frontiers in Agronomy

TYPE Original Research
PUBLISHED 02 March 2026
DOI 10.3389/fagro.2026.1766927

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OPEN ACCESS

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RECEIVED 13 December 2025
REVISED 04 February 2026
ACCEPTED 04 February 2026
PUBLISHED 02 March 2026

CITATION
Gong L, Wang Y, Jiang W and He X (2026) Integrated application of green manure and mushroom residue improves soil quality and carbon-nitrogen fraction, and pepper yield and quality.
Front. Agron. 8:1766927.
doi: 10.3389/fagro.2026.1766927

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Integrated application of green manure and mushroom residue improves soil quality and carbon-nitrogen fraction, and pepper yield and quality

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Greenhouse vegetable systems face degradation from intensive inputs and weak organic matter recycling. The synergistic effect of combining green manure and mushroom residue on soil fertility, especially regarding soil carbon-nitrogen fractions, and yield-quality characteristic is poorly understood. This study investigate the impacts of green manure and mushroom residue on soil quality and pepper yield and quality. A randomized block design with five treatments, including CK (control), GM (green manure 3000 kg ha^{-1}), MR (mushroom residue 7500 kg ha^{-1}), GMS (green manure 3000 kg ha^{-1} and mushroom residue 3750 kg ha^{-1}), and GMMR (green manure 3000 kg ha^{-1} and mushroom residue 7500 kg ha^{-1}). The GM, MR, GMS and GMMR significantly improved the contents of total nitrogen (TN), total phosphorus (TP), total potassium (TK), alkaline hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and soil organic carbon (SOC), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), soil inorganic nitrogen (SIN), microbial biomass carbon (MBC), easily oxidizable organic carbon (EOC) compared to CK treatments with the order GMMR > GMS > MR > GM > CK in both soil layers. The SOC storage in the GMMR, GMS, MR and GM treatment in the 0-10 cm increased by 24.25%, 24.49%, 17.56% and 4.39% compared with CK, which increased by 54.28%, 31.55%,25.31% and 9.46% compared with the CK treatment in 10-20 cm layers, respectively. The SOC storage and carbon sequestration efficiency under different treatments followed the order: GMMR > GMS > MR > GM. Compared to CK, the GMMR and GMS treatment achieved the highest pepper yield of 48797 and 46708 kg ha^{-1}, representing a significant increase of 17.55% and 12.52%. The random forest model showed MBC is key soil factors affecting yield and soluble sugar, and NO_3^+-N is key soil factors affecting soluble protein. The GMMR treatment emerges as the optimal organic amendment strategy for similar greenhouse conditions in semi-arid regions.

KEYWORDS
carbon sequestration efficiency, microbial biomass carbon, nutritional quality, soil organic carbon storage, yield

1 Introduction

With the ongoing transformation of crop cultivation patterns in China, facility-based agriculture has experienced rapid expansion (Liu et al., 2024). As a key component of this system, Global greenhouse vegetable production has expanded in recent years, driven by rising demand for year–round supply and the adoption of resource-efficient cultivation practices. However, the absence of science-based fertilization practices frequently leads to overapplication of chemical fertilizers, contributing to a range of environmental problems including soil degradation, acidification, and greenhouse gas emissions (Zhang et al., 2024; Ren et al., 2024). These issues pose serious threats to the stability of agricultural ecosystems and long-term sustainability, underscoring the urgent need to optimize soil fertility management in protected chili cultivation.

Green manure represents a traditional organic amendment that enhances soil structure by loosening soil texture, increasing porosity, and stimulating microbial activity via root exudates, thereby elevating soil organic matter content (Li et al., 2025). Furthermore, leguminous varieties contribute additional nitrogen fixation, which can partially replace chemical fertilizers and reduce production costs (Fan et al., 2026; Liu et al., 2025). Concurrently, the utilization of agricultural waste has become a vital component of sustainable agriculture (Liang et al., 2024). For example, mushroom residue, which is rich in humus and diverse nutrients, supplies substantial nourishment to soils (Jiang et al., 2025). The application of Stropharia rugannoannulata residue in forestry systems has been shown to enhance soil fertility and advance the recycling of agricultural waste. Research confirms that combined microbial fermentation and enzymatic hydrolysis can transform mushroom residue into a nutrient-rich growth medium with improved aeration, water retention, and fertility, ultimately establishing an efficient ecological recycling system (Singh et al., 2019).

Soil organic carbon (SOC), as the most dynamic component of the soil carbon pool, serves as a critical mediator of ecosystem carbon balance (Bastida et al., 2021; He et al., 2024; Luo et al., 2020). It exerts substantial influence on soil structure, moisture, nutrient availability, and microbial activity (Jiao et al., 2020; Just et al., 2023). Changes in SOC content directly affect the size and stability of the soil carbon stock, thereby influencing the carbon sequestration potential of terrestrial ecosystems (Chen et al., 2025; Cui et al., 2024; Fan et al., 2025a). The physical occlusion of SOC significantly enhances carbon storage capacity and reduces the susceptibility of SOC to microbial decomposition. Both green manure and mushroom residue are valuable organic amendments that enhance SOC levels and improve multiple soil properties. They not only directly modify soil structure and characteristics but may also directly or indirectly affect the abundance and composition of soil aggregates (Shi et al., 2025; Ren et al., 2024). However, current research has predominantly emphasized the impact of external disturbances on aggregate stability, often overlooking the specific influence of organic inputs such as green manure and mushroom residue on aggregate formation and characteristics. Moreover, while green manure and mushroom residue are two important sources of organic fertilization, their separate application each with distinct C and N compositions, which may lead to agronomic and biochemical limitations. These limitations could constrain the improvement of soil carbon storage and undermine the long-term enhancement of soil quality.

