

RESEARCH ARTICLE

Carbon and Nitrogen Mineralization in Relation to Soil Particle-Size Fractions after 32 Years of Chemical and Manure Application in a Continuous Maize Cropping System

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Abstract

Long-term manure application is recognized as an efficient management practice to enhance soil organic carbon (SOC) accumulation and nitrogen (N) mineralization capacity. A field study was established in 1979 to understand the impact of long-term manure and/or chemical fertilizer application on soil fertility in a continuous maize cropping system. Soil samples were collected from field plots in 2012 from 9 fertilization treatments (M_0 CK, M_0 N, M_0 NPK, M_{30} CK, M_{30} N, M_{30} NPK, M_{60} CK, M_{60} N, and M_{60} NPK) where M_0 , M_{30} , and M_{60} refer to manure applied at rates of 0, 30, and 60 t ha⁻¹ yr⁻¹, respectively; CK indicates no fertilizer; N and NPK refer to chemical fertilizer in the forms of either N or N plus phosphorus (P) and potassium (K). Soils were separated into three particle-size fractions (2000–250, 250–53, and <53 μm) by dry- and wet-sieving. A laboratory incubation study of these separated particle-size fractions was used to evaluate the effect of long-term manure, in combination with/without chemical fertilization application, on the accumulation and mineralization of SOC and total N in each fraction. Results showed that long-term manure application significantly increased SOC and total N content and enhanced C and N mineralization in the three particle-size fractions. The content of SOC and total N followed the order 2000–250 μm > 250–53 μm > 53 μm fraction, whereas the amount of C and N mineralization followed the reverse order. In the <53 μm fraction, the M_{60} NPK treatment significantly increased the amount of C and N mineralized (7.0 and 10.1 times, respectively) compared to the M_0 CK treatment. Long-term manure application, especially when combined with chemical fertilizers, resulted in increased soil microbial biomass C and N, and a decreased microbial metabolic quotient. Consequently, long-term manure fertilization was beneficial to both soil C

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and N turnover and microbial activity, and had significant effect on the microbial metabolic quotient.

Introduction

Soil organic carbon (SOC) and nitrogen (N) mineralization are fundamental biogeochemical processes that underpin soil fertility and crop production. A better understanding of the mineralization of SOC and N in soils is necessary to improve management of soil fertility, thereby contributing to food security and climate change mitigation. Application of organic amendments, with/without chemical fertilizers [including N, phosphorus (P), and potassium (K)], is recommended as a proven practice to enhance both the accumulation and mineralization capacity of soil organic matter in agricultural systems [1–3]. Manure application can improve soil structure [4] and increase the amount and availability of nutrients for microbial processing [5]. A number of studies have shown that long-term application of organic manure can significantly increase SOC and total N both in bulk soil and particle-size fractions [6, 7]; especially when applied in combination with chemical fertilizers.

The mineralization of SOC and total N in different particle-size fractions is largely regulated by soil organic matter quality (e.g., lignin content and C/N ratio) and the stability which is largely determined by soil inherent properties (e.g., texture and mineralogy) [8]. For example, sands exhibit weak bonding affinities to organic molecules, while organic molecules associated with clay particles (e.g. sesquioxides, layer silicates) can be absorbed by strong ligand exchange and polyvalent action bridges over large surface areas and at numerous reactive positions [9]. Slower SOC turnover times in the clay fraction may be explained by the combined action of an increase in spatial inaccessibility (e.g., due to micro-aggregation) that restricts microbial decomposition, the adsorption of SOC on mineral surfaces, and the consequent chemical change in SOM quality [10–12]. Consequently, SOC within the sand fraction is allocated to the active pool, whereas SOC in silt and clay fractions are defined as the intermediate and passive pools. Various ¹³C natural abundance studies have revealed turnover times of about 15–50 years for SOC stored in the >250 μm fraction and 100–300 years for SOM in the <250 μm fraction [13, 14].

Field management practices can impact on SOC stabilization process through altered aeration associated with mechanical soil disturbance and increased nutrient availability from fertilizer application [1, 15]. A number of studies have shown that long-term application of organic manure significantly increased SOC and total N not only in bulk soil, but also in soil particle-size fractions [6, 7]; especially when manure was applied in combination with chemical fertilizers. Long-term compost application significantly increased the stability of SOC in the 53–250 μm and <53 μm fractions, but not in the >250 μm fraction [16]. It is also reported that the mineralization of SOC and N within particle-size fractions varies with land management practices. Hernández-Hernández and López-Hernández [17] showed that more N was mineralized in the >250 μm fraction than in the <250 μm fraction in cultivated soils, whereas SOC mineralization was significantly higher in the >250 μm fraction than in the <250 μm fraction of the non-cultivated soil. Although, Elliott [18] and Gupta and Germida [19] reported that more SOC and N was mineralized in the >250 μm fraction than in the <250 μm fraction in both cultivated and non-cultivated soils. Hence, there is little available information on the relative contribution of SOC and N mineralization from different particle-size fractions to bulk soil under long-term chemical fertilizer application compared with manure. The objective of this study

were to (1) quantify the impact of long-term (32 years) manure application (with/without chemical fertilizer) on the accumulation and distribution of SOC and total N in different particle-size fractions, (2) explore the responses of SOC and N mineralization to manure application in different particle-size fractions, and (3) evaluate the impact of long-term manure application on soil microbial biomass and activity in various soil particle sizes. We focused the study on Mollisols as this soil type plays an important role in grain production in China and covers 75% of the total area (5.9×10^6 ha) cultivated for agricultural production. The overall target was to improve the understanding of manure application on soil fertility improvement within this agro-ecosystem.

