

Manure and mineral fertilizer synergistically drive soil organic carbon recovery in reclaimed cropland through aggregate turnover and plant-derived inputs

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Abstract

Reclaimed croplands often have depleted soil organic carbon (SOC) and impaired soil structure, yet the microbial and plant mediated mechanisms governing SOC recovery under long-term fertilization remain poorly understood. We conducted a 10-year field experiment with four fertilization treatments, including no fertilization (CK), mineral fertilizer (NPK), manure alone (M), and combined manure and mineral fertilizer (MNPK), to investigate how fertilization regulates carbon inputs, aggregate-associated SOC, microbial transformation, and SOC recovery in reclaimed cropland. and enhanced total lignin phenol concentrations by 13%–30%, indicating greater incorporation of plant-derived carbon. Manure treatments also increased SOC across aggregate-size fractions, with MNPK increasing SOC in > 2 mm and 0.25–2 mm aggregates by 69%–124% and 57%–62%, respectively. However, SOC accumulation occurred despite limited improvement or even declines in aggregate stability, suggesting a decoupling between carbon retention and aggregate stability during the early recovery of structurally degraded reclaimed soils. Microbial necromass carbon showed contrasting responses between years, while its contribution to SOC declined under manure treatments, remaining below 30%, indicating a dynamic but non-dominant role in SOC accumulation during the recovery process. In contrast, lignin-derived carbon showed a progressive increase relative to SOC during reclamation, suggesting an increasingly important contribution of plant-derived carbon to SOC formation. Overall, long-term manure application promoted SOC recovery primarily by increasing plant-derived carbon inputs and enhancing carbon retention within soil aggregates, with MNPK providing the strongest combined benefits for crop productivity and SOC accumulation. These findings highlight the importance of integrating carbon inputs with aggregate-associated retention when designing fertilization strategies for the restoration of degraded and reclaimed agricultural soils.

1 Introduction

Mining activities, intensive land use, and other anthropogenic disturbances have resulted in extensive soil degradation and substantial losses of soil organic carbon (SOC) stocks worldwide [47], threatening both agricultural productivity and ecosystem services. The scale of mining-induced land disturbance is substantial: global mapping has identified approximately 10.16 million ha of mining-related land across 145 countries [33]. This challenge is particularly pronounced in China, where underground coal mining has damaged approximately 3.16 million ha of land, with an additional 65,000 ha affected annually. The extensive overlap between coal-producing regions and cultivated land further underscores the need to restore soil functions and carbon stocks in mining-affected croplands [4]. Although reclamation can restore vegetation and crop production, reclaimed soils often remain characterized by low SOC stocks, weak aggregate development, nutrient limitations, and incompletely recovered microbial communities [28, 41]. Restoring degraded soils has therefore become an important strategy for recovering ecosystem functions and enhancing terrestrial carbon sequestration. Consequently, rebuilding SOC is a central objective of reclaimed cropland restoration because it can simultaneously improve soil fertility, structural development, and long-term carbon storage [5, 27]. However, SOC recovery in degraded soils is often constrained by multiple interacting factors, including insufficient carbon inputs, impaired soil structure,

nutrient limitations, and delayed recovery of biological processes [23, 38, 51], any of which may act as a rate-limiting step depending on the degradation history and restoration approach.

Among various degradation scenarios, mining-induced land degradation represents a particularly challenging restoration context because reclamation involves soil excavation, topsoil removal, and reconstruction of soil profiles, which fundamentally disrupts soil physical, chemical, and biological properties [15]. These persistent deficits indicate that reclamation does not immediately restore SOC cycling processes, and long-term management interventions are required to rebuild SOC pools and associated ecological functions. Therefore, reclaimed croplands provide an important system for understanding how SOC is re-established after severe disturbance, offering insights that may inform restoration strategies across diverse degraded ecosystems.

Long-term manure application has been widely used to enhance SOC accumulation and improve soil quality. Meta-analysis and long-term field experiments have demonstrated that manure inputs generally increase SOC concentrations and improve soil properties, although the magnitude of SOC responses varies among soil types, climatic conditions, and management practices [17, 32, 53, 54]. The combination of manure and mineral fertilizers may be particularly effective because mineral nutrients can alleviate nutrient constraints and sustain plant growth, thereby increasing the quantity of carbon entering the soil [52]. However, increased carbon input does not necessarily translate directly into persistent SOC accumulation. The fate of newly added carbon depends on its transformation and subsequent retention within soil. Thus, understanding SOC recovery requires consideration not only of how much carbon enters reclaimed soil, but also of how that carbon is transformed and stabilized.

Three complementary pathways are generally recognized to contribute to SOC formation and persistence: Firstly, plant-derived carbon inputs are considered a primary pathway of SOC formation because increased crop productivity and residue return provide continuous carbon substrates for soil organic matter formation [9, 11]. After entering soil with plant residues, lignin undergoes progressive decomposition and oxidative transformation, and lignin-derived compounds with different degrees of decomposition may subsequently follow distinct stabilization pathways. Recent long-term evidence showed that less-decomposed lignin-derived compounds preferentially accumulated in particulate organic matter, whereas more-decomposed lignin-derived compounds were increasingly retained in mineral-associated organic matter, with these pathways regulated by the chemical quality of organic inputs and the availability of dissolved C and N [6]. Second, microbial transformation processes contribute to SOC persistence through the formation and accumulation of microbial necromass, which has been recognized as an important component of stable SOC pools, particularly in mineral-associated organic matter [12, 30]. This process is conceptualized as the microbial carbon pump, whereby microorganisms assimilate and transform plant-derived substrates into microbial biomass and subsequently necromass, facilitating the transfer of relatively labile plant C into more persistent soil C pools [8, 21]. Microbial necromass is commonly quantified using amino sugars as biomarkers, particularly glucosamine and muramic acid for estimating fungal- and bacterial-derived necromass C, respectively, and global syntheses indicate that microbial necromass can account for approximately

