



Optimizing soybean production and emission reduction through biogas slurry substitution and straw incorporation: A five-year field study in northeast China's black soil region



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ABSTRACT

To address the dual challenges of enhancing soybean yield and mitigating greenhouse gas (GHG) emissions in sustainable agriculture, this five-year field study (2020–2024) in Northeast China's black soil region evaluated the synergistic effects of biogas slurry substitution (0–100 % nitrogen replacement) and straw incorporation methods (deep ploughing vs. surface covering) on soil carbon-nitrogen dynamics, GHG emissions, and agronomic performance. Results demonstrated that a 75 % biogas slurry substitution (R3) combined with deep ploughing maximized soybean yield (4.24–18.36 % increase over conventional nitrogen fertilization) while significantly improving soil organic carbon (SOC, +10.52–25.72 %), dissolved organic carbon (DOC, +1.36–5.58 %), and nitrogen availability (NO_3^- -N: +11.47–25.27 %; NH_4^+ -N: +10.04–22.74 %). Crucially, deep ploughing with R3 reduced GHG emissions by 4.29–18.63 % (CO_2 , N_2O , CH_4), global warming potential (GWP) by 8.96–17.63 %, and emission intensity (GHGI) by 9.26–16.29 % compared to surface covering. Structural equation modeling revealed that elevated SOC and NO_3^- -N mediated the trade-off between yield enhancement and emission reduction. These findings highlight that integrating 75 % biogas slurry substitution with deep straw incorporation optimizes the carbon-nitrogen cycle, achieving synergistic yield growth (13.31 % maximum), carbon sequestration (SOC, +25.72 %), and GHG mitigation (CH_4 , –18.63 %), providing a transformative strategy for climate-resilient soybean production in temperate agroecosystems.

1. Introduction

Agricultural production is a significant source of greenhouse gas (GHG) emissions, particularly in traditional agricultural systems dominated by fertilizers (Fernández-Ortega et al., 2024). The excessive use of nitrogen fertilizers not only leads to inefficient nitrogen use but also exacerbates global warming by emitting large amounts of N_2O through the nitrification-denitrification process (Tamale et al., 2021). Additionally, agricultural activities can affect soil carbon and nitrogen cycling, disrupting the dynamic balance of soil carbon and nitrogen

forms, which in turn has a profound impact on soil fertility, crop yields, and ecosystem sustainability (Pareja-Sánchez et al., 2024). Therefore, exploring green, low-carbon, and efficient agricultural management practices to reduce greenhouse gas emissions and improve resource utilization efficiency has become a key research focus for achieving sustainable agricultural development. Among these practices, straw returning to the field is not only an effective method for recycling agricultural waste but also plays a vital role in soil improvement and greenhouse gas emission reduction.

As an important crop for both food and oil, soybean has a unique

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nitrogen fixation ability through rhizobia, making it an ideal crop for building a low-carbon agricultural system (Reimer et al., 2024). By reducing dependence on nitrogen fertilizer, soybean cultivation also requires a balanced approach to soil nutrients, providing an excellent platform for studying agricultural resource management practices. Straw returning, a key method for recycling agricultural waste, plays an important role in improving soil structure, increasing organic matter content, and enhancing soil fertility (Guo et al., 2024). China, the world's largest agricultural producer, generates more than 800 million tons of straw annually, most of which is either incinerated or discarded, leading to air pollution and resource waste (Zhang et al., 2024). In contrast, straw returning not only improves soil structure and increases organic matter but also reduces air pollution from burning and lowers the intensity of chemical fertilizer use, addressing the dual challenges of resource waste and environmental pollution (Zhang et al., 2024). However, the effects of different straw returning methods on soil carbon and nitrogen dynamics, greenhouse gas emissions, and crop yields vary significantly. Common methods include deep straw ploughing and surface mulching (Kan et al., 2023). Deep ploughing buries the straw deep in the soil, promoting decomposition and nutrient release, but may increase N_2O emissions due to soil disturbance (Li et al., 2021). Surface mulching improves the soil microenvironment by reducing evaporation, inhibiting weed growth, and alleviating temperature fluctuations (Momesso et al., 2022), though the mechanisms behind its impact on soil nitrogen conversion and greenhouse gas emissions remain unclear. Compared to deep ploughing, surface mulching has a slower effect on soil carbon and nitrogen cycling, and its potential impact on greenhouse gas emissions is still debated. Therefore, the ecological effects of different straw returning methods in crop systems must be evaluated through long-term field experiments to better understand their specific contributions and regulatory mechanisms related to soil carbon and nitrogen dynamics and greenhouse gas emissions.

Biogas slurry, a liquid organic fertilizer derived from anaerobic fermentation, is increasingly recognized as a sustainable alternative nutrient source due to its rich mineral content, including NH_4^+ -N, soluble carbon (DOC), and phosphorus and potassium (Xu et al., 2024). The application of biogas slurry can reduce the need for chemical fertilizers, improve soil nutrient availability, and stimulate microbial activity, thereby enhancing soil fertility through increased soil carbon input (Lu et al., 2024; Wang et al., 2024). However, the high nitrogen and carbon content in biogas slurry may influence greenhouse gas emissions. For example, NH_4^+ -N may produce more N_2O through nitrification, while DOC input could promote soil carbon mineralization and increase CO_2 emissions (Kong et al., 2023). Additionally, the effect of biogas slurry on soil nitrogen states may be reflected in the dynamic balance of nitrate nitrogen (NO_3^- -N), ammonium nitrogen (NH_4^+ -N), and microbial available nitrogen (MBN), and it remains unclear whether these changes can simultaneously increase soybean yield and reduce greenhouse gas emissions (Reimer et al., 2024). The long-term impact of different straw returning methods on the mechanisms of nitrogen slurry substitution, soil carbon and nitrogen indices, greenhouse gas emissions, and crop yields needs further investigation. Moreover, the performance of biogas slurry as a substitute for nitrogen fertilizer may vary under different straw returning methods. While biogas slurry application may enhance crop yield through nitrogen supplementation, it remains to be determined whether its synergistic effect with straw returning can significantly improve soil carbon and nitrogen balance.

Greenhouse gas emission intensity (GHGI) and global warming potential (GWP) are important indicators for assessing the carbon footprint and environmental sustainability of agricultural systems (Kamran et al., 2023). In soybean cropping systems, the balance of soil carbon and nitrogen, along with carbon sequestration capacity, has significant implications for ecosystem stability (Yan et al., 2023). The unique nitrogen fixation ability of rhizobia in soybean reduces reliance on nitrogen fertilizers, but it still requires a reasonable supply of nitrogen to meet its nutrient needs (Zhang et al., 2023). Straw returning and biogas slurry

application should be studied as typical green agricultural management practices from the perspectives of greenhouse gas emission reduction and optimization of carbon and nitrogen nutrient cycling (Chen et al., 2024). Straw returning may increase the size of the soil carbon pool by enhancing soil organic carbon (SOC), microbial biomass carbon (MBC), and DOC, while biogas slurry directly influences nitrogen speciation and microbial activity through the input of available nitrogen and soluble organic carbon (Mohanty et al., 2024). At the same time, straw returning may amplify or mitigate the effects of biogas slurry on soil, crops, and the environment (Huang et al., 2021). Therefore, it is crucial to clarify the combined impact of these two resource management practices on yield, GHGI, and GWP in soybean farming systems to achieve low-carbon agriculture (Deng et al., 2023). However, there is still insufficient research on the long-term effects of different straw returning methods and biogas slurry application on soybean yields, especially under continuous multi-year tillage conditions. Additionally, the long-term impacts of these management practices on soil fertility and crop yields remain unclear.

This study focused on the soybean planting system to explore the comprehensive effects of biogas slurry as a replacement for nitrogen fertilizer on farmland greenhouse gas emissions, soil carbon and nitrogen indices, and soybean yield under different straw returning methods (deep straw ploughing and surface mulching) over multiple years. The main objectives of this study are: (1) to clarify the differential effects of deep straw ploughing and surface mulching on soil carbon and nitrogen conversion and greenhouse gas emissions; (2) to reveal the synergistic effect of biogas slurry replacing nitrogen fertilizer on soil nutrient supply and soybean yield under different straw returning methods; and (3) to explore the combined effects of straw returning and biogas slurry application on the sustainability of agroecosystems. By systematically analyzing the synergistic effects of these two practices, this study aims to provide a scientific basis for a low-carbon, efficient soybean planting management system and offer theoretical support for straw resource utilization and the promotion of biogas slurry as a fertilizer.

