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Long-term effect of fertilizer and manure application on soil-carbon sequestration and soil fertility under the wheat-wheat-maize cropping system in northwest China

Yong-Zhong Su · Fang Wang · Dong-Rang Suo · Zhi-Hui Zhang · Ming-Wu Du

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Abstract Maintenance of soil organic carbon (SOC) is important for the long-term productivity of agroecosystems. An investigation was conducted to study the effects of long-term application of inorganic fertilizers and farmyard manure (FYM) on soil organic carbon (SOC), nitrogen, phosphorus, and potassium nutrient content, water-stable aggregate distribution, and aggregateassociated carbon in a field experiment started in 1982 in an arid region of northwest China. Application of inorganic fertilizer alone (N, NP, or NPK treatments) did not increase SOC concentrations compared with no application of fertilizers (CK) and SOC concentration was significantly reduced, by 18% on average, compared with the initial value at the beginning of the experiment. Application of imbalanced inorganic fertilizer (N and NP), especially, resulted in a significant decrease in available phosphorus and potassium nutrients at a depth of 20 cm. This indicates that long-term application of inorganic

D.-R. Suo Zhangye Institute of Agricultural Science, Gansu Province 734000, China fertilizers were inadequate to maintain levels of SOC and nutrients under conventional management with no aboveground crop residues returning to the soil. Long-term application of FYM alone or combined with inorganic fertilizers (M (FYM), MN, MNPK, or MNPK treatments), however, improved SOC and total nitrogen concentrations from initial values of 12.1 and 0.76 g kg^{-1} , respectively, to 15.46 and 1.28 g kg⁻¹, on average, and also enhanced available nitrogen, phosphorus, and potassium concentrations by 47, 50, and 68%, respectively, during the 23-year period. Treatment with FYM resulted in a 0.48 mm greater average mean weight diameter (MWD) of aggregates and a higher percentage of macro-aggregates (>2 mm) and small macroaggregates (2-0.25 mm) than treatment without FYM. The MWD increased with increasing SOC concentration (R^2 =0.75). The SOC concentration was highest in small macro-aggregates, intermediate in macro-aggregates, and lowest in micro-(0.25–0.05 mm). Approximately aggregates 54-60% of total SOC was stored in micro-aggregates (0.25–0.05 mm) and sand+silt fractions (<0.05 mm) after treatment without FYM but 57-64% of total SOC was stored in macroaggregates (>0.25 mm) after treatment with FYM. MNPK treatment had the greatest effect on improving the levels of SOC and NPK nutrients and in enhancing the formation and stability of macro-aggregates.

Y.-Z. Su (⊠) · F. Wang · Z.-H. Zhang · M.-W. Du Linze Inland River Basin Comprehensive Research Station, Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China e-mail: suchengyang@vahoo.com.cn

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Introduction

Maintenance of a high concentration of soil organic carbon (SOC) is important for several reasons. First, SOC has a profound effect on soil quality. It encourages aggregation, increases water retention, nutrient supply, and soil organism activity, and improves soil fertility and productivity (Karlen et al. 1997), thereby ensuring the long-term sustainability of an agroecosystem. Soil can also be a sink for atmospheric carbon dioxide (CO₂) and increased sequestration of carbon in agricultural soils has the potential to mitigate the global increase in atmospheric greenhouse gases (Young 2003).

Increasing carbon sequestration in agricultural soils and making soil a net sink for atmospheric carbon can be achieved by adoption of the best management practices, for example conservation tillage, application of fertilizers and bio-solids or organic amendments, crop rotation, and improved residue management (Lal 2003). Among these practices, the benefits of balanced application of mineral fertilizers (combined application of nitrogen, phosphorus, and potassium fertilizer) and manures in maintaining and increasing levels of SOC in agricultural soils have been well documented (Rudrappa et al. 2006). Many long-term fertilizer experiments worldwide have proved that balanced fertilization using mineral fertilizers with organic manures can improve the nutrient status of the soil and maintain high crop yields and high levels of residues that can be returned to the soil to increase the SOC concentration (Holeplass et al. 2004). In long-term experiments in Canada, SOC sequestration rates were 50-75 g C m⁻ ² year⁻¹ in well-fertilized soils with the optimum cropping system (Dumanski et al. 1998). In Australia, Dersch and Böhm (2001) reported that nitrogen, phosphorus, and potassium fertilizers combined with FYM application (10 t ha⁻¹ year⁻¹ ¹) enhanced carbon storage to approximately 5.6 Mg ha⁻¹ after 21 years, and that optimum

