The Journal of Animal & Plant Sciences, 29(5): 2019, Page: 1383-1389E
ISSN: 1018-7081

LONG-TERM FERTILIZATION ALTERED LABILE SOIL ORGANIC CARBON FRACTIONS IN UPLAND SOILS OF CHINA

R. Nazia[1], F. Liang[1, 2], S. Huang[3], B.Wang[1], M. Xu[1], J. Li[1,4], H. Gao[5] and W. Zhang[1]

[1]National Engineering Laboratory for Improving Quality of Arable Land, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, 100081, P. R. China
[2]Key laboratory of Poyang Lake Watershed *Agricultural* Resources and Ecology of Jiangxi Province, Academy of Land Resource and Environment, Jiangxi Agricultural University, Nanchang 330045, China
[3]Institute of Plant Nutrition and Agricultural Resources, Henan Academy of Agricultural Sciences, Zhengzhou, Henan 450002, China.
[4]Department of Agricultural and Environmental Sciences, Tennessee State University, Nashville TN 37209 United States.
[5]Agricultural Resources and Environment Research Center, Jilin Academy of Agricultural Sciences, Changchun 130124, P. R. China
Corresponding author, e-mail: zhangwenju01@caas.cn

ABSTRACT

Labile soil organic carbon (SOC) plays key roles in the assessment of improving soil fertility and structure for agricultural land sustainability, but changes in labile SOC fractions at different soil profiles with long-term fertilization are limited. In this study, we investigated the effect of long-term fertilization on the labile SOC fractions with various potassium permanganate oxidizable C ($KMnO_4$–C) concentrations at three different long-term experimental sites. Soil depths were 0-40cm at GZL and 0-100cm at ZZ and QY. The four fertilization treatments included cropping without fertilization (CK), inorganic fertilizer nitrogen, phosphorus and potassium (NPK), inorganic fertilizer with manure (NPKM) and inorganic fertilizer with straw (NPKS). Results showed that SOC storage in 0-20cm soil layer increased under all treatments in the order of NPKM > NPKS >NPK relative to CK at all sites except GZL (NPKM>NPKS, NPK). The SOC storage in whole soil profile was greater under all treatments in the order of NPKM >NPKS> NPK relative to CK at GZL, ZZ and QY. Moreover, long-term fertilization increased topsoil (0-20 cm) labile SOC storage pool which declined sharply with the increasing soil depth at each experimental site. The average SOC storage of less, mid and highly labile fraction in manure with balanced fertilizer (NPKM) increased by113.3, 183.7 and 274.5% at 0-40cm depth of GZL and by 153.4, 76.0, 67.6, 105.5, 129.8 and 118.7% at depth of 0-100 cm of ZZ and QY, respectively, as compared to CK. Consequently, this study suggested that the application of combined NPK with manure or straw can improve soil carbon storage and SOC liable fraction along soil profile across various upland region of China.

Keywords: Long-term fertilization， labile soil organic carbon, soil depths.

INTRODUCTION

Soil organic carbon (SOC) is essential in soil feature assessment due to its vital role in altering soil fertility and quality (Baldock *et al*., 2004; Haynes 2005; Liu *et al*., 2006). Great amount of SOC have favorable effects on crop production (Rasmussen and Parton 1994; Smith *et al*., 2000; Pan *et al*., 2003; Yang *et al*., 2012). Hence, maintaining a suitable SOC content is a key factor for the productivity and sustainability of terrestrial ecosystems (Reeve, 1997). SOC pool shows a significant role in the global C cycle and has a beneficial impact on agricultural sustainability and environmental quality (Jenkinson *et al*., 1991; Stevenson, 1994). According to their decomposition rate and their replacement, these pools were generally divided into three groups, i.e labile, stable and recalcitrant pool. Labile or energetic pool has less turnover time with harsh disintegration (e.g. <5 year);stable or humus pool takes longer time with slower disintegration (e.g. 20-40 years) and recalcitrant pool has a much longer turnover time (e.g. hundreds to thousands of years) (Haynes, 2005). Labile SOC tends to decompose very quickly in the soil and then survive for a period of time. Due to its fast cycling, the active part of SOC is more sensitive to changes in the assessment of management practices (Vieira *et al*., 2007; Campos *et al*., 2011). Potassium permanganate oxidizable C ($KMnO_4$-C), considered to be the labile fractions extracted by chemical oxidation method (Blair *et al*., 1995), actsas a sensitive key tolong-term fertilization or the influence of organic resources on the dynamics of the labile SOC fraction (Mtambanengwe and Mapfumo, 2008; Xu *et al*., 2011).

Inorganic and manure fertilization to agricultural soils is extensively applied as a common management practice to improve soil productivity and soil organic C storage. In general, manure fertilization application with or without inorganic fertilizers increased SOC storage

(Blair *et al*., 2006; Bhattacharyya *et al*., 2010; Ding *et al*., 2012; Maillard *et al*., 2015), although inorganic fertilization alone yielded inconsistent products (Purakayastha *et al*., 2008; Lemke *et al*. 2010; He *et al*., 2015).Despite a clear documentation of SOC increase and losses through fertilization, only conflicting data are available for the effects of long-term fertilization strategies on SOC that could affect SOC chemical composition (Leinweber and Schulten, 1995).

Nowadays, several studies have been conducted on labile SOC pools affected by management practices (Weil and Magdoff, 2004; Smith, 2004; Hutchinson *et al*., 2007), including tillage practices, cropping intensity and rotations management (Six *et al*., 2002). However, little information is available on the impact of labile organic C after long-term fertilization under typical Chinese croplands along with soil depth.

Long-term fertilization may lead to significantly different qualitative and quantitative changes in SOC. Numerous studies have demonstrated the performance of the labile fraction under different fertilizations (Loss *et al*., 2014). Though, most researchers are mainly focusing on the dynamics of total SOC, its relationship with the total C, and labile fractions of SOC in top soil. Few studies were conducted to examine the labile fraction of SOC in different soils, much or less along further soil profile or under long-term application of manure and chemical fertilizers. Thus, in the current study, we tend to evaluate the effects of long-term fertilization on labile organic carbon fraction. We hypothesize that fertilization would increase SOC storage and its labile fractions across soil profile in each site of upland in China.

MATERIALS AND METHODS

Description of experimental sites: Three experimental sites, starting in 1990 in China and locating from north to south with varying latitudes, were selected, i.e. Gongzhuling (GZL) in Jilin province, Zhengzhou (ZZ) in Henan province and Qiyang (QY) in Hunan province. Situated in different climatic zones, they are different in average annual temperature (up to >20 °C difference), mean annual precipitation (MAP) (about 3 times difference). Cropping system also varied i.e mono-cropping cultivation of maize in GZL site and double cropping of cultivation (wheat-maize) in ZZ and QY sites (Table 1).