2. Materials and methods

2.1. Study area

The experiment will be conducted from 2020 to 2024 at the Modern Agricultural High-tech Demonstration Park ($45^\circ 63' \text{N}$, $125^\circ 44' \text{E}$, average altitude of 162 m) in Harbin City, Heilongjiang Province. The experimental site has a cold temperate continental climate, characterized by warm and rainy summers with an average annual rainfall of 553.2 mm, primarily concentrated from June to August. The average annual evaporation is 796 mm, and the winters are cold and dry, with an average annual temperature of 3.46°C . The groundwater depth at the experimental field is less than 5 m, and the soil type is black soil. The basic physical and chemical properties of the 0–60 cm soil layer are as follows: soil particle size fraction less than 0.01 mm is greater than 22 %, soil bulk density is 1.48 g cm^{-3} , pH is 6.09, organic matter content is 41.26 g kg^{-1} , available nitrogen is 65.75 mg kg^{-1} , available phosphorus is 28.57 mg kg^{-1} , available potassium is 98.62 mg kg^{-1} , field water holding capacity mass ratio is 30.54 %, and wilting coefficient mass ratio is 12.72 %. Changes in rainfall and temperature during the soybean growth period are shown in Fig. 1.

2.2. Experimental design

In this study, soybean was used as the test crop, and the total amount of straw returning and the ratio of biogas slurry were considered as the experimental factors. A two-factor orthogonal design was used for the experiment. During the soybean growth period, two straw returning methods were set (S1: deep ploughing and returning to the field; S2: surface covering return), and five biogas slurry ratio levels were applied (R0: 100 % CF; R1: 25 % Bs + 75 % CF; R2: 50 % Bs + 50 % CF; R3:

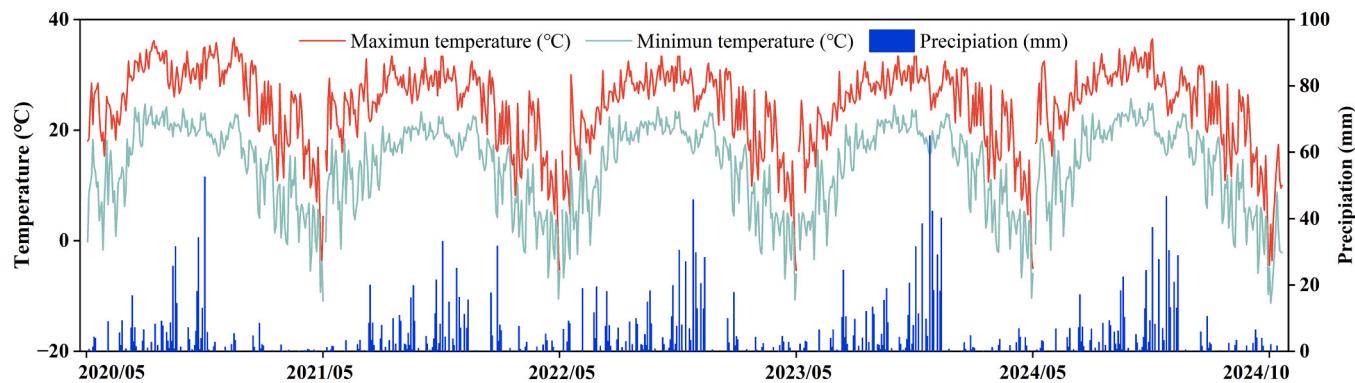


Fig. 1. Changes in rainfall and temperature during crop growth period.

75 % Bs + 25 % CF; R4: 100 % Bs; where Bs refers to the amount of nitrogen fertilizer in the biogas slurry in kg ha^{-1} , and CF represents the recommended nitrogen application rate of 80 kg ha^{-1}). A total of 10 treatments were conducted, each treatment replicated 3 times, with each plot having an area of 100 m^2 ($10 \times 10 \text{ m}$). Based on the principle of equal nitrogen application, the biogas slurry dosage was adjusted, and the biogas slurry amounts for each treatment are shown in Fig. 2 and Table S1. Every year, nitrogen fertilizer (with a base-to-chase ratio of 1:4), phosphate fertilizer, and potassium fertilizer were applied in the form of urea ($\text{CO}(\text{NH}_2)_2$), superphosphate (P_2O_5), and potassium chloride (K_2O) before soybean planting, with the remaining nitrogen fertilizer applied continuously during the soybean growth period. The plots were planted using a typical local micro-ridge planting pattern, with a 3 m wide protective row separating each plot. After soybean harvest, the straw was crushed using a pulverizer and evenly spread across the soil surface. For straw that needed to be deep-turned and returned to the field, a rotary tiller was used to incorporate it into a 0.3 m soil layer. The basic physical and chemical properties of biogas slurry were as follows: pH value of 7.33, organic matter content of 9.75 g L^{-1} , total nitrogen of 1.01 g L^{-1} , total phosphorus of 0.63 g L^{-1} , and total potassium of 1.09 g L^{-1} . Agronomic management practices, including pest, disease, and weed control, followed those commonly used by local farmers.

2.3. Indicator measurement and methodology

2.3.1. Collection and measurement of soil indicators

The five-point sampling method was used to collect soil samples from the 0–0.3 m tillage layer before and after planting crops in each treatment area. The samples were then thoroughly mixed and sieved. To comprehensively evaluate the effects of straw returning and biogas slurry application on soil carbon and nitrogen cycling, the following key indicators were selected to reflect the dynamic changes in soil carbon and nitrogen pools and their regulatory effects on microbial activity and nitrogen speciation: SOC, DOC, MBC, NO_3^- -N, NH_4^+ -N, and MBN. SOC was determined using the potassium dichromate oxidation method (Zhang et al., 2024); DOC was measured using the potassium dichromate external heating method (Mohanty et al., 2024); MBC was determined by chloroform fumigation extraction (Reimer et al., 2024); NO_3^- -N was determined by ferrous sulfate reduction photometry (Yan et al., 2023); NH_4^+ -N was determined by Nessler's reagent colorimetric method; and MBN was measured by chloroform fumigation (Yan et al., 2023).

The formula for calculating the soil-related index reserves is (Guo et al., 2024):

$$R_{\text{index}} = 10\rho_{\text{soil}}H_{\text{soil}}C_{\text{index}} \quad (1)$$

where R_{index} represents the storage of soil SOC (t ha^{-1}), NO_3^- -N (t ha^{-1}), NH_4^+ -N (t ha^{-1}), DOC (t ha^{-1}), MBN (t ha^{-1}), and MBC (t ha^{-1}),

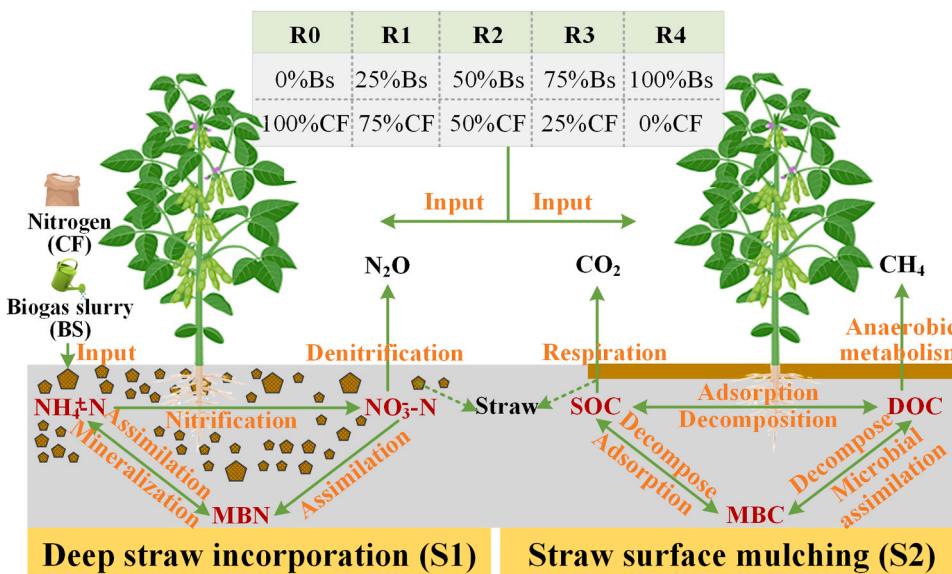


Fig. 2. Experimental design and schematic diagram.

respectively. H_{soil} is the depth of the sampling layer, 0.3 m. G_{index} represents the reserves of soil SOC (g kg^{-1}), NO_3^- -N (mg kg^{-1}), NH_4^+ -N (mg kg^{-1}), DOC (mg kg^{-1}), MBN (mg kg^{-1}), and MBC (mg kg^{-1}), respectively. ρ_{soil} is the bulk density of the soil (g cm^{-3}), and 10 is the unit conversion coefficient.

