Contrasting impacts of manure and inorganic fertilizer applications for nine years on soil organic carbon and its labile fractions in bulk soil and soil aggregates

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Abstract

Careful nutrient management to increase soil organic carbon (SOC) content is important in increasing agricultural productivity and maintaining ecosystem health. A field experiment was conducted for nine years to investigate the effects of manure (M) and inorganic fertilizer (NPK) on SOC and its labile fractions within the bulk soil and in soil aggregates in a wheat-maize rotation on the North China Plain. Nine treatments were included: control (CK) with no fertilization, cattle manure (M, applied at rates of 3000, 6000, 9000, and 12000 kg ha^{-1} $crop^{-1}$), and inorganic NPK fertilizer applied to give equivalent rates of N, P and K. Straw was returned to all plots. Results showed that fertilization significantly increased SOC sequestration and the concentrations of SOC and labile SOC fractions (cold water - extractable SOC, hot water-extractable SOC, microbial biomass C, and dissolved organic C within the bulk soil and soil aggregates). The values increased with increasing application rate of manure but not with increasing NPK fertilizer rate. The differences between manure and NPK fertilizer were apparent at rates equivalent to 9000 and 12000 kg manure ha^{-1}. Labile SOC fractions were linearly correlated with SOC within the bulk soil and aggregates and hot water-extractable C was the labile C fraction most sensitive to changes in SOC content. Aggregate stability was significantly positively correlated with SOC content and its labile fractions in both bulk soil and aggregates. The results indicate that straw return and/or combined application of fertilizers and manures may be feasible in achieving the '4 per 1000' initiative, with manure application particularly effective. Manure application at a rate of 9000 kg ha^{-1} $crop^{-1}$ may be the optimum strategy to sequester C and maintain high crop productivity. Periodic high application rates of manures should be integrated with appropriate inorganic fertilizer application rates to optimize nutrient management strategies on calcareous soils.

Key words: Fertilization; Organic carbon; Aggregate stability; North China Plain

1. Introduction

Soils store more than three times as much carbon (C) as the atmosphere or terrestrial vegetation globally (Lehmann and Kleber, 2015), and arable soils contain nearly 10% of the global soil C stocks in the top 1-m of the soil profile (Jobbagy and Jackson, 2000). Soil organic carbon (SOC) plays an important role in increasing crop productivity and maintaining soil health, improving soil structure, mitigating climate change, and supporting other ecosystem services (Zhang et al., 2012). Strategies to increase SOC storage in arable soils include C inputs in the form of crop residues, composts or manures to increase the equilibrium level of organic C inputs and decomposition processes (Cooper et al., 2011). Of these, manure application is effective in increasing SOC content through direct C inputs and/or indirect increase in belowground C inputs. However, the effectiveness of manure application in increasing SOC content depends on the manure application rate (Franzluebbers et al., 2001), manure type (farmyard manure/slurry) (Grignani et al., 2007), initial SOC content (Dersch and Böhm, 2001), duration of organic fertilizer application, and mineralogy of the soil clay fraction (Singh et al., 2018).

The use of manures in farming practice started in China nearly 4000 years ago (Dormaar et al., 1988). However, during the past two decades the substantial yield gains and economic benefits obtained from the application of chemical fertilizers have led to a lack of interest in the application of manures to agricultural land. The annual production of livestock manures is > four billion tonnes in China, but only a small proportion is applied to arable land because of the high costs involved in manure transport and spreading (Fan et al., 2017). The effects of inorganic fertilizers on SOC content are smaller than those of manure inputs and the results are contradictory, being positive (Gong et al., 2009), negative (Neff et al., 2002; Mack et al., 2004; Li J. et al., 2018) or neutral (Brown et al., 2014; He et al., 2015; Xu et al., 2016). The discrepancies are attributable to the experimental duration, soil type, climate, C inputs (He et al., 2015; Han et al., 2016; Li J. et al., 2018) and cropping systems (He et al., 2015). It is therefore important to assess C sequestration in the context of specific soil-crop-environment systems under different fertilization management strategies.

A global annual C sequestration rate of 0.2-0.5 t C ha^{-1} might be feasible (Minasny et al., 2017) based on the '4 per 1000' soil C initiative (French Ministry of Agriculture and Food, 2018). Studies in China indicate average C sequestration rates ranging between 0.2 and 0.8 t C ha^{-1} yr^{-1} (Xie et al., 2007). Two manure management practices are commonly used to increase SOC sequestration, i.e. high manure inputs over the short term or low inputs over long-term increments. The key question is whether there is an upper limit or 'saturation level' for soil C storage (Six et al., 2002) and, if so, the duration required to attain this maximum. The arguments for both C saturation and unsaturation have long been discussed (Stewart et al., 2007; Du et al., 2014; Di et al., 2018; Poulton et al., 2018). Meta-analysis of 16 long-term experiments in the UK show that low rates or periodic application of farmyard manure increased SOC by 3-8 per mille per year over several decades and high annual application rates of farmyard manure (3.2 Mg C ha^{-1} yr^{-1}) greatly increased SOC content over the first 20 years. Hence, understanding the response of the SOC pool to varying manure and NPK fertilizer applications may help to provide appropriate manure management strategies to increase SOC for better soil health and delivery of soil functions.

The total C losses or gains in response to agricultural management are difficult to detect both temporally and spatially. In contrast, SOC labile fractions are more sensitive early indicators and more responsive to changes in land management (Li J. et al., 2018). The labile C is easily decomposable and has fast turnover times (several days to months) and this has a great impact on nutrient cycling and biologically related soil attributes (Xu et al., 2011). Several labile SOC fractions have been proposed as indicators for assessing the effects of soil management on SOC stocks (Iovieno et al., 2009). Of these, cold (WSC) and hot water - extractable SOC (HWSC), microbial biomass C (MBC) and dissolved organic C (DOC) have been well studied.

Aggregate protection of SOC is also an important C stabilization and sequestration mechanism (Tisdall and Oades, 1982). Macroaggregates of size > 250 μm provide minimal physical protection (Oades, 1984) and are sensitive to land management and mechanical disruptive forces such as tillage. Microaggregates (< 250 μm) are important to long-term SOM stabilization (Totsche et al., 2017). The effects of inorganic fertilizer and manure inputs on aggregate stability and SOC within aggregates are inconsistent (Abiven et al., 2009). For instance, repeated application of composted pig manure at a high rate (27 Mg ha^{-1} yr^{-1}) over 30 years decreased aggregate stability but increased it at a low rate (13.5 Mg ha^{-1} yr^{-1}) as a result of the increase in glomalin-related soil protein in small macroaggregates (0.25-2.0 mm) (Xie et al., 2015). Zhou et al. (2017) found that the water stability of aggregates decreased by 55.3% and 36.9%, respectively, after 12 years of application of NPK and fresh swine manure compared to the control. Xie et al. (2017) found that the application of chemical fertilizers with or without dairy manure for 21 years to a wheat-maize rotation on a calcareous soil affected neither aggregate stability nor aggregate-associated OC, mainly due to the similar mineralization rates in different aggregates. Aggregation is a complex and continuous process and the effects of fertilization on aggregate stability and aggregate-associated OC may vary with soil type and climatic conditions.