Soil sampling and fertilization: Soil samples were collected in September-October 2015 after harvesting at all sites from top (0-20 cm) to deep (80-100 cm) soil i.e under depth of 0-40cm (0-20, 20-40cm) from GZL and 0-100cm (0-20, 20-40, 40-60, 60-80 and 80-100 cm) from ZZ and QY. Four treatments were selected: control (CK) without fertilization, inorganic balanced nitrogen, phosphorus and potassium fertilizers (NPK), manure along inorganic fertilizer (NPKM) and straw along inorganic fertilizer (NPKS). Nitrogen and potassium fertilizers were sourced from urea and potassium chloride at GZL, ZZ and QY. However, Phosphorus fertilizer came from calcium superphosphate at ZZ, QY and diammonium phosphate at GZL. The annual application rates of NPK at GZL, ZZ and QY site were 112-36-69, 165-36-69 and 190-16-30 kgha^{-1}, respectively (Table 1). Moreover, 30~40% of total nitrogen was obtained from inorganic fertilizer, other was derived from manure of pig or cow wastes for NPKM treatment. These same manures were applied as base fertilizer before seeding at GZL and QY and as basal one in each autumn earlier to wheat planting at ZZ. 1/3 of nitrogen fertilizer was applied as basal fertilizer before seeding and the remaining as top dressing at the jointing stage (Liang *et al*., 2016). Phosphorus and potassium fertilizers were applied together as base fertilizers before sowing. 398 and 414g kg^{-1}organic carbon of pig manure were applied for GZL and QY, respectively, 368 g kg^{-1}organic carbon of cow manure at ZZ. Above ground biomass was removed from the field except in the plot under NPKS treatment, where entire straw was returned to the plots (Table 2).

Physiochemical analysis: Soil physiochemical properties (0-20 cm) were measured before starting experiment at three sites. Soil samples were air-dried, removed root materials and stones, and then sieved through a 2mm sieve. After that, dispersed soil samples were collected to measure the soil bulk which was the highest in ZZ (1.24 g cm^{-3}) then in GZL (1.19 g cm^{-3}) and QY (1.10 g cm^{-3}) (Blake and Hartge, 1986).Soil organic C and soil total nitrogen (TN) content of surface soil (0-20 cm) were higher at GZL site than those at QY and ZZ sites. Soil C: N ratio varied from 8.0-10 at QY and ZZ sites, respectively. Soil pH was acidic at QY (5.7), nearly neutral at GZL (7.2) and ZZ (8.3). Clay content (41%) of QY soil was greater than that of the other sites (13-32%) (Table3).Total SOC content was measured in the laboratory through wet acid dichromate oxidation method (Islam and Weil, 1998; Walkley and Black, 1934). SOC was oxidized by 333mM (less labile), 167mM (Mid labile) and 33mM (Highly labile). $KMnO_4$ concentration had been considered as a useful index of labile SOC (Blair, 1995).This reagent appeared to react with a relatively labile pool of soil C and changed $KMnO_4$ concentration. So it was used to indicate the amount of C oxidized, assuming that 1mM $KMnO_4$was consumed in the oxidation of 0.75mM or 9mg of C (Blair *et al*., 2001). SOC storage of profile for all depths i.e0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm and 80-100 cm was calculated by the formula given below:

SOC storage (Mg ha^{-1}) = SOC concentration (g kg^{-1}) × Bulk Density (Mg m^{-3}) × Soil Depth×10 (factor).

Statistical analysis: Microsoft excel 2010 (Microsoft Corporation, USA) was used to convey data processing and statistical analysis (ANOVA). Two-way ANOVA was used to test the effects of fertilization treatments (CK, NPK, NPKM and NPKS) within each depth (0-20, 20-40, 40-60, 60-80 and 80-100cm) of each site on BD, SOC storage and its labile SOC fractions (less labile, mid labile and highly labile) and one-way ANOVA was used to measure fertilization effectiveness on whole soil profile of each site ($P<0.05$). SPSS version 21.0 was used to analyze the relationship among SOC, labile fraction and soil properties.

RESULTS

Soil bulk density (BD): BD (g m^{-3}) ranged from 1.17~1.34, 1.29~1.51 and 1.16~1.76 for all treatments of each site, i.e GZL, ZZ and QY. Long-term application of different fertilization significantly increased soil BD with increasing soil depth but with very few quantity, i.e 1.17-1.34 in 0-20 cm and 1.27-1.30 in 20-40 cm of GZL, 1.29-1.41, 1.16-1.28 in 0-20 cm and 1.40-1.51, 1.62-1.74 in 80-100 cm of ZZ and QY (Table4).

Influence of long-term fertilization on SOC storage: SOC storage ranged from 50.0, 30.0 and 36.0Mg ha^{-1}in top layer to 23.0, 7.0 and 6.0Mg ha^{-1} in deep layer of GZL, ZZ and QY. These sites had significant effect of fertilization, interaction between fertilizers and accumulations on SOC storage which indicated varied treatment effects across the soil depth (Table 5). The greatest accumulation of total SOC storage was observed in integrated manure with balanced fertilizer (NPKM) treatment, compared to integrated straw with inorganic fertilizer (NPKS) and balanced fertilizer (NPK).Control plot showed the lowest value in 0-20cm of each site, whereas in comparison to sites with same treatment, highest SOC storage increase was found in topsoil (0-20cm) of GZL site (50.0Mg ha^{-1}) followed by QY (36.0 Mg ha^{-1}) and ZZ (30.0Mg ha^{-1}) sites (Fig. 1).

Fertilization effect decreased sharply with increasing soil depth in all three sites. NPKS and NPK did not significantly showed effect under 20-40cm soil layer except ZZ site. Moreover, SOC storage between all treatments were of no significant difference below 40cm of QY and ZZ sites (Fig. 1). Furthermore, significant effect was recorded among different treatments under whole soil profile of each site as compared to control (CK). NPKM, NPKS and NPK treatment increased SOC storage in soil profile of 0-40cm by 74.0, 36.0 and 14.0% in GZL, 88.0, 51.0 and 24.0% in ZZ and 84.0, 39.0 and 37.0% in QY sites respectively. Two different sites were selected under whole soil profile (0-100cm) and observed thatSOC storage increased the highest under NPKM treatment (85.0-84.0%), then NPKS (39.0-44.0%) and the least under NPK (6.0-33.0%) as compared to control treatment of ZZ and QY sites, respectively (Fig. 2).

Influence of long-term fertilization on labile SOC storage: Potassium permanganate-oxidizable carbon ($KMnO_4$-C) was considered to be labile C fractions, which was oxidized by different concentrations of 333, 167 and 33mM $KMnO_4$and referred to as less, mid and highly labile C fraction, respectively. Labile SOC storage fractions (less, mid and highly) were affected by fertilization, depth and their interaction in each site (Table 5).

Influence of long-term fertilization on less labile SOC storage: Less labile storage fraction was highly accumulated on surface soil (0-20cm) but it declined with increasing soil depth of each site and their proportion in SOC storage (Table 6, 7 and 8). Manure treatment with balanced fertilizer for 0-20 and 20-40 cm significantly increased labile SOC storage as compared to other treatments and remained the lowest in control treatment in each site respectively. The NPK and NPKS were of non-significant change below 40cm of ZZ site, however, in QY site that had significant change between NPK and NPKS treatment but no-significant change between CK and NPK treatment (Table 7 and 8). Furthermore, compared to control treatment, mineral fertilization combined with manure increased less labile SOC storage up to 104.9, 97.8 and 82.7% in surface soil of GZL, ZZ and QY sites, respectively. As to whole soil profile (0-40cm), less labile storage C was accumulated the highest in GZL followed by ZZ and QY under NPKM treatment. Furthermore, selected whole soil profile of two different sites (0-100cm) had recorded a higher accumulation of labile storage in QY than in ZZ under NPKM treatment (Fig. 3).