35%–51% of total SOC across major terrestrial ecosystems, with an average contribution of about 51% in cropland topsoils [57]. Third, physical protection through soil aggregation can reduce carbon accessibility to microorganisms and enhance SOC stabilization by promoting the retention of organic matter within aggregates [26, 36, 43]. These three pathways plant-derived inputs, microbial transformation, and aggregate-mediated protection are not mutually exclusive but interact dynamically to regulate SOC dynamics [39, 55]. However, the relative contribution of each pathway during SOC recovery in reclaimed soils remains poorly understood. Unlike mature agricultural soils, reclaimed soils develop under conditions of initially depleted carbon pools, disrupted soil structure, and incomplete biological recovery. Therefore, mechanisms identified in conventional croplands may not fully explain SOC accumulation in reclaimed ecosystems. Specifically, it remains unclear whether manure-induced SOC recovery in reclaimed soils is primarily driven by (i) enhanced plant-derived carbon inputs, increased microbial necromass formation, or improved aggregate-mediated carbon retention, (ii) which of these mechanisms represents the rate-limiting step constraining SOC recovery; and (iii) how combined manure and mineral fertilizer application influences these pathways relative to manure alone. Here, we investigated a ten-year fertilization experiment established in reclaimed cropland in the Loess mining area with four treatments: no fertilization (CK), mineral fertilizer (NPK), manure alone (M), and manure combined with mineral fertilizer (MNPK). We employed a multi-biomarker approach integrating measurements of: (i) SOC and aggregate-associated SOC across four aggregate size fractions to assess carbon retention; (ii) lignin phenol biomarkers to trace plant-derived carbon inputs and decomposition; (iii) amino sugar biomarkers (glucosamine and muramic acid) to quantify microbial necromass carbon and its contribution to SOC; and (iv) soil enzyme activities (urease, β -glucosidase, catalase, and sucrase) to assess microbial activity and nutrient cycling. Measurements from two consecutive years were further used to assess whether these responses were temporally consistent. We hypothesized that (i) long-term manure application would enhance SOC recovery primarily by increasing plant-derived carbon inputs through improved crop productivity; and (ii) manure combined with mineral fertilizer would strengthen these processes by improving nutrient availability and crop productivity, resulting in greater SOC accumulation than mineral fertilization alone.

2 Materials and Methods

2.1 Study site

The long-term fertilization experiment was conducted at the reclaimed coal gangue disposal area of Tunlan Mine in Gujiao City, Shanxi Province, China (37°53'15"N, 112°06'42"E; elevation 1020 m a.s.l.). The site is located in the eastern Loess Plateau, a region characterized by severe soil erosion and extensive coal mining activities. The surrounding landscape consists of gently rolling hills with adjacent land uses including active mining operations, rehabilitated forestland, and rainfed cropland. Coal gangue (a byproduct of coal washing) was continuously discharged into natural ravines since 2002. After waste disposal ceased in 2012, topsoil was imported from nearby areas to cover the dump surface, and the land was reclaimed as cropland following 2 years of natural recovery. The region has a warm-temperate,

semi-arid continental monsoon climate with a mean annual temperature of $\sim 9.5^{\circ}\text{C}$. The soil is classified as a Calcaric Cambisol (IUSS Working Group WRB 2015). At experiment initiation, the 0–20 cm soil layer had 1.49 g cm^{-3} bulk density (BD), 7.05 g kg^{-1} soil organic matter (SOM), 0.34 g kg^{-1} total N (TN), 3.03 mg kg^{-1} available P (AP), 102.17 mg kg^{-1} available K (AK), and pH 8.46 (mean across plots).

2.2 Experimental design

The experiment followed a randomized complete block design with three replicates per treatment. Each plot was $5\text{ m} \times 2.5\text{ m}$ and planted with spring maize (*Zea mays* L.) in a single-crop-per-year system, which is typical for the region. Four treatments were included: (1) control (CK, no fertilizer); (2) mineral fertilizers (NPK); (3) organic manure (M); and (4) manure plus mineral fertilizers (MNPK). Mineral fertilizer sources were urea (46% N), superphosphate (12% P_2O_5) and potassium chloride (60% K_2O). Application rates in NPK were 190 kg N ha^{-1} , $75\text{ kg P}_2\text{O}_5\text{ ha}^{-1}$ and $100\text{ kg K}_2\text{O ha}^{-1}$. The M treatment received $11,250\text{ kg ha}^{-1}\text{ yr}^{-1}$ chicken manure (from Honghao livestock breeding professional cooperative in Taigu District), while MNPK received twice this manure rate, the same P and K rates, and half the N rate of the NPK treatment. The manure compost contained 29.43% organic C, 8.40% total N, 0.54% P_2O_5 and 1.56% K_2O (dry mass basis).

In treatments receiving mineral fertilizers, urea was split between basal application before sowing and topdressing at the 12-leaf (V12) and silking (R1) stages; P and K fertilizers were applied as basal inputs prior to ploughing. Spring maize was sown in early April and harvested in late September to early October. The variety of maize is Xianyu 335. The planting density is 14,400 plants/ha, with a plant spacing of $40\text{ cm} \times$ row spacing. No irrigation is required throughout the growing season. Maize straw from all treatments was returned to the field to reduce erosion, consistent with local management on the Loess Plateau. Basic soil chemical properties for each treatment are shown in Table 1.

Table 1
Soil chemical properties under long-term fertilization from 2020 to 2021

Reclamation year	Treatment	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	AP (mg kg ⁻¹)	AK (mg kg ⁻¹)
2020	CK	5.21 ± 0.34 c	0.40 ± 0.02 c	27.26 ± 4.37 bc	161.80 ± 13.87 b
	NPK	4.96 ± 0.20 c	0.45 ± 0.02 bc	24.59 ± 1.19 c	195.81 ± 45.49 b
	M	6.33 ± 0.20 b	0.49 ± 0.04 ab	34.51 ± 1.21 ab	320.97 ± 47.63 a
	MNPK	8.61 ± 0.71 a	0.52 ± 0.06 a	37.84 ± 5.39 a	353.62 ± 67.34 a
2021	CK	6.53 ± 0.41 c	0.48 ± 0.04 c	4.58 ± 1.09 c	284.40 ± 0.11 c
	NPK	5.92 ± 0.55 c	0.51 ± 0.04 c	5.63 ± 0.26 c	377.11 ± 15.89 b
	M	9.31 ± 0.29 b	0.65 ± 0.00 b	17.46 ± 2.05 a	459.43 ± 32.34 a
	MNPK	11.91 ± 0.84 a	0.73 ± 0.02 a	11.57 ± 3.39 b	360.65 ± 57.03 b
Effect	Y (df = 1)	**	**	**	**
	T (df = 3)	**	**	**	**
	Y×T (df = 3)	**	*	ns	*

Note: SOC: soil organic carbon; TN: total nitrogen; AP: available phosphorus; AK: available potassium. Results of two-way ANOVA are shown as Year (Y), Treatment (T), and their interaction (Y × T): * $P < 0.05$, ** $P < 0.01$, ns indicates no significant differences at $P < 0.05$. Different lowercase letters indicate significant differences among treatments ($P < 0.05$).