The North China Plain (NCP) is the largest and most important agricultural region in China. It produces > 79% and 30% of the national wheat and maize crops, respectively (China Statistical Bureau, 2018). However, the SOC content in north China is the second lowest (0.5-0.8%) compared to the mean SOC content (1.08%) of Chinese dry arable land (Pan et al., 2010;

Cheng et al., 2013). This can be ascribed to two main factors. Firstly, manure application rates have remained unchanged since the 1980s but the application rates of chemical fertilizers have increased rapidly due to the affordability of chemical fertilizers (Gong et al., 2009). In addition, unbalanced fertilization, particularly excess N without appropriate straw return or organic amendments, reduces SOC sequestration (Brown et al., 2014; Yang et al., 2015). Crop straw was customarily burned by local farmers and straw return is slowly becoming more common after the launch of the Straw Comprehensive Utilization Project. Previous studies show that organic manures have significant impacts on the dynamics of SOC concentrations (Fan et al., 2014). However, little information is available regarding the application rates of organic manures required to increase SOC storage and sensitive indicators for the assessment of SOC have not been established. Few studies have compared the contribution of manures and inorganic fertilizers to different SOC fractions. The objectives of the current study were to 1) assess whether long-term manure and NPK fertilizer applications increase SOC sequestration at a rate of 4 per mille; and 2) contrast fertilization effects on SOC concentrations and its labile fractions in bulk soil and soil aggregates and on aggregate stability. We hypothesized that appropriate rates of manure application are important to promote SOC sequestration and the build-up of soil structure and that labile C fractions can act as important indicators of good SOC management strategies.

2. Materials and methods

2.1. Site description and experimental design

A long-term field experiment was established in 2007 at the experiment station of China Agricultural University (36°52′ N, 114°01′ E, 40 m a.s.l.) in Quzhou county, Hebei province.

The climate is semi-humid temperate continental monsoon, with a mean annual temperature of 13.4 °C and an annual precipitation of 791.7 mm (2007-2016). Approximately 60% of the annual precipitation occurs during the maize growth period (from July to September). The soil is classified as a Cambisol with a silt loam texture (clay 28%, silt 54% and sand 19%). Prior to the onset of the experiment the surface soil (0-20 cm depth) contained 7.05 g kg^{-1} SOC, 0.80 g kg^{-1} total N, 11.2 mg kg^{-1} available P ($NaHCO_3$), 122.0 mg kg^{-1} available K (NH_4OAc), and the pH (H_2O) value was 8.58.

The experiment consisted of 27 plots, each 10 m × 10 m (Fig. 1). There were eight fertilization treatments and one control (CK), each with three replicates arranged in a randomized block design. The eight fertilization treatments were: cattle manure at four application rates (3000 (L1), 6000 (L2), 9000 (L3), and 12000 (L4) kg ha^{-1} crop^{-1}). The mean annual manure carbon inputs were 1.91, 3.82, 5.72 and 7.60 Mg C ha^{-1} yr^{-1} respectively. The corresponding inorganic fertilizer treatments were L1, L2, L3, L4, with application rates approximately equivalent to the amounts of N, P and K in the respective manure application treatments (Table 1). Cattle manure from a local livestock farm was air dried and composted naturally. Fertilization started in 2007 and the fertilizer and manure were applied twice a year after the harvest of maize and wheat. The average C, N, P, and K concentrations of the manure were 31.8, 1.88, 0.66 and 1.59% respectively. The N, P, and K fertilizers (NPK treatments) were urea, calcium superphosphate, and potassium sulfate, respectively. The basal fertilizers (all the manure and part of the inorganic fertilizers) were incorporated into the plow layer (0-25 cm depth) by deep plowing/rotary tillage before sowing of wheat or maize. Control plots were plowed in the same fashion. Briefly, one-third of the urea was applied as basal fertilizer and two-thirds were top-dressed at the jointing stage of wheat or the bell stage of maize. The supplementary fertilizer was surface-applied by hand with irrigation water. Calcium superphosphate and potassium sulfate were used as basal fertilizers in both the wheat and maize seasons.

Each year winter wheat (cv. ‘Liangxing 99’) was sown at a density of 2.25 kg seeds ha^{-1} in early October and harvested in mid-June the following year. Summer maize (cv. ‘Zhengdan 958’) was sown at a density of 75,000 seeds ha^{-1} with a row spacing of 60 cm and intra-row spacing of 22 cm in mid-June and harvested in early October. All straw was returned to the soil in the individual plots. Herbicides, insecticides, and irrigation were used according to local conventional farming practice (Zhang Y. et al., 2016).

2.2. Soil sampling

Topsoil (0-15 cm) samples were taken using an auger (8 cm diameter) on two occasions in 2016 in June (wheat harvest) and October (maize harvest). The samples were taken after removal of crop residues from the soil surface. A composite soil sample was obtained from each plot by taking five cores randomly, placing the cores in a sterile polypropylene bag and storing at 4 °C until further processing. Field-moist soils were cool-dried at 4 °C until they reached a gravimetric water content of 8% for subsequent aggregate-size separation (Schutter and Dick, 2002).

The soil samples were mixed thoroughly and prepared for aggregate analysis by removal of visible crop residues and stones and passing through an 8-mm sieve by gently breaking the large clods by hand along the natural planes of fracture. The samples were then passed through a 2-mm sieve and divided into two parts. Approximately 50 g were stored at -20 °C for determination of MBC and DOC within one week of sieving and the remainder was air-dried for determination of soil physicochemical properties.

2.3. Aggregate-size fractionation

Dry and wet-sieving methods were used to isolate different sizes of aggregate fractions. Briefly, 200 g of pre-sieved (8 mm) soil were passed through 2.0 mm and 0.25 mm sieves to obtain three size fractions (Schutter and Dick, 2002): > 2.0 mm (large macroaggregates), 0.25-2.0 mm (small macroaggregates) and < 0.25 mm (microaggregates and silt and clay fractions). The sieves were placed for 2 min on an aggregate analyzer (DM185, Information Technology Inc., Dimart, Shanghai, China) operating with a circular motion at 1450 rpm. These separated aggregates were divided into two parts, one of which was air dried for determination of aggregate-associated OC and the other stored at -20 °C to determine MBC and DOC. In addition, a portion of each sub-sample of the pre-sieved (8 mm) soil was used for wet sieving (Elliott, 1986).

Briefly, 30 g soil were wet-sieved into the same three size fractions as by dry sieving. The stacked sieves were placed in a bucket and connected to a motor. Soil samples were spread evenly on the top sieve, immersed in deionized water for 3 min, and the sieves were raised and lowered by 4.0 cm at a speed of 30 times min^{-1} for 2 min. Floating organic material was decanted off and the aggregate fractions retained on each sieve were transferred to a container, dried at 65 °C in a vacuum oven for 48 h, and the weights of the aggregate fractions were recorded. Soil recovery after wet sieving was > 90%. The water-stability of soil aggregates was represented by mean weight diameter (MWD, mm) calculated as (van Bavel, 1950):

$$MWD = \Sigma(d \times m) \tag{1}$$

where d is the mean diameter between two sieves (mm) and m is the mass fraction of aggregates remaining on each sieve (%).

2.4. SOC and labile SOC fractions in bulk soil and aggregates

SOC of air-dried ball-milled soil samples was determined. Ball-milled samples were pretreated with 0.5 M HCl to remove carbonates and washed with deionized water (Chen et al., 2009). Bulk soil samples and samples originating from dry-sieving were acid-washed and analyzed for SOC by dry combustion using an elemental analyzer (Vario Macro CNS analyzer, Elementar, Langenselbold, Germany). Dry combustion is more efficient and less time-consuming than wet oxidation for the determination of SOC in calcareous soils (Santi et al., 2006).

