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## Effect of chemical and organic fertilization on soil carbon and nitrogen accumulation in a newly cultivated farmland



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### Abstract

Increased food demand from the rapidly growing human population has caused intensive land transition from desert to farmland in arid regions of northwest China. In this developing ecosystem, the optimized fertilization strategies are becoming an urgent need for sustainable crop productivity, efficient resources use, together with the delivery of ecosystems services including soil carbon (C) and nitrogen (N) accumulation. Through a 7-year field experiment with 9 fertilization treatments in a newly cultivated farmland, we tested whether different fertilizations had significant influences on soil C and N accumulation in this developing ecosystem, and also investigated possible mechanisms for this influence. The results showed that applying organic manure in cultivated farmland significantly increased the soil C and N accumulation rates; this influence was greater when it was combined with chemical fertilizer, accumulating 2.01 t C and 0.11 t N ha<sup>-1</sup> yr<sup>-1</sup> in the most successful fertilization treatment. These high rates of C and N accumulation were found associated with increased input of C and N, although the relationship between the N accumulation rate and N input was not significant. The improved soil physical properties was observed under only organic manure and integrated fertilization treatments, and the significant relationship between soil C or N and soil physical properties were also found in this study. The results suggest that in newly cultivated farmland, long term organic manure and integrated fertilization can yield significant benefits for soil C and N accumulation, and deliver additional influence on physical properties.

**Keywords:** C and N accumulation, sandy farmland, chemical fertilizer, manure, soil physical property

## 1. Introduction

Globally, soils are the largest terrestrial carbon (C) and

nitrogen (N) reservoir, but there is compelling evidence that over the last few decades large amounts of C and N have been lost from soil of natural and agricultural ecosystems through erosion, leaching and accelerated soil respiration (Di and Cameron 2002; Bellamy *et al.* 2005; Quinton *et al.* 2010). To help mitigate rising atmospheric CO<sub>2</sub> levels and improve farmland soil nutrient status and sustainability, the management of ecosystems to deliver ecosystem services such as C and N storage has recently become an important aim of agri-environment schemes in many regions (Stewart *et al.* 2007; Yan *et al.* 2007; Sainju *et al.* 2008). Commonly

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recommended management practices to increase and maintain the soil C and N stocks in farmland ecosystems include soil tillage, nutrient and fertilizer management, cover crops, efficient irrigation, and agroforestry practices (Lal 2004), among them, the balanced fertilization has been shown to be an efficient practice (Tong *et al.* 2009; Zhou *et al.* 2013).

The rational fertilization can improve the soil nutrient status and maintain high crop productivity which can directly alter the amount and quality of C and N input from crop residues (Dersch and Böhm 2001; Holeplass *et al.* 2004). The fertilization also influence soil C and N accumulation through change on soil structure, soil organic C and N component (Hai *et al.* 2010), and soil aggregation (Su 2007). Soil C and N accumulation in soils of different textures could have different responses to fertilization, because the different saturation and initial soil C and N levels could limit the soil C and N sequestration capacity (Six *et al.* 2002; Kong *et al.* 2005). Compared with the studies in other soil texture (Rudrappa *et al.* 2006; Tong *et al.* 2009; Zhou *et al.* 2013), the study about the effects of fertilization on soil C and N accumulation in sandy texture farmland developed from desert are much more limited.

Desert soil is widely distributed in the inland arid regions of northwest China, which cover about one-fifth of China's land surface (Ogle *et al.* 2012). Over the past several decades, increasing population and food demands have caused intensive land exploitation in these arid regions (Luo *et al.* 2003; Huang *et al.* 2007; Li *et al.* 2009), which led to frequent land transition from desert to farmland (Su *et al.* 2010). To maintain environmental stability and the sustainable productivity through rational agricultural practice is becoming an important issue in this developing ecosystem. However, local farming practices tend to adopt an unbalance fertilization practice, in which excessive chemical fertilizer is applied, but no organic manure, which result in some ecological problems, such as excessive resource consump-

tion, soil nutrient leaching. Thus, the optimized fertilization strategies in here are becoming a need for sustainable crop productivity and efficient resources use, and the delivery of ecosystems services including soil C and N accumulation. In this paper, we quantified the influence of long-term fertilization on soil C and N stock and their accumulation rate in such a developing ecosystem by using a 7-year field experiment. We also estimated treatment effects on the C and N input, N output, soil physical properties; and their relationship with soil C and N to explore the mechanisms underlying the influence.

