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Synergistic effects of slow-release fertilizer and biochar on crop yield and soil carbon-nitrogen dynamics under reduced nitrogen input

Lianhao Zhao [a], Wen Yin [a,b,*], Qiang Chai [a,b,*], Pan Li [b], Zhipeng Guo [b], Zhilong Fan [a,b], Falong Hu [a,b], Cai Zhao [a], Aizhong Yu [a,b], Pingxing Wan [b], Wei He [a], Hong Fan [a], Yali Sun [a], Feng Wang [b]

[a] State Key Laboratory of Aridland Crop Science, Lanzhou 730070, China
[b] College of Agronomy, Gansu Agricultural University, Lanzhou 730070, China

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ABSTRACT

Reducing fertilizer input while improving crop yield and enhancing soil carbon and nitrogen storage in agricultural ecosystems remains a serious challenge. However, it remains unclear whether the addition of exogenous additives with various functions can enhance soil carbon (C) and nitrogen (N) stocks following nitrogen fertilizer reduction. Here, we report results from a wheat-maize rotation system with multiple cropping green manure designed to understand the response mechanism of soil C and N storage to slow-release fertilizer (SRF) and soil amendments (biochar and attapulgite) combination in two nitrogen level agricultural systems (no nitrogen reduction: NNR, and 30 % nitrogen reduction: NR30). We found that SRF and biochar combination significantly increased crop yield, soil nutrients (ammonium nitrogen and nitrate nitrogen), soil microbial biomass carbon (MBC) and nitrogen (MBN) in both systems, and the increase magnitude was notably greater in the NR30 system (+19.5 % for yields, +60 %~85 % for nutrients, and +18 %~44 % for MBC and MBN) compared with the NNR system (+17.0 % for yields, +31 %~35 % for nutrients, and +16 %~40 % for MBC and MBN) by inducing soil negative priming effects and ensuring the continuous nitrogen supply. The combination of SRF and biochar could reduce nitrogen input by at least 30 % while still increasing soil C (1.98 %~7.90 %) and N storage (49.83 % ~56.61 %), which attributed to the dual control mechanism that nitrogen reduction alleviated the 'nitrogen repression' of green manure and biochar enhanced the sensitivity of soil nitrogen-related extracellular enzyme activities to changes in MBN under NR30 system. This finding offers new insights for sustainable dryland agriculture.

1. Introduction

Under the severe challenges of resource constraints and environmental pressure on global agriculture, maintaining crop yield and enhancing the carbon and nitrogen storage capacity of farmland ecosystem is an important basis for ensuring the resource balance of farmland ecosystem and achieving sustainable agricultural development (Nazir et al., 2024), which has far-reaching impacts on fertilizer utilization rate, global food security and ecological security (Wang et al., 2022). However, the traditional and rough agricultural management measures and monoculture structure jointly restrict the improvement of soil carbon and nitrogen storage and hinder the increase of crop yield (Clermont-Dauphin et al., 2018).

Traditional farmland management in water-limited regions often relies on excessive nitrogen fertilizer inputs to achieve high crop yields (Shi et al., 2024). Nevertheless, this strategy resulted in excessive loss of soil nitrogen, and given rise to numerous ecological and environmental challenges, including nutrient supply imbalance and soil degradation (Schulte-Uebbing et al., 2022). These adverse effects will compromise soil quality, alter microbial community composition, and inhibit soil carbon and nitrogen fixation, ultimately hindering crop yield improvement (Zang et al., 2024). Due to the different crop types and environmental conditions, the previous results on the effect of nitrogen reduction on crop yield were not consistent (Moises et al., 2024). Wang et al. (2024) found that reducing nitrogen fertilizer application by 25 % would not reduce maize grain yield in wheat-maize rotation system

* Corresponding authors at: College of Agronomy, Gansu Agricultural University, Lanzhou 730070, China.
E-mail addresses: yinwen@gsau.edu.cn (W. Yin), chaiq@gsau.edu.cn (Q. Chai).

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under the condition of multiple cropping common vetch. Some studies have also highlighted the benefits of nitrogen reduction-such as reducing ammonia volatilization and N_2O emissions, and improving nitrogen use efficiency (NUE) (Zhang et al., 2024; Li et al., 2025). Other researchers, however, have found that chemical nitrogen reduction significantly reduced crop yields, thereby reducing smallholder income and threatening global food security (Moises et al., 2024). This discrepancy underscores a critical gap in understanding how to balance nitrogen reduction with sustainable yield maintenance.

Alternatively, the integration of soil amendments with slow-release fertilizers (SRFs) offered a promising strategy to counteract the decline in crop yield resulting from reduced chemical nitrogen fertilizer application. Soil amendments, such as attapulgite and biochar, have demonstrated significant potential to enhance soil carbon (C) and nitrogen (N) storage through multiple mechanisms (Ding et al., 2016; Mo et al., 2021). Biochar could alter soil physicochemical properties, such as pH and water retention, while stimulating microbial activity and influencing microbial community composition and assembly processes. These changes promote the decomposition and resynthesis of soil organic matter, ultimately enhancing the stability of soil C and N pools (Bai et al., 2024; Hu et al., 2024; Zhang et al., 2024). Similarly, attapulgite, a natural clay mineral, could improve soil structure and nutrient retention, further supporting microbial activity and nutrient cycling. SRFs can significantly minimize nutrient losses and environmental pollution caused by excessive single-dose fertilization by regulating the nutrient release rate (Wang et al., 2022). This synchronization with the crop growth cycle enhances the absorption and transmission of resources by the crop roots, thereby boosting crop growth vigor, improving fertilizer use efficiency, and reducing nutrient losses (Li et al., 2021). Previous research on improving soil carbon and nitrogen storage under reduced nitrogen fertilization has predominantly focused on single variables, such as SRFs or soil amendments, and frequently neglecting their impacts on crop yield. However, the regulatory mechanism of the coupling effects of SRF and soil amendment on crop yield, soil carbon and nitrogen storage under nitrogen fertilizer reduction in arid regions remain unclear.

Northwestern China, characterized by arid and semi-arid climates (Li et al., 2021; Zhao et al., 2023), has seen the local government promote multiple cropping of green manure crops, such as common vetch, after wheat harvesting in traditional wheat-maize rotation systems to enhance biodiversity and improve farmland ecological conditions (Gou et al., 2022; Wang et al., 2024). Numerous researchers have found that multiple cropping green manure can maintain or even increase crop yields while reducing chemical nitrogen fertilizer use in arid regions (Wang et al., 2024), but its effect on increasing soil carbon and nitrogen storage capacity is not well documented. Therefore, the coupling effects of SRFs and soil amendment on soil carbon and nitrogen storage of wheat-maize rotation system with multiple cropping green manure under nitrogen reduction requires in-depth studies.

During a three-year field experiment (2021 is a preliminary trial) in a wheat-maize rotation with multiple cropping green manure in the northwest arid regions of China, we set up two agricultural systems with and without nitrogen reduction and measured the soil properties (including soil bulk density, soil moisture, soil temperature, pH, soil salt content and electrical conductivity), soil organic carbon and total nitrogen, soil MBC and MBN in wheat-maize rotation system with multiple cropping green manure. We aimed to understand how the coupling effects of SRF and soil amendment affect crop yield, soil carbon and nitrogen storage in wheat-maize rotation with multiple cropping green manure under nitrogen reduction in arid regions. We hypothesize that:

1) The nitrogen reduction agricultural system would significantly reduce crop yield due to the nitrogen limitation compared with the no nitrogen reduction agricultural system. In addition, because soil amendments (biochar and attapulgite) had high porosity and strong adsorption capacity, and SRF could effectively match the different nutrient requirements of crops at different growth periods, the coupling effect of the SRF and soil amendment would improve soil structure and hydrothermal conditions, thereby increasing crop yield in two nitrogen level agricultural systems.
2) Based on hypothesis 1, the improvement of soil hydrothermal conditions by SRF and soil amendment in two systems, which could stimulate microbial activity, metabolic processes and the activity of soil carbon and nitrogen turnover-related enzymes, and then lead to the increase of soil MBC, MBN, and soil nutrient content. The nitrogen reduction system may be affected by nitrogen limitation, which markedly decreases microbial activity and simultaneously reduces MBC and MBN compared with no nitrogen reduction system.
3) Based on hypothesis 2, the increase of soil MBC and MBN mean that more carbon and nitrogen were locked in microorganisms, which would increase soil carbon and nitrogen storage. However, due to the influence of microbial nitrogen limitation, the coupling effect of SRF and soil amendment had limited effect on the increase of soil carbon and nitrogen storage under nitrogen reduction environment compared with the no nitrogen reduction system.

2. Materials and methods

2.1. The study site

This study was carried out on Wuwei Oasis Comprehensive Experimental Station (37°30′N, 103°05′E, 1600 m a. s. l.), in Wuwei Cite, Gansu Province, China, the area being representative of a typical temperate continental arid climate zone (Guo et al., 2019). The historical average temperature in this area is 7.1 °C. The effective accumulated temperature above 10 °C is more than 2910 °C. The sunshine duration is more than 2940 h. The mean annual precipitation (MAP) in this area is 155 mm (Yin et al., 2021).

2.2. Field experimental design

To understand the coupling effect of SRF and soil amendment on soil carbon and nitrogen storage capacity in typical wheat-maize rotation system (Starting from 2021) with multiple cropping green manure under nitrogen reduction environment, we set up two types of agricultural systems: nitrogen reduction (NR30) system (achieved by reducing the amount of nitrogen applied by local smallholder farmers by 30 %, N1) and no nitrogen reduction (NNR) system (customary nitrogen application by local smallholder farmers, N2). At the same time, we designed three soil amendment treatments: biochar (B) and attapulgite (A) and a control group without soil amendments (C), and applied two types of chemical fertilizers: traditional N fertilizer (T) and slow-release N fertilizer (S) in each system. We implemented a split-plot design comprising twelve treatments (N1TA, N1TB, N1TC, N1SA, N1SB, N1SC, N2TA, N2TB, N2TC, N2SA, N2SB and N2SC), each replicated three times to meet the minimum statistical replication requirements and ensure statistical significance. Soil samples from the 0–30 cm layer were collected in an S-shaped pattern within each replicate plot, thoroughly mixed, and a quarter of the mixture was selected using the quartering method for analysis. Each index had a total of 36 samples. Each plot had a test area of 31.2 m (6.5 m length and 4.8 m width), the total area of the test area was 2850 m. Specific experimental layouts are shown in Fig. 1. To shorten the crop rotation cycle and eliminate the climatic differences between years, double sequences experiments were designed in the same experimental field, which were wheat—green manure→maize rotation (Seq.1) and maize→wheat—green manure rotation (Seq.2) (Fig. 1). All the detection indexes were represented by the mean value of the double sequences experiments.

