

Two-decade long fertilization induced changes in subsurface soil organic carbon stock vary with indigenous site characteristics

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ABSTRACT

Soil organic carbon (SOC) sequestration at subsurface layers (i.e. > 20 cm) remains unclear under long-term fertilization practices. Based on long-term datasets of fertilization experiments in four typical Chinese croplands, representing soils with high fertility at Gongzhuling (GZL, black soil) and Chongqing (CQ, purple soil), and low fertility at Zhengzhou (ZZ, aquatic Chao soil) and Qiyang (QY, red soil), we calculated SOC storage, its change relative to initial condition (Δ SOC) in 0–20, 20–40 and 40–60 cm. We also obtained annual organic C inputs (OCI; stubble, roots and manure amendment) and derived soil C sequestration efficiency (CSE: the ratio of Δ SOC over OCI) in 0–20 cm and 0–60 cm. The fertilization treatments include cropping with no fertilization (CK), chemical nitrogen, phosphorus and potassium fertilizers (NPK) and combined chemical fertilizers and manure (NPKM). Results showed SOC stock significantly increased with fertilizations (i.e. initial, CK < NPK < NPKM). Relative to initial condition, surface (0–20 cm) and subsurface (20–60 cm) SOC stocks significantly decreased under CK at all sites except GZL, a site with elevated SOC stocks under all fertilizations and depths. Subsurface SOC stocks significantly increased at high fertility soils (i.e., GZL and CQ) but remained no change or significantly decreased at low fertility soils (i.e., ZZ and QY) under NPK and NPKM. Accordingly, CSE derived in 0–60 cm was consistently higher than that in 0–20 cm in high fertility soils but lower in low fertility soils. These results demonstrated that subsurface soils (20–60 cm) remained as C sinks in indigenously high fertility sites but experienced substantial C depletions in low fertility sites. This study informed the need to account for subsurface soil carbon changes for accurate estimates of soil C sequestration capacity under long-term fertilization.

1. Introduction

Soil organic carbon (SOC) is a key feature of soil biogeochemistry for maintaining soil ecosystem services such as healthy soil microbiomes, nutrient cycling, and erosion control (Jimenez et al., 2002; Weil and Magdoff, 2004). SOC represents the largest terrestrial C pool with a storage ranging from 2376 to 2456 Pg C (1 Pg C = 10^{15} g C) at top 2 m globally (Baties, 1996), about triple the amount of C stored in vegetation biomass or in the atmosphere (Post et al., 1990). Of the

global SOC storage in top 1 m (i.e. 1220–2000 Pg C), about 46–61% is located deeper than 30 cm (Baties, 1996; Tarnocai et al., 2009). Most studies of soil C sequestration focused on the surface soil layers (0–20 or 30 cm), or plow layer in croplands, rarely accounting for soil C change below the plow layer or subsurface horizons at depth deeper than 20 cm (Richter et al., 2007; Rumpel and Kögel-Knabner, 2011; Dungait et al., 2012; Li et al., 2013).

SOC storages were changed by fertilization practices at both surface and subsurface soils. Relative to cropping without fertilization (CK),

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long-term application of chemical nitrogen, phosphorus and potassium fertilizers (NPK) increased SOC stock by 11–66% in 0–20 cm and 5–43% in 20–60 cm (Liu et al., 2014; Benbi et al., 2015; Wang et al., 2015). In other studies, NPK treatment however little affected SOC storage in both surface and subsurface soils (Su et al., 2006; Nayak et al., 2012; Maillard et al., 2015). NPK treatment even decreased SOC stock in 60–300 cm by 5–6% in a Chinese Aridisols (Li et al., 2013). Long-term chemical fertilizers combined with manure input (NPKM) increased SOC stock in 0–60 cm by 12–113% (Zhou et al., 2013; Chai et al., 2015; Zhang et al., 2015). NPKM is however found to little affect SOC storage in 15–60 cm (Gami et al., 2009) and 60–100 cm (Liu et al., 2013). Relative to the initial condition, CK could lead to SOC changes by –2.1% to 2.4% based on decade-long Chinese and European fertilization experiments (Körschens et al., 2013; Zhou et al., 2013). In summary, effects of long-term fertilization on SOC stock still entail high uncertainty particularly in subsurface soils (e.g. > 20 cm).

SOC storage depends on the balance of organic carbon inputs and outputs (Six et al., 2002; Sistla et al., 2013). Long-term fertilization usually elevated organic C inputs through crop stubbles, roots and manure (Kundu et al., 2007; Banger et al., 2008). On the other hand, decomposition of SOC was likely enhanced due to priming effect driven by microbial utilization of exudate C from plant roots (Fontaine et al., 2007; Cheng, 2009). To evaluate to what extent SOC storage change under long-term fertilizations, carbon sequestration efficiency (i.e. CSE), defined as C stock change per unit C input (Stewart et al., 2007), was employed to account for both SOC storage change and OC input under long-term fertilizations (Liang et al., 2016). CSE, derived for soils at 0–20 cm, was negative, positive or little changed under decade-long fertilizations (Liang et al., 2016; Zhang et al., 2010). From our knowledge, very few studies have strived to obtain CSE at subsurface soils (> 20 cm). The reasons are likely due to scarcity in subsurface soil C data and the difficulty quantifying organic C input allocated between surface and subsurface soil layers. Nevertheless, only accounting for soil C change at surface soil layer may have misrepresented C sequestration capacity across the soil profile. Furthermore, the underlying edaphic drivers for explaining variations of CSE were recently investigated at surface soil layers only (Liang et al., 2016). Although subsurface soil C stock (> 20 cm) represents a potentially important C pool under long-term fertilization, its change remains poorly understood.

Since 1970s, > 80 long-term fertilization experiments and trials have been established in China varying in climate, management practices and soil types (Xu et al., 2015). Monitored continuously for more than a few decades, these experiments accumulated soil C records mostly available in 0–20 cm or 0–30 cm. Based on an extensive literature review and data compilation, the current study synthesized long-term records of SOC (0–20, 20–40 and 40–60 cm) and manure inputs at four of these sites representing high fertility sites (i.e., black soil and purple soil) and low fertility sites (i.e., Chao aquatic soil and red soil). The site characteristics were presented in Tables 1 and 2.

This study aims to examine changes of SOC storage in surface

(0–20 cm) and subsurface (20–60 cm) soil layers under different fertilization treatments. By simulating the allocation of organic C input between surface and subsurface soil layers, the study strived to elucidate whether soil CSE was significantly different between surface layer (0–20 cm) and soil profile (0–60 cm). Given the data availability, the variance partition analysis was conducted at the Chao aquatic soil to explore the driving factors in explaining CSE variations derived in 0–20 cm and 0–60 cm. We hypothesized that relative to initial condition, SOC storages significantly decreased under CK and increased under NPK and NPKM at both surface and subsurface soil layers and the patterns were evident at both low and high fertility sites; Second, after accounting for allocation patterns of OC input, CSE little differed between 0–20 cm and 0–60 cm and the pattern was evident at both low and high fertility sites.