2.3 Soil sampling and analyses

Soil samples were collected from the 0–20 cm layer immediately before maize harvest in October 2020 and October 2021. For aggregate analysis, three undisturbed soil cores (10 cm internal diameter) were collected from each plot, sealed in plastic containers, gently broken along natural fracture planes, and passed through an 8-mm sieve. The sieved samples were air-dried at room temperature before aggregate fractionation by wet sieving. In parallel, after harvest, three additional auger samples (2.5 cm internal diameter; 0–20 cm) were collected per plot, composited, and used for measurements of basic soil properties, amino sugars, lignin phenols, and enzyme activities.

Water-stable aggregates were separated by wet sieving following Elliott[13]. Organic C in bulk soil and aggregate fractions was quantified using an elemental analyzer (vario MACRO cube; Elementar, Hanau,

Germany). Amino sugars were determined by gas chromatography after hydrolysis and derivatization [56]. Lignin phenols were quantified by alkaline CuO oxidation [19]. Enzyme activities, including urease (UA) and beta-glucosidase (BG); catalase (CA) and sucrase (SA), were assayed using standard colorimetric protocols [16].

2.4 Data calculations and statistical analysis

Lignin phenols were grouped into vanillyl (V), syringyl (S) and cinnamyl (C) units, calculated as sums of their corresponding CuO oxidation monomers.

V = vanillin + vanillic acid + acetovanillone (1)

S = syringaldehyde + syringic acid + acetosyringone (2)

C = p-coumaric acid + ferulic acid (3)

Total lignin phenols = V + S + C (4)

The concentration of plant-derived carbon, expressed as a percentage of SOC, was estimated from lignin phenol concentrations using the conversion coefficients proposed by Hautala et al.[18] and Chen et al. [10]:

Plant derived carbon = $[(V/33.3\% + S/90\% + C)/(8\% \times \text{SOC})] \times 100\%$ (5)

Microbial necromass C was calculated according to Appuhn and Joergensen [3], Engelking et al. [14] and Liang et al. [29].

Fungal necromass C = $(\text{GluN} - 2 \times \text{MurN}) \times 179.2 \text{ g mol}^{-1} \times 9$ (6)

Bacterial necromass C = $\text{MurN} \times 45$ (7)

Microbial necromass C = Fungal necromass C + Bacterial necromass C (8)

where GluN and MurN (mmol g^{-1} soil) are glucosamine and muramic acid concentrations in the amino sugar pool; 179.2 is the molecular weight of GluN; the factor 2 assumes a 2:1 molar ratio of GluN to MurN in bacterial cells; 9 converts fungal GluN to fungal necromass C; and 45 converts MurN to bacterial necromass C.

The contribution of microbial necromass C to SOC was calculated using the following Eq. (9):

Microbial necromass C in SOC (%) = $\text{Microbial necromass C} / \text{SOC} \times 100$ (9)

IBM SPSS Statistics 27.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. For soil fertility properties, aggregate metrics, biomarker indicators and enzyme activities measured in 2020 and 2021, a two-way analysis of variance (ANOVA) with Year (Y), Treatment (T) and their interaction (Y×T) was performed. When ANOVA was significant, means were separated using the least significant difference

(LSD) test at $P < 0.05$. For maize yield, treatment and year effects (and their interaction) were evaluated using two-way ANOVA on plot-level yields. Because replication was incomplete in 2014, 2015 and 2021 (< 3 field replicates per treatment), inferential tests for yield were based on fully replicated years (2016–2020), while yields from all years (2014–2021) are presented descriptively (Fig. 1). Yield stability was summarized as the coefficient of variation (CV) of annual treatment means.

3 Results

3.1 Maize yield

Across the experimental period (2014–2021), fertilization consistently increased maize yield relative to the unfertilized control (CK) (Fig. 1). Averaged across years, maize yield was significantly higher under NPK (3090 kg ha^{-1}), M (4226 kg ha^{-1}), and MNPK (4142 kg ha^{-1}) than under CK (1180 kg ha^{-1}), representing increases of 162%, 258%, and 251%, respectively (Table 2). Among the fertilized treatments, M and MNPK produced significantly greater yields than NPK, whereas no significant difference was observed between M and MNPK. Despite interannual variation, the temporal patterns of maize yield were similar between the two manure treatments, and both consistently maintained higher yields than NPK and CK throughout the experimental period (Fig. 1). The coefficient of variation (CV) of annual maize yield ranged from 2.63% in CK to 12.77%–13.70% in the fertilized treatments (Table 2).

Table 2

Mean maize yield and coefficient of variation (CV) under long-term fertilization from 2014 to 2021

Treatment	Maize yield (kg ha^{-1})	CV (%)
CK	$1180.30 \pm 31.01 \text{ c}$	2.63%
NPK	$3089.89 \pm 396.82 \text{ b}$	12.84%
M	$4226.20 \pm 579.17 \text{ a}$	13.70%
MNPK	$4141.63 \pm 529.08 \text{ a}$	12.77%
Note: Different lowercase letters indicate significant differences among treatments ($P < 0.05$).		

3.2 Aggregate distribution and stability

Long-term fertilization significantly affected aggregate-size distribution (Fig. 2a) and aggregate stability (Fig. 2b). Aggregate-size distribution was influenced by both year and fertilization treatment, whereas significant year $\times$ treatment interactions ($Y \times T$) were observed only for some aggregate fractions. Across both years, fertilization generally reduced the proportion of the $> 2 \text{ mm}$ fraction while increasing the proportions of the $0.25\text{--}2 \text{ mm}$ and $0.053\text{--}0.25 \text{ mm}$ fractions. Compared with CK, manure treatments (M and MNPK) significantly reduced the $> 2 \text{ mm}$ fraction by 29%–49% in 2020 and 31%–56% in 2021, respectively. However, the $0.053\text{--}0.25 \text{ mm}$ fraction was significantly enriched by 20%–30% in 2020 and

29%-40% in 2021, respectively. In contrast, the < 0.053 mm fraction was affected mainly by year rather than fertilization treatment (Fig. 2a).

Aggregate stability, expressed as mean weight diameter (MWD), was significantly affected by both year and fertilization treatment, whereas no significant $Y \times T$ was detected (Fig. 2b). Across both years, CK consistently exhibited the highest MWD, whereas all fertilized treatments showed lower values. In 2020, M showed the lowest MWD, while in 2021 NPK and MNPK remained significantly lower than CK, with M showing intermediate values.

