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Li Juan, Wen Yanchen, Xuhua Li, Yanting Li ...+5 more authors

Institutions: Shandong Agricultural University, Newcastle University

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Juan Li[a], Yanchen Wen[a], Xuhua Li[b], Yanting Li[a], Xiangdong Yang[a], Zhian Lin[a], Zhenzhen Song[b], Julia Mary Cooper [c *], Bingqiang Zhao [a **]

[a] *Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture/Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China;*

[b] *National Engineering Laboratory for Efficient Utilization of Soil and Fertilizer Resources/College of Resources and Environment, Shandong Agricultural University, Taian, Shandong 271018, China;*

[c] *School of Agriculture, Food and Rural Development, Newcastle University, Newcastle Upon Tyne, NE1 7RU, UK*

* Corresponding Author 1

Tel: +44 191 208 6883

Postal address: School of Agriculture, Food and Rural Development, Newcastle University, Kings Road, Newcastle Upon Tyne NE1 7RU UK

E-mail address: julia.cooper@newcastle.ac.uk

** Corresponding Author 2

Tel.: +86-10-82108658

Postal address: Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

E-mail address: zhaobingqiang@caas.cn (B. Zhao)

ABSTRACT

To improve C sequestration in soils and mitigate climate change, it is essential to understand how nutrient management strategies impact on soil organic carbon (SOC) stocks and labile fractions. This study was designed to explore changes in soil bulk density (BD), SOC concentrations, SOC stocks and soil labile organic C fractions (mineralizable C (Cmin), microbial biomass C (MBC), dissolved organic C (DOC), particulate organic C (POC), light fraction organic C (LFOC) and permanganate oxidizable C ($KMnO_4$-C)) under 26-year fertilization regimes in a wheat-maize rotation system in the North China Plain. Soil from the following six treatments was analyzed: (1) Control with no amendment addition (CK); (2) Standard rate of mineral fertilizer treatment (SMF) reflecting local farmers' practice; (3) Standard rate of organic manure treatment (SMA) with total N input equal to SMF; (4) Half the standard rate of mineral fertilizer plus half the standard rate of organic manure treatment (1/2 SMF+1/2 SMA); (5) Double standard rate of mineral fertilizer treatment (DMF); (6) Double standard rate of organic manure treatment (DMA). Results showed that all long-term fertilization regimes significantly decreased BD in topsoil compared to CK except for SMF, with treatments that included organic manure resulting in the lowest BDs. Treatments that included organic manure had significantly higher SOC concentrations and stocks than mineral or unfertilized treatments. The organic manure treatments also had higher concentrations of non-labile C but at the same time a higher proportion of labile C than the mineral or unfertilized treatments. This was confirmed by the carbon management index (CMI) which was significantly increased by organic manure addition. Control and mineral fertilized treatments had higher efficiencies of C retention (RE) from added inputs (crop residues only). Differences in Cmin, POC and $KMnO_4$-C were affected by differences in MA-C, however, changes in rhizodeposition-C, stubble-C and root-C significantly affected DOC, MBC and LFOC. This study demonstrates that fertilization strategies that include organic manure can increase the pool of stable C in the surface soil layer, while at the same time increasing concentrations and proportions of labile C. Organic manure use can therefore contribute to improved nutrient cycling services and higher soil quality in the North China Plain.

Keywords: long-term fertilization; soil organic carbon; labile organic carbon fractions

Abbreviations

BD: bulk density;

SOC: soil organic carbon;

MBC: microbial biomass carbon;

DOC: dissolved organic carbon;

POC: particulate organic carbon;

$KMnO_4$-C: permanganate oxidizable carbon;

NCP: North China Plain;

L: Lability;

LI: Lability Index;

CPI: Carbon Pool Index;

CMI: Carbon Management Index;

RE: retention efficiency;

1. Introduction

The accumulation of carbon (C) in soils is a function of the relationship between C inputs in the form of crop residues and organic fertilizers, and the rate of soil C breakdown (decomposition) as mediated by soil microorganisms and the environment (soil type, temperature) (Cooper et al., 2011). Regular inputs of residues, compost, or manure can increase total soil organic C (SOC) until it reaches a higher equilibrium level, related to the balance between C inputs and decomposition processes. This equilibrium level is also affected by the types of C inputs to the system and how these are converted into stable C in the soil by microbial communities (Kallenbach et al., 2016). Crop residues may be relatively labile and not increase levels of stable C in the soil, while materials like biochar are recalcitrant and can have a much longer half-life in the soil (Lorenz et al., 2007; Steinbess et al., 2009). The impacts of historic additions of different quantities and qualities of C inputs have been observed in long-term organic matter addition experiments including the Broadbalk Experiment at Rothamsted, UK (Blair et al., 2006a), the DOK trial in Switzerland (Mäder et al., 2002) and various experiments in China (Cai and Qin 2006; Lou et al., 2011; Ding et al., 2012; Yang et al., 2012; Liu et al., 2013).

SOC is recognized as vital for the delivery of multiple ecosystem services including not only climate regulation i.e. soil C sequestration (Plaza-Bonilla et al., 2014), but also the supporting service of nutrient cycling (Duru et al., 2015). However, the properties of the SOC pool required for these two ecosystem services may not be the same. For soil C to contribute to climate regulation by sequestration, it needs to be in a stable, non-labile form that will not be susceptible to losses should the system be perturbed by a change in tillage (Powlson et al., 2012), small changes in C inputs (Liu et al., 2013), or by climatic changes that increase microbial activity e.g. rising temperatures (Lal et al., 2004).

Soil C pools that promote microbial activity and nutrient cycling are primarily the labile pools (Kaye and Hart, 1997), a series of small, but variable, proportions of SOC with turnover times of a few days to months. These pools have been suggested as early sensitive indicators of soil quality which influence soil function in specific ways (Cambardella et al., 1998; Yang et al., 2005; Rudrappa et al., 2006; Xu et al., 2011; Blanco-Moure et al., 2016). Various techniques are used to estimate the size of the labile C pools. Cmin, which is biologically respired CO_2, indicates the total metabolic activity of the heterotrophic microorganisms in the soil that are decomposing organic matter (Haynes, 2005). Accurate identification of the mineralizable C pool is essential for modelling soil C dynamics and ecosystem responses to changing environmental factors (Saviozzi et al., 2014). POC and LFOC obtained by particle size or density fractionation methods have been used to identify the effects of fertilization practices on soil organic matter in many studies (Wander, 2004). The POC and LFOC concentrations have been found to be elevated in farming systems relying on organic fertility compared with those using synthetic fertilizers (Wander et al., 1994; Fortuna et al., 2003; Nissen and Wander, 2003). Dissolved organic C can be extracted using a weak salt solution (Jensen et al., 1997), and is a measure of carbon easily transportable within ecosystems and the formation of SOC (Neff and Asner, 2001). Organic matter (organic manure or crop residues) additions to soil over time have been demonstrated to increase DOC contents (Gong et al., 2009a; Xu et al., 2011; Liu et al., 2013; Li et al., 2015). Microbial biomass measurement, particularly MBC, which serves as a sink for labile nutrients or a source of nutrients for biota, has been extensively used to assess soil fertility under long-term fertilization regimes (Li et al., 2008; Liu et al., 2013). $KMnO_4$-C, the fraction of labile C which is obtained from chemical oxidation methods using $KMnO_4$ (Blair et al., 1995), has since been considered as an early sensitive index for the impacts of long-term applications of fertilizers or organic resources on the dynamics of the active SOC fraction (Mtambanengwe and Mapfumo, 2008; Xu et al., 2011).