Despite their individual benefits, the combined effects of integrating green manure and mushroom residue into protected culture systems remain largely unexplored for chili production. Most existing studies focus separately on either green manure cropping sequences or single-application scenarios of mushroom residues like stropharia rugosoannulata spent substrate (Li et al., 2020; Zhang et al., 2024). Few investigations have examined how these amendments interact when applied jointly, particularly regarding their synergistic influence on soil physicochemical properties, carbon fraction, yield and aggregate stability under greenhouse conditions. This study aims to achieve the following research objectives through field experiments: (i) systematically investigate the synergistic effects of combined green manure and mushroom residue on soil physicochemical properties, organic carbon and nitrogen fractions, aggregate size distribution and stability characteristics; (ii) elucidate the regulatory mechanisms governing the effects of combined green manure and mushroom residue on yield and quality; and (iii) establish quantitative relationships between soil parameters and yield. The anticipated outcomes of this research will contribute to China's strategic initiatives for agricultural waste resource utilization and provide technical support to mitigate the environmental pressures resulting from the excessive application of chemical fertilizers.

2 Materials and methods

2.1 Experimental site

The field experiments were conducted in a greenhouse of Gaoqiao town, Ansai district (36°38' N, 109°16' E) from 2023 to 2024. The site is situated at an elevation of 1192 m and characterized by a semi-arid temperate monsoonal climate, featuring a mean annual temperature of 8.8 °C and annual precipitation of 540 mm. Rainfall occurs predominantly between June and September, accompanied by high annual evaporation reaching 1800 mm. Sunshine duration totals 2400 hours annually, accumulated temperatures range from 3500 to 4600 °C, and the frost-free period lasts 160–200 days. The dominant soil type is sandy loam (plow layer properties): pH 7.9, organic matter content of 8.7 g kg^{-1}, total nitrogen of 0.56 g kg^{-1}, alkaline hydrolyzable nitrogen of 58.76 mg kg^{-1}, available phosphorus of 13.98 mg kg^{-1}, available potassium of 111.34 mg kg^{-1}, and average dry bulk density of 1.2 g cm^{-3}.

2.2 Experimental materials

The aboveground plant residues of Astragalus sinicus (Chinese milk vetch) were harvested and used as green manure, which the nutrient composition of 2.13–2.56% N 0.86–1.07% P, 1.53–1.89% K, and 12.1–15.4 C/N ratio. Mushroom spent substrate was collected after removing the plastic film from discarded spent mushroom,

which include of 1.82–2.21% N, 0.62–0.94% P, 1.27–1.65% K, 10-13 C/N ratio for mushroom residue. The variety of pepper (Capsicum annuumL.) is Yan'an University pure No. 8.

2.3 Experimental design

The experiment was carried out in a controlled greenhouse (plastic tunnel structure, 30 m × 8 m, with temperature control at 20-25 °C ensuring applicability to protected cultivation systems. A randomized block design with five treatments involving green manure and mushroom residue applications for two years (2023-2024): CK (control without amendments), GM (3000 kg/ha milk vetch green manure only), MR (7500 kg ha^{-1} mushroom residue only), GMS (3000 kg ha^{-1} milk vetch plus 3750 kg ha^{-1} mushroom residue), and GMMR (3000 kg ha^{-1} milk vetch plus 7500 kg ha^{-1} mushroom residue). Each treatment was replicated three times in 30 m^2 plots separated by 1m isolation zones. All amendments were incorporated into the 0–20 cm soil layer using a rotary tiller for homogeneous mixing. Conventional chemical fertilizers were applied at rates of 180 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1}, and 120 kg K_2O ha^{-1} for all treatments. Pepper were grown under uniform groundwater irrigation at 50 mm per event, with pest and weed management following local practices.

2.4 Sample collection and measurement

Soil samples were collected in havesting stage from ten points within each treatment plot, where soil blocks were carefully fractured along natural cracks and mix them together to form one soil sample. After removing roots, gravel, and plant or animal debris while sealed in plastic containers. Upon transportation to the laboratory, three subsamples from the same plot were pooled to form three composite samples per treatment. A portion of each composite sample was stored at 4 °C for analyzing soil carbon and nitrogen fractions, while the remainder was air-dried, sieved, and used for determining basic soil physicochemical properties.

Soil pH was measured using a pH meter with a water-to-soil ratio of 5:1. Nitrate nitrogen and ammonium nitrogen were determined by spectrophotometry after KCl extraction and indophenol blue colorimetry, respectively. The content of total nitrogen (TN) was measured using a Kjeldahl nitrogen apparatus. Total phosphorus (TP) was determined by molybdenum-antimony anti-colorimetry after sodium hydroxide fusion. Total potassium (TK) was measured by flame photometry after sodium hydroxide fusion. Alkali-hydrolyzable nitrogen (AN) was assessed using the alkali diffusion method. Available phosphorus (AP) was measured by the sodium bicarbonate extraction-molybdenum antimony anti-colorimetry method, while available potassium (AK) was determined by the ammonium acetate extraction-flame photometry method. Soluble protein, soluble sugar and vitamin C were measured by coomassie brilliant blue assay, anthrone colorimetry, and 2,6-dichlorophenolindophenol titration methods, respectively. Easily oxidizable organic carbon (EOC) was analyzed via potassium permanganate oxidation and colorimetry. Dissolved organic carbon (DOC) was extracted with distilled water and subsequently measured. Microbial biomass carbon (MBC) was assessed using the chloroform fumigation-extraction technique. The content of SOC was determined by the potassium dichromate oxidation-external heating method.