mineral N fertilizer input for 36 years increased the SOC pool to an average of 2.1 Mg ha⁻¹ compared with no nitrogen fertilization. In an experiment in north China, Meng et al. (2005) reported that balanced application of NPK fertilizers and organic manure significantly increased SOC accumulation, to averages of 0.1 Mg ha⁻¹ and 1.01 Mg ha⁻¹, respectively, in a 13-year period. Jiang et al. (2006) reported that continuous application of FYM and NPK mineral fertilizers increased SOM by 80 and 10% over 20 years in south China. FYM was more effective than inorganic fertilizers at increasing the SOC stock.

Application of fertilizer often increases soil aggregation, as a result of its effect on crop production, because more residues are returned to fertilized than unfertilized soils (Campbell et al. 2001). Whalen et al. (2003) found that application of up to 45 Mg ha⁻¹ year⁻¹ composted manure for 2 years increased SOM and led to more waterstable macro-aggregates (>2 mm) in conventional and no-tillage systems. Soil aggregate stability correlates significantly with SOC, because of the binding action of humic substances and other microbial by-products (Goh 2004). SOC can also be physically protected from microbial attack within soil aggregates and contributes to the productivity and structural improvement of soils (Campell et al. 2001). Aggregate protection of SOC is high when aggregate stability is high and aggregate turnover is low (Amelung and Zech 1996). Macro-aggregates often form around particles of SOM, protecting them from mineralization (Six et al. 2000). Stable macro-aggregates in cultivated soils have been shown to contain more carbon, and relatively younger carbon, than the carbon in micro-aggregates (Six et al. 2000; Goh 2004). An increasing proportion of stable macroaggregates can protect SOC from degrading processes, thus increasing the SOC concentration (Holeplass et al. 2004).

Several long-term field experiments have recently been conducted to investigate changes in the sustainability of crop production and in the physical, chemical, and biological properties of soils (Hao et al. 2005; Suo 2005; Jiang et al. 2006), and to determine the effects of mineral fertilizers and manure application on the SOC pool (Yang et al. 2003; Jiang et al. 2006) and on carbon sequestration in China (Meng et al. 2005). Few studies have been performed on the distribution of carbon among soil aggregates in relation to long-term application of inorganic fertilizers either alone or with FYM, however.

The objective of this study was to determine how soil carbon and nitrogen accumulation, the formation of water-stable aggregates, and the quantity of aggregate-associated C was affected by long-term application of fertilizers and manure in an irrigated wheat–wheat–maize cropping system in arid northwestern China.

Materials and methods

Site description and experimental design

The research site is located at the experimental farm of the Zhangye Institute of Agricultural Science, Gansu Province, in the middle of Hexi Corridor, northwestern China (latitude 38°54' N, longitude 100°21' E, elevation 1540 m asl.). The climate is typified by cold winters and dry, hot summers with an average annual temperature of 7.6°C. Mean annual precipitation is 120 mm. The frost-free season lasts 165 days on average. Accumulated temperature of ≥10°C is approximately 3,088°C. The experiment was established in 1982 on a silt loam soil identified as an anthropogenic-alluvial soil according to the Chinese soil classification system (Chinese Soil Taxonomy Cooperative Research Group 1995); this is equivalent to fluvent according to the FAO-UNESCO system (Zhang 2001). Before establishment of the experiment in 1982, a composite soil sample (20 sampling points) was obtained from the 0-20 cm plough layer and SOC and nutrient concentrations were determined. The results are reported in Table 1.