## 2. Results

### 2.1. C, N input, and N output rates under different treatments

Fertilizer C input rates ranged from 0 to 2401 kg ha<sup>-1</sup> yr<sup>-1</sup> (Table 1) with maximum values under high organic manure (M3), low chemical N, phosphorus (P) levels combined with high organic manure level (NP1M3), and low chemical N, P, potassium (K) levels combined with high organic manure level (NPK1M3) treatments, and minimum values under high chemical N and P levels (NP3, CK), low chemical N, P, and K (NPK1), medium chemical N, P, and K levels (NPK2), and high chemical N, P, and K levels (NPK3) treatments. Fertilizer N input rates ranged from 120 to 311 kg ha<sup>-1</sup> yr<sup>-1</sup> with maximum values under the high chemical N, P, and K levels combined with low organic manure level (NPK3M1) treatment and minimum values under the NPK1 treatment. Average crop C input rate of 756 kg ha<sup>-1</sup> yr<sup>-1</sup>, with maximum values under the high chemical N, P, and K levels combined with low organic manure level (NPK2M2) treatment and minimum values under the M3 treatment, was 40% of average C input rate (1891 kg ha<sup>-1</sup> yr<sup>-1</sup>). Average crop N input rate of 25 kg ha<sup>-1</sup> yr<sup>-1</sup>, with maximum values under the NPK1M3

**Table 1** Estimated average annual C and N input and N output under different treatments

Treatments <sup>1)</sup>	Fertilizer input (kg ha <sup>-1</sup> )		Crop input (kg ha <sup>-1</sup> )		Crop output (kg ha <sup>-1</sup> )	
	C	N	C	N	C	N
M3	2401	134	641	17	–	177
NP3 (CK)	0	244	739	23	–	248
NPK1	0	120	713	21	–	241
NPK2	0	182	768	24	–	266
NPK3	0	244	754	24	–	266
NP1M3	2401	254	736	21	–	252
NPK1M3	2401	254	778	46	–	264
NPK2M2	1801	283	858	25	–	294
NPK3M1	1201	311	813	25	–	282

<sup>1)</sup> M3, high organic manure level; NP3, high chemical N and P levels (CK, which represented the local fertilization practice); NPK1, low chemical N, P, and K levels; NPK2, medium chemical N, P, K levels; NPK3, high chemical N, P, K levels; NP1M3, low chemical N, P levels combined with high organic manure level; NPK1M3, low chemical N, P, K levels combined with high organic manure level; NPK2M2, medium chemical N, P, K levels combined with medium organic manure level; NPK3M1, high chemical N, P, K levels combined with low organic manure level. The same as below.

treatment and minimum values under the M3 treatment, was only 11% of average fertilizer N input rate ( $225 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ) and 10% of average crop N output rate ( $254 \text{ kg ha}^{-1} \text{ yr}^{-1}$ ).

## 2.2. Soil property under different treatments

Soil property variables were strongly influenced by the treatments (Table 2). In 2012, soil C under the M3 treatment was the greatest and 79% higher than that under CK. Soil C under integrated fertilization treatments were 22–54% higher than that under CK. There was no significant soil C difference between only chemical fertilizer application treatments. Similarly, soil N under the M3 treatment was 50% higher than that under CK. Soil N under integrated fertilization treatments were 9–34% higher than that under CK. There was also no significant soil N difference between chemical fertilizer application treatments.