Materials and Methods

Site description and soil sampling

A long-term agricultural site was established in 1979 by Jilin Academy of Agricultural Sciences. The site is located in Gongzhuling ($148^{\circ}57'E$, $42^{\circ}57'N$), Jilin province, Northeast China. The climate is classified as temperate semi-humid monsoon, with mean annual air temperature of $4.5^{\circ}C$ and annual precipitation from 450 to 600 mm, with about 70% of precipitation occurring between June and August. This site has 125 to 140 frost-free days and an average 2500 to 2700 h of sunshine each year. Prior to the establishment of the long-term experiment, the field had been intensively cultivated for more than 50 years. The initial (in 1979) topsoil (0–20 cm) had a SOC of 16.1 g kg^{-1} , total N of 1.9 g kg^{-1} , total P of 1.39 g kg^{-1} , and total K of 22.1 g kg^{-1} . Available N (alkali-hydrolyzable), P (Olsen-P), and K ($1 \text{ mol L}^{-1} \text{ NH}_4\text{OAc}$) were 114, 27, and 190 mg kg^{-1} , respectively. Soil pH (1:2.5 w/v, distilled water) was 7.6 and soil clay content ($<2 \mu\text{m}$) was 29.3%.

The long-term field trial consisted of main plots for manure treatments (M_0 , M_{30} and M_{60}) and subplots for chemical fertilizer applications (control, N only or NPK). Nine treatments were chosen for this study: $M_0\text{CK}$, $M_0\text{N}$, $M_0\text{NPK}$, $M_{30}\text{CK}$, $M_{30}\text{N}$, $M_{30}\text{NPK}$, $M_{60}\text{CK}$, $M_{60}\text{N}$, and $M_{60}\text{NPK}$. The CK indicates no fertilizer; M_0 , M_{30} , and M_{60} refer to livestock manure applied at 0, 30, and 60 t manure $\text{ha}^{-1} \text{ yr}^{-1}$ on a fresh weight basis, respectively; and NPK represents chemical N (urea), P (multiple superphosphate), and K (potassium sulfate) fertilizer, which were applied at 150, 33, and $62 \text{ kg ha}^{-1} \text{ yr}^{-1}$, respectively. The manure used in this experiment was fresh pig and cattle manure compost. The organic carbon content of manure was 10%–20% and total N content was 0.5%–1%. Water content of manure was around 68.7%. Straw was removed at harvest. Each treatment plot (400 m^2 in area) was in a randomized block design with no replication and managed as a continuous maize cropping system.

During November 2012, we collected three sub-samples of topsoil (0–20 cm) with each sub-sample consisting of 20–40 cores that were collected using a 5-cm-diam auger, randomly sampled from field plots for each treatment [20]. Crop residues on the soil surface were carefully removed before soil sampling. The fresh bulk soil samples (about 2 kg per plot) were gently broken apart by hand and were passed through a 2 mm sieve. Then, a third of the sieved soil was stored at $4^{\circ}C$ for subsequent soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) measurement. The remaining subsample of soil was air-dried for particle-size fractionation and subsequent SOC and N mineralization assays. Subsamples were also sieved $<0.15 \text{ mm}$ for SOC and total N content measurement.

Soil particle-size separation

Soil samples from each field plot were separated into three size fractions (2000–250, 250–53, and $<53 \mu\text{m}$) by the wet-sieving method [21]. Briefly, about 50 g air-dried bulk soil samples ($<2 \text{ mm}$) were wetted slowly up to saturation and kept overnight. Then samples were sieved

through 250 μm and 53 μm sieves using automated sieving equipment. The wetted soil samples were then shaken for 30 min under water and the material remaining on each of the sieves was collected; with the $<53\mu\text{m}$ fraction being collected by centrifugation. Each collected fraction was oven-dried at 60°C for 48 hours and weighed for the fraction percentage. A sub-sample of each particle-size fraction was then ground to 0.15 mm for SOC and total N content measurement. The remaining soil (not ground) was kept to measure SOC, N mineralization and soil microbial biomass by incubation. SOC and total N of bulk particle-size soil fractions were analyzed using a C/N/H/S-analyzer (EA3000, Italy).

Incubation experiment for SOC and N mineralization

For each treatment from the long-term trial, the C and N mineralization of different particle-size fractions and bulk soil were determined from laboratory incubations (in triplicate) using the method reported by Steinweg, et al. [22]. Briefly, a 25 g sample of each particle-size soil fraction was mixed with 25 g field-moist bulk soil to provide a source of microbial inoculums from the same field treatment plot. The soil mixture was then pre-incubated at 25°C for 7 days in a 200 mL jar that contained 5 mL of distilled water that was kept separated to the soil and used to increase humidity. After the pre-incubation period, a 10 g subsample of the soil mixture from each jar was extracted with 50 mL of 2 M KCl to examine the initial ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, which were quantified using a flow injection analyzer (FIAstar 5000, Sweden). The remaining soils were then incubated at 25°C for 12 days in the closed jars, containing an alkali trap (a 50 mL plastic cup containing 20 mL of 0.25 M NaOH). Each jar was regularly opened and ventilated for 5 minutes. After incubation, the final soil samples were subjected to the same KCl extraction to measure NH_4^+ and NO_3^- concentrations. Net N mineralization rates were calculated as the difference between the final and initial extractable inorganic N ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) concentrations, after adjustment soil from fresh dry weight. SOC mineralization was calculated as the $\text{CO}_2\text{-C}$ absorbed in NaOH, as measured by total oxidizable carbon (Multi N/C 3100, Switzerland). SMB-C of bulk soil and particle-size fractions as well as SMB-N of bulk at the end of incubation were measured using the chloroform fumigation—extraction (K_2SO_4) method [23].