The formula for calculating the sequestration rate of each soil index is as follows (Guo et al., 2024):

$$CR_{index} = \frac{\Delta R_{index}}{t} \quad (2)$$

where CR_{index} represents the retention rate of soil SOC ($\text{t}(\text{ha}\cdot\text{y})^{-1}$), NO_3^- -N ($\text{t}(\text{ha}\cdot\text{y})^{-1}$), NH_4^+ -N ($\text{t}(\text{ha}\cdot\text{y})^{-1}$), MBC ($\text{t}(\text{ha}\cdot\text{y})^{-1}$), MBN ($\text{t}(\text{ha}\cdot\text{y})^{-1}$), and DOC ($\text{t}(\text{ha}\cdot\text{y})^{-1}$), respectively. ΔR_{index} represents the changes in soil SOC (t ha^{-1}), NO_3^- -N (t ha^{-1}), NH_4^+ -N (t ha^{-1}), MBC (t ha^{-1}), MBN (t ha^{-1}), and DOC (t ha^{-1}) during the test period. t represents the number of years in the trial.

2.3.2. Greenhouse gas collection and index calculation

In this study, greenhouse gases (CO_2 , N_2O , CH_4) were collected using static chamber-gas chromatography. Emission fluxes and cumulative emissions were then calculated. The static chamber ($0.40 \times 0.40 \times 0.60$ m) is equipped with a thermometer and an air extraction pipe. During sampling, the chamber is wrapped with aluminum foil. The base ($0.45 \times 0.30 \times 0.10$ m) is installed at the center of each treatment plot, and a water seal method is used to form an airtight seal. Sampling was conducted between 9:00 and 11:30 a.m., with gas concentrations measured at 0, 10, 20, and 30 min, and sampling occurred every 7 days. After rainfall, top-dressing, or spraying, the sampling frequency was increased to every 3 days. The collected gas samples were analyzed in the laboratory using a gas chromatograph (Shimadzu GC-2010PLUS, Japan) to determine the gas concentration. The gas emission fluxes for CO_2 , N_2O , and CH_4 were calculated as follows (Chen et al., 2024):

$$f = \rho H_{box} \frac{\Delta c}{\Delta t} \frac{273P}{(273 + T)P_0} \quad (3)$$

Where f represents the greenhouse gas emission flux, $\text{mg}(\text{m}^2\cdot\text{h})^{-1}$. ρ represents the density of the standard gas, kg m^{-3} . H_{box} represents the height of the static box, m. $\frac{\Delta c}{\Delta t}$ represents the rate of change of greenhouse gas concentration, $\text{ml}(\text{m}^3\cdot\text{h})^{-1}$. T represents the temperature in the chamber at the time of sampling, $^\circ\text{C}$. P represents the pressure in the chamber at the time of sampling, kPa. P_0 represents the standard atmospheric pressure, kPa.

The formula for calculating cumulative gas emissions is as follows:

$$F = 24 \sum_{i=1}^n \left(\frac{f_i + f_{i+1}}{2} \right) (t_{i+1} - t_i) \quad (4)$$

where F represents the cumulative greenhouse gas emissions, kg ha^{-1} . f_i and f_{i+1} represent the gas emissions at the i th and $(i+1)$ th times, $\text{mg}(\text{m}^2\cdot\text{h})^{-1}$. t_i and t_{i+1} represent the sampling times for the i th and $(i+1)$ th measurements, d. n represents the number of samples, and 24 represents the conversion factor.

The global warming potential (GWP, 10^3 kg ha^{-1}) of greenhouse gases was calculated over a 100-year time scale as follows:

$$GWP = 298F_{N_2O} + 25F_{CH_4} \quad (5)$$

Where F_{N_2O} , and F_{CH_4} represent the cumulative emissions of N_2O , and CH_4 , in kg ha^{-1} , respectively.

The formula for calculating the greenhouse gas emissions intensity (GHGI) is as follows (Zhang et al., 2023):

$$GHGI = \frac{GWP}{Y} \quad (6)$$

where $GHGI$ is the greenhouse gas emission intensity, kg kg^{-1} ; and Y is the crop yield, in 10^3 kg ha^{-1} .

2.4. Data processing

Multi-year data were summarized using Excel 2022 software. A three-way analysis of variance (ANOVA) was conducted to explore the effects of straw returning method, biogas slurry dosage, planting year, and their interactions on the indices using SPSS 22.0 software, with the least significant difference (LSD) test applied for multiple comparisons ($P < 0.05$). Graphs were generated using Origin 2024.

3. Results

3.1. Effects of straw and biogas slurry returning on CO_2 , N_2O and CH_4 emissions

The CO_2 emission fluxes of different treatments displayed a dynamic multimodal curve, with short-term peaks following topdressing and rainfall, and the trend was generally similar across multiple years (Fig. 3a). Under the same biogas slurry application conditions, the CO_2 emission flux for the S1 treatment was significantly reduced by 4.29–16.27 % compared to the S2 treatment ($P < 0.05$). Among them, the emission flux of the R3 treatment was the lowest, significantly lower by 4.68–14.81 % than that of the other treatments ($P < 0.05$). Under the same straw returning method, the CO_2 emission flux of biogas slurry initially decreased and then increased, with the R3 treatment showing a significant reduction of 4.02–14.19 % compared to the other treatments ($P < 0.05$). There was a nonlinear correlation between biogas slurry application and CO_2 accumulation (Fig. 3b), suggesting that higher biogas slurry use could inhibit the soil's carbon sequestration capacity.

The N_2O emission fluxes of different treatments exhibited a similar trend, particularly after topdressing and rainfall (Fig. 3c). The ranking of N_2O emission flux in recent years was 2020 > 2021 > 2022 > 2023 > 2024, with changes ranging from 32.65 to 195.69 $\mu\text{g}\cdot\text{m}^{-2}\text{h}^{-1}$. Significant differences were observed in the effects of straw, biogas slurry, and their interactions on N_2O emission flux ($P < 0.05$). Under the same straw returning method, the N_2O emission flux of biogas slurry decreased, with the R3 treatment showing a significant reduction of 3.28–11.01 % compared to the other treatments ($P < 0.05$). There was a nonlinear correlation between the amount of biogas slurry and N_2O accumulation (Fig. 3d), suggesting that higher biogas slurry use may reduce the nitrification-denitrification process of nitrogen in the soil.

The CH_4 emission fluxes of different treatments exhibited a +/- trend over time, indicating that the soil was absorbing CH_4 during emission periods, although it was generally dominated by absorption (Fig. 3e). The ranking of CH_4 emission flux in recent years was 2020 > 2021 > 2022 > 2023 > 2024, with changes ranging from –0.62–0.43 $\mu\text{g}\cdot\text{m}^{-2}\text{h}^{-1}$. Significant differences were observed in the effects of straw, biogas slurry, and their interactions on CH_4 emission flux ($P < 0.05$). Under the same biogas slurry application conditions, the CH_4 emission flux in the S1 treatment was significantly reduced by 4.21–18.63 % compared to the S2 treatment ($P < 0.05$). There was a nonlinear correlation between the amount of biogas slurry and CH_4 accumulation (Fig. 3f), suggesting that higher biogas slurry use may reduce the soil's capacity to absorb CH_4 .

