



Article

The Inorganic Carbon Fixation Improved by Long-Term Manure Fertilization in Kastanozems under Rotation System of North China

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Abstract: In China, manure is the most abundant organic fertilizer product. Understanding the changes in soil inorganic carbon (SIC) resulting from long-term manure fertilization is vital for accurately evaluating agricultural soil carbon stocks and predicting global change. However, a comprehensive and quantitative understanding of the impacts of long-term fertilization on SIC is lacking. This study was conducted to assess the effects of SIC changes in topsoil (0–20 cm), aggregates of kastanozems and influencing factors under the potato-rape-naked oats cultivation system after 16 years of long-term different fertilization in Wuchuan station. The results showed that 16 years of application of manure promoted the fixation of SIC by 2.25 t ha^{−1}–3.25 t ha^{−1}. As soil organic carbon (SOC) content, exchangeable calcium and magnesium concentrations in free coarse particulate organic matter (cfPOM) increased, the crystallization of carbonate was promoted at the aggregate level. The distribution proportion of free-coarse particulate organic matter (cfPOM) and microaggregates in mineral-related organic matter (iMOM) were also increased which affected the content of SIC. However, the pH value of NPKM treatment was lower than that of M treatment, which reduced the amount of carbonate crystallization. Thus, the application of manure alone was the best way to promote the fixation of SIC in topsoil rather than manure combined with chemical NPK fertilize. This work provides a new insight into the conversion of inorganic carbon, which is beneficial to promote the sequestration of inorganic carbon.

Keywords: long-term fertilization; soil inorganic carbon fixation; manure; aggregate; kastanozems



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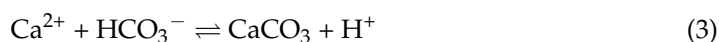
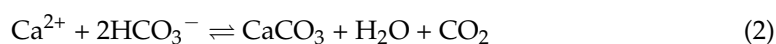
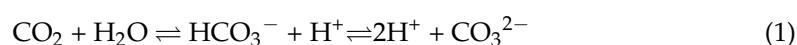
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1. Introduction

As the largest terrestrial carbon pool, soil plays a great role in soil fertility release and global carbon cycle [1–3]. Soil carbon pools are divided into organic and inorganic carbon pools [4]. At a depth of 0–2 m, global soil organic carbon (SOC) and soil inorganic carbon (SIC) storages were approximately 1460–1550 Pg C and 700–950 Pg C, respectively [5]. SIC mainly includes pedogenic carbonates and petrogenic carbonates. Most of SIC is sequestered in the 1–2 m soil layer and weathered bedrock [5]. The storage of SIC in topsoil could account for 21% in the profile [6,7]. It was very active in soil carbon cycle due to the involvement of crops and microorganisms, etc. Recently, a study using ¹³C radiological found that planting resulted in faster crop turnover and incorporation of new crop carbon into SIC pools under field conditions [8]. Calcareous soil respiration measurements might

be overestimated if CO₂ from the decomposition of inorganic carbon was not considered [9]. Therefore, it is very important for us to study the fixation of SIC in topsoil.

However, recent studies have found that SIC storage in surface soil has decreased on average in different ecosystems. A meta-analysis and nationwide survey datasets were used to investigate changes in the SIC storage in China. This study estimated that the inorganic carbon loss of China farmland soil was 11.33 g C m⁻² yr⁻¹ [9]. According to the data of China Statistical Yearbook (2022), the arable land area of China is 1.28 × 10⁸ ha, and the chemical fertilizer application amount is 5.19 × 10⁷ t. Widespread soil acidification due to atmospheric acid deposition and agricultural fertilization may greatly accelerate soil carbonate dissolution and CO₂ release [10]. Fertilization and irrigation also changed the potential of soil pedogenic carbonates [11,12]. The dissolution balance of inorganic carbon in soil is affected by the partial pressure of CO₂ in soil, soil pH, soil moisture, and the content of calcium and magnesium ions [11,13,14]. Taking calcium ions as an example, inorganic carbon has the following transformation equilibrium in soil:



The loss of SIC can be reduced by alkaline regeneration. Under the action of microorganisms, soil organic fertilizer mineralization can release CO₂ and alkaline cations such as Ca²⁺ and Mg²⁺ [15]. Moreover, by delaying carbonate recrystallization, microorganisms obviously affect the balance between soil CO₂, HCO₃⁻ and CaCO₃ [16], which lead to the direction of inorganic carbon precipitation.

Nonetheless, people often apply manure and chemical fertilizer together. Despite the potential for soil acidification, long-term N fertilization not only directly contributes to soil N resources, but also indirectly improves soil structure through the formation of large macroaggregates, accelerating SOC turnover, and shifting the localization of microorganisms to the macroaggregates [17,18]. In addition, it has also been shown that precipitation of soil secondary carbonates can enhance soil polymerization [19]. Soil aggregate fractions presented different sensitivity to changes in different fertilization management. Results in Mollisol soil showed that physical, physico-biochemical and physico-chemical protection were the predominant mechanisms to sequester carbon in the whole profile, whereas the biochemical protection mechanisms were only relevant in the topsoil [20]. An 18-year long-term experiment indicated that the particulate organic carbon (POC) fraction had the greatest sensitivity to changes in agricultural management practices [21]. Another result indicated that the most efficient fertilization practice for sequestering C in each fraction in red soil was continuous applications of either manure or manure plus mineral fertilizers, and mineral-associated organic carbon (MOC) was the primary fraction of C sequestration in red soil [22]. However, these studies did not consider the presence of inorganic carbon in alkaline soils.

It was said that SIC source CO₂ accounted for about 20% of the total CO₂ emissions in the surface soil [8]. Moreover, calcareous soils contain 5% or more inorganic carbon or carbonate calcium equivalents [23] because of the high pH, Ca, Mg, K, P and ash contents in the soil [15]. Therefore, the fixation of SOC in soil aggregates would affect the fixation of SIC. Unfortunately, previous studies mostly focused on SIC fixation in bulk soils. To date, few studies have addressed the changes in SIC in soil aggregates.

As an alkaline soil, the inorganic carbon pool of kastanozems is very important for carbon fixation. The objective of this study was to investigate the characteristics of SIC changes in aggregates under a potato-rape-naked oats cultivation system after 19 years of long-term different fertilization. We hypothesized that the long-term application of manure alone or the application of manure combined inorganic fertilizer could: (1) increase SIC content; (2) change the distribution of aggregates and increase the content of inorganic

carbon in soil aggregates. Moreover, the fixation of SIC is affected by microbial activity and basic soil properties.

2. Materials and Methods

2.1. Study Site

The study site was established in Wuchaun City, Hohhot, Inner Mongolia Autonomous Region, China (41°08′22.8″ N, 111°17′43.6″ E). This region has a semi-arid continental climate, with a mean annual temperature of 7.3 °C and mean annual precipitation of 400 mm (1 January 2004 to 31 December 2019) (Figure A1) (data from China meteorological sharing service system, <http://cdc.cma.gov.cn/>, accessed on 1 January 2020). The soil is classified as kastanozems (IUSS, 2022) and characterized by a 2:1 clay mineralogy dominated by montmorillonite [24].