3.3 Aggregate-associated SOC contents

Aggregate-associated SOC was significantly affected by fertilization treatment across all aggregate-size fractions, whereas year effects were comparatively weaker (Fig. 3). Across both years, M and MNPK consistently exhibited higher aggregate-associated SOC than CK and NPK in all aggregate-size fractions. Among the fertilized treatments, MNPK generally showed the highest aggregate-associated SOC contents. Compared with CK, MNPK treatment significantly increased the SOC contents within > 2 mm and 0.25-2 mm by 69%-124% and 57%-62% in both years. MNPK treatment also significantly increased the SOC contents within 0.053–0.25 mm and < 0.053 mm by 58% and 65% in 2021, respectively. The positive effect of manure application on aggregate-associated SOC was observed across all aggregate-size fractions, indicating a broad enrichment of SOC within soil aggregates under long-term manure application.

3.4 Plant-derived carbon

Lignin phenol composition was significantly affected by reclamation year, fertilization treatment, and their interaction, except that the interaction effect was not significant for cinnamyl monomers (Fig. 4a-d). Across all treatments, the contents of vanillyl (V), syringyl (S), cinnamyl (C) monomers and total lignin phenols (TLP) were significantly higher in 2021 than in 2020. In both years, manure-amended treatments (M and MNPK) significantly increased lignin phenols by 24%-30% in 2020 and 13%-20% in 2021 than CK, respectively, with MNPK generally exhibiting the highest values, whereas no significant differences were observed between CK and NPK. Lignin composition and degradation indices also differed among fertilization treatments (Fig. 4e, f). Compared with CK, manure-amended treatments exhibited higher S/V, C/V and (Ad/Al)/V ratios in 2020, but showed lower C/V, (Ad/Al)/V and (Ad/Al)/S ratios in 2021.

3.5 Soil enzyme activities

Soil enzyme activities were significantly affected by reclamation year and fertilization treatment (Fig. S1). Urease activity (UA) was significantly affected by year, fertilization treatment, and their interaction ($P < 0.01$), whereas β -glucosidase activity (BG) showed significant effects of year and fertilization treatment but no significant interaction. Compared with CK, M and MNPK treatments significantly increased UA activity by 43%-80% and 55%-61%, meanwhile M and MNPK treatments significantly

increased BG activity by 59%-78% and 61%-87% across both years. Catalase (CA) and sucrase (SA) exhibited response patterns generally consistent with those of UA and BG.

3.6 Microbial necromass carbon

Microbial necromass carbon (MNC) was significantly affected by reclamation year and fertilization treatment (Fig. 5a). Total amino sugar (TAS) (Table S1) and MNC contents were generally higher in 2021 than in 2020. Compared with CK, M and MNPK significantly increased MNC content by 17% and 33% in 2020, respectively, whereas application of manure significantly decreased MNC content by 4%-20% in 2021. The relative contribution of microbial necromass to SOC (MNC/SOC) was consistently lower under manure-amended treatments than under CK in both years (Fig. 5b), especially under MNPK, the decline were 17% and 58% in 2020 and 2021, respectively. Both bacterial necromass carbon (BNC) and fungal necromass carbon (FNC) exhibited response patterns generally consistent with those of MNC (Fig. S2).

4 Discussion

4.1 Plant-derived carbon inputs promoted SOC accumulation in reclaimed soil under long-term manure application

Continuous carbon input from vegetation is fundamental to SOC formation and accumulation, particularly in reclaimed soils where severe disturbance and low organic matter availability constrain carbon replenishment. Plant-derived organic matter provides the primary substrate for SOC formation, and insufficient carbon input is widely recognized as a major limitation for SOC recovery in degraded ecosystems [12, 9, 55]. In this context application of external organic materials has been recognized as a major strategy to improve crop productivity and hence bring more residues in soil eventually resulting in higher carbon input. In our study, long-term fertilization significantly increased maize productivity compared with CK, and manure-amended treatments (M and MNPK) exhibited the greatest yield enhancement (Fig. 1). Increased crop productivity generally promotes greater aboveground residue return and belowground carbon inputs through root biomass and rhizodeposition, thereby increasing the amount of plant-derived carbon entering soil [45]. Recent long-term fertilization studies have similarly demonstrated that manure application enhances SOC accumulation by increasing organic carbon inputs and promoting carbon retention processes [1, 37, 54].

Compared with CK, manure-amended treatments significantly increased vanillyl (V), syringyl (S), cinnamyl (C) monomers and total lignin phenols (TLP) in two study years (Fig. 4), indicating greater incorporation of plant-derived organic matter into reclaimed soils. Lignin phenols are derived primarily from vascular plant tissues and are widely used as molecular indicators for tracing plant-derived carbon inputs and transformation in soils [25, 49]. Although lignin composition and degradation indices varied between reclamation years, the consistently higher lignin phenol concentrations under manure treatments indicate that enhanced plant-derived carbon input was the dominant response to long-term

manure application. These results suggest that manure application promoted carbon accumulation mainly by increasing the quantity of organic carbon entering the soil rather than through a consistent alteration of lignin decomposition pathways. However, increased carbon input alone does not necessarily result in long-term SOC accumulation because newly incorporated organic matter remains vulnerable to microbial decomposition [48]. A recent study has shown that manure-induced increases in carbon inputs are often accompanied by enhanced carbon retention within soil aggregates, highlighting the importance of subsequent stabilization processes [1]. Therefore, understanding how this additional plant-derived carbon is retained and stabilized within soil aggregates is essential for explaining SOC recovery under long-term manure application.

4.2 Aggregate-mediated carbon retention enhanced SOC stabilization in reclaimed soil

Soil aggregates are fundamental physical structures regulating the incorporation, protection, and persistence of organic carbon in soils [35]. The formation and turnover of aggregates determine the accessibility of organic matter to microbial decomposition and influence SOC stabilization through physical occlusion and reduced substrate accessibility [36, 44, 50]. Our study showed that long-term fertilization significantly altered aggregate-size distribution and increased SOC concentrations across all aggregate fractions (Figs. 2 and 4), suggesting that manure application promoted carbon retention through changes in aggregate dynamics rather than simply increasing aggregate stability.