The calculation method for SOC storage (t ha^{-1}), change in SOC storage (t ha^{-1}) and soil carbon sequestration rate (t ha^{-1} a) using the following equations:

SOC storage for each soil layer (SOC storage) = SOC × BD × h × 0.1.

Change in SOC storage (t ha^{-1}) =SOC_{st} − SOC_{sk}.

Soil carbon sequestration rate (t ha^{-1} a)=Change in SOC storage/t.

Where SOC is the soil organic carbon content (g kg^{-1}), BD is the soil bulk density (g cm^{-3}), h is the thickness of the soil layer (cm), SOC_{st} (t ha^{-1}) is the SOC storage under treatment conditions, SOC_{sk} is the SOC storage under CK, t is the duration of the treatment (years).

2.5 Statistical analysis

Descriptive statistical analysis was conducted using IBM SPSS Statistics 22 (IBM Corp., Armonk, N.Y., USA) to evaluate the mean and standard deviation of each parameter. ANOVA was conducted to assess significant differences among treatments in the same layer (at $p < 0.05$), followed by the Duncan method for pairwise comparisons. All graphs were drawn using Origin 2025 (Origin Lab Corporation, Northampton, MA, USA). Pearson's correlation analysis was used to reveal the relationships among the measured parameters. A random forest model was employed to reveal the key soil factors influencing pepper yield, yield, soluble protein, soluble sugar, and vitamin C. These relationships were calculated and visualized using R software, with the ggplot2 package for plotting.

3 Results

3.1 Soil physicochemical properties

The application of green manure and mushroom residue significantly enhanced soil fertility by increasing the contents of SOC, total and available nitrogen, phosphorus, and potassium. Significant differences in these soil properties among the different treatments across both the 0-10 cm and 10-20 cm soil layers (Figure 1). In the topsoil (0-10 cm), the combined GMMR treatment consistently yielded the highest values for all measured indicators. Specifically, compared to the CK treatment, the GMMR treatment significantly increased the contents of total nitrogen (TN), total phosphorus (TP), total potassium (TK), alkaline hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and soil organic carbon (SOC) by 17.9%, 56.5%, 27.2%, 88.9%, 34.5%, 46.2%, 53.0%, 71.5%, and 26.4%, respectively (Figure 1). Consequently, the ranking for all soil nutrient levels in the 0-10 cm layer was consistently observed as GMMR > GMS > MR > GM > CK. This trend persisted in the subsurface soil (10-20 cm), where the GMMR treatment also demonstrated superior performance, increasing the content of TN, TP, TK, AN, AP, AK, NH_4^+-N, NO_3^-N, and SOC by 25.4%, 61.5%, 45.4%, 48.7%, 45.1%,