2.2. Experimental Design and Soil Sampling

Long-term localization trials began in 2004. The texture was loam soil (by sedimentation method) with 53.3% sand, 31.1% silt and 15.6% clay according to the American system texture type in 2013 [25]. The top soil (0–20 cm) comprised 8.70 g kg^{−1} of soil organic carbon measured by K₂Cr₂O₇ oxidation method, 48.5 mg kg^{−1} of alkali-hydrolysable nitrogen by alkaliolytic diffusion method, 9.2 mg kg^{−1} of available phosphorus (AP) by NaHCO₃ extraction method, and 39.1 mg kg^{−1} of available potassium (AK) by flame photometry method, with pH (1:2.5) (*w/v*) 8.50 in the initial year. The cation exchange capacity was 1.92 cmol kg^{−1} by EDTA-NH₄OAc method and the total salt content (5:1) (*w/v*) was 0.83 g kg^{−1} by 0.1% sodium metaphosphate solution. The average content of CaCO₃ in the surface soil was 8.4 g kg^{−1} [26]. Pedogenic carbonates accounted for 87.7% and lithogenic carbonates accounted for 12.3% in topsoil (0–20 cm) [25]. The soil bulk density was 1.44 g cm^{−3} [25]. The treatment size was 6 m × 8 m. The crop rotation consisted of potato, rape and naked oats. Potato was planted in the initial year.

Four treatments were employed in a randomized block design with three replications: (1) unfertilized control (CK); (2) balanced fertilization of nitrogen, phosphorus and potassium (NPK); (3) manure alone (M); and (4) manure combined with chemical NPK fertilizer (NPKM). The chemical fertilizers (N, P and K) were applied as urea, calcium superphosphate and potassium chlorate, respectively. Chemical fertilizers and manure used as a substrate were applied on the surface before sowing crops every year, and then ploughed into the soil. The manure was composed of dry pure sheep manure containing 450 g kg^{−1} total organic C, 3.9 g kg^{−1} total N, 15 g kg^{−1} P₂O₅ and 7.4 g kg^{−1} K₂O before 2014 and 466 g kg^{−1} total organic C, 9.5 g kg^{−1} total N, 51 g kg^{−1} P₂O₅ and 19.5 g kg^{−1} K₂O since 2014. The amount of fertilizer applied under different treatments is shown in Table A1 [26].

After the potatoes were harvested, the stems and leaves of the above-ground part were all returned to the field. The stubble of rapes and naked oats were not included in the carbon input as the soil was ploughed and harrowed before sowing the next year.

2.3. Measurements of Soil Basic Chemical Properties

Soil samplings were performed from the 0 to 20 cm soil layer in 2019 after crop harvest. Each subsample was divided into three parts by the quad method. Before dividing, we need to mix each soil sample individually. Fresh samples were kept cold with ice packs, then immediately transferred to the laboratory, stored at 4 °C before analysis. Roots and stones in the soil need to be removed. Air-dried samples were air dried at room temperature and used to measure soil basic physical and chemical properties after passing through sieves of different sizes. The air-dried soil samples were divided into three quartiles by quartering from mixed soils, one for SOC and total nutrient content through a 0.15 mm sieve, one for available nutrient content through a 2 mm sieve, and one for aggregate grading through a 5 mm sieve.

The basic physicochemical properties of the soil samples were characterized according to the analytical methods for soil and agro-chemistry published by China agricultural science and technology press [27] (Table 1). Soil pH (1:2.5) (*w/v*) was measured by pH meter (FE20, Mettler Toledo, Shanghai, China) [28]. The soil NO_3^- -N and NH_4^+ -N were extracted by 2 mol L⁻¹ KCl solution and analyzed using a Skalar flow analyzer (San++ 5000, Breda, The Netherlands). Soil microbial biomass C and N (SMBC and SMBN) were determined after 24 h of chloroform fumigation extraction using Network GC System (6890N, Agilent Technologies, Inc., Santa Clara, CA, USA). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were measured using a TOC/TN total organic carbon/total nitrogen analyzer (Multi N/C3100, Analytikjena AG, Jena, Germany). Exchangeable calcium and magnesium were extracted by 70% ethanol—1 mol L⁻¹ NH_4Cl solution and analyzed by ICP-OES (Varian 715-ES, Agilent Technologies, Inc., Santa Clara, CA, USA) [28]. Soil total phosphorus (TP) and total potassium (TK) were extracted by NaOH in Muffle furnace. Then, TK was measured by flame photometer (410, Sherwood Scientific Ltd., Cambridge, UK). After 7.5 mol L⁻¹ molybdenum antimony was extracted for 30 min, TP was measured at 700 nm using an UV-visible spectrophotometer (752N, Shanghai Jingke Industrial Co., Ltd., Shanghai, China) [27]. AP was obtained by 0.5 mol L⁻¹ NaHCO_3 solution after shocked for 30 min, and measured at 660 nm using an UV-visible spectrophotometer. AK was obtained by 1 mol L⁻¹ NH_4OAc solution after shocked for 30 min and measured by flame photometer [27].

Table 1. Soil basic properties under different treatments after 16 years of fertilization.

Treatments	CK	NPK	M	NPKM
TN (g kg ⁻¹)	0.94 b	0.98 b	1.45 a	1.50 a
SOC (g kg ⁻¹)	7.93 b	8.18 b	11.60 a	12.75 a
pH (1:2.5)	8.26 a	8.27 ab	8.27 a	8.04 b
DOC (g kg ⁻¹)	60.03 c	61.32 c	131.46 b	169.02 a
DON (g kg ⁻¹)	16.83 c	28.02 bc	39.62 b	66.40 a
SMBC (g kg ⁻¹)	165.15 c	226.46 b	361.94 a	319.14 a
SMBN (g kg ⁻¹)	36.57 b	50.20 b	82.51 a	67.17 a
TK (g kg ⁻¹)	22.61 ab	19.54 b	24.31 a	22.69 ab
AK (g kg ⁻¹)	87.3 c	109.7 c	206.7 b	328.0 a
TP (g kg ⁻¹)	0.38 b	0.35 b	0.53 a	0.37 b
AP (g kg ⁻¹)	2.63 c	8.90 b	12.07 b	33.00 a

The four treatments were: (1) unfertilized control (CK); (2) balanced fertilization of nitrogen (N), phosphorus (P) and potassium (K) (NPK); (3) manure alone (M); and (4) manure combined with chemical NPK fertilizer (NPKM). Means with similar lower-case letters within a column are not significantly different at $p < 0.05$. Abbreviations: TN, total nitrogen content; SOC, soil organic matter; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; SMBC, soil microbial biomass carbon; SMBN, soil microbial biomass nitrogen; TK, total potassium; AK, available potassium; TP, total phosphorus; AP, available phosphorus.