Wheat and maize varieties, planting densities, sowing dates and harvest dates could be seen elsewhere (Wang et al., 2024). Green manure (common vetch: variety Lan-jian No. 2 with density 1.50 million plants ha^{-1}) was sown in July each year, and turned over and returned

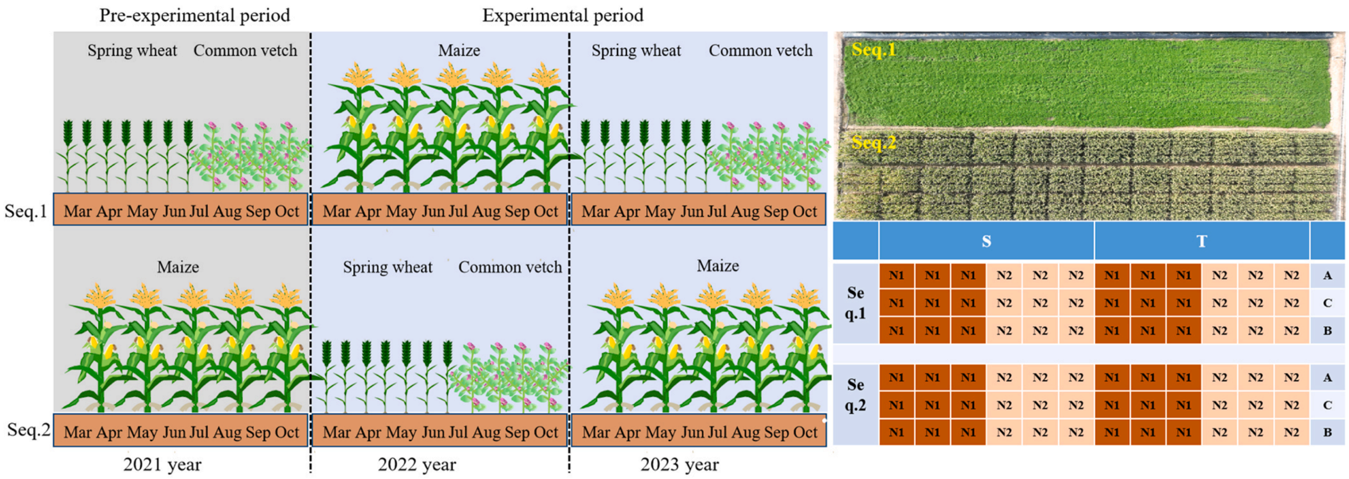

Fig. 1. Layout of common vetch and crops (Maize and Spring wheat) in the experimental plot; Seq.1 Spring wheat—common vetch→maize rotation sequence. Seq.2. Maize→spring wheat—common vetch rotation sequence. N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer.

to the field as green manure in October of each year. The nitrogen application rates were 126 kg ha^{-1} and 180 kg ha^{-1} for spring wheat, respectively, and maize were 252 kg ha^{-1} and 360 kg ha^{-1} in NR30 system and NNR system, respectively. Common vetch did not apply nitrogen. The traditional nitrogen fertilizer of maize was applied in stages according to pre-sowing (base fertilizer), big flare stage (topdressing), and filling stage (topdressing) with a ratio of 3:5:2. Traditional nitrogen fertilizer, as base fertilizer, was applied at one time for wheat. The SRF for wheat and maize were all applied as base fertilizers at the time of sowing, and the application rate was consistent with the traditional N fertilizer in the two systems. Phosphorus fertilizer was applied as base fertilizer when crops were sown, and the phosphorus application rates of wheat and maize were 90 kg ha^{-1} and 180 kg ha^{-1}, respectively. Drip irrigation was used to irrigate 700, 900 and 700 m ha^{-1} at seedling, booting and filling stage for spring wheat, respectively. Maize was irrigated with 900, 700, 850, 700 and 700 m ha^{-1} at jointing, trumpeting, tasseling, flowering and filling stages, respectively. Two kinds of soil amendments were applied at one time when crops were sown, the biochar (maize straw biochar) additions were 15 and 20 t ha^{-1}, and the attapulgite (attapulgite clay) additions were 30 and 45 kg ha^{-1} for wheat and maize plots, respectively. The basic properties of biochar and attapulgite were shown in Table S1 and Table S2 in Supplementary.

2.3. Crop yield and yield components

All wheat and maize in each plots were harvested in July and October, respectively, air-dried and determined for crop yields. In each plot, a quadrat (1 m ×1 m) was randomly selected to measure the ear number of wheat and maize. Fifteen wheat and ten maize plants were selected to measure kernels per ear and 1000-grain weight in each quadrat.

2.4. Soil environmental factors

We measured the soil temperature and soil moisture in the top 30 cm soil layer of each treatment every two weeks during the crop growth period. Soil temperature was determined with a curved tube geothermometer, and soil moisture was measured by oven-drying method. Other environmental factors, including bulk density, pH, EC and salt content, were measured after maize harvest each year. The soil bulk density was measured by using the method of standard ring knife. Eight grams of naturally air-dried soil was weighed and ground through a 2 mm screen to prepare a 1:5 soil-water ratio extract, and then the pH was determined by electrode method, the EC and total salt content of the other extract were determined by DDSJ-319L conductivity meter.

2.5. Soil NO_3^--N and NH_4^+-N

Soil samples were collected annually for each treatment during key maize growth stages, including emergence (V1), jointing (V6), trumpet (V12), silking (R1), filling (R2), and maturity (R6) stage, to capture dynamic changes in soil properties across the growing season. Weighed 10 g of the naturally air-dried soil samples (0.25 mm) and put it into a 150 mL plastic bottle, then added 100 mL of potassium chloride solution (the concentration was 1 mol L^{-1}) for leaching (soil to water ratio 10:1), then filtered the leaching solution with qualitative filter paper. Finally, the continuous flow analyzer (AA3, Bran Luebbe GmbH, Germany) was used to detect the filtered clear extract, and the NO_3^--N and NH_4^+-N content were obtained.

2.6. Soil MBC and MBN

We extracted the soil MBC and MBN by chloroform fumigation-potassium sulfate extraction method. Five grams of fresh soil was weighed into a beaker and placed in a vacuum dryer. The fumigation soil samples in the beaker were taken out, and the soil samples of each beaker were extracted with 20 mL 0.5 mol L^{-1} potassium sulfate solution. Another soil sample was directly extracted by potassium sulfate, shocked, centrifuged and filtered without fumigation. The TOC analyzer (TOC-VCPH/CPN, Shimadzu, Japan) was used to determine dissolved organic carbon (DOC) content, and the continuous flow analyzer was used to detect the NO_3^--N and NH_4^+-N content.

$$MBC= \Delta E_C/K_C$$

$$MBN= \Delta E_N/K_N$$

Where ΔE_C was the difference between fumigated and unfumigated soil DOC content, and K_C was the conversion coefficient (0.45); ΔE_N was the difference in the sum of NO_3^--N and NH_4^+-N content between fumigated and unfumigated soil, and K_N was the conversion coefficient (0.54).

2.7. Soil enzyme activity

Six soil enzymes related to the carbon and nitrogen turnover were measured by using the colorimetric method. The determination of urease activity referred to the method of Liu et al. (2017) by using the indophenol blue colorimetric method (details were in Notes 2 in

Supplementary). Nitrate reductase (NR) and nitrite reductase (NiRs) were measured by using the phenol disulfonic acid colorimetric (Wang et al., 2022)(details were in Notes 1 in Supplementary). Soil sucrase was extracted using the S-SC activity detection kits (Beijing Soleibao Technology Co., Ltd., China.) (Li et al., 2022). Soil α-glucosidase was determined using 4-methylumbelliferyl substrate (Ni et al., 2021). Soil RubisCO activity was determined by the method described by Wang et al..

2.8. Soil carbon and nitrogen storage

We determined soil organic carbon (SOC) and total nitrogen (TN) content in the top 30 cm soil layer of each treatment at pre-sowing, flowering and post-harvest for maize each year. Soil samples were naturally air-dried and sieved (0.15 mm) for the measurement of SOC and TN. SOC and TN were determined by using the K_2CrO_7–H_2SO_4 oxidation method and Kjeldahl digestion, respectively. Soil carbon and nitrogen storage were calculated as below:

$$SCS=SOC_{content}\times BD\times H\times 10^{-1}$$

$$SNS=NT_{content}\times BD\times H\times 10^{-1}$$

where SCS and SNS were soil carbon storage (Mg ha^{-1}) and nitrogen storage (Mg ha^{-1}), $SOC_{content}$ and $NT_{content}$ represented SOC content (g kg^{-1}) and soil TN (g kg^{-1}). BD was soil bulk density(g cm^{-3}) , H was the soil depth (cm). The soil C:N ratio was obtained by the ratio of SOC concentration to soil TN concentration.