2. Materials and methods

2.1. Site characteristics and fertilization treatments

Located from northern cool to southern warm regions in China, the four experimental sites are referred to Gongzhuling (GZL) in Jilin province, Zhengzhou (ZZ) in Henan Province, Chongqing (CQ) autonomous region, and Qiyang (QY) in Hunan province (Fig. 1). GZL and CQ showed higher indigenous soil fertility than ZZ and QY. The two groups were hereafter referred to high and low fertility sites, respectively. Representing distinct climatic zones, the four sites differ in mean annual temperature (MAT), mean annual precipitation (MAP), soil type and cropping system (Tables 1 and 2). The fertilization experiments were initiated in 1990 (GZL, ZZ and QY) and in 1991 (CQ). The plot dimension is 25 m × 16 m (GZL, ZZ), 10 m × 20 m (QY), and 10 m × 12 m (CQ). Despite diverse fertilization treatments, three fertilization treatments were common across sites including cropping with no fertilizer input (CK), chemical N, P and K fertilizers (NPK), and NPK with animal manure input (NPKM). The chemical N, P and K fertilizers were referred to urea, calcium superphosphate and potassium chloride, respectively. In the NPKM treatment, 30–40% of total N was applied as chemical N fertilizer and the rest substituted by N input in the manure. The source of amended manure was of cow or pig wastes. Manure was applied once a year before seeding of wheat at ZZ and CQ, and before seeding of maize at GZL. The manure was applied twice a year before seeding of wheat and maize at QY. The fertilization rates in each site were listed in Table 3.

2.2. Soil collection and physiochemical analysis

Soil samples (0–20 cm, 20–40 cm and 40–60 cm) were collected during August and October in each site in the initial year and during the soil sampling campaign in 2009 or 2010 (Table 1). Three soil samples were obtained randomly from each replicated plot by using 5-cm diameter auger, then composited and homogenized into one sample in

Table 1
Site characteristics of long-term fertilization experiments at four typical croplands in China.

Site	Coordinate	Altitude (m)	MAT (°C)	EAT (°C)	MAP (mm)	MAE (mm)	Climatic zone in China	Cropping system	Experiment Initiation	Sampling	Duration
GZL	43°30'23"N 124°48'34"E	220	4.5	1700	589	1400	Mild-Temperate, Semi-Humid	Mono-cropping Maize	1990	2010	20
ZZ	35°50'00"N 113°42'00"E	59	14.5	2661	615	1450	Warm-Temperate, Semi-Humid	Double-cropping Wheat-maize	1990	2009	20
CQ	30°26'00"N 106°26'00"E	266	18.5	3271	1110	990	Sub-Tropical, Humid Monsoon	Double-cropping Wheat-rice	1991	2010	20
QY	26°45'00"N 111°52'00"E	120	18.5	3429	1255	1470	Sub-Tropical, Humid	Double-cropping Wheat-maize	1990	2010	20

GZL: Gongzhuling in Jilin province; ZZ: Zhengzhou in Henan province; CQ: Chongqing autonomous region; QY: Qiyang in Henan province. MAT: mean annual temperature; EAT: effective annual temperature; MAP: mean annual precipitation; MAE: mean annual evaporation.

Table 2
Soil characteristics (0–20 cm) at the initiation of long-term fertilization experiments at four typical croplands along a latitudinal transect in China.

Site	SOC g·kg ⁻¹	TN g·kg ⁻¹	C:N	AN mg·kg ⁻¹	TP g·kg ⁻¹	Olsen-P mg·kg ⁻¹	TK g·kg ⁻¹	AK mg·kg ⁻¹	pH	BD g·cm ⁻³	Clay %	Soil type ^a	Soil type ^b
GZL	13	1.42	9.2	131	1.5	23	24.6	160	7.2	1.19	32	Black soil	Luvic Phaeozems
ZZ	6.7	0.67	10	51	0.6	6.5	16.9	74	8.3	1.24	13	Fluvo-aquic soil	CalcricCambisol
CQ	14	1.25	11	93	0.67	4.3	21.1	88	7.7	1.38	30	Neutral purple soil	Chromic Cambisol
QY	8.6	1.07	8.0	79	0.5	11	13.3	122	5.7	1.10	41	Red soil	EutricCambisol

SOC: soil organic carbon; TN: total nitrogen; AN: available nitrogen; TP: total phosphorus; TK: total potassium; AK: available potassium. BD: bulk density; SP: soil porosity.

^a Based on China soil taxonomy.

^b Based on United Nations FAO soil taxonomy.

each plot. The soil samples were stored in 4 °C cooler and then were transported to the laboratory subjected to physiochemical analysis in two weeks. Crop residues, root material and gravels were identified and removed from the soil sample. Prior to analysis, soil samples were air-dried, ground with wooden blocks and passed through a 2-mm sieve.

To minimize the influence of field viabilities on bulk density and SOC (Li et al., 2010), soils were tilled heavily for two continuous years prior to the establishment of the long-term soil experiment at each site. This measure homogenized soils and enabled one composited sample to produce values in both bulk density and SOC close to the overall mean at the initial year (Li, 2018), which were used to derive the initial SOC stock in each site. At the initial year, one soil sample was obtained for SOC in each depth by compositing multiple soil samples randomly collected in each site. The soil sample collected for bulk density was described in the following section.

The determination of SOC concentrations in soil samples at the four sites collected at the initial year and in 2010 was based on the Walkley-Black chromic acid wet oxidation method (Allison, 1965; Lu, 1999).

Oxidisable matter in the soil is oxidized by 1 N K₂Cr₂O₇ solution assisted by the heat generated when H₂SO₄ is mixed with the dichromate. The remaining dichromate is titrated with ferrous sulphate. The amount of ferrous sulphate consumed in titration is inversely related to the amount of C present in the soil sample. The alkaline solution diffusion, 0.5 mol L⁻¹ H₂SO₄ extracting-molybdenum antimony, and NH₄OAc extracting-flame photometric method were applied to quantify available N, P and K in soil samples (Lu, 1999). Total P and K concentrations were quantified using NaOH melted: molybdenum antimony method and flame photometry method, respectively (Lu, 1999). The soil pH (1:1 soil: water) was measured by potentiometry (Thomas et al., 1996).