Non-labile C can be estimated as the difference between SOC and $KMnO_4$-C (Blair et al., 2006a). The Carbon Management Index (CMI) can be calculated to give an indication of the changes in the C dynamics of each system and ecosystem response relative to a paired reference soil (Blair et al., 1995). The CMI increases when either or both the treatment total C or labile C increase as a proportion of the reference. The CMI can also be a useful parameter for assessing the potential of long-term manure addition, straw incorporation or conservation agriculture to improve soil quality and thus optimizing practices that impede soil degradation (Xu et al., 2011; Wang et al., 2015a; Ghosh et al., 2016).

The North China Plain (NCP) region, referred to as "China's breadbasket" is a highly productive agricultural area with the main cropping system of a winter wheat-summer maize double-cropping rotation. It is essential to optimize fertilization to maintain crop yields while reducing negative impacts on environment in this region with many researchers focusing on this challenge (Chen et al., 2014). Lin et al. (2009) showed that substituting 100% or 50% of mineral fertilizers with organic manure over 15 years could maintain crop yields and increase SOC compared to equivalent mineral fertilizer treatments in a trial in the NCP region. However it is currently still not known how the different fertilizer treatments in this trial have affected soil labile and non-labile organic C fractions under the 26-year fertilization regimes. Therefore, this study was conducted to investigate how different fertilizer treatments over the 26 year experiment had impacted on the proportions of labile (Cmin, MBC, DOC, POC, LFOC and $KMnO_4$-C) and non-labile C fractions in each treatment.

2. Materials and methods

2.1. Site description and experimental design

This study was carried out on a long-term fertilization experiment started in 1986 at Dezhou Experimental Station (116°34′ E，36°50′ N, altitude: 20m), Chinese Academy of Agricultural Sciences (CAAS), Yucheng, Shandong, China. The full site description and experimental design are described in Li et al. (2015). Briefly, this region belongs to a semi-humid warm temperate continental monsoon climate zone with an average annual temperature of 13.4℃. The annual average sunshine period is 2640 h and the annual average period free of frost is 206 days. The mean annual precipitation is 569.6 mm, and more than 70% of the rainfall falls between June and September. The soil is a Fluvo-aquic type formed from the sediments of the Yellow River with light loam texture (clay 21.4%; silt 65.6%; sand 13.0%). Soil initial chemical properties prior to the beginning of the experiment in 1986 were 3.93 g total soil organic carbon kg^{-1}, 0.51 g total nitrogen kg^{-1}, 7.50 mg Olsen P kg^{-1}, 73.00 mg ammonium acetate-extractable K kg^{-1} and 0.96 g soluble salt kg^{-1}. The experiment mimics the standard winter wheat-summer maize double cropping system which is widely used in the NCP. Standard commercial tillage and irrigation regimes are used.

Six treatments are arranged in a randomized complete block design with four replications (total 24 plots). Each plot is 28 m^2 (4 m×7 m) with a 0.8 m concrete slab separating the plots. The six treatments are: (1) Control with no amendment addition (CK); (2) Standard rate of mineral fertilizer treatment (SMF) that reflects local farmers' practice; (3) Standard rate of organic manure treatment (SMA) with N input rate equal to SMF; (4) Half the standard rate of mineral fertilizer plus half the standard rate of organic manure treatment (1/2 SMF+1/2 SMA); (5) Double standard rate of mineral fertilizer treatment (DMF); (6) Double standard rate of organic manure treatment (DMA). The organic manure is cattle manure from the dairy industry nearby and it is composted by regular turning (3-4 times) over a 4 month period before application. Typical compost nutrient concentrations are 1.00-1.84% N, 0.58-1.02% P_2O_5 and 0.98-1.15% K_2O. All N (mineral fertilizer or cattle manure) application rates are based on total N contents. Fertilizer N, P and K sources are urea (47% N), mono-calcium phosphate (17% P_2O_5) and potassium sulphate (50% K_2O) with the standard application rates of 375-450 kg N ha^{-1}, 225-300 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1} per year, respectively. Organic manure and total mineral fertilizer P and K are applied once before winter wheat sowing. Total mineral fertilizer N is applied twice per year: half is applied in October before winter wheat sowing and the other half is applied in June before summer maize sowing. For winter wheat, the N application is split with 40% N applied before sowing and 60% N applied to the soil surface between the rows at jointing stage of winter wheat. For summer maize, 40% N is applied before sowing, and 60% N is applied to the soil surface between the rows at the elongation stage. The basal application of N, total P, total K and organic manure are uniformly broadcast onto the topsoil before plowing the soil.

2.2 Soil sampling, chemical analysis and calculations

Soil samples were collected from the surface soil layer (0-20 cm soil depth) of each plot at the end of September 2012 after summer maize harvest. At least 5 soil cores were taken with a 5 cm diameter auger per plot and mixed together, and immediately stored in an ice chest until they were transported to the laboratory. After removing the visible organic materials, stones and fine roots by hand, the samples were divided into two parts. One part of the fresh soil sample was passed through a 2mm mesh sieve and was kept at 4°C for measurement of soil DOC, MBC and Cmin within 2 weeks, and the other part was air-dried and sieved through either a 0.15mm mesh for the estimation of SOC and $KMnO_4$-C (Shang et al., 2011) or a 2mm mesh prior to the measurement of POC and LFOC.

For bulk density (BD) measurement soil cores (0-20 cm, 3 replicates in each plot) were collected using a soil core sampler (the volume is 100 cm^3) and were dried in an oven at 105°C for 24 h before weighing. BD was calculated as the ratio of the dry weight of the soil core and the internal volume of the metallic core (Lu, 2000).

