for FA than for RY (Table II). Both in 2020 and 2021, the soybean biomass was significantly affected by the interaction among tillage, cover crop, and biochar treatments (Table III). In 2020, the NT-RY-NB treatment resulted in the highest soybean biomass (10.91 Mg ha^{-1}), exceeding that of MP-FA-NB by 39.5% ($P = 0.32$). In 2021, the highest soybean biomass (*ca.* 6.50 Mg ha^{-1}) was observed in both MP-RY-NB and NT-RY-WB, exceeding that of MP-FA-NB by 28.0% ($P = 0.73$). The soybean yield was 42.0% and 29.8% higher with MP than with NT in 2020 and in 2021, respectively, although the increase showed no significance ($P > 0.05$). A significant effect of tillage was found on harvest index, with MP being higher by 92.8% in 2020 and 34.2% in 2021 compared with the values obtained for NT.

Soil BD and SOC stock

In this study, the crop residues consisting of soybean leaves and cover crop acted as the major source of C and N input to the soil (Fig. 1). Moreover, the cover crop residue was the principal part of the above C input. Compared with RY, FA showed lower C input by 48.2% in 2020 and 52.6% in 2021. For N input, RY contributed more by 25.8% in 2020 and 20.0% in 2021, although the difference was less pronounced than for C. In both 2020 and 2021, SOC content was related to the management of tillage, cover crop, and

TABLE II

Effects of tillage (Til), cover crop (CC), and biochar (Bio) on plant[a] biomass in 2020 and 2021 and one-way analysis of variance (ANOVA) results of a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan

Treatment[b]	Plant biomass	
	2020	2021
	Mg ha^{-1}	
MP-FA-WB	2.8bc[c]	4.0bc
MP-FA-NB	1.0c	0.8c
MP-RY-WB	8.1ab	7.1ab
MP-RY-NB	9.7a	9.4a
NT-FA-WB	4.3abc	3.0bc
NT-FA-NB	3.4bc	2.2c
NT-RY-WB	5.7abc	3.6bc
NT-RY-NB	5.4abc	4.3bc
ANOVA		
Til	ns[d]	*
CC	**	***
Bio	ns	ns
Til × CC	*	*
Til × Bio	ns	ns
CC × Bio	ns	*
Til × CC × Bio	ns	ns

*, **, and ***Significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

[a] Weeds in the fallow (FA) treatments and rye in the cover crop (RY) treatments growing from November to next May before soybean was sown.

[b] MP = moldboard plowing; NT = no-tillage; WB = biochar application; NB = no biochar application.

[c] Different letters within the same column indicate significant differences between treatments at $P < 0.05$ according to the Tukey test.

[d] Nonsignificant.

TABLE III

Effects of tillage (Til), cover crop (CC), and biochar (Bio) on soybean biomass, soybean yield, and harvest index (HI) and one-way analysis of variance (ANOVA) results of a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan

Treatment[a]	Soybean biomass		Soybean yield		HI	
	2020	2021	2020	2021	2020	2021
	Mg ha^{-1}					
MP-FA-WB	5.83b[b]	6.02a	3.43a	2.51a	0.58a	0.41a
MP-FA-NB	7.82ab	5.08a	4.11a	2.06a	0.55ab	0.41a
MP-RY-WB	5.32b	5.79a	2.63a	2.31a	0.49ab	0.39a
MP-RY-NB	6.38b	6.48a	3.53a	2.52a	0.55ab	0.39a
NT-FA-WB	8.69ab	5.06a	1.86a	1.62a	0.23b	0.31a
NT-FA-NB	6.30b	6.14a	1.50a	2.10a	0.26ab	0.33a
NT-RY-WB	8.45ab	6.52a	3.56a	1.67a	0.41ab	0.25a
NT-RY-NB	10.91a	6.23a	2.73a	1.85a	0.23b	0.31a
ANOVA						
Til	*	ns[c]	ns	ns	*	*
CC	ns	ns	ns	ns	ns	ns
Bio	ns	ns	ns	ns	ns	ns
Til × CC	ns	ns	ns	ns	ns	ns
Til × Bio	ns	ns	ns	ns	ns	ns
CC × Bio	ns	ns	ns	ns	ns	ns
Til × CC × Bio	*	*	ns	ns	ns	ns

*Significant at $P < 0.05$.

[a] MP = moldboard plowing; NT = no-tillage; FA = fallow; RY = rye; WB = biochar application; NB = no biochar application.

[b] Different letters within the same column indicate significant differences between treatments at $P < 0.05$ according to the Tukey test.

[c] Nonsignificant.

biochar (NT > MP, RY > FA, WB > NB). The NT-RY-WB treatment had the highest SOC content (55 g kg^{-1} in 2020 and 58 g kg^{-1} in 2021) at the 0–30 cm depth. Compared with MP-FA-NB, NT-RY-WB significantly increased the SOC content by 65.6% in 2020 and 62.2% in 2021. Total N content in the 0–30 cm soil was affected by tillage, and it was 19.8% and 15.2% higher under NT compared with MP in 2020 and 2021, respectively. In 2021, the soil TN content under NT-RY-WB significantly surpassed that under MP-FA-NB by 32.1%.

Tillage method had a significant effect on soil BD in the 0–30 cm layer, with the soil BD values in MP significantly surpassing those in NT by 5.5% in 2020 ($P < 0.05$) and by 8.5% in 2021 ($P < 0.01$) (Table IV). Additionally, soil BD in 2021 was also significantly affected by cover crop treatment, with it being 4.4% higher ($P < 0.05$) in FA than in RY. Biochar also showed a significant effect on soil BD in 2021, with a 6.4% reduction ($P < 0.01$) in WB compared with NB.