3.2. Effects of straw and biogas slurry returning on SOC, MBC, DOC and carbon conversion

The content, storage, and sequestration rates of SOC, MBC, and DOC were significantly increased by straw and biogas slurry ($P < 0.05$). As shown in Fig. 4a and S1a, the SOC content and reserves of S1 increased by 11.63–25.72 % and 10.52–22.16 %, respectively, compared to S2 ($P < 0.05$). The SOC content and storage under biogas slurry increased by 4.62–12.54 % and 6.58–13.28 %, respectively, under the S1 method ($P < 0.05$). Under the same biogas slurry application conditions, the SOC retention rate for S1 significantly increased by 5.16–12.46 %

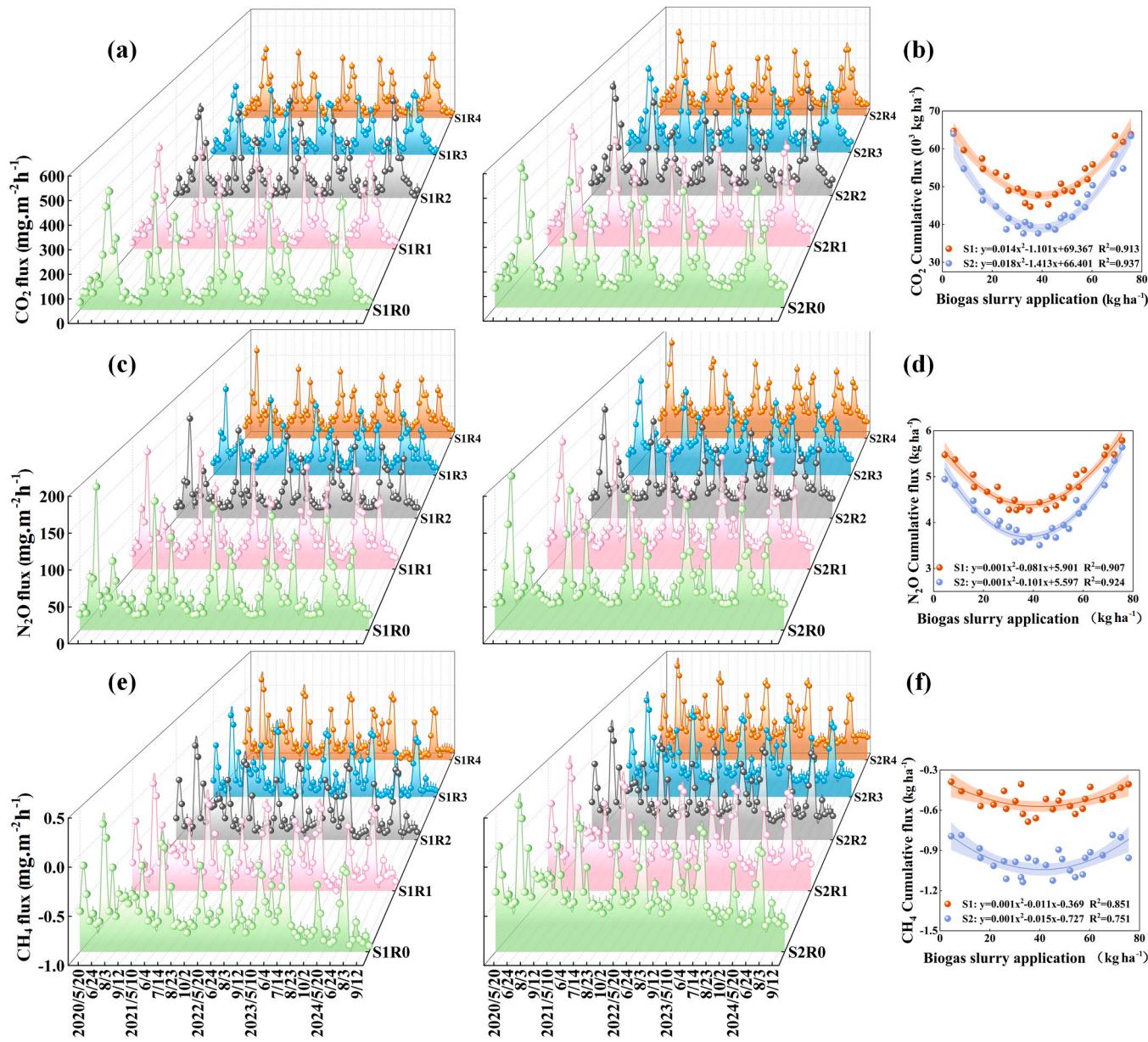


Fig. 3. CO_2 , N_2O and CH_4 emissions under different treatments and their fitting analysis with biogas slurry dosage. The shaded part indicates the confidence interval under different straw returning methods.

($P < 0.05$). The SOC content and reserves followed the trend: 2023 > 2024 > 2022 > 2021 > 2020. As shown in Fig. 4b and S1b, S1 significantly increased the content and reserves of MBC ($P < 0.05$). From 2020–2024, MBC content increased from $1.42\text{--}2.21 \text{ mg kg}^{-1}$ to $1.65\text{--}22.64 \text{ mg kg}^{-1}$, and the MBC reserve increased by $3.52\text{--}10.52 \text{ %}$. Both the application of biogas slurry and its interaction with straw significantly increased the MBC sequestration rate ($P < 0.05$), with S1 increasing by $4.12\text{--}10.45 \text{ %}$ compared to S2. MBC content and reserves followed the trend: 2023 > 2024 > 2022 > 2021 > 2020. Fig. 4c shows that the DOC content of S1 increased significantly by $1.36\text{--}5.58 \text{ %}$ ($P < 0.05$). The reserves and retention rates of DOC under increased biogas slurry in the S1 method increased by $5.26\text{--}10.25 \text{ %}$ and $3.52\text{--}12.86 \text{ %}$, respectively ($P < 0.05$) (Fig. S1c). DOC content and reserves followed the trend: 2023 > 2024 > 2022 > 2021 > 2020. Additionally, the results showed that biogas slurry application had direct positive effects on SOC, DOC, and MBC, and direct or indirect effects on CO_2 and CH_4 ($P < 0.05$) (Fig. 4d). Straw returning not only had indirect effects on DOC and MBC, but also had direct or indirect effects on CO_2

and CH_4 ($P < 0.05$). Furthermore, SOC and DOC had a direct positive effect on CO_2 ($P < 0.05$), while SOC had an indirect inhibitory effect on CH_4 . MBC had no significant effect on CO_2 or CH_4 , and DOC had a direct positive effect on CO_2 but no significant effect on CH_4 .

3.3. Effects of straw and biogas slurry returning on NO_3^- -N, NH_4^+ -N, MBN, and nitrogen conversion

The content, storage, and sequestration of NO_3^- -N, NH_4^+ -N, and MBN were significantly increased by straw and biogas slurry and their interactions ($P < 0.05$). As shown in Fig. 5a-b, the contents of NO_3^- -N and NH_4^+ -N in S1 increased by $10.41\text{--}24.10 \text{ %}$ and $9.27\text{--}24.16 \text{ %}$, respectively, compared to S2 ($P < 0.05$). The contents of NO_3^- -N and NH_4^+ -N initially increased and then decreased with the increasing dosage of biogas slurry, with the ratio for R3 being the highest, increasing by $11.47\text{--}25.27 \text{ %}$ and $10.04\text{--}22.74 \text{ %}$, respectively, compared to other treatments ($P < 0.05$). The contents and reserves of NO_3^- -N and NH_4^+ -N followed the trend: 2023 > 2024 > 2022 > 2021 > 2020. As seen in