The bulk density data at the initial year and in 2010 were adapted from published dataset (Ma and Li, 2010). The same sampling method has been applied in all sites. Bulk density was quantified by collecting a known volume of soil using a metal ring pressed into the soil (intact core), and determining the weight after drying (Lu, 1999; Bellamy et al., 2005). Soil profiles up to 100 cm were excavated for sampling purpose in each site. Three replicate soil samples were collected at each

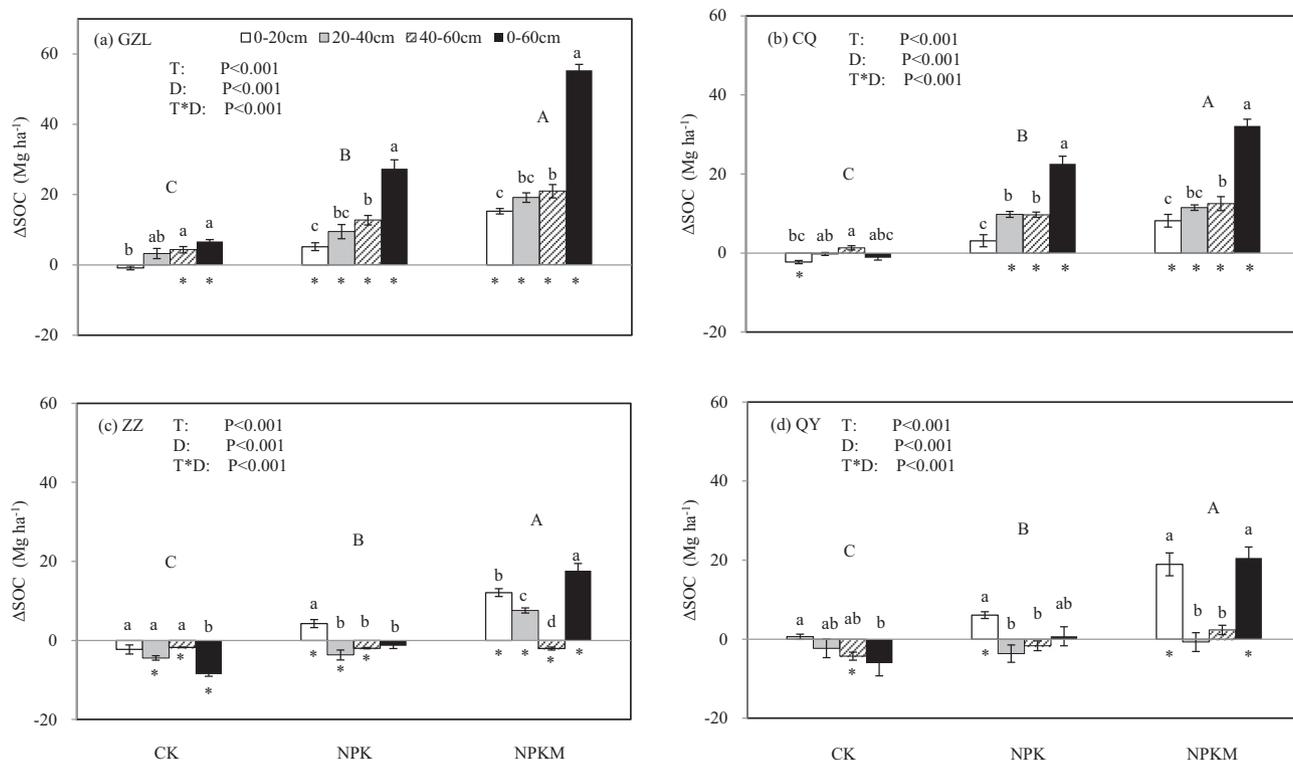


Fig. 1. The mean Δ SOC (\pm SE, Mg C ha⁻¹) at 0–20, 20–40, 40–60 and 0–60 cm under CK, NPK and NPKM treatments at four sites along a latitudinal transect in China. SOC Data is unavailable at 40–60 cm in ZZ. In each panel, different uppercase letters denote significant difference between fertilization treatments in each site at p-value < 0.05. Lowercase letters denote significant difference between four depths under each fertilization treatment. * denotes Δ SOC is significantly different from zero. T: fertilization treatment; D: depth.

Table 3
Input rates of chemical fertilizer (kg ha⁻¹) and manure (t ha⁻¹) in long-term fertilization experiments at four typical croplands in China.

Site	Fertilizer	CK		NPK		NPKM	
		Crop1 ^a	Crop2	Crop1	Crop2	Crop1	Crop2
GZL	N-P ₂ O ₅ -K ₂ O	0-0-0	No	165-36-69	No	50-36-69	No
	Manure	0		0		23	
ZZ	N-P ₂ O ₅ -K ₂ O	0-0-0	0-0-0	165-36-68	188-41-78	50-36-68	188-41-78
	Manure	0	0	0	0	15 ^b	0
CQ	N-P ₂ O ₅ -K ₂ O	0-0-0	0-0-0	150-33-63	150-33-63	150-33-63	150-33-63
	Manure	0	0	0	0	22.5	0
QY	N-P ₂ O ₅ -K ₂ O	0-0-0	0-0-0	90-16-30	210-37-70	27-16-30	63-37-70
	Manure	0	0	0	0	10-15 ^c	25-35

^a Denotes the cropping system at each site described in Table 1.

^b Calculated based on C/N (i.e. 25) and carbon content (i.e. 19.8%) in cow manure.

^c Denotes a range.

layer of 0–20 cm, 20–40 cm, and 40–60 cm during soil sampling campaign in 2009 or 2010. Only one sample was collected for each depth at the initial year at all sites. At ZZ, bulk density at 0–27 cm and 27–50 cm were used to represent the bulk density at 0–20 cm and 20–40 cm, respectively (personal communication with Dr. S Huang at the site).

To examine the influence of substrate quality on soil C stock change, soil samples (0–20 cm and 20–40 cm) and crop materials (aboveground whole plant and belowground materials) were collected in a field sampling campaign in July 2015 at QY and in September in 2015 at GZL. A composited soil or plant sample was obtained by homogenizing three soil or plant samples randomly collected in the same treatment. Prior to analysis, soil and plant samples were air-dried, ground with wooden blocks and passed through a 0.125-mm sieve. Samples were quantified for their stable carbon isotope (¹³C) and radiocarbon (¹⁴C) contents by 3MV multi-element AMS system (Model 4130-AMS, HVEE, Netherlands) at Xi'an Accelerator Mass Spectrometry Center (XAAMS) in Shan'xi province, China.

Rate of SOC change (Δ SOC) and soil carbon sequestration efficiency (CSE).

The soil C storage (Mg ha⁻¹) was calculated based on the following equation:

$$C_{storage} = SOC \times \rho \times D \times 10 \quad (1)$$

where SOC is the soil organic carbon concentration (g kg⁻¹), ρ is soil bulk density (g cm⁻³), and D is the soil depth (m). In each site, we extracted the soil bulk density data of soil samples collected at each depth under each fertilization treatment in the initial year and 2010 (Table S1).