In 2020, while not significant, NT, RY, and WB tended to increase SOC stock, resulting in the SOC stock of the 0–30 cm depth in NT-RY-WB being 23.2% higher ($P < 0.05$) than that in MP-FA-NB (Table IV). In 2021, tillage, cover crop, and biochar significantly affected SOC stock in the 0–30 cm depth, with the SOC stock in NT-RY-WB being significantly ($P < 0.01$) higher by 30.2% compared with that in MP-FA-NB.

Soil WFPS and GHG fluxes

Except for very rare cases, NT tended to have a higher WFPS than MP, and the maximum gap reached 74.3% ($P < 0.05$) (Fig. 2). From December 2020 to June 2021, FA showed a lower WFPS than RY, although not significant at $P = 0.05$. In August 2020, November 2020, and August 2021 to November 2021, the WFPS in WB markedly exceeded that in NB, but this trend reversed in September 2020.

We calculated the annual CH_4 emissions based on the daily CH_4 emission data (Fig. S2, see Supplementary Material for Fig. S2). In 2020, biochar, tillage-cover crop interaction, and tillage-biochar interaction had a significant effect on annual CH_4 emissions (Table V). Compared with NB, WB emitted more CH_4 in MP conditions (13.0 *vs.* −7.0 kg ha^{-1} year^{-1}), but the difference under NT conditions was minimal (−3.4 kg ha^{-1} year^{-1} for WB *vs.* −2.3 kg ha^{-1} year^{-1} for NB). In 2020, MP (except MP-RY-NB) resulted in CH_4 emission, whereas in 2021, CH_4 emission was observed in MP without RY and in NT with RY. The interaction of tillage with cover crop was significant in both 2020 and 2021. In MP, the presence of RY decreased CH_4 emission

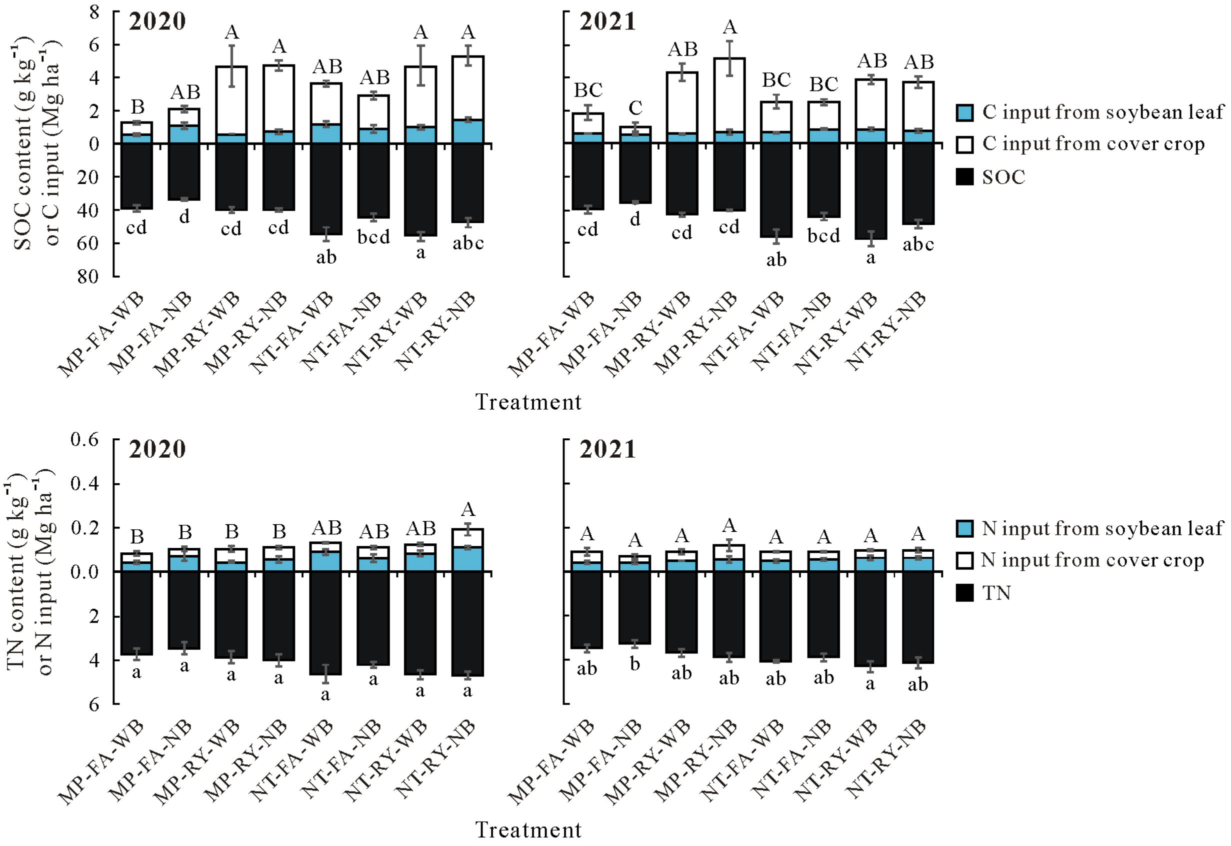

Fig. 1 Inputs of C and N from soybean leaf and cover crop (rye) residues and soil organic C (SOC) and total N (TN) contents (0–30 cm) in 2020 and 2021 in the different tillage, cover crop, and biochar treatments of a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan. Different uppercase letters indicate significant differences in C or N input, and different lowercase letters indicate significant differences in SOC or TN content between treatments at $P < 0.05$ according to the Tukey test. MP = moldboard plowing; NT = no-tillage; FA = fallow; RY = rye; WB = biochar application; NB = no biochar application.