Soil bulk density (BD) has a maximum value of  $1.42 \text{ g cm}^{-3}$  under the NPK3 treatment and a minimum value of  $1.21 \text{ g cm}^{-3}$  under the M3 treatment. On average, BD was 16.5% lower under the M3 treatment and 9.1% lower under integrated fertilization treatments than that under CK. There were also no significant BD differences between only chemical fertilizer application treatments. Field capacity (FC) and saturated soil water content ( $\theta_s$ ) ranged from 23.6 to 39.0% and from 28.1 to 41.6%, respectively, which were both higher under M3 and integrated fertilization treatments. In addition, BD had a decreasing trend with increasing organic manure rates, and FC,  $\theta_s$ , soil C, and N had an increasing trend with increasing organic manure rates.

## 2.3. Soil C and N accumulation rate

The C and N stocks increased in all treatments (Fig. 1). The C stock mean value in 2012 was  $20.9 \text{ t ha}^{-1}$  (range:  $14.2$  to  $27.9 \text{ t ha}^{-1}$ ), which was 40% higher than that in 2006, when

it was  $15.0 \text{ t ha}^{-1}$  (range:  $12.0$  to  $18.8 \text{ t ha}^{-1}$ ). Likewise, the N stock mean value in 2012,  $2.07 \text{ t ha}^{-1}$  (range:  $1.56$  to  $2.53 \text{ t ha}^{-1}$ ), was 19% higher than the mean in 2006,  $1.74 \text{ t ha}^{-1}$  (range:  $1.50$  to  $2.14 \text{ t ha}^{-1}$ ). It can be readily seen that both C and N stocks were the highest under the M3 treatment, followed by integrated fertilization treatments, and then by only chemical fertilizer treatments.

The annual C accumulation rate had a maximum value of  $2.01 \text{ t ha}^{-1} \text{ yr}^{-1}$  under the NPK1M3 treatment, and a minimum value of  $0.09 \text{ t ha}^{-1} \text{ yr}^{-1}$  under the NPK3 treatment. The annual N accumulation rate had a maximum value of  $0.112 \text{ t ha}^{-1} \text{ yr}^{-1}$  under the NPK1M3 treatment, and a minimum value of  $0.010 \text{ t ha}^{-1} \text{ yr}^{-1}$  under the NPK2 treatment. Organic manure had a positive influence on soil C and N accumulation, especially when combined with chemical fertilizer. C accumulation rates under M3 treatment and integrated fertilization treatments were 1.9 and 1.3–2.9 times higher than that under CK. C accumulation rates under only chemical fertilizer application treatments were 21–83% lower than that under CK. N accumulation rates under M3 treatment and integrated fertilization treatments were 33.3% and 31–167% higher than that under CK. N accumulation rates under only chemical fertilizer application treatments were 50–76% lower than that under CK.

