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Effects of long-term fertilization on oxidizable organic carbon fractions on the Loess Plateau, China

DING Shaonan[1], XUE Sha[1,2], LIU Guobin[1,2*]
[1] College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, China;
[2] Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling 712100, China

Abstract: The effects of long-term fertilization on pools of soil organic carbon (SOC) have been well studied, but limited information is available on the oxidizable organic carbon (OOC) fractions, especially for the Loess Plateau in China. We evaluated the effects of a 15-year fertilization on the OOC fractions (F1, F2, F3 and F4) in the 0–20 and 20–40 cm soil layers in flat farmland under nine treatments (N (nitrogen, urea), P (phosphorus, monocalcium phosphate), M (organic fertilizer, composted sheep manure), N+P (NP), M+N (MN), M+P (MP), M+N+P (MNP), CK (control, no fertilizer) and bare land (BL, no crops or fertilizer)). SOC content increased more markedly in the treatment containing manure than in those with inorganic fertilizers alone. F1, F2, F4 and F3 accounted for 47%, 27%, 18% and 8% of total organic carbon, respectively. F1 was a more sensitive index than the other C fractions in the sensitivity index (SI) analysis. F1 and F2 were highly correlated with total nitrogen (TN) and available nitrogen (AN), F3 was negatively correlated with pH and F4 was correlated with TN. A cluster analysis showed that the treatments containing manure formed one group, and the other treatments formed another group, which indicated the different effects of fertilization on soil properties. Long-term fertilization with inorganic fertilizer increased the F4 fraction while manure fertilizer not only increased labile fractions (F1) in a short time, but also increased passive fraction (F4) over a longer term. The mixed fertilizer mainly affected F3 fraction. The study demonstrated that manure fertilizer was recommended to use in the farmland on the Loess Plateau for the long-term sustainability of agriculture.

Keywords: long-term fertilization; oxidizable organic carbon fractions; farmland soil; Loess Plateau

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Because soil organic carbon (SOC) in agricultural soils has a turnover time from decades to centuries, the gross contents of SOC change very slowly (Stevenson, 1994). As a result, the measurement of SOC alone does not adequately reflect the changes in soil quality and nutrient status (Bolinder et al., 1999; Banger et al., 2010; Liu et al., 2010). Identifying alternative C fractions that are more sensitive or indicative of changes in C contents than SOC would, therefore, be useful (Wang and Wang, 2011; Zhang et al., 2012). Oxidizable organic carbon (OOC) has received researchers' attention due to its sensitivity and ease of detection, which characterizes SOC both quality and quantity (Barreto et al., 2011). Chan et al. (2001) proposed a modified procedure based on Walkley-Black method (Walkley, 1947), which only uses half amount of

*Corresponding author: LIU Guobin (E-mail: gbliu@ms.iswc.ac.cn)
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sulfuric acid, is a more sensitive indicator for the improvement in soil quality parameters, and can separate OOC into four fractions (F1, F2, F3 and F4) by its degree of oxidation. The use of modified Walkley-Black procedure is well documented in different regions (Majumder et al., 2007, 2008; Xavier et al., 2009; Barreto et al., 2011; Bhattacharyya et al., 2011). The fractions F1 and F2 are associated with the availability of nutrients and the formation of macro-aggregates (Chan et al., 2001). F1 is the most labile fraction in soil and is highly correlated with the free light fraction of soil organic matter (SOM) (Maia et al., 2007). F3 and F4, however, are associated with compounds of greater chemical stability and higher molecular weight which originate from the decomposition and humification of SOM (Guareschi et al., 2013).

OOC, like SOC, is influenced by many factors, such as soil parental material, climatic conditions, land use and cover change (Lefroy et al., 1993; Campbell et al., 1996; Verma et al., 2010; Zhang et al., 2012, 2013). The ratios of the four fractions differ in different types of ecosystems, and changes in the use of soils or the state of the vegetation may quickly alter the processes of the accumulation or loss of organic matter in soils, thereby affecting the total amount of carbon stored (Patra et al., 2014). Chan et al. (2001) compared the effectiveness of different pasture species in maintaining OOC fractions in a semi-arid region of Australia, and other studies subsequently explored OOC changes in various agro-ecosystems in different regions (Chan et al., 2002; Loss et al., 2010; Bhattacharyya et al., 2011; Pereira et al., 2013). The effects of fertilization on OOC fractions in farmland soil, however, have been rarely studied, especially the changes over varying durations of fertilization. Long-term field experiments can overcome the effects of inter-annual climatic variation on soil fertility (Yang et al., 2006) and provide critical insights into factors that influence SOC management and their contributions to agricultural sustainability (Rasmussen et al., 1998).

