Liu *et al. Carbon Research (2024) 3:29*
https://doi.org/10.1007/s44246-024-00121-4

Carbon Research

ORIGINAL ARTICLE

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Soil organic carbon pools under long-term mineral and organic amendments: a multisite study

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Abstract

Soil organic carbon (SOC) has various pools with different stabilization mechanisms. It is unclear how these SOC pools respond to various mineral and organic amendments depending on a large climate-soil gradient. Here, we studied in three zonal soils: Ferralic Cambisol (subtropic), Calcaric Cambisol (warm-temperate) and Luvic Phaeozem (mid-temperate) under 23-year mineral, straw and manure amendments. Six SOC sub-pools were isolated: unprotected, physically, chemically, biochemically, physico-chemically and physico-biochemically protected pools. Compared to initial level, SOC and most sub-pools increased in the three soils under manure application ($p < 0.05$), but little under straw and mineral amendments. The Luvic Phaeozems had much higher sequestration efficiencies of bulk SOC (27%) and its five sub-pools (5–7%) more than the Calcaric Cambisol (9%, 1–2%) and Ferralic Cambisol (9%, 0.5–1%). In contrast, Ferralic Cambisol had highest sequestration efficiency of unprotected pool (7%). The Calcaric Cambisol had divergent patterns of the six SOC pools compared with Luvic Phaeozems and Ferralic Cambisol, due to the low clay content. With the build-up of bulk SOC, the building-up abilities of non-protected, physically-, chemically- and biochemically-protected pools depended on soil type, while the building-up abilities of physico-chemically- and physico-biochemically-protected pools were convergent (12–19%) among soils. In conclusion, the Luvic Phaeozems had much higher build-up ability of bulk SOC and most sub-pools than the other two soils. With the build-up of SOC, the physico-chemically- and physico-biochemically-protected pools (most stable) had convergent response rates among soils, while the other pools had divergent response rates.

Highlights

- Manure amendments fed SOC and most pools much better than straw and mineral fertilizers.
- Luvic Phaeozems had higher build-up ability of SOC pools than the other two soils.
- Physico-chemical and physico-biochemical pools had convergent build-up abilities among soils.
- Calcaric Cambisol has dissimilar fractionation pattern of SOC with other two soils.

Handling Editor: Fengchang Wu.

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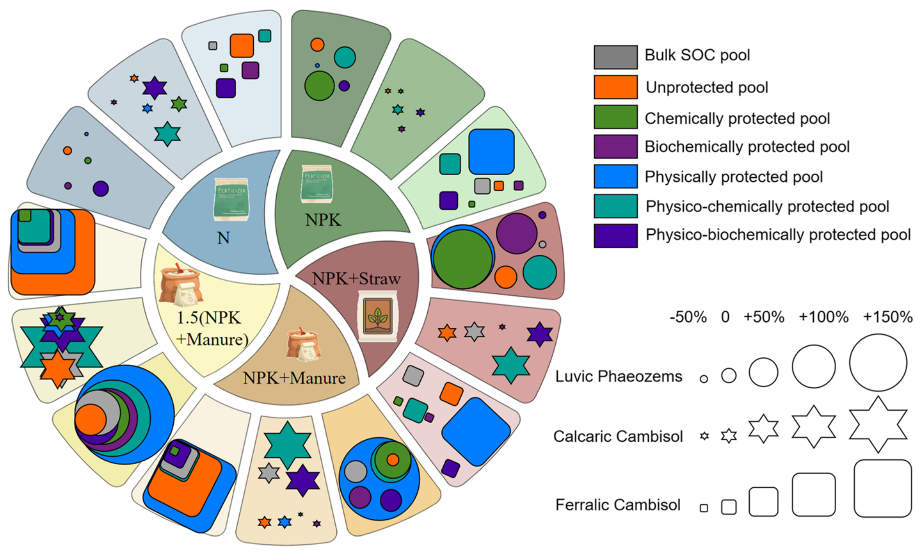
Keywords Manure amendment, Microaggregate, Particulate organic matter, Soil aggregation, Soil C sequestration, Soil organic matter

Graphical Abstract



1 Introduction

Soil organic carbon (SOC) is fundamental for the C cycling and health of terrestrial ecosystems. SOC has multiple conceptual and measurable pools with various inherent formation pathways and stabilization mechanisms (Poeplau et al. 2018; Stewart et al. 2008, von Lützow et al. 2007, Zhu et al. 2020). Using physical and chemical fractionation methods, six measurable pools linking stabilization mechanisms, unprotected, physically, physico-chemically, chemically, physico-biochemically and biochemically protected, were usually isolated (Stewart et al. 2008, 2009). The non-protected pool, usually accounting for 10–25% of SOC (Just et al. 2023), is mainly in the form of crop residue-origin free coarse particulate (cPOC, > 0.25 mm) and fine particulate OC (fPOC, 0.053–0.25 mm) with labile feature and short mean residence time (Six et al. 2002; Stewart et al. 2008; Wang et al. 2021b, Zhang et al. 2022). The fPOC is generally derived from cPOC and served as the transitional component to subsequently protected sub-pools. The physically protected pool refers to the POC enveloped by microaggregates, which is from free fPOC but has longer persistence (Totsche et al. 2018). In contrast, the mineral-associated OC (MAOC), mainly from microbial necromass as well as plant legacy (Angst et al. 2021, Chen et al. 2023), is more stable and persistent than free and protected POC (Lehmann and Kleber 2015). Within the MAOC, four sub-pools can also be separated based on their acid hydrolysable and aggregating features. The chemically protected and biochemically protected pools are hydrolysable and nonhydrolyzable parts of easily dispersed MAOC (free mineral particles that have not formed aggregates), respectively; while the physico-chemically protected and physico-biochemically protected pools are inside microaggregates. Compared with the chemically-protected SOC, the biochemically-protected pool is supposed to be more complex in chemical structure and have higher inherent recalcitrance and stability (Stewart et al. 2008).

Nutrient replenishments by mineral, straw and manure amendments are fundamental for sustainable grain productivity. The straw and manure incorporations generally enhanced SOC pool (Berhane et al. 2020, Wang et al. 2015, 2021a), while chemical fertilization usually had little impact on SOC accumulation (Chen et al. 2023, Tian et al. 2017, Wang et al. 2018). Among the SOC pools linking stabilization mechanisms, the unprotected sub-pool, especially cPOC, was usually most sensitive to long-term amendments, particular the straw and manure amendments (Abrar et al. 2020, Ali Shah et al. 2021, Mandal et al. 2019, Tian et al. 2017, Yang et al. 2018). The pure physical protection pool was also sensitive to the straw and manure amendments (Abrar et al. 2020, Ali Shah et al. 2021, Mandal et al. 2019). The responses of physico-chemically and physico-biochemically protected sub-pools to organic amendments were similar in the soils of Eumorthic Anthrosolsh and Udic Ustochreptsx but different in black soils (Abrar et al. 2020, Ali Shah et al. 2021, Mandal et al. 2019). Compared with above sub-pools, the chemically protected and biochemically protected sub-pools generally respond less to straw and manure amendments (Abrar et al. 2020, Ali Shah et al. 2021, Mandal et al. 2019, Tang et al. 2021). Even though different responses of the SOC sub-pools to mineral and organic amendments were reported in several sites, the experimental durations ranged from 4 to 35 years, which may bias the comparison results based on these individual studies. Thus, a comparative multisite study with similar long-term mineral and organic amendment regimes is needed.