Soil total (SC) and soil organic carbon (SOC) were analyzed using the C/N EuroVextor elemental analyzer (EA3000, Via, Tortona 5, Milano, Italy) after processing through a 0.15 mm sieve. The inorganic carbon in the soil was removed with 5 mL 1 mol L⁻¹ hydrochloric acid (HCl) solution and dried at 60 °C [29]. Then, the carbon content was measured by carbon and nitrogen element analyzer. Total soil inorganic carbon was then determined by subtracting soil organic carbon (SOC) concentrations from SC.

2.4. Soil Fractionation and Analysis

Once in the laboratory, the soil samples were gently air dried at room temperature and sieved with a 5 mm sieve. In this experiment, the soils were grouped according to the grouping of aggregate organic matter [22,30]. The detailed process can be seen in Figure A2.

Thus, mean weight diameter (MWD), geometric mean diameter (GMD) under different fertilization treatments could be calculated from the following equation:

$$\text{MWD} = \sum X_i \times W_i \quad (4)$$

$$\text{GMD} = \exp[(\sum W_i \times \lg X_i) / (\sum W_i)] \quad (5)$$

where X_i is the mean diameter (mm) of the soil aggregate size fractions; W_i is the proportion of each aggregate size with respect to the total sample weight.

2.5. Statistical Analyses

All data were subjected to one-way analysis of variance (ANOVA) according to Duncan's multiple range test ($p < 0.05$). Data and correlation analyzes were processed by Microsoft Excel 2010 and mean separations were performed by SPSS 26 software (IBM SPSS Inc., Chicago, IL, USA). Figures were generated using Origin 8.5 software (OriginLab Corporation, Northampton, MA, USA). Redundancy analyzes (RDA) and random forest (RF) analyzes were generated using Canoco 5.0 software (Microcomputer Power, Ithaca, NY, USA).

3. Results

3.1. Soil Basic Properties

After 16 years of fertilization, compared to CK treatment, the pH in M treatment decreased by 0.22. Meanwhile, the contents of AK, AP and exchangeable Mg^{2+} in the soil under M and NPKM treatments were significantly increased by 136.6–275.6%, 3.58–11.53 times and 31.4–32.5% compared with CK, respectively (Table 1). The pH value in M treatment was 0.23 higher than that in NPKM treatment (Table 1).

The total nitrogen content under M and NPKM treatments was significantly increased by 54.8% and 60.3% compared with CK, respectively, and the contents of different forms of nitrogen also increased (Table 1). The soil DOC and DON contents under different long-term fertilization are presented in Table 1. The content of soil DOC in M and NPKM treatments significantly increased by 119.0–181.6% and 114.4–175.6%, respectively, compared with CK and NPK treatments, respectively. The content of soil DON under NPKM treatment was significantly higher than that under CK and NPK treatments, 135.4% and 137.0% higher, respectively. The DON content under M treatment was significantly higher, at 135.4%, than that under CK treatment. DOC and DON contents were not significantly different between CK and NPK treatments. The content of soil SMBC under NPK, M and NPKM treatments was significantly higher 37.1–119.2% than that under CK treatment (Table 1). The content of soil SMBN was significantly higher under M and NPKM treatments than that under CK treatment, namely 125.6% and 83.7% higher, respectively.

3.2. Aggregate Distribution and Stability

The mass distribution of soil dry matter in the different physically isolated fractions varied considerably under different treatments (Table 2). The cfPOM and the ffPOM fractions (unprotected fractions) accounted for 34.5–38.3% and 0.29–0.74% of the total whole soil dry matter under different fertilization treatments, respectively, while the iPOM fraction accounted for 11.8–13.5%, and iMOM and oMOM (biochemically protected) accounted for 28.4–30.2% and 17.4–24.4%, respectively.

Table 2. Soil aggregate distribution (%) under different fertilization treatments.

Treatments	>0.25 mm	0.053–0.25 mm				<0.053 mm	MOM
	cfPOM	0.053–0.25 mm	ffPOM	iPOM	iMOM	oMOM	
CK	34.47 b	41.18 a	0.29 c	12.45 a	28.44 b	24.35 a	52.78 a
NPK	34.24 b	42.64 a	0.46 bc	12.56 a	29.61 ab	23.12 a	52.73 a
M	36.53 ab	44.94 a	0.74 a	11.84 a	32.35 a	18.53 b	50.89 ab
NPKM	38.26 a	44.39 a	0.68 ab	13.50 a	30.21 ab	17.36 b	47.57 b

cfPOM means coarse free particulate organic matter fraction, iPOM means particulate organic matter inner microaggregate, iMOM means mineral-associated organic matter inner microaggregate, and oMOM means mineral-associated organic matter outer fraction. MOM means mineral-associated organic matter fraction. Means with similar lowercase letters within same color aggregate were not significantly different at $p < 0.05$.

After 16 years of fertilization, the cfPOM composition was 6.0% and 11.0% higher under M and NPKM treatments, respectively, compared to CK treatments. The ffPOM composition under M and NPKM treatments were 132.8–154.7%, significantly greater than that under CK treatment. Nevertheless, the iPOM content in four treatments was not significantly different. Interestingly, the biochemically protected matter iMOM and the oMOM fractions showed an opposite trend in soil surface. Compared to CK treatment, aggregate distribution of iMOM in M treatment increased by 13.8%, while oMOM in M and NPKM treatments decreased by 23.9–28.7%. Thus, there was no significant difference in MOM content under M treatment and a significant decrease in MOM content under NPKM treatment compared to CK.

Mean weight diameter (MWD) and geometric mean diameter (GMD) of the aggregates varied considerably among treatments (Figure 1). After 16 years of different types of fertilization, the soil structural stability in NPKM treatment was improved compared to that in CK treatment. The MWD and GMD values under NPKM treatment were significantly higher than those under CK and NPK treatments in the 0–20 cm soil layer. The MWD and GMD values under NPKM treatment were significantly higher than those of CK by 10.5% and 19.7%, respectively. The MWD and GMD values under M treatment were higher, but not significantly, by 6.0% and 12.9%, respectively. Similarly, there were no significant differences in aggregate composition, as the MWD and GMD values under CK and NPK treatments were similar.