The semi-arid Loess Plateau is a highly fragile ecosystem where the soil has degraded over vast areas in recent decades (Shi and Shao, 2000). Dryland farming has prevailed for several decades in this region. The main soils of the Loess Plateau develop from calcareous loessial parental material and have low SOC contents, likely due to the dry climate, sparse vegetation and intensive farming (Janzen et al., 1997). For improving the ecological environment, the Chinese government launched the nationwide “Grain for Green” project in 1999, which converted unsuitable sloping farmland to forests or grassland (Zhang et al., 2011). The flat farmland between valleys has thus become of great importance to regional farming due to the loss of the sloped farmland. Low soil fertility, however, is hindering dryland farming on the Loess Plateau, so fertilization is required to guarantee yields (He and Lin, 1992), which consequently affects the soil properties (Fan et al., 2008, Liu et al., 2010). The study of the influence of fertilization on the SOC and OOC fractions in this special region has been neglected.

We thus hypothesized that various types of fertilization would differentially influence the SOC and OOC fractions. We tested this hypothesis with a 15-year fertilization experiment in the farmland on the Loess Plateau. Our objective was to compare the effects of various fertilizations on the OOC fractions and related soil properties for identifying the best practice influencing labile OC fractions and the regional farming activity.

1 Materials and methods

1.1 Study area

The study was carried out at the Ansai Station of the Northwest Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resource in northern Shaanxi province, a typically hilly region of the Loess Plateau (36°51′30″N, 109°19′23″E; 1,032 m asl). This semi-arid region has a gully density of 4.2–8.0 km/km^2 and erosion rate of 13.5×10^3 t/(km^2·a). The climate is warm and temperate with an annual mean temperature of 8.8°C, accumulated temperature of 3,114°C (≥10°C), a frost-free period of 159 d, 2,416 h of sunshine annually and total radiation of 548×10^3 J/cm^2. Annual precipitation, evaporation and aridity index are 535 mm, 146 mm and 1.46, respectively. The soil is mainly wind-deposited Huangmian soil, characterized by a yellow color, an absence of bedding, a silty texture, looseness and wetness-induced collapsibility (Zhang et al., 2011). The vegetation of the region belongs to the temperate forest steppe, but the original vegetation has been destroyed. Because there is no irrigation conditions, the farmland just rely on natural rainfall.

1.2 Experimental design

Experimental plots were established in 1997 for investigating the effects of fertilization on soil properties. The crop was planted in soybean-corn rotation, and it became corn in 2011. Before the plots, the land was planted to millet. The plots were 2.33 m×6 m in size and were arranged in a completely randomized block design with three replicates of nine treatments: N (nitrogen, urea), P (phosphorus, monocalcium phosphate), M (organic fertilizer, sheep manure, C/N=15), N+P (NP), M+N (MN), M+P (MP), M+N+P (MNP), CK (control, no fertilizer), and bare land (BL, no crops or fertilizer). Every two plots were separated by a 0.5-m strip. All plots were managed in the same way except fertilization treatment. The rates of N, P and manure fertilization were 97.5, 75 and 7,500 kg/hm^2, respectively. The manure and phosphate fertilizer were applied once as the seed fertilizer, and 20% of the urea was applied as a seed fertilizer, the remaining 80% of urea was applied during the bell-mouthed and tasselling stages. Weeding was done twice. Corn (*Zea mays* L., cultivar Shuang-hui II) was planted on 27 April and harvested on 19 October 2011. Row spacing and plant spacing was 58 cm×35 cm.

1.3 Soil sampling and analysis

Five soil samples were collected and bulked after the harvest with a core sampler using the S-sampling method from the 0–20 and 20–40 cm layers. The samples were packed in fabric bags, immediately transported to the laboratory, air-dried at room temperature, ground and sequentially passed through 2-, 1- and 0.25-mm sieves for the analysis of physical and chemical properties and the content of OOC fractions.

The physical-chemical properties were analyzed using standard procedures of the Chinese Ecosystem Research Network (Editorial Committee, 1996) and the Soil Science Society of China (1999). SOM content was determined by wet digestion with a mixture of potassium dichromate and concentrated sulfuric acid, and total organic carbon (TOC) content was calculated as SOM/1.724. Total N (TN) content was measured by the semi-micro Kjeldahl method, and total P (TP) content was colorimetrically determined by wet digestion with H_2SO_4+$HClO_4$. Available N content (AN) was determined with a micro-diffusion technique after alkaline hydrolysis. Available P (AP) content was determined by the Olsen method. Soil pH was determined by an automatic titrator in 1:2.5 soil:water suspensions (Wang et al., 2012). The results are shown in Table 1.