Compared with CK, manure application reduced the proportion of > 2 mm aggregates while increasing the proportions of 0.25–2 mm and 0.053–0.25 mm aggregates (Fig. 2a). Although MWD was lower under fertilized treatments than CK (Fig. 2b), this does not necessarily indicate weakened SOC protection, because aggregate stability and carbon retention represent different aspects of soil structural functioning. Aggregate formation is a dynamic process involving continuous disruption, reorganization, and stabilization of soil particles, during which newly incorporated organic carbon can be redistributed among different aggregate fractions [42]. A recent study has demonstrated that fertilization-induced SOC accumulation is often associated with changes in aggregate-carbon distribution rather than a simple increase in aggregate stability, highlighting the importance of aggregate dynamics in regulating soil carbon persistence [31]. Therefore, the reduced proportion of large aggregates under manure application may reflect enhanced aggregate turnover and carbon redistribution rather than impaired soil structure.

The enrichment of aggregate-associated SOC provides further evidence that manure application promoted carbon retention within soil aggregates. Compared with CK, MNPK significantly increased SOC contents within > 2 mm and 0.25–2 mm aggregates by 69%–124% and 57%–62%, respectively, across both study years, while SOC enrichment was also observed in smaller aggregate fractions (Fig. 3). This broad accumulation pattern indicates that newly incorporated carbon was distributed throughout the aggregate hierarchy rather than being restricted to a specific aggregate class. Long-term organic amendments have been shown to enhance SOC sequestration by increasing carbon storage within

aggregate-associated fractions and promoting the stabilization of newly incorporated organic matter [22, 34, 40].

According to the aggregate-mediated stabilization framework, plant-derived carbon entering soil can be progressively incorporated into aggregates, where physical occlusion reduces microbial accessibility and slows decomposition [26, 27, 36]. Thus, the increased aggregate-associated SOC observed under manure application likely resulted from the combined effects of enhanced plant-derived carbon inputs (Fig. 4) and improved retention of newly incorporated carbon within soil aggregates. These results demonstrate that aggregate dynamics and carbon retention, rather than aggregate stability alone, play a critical role in SOC accumulation during soil reclamation.

4.3 Microbial transformation regulated SOC dynamics during recovery of reclaimed soil

Microbial transformation plays an important role in linking plant-derived carbon inputs with SOC formation, as microorganisms convert fresh organic substrates into microbial-derived carbon that can contribute to persistent SOC pools [12, 30]. In our study, manure application significantly increased urease and β -glucosidase activities compared with CK (Fig. S1), indicating enhanced microbial processing of newly incorporated organic matter. Increased extracellular enzyme activities suggest that manure inputs stimulated microbial acquisition of carbon and nutrients, thereby accelerating the transformation of plant-derived substrates during soil reclamation [7].

However, microbial necromass carbon (MNC) exhibited a temporal-dependent response to manure application rather than a consistent increasing trend (Fig. 5a). Compared with CK, M and MNPK increased MNC by 17% and 33% in 2020, respectively, whereas manure application decreased MNC by 4%-20% in 2021. This temporal variation indicates that microbial necromass accumulation was not solely determined by external carbon inputs but was also regulated by microbial growth efficiency, substrate utilization, and soil environmental conditions. Recent studies have demonstrated that microbial necromass accumulation and its contribution to SOC vary among ecosystems because microbial turnover, residue stabilization, and environmental constraints jointly regulate necromass formation [20, 24].

The contrasting responses of bacterial and fungal necromass further indicated that microbial-derived carbon formation was closely linked to the recovery trajectory of reclaimed soils. Reclaimed soils generally contain depleted organic carbon pools and limited substrate availability after topsoil removal and soil reconstruction, which may constrain microbial growth and necromass formation. In our study, fungal necromass carbon (FNC) showed a stronger response to manure application than bacterial necromass carbon (BNC), particularly in 2020, when M and MNPK significantly increased FNC compared with CK (Fig. S2). This pattern may reflect the greater ability of fungi to utilize complex plant-derived substrates, including lignin-rich residues, during the early stages of carbon recovery [46]. However, the subsequent decline of FNC under manure-amended treatments in 2021 suggests that microbial necromass accumulation was not solely controlled by carbon inputs, but also depended on the

stabilization capacity of reclaimed soils and environmental constraints affecting microbial turnover. Therefore, although manure inputs stimulated microbial transformation processes, the incomplete recovery of soil structure and carbon protection capacity may have limited the persistent accumulation of microbial-derived carbon in reclaimed soils.

A particularly important finding was that manure application consistently decreased the contribution of microbial necromass to SOC (MNC/SOC), with the strongest decline observed under MNPK treatment (Fig. 5b). This result suggests that SOC accumulation occurred faster than microbial necromass formation, indicating that microbial-derived carbon was not the dominant contributor to SOC increases during reclamation. Similar findings have shown that microbial necromass content and its relative contribution to SOC are not always positively correlated, because the proportion of microbial-derived carbon depends on the balance between carbon input pathways, microbial turnover, and stabilization processes [2, 58]. Therefore, the decline in MNC/SOC under manure application should not be interpreted as a reduced role of microorganisms in SOC formation. Instead, microbial transformation likely functioned as an intermediate pathway that converted plant-derived carbon into microbial products, while the overall SOC increase was primarily associated with enhanced plant-derived carbon inputs (Fig. 4) and aggregate-mediated carbon retention (Fig. 3). Together, these results indicate that SOC recovery in reclaimed soils was driven mainly by increased carbon input and stabilization, whereas microbial necromass contributed dynamically to carbon transformation rather than serving as the primary driver of SOC accumulation.

Collectively, our results suggest that long-term fertilization promoted SOC recovery in reclaimed soils through the coordinated regulation of carbon input, carbon retention, and microbial transformation processes. Manure application provided external organic C inputs and initiated SOC recovery by increasing plant-derived carbon inputs, as indicated by enhanced lignin phenol accumulation and improved crop productivity. The addition of mineral fertilizer further enhanced this recovery process by improving nutrient availability, stimulating plant growth, and increasing carbon retention potential through greater aggregate-associated SOC accumulation. Although microbial activity was stimulated under manure-amended treatments, the reduced relative contribution of microbial necromass to SOC suggested that microbial transformation mainly regulated C cycling rather than serving as the dominant source of SOC accumulation. Therefore, we propose that long-term MNPK application provides a more effective pathway for SOC recovery and stabilization in reclaimed soils by integrating enhanced C inputs with improved carbon retention mechanisms.