2.9. Statistical analysis

The Shapiro-Wilks normality test was applied to test the normality of all measured variables. We analyzed differences in crop yield, soil properties, soil nutrients, soil enzyme activities, MBC and MBN, and SCS and SNS, and other parameters between treatments using multi-factor variance analysis using SPSS version 22.0. The *post-hoc* analysis was conducted by using Tukey's honestly significant difference (HSD) test, considering α= 0.05 as the threshold for statistical significance. Principal component analysis (PCA) was applied to explain the mechanism of the effects of SRF, soil amendment and environmental factors on SCS and SNS in two nitrogen level systems using SPSS 22.0 software, and the Mantel' test was applied to analyze the correlation among SCS, SNS and environmental factors. Furthermore, the structural equation modeling (SEM) was conducted to describe the standardized total effects of SRF and soil amendment on SCS and SNS, utilizing the Structural Equation Modeling framework with AMOS version 23.0 (Zhao et al., 2023), and the goodness of fit index (GFI) and comparative fit index (CFI) were selected to evaluate the model simulation performance, with values greater than 0.8 for both indices indicating reliable simulation results. The selected influencing variables included soil properties (e.g., soil temperature, soil moisture, pH, EC and salt content), enzyme activities (e.g., NR, NiRs, soil sucrase, soil α-glucosidase and soil RubisCO activity), and microbial biomass carbon and nitrogen.

3. Results

3.1. Grain yield of crops

Although a 30 % nitrogen reduction significantly (-5.7 %, $p < 0.05$) decreased maize grain yield compared with NNR system, the combined application of SRF and soil amendment offset this decline, with the N1SB treatment increasing yield by 8.7 % compared to the control (N2TC). N2SB treatment recorded the highest yield of maize in all treatments (Table S3). Similarly, the combined application of SRF and soil amendment significantly ($p < 0.01$) mitigated wheat yield losses under nitrogen reduction, with N1SB and N1SA treatments increasing yields by 18.02 % and 16.16 %, respectively, compared to N2TC (Table S3). The highest wheat yield was obtained in the N2SB treatment, which was significantly ($p < 0.01$) increased by 21.07 % compared with control treatment (N2TC). Overall, SRF and soil amendment maintained high maize and wheat yields while reducing nitrogen fertilizer by 30 %.

3.2. Soil physicochemical characteristics

Nitrogen reduction progressively increased soil temperature (Fig. 2n). SRF improved soil hydrothermal conditions at the same nitrogen level (Fig. 2j, k), while soil amendments (notably biochar) significantly increased soil temperature/moisture and reduced bulk density (Fig. 2i). Consequently, the N2SB treatment achieved optimal hydrothermal status (highest soil temperature/moisture, lowest bulk density), followed by N2SA compared with control (N2TC). Nitrogen reduction decreased soil salt content (SSC) and soil electrical conductivity (EC) by 18.24 % and 14.25 % versus traditional nitrogen levels (Fig. 3m-o). SRF further reduced these parameters by 28.75 % (SSC) and 26.64 % (EC). Biochar uniquely lowered SSC/EC among amendments, with N1SB showing minimal values. Although 30 % nitrogen reduction maintained soil pH, the combined use of SRF and biochar significantly increased the soil pH value under nitrogen reduction (Fig. 3i, l).

A 30 % nitrogen reduction decreased soil NH_4^+-N by 12.35 % compared with the NNR system ($p < 0.05$) (Fig. 4). SRF significantly ($p < 0.01$) increased NH_4^+-N in both systems (28.65 % in NR30 and 26.01 % in NNR systems over traditional fertilizer). Soil amendments also significantly boosted the NH_4^+-N. Averaged over two years, the average NH_4^+-N contents of added biochar and attapulgite treatments were 11.15 mg kg^{-1} and 10.63 mg kg^{-1}, respectively, which were 11.76 % and 6.48 % higher than those without amendment in both systems. Therefore, the N2SB treatment achieved peak NH_4^+-N (13.06 mg kg^{-1}). Soil NO_3^--N followed similar trends except for insignificant amendment effects. N2SB treatment also recorded the highest NO_3^--N values (33.23 mg kg^{-1}) across all treatments (Fig. 4).

3.3. Soil MBC, MBN, and enzyme activities

Nitrogen reduction did not significantly affect soil microbial biomass carbon (MBC) at pre-sowing, flowering and post-harvest stages for maize (Fig. 5a,b). However, SRF significantly increased soil MBC by 4 % (2022) and 5 % (2023) versus conventional fertilizers in both systems. The combined application of SRF and soil amendment further enhanced soil MBC, where N2SB yielded peak values (15 % increase over N2TC), followed by N2SA (14 %). Conversely, nitrogen reduction reduced soil microbial biomass nitrogen (MBN) during the maize growth period (Fig. 5c,d). SRF significantly increased the soil MBN by 15 % (2022) and 12 % (2023) relative to traditional fertilizers. The synergic effect of SRF and soil amendment further improved soil MBN under nitrogen reduction, with N1SB and N1SA treatments consistently showing higher MBN values throughout the study period.

Nitrogen reduction (NR30) decreased soil urease, nitrate reductase (NR), and nitrite reductase (NiRs) by 2.78 %, 8.86 %, and 4.79 % versus NNR system (Fig. 6). The application of SRF and soil amendment, particularly their combined use, significantly increased urease content while reducing NR and NiRs. The combination of SRF and biochar yielded the highest urease activity, averaging 48.87 and 50.15 μmol d^{-1} g^{-1} in the NR30 and NNR system, respectively, while maintaining the lowest NR and NiRs values (6.18 and 0.24 μmol d^{-1} g^{-1} in the NR30; 0.25 and 7.45 μmol d^{-1} g^{-1} in the NNR). Nitrogen reduction did not affect soil sucrase but reduced β-glucosidase and RubisCO by 12.20 % and 30.06 %, respectively (Fig. 7). Soil amendments enhanced sucrase, while SRF improved β-glucosidase and RubisCO. The SRF-biochar synergy maximized enzyme activities, peaking at 5.64 mg d^{-1} g^{-1} sucrase, 32.76 μmol d^{-1} g^{-1} β-glucosidase and 1.94 nmol d^{-1} g^{-1} RubisCO in the NR30 system, and 5.66 mg d^{-1} g^{-1}, 33.07 μmol d^{-1} g^{-1}, and 2.02 nmol d^{-1} g^{-1} in the NNR system, respectively.

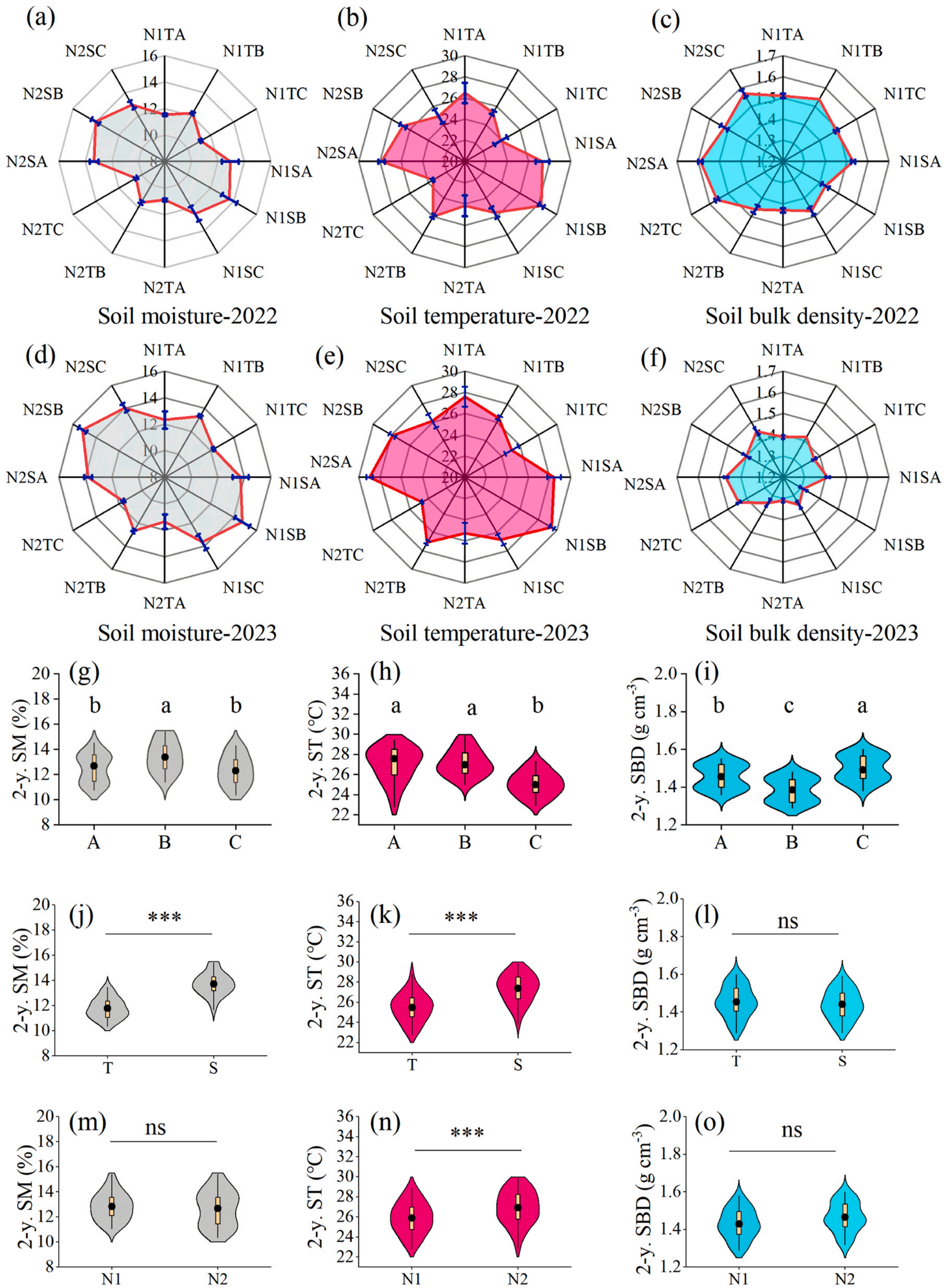

Fig. 2. The coupling effects of slow-release fertilizer and soil amendment on soil moisture, soil temperature, and soil bulk density under nitrogen reduction. N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer. Different small case letters indicate statistically significant differences ($p < 0.05$) among treatments. ns denotes no significant difference treatments; ***denotes significant difference at $p < 0.001$.