Climate (e.g., precipitation and temperature) and soil conditions (e.g., clay content, texture and pH) across climate-soil gradient should affect the responses of SOC sub-pools with various protection mechanisms to long-term mineral and organic amendments. Our previous study showed that post-agriculture natural restoration reduced the physico-biochemically protected SOC in two temperate soils (Calcaric Cambisol and Luvic Phaeozem) but had little impact on that in a subtropical soil (Ferralic Cambisol), indicating a divergent restoring trend among soils across a large-scale climate-soil gradient (Wang et al. 2021b). And the variations of bulk SOC and most sub-pools with different protection mechanisms (unprotected, physico-chemical, physico-biochemical and biochemical) were dependent on wetness (precipitation/temperature ratio) (Wang et al. 2021b). Furthermore, coarse soil texture, with lower stabilization sites for SOC, hampered the builds up rates of bulk SOC and sub-pools, while subtropical humid climate restricted accumulation of pure physically protected SOC (Wang et al. 2021b). Furthermore, clay minerals also regulate the protection patterns of SOC pools through organo-mineral associations (Song et al. 2022). To date, it is unclear how these SOC sub-pools linking stabilization mechanisms respond to long-term mineral and organic amendments across a large-scale climate-soil gradient. Based on this, we studied this issue at three typical soils, with same experimental durations and similar amendment regimes, across a climate-soil gradient in eastern China. We hypothesized that (i) the build-up efficiency of bulk SOC under longterm mineral and organic amendments was climate- and soil-dependent, with higher values in temperate soil rather than subtropical soil; (ii) with the build-up of bulk SOC, the SOC sub-pools generally had divergent responses to amendment-derived C input and bulk SOC changes, with reverse relationship between the protection degree and response sensitive.

2 Materials and methods

2.1 Research sites

This study was conducted using three soils under different climates: Luvic Phaeozems (mid-temperate), Calcaric Cambisol (warm-temperate), and Ferralic Cambisol (subtropics) across China. The background information of the three soils is given in Table 1.

The Luvic Phaeozems soil is at the National Soil Fertility and Fertilizer Efficiency Monitoring Station in Gongzhuling City (altitude 220 m, 43°30′ N, 124°48′ E). The site has a cool-temperate monsoon climate, with a mean annual precipitation (MAP) of 525 mm yr^{-1} and a mean annual temperature (MAT) of 4.5 °C. The topography is flat and well-drained, soil pH is 7.6, and the texture is clay loam (39% of sand, 29% of silt, and 29% of clay) (Li et al. 2018, Wang et al. 2021b). The corn has been cropped since 1990.

The Calcaric Cambisol soil is at the National Soil Fertility and Fertilizer Efficiency Monitoring Station in Zhengzhou City (altitude 90 m, 35°01′ N, 113°40′ E). The site has a warm temperate monsoon climate, with MAP of 632 mm yr^{-1}, MAT of 14.3 °C, and frost-free period of 224 days yr^{-1}. The terrain is flat and well-drained, soil pH is 8.3, and the texture is sandy loam (67% of sand, 19% of silt and 10% of clay) (Li et al. 2018, Wang et al. 2021b). The wheat-corn rotation has been cropped since 1990.

The Ferralic Cambisol soil is at the National Soil Fertility and Fertilizer Efficiency Monitoring Station in Qiyang City (26°45′ N, 111°52′ E, altitude 1003 m). The area has a subtropical humid monsoon climate, with MAP of 1250 mm yr^{-1}, MAT of 18.0 °C, frost-free period of 300 days yr^{-1}, and sunshine duration of 1613 h yr^{-1}. Soil pH is 5.7, and the texture is clay (19% of sand, 32% of silt, and 44% of clay) (Li et al. 2018, Wang et al. 2021b). The wheat-corn rotation has been cropped since 1990.

2.2 Experimental design

At the three experimental sites, field researches were all established in 1990, with six mineral and organic fertilizations: no fertilizer (control), nitrogen addition (N), N + phosphorus (P) + potassium (K) applications (NPK), NPK plus straw incorporation (NPK + Straw), NPK plus manure incorporation (NPK + Manure), and 1.5 × (NPK + Manure) (1.5(NPK + Manure)). In the Calcaric Cambisol, each treatment had six replicate plots (4 × 10 m), while in the Luvic Phaeozems and Ferralic Cambisol, each treatment had one big plot with an area of 400 m^2 (16 × 25 m) and 200 m^2 (10 × 20 m), respectively, for more unified and convenient field management. Based on this, we artificially designated 6 duplicate plots in the Luvic Phaeozems and Ferralic Cambisol, with a size of 4 × 10 m and 4 × 5 m, respectively. Before the experiments, the three experimental sites had been cultured

Table 1 The site information and initial physico-chemical properties of the three soils in 1990 (pre-experiment)

WRB soil classification[a]	Locations	MAT (°C)	MAP (mm yr^{-1})	Cropping system	Clay content (%)	pH	SOC (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})
Luvic Phaeozem (Black Soil)	43º30′ N, 124°48′ E	4.5	525	Single cropping of corn	29.3	7.6	13.5	1.40	1.39	23.1
Calcaric Cambisol (Fluvo-Aquic Soil)	34°47′ N, 113°40′ E	14.3	632	Double cropping of wheat–corn	10.1	8.3	6.7	0.65	0.64	17.7
Ferralic Cambisol (Red Soil)	26°45′ N, 111°52′ E	18.0	1250	Double cropping of wheat–corn	43.9	5.7	7.9	1.07	1.03	13.3

[a] The soil names in brackets are based on Chinese Soil Classification

under mineral fertilization for more than 20 years. During 1988–1990, all the three sites were routinely and continuously cultivated without fertilization. Soil properties (0–20 cm) of the three sites in 1990 (pre-experiment) are listed in Table 1.

The mineral and organic fertilization rates in each site are shown in Table 2. The mineral fertilizers were urea, calcium superphosphate and potassium chloride. The organic amendments were local animal manure and straw. In Luvic Phaeozems, all mineral NPK were used as basal fertilizers in each growing season and the organic fertilizer was pig manure. In the Calcaric Cambisol, the annual application quota of mineral N, P and K was 47% for wheat and 53% for corn, respectively. In each growing season of wheat/maize, 60% of N, all phosphorus and potassium were added as basal fertilizer; while the rest of N (40%) was used as surface fertilizer and the organic fertilizer was horse manure (1991–1999) and cattle manure (2000–2010). In Ferralic Cambisol, the yearly fertilization quota of N, P and K was 30% for wheat and 70% for corn, respectively. Before planting wheat and maize, all mineral N, P and K were used as basal fertilizer, and manure was also pig manure.

2.3 Soil sampling

After harvest in the fall of 2013, composite soil samples (mixed from 5 sub-samples) were collected by a soil auger from 0–20 cm depth in each replicate plot. The timing of samples were chosen to minimize the effects of the growth season, including plant grow and the fertilization. The fresh bulk soil samples are packaged and transported cautiously to laboratory to preserve the soil structure and aggregates, and then lightly beaten into small portions by hand. Subsequently, soils were air-dried at room temperature and subsequently passed through 2-mm sieve for separation of SOC sub-pools. All visible plant residues, animals and stones were taken out during the sieving.

2.4 Separation of SOC pools

The isolation method of SOC sub-pools was conducted following Stewart et al. (2008) (Fig. 1). First, 20 g soil was dispersed physically into three size fractions using a wet sieve method. The > 250 μm unprotected coarse particulate OC (cPOC), 53–250 μm microaggregates (μagg), and < 53 μm readily dispersible silt and clay (dSilt and dClay) were separated using a microaggregate isolator with 250-μm and 53-μm screens (Six et al. 2000). The cPOC (> 250 μm, including sand) was got with 50 glass beads oscillating at 120 rpm for 10 min in running water. The material passing through the 250-μm sieve was further wet sieved manually with 53-μm sieve for 50 passes in 2 min to separate μagg (53–250 μm) from the dSilt and dClay (Elliott 1986). The dSilt fractions were separated from the dClay by centrifugation at 127 g for 7 min. Subsequently, 25 mL of 0.25 M $CaCl_2$ was put into suspension, which was then centrifuged at 1730 g for 15-min to get an easily-dispersed clay-sized pool (dClay).