The OOC fractions were determined by a modified Walkley-Black method (Walkley and Black, 1934) as described by Chan et al. (2001) using 5, 10 and 20 mL of concentrated H_2SO_4 to produce three acid-aqueous solutions of 0.5:1, 1:1 and 2:1 (corresponding to 6, 9 and 12 mol/L H_2SO_4, respectively), separating TOC into the following four fractions of variable oxidizability: F1, OOC under 6 mol/L H_2SO_4; F2, the difference in OOC extracted between 6 and 9 mol/L H_2SO_4; F3, the difference in OOC extracted between 9 and 12 mol/L H_2SO_4. The 12 mol/L H_2SO_4 is equivalent to the standard Walkley-Black method; F4, residual organic carbon after reaction with 12 mol/L H_2SO_4.

A sensitivity index (SI) for identifying the best SOC indicator was calculated for each C fraction as:

$$SI=(C_{\text{fraction in fertilization}}-C_{\text{fraction in control}})/C_{\text{fraction in control}}\times 100, \quad (1)$$

The C content in F1 was considered labile C (C_L), and the non-labile C (C_{NL}) was determined by C_{NL}=SOC–C_L. We calculated the following indices based on differences in C content between the fertilization and control treatments:

$$CPI=C_{\text{fertilization}}/C_{CK}, \quad (2)$$

$$\text{Lability (L)}=C_L/C_{NL}, \quad (3)$$

$$\text{Lability Index (LI)}=L_{\text{fertilization}}/L_{CK}, \quad (4)$$

$$\text{Carbon Management Index (CMI)}=CPI\times LI\times 100. \quad (5)$$

1.4 Data analysis

All statistical analyses were performed with SPSS for Windows (version 13.0), and analysis of variance was performed using ANOVA procedure. The treatment means were compared using least significant differences (LSD) (P<0.05). Relationships between the C fractions and the soil physical-chemical properties were compared by correlation analysis (n=27). A cluster analysis was also conducted using SPSS.

Table 1 Soil properties of different fertilization treatments in the 0–20 and 20–40 cm soil layers

Index	Soil layer (cm)	Organic matter (g/kg)	Total N (g/kg)	Total P (g/kg)	Available N (mg/kg)	Available P (mg/kg)	pH
BL	0–20	9.75±0.01^{d}	0.56±0.07^{b}	0.65±0.03^{c}	43.6±0.94^{b}	8.01±1.99^{c}	8.57±0.02^{abc}
	20–40	6.71±0.35^{B}	0.44±0.06^{BCD}	0.57±0.02^{C}	24.6±5.21^{BC}	3.03±0.24^{B}	8.68±0.03^{B}
CK	0–20	10.66±0.12^{c}	0.60±0.13^{b}	0.64±0.03^{c}	35.2±5.67^{c}	2.91±0.95^{d}	8.60±0.01^{a}
	20–40	6.92±0.46^{B}	0.45±0.05^{ABCD}	0.58±0.01^{BC}	21.8±1.77^{C}	1.38±0.10^{B}	8.74±0.05^{A}
N	0–20	10.31±0.39^{cd}	0.59±0.01^{b}	0.64±0.01^{c}	39.7±8.59^{bc}	2.57±0.70^{d}	8.55±0.02^{bcd}
	20–40	6.86±0.05^{B}	0.43±0.04^{D}	0.58±0.01^{BC}	27.6±1.26^{AB}	1.35±0.42^{B}	8.74±0.04^{A}
P	0–20	11.01±0.44^{c}	0.64±0.04^{b}	0.82±0.04^{ab}	36.1±3.22^{c}	22.01±3.43^{b}	8.46±0.01^{e}
	20–40	6.56±0.73^{B}	0.44±0.01^{CD}	0.60±0.03^{AB}	22.4±3.16^{C}	2.58±1.65^{B}	8.74±0.02^{A}
NP	0–20	10.64±0.33^{cd}	0.62±0.02^{b}	0.80±0.05^{bc}	37.2±5.21^{c}	18.01±2.56^{b}	8.61±0.03^{a}
	20–40	6.57±0.21^{B}	0.44±0.03^{CD}	0.59±0.02^{B}	24.0±5.17^{BC}	2.83±0.74^{B}	8.72±0.01^{AB}
M	0–20	14.54±0.57^{ab}	0.86±0.06^{a}	0.68±0.01^{c}	54.1±7.06^{a}	5.9±0.79^{cd}	8.53±0.02^{cd}
	20–40	7.73±0.16^{A}	0.49±0.02^{AB}	0.60±0.02^{AB}	29.5±4.87^{A}	2.07±0.15^{B}	8.73±0.01^{A}
MN	0–20	15.21±0.26^{a}	0.83±0.05^{a}	0.66±0.02^{c}	55.8±0.64^{a}	5.41±0.87^{cd}	8.52±0.06^{d}
	20–40	7.69±0.48^{A}	0.48±0.01^{ABC}	0.60±0.03^{AB}	27.7±5.01^{AB}	1.80±0.20^{B}	8.75±0.01^{A}
MP	0–20	14.16±1.32^{ab}	0.85±0.02^{a}	0.88±0.10^{a}	55.7±2.60^{a}	45.19±2.32^{a}	8.59±0.02^{ab}
	20–40	7.27±0.78^{AB}	0.48±0.02^{ABCD}	0.60±0.01^{AB}	27.4±2.03^{AB}	6.68±3.22^{A}	8.74±0.02^{A}
MNP	0–20	13.80±0.17^{b}	0.83±0.10^{a}	0.83±0.07^{ab}	53.2±3.73^{a}	40.97±6.24^{a}	8.57±0.03^{abc}
	20–40	7.68±0.40^{A}	0.49±0.03^{A}	0.61±0.03^{A}	30.3±4.11^{A}	6.47±0.28^{A}	8.75±0.01^{A}