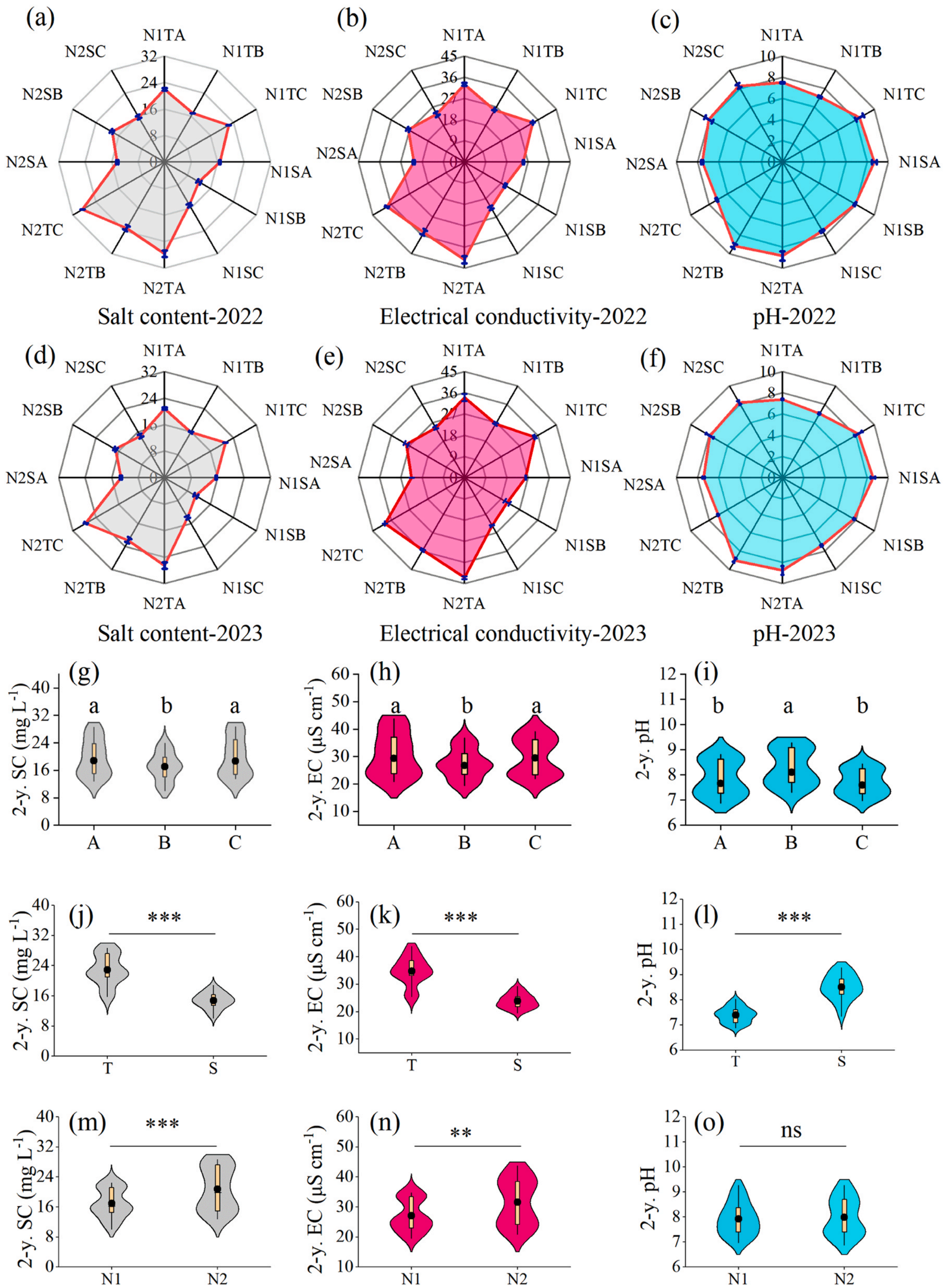

Fig. 3. The coupling effects of slow-release fertilizer and soil amendment on soil salt content, soil electrical conductivity, and soil pH under nitrogen reduction. N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer. Different small case letters indicate statistically significant differences ($p < 0.05$) among treatments. ns denotes no significant difference treatments; ** and ***denote significant difference at $p < 0.01$ and $p < 0.001$, respectively.

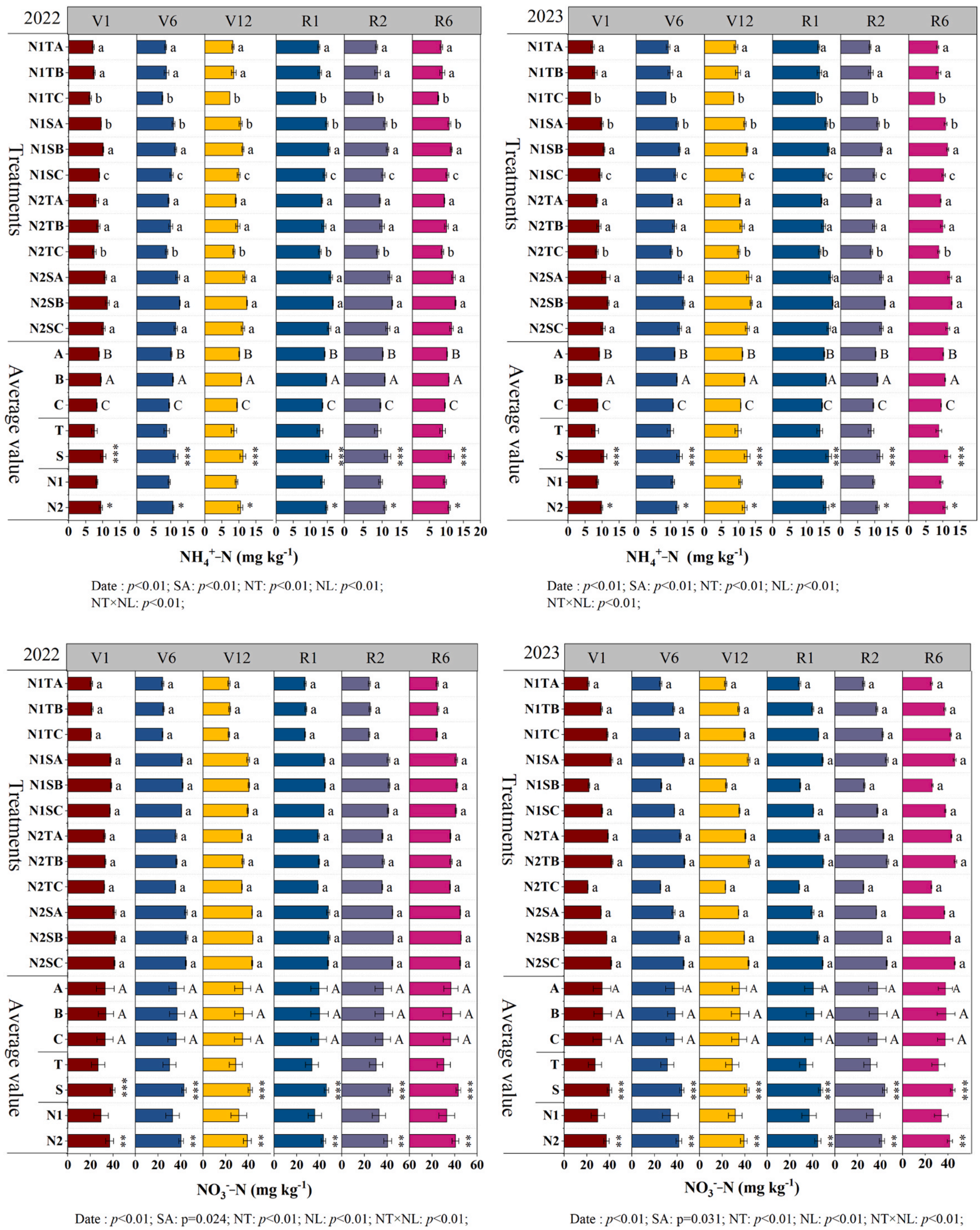

Fig. 4. The changes of soil ammonium nitrogen and nitrate nitrogen in different treatments at different growth stages of maize.N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer. Different color bars represent different growth stages of maize.Different capital and small case letters indicate statistically significant differences ($p < 0.05$) among soil amendments and treatments, respectively. *, **, and ***denote significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

3.4. *Soil organic carbon and total nitrogen storage*

Although 30 % nitrogen reduction significantly ($p < 0.01$) reduced SOC, the combined use of SRF and soil amendment significantly ($p < 0.01$) increased SOC at pre-sowing, flowering and post-harvest for maize (Fig. S1). The N2SB treatment recorded the highest soil carbon storage, reaching a two-year average of 44.70 Mg ha^{-1} (Fig. 8), an increase of 10.97 % compared with the control treatment (N2TC). The effect of attapulgite treatment on SOC was not significant. Similarly, the application of SRF and the addition of biochar significantly ($p < 0.01$) increased soil total nitrogen content throughout the sampling period (Fig. 9 and Fig. 10c,d). The soil nitrogen storage of N1SA and N2SB treatments were still higher compared with the control treatment (N2TC), increasing by 22.12 % and 27.05 %, respectively, indicating that the coupling effects of SRF and biochar can offset the reduction of soil nitrogen storage by nitrogen reduction.