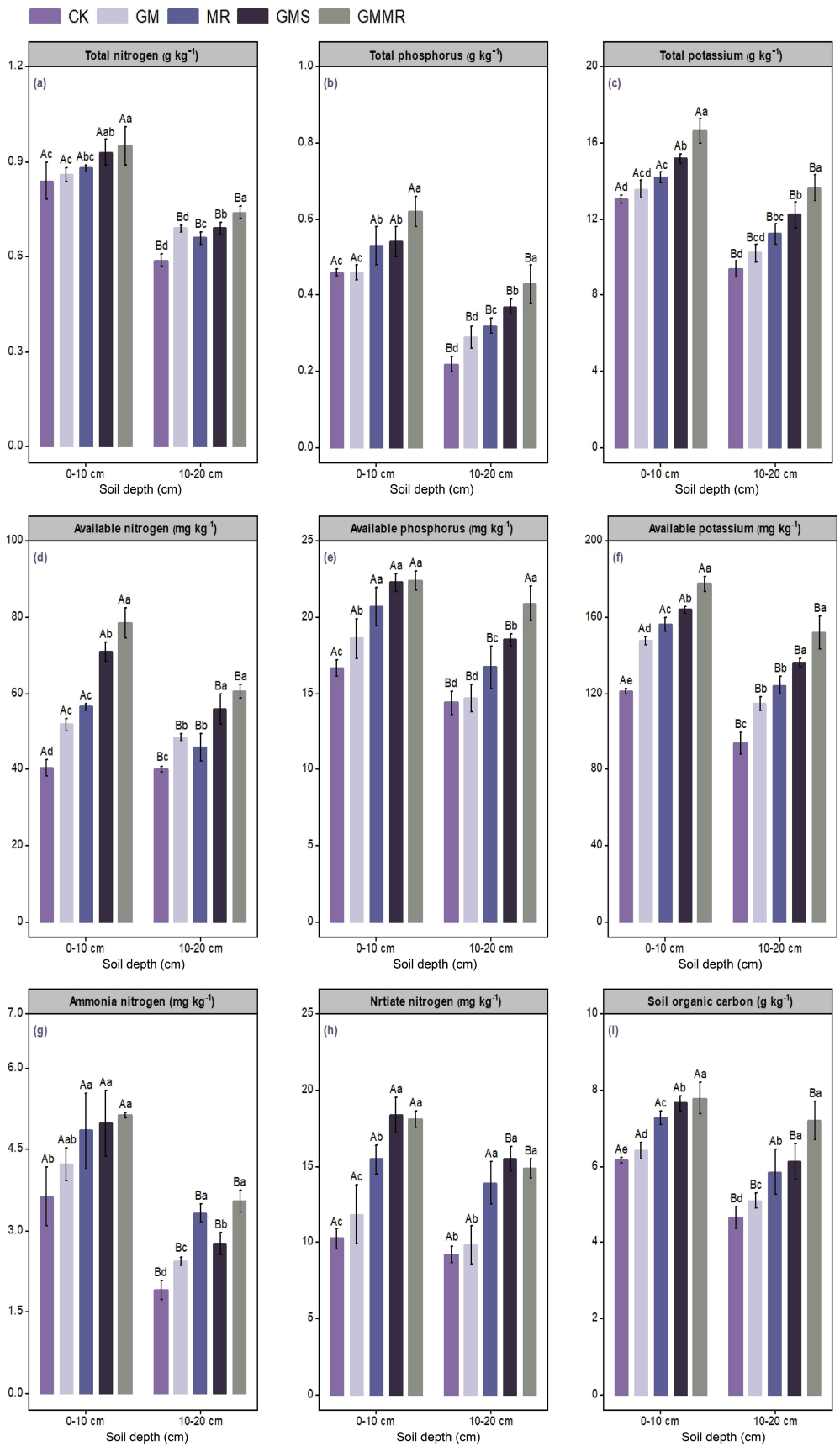

FIGURE 1
Changes in soil physicochemical properties **(a–i)** under different treatments. Distinct uppercase letters denote significant differences in the same treatment across different soil layers ($P < 0.05$), while the lowercase letters represent significant differences between various treatments within the same soil layer ($P < 0.05$).

65.3%, 87.7%, 53.9%, and 54.2% compared to CK (Figure 1). Furthermore, as expected, the contents of all soil nutrients exhibited a decreasing trend with increasing soil depth.

3.2 Dissolved organic carbon, dissolved organic nitrogen, and soil inorganic nitrogen

The content of soil dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and soil inorganic nitrogen (SIN) varied significantly among treatments in both soil layers (Figures 2a–c). The content of DOC, DON and SIN was consistently higher at the soil depth of 0-10 cm than 10-20 cm, with the order GMMR > GMS > MR > GM > CK in both soil layers (Figures 2a–c). In the 0-10cm layer, the GM, MR, GMS, and GMMR treatments increased DOC by 7.8%, 12.6%, 29.0%, and 38.8%, with increased DON by 3.0%, 12.3%, 21.5%, and 41.3%, which increased SIN by 13.6%, 29.6%, 34.6%, and 63.1% compared to CK. In the 10-20 cm layer, all treatments also significantly enhanced DOC, DON and SIN, which improvements ranging from 9.2%-54.2%, 7.2%-40.4%, and 8.6%-57%.

3.3 Soil microbial biomass carbon and easily oxidizable organic carbon

The effects of green manure and mushroom residue on soil microbial biomass carbon (MBC) and easily oxidizable organic carbon (EOC) are shown in Figures 2d, e. Significant differences in MBC content were observed among treatments, with the overall order were GMMR was highest. The content of MBC in GMS treatments increased by 8.2%, 18.8%, 25.7% than GM, MR, CK treatment in the 0-10 cm, which 4.5%, 32.9%, 31% higher than GM, MR, CK treatment in the 10-20 cm soil layer (Figure 2d). The content of EOC in GM, MR, GMS, and GMMR treatments increased by 5.5%, 17.4%, 19.4%, and 27.4% compared to CK, respectively (Figure 2e). In the 10-20 cm layer, the content of CK were lower by 39.2%, 48.8%, 48.5%, and 50.6% than GM, MR, GMS, and GMMR treatments, respectively (Figure 2e). These results demonstrate that the synergistic application of green manure and mushroom residue (particularly the GMMR treatment) can effectively enhance the relatively active easily oxidizable organic carbon fraction in the soil, contributing positively to soil fertility and carbon pool quality.

3.4 Soil carbon storage and carbon sequestration efficiency

The effects of green manure and mushroom residue applications on SOC storage (Figures 3a, b) and changes in SOC storage (Figures 3c, d) in the 0-10 cm and 10-20 cm soil layers are illustrated in Figures 3a–d. SOC storage showed a decreasing trend with the increase in soil depth (Figures 3a–d). Green manure and mushroom residue significantly affected SOC storage in both soil layers. SOC storage in GMMR, GMS, MR, and GM treatments was significantly higher than that under the CK treatment ($p < 0.05$), while no significant difference was observed between the MR and GM treatments ($p>0.05$) (Figures 3a, b). The SOC storage in the GMMR, GMS treatment in the 0-10 cm increased by 24.25%, 24.49%, 17.56% and 4.39% compared with the CK treatment, which increased by 54.28%, 31.55%,25.31% and 9.46% compared