Then, using density flotation and complete dispersion, the μagg was further isolated into unprotected fine POC (fPOC), μagg-protected POC (iPOC), and μagg-origin silt and clay portions (μSilt and μClay) (Six et al. 2000). The fPOC was accessed by density flotation using sodium polytungstate (1.85 g cm^{-3}) and a 0.45-μm glass fiber filter. The heavy portions (more than 1.85 g cm^{-3}) were dispersed overnight by shaking with 12 glass beads and 60 mL 5 $g{\cdot}L^{-1}$ sodium hexametaphosphate and subsequently passed through a 53-μm sieve, separating the iPOC (53–250 μm) from mineral portions (μSilt and μClay). The μSilt portion was also separated by 127 g centrifugation for 7 min from the μClay portion. The suspension, put in 25 mL of 0.25 M $CaCl_2$, was centrifuged for 15 min at 1730 g to get the μClay.

Table 2 Annual mineral and organic fertilization rates (kg ha^{-1} $year^{-1}$) of the three regional soils: Luvic Phaeozem, Calcaric Cambisol and Ferralic Cambisol since 1990

Soil types	Control (CK)	N (kg N ha^{-1})	NPK (kg N/P/K ha^{-1})			NPK + Straw				NPK + Manure			
			N	P	K	N	P	K	Straw (t ha^{-1} $year^{-1}$)	N	P	K	Fresh manure[a] (t ha^{-1} $year^{-1}$)
Luvic Phaeozem	0	165	165	36	68	112	36	68	8.2	50	36	68	30
Calcaric Cambisol	0	353	353	77	146	237	77	146	17.8	237	77	146	22.5
Ferralic Cambisol	0	300	300	52	100	300	52	100	5.3	90	52	100	42

(1) Compared with the NPK, the reductions of the N application rate of the NPK + Straw and NPKM were compensated by the straw- and manure-derived N, respectively. (2) The application rate of 1.5NPKM treatment is 1.5 times of the NPKM treatment, including all parameters of N, P, K and manure. [a] The manures are pig manures as base manures in the Luvic Phaeozem and Ferralic Cambisol, but have different C contents. The manure is horse manure (1990–1999) and cow manure (since 2000) as base manure in Calcaric Cambisol

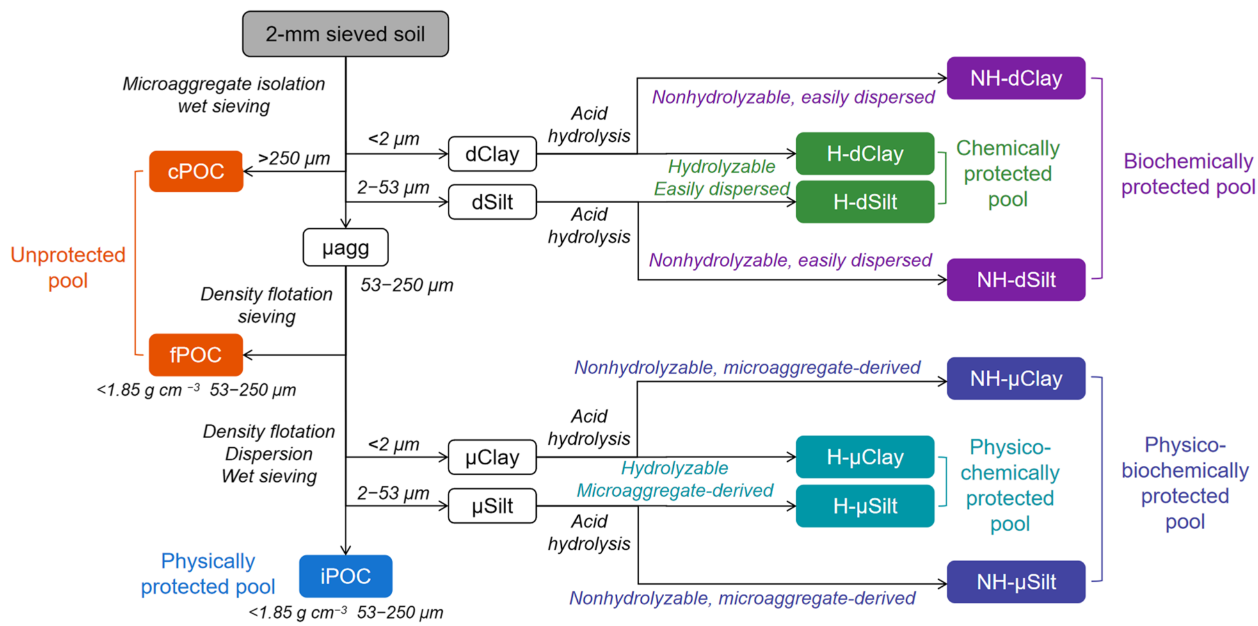


Fig. 1 Separation scheme of SOC pools based on protection mechanisms following Stewart et al. (2008). See details in Materials and methods

Finally, each separated silt and clay (dSilt, dClay, μSilt and μClay) was subjected to acid hydrolysis in 25 mL 6 mol L^{-1} HCl (refluxing at 95 °C for 16 h) (Plante et al. 2006). Then the suspension was washed using deionized water on a 0.45-μm glass fiber filter. The residues represent the non-hydrolyzable SOC sub-pools: NH-dSilt, NH-dClay, NH-μSilt, and NH-μClay. Correspondingly, the hydrolyzable SOC pools (H-dSilt, H-dClay, H-μSilt, and H-μClay) are estimated from the difference between the whole pools and the non-hydrolyzable pools. Dilute HCL (0.5 mol L^{-1}) was used to the unsorted soil (bulk MAOC pools) to remove carbonates, then weighed after oven-dried at 60 °C. The bulk SOC and its sub-pools were analyzed by an elemental analyzer (EA3000, Euro Vector, Milan, Italy).

Based on the stabilization mechanisms following Stewart et al. (2008), six SOC protection pools were grouped: (1) unprotected sub-pool consisting of cPOC and fPOC; (2) chemically-protected sub-pool corresponding to the hydrolyzable silt and clay portions separated during the initial dispersion (H-dSilt and H-dClay); (3) biochemically-protected sub-pool consisting of non-hydrolyzable respective isolated silt- and clay-sized portions (NH-dSilt and NH-dClay); (4) physically-protected sub-pool referring to iPOC; (5) physico-chemically protected sub-pool consisting of microaggregate-derived hydrolyzable silt and clay portions (H-μSilt and H-μClay); and (6) physico-biochemically protected sub-pool including microaggregate-derived non-hydrolyzable silt and clay portions (NH-μSilt and NH-μClay).

2.5 Estimation of C input from crop residue, straw and manure

In each experimental plot, crop grain and straw was harvested and dry-weighted, respectively, which was summed as aboveground biomass. The 23-year total crop residue C input was estimated by the Eqs. (1)–(3). The 23-year total C inputs from the manure and straw were calculated using the Eqs. (4) and (5), respectively.

$$\text{Input} - C_{\text{residue}} = \sum \text{Input} - C_{\text{root}} + \sum \text{Input} - C_{\text{stubble}} \quad (1)$$

$$\sum \text{Input} - \mathrm{C_{root}} = \sum \mathrm{B_a} \times (1 - \mathrm{W_a}) \times \mathrm{R_{r-s}} \times \mathrm{C_r} \times \mathrm{R_{20}} \quad (2)$$

$$\sum Input - C_{stubble} = \sum B_a \times (1 - W_a) \times R_{s-s} \times C_s \quad (3)$$

$$Input - C_{manure} = M_f \times (1 - W_m) \times C_m \times 23 \quad (4)$$

$$\text{Input} - \mathrm{C_{straw}St_f} \times (1 - \mathrm{W_{st}}) \times \mathrm{C_{st}} \times 23 \quad (5)$$

where Input-$C_{residue}$ is the 23-year cumulative C input from the stubble and root (t C ha^{-1}); ΣInput-C_{root} represents the cumulative root-C input from the wheat and corn; ΣInput-$C_{stubble}$ is cumulative stubble C input from the wheat and corn; ΣB_a refers to the cumulative aboveground biomass (grain + straw) (t C ha^{-1}); W_a refers to water content of aboveground biomass; R_{r-s} is root/shoot ratio of the wheat (0.3) and corn (0.26); C_r is the C content of maize root (45% in dry weight) (Zhang et al.