SOC was determined using vitriol acid-potassium dichromate wet oxidation method (Walkey and Black, 1934) with additional heat provided (170-180°C for 5 minutes) and application of a correction factor of 1.1 to account for incomplete digestion (Heanes, 1984). SOC stocks (Mg C ha^{-1}) in the corresponding soil layer were calculated as:

SOC stocks (Mg ha^{-1}) = SOC concentration (g kg^{-1}) × bulk density (Mg m^{-3}) × soil depth (m) × 10 (Liu et al., 2013).

Permanganate oxidizable C ($KMnO_4$-C) was measured as described by Blair et al. (1995) with the change in concentration of $KMnO_4$ used to estimate the amount of carbon oxidized assuming that 1.0 mmol L^{-1} of MnO_4^- was consumed ($Mn^{7+} \rightarrow Mn^{2+}$) in the oxidation of 0.75 mmol L^{-1} (9.0 mg) of carbon.

Particulate organic C (POC) was determined with modifications of the method described by Cambardella and Elliott (1992). In brief, 10 g air-dry soil and 30 mL Na hexametaphosphate solution (5g L^{-1}) were added to a 100 mL centrifuge tube, and shaken for approximately 18 h. The soil suspension was poured over a 53-µm screen and the retained coarse fraction was rinsed with a weak stream of distilled water. All material remaining on the screen was washed into a dry dish, oven dried at 60 °C for 48 h, and ground to determine C content using the modified Walkley-Black method described above.

Microbial biomass C (MBC) was determined by the $CHCl_3$ fumigation–extraction method (Vance et al., 1987). Extract C concentration was determined using a Multi 2011 N/C TOC analyzer (Analytik Jena, Germany). Extracted C was converted to microbial biomass C as follows: MBC (mg C kg^{-1}) = (fumigated C-non fumigated C)/0.38 (Vance et al., 1987).

Dissolved organic C (DOC) was determined by the method of Jones and Willett (2006). C concentrations in the extracts were measured using the Multi N/C 2100 Analyzer as described above.

Light fraction organic C (LFOC) was determined using the density fractionation method as described by Janzen and Campbell (1992). C concentrations in the extracts were measured using the Multi N/C 2100 Analyzer as described above.

The carbon management index (CMI) was obtained according to the method of Blair et al. (1995). CMI was calculated as follows:

CMI=Carbon Pool Index (CPI) × Lability Index (LI) ×100, where

CPI = Total C content of sample soil / Total C content of reference soil

LI =Lability of C in sample soil / Lability of C in reference soil

The C lability is the ratio of labile C ($KMnO_4$-C) to non-labile C, and non-labile C is determined as the difference between total C content and labile C ($KMnO_4$-C) content of soil. In this study, the soil sampled in CK is used as the reference soil.

2.3 Annual carbon input calculations

The mean annual carbon input supplied by each source was estimated by summing up manure additions from the beginning of the experiment as well as estimated inputs from rhizodeposition, stubble (above-ground residues below harvest height), roots and straw (Table 1). In this experiment, straw refers to above-ground crop residues above harvest height, which are typically removed from the field in this region, so the C input from straw was zero.

Both grain (wheat and maize) yields and organic manure C (MA-C) were measured annually; the biomass of above-ground residues (straw and stubble) and below-ground residues (mainly root biomass) were assumed to be proportional to the crop grain yield using the ratio of 43:37:20 for grain:above-ground residues:below-ground residues for both wheat and maize, as reported by Tang et al. (2012). Stubble was assumed to represent 10% of the straw (Liu et al., 2013). Hence the above-ground residues, root biomass and stubble were calculated as 86%, 47% and 8% of the grain yields, respectively. According to the local crop management protocol, the stubble and roots of wheat are left in the soil while the stubble and roots of maize are removed from the ground; hence, we only included the average root and stubble biomass of the wheat. Total rhizodeposition (root exudates) represented 15% of above-ground biomass (crop grain yield plus above-ground residues) at maturity (Liu et al., 2013). The concentrations of C in the dry matter were assumed to be 44% in the above ground residues and 38% in root (Tang et al., 2012).

The retention efficiency (RE) of the added C inputs was calculated as the average change in C stocks per year divided by the average annual C inputs, expressed as a percentage.

2.6 Statistical analysis

The SAS (SAS Systems, Cary, NC, USA) and Microsoft excel 2007 (Microsoft Corporation, USA) were used to carry out data processing and statistical analysis (ANOVA). The effects of long-term fertilization regimes on soil BD, SOC concentrations, SOC stocks, soil labile organic C fractions (Cmin, MBC, DOC, POC, LFOC and $KMnO_4$-C) and CMI were analyzed using one-way ANOVA with separation of means by least significant difference (LSD) test ($P < 0.05$). Moreover, redundancy analysis (RDA) was used to analyze the relationship between the proportion of each labile organic C fraction to total SOC and the proportion of C from each source to total C input with Canoco version 4.5.

3. Results

3.1 Soil BD

Soil BD ranged between 1.12 and 1.35 Mg m^{-3} in all treatments and was significantly lower in soils fertilized with organic manure (DMA, SMA and 1/2SMF+1/2SMA) compared to soils treated with mineral fertilizers (DMF and SMF); the control treatment (CK) had the highest BD (Table 2). Increasing organic manure input rates significantly decreased BD (1.12 Mg m^{-3} for DMA, 1.19 Mg m^{-3} for SMA and 1.24 Mg $m^{-3}$for 1/2SMF+1/2SMA). There was no statistically significant difference in BD between the SMF and DMF treatments.

3.2 SOC concentrations and SOC stocks

Long-term continuous fertilizer input significantly increased SOC concentrations and stocks after 26 years for all treatments (Table 2). Treatments which received organic manure (DMA, SMA, 1/2SMF+1/2SMA) had significantly higher SOC concentrations and stocks compared to the two mineral and control treatments, storing as much as 35.39 Mg ha^{-1} more C in the top 20cm than the CK treatment. Furthermore, SOC concentrations and stocks significantly increased with increasing manure input rates, while there was no difference in SOC stocks between the two rates of mineral fertilizers (DMF and SMF) ($P < 0.05$). The lowest SOC concentrations and SOC stocks after 26 years were observed in the CK treatment.

The control treatment had the highest retention efficiency (~37%) while the lowest RE was for treatments that included manure (10-11%). Treatments that included mineral fertilizer only had REs of 16% for the standard rate of fertilizer and 19% where double the standard fertilizer rate was used.

3.3 Soil labile organic C fractions

The effects of different fertilizer treatments for 26 years on soil labile organic C fractions (Cmin, MBC, DOC, POC, LFOC and $KMnO_4$-C) are shown in Fig.1 (P < 0.05). Table 3 lists the proportion of each labile organic C fraction relative to total SOC under long-term fertilization regimes.