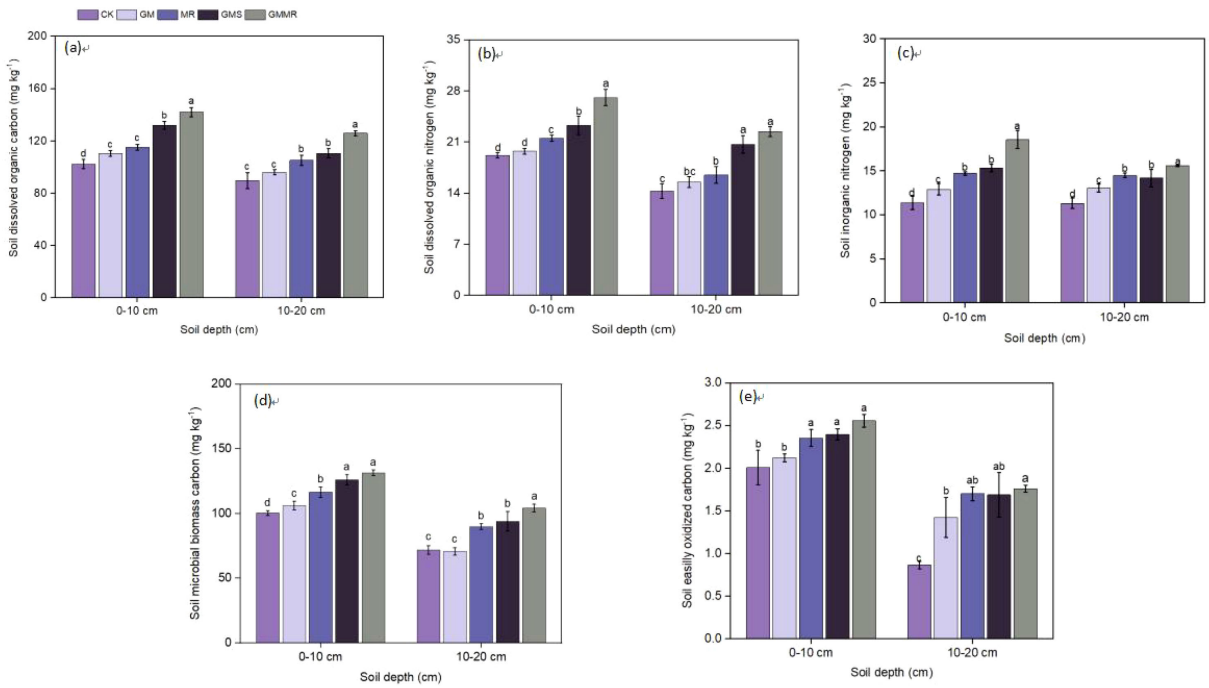

FIGURE 2
The content of dissolved organic carbon **(a)**, dissolved organic nitrogen **(b)**, soil inorganic nitrogen **(c)**, soil microbial biomass carbon **(d)** and easily oxidizable organic carbon **(e)** under different treatments in both soil layers. The lowercase letters represent significant differences between various treatments within the same soil layer.

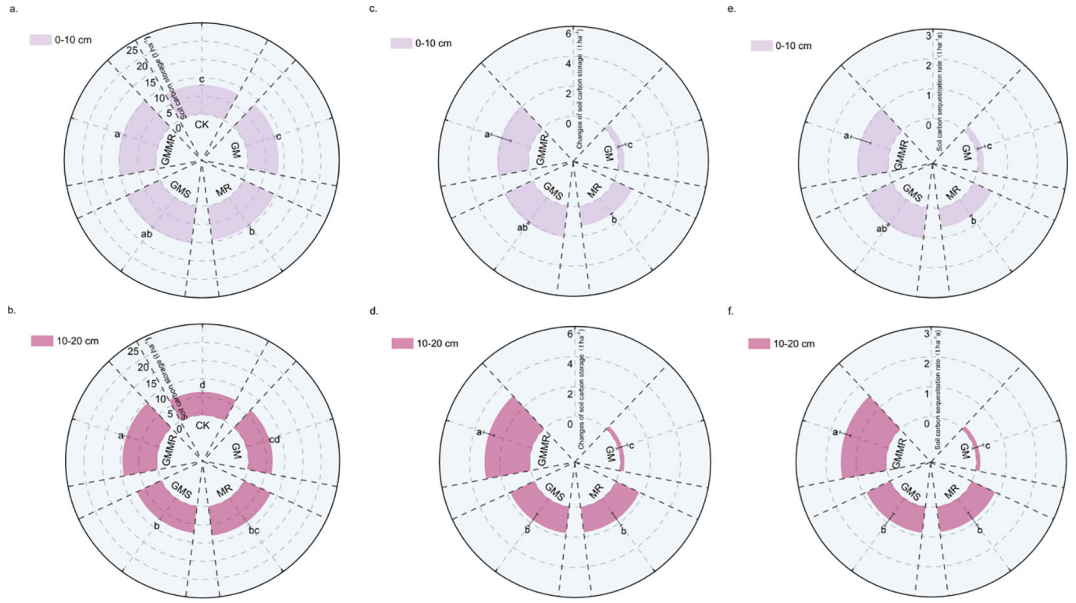

FIGURE 3
Soil organic carbon storage **(a, b)**, changes of soil organic carbon storage **(c, d)**, carbon sequestration efficiency **(e, f)** under different green manure and mushroom residue treatments. The lowercase letters represent significant differences between various treatments within the same soil layer.

with the CK treatment in 10-20 cm layers, respectively. Overall, the SOC storage under different treatments followed the order: GMMR > GMS > MR > GM.

Soil carbon sequestration efficiency reflects the conversion degree of unit exogenous organic carbon in soil and is used to characterize the evolution characteristics of SOC. Green manure and mushroom residue applications had significant effects on the SOC sequestration rate in the 0-10 cm and 10-20 cm soil layers (Figures 3e, f). In both soil layers, the carbon sequestration efficiency gradually increased with the increase in the application rate of green manure and mushroom residue. The soil carbon sequestration efficiency of the GMMR, GMS, MR and GM treatments was 1.07 t ha^{-1} a, 0.98 t ha^{-1} a, 0.703 t ha^{-1} a, and 0.17 t ha^{-1} a in the 0-10 cm soil layer, respectively, which were 1.7 t ha^{-1} a, 0.98 t ha^{-1} a, 0.79 t ha^{-1} a, and 0.29 t ha^{-1} a in the 10-20 cm soil layer.