(negative flux), whereas in NT, the presence of RY increased CH_4 emission.

A seasonal fluctuation in N_2O daily emission was observed among treatments, with a high peak observed in July. In most instances, the N_2O daily emission under MP was slightly lower than that under NT (Fig. 3). From December 2020 to March 2021, the N_2O daily emission under FA slightly exceeded that under RY, but this trend was opposite during most of the experimental period. In the first-year experiment, N_2O daily emission under NB tended to exceed that under WB. However, most of the N_2O daily emissions under WB exceeded those under NB in the second-year experiment. In 2020, tillage, cover crop, and biochar had no significant effect on annual total N_2O emission, but WB tended to reduce the emission compared with NB in NT by 33% (Table V). In 2021, cover crop significantly increased the annual total N_2O emission by 29% compared with FA. Specifically, the annual N_2O emission under MP-RY-NB and NT-RY-NB reached 1.2 kg ha^{-1} year^{-1}, on average 50% higher than that under MP-FA-NB.

NGWP and GHGI

The tillage-cover crop interaction and tillage-biochar interaction significantly affected the GWP in 2020 (Table VI). In 2020, FA and WB increased the GWP under MP but reduced it under NT. A similar effect of tillage, cover crop, and biochar on GWP was observed in 2021, although it was not significant. Both in 2020 and 2021, NCDR was lowest under MP-FA-NB, which was the only negative value among all treatments. Compared with FA, RY tended to have a higher NCDR in both 2020 ($P = 0.58$) and 2021 ($P = 0.24$). Moreover, both in 2020 ($P = 0.18$) and 2021 ($P = 0.11$), the NCDR was higher in WB than in NB. Both in 2020 ($P = 0.46$) and 2021 ($P = 0.30$), compared with FA, the NGWP in RY was more negative, although not significantly. Similarly, WB tended to have a lower NGWP compared with NB. In addition, MP-FA-NB contributed the highest NGWP in 2020 and 2021. In 2021, biochar significantly affected GHGI, with a 145% decrease under WB compared with NB ($P = 0.04$). Both in 2020 and 2021, the highest GHGI was observed in MP-FA-NB.

Correlations between total C and N inputs, SOC, soil TN, and N_2O emission

Significant positive correlations were observed between N_2O emission and total C and N inputs as well as SOC

TABLE IV

Effects of tillage (Til), cover crop (CC), and biochar (Bio) on soil bulk density (BD) and organic C (SOC) stock (0–30 cm) and one-way analysis of variance (ANOVA) results of a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan

Treatment[a]	BD		SOC stock	
	2020	2021	2020	2021
	g cm^{-3}		Mg ha^{-1}	
MP-FA-WB	0.65ab[b]	0.68ab	86.7ab	88.5ab
MP-FA-NB	0.67ab	0.70a	77.9b	79.1b
MP-RY-WB	0.70a	0.62bc	90.9ab	95.5a
MP-RY-NB	0.65ab	0.67abc	88.1ab	90.6ab
NT-FA-WB	0.61b	0.59bc	93.2ab	97.7a
NT-FA-NB	0.65ab	0.65abc	88.6ab	92.4ab
NT-RY-WB	0.64ab	0.59c	96.0a	103.0a
NT-RY-NB	0.63b	0.63abc	89.6ab	94.9a
		ANOVA		
Til	*	**	ns[c]	*
CC	ns	*	ns	*
Bio	ns	**	ns	**
Til × CC	ns	ns	ns	ns
Til × Bio	ns	ns	ns	ns
CC × Bio	ns	ns	ns	ns
Til × CC × Bio	ns	ns	ns	ns

*, **Significant at $P < 0.05$ and $P < 0.01$, respectively.

[a] MP = moldboard plowing; NT = no-tillage; FA = fallow; RY = rye; WB = biochar application; NB = no biochar application.

[b] Different letters within the same column indicate significant differences between treatments at $P < 0.05$ according to the Tukey test.

[c] Nonsignificant.

and soil TN contents (Fig. 4). Furthermore, SOC stock exhibited significant positive correlations with SOC and soil TN contents and N_2O emission.

DISCUSSION

Effects of NT, cover crop and biochar on crop performance

In general, adopting NT practice singly reduces crop yield compared with conventional tillage (Pittelkow *et al.*, 2015; Xiao *et al.*, 2019). Our data showed that tillage method had no significant effect on soybean yield, while the soybean biomass under NT was higher compared with MP, resulting in a higher harvest index in MP. We speculate that NT could improve the vegetative growth of soybean but showed no effect on the reproductive growth. Another study reported that NT combined with cover crop increases soybean yield (Wells *et al.*, 2016). In the present study, compared with NT-FA, the soybean yield of NT-RY was slightly higher ($P > 0.05$) in 2020, indicating that RY tended to alleviate yield reduction in the NT plots. In barren tropical regions, biochar showed a significant effect on crop yield improvement (Schulz *et al.*, 2013; Kätterer *et al.*, 2019). Benefiting from a large surface area and porous microstructure, biochar effectively improves soil physical condition and enhances soil nutrient availability, thereby promoting crop growth (Tanure *et al.*, 2019; Zhang *et al.*, 2020). However, based on our data, biochar had no significant effect on soybean yield, irrespective of whether applied singly or combined with NT and RY (Table III). Studies conducted in rice-wheat and maize systems documented that the effect of short-term biochar application on crop yield was limited (Major *et al.*, 2010; Zhang *et al.*, 2020). Since the biochar application had only been implemented for two years in this study, its effect on crop performance was still undetectable.