Calculation and statistical analysis

Cumulative $\text{CO}_2\text{-C}$ and net N mineralization rates were calculated as the percentage of C or N mineralized compared with the total bulk soil SOC and total N content. The microbial quotient was calculated as the percentage of SMB-C to total SOC, while the metabolic quotient ($q\text{CO}_2$; i.e., microbial efficiency) was calculated as the rate of $\text{CO}_2\text{-C}$ evolved per unit of SMB-C. The change in SOC and total N of bulk soil and particle-size fractions (as well as the percentage change) under different treatments were analyzed using analysis of variance (ANOVA) and LSD test at 5% level of significance. Statistical analysis was conducted using SPSS 11.5. A linear regression was used to determine the relationships between the SOC (total N) storage and potential C (N) mineralization in bulk soil and different particle-size fractions after 32 years of manure fertilization.

Results

Change in SOC, total N and soil microbial biomass after 32 years of manure application

The SOC and total N of the control ($M_0\text{CK}$) remained unchanged over the 32 years of field treatments (16.1 vs. 16.9 g C kg^{-1} ; 1.9 vs. 1.8 g N kg^{-1} ; [Table 1](#)). Long-term manure application

Table 1. The soil organic carbon (SOC), total nitrogen (N), soil microbial biomass carbon (SMB-C), soil microbial biomass nitrogen (SMB-N), potential C (C_{min}) and N (N_{min}) mineralization, SMB-C/SMB-N, SMB-C/SOC, and CO_2 /SMB-C (qCO_2) in bulk soil from the control (CK) or soil that has received 32 years of chemical fertilizer application (N, P: phosphorus, K: potassium) and either 0 (M_0), 30 (M_{30}) or 60 (M_{60}) t manure $ha^{-1} yr^{-1}$.

Treatment	SOC	Total N	SMBC	SMBN	C_{min}	N_{min}	SMBC:SMBN	SMBC:SOC	qCO_2
	$g\ kg^{-1}$							$mg\ kg^{-1}$	
CK									
M_0	16.9e	1.76d	285e	52.1c	151.0a	10.8de	5.5ef	1.7cd	0.54a
M_{30}	23.8d	2.29c	611bcd	99.7a	136.1ab	17.3cd	6.1def	2.6a	0.22b
M_{60}	27.5b	2.75b	641bc	72.3bc	123.3ab	32.4a	8.9bc	2.3ab	0.19b
N									
M_0	16.6e	1.79d	349e	55.5bc	155.5a	10.9de	6.3def	2.1abcd	0.45a
M_{30}	25.5c	2.54bc	512d	63.7bc	99.2b	14.4cd	8.0cd	2.0bcd	0.19b
M_{60}	30.8a	3.16a	687ab	61.8bc	125.6ab	20.2bc	11.1ab	2.2abc	0.18b
NPK									
M_0	17.0e	1.80d	267 e	53.0c	122.5ab	16.7cd	5.0f	1.6d	0.46a
M_{30}	22.7d	2.43bc	544cd	77.2ab	139.6ab	7.0e	7.0de	2.4ab	0.25b
M_{60}	30.6a	3.45a	791a	68.6bc	138.6ab	26.1ab	11.5a	2.6a	0.18b

Note: Different letters indicate significant differences at 5% probability level under different treatments.

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increased SOC by 34–82% and total N by 30–96%, while the application of chemical fertilizer alone (M_0N and M_0NPK) had no effect on SOC or total N content, compared with the M_0CK . The size of the SMB-C increased by 46–196% (and SMB-N increased by 11–91%), in response to 32 years of manure application, whereas there was no effect of chemical fertilizers application alone (Table 1). Nitrogen mineralization significantly increased with increase in manure application rate, while chemical fertilizer or manure application did not influence the C mineralization rate (Table 1). There was no impact of chemical fertilizer application on the C:N of soil microbial biomass (SMB), while increasing manure application resulted in a significant increase in this ratio from a bacterial (M_0 ; SMB C:N ratio = 5.6:1) to a fungal (M_{60} ; SMB C:N ratio = 10.5:1) dominated system (Table 1). There was a tendency for the microbial quotient (qCO_2) to be higher in the plots that did not received manure; this was significant within the CK and NPK treatments (Table 1). Regardless of chemical fertilizer application, the qCO_2 was significantly higher (approximately double) in the no manure treatments (M_0) compared with plots that received manure application. However, there was no significant difference in qCO_2 between the treatments with M_{30} vs. M_{60} . Compared with the initial year soil values, the treatments with M_{30} and M_{60} significantly increased SOC by 7.7 and 11.4 $g\ kg^{-1}$, and total N by 0.39 and 0.85 $g\ kg^{-1}$, respectively (Fig 1). This increments represented conversion efficiencies C of 9.16% (M_{30}) and 6.80% (M_{60}) from manure C to SOC, and of 9.3% (M_{30}) and 10.1% (M_{60}) from manure-N to soil total N. The effect of combined application of manure and chemical fertilizer was better for SOC and total N sequestration than application manure alone.