Both cold water- and hot water-extractable SOC (WSC/HWSC) were determined on fresh field-moist soil samples using a modification of the method of Ghani (Ghani et al., 2003). Briefly, the equivalent of 3 g oven dry weight of each soil was weighed into a 50 mL polypropylene centrifuge tube and extracted with deionized water at a soil:water ratio of 1:10, w/v. The tubes were shaken at 180 rpm for 30 min and centrifuged at 4000 rpm for 20 min. The extracts were filtered through 0.45-μm pore cellulose nitrate membrane filters and the supernatants were used for C analysis (TOC-Vcph, Shimadzu, Kyoto, Japan). This fraction of the SOC is the cold water-extractable soil organic C (WSC). Deionized water was added to the same tubes at a soil:water ratio of 1:10, w/v for further extraction of the soil residues. The tubes were shaken for 10s to suspend the soil in the water and then placed in a hot water bath at 80 °C for 16 h. Each tube was then shaken on a vortex shaker for 10 s to ensure that HWSC released from the SOM was fully suspended in the extract and centrifuged at 4000 rpm for 20 min. The extracts were passed through 0.45-μm pore cellulose nitrate membrane filters. The extracts were analyzed for total OC (TOC-VCPH, Shimadzu, Kyoto, Japan). This fraction of the SOC was the hot water-extractable soil organic C (HWSC).

MBC was determined by chloroform fumigation-extraction (Brookes et al., 1985). Briefly, 10 g of chloroform-fumigated and unfumigated fresh soil samples were extracted with 0.5 M K_2SO_4 at a 1:4, w/v ratio after 24 h of incubation. The K_2SO_4 extracts were analyzed for C with a liquid analyzer (TOC-VCPH; Shimadzu, Kyoto, Japan). MBC was calculated as the difference between C concentrations in chloroform-fumigated and unfumigated samples. The microbial C was not completely extracted with K_2SO_4 and a correction factor was used to convert microbial C to MBC, i.e., $K_C = 0.45$ (Joergensen, 1996). DOC refers to the C concentration determined in a 200-mL aliquot of each extract from unfumigated samples that were filtered through 0.45-μm pore membrane filters.

2.5. Soil physiochemical properties

Sieved soil samples (2 mm) were used to determine pH (1:2.5 soil:H_2O, w/v), mineral nitrogen (N_{min}, 0.01M $CaCl_2$-extractable), available P (Olsen-P, 0.05M $NaHCO_3$-extractable), and available K (AK, 1M NH_4OAc-extractable) using standard methods. Bulk soil and aggregate soils were prepared as soil-water mixtures at a 1:5 ratio (w/v) to determine water-soluble Ca^{2+}, Mg^{2+}, K^+ and Na^+ by ICP-OES (Optima 3300 DV, Perkin-Elmer, Waltham, MA).

2.7. Carbon inputs and SOC sequestration rates

Mean annual C inputs were assessed on 9 years of data (2007-2016), including the amounts of C derived from straw, stubble, roots, rhizodeposition and manure (Fan et al., 2014). Carbon contents in both wheat and maize were assumed to be approximately 0.40% (Johnson et al., 2006). The amounts of root-C and stubble-C remaining in the plots were evaluated based on the ratio of root-to-shoot biomass (wheat, ~ 22% and maize, ~ 23%) and the stubble-to-shoot biomass ratio (both crops, ~ 26%), respectively (Kong et al., 2005; Rasse et al., 2006). Rhizodeposition C was estimated to be equivalent to the root biomass C in both wheat and maize (Bolinder et al. 1999). Manure-C was calculated by the input amounts multiplied by the average C concentration in the manure.

The SOC stock was calculated according to Fan et al. (2014) as follows:

$$SOC\ stock\ (Mg\ C\ ha^{-1}\ yr^{-1}) = SOC\ concentration\ (g\ kg^{-1}) \times bulk\ density\ (Mg\ m^{-3}) \times depth\ (m) \times 10, \qquad (2)$$

where bulk density was estimated according to Zhao et al. (2015) as follows:

$$BD = 100/((OM/0.244) + ((100-OM)/1.64)), \qquad (3)$$

where OM was the concentration of soil organic matter (%) and was estimated by SOC divided by 0.58.

Mean annual C sequestration rate was calculated according to Fan et al. (2014) as follows:

$$C\ sequestration\ rate\ (Mg\ C\ ha^{-1}\ yr^{-1}) = [SOC\ stock(t2) - SOC\ stock\ (t1)]/t, \qquad (4)$$

where $t2$ and $t1$ are the SOC stocks in October 2016 and 2007, respectively, and t denotes the interval (years) between two soil samples collected in the same plot.

Annual SOC increase rate was calculated as (Poulton et al., 2018):

$$Annual\ SOC\ increase\ rate = (SOC_{stock2016} - SOC_{stock2007})/SOC_{stock2007}/9 \times 1000‰, \qquad (5)$$

Where ‰ is the permillage, based on the goal of global carbon increase of 4‰.

2.8. Statistical analysis

Statistical analysis was conducted using the SPSS 21.0 software package. All data were tested to satisfy normal distribution and variance homogeneity. The effects of fertilization type and application rate on SOC and its labile fractions and basic soil physicochemical properties were analyzed using two-way analysis of variance (ANOVA). Other treatment effects were analyzed using one-way ANOVA and significant differences between means were compared using Duncan's multiple range test at the 5% level. All data are presented as mean ± standard error (SE). Linear regression analyses were conducted to evaluate the relationships between mean annual C input and C sequestration rate and between SOC and labile OC forms (HWSC, MBC, DOC, WSC). Pearson correlation analyses were used to evaluate the relationships between mean weight diameter (MWD) and SOC and its labile OC fractions (HWSC, MBC, DOC, WSC).

3. Results

3.1. Yields and aboveground biomass

Fertilization significantly increased mean grain yields (2014-2016) over the control. The response of grain yield and aboveground biomass to fertilization rates differed between wheat and maize (Fig. 2). Yields and biomass of wheat increased with increasing application rates of both manure and fertilizer, but no significant difference was found between rates L3 and L4. Minimum yield and biomass of maize occurred at L1 and no significant difference was found among the other three fertilization rates. Mean wheat yields ranged from 3.35 to 7.07 Mg ha^{-1} in the M treatments, and 4.12 to 8.15 Mg ha^{-1} in the NPK treatments. NPK application increased wheat yields by 23% at L1 and 38% at L2 compared to the corresponding M treatments. Maize yields ranged from 6.97 to 8.83 Mg ha^{-1} in the M treatments and 7.10 to 8.79 Mg ha^{-1} in the

NPK treatments.

Wheat aboveground biomass ranged from 7.60 to 15.0 Mg ha^{-1} in the M treatments and 9.12 to 16.5 Mg ha^{-1} in the NPK treatments. NPK application increased wheat biomass by 20% at L1 and 23% at L2 compared to the corresponding M treatments. Maize aboveground biomass ranged from 13.2 to 15.5 Mg ha^{-1} in the M treatments and 13.0 to 16.0 Mg ha^{-1} in the NPK treatments.

3.2. Carbon inputs and carbon sequestration

The total input of C averaged 6.20 Mg C ha^{-1} yr^{-1} in the control, 10.0 to 13.1 Mg C ha^{-1} yr^{-1} in the fertilizer plots and 10.4 to 19.8 Mg C ha^{-1} yr^{-1} in the manure treatments (Table 2). The C inputs were derived from straw, stubble, and roots in the control and the fertilizer treatments but from plant materials in combination with the manure in the manure treatments. Manure application significantly increased mean annual C inputs compared to the corresponding fertilizer rates by 19, 34 and 53% at L2, L3 and L4 respectively. The SOC sequestration rate in the top 15 cm of the soil profile averaged 0.13 Mg C ha^{-1} yr^{-1} in the control, 0.44 to 0.64 Mg C ha^{-1} yr^{-1} in the fertilizer treatments, and 0.89 to 1.66 Mg C ha^{-1} yr^{-1} in the manured plots. Manure application substantially increased mean annual SOC sequestration rates by 34 and 53% at rates L3 and L4 over the corresponding fertilizer rates. No difference was observed between the manure and fertilizer at rates L1 and L2. Mean annual SOC sequestration rates were significantly correlated with mean annual C inputs (Fig. 3). Calculations indicated that at least 5.83 Mg C ha^{-1} yr^{-1} was required to maintain the SOC stock, i.e. a sequestration rate of zero.