The rate of SOC change (Δ SOC, Mg ha⁻¹ yr⁻¹) was calculated in each of three depths (0–20 cm, 20–40 cm and 40–60 cm) based on the following equation:

$$\Delta SOC = \frac{SOC_n \times \rho_n \times D \times 10 - SOC_{initial} \times \rho_{initial} \times D \times 10}{N} \quad (2)$$

where SOC_n and $SOC_{initial}$ denote SOC concentrations at the n^{th} year and the initial year (g kg⁻¹), respectively. ρ_n and $\rho_{initial}$ are the bulk density determined at the n^{th} year and the initial year (g cm⁻³), respectively. D is 20 cm for all sites. The constant value (i.e., 10) is the conversion factor due to unit transformations in area (1 ha = 10⁴ cm²) and weight (1 kg = 10³ g). N is the number of years of experimental duration between the initial year of the experiment and the n^{th} year (i.e. 2009 in ZZ and 2010 in other sites). Δ SOC is positive given soil C accumulation, and negative given soil C depletion.

Under all fertilization treatments, the aboveground materials (grain and straw) were removed but the stubble and root remained in the maize and wheat plots (only root remained in a rice plot). Given the relationship between aboveground standing biomass (grain and straw) and belowground root biomass as well as relationship between stubble and straw, the plant C input to soil via crop stubble and root (PCI,

Mg C ha⁻¹ yr⁻¹) was calculated based on the following equations:

$$PCI_{maize} = \left((Y_{grain} + Y_{straw}) \times \left(\frac{26\%}{74\%} \right) + Y_{straw} \times \frac{3\%}{100\%} \right) \times (1 - 14\%) \times 0.444 \quad (3)$$

$$PCI_{wheat} = \left((Y_{grain} + Y_{straw}) \times \left(\frac{30\%}{70\%} \right) + Y_{straw} \times R_s \right) \times (1 - 14\%) \times 0.399 \quad (4)$$

$$PCI_{rice} = \left((Y_{grain} + Y_{straw}) \times \left(\frac{30\%}{100\%} + \frac{5.6\%}{100\%} \right) \right) \times (1 - 14\%) \times 0.418 \quad (5)$$

where PCI_{maize} , PCI_{wheat} and PCI_{rice} are the organic C input from plant stubble and root (Mg C ha⁻¹ yr⁻¹) of maize, wheat and rice in each site in each year, respectively. For each crop, the mean annual plant C input was obtained averaged during the initial year and n^{th} year. Y_{grain} is the grain yield biomass (kg·ha⁻¹); Y_{straw} is the straw yield biomass (kg·ha⁻¹); $\frac{26\%}{74\%}$, $\frac{30\%}{70\%}$ and $\frac{30\%}{100\%}$ are the relative ratio of root biomass over grain and straw biomass for maize, wheat and rice respectively; $\frac{3\%}{100\%}$ and R_s are the relative ratio of stubble biomass over straw biomass for maize, and wheat, respectively, and R_s varies from 13.1% to 18.3% in different fertilization treatments (Jiang, 2013). $\frac{5.6\%}{100\%}$ is the relative ratio of stubble biomass over grain and straw biomass for rice. 14% is the average moisture content of air-dried plant sample; 0.444, 0.399 and 0.418 are the proportions of organic carbon content of dried biomass for maize, wheat and rice, respectively.

Under NPKM treatments, the manure-derived organic C input (MCI, Mg C ha⁻¹ yr⁻¹) was calculated based on the following equation:

$$MCI = M_{total} \times P_c / 1000 \quad (6)$$

where M_{total} is the amount of total manure on average (Mg ha⁻¹ yr⁻¹); P_c is the carbon content of manure (g kg⁻¹), 1000 is the conversion factor.

The carbon sequestration efficiency (i.e. CSE) was derived using the following equation:

$$CSE = \frac{\Delta SOC}{PCI + MCI} \quad (7)$$

where $MCI = 0$ under CK treatment.

The PCI and MCI allocated in 0–20 cm and 0–60 cm were required to derive CSE in the respective depth. Based on our literature review, the plant and manure C input allocated at depth deeper than 60 cm is negligible (Vogt and Bloomfield, 1991; Lauenroth and Gill, 2003; Watt et al., 2006). Most root biomass (e.g. 75% to 93%) was found at 0–20 cm for wheat, maize and rice (Liao et al., 2014; Guan et al., 2015). To derive the CSE in 0–20 cm and 0–60 cm, a model simulation was conducted in this study. The proportion of plant and manure C input in 0–20 cm (γ) was thus assumed to be 0–100% of C input in 0–60 cm. CSE

derived 0–20 cm and 0–60 cm was compared and plots of *CSE* against the proportion (γ) were produced under all treatments and sites. The calculations were listed in Eqs. (8)–(10).

$$CSE_{0-20} = \frac{\Delta SOC_{0-20}}{OCI_{0-20}} \quad (8)$$

$$CSE_{0-60} = \frac{\Delta SOC_{0-60}}{RCI + MCI} \quad (9)$$

$$OCI_{0-20} = (RCI + MRI) \times \gamma \quad (10)$$

$$\gamma \in (0, 1)$$

where CSE_{0-20} and CSE_{0-60} denote *CSE* at two depths; OCI_{0-20} denotes the amount of OC input at 0–20 cm, which is proportional to the total OC input at 0–60 cm (i.e. γ).

2.3. Statistical analysis

The one-way ANOVA was used to test effect of site on SOC stock, Δ SOC and *CSE*. In each site, the two-way ANOVA was used to test the main and interactive effects of fertilization treatment and soil depth on SOC stock, Δ SOC and *CSE*. Grouped as year, the repeated-measure ANOVA was used to test the main and interactive effects of fertilization treatment and sites on OCI. Post hoc tests were conducted to achieve multiple comparisons between means. At ZZ, the variance partitioning analysis (VPA) was used to identify the relative contributions of underlying driving factors of edaphic, climatic, C input and their interactions that explain the variations of *CSE* at 0–20 cm and 0–40 cm, respectively. To derive *CSE* at 0–20 and 0–40 cm at ZZ, the proportion of OC input allocated in 0–20 cm (γ) was chosen to be 50%, 60%, 70%, 80% and 90%, while the proportion allocated in 0–40 cm was assumed to be 75%, 80%, 85%, 90% and 95%. The VPA method was described in detail in Liang et al. (2016). All analyses were conducted using R program version 3.3.1 (R Core Team, 2015). The significance level is set at $P < 0.05$.

3. Results

3.1. Effects of long-term fertilizations on SOC and Δ SOC at surface and subsurface layers

SOC stocks generally decreased with soil depth in the initial condition and all treatments (0–20 cm > 0–40 cm > 0–60 cm) and increased significantly with fertilizations (CK < NPK < NPKM) (Fig. S1). SOC stocks varied significantly between high and low fertility sites (GZL, CQ > ZZ, QY) ($P < 0.01$, Fig. S1). Under CK, Δ SOC was negative in CQ, ZZ and QY but positive in GZL (see * and hereafter, p -value < 0.05, Fig. 1). Under NPK and NPKM, Δ SOC was always positive in GZL and CQ (p -value < 0.05, Fig. 1a, b) but was either positive or negative in ZZ and QY (p -value < 0.05, Fig. 1c, d). In particular, Δ SOC was negative at subsurface soil layers in ZZ (p -value < 0.05, Fig. 1c). From surface to subsurface layers, Δ SOC (positive values) further increased in GZL and CQ (Fig. 1a, b) but Δ SOC (positive values) decreased or Δ SOC (negative values) were further amplified (more negative) in ZZ and QY (Fig. 1c, d). Δ SOC significantly increased with fertilizations (CK < NPK < NPKM, Fig. 1).