2015); R_{20} refers to the mass proportion of surface layer root (0–20 cm) to total root of the wheat (75%) and corn (85%); $R_{s\text{-}s}$ represents the ratio of stubble/shoot of wheat (13%) and corn (3%); C_s refers to the stubble C content of wheat (40%) and maize (45%); Input-C_{manure} refers to the total manure-C input (t C ha^{-1}); the M_f refers to application rate of fresh manure; the W_m is moisture content of manure (71%, 68% and 75% for pig manure, horse and cow manure, respectively); the C_m is C content of dry manure (389, 361, 368 g kg^{-1} dry weight for pig manure, horse and cow manure, respectively); the number 23 is the experimental years; Input-C_{straw} refers to total straw-C input (t C ha^{-1}); the St_f is application rate of straw; W_{st} refers to the moisture of fresh straw (14%); and C_{st} represents C content of dry straw (40% and 44% for wheat and corn, respectively).

2.6 Statistical analysis

Based on the weight and measured SOC content of fraction, the SOC sub-pool in bulk soil (g kg^{-1} bulk) was estimated using Eq. (6). Two-way analysis of variance with Tukey's HSD post hoc was used to determine differences among the three soils and among the six amendment regimes. Non-metric multidimensional scaling (NMDS) was used to categorize the sub-pools after downscaling analysis. Linear regressions (Eq. [7]) and nonlinear regressions (Eqs. [8] and [9]; Six et al. 2002) were used to fit the correlations between bulk SOC and each sub-pool by SPSS 20.0 (SPSS Inc, Chicago). Linear Eq. (10) was used to fit the response of SOC pool to cumulative C input. Variance partitioning analysis (MAP and MAT for climate; and pH and clay content for soil type) was conducted with RStudio (package 'vegan', R Development Core Team, 2015). Graphs were prepared in ORIGIN 8.0 (OriginLab Inc, Northampton, MA) and R Studio.

$$\mathrm{SOC_{pool}} = SOC_{\mathrm{measured}} \times \mathrm{W_{fraction}}/\mathrm{W_{bulk}} \quad (6)$$

$$\mathrm{SOC_{fraction}} = \alpha \times \mathrm{SOC} + \mathrm{R} \quad (7)$$

$$\mathrm{SOC_{fraction}} = \beta \times \ln(\mathrm{SOC}) + \Gamma \quad (8)$$

$$\mathrm{SOC_{fraction}} = \mathrm{V_{max}} \mathrm{SOC}^{\mathrm{n}}/K^{\mathrm{n}} + \mathrm{SOC}^{\mathrm{n}}) \quad (9)$$

$$\mathrm{SOC_{pool/fraction}} = \kappa \times \mathrm{Input} - \mathrm{C} + \eta \quad (10)$$

where $SOC_{measured}$ is SOC content of each sub-pool (g kg^{-1} fraction); SOC_{pool} represents calculated SOC sub-pool in bulk soil (g kg^{-1} bulk); $W_{fraction}$ and W_{bulk} are weights (g) of the sub-pool and bulk soil, respectively; SOC refers to total SOC content (g kg^{-1}); R is residual SOC (Stewart et al. 2007); α, β and Γ represent fitted parameters, the parameter α refers to the build-up ability; and κ and η are fitted parameters; V_{max}, K and n are fitted parameters; the parameter κ refers to sequestration efficiency.

3 Results

3.1 Bulk SOC and sub-pools

Compared with control, the straw (NPK + Straw) and manure additions (NPK + Manure, 1.5[NPK + Manure]) increased the bulk SOC in the three soils, while the pure mineral amendments had much less effects (Fig. 2a). Compared with manure amendment, however, the straw incorporation promoted the bulk SOC in the Luvic Phaeozems and Calcaric Cambisol with lower extent (6.4 – 27.4%) but had little effect in the Ferralic Cambisol (2.1%) (Fig. 2a). Furthermore, the manure-derived increments of bulk SOC (refer to [NPK + Manure – NPK]/NPK) in the Luvic Phaeozems (52.2%) and Ferralic Cambisol (47.6%) were higher than that in the Calcaric Cambisol (30.2%) (Fig. 2a). The soil type and amendment had significant interactions ($p < 0.05$). Compared to the level in 1990, the SOC under various amendments all increased in the Luvic Phaeozems and Calcaric Cambisol, especially under the manure amendments; while the control and N addition reduced the SOC pool in the Ferralic Cambisol (Fig. 2b).

In the Luvic Phaeozems, the six SOC sub-pools with various stabilization mechanisms were mainly sensitive to straw and manure amendments, but little for the N and NPK amendments (Fig. 3a–f). It was interesting that the unprotected SOC pool decreased under the NPK and straw amendments (Fig. 3a). In the Calcaric Cambisol, the non-protected, chemically-, physically-, physico-chemically- and physico-biochemically-protected sub-pools increased mainly under the 1.5(NPK + Manure) amendment, while the physico-chemical and physico-biochemical sub-pools increased under the NPK + Straw and NPK + Manure amendments (Fig. 3g–l). In the Ferralic Cambisol, the non-protected, physically-, physico-chemically- and physico-biochemically-protected sub-pools increased mainly under the NPK + Manure and 1.5(NPK + Manure) amendments (Fig. 3m–r), and the increase in the physically protected sub-pools was more obvious under the NPK + Manure and 1.5(NPK + Manure) amendments, rather than NPK and NPK + Straw amendments (Fig. 3p).

Based on the NMDS analysis of the relative abundances of the 6 protection SOC pools and 11 specific pools, the fractionation pattern of the Calcaric Cambisol was dissimilar to the Ferralic Cambisol and Luvic Phaeozem, and the latter two had a convergent fractionation pattern (Fig. 4a, b and S1). In the Calcaric Cambisol, the various amendments had generally convergent fractionation pattern. In contrast, the NPK and manure amendments