Treatments which received organic manure alone evolved greater cumulative amounts of CO_2-C (Cmin) from soils after 21 days of incubation (467 mg CO_2-C g^{-1} soil for DMA and 279 mg CO_2-C g^{-1} soil for SMA) than other treatments and the lowest Cmin concentrations (109 mg CO_2-C g^{-1} soil) were found in the CK treatment. Application of mineral fertilizers combined with organic manure (1/2SMF+1/2SMA) significantly increased C mineralization by 81% compared to the SMF treatment and by 32% compared to the DMF treatment. Mineralizable C in SMF and CK treatments were not significantly different from each other (Fig.1). Cmin comprised very small proportions (1.49-1.89%) of the total SOC, and the proportions of Cmin to SOC in DMA, DMF, SMA and 1/2SMF+1/2SMA were significantly higher than those in SMF and CK treatments (Table 3).

MBC ranged from 168.84 to 471.04 mg kg^{-1}, constituting about 1.74-2.32% of total SOC (Fig.1 and Table 3). The long-term application of N through organic manure alone (DMA and SMA) resulted in a significant increase in MBC compared to mineral-fertilized plots (DMF and SMF) and CK, meanwhile, the MBC concentration significantly increased with increasing rate of organic manure application. Similarly, substitution of 50% N through manure (1/2SMF+1/2SMA) also increased the POC concentration compared to SMF. MBC was lowest in CK and SMF treatments with no statistical difference between each other. The values for MBC to SOC as influenced by different fertilizer treatments showed an opposite trend to the MBC concentrations with higher proportions under long-term application of mineral fertilizer alone and CK, and lower proportions under long-term application of organic manure alone.

DOC concentrations in soils followed a pattern similar to Cmin and MBC concentrations among all treatments. DOC was highest in only manure-treated soils and lowest in soils treated with only mineral fertilizer or the control soil. Increasing manure input levels resulted in higher levels of DOC with a concentration 1.17 times higher under DMA compared with SMA. The integrated treatment (1/2SMF+1/2SMA) markedly increased DOC content compared to CK. DOC comprised the smallest proportion (0.84-1.19%) of SOC and was significantly affected by different fertilizer treatments, with the highest proportion in the SMA treatment and the lowest in the 1/2SMF+1/2SMAtreatment.

Pure organic manure treatments (DMA and SMA) showed significantly higher concentrations of POC as compared to integrated (1/2SMF+1/2SMA) and mineral-fertilized plots (DMF and SMF) ($P \leqslant 0.05$). POC constituted 10.20 to 23.65% of total SOC with a mean value of 16.43%. Highest proportion of POC was observed under DMA, followed by SMA, which was not significantly different from DMF; 1/2SMF+1/2SMA and SMF had a lower proportion of POC and the lowest proportion was found in the CK treatment.

In the surface soil (0-20cm), the LFOC concentration was 60% higher under DMA than under CK. Other treatments showed no significant effects on LFOC concentrations relative to CK. At the same standard N input level, SMA contained 27% higher organic C in LFOC than SMF, however, there was no significant difference between 1/2SMF+1/2SMA and SMF. Long term application of mineral fertilizers alone slightly decreased LFOC concentrations relative to CK, but this effect was not statistically significant. The fraction of SOC as LFOC ranged from 1.73 to 3.29% with an average value of 2.56% in SOC, and exhibited a pattern similar to MBC.

The plots receiving organic manure had significantly higher $KMnO_4$-C compared to mineral-fertilized plots and CK ($P < 0.05$, Fig.1). $KMnO_4$-C concentrations were higher when double the rate of either manure or mineral fertilizer was used ($P < 0.05$, Fig 1). Moreover, $KMnO_4$-C accounted for the highest proportion of SOC compared with other labile organic C fractions. The proportion of $KMnO_4$-C varied from 14.48 to 21.89% with the mean value of 18.39% of total SOC. The impacts of different fertilizer treatments on the proportion of $KMnO_4$-C were similar to POC, with highest proportions in DMA and lowest in CK.

3.4 Carbon pool index and carbon management index

There were significant differences among all fertility treatments for soil carbon pool index (CPI) and carbon management index (CMI) (Table 4). Changes in CPI under different fertilizer treatments decreased in the order DMA >SMA >1/2SMF+1/2SMA >DMF >SMF, with values ranging from 1.17 to 3.35. With reference to CMI, it showed a similar trend to CPI as influenced by different fertilizer treatments. The highest values of CMI were associated with the treatments where the entire amount of nitrogen was applied through organic manure, followed by 1/2SMF+1/2SMA treatment. There was also a significant improvement in CMI under DMF treatment compared with SMF treatment.

3.5 Correlations between soil labile organic C fractions and estimated annual C inputs

Redundancy analysis showing the proportion of estimated annual C input from each source to total C input as drivers and the proportion of each labile organic C fraction to SOC as well as CMI as responses was conducted to help us to understand which types of C are causing the changes in the labile organic C fractions (Fig. 2). The first two axes accounted for 49.79% of total variation and all the explanatory variables had significant impacts on the proportion of each labile organic C fraction in SOC and CMI ($P < 0.01$). Fig. 2 showed that changes in the proportion of MBC, LFOC and DOC to SOC were positively correlated with the changes in stubble-C, rhizodeposition-C and root-C along axis 1. Changes of Cmin, POC and $KMnO_4$-C in total SOC were closely related to additions of MA-C as shown by their alignment along the negative axis of axis 1; this was also the case for CMI. The organic manure fertilized treatments (DMA, SMA and 1/2SMF+1/2SMA) were significantly differentiated from other treatments along the negative axis of axis 1 and all of them were clearly separated from each other. The control treatment was significantly separated from other treatments along the positive axis of axis 1 and correlated with higher MBC and LFOC contents. The SMF and DMF treatments were not separated by the RDA.

4. Discussion

4.1 Effect of long-term fertilization regimes on soil BDs, SOC concentrations and stocks

The dominant effect in all the fertilized plots was the development of porosity as indicated by the significant reduction in BDs, especially in manure-treated plots. BDs decreased as rates of organic manure addition increased; this could be due to the formation of macro-pores and macro-aggregates induced by the cementing action of organic acids and polysaccharides excreted by microorganism during the decomposition of the added organic manure (Rasool et al., 2008; Yu et al., 2012; Brar et al., 2013). Mineral fertilization decreased soil BD relative to the control treatment, a finding supported by Liu et al. (2014). These decreases may reflect higher levels of stubble, wheat root and rhizodeposition inputs when mineral fertilizer is used compared to the control (Table 1).

This pattern reflected changes in SOC; highest SOC was correlated with the lowest BD in the manure-fertilized treatments, with the mineral fertilized treatments having moderate levels of SOC and BD, and lowest SOC and highest BD in the CK treatment. Loss of organic matter often results in increased BD of the surface soil because organic matter stabilizes soil aggregates against slaking, dispersion and collapse (Logsdon and Karlen, 2004), which is consistent with our results that increases in soil SOC were associated with decreased BD in different fertilizer treatments (Table 2).