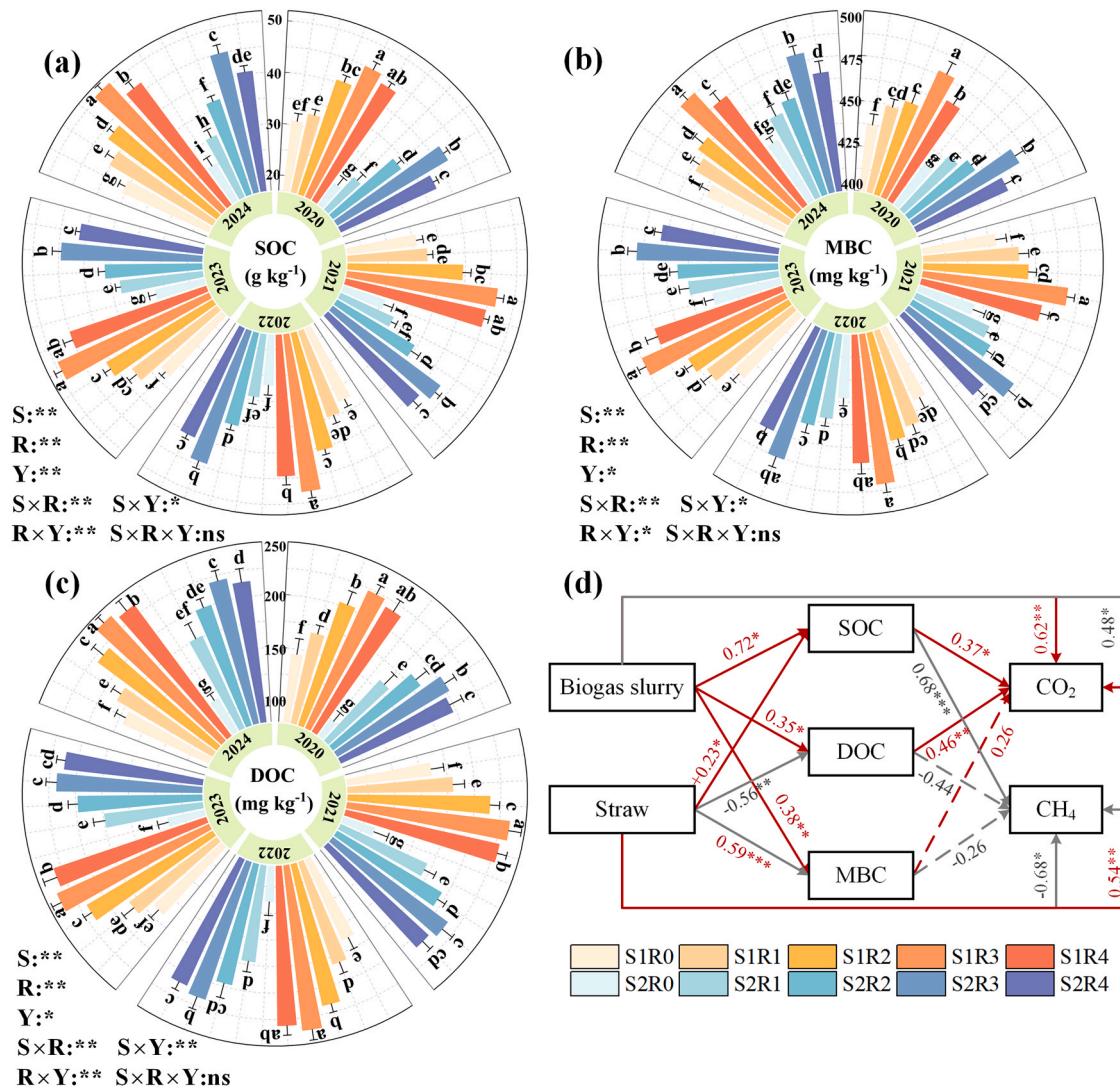


Fig. 4. shows the changes and through-passage analysis of SOC, MBC, and DOC under different treatments. 'S' represents the straw application method, 'R' denotes the proportion of biogas slurry, and 'Y' indicates the year of the test. Different letters indicate significant differences between treatments in different years ($P < 0.05$). ** represents $P < 0.01$, * represents $P < 0.05$, and 'ns' indicates no significant difference. Red and gray indicate direct/indirect correlations, while dotted lines indicate no significant correlation.

Figure S2a-b, the reserves and sequestration rates of NO_3^- -N and NH_4^+ -N decreased linearly with increasing biogas slurry dosage, and increased by 5.29–14.27 %, 4.69–16.58 %, 4.65–12.06 %, and 5.68–15.21 %, respectively, compared to R0 ($P < 0.05$). From Fig. 5c and S2c, the MBN content and reserves of S1 increased by 1.36–7.58 % and 2.45–10.96 %, respectively, compared to S2 ($P < 0.05$). The MBN content and reserves under biogas slurry increased by 2.46–5.69 % and 3.07–9.15 %, respectively, under the S1 method ($P < 0.05$). Under the same biogas slurry application conditions, the MBN retention rate of S1 increased significantly by 2.63–8.62 % ($P < 0.05$). The content and reserves of MBN followed the trend: 2023 > 2024 > 2022 > 2021 > 2020. The effects of straw returning and biogas slurry application on soil NO_3^- -N, NH_4^+ -N, MBN, and N_2O were analyzed (Fig. 5d). The results showed that biogas slurry application had a direct positive effect on soil NO_3^- -N, NH_4^+ -N, MBN, and N_2O emissions. The content of NO_3^- -N in the soil also had a direct positive effect on N_2O emissions, while soil NH_4^+ -N indirectly affected N_2O emissions. Straw returning directly affected the content of MBN, but had no significant effect on soil NO_3^- -N and NH_4^+ -N.

3.4. Effects of straw and biogas slurry returning on yield, GWP and GHGI

The effects of straw and biogas slurry and their interactions on crop yield were highly significant ($P < 0.01$), ranging from $2.41\text{--}3.08 \times 10^3 \text{ kg ha}^{-1}$ (Fig. 6a). Under the same biogas slurry application conditions, the yield of S1 increased by 2.16–13.31 % compared with S2 treatment ($P < 0.05$). Among the treatments, the yield of S1R3 was the highest, increasing by 4.24–18.36 % compared to S2R3. The yield under biogas slurry application increased by 3.26–15.17 % with the straw deep-turning method and by 2.21–10.15 % with the straw surface mulching method ($P < 0.05$). In comparison with other treatments, S1R0 and S2R0 treatments decreased by 3.08–12.61 % and 2.68–11.06 %, respectively, suggesting that reasonable biogas slurry application contributes to increased crop yield. Additionally, the planting year had a significant effect on crop yield ($P < 0.05$), with the yield peaking in 2024, ranging from $2.49\text{--}3.08 \times 10^3 \text{ kg ha}^{-1}$, an increase of 2.56–9.02 % compared with other years.

As shown in Fig. 6b, under the same biogas slurry application conditions, the GWP of S1 treatment was significantly reduced by 8.96–17.63 % compared to S2 treatment ($P < 0.05$). Among the treatments, the GWP of S1R3 was the lowest, 9.26–15.38 % lower than that

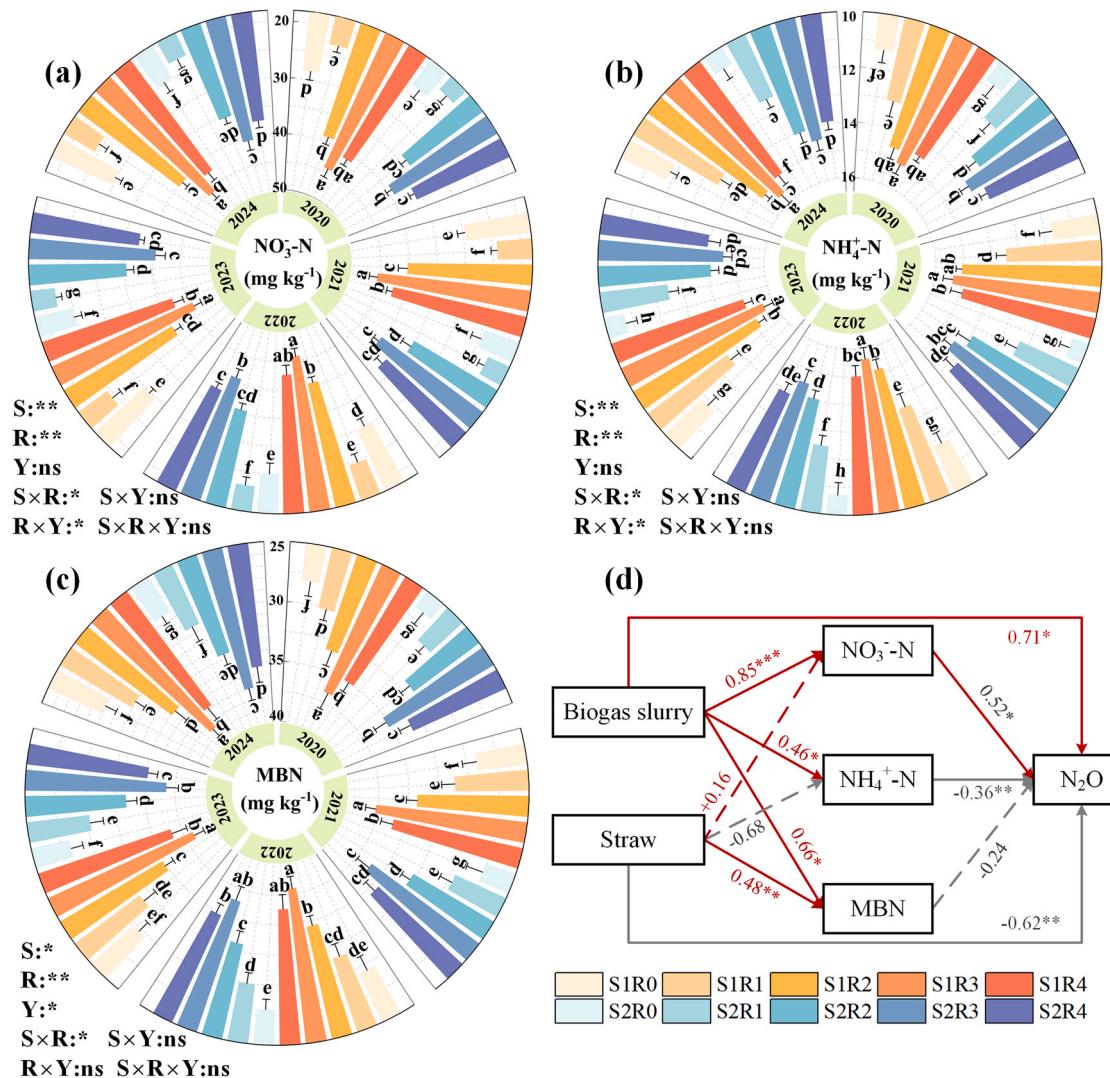


Fig. 5. shows the changes and analysis of NO_3^- -N, NH_4^+ -N, and MBN under different treatments. 'S' represents the straw application method, 'R' denotes the proportion of biogas slurry, and 'Y' indicates the year of the test. Different letters indicate significant differences between treatments in different years ($P < 0.05$). ** represents $P < 0.01$, * represents $P < 0.05$, and 'ns' indicates no significant difference. Red and gray indicate direct/indirect correlations, while dotted lines indicate no significant correlation.