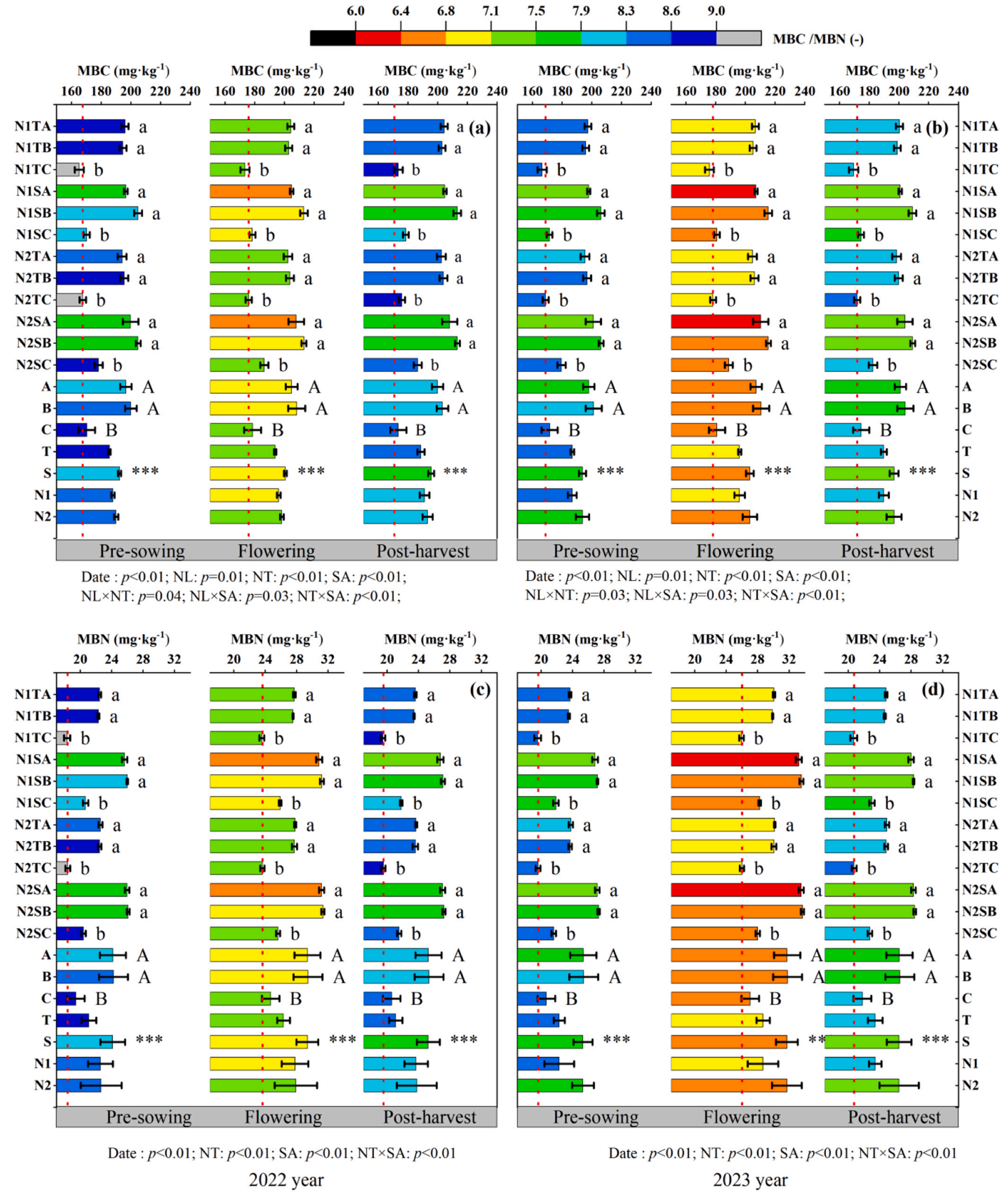

Fig. 5. The soil microbial biomass C and N in different treatments at different growth stages of maize. N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer. Different capital and small case letters indicate statistically significant differences ($p < 0.05$) among soil amendments and treatments, respectively. ** and ***denote significant difference at $p < 0.01$ and $p < 0.001$, respectively.

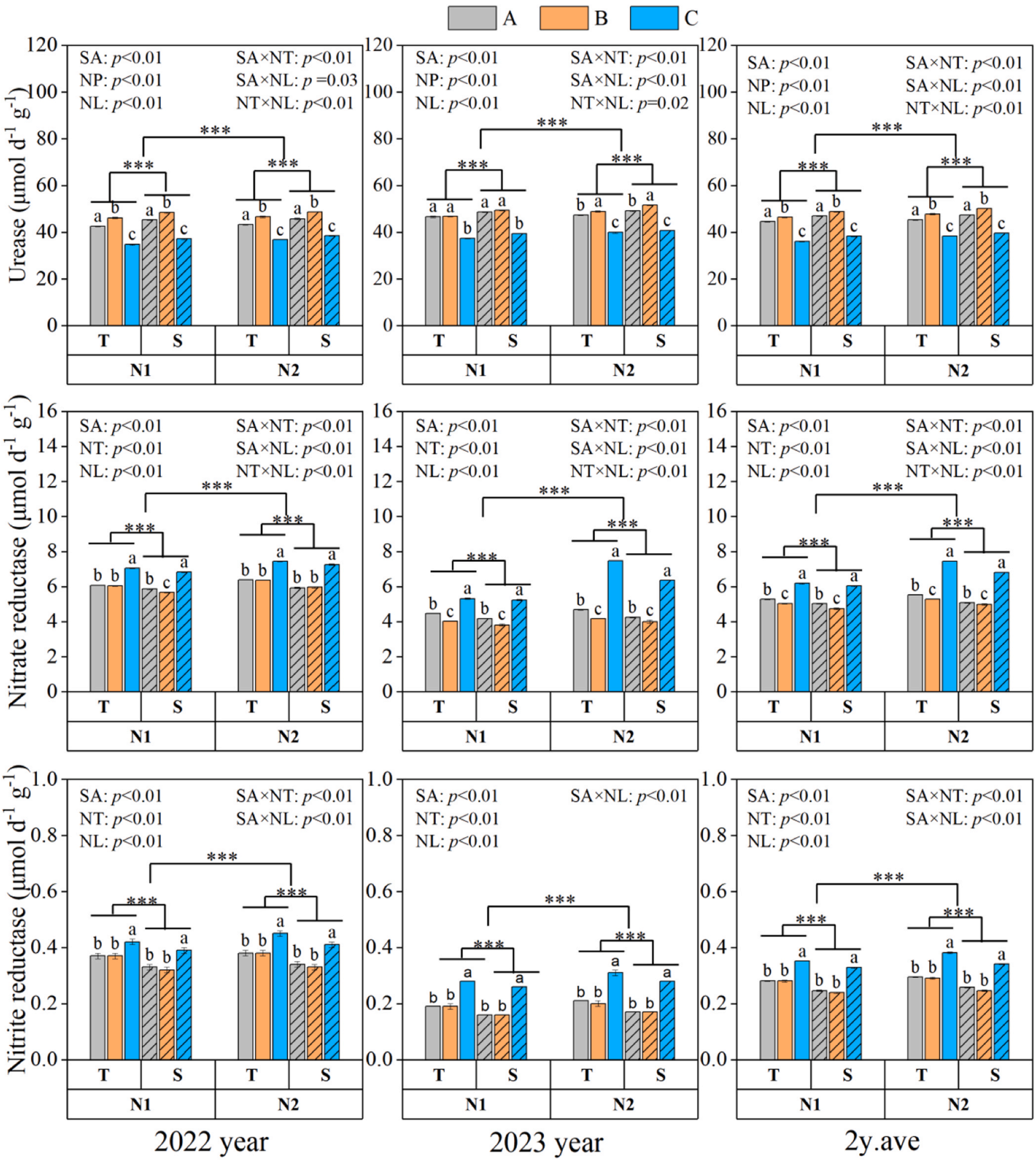

Fig. 6. The effects of slow-release fertilizer and soil amendment on soil nitrogen enzyme activity under nitrogen reduction. N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer. Different small case letters indicate statistically significant differences ($p < 0.05$) among ***denotes significant difference at $p < 0.001$.

4. Discussion

4.1. *Responses of grain yield, soil environmental factors to the coupling effects of SRF and soil amendment under nitrogen reduction*

As an indispensable nutrient element for crop photosynthetic assimilation and growth, nitrogen is crucial to the sustainability and stability of farmland ecosystems in arid regions (Xia et al., 2024). Due to the different crop types and environmental conditions, the previous results on the effect of nitrogen reduction on crop yield were not consistent, and most studies found that nitrogen reduction would directly lead to crop yield reduction (Moises et al., 2024). However, Wang et al. (2024) found that reducing nitrogen fertilizer application by 25 % would not reduce maize grain yield in wheat-maize rotation