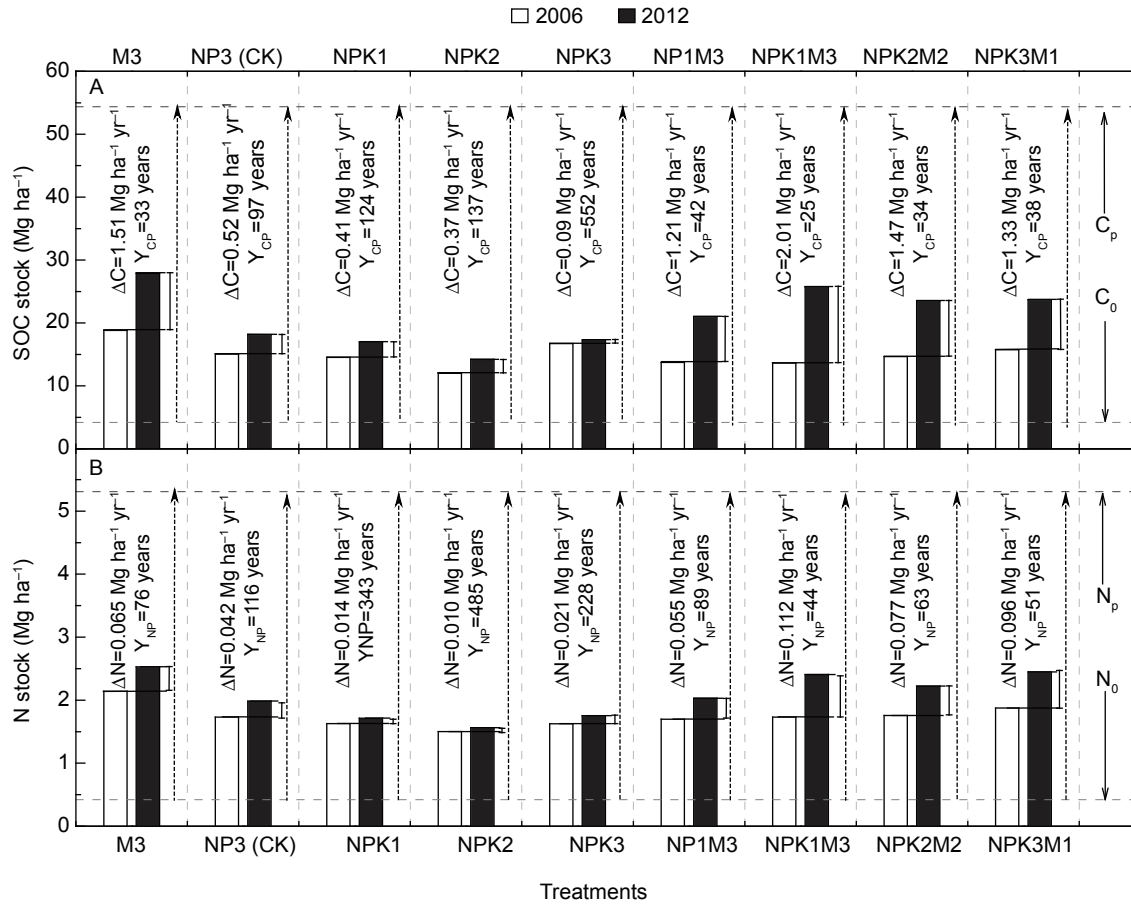
The potential soil C and N levels ( $C_p$  and  $N_p$ ) are deemed as the maximum soil C and N levels in the farmland of study area, the initial soil C and N levels ( $C_0$  and  $N_0$ ) are the soil C and N levels in the first few years when farmland was being reclaimed from desert, according to our another investigation study (Su *et al.* 2010; Liu *et al.* 2011). The time needed to reach potential soil C and N stock levels from initial soil C and N levels across the seven treatments are also presented in Fig. 1. To reach the potential soil C stock level, the soil with the organic manure application needed 33 years, with integrated fertilizations needed 25–42 years, and with only chemical fertilizer needed 97–552 years. To reach the