3.5 Effects of green manure and mushroom residue on pepper yield and quality

The application of green manure and mushroom residues significantly influenced crop yield and quality. All treatments exhibited varying degrees of enhancement Compared to CK, with the combined application of green manure and mushroom residues (GMS and GMMR treatment) showed the best performance. Compared to CK, the GMMR and GMS treatment achieved the highest pepper yield, representing a significant increase of 17.55% and 12.52%.The MR and GM treatment increased yield by 8.91% and 3.39% compared to CK, which no significant difference was observed between these two treatments (Table 1).

Regarding quality-related physiological indicators, the highest soluble protein content in the GMS treatment, which represented a 10% increase over CK, and also showed the greatest content of vitamin C, with an increase of 25.84% (Table 1). The GMMR treatment exhibited a content of soluble sugar similar to that of the MR treatment, which was 4.87% higher than CK. Meanwhile, the content of vitamin C under the GMS treatment was notably higher than that in CK, with an increase of 25.13% (Table 1). Overall, the effects of different treatments on the measured indicators followed a consistent trend was GMMR ≥ GMS > MR > GM > CK. This indicates that the combined application of green manure and mushroom residues not only significantly improves pepper yield and quality but also synergistically enhances plant physiological metabolism and quality formation. Among the treatments, GMMR showed greater advantages in yield enhancement, while GMS performed better in certain quality-related indicators.

3.6 Correlation among variables based on the spearman correlation analysis and random forest model

According to the spearman correlation analysis (Figure 4a), there are varying degrees of significant correlations between soil nutrients, carbon-nitrogen components, and organic carbon storage under treatments with fungal residue and green manure and the soluble protein, soluble sugar, VC, and yield. Soil nutrient indicators (AP, AK, NH_4^+-N) also exhibit highly significant strong positive correlations with yield, soluble protein, soluble sugar, and VC. This indicated that the supply of available soil nutrients is fundamental to ensuring both high quality and high pepper yield. The content of SOC, DOC, MBC and soluble protein,

TABLE 1 Changes of pepper yield and quality under different green manure and mushroom residue treatments.

Plant physiological indicators	Treatments				
	CK	GM	MR	GMS	GMMR
Yield (kg ha^{-1})	41514 ± 632.7^{d}	42920.33 ± 838.3^{d}	45209.33 ± 1050.8^{c}	46708 ± 471.02^{b}	48797 ± 965.15^{a}
Soluble protein (mg/kg)	0.40 ± 0.03^{b}	0.41 ± 0.01^{ab}	0.42 ± 0.01^{ab}	0.44 ± 0.01^{a}	0.43 ± 0.02^{a}
Soluble sugar (%)	6.16 ± 0.12^{b}	6.29 ± 0.05^{ab}	6.45 ± 0.11^{a}	6.42 ± 0.09^{a}	6.46 ± 0.08^{a}
Vitamin C (mg $100g^{-1}$)	147.73 ± 4.72^{c}	166.3 ± 1.12^{b}	172.02 ± 2.04^{b}	185.91 ± 1.90^{a}	184.89 ± 4.53^{a}

Different lowercase letters indicate significant differences between treatments at $p < 0.05$. CK (control), GM (green manure 3000 kg ha^{-1}), MR (mushroom residue 7500 kg ha^{-1}), GMS (green manure 3000 kg ha^{-1} and mushroom residue 3750 kg ha^{-1}), and GMMR (green manure 3000 kg ha^{-1} and mushroom residue 7500 kg ha^{-1}).

soluble sugar, VC, and yield all show extremely significant positive correlations ($p \leq 0.001$, Figure 4a), with mostly moderate to strong correlation strengths (Mantel's r≥0.25). This demonstrated that the richness and activity of the soil organic carbon storage directly promote the formation of pepper quality traits and the increase in yield (Figure 4a).

The random forest model showed the soil factors explained 97.4%, 88.9%, 93.8%, and 96.9% and of the variability in the yield, soluble protein, soluble sugar, and VC, respectively (Figure 4b), whereas MBC is key soil factors affecting yield and soluble sugar, and NO_3^+-N is key soil factors affecting soluble protein. Addition, AP is key soil factors affecting VC ($p < 0.05$, Figure 4b).