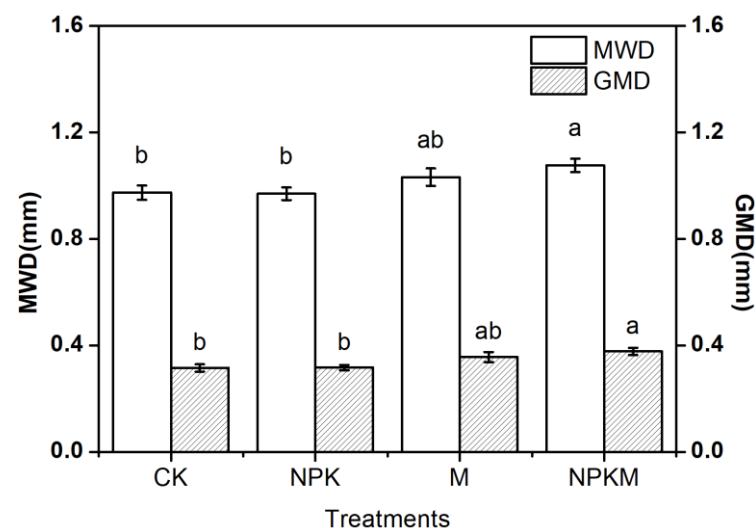


Figure 1. Soil mean equivalent diameter (MWD) and geometric mean diameter (GMD) under different fertilization treatments. Means with similar lowercase letters indicate that values in different treatments were not significantly different at $p < 0.05$.

3.3. Soil Carbon Content

Compared with the CK, long-term application of manure fertilizer could significantly increase the SOC and SIC contents. After 16 years of fertilization, the SIC content under M and NPKM treatments increased by 275.0% and 145.1% compared with that of CK, respectively (Figure 2a). Meanwhile, compared to CK, SOC content under M and NPKM treatments also increased by 46.3% and 60.8%, respectively (Table 1). Interestingly, SIC content in M treatment was significantly increased by 53.0% compared with that in NPKM treatment while SOC content in NPKM treatment was significantly 9.9% higher than that in M treatment. The SIC/SOC value of topsoil (0–20 cm) under M treatment was significantly higher than another three treatments by 69.3–158.2% (Figure 2b). There was no significant difference in SIC content between NPK and CK treatments.

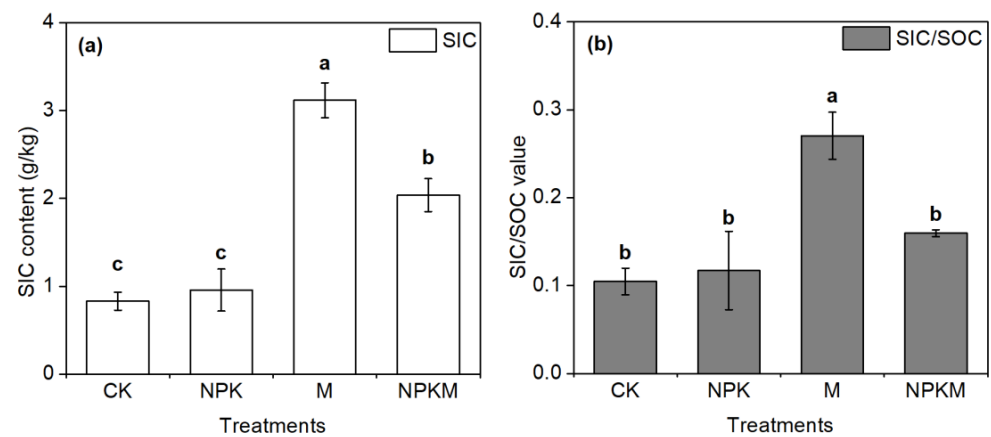


Figure 2. Soil carbon content under different long-term fertilization: (a) SIC content; (b) value of SIC/SOC. Error bars represent the standard deviations of the mean ($n = 3$). Different characters above bars indicate significant differences among the treatments at $p < 0.05$.

3.4. Soil Carbon Content in Aggregate Soils

M and NPKM significantly increased the content of SIC in different aggregates, while there was no significant difference between NPK and CK (Figure 3). Compared to CK, SIC contents of cfPOM, iMOM, oMOM under M treatment were significantly increased by 178.0%, 236.4% and 181.9%, respectively. SIC contents of cfPOM and iMOM under NPKM treatment were significantly increased by 99.5% and 229.6%, respectively. In addition, SIC content of cfPOM was significantly increased by 11.4% in M treatment than that in NPKM treatment.

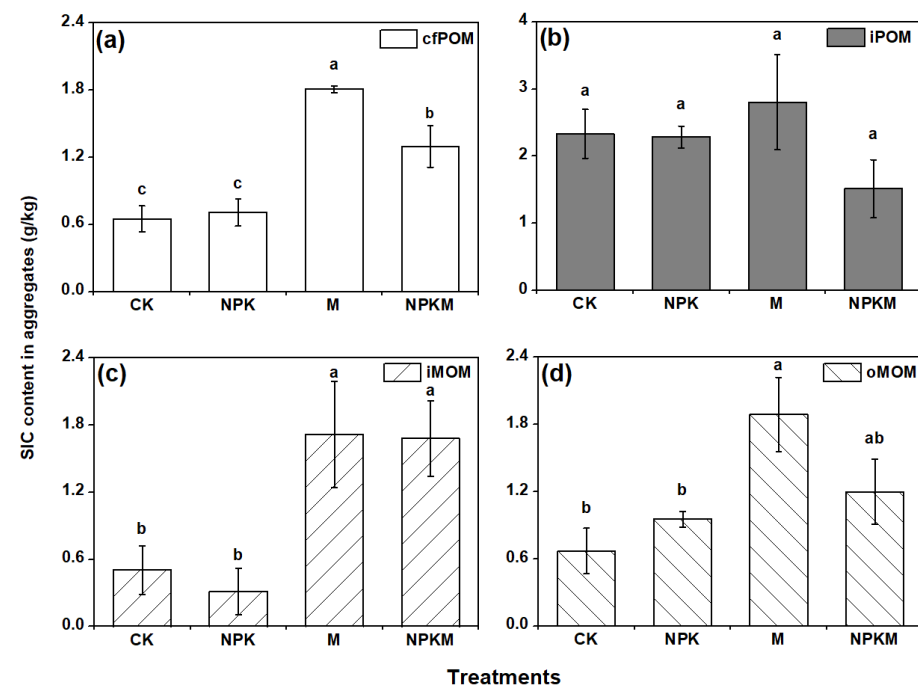


Figure 3. SIC content in different aggregates: (a) cfPOM; (b) iPOM; (c) iMOM; (d) oMOM. cfPOM means coarse free particulate organic matter fraction, iPOM means particulate organic matter inner microaggregate, iMOM means mineral-associated organic matter inner microaggregate, and oMOM means mineral-associated organic matter outer fraction. Error bars represent the standard deviations of the mean ($n = 3$). Different characters above bars indicate significant differences among the treatments at $p < 0.05$.

At the topsoil (0–20 cm), the value of SIC/SOC of oMOM was changed. It was 27.8% significantly higher in M treatment than CK (Figure 4d). There was no significant difference in the value of SIC/SOC in the other three aggregates (Figure 4a–c) after 16 years of long-term fertilization.