Influence of long-term fertilization on mid labile SOC storage: Compared with less labile, the mid labile had a low storage of labile C but still had the highest accumulation of labile C storage followed by other treatments, with the lowest accumulation in control on surface soil of each site. This trend decreased with increasing soil depth and their proportion in SOC storage (Table 6, 7 and 8). Moreover, NPK and NPKS were not significant in ZZ site at20-40cm soil layer and NPKM and NPKS in QY site were not significant at 40-60 and 60-80 cm of soil layer (Table 7 and 8). As compared to CK, treatment of manure with inorganic fertilizer increased 228.6, 58.5 and 112.3% in surface soil of GZL, ZZ and QY, respectively. Similarly, in whole soil profile (0-40cm), mid labile storage C was accumulated the highest in GZL and then in ZZ and QY under NPKM treatment. Furthermore, selected whole soil profile of two different sites (0-100cm) had recorded a higher accumulation of labile C storage in QY than in ZZ under NPKM treatment (Fig. 3).

Effect of long-term fertilization on highly labile SOC storage: Lower storage C in highly labile fraction was found in less and mid labile fractions. Whereas, NPKM treatment had the highest accumulation of labile C storage and CK had the lowest in relation to surface soil of each site and their proportion in SOC storage (Table 6, 7 and 8). However, NPK and NPKS treatment below 40cm were of no significant change in ZZ and QY site (Table 7 and 8). Manure with balanced fertilizer accumulated the highest SOC storage. Similarly, it increased 283.9, 45.1 and 91.3% in surface layer of GZL, ZZ and QY sites as compared to CK, respectively. As in whole soil profile (0-40cm), highly labile storage C was accumulated the highest in GZL and then in ZZ and QY under NPKM treatment. Furthermore, selected whole soil profile of two different sites (0-100cm) had recorded a higher accumulation of labile C storage in QY than in ZZ under NPKM treatment (Fig. 3).

Table 1. Site characteristics of long-term fertilization in initiation (1990) of experiments at three typical croplands in China.

Particular	Gongzhuling (GZL)	Zhengzhou (ZZ)	Qiyang (QY)
Coordinate	43°30’23”N; 124°48’34”E	35°50’00”N; 113°45’00”E	26°45’00”N; 111°52’00”E
Altitude (m)	220	59	120
MAT (℃)	4.5	14.5	18.5
EAT (℃)	1700	2661	3429
MAP (mm)	589	615	1255
MAE (mm)	1400	1450	1470
Climatic zone	Mid-Temperate, Semi-Humid	Warm-Temperate, Semi-Humid	Sub-Tropical, Humid
Cropping system	Mono-cropping	Double cropping	Double cropping

Note: MAT, Mean annual temperature; EAT, Effective annual temperature; MAP, Mean annual precipitation and MAE, mean annual evaporation.

Table 2. Input rates of chemical fertilizers (kg ha^{-1}) and manure (t ha^{-1}) in long-term fertilization experiments at three typical croplands in China

Site	Fertilizer	CK		NPK		NPKM		NPKS	
		Wheat	Maize	Wheat	Maize	Wheat	Maize	Wheat	Maize
GZL	N-P-K	NA	0-0-0[a]	NA	165-36-69	NA	50-36-69	NA	112-36-69
	manure	0	0	NA	0	NA	23	NA	0
	Straw	NA	0	NA	0	NA	0	NA	7.5
ZZ	N-P-K	0-0-0	0-0-0	165-36-68	188-41-78	50-36-68	188-41-78	50-36-68	0
	manure	0	0	0	0	15[b]	0	0	0
	Straw	0	0	0	0	0	0	116-64-83	188-41-78
QY	N-P-K	0-0-0	0-0-0	90-16-30	210-37-70	27-16-30	63-37-70	300-53-104	
	manure	0	0	0	0	10~15[c]	25~35	0	0
	Straw	0	0	0	0	0	0	6.6[d]	0

Note: NA, no crop. a Denotes fertilization rates are zero for all three fertilizers; b is calculated based on C/N(i.e. 25) and carbon content (i.e.19.8%) in cow manure; c Denotes a range

Table 3. Soil characteristics (0~20 cm) at the initiation of long-term fertilization experiments at three typical croplands in China.

Soil Properties	Gongzhuling (GZL)	Zhengzhou (ZZ)	Qiyang (QY)
SOC (g kg^{-1})	13	6.7	8.6
TN (g kg^{-1})	1.42	0.67	1.07
C:N	9.2	10	8
AN (mg kg^{-1})	131	51	79
TP (g kg^{-1})	1.5	0.6	0.5
Olsen p (mg kg^{-1})	23	6.5	11
TK (g kg^{-1})	24.6	16.9	13.3
AK (mg kg^{-1})	160	74	122
pH	7.2	8.3	5.7

BD (g cm^{-3})	1.19	1.24	1.1
Clay %	32	13	41
Soil type[a]	Black	Fluvo-aquic	Red
Soil type[b]	LuvicPhaeozems	Calcariccambi soil	Eutriccambi soil

Note: SOC: Soil organic carbon, TN: Total nitrogen, AN: Available nitrogen, TP: Total phosphorus, TK: Total potassium, AK: Available potassium. BD: Bulk density, Clay %: clay content, SP: soil porosity, Soil type[a]: Based on China soil taxonomy and Soil type[b] : Based on United Nations FAO soil taxonomy.

Table 4. Effect of long-term fertilization on soil bulk density (g cm^{-3}) under different site at three typical croplands in China.

Site	Treatment	0-20cm	20-40cm	40-60cm	60-80cm	80-100cm
G ZL	CK	1.17cB	1.27abA			
	NPK	1.34aB	1.30aA			
	NPKM	1.21bB	1.28abA			
	NPKS	1.20bA	1.22bA			
ZZ	CK	1.41aD	1.43aC	1.48aB	1.49aB	1.51aA
	NPK	1.40aE	1.41abD	1.44bC	1.46bB	1.49bA
	NPKM	1.34bD	1.36bC	1.39cB	1.41cA	1.42cA
	NPKS	1.29c	1.32c	1.36d	1.39d	1.40d
QY	CK	1.28aD	1.61aC	1.67aB	1.76aA	
	NPK	1.26abE	1.59abD	1.64bC	1.70bB	1.74aA
	NPKM	1.16bcE	1.48bD	1.54dC	1.60dB	1.62cA
	NPKS	1.18bE	1.49bD	1.58cC	1.64cB	1.69bA

Note: The lower-case represents the difference between the treatments at the same layer on the same site ($P<0.05$). The upper case represents the difference between the layers at the same treatments on the same site($P<0.05$).