4 Discussion

4.1 Effect of green manure and mushroom residue on soil physical and chemical properties

In this study, GMMR treatment significantly increased TN, TP, TK, AN, AP, AK, NH_4^+-N, NO_3^-N, and SOC in the 10-20 cm soil layer, with the same ranking as in the 0-10 cm layer (GMMR > GMS > MR > GM > CK). This aligns with Wang et al. (2021) in their study of Rational utilization of Chinese milk vetch improves soil fertility and production in cropping systems, which concluded that leguminous green manure can simultaneously provide active carbon sources and nitrogen fixation while systemically enhancing soil fertility and crop productivity. Additionally, mushroom residue as an organic amendment rich in organic matter and mineral elements, which can establish a more stable nutrient pool and slow-release effect, thereby amplifying the synergistic benefits of combined application (Martín et al., 2023). This study demonstrated that all treatments significantly increased the contents of AP and AK besides for the CK treatment. The content of TN, TP, TK, AN, AP, AK, NH_4+-N, NO_3^-N, and SOC exhibit a gradual decrease with increasing soil depth. This is because the topsoil layer serves as the primary site for organic matter input and microbial activity, while downward migration is restricted (Jobbágy and Jackson, 2000). However, mechanical incorporation methods can still maintain significant soil improvement effects in the 10-20 cm layer. Furthermore, adequate organic inputs enhance aggregate stability and cation exchange capacity, thereby improving nutrient retention and buffering capacity for elements such as phosphorus and potassium (Annabi et al., 2011; Abiven et al., 2009). Consequently, this provides a structural explanation for the sustained increases in the content of TN, TP, TK, AN, AP, AK, NH_4^+-N, NO_3^-N, and SOC in both soil layers.

4.2 Effect of green manure and mushroom residue on organic carbon-nitrogen fraction

Significant differences in the treatments of green manure and mushroom residue application on the content of DOC, DON, SIN, MBC, and EOC showed the potential of combined applications of green manure and mushroom residue to improve soil health and fertility. The content of DOC in the soil varied significantly among different application treatments. Notably, in the 0-10 cm layer, the GMMR treatment demonstrated the most significant increase (47.69%), while in the 10-20 cm layer, it achieved a 42.11% improvement. This finding supports the research by Ren et al. (2024), which pointed out that the application of organic materials can significantly enhance the content of soluble organic carbon in the soil, thereby benefiting the functionality of soil ecosystems. The content of DON also reached its maximum value under the GMMR treatment, showing increases of 68.42% and 65.00% compared to CK, respectively. This phenomenon aligns with the findings of Yao et al. (2021), which showed that the introduction of organic nitrogen sources, particularly through green manure and mushroom residue, effectively increases the available nitrogen in the soil for plant growth.

The GMMR treatment exhibited the highest content of SIN, with increases of 35.29% and 41.18% in the 0-10 cm and 10-20 cm layers, respectively. This indicates that the combined application significantly enhances the content of SIN readily available for crop uptake, consistent with the findings of Liu et al. (2020), which demonstrated that the combined use of various organic fertilizers can promote the accumulation of inorganic nitrogen in the soil. Similarly, the GMMR treatment showed the best performance in the content of MBC, with an increase of 48.21%in the topsoil layer. This suggests that the application of green manure and mushroom residue can provide a rich source of available carbon for soil microorganisms, enhancing their activity and biomass. According to Feng et al. (2024),