of other treatments, and 8.96 % lower than that of S2R3, indicating that the GWP emission was lowest when 75 % biogas slurry was applied under the straw deep-turning method. Under both straw returning methods, GWP was reduced with increased application of biogas slurry. Compared to R0 treatment, R1, R2, R3, and R4 treatments decreased GWP by 10.26–14.38 %, 11.68–16.02 %, and 11.95–17.52 %, respectively. Additionally, there were significant differences in GWP across years ($P < 0.05$), with GWP in 2024 decreasing by 10.84–19.12 % compared to other years ($P < 0.05$). The results indicate that the long-term application of biogas slurry under the deep-turning method of straw helps reduce greenhouse gas emissions.

The effects of straw and biogas slurry and their interactions on GHGI were significant ($P < 0.05$), ranging from 1.40–2.83 kg kg^{-1} (Fig. 6c), while the planting year had no significant effect on GHGI ($P > 0.05$). Under the same biogas slurry application conditions, the GHGI of soil in S1 was 9.26–16.29 % lower than that of S2 treatment ($P < 0.05$). Under the same straw returning method, the GHGI of different biogas slurry treatments followed the order: R0 > R1 > R2 > R4 > R3 (except in 2024). Among these, the GHGI of S2R3 was the lowest, 15.26–27.85 % lower than that of other treatments, and 27.85 % lower than that of S2R0, indicating that the GHGI was smallest when 75 % biogas slurry

was applied under the straw deep-turning method, which helped reduce greenhouse gas emissions. Additionally, the synergy degree of multi-year targets showed that S1R3 had the highest value, increasing by 5.42–12.65 % compared to other treatments, suggesting that the application of 75 % biogas slurry under the straw deep-turning method was beneficial for increasing yield and emission reduction (Fig. 6d). As seen in Fig. 6e-g, there was a nonlinear correlation between biogas slurry dosage, yield, GWP, and GHGI under different straw returning methods. This indicates that an appropriate amount of biogas slurry can promote yield improvement and optimize greenhouse gas emission reduction, but excessive application may reduce marginal benefits and increase greenhouse gas emission intensity, thereby decreasing environmental benefits and resource use efficiency. Furthermore, the soil C/N ratio was significantly influenced by straw incorporation methods and biogas slurry application rates ($P < 0.05$), exhibiting nonlinear relationships with crop yield, GWP, and GHGI (Fig. 6h-j). Specifically, under deep plowing straw return combined with 75 % biogas slurry application (R3), the soil C/N ratio reached an optimal state, resulting in a significant 4.24–18.36 % increase in crop yield. Concurrently, this treatment substantially decreased GWP and GHGI, demonstrating a synergistic effect of yield enhancement and emission reduction. Conversely,

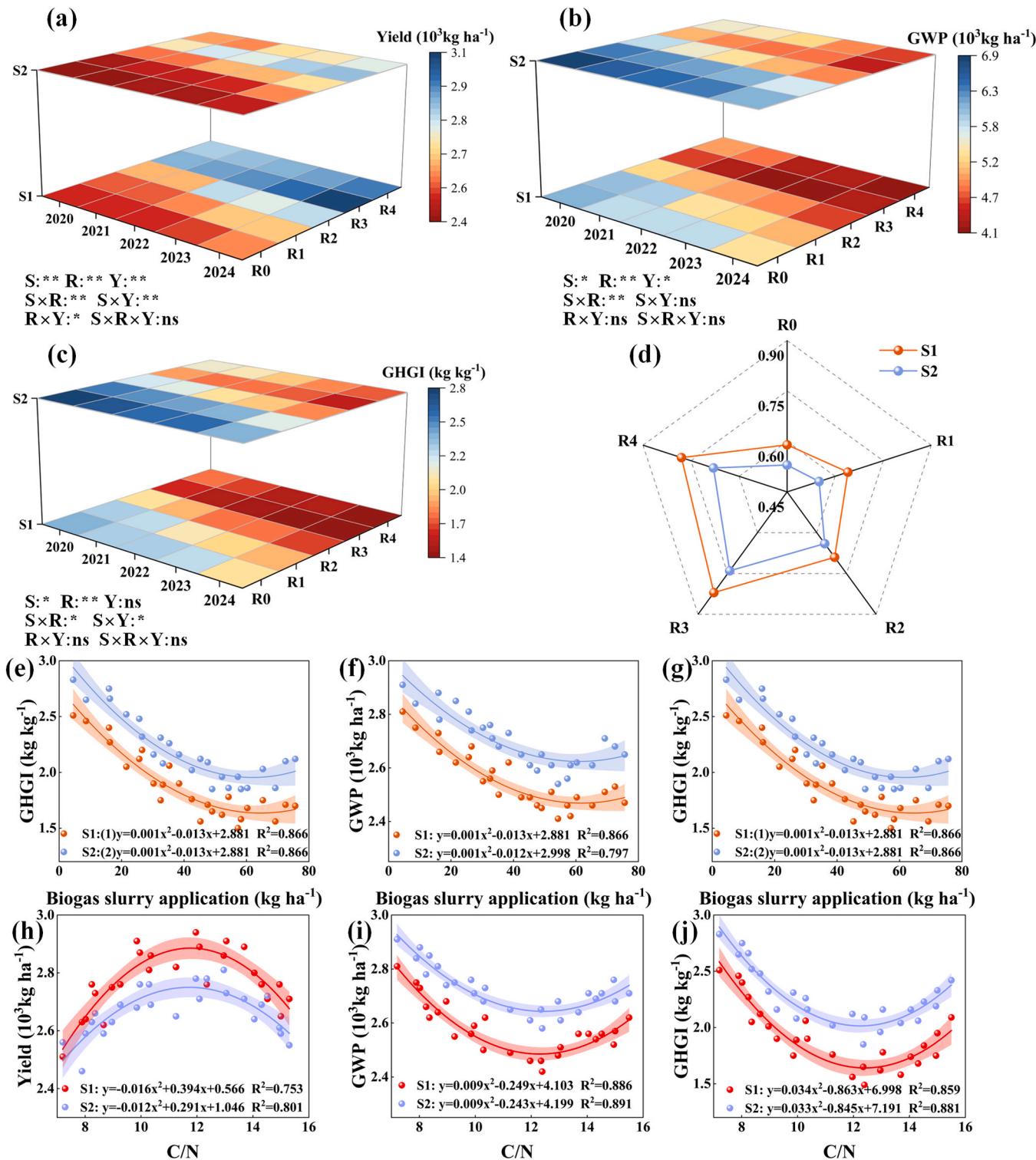


Fig. 6. shows changes in yield, GWP, and GHGI under different treatments. S represents the straw application method; R represents the proportion of biogas slurry; Y represents the year of the test. Different letters indicate significant differences between treatments across different years ($P < 0.05$). ** indicates $P < 0.01$, * indicates $P < 0.05$, and ns indicates no significant difference.

imbalanced C/N ratios, whether excessively high or low, led to reduced yields and elevated greenhouse gas emissions, posing constraints to sustainable agricultural practices.