Numerous studies have reported that changes in carbon sequestration pools and dynamics were induced by soil management practices, such as afforestation (Laik et al., 2009), conservation tillage (Liu et al., 2014; Wang et al., 2014), and also fertilization practices (Yan et al., 2007; Xu et al., 2011; Yang et al., 2012; Wang et al., 2015b). The amount of C sequestered and the rate of C sequestration is also related to the type and quality of C added i.e. which pool it contributes to. Additions to labile pools may not benefit C sequestration because they are rapidly mineralized by soil organisms (Powlson et al., 2012).

In our report, SOC concentrations in unfertilized soil were greatly increased after 26 years of maize-wheat double-cropping compared to initial soil levels (3.93g kg^{-1}). This can be attributed to two factors. First, an increase in root exudates from modern higher yielding varieties, and second, from the C contained in the stubble which is returned to the soil each year. Historically on the NCP all stubble and even the roots of crops were removed from the field and used as fuel by peasant farmers (Mu et al., 2016). In today's farming system, roots are left in the soil and a no-till approach to summer maize seeding has been adopted; these may be contributing to the increase in soil C contents in the control plots even though most of the above-ground residues are removed from the land. The C contained in the stubble and root exudates provides a substrate for the production of relatively stable end-products, since the retention efficiency of C inputs in the control treatment was ~37%.

Soil C levels in the mineral fertilized treatments also increased marginally relative to the control plots. There are conflicting reports about the impact of mineral fertilization on SOC sequestration (Khan et al., 2007; Reid, 2008); some reports demonstrated that the use of synthetic N fertilization induced a net loss of SOC (Khan et al., 2007; Mulvaney, 2009; Lou, 2011), however, other reports indicated that long-term mineral fertilization could increase SOC stocks in the topsoil layer (Johnston et al., 2009; Gong et al., 2012; Fan et al., 2014). Such differences seem to depend on the initial soil C status, the ecosystem under study, the quantity and quality of residues returned and the nature, quantity and duration of fertilizer application (Reid, 2008; Hamer et al., 2009). The increases in SOC concentration and stocks in our study may be due to more available nutrients being provided for better crop growth, resulting in increased root debris and exudates being returned to the soil (Table 1). This has been supported by many reports from other long-term field experiments (Blair et al., 2006b; Fan et al., 2014; Kätterer et al., 2014). There were no statistical differences in DMF and SMF SOC concentrations and stocks, which reflects the similar annual C inputs from crop residues in these treatments (Table 1).

SOC concentrations and stocks increased considerably with organic manure incorporation rates, which is possibly attributed to a larger proportion of recalcitrant organic compounds in manure (Drinkwater et al., 1998; Liu et al., 2014), farmyard manure application can result in an increase in lignin and lignin-like products, which are major components of the resistant C pool in the soil (Lima et al., 2009). Crop production was also enhanced by the manure inputs, which lead to higher total C inputs from rhizodeposition, root biomass and stubble return (Table 1) (Lin et al., 2009). The significant increases in non-labile C in the manure-fertilized soils (Table 4) indicate that manure addition could be a strategy to improve SOC stabilization in the long term (Ding et al., 2012).

4.2 Effect of long-term fertilization regimes on soil labile organic C fractions

It has been widely accepted that application of organic manure markedly increases labile organic C fractions (Gong et al., 2009a; Ding et al., 2012; Liang et al., 2012) directly or indirectly, which is consistent with our findings. This effect can be explained by 2 factors. First, through directly contributing to the soil's labile organic C pool and second, by enhancing microbial activities in organically amended treatments thereby increasing the conversion of plant residue-C into labile forms of organic C (Aita et al., 1997; Poirier et al., 2013; Whalen et al., 2014).

Cmin and DOC are produced from decomposition of soil organic matter mainly driven by soil microbes (Marschner et al., 2002), and MBC is indicative of the size of the microbial biomass that does the decomposing (Powlson et al., 1987). Although they account for only a small proportion of SOC (generally 0.80-12.00% for Cmin 0.05-0.50% for DOC and 0.30-4.00% for MBC) in agricultural soils, these measures of soil C are considered good indicators of the soil's potential to cycle nutrients, a key ecosystem service (Kaur et al., 2005; Haynes, 2005; Moharana et al., 2012; Benbi et al., 2015). Significant increases in Cmin, DOC and MBC were observed after organic manure addition, suggesting that organic manure alone or combined with mineral fertilizers had beneficial effects on the activity of microorganisms probably by providing a readily-available source of C substrate and improving the soil physical environment e.g. porosity (Lou et al., 2011; Yang et al., 2012). Inconsistent effects of mineral fertilizers on MBC have been reported, with positive, negative and no impacts (Xue et al., 2006; Gong et al., 2009b; Lou et al., 2011). In our study MBC as a proportion of SOC was highest in the two mineral fertilized treatments and control (Table 3), suggesting that inputs of C from roots/stubble/rhizodeposition in these three treatments promoted growth of the microbial biomass but did not contribute as much to the total SOC. A negative relationship between the MBC:SOC ratio and total SOC concentrations was also reported in Jiang et al. (2006) in a seeded alfalfa grassland on the Loess Plateau of China. Results in Clemente et al. (2013) indicate that root amendment may enhance contributions from microbial-derived OM, which corresponds with our investigation since the DMF, SMF and CK treatments included higher proportions of root-C to total C input (Table 1).

Soil DOC represents the most bioavailable source of C substrates (Marschner and Kalbitz, 2003) and a key source of C for microbial metabolic maintenance needs (Xu et al., 2011). This explains why in our study soils with the highest DOC also had highest Cmin and MBC (Montaño, 2007). However, long-term use of mineral fertilizers alone had no significant effect on DOC regardless of the fertilizer addition rates, which is in agreement with Zsolnay and Görlitz (1994), confirming that the primary source of DOC is manure inputs. While DOC as a proportion of SOC was highest for the SMA treatment, it was lowest for the DMA treatment, possibly reflecting the higher levels of undecomposed organic C in the DMA treatment, as evidenced by its high proportion of POC (Wright et al., 2005).