Effects of NT, cover crop and biochar on SOC

Notably, NT can reduce soil disturbance and protect organic C in soil aggregates, improving SOC stock (Six and Paustian, 2014; Du *et al.*, 2017). Meanwhile, compared with conventional tillage, NT provides higher soil moisture and lower soil temperature, decelerating the decomposition rate of soil organic materials and promoting soil C sequestration (Jin *et al.*, 2017; Alskaf *et al.*, 2021). Compared with MP, which inverts soil surface and distributes SOC in the deep soil profile (Xue *et al.*, 2015), NT exhibited a higher SOC content at the surface soil (0–30 cm). High field moisture decreases C mineralizability, thus enhancing SOC stabilization (Das *et al.*, 2019). The enhanced SOC stock under NT may be considerably linked to the high WFPS under NT (Fig. 2).

Cover crop incorporation induces a large amount of C in the soil, leading to a complement of SOC (Hubbard *et al.*, 2013; Paustian *et al.*, 2016). From the perspective of microorganisms, crop residues incorporated into the soil provide some nutrients and energy sources, thereby facilitating the conversion of residue C to microbial C and resulting in SOC stock improvement and C sequestration (Mendes *et al.*, 1999). Prior research conducted within the identical experimental field showed that RY provided a larger biomass source to soil than FA (Higashi *et al.*, 2014; Gong *et al.*, 2021), and our data showed the same trend. A higher cover crop biomass means a higher C input from crop residues and a higher SOC content, which was positively correlated with SOC stock (Fig. 4). Thus, the SOC stock (0–30 cm) under RY exceeded that under FA in the present study, although the difference was only significant in 2021 (Table IV). As crop residues decompose, a large amount of labile organic C is released into the soil, which accelerates macroaggregate construction (Xu *et al.*, 2011) and increases the ratio of C in the soil organic matter fraction (Bu *et al.*, 2020), thereby increasing SOC stock (West *et al.*, 2020). However, compared with NT, the macroaggregates and SOC under conventional tillage are at high risk of exposure and breakdown (Fiedler *et al.*, 2016). No-tillage and cover crop could synergistically enhance soil C sequestration (Huang *et al.*, 2020), resulting in higher SOC accumulation (Higashi *et al.*, 2014; Bai *et al.*, 2019), which is consistent with our findings for NT-RY as compared with MP-FA.