3.2. OC input under long term fertilizations

The amount of annual OC input remained relatively stable over two decades (Fig. 2a–d) and followed an ascending order with fertilization treatments (i.e. CK < NPK < NPKM, Fig. 2e). Under NPKM, the amount of OC input from manure was generally larger than or similar to that from plant residual and root, with a proportion of 67% at QY, 53% at GZL, 48% at ZZ, and 44% at CQ, respectively.

3.3. *CSE* at 0–20 cm and 0–60 cm under long term fertilizations

In the high fertility sites (i.e., GZL and CQ), *CSE* at 0–20 cm was consistently lower than that at 0–60 cm under all fertilization treatments when $\gamma > 0.28$ (Fig. 3a–f). The same trend was evident for the full range of γ for both sites under CK, $\gamma > 0.19$ (GZL) and $\gamma > 0.14$ (CQ) under NPK, and $\gamma > 0.28$ (GZL) and $\gamma > 0.25$ (CQ) under NPKM (Fig. 3a–f). In the lower fertility sites (i.e., ZZ and QY), *CSE* at 0–20 cm was consistently larger than that at 0–60 cm under all fertilization treatments when $\gamma > 0.34$ (Fig. 3g–l). At ZZ, the same trend was evident when $\gamma > 0.34$ under CK, and $\gamma > 0.25$ under NPK and NPKM (Fig. 3g–i). At QY, the same trend remained for the full range of γ under all fertilization treatments (Fig. 3j–l).

3.4. Driving factors for explaining Variations in *CSE*

At ZZ, comparing the percentile contributions to *CSE* in 0–20 cm and 0–40 cm from each individual factor or interactive terms, the same conclusion was reached under each of the five selected γ (Tables 4 and S2). Take $\gamma = 50\%$ as an example, the total variation in *CSE* explained by all factors was 71% and 66% in 0–20 cm and 0–40 cm, respectively (Table 4, p -value < 0.01). About 42% of variation in *CSE* in 0–20 cm and 44% in 0–40 cm were explained by the interaction of edaphic factor and OC input, but < 3% was explained by any other two or three interaction terms in both depths (Table S2).

The percentiles of variation in *CSE* explained by edaphic, OC input or climate factors were 18%, 5.2% and 1.3% in 0–20 cm, and 12%, 5.0%, and 1.2% in 0–40 cm (Table 4). Soil bulk density was identified as the most important individual factor explaining variation in *CSE* in 0–20 cm (~9.8%), and stubble carbon was the counterpart in 0–40 cm (~4.2%; Table 4).

4. Discussions

4.1. SOC stock generally decreased in both surface and subsurface layers under no fertilization treatment

Relative to initial condition, SOC stocks generally decreased under no fertilization (CK) in all sites except GZL, where SOC stocks little changed in 0–20 cm and 20–40 cm and even significantly increased in 40–60 cm under CK. The generally depleted SOC stocks under CK were likely driven by rapid litter and soil decomposition and concomitantly limited quantity of crop residues return to soil (Körschens et al., 2013; Cong et al., 2014). However, the no change or increase in SOC stock as observed in GZL contrasted with C depletions in other sites suggesting a slow C accumulation at GZL likely due to the constrained decomposition in much lower mean annual temperature (MAT) at GZL than that at other sites (4.5 °C vs. 14.5–18.5 °C). This is somewhat analogous to the large C accumulation in high latitude soils due to even lower soil temperature (Tarnocai et al., 2009). It is also interesting to speculate that the habit of tillage to a deeper depth (i.e. up to 35 cm) at GZL may have also contributed to C accretion at subsurface layer lower than 35 cm, while the tillage depth in other sites was generally at surface layer (i.e. 20 cm or shallower) (Xu et al., 2015).

4.2. SOC stock increased in surface layers under long-term fertilization

Relative to initial condition, SOC stocks consistently increased at surface layers in all sites under NPK and NPKM. The effect was significantly more pronounced under NPKM than that under NPK. These have been likely driven by the elevated amount of plant residues and root C inputs to soils (Johnson et al., 2006; Shen et al., 2013), demonstrating the advantage of chemical fertilizers in not only improving crop productivity and maintaining soil fertility but climate change mitigation at a broad spatiotemporal scales (Smith, 2004; Lal, 2011, 2013). The balanced chemical fertilizers amendments with manure (i.e.