Haynes (2005) reported values of 20-45% for POC and 2-18% for LFOC as a proportion of SOC in agricultural soils and Wander (2004) reported that the proportion of POC and LFOC varied from 2-30%. Our results are at the low end of these ranges, reflecting the relatively low annual inputs of fresh C in our treatments (Table 3). Soil POC and LFOC belong to physically uncomplexed organic matter which is isolated on the basis of particle size and/or density using physical fractionation techniques (Gregorich et al., 2006). Both the POC and LFOC are considered to include decomposing plant and animal residues that are rapidly turned over, and hence are important sources of plant nutrients (Wander et al., 1994). A meta-analysis of data from over 150 experiments has confirmed that both POC and LFOC can be used to predict long-term changes in total SOC very well (Gosling et al., 2013). Our results are supported by numerous reports (Yan et al., 2007; Yang et al., 2012; Ibrahim et al., 2015), indicating that higher C input induced by fertility management practices resulted in significantly larger physically uncomplexed organic carbon (POC and LFOC) pools (Fig.1). Gosling et al (2013) also indicated that POC and LFOC were strongly influenced by factors related to the recent history of organic matter addition. In our study the proportion of organic C in POC was greater than that in LFOC, which is consistent with previous reports (Gregorich et al., 2006; Yan et al., 2007). Significant quantities of C from organic manure are retained in soil particulate fractions as suggested by Whalen et al. (2003) while LFOC contains more lignin derivatives, carbohydrate constituents, and aliphatic compounds than POC and is much more closely related to plant residues (Gregorich et al., 2006; Yan et al., 2007). This also explains why the highest proportion of POC was found in the DMA treatment while the highest proportion of LFOC was found in the CK, SMF and DMF treatments (Table 3). Fig. 2 also indicated that changes in POC were closely linked to changes in MA-C, however, changes in LFOC resulted from changes in rhizodeposition-C, stubble-C or root-C.

C extractable with $KMnO_4$-C consists of amino acids, simple carbohydrates, a portion of soil microbial biomass and other simple organic compounds and is the fraction of SOC with a rapid turnover time (Zou et al., 2005). Since $KMnO_4$-C can respond rapidly to changes in C supply, it is considered an important indicator of soil quality (Haynes, 2005; Xu et al., 2011). Our study indicated that $KMnO_4$-C was higher in total and as a proportion of the total C in manure-treated soils compared to mineral fertilized soils and unfertilized soils, confirming that manure inputs drive changes in pools of labile C as already discussed for the other measures of labile C.

4.3 Effect of long-term fertilization regimes on CMI

It has been confirmed that the carbon management index (CMI) is a useful parameter to assess the capacity of management systems to improve soil quality (Blair et al., 1995; Diekow et al., 2005). The index incorporates a measure of the impact on total soil C (CPI) and the lability of that C (LI) thus reflecting both C sequestration and nutrient cycling potential. Since the index is the product of these two measures, only treatments that score highly on both will have a high CMI. In our research, the effects of fertilization regimes on CMI were significant with CMI increasing in treatments receiving organic manure compared to those treatments receiving mineral fertilizers. This is confirmed by numerous studies on long-term fertilization systems (Xu et al., 2011; Ghosh et al., 2012). Tirol-Padre and Ladha (2004) explained that variations of CMI in different fertilizer treatments are attributed to the increase in annual C addition and the changes in organic matter quality, thus affecting the susceptibility of C to $KMnO_4$ oxidation. CMI was highly correlated with the amount of each labile organic C fraction and total SOC ($P < 0.05$, data not shown). Our reports also indicated that the proportion of MA-C in the total C input significantly increased CMI (Fig. 2).

5. Conclusions

Our results clearly indicated that 26 years of organic manure addition or combined manure-mineral fertilizer application significantly decreased soil bulk density, increased total SOC concentrations, SOC stocks and labile organic C fractions (Cmin, MBC, DOC, POC, LFOC and $KMnO_4$-C) compared to mineral fertilizers or the unfertilized control in a wheat-maize rotation system in the North China Plain. Results were additive with higher rates of organic manure resulting in further beneficial effects. Great improvement in the values of CMI under organic or integrated organic-mineral fertilized treatments indicated enhanced delivery of C sequestration and nutrient cycling soil ecosystem services compared to mineral fertilized treatments. In conclusion, organic manure fertilization or integrated organic manure with mineral fertilization could be important strategies for improving SOC status and maintaining soil quality in soils in the North China Plain.

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Table 1 Estimated mean annual carbon input (Mg C ha^{-1} y^{-1}) with % of total in parentheses in different fertilizer treatments based on analysis of added material each year.

Treatments	Manure-C	Stubble-C (wheat)	Root-C (wheat)	Rhizodeposition-C	Total C input
CK	0 (0)	0.06 (6)	0.32 (33)	0.59 (61)	0.97
SMF	0 (0)	0.20 (7)	1.04 (37)	1.59 (56)	2.83
SMA	6.75 (73)	0.17 (2)	0.85 (9)	1.53 (16)	9.30
1/2SMF+1/2SMA	3.38 (55)	0.20 (3)	1.00 (16)	1.57 (26)	6.14
DMF	0 (0)	0.20 (7)	1.00 (36)	1.57 (57)	2.77
DMA	13.50 (83)	0.20 (1)	0.99 (6)	1.62 (10)	16.31

CK: control with no amendment addition; SMF: standard rate of mineral fertilizer; SMA: standard rate of organic manure; 1/2SMF+1/2SMA: half the standard rate of mineral fertilizer plus half the standard rate of organic manure; DMF: double standard rate of mineral fertilizer; DMA: double standard rate of organic manure

Table 2 Effects of long-term fertilization regimes on soil bulk density (BD), soil organic carbon (SOC) and SOC stocks under a wheat–maize rotation in China (0-20cm). Long term averages (mean ± SE) followed by the same letter in the same column are not significantly different (LSD, $P < 0.05$) in different fertilizer treatments.

Treatments	**BD ($Mg\ m^{-3}$)**	**SOC ($g\ kg^{-1}$)**	**SOC stocks ($Mg\ ha^{-1}$)**
CK	1.35±0.03a	7.34±0.37e	19.88±1.37e
SMF	1.30±0.05ab	8.57±0.72d	22.28±2.51de
SMA	1.19±0.02c	15.68±0.54b	37.17±1.47b
1/2SMF+1/2SMA	1.17±0.08c	12.24±1.22c	28.63±4.47c
DMF	1.26±0.03b	9.56±0.62d	24.15±1.84d
DMA	1.12±0.05c	24.61±0.28a	55.27±2.93a

CK: control with no amendment addition; SMF: standard rate of mineral fertilizer; SMA: standard rate of organic manure; 1/2SMF+1/2SMA: half the standard rate of mineral fertilizer plus half the standard rate of organic manure; DMF: double standard rate of mineral fertilizer; DMA: double standard rate of organic manure.