The rate of increase in mean annual SOC stock in the top 15 cm of the soil profile was calculated over the nine years of the field experiment based on the ‘4 per mille’ soil C initiative

(Fig. S1). Rates of increase were 8‰ in the control, 54‰ to 101‰ (74‰ on average, 1.22 Mg C ha^{-1} yr^{-1}) in the manure treatments, and 27‰ to 39‰ (31‰ on average, 0.51 Mg C ha^{-1} yr^{-1}) in the fertilizer treatments. The annual SOC increase rate was increased with increasing cattle manure application rate but was relatively unaffected by fertilizer rate.

3.3. Soil organic carbon concentrations in bulk soil and aggregates

Nine years of fertilization significantly increased SOC concentrations over the control (Fig. 4). SOC concentrations in bulk soil and aggregates (except < 0.25 mm aggregates under maize) increased significantly at both wheat and maize harvests with increasing application rate of manure but not of fertilizer. The SOC concentrations at rates L3 and L4 in the manure treatments were significantly higher than at L1 and L2. At wheat harvest, SOC concentrations in the manure treatments within the bulk soil and > 2.0 mm and 0.25-2.0 mm aggregates increased by 20, 18 and 23%, respectively, compared to the fertilizer treatments but no increase was observed in the < 0.25 mm aggregates. At maize harvest the corresponding values were 35, 27, 38 and 40%, respectively.

3.4. Labile organic carbon concentrations in bulk soil and aggregates

Labile SOC fraction dynamics were similar to the patterns in total SOC in the bulk soil and aggregate fractions (Fig. S2-S3). The labile C contents increased significantly with increasing application rate only in the manure treatments and not in the fertilizer treatments (Table 3 and Fig. S2-S3). The amounts of the labile SOC fractions (i.e. HWSC, MBC, DOC and WSC) at rates L3 and L4 in the manure treatments were higher than those at L1 and L2. In general, the amounts of the labile SOC fractions followed the sequence HWSC > MBC > DOC > WSC. All labile SOC fractions were positively correlated with SOC in the bulk soil and aggregate fractions at both wheat and maize harvests (except in the microaggregates at the wheat harvest) (Fig. 5). There were significant correlations between SOC and all labile C fractions, and the slopes mostly followed the sequence HWSC > MBC > WSC = DOC.

3.5. Aggregate fractions and water stability

Soil microaggregate fractions (47-61%) dominated at harvests of both crops followed by small macroaggregates (21-30%), with large macroaggregates (9-24%) forming the smallest fraction (Table 4). The effect of fertilization on aggregate size distribution differed between the maize and wheat harvests and showed different trends among different fractions. Nutrient application increased the large macroaggregate mass fraction at wheat harvest and the effect was significant at rates L3 and L4 of manure. The percentage of small macroaggregates remained relatively stable but showed a tendency to increase with increasing application rate of manure. In contrast, the percentage of microaggregates decreased with increasing application rate of both manure and fertilizer. Mean weight diameter (MWD) increased with application rate but was significantly enhanced (10% increase) only at rate L4 of manure compared to the corresponding fertilizer treatments. At maize harvest the three aggregate fractions in the manure and fertilizer treatments did not differ significantly among different application rates, except that at rate L4 of manure the percentage of large macroaggregate increased at the cost of microaggregates. In the manure treatments at rates L3 and L4 the values of MWD increased by 10 and 24%, respectively, compared to the corresponding fertilizer rates.

Mean weight diameter (MWD) was mostly (and positively) correlated with SOC concentration and its labile fractions in both bulk soil and aggregates at the harvests of both crops (Table S1).

3.6. Effects of fertilization on soil physicochemical properties

Applications of both manure and fertilizer generally decreased soil pH and increased soil nutrient concentrations (Table S2). Soil pH decreased with increasing rate of both fertilizer and manure, the difference between which was observed only at rate L4 at the wheat harvest. The concentrations of N_{min}, Olsen-P, and AK increased with increasing application rate at the harvests of both crops. Manure application significantly decreased mineral N (N_{min}) concentrations at wheat harvest compared to the fertilizer across all three rates (L2, L3 and L4). Only at L3 did manure increase the concentration of AK compared to the corresponding fertilizer rate. Manure application significantly increased soil N_{min} concentrations and AK at L4 and Olsen-P concentrations at rates L3 and L4 at the maize harvest compared to fertilizer application.

Fertilization significantly increased water-extractable Ca^{2+}, Mg^{2+}, and K^+ but decreased Na^+ compared to the control (except manure at rate L1 at the wheat harvest). In general, manure application increased soil water-extractable Mg^{2+}, K^+, and Na^+ concentrations by 23, 25 and 16%, respectively, at the wheat harvest compared to fertilizer application. Manure application appeared to increase soil water-extractable Mg^{2+}, K^+, and Na^+ concentrations by 33, 36, and 28%, respectively, at the maize harvest with lower concentrations of soil water-extractable Ca^{2+} than the fertilizer treatments but there were no significant differences in Ca^{2+} between manure and fertilizer at the same nutrient application rates. Manure application increased soil water-extractable Mg^{2+} (at L3), K^+ (at L3 and L4) and Na^+ (at L1 and L4) at the wheat harvest compared to fertilizer application. Manure application increased soil water-extractable Mg^{2+}

(at L2 and L4) and Na^+ (at L4) at the maize harvest compared to fertilizer application.

4. Discussion

4.1. Effects of fertilization on soil organic carbon sequestration

Our nine-year fertilization field study indicates that straw return (control plots) or fertilization (either manure or inorganic fertilizer) plus straw return can increase SOC sequestration and achieve a '4 per 1000' increase in SOC stock and, as expected, the increase was larger in the manured plots. Evaluation of 16 long-term experiments over 7-157 years in the southeast of the UK indicates that SOC increases occurred at rates of > 7‰ per year in the top 23 cm of the soil profile in 65% of cases, approximately equivalent to 4‰ per year to a depth of 40 cm (Poulton et al., 2018). The C stock to a depth of 30 cm in agricultural soils is mostly affected by plant roots and agricultural management practices (Minasny et al., 2017). Here, SOC increases of > 7‰ per year occurred to a depth of 15 cm, approximately equivalent to 4‰ per year in the top 30 cm of the soil profile. Return of straw alone in the control increased SOC at an annual rate of 8‰ (0.13 Mg C ha^{-1} yr^{-1}) (Fig. S1). A previous study found that the rate of SOC increase was higher in a high clay soil with low initial SOC stock during the first several years of experiments (Poulton et al., 2018). Our results indicate that straw return is a feasible approach in realizing the '4 per 1000' initiative. Similarly, in north China the increase in SOC in the top 40 cm of the soil reached 17.6‰ after 7 years of straw return (Zhao et al., 2018). However, our rate of increase in SOC was independent of the fertilization rate of NPK (Fig. S1). This may be attributable to the growth stagnation of crops with excessive nutrients, as shown for maize and wheat in the present study (Fig. 2) and in other studies (Han et al., 2018). In addition, high soil N availability may also promote the decomposition of SOM

(Kirkby et al., 2014; Li J. H. et al., 2018). Manure application significantly enhanced the rates of SOC increase (54 to 101‰, 0.89 to 1.66 Mg C ha^{-1} yr^{-1}) with increasing application rate. Our results agree with previous studies (Smith et al. 1997; Su et al. 2006; Lee et al. 2007). Similarly, the average C sequestration rates were 0.255 Mg C ha^{-1}yr^{-1} in the NPK and 0.465 Mg C ha^{-1}yr^{-1} in NPK + manure treatments after 20 years of fertilization on the North China Plain (Fan et al., 2014). Mean C sequestration rates were 1.09 Mg C ha^{-1} yr^{-1} under NPK + manure applied over a period of > 21 years of application on silty clay loam soils (Xie et al., 2017). The increase in SOC content in our manure treatments might be explained by the regular organic inputs which may be effective in promoting soil organic carbon content on the North China Plain.