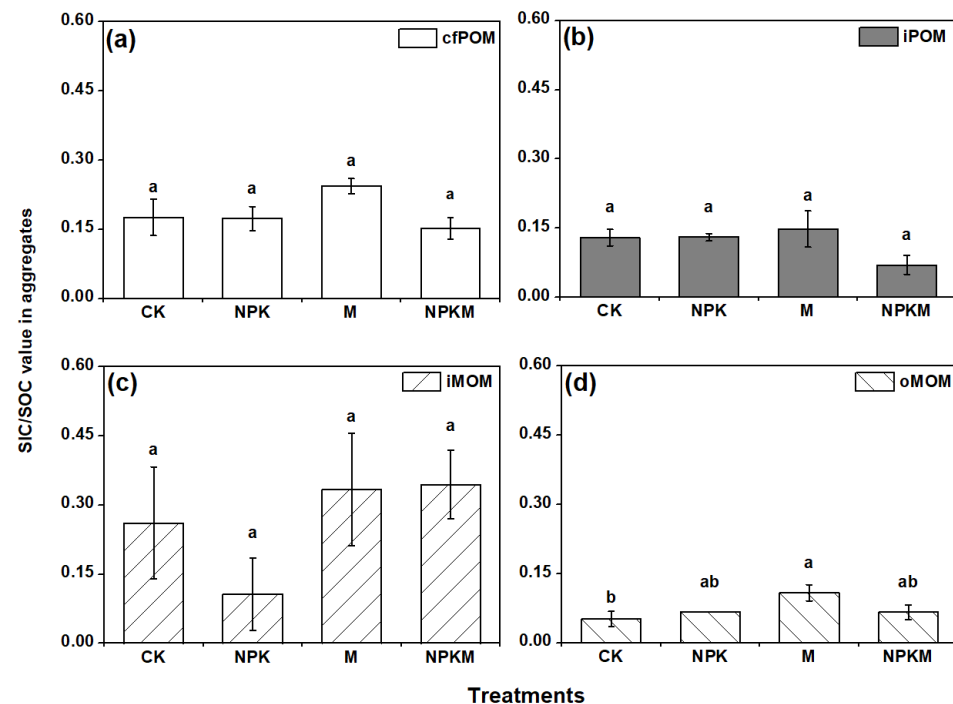


Figure 4. The value of SIC/SOC in different aggregates: (a) cfPOM; (b) iPOM; (c) iMOM; (d) oMOM. cfPOM means coarse free particulate organic matter fraction, iPOM means particulate organic matter inner microaggregate, iMOM means mineral-associated organic matter inner microaggregate, and oMOM means mineral-associated organic matter outer fraction. Error bars represent the standard deviations of the mean ($n = 3$). Different characters above bars indicate significant differences among the treatments at $p < 0.05$.

3.5. Exchangeable Calcium and Magnesium in Aggregate Soils

After 16 years of long-term fertilization, the concentrations of exchangeable Ca^{2+} and Mg^{2+} in the aggregates were different under different treatments (Figure 5). In cfPOM, compared to CK, the exchangeable Ca^{2+} concentrations under NPK, M and NPKM treatments were significantly increased by 37.53%, 40.23% and 48.37%, respectively, and the exchangeable Mg^{2+} concentrations were significantly increased by 54.49%, 105.52% and 97.77%, respectively (Figure 5a). Meanwhile, in oMOM, the exchangeable Ca^{2+} concentration under NPKM treatment was significantly decreased by 26.74%, and the exchangeable Mg^{2+} concentration under NPKM treatment was significantly decreased by 26.74% compared with CK (Figure 5d). In addition, there was no significant difference in the concentrations of exchangeable Ca^{2+} or Mg^{2+} in iPOM and iMOM under different treatments (Figure 5b,c).

3.6. RDA Analysis of Aggregate Distribution and Soil Properties

RDA was used to study the effects of treatment on soil basic properties on the distribution of soil aggregates. RDA showed that SOC and pH could be considered as the main factors driving the aggregate distribution variation, which accounted for 68.9% of the aggregate distribution variation (Figure 6).

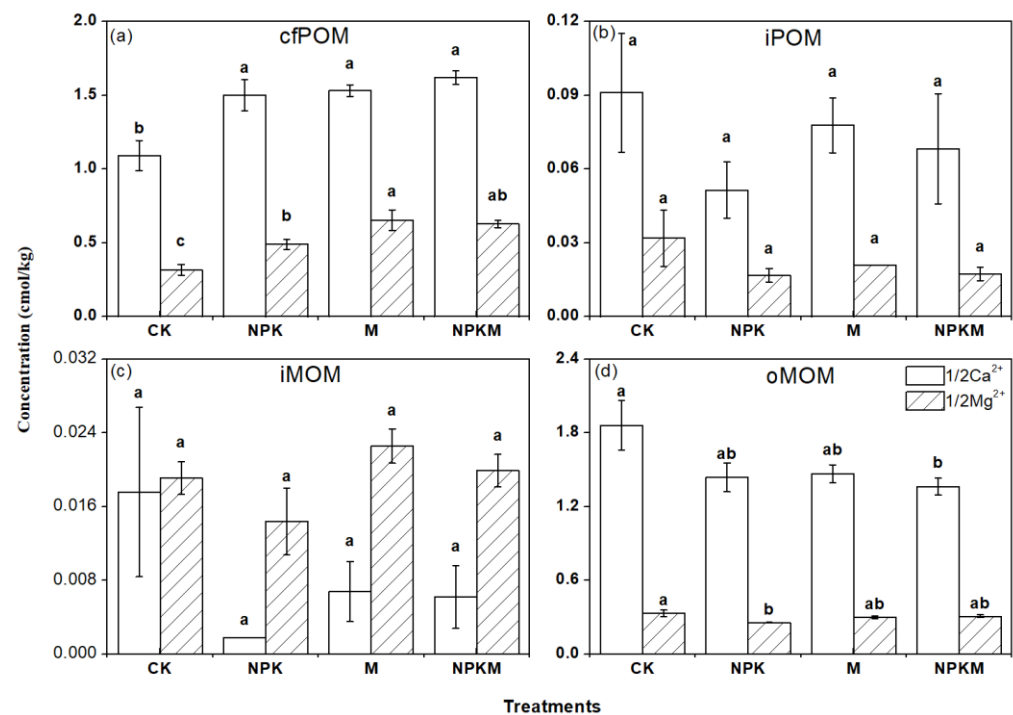


Figure 5. The value of SIC/SOC in different aggregates: (a) cfPOM; (b) iPOM; (c) iMOM; (d) oMOM. cfPOM means coarse free particulate organic matter fraction, iPOM means particulate organic matter inner microaggregate, iMOM means mineral-associated organic matter inner microaggregate, and oMOM means mineral-associated organic matter outer fraction. Error bars represent the standard deviations of the mean ($n = 3$). Different characters above bars indicate significant differences among the treatments at $p < 0.05$.