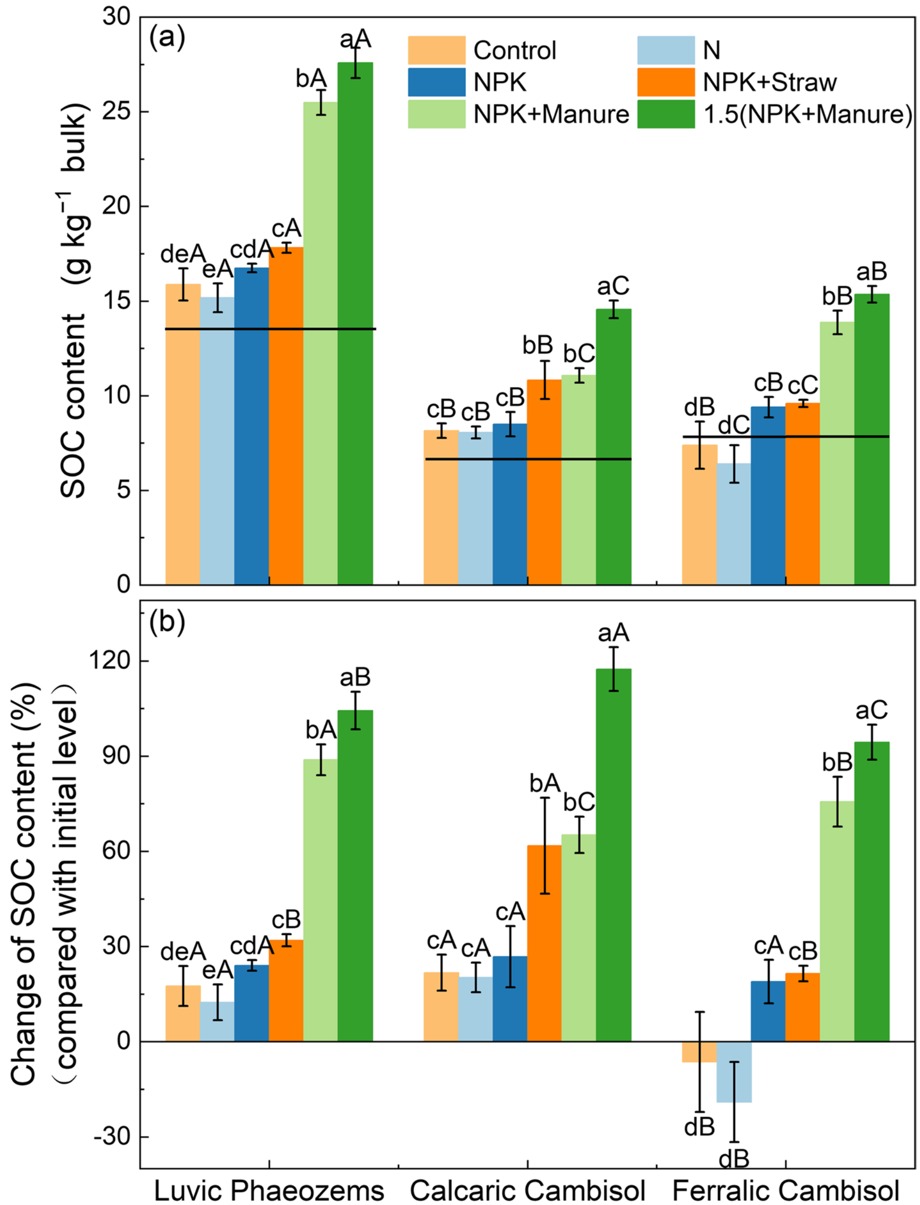


Fig. 2 SOC contents depending on different mineral and organic amendments (**a**) and the changes of SOC compared with initial level in 1990 (**b**) of the three soils. Values are mean ± standard deviation (SD). Lower- and upper-case letters refer to differences among the six fertilizations and soil types at $p < 0.05$, respectively. Three solid lines (**a**) represent initial SOC contents in 1990

generally had dissimilar fractionation patterns in the Ferralic Cambisol and Luvic Phaeozem, respectively, compared with other fertilization regimes (Fig. 4a, b and S1).

3.2 Relationships among bulk SOC, sub-pools and cumulative C inputs

The Luvic Phaeozems had 3 times sequestration efficiency (27%) of bulk SOC more than the Calcaric Cambisol and Ferralic Cambisol, while the latter two had similar build-up efficiencies (Fig. 5). The conversion efficiencies from the cumulative input-C (manure + plant residue) to SOC in the Luvic Phaeozems, Calcaric Cambisol and Ferralic Cambisol were 27%, 9% and 9%, respectively (Fig. 5). Within the physically-, chemically-, biochemically-, physico-chemically-, and physico-biochemically-protected sub-pools, the Luvic Phaeozems similarly had much higher build-up efficiencies than the Ferralic Cambisol and Calcaric Cambisol (Fig. 6b–f and S2 d–p). Within the unprotected pool, however, the Ferralic Cambisol had much higher build-up efficiency more than the

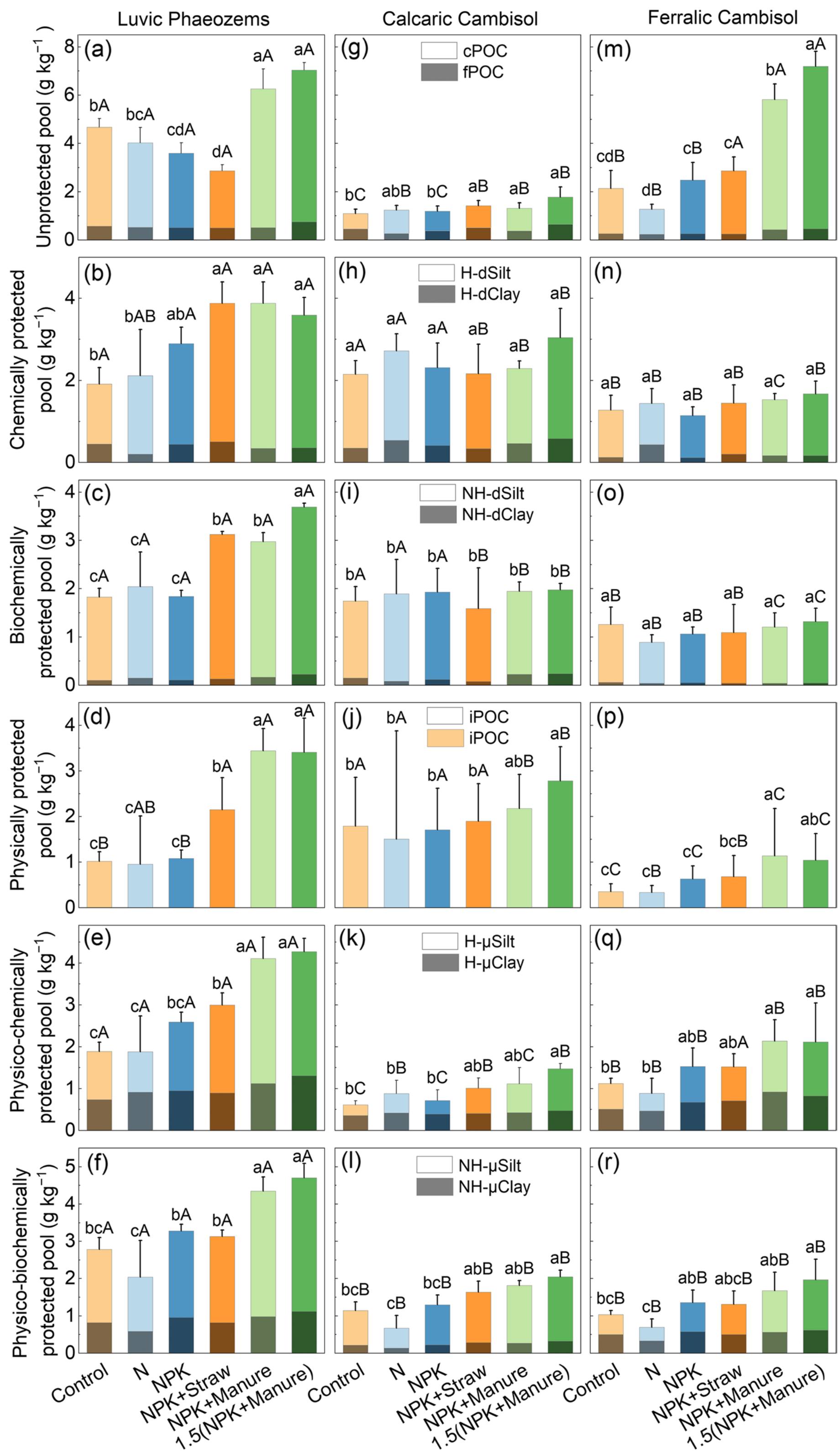


Fig. 3 SOC sub-pools of the three soil types: Luvic Phaeozem (**a**–**f**), Calcaric Cambisol (**g**–**l**) and Ferralic Cambisol (**m**–**r**) under different mineral and organic amendments. Values are mean ± SD. Lower- and upper-case letters indicate differences among the six fertilizations and soils at $p < 0.05$, respectively