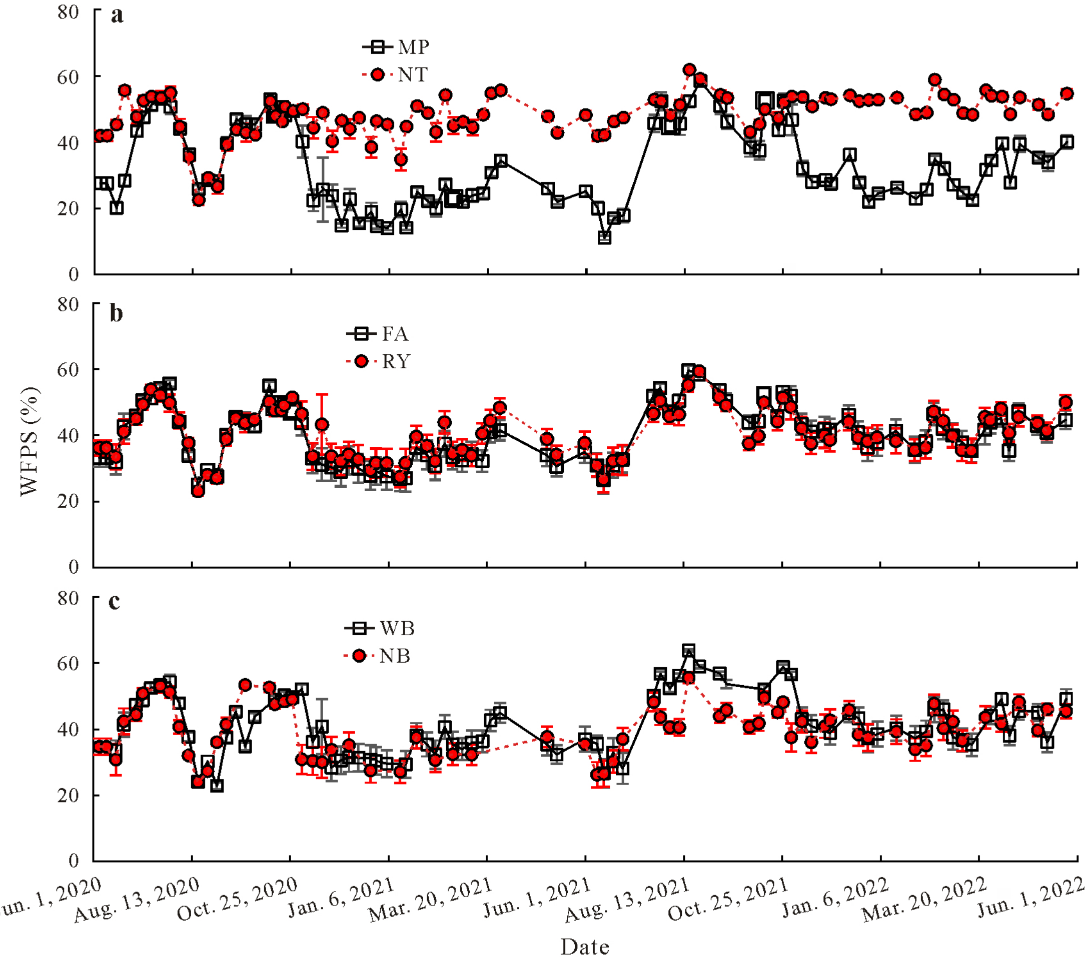

Fig. 2 Changes of soil water-filled pore space (WFPS) (0–15 cm) in the treatments under different tillage (a), cover crop (b), and biochar (c) practices during a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan. Error bars are standard errors of the means ($n = 4$). MP = moldboard plowing; NT = no-tillage; FA = fallow; RY = rye; WB = biochar application; NB = no biochar application.

Numerous studies have demonstrated how biochar increases and stabilizes SOC (Backer *et al*., 2016; Dong *et al*., 2022). A global meta-analysis focusing on SOC sequestration after biochar application also indicated that biochar possesses a high potential for SOC stock improvement (Gross *et al*., 2021), corroborating our finding that WB provided a higher SOC stock than NB. Biochar changes soil aggregation and accelerates the formation of microaggregates *via* organo-mineral interactions, thereby stabilizing and increasing SOC (Weng *et al*., 2017; Sun *et al*., 2020). Notably, based on the regulation of microorganism community diversity and composition, biochar can regulate the usage efficiency of SOC, enhancing SOC sequestration potential and improving SOC stock (Zhang *et al*., 2022). Biochar amendment would induce the SOC pool to accumulate more recalcitrant organic C fraction, protecting SOC from microbe-mediated biodegradation (Zheng *et al*., 2022). In the NT-RY-WB system, rye residue acted as a C source, providing a large amount of C input to the soil. Meanwhile, NT and WB created a suitable environment for SOC sequestration, preventing rapid SOC decomposition. Based on this combined effect, the SOC stock under NT-RY-WB was significantly increased, suggesting that this particular combination could be an effective way of enhancing SOC sequestration in the 0–30 cm soil layer.

Effects of NT, cover crop and biochar on CH_4 and N_2O fluxes

The CH_4 production in soil is regulated by methanogens. Meanwhile, CH_4 in soil can also be consumed by methanotrophic microorganisms, resulting in the soil's capacity to both release and absorb CH_4 (Dutaur and Verchot, 2007; Serrano-Silva *et al*., 2014). A chronosequence study of NT claimed that fields under NT were net CH_4 sinks in agriculture (Bilen *et al*., 2022). The annual total CH_4 emissions under NT exhibited negative values in 2020, indicating that the soil under NT in this study could be a sink for CH_4, which corroborates other findings that NT decreased CH_4 emission compared with conventional tillage (Liu *et al*., 2015; Yeboah *et al*., 2016). Some studies interpreted this as being a consequence of better aeration and less soil degradation in NT,

TABLE V

Effects of tillage (Til), cover crop (CC), and biochar (Bio) on annual CH_4 and N_2O emissions and one-way analysis of variance (ANOVA) results of a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan

Treatment[a]	Annual CH_4 emission		Annual N_2O emission	
	2020	2021	2020	2021
	$kg\ ha^{-1}\ year^{-1}$			
MP-FA-WB	18.9a[b]	5.7a	0.7a	0.7a
MP-FA-NB	1.2abc	−0.6a	0.6a	0.8a
MP-RY-WB	7.1ab	−0.6a	0.7a	0.7a
MP-RY-NB	−15.1c	0.0a	0.8a	1.2a
NT-FA-WB	−6.6bc	−5.5a	0.6a	1.0a
NT-FA-NB	−1.6abc	−4.9a	0.9a	0.7a
NT-RY-WB	−0.1abc	3.6a	0.6a	1.0a
NT-RY-NB	−3.0abc	1.7a	0.9a	1.2a
	ANOVA			
Til	ns[c]	ns	ns	ns
CC	ns	ns	ns	*
Bio	*	ns	ns	ns
Til × CC	*	*	ns	ns
Til × Bio	*	ns	ns	ns
CC × Bio	ns	ns	ns	ns
Til × CC × Bio	ns	ns	ns	ns

*Significant at $P < 0.05$.

[a] MP = moldboard plowing; NT = no-tillage; FA = fallow; RY = rye; WB = biochar application; NB = no biochar application.

[b] Different letters within the same column indicate significant differences between treatments at $P < 0.05$ according to the Tukey test.

[c] Nonsignificant.

enhancing methanotrophic activities in soil and resulting in higher CH_4 uptake (Liu *et al*., 2015; Yeboah *et al*., 2016). On the other hand, some authors suggested that cover crops had no effect on CH_4 emission in no-flood land (Guardia *et al*., 2016; Wegner *et al*., 2018). In the present study, CH_4 emission was affected by the interaction of tillage and cover crop, being higher with FA under MP and higher with RY under NT. The rye residues were buried in the soil in the MP plots, whereas in the NT plots, they were left on the soil surface. We speculated that the rye residues formed a mulch on the surface soil, weakening the aeration in NT soil. Therefore, the NT-RY management increases anoxic conditions in soil, which could promote CH_4 production and/or decrease CH_4 oxidation, thereby exacerbating CH_4 emission.