SOC and total N in particle-size fractions

Fractionation recovery rate of total soil weight of the three fractions ranged from 96.1–101.7%; the recovery rate of SOC in the three fractions ranged from 90.2–101.8%, and that of total N ranged from 89.0–103.4%. Long-term manure with/without chemical fertilizer application significantly increased the content of SOC and total N in all soil particle-size fractions, while application of chemical fertilizer on its own (M_0N , M_0NPK) did not alter SOC or total N

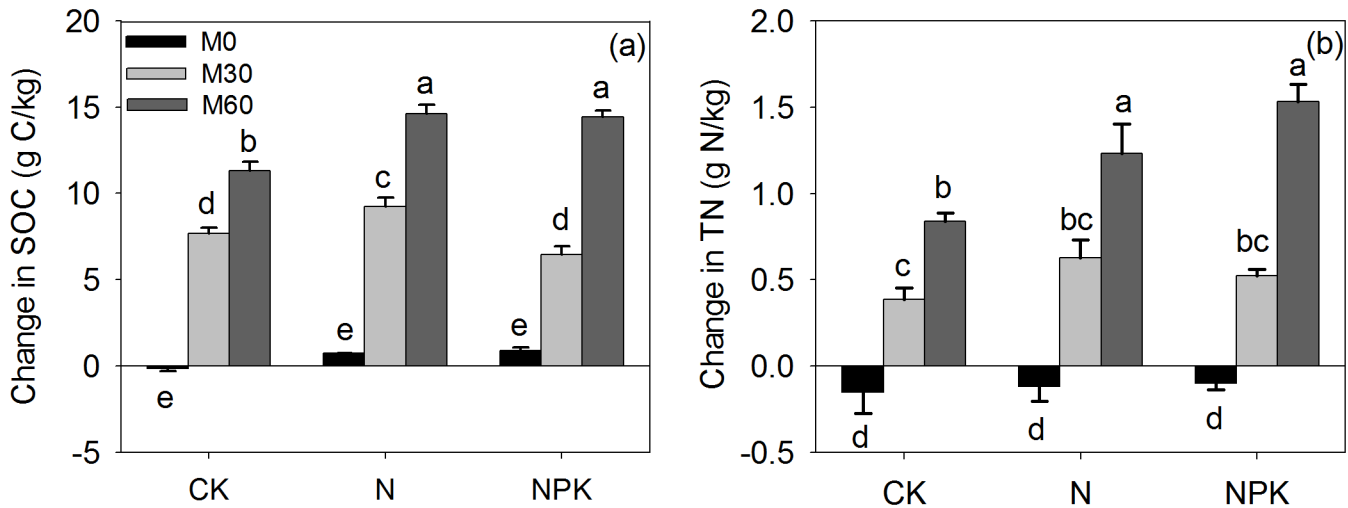


Fig 1. Change in soil organic carbon (SOC, a) and total nitrogen (N) (b) in bulk soil from the control (CK) or soil that has received 32 years of chemical fertilizer application (N, P: phosphorus, K: potassium) and either 0 (M_0), 30 (M_{30}) or 60 (M_{60}) t manure $\text{ha}^{-1} \text{yr}^{-1}$. Note: Different letters above the bars indicate significant differences at 5% probability level. Capped bars are standard error of the mean ($n = 3$).

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content in any soil particle-size fractions compared with the control (CKM_0) (Fig 2). Compared with no manure application (M_0), the 30 and 60 t ha^{-1} annual manure application rates increased the SOC in soil fractions by 20–60% (M_{30}) and 52–115% (M_{60}), and increased total N by 34–57% (M_{30}) and 61–99% (M_{60}). The greatest amount of SOC (45.1 g C kg^{-1} fraction) and total N (4.1 g N kg^{-1} fraction) occurred in the 2000–250 μm particle-size fraction under the M_{60} NPK treatment. There was no significant difference in the soil C:N ratio of the 2000–250 μm fraction among treatments (C:N = 10.5:1), while manure combined with NPK application decreased the soil C:N ratio of the 250–53 μm (C:N = 9.8:1) and <53 μm fractions to some extent (C:N = 8.8:1). The 2000–250 μm and 250–53 μm particle-size fractions contained approximately 37% of the total SOC and total N pools among the three particle-size fractions, regardless of the difference in management treatments (Fig 3).

The amount of SOC and N mineralized differed among particle-size fractions and under different fertilizer application treatments (Table 2). For the CK treatments, there were no differences among the three soil fractions under manure application. However, there was more C mineralized from the <53 μm fraction with manure applied at 60 $\text{t ha}^{-1} \text{yr}^{-1}$ (M_{60}), compared with no manure (M_0 , Table 2). As a general trend N mineralization increased with manure application, showing more N mineralized from <53 μm fraction under the M_{30} NPK and M_{60} NPK treatments. There was no relationship between the storage of SOC or total N and the C or N mineralization from the bulk soil (Fig 4). Interestingly, N mineralization was strongly related to total N storage in the 250–53 μm and <53 μm fractions and C mineralization was significantly positive related to SOC storage in the 2000–250 μm and <53 μm fractions.

Long-term application of manure significantly increased SMB-C in all soil fractions with and without manure. However, chemical fertilizer alone application showed no effect on SMB-C (Table 3). Compared with long-term application of chemical fertilizer, manure application had significantly decreased the microbial metabolic quotient (Table 1). The phenomenon of microbial metabolic quotient decreasing mainly occurred in the 250–53 μm and <53 μm fractions (Table 3).