The significant linear relationship between SOC sequestration rate and carbon input (Fig. 3) indicates that SOC was not saturated after nine years of inputs of crop residues (in the CK and NPK treatment) and manure/crop residues (in the M treatment). Long-term experimental monitoring at Rothamsted Research in the UK shows that the SOC stock increased rapidly after annual application of 35 Mg ha^{-1} farmyard manure for the first 20 years, and remained > 7‰ per year for 40-60 years, and thereafter the SOC stock tended to reach an equilibrium (Poulton et al., 2018). Here, the duration of the experiment was short-term as summarized in a global meta-analysis study which reported that SOC saturation occurs after 12 years under straw return (Liu et al., 2014). SOC contents on the North China Plain had reached an equilibrium in chemical fertilizer treatments (fertilizer N, NPK, N+straw) after 10 years of cropping but not in the manure plus fertilizer N treatments (Yang et al., 2015). Soil physicochemical properties provide the protective capacity of stable SOM pools associated with soil aggregates and clay minerals, limiting increases in SOM even with increased external or internal inputs (Six et al.,2002; Stewart et al.,2007). The soil in the present study has a high capacity to preserve C as it is a silt loam with 82% clay and silt content. Empirical evidence on the North China Plain shows that < 53 μm particle-associated OC concentration preferentially reached C saturation when the manure application rate was increased to 7.5 or 15 Mg ha^{-1}, but no saturation in macroaggregate-associated OC in a winter wheat-summer maize rotation was observed after 17 years of fertilization (Du et al., 2014). Clay minerals have a finite surface area and the fine fraction is more likely to reach C saturation than the whole soil where OC can readily accumulate in the form of particulate OC (Stewart et al., 2008). The lowest carbon input (5.83 Mg ha^{-1} yr^{-1}) to maintain the initial SOC content here was much higher than in other studies on the North China Plain (Fan et al., 2014) in which an input of ≥ 2.04 Mg C ha^{-1} yr^{-1} was recommended to maintain the SOC stock in a wheat-maize rotation.

4.2. Effects of fertilization on soil organic carbon and its labile fractions

Application of organic manures markedly increases SOC content (Fan et al., 2014; Li J. et al., 2018) and its labile fractions (Liang et al., 2012; Li J. et al., 2018) and our results (Fig. 4 and Fig. S2-S3) correspond with this. The dynamics of labile C fractions were similar to those of SOC and mostly exhibited positive correlation with SOC in the bulk soil and aggregates (Fig. 5). Hot water-extractable C was the labile SOC form showing the highest correlations with changes in SOC. The HWSC contains copious readily decomposable C for microbial utilization (Ghani et al., 2003) and it responds more quickly than total SOC content to soil management practices (Xu et al., 2011). Similarly, cattle manure application over 26 years on the North China Plain significantly increased the SOC and its labile fractions (DOC, MBC, particulate OC, light fraction OC) (Li J. et al., 2018). Manure application either directly contributes to the labile OC pool and/or indirectly affects the conversion of plant residue-C into labile forms by enhancing microbial activity (Aita et al., 1997; Poirier et al., 2013; Whalen et al., 2014).

Interestingly, the concentrations of SOC and its labile fractions were not significantly altered by the application rate of the NPK fertilizer (Fig. 4 and Fig. S2-S3). Appropriate N addition enhances net primary production and soil C saturation, and higher N application rates (225 kg ha^{-1}) reduce the contents of DOC due to increased C consumption by soil microbes (Tian et al., 2013; Li J. H. et al., 2018). Long-term NPK fertilizer application on the North China Plain enhanced SOC mineralization and led to concentrations of labile SOC fractions similar to that in the control and that were independent of NPK fertilizer rates (Li J. et al., 2018). The use of synthetic fertilizer N induced a net loss of SOC over different rotation systems due to accelerated residue breakdown and initial SOC decomposition (Khan et al., 2007). Here, the unresponsiveness of SOC to NPK fertilizer application rates may be associated with several processes: 1) the amount of C input as crop residues may be used for microbial processes (Gong et al., 2009; Brown et al., 2014); and 2) high nutrient concentrations (in particular N) in the NPK fertilizer accelerate the decomposition of both litter and SOM (Khan et al., 2007). Here, the amount of N applied at rate L4 (453 kg ha^{-1}) was higher than the recommended value for the cropping system in this region (Cui et al., 2010), and this may have accelerated the decomposition of both crop residues and native SOC (Khan et al., 2007). Similarly, NPK fertilizer over 12 years decreased the water stability of soil aggregates by 55% compared to the controls due to acceleration of SOM turnover (Zhou et al., 2017). However, it should be remembered that in practice high application rates (L3 and L4) of manure are not feasible for smallholder farmers due to the low economic benefits of manure applications, despite the fact that the SOC benefits of manure application were substantial in these two treatments. In addition, high manure application rates (L4) increased the concentrations of mineral N, available P and available K, resulting in a risk of nutrient leaching and greenhouse gas emissions (Charles et al., 2017).

4.3. Effects of fertilization on soil aggregate distribution and stability

Long-term fertilization altered soil aggregate distribution and water stability. Manure application increased the percentage of large macroaggregates at the cost of microaggregates, contributing to higher water stability of aggregates, but only at higher application rates (L3 or L4). Furthermore, MWD values in the manure treatments were only slightly higher than that in the control (Table 4). Previous studies show that organic materials enhance macro-aggregate formation and this in turn enhances the physical protection of OC in the macro-aggregates (Du et al., 2014). Macroaggregates are associated with the exudation of organic acids and polysaccharides by soil microorganisms during decomposition processes (Bronick and Lal, 2005; Yu et al., 2012). The minor effect of fertilization on aggregates and MWD may be related to the change in calcium carbonate content. Carbonates were shown to increase the stability of macroaggregates but decrease the stability of microaggregates (Boix-Fayos et al., 2001). Organic acids present in the manure treatment and the increased acidity due to fertilizer N would decrease calcium carbonate, perhaps offsetting the beneficial effect on aggregate stability (Fan et al., 2014). Similarly, Domingo-Olivé et al. (2016) found that the application of pig manure (22.5 Mg ha^{-1} yr^{-1}) to an Entisol for 12 years did not affect soil aggregation due to the "transient effect" of pig manure as a binding agent, which may be insufficient to enhance aggregate stability. Furthermore, the increased concentration of water-soluble Na^{+} in the manure treatments compared to NPK fertilizer (Table S2) may offset the improvement effect of MWD by manure addition, as Na^{+} acts as a dispersing agent in the soil aggregation process. Similarly, pig and cattle manure can increase SOC and biological binding but restrict the positive effect of aggregation because of the simultaneous addition of sodium (Guo et al., 2018;

Guo et al., 2019). In addition, high soil available P content due to manure application may decrease the diversity and growth of arbuscular mycorrhizal fungi (AMF), resulting in lower percentages of large macroaggregates (Zhang S.L. et al., 2016).