Many studies have shown that biochar application to agricultural soil can increase CH_4 uptake (Karhu *et al*., 2011), decreasing CH_4 emission from soil (He *et al*., 2020). However, some studies found opposite results, indicating that biochar application led to higher CH_4 flux (Ribas *et al*., 2019; Chen *et al*., 2020). With its high porosity, biochar can retain water in small pores while facilitating water flow through the large pores after intense rainfall from topsoil to deeper soil layers (Asai *et al*., 2009). Since biochar can increase the total porosity and water-holding capacity of soil at the same time, it is possible to reach the anaerobic conditions in biochar-amended soils in high-humid environments (Karhu *et al*., 2011), allowing high net CH_4 emission (Ribas *et al*., 2019; Chen *et al*., 2020). Based on our data, in the first-year experiment, CH_4 emission of WB under MP was higher, whereas little difference was observed under NT. Biochar can release labile organic C and provide it to methanogens, thereby enhancing the production of CH_4 (Knoblauch *et al*., 2011). With reduced soil disturbance, labile organic C can be sequestrated within stable soil aggregates in the NT plots (Six and Paustian, 2014; Du *et al*., 2017). However, in the MP plots, the labile organic C could be easily used by methanogens, thereby promoting CH_4 production. Moreover, in 2021, no significant effect of biochar on CH_4 emission was detected. The ability of biochar to promote or inhibit CH_4 oxidation depends on its physicochemical properties (Nan *et al*., 2021; Lu *et al*., 2022). After the first-year experiment, biochar properties and accumulation in our field may have changed, leading to the different effects observed in 2020 and 2021.

The emission of N_2O is primarily driven by microbial processes like nitrification and denitrification, with functional genes such as *nirS*, *nirK*, and *nosZ* playing a significant role in these processes (Kumar *et al*., 2020). Variations in the abundance and activity of these genes are directly affected by soil properties (Li *et al*., 2020), while tillage can significantly affect these properties, such as WFPS (Rahmawati *et al*., 2015; Çelik *et al*., 2019). No-tillage contributes less soil disturbance and more residue mulch, decreasing evapotranspiration and resulting in higher water storage, while MP inverts the soil surface and increases evapotranspiration, resulting in less water storage (Kan *et al*., 2020). In a global meta-analysis, NT led to 14.7% higher N_2O emission than conventional tillage (Shakoor *et al*., 2021b). A previous study conducted on legume crops found that long-term NT increased soil N_2O emission (Badagliacca *et al*., 2018). Another long-term NT study conducted in northern France documented that NT changed WFPS, resulting in elevated N_2O emission (Oorts *et al*., 2007). According to our data (Fig. 2), NT was associated with a higher WFPS, which may lead to increased soil N_2O production. However, the effect of tillage on N_2O emission was not significant in this study, although N_2O emission was slightly higher with NT than with MP.

The effect of cover crop rye on soil N_2O emission manifested two stages. First, due to mineral N consumption during active growth, RY can reduce N_2O production and emission in soil (Parkin *et al*., 2016; Fiorini *et al*., 2020). Second, after termination, residue from rye provides a large amount of available nutrients and energy to produce N_2O, resulting in high N_2O emission (Sarkodie-Addo *et al*., 2003). In the present study, the N_2O emissions under RY exceeded those under FA, mainly in 2021. A meta-analysis revealed