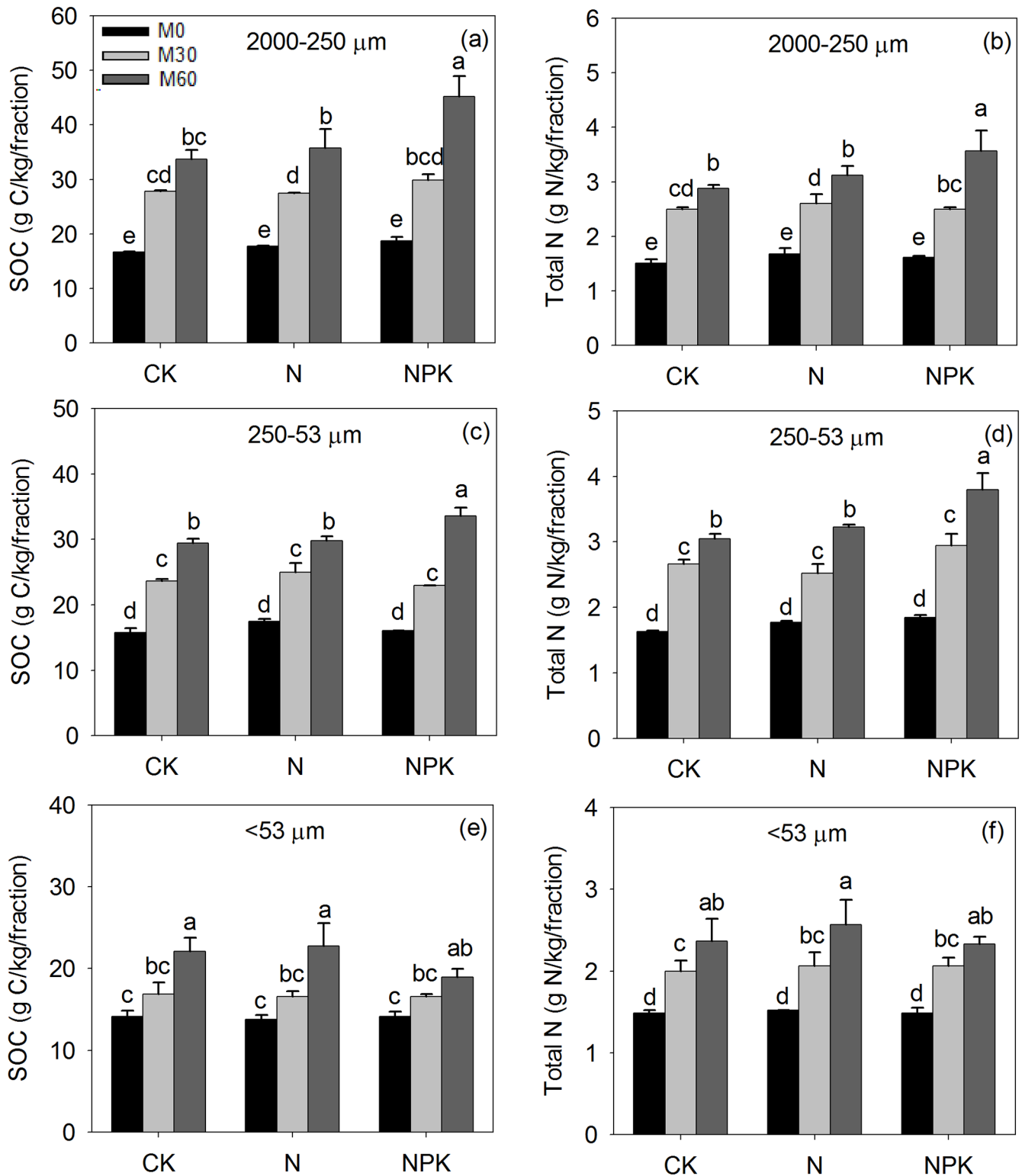


Fig 2. The content of soil organic carbon (SOC) in 2000–250 μm (a), 250–53 μm (c), <53 μm (e) fraction and total nitrogen (N) in 2000–250 μm (b), 250–53 μm (d), <53 μm (f) fraction from the control (CK) or soil that received 32 years of chemical fertilizer application (N, P: phosphorus, K: potassium) and either 0 (M₀), 30 (M₃₀) or 60 (M₆₀) t manure ha⁻¹ yr⁻¹. Note: Different letters above the bars indicate significant differences at 5% probability level. Capped bars are standard error of the mean (n = 3).