Table 5. Variance analysis of two-way ANOVA for the effect of long-term fertilization (T), soil depth (D) and their interaction (D^*T) on SOC storage and labile storage (Mg ha^{-1}) of different sites of China.

Site	Effect	***P* - value**			
		SOC	**Less labile**	**Mid labile**	**Highly labile**
GZL	D	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	T	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	D × T	0.015	0.227	**<0.025**	**<0.034**
ZZ	D	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	T	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	D × T	**<0.001**	**<0.007**	0.113	0.204
QY	D	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	T	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	D × T	**<0.001**	**<0.001**	**<0.001**	0.843

Note: Bold value represent significance

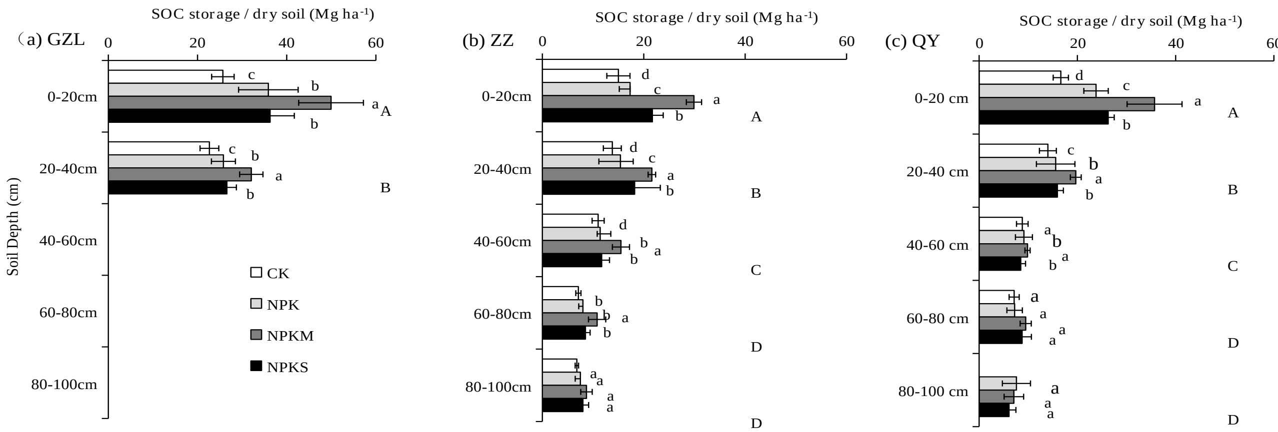

Fig. 1. Mean (±SD) (n=9) SOC storage (Mg ha^{-1}) at different depth of GZL (0-20cm and 20-40cm), ZZ and QY (0-20cm, 20-40cm, 40- 60cm, 60-80cm and 80-100cm) sites under CK, NPK, NPKM and NPKS treatments in China. Lowercase letter is the significant difference between four treatments at (p<0.05) and the uppercase is the significance difference between soil layers.

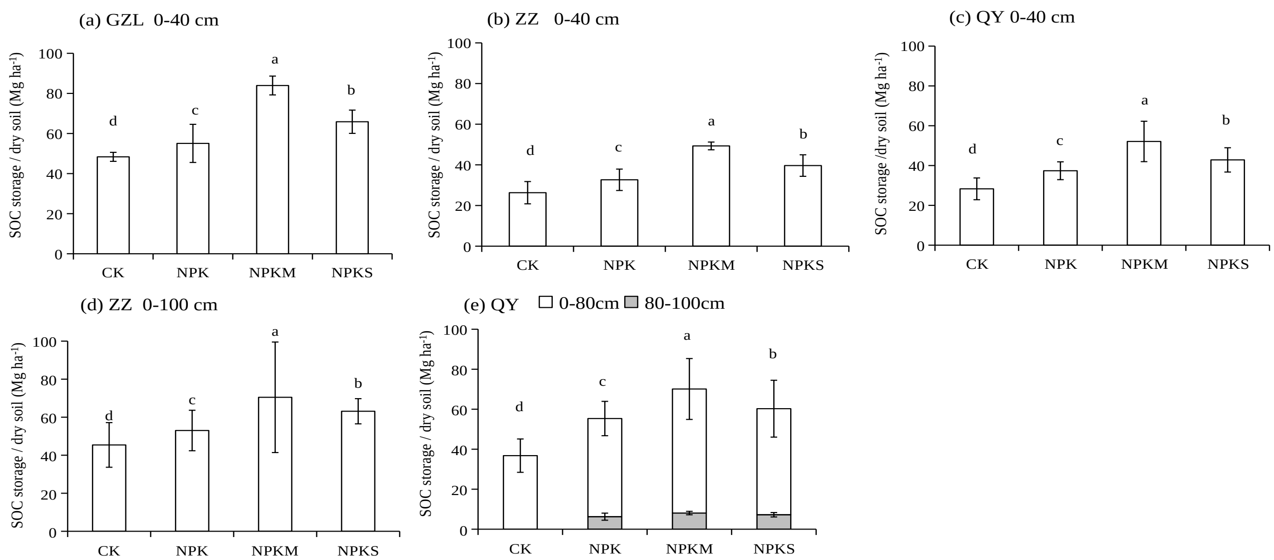

Fig. 2. Mean (±SD) (n=9) one way ANOVA of long-term fertilization effect on SOC storage (Mg ha^{-1}) at 0-40 cm soil layer of GZL,ZZ and QY site 0-100 cm soil layer of ZZ, 0-80cm and 80-100cm soil layer of QY sites under CK, NPK, NPK+M and NPK+S treatments in China. Lowercase letters are the significant difference between four treatments at p-value <0.05.

Table 6. Effect of long-term fertilization treatment on storage of various labile fraction carbons in Gongzhuling site.

Soil Depth (cm)	Treatment	Less labile/SOC (Mg ha^{-1})	Mid labile/SOC (Mg ha^{-1})	Highly labile/SOC (Mg ha^{-1})
0-20cm	CK	9.5d(37)	4.7d(5)	3.1d(12)
	NPK	13.1c(45)	8.3c(8)	7.1c(20)
	NPKM	19.6a(39)	15.4a(15)	11.8a(24)
	NPKS	16.5b(47)	10.6b(10)	8.2b(23)
20-40cm	CK	7.4d(32)	4.6d(5)	2.5d(11)
	NPK	10.5c(40)	6.5c(6)	4.9c(18)
	NPKM	16.5a(51)	10.9a(10)	8.6a(27)
	NPKS	14.6b(55)	9.3b(9)	6.3b(23)

Note: Values given in Mg ha^{-1} are labile SOC storage, lower case letter in the same column is the significant difference between treatments and values present in the brackets are percentage of labile storage in same treatment of SOC

Table 7. Effect of long-term fertilization treatment on storage of various labile fraction carbons in Zhengzhou site.

