

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

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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 (KMnO₄-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 by 113.3, 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 labile 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 (Baldoek *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 (KMnO₄-C), considered to be the labile fractions extracted by chemical oxidation method (Blair *et al.*, 1995), acts as a sensitive key to long-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; Quanying *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. monocropping 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 kg ha⁻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 414 g 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%) (Table 3). 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). KMnO₄ 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 KMnO₄ concentration. So it was used to indicate the amount of C oxidized, assuming that 1mM KMnO₄ was consumed in the oxidation of 0.75mM or 9mg of C (Blair *et al.*, 2001). SOC storage of profile for all depths, i.e. 0-20 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 (Table 4).

Influence of long-term fertilization on SOC storage: SOC storage ranged from 50.0, 30.0 and 36.0 Mg ha^{-1} in top layer to 23.0, 7.0 and 6.0 Mg 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 (50.0 Mg ha^{-1}) followed by QY (36.0 Mg ha^{-1}) and ZZ (30.0 Mg 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 that SOC 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 ($\text{KMnO}_4\text{-C}$) 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 at 20-40cm 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).

DISCUSSION

Influence of long-term fertilization on SOC storage: Management practices and soil type affected SOC accumulation (Moreno *et al.*, 2006; Laiket *et al.*, 2009; Zhao *et al.*, 2014) among which fertilization application (Yan *et al.*, 2007; Xuet *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 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⁻¹yr⁻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 KMnO₄-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 oxidizable 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 KMnO₄-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 trends 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.

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