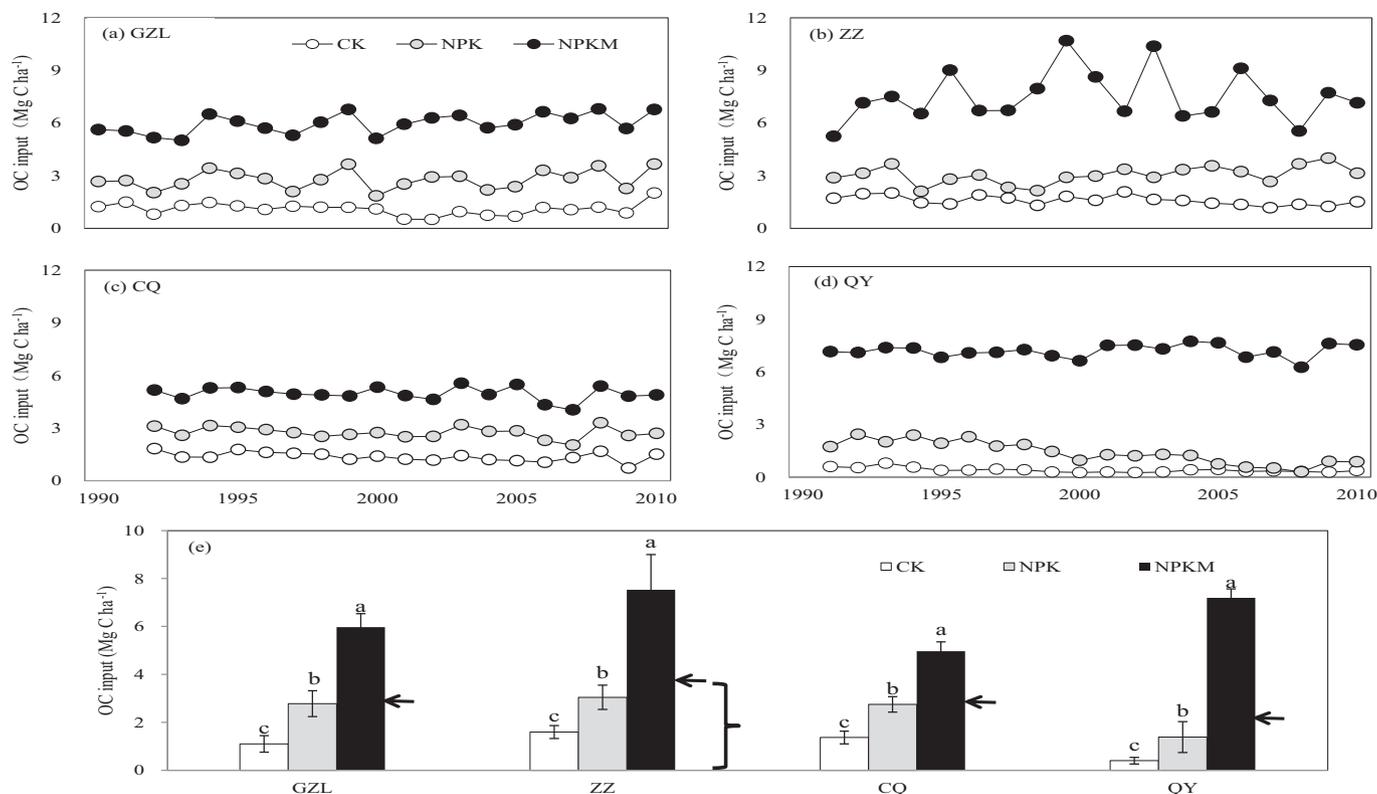


Fig. 2. Annual organic C input to 0–60 cm soil (Mg C ha^{-1}) under CK (via crop residual and root), NPK (via crop residual and root) and NPKM (via crop residual, root and manure application) treatments at four sites along a latitudinal transect in China (a–d), and the yearly mean (\pm SE) OC input ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$) at each site (e). In (e), each arrow denotes the yearly mean OC input via crop residual and root under NPKM treatments; different lowercase letters denote significant fertilization treatment effect at each site. Below the arrows represent the carbon inputs from the stubble.

NPKM) further mediated the adverse effects of soil acidification and reduction in base saturation caused by chemical fertilizers input alone (Han et al., 2016; Zeng et al., 2017). Thus, organic manure amendments have long been recommended in agricultural practice (Paustian et al., 1992).

4.3. SOC stock changes in subsurface layers vary in both signal and magnitude under long-term fertilization

At the subsurface layers (20–60 cm), the aforementioned SOC accretions under fertilizations (NPK and NPKM) were not evident in either ZZ or QY. In fact, SOC stocks significantly decreased in 20–40 cm and 40–60 cm under NPK and in 40–60 cm under NPKM in ZZ. SOC stocks also declined in subsurface layers under NPK and NPKM in QY. As a result, such reductions in SOC in the subsurface layers diminished or even reversed the C accretions in surface layers, leading to minor SOC accretions under NPKM or nearly zero net changes in 0–60 cm under NPK in both ZZ and QY. Our findings were supported by other studies. For instance, a study reported SOC storage increased in 20–40 cm under NPKM in a black soil (Dou et al., 2016). The change in SOC storage was not detectable in 25–100 cm under NPK in a forty-year long fertilization experiment (Walia et al., 2017). These results demonstrated that sites with high and poor fertility were distinct in signal and magnitude in changes of SOC stock in response to long-term fertilization. This filled the knowledge gap as very few studies explicitly identified this distinction among sites given their indigenous fertility.

4.4. Multiple drivers for the site-specific changes in SOC stock and sequestration

The most important edaphic factor in driving soil C sequestration was identified different in surface and subsurface layers in one site (i.e.,

ZZ). Furthermore, consistently higher C sequestration rate was revealed in 0–60 cm than that in 0–20 cm in high fertility sites but lower in poor fertility sites, corroborating the site-specific feature of CSE between surface and subsurface layers. We speculate that sites with indigenously high and low fertility differ in the turnover, formation and accumulation of SOC driven by multiple soil characteristics (Sollins et al., 1996, 2007). Of particular importance are the nature of soil substrate, the mineral type, soil C saturation potential and their interactions in surface and subsurface layers. First, ¹⁴C age was generally larger in 20–40 cm than that in 0–20 cm (Table 5), suggesting a longer residence time of SOC at the subsurface layer (Trumbore, 2009). However, the differences in ¹⁴C age between depths are more pronounced in QY (i.e., low fertility site) than that in GZL (i.e., high fertility site), indicating a relatively much more rapid replenishment of organic C in subsurface layers in the high fertility site or more rapid depletion of organic C in subsurface layers in the low fertility site. Also, soil surface area is twice large in GZL than in ZZ (Table 6a) leading to strong associations of surface area and sorption of organic matter within soil compartments (Kaiser and Guggenberger, 2003), supporting the relatively greater SOC accumulation at surface soil in the high fertility site than the low fertility site.

The mass proportion of fine soil particles (< 0.02 mm; referred to P hereafter) can be used to predict the maximal SOC stocks (C_{max}) based on a linear relationship with a slope of α , i.e., $C_{\text{max}} = P \times \alpha$ (Di et al., 2017). The same study suggested that α is about 0.84 and 0.43 for 2:1 or 1:1 clay mineral soils, respectively. Soils in GZL and QY are dominated by 2:1 and 1:1 clay minerals, respectively (Table 6a), and clay and silt contents are higher in the high fertility sites (i.e., GZL and CQ) than that in the low fertility site (ZZ; Table 6b). Despite a relatively high clay and silt content in the low fertility site (QY), the product of clay content and α still lead to larger stable SOC deficit and relatively large soil C saturation potentials in the high fertility site than the low