The experimental design was a split-plot within a randomized complete block. FYM treatment served as the main plot and inorganic fertilizer treatment was the sub plot. Treatments consisted of: (1) CK (no fertilizer or manure), (2) N, (3) NP, (4) NPK, (5) M (manure), (6) MN, (7) MNP, and (8) MNPK. The inorganic fertilizer dose was 120–150 kg N ha⁻¹, 60–75 kg P₂O₅ ha⁻¹, and 60–75 kg K₂O ha⁻¹ for

spring wheat and 240–300 kg N ha⁻¹, 120–150 kg P_2O_5 ha⁻¹, and 120–150 kg K₂O ha⁻¹ for maize. The nitrogen, phosphorus, and potassium were applied as urea or ammonium nitrate/superphosphate and potassium muriate, respectively. FYM of average composition 190 g kg⁻¹ organic carbon and 21 g kg⁻¹ nitrogen was applied at a level of approximately 30-45 t ha⁻¹ each year before sowing. Each treatment was repeated three times and the plot size was 33 m^2 $(6.6 \text{ m} \times 5 \text{ m})$. The plots were separated by ridges 0.3 m wide and 0.3 m high. Initially, in 1982, the experiment was started with spring wheat-spring wheat-maize three-year rotation. Conventional tillage practice was used. Crops were manually harvested and the aboveground crop residues were removed from the field. After harvesting for wheat in August and for maize in October the plots were ploughed manually to a depth of 20 cm, using a spade, and the maize roots were removed, which is common practice in the area studied. Before sowing in spring, FYM was incorporated by harrowing.

Soil sampling and analysis

Soil was sampled in July 2004 after spring wheat harvesting. Five soil samples per plot were collected from the 0–20 cm layer. The samples were mixed by hand and a 2-kg composite sample was obtained from each plot. Additional triplicate samples were taken using a cutting ring (volume 100 g cm⁻³), for measurement of bulk density. The air-dried samples were passed through a 8-mm sieve and visible pieces of crop residues and roots were removed. Parts of these samples were then passed through a 2-mm sieve and ground further to pass a 0.25-mm sieve for determination of SOC and other properties.

The method used for aggregate separation was adapted from Elliott and Cambardella (1991). Briefly, aggregates were separated by wet sieving the air-dried soil through a series of three sieves (2, 0.25, and 0.05 mm). The air-dried soil was quickly submerged in deionized water on top of the 2-mm sieve, resulting in slaking of the soil. Aggregate separation was achieved by manually moving the sieve approximately 3 cm up and down 50 times during a period of 2 min. Water-stable aggregates

Treatment	SOC (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N	Total P (g kg ⁻¹)	Available N (mg kg ⁻¹)	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	Bulk density (g cm ⁻³)
At the beginn	ing of the experimen. 12.1	t in 1982 0.76	15.9	0.36	28.1	12.7	66	1.45
After 23 year. CK	s of the experiment in 10.08 + 0.46 c	$i \ 2003 \ 0.80 + 0.05 \ c$	12.6 ± 0.7 b	0.25 ± 0.0 c	17.4 + 4.1 d	1.2 + 0.2 c	88 + 5 d	1.45 + 0.02 a
Z	$10.34 \pm 0.85 c$	$0.85 \pm 0.07 c$	$12.2 \pm 0.2 b$	0.28 ± 0.05 b c	$23.3 \pm 1.6 c d$	$2.1 \pm 1.0 c$	$78 \pm 5 d$	1.42 ± 0.03 a
NP	$10.12 \pm 0.67 c$	$0.82 \pm 0.06 \text{ c}$	$12.3 \pm 0.2 b$	0.30 ± 0.05 b c	$26.8 \pm 3.0 c$	$7.3 \pm 0.8 \text{ b c}$	$61 \pm 5 d$	1.44 ± 0.03 a
NPK	$10.51 \pm 0.91 c$	$0.83 \pm 0.04 \text{ b}$	$12.6 \pm 1.1 \text{ b}$	0.34 ± 0.01 a b	$26.6 \pm 4.7 c$	$10.7 \pm 2.4 \text{ b c}$	83 ± 8 d	1.39 ± 0.02 a b
Mean	10.26 ± 0.66 B	$0.83 \pm 0.05 \text{ B}$	$12.4 \pm 0.1 \text{ A}$	$0.29 \pm 0.05 \text{ A}$	$23.5 \pm 5.0 \text{ B}$	$5.3 \pm 4.2 \text{ B}$	$77 \pm 12 \text{ B}$	$1.43 \pm 0.03 \text{ A}$
M	$14.79 \pm 0.82 \text{ b}$	$1.25 \pm 0.08 \text{ b}$	11.9 ± 0.3 a	0.29 ± 0.01 b c	$30.1 \pm 0.5 c$	$16.4 \pm 4.5 \text{ b}$	$243 \pm 33 b$	$1.31 \pm 0.04 \text{ c}$
MN	$14.69 \pm 0.70 \text{ b}$	$1.23 \pm 0.06 \text{ b}$	$11.9 \pm 0.8 \text{ a}$	$0.33 \pm 0.06 \text{ b}$	$40.8 \pm 2.9 \text{ b}$	$13.6 \pm 0.4 \text{ b}$	$185 \pm 10 c$	$1.32 \pm 0.05 c$
MNP	15.86 ± 0.92 a b	1.30 ± 0.06 a b	$12.2 \pm 0.2 a$	0.39 ± 0.01 a	$44.0 \pm 6.0 \text{ a b}$	50.3 ± 11.8 a	$271 \pm 50 \text{ b}$	1.35 ± 0.06 b c
MNPK	16.54 ± 0.66 a	1.40 ± 0.25 a	$11.8 \pm 0.5 \text{ a}$	0.39 ± 0.01 a	$49.7 \pm 5.5 a$	46.7 ± 13.2 a	$360 \pm 34 a$	$1.31 \pm 0.05 c$
Mean	$15.46 \pm 1.04 \text{ A}$	$1.29 \pm 0.10 \text{ A}$	$12.0 \pm 0.5 \text{ A}$	$0.35\pm0.05~{\rm A}$	$41.2\pm8.3~\mathrm{A}$	$31.7 \pm 19.2 \text{ A}$	265 ± 72 A	$1.33\pm0.04~\mathrm{B}$
Values follow $P < 0.05$) betv	ed by the same lower veen treatments	case letter indicate t	hat mean values c	of SOC, total N, C/N	, available N, P, and	d K, and bulk densi	ty are not signific	antly different (at
Values follow different (at J	red by the same UPP $P < 0.05$) between tree	ERCASE letter ind atments with FYM	icate that the mean and treatments w	an values of SOC, to ithout FYM	otal N, C/N, availat	ole N, P, and K, and	d bulk density ar	e not significantly