Note: Means with lowercase and capital letters within a column indicate significantly different in the 0–20 and 20–40 cm soil layers at P<0.05 level. Mean±SD (n=3). BL, bare land; CK, control; N, nitrogen; P, phosphorus; M, organic fertilizer. NP, MN, MP and MNP indicate the combination of different fertilizers. The following abbreviations are the same.

2 Results

2.1 SOC

The SOC content was higher in the 0–20 cm layer than in the 20–40 cm layer for all treatments (Table 2), and varied from 5.98 to 8.82 g/kg in the 0–20 cm layer and from 3.64 to 4.46 g/kg in the 20–40 cm layer. The average SOC content in the treatments with inorganic fertilizers was 6.18 g/kg, with the lowest content in the N treatment (5.98 g/kg). SOC contents did not differ significantly between CK and the treatments containing only inorganic fertilizers. In contrast, SOC contents were significantly higher in the treatments containing manure than in CK by an average of 35% in the 0–20 cm layer and of 9% in the 20–40 cm layer.

2.2 OOC fractions

The fractions of F1, F2 and F4 were the lowest in the 0–20 cm layer in BL, with F3 similar in all fertilization treatments except NP and MN treatments. F1, F2 and F4 were not significantly higher in the treatments containing only inorganic fertilizers than in CK, but F1 and F2 were significantly higher in the treatments containing manure. F3 was significantly higher than in CK in only M and MN treatments. F4 was significantly higher in MN than in CK, with the other treatments varying only slightly.

F1 was significantly higher in the subsurface layer in MN and MNP treatments than in CK, and F2 was significantly higher in the treatments containing manure, except the MN treatment. F3, however, did not differ significantly between the organic and inorganic treatments. F4 also did not differ significantly but was the lowest in P treatment and highest in MN treatment (Table 2).

Table 2 The oxidizable organic carbon fractions and soil organic carbon content in the 0–20 and 20–40 cm soil layers

Treatment	F1 (g/kg)		F2 (g/kg)		F3 (g/kg)		F4 (g/kg)		SOC (g/kg)	
	0–20 cm	20–40 cm	0–20 cm	20–40 cm	0–20 cm	20–40 cm	0–20 cm	20–40 cm	0–20 cm	20–40 cm
Value in 1997	2.68	1.88	1.48	1.05	0.48	0.65	0.92	0.53	5.57	4.12
BL	2.63^{d}	1.80^{cd}	1.53^{c}	0.95^{cde}	0.56^{bc}	0.43^{a}	0.94^{d}	0.72^{bc}	5.66^{d}	3.89^{cd}
CK	2.77^{cd}	1.84^{bcd}	1.71^{bc}	0.93^{de}	0.44^{cd}	0.39^{ab}	1.27^{bc}	0.84^{ab}	6.18^{cd}	4.01^{bcd}
N	2.83^{cd}	1.84^{bcd}	1.59^{c}	1.00^{bcde}	0.46^{cd}	0.41^{ab}	1.10^{cd}	0.73^{bc}	5.98^{cd}	3.98^{bcd}
P	2.97^{c}	1.78^{cd}	1.61^{c}	0.89^{e}	0.64^{b}	0.37^{ab}	1.16^{bcd}	0.60^{c}	6.38^{c}	3.64^{d}
NP	2.64^{d}	1.65^{d}	1.96^{b}	1.12^{abcd}	0.40^{d}	0.32^{ab}	1.16^{bcd}	0.72^{bc}	6.17^{cd}	3.82^{cd}
M	4.29^{a}	2.03^{ab}	2.37^{a}	1.20^{ab}	0.69^{b}	0.42^{a}	1.09^{cd}	0.72^{bc}	8.43^{ab}	4.37^{ab}
MN	4.01^{b}	2.05^{a}	2.37^{a}	1.10^{abcd}	0.86^{a}	0.37^{ab}	1.58^{a}	0.94^{a}	8.82^{a}	4.46^{a}
MP	4.02^{b}	1.97^{abc}	2.40^{a}	1.22^{a}	0.44^{cd}	0.26^{b}	1.35^{b}	0.76^{bc}	8.21^{b}	4.21^{abc}
MNP	3.82^{b}	2.05^{a}	2.31^{a}	1.14^{abc}	0.50^{cd}	0.38^{ab}	1.38^{ab}	0.87^{ab}	8.01^{b}	4.45^{a}

Note: Means with the same letter within a column are not significantly different at $P<0.05$ level.