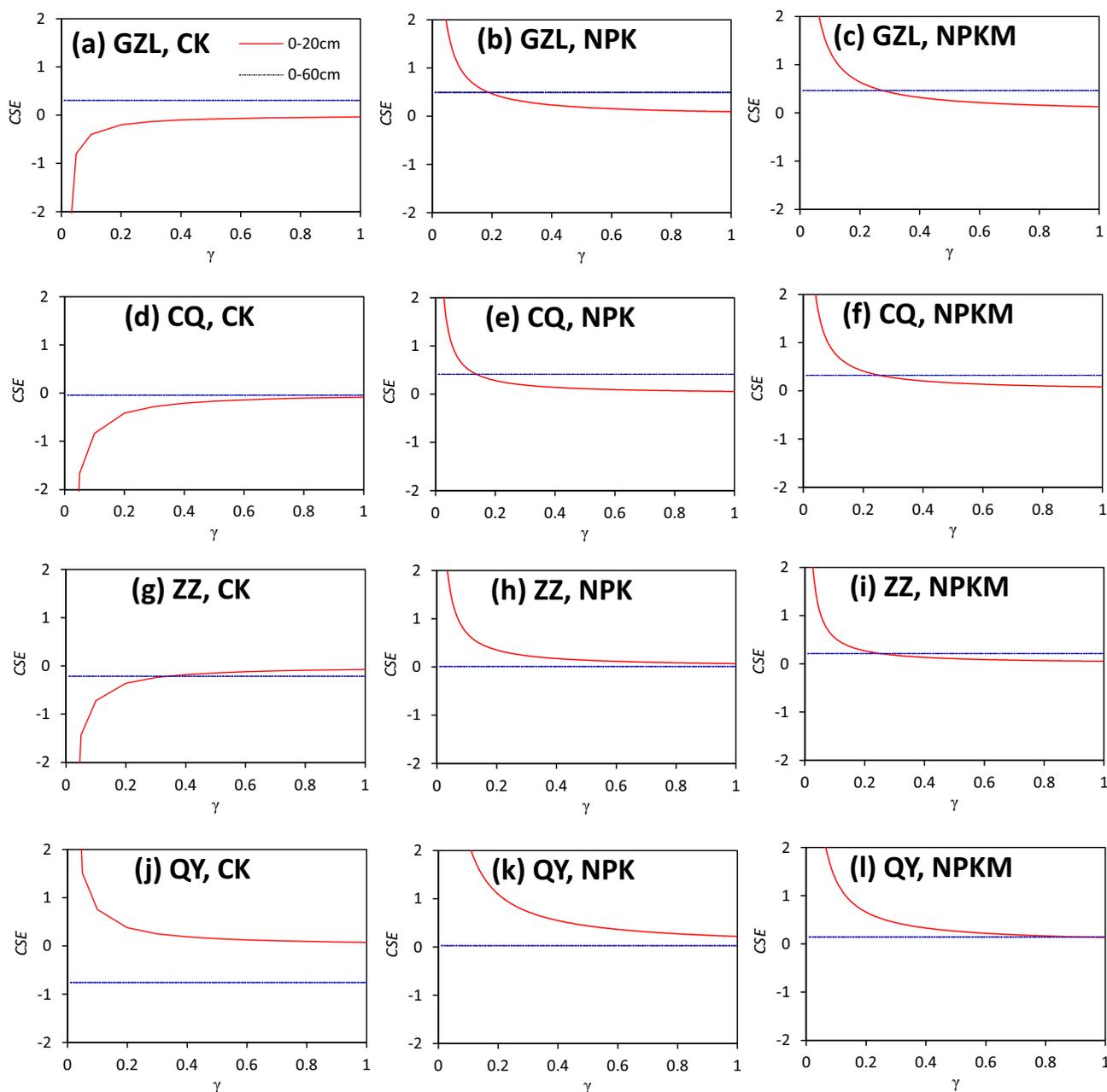


Fig. 3. (a–l) CSE derived in 0–20 cm (red line) and 0–60 cm (blue dashed line) varying with the proportion of OC input allocated in 0–20 cm over total OC input in 0–60 cm (i.e. γ) under CK, NPK and NPKM treatments at GZL, ZZ, CQ and QY. CSE calculations were presented in *Methods and Materials*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fertility site (Di et al., 2017). This is consistent with a low saturation deficit, consequently relatively more rapid C saturation in the fine soil particles (Li et al., 2013), and a more rapid SOC destabilization than formation at these poor fertility sites (Lal, 2015, 2016). Whereas, soils in the high fertility sites will continue to act as C sinks because their current C concentrations stay far below C saturations due to their large C saturation deficits as well as their high fraction of fine soil particles (Six et al., 2002; Stewart et al., 2007; Feng et al., 2014).

4.5. Implication for agricultural soil carbon sequestration capacity

Agricultural soils could act as an important C sink to offset fossil fuel emission (Follett, 2001; Lal, 2004; Smith, 2004) but its overall effect was also debated recently (Pan et al., 2004; Lal, 2015, 2016). It was estimated that Chinese agricultural soils can sequester 2.7 Pg C (Qin

et al., 2013). However, these estimates were based on SOC stocks at surface horizons or plow layers (i.e. 0–30 cm) (Pan et al., 2010; Qin et al., 2013). This study demonstrated that after more than two-decade fertilizations, SOC stocks in subsurface soil layers (i.e. 20–60 cm) were altered significantly, either increased or decreased, in a magnitude similar or even larger than that at surface soil horizons. Therefore, our study suggested that former estimates of soil C sequestration capacity may likely have been underestimated in the agricultural soils with indigenously high fertility, but overestimated in the agricultural soils with indigenously poor fertility. Accounting for soil carbon changes in subsurface layers will enable more accurate estimates of soil C sequestration capacity in cropland soils under long-term fertilizations in China and worldwide.

Table 4

The percentile contributions (%) of individual and total edaphic factor, climate, and C input on variance of CSE derived in 0–20 cm and 0–40 cm based on VPA method in long-term fertilization experiments at ZZ. The proportions of OC input at 0–20 cm and 0–40 cm were based on the assumption that all OC input were distributed at 0–60 cm.

Category	Indictors	0–20 cm						0–40 cm					
		50%	60%	70%	80%	90%	100%	75%	80%	85%	90%	95%	100%
Edaphic factor	TN	0.1	0.1	0.1	0.1	0.1	0.1	0.6	0.6	0.6	0.6	0.6	0.6
	AN	0.1	0.1	0.1	0.1	0.2	0.1	1.2	1.1	1.2	1.2	1.2	1.1
	TP	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
	AP	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	TK	0.5	0.5	0.5	0.5	0.5	0.5	2.9	2.9	2.9	2.9	2.9	3.0
	AK	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	pH	5.3*	5.2*	5.1*	5.2*	5.2*	5.0*	2.3	2.2	2.2	2.3	2.3	2.2
	BD	9.8**	9.7**	9.7**	10.1**	9.6**	9.8**	3.2	3.3	3.2	3.3	3.2	3.2
	Total ^a	17.6 ^a	17.5 ^a	17.6 ^a	17.8 ^a	17.6 ^a	17.6 ^a	12.2	12.2	12.2	12.3	12.1	12.4
C input	SC	2.8	2.9	2.8	2.8	2.9	2.7	4.2 ^c	4.1 ^a	4.3 ^a	4.2 ^a	4.2 ^a	4.2 ^a
	MC	2.1	2.1	2.0	2.2	2.0	2.1	0.8	0.8	0.8	0.8	0.8	0.8
	TC	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
	Total	5.2	5.2	5.2	5.4	5.2	5.1	5.0	4.9	5.2	5.0	5.1	5.0
Climate	MAP	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.3	0.3	0.3
	MAT	1.3	1.3	1.3	1.3	1.3	1.3	0.5	0.4	0.4	0.4	0.5	0.5
	Total ^a	1.3	1.3	1.3	1.3	1.3	1.3	1.2	1.2	1.2	1.1	1.2	1.1
Total ^b	–	70.8**	70.7**	70.8**	70.9**	70.9**	70.7**	65.8**	65.4**	65.9**	65.7**	65.9**	65.6**

Abbreviations: SC: stubble carbon input; MC: manure C input; TC: total OC input as the sum of MSC, WMS plus MC. Other abbreviations were presented in Table 2.