3.5. Variable analysis of productivity and carbon emissions from straw and biogas slurry returning to the field

The Mantel test revealed the correlation between SOC, MBC, DOC, NO_3^- -N, NH_4^+ -N, MBN, CO_2 , N_2O , CH_4 , yield, GWP, and GHGI under different straw returning methods (Fig. 7). SOC was significantly correlated with DOC, MBC, NO_3^- -N, MBN, CH_4 , yield, and GWP under

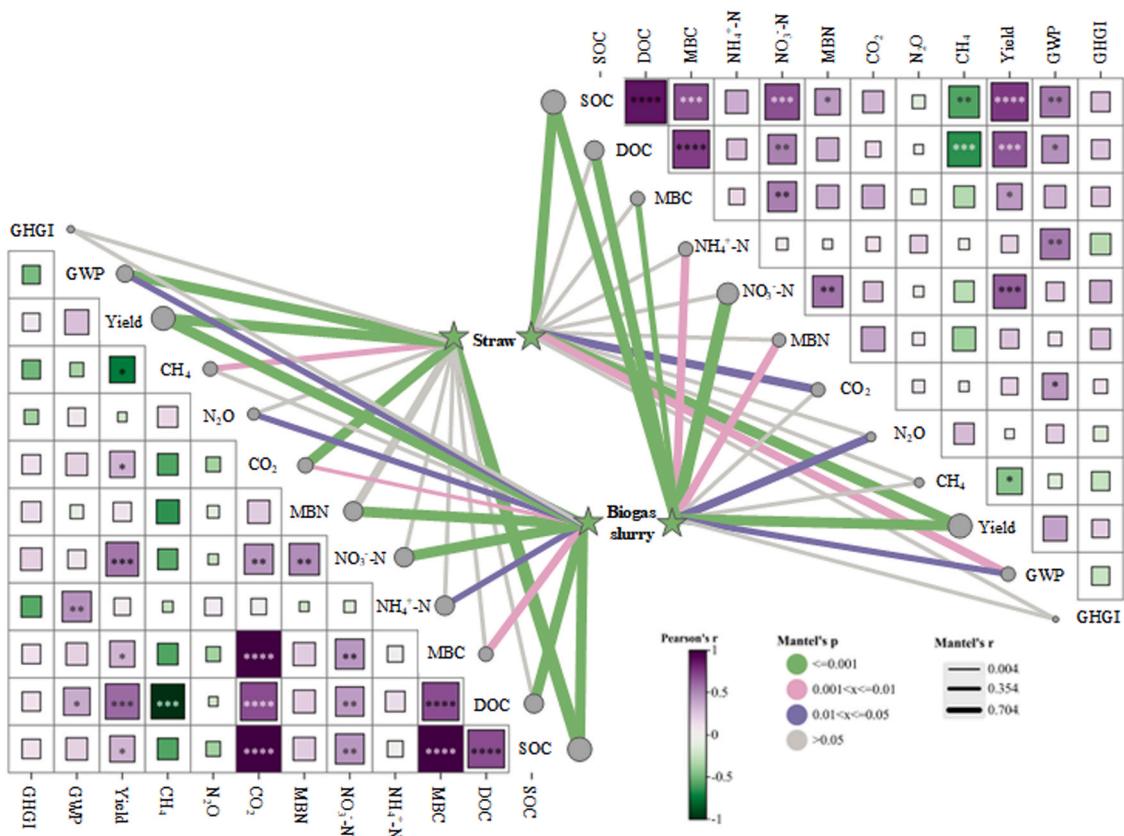


Fig. 7. Mantel relationship between straw and biogas slurry treatments and soil and gas indices under different straw returning methods. *** denotes $P < 0.001$, ** denotes $P < 0.01$, * denotes $P < 0.05$. S1 represents straw deep ploughing and returning to the field, while S2 represents straw surface cover returning to the field.

the straw deep ploughing method (Fig. 7a), while SOC was significantly correlated with DOC, MBC, $\text{NO}_3^-\text{-N}$, and yield under the straw surface mulching method ($P < 0.05$) (Fig. 7b). Crop yield in both straw returning methods was significantly correlated with SOC, DOC, MBC, $\text{NO}_3^-\text{-N}$, CO_2 , and CH_4 ($P < 0.05$), but not with $\text{NH}_4^+\text{-N}$, MBN, N_2O , GWP, or GHGI ($P > 0.05$). CH_4 was significantly correlated with DOC ($P < 0.05$), but not significantly correlated with other indices ($P > 0.05$). Besides, SOC, yield, and GWP were significantly affected by the amount of straw and biogas slurry ($P < 0.01$), while MBC, DOC, $\text{NO}_3^-\text{-N}$, $\text{NH}_4^+\text{-N}$, MBN, and N_2O were significantly affected by the amount of biogas slurry ($P < 0.05$). The amount of straw and biogas slurry had no significant effect on CH_4 and GHGI ($P > 0.05$). Under the straw surface mulching method, biogas slurry application had significant effects on SOC, MBC, DOC, $\text{NO}_3^-\text{-N}$, $\text{NH}_4^+\text{-N}$, MBN, CO_2 , N_2O , yield, and GWP ($P < 0.05$), but no significant effect on CH_4 and GHGI ($P > 0.05$). Straw dosage significantly affected SOC, CO_2 , CH_4 , yield, and GWP ($P < 0.05$), but had no significant effect on MBC, DOC, $\text{NO}_3^-\text{-N}$, $\text{NH}_4^+\text{-N}$, MBN, N_2O , or GHGI ($P > 0.05$). The Mantel test revealed the differences in the correlation between various indicators under different straw returning and biogas slurry application methods, which provides a key reference for formulating more scientific and effective agricultural strategies for soybean planting systems.

4. Discussion

4.1. Effects of straw and biogas slurry returning on CO_2 , N_2O and CH_4 emissions

In agricultural production, the application of straw and biogas slurry provides additional organic carbon and nutrients to the soil, significantly influencing soil microbial activity and the carbon and nitrogen cycling processes, thereby affecting greenhouse gas emissions in the soil

(Yue et al., 2023). The results showed that CO_2 , N_2O , and CH_4 emissions followed a dynamic and multimodal trend under different straw returning methods and biogas slurry applications, with rainfall and topdressing having a significant impact on the peak emissions of greenhouse gases (Fig. 3a, c, e). This is likely because rainfall and topdressing activities introduce additional carbon sources and nutrients, which accelerate the decomposition of organic matter and gas production (Wang et al., 2021). Moreover, different straw returning methods and biogas slurry applications altered the physicochemical and biological properties of the soil, thereby affecting its carbon emission and sequestration capacity. There was a nonlinear relationship between the cumulative emissions of CO_2 , N_2O , and CH_4 and the amount of biogas slurry, indicating that rational biogas slurry application reduced their emissions, while excessive biogas slurry application increased them (Fig. 2b, d, f). This may be due to the abundance of organic matter and nutrients in biogas slurry, which stimulates soil microbial activity, enhancing the carbon sink function of the soil while reducing nitrogen nitrification and denitrification (Meng et al., 2022). The study also found that under the same biogas slurry application conditions, CO_2 , N_2O , and CH_4 emissions were significantly lower with the straw deep turning method compared to the surface mulching method, and emissions decreased over the course of the experiment. This could be due to deep ploughing improving soil structure, increasing organic matter content, and creating a more aerobic environment, which promotes soil porosity and aggregation, thereby enhancing soil carbon sequestration capacity (Wang et al., 2025). Additionally, the passage of time contributed to the decomposition and humification of straw in the soil, further boosting the soil's carbon sink function. On the other hand, deep ploughing may help reduce greenhouse gas emissions by increasing soil nutrient content and modifying soil physicochemical properties, such as raising SOC, DOC, and MBC content (Li et al., 2024). Furthermore, the study found that a 75 % biogas slurry ratio resulted in significantly

lower greenhouse gas emissions than other treatments under the same straw returning method. This is likely due to the fact that an appropriate amount of biogas slurry promotes straw decomposition, enhances the stability of the soil carbon pool, and optimizes nitrogen and carbon conversion efficiency.