The percentage of F1 accounting for TOC was the largest under all treatments and varied from 43% to 51% (Fig. 1). When manure was applied alone, F1 percentage significantly increased in the 0–20 cm layer, and the percentage of F4 declined correspondingly. Little difference was found in the other two fractions. However, the percentage of F1 declined in the NP treatment compared with other treatments. The four fractions F1, F2, F4 and F3 accounted for 47%, 27%, 18% and 8% of TOC, respectively.

NP, MN and MP had significant effects on F3 ($P<0.05$; Table 3), indicating that these fertilizers had an interactive effect and mainly influenced F3 in the 0–20 cm layer and that MN

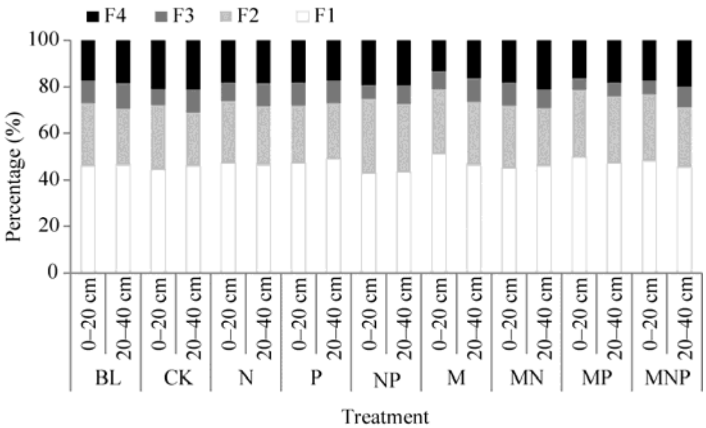

Fig. 1 The percentage of OOC fractions (F1–F4) accounting for TOC under different fertilization treatments

and MNP treatments had an interactive effect on F4. In the 20–40 cm layer, only MN had an interactive effect on F2.

2.3 Sensitivity index of OOC and SOC

Sensitivity index (SI) of the OOC fractions significantly differed among the treatments (Fig. 2). In the 0–20 cm layer, the SI of organic fertilizer involved was greater than inorganic fertilizer as a whole. SI of F1 and F2 seemed more sensitive than SOC, especially in the treatments with organic fertilizer. SI was the highest for F2 in the 20–40 cm layer. The application of P fertilizer had an adverse effect on SI in all fractions.

Table 3 Interaction of N, P and M fertilizers on SOC and OOC fractions

Soil layer (cm)		N	P	M	N×P	N×M	P×M	N×P×M
0–20	SOC	0.669	0.254	0.000	0.283	0.288	0.019	0.299
	F1	0.008	0.088	0.000	0.230	0.398	0.076	0.072
	F2	0.664	0.442	0.000	0.232	0.310	0.330	0.090
	F3	0.919	0.003	0.001	0.010*	0.003*	0.000*	0.294
	F4	0.223	0.953	0.024	0.298	0.025*	0.695	0.036*
20–40	SOC	0.278	0.112	0.000	0.415	0.667	0.407	0.894
	F1	0.924	0.155	0.000	0.750	0.286	0.360	0.329
	F2	0.528	0.528	0.003	0.377	0.033*	0.935	0.508
	F3	0.757	0.107	0.757	0.482	0.509	0.791	0.146
	F4	0.108	0.173	0.057	0.540	0.114	0.254	0.095

Note: * indicates significance at $P<0.05$ level.