Table 1 SOC and nutrient concentrations and bulk density at the beginning of the experiment, in 1982, and after 23 years of the experiment, in 2003

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retained by the sieves were backwashed into preweighed containers, over dried at 50°C for 3 days, and weighed. Soil that passed the 0.05 mm sieve was not collected but the amount of this fraction was determined by calculation from the difference between whole soil and the sum of the three aggregate-size fractions (>2 mm, 2–0.25 mm, and 0.25–0.05 mm). Aggregate-size fractions included macro-aggregates (>2 mm), small macro-aggregates (2–0.25 mm), and micro-aggregates (0.25– 0.05 mm). Subsamples of aggregate-size fractions were ground to pass a 0.25-mm sieve and analyzed for SOC.

The mean weight diameter (MWD) of waterstable aggregates was calculated as:

$MWD = \Sigma x_i y_I$

where y_I is the proportion of each size class of the total sample and x_I the mean diameter of the size class.

SOC concentrations for whole soil, aggregatesize fractions, and nutrient concentrations for whole soil were determined by the methods used at the beginning of experiment in 1982. SOC concentration was measured by the Walkley– Black method (Nelson and Sommers 1982), total nitrogen by the Kjeldahl procedure, and total phosphorus by means of a UV-2450 spectrophotometer after H_2SO_4 –HCIO₄ digestion (Institute of Soil Science 1978). Available soil nitrogen was determined by the alkalizable diffusion method, and available potassium by the NH₄OAc extraction method (Institute of Soil Science 1978).

Statistical analysis

The data were analyzed as for the split-plot design, with FYM as the main-plot treatment and inorganic fertilizer as the sub-plot treatment, using the analysis of variance (ANOVA) procedure of SPSS 11.5. For a significant *F*-value, the least significant difference (LSD) was used to compare the means of the variables determined. Simple linear regression was performed to characterize the relationship between MWD and SOC concentration. Differences were considered significant at P < 0.05.