Soil Depth(cm)	Treatment	Less labile/SOC (Mg ha^{-1})	Mid labile/SOC (Mg ha^{-1})	Highly labile/SOC (Mg ha^{-1})
0-20 cm	CK	7.1d(47)	4.7d(31)	3.0d(21)
	NPK	10.0c(59)	5.2c(29)	3.4c(19)
	NPKM	14.0a(47)	7.5a(25)	4.6a(15)
	NPKS	11.9b(54)	6.6b(30)	4.1b(18)
20-40 cm	CK	4.3d(30)	3.1c(22)	2.1c(14)
	NPK	6.8c(56)	4.0b(28)	2.7b(17)
	NPKM	10.0a(72)	6.0a(43)	4.1a(30)
	NPKS	7.6b(54)	3.9b(28)	2.9b(21)
40-60 cm	CK	3.8c(34)	2.5c(24)	1.5c(15)
	NPK	5.5b(49)	3.1b(28)	1.9b(17)
	NPKM	7.3a(66)	4.6a(41)	2.9a(26)
	NPKS	4.6b(41)	2.9b(26)	2.1b(19)
60-80 cm	CK	2.7c(37)	1.9c(26)	0.9b(13)
	NPK	3.5b(43)	2.0b(28)	0.9b(13)
	NPKM	5.7a(80)	3.5a(49)	1.8a(25)
	NPKS	4.0b(56)	2.3b(35)	1.2b(19)
80-100 cm	CK	2.4c(35)	1.7b(24)	0.8b(11)
	NPK	3.2b(46)	1.9b(28)	0.9b(13)
	NPKM	4.8a(70)	2.7a(39)	1.4a(17)
	NPKS	3.4b(49)	2.0b(29)	1.1b(16)

Note: Values given in Mg ha^{-1} are labile SOC storage, lower case letter in the same column is the significant difference between treatments and values present in the brackets are percentage of labile storage in same treatment of SOC

Table 8. Effect of long-term fertilization treatment on storage of various labile fraction carbons in Qiyang site.

Soil Depth(cm)	Treatment	Less labile/SOC (Mg ha^{-1})	Mid labile/SOC (Mg ha^{-1})	Highly labile/SOC (Mg ha^{-1})
0-20cm	CK	8.7d(51)	5.9d(35)	2.3d(14)
	NPK	10.7c(45)	7.8c(33)	2.7c(11)
	NPKM	15.9a(45)	12.5a(35)	4.4a(12)
	NPKS	12.6b(55)	9.2b(42)	3.3b(12)
20-40cm	CK	5.9d(42)	4.6d(33)	1.0d(7)
	NPK	6.4c(43)	5.0c(34)	1.2c(9)
	NPKM	12.0a(61)	10.2a(51)	2.6a(13)
	NPKS	7.8b(49)	6.4b(40)	1.6b(8)
40-60cm	CK	2.3c(41)	1.4c(26)	0.7c(12)
	NPK	3.9c(47)	2.7b(32)	1.1b(13)
	NPKM	5.4a(54)	3.5a(35)	2.1a(16)
	NPKS	4.8b(53)	3.6a(39)	1.2b(24)
60-80cm	CK	2.0c(44)	1.1b(24)	0.5c(10)
	NPK	3.9c(57)	2.8a(37)	0.7b(15)
	NPKM	4.8a(51)	3.2a(34	1.4a(15)
	NPKS	4.6b(57)	3.4a (41)	0.8b(20)
80-100cm	CK	---	---	----
	NPK	3.0c(59)	2.1a(40)	0.7b(14)
	NPKM	4.4a(64)	2.8a(40)	1.3a(17)
	NPKS	3.8b (57)	2.3a(35)	0.8b(12)

Note: Values given in Mg ha^{-1} are labile SOC storage, lower case letter in the same column is the significant difference between treatments and values present in the brackets are percentage of labile storage in same treatment of SOC

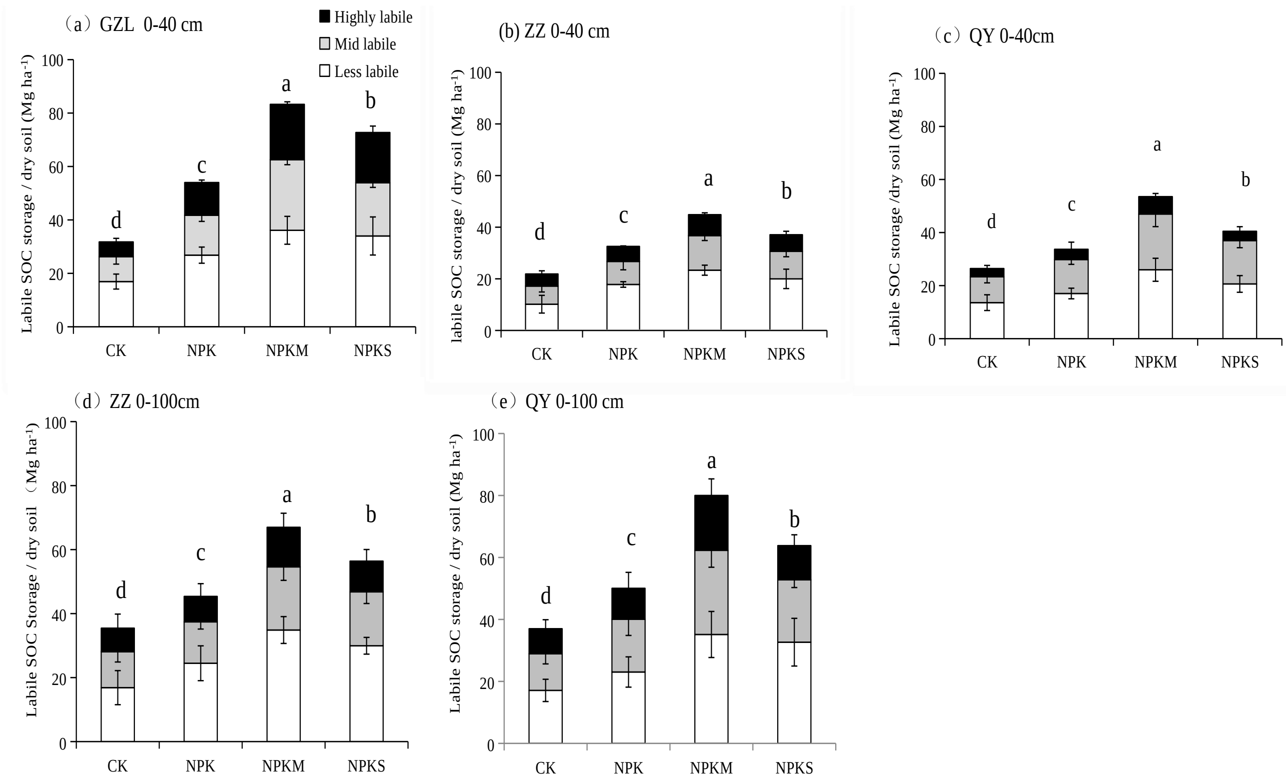

Fig. 3. Mean (±SD) (n=6) one way ANOVA of long-term fertilization effect on labile (less, mid and highly labile)SOC storage (Mg ha^{-1}) at 0-40 cm soil layer of GZL,ZZ and QY site at 0-100 cm soil layer of ZZ and QY sites under CK, NPK, NPKM and NPKS treatments in China. Lowercase letters are the significant differences between four treatments at p-value <0.05.