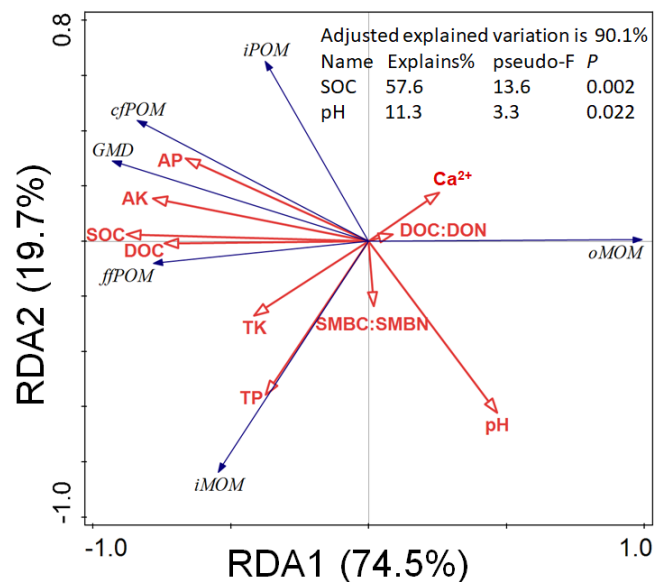


Figure 6. RDA analysis of aggregate distribution and soil properties. cfPOM means coarse free particulate organic matter fraction, iMOM means mineral-associated organic matter inner microaggregate, and MOM means mineral-associated organic matter fraction. Abbreviations: TN, total nitrogen content; SOC, soil organic matter; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; SMBC, soil microbial biomass carbon; SMBN, soil microbial biomass nitrogen; TK, total potassium; AK, available potassium; TP means total phosphorus; AP, available phosphorus.

3.7. Correlation and RF Analysis of SIC Content in Aggregates and Soil Properties

The correlation analysis showed that the SIC content of soil aggregates was linearly correlated with soil properties (Table 3). SIC content of cfPOM was significantly correlated with Mg^{2+} , SOC, SMBC, SMBN and DOC content in bulk soil. SIC content of iMOM was significantly correlated with SOC content in bulk soil. SIC content of MOM was significantly correlated with SOC and SMBN content in bulk soil (Table 3).

Table 3. Correlation between SIC content and soil properties.

Treatments	cfPOM	iPOM	iMOM	oMOM	MOM
SOC	0.604 *	0.003	0.594 *	0.106	0.597 *
Ca^{2+}	0.018	0.005	0.042	0.068	0.031
Mg^{2+}	0.699 **	0.208	0.465	0.001	0.511
DOC	0.601 *	0.091	0.413	0.080	0.515
DON	0.335	0.209	0.208	0.024	0.297
SMBC	0.830 **	0.324	0.433	0.001	0.634 *
SMBN	0.727 **	0.029	0.532	0.311	0.680 *

cfPOM means coarse free particulate organic matter fraction, iMOM means mineral-associated organic matter inner microaggregate, and MOM means mineral-associated organic matter fraction. “*” indicates the linear correlation was significantly different at $p < 0.05$, “**” indicate the linear correlation was significantly different at $p < 0.01$ ($n = 12$).

Random forest (RF) analysis was used to study the effects of treatment on soil basic properties on the SIC content in aggregates (Figure A3). RF analysis showed that SIC content was correlated with soil properties. In bulk soil, soil properties were most relevant in order of SMBC, AK, SMBN, GMD, DOC, SOC, Mg^{2+} , DON, pH (var explained: 59.8%). In cfPOM aggregates, soil properties were most relevant in order of SMBC, SMBN, SOC, Mg^{2+} , DOC, AK, DON, pH (var explained: 66.2%). In iMOM aggregates, soil properties were most relevant in order of SMBN, DOC, Mg^{2+} , AK, SMBC, DON, pH (var explained: 26.2%). In oMOM aggregates, soil properties were most relevant in order of SMBN, SMBC, Mg^{2+} , SOC, DON, AK, DOC, pH (var explained: 43.5%).

4. Discussion

4.1. Effect of Long-Term Fertilization on SIC and SOC Content in Bulk Soil

Data from China’s Ministry of Agriculture and Rural Affairs showed that 5.5×10^7 t of manure from livestock and poultry was produced in 2021, accounting for 42 percent of China’s organic fertilizer products. As an effective carbon sequestration measure, the manure application could not only improve soil organic carbon, but also affect soil inorganic carbon sequestration. It showed that manure (M) and manure combined with chemical NPK fertilizer (NPKM) amendment significantly increased SIC and SOC contents by $1.76\text{--}2.56 \text{ t ha}^{-1}$ and $7.47\text{--}9.44 \text{ t ha}^{-1}$ in the topsoil (0–20 cm) relative to the unfertilized treatment, respectively (Table 1, Figure 1), which was consistent with Tian et al. [31]. In this experiment, long-term application of nitrogen fertilizer had no significant effect on SOC content of NPKM treatment was increased by 3.30 t ha^{-1} than that of M treatment (Table 1). This is in agreement with the results of an analysis [32] of 90 long-term field trials (20–37 years) in northwest Chinese croplands [32]. To enhance and sustain SOC storage, a balanced combination of mineral and organic fertilizers appears to be the most appropriate nutrient management strategy across all climates in China [33]. Interestingly, although long-term application of organic fertilizer and combined application of organic and inorganic fertilizers significantly increased SIC content, the SIC content of M treatment significantly increased by 3.11 t ha^{-1} compared with that of NPKM treatment (Figure 2a). The SIC/SOC of M treatment was significantly higher than that of other treatments (Figure 3b). It may be because the soil acidification with nitrogen fertilizer accelerated SIC loss under continuous and intensive N applications [10]. In conclusion, long-term application of manure fertilizer significantly increased the storage of SOC and SIC in kastanozems. The application of

manure alone was more beneficial to promote the fixation of SIC in topsoil rather than manure combined with chemical NPK fertilizer.

4.2. Effect of Long-Term Fertilization on SIC and SOC Content in Aggregate

The 16-year manure fertilization showed significant effects on aggregate-associated SIC fixation. We found that the SIC storage of coarse free particulate organic matter (cfPOM) and mineral-associated organic matter inner microaggregate (iMOM) was significantly increased by $0.66\text{--}1.14\text{ ha}^{-1}$ and $1.36\text{--}1.37\text{ t ha}^{-1}$ under M and NPKM treatment, respectively. Moreover, the SIC content in mineral-associated organic matter outer fraction (oMOM) under M treatment was also significantly increased by 0.46 t ha^{-1} compared to CK, which led to a higher SIC content in bulk soil (Figure 2). On the other hand, there was no significant difference in SIC/SOC ratio among different treatments of cfPOM, particulate organic matter inner microaggregate (iPOM) and iMOM aggregates after long-term fertilization (Figure 4), indicating that SOC content also significantly increased after long-term application of organic fertilizer. The content and composition of SOC differs among aggregate size classes and density fractions [34]. The hierarchical organization of soil aggregates is postulated to play a crucial role for SOC stabilization [35,36]. Aggregates play a key role in protecting SOC from microbial decomposition. Fractionation of SOC is crucial for mechanistic understanding and modeling of soil organic matter decomposition and stabilization processes. The results indicated that the most efficient fertilization practice for sequestering C in each fraction in red soils was the continuous application of either manure alone or manure plus mineral fertilizers, and MOC was the primary fraction of C sequestration in red soils [22]. Findings in studies [37,38] highlighted the importance of fine microaggregates and organo-mineral interactions for SOC stabilization, from which the concept of C saturation evolved [39]. In this challenged concept, the amount of SOC that can be stored in a soil is limited by the amount of silt and clay minerals. In agricultural soils, this fraction can store up to 90% of the total SOC [38,40].