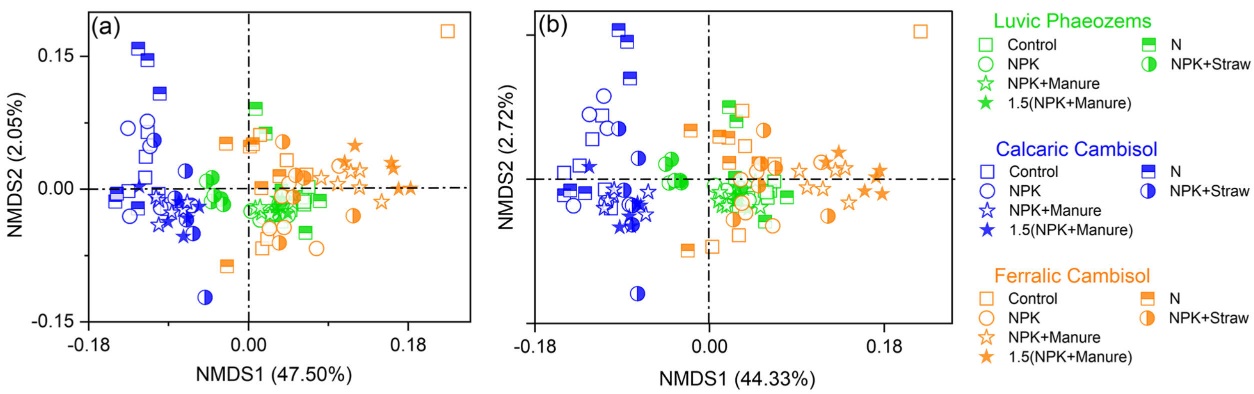


Fig. 4 Nonmetric multidimensional scaling (NMDS) of relative abundances of the six SOC protected pools (unprotected, physical, chemical, biochemical, physico-chemical and physico-biochemical) (**a**) and 11 specific pools (cPOC, fPOC, iPOC, H-dClay, H-dSilt, H-μClay, H-μSilt, NH-dClay, NH-dSilt, NH-μClay and NH-μSilt) (**b**). Bray–Curtis distance was used to calculate the dissimilarity

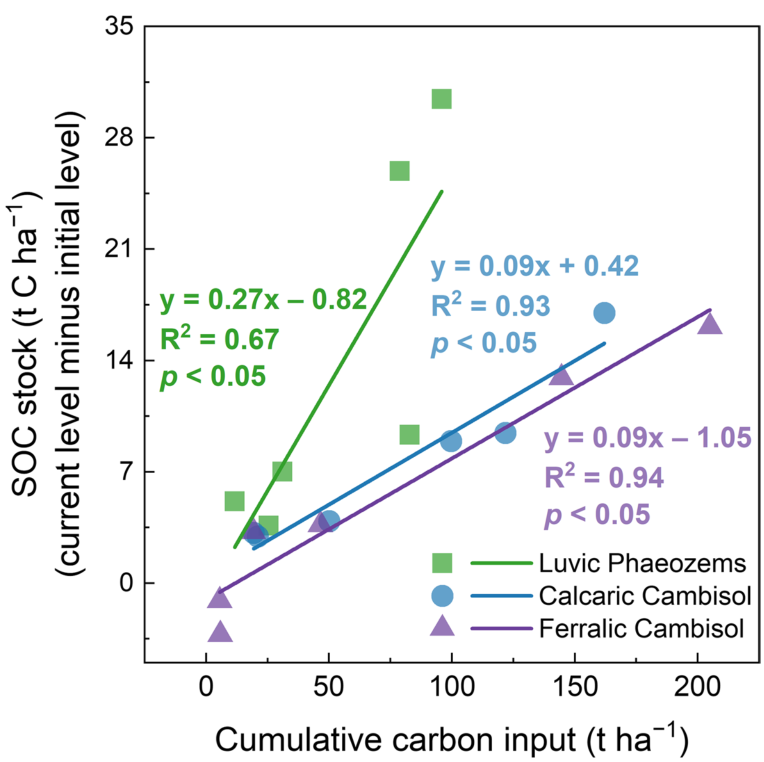


Fig. 5 Relationships between SOC pool changes and total C inputs for the three soils under different mineral and organic amendments. Equivalent soil mass corrections were applied to the estimation of SOC stock. Each value represents the mean of each amendment with six replicates

Calcaric Cambisol, whereas the Luvic Phaeozems had no linear response to cumulative C inputs because of the low build-up efficiency under the NPK + Straw amendment (Fig. 6a and S2 a–c). With the build-up of bulk SOC, the changes of unprotected, physically-, chemically-, and biochemically-protected sub-pools were dependent on soil type (Fig. 6g–j and S3 a–j). Within the unprotected pool, the Ferralic Cambisol had highest building-up slope with the build-up of bulk SOC, followed by the Luvic Phaeozems and Calcaric Cambisol (Fig. 6g and S3 a–c). Within the physically protected pool, the Luvic Phaeozems had highest building-up slope with the build-up of bulk SOC, followed by the Calcaric Cambisol and Ferralic Cambisol (Fig. 6j and S3 j). Within the chemically and biochemically protected pool, only the Luvic Phaeozems had nonlinear and linear relationships with the

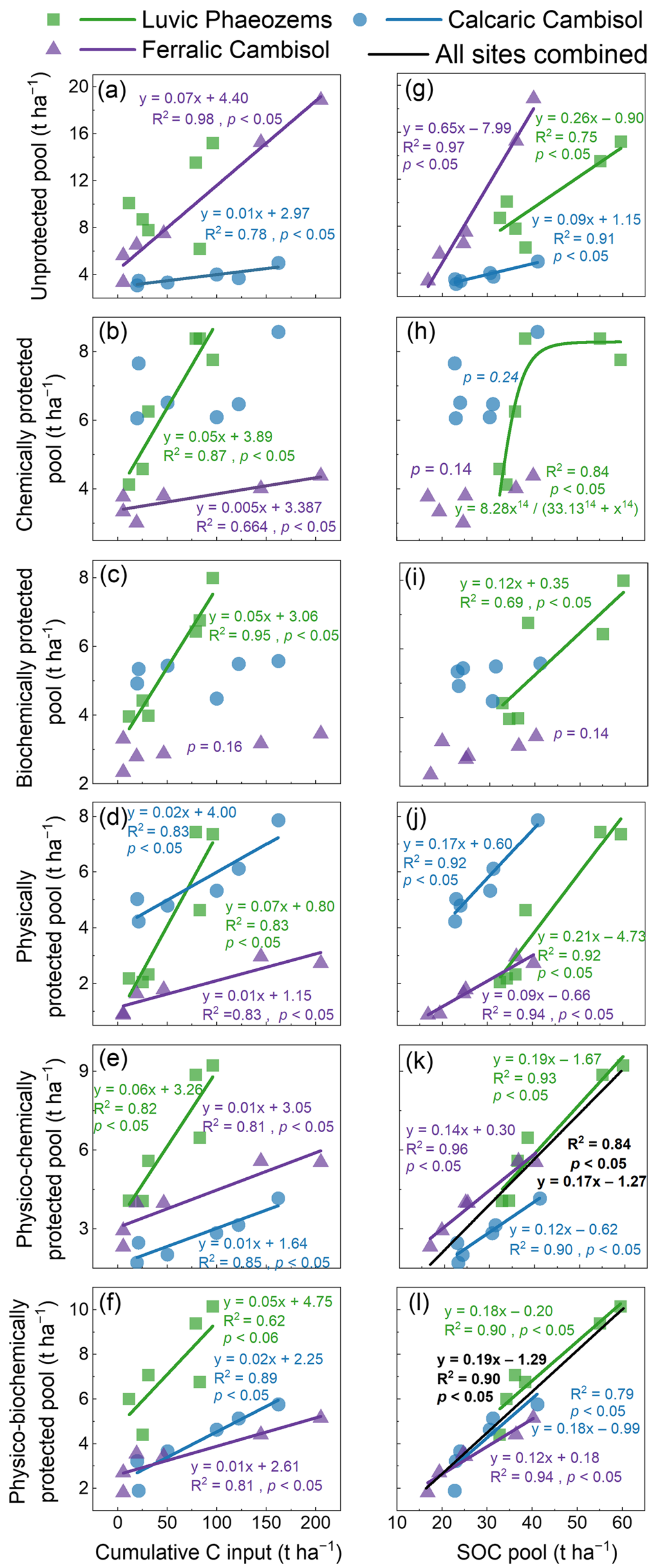


Fig. 6 Responses of the six SOC sub-pools to cumulative C input (**a**–**f**) and bulk SOC pool (**g**–**l**) in the three soils

build-up of bulk SOC, respectively (Fig. 6h, i and S3 d–i). In contrast, the building slopes of physico-chemically (0.12–0.19) and physico-biochemically protected pools (0.12–0.18) were convergent among the three soil types (Fig. 6k, l and S3 k–p).