Table 3 Labile organic C fractions including mineralizable C (Cmin), microbial biomass C (MBC), dissolved organic C (DOC), particulate organic C (POC), light fraction organic C (LFOC), and permanganate oxidizable C ($KMnO_4$-C) as a proportion of total SOC (%) in different fertilizer treatments. Averages (mean ± SE) followed by the same letter in the same column are not significantly different (LSD, $P < 0.05$).

Treatments	Cmin/SOC	MBC/SOC	DOC/SOC	POC/SOC	LFOC/SOC	$KMnO_4$-C/SOC
CK	1.49±0.06b	2.30±0.17a	1.06±0.05b	10.20±1.45e	3.29±0.63a	14.48±0.43d
SMF	1.40±0.17b	2.13±0.24ab	0.96±0.08cd	13.52±1.78d	3.02±0.14ab	16.41±1.74c
SMA	1.78±0.07a	1.74±0.02c	1.19±0.03a	19.03±1.52b	2.13±0.18de	19.50±0.73b
1/2SMF+1/2SMA	1.78±0.03a	1.99±0.09b	0.84±0.08e	15.31±0.61cd	2.51±0.11cd	19.33±1.17b
DMF	1.73±0.07a	2.32±0.16a	1.04±0.04bc	16.84±1.99bc	2.69±0.20bc	18.74±1.20b
DMA	1.89±0.20a	1.91±0.14bc	0.89±0.09de	23.65±3.10a	1.73±0.33e	21.89±0.16a

CK: control with no amendment addition; SMF: standard rate of mineral fertilizer; SMA: standard rate of organic manure; 1/2SMF+1/2SMA: half the standard rate of mineral fertilizer plus half the standard rate of organic manure; DMF: double standard rate of mineral fertilizer; DMA: double standard rate of organic manure.

Table 4 Soil Carbon pool index (CPI), Non-labile C, Lability of C (L), Lability index (LI), and Carbon management index (CMI) of different fertilizer treatments. Long term averages (mean ± SE) followed by the same letter in the same column are not significantly different (LSD, $P <$ 0.05) in different fertilizer treatments. "-" indicate no available data.

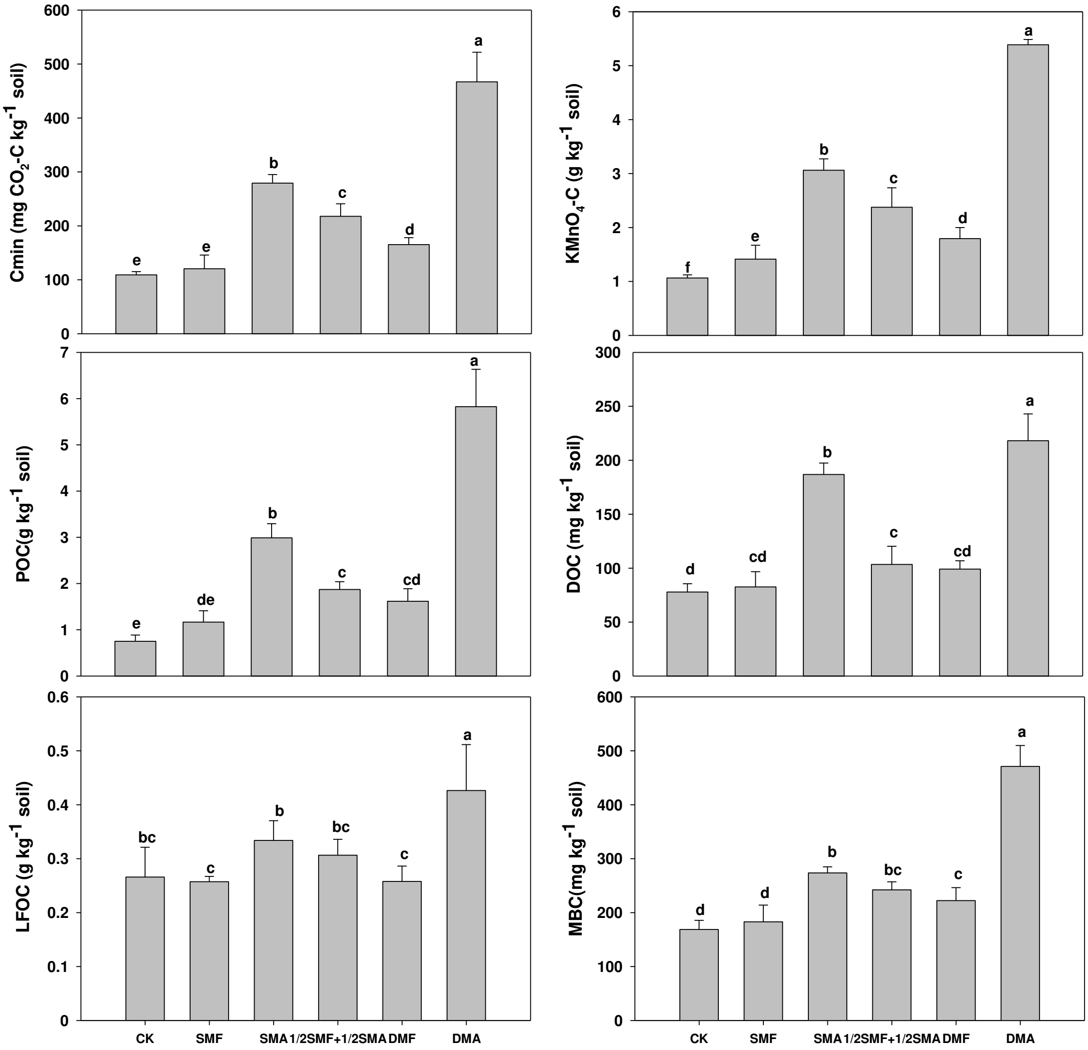
Treatments	CPI	Non-labile C ($g\ kg^{-1}$)	L	LI	CMI
CK	-	6.28±0.29e	0.17±0.01d	-	-
SMF	1.17±0.05e	7.16±0.49d	0.20±0.03c	1.16±0.12c	135.41±18.44e
SMA	2.14±0.07b	12.62±0.33b	0.24±0.02b	1.42±0.03b	304.68±12.65b
1/2SMF+1/2SMA	1.66±0.12c	9.87±0.87c	0.24±0.02b	1.41±0.06b	234.90±23.77c
DMF	1.30±0.07d	7.77±0.41d	0.23±0.02b	1.36±0.07b	176.46±8.47d
DMA	3.36±0.12a	19.23±0.19a	0.28±0.00a	1.65±0.07a	554.19±37.67a

CK: control with no amendment addition; SMF: standard rate of mineral fertilizer; SMA: standard rate of organic manure; 1/2SMF+1/2SMA: half the standard rate of mineral fertilizer plus half the standard rate of organic manure; DMF: double standard rate of mineral fertilizer; DMA: double standard rate of organic manure