 Table 2 Changes in SOC stock (0–20 cm) during the 23-year period of the experiment

Treatment	SOC change (Mg ha ⁻¹)	Rate of SOC change (Mg ha ⁻¹ year ⁻¹)
СК	-5.90 ± 1.60	-0.26 ± 0.07
Ν	-5.57 ± 2.23	-0.24 ± 0.10
NP	-6.03 ± 1.65	-0.26 ± 0.07
NPK	-5.80 ± 2.67	-0.25 ± 0.12
Mean	-5.82 ± 1.78	-0.25 ± 0.08
М	3.70 ± 1.91	0.16 ± 0.08
MN	3.60 ± 2.05	0.16 ± 0.09
MNP	6.63 ± 2.10	0.29 ± 0.09
MNPK	9.67 ± 3.10	0.42 ± 0.06
Mean	5.90 ± 2.53	0.26 ± 0.11

Results

Soil organic carbon concentration and stock

Among the treatments, MNPK treatment resulted in the highest SOC concentration and CK treatment (no fertilizer and FYM) resulted in the lowest (Table 1). Under conditions without FYM application, no significant differences between SOC concentrations were observed among the treatments, indicating that long-term application of inorganic fertilizers has no significant effect on SOC accumulation. Under conditions with FYM application, the SOC concentration was significantly higher in after MNPK treatment than after MN and M treatments. Compared with soils that received no FYM, treatment with FYM application significantly increased SOC concentration, by 50.7% on average.

Soil bulk density was used to calculate SOC stocks on a per-hectare-by-depth basis (Table 1). The results showed there were no significant differences between bulk density among CK, N, NP, and NPK treatments or among M, MN, MNP, and MNPK treatments. Under conditions with FYM treatment, however, bulk density was, on average, 1.33 g cm^{-3} , significantly lower than for treatments without FYM (1.43 g cm⁻³). SOC stocks ranged from 29.2 Mg ha⁻¹ for CK treatment to 29.5 Mg ha⁻¹ for N treatment, average 29.3 Mg ha⁻¹, for treatments without FYM, and from 38.8 Mg ha⁻¹ for M treatment to 44.8 Mg ha⁻¹ for MNPK treatment, average 41.0 Mg ha⁻¹, for

Treatment	Aggregate distri	bution (%)		Silt+clay (%)	MWD
	>2 mm	2–0.25 mm	0.25–0.05 mm	<0.05 mm	(mm)
СК	13.1 ± 4.7 c	15.1 ± 4.4 c	33.4 ± 3.9	38.4 ± 6.3	0.83 ± 0.12
Ν	$17.0 \pm 3.7 \text{ c}$	$16.7 \pm 2.8 \text{ c}$	30.4 ± 2.3	36.0 ± 2.8	0.88 ± 0.13
NP	14.9 ± 4.4 c	$16.3 \pm 2.0 \text{ c}$	38.7 ± 1.9	30.1 ± 4.5	0.85 ± 0.17
NPK	$13.0 \pm 1.3 \text{ c}$	$16.6 \pm 4.9 \text{ b}$	31.4 ± 3.0	39.1 ± 5.9	0.76 ± 0.10
Mean	$14.5 \pm 3.6 \text{ B}$	$16.2 \pm 3.0 \text{ B}$	33.5 ± 4.2	35.8 ± 5.7	0.83 ± 0.12
М	26.5 ± 5.4 b	24.5 ± 5.1 b	25.4 ± 1.7	23.6 ± 3.9	1.38 ± 0.40
MN	$22.2 \pm 3.0 \text{ b}$	$23.8 \pm 2.3 \text{ b}$	24.7 ± 3.7	26.6 ± 5.4	1.21 ± 0.13
MNP	24.2 ± 3.1	30.4 ± 2.9 a b	25.8 ± 2.4	19.0 ±4.8	1.35 ± 0.12
MNPK	23.5 ± 1.3 a	28.0 ± 2.3 a	27.9 ± 1.4	20.6 ± 1.9	1.30 ± 0.35 a
Mean	$24.1 \pm 4.7 \text{ A}$	$26.6 \pm 4.0 \ \mathrm{A}$	26.6 ± 2.4	22.5 ± 4.7	$1.31\pm0.13~\mathrm{A}$

Table 3 Affect of different fertilizer treatments on soil aggregate distribution and mean weight diameter (MWD)

Values followed by the same lowercase letter indicate that the means of aggregate-size fractions and mean weight diameter (MWD) are not significantly different (at P < 0.05) between treatments

Values followed by the same UPPERCASE letter indicate that the means of aggregate-size fractions and mean weight diameter (MWD) are not significantly different (at P < 0.05) between treatments with FYM and treatments without FYM

treatments with FYM. The initial SOC stock was 35.1 Mg ha^{-1} at the beginning of the experiment in 1982. After long-term application of FYM alone or combined with chemical fertilizers, SOC sequestration was $3.6-9.7 \text{ Mg C ha}^{-1}$, average 5.9 Mg C ha^{-1} , with a sequestration rate of 0.28 Mg ha⁻¹ year⁻¹. For soils that received no FYM, however, an average SOC loss of 5.82 Mg ha^{-1} was observed over the 23-year period (Table 2).