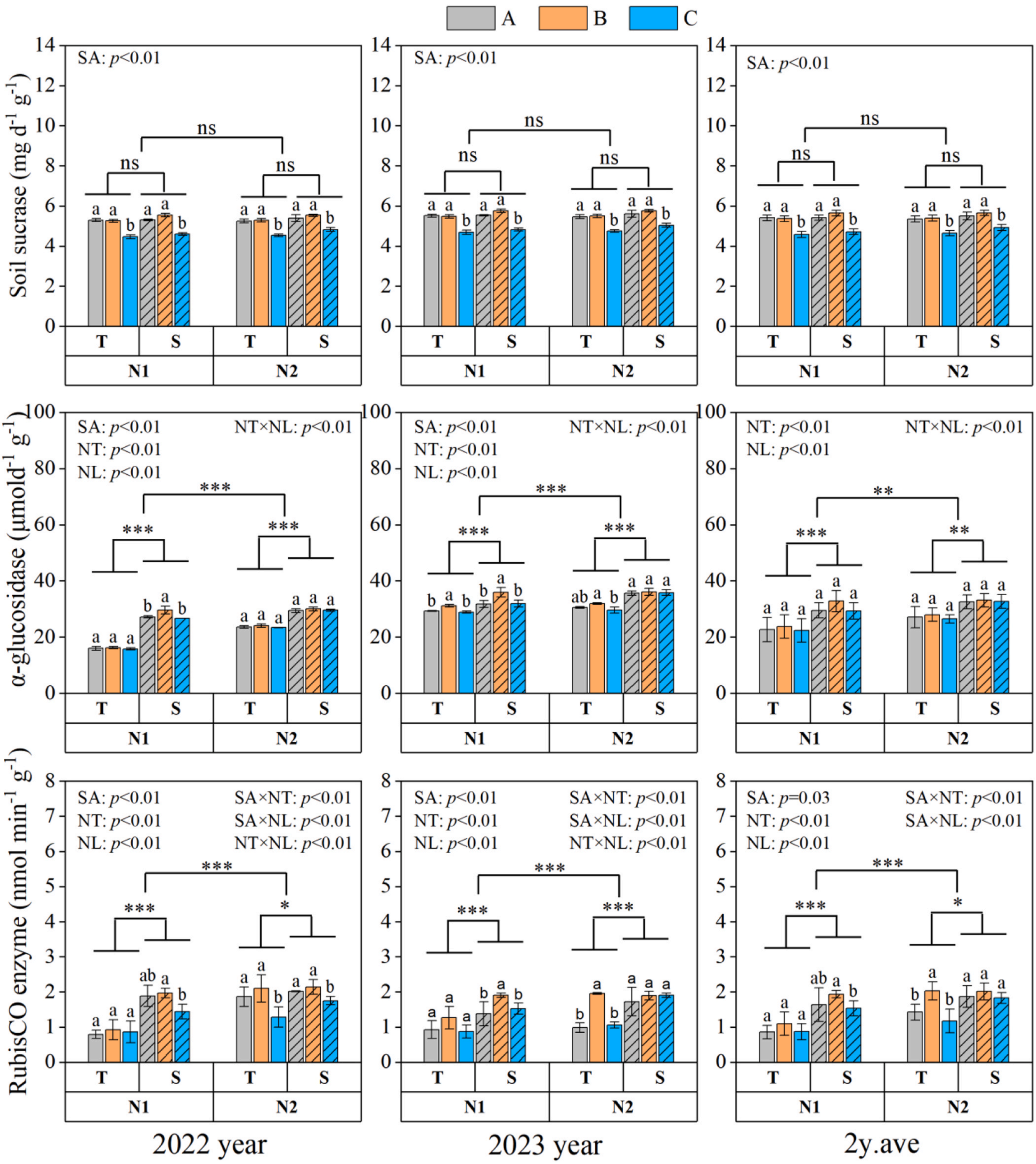

Fig. 7. The effects of slow-release fertilizer and soil amendment on soil carbon enzyme activity under nitrogen reduction. N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer. Different small case letters indicate statistically significant differences ($p < 0.05$) among treatments. *, **, and ***denote significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

system under the condition of multiple cropping common vetch. Our study found that the grain yields of both wheat and maize in rotation system with multiple cropping green manure in the NR30 system were significantly lower compared with the NNR system when only traditional nitrogen fertilizer was applied (Table S3, S4), which is consistent with our first hypothesis. The contradictory phenomenon of our findings with the former was attributed to different degrees of nitrogen reduction in the agricultural system. Our experiment reduced nitrogen by 30 %, much higher than the nitrogen reduction designed by Wang et al. (2024). Previous studies have reported that the addition of SRF and soil amendments (biochar and attapulgite) could improve crop yield in farmland ecosystems, which mainly depends on the improvement of soil aggregate structure and hydrothermal conditions suitable for crop root growth by exogenous additives (Bai et al., 2023; Zhang et al., 2024).

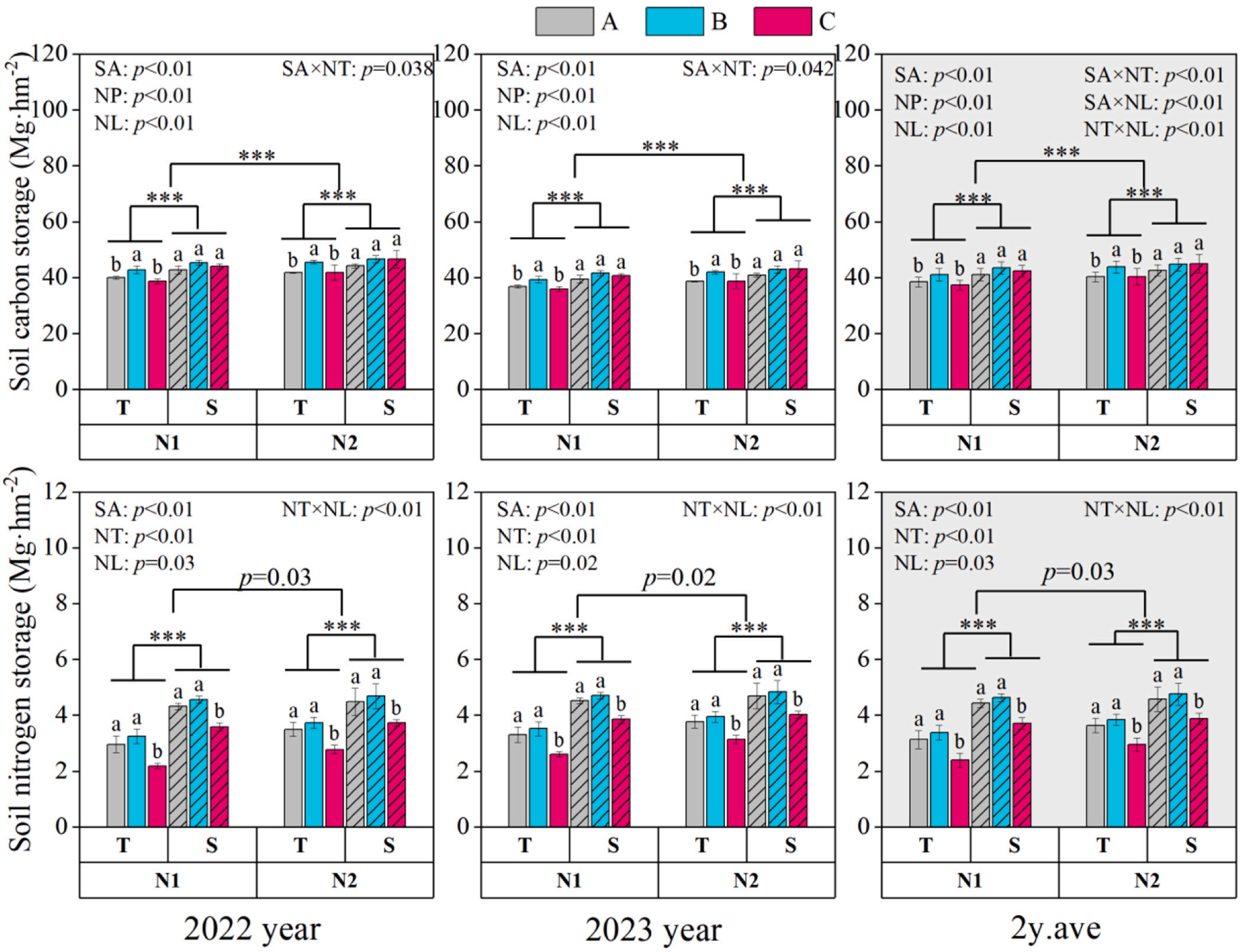

Fig. 8. The effects of slow-release fertilizer and soil amendment on soil carbon and nitrogen storage under nitrogen reduction. N1: nitrogen reduction system, N2: no nitrogen reduction system, A: attapulgite, B: biochar, C: no soil amendment, S: slow-release N fertilizer, T: traditional N fertilizer. Different small case letters indicate statistically significant differences ($p < 0.05$) among treatments. ***denotes significant difference at $p < 0.001$.

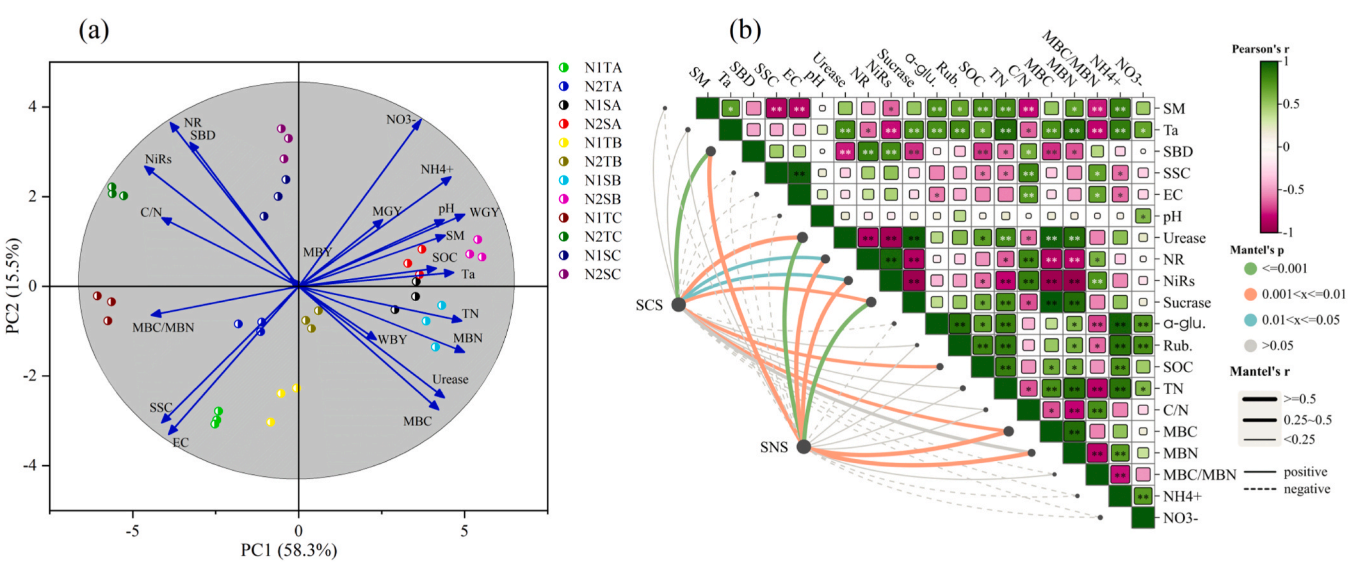

Fig. 9. Principal component analysis and Mantel's test between soil carbon and nitrogen storage and environmental factors.