Table 9. Person correlation among selected parameter at 0-20 cm and 20-40cm of GZL site.

	SOC	Less labile	Mid labile	Highly labile	TP	TN	pH
0-20 cm							
SOC	1					.	
Less labile	.716**	1					
Mid labile	.739**	.822**	1				
Highly labile	.778**	.830**	.894**	1			
TP	.685*	.706*	.901**	.934**	1		
TN	.691*	.635*	.885**	.858**	.957**	1	
pH	0.2	0.29	0.09	0.238	0.006	-0.091	1
20-40 cm							
SOC	1						
Less labile	.791**	1					
Mid labile	.874**	.892**	1				
Highly labile	.870**	.870**	.937**	1			
TP	.818**	.973**	.885**	.911**	1		
TN	.652*	.848**	.869**	.855**	.894**	1	
pH	-0.069	-0.279	0.046	-0.043	-0.369	-0.249	1

** Correlation is significant at the 0.01 level
* significant at the 0.05 level

DISCUSSION

Influence of long-term fertilization on SOC storage: Management practices and soil type affected SOC accumulation (Moreno *et al.*, 2006; Laik *et al*., 2009; Zhao *et al.*, 2014) among which fertilization application (Yan *et al*., 2007; Xu*et al*., 2011; Yang *et al*., 2012; Wang *et al*., 2015) was a vital practice to improve soil productivity and augment crop production (Edmeades, 2003; Manna *et al*., 2007). In this research, deep soil profile with less SOC in all treatments of each site (Fig.1) indicated that in contrast to the lack of residues input into the deep soil, surface soil, with its higher amount of residues input, might contribute to significant increase of SOC storage under all fertilizer treatments at each site. Long-term application of compost (Banger *et al*., 2010; He *et al*., 2015), straw (Liu *et al*., 2014) and inorganic fertilizer (Maillard *et al*., 2015) enhanced a significant SOC storage due to their direct application into the soil. In addition, fertilization encompassing straw and dung increased SOC sequestration more than inorganic fertilization, suggesting that it would be less effective if without the application of straw and manure (He *et al*., 2018). Similarly, it has been reported that the application of animal manure with inorganic fertilizers had augmented the SOC storage (Gong *et al*., 2009; Li *et al*., 2010; Zhang *et al*., 2010, 2012). Moreover, long-term trials conducted at Rothamsted, UK, had predicted a gradual rise in OC at the rate of 35 Mg $ha^{-1}yr^{-1}$due to manure with inorganic treatment under 150 years field experiments (Powlson *et al*., 1998). Another previous study indicated that the highest SOC contents in NPKM amended soils in three long-term fertilizer trials in eastern cereal belt of India (Ghosh *et al*., 2010). Furthermore, manure application also created the physically/ biologically favorable soil environment for crop growth (Purakayastha *et al*., 2008). The enhancement of nutrient resources through the accumulation of fertilizers and animal manure caused the increase in crop residues in the form of roots and stubble, which in turn increased the storage of SOC (Malhi and Gill, 2002). Additionally, Ding *et al*. (2012) reported that the C storage was increased by 12-18% by the combined application of inorganic and organic fertilizers. Furthermore, in this study treatment effect was not observed below 40cm significantly. These results suggested that fertilization practice had limited beneficial effect below surface soil. Hernandez-Ramirez *et al*. (2007) and Chen *et al*. (2009) similarly reported that SOC concentration through management was mostly enhanced in the 15 or 30cm of surface layer, and their accumulation in deep soil was unaffected. The results of this study demonstrated that 25 years of fertilization significantly improved SOC, which was consistent with our hypothesis.

Moreover, long-term fertilization has been extensively reported in previous studies by Moharana *et al*. (2012) and Wang *et al*. (2014) who concluded that fertilizer application raised $KMnO_4$-C in the soil profile. Consequently, in our study we found similar results for SOC storage through the application of balanced fertilization (NPK) and supplemented manure. Whereas, straw increased the oxidizable OC content as compared to control treatment with no fertilization in the upper soil profile (0-20 and 20-40cm) which reduced oxidaizable OC content with increasing soil depth. This might be associated with the increased yield of crop residues and root biomass C returning to the soil and the straight application of organic substance from straw as well as from manure (Rudrappa *et al*., 2006; Mandal *et al*., 2008). Furthermore, it stated that there was no significant change in $KMnO_4$-C storage among straw and dung manure after twenty years field trial. Similar to soil OC, oxidizable OC content was also gradually decreased with the increase of soil depth (Yang *et al*., 2012). The highest storage of oxidizable OC in the top layer as compared to deep layers was possibly linked to the enhanced activity of roots in the surface soil. Majumder *et al*. (2007) similarly detected a decrease in the oxidizable OC content along with depth. Reportedly, to better understand the mechanism through C deposited, researchers separated soil organic C into various oxidizability pools (Chan *et al*., 2001). Our findings are correlated with hypothesis that long-term fertilization of integrated animal manure with inorganic fertilizer increased active C pools as compared to the integrated straw with balanced fertilizer, chemical fertilizer alone or control treatments also reported by Bhattacharyya *et al*. (2011). Results from this study also documented the findings of Mandal *et al*. (2008) that, 51% labile C in total SOC was recorded from 36-year-old rice-rice cropping system experiment in semi-humid tropical climatic circumstances of sandy loam soil in India. Though Chan *et al*. (2001) associated the efficiency of changed grassland species with maintaining the labile pools of SOC and exposed that 65% of total SOC was in the labile pool of semiarid areas in Australia. The continuous cultivation of land could cause reduction in labile C fraction due to mineralization (Shrestha *et al*., 2008).

Relationship of SOC and labile fraction with soil properties: SOC content and labile fraction positively related to soil properties except for pH, which was not correlated at 0-20 cm and 20-40 cm of GZL (Table 9). In general, similar trend were observed in ZZ and QY except pH, which was negatively correlated at each soil depth (data not shown). These relationships suggested that soil properties were the key factor regulating SOC and labile fraction. Liang *et al*. (2019) also reported that soil properties related to SOC content.

Conclusion: Our findings indicated that integrated animal manure application with inorganic fertilizer (NPKM) significantly improved the total SOC storage, including labile organic C fractions compared to chemical fertilizer alone (NPK) or untreated control (CK) in all three experimental sites on top soil but it decreased with the increasing soil depth. In conclusion, we need to incorporate inorganic fertilization application with manure and straw to improve SOC and its labile fractions for better productivity of upland plains of China.

Acknowledgments: Financial supports were received from the National Natural Science Foundation of China (41601326, 41877105) and the National Key Research and Development Plan (2016YFE0112700). We acknowledge our colleagues for their unremitting efforts to maintain the long-term experiments.

REFERENCES

Baldock, J.A., C.A. Masiello, and Y. Ge (2004). Cycling and composition of organic matter in terrestrial and marine ecosystems. J. Marchem. 92: 39–64.

Banger, K., G.S. Toor, A. Biswas, S.S. Sidhu, and K. Sudhir (2010).Soil organic carbon fractions after 16-years of applications of fertilizers and organic manure in a Typic Rhodalfs in semi-arid tropics. Nutrient Cycling in Agroecosystems. 86: 391–399.