Figure Legends:

Figure 1 Effects of long term fertilization regimes on contents of soil organic carbon (SOC) and labile organic C fractions (Cmin, cumulative carbon mineralization of 21 days in a 21-day incubation experiment; $KMnO_4$-C, permanganate oxidizable carbon; POC, particulate organic carbon; DOC, dissolved organic carbon; LFOC, light fraction organic carbon; MBC, microbial biomass carbon) in soil at 0-20 cm depth in intensive Chinese maize/wheat rotations. (**CK**, control with no amendment addition; **SMF**, standard rate of mineral fertilizer treatment that reflect local farmer practice; **SMA**, standard rate of organic manure treatment with N input rate equal to SMF; **1/2SMF+1/2SMA**, half the standard rate of mineral fertilizer plus half the standard rate of organic manure treatment; **DMF**, double standard rate of mineral fertilizer treatment; **DMA**, double standard rate of organic manure treatment.) Long term averages (mean ± SE) followed by the same letter are not significantly different (LSD, $P < 0.05$) in different fertilizer treatments.

Figure 2 Redundancy analysis (RDA) of the proportion of each labile organic C fraction (Cmin, MBC, DOC, POC, LFOC, $KMnO_4$-C) to SOC as well as CMI constrained by the proportion of estimated amount of annual C input from each source (MA-C, stubble-C, rhizodeposition-C and root-C) to total C input under long-term fertilization regimes. (● CK: control with no amendment addition; ◀ SMF: standard rate of mineral fertilizer treatment that reflect local farmer practice; ◆ SMA: standard rate of organic manure treatment with N input rate equal to SMF; ▼ 1/2SMF+1/2SMA: half the standard rate of mineral fertilizer plus half the standard rate of organic manure treatment; ×DMF: double standard rate of mineral fertilizer treatment; ▲DMA: double standard rate of organic manure treatment).



Cmin (mg CO_2-C kg^{-1} soil)
$KMnO_4$-C (g kg^{-1} soil)
POC(g kg^{-1} soil)
DOC (mg kg^{-1} soil)
LFOC (g kg^{-1} soil)
MBC(mg kg^{-1} soil)
CK
SMF
SMA
1/2SMF+1/2SMA
DMF
DMA

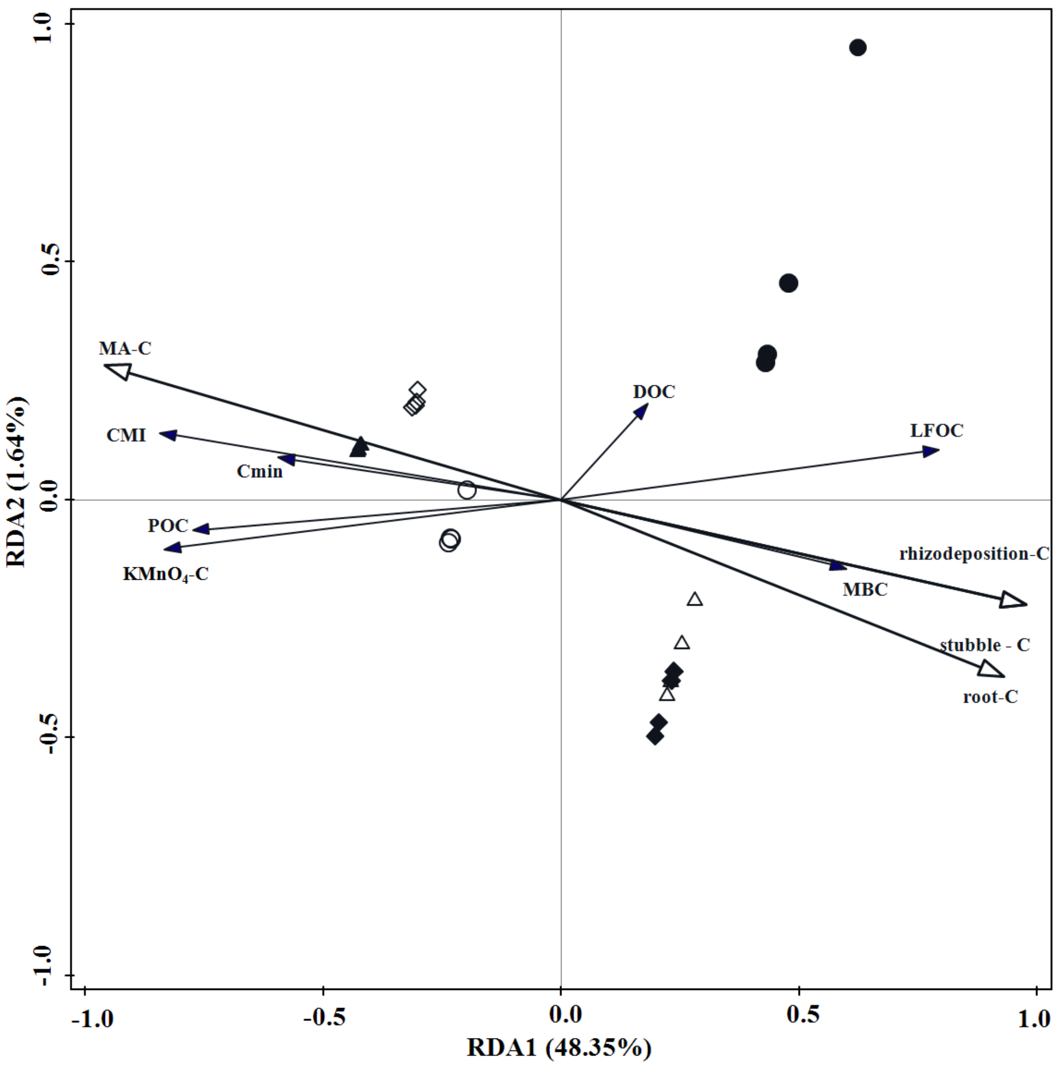


Fig.2 Redundancy analysis (RDA) of the proportion of each labile organic C fraction (Cmin, MBC, DOC, POC, LFOC, $KMnO_4$-C) to SOC as well as CMI constrained by the proportion of estimated amount of annual C input from each source (MA-C, stubble-C, rhizodeposition-C and root-C) to total C input under long-term fertilization regimes. (● CK: control with no amendment addition; ◆ SMF: standard rate of mineral fertilizer treatment that reflect local farmer practice; ◇ SMA: standard rate of organic manure treatment with N input rate equal to SMF; ○ 1/2SMF+1/2SMA: half the standard rate of mineral fertilizer plus half the standard rate of organic manure treatment; △ DMF: double standard rate of mineral fertilizer treatment; ▲ DMA: double standard rate of organic manure treatment).