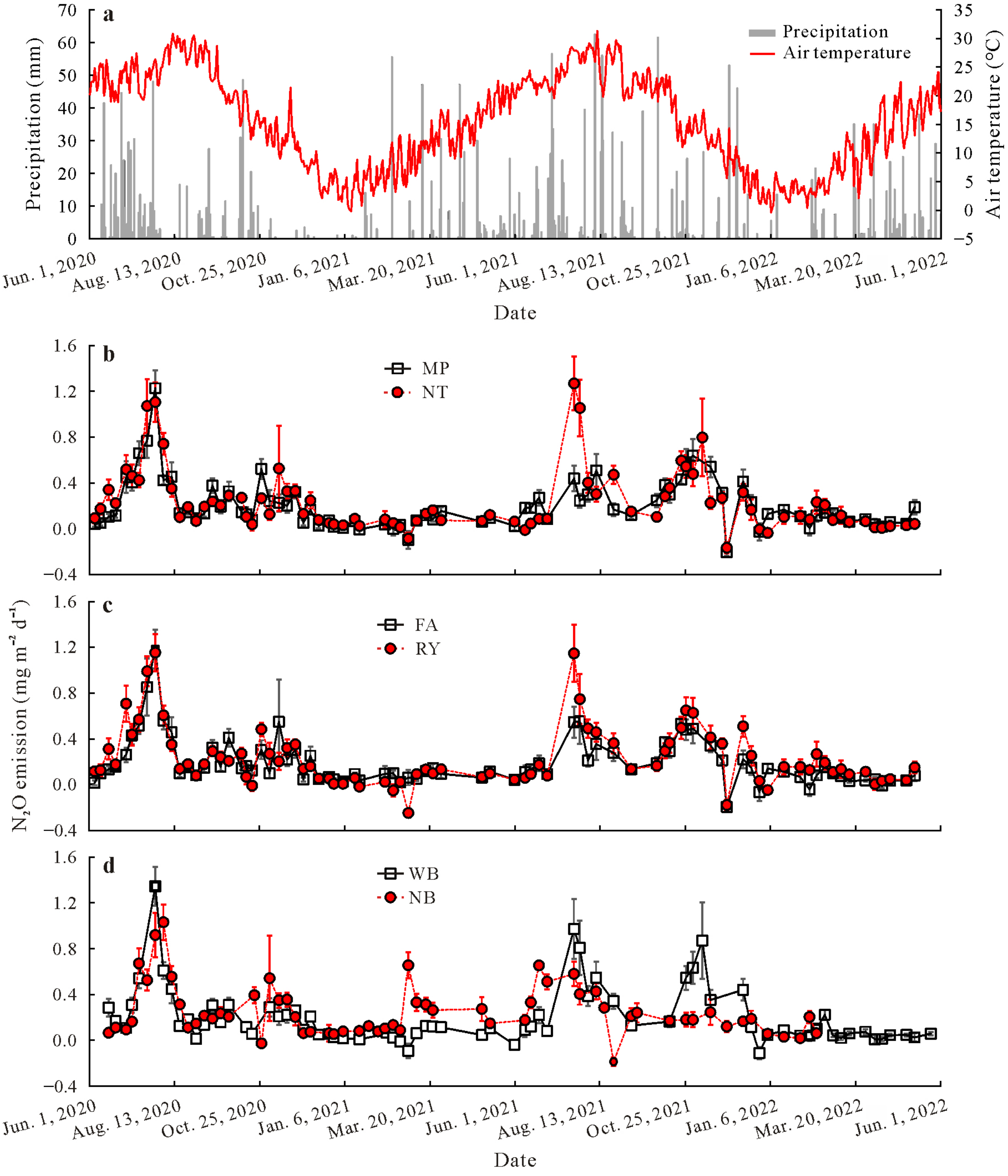

Fig. 3 Daily precipitation and mean air temperature (a) and N_2O emission in the treatments under different tillage (b), cover crop (c), and biochar (d) practices during a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan. Error bars are standard errors of the means (n = 4). MP = moldboard plowing; NT = no-tillage; FA = fallow; RY = rye; WB = biochar application; NB = no biochar application.

that cover crops may increase soil N_2O emissions when incorporated into the soil (Basche *et al.*, 2014), probably because all residues returned to the field supplied more available C and N, enhancing N_2O production (Mitchell *et al.*, 2013). Then, the high biomass input from rye residue incorporated into the soil could have caused the high N_2O emissions in the RY plots.

The N_2O flux was significantly affected by WFPS, with a linear relationship for N_2O production between WFPS values of 30% and 70% (Linn and Doran, 1984). Pimentel *et al.* (2015) found that N_2O emission under 70% WFPS was significantly higher than that under 40% WFPS following cover crop residue incorporation. In 2020, the total rainfall was 1 121 mm, while in 2021, the total rainfall was 1 422 mm. In our study, the higher N_2O emissions observed in 2021 compared with 2020 could be partly explained by the

TABLE VI

Effects of tillage (Til), cover crop (CC), and biochar (Bio) on global warming potential (GWP), net GWP (NGWP), net CO_2 retention (NCDR), and greenhouse gas intensity (GHGI) and one-way analysis of variance (ANOVA) results of a field experiment conducted from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan

Treatment[a]	GWP		NGWP		NCDR		GHGI	
	2020	2021	2020	2021	2020	2021	2020	2021
	kg CO_2-eq[b] ha^{-1} $year^{-1}$				kg ha^{-1} $year^{-1}$		kg CO_2-eq Mg^{-1} yield	
MP-FA-WB	713a[c]	337ab	−1 445ab	−2 131ab	2 158a	2 469a	−762ab	−775ab
MP-FA-NB	200ab	197ab	629a	270a	−429b	−72a	166a	161a
MP-RY-WB	385ab	181ab	−1 071ab	−2 375ab	1 456ab	2 555a	−491ab	−1 109ab
MP-RY-NB	−219b	314ab	−1 826b	−1 822ab	1 607ab	2 136a	−582ab	−927ab
NT-FA-WB	−25b	115ab	−1 441ab	−2 382ab	1 416ab	2 498a	−157a	−1 713b
NT-FA-NB	189ab	45b	−588ab	−1 686ab	777ab	1 731a	−1 721b	−309ab
NT-RY-WB	169ab	361a	−1 231ab	−2 767b	1 399ab	3 128a	−356ab	−1 825b
NT-RY-NB	160ab	356ab	−1 012ab	−2 112ab	1 172ab	2 468a	−1 041ab	−1 147ab
				ANOVA				
Til	ns[d]	ns	ns	ns	ns	ns	ns	ns
CC	*	ns	ns	ns	ns	ns	ns	ns
Bio	ns	ns	ns	ns	ns	ns	ns	*
Til × CC	**	ns	ns	ns	ns	ns	ns	ns
Til × Bio	*	ns	ns	ns	ns	ns	ns	ns
CC × Bio	ns	ns	ns	ns	ns	ns	ns	ns
Til × CC × Bio	ns	ns	ns	ns	ns	ns	ns	ns

* and **Significant at $P < 0.05$ and $P < 0.01$, respectively.

[a] MP = moldboard plowing; NT = no-tillage; FA = fallow; RY = rye; WB = biochar application; NB = no biochar application.

[b] CO_2 equivalent.

[c] Different letters within the same column indicate significant differences between treatments at $P < 0.05$ according to the Tukey test.