Bhattacharyya, R., S. Kundu, A.K. Srivastva, H.S. Gupta, V. Prakash, and J.C. Bhatt (2011). Long term fertilization effects on soil organic carbon pools in a sandy loam soil of the Indian sub-Himalayas. Plant Soil. 341: 109–124.

Bhattacharyya, R., V. Prakash, S. Kundu, A.K. Srivastva, H.S. Gupta, and S. Mitra (2010). Long-term effects of fertilization on carbon and nitrogen sequestration and aggregate associated carbon and nitrogen in the Indian sub-Himalayas. Nutrient Cycling in Agroecosystems. 86: 1–16.

Blair, G.J., R.D.B. Lefroy, and L. Lisle (1995). Soil carbon fractions based on their degree of oxidation and the development of a carbon management index for agricultural systems. Australian J. Agri. Res., 46: 1459-1466.

Blair, N., R. D. Faulkner, A. R. Till, and P.R. Poulton (2006). Long-term management impacts on soil C, N and physical fertility-Part I: Broad balk experiment. Soil Tillage Research. 91: 30-38.

Blake, G.R. and K.H. Hartge (1986).Bulk density. In: Klute A. (ed.) Methods of soil analysis. Part 1.Physical and mineralogical methods.(2nd Ed.).Agronomy Monograph 9, ASA and SSSA, Madison, WI. 1986: 363-375.

Campos, B. H. C., T. J. C. Amado, C. Bayer, R.S. Nicoloso, and J. E. Fiorin (2011). Carbon stock and its compartments in a subtropical oxisol under long-term tillage and crop rotation systems. Revista Brasileira de Ciência do Solo. 35: 805-817.

Chan, K. Y., A. Bowman, and A. Oates (2001). Oxidizible organic carbon fractions and soil quality changes in an oxicpaleustalf under different pasture leys. Soil Science. 166(1): 61–67.

Chen, H. Q., R. X. Hou, Y. S. Gong, H. W. Li, M. S. Fan, and Y. Kuzyakov (2009). Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China. Soil and Tillage Research.106: 85–94.

Ding, X. X., Y. Han, Y. Liang, and Qiao (2012). Changes in soil organic carbon pools after 10years of continuous manuring combined with chemical fertilizer in a Mollisol in China. Soil and Till Research.122: 36-41.

Edmeades, D. C. (2003). The long-term effects of manures and fertilizers on soil productivity and quality: a review. Nutrient Cycling in Agroecosystems. 66(2): 165–180. doi:10.1023/a: 10239 99816690

Ghosh, S., B. R. Wilson, B. Mandal, S. K. Ghoshal, and I. Growns (2010). Changes in soil organic carbon pool in three long-term fertility experiments with different cropping systems and inorganic and organic soil amendments in the eastern cereal belt of India. Australian J. Soil Research. 48: 413–420.

Gong, W., X. Y. Yan, J. Y. Wang, T. X. Hu, and Y. B. Gong (2009). Long-term manuring and fertilization effects on soil organic carbon pools under a wheat–maize cropping system in North China Plain. Plant Soil. 314: 67–76.

Haynes, R. J. (2005). Labile organic matter fractions as central components of the quality of agricultural soils: An Overview. Advances in Agronomy.85: 221–268.

He, Y., W. Zhang, M. Xu, X. Tong, F. Sun, J. Wang, S. Huang, P. Zhu, and X.He (2015). Long-term combined chemical and manure fertilization increase soil organic carbon and total nitrogen in aggregate fractions at three typical cropland soils in China. Sci. Total Environ.532: 635–644.

He, Y. T., X. H. He, M.G. Xu, W. J. Zhang, X. Y. Yang, and S. M. Huang (2018). Long-term fertilization increases soil organic carbon and alters its chemical composition in three wheat-maize cropping sites across central and south China. Soil and Tillage Research. 177: 79–87.

Hernandez-Ramirez, G., S. M. Brouder, D. R. Smith, and G. E. Van Scoyoc(2007). Carbon and nitrogen dynamics in an eastern corn belt soil: nitrogen source and rotation. Soil SciSoc Am J. 73:128–137.

Hutchinson, J.J., C.A. Campbell, and R.L. Desjardins (2007).Some perspectives oncarbon sequestration in agriculture. Agr. Forest Meteorol.142:288–302.

Islam, K.R., and R.R. Weil (1998). A rapid microwave digestion method for colorimetric measurement of soil organic C. Commun. Soil Sci. Plant Anal. 29: 2269-2284.

Jenkinson, D.S., D. E. Adams, and A. Wild (1991). Model estimates of CO2 emission from soil in response to warming. Nature.351:304-306.

Laik, R.,K.Kumar,D. K. Das, and O.P. Chaturved (2009). Labile soil organic matter pools in a calciorthent after 18 years of afforestation by different plantations. Appl. Soil Ecol. 42: 71–78.

Leinweber, P., and H.R. Schulten(1995). Composition, stability, and turnover of soil organic matter: investigations by off-line pyrolysis and direct pyrolysis-mass spectrometry. J. Anal. Appl. Pyrolysis.32: 91–110.

Lemke, R., A. Vandenbygaart, C. Campbell, G. Lafond, and B. Grant (2010). Crop residue removal and fertilizer N: effects on soil organic carbon in a long-term crop rotation experiment on a Udic Boroll. Agric. Ecosyst. Environ. 135: 42–51.

Li, Y.F., P.K. Jiang, S. X. Chang, J. S. Wu, and L. Lin (2010). Organic mulch and fertilization affect soil carbon pools and forms under intensively managed bamboo (Phyl- lostachys praecox) forests in southeast China. J. Soil. Sedi-ment.10:739–747.

Liang, F., J. Li, X. Yang, S. Huang, Z. Cai, H. Gao, J. Ma, X. Cui, and M. Xu (2016). Three-decade long fertilization induced soil organic carbon sequestration depends on edaphic characteristics in six typical croplands. Scientific Reports.6: 30350

Liang, F., J. Li, S. Zhang, H. Gao, B. Wang, X. Shi,S. Huang, and M. Xu (2019). Two-decade long fertilization induced changes in subsurface soil organic Carbon stock vary with indigenous site characteristics. Geoderma. 337: 853–862

Liu, E. K., S. G. Teclemariam, C.R.Yan, J. M. Yu, R. S. Gu, S. Liu, W.Q. He and Q. Liu (2014). Long-term effects of no-tillage management practice on soil organic carbon and its fractions in the northern China. Geoderma.213: 379–384.

Liu, M., W.T. Yu, and Z.S. Jiang (2006).A research review on soil active organic carbon. Chinese J. Ecology. 25(11): 1412-1417. (In Chinese)

Loss, A., M.G. Pereira, E.M. Costa, and S.J. Beutler (2014). Soil fertility, physical and chemical organic matter fractions, natural 13C and 15N abundance in biogenic and physic-genic aggregates in areas under different land use systems. Soil Research. 52: 685-697.

Maillard, E., D.A. Angers, M. Chantigny, S. Bittman, P. Rochette, G. Lévesque, D. Hunt, and L.E. Parent (2015). Carbon accumulates in organo-mineral complexes after long- term liquid dairy manure application. Agric. Ecosyst. Environ. 202:108–119.