5 Conclusions

This 10-year field experiment demonstrates that long-term manure application can substantially accelerate SOC recovery in reclaimed cropland, but the mechanisms differ from those typically emphasized in mature agricultural soils. The study provides three key insights into the mechanisms of SOC recovery. First, manure application markedly increased crop productivity and plant-derived carbon inputs, as indicated by higher maize yield and lignin phenol concentrations, providing a major carbon

source for SOC accumulation. Second, manure increased SOC across aggregate-size fractions despite lower aggregate stability, indicating that SOC retention was associated more strongly with changes in aggregate distribution and carbon redistribution than with greater aggregate stability. Third, manure stimulated microbial activity, but microbial necromass showed strong interannual variation and contributed a smaller proportion of the expanding SOC pool, suggesting that microbial transformation acted primarily as a dynamic intermediary rather than the dominant source of SOC accumulation during the observed recovery period. Among the fertilization strategies, MNPK provided the strongest overall combination of crop productivity and SOC accumulation, particularly through enhanced plant-derived carbon inputs and greater aggregate-associated SOC. These findings indicate that successful restoration of degraded cropland requires management strategies that simultaneously increase carbon inputs and promote its retention within developing soil structural networks.

Declarations

Compliance with Ethical Standards

This article does not contain any studies of human participants or animals performed by any of the authors.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Author Contribution

Junyu Xie: Writing-original draft & editing, Methodology, Investigation, Data curation, Conceptualization. Hanbing Cao: Investigation, Resources. Mengru Cao: Data curation. Jiahui Li: Formal analysis. ZhanXiang Wu: Formal analysis, Data curation. Lina Li: Data curation. Minggang Xu: Supervision, Writing–review & editing. Hanqiong He: Formal analysis. Jie Zhang: Formal analysis. Huisheng Meng:

Formal analysis. Jianping Hong: Resources, Supervision. Tingliang Li: Conceptualization, Supervision, Funding acquisition. Adnan Mustafa: Supervision, Writing–review & editing.

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Data Availability

All data available within the article.

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Figures

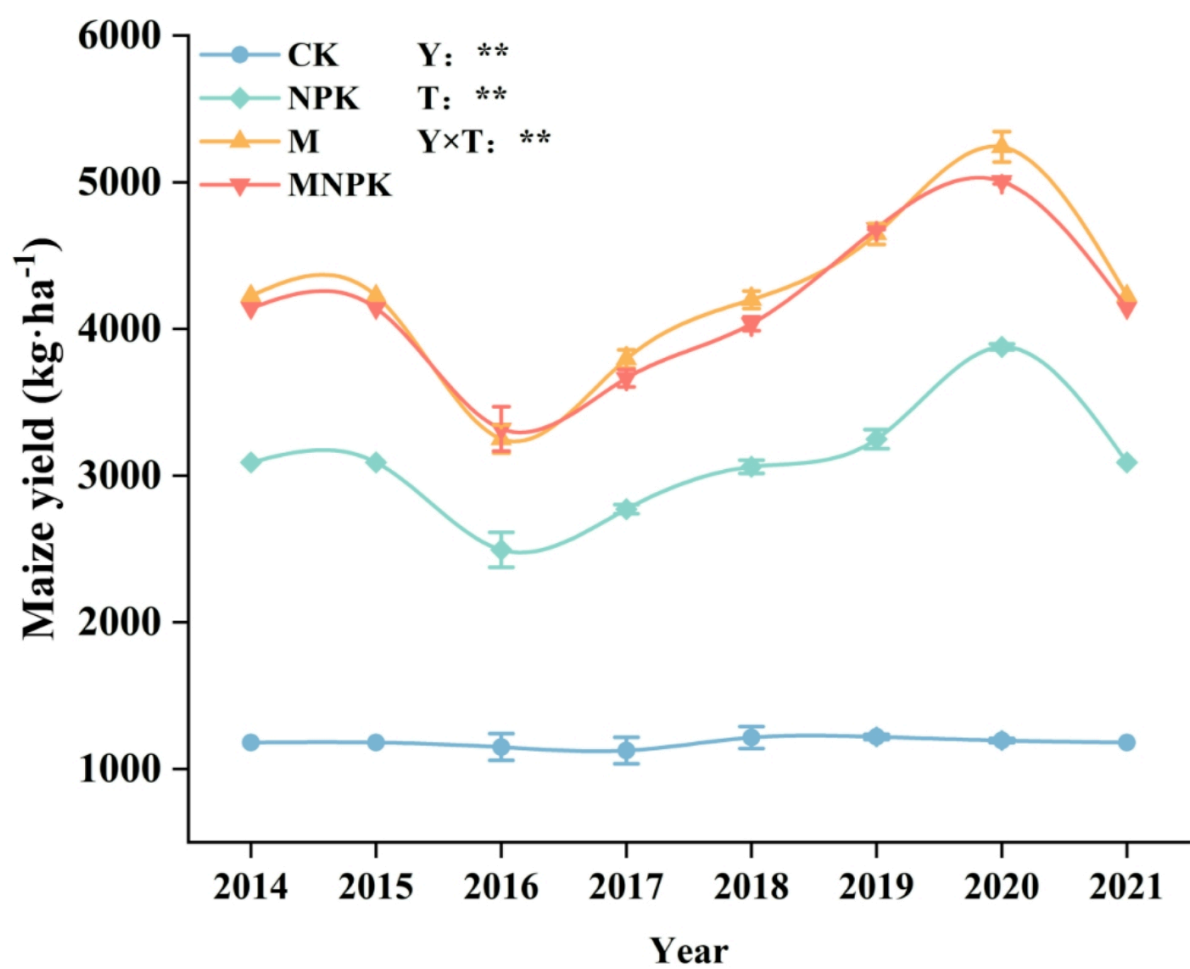


Figure 1

Interannual variation in maize yield under long-term fertilization from 2014 to 2021. CK, no fertilization; NPK, chemical fertilizers; M, manure alone; MNPK, manure combined with chemical fertilizers. Symbols represent annual treatment means, and error bars indicate the standard deviation (SD) among field replicates within a given year ($n = 3$ when available; error bars are omitted for years with incomplete replication). Results of two-way ANOVA are shown as Year (Y), Treatment (T), and their interaction ($Y \times T$): ** $P < 0.01$.