4.3. Effect of Long-Term Fertilization on Aggregate Distribution and Stability

Soil aggregation has been recognized as an important mechanism of soil stabilization against the decomposition of SOC [30,41]. The results showed that the macroaggregate turnover is the key process affecting the stability of SOC [30]. Long-term application of organic fertilizer could significantly improve the stability of soil aggregates (Figure 6) and macroaggregate proportion (Table 2). In this study, mean weight diameter (MWD) and geometric mean diameter (GMD) were improved by 6.0–12.9% and 10.5–19.7%, respectively, after 16 years of long-term organic fertilization. The proportion of macroaggregate (cfPOM) was increased by 6.0–11.0% which was similar to fertilization experiments by applying manure or straw in different types of soils [42–45]. However, in these studies, the proportion change of mineral-associated organic matter microaggregate (MOM) in soil was opposite [42,44,45]. We divided MOM components into iMOM and oMOM according to their position in aggregates. The data showed that the proportion of oMOM was significantly reduced by 23.9–28.7%, but the proportion of iMOM increased by 6.2–13.8% in M and NPKM treatments. Therefore, changes in the distribution of inner and outer microaggregates of mineral-associated organic matter differed after long-term fertilization.

4.4. Factor Analysis of Aggregate Distribution and Stability

RDA analysis showed that the distribution of aggregates was affected by SOC and pH (Figure 6). Previous studies have shown that soil SOC has a significant relationship with soil stability [46]. Studies have shown that SIC was also an important cementing material for aggregates [19], which affected by the partial pressure of CO_2 in soil, soil pH, soil moisture, and the content of calcium and magnesium ions [13,16,47]. It has also been shown that precipitation of soil secondary carbonates can enhance soil polymerization [19].

RF analysis showed that the fixation of SIC in bulk soil was affected by soil properties as microbial activity (SMBC, SMBN), soil structural stability (GMD), soil carbon sources

(DOC, SOC), soil cations (AK, Mg^{2+}) and soil pH (Figure A3). After 16 years of long-term fertilization, soil properties and structural stability changed (Table 1), which may affect soil inorganic carbon fixation.

Previous studies also showed that manure fertilization significantly increased SOC sequestration and labile SOC fractions (SMBC, and DOC within the bulk soil and soil aggregates), which can improve the availability of soil nutrients [22,34,48,49]. Long-term manure fertilization had a significant effect on the relative abundances of PLFAs in soil aggregates [29]. The soil microbial biomass carbon (SMBC) was significantly related to the composition of microbial communities [31]. A study [49] showed that bacteria and fungi were highly involved in the large aggregate accumulation after nitrogen addition and regulated the loss of SOC by enhancing soil large aggregate content in SOC mineralization [47]. DOM (dissolved organic matter) is considered to have a major role in the transport and supply of C and N to microbial populations within the soil solution [50,51]. DOC, DON, SMBC and SMBN contents were significantly higher after organic fertilizer application than those of the non-fertilizer treatment (Tables 1 and 2), which is in agreement with the findings of Zhang M [52]. It provided material and energy for microbial activity. The effect on the mineralization of microorganisms in aggregates was greater than that on the distribution of soil aggregates [35]. A temperature test indicated that DOC is not primarily litter-derived, but is in equilibrium with the mineral-associated fraction through sorption and desorption [53]. Large aggregates have strong microbial activity, which reduce substrate content and bacterial diversity, and are conducive to carbon mineralization [54]. Microaggregates have weak microbial activity, high substrate content and high substrate utilization, which are conducive to carbon storage [54]. Thus, CO_2 produced by SOC mineralization is conducive to recrystallization and precipitation of inorganic salts in the soil [14,55].

In our experiment, the effect caused by irrigation was excluded as field experiments were managed uniformly except for fertilization. However, the application of inorganic phosphate fertilizer brings calcium ions into soil, just as the application of organic fertilizer brings calcium and magnesium ions into soil [11]. Thus, we can conclude that long-term application of organic fertilizer could not only increase the exchangeable Ca^{2+} and Mg^{2+} , but also facilitate the fixation of SIC in the soil (Tables 1 and 3), which was beneficial for the crystallization of carbonate in the soil.

5. Conclusions

This study supports that the 16-year manure application promoted the fixation of SIC by 2.25–3.25 t ha⁻¹ in topsoil (0–20 cm) of kastanozems. The contents of cfPOM, iMOM, oMOM were increased 0.66–1.14 t ha⁻¹, 1.36–1.37 t ha⁻¹, 0.13–0.46 t ha⁻¹, respectively. In cfPOM, compared to CK, the exchangeable Ca^{2+} concentrations under M and NPKM treatments were significantly increased by 40.23% and 48.37%, respectively, and the exchangeable Mg^{2+} concentrations were significantly increased by 54.49%, 105.52% and 97.77%, respectively. In addition, the contents of SMBC, SMBN, DOC, DON also increased in manure application treatments. These promoted the crystallization of carbonate at the aggregate level. Moreover, long-term manure application changed aggregate distribution which would affect the content of SIC. However, the pH value of NPKM treatment was lower than that of M treatment, which can reduce the amount of carbonate crystallization. Thus, the application of manure alone was the best way to promote the fixation of SIC in topsoil rather than manure combined with chemical NPK fertilizer.

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Appendix A

Table A1. The amount of fertilizer applied under different treatments (kg ha^{-1}).

Year	Nutrient (kg ha^{-1})	CK	NPK	M	NPKM
2004–2005	N	0	45	37.5	82.5
	P_2O_5	0	30	15	45
	K_2O	0	30	55.5	85.5
2006–2013	N	0	60	37.5	97.5
	P_2O_5	0	45	15	60
	K_2O	0	30	55.5	85.5
2014–2015	N	0	150	71.25	221.25
	P_2O_5	0	45	38.25	83.25
	K_2O	0	75	146.25	221.25
2016–2019	N	0	150	142.5	292.5
	P_2O_5	0	45	76.5	121.5
	K_2O	0	75	292.5	367.5