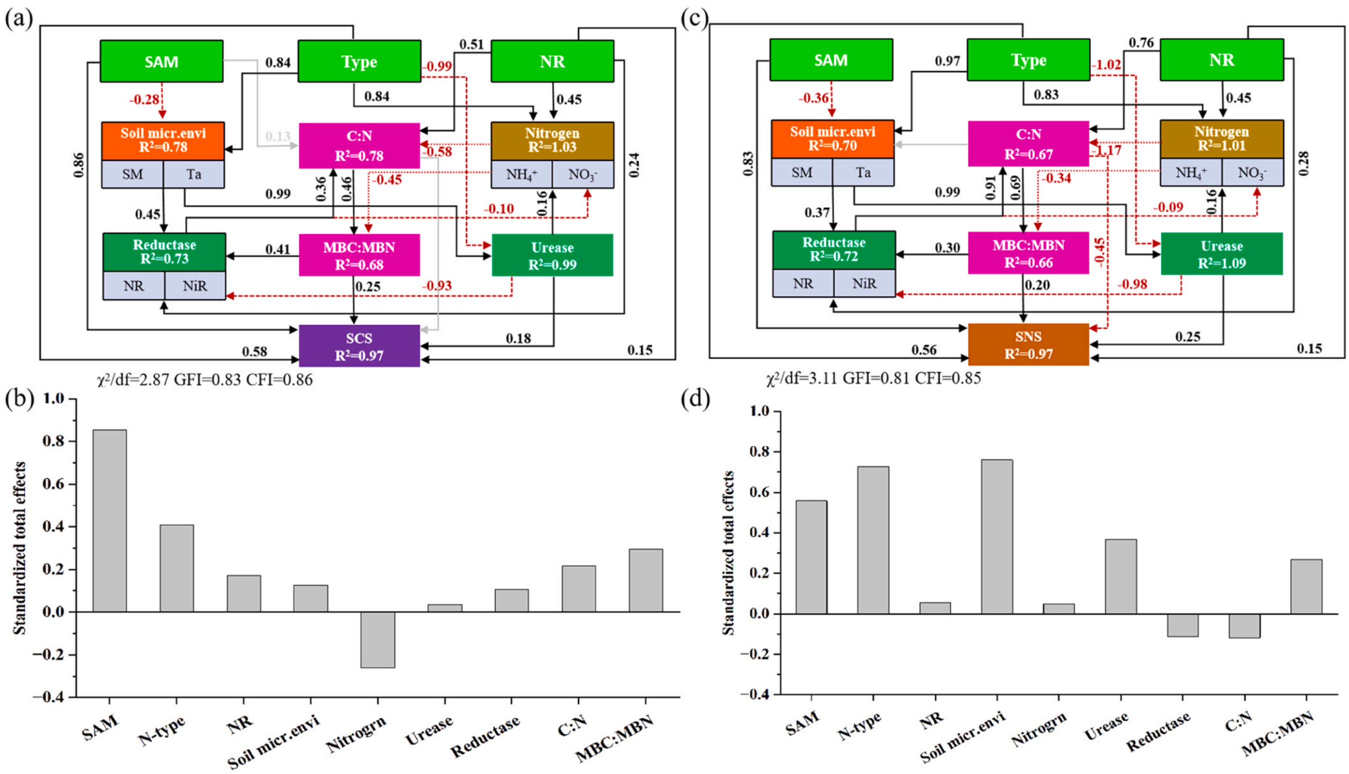

Fig. 10. Structural equation modeling (SEM) to examine the multivariate effects of slow-release fertilizer and soil amendment on soil carbon and nitrogen storages under nitrogen reduction. The solid and dashed lines indicate positive and negative coefffcients, respectively; the thickness of the arrows indicates the magnitude of standardized path coefffcient; R^2 values indicate the proportion of variance explained for each endogenous variable. GFI, goodness of fit index; CFI, comparative fit index.

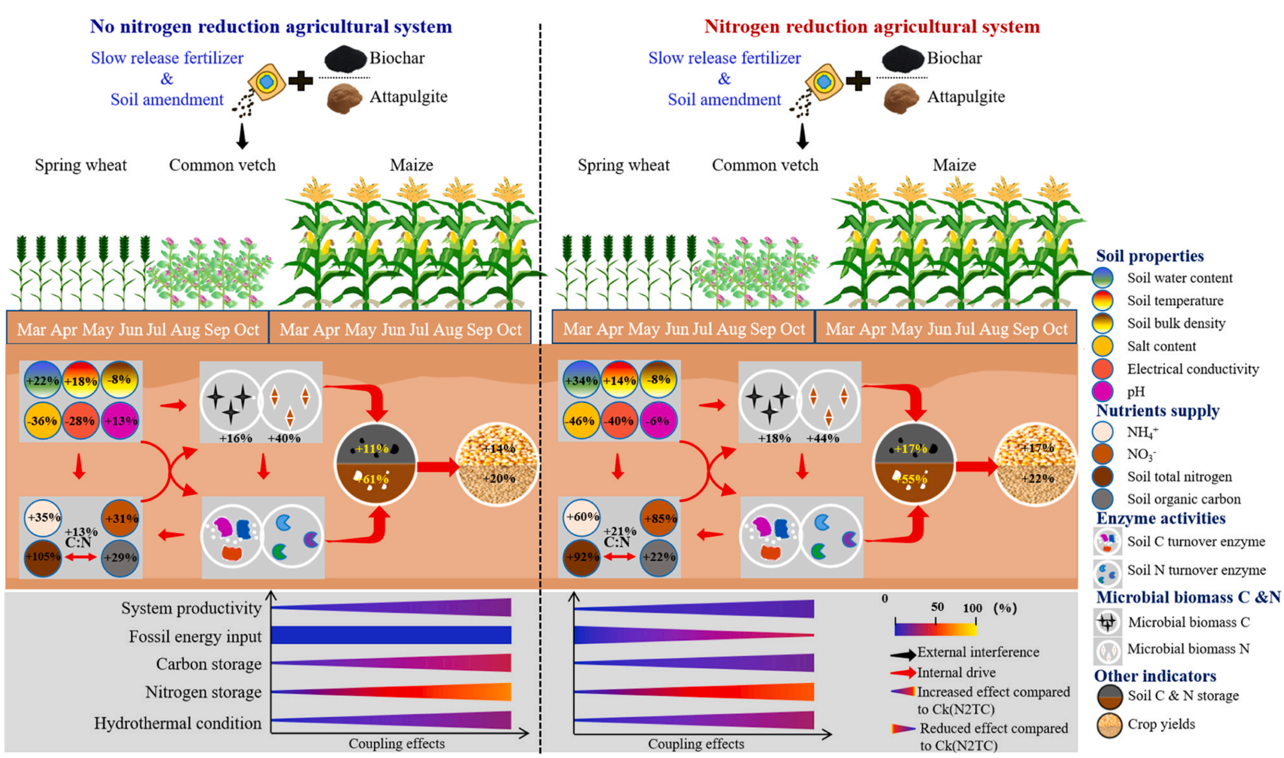

Fig. 11. A conceptual diagram of the impact of slow-release fertilizer and soil amendment on soil properties, nutrients supply, soil enzyme activities and soil C & N storage in NR30 and NNR agricultural systems.

Consistent with first hypothesis, the coupling effect of SRF and soil amendment significantly enhanced the yields for wheat and maize in both NR30 and NNR systems. Moreover, the combined application of SRF and soil amendment in the NR30 system resulted in a significantly higher crop yield increase ratio compared to the NNR system (Table S3, S4 and Fig. 11), with biochar demonstrating superior efficacy due to its high C:N ratio, as evidenced by the experimental results.(Fig. S1). This indicated that crop yields in the rotation system in low-nitrogen environments were more sensitive to the coupling effect of SRF and biochar compared with conventional nitrogen application environments. The primary reason for this phenomenon was that the external biochar containing high C:N ratio (Fig. S1), promoted soil nitrogen accumulation, and SRF could continuously supply the nitrogen required by crops (Fig. 4), effectively compensating for the nitrogen limitation caused by system nitrogen reduction (Yao et al., 2022).

Numerous studies have reported that the combined use of soil amendment such as biochar and attapulgite clay with SRF improved soil environmental quality and fertility, thereby creating superior soil conditions for crop growth (Bai et al., 2024; Hu et al., 2024). In our case, experimental results demonstrated that the combined application of SRF and soil amendment significantly increased soil temperature and moisture while reducing soil bulk density in both the NR30 and NNR systems (Fig. 2g-i). This can be attributed to three key mechanisms: firstly, the high porosity and strong adsorption capacity of biochar and attapulgite clay enhanced soil aggregate formation, thereby improving soil aeration and water retention capacity (Zhang et al., 2024). Secondly, the large specific surface area of biochar enabled it to absorb and retain more solar radiation, directly contributing to the observed increase in soil temperature (Fig. 2h). Lastly, SRF released nutrients in synchrony with crop growth stages, enhancing root activity and respiration, which further improved soil hydrothermal conditions (Li et al., 2021; Fig. 2g-h). These findings robustly support our first hypothesis, highlighting the synergistic effects of SRF and soil amendments on soil physical properties. In NR30 system, the combined use of SRF and biochar significantly increased soil pH and reduced soil salt content and electrical conductivity compared with NNR system (Fig. 3). This phenomenon indicated that the combination of SRF and biochar could effectively prevent soil salinization, thereby enhancing soil quality. The coupling effect of SRF and soil amendment on improving soil hydrothermal conditions, as well as enhancing soil quality, further supported the reasons for increased crop yields.

4.2. Impact of SRF and soil amendment on soil nutrient, microbial biomass carbon and nitrogen under nitrogen reduction

SRF and soil amendments (such as biochar and attapulgite) elevates soil nutrients through enhanced adsorption capacity reducing nutrient leaching and mitigating gaseous nutrient losses (Wang et al., 2022), while amendments conserve native nutrients via organic-mineral interactions and aggregate-induced negative priming (Chen et al., 2024). Concurrently, these inputs improve soil structure by increasing macroaggregates and modifying C:N stoichiometry, stimulating microbial-mediated positive priming on SOC mineralization that augments nutrient availability (He et al., 2024). Consistent with the second hypothesis, our case found that the combined application of SRF and soil amendment significantly increased soil NO_3^--N and NH_4^+-N in both systems (Fig. 4 and Fig. 11). Notably, the magnitude of increase in NO_3^--N and NH_4^+-N was significantly higher in the NR30 system compared with the NNR system, with the combination of SRF and biochar showing the most pronounced enhancement effect on soil nutrients (NO_3^--N and NH_4^+-N), followed by attapulgite (Fig. 4). This phenomenon can be explained by several mechanisms: biochar improved soil hydrothermal conditions and physical structure, while SRF synchronized nutrient release with crop growth stages (Fig. 2g,h and Fig. 4). Additionally, the low nitrogen environment in the NR30 system facilitated nodule nitrogen fixation in common vetch (Fig. 2g,h and Fig. 10), and the availability of soil nitrogen relative to carbon input mediated microbial growth, alleviating microbial nitrogen limitation by reducing the soil C/N ratio (Zhang et al., 2022). Furthermore, the combined use of SRF and biochar also had a higher potential to enhance soil nutrients, primarily by promoting the negative priming effect due to the physical adsorption of biochar, thus reducing the leaching and loss of nutrients and improving soil nutrient levels in the NNR system.These findings robustly support our second hypothesis.