Soil nutrients

Table 1 shows there are significant differences between total nitrogen and available nitrogen,

phosphorus, and potassium concentrations after treatments with and without FYM application. Among treatments without FYM there were no significant differences between soil nitrogen, phosphorus, and potassium nutrients except for available phosphorus. Long-term application of FYM alone and combined with inorganic fertilizers resulted in large increases in soil nitrogen, phosphorus, and potassium nutrients, however, except for total phosphorus, compared with soils that received no FYM. Compared with the nutrient conditions at the beginning of the experiment in 1982 (Table 1), total nitrogen increased slightly but total phosphorus and available nitrogen,

Table 4 Effect of different fertilizer treatments on the soil organic carbon content of aggregate-size fractions

Treatment	Aggregate carbon concentration (g kg ⁻¹ aggregates)				
	>2 mm	2-0.25 mm	0.25–0.05 mm		
СК	13.6±1.7 b	16.1±1.5 b c	9.7±0.3 b		
Ν	11.6±0.8 b c	16.6±2.8 b c	8.8±0.6 b		
NP	12.9±3.2 b	14.1±2.1 c	9.4±0.8 b		
NPK	12.7±2.0 b c	15.9±2.5 c	9.3±0.9 b		
Mean	12.7±2.0 B	15.6±2.2 B	9.3±0.6 B		
М	15.1±0.3 b	21.4±0.2 a	12.4±0.8 a		
MN	17.2±3.0 a b	19.0±2.3 a b	13.4±1.8 a		
MNP	18.9±1.3 a	18.5±2.7 a b	12.4±0.7 a		
MNPK	20.4±1.5 a	19.8±1.4 a b	13.6±0.4 a		
Mean	17.9±2.6 A	19.7±2.0 A	13.0±1.1 A		

Values followed by the same lowercase letter indicate that the means of aggregate carbon concentrations are not significantly different (at P < 0.05) between treatments

Values followed by the same UPPERCASE letter indicate that the means of aggregate carbon concentrations are not significantly different (at P < 0.05) between treatments with FYM and treatments without FYM



Fig. 1 The distribution of SOC stocks among different aggregate-size fractions under different fertilization treatments. *Error bars* represent the standard error of the mean

phosphorus, and potassium decreased to different extents after application of chemical fertilizers alone, even after application of balanced NPK fertilizers. Under conditions of FYM application, Total nitrogen, and available nitrogen, phosphorus, and potassium concentrations increased, on average, by 70, 47, 50, and 68%, respectively, compared with the initial values.

Aggregate distribution

There were no significant differences between the different classes of aggregates and MWD among the treatments CK, N, NP, and NPK or among the treatments M, MN, MNP, and MNPK (Table 3). Under conditions without FYM application, the aggregate-size distribution was dominated (69%) by micro-aggregates (0.25–0.05 mm) and silt+clay fractions. FYM application increased the proportion of macro-aggregate classes (>2 mm and 2–0.25 mm) by 60%, on average, compared with treatments without FYM, and there was a corresponding decrease in the proportion of the micro-aggregate fractions. The MWD of the aggregates was, on average, 0.48 mm greater for treatments with FYM than for treatments without FYM.

Aggregate-associated carbon

The SOC concentrations in the three aggregatesize fractions followed a trend similar to that in bulk soil and increased with increasing total SOC

(Table 4). Among treatments concentration without FYM, no significant differences were found between organic carbon concentrations in these aggregate classes. The highest SOC concentration in all aggregate fractions occurred after MNPK treatment, but no significant difference was found among treatments M, MN, MNP, and MNPK. Long-term application of FYM resulted in a significant increase of SOC concentrations in the three aggregate fractions compared with treatments without FYM. We found the SOC concentration to be highest in the small $(17.7\pm2.9 \text{ g kg}^{-1}),$ macro-aggregate fraction intermediate in the macro-aggregate fraction $(15.3\pm3.5 \text{ g kg}^{-1})$, and lowest in the micro-aggregate fraction $(11.1\pm2.1 \text{ g kg}^{-1})$, and the differences were significant (F=3.18, P<0.0001).