[d] Nonsignificant.

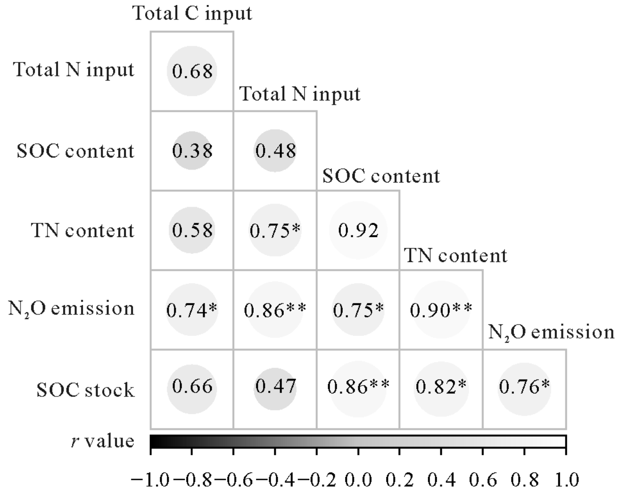

Fig. 4 Correlation coefficients between total C input, total N input, soil organic C (SOC) content (0–30 cm), soil total N (TN) content (0–30 cm), N_2O emission, and SOC stock (0–30 cm) in a field experiment conducted on the influences of tillage, cover crop, and biochar on these parameters from June 2020 to June 2022 at the Center for International Field Agriculture Research and Education, Ibaraki University, Japan. Asterisks * and ** indicate significances at $P < 0.05$ and $P < 0.01$, respectively.

increased rainfall, which created anoxic conditions favorable for denitrification. This process is mediated by denitrifying bacteria harboring *nirS* and *nirK* genes, leading to increased N_2O production (Kumar *et al.*, 2020). Meanwhile, evenly distributed crop straw on the soil surface can effectively improve soil moisture, creating conditions conducive to N_2O emission (Hu *et al.*, 2019; Li *et al.*, 2019). Our findings substantiate this perspective that NT combined with RY increased WFPS, resulting in an enhancement of N_2O emission.

In our study, no significant effect of biochar on N_2O emissions was observed. While there was an observed trend for lower emissions with biochar in NT in 2020, it did not reach statistical significance. This indicates that, within the conditions of our experiment, biochar did not have a definitive impact on N_2O emissions. Future studies with larger sample sizes or different experimental conditions may be needed to detect any potential effects more conclusively.

Effects of NT, cover crop and biochar on NGWP and GHGI

Based on our data, there was a significant interaction of tillage with cover crop and biochar on GWP. In terms of tillage method, NT tended to decrease GWP relative to MP, corroborating previous findings obtained from the same experimental field, in which NT generated a lower GWP than MP (Gong *et al.*, 2021). The reduction in CH_4 emission contributed to a low GWP in the NT system, which was supported by other researchers (Zhang *et al.*, 2015; Guo *et al.*, 2021). Notably, NCDR showed a significant effect on NGWP (Sainju *et al.*, 2014). In our study, the lowest NCDR was found under MP-FA-NB in 2020 and 2021, which was the only negative value among treatments, meaning that C output exceeded its input under this system. Apart from the MP-FA-NB treatment, all NGWPs were negative. This suggests that non-CO_2 emissions can be covered by the NCDR when

NT, RY, and WB are used as a field management practice. The combined use of these three factors in the present study significantly improved SOC accumulation, resulting in a corresponding negative NGWP value, as reported by Wolff *et al*. (2018).

The GHGI serves as a valuable indicator for assessing the effect of various agricultural management practices on the balance between global warming and crop production (Lee *et al*., 2019). The lower the GHGI values, the fewer the GHG emissions generated in each unit of crop production. A previous study conducted in this site indicated that NT and rye cover crop effectively decreased the GHGI of soybean production (Gong *et al*., 2021), corroborating our findings that GHGI under NT with RY was lower than that under MP with FA. Moreover, biochar application also had a positive effect on GHGI reduction, particularly in 2021. While having a limited effect on improving soybean yield, the combination of NT, RY, and WB effectively reduced GHG emissions, resulting in the lowest GHGI. Therefore, we confirm that the synergistic utilization of NT, RY, and WB is a promising strategy for mitigating climate warming.

CONCLUSIONS

To explore the sustainable practice for organic soybean production, this study compared the effects of various agricultural managements on SOC sequestration, GHG emission, NGWP, and GHGI. No-tillage emitted more CH_4 in the presence of RY, while MP emitted more CH_4 under FA. Cover crop (rye) contributed a higher N_2O emission, thus increasing the GWP from N_2O. However, WB could partly offset the increased N_2O emission attributed to the adoption of NT and RY in the combined system. These findings indicated that the combination of NT, RY, and WB tended to maintain low GHG emissions and induced significant improvement in SOC stock, suggesting strengthened C sequestration. The NCDR in the NT-RY-WB treatment fully offset the non-CO_2 emissions, resulting in negative NGWP and GHGI in this system. With its low GHG emission and efficient C sequestration ability, the NT-RY-WB system effectively increased SOC stock and acted as a long-term sink for NGWP, indicating that this system will be an effective sustainable practice in Asia for alleviating climate change while maintaining crop yield. Considering that the effects of NT, cover crop, and biochar application on crop yield, SOC stock, and NGWP were not just simple stacking or offsetting, investigation on the specific mechanisms of their interaction will be necessary in further research.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article can be found in the online version.

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