**Table 2** Treatment effects on soil properties in 2012<sup>1)</sup>

Treatment	BD ( $\text{g cm}^{-3}$ )	FC (%)	$\theta_s$ (%)	Soil C content ( $\text{g kg}^{-1}$ )	Soil N content ( $\text{g kg}^{-1}$ )
M3	$1.21 \pm 0.04$ c	$39.0 \pm 4.0$ a	$41.6 \pm 3.4$ a	$11.53 \pm 1.20$ a	$1.05 \pm 0.12$ a
NP3 (CK)	$1.41 \pm 0.02$ a	$23.6 \pm 0.5$ d	$28.3 \pm 0.3$ c	$6.43 \pm 0.88$ de	$0.70 \pm 0.10$ bcd
NPK1	$1.40 \pm 0.01$ a	$24.7 \pm 0.7$ cd	$28.9 \pm 0.1$ c	$6.04 \pm 0.44$ de	$0.61 \pm 0.04$ d
NPK2	$1.41 \pm 0.02$ a	$26.0 \pm 1.0$ cd	$29.0 \pm 1.6$ c	$5.02 \pm 0.38$ e	$0.55 \pm 0.03$ d
NPK3	$1.42 \pm 0.00$ a	$25.7 \pm 1.3$ cd	$28.1 \pm 1.2$ c	$6.09 \pm 0.25$ de	$0.62 \pm 0.03$ cd
NP1M3	$1.34 \pm 0.02$ b	$28.5 \pm 1.4$ bcd	$32.8 \pm 1.7$ bc	$7.85 \pm 0.61$ cd	$0.76 \pm 0.02$ bcd
NPK1M3	$1.30 \pm 0.03$ b	$33.5 \pm 4.0$ ab	$37.2 \pm 4.7$ ab	$9.91 \pm 0.36$ ab	$0.93 \pm 0.02$ ab
NPK2M2	$1.31 \pm 0.04$ b	$31.1 \pm 1.5$ bc	$35.0 \pm 1.6$ abc	$8.99 \pm 0.68$ bc	$0.85 \pm 0.07$ abc
NPK3M1	$1.35 \pm 0.01$ b	$28.0 \pm 0.6$ bcd	$31.0 \pm 0.9$ bc	$8.77 \pm 0.39$ bc	$0.91 \pm 0.12$ abc
Summary of ANOVA					
F-value	8.2	4.50	5.31	11.24	5.43
Significant level	0.000**	0.004**	0.002**	0.000**	0.001**

<sup>1)</sup>BD, bulk density; FC, field capacity;  $\theta_s$ , saturated soil water content.

Data are means  $\pm$  SE. Different lowercase letters indicate significant difference ( $P < 0.05$ ) between treatments within a variable. \*\*, significant difference at  $P < 0.01$ .



**Fig. 1** Changes of soil carbon (C) (A) and nitrogen (N) stock (B) between 2006 and 2012 under different treatments.  $\Delta C$  and  $\Delta N$ , soil C and N accumulation rate;  $C_0$  and  $N_0$ , initial soil C and N stock level in newly cultivated farmland;  $C_p$  and  $N_p$ , potential soil C and N stock level;  $Y_C$  and  $Y_N$ , the year to reach potential soil C and N stock level. M3, high organic manure level; NP3, high chemical N and P levels (CK, which represented the local fertilization practice); NPK1, low chemical N, P, and K levels; NPK2, medium chemical N, P, K levels; NPK3, high chemical N, P, K levels; NP1M3, low chemical N, P levels combined with high organic manure level; NPK1M3, low chemical N, P, K levels combined with high organic manure level; NPK2M2, medium chemical N, P, K levels combined with medium organic manure level; NPK3M1, high chemical N, P, K levels combined with low organic manure level. The same as below.

potential soil N stock level, the soil with the organic manure application needed 76 years, with integrated fertilization needed 44–89 years, and with only chemical fertilizer needed 116–485 years.