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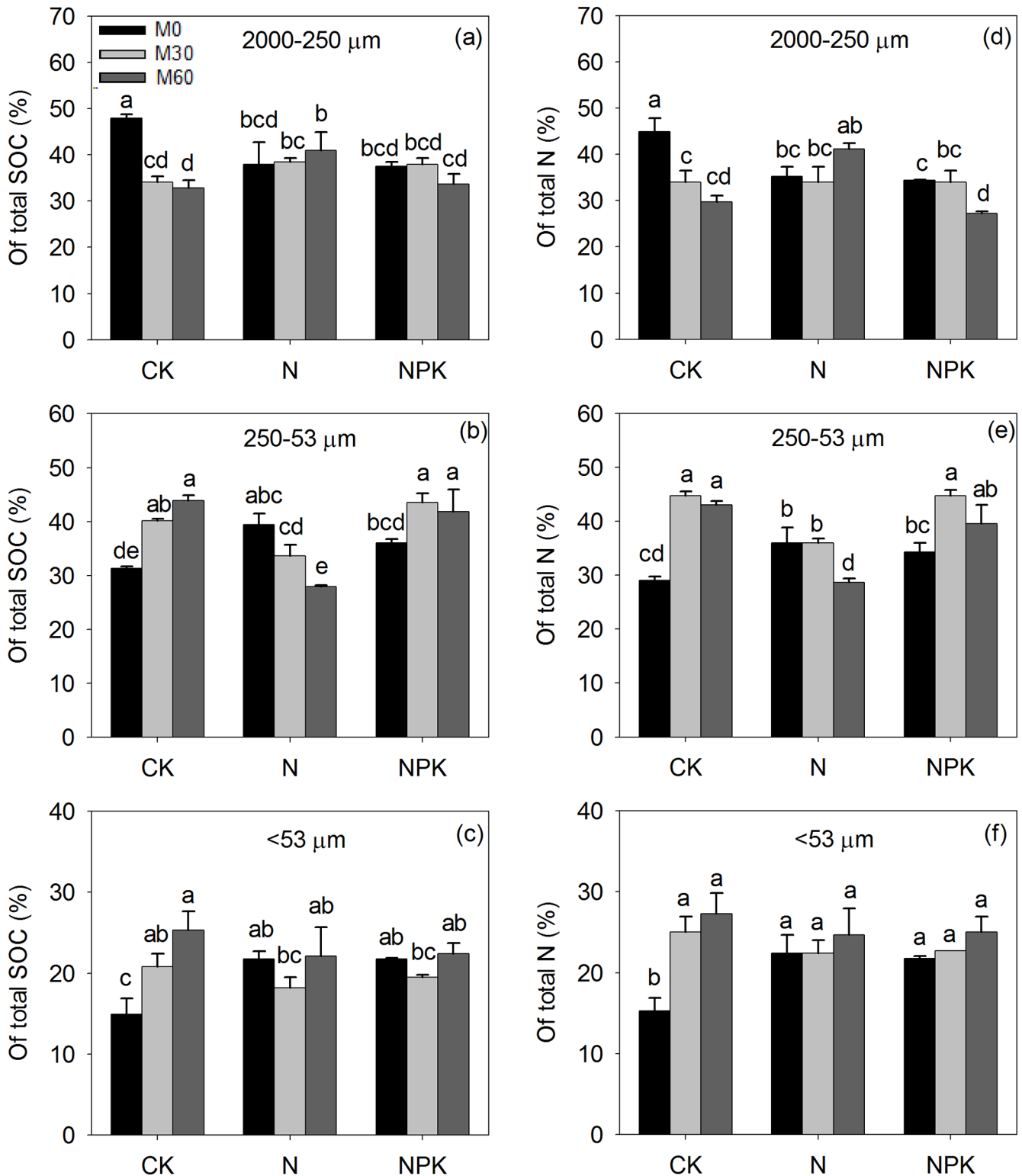


Fig 3. The percent (%) of soil organic carbon (SOC) in 2000–250 μm (a), 250–53 μm (c), <53 μm (e) fraction and total nitrogen (N) in 2000–250 μm (b), 250–53 μm (d), <53 μm (f) fraction from the control (CK) or soil that received 32 years of chemical fertilizer application (N, P: phosphorus, K: potassium) and either 0 (M₀), 30 (M₃₀) or 60 (M₆₀) t manure ha⁻¹ yr⁻¹. Note: Different letters above the bars indicate significant differences at 5% probability level. Capped bars are standard error of the mean (n = 3).

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Table 2. The potential pool of soil organic carbon (SOC) and nitrogen (N) mineralized in different particle-size fractions from the control (CK) or soil that received 32 years of chemical fertilizer application (N, P: phosphorus, K: potassium) and either 0 (M₀), 30 (M₃₀) or 60 (M₆₀) t manure ha⁻¹ yr⁻¹ (mg kg⁻¹ soil).

Treatment	SOC mineralization			Soil N mineralization		
	2000–250 μm	250–53 μm	<53 μm	2000–250 μm	250–53 μm	<53 μm
CK						
M ₀	30.5bc	27.1abcd	8.5e	1.1bc	1.2abc	0.9cd
M ₃₀	25.9bc	36.3abc	34.0bc	2.5bc	5.7a	6.6abc
M ₆₀	24.1c	43.9a	34.6bc	2.4bc	3.5abc	2.5cd
N						
M ₀	23.9c	26.6abcd	20.6cde	1.5bc	- 1.2c	3.1bcd
M ₃₀	42.2ab	24.5bcd	28.3bcd	7.7a	5.8a	4.7abcd
M ₆₀	48.6a	34.1abc	38.7b	5.2ab	1.2abc	6.2abcd
NPK						
M ₀	23.1c	22.3cd	19.0de	- 0.7c	- 0.6bc	0.5d
M ₃₀	29.1bc	13.6d	16.7de	3.9ab	4.7ab	8.7ab
M ₆₀	29.6bc	42.3ab	59.1a	1.7bc	6.3a	9.5a
Average ^a	33.3	32.5	35.2	3.9	4.5	6.4

Note: Different letters indicate significant differences at 5% probability level within the same particle-size fraction.

^a the average represent manure treatments.

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Discussion

The effect of manure application on SOC and total N distribution

Long-term manure application, with/without chemical fertilizer application, significantly increased SOC and total N in bulk soil and all particle-size fractions, indicating that manure application was effective at building new soil organic matter in this agro-ecosystem [24, 25]. The SOC and total N content in the 2000–250 μm and <53 μm fractions (and thus bulk soil) was further increased when manure was applied in combination with chemical nutrition of N, P, and K (M₆₀NPK) compared to manure alone (M₆₀CK). Therefore, for soil fertility, the inorganic nutrient status played an important role in the extent of soil C sequestration. Our findings were consistent with findings from Zhu, et al. [26], who showed that while mineral fertilizer application can maintain high yields, a combination of chemical fertilizers plus manure was required to enhance soil C sequestration. The content of SOC and total N in soil particle-size fractions declined with a decrease in particle-size, indicating that larger-size soil fractions were the main pool of SOC and total N [27], with the less decomposable SOC and N associated with fine soil particles [28].