4.4. Limitations and implications for future studies

Soil degradation and environmental damage due to high inputs of inorganic fertilizers have been frequently reported on the North China Plain (Cui et al., 2010) and other regions in China (Guo et al., 2010). Organic inputs are often encouraged as they have increased the SOC stock in Chinese croplands in recent decades (Tao et al., 2019). Similarly, our results show that manure application is a powerful strategy to enhance SOC storage in croplands. However, there are drawbacks in the present study. Firstly, the carbon storage was calculated only in the surface soils. This may have underestimated total SOC storage and sequestration in the field because it has been suggested that deeper zones of soils are important in carbon sequestration (Lal, 2018). Secondly, carbon flux can vary greatly, and temporal monitoring of carbon flux may provide better prediction of carbon dynamics in the system. In addition, we did not measure the belowground biomass and the carbon input was estimated based on the root:shoot ratio (Kong et al., 2005; Fan et al, 2014). The importance of roots in modifying the carbon pool has recently been highlighted, as roots modify the carbon pool by interacting with soil microbes (Sokol et al., 2019). A mechanistic understanding of soil C dynamics needs to be explored in future studies. Thirdly, in the current study we did not have a combined NPK fertilizer and manure treatment. Previous study in this region shows that combined use of inorganic fertilizers and manures can meet the nutrient demand of crops, especially during the rapid growth period of crops (Zhang Y. et al., 2016). In contrast, manures have been found to be important in improving soil quality and other beneficial effects aside from C sequestration (Ghosh et al., 2018; Blundell et al., 2020). On the other hand, rational manure management is required because excessive rates of manure application result in high soil available P (Zhang S.L. et al., 2016) and Na (Guo et al., 2018) contents that may threaten soil and environmental quality. Finally, local farmers are unwilling to apply manures according to the recommendations due to higher expenditure and labor compared with chemical fertilizers (Zhang W. et al., 2016). Incentives are therefore necessary to encourage farmers to change from intensive-managed agriculture with high reliance on inorganic fertilizers to sustainable agriculture based on balanced management of both manures and inorganic fertilizers.

5. Conclusions

The present study quantifies the changes in soil organic C and its labile fractions in bulk soil and aggregates, providing useful information on nutrient and carbon management on the North China Plain. The results indicate that straw return and/or fertilization is a good approach for achieving the ‘4 per 1000’ C initiative, and the effects are noteworthy when manures are used instead of inorganic fertilizers. Manure addition significantly increased SOC concentrations and labile SOC fractions (HWSC, WSC, MBC, and DOC) within the bulk soil and soil aggregates compared to inorganic fertilizer or the unfertilized control with straw return only. The effects of fertilization rate were significant only in the manured plots and not in the NPK fertilizer treatments. The difference in SOC concentration and its labile fractions within bulk soil and aggregates between manure and fertilizer treatments was significant at high application rates (L3 and L4). Labile SOC fractions were linearly correlated with SOC within the bulk soil and soil aggregates, of which hot water-extractable C was the most sensitive to the changes in SOC. Aggregate stability was positively correlated with SOC and its labile fractions in both bulk soil and aggregates. The results indicate that 9000 kg manure ha^{-1} crop^{-1} can promote SOC sequestration and soil aggregation compared to NPK fertilizer in calcareous soils. Continual high application rates of manures may not be feasible and periodic high application rates of manures may be combined with balanced rates of inorganic fertilizers to produce an integrated fertilization management strategy for calcareous soils.

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Highlights

- Concentrations of SOC and its labile forms increased with manure doses but not NPK.
- Manure contributed to aggregation only at the higher application rates.
- Hot water extractable SOC was a more sensitive indicator for SOC change.
- Periodical heavy manure and proper NPK should be integrated to increase soil SOC.

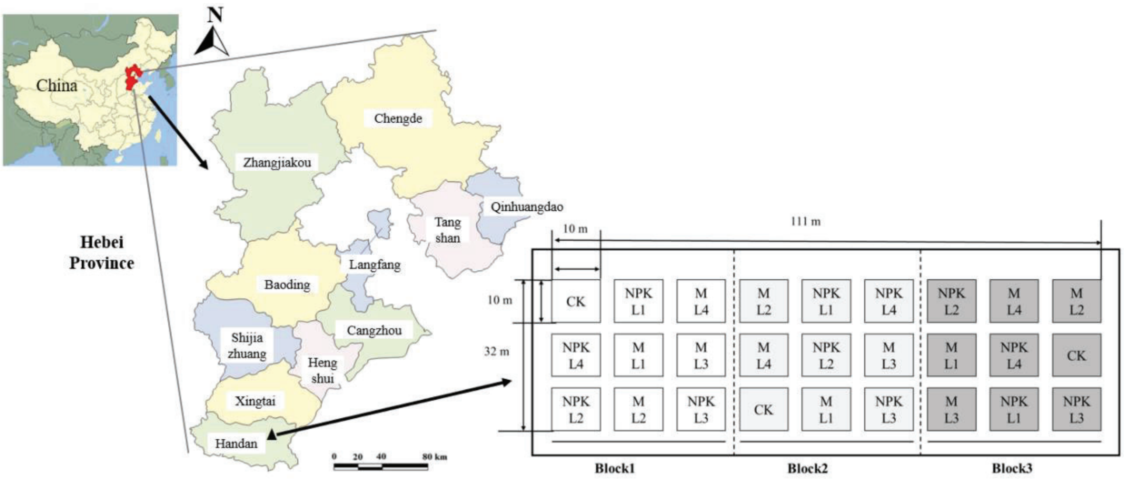

Fig. 1. The location of the study site and the layout of the long-term experimental plots.

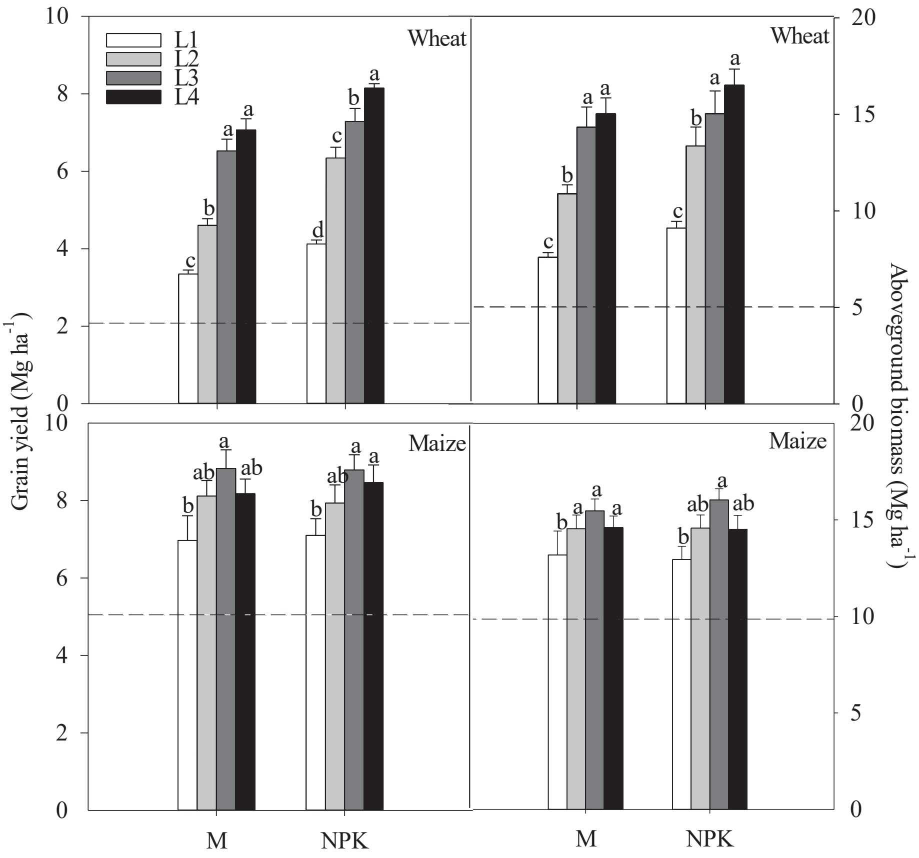

Fig. 2. Mean grain yield and aboveground biomass of wheat and maize from 2014 to 2016. Dashed lines indicate the value of the Control. L1, L2, L3, L4 indicated the annual application rates of manure (M) at 3000, 6000, 9000, and 12000 kg ha^{-1}crop^{-1} respectively, and the equivalent amounts of nutrients in the inorganic (NPK) treatments. Lowercase letters indicate significant differences among fertilizer application rates of the same fertilizer type (P<0.05).