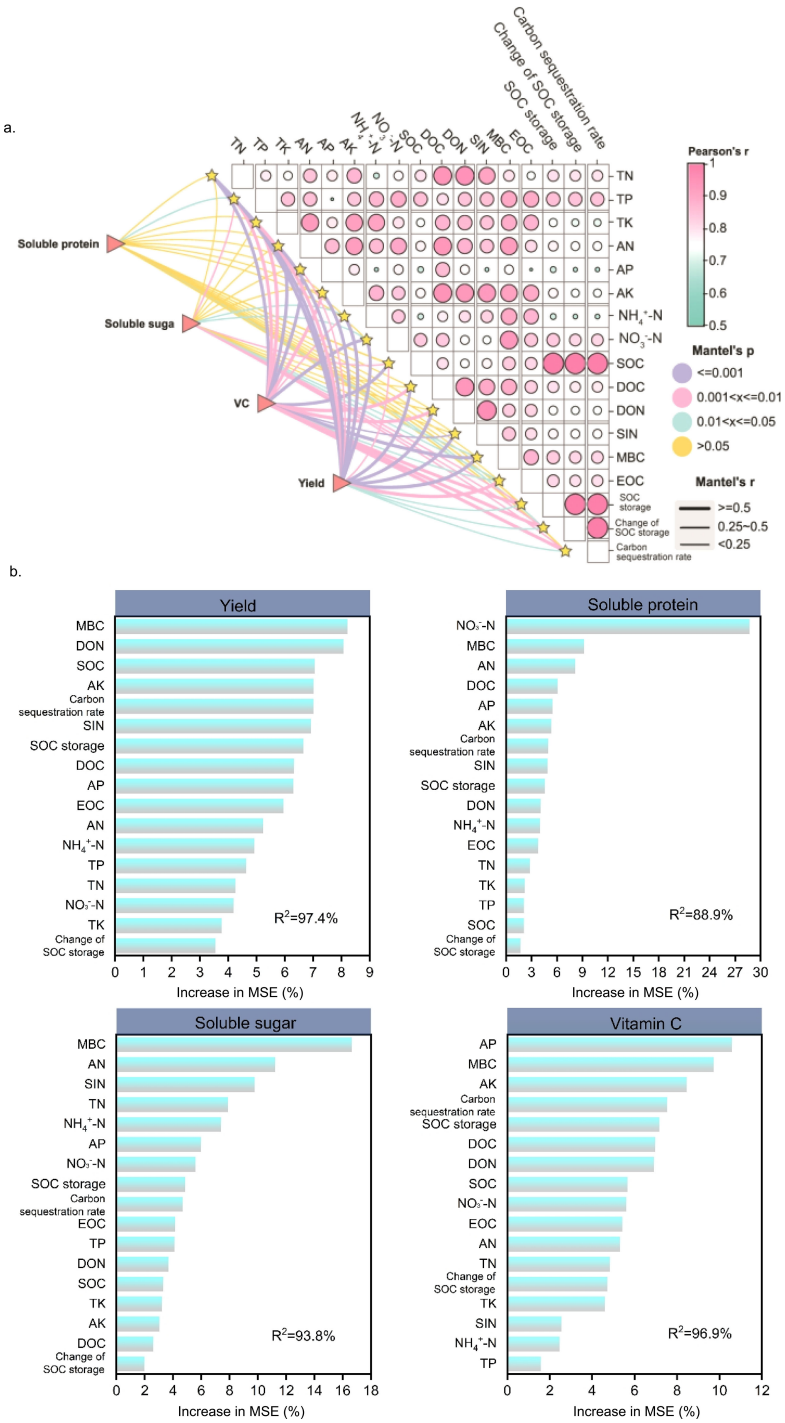

FIGURE 4
(a) Pearson's correlation analysis of total nitrogen (TN), total phosphorus (TP), total potassium (TK), alkaline hydrolyzable nitrogen (AN), available phosphorus (AP), available potassium (AK), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), soil organic carbon (SOC), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), soil inorganic nitrogen (SIN), microbial biomass carbon (MBC), easily oxidizable organic carbon (EOC), soil organic carbon storage (SOC storage), change of SOC storage, and carbon sequestration efficiency. **(b)** Percent increase in mean square error (MSE) for yield, soluble protein, soluble sugar, and vitamin C based on random forest model.

microbial activity is closely related to the addition of organic carbon, which further supporting our findings. In the 0-10 cm layer, EOC increased by 46.67% under this treatment. This is particularly important for improving soil fertility and enhancing the quality of the carbon pool, in line with the conclusions of Liptzin et al. (2022), which indicated that easily oxidizable organic carbon components are crucial indicators for assessing soil health.

4.3 Effect of green manure and mushroom residue on soil physicochemical properties and their correlations with yield and quality

Integrated application of green manure and mushroom residue, with the GMMR treatment showing the most pronounced effects, systematically enhances soil physicochemical properties and

biological activity. These improvements collectively establish a synergistic physical chemical biological basis for increasing both pepper yield and quality. In this study, random forest model and correlation analyses jointly indicated that the AP exhibits the strongest driving relationship with vitamin C, whereas NO_3^-N showed the closest association with soluble protein content (Figure 4). This confirms that the form and intensity of nutrient supply directly regulate secondary metabolic pathways in plants. Green manure contributes biological nitrogen fixation and readily mineralizable nitrogen sources, while mushroom residue provides slow-release phosphorus, potassium, and other trace elements, collectively established a nutrient supply regime that integrates immediate availability with sustained release (Han et al., 2026; Liang et al., 2024; Li et al., 2020). This integrated nutrient supply regime not only satisfies the substantial nitrogen, phosphorus, and potassium requirements of peppers to support high yield, but also offers targeted nutritional support for the synthesis of quality-related constituents (Lenka et al., 2022). These include vitamin C, which relies on phosphorus-mediated photophosphorylation and reducing equivalents, as well as soluble proteins.

Furthermore, our research found that the significant positive correlations observed among SOC, MBC, DOC, and all yield and quality indicators reveal a deeper regulatory mechanism (Figure 4). The addition of organic materials not only elevates the total carbon pool but also specifically promotes the formation of active carbon fractions (Mustafa et al., 2020; Zhang et al., 2023). Consequently, the highest carbon sequestration efficiency and active carbon content observed under the GMMR treatment not only represent superior carbon storage potential but also signify a more functional and plant-growth-promoting rhizosphere microenvironment.

5 Conclusions

The application of green manure and mushroom residue significantly enhanced soil fertility by increasing the contents of TN, TP, TK, AN, AP, AK, NH_4^+-N, NO_3^-N, and SOC, with the same ranking as in the 0-10 cm and 10-20cm soil layer. The content of DOC, DON, SIN, MBC and EOC was consistently higher at the soil depth of 0-10 cm than 10-20 cm, with the order GMMR > GMS > MR > GM > CK in both soil layers. The SOC storage in the GMMR, GMS treatment in the 0-10 cm increased by 24.25%, 24.49%, 17.56% and 4.39% compared with the CK treatment, which increased by 54.28%, 31.55%,25.31% and 9.46% compared with the CK treatment in 10-20 cm layers, respectively. In comparison to the control (CK), the GMMR and GMS treatments resulted in the highest pepper yields of 48,797 kg ha^{-1} and 46,708 kg ha^{-1}, reflecting substantial increases of 17.55% and 12.52%, with the order being GMMR ≥ GMS > MR > GM > CK. The random forest analysis identified MBC as a key soil factor influencing both yield and soluble sugar, while NO_3^--N was crucial for soluble protein content. Practically, adopting integrated application of green manure 3000 kg ha^{-1} plus 7500 kg ha^{-1} mushroom residue (GMMR) in greenhouse systems can promote sustainable management by reducing synthetic fertilizer use and enhancing long-term soil health.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LG: Writing – original draft, Conceptualization, Data curation, Methodology. YW: Funding acquisition, Writing – review & editing, Conceptualization. WJ: Validation, Funding acquisition, Writing – review & editing, Methodology. XH: Methodology, Writing – review & editing, Software.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work is funded by the National Natural Science Foundation of China, Grant No. 32560774, Shaanxi Provincial Education Department (Grant No.24JS059), Shaanxi Provincial Innovative and Entrepreneurial for college student Project (S202410719119), and Shaanxi Provincial Science and Technology Department key research plan project (Grant No.2025JC-YBMS-228).

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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