^a Represents total variance explained by all individual and interactive edaphic factors.

^b Represents total variance explained by all single and interactive edaphic, C input and climate factor.

^c Denotes marginal significance at 0.05 < P < 0.1.

* P < 0.05.

** P < 0.01.

5. Conclusions

Surface and subsurface soil C stocks are potentially important C reservoir but their changes under long-term fertilizations were unclear in a wider spatiotemporal scale. By synthesizing SOC data at three different depths (i.e. 0–20, 20–40 and 40–60 cm) in four typical long-term cropland soil experiments, this study showed that SOC stocks

generally decreased under no fertilization treatment (CK) in both surface and subsurface layers, and increased under two-decade long fertilizations (i.e. NPK and NPKM) in surface layers. One exception is that SOC stock increased in both surface and subsurface layers under CK in black soil (i.e., indigenously high fertility site). In particular, this study elucidated subsurface SOC stocks significantly increased under NPK and NPKM at the indigenously high fertility sites, but significantly

Table 5

The $\delta^{13}C$ (‰), percentile of mass C (pMC, %) and ^{14}C age (yr, BP) in aboveground and belowground plant samples and soil samples (0–20 cm and 20–40 cm) under three long-term fertilization treatments at GZL and QY.

Site	Treatment	Sample	$\delta^{13}C$ (‰)		pMC (%)		^{14}C Age (yr, BP)	
			$\delta^{13}C$	Error (1 σ)	pMC	Error (1 σ)	^{14}C Age	Error (1 σ)
GZL	CK	AG tissue	–13.78	0.16	97.64	0.30	190	25
		BG tissue	–12.03	0.15	98.75	0.23	100	20
		Soil: 0–20 cm	–23.70	0.13	40.22	0.14	7320	30
		Soil: 20–40 cm	–24.35	0.13	42.47	0.16	6880	30
	NPK	AG tissue	–16.21	0.18	97.59	0.24	200	20
		BG tissue	–13.42	0.24	98.62	0.21	110	20
		Soil: 0–20 cm	–24.76	0.16	34.88	0.14	8460	30
		Soil: 20–40 cm	–28.28	0.45	27.57	0.11	10,350	30
	NPKM	AG tissue	–11.70	0.15	99.36	0.21	50	20
		BG tissue	–10.99	0.27	99.68	0.22	25	20
		Soil: 0–20 cm	–22.73	0.41	72.27	0.18	2610	20
		Soil: 20–40 cm	–22.75	0.19	66.55	0.17	3270	20
QY	CK	AG tissue	–9.76	0.17	99.21	0.27	63	22
		BG tissue	–9.99	0.24	99.61	0.26	31	21
		Soil: 0–20 cm	–24.17	0.24	65.31	0.21	3422	26
		Soil: 20–40 cm	–23.84	0.17	40.59	0.16	7243	32
	NPK	AG tissue	–12.46	0.18	99.85	0.26	12	21
		BG tissue	–13.19	0.31	100.17	0.29	modern	NA
		Soil: 0–20 cm	–21.48	0.17	70.24	0.28	2837	32
		Soil: 20–40 cm	–25.39	0.21	38.80	0.16	7606	33
	NPKM	AG tissue	–11.53	0.16	99.26	0.25	60	21
		BG tissue	–11.57	0.46	99.10	0.28	73	23
		Soil: 0–20 cm	–24.61	0.16	66.73	0.21	3250	25
		Soil: 20–40 cm	–24.36	0.15	45.24	0.18	6371	32

Note: AG: aboveground; BG: belowground; “modern” is defined as 95% of the radiocarbon concentration (in 1950 CE) of NBS Oxalic Acid I (SRM 4990B, OX-I) normalized to $\delta^{13}C_{VPDB} = -19$ per mil (Stuiver and Polach, 1977); NA: not available.

Table 6

(a) The soil parent material and mineral properties at 0–20 cm and (b) soil texture at 0–60 cm at the initiation of long-term fertilization experiments at four typical croplands along a latitudinal transect in China.

(a)						
Soil fertility	Site name	Soil parent material	Soil mineral		Clay type	SSA ^b , m ² g ⁻¹
High fertility soil	GZL	Quaternary loess	Montmorillonite, Illite		2:1	37.4
	CQ	Slope and residual matter of Weathering of Purple Mudstone in Jurassic Shaximiao Formation	Illite-Whewellite ^a Montmorillonite ^a , Kaolinite ^a		2:1,1:1	–
Low fertility soil	QY	Quaternary red earth	Kaolinite, Iron-aluminum oxide		1:1	34.5
	ZZ	Alluvial of Yellow River	Montmorillonite, Hydromica		2:1	13.7

(b)						
Soil fertility	Site name	Depths	Sand 2–0.02 mm(%)	Silt 0.02–0.002 mm(%)	Clay < 0.002 mm(%)	Soil texture
High fertility soil	GZL	0–20 cm	38.3	29.9	31.1	Loam clay ^c
		20–40 cm	36.0	37.2	27.2	Loam clay ^c
		40–60 cm	40.5	45.3	13.0	Silty loam ^c
	CQ	0–20 cm	47.2(1–0.01 mm)		52.8(< 0.01 mm)	Heavy loam ^d
		20–40 cm	47.4(1–0.01 mm)		52.6(< 0.01 mm)	Heavy loam ^d
		40–60 cm	41.3(1–0.01 mm)		58.7(< 0.01 mm)	Heavy loam ^d
Low fertility soil	ZZ	0–20 cm	73.9(1–0.01 mm)		26.1(< 0.01 mm)	Light loam ^d
		20–40 cm	75.0(1–0.01 mm)		25.0(< 0.01 mm)	Light loam ^d
		40–60 cm	68.8(1–0.01 mm)		31.2(< 0.01 mm)	Medium loam ^d
	QY	0–20 cm	3.7		61.4	Clay ^c
		20–40 cm	3.6	34.9	70.2	Heavy clay ^c
		40–60 cm	5.8	25.5	68.7	Heavy clay ^c

^a The soil mineral at CQ were from Soil Society of China Soil Classification and Soil Geography Professional Committee (1989), and the soil mineral is mainly Illite-Whewellite in the 0–20 cm, and Montmorillonite and Kaolinite below the 20 cm.

^b The data of soil mineral specific surface area(SSA) of the silt + clay fraction were from Feng et al. (2014).

^c Based on United Nations FAO soil taxonomy.

^d Based on soviet system soil taxonomy.

decreased under the same treatments at the indigenously poor fertility sites. Consequently, CSE derived in the soil profile (0–60 cm) was consistently higher than that in surface layer (0–20 cm) in high fertility soils but lower in low fertility soils. These patterns suggest distinct turnover, formation and accumulation of SOC likely driven by multiple edaphic characteristics. This study informs the need to account for subsurface soil carbon changes to achieve more accurate estimates of soil C sequestration capacity in Chinese cropland soils and globally.

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Appendix A. Supplementary data

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