The effect of manure application on SOC and N mineralization

The amount of SOC and N mineralization differed according to the size of soil particle-size fractions. Our study revealed that the amount of SOC and N mineralization followed the order: 53 μm > 250–53 μm > 2000–250 μm fraction. However, our findings contrasted with findings that reported organic C in soil aggregates to be more stable in small particle-size fraction than in larger particle-size fraction [29]. A similar amount of C was mineralized from the soil particle-size fractions. A number of studies have shown that SOC stability in aggregates generally increases with decreasing aggregate size [30, 31], primarily due to physical protection in micro aggregates and/or physico-chemical stabilization in the silt + clay fraction [32, 33]. Therefore,

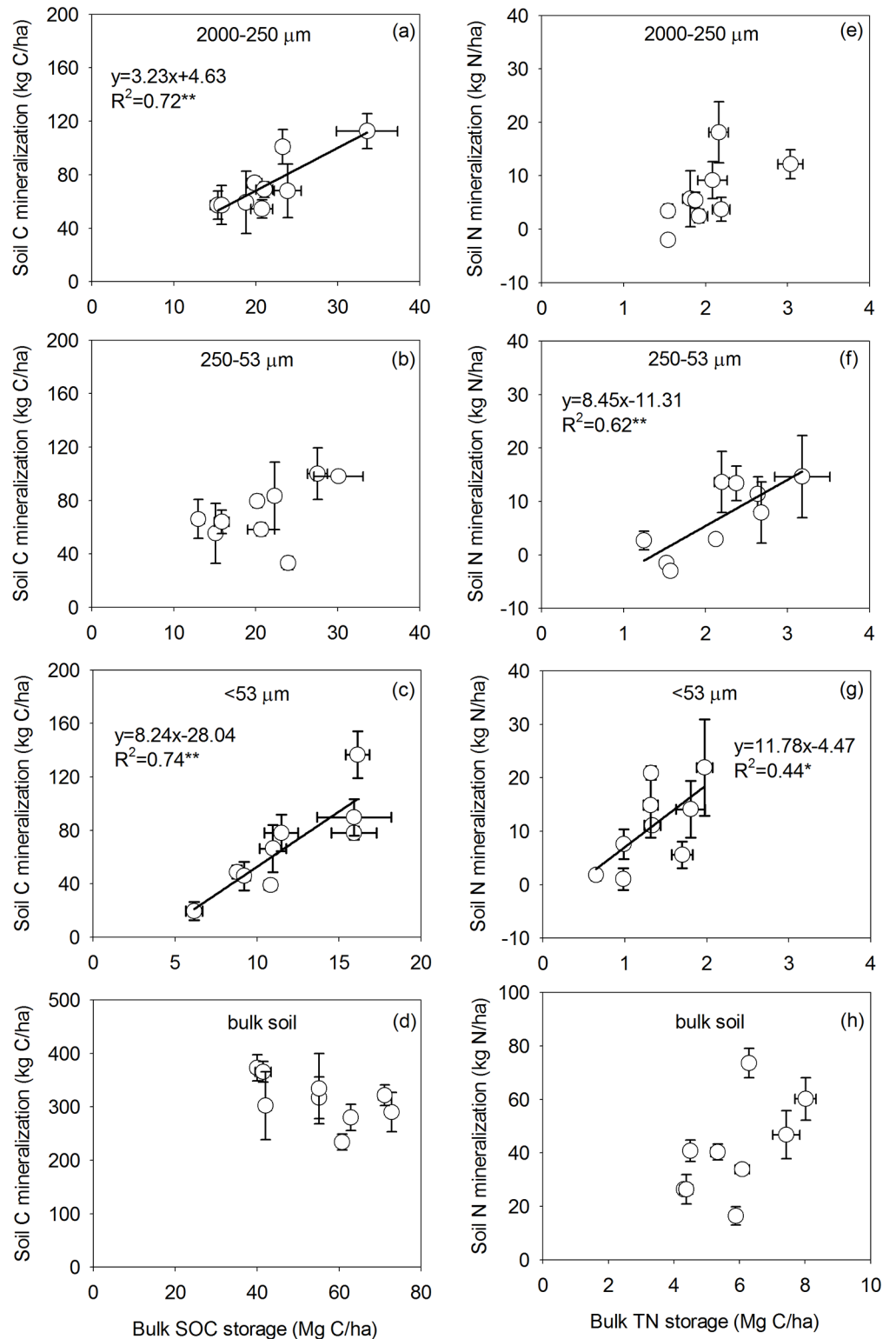


Fig 4. Relationship between soil organic carbon (SOC) storage vs potential carbon (C) mineralization, and total N storage, potential nitrogen (N) mineralization in 2000–250 μm , 250–53 μm , <53 μm fraction and bulk soil after 32 years of manure fertilization.

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Table 3. Soil microbial biomass carbon(SMB-C) and CO₂/SMB-C (qCO₂) in different particle-size fractions from the control (CK) or soil that received 32 years of chemical fertilizer application (N: nitrogen, P: phosphorus, K: potassium) and either 0 (M₀), 30(M₃₀) and 60 (M₆₀) t manure ha⁻¹ yr⁻¹.