Majumder, B., B. Mandal, and P.K. Bandyopadhyay (2007). Soil organic carbon pools and productivity relationships for a 34 year old rice–wheat–jute agroecosystem under different fertilizer treatments. Plant and Soil.297(1–2): 53–67.

Malhi, S.S., and K.S. Gill (2002). Fertilizer N and P effects on root mass of brome grass, alfalfa and barley. J. Sustain Agr. 19: 51–63.

Mandal, B., B. Majumder, T.K.Adhya, P.K. Bandyopadhyay, A. Gangopadhyay, D. Sarkar,M.C.Kundu, S.G. Choudhury, G.C.Hazra, S. Kundu, R.N. Samantaray, and A.K. Misra (2008). The potential of double-cropped rice ecology to conserve organic carbon under subtropical climate. Global Change Biology.14: 2139–2151.

Manna, M.C., A. Swarup, R. H. Wanjari, B. Mishra, and D. K. Shahi (2007). Long-term fertilization, manure and liming effects on soil organic matter and crop yields. Soil Till Research. 94: 397–409.

Moharana, P.C., B.M. Sharma, D.R. Biswas, B.S. Dwivedi, and R.V. Singh (2012). Long-term effect of nutrient management on soil fertility and soil organic carbon pools under a 6-year-old pearl millet–wheat cropping system in an Inceptisol of subtropical India. Field Crop Research. 136: 32–41.

Moreno, F., J.M. Murillo, F. Pelegrın, andI.F.Giro’n (2006). Long-term impact of conservation tillage on stratification ratio of soil organic carbon and loss of total and active CaCO3.Soil Tillage Research. 85: 86–93.

Mtambanengwe, F., and P. Mapfumo (2008). Smallholder farmer management impacts on particulate and labile carbon fractions of granitic sandy soils in Zimbabwe. Nutrient Cycling in Agroecosystems. 81: 1–15.

Pan, G.X., L. Q. Li, L.S. Wu, and X.H. Zhang (2003).Storage and sequestration potential of topsoil organic carbon in China’s paddy soils. Global Change Biology. 10: 79–92.

Powlson, D.S., P. Smith, K. Coleman, J. U. Smith, M.J. Glendining, M. Korschens, and U. A. Franko (1998). European network of long-term sites for studies on soil organic matter. Soil and Tillage Research , 47, 263–274.

Purakayastha, T.J., L. Rudrappa, D. Singh, A. Swarup and S. Bhadraray (2008). Long-term impact of fertilizers on soil organic carbon pools and sequestration rates in maize-wheat- cowpea cropping system. Geoderma.144: 370–378.

Wang, Q.,Y. Wang, Q. Wang, and J. Liu(2014).Impacts of 9 years of a new conservational agricultural management on soil organic carbon fractions. Soil and Tillage Research. 143: 1-6.

Rasmussen, P.E., and W.J. Parton (1994). Long-term effects of residue management in wheat-fallow: Inputs, yield, and soil organic matter. Soil Science Society of America J. 58: 523–530.

Reeves, D.W. (1997).The role of soil organic matter in maintaining soil quality in continuous cropping system.Soil and Tillage Research. 43: 131–167.

Rudrappa, L., T. J. Purakayastha, D. Singh, and S. Bhadraray (2006). Long-term manuring and fertilization effects on soil organic carbon pools in a TypicHaplustept of semi-arid sub-tropical India.Soil Till.Research. 88: 180–192.

Shrestha, B.M., G. Certini, C. Forte, and B.R. Singh (2008).Soil organic matter quality under different land uses in a mountain watershed of Nepal. Soil Science Society of America J. 72: 1563–1569.

Six, J.,P. Callewaert, S. Lenders, S.D. Gryze, S.J. Morris, E.G. Gregorich, E.A. Paul, and K. Paustian (2002). Measuring and understanding carbon storage in afforested soils by physical fractionation. Soil Science Society of America J. 66: 1981- 1987.

Smith, P. (2004). How long before a change in soil organic carbon can be detected?. Global Change Biol.10:1878–1883.

Smith, P., D.S. Powlson, J.U. Smith, P. Fallon, and K. Colemn (2000). Meeting the UKs climate change commitments: options for carbon mitigation on agriculture land. Soil use and Management. 16(1): 1-11.

Stevenson, F.J. (1994). Humus Chemistry: Genesis, Composition, Reactions.2nd Ed. John Wiley and Sons, Inc., New York.

Vieira, F.C., B. C. Bayer, and J.A. Zanatta (2007). Carbon management index based on physical fractionation of soil organic matter in an Acrisol under long-term no-till cropping systems. Soil and Tillage Research. 96(1-2): 195-204.

Walkley, A., and I.A. Black (1934).An examination of the Dedtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-38.

Wang, F., Y. A. Tong, P.C. Gao, J.S. Zhang, R.R. Weil, and J. N. Coffie (2014). Organic amendments to a wheat crop alter soil aggregation and labile C on the loess plateau China. Soil Sci.179: 166–173.

Wang, Y., N. Hu, M. Xu, Z. Li, Y. Lou, Y. Chen, C.Y. Wu, and Z.L. Wang. (2015). 23-year manure and fertilizer application increases soil organic carbon sequestration of a rice–barley cropping system. Biol. Fertil. Soils. 51 (5): 583–591.

Weil, R.,F. Magdoff, and Ray (2004).Significance of soil organic matter to soil quality and health. Soil organic matter in sustainable agriculture. CRC Press. 1-43.DOI: 10.1201/9780203496374.ch1

Xu, M., Y. Lou, X.Sun, W. Wang, M. Baniyamuddin, and K. Zhao (2011). Soil organic carbon active fractions as early indicators for total carbon change under straw incorporation. Biology and Fertility of Soils. 47: 745-752.

Yan, D.Z., D.J. Wang, and L.Z. Yang (2007).Long-term effect of chemical fertilizer, straw, and manure on labile organic matter fractions in a paddy soil. Biol. Fertil. Soils. 44: 93–101.

Yang, X., W. Ren, B. Sun, and S. Zhang (2012). Effects of contrasting soil management regimes on total and labile soil organic carbon fractions in a loess soil in China. Geoderma. 177–178: 49–56.

Zhang, W., M. Xu, X. Wang, Q. Huang, J. Nie, Z. Li, S. Li, S. Hwang and K. Lee (2012). Effects of organic amendments on soil carbon sequestration in paddy fields of subtropical China. J. Soils Sediments.1–14.doi:10.1007/s11368-011-0467-8.

Zhang, W.J., X.J. Wang, M.G. Xu, S.M. Huang, H.Liu, and C. Peng (2010). Soil organic carbon dynamics under long-term fertilizations in arable land of northern China. J. Biogeosciences. 7: 409–425.

Zhao, F.Z., G.H. Yang, X.H. Han, Y.Z. Feng, and G.X. Ren (2014). Stratification of carbon fractions and carbon management index in deep soil affected by the grain-to- green program in China. PLoS One. 9 (6): 99657.