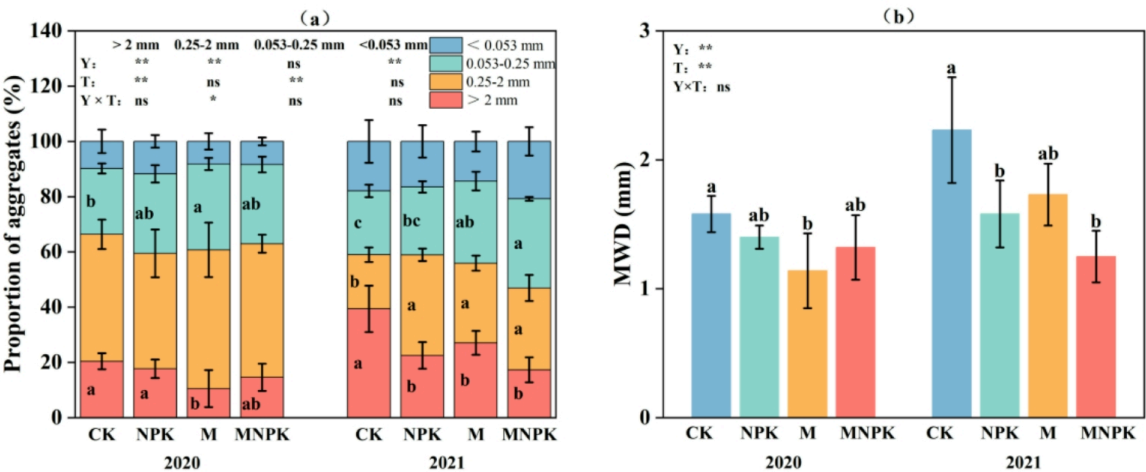


Figure 2

Proportion of water stable aggregates (a) and mean weight diameter (MWD) of water stable aggregates (b) in 2020 and 2021 under CK, NPK, M, and MNPK. Bars show mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among treatments within the same year (LSD, $P < 0.05$). Results of two-way ANOVA are shown as Year (Y), Treatment (T), and their interaction ($Y \times T$): * $P < 0.05$, ** $P < 0.01$, ns indicates no significant differences at $P < 0.05$.

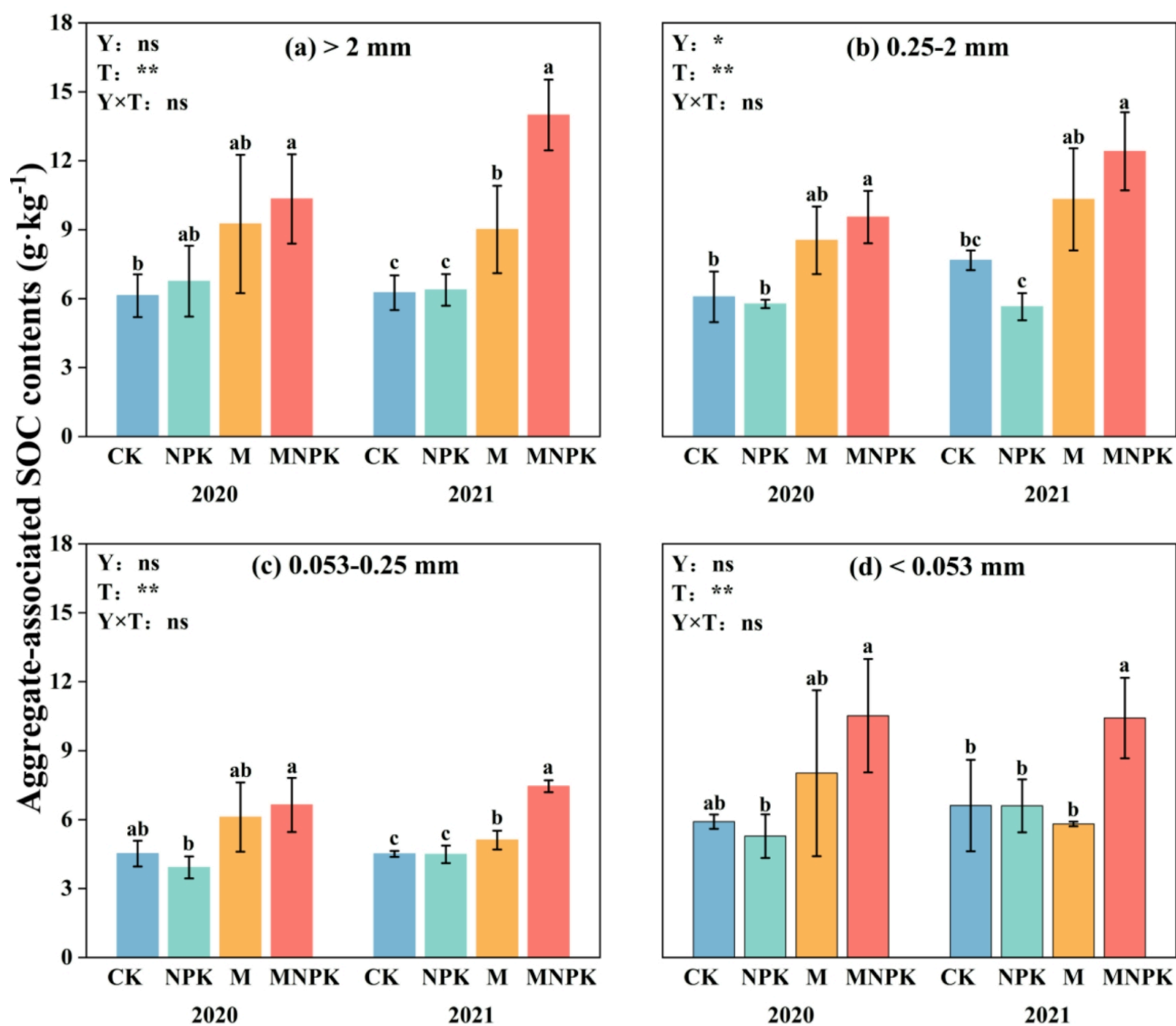


Figure 3

Water stable aggregate-associated SOC contents in 2020 and 2021 under CK, NPK, M, and MNPK. Bars show mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among treatments within the same year (LSD, $P < 0.05$). Results of two-way ANOVA are shown as Year (Y), Treatment (T), and their interaction ($Y \times T$): * $P < 0.05$, ** $P < 0.01$, ns indicates no significant differences at $P < 0.05$.