Treatment	SMB-C (mg kg ⁻¹ soil)			CO ₂ /SMB-C		
	2000–250 μm	250–53 μm	<53 μm	2000–250 μm	250–53 μm	<53 μm
CK						
M ₀	68cd	32de	18e	0.46b	0.84b	0.49c
M ₃₀	64cd	56cd	55bc	0.38b	0.64bc	0.61bc
M ₆₀	79bc	99ab	81b	0.31b	0.45cd	0.43c
N						
M ₀	31e	22e	25de	1.11a	1.34a	1.06ab
M ₃₀	104ab	82bc	63b	0.41b	0.31cd	0.51c
M ₆₀	108a	62cd	34cde	0.46b	0.55bc	1.15a
NPK						
M ₀	45de	42de	31cde	0.50b	0.50bcd	0.69abc
M ₃₀	84abc	78bc	53bcd	0.36b	0.18d	0.33c
M ₆₀	68cd	125a	126a	0.47b	0.37cd	0.51c

Note: Different letters indicate significant differences at 5% probability level within the same particle-size fraction.

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soil C sequestration was variable due to physical protection in the <250 μm fraction or physico-chemical stabilization in the <53 μm fraction, depending on both soil types and management measures [34, 35]. The N mineralized in the <53 μm fraction was about 1.5 times greater than that in the other two fractions, suggesting that smaller particle-size fractions contain a larger proportion of readily mineralizable organic N than larger particle-size fraction in Mollisols. The significant linear relationships between SOC storage and C mineralization (and similarly total N versus N mineralization) in the <53 μm particle-size fraction indicated that this fraction had the greatest capacity for SOC and N mineralization.

The effect of manure application on soil microbial biomass and qCO₂

Our findings clearly indicated that the addition of manure increased SMB-C within all fractions and bulk soil. However, the highest level of SMB-C occurred in treatment plots that received the highest manure level (M₆₀) in combination with chemical fertilizers (M₆₀N; M₆₀NPK), indicating a nutrient limitation to the size of the microbial biomass in other treatments. We measured more SMB-C in the larger particle-size fractions, which supported that microbial biomass had different distributions in different particle-size fractions [36]. Similarly, Franzluebbbers and Arshad [37] have also reported higher SMB-C in the >250 μm fraction than in the <250 μm fraction, probably due to differences in microbial accessibility to substrates resulting from fraction stability. On the contrary, other researcher has reported same or higher microbial activity in the <250 μm fraction [38]. The average ratio of SMB-C to SMB-N of fungi is about 15:1, versus 6:1 for bacteria. We found 32 years of manure application caused a shift from a bacterial to a fungal dominated population, which promoted the transformation of soil organic C and N storage [39, 40].

Long-term manure and chemical fertilizer application had a significant influence on the SMB-C and SOC mineralization. Manure application stimulates soil microbial activity due to the increased annual additions of fresh C inputs [41]. The qCO₂ provides a measure of the specific metabolic activity, which varies according to the composition and physiological state of the microbial community, the availability of substrates, and various abiotic factors. Our finding

that reduction by the addition of manure compared with without manure in $q\text{CO}_2$ indicated lower catabolic demand of the soil microbial community [42]. It is believed that high soil $q\text{CO}_2$ might be attributed to lower availability of soil nutrients [43], whereas the lower $q\text{CO}_2$ under organic practices might be due to the protective capacity of the microbial biomass [44]. Either way, results suggested a more stable environment for microbial activity when manure was applied to soil. Our findings illustrate that long-term manure application enhanced SOC and total N stabilization, especially when it was applied in combination with chemical fertilizers to enable improved microbial efficiency.

The conversion efficiency from manure C into SOC in Mollisols ($9.4 \pm 3.1\%$ for M_{30} and $8.1 \pm 2.7\%$ for M_{60}) was at the range of global manure C retention coefficient of $12 \pm 4\%$ [45]. But it was much lower than the result from similar temperate climate with high clay content ($23 \pm 15\%$) [46, 47]. The similar soil organic carbon sequestration efficiency for both the 30 and 60 t manure $\text{ha}^{-1} \text{yr}^{-1}$ application rates used in our study indicate that this soil has not yet reached its soil C saturation capacity and thus can sequester more CO_2 as SOC.

Supporting Information

S1 Dataset. S1 Dataset was the data of SOC, total N, potential C and N mineralization, soil microbial biomass carbon, nitrogen and metabolic quotient. This data contained two parts: (1). Soil organic carbon, total nitrogen, potential C and N mineralization in bulk soil and different particle-size fractions from the control (CK) or soil that received 32 years of chemical fertilizer application (N: nitrogen, P: phosphorus, K: potassium) and either 0 (M0), 30 (M30) or 60 (M60) t manure $\text{ha}^{-1} \text{yr}^{-1}$. (2). Soil microbial biomass carbon and microbial metabolic quotient in bulk soil and different particle-size fractions from the control (CK) or soil that received 32 years of chemical fertilizer application (N: nitrogen, P: phosphorus, K: potassium) and either 0 (M0), 30 (M30) and 60 (M60) t manure $\text{ha}^{-1} \text{yr}^{-1}$. (XLSX)

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Author Contributions

Conceived and designed the experiments: WJZ MGX. Performed the experiments: ADC XFS PZ HX. Analyzed the data: XFS HX. Contributed reagents/materials/analysis tools: ADC XFS PZ WJZ MGX. Wrote the paper: ADC XFS PZ WJZ MGX DVM.

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