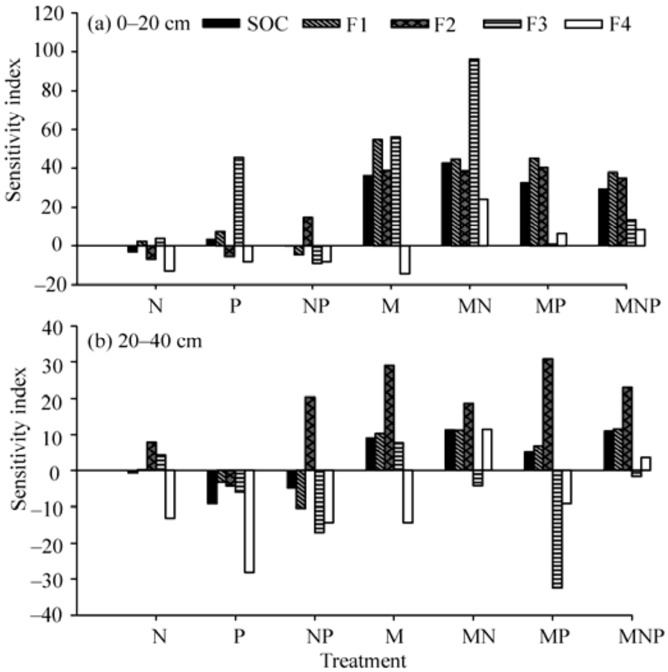

Fig. 2 Comparison the sensitivity of OOC fractions and SOC in the 0–20 (a) and 20–40 cm (b) soil layers under different fertilization treatments

2.4 The carbon content and OOC fractions after 15-year fertilization

After 15-year cultivation with different fertilization, the SOC contents increased in all fertilization treatments in the 0–20 cm layer, while the average increase level was different in inorganic fertilizer and organic manure, 11% and 50%, respectively (Fig. 3). As for OOC fractions, the average increases of F1, F2, F3 and F4 were 5%, 16%, 4% and 24% in the treatment with inorganic fertilizer and 50%, 60%, 29% and 46% in the treatment with organic manure, respectively. On the whole, organic fertilizer increased greater than inorganic fertilizer in different fractions. While in the 20–40 cm layer, carbon content with inorganic fertilizer decreased in most fractions except in F4, which increased in all treatments, and the average increases for inorganic fertilizer and organic manure were 29% and 55%, respectively. For the soil with organic fertilizer, the carbon content of SOC, F1 and F2 increased about 8%, by contrast, F3 decreased by 40% averagely in all treatments.

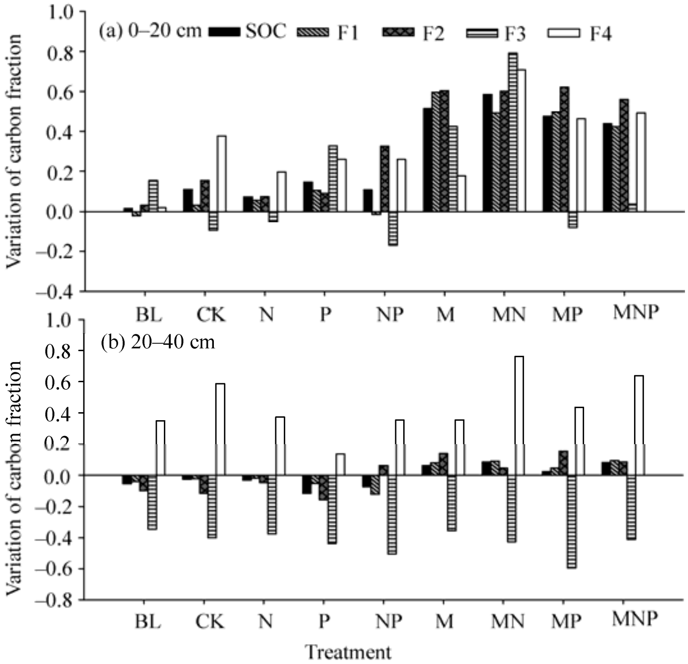

Fig. 3 Variation of the carbon fractions in the 0–20 (a) and 20–40 cm (b) soil layers after 15-year fertilization

2.5 Carbon management index

Carbon management index (CMI) is an important index for evaluating the changes in C status. CMI was significantly higher in the 0–20 cm layer in the treatments containing manure than in those with inorganic fertilizer (Table 4) and was highest in M treatment (174.16). CMI did not

Table 4 The values of CPI, L, LI and CMI under different treatments

Index	0–20 cm				20–40 cm			
	CPI	L	LI	CMI	CPI	L	LI	CMI
BL	0.92[d]	0.87[bc]	1.07[bc]	97.96[cd]	0.97[cd]	0.87[ab]	1.02[abc]	98.56[ab]
N	0.97[cd]	0.90[bc]	1.11[bc]	107.56[cd]	0.99[bcd]	0.86[abc]	1.01[abc]	100.74[a]
P	1.03[c]	0.87[bc]	1.07[bc]	110.96[c]	0.91[d]	0.96[a]	1.12[a]	102.11[a]
NP	1.00[cd]	0.75[d]	0.93[d]	92.38[d]	0.95[cd]	0.76[c]	0.90[c]	85.27[ab]
M	1.36[ab]	1.03[a]	1.28[a]	174.16[a]	1.09[ab]	0.87[ab]	1.02[ab]	111.15[a]
MN	1.43[a]	0.83[cd]	1.03[cd]	146.67[b]	1.11[a]	0.85[bc]	1.00[bc]	110.90[a]
MP	1.33[b]	0.96[ab]	1.18[ab]	157.04[b]	1.05[abc]	0.88[ab]	1.03[ab]	108.21[a]
MNP	1.30[b]	0.91[bc]	1.13[bc]	145.94[b]	1.11[a]	0.86[bc]	1.01[bc]	111.85[a]

Note: Means with the same letter within a column are not significantly different at $P<0.05$ level. CPI, carbon pool index; L, lability; LI, lability index; CMI, carbon management index.

differ significantly in the 20–40 cm layer between the treatments containing manure and those containing inorganic fertilizer.