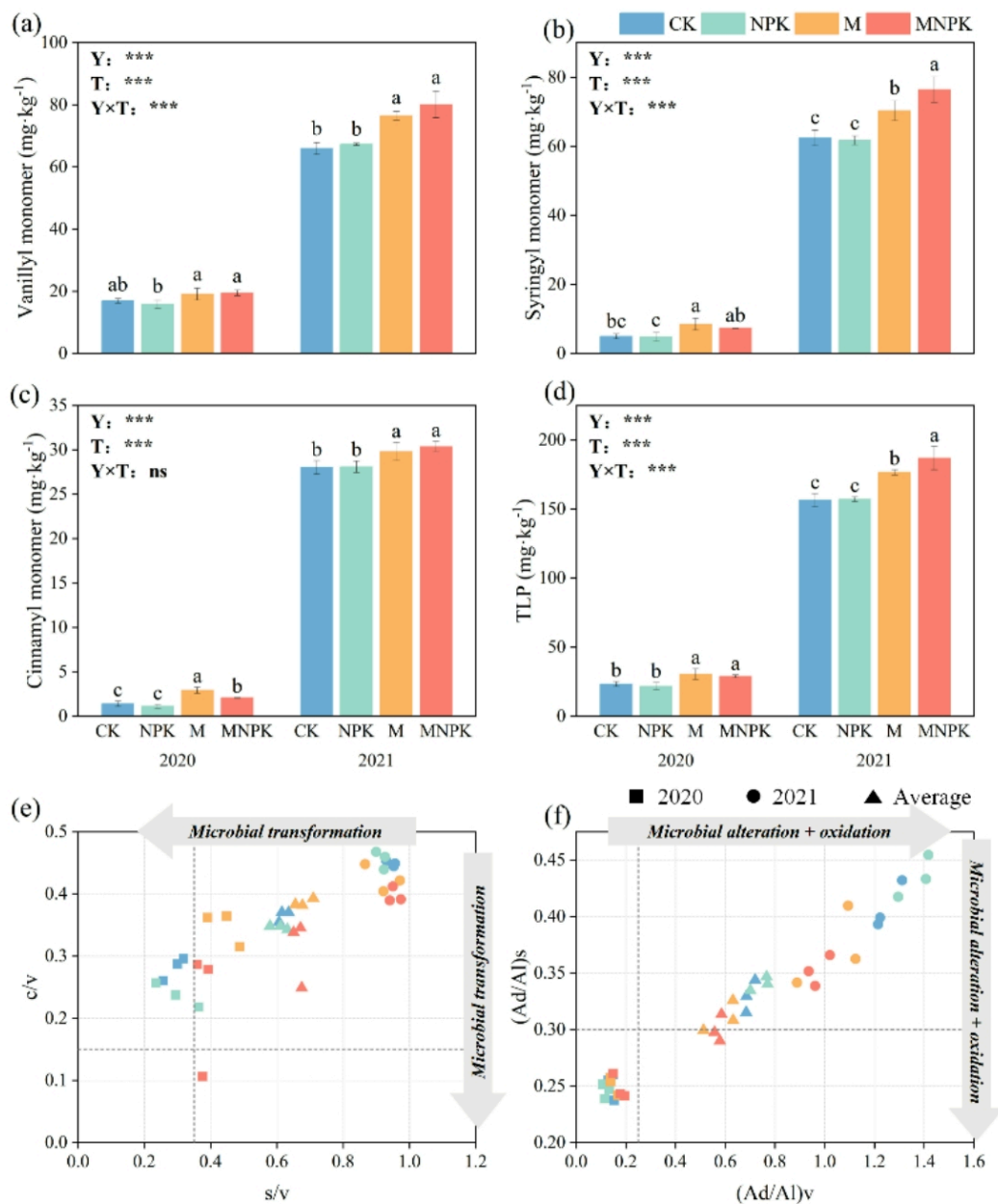


Figure 4

Individual and total lignin phenol contents (TLP) (a-d) and (e) the ratio of syringyl-type to vanillyl-type monomer (S/V) and the ratio of cinnamyl-type to vanillyl-type monomer (C/V); (f) the acid-to-aldehyde ratio of vanillyl-type lignin monomer (Ad/Al)_v and the acid-to aldehyde ratio of syringyl-type monomer (Ad/Al)_s. Bars show mean ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within the same year (LSD, $P < 0.05$). Results of two-way ANOVA are shown as Year

(Y), Treatment (T), and their interaction ($Y \times T$): *** $P < 0.001$, ns indicates no significant differences at $P < 0.05$.

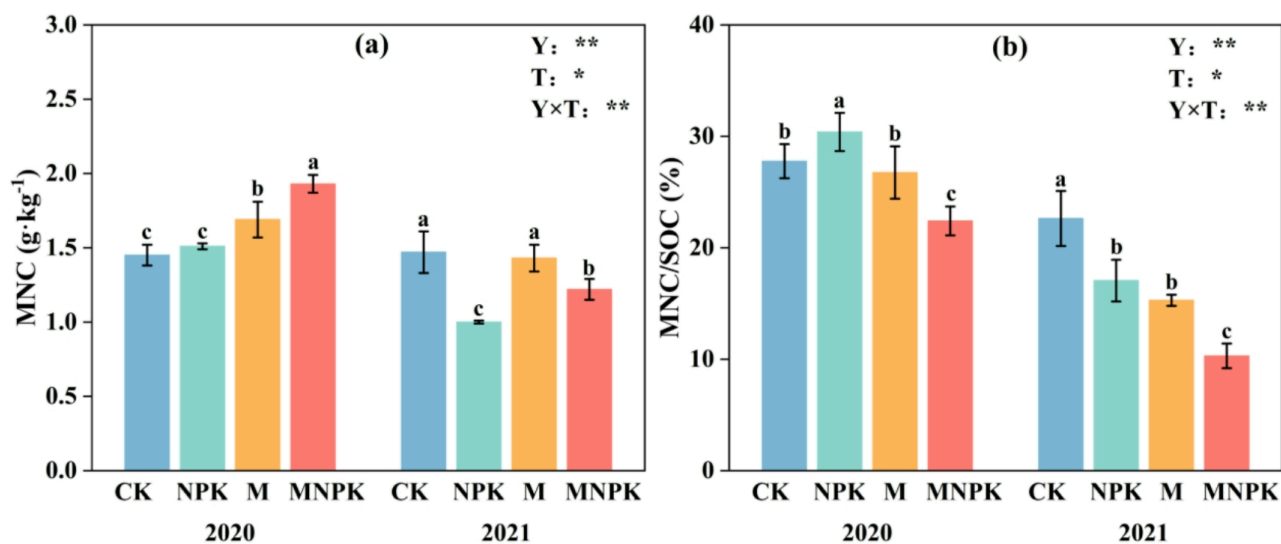


Figure 5

Microbial necromass carbon (MNC) (a) and its contribution to soil organic carbon (MNC/SOC) (b) in 2020 and 2021 under CK, NPK, M, and MNP. Bars show mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among treatments within the same year (LSD, $P < 0.05$). Results of two-way ANOVA are shown as Year (Y), Treatment (T), and their interaction ($Y \times T$): * $P < 0.05$, ** $P < 0.01$, ns indicates no significant differences at $P < 0.05$.

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