#### 2.4. Factors influencing soil C and N

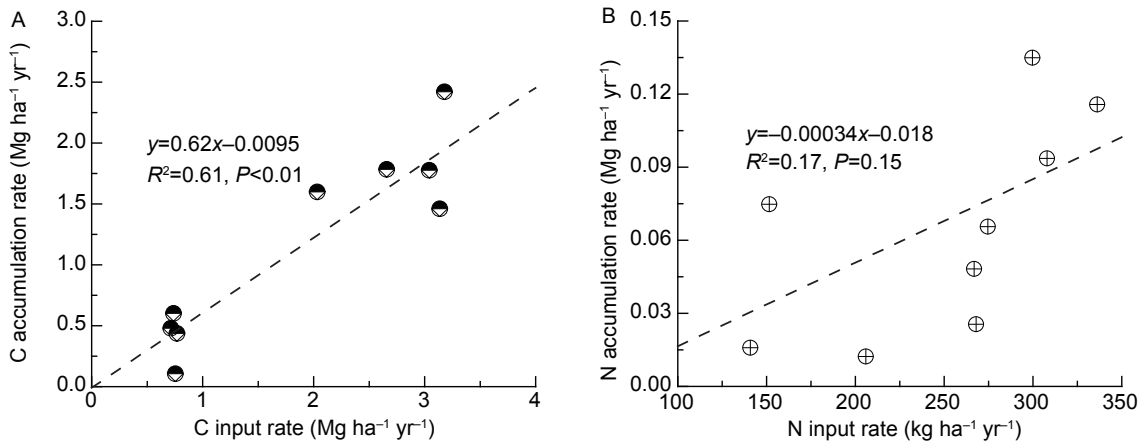
The soil C accumulation rate increased with increasing C input ( $R^2=0.61$ ,  $P<0.01$ ), indicating that the C input was potentially a main factor influencing soil carbon accumulation in this newly cultivated sandy farmland. The soil N accumulation rate also had an increase trend with the increasing N input, but the relationship was not significant ( $R^2=0.17$ ,  $P=0.15$ ) (Fig. 2).

Soil C and N contents were significantly negatively related to BD ( $R^2=0.59$ ,  $P<0.01$ ;  $R^2=0.65$ ,  $P<0.01$ ). In contrast, they were significantly positively related to FC ( $R^2=0.64$ ,

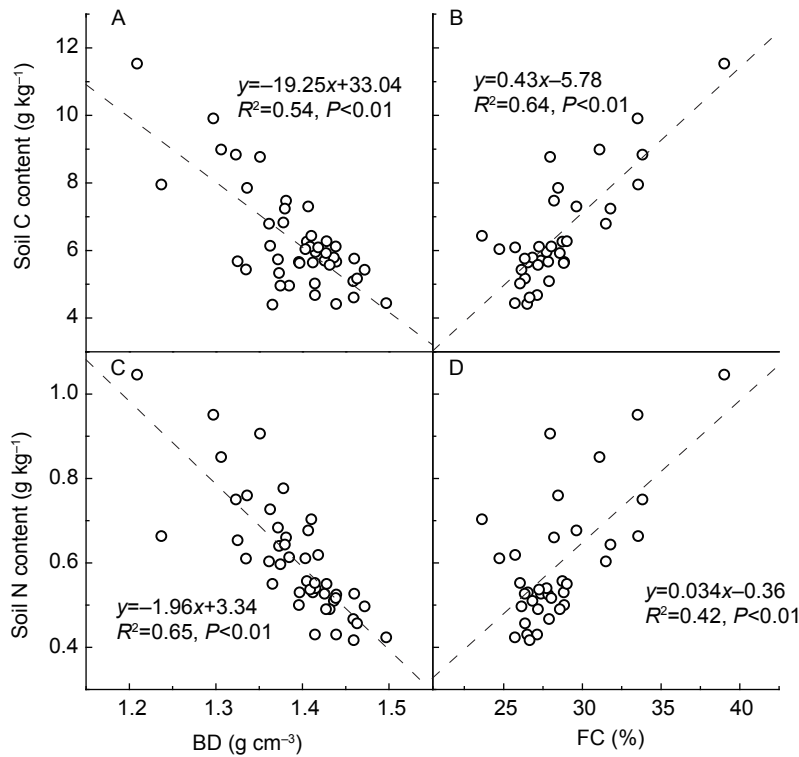
$P<0.01$ ;  $R^2=0.42$ ,  $P<0.01$ ) (Fig. 3). Soil C and N contents were significantly negatively related to soil sand content ( $R^2=0.22$ ,  $P<0.01$ ;  $R^2=0.17$ ,  $P<0.05$ ). In contrast, they were significantly positively related to soil silt content ( $R^2=0.30$ ,  $P<0.01$ ;  $R^2=0.25$ ,  $P<0.01$ ) (Fig. 4).