2.6 Relationships between the OOC fractions and soil properties

We conducted correlation analysis for better understanding the relationship between OOC fractions and soil physical-chemical properties (Table 5). All OOC fractions were highly correlated with SOC. F1 and F2 were highly correlated with TN and AN; F3 was negatively correlated with pH and F4 was weakly correlated with TN.

Table 5 Correlation coefficient between OOC fractions and soil properties

	SOC	F1	F2	F3	F4	TN	TP	AN	AP	pH
SOC	1.00									
F1	0.96**	1.00								
F2	0.91**	0.84**	1.00							
F3	0.50**	0.49*	0.22	1.00						
F4	0.62**	0.44*	0.52**	0.20	1.00					
TN	0.88**	0.88**	0.89**	0.33	0.38*	1.00				
TP	0.25	0.23	0.31	–0.27	0.31	0.30	1.00			
AN	0.81**	0.83**	0.79**	0.38	0.28	0.84**	0.13	1.00		
AP	0.35	0.35	0.43*	–0.29	0.30	0.42*	0.88**	0.33	1.00	
pH	–0.18	–0.21	0.06	–0.60**	–0.05	–0.03	0.05	0.03	0.14	1.00

Note：* and ** indicate significant correlations at $P<0.05$ and $P<0.01$ levels, respectively; n=27. SOC, soil organic carbon; F1–F4 indicate the fractions of OOC; TN, total nitrogen; TP, total phosphorous; AN, available nitrogen; AP, available phosphorous.

2.7 Cluster analysis

The results of the cluster analysis with the OOC fractions and SOC as variables are shown in Fig. 4. The analysis identified two groups, one containing CK, BL, N, P, and NP and the other containing the treatments with manure. In addition to BL and CK, the cluster analysis thus indicated a significant difference between the inorganic and organic fertilizations.

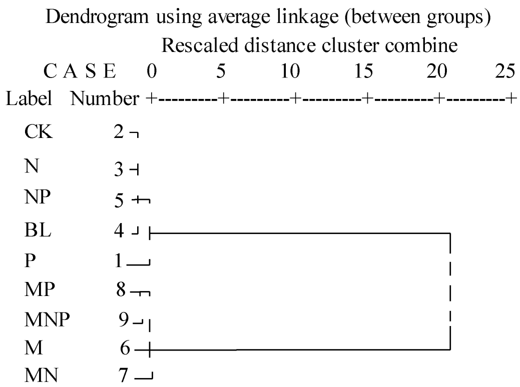

Fig. 4 Cluster analysis for different treatments

3 Discussion

3.1 SOC

Cultivation over 15 years did not cause a significant decrease in SOC content (CK-BL; Table 2), which is inconsistent with the result of Majumder et al. (2007) in India. Variations in the rate of decomposition of crop residues in soils under different climatic conditions (semi-arid vs sub-tropic) might be responsible for such differences (Majumder et al., 2008). The BL soil did not change significantly over the years without the input of organic matter; its fertility was mainly maintained by a relative dynamic balance. Also, the corn in CK treatment consumed some nutrients, and its roots generate more root exudates or crop residues for conversion to SOM by biochemical decomposition and microbial activity (Wander, 2004).

SOC content increased more with organic than inorganic fertilization. SOC content did not differ significantly between CK and the treatments containing only inorganic fertilizer, indicating that application of inorganic fertilizer did not contribute to SOC stabilization in the soil, as previously reported (Lou et al., 2011). The lowest SI under P treatment also confirmed this result. SOC content, though, increased significantly relative to CK with a combination of manure and N or P even with manure alone (Blair et al., 2006). The sheep manure in our study contained abundant nutrients, including 24% SOM, 0.923% N, 0.167% P and 2.669% potassium. In addition to the high input of organic fertilizer, the crops in these treatments had strong and extensive root systems, so an increase in nutrient supply by the addition of fertilizers would increase crop residues in the form of roots and stubble, which in turn would increase the SOC content (Malhi and Gill, 2002; Sherrod et al., 2005). Fertilization with manure for 15 years in our study increased SOC content by 35% compared to CK. While Blair et al. (2006) reported a 47% increase in conventional tillage as a result of the highest farmyard manure applications compared with the control during 96-year of a static experiment Kirchmann and Gerzabek (1999) reported a 75% increase in conventional tillage with the application of animal manure for 41-year compared with no fertilizer for the Ultano, long-term experiment in Sweden. These studies showed that such an increase in SOC levels is directly linked to the time of fertilization. SOC content in our study was highest with a combination of manure and N fertilizer, which demonstrated that N fertilizer is indispensable to soil fertility, and was consistent with the results reported by Purakayastha et al. (2008).