3.3 Factors influencing SOC pools

The physically-, chemically-, biochemically-, physico-chemically-, and physico-biochemically-protected SOC sub-pools were negatively related with MAT and MAP (Fig. 7). In contrast, the unprotected SOC pools increased with the ratio of MAP/MAT (indices of wetness) and clay content. The physically-, chemically- and biochemically-protected SOC sub-pools increased with pH (Fig. 7). The proportion of non-protected and physico-chemically protected SOC sub-pools increased with clay content and decreased with pH (Fig. 7). On the contrary, the proportions of physically-, chemically- and biochemically-protected SOC sub-pools decreased with clay content and increased with pH (Fig. 7). However, the proportion of physico-biochemically protected SOC pools increased with the ratio of MAP/MAT and decreased with MAT and MAP (Fig. 7). Based on the variance partitioning analysis, climate was the primary factor for the variation in bulk SOC and sub-pools at the three sites (Fig. S4).

4 Discussion

4.1 Bulk SOC under various mineral and organic amendments among the three soils

Compared with mineral fertilization, manure amendments greatly increased SOC stock in the three soils (Fig. 2), which is widely known (e.g., Dutta et al. 2022, Jiang et al. 2018, Just et al. 2023, Tian et al. 2017, Wang et al. 2015, Zhang et al. 2022), suggesting that no matter what kind of climate and soil type, manure addition is a good practical strategy to build soil health and fertility. Furthermore, the Calcaric Cambisol had lower pure manure-derived SOC build-up (30.2%) than the Luvic Phaeozems (52.2%) and Ferralic Cambisol (47.6%) (Fig. 2a), which might be attributed to the sandy loam texture with lower stabilization sites for SOC, indicating that coarse texture limited the soil fertility and health conservation. Compared with manure, the straw return alone increased the bulk SOC in Calcaric Cambisol and Luvic Phaeozems to a low extent but had no significant effect in the Ferralic Cambisol (Fig. 2), which is in support of previous studies (Berhane et al. 2020, Wang et al. 2021a, Yan et al. 2022), suggesting that straw incorporation has little efficiency on building soil health and fertility. The different straw-derived effects may be ascribed to the various climatic conditions. Compared with the two temperate soils (Calcaric Cambisol and Luvic Phaeozems), the subtropical warm and humid climate soil (Ferralic Cambisol) generates faster mineralization and higher degree decomposition of organic matter, which intensively depletes the returning straw and certainly contributes little to bulk SOC pool. However, it is worth noting that the stability of mineral-organic carbon complexes is also influenced by mineral types. The Ferralic Cambisol, with higher clay content, contains more amorphous iron/aluminum oxides/hydroxide than the Luvic Phaeozems (Huang et al. 2021), which has a greater capacity for binding OC. Additionally, long-term manure application could enhance the presence of amorphous metal oxides and increase the association of OC with minerals. Therefore, despite greater capacity of iron/aluminum-binding OC, the lower SOC in the Ferralic Cambisol is primarily attributed to the faster mineralization and higher degree decomposition.

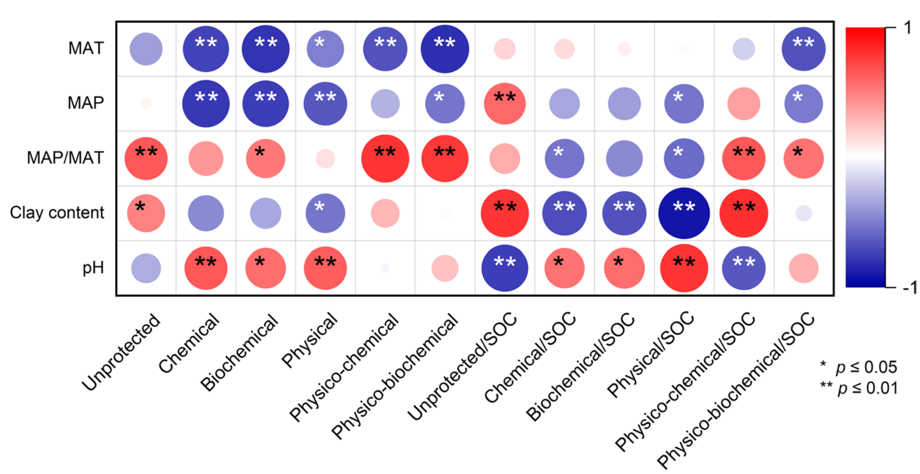


Fig. 7 Correlations of the six SOC sub-pools and environmental and soil variables (MAT, MAP, MAT/MAP, clay content and pH)

The Calcaric Cambisol and Ferralic Cambisol had similar build-up efficiencies of bulk SOC (9%) (Fig. 5), which is relatively low and comparable to the soils in the warm temperate regions (Xuzhou, 7%; Changping, 7.7%; and Davis of USA, 7.6%) (Kong et al. 2005, Zhang et al. 2010) and subtropical region (Nanchang, 6.8%) (Chen et al. 2023), suggesting lower rebuilt of SOC in these two soils rather than the Luvic Phaeozems even under manure amendments. These low conversion efficiencies were likely attributed to the low clay contents in temperate regions (6–10.2%, or silt loam texture) that have low organo-mineral associating efficiencies and subtropical warm climate-induced higher decomposition rate and degree of organic matter. In contrast, the Luvic Phaeozems had 3 times build-up efficiency (27%) more than the above two soils (Fig. 5), which is high and comparable to the soils in the temperate non-monsoon arid/semiarid regions in Urumqi region (26.7%), Zhangye region (31%) (Zhang et al. 2010), the North China Plain (14.1%) (Fan et al. 2014), Alberta area of Canada (26.8%, clay loam) (Malhi et al. 2011), USA temperate semiarid regions (14–21%) (Rasmussen and Collins 1991), and south-west monsoon soils in Meerut of India (Dutta et al. 1991). These high conversion efficiencies were ascribed to the cool and arid climate-induced slower mineralization and decomposition of organic matter and high clay contents (15–29.3%) that can more strongly adsorb and protect organic carbon. Therefore, the soils in Northeast China such as Luvic Phaeozems are prioritized for SOC sequestration when under similar mineral and organic amendments.

4.2 SOC sub-pools linking stabilization mechanisms under long-term amendments among the three soils

Manure application enhanced the unprotected SOC sub-pools in all the three soils (Fig. 3), which is reasonable and consistent with previous studies (Abrar et al. 2020, Ali Shah et al. 2021, Mandal et al. 2019, Yang et al. 2018), suggesting that the most active SOC sub-pool was sensitive to manure addition. In the Luvic Phaeozems, however, the NPK and NPK + Straw both decreased the unprotected SOC (Fig. 3a), which may be attributed to (1) the higher priming effect derived from more root and residue input (Kuzyakov 2010, Zhang et al. 2022), (2) faster decomposition of crop residue (stubble and root) and straw under NPK addition (Cardinael et al. 2015, Lavallee et al. 2020, Liang et al. 2018), and (3) most residues were broken down into small particles and then transferred to macromolecules, oligomers and microbial necromass associated with minerals to form more stable SOC forms. Similarly, it is also unexpected that the N application decreased the unprotected SOC sub-pool in the Ferralic Cambisol (Fig. 3m), which might be ascribed to reduced pH and inhibition of N-mining for microorganism (Kuzyakov 2010). The NPK, straw and manure all increased the chemically and biochemically protected SOC sub-pools in the Luvic Phaeozems, but not for the other two soils (Fig. 3), which may be attributed to the faster decomposition of organic matter in the latter two because of the higher MAT and higher sand content against microaggregates formation in the Calcaric Cambisol. In the Luvic Phaeozems and Ferralic Cambisol, organic amendments (especially manure) all increased the physically protected SOC pools (Fig. 3d, p), which is in support of many studies (Huang et al. 2010, Kou et al. 2012, Yang et al. 2018). In contrast, in the Calcaric Cambisol, only 1.5(NPK + Manure) increased the physically protected SOC pools (Fig. 3j), in which the lower clay content (10%) may be responsible for the less formation of microaggregates even under straw and manure amendments.