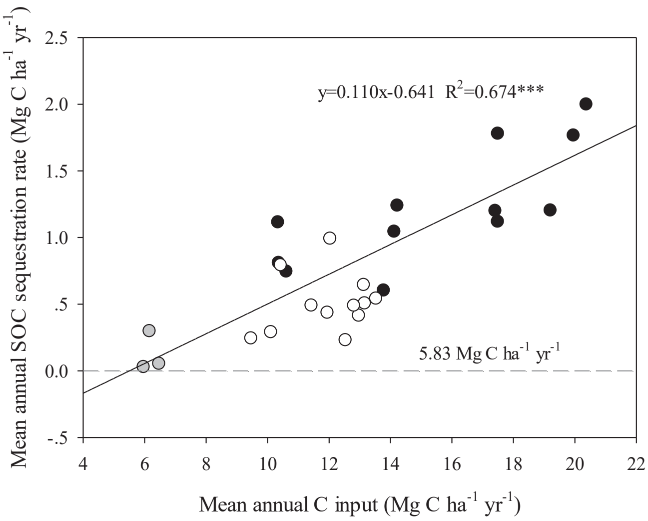

Fig. 3. Regression between mean annual SOC sequestration rate and mean annual C input in all the treatments in the 0-15 cm soil layer at maize harvest of in 2016. Gray, white and black dots represented values in the CK, NPK and M treatments at maize harvest e in 2016, respectively. *** P <0.001.

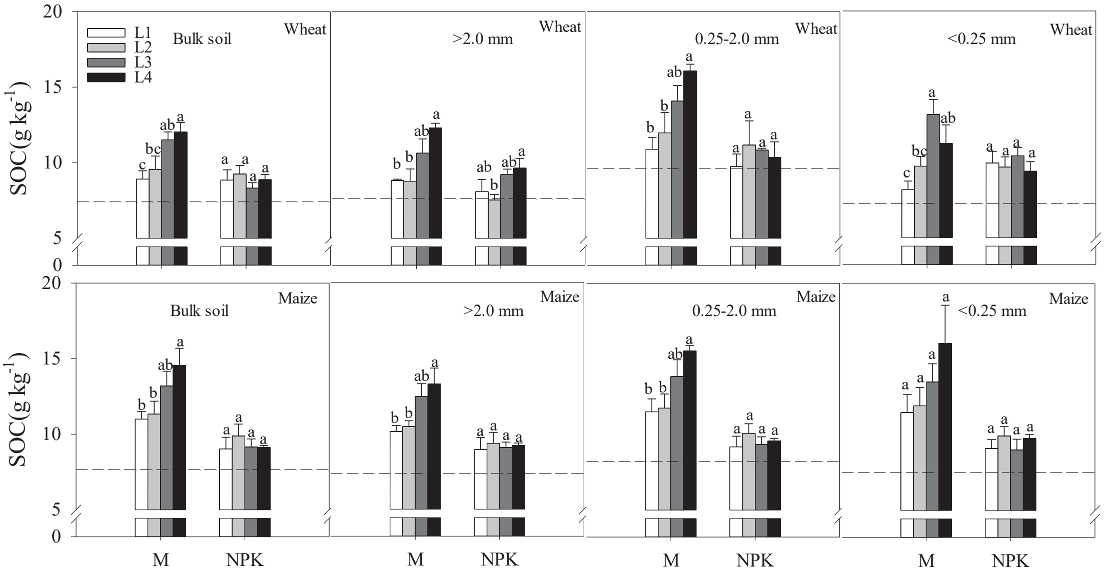

Fig.4. Soil organic carbon (SOC) concentrations in bulk soil and different aggregate-associated fractions under different fertilizer treatments at the harvests of wheat and maize. Data are mean ± SE (n=3). Dashed lines indicate the value of the Control. L1, L2, L3, L4 indicated the annual application rates of manure (M) at 3000, 6000, 9000, and 12000 kg ha^{-1} crop^{-1} respectively, and the equivalent amounts of nutrients in the inorganic (NPK) treatments. Lowercase letters indicate significant differences among fertilizer application rates of the same fertilizer type ($P < 0.05$).

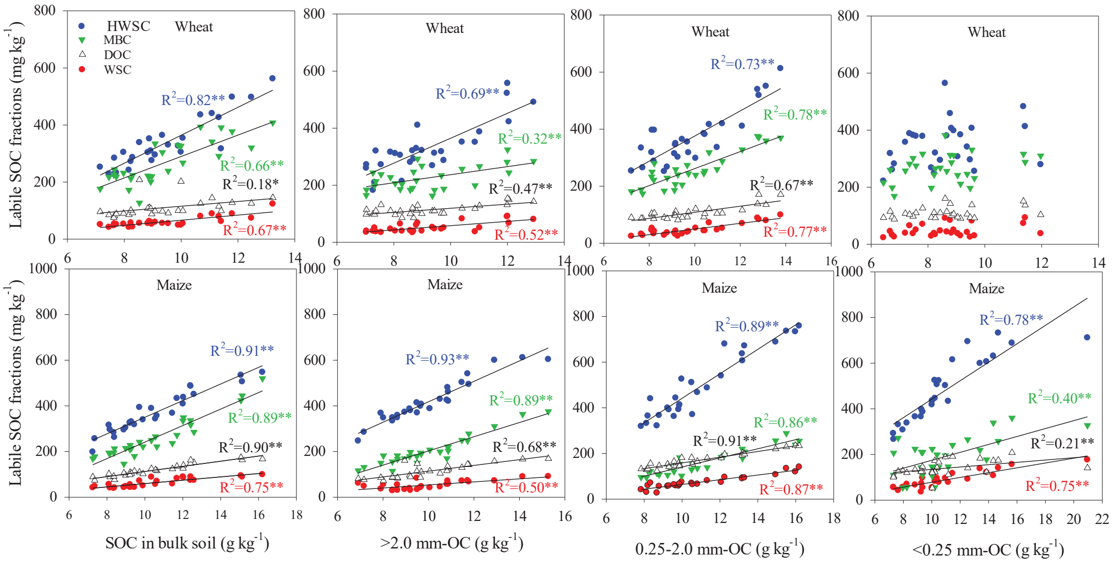

Fig. 5. Relationships between different labile organic carbon fractions (HWSC, WSC, MBC, DOC) and SOC in bulk soil and different aggregate size fractions in the topsoil of 0-15 cm at harvests of wheat and maize, respectively. HWSC-hot water extractable soil organic carbon; WSC-cold water extractable soil organic carbon; MBC-microbial biomass carbon; DOC-dissolved organic carbon. * $P < 0.05$, ** $P < 0.01$.

Table 1 Mean annual application rates of manure (M) and inorganic (NPK) fertilizers from 2007 to 2016. The NPK application rates were equivalent to the amounts of nutrients (N, P_2O_5 and K_2O) in the corresponding manure applications.

Fertilizer types	Application rates	Wheat (kg ha^{-1}yr^{-1})				Maize (kg ha^{-1}yr^{-1})			
		Manure	N	P_2O_5	K_2O	Manure	N	P_2O_5	K_2O
Control	CK	0	0	0	0	0	0	0	0
M	L1	3000	0	0	0	3000	0	0	0
	L2	6000	0	0	0	6000	0	0	0
	L3	9000	0	0	0	9000	0	0	0
	L4	12000	0	0	0	12000	0	0	0
NPK	L1	0	56	46	52	0	57	45	63
	L2	0	112	92	104	0	114	90	126
	L3	0	168	138	156	0	171	135	189
	L4	0	224	184	208	0	228	180	252

Ck, no fertilizer input; L1, L2, L3, L4 indicated the annual application rates of manure (M) at 3000, 6000, 9000, and 12000 kg ha^{-1} crop^{-1} respectively, and the equivalent amounts of nutrients in the inorganic (NPK) treatments.