The release, fixation, and cycling of nutrients in agricultural soils primarily depend on soil microbial activities (Ning et al., 2021). In our case, the combined use of SRF and soil amendment significantly increased soil MBC and MBN in both nitrogen-level systems, with the combination of biochar and SRF eliciting a stronger positive response in microbial activity, particularly in the NR30 system, where the highest MBC and MBN values were recorded (Fig. 5). This finding contradicted our second hypothesis. The observed phenomenon can be attributed to several key factors: on one hand, the principal component analysis (PCA) revealed that soil MBC and MBN were significantly positively correlated with soil moisture across all treatments (Fig. 9a), SRF and biochar combination had the most pronounced effect on increasing soil moisture in the NR30 system (Fig. 2 and Fig. 11), with optimal moisture levels being a primary driver of increased microbial activity. On the other hand, soil total nitrogen (TN) showed a significant positive correlation with MBC and MBN (Fig. S9a,b), and structural equation modeling (SEM) indicated that soil TN had the strongest influence on MBC and MBN in the nitrogen reduction system (Fig. 10a,c). This suggested that nitrogen limitation, rather than soil amendment alone, was the main controlling factor for changes in MBC and MBN, and the addition of exogenous biochar and SRF provided essential resources for soil microorganisms. Based on the above results and the principle of soil nutrient balance, there was a potential competition for soil nitrogen between soil microbes and crops in the NR30 system (Liu et al., 2021), which was another important explanation for the lower crop yield in NR30 system compared with the NNR system.

4.3. Regulation mechanism of SRF and soil amendment on soil carbon and nitrogen storage under nitrogen reduction

Farmland soil carbon and nitrogen stocks are crucial components of terrestrial ecosystem carbon and nitrogen pools, owning a significant role in the global carbon and nitrogen cycles (Francesca et al., 2021). Regulated by the stoichiometric balance of elements, increased external carbon inputs and coordinated nitrogen management in agriculture systems with varying nitrogen additions significantly impact soil organic carbon and soil nitrogen pools (Zang et al., 2024). Consistent with the third hypothesis, our study found that the SOC in the NR30 system was consistently lower than in the NNR system throughout the study period (Fig. S1). This was primarily because reduced nitrogen input increased the mineralization of the labile organic carbon pool, decreased soil RubisCO enzyme activity (Fig. 7), and increased CO_2 release, leading to a reduction in SOC. This indicated that reduced nitrogen input was detrimental to soil carbon sequestration, which was in line with the research results of Zang et al. (2024). The combined use of SRF and biochar significantly increased SOC in both systems (Fig. S1 and Fig. 11), and the soil carbon storage (SCS) of the combination of SRF and biochar in the NR30 system (N1SB) was significantly higher compared with the NNR system with conventional nitrogen fertilizer alone (N2TC) (Fig. 8). This phenomenon suggested that the combined application of biochar and SRF could effectively compensate for the loss of SCS due to reduced nitrogen input, which was mainly because biochar increased carbon storage capacity by providing easily degradable organic substrates, enhancing soil sucrase and α-glucosidase activities and microbial carbon, thus promoting the efficient transformation of labile organic substrates into stable organic components (Kalu et al., 2024) and reducing CO_2 emission (Fig. 7). The significant increase in soil MBC in the NR30 system also supported this view (Fig. 5 and Fig. 9b). Additionally, we found that SRF and biochar combinationr significantly increased SCS even in the NNR system (Fig. 11), which indicated that in addition to the combined use of SRF and biochar to increase the effectiveness of soil carbon pool, the inhibition of nitrogen input on mineralization was also a crucial factor for the stability of soil carbon pools in arid regions.

The reduction in nitrogen input is bound to affect the soil nitrogen storage (SNS) of the entire farmland ecosystem (Wang et al., 2024). We found that soil total nitrogen (TN) in the NR30 system was significantly lower compared with the NNR system (Fig. S1). Nevertheless, contrary to the third hypothesis, the combined use of SRF and soil amendment significantly increased TN in the NR30 system at each sampling period (Fig. S1). Specifically, the combined use of SRF and biochar increased TN in the NR30 system to the level of the NNR system. The reasons for this surprising phenomenon could be attributed to the following three aspects: firstly, the reduction in nitrogen input could significantly alleviate the green manure 'nitrogen repression' phenomenon caused by excessive nitrogen input, thereby enhancing the biological nitrogen fixation capacity of green manure. Secondly, SRF and biochar combination improved soil hydrothermal conditions, thereby activating urease activity (Zhang et al., 2024), and high C/N ratio was conducive to soil nitrogen retention, significantly inhibiting the contents of nitrate reductase and nitrite reductase (Fig. 6), effectively reducing the emission of the greenhouse gas N_2O (Yao et al., 2022). Lastly, our findings revealed that soil nitrogen storage (SNS) was closely associated with urease activity, nitrate reductase, nitrite reductase, and soil sucrase. The combined application of SRF and biochar enhanced the sensitivity of urease activity and soil sucrase to microbial biomass nitrogen (MBN) in the NR30 system compared with the NNR system (Fig. 9 and Fig. S2e,h), which was more conducive to increasing SNS. It was also an important reason for the increased sensitivity of soil nutrients and crop yield to the coupling effect of SRF and biochar in the NR30 system.These results suggested that the combined use of SRF and biochar could replace part of the nitrogen fertilizer and maintain SNS levels, which attributed to the dual control mechanism that nitrogen reduction alleviated the 'nitrogen repression' of green manure and biochar altered soil C/N ratio, thereby regulating soil carbon and nitrogen storage under the dominance of microorganisms (Fig. 9b). Our findings show that SRF and biochar adoption in arid regions is economically viable, enhancing soil fertility and reducing nitrogen costs. SRF improves nutrient efficiency, while biochar boosts soil health and carbon sequestration. Despite higher initial costs, long-term savings from reduced inputs and higher yields outweigh expenses. Government subsidies could further promote adoption. However, SRF's energy-intensive production may offset some carbon benefits. A balanced approach integrating local biomass, renewable energy, and policy support is essential to maximize economic and ecological gains while minimizing trade-offs. To sum up, our findings highlight three key points: 1) in our case, the combined use of SRF and soil amendment is beneficial for the stability and enhancement of soil carbon and nitrogen pools, with biochar showing absolute potential advantages in NR30 agricultural systems. This phenomenon is mainly due to the alleviation of common vetch 'nitrogen inhibition' and the modification of soil C/N ratio by biochar, which regulates microbial activity and carbon-nitrogen turnover, providing a new pathway for forming more stable long-term carbon and nitrogen pools. 2) Based on our research results, we speculate that SRF and biochar combination can replace over 30 % of chemical nitrogen fertilizers, with the extent of replacement depending on biochar application rates and green manure planting density. 3) Our study only covers a 3-year system; longer-term experiments are necessary to verify the effectiveness of the combined application of SRF and biochar in enhancing soil carbon and nitrogen storage under nitrogen reduction in arid regions. Therefore, future research should also explore the interactions between biochar application rates, green manure species, and crop rotation systems to optimize sustainable nitrogen management strategies. Additionally, investigating the economic and ecological trade-offs of large-scale biochar production and application in arid regions will be critical for practical implementation.

5. Conclusion

In general, the combined use of SRF and soil amendment improved soil environmental quality and fertility under both nitrogen levels, with the SRF and biochar combination showing the most significant effects by inducing soil negative priming effects and ensuring continuous nitrogen supply. In the NR30 system, soil nutrients and crop yields were more sensitive to the coupling effect of SRF and biochar compared with the NNR system, primarily due to enhanced sensitivity of urease activity and soil sucrase to MBN, thereby improving soil nutrients and increasing crop yields. Nitrogen limitation was the main driver of changes in microbial biomass carbon (MBC) and MBN, with the SRF and biochar combination eliciting a stronger positive response in MBC and MBN in the NR30 system, albeit increasing competition between soil microorganisms and crops for nitrogen. The combined application of SRF and soil amendment could replace over 30 % of chemical nitrogen fertilizers while enhancing soil carbon and nitrogen storage stability, and the combination of SRF and biochar showed significant potential in this regard, mainly due to the alleviation of common vetch 'nitrogen repression' by reducing nitrogen and the alteration of soil C/N ratio by biochar which regulated microbial activity and carbon-nitrogen turnover. These findings provide new insights into the mechanisms of carbon and nitrogen pool enhancement in arid farmland ecosystems.

CRediT authorship contribution statement

Wen Yin: Writing – review & editing, Project administration, Conceptualization, Supervision, Methodology. **Lianhao Zhao:** Methodology, Conceptualization, Writing – original draft, Data curation. **Pingxing Wan:** Data curation. **Zhipeng Guo:** Investigation, Validation, Data curation. **Pan Li:** Validation, Investigation. **Qiang Chai:** Eediting and Supervision. **Zhilong Fan:** Data curation. **Falong Hu:** Validation, Investigation. **Cai Zhao:** Data curation. **Aizhong Yu:** Methodology. **Wei He:** Methodology. **Hong Fan:** Validation. **Yali Sun:** Data curation. **Feng Wang:** Investigation.

Declaration of Competing Interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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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.2025.109849.

Data availability

Data will be made available on request.

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