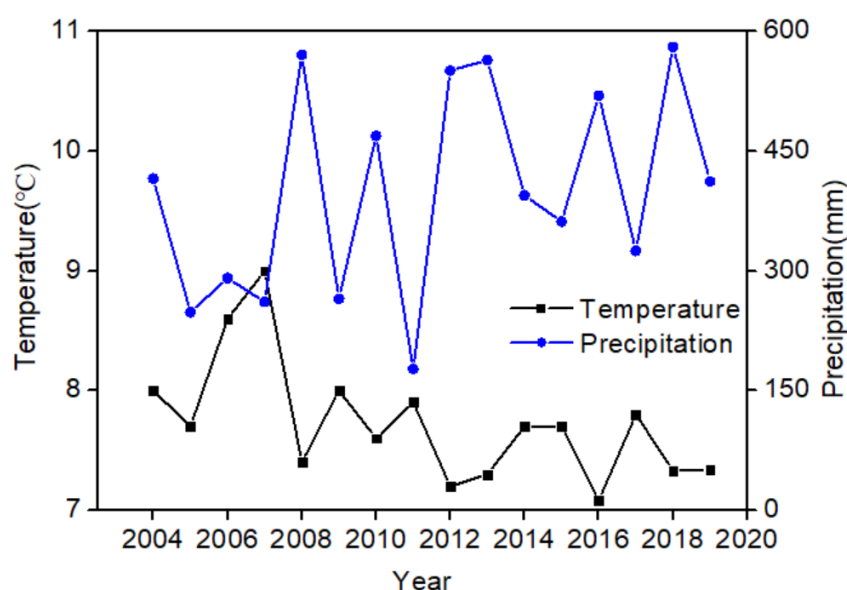


Figure A1. The annual temperature and precipitation of Wuchuan station.

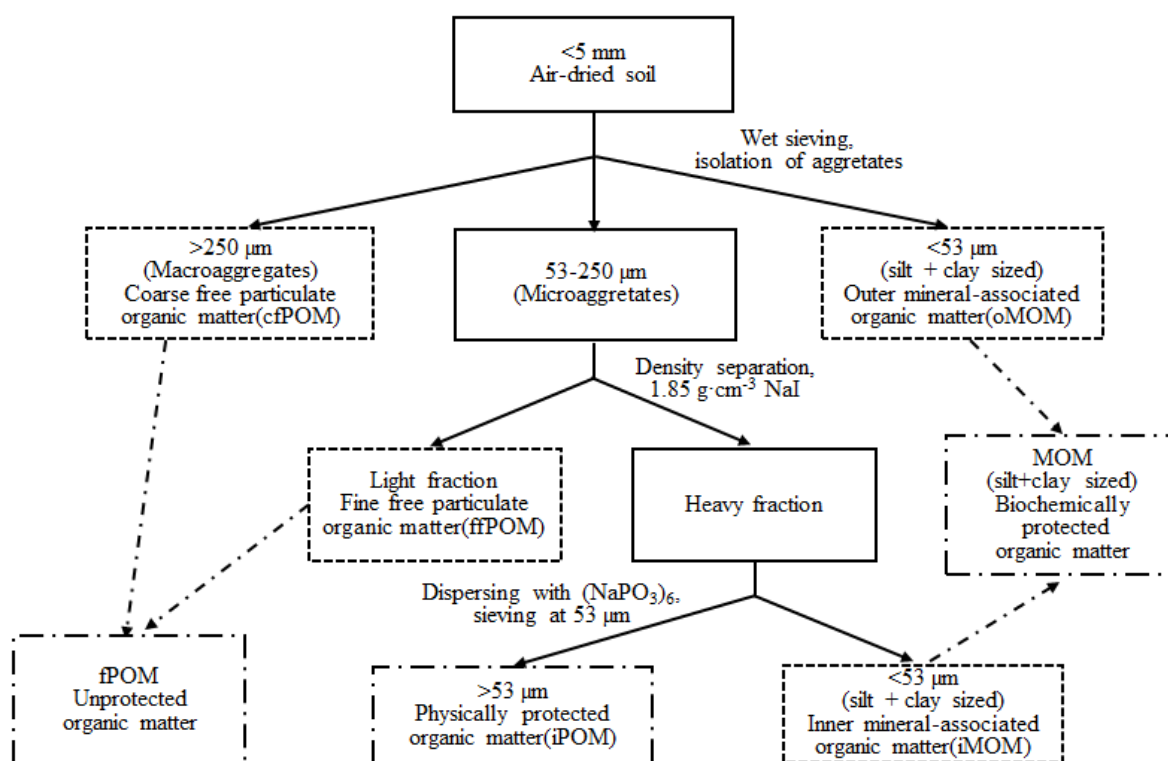


Figure A2. Schematic of the applied physical fractionation procedure for the isolation of coarse-free particulate organic matter (cfPOM, unprotected organic matter), fine free particulate organic matter (ffPOM, unprotected organic matter), particulate organic matter inner microaggregate (iPOM, physically protected organic matter), mineral-associated organic matter inner microaggregate (iMOM, biochemically protected organic matter), and mineral-associated organic matter outer fraction (oMOM, biochemically protected organic matter). The percentage of each fraction in the total soil (PEF) was calculated by weight.

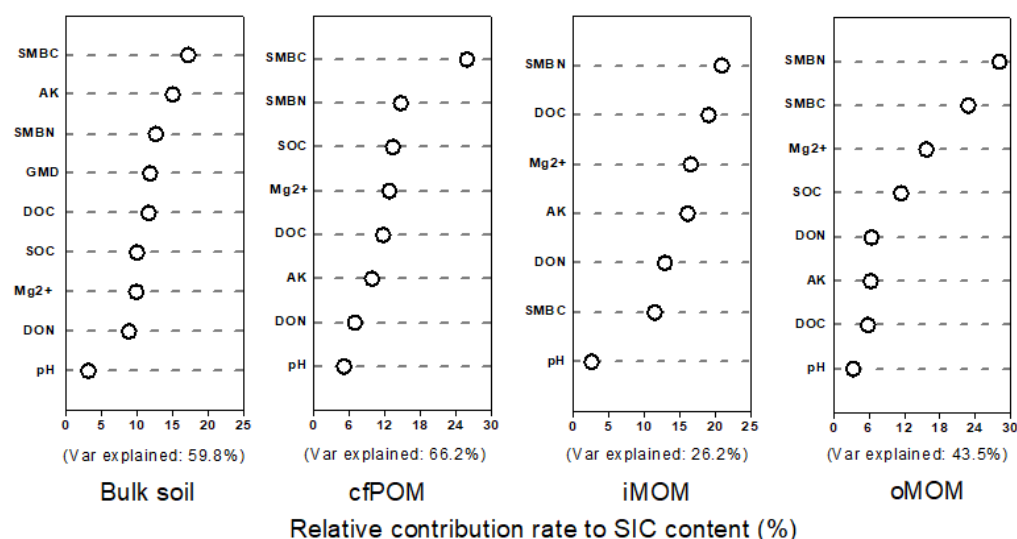


Figure A3. RF analysis of relative contribution rate to SIC content. cfPOM means coarse free particulate organic matter fraction, iPOM means particulate organic matter inner microaggregate, iMOM means mineral-associated organic matter inner microaggregate, and oMOM means mineral-associated organic matter outer fraction. MOM means mineral-associated organic matter fraction.

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