4.2. Effects of straw and biogas slurry returning on SOC, MBC, DOC and carbon

Carbon is a key factor affecting soil fertility and crop growth in agricultural production and plays a crucial role in nutrient supply for crop growth. The results indicated that different straw returning methods combined with biogas slurry had significant effects on SOC, MBC, and DOC ($P < 0.05$). This may be due to the fact that straw returning and fertilization treatments alter the distribution of SOCs and their active components (DOC and MBC) in soil aggregates (Li et al., 2023). Exogenous straw input not only directly increases the content of soil organic carbon components but also promotes the formation of aggregates as organic cementing substances. The physical protection provided by aggregates effectively prevents the encapsulated organic carbon from being preferentially decomposed by microorganisms, leading to the gradual accumulation of organic carbon components in aggregates (Zhang et al., 2024). At the same time, it was observed that under the same biogas slurry application conditions, deep straw turning significantly increased the content, storage, and retention rate of SOC, MBC, and DOC in the soil compared to surface mulching (Fig. 4a-c), with the highest levels reached in 2023 (Fig. S1). On one hand, straw is a rich source of carbon, and deep turning helps promote its decomposition in the soil, accelerating the mineralization process of soil organic matter and providing energy and nutrients for microbial activities (Li et al., 2024). On the other hand, biogas slurry is rich in various nutrients, significantly enhancing soil microbial activity and nutrient availability for agricultural purposes. The application of biogas slurry improves soil mineralization properties and enhances soil carbon sequestration capacity, thus positively influencing soil carbon cycling and crop growth (Chen et al., 2024). Additionally, the study found that a 75 % biogas slurry ratio effectively increased the content of SOC, MBC, and DOC in the soil. This is likely due to biogas slurry's high organic matter and nutrient content, which provides carbon sources and nutrients for soil microorganisms, significantly increasing the content of soluble DOC and MBC, thereby improving soil microbial activity and SOC retention capacity (Cheng et al., 2020). Furthermore, biogas slurry application also increases nutrient concentrations in the soil, accelerates straw decomposition, and alters the metabolic activity of indigenous microorganisms, promoting soil carbon recycling and sequestration. Therefore, the combination of straw returning and biogas slurry significantly reduced CO₂ and CH₄ emissions ($P < 0.05$). SOC and DOC had a direct positive effect on CO₂ ($P < 0.05$), while SOC had an indirect inhibitory effect on CH₄. MBC had no significant effect on CO₂ and CH₄, and DOC had a direct positive effect on CO₂ but no significant effect on CH₄ (Fig. 4d). CH₄ emissions were significantly correlated with SOC and DOC contents in the soil ($P < 0.05$) (Fig. 7a). Under surface cover, CO₂ emissions were significantly correlated with SOC, MBC, and DOC contents in the soil ($P < 0.05$) (Fig. 7b). These findings suggest that different straw returning methods combined with biogas slurry significantly impact carbon emissions, consistent with the study by Yue et al. (2023).

4.3. Effects of straw and biogas slurry returning on NO₃⁻-N, NH₄⁺-N, MBN and nitrogen

Nitrogen is a key nutrient for crop growth and a major driver of greenhouse gas emissions. The results showed that under the same biogas slurry application conditions, deep straw turning significantly increased the contents of NO₃⁻-N, NH₄⁺-N, and MBN in soil compared to surface mulching (Fig. 5a-c). This may be because biogas slurry is rich in organic matter and nutrients, which, when returned to the field, directly

increase the storage capacity of easily mineralized and refractory nitrogen pools in the soil, thereby promoting the rate of soil organic nitrogen mineralization (Wang et al., 2024). On the other hand, deep turning of straw provides more carbon substrates for the tillage layer, promotes soil nitrification and denitrification processes, and thus increases the content of NO₃⁻-N and NH₄⁺-N in the soil. Additionally, straw, as a rich carbon source, provides a large amount of SOC to the soil after deep ploughing and decomposition, enhancing the nitrogen mineralization process and reducing N₂O emissions (Sun et al., 2024). The study also found that the content, storage, and retention rate of NO₃⁻-N, NH₄⁺-N, and MBN increased first and then decreased with the progression of the experimental years, peaking in 2023 (Fig. S2). This could be related to the straw returning method and the proportion of biogas slurry. Deep ploughing of straw increases organic carbon input into the soil, promotes straw decomposition by soil microorganisms, accelerates the mineralization of soil organic matter, and provides energy and nutrients for microbial activity (Wang et al., 2024). Moreover, biogas slurry application can enhance the content and types of NO₃⁻-N, NH₄⁺-N, and MBN in the soil, improving soil mineralization characteristics and promoting the mineralization and fixation of nitrogen, thereby reducing N₂O emissions (Yue et al., 2023). Additionally, the study found that, under the same straw returning method, a 75 % biogas slurry ratio could effectively increase the content, storage, and retention rate of NO₃⁻-N, NH₄⁺-N, and MBN in the soil, which differed from the findings of Mohanty et al. (2024). On the one hand, it may be due to the fact that biogas slurry is a composite liquid organic fertilizer, and later topdressing has more diverse nutritional components and more complex bioactive substances compared to traditional nitrogen fertilizers. On the other hand, with the long-term and heavy metal rich input of biogas slurry, the accumulation of heavy metal content in the soil will occur, which will interfere with the normal ecological functions of the soil and also affect the absorption and transport of nutrients by crops. This may be due to biogas slurry being a compound liquid organic fertilizer, with more diverse nutrients and complex bioactive substances compared to traditional nitrogen fertilizers. Furthermore, biogas slurry application had a direct positive effect on soil NO₃⁻-N, NH₄⁺-N, MBN, and N₂O emissions (Fig. 5d). A positive correlation was observed between soil NO₃⁻-N and CO₂ under different straw returning methods, but the correlation was not statistically significant ($P > 0.05$) (Fig. 7).

4.4. Effects of straw and biogas slurry returning on yield, GWP and GHGI

Crop yield, GWP, and GGHGI are key indicators for assessing the environmental impact and sustainability of soybean farming systems. The results showed that combining straw deep turning with biogas slurry significantly increased crop yield and reduced the comprehensive greenhouse effect of farmland (Fig. 6). On one hand, deep turning of straw improves soil porosity and water content, promoting root growth (Li et al., 2024). Additionally, it enhances microbial biomass and soil enzyme activity, improving the microbial community structure and facilitating better nutrient uptake and utilization by crops (Wang et al., 2024). On the other hand, the application of straw combined with biogas slurry can significantly improve the decomposition rate of straw and the mineralization rate of soil organic carbon, which is closely related to the regulation of C/N ratio. The addition of biogas slurry supplemented nitrogen, optimized soil C/N ratio, accelerated straw decomposition and nutrient release, and enhanced soil fertility. Furthermore, the trial year was positively correlated with crop yield and negatively correlated with GWP, peaking in 2024. This may be due to the cumulative effect of long-term application of straw and biogas slurry. As the years increase, the soil organic matter and nutrient content continue to increase, and the C/N ratio gradually tends to optimize, promoting soil carbon sequestration and microbial community stability, thereby reducing GWP (Huang et al., 2021). The results also showed that GHGI was generally lower under the straw deep turning method compared to the surface mulching method, with the lowest GHGI observed at a 75 % biogas

slurry ratio. This could be due to GHGI being influenced by the combined effects of crop yield and GWP; biogas slurry application boosts crop yields while impacting soil nutrient content and microbial community structure, thereby reducing greenhouse gas emission intensity per unit of yield (Bai et al., 2023). Additionally, it was found that the application of straw combined with biogas slurry under different straw returning methods significantly increased crop yield and GWP, but there was no significant correlation with GHGI (Fig. 7). Finally, the multi-year target for the 75 % biogas slurry ratio was highest under the straw deep turning method, indicating that the application of 75 % biogas slurry under this method is beneficial for synergistically improving both soybean yield and emission reduction goals.

5. Conclusion

In this study, the effects of different straw returning methods combined with biogas slurry on the soybean planting system were investigated through five consecutive years of field experiments. The results showed that CO₂, N₂O, CH₄, GWP, and GHGI initially decreased and then increased with increasing biogas slurry dosage. Compared with surface covering, deep tillage significantly reduced the emissions of CO₂ (4.29–16.27 %), N₂O (3.28–11.01 %), and CH₄ (4.21–18.63 %), as well as GWP (8.96–17.63 %) and GHGI (9.26–16.29 %). Meanwhile, yield, SOC, MBC, DOC, NO₃⁻-N, NH₄⁺-N, and MBN increased initially and then decreased as the biogas slurry dosage increased. Deep ploughing significantly increased the contents of SOC (10.52–25.72 %), MBC (3.52–10.52 %), DOC (1.36–5.58 %), NO₃⁻-N (10.41–24.10 %), NH₄⁺-N (9.27–24.16 %), MBN (2.46–5.69 %), and soybean yield (2.16–18.36 %). Among these, the 75 % biogas slurry ratio significantly increased crop yield (4.24–18.36 %) while reducing CO₂, N₂O, and CH₄ emissions, with yield increasing over the experimental years. Overall, deep straw turning combined with 75 % biogas slurry, as a substitute for nitrogen fertilizer, not only effectively reduced greenhouse gas emissions (4.21 %–18.63 %) but also significantly improved soil nutrient levels (4.24 %–24.16 %) and crop yield (2.16 %–13.31 %). Therefore, this model offers a synergistic and efficient sustainable development path for increasing yield, sequestering carbon, and reducing emissions in soybean planting systems.

CRediT authorship contribution statement

Mo Li: Software, Resources, Project administration, Methodology, Investigation, Funding acquisition. **Yan Sha:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology. **Lihong Wang:** Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Xiaofang Wang:** Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Aizheng Yang:** Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Pingan Zhang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Data curation, Conceptualization.

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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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109853.

Data availability

Data will be made available on request.

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