The distribution of total organic carbon among the aggregate-size fractions had a pattern similar to that of the soil mass in aggregate-size classes (Fig. 1). Under conditions of treatments without FYM application, the SOC stored in the microaggregate and silt+clay fraction (54–60%) was larger than that in the macro-aggregate fractions. In contrast, 57–64% of the total SOC was stored in the macro-aggregate fractions (>0.25 mm).

Discussion

Soil organic carbon and nutrients

The long-term fertilization experiment in this arid region of northwest China showed that application of inorganic fertilizers alone had no significant effect on soil carbon sequestration compared with CK (no fertilizers) but resulted in a decrease of SOC concentration by 18%, on average, compared with the value at the beginning of the experiment. This was consistent with results from a long-term fertilization experiment in northeast China by Yang et al. (2003) who reported that inorganic N and NPK fertilization were inadequate for maintaining SOC levels under conditions of conventional management associated with no aboveground crop residues returning to the soil. In general, increased crop yields and residue returns with long-term inorganic fertilizer application can result in a higher SOC content than when no

fertilizers are applied (Haynes and Naidu 1998). In long-term experiments, however, other factors, for example the rate of application of fertilizers, rotation regime, and management practices, are also found to be important in the maintenance of SOC content. In the US, for example, Raun et al. (1998) reported that SOC increased with increasing nitrogen applied, but when nitrogen was applied at rates ≥ 90 kg ha⁻¹, surface soil organic carbon was equal to that of the control (no nitrogen applied) or slightly greater. Long-term experiments in the northern Great Plains have shown that fertilizer nitrogen increased crop residue returns but did not usually increase SOC sequestration (Halvorson et al. 2002). Russell et al. (2005) also reported that nitrogen fertilization usually had a net negative effect on carbon sequestration. Our result showed that application of FYM alone or with chemical fertilizers significantly increased SOC concentration; on average the SOC stock increased by 5.9 Mg ha⁻¹ in the 23-year period. This suggested that application of manure has strong effect on SOC maintenance and accumulation in cropping systems; this is consistent with results obtained from long-term experiments elsewhere (Kanchilkerimath and Singh 2001; Rudrappa et al. 2006). The results of this experiment showed that the SOC sequestration was $0.16-0.42 \text{ Mg ha}^{-1} \text{ year}^{-1}$, average rate 0.26 Mg ha⁻¹ year⁻¹, after application of FYM. In comparison with long-term experiments in humid regions, SOC sequestration was more significant in the arid region after application of FYM. In southeastern Norway, for example, Holeplass et al. (2004) reported that the SOC sequestration rate was 0.04-0.162 Mg ha⁻¹ year⁻¹.

The level of organic carbon in soil is believed to be a function of the net input of organic residues by the cropping system (Gregorich et al. 1996). Soil and crop-management practices such as crop rotation, residue management, and fertilization therefore have a substantial effect on the level of SOC over time. Lal (2003) reported application of inorganic fertilizer is important to obtaining high yields, but may have little impact on SOC concentration unless used in conjunction with no-till and residue management. In the current study conventional tillage practice was performed, and the aboveground biomass of wheat and maize and the roots of maize were removed. The only input of organic materials was through wheat root biomass, so use of chemical fertilizers without application of FYM did not maintain the long-term level of SOC. This agrees with results from work by Nardi et al. (2004), who reported that incorporation of limited crop residues had little effect on TOC level.

Our results also showed that soil nutrient content was significantly affected by different fertilization regimes. A previous study suggested that use of NPK fertilizers can maintain a high level of crop production throughout the period of the experiment (Suo 2005). The results in the current study showed that long-term application of chemical fertilizers without FYM did not significantly increase soil nutrient content. Imbalanced fertilization (no input of K or PK fertilizer), especially, resulted in a significant decreases in soil phosphorus and potassium nutrients. This demonstrated that nutrient depletion and deterioration of soil fertility is inevitable when only chemical fertilizers are added.