Table 2 Estimates of the mean annual carbon (C) inputs into the soil from manure and crop (wheat + maize) residues, C sequestration rate and C stock.

Application rates	Fertilizer types	Mean annual C input (Mg C ha^{-1} yr^{-1})					C Sequestration	C Stock#
		Manure-C	Straw-C	Stubble-C	Roots-C	Total-C	rate* (Mg C ha^{-1} yr^{-1})	(Mg C ha^{-1})
CK	Control	0.00	2.51 ± 0.05	1.35 ± 0.04	2.35 ± 0.07	6.20 ± 0.15	0.13 ± 0.09	17.52 ± 0.77
L1	M	1.91	3.43 ± 0.04	1.86 ± 0.02	3.24 ± 0.03	10.44 ± 0.09 a	0.89 ± 0.11a	24.39 ± 1.03 a
	NPK	0.00	3.97 ± 0.14	2.20 ± 0.05	3.83 ± 0.09	10.00 ± 0.28 a	0.44 ± 0.18a	20.36 ± 1.58 a
L2	M	3.82	4.09 ± 0.06	2.24 ± 0.03	3.90 ± 0.05	14.04 ± 0.14 a	0.96 ± 0.19a	25.05 ± 1.70 a
	NPK	0.00	4.68 ± 0.08	2.60 ± 0.04	4.52 ± 0.08	11.80 ± 0.19 b	0.64 ± 0.18a	22.14 ± 1.59 a
L3	M	5.72	4.61 ± 0.02	2.61 ± 0.00	4.53 ± 0.01	17.47 ± 0.03 a	1.37 ± 0.21a	28.68 ± 1.87 a
	NPK	0.00	5.23 ± 0.12	2.86 ± 0.06	4.97 ± 0.11	13.07 ± 0.29 b	0.47 ± 0.12b	20.63 ± 1.12 b
L4	M	7.63	4.85 ± 0.15	2.69 ± 0.07	4.67 ± 0.12	19.85 ± 0.34 a	1.66 ± 0.24a	31.29 ± 2.12 a
	NPK	0.00	5.18 ± 0.07	2.85 ± 0.01	4.95 ± 0.02	12.98 ± 0.10 b	0.47 ± 0.03b	20.61 ± 0.25 b

*Carbon sequestration rate was the annual rate from 2007 to 2016;

#Carbon stock was the value in 2016.

Ck, no fertilizer input; L1, L2, L3, L4 indicated the annual application rates of manure (M) at 3000, 6000, 9000, and 12000 kg ha^{-1} crop^{-1} respectively, and the equivalent amounts of nutrients in the inorganic (NPK) treatments. Lowercase letters indicate significant differences between fertilizer types at the same application rate (*P*<0.05).

Table 3 Two-way ANOVA of the effect of different parameters on SOC and its labile fractions in bulk soil and aggregate fractions at harvests of wheat and maize.

	Factors	Wheat					Maize				
		SOC	HWSC	MBC	DOC	WSC	SOC	HWSC	MBC	DOC	WSC
Bulk soil	T	***	***	*	ns	***	***	***	***	***	***
	R	ns	*	*	ns	**	ns	**	ns	*	ns
	T×R	*	ns	ns	ns	*	ns	**	*	*	*
>2.0 mm	T	**	***	***	*	**	***	***	***	***	***
	R	***	***	ns	**	ns	ns	**	*	**	ns
	T×R	ns	***	*	**	*	ns	**	*	ns	ns
0.25-2.0 mm	T	**	**	***	***	***	***	***	***	***	***
	R	ns	***	**	***	**	*	***	**	*	**
	T×R	ns	**	*	*	**	*	**	*	*	**
<0.25 mm	T	ns	ns	ns	***	**	***	***	*	***	***
	R	*	*	*	**	*	ns	*	ns	*	ns
	T×R	ns	*	ns	*	ns	ns	*	ns	ns	**

T: fertilizer types, R: fertilizer application rates, HWSC - hot water extractable soil organic carbon; MBC - microbial biomass carbon; DOC - dissolved organic carbon; WSC - cold water extractable soil organic carbon. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns: non-significant.

Table 4 Effects of 9 years of manure (M) and inorganic fertilizer (NPK) application on aggregate distribution and mean weight diameter (MWD) at harvests of wheat and maize after wet sieving

Crop species	Application rates	Fertilizer types	Aggregate size distribution (%)			MWD (mm)
			Large Macroaggregate >2.0 mm	Small Macroaggregate 0.25-2.0 mm	Microaggregate <0.25 mm	
Wheat	CK	Control	11.80 ± 1.10	22.31 ± 1.28	57.83 ± 0.80	0.63 ± 0.02
	L1	M	12.76 ± 0.67aC	20.67 ± 0.97aB	58.67 ± 0.43aA	0.63 ± 0.01aC
		NPK	8.64 ± 0.85b	22.89 ± 0.17a	60.89 ± 0.82a	0.58 ± 0.02a
	L2	M	12.77 ± 0.67aBC	25.22 ± 0.51aA	56.20 ± 0.59aB	0.68 ± 0.01aB
		NPK	10.89 ± 1.11a	27.16 ± 1.96a	53.90 ± 0.50b	0.66 ± 0.00a
	L3	M	14.13 ± 0.18aB	22.08 ± 1.24aB	56.71 ± 2.16aAB	0.67 ± 0.01aB
		NPK	11.97 ± 0.70b	22.13 ± 1.11a	58.84 ± 1.58a	0.64 ± 0.02a
	L4	M	17.78 ± 0.89aA	23.17 ± 1.88aB	52.64 ± 2.50aB	0.75 ± 0.01aA
		NPK	13.82 ± 0.63b	23.10 ± 1.25a	56.67 ± 1.37a	0.68 ± 0.01b
Maize	L0	Control	23.46 ± 1.34a	22.42 ± 0.56	54.12 ± 1.53	0.82 ± 0.02
	L1	M	22.04 ± 1.39aA	26.61 ± 1.74aAB	51.36 ± 0.97aA	0.84 ± 0.01aA
		NPK	22.45 ± 1.60a	25.72 ± 0.19a	51.83 ± 1.42a	0.84 ± 0.03a
	L2	M	22.10 ± 1.58aA	29.01 ± 1.60aA	48.89 ± 0.71aA	0.85 ± 0.02aA
		NPK	18.21 ± 1.47a	30.43 ± 3.64a	51.35 ± 2.31a	0.81 ± 0.01a
	L3	M	23.34 ± 1.27aA	26.28 ± 1.29aB	50.39 ± 0.59bA	0.86 ± 0.01aA
		NPK	19.92 ± 0.50a	23.86 ± 1.49a	56.22 ± 1.00a	0.78 ± 0.01b
	L4	M	24.34 ± 1.44aA	28.67 ± 1.66aAB	46.99 ± 3.10bA	0.89 ± 0.04aA
		NPK	16.49 ± 1.73b	23.48 ± 1.85a	60.03 ± 2.55a	0.72 ± 0.03b

CK, no fertilizer input; L1, L2, L3, L4 indicated the annual application rates of manure (M) at 3000, 6000, 9000, and 12000 kg ha^{-1} crop^{-1} respectively, and the equivalent amounts of nutrients in the inorganic (NPK) treatments. Lowercase letters indicate significant differences between fertilizer types at the same application rate for each crop; Capital letters indicate significant differences among different application rates (P<0.05).