3.2 OOC fractions

OOC did not differ significantly between inorganic fertilization and CK, either in the 0–20 or 20–40 cm soil layer. The four fractions of OC extracted under a gradient of oxidizing conditions significantly differed among the treatments (Table 2), with proportions following the order F1 (47%)>F2 (27%)>F4 (18%)>F3 (8%). Chan et al. (2001), comparing the effectiveness of different pasture species in maintaining labile pools of SOC, reported a relatively high proportion (65%) of TOC in the F1 and F2 pools in semi-arid areas of Australia, and Majumder (2007) reported that of 56.1% in India. For our result the TOC was higher, ranging from 70% to 79%. The probable reason may assume two aspects. The manure in our experiment contained a lot of labile matter, leading directly to high F1 and F2 contents. On the other hand, due to dry climate condition, SOM was easily to be decomposed, which means that its labile fractions were higher than passive pool. Namely its passive pool or stabilized C is relatively low. Variations in the rate of decomposition of crop residues under different climatic conditions may contribute to the differences in the magnitude of the pools (Maia et al., 2007).

Chan et al. (2001) observed that most differences in soil quality among pastures were in the more easily oxidizable fractions (F1), and only small differences were found in other fractions. F1 contained a quickly reactive labile organic matter, which provides energy and nutrients for soil micro-organisms and releases part of the nutrients for plant usage with a short-term turnover. Its half-life is between days and few years (Strosser, 2010). The result that F1 percentage in M treatment in our study (51%) was significantly higher than other treatments also confirmed his view and indicated that application of manure fertilizer could increase F1 fraction (Bhattacharyya et al., 2011). While F1 percentage decreased in NP treatment, the reason was that crop under this treatment grew fast and consumed lots of labile matter, without exogenous labile matter input, and then its F1 fraction decreased somehow (Soon et al., 2009). Majumder et al. (2008) reported that F1 was highly correlated with the production of corn and rice and concluded that F1 could be considered as a good indicator of the sustainability of a crop system. The higher variations in F1 in our study demonstrated that it can be used as an indicator for monitoring the changes in SOC quality in agricultural systems.

Our result also indicated that long-term fertilization with inorganic fertilizer increased F4 fraction, especially obvious in deeper soil. This result suggests that recalcitrant fraction tended to stabilize in deeper layer and was consistent with the result of Ghosh et al. (2010). However, this speculation needs to be verified in future study. F4 is the most resistant soil fraction and is known as the "passive component" in the simulation models of SOM. The turnover time of the passive pool, which is inert, is about 2,000 years. The labile pool is thus a more sensitive indicator than the passive pool of changes in SOC resulting from different management practices (Chan et al., 2001).

3.3 CMI evaluation

Low CMI values (<100) indicate a negative impact of a management practice on SOC content and soil quality (Blair et al., 1995). The values of CMI in the study were greater than 100 in the 0–20 cm layer for the treatments containing manure but were lower in both layers for the treatments with inorganic fertilizer. The cluster analysis result also confirmed this difference. Blair et al. (2006) reported that fertilization with manure and manure combined with an inorganic fertilizer significantly increased CMI value relative to other chemical fertilizer treatments in a long-term experiment. Gong et al. (2009) also reported that 18-year of organic manure addition (alone and in combination with N fertilizer) was more effective for increasing CMI than chemical fertilizer alone in a wheat-maize system.

4 Conclusions

The study demonstrated that applications of manure fertilizer increased SOC content much more than inorganic fertilizer in agricultural soils, and differences in fertilization significantly influenced SOC pool at the surface layer. And fertilization time is an important factor to determine SOC content. The labile fractions (F1 and F2) showed advantages in quantity over passive fractions (F3+F4) in this region. F1 was a more sensitive index than the other C fractions through SI analysis, and could reflect the labile C fractions changes. Long-term fertilization with inorganic fertilizer could increase F4 fraction and F4 tend to be stabilized in deeper soil layers. The manure fertilizer not only increased labile fractions (F1) in a short time, but also increased passive fraction (F4) in a long term. The mixed fertilizer mainly influenced F3. Farmland on the Loess Plateau thus should use more manure fertilizer for the long-term soil sustainability.

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