It is known that long-term application of FYM can maintain soil nutrient levels and stimulate different aspects of soil fertility, because FYM ensures the largely constant presence of active microorganisms and the regular dynamics of biomass carbon (Nardi et al. 2004). In line with this concept, our results showed that combination of FYM with inorganic fertilizers resulted in a substantial increase of nitrogen, phosphorus, and potassium in the soil, indicating the importance of adding manure when straw is removed.

Distribution of aggregates and carbon concentration and stocks in aggregates

Our results showed that long-term application of inorganic fertilizers alone had no significant role in the formation and stability of soil aggregates and, in turn, in maintenance and improvement of soil structure. Long-term application of FYM, however, substantially increased the proportion of the macro-aggregate fractions (>2 mm and 2–0.25 mm), compared with soils that received no FYM. Similarly, Aoyama et al. (1999) found an increase in SOC on addition of manure and, consequently, the formation of slaking-resistant macro-aggregates (0.25–1 mm diameter). Shirani et al. (2002) reported a significant increase in MWD on addition of FYM. This indicated that long-term application of FYM can markedly enhance soil aggregation and, therefore, improve soil structure.

Soil aggregation is affected by a variety of factors in agroecosystems, for example tillage regime (Shirani et al. 2002; Zotarelli et al. 2005), crop rotation system (Holeplass et al. 2004; Zotarelli et al. 2005), crop species (Wright and Lons 2005), residue management (Saroa and Lal 2001), and fertilization regime (Holeplass et al. 2004); soil organic carbon is believed to be a basic factor affecting aggregation stability (Elliott and Cambardella 1991). Beare et al. (1994) reported that aggregates from 2 mm to 0.25 mm in size must be protected by organic carbon, otherwise, under heavy and intensive cultivation the aggregates are disrupted. In this study conventional tillage practice was performed, and wheat and maize straw and maize roots were removed. Thus, residue incorporated into the soil by tillage was very limited and had a small effect on the formation of macro-aggregates. In these circumstances application of FYM is important for SOC accumulation and aggregate stability.

Most of the literature reports that improved soil aggregation is accompanied by more SOC in agroecosystems (Holeplass et al. 2004; Jiao et al. 2005). Similarly, we found increasing aggregate size with increasing SOC concentration and there was a close correlation between the SOC concentration and MWD, with a coefficient of determination of 0.75 (Fig. 2).



Fig. 2 The relationship between SOC concentration and mean weight diameter (MWD)

The result also showed that application of FYMsignificantly affected the concentration of SOC in whole soil and aggregate fractions, with most of the SOC stored in the macro-aggregates (>0.25 mm) under conditions of treatment with FYM. This finding was consistent with that of Whalen et al. (2003) and Jiao et al. (2005), who suggested that significant quantities of carbon from FYM were retained in whole soil and aggregate fractions. Use of inorganic fertilizer alone had a minimal effect on SOC concentration in the three aggregate-size classes, however. The results showed that the SOC concentration was highest in the small macro-aggregates (2-0.25 mm) and lowest in the macro-aggregate fraction (>2 mm). This was in accordance with several studies (Saroa and Lal 2001; Tisdall and Oades 1982) which revealed that macro-aggregates (>0.25 mm) contain higher concentrations of SOC than micro-aggregates (< 0.25-0.05 mm) because micro-aggregates are bound together by organic matter. The inter-microaggregate binding agents are the principal components of the organic carbon lost when soil is cultivated (Tisdall and Oades 1982) and the stability of aggregates protects SOC against mineralization under intensive tillage (Unger 1997). In the current study the SOC concentration in the micro-aggregates after all treatments was among the lowest, suggesting that macro-aggregates play an important role in stabilizing SOC concentration.

Conclusions

The results from this long-term fertilization experiment indicate that application of inorganic fertilizer alone is not sufficient to maintain levels of SOC and nutrients under conditions of conventional management in which no aboveground crop residues are returned to the soil. Application of FYM alone and combined with inorganic fertilizers increased SOC, soil nitrogen, phosphorus, and potassium concentrations, reduced soil bulk density, and improved the formation and stability of macro-aggregates. The SOC concentration was higher in macro-aggregates than in micro-aggregates. The study emphasizes the importance of applying FYM with inorganic NPK fertilizers in maintaining the sustainability of soil fertility when the straw is removed.

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