The unprotected SOC pool increased with cumulative C input in the Calcaric Cambisol and Ferralic Cambisol, which is reasonable because of the inputs of residue (stubble & root), straw and manure (Abrar et al. 2020, Tian et al. 2017). In the Luvic Phaeozems, however, it was unexpected that the abnormally low amount of unprotected SOC pool even under 23-year abundant straw incorporation (Fig. 6a), which might be ascribed to the NPK-induced easier decomposition of straw with low quality. In contrast, the other five SOC pools had similar responses to cumulative C input in the Luvic Phaeozems (Fig. 6b–f), suggesting that these five pools had convergent sequestration efficiencies and contribution to soil fertility under various mineral and organic amendments. The Luvic Phaeozems had much higher sequestration efficiencies of the microaggregate-related (physical, physico-chemical and physico-biochemical) SOC sub-pools than the other two soils (Fig. 6d–f), which might be attributed to the lower decomposition of OC due to the lower MAT (Wang et al. 2021a, b) and plenty clay content with high association with OC. Furthermore, the Luvic Phaeozems also had much higher sequestration efficiencies of the chemically- (hydrolyzable silt and clay portions) and biochemically-protected SOC sub-pools (non-hydrolyzable silt and clay portions) than the other two soils (Fig. 6b–c), which might be attributed to its lower decomposition of OC due to the lower MAT compared with the latter two soils (Wang et al. 2021a, b) and plenty silt and clay contents with high association with OC compared to the Calcaric Cambisol.

With the build-up of bulk SOC, it is interesting that the Ferralic Cambisol had the highest sensitivity of unprotected pool than the other two soils (Fig. 6g), suggesting that unprotected pool preferentially accumulated during building-up of bulk SOC in this soil. As known, the decomposition of input organic matter (stubble, root, straw and manure) is highest in the Ferralic Cambisol compared with the other two soils (Wang et al. 2021a, b). Based on this situation, the soil matrix in the Ferralic Cambisol received less residual OC due to the high microbial decomposition of organic amendments compared with the other two soils. Therefore, the result above is reasonable. Furthermore, the lower sensitivity of unprotected sub-pool in the Calcaric Cambisol, compared with the Luvic Phaeozems (Fig. 6g), might be attributed to the low content of clay that greatly accommodated unprotected pool (cPOC and fPOC) (Abramoff et al. 2021, Abrar et al. 2020, Six et al. 2002, Stewart et al. 2008). In chemically-protection sub-spool, we found that the Luvic Phaeozems reached saturation under straw and manure incorporation (Fig. 6h), indicating that this sub-pool had low capacity in storing SOC. In the biochemically protection pool, however, the Luvic Phaeozems did not reach saturation (Fig. 6i), suggesting that this sub-pool had high capacity in storing SOC. In contrast, the Ferralic Cambisol and Calcaric Cambisol had little response in both chemically- and biochemically-protection pools under various amendments (Fig. 6h, i), suggesting that these two sub-pools had low sensitivity to input organic matter (stubble, root, straw and manure). In the physically protected pool, the Ferralic Cambisol had lower sensitivity than the other two soils (Fig. 6j), which is attributed to the climate-induced faster decomposition. Within the physico-chemical and physical-biochemical pool, it is interesting that the three soil had convergent response rates (17% and 19%) with the build-up of bulk SOC under various amendments (Fig. 6k, l), respectively. These results suggested that these two microaggregate-associated SOC pools had convergent building-up abilities, which was independent with soil types. The microaggregates consisted of clay, silt and SOC (Six et al. 2002, Totsche et al. 2018), suggesting that the formation of microaggregates simultaneously brought SOC accumulation. Based on the unsaturation of three soils, it is harmony that the formation of microaggregates was consistent with the build-up of bulk SOC. Thus, it is reasonable that these two microaggregate-induced SOC sub-pools (physico-chemical and physical-biochemical pools) had convergent building-up abilities among soils across a climate gradient.

In this present study, the unprotected SOC pools raised with wetness and clay content, while the five protected SOC sub-pools (physical, chemical, biochemical, physico-chemical, and physico-biochemical) were negatively correlated with MAT and MAP, suggesting that the climatic and edaphic factors regulated the dynamics of SOC sub-pools linking stabilization mechanisms. However, it should be noted that the climate and soil information here should only be three data sets, so the results obtained may be subject to chance and further site studies are needed.

5 Conclusions

In the three soils from mid-temperate to subtropics, manure amendment greatly increased the whole SOC and most sub-pools compared with the initial level, but those under the straw and mineral amendments were much lower. The Luvic Phaeozems had much higher sequestration efficiencies of bulk SOC and most sub-pools (except for unprotected pool) than the Calcaric Cambisol and Ferralic Cambisol. In regard to the unprotected pool, however, the Ferralic Cambisol had the highest sequestration efficiency as the Luvic Phaeozems (just not linearly due to abnormally low amount under straw incorporation). The Calcaric Cambisol had divergent patterns of the SOC sub-pools compared with the Luvic Phaeozems and Ferralic Cambisol, due to the low clay and high sand contents. With the build-up of bulk SOC, the physico-chemically and physico-biochemically protected pools (most stable pool) had convergent response rates among soil types, while the building-up abilities of the other pools were dependent on soil type.

Abbreviations

cPOC	Coarse particulate organic carbon
fPOC	Fine particulate organic carbon
H-dClay	Hydrolyzable, easily dispersed, clay-sized pool (acid soluble, < 2 μm)
H-dSilt	Hydrolyzable, easily dispersed, silt-sized pool (acid soluble, 2–53 μm)
H-μClay	Hydrolyzable, microaggregate-derived clay-sized pool (acid soluble, < 2 μm)
H-μSilt	Hydrolyzable, microaggregate-derived silt-sized pool (acid soluble, 2–53 μm)
iPOC	Microaggregate-protected POC
MAOC	Mineral-associated organic carbon
MAP	Mean annual precipitation
MAT	Mean annual temperature
NH-dClay	Nonhydrolyzable, easily dispersed, clay-sized pool (acid resistant, < 2 μm)
NH-dSilt	Nonhydrolyzable, easily dispersed, silt-sized pool (acid resistant, 2–53 μm)
NH-μClay	Nonhydrolyzable, microaggregate-derived clay-sized pool (acid resistant, < 2 μm)
NH-μSilt	Nonhydrolyzable, microaggregate-derived silt-sized pool (acid resistant, 2–53 μm)
POC	Particulate organic carbon
SOC	Soil organic carbon

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44246-024-00121-4.

Supplementary Material 1: Fig. S1. Relative abundance of each SOC sub-pool to bulk SOC pool.

Supplementary Material 2: Fig. S2. Responses of the SOC sub-pools to total C input. All regression lines are significant at p<0.05.

Supplementary Material 3: Fig. S3. Correlations of the SOC sub-pools to bulk SOC pool. All regression lines are significant at p<0.05.

Supplementary Material 4: Fig. S4. The variance partitioning analysis of the effects of climate and soil conditions on bulk SOC and sub-pools at the three sites.

Acknowledgements
Not applicable.

Author's contributions
Yidong Wang and Yilai Lou contributed to the study conception and design and funding acquisition. Huimin Zhang, Ping Zhu, Dongchu Li, Hongjun Gao and Shuiqing Zhang contributed to the resources and material preparation. The investigation, data collection and analysis were performed by Yiping Liu, Limin Zhang, Ning Hu, Zhongfang Li, Yilai Lou and Yidong Wang. The first draft of the manuscript was written by Yiping Liu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding
This research was supported by the National Natural Science Foundation of China (41930862, 31300381 and 31570523), Guangxi Natural Science Foundation of China (GXNSFAA220039), Jiangxi Natural Science Foundation of China (20224ACB205003), Strategic Academic Leadership Program "Priority 2030" of the Kazan Federal University, and the RUDN University Strategic Academic Leadership Program.

Availability of data and materials
The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Consent for publication
All authors declare that they are consent for publication in the journal of Carbon Research.

Competing interests
All authors declare that there are no competing interests.

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Received: 10 September 2023 Revised: 8 March 2024 Accepted: 15 March 2024
Published: 27 March 2024

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