### 3. Discussion

The study indicated that in newly cultivated sandy farmland of the Hexi Corridor Oasis, soil C and N accumulation were strongly influenced by the different fertilization treatments, and the organic manure especially combined with chemical fertilizer had a positive influence on soil C and N accumulation. The conclusion has also been confirmed by many previous studies (Holeplass et al. 2004; Rudrappa et al. 2006; Su et al. 2006). However, we found that the organic manure's influence on soil C and N accumulation



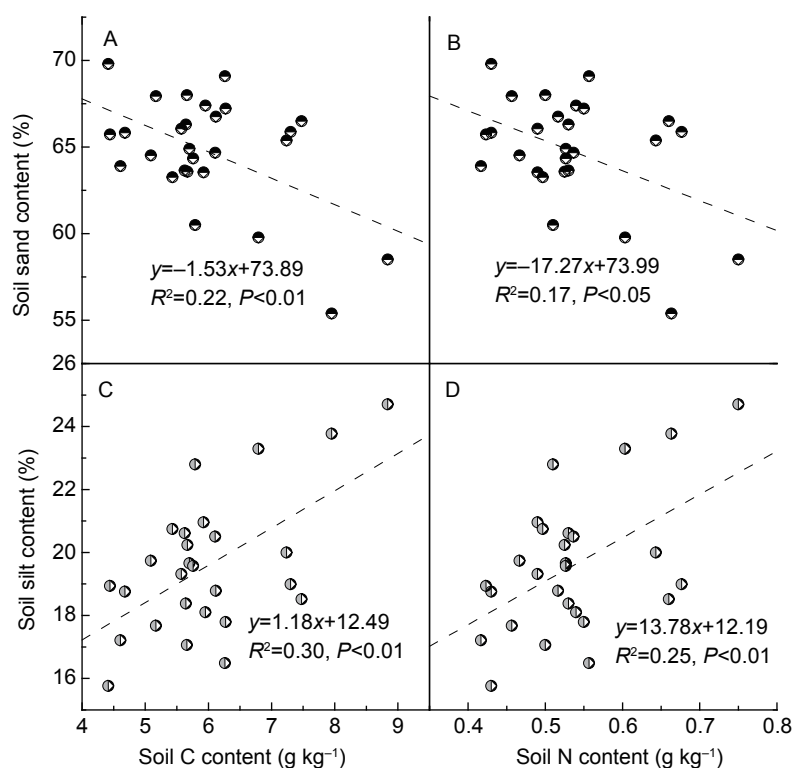
**Fig. 2** Relationships between C input rate and C accumulation rate (A) and N input rate and N accumulation rate (B).



**Fig. 3** Relationships between soil C and bulk density (BD) (A) or field capacity (FC) (B); soil N and BD (C) or FC (D).

was particularly strong in this study. The soil C and N accumulation rates were nearly four and three times higher in organic manure and integrated fertilization treatments than in chemical fertilizer treatments, respectively. There may be several factors contributing to the high C and N accumulation rates in our study, including (1) the low initial soil C and N stocks in cultivated sandy farmland which suggested a high probability for increasing soil C and N stocks; and (2) dry and windy climate conditions which can decelerate the decomposition and release of soil organic matter.

As soil C and N levels are a dynamic balance between soil C and N inputs and outputs (Qin and Huang 2010), an important issue which needs to be discussed is whether the differences in C and N inputs and outputs between treatments can explain the actual difference in soil C and N accumulation in our study. During the experiment period, all aboveground biomass and much of the underground biomass in these plots were removed according to local cultivation habits. Thus, the C input in the soil of only chemical fertilizer treatments were scarce. This appears



**Fig. 4** Relationships between soil C and soil sand contents (A) or soil silt content (C); soil N and soil sand contents (B) or soil silt content (D).

to be the primary reason for the low C accumulation in these treatments, which can be concluded from the linear relationship between the C input and C accumulation rate in Fig. 2. This linear relationship has also been reported in other studies (Li *et al.* 2010; Ghosh *et al.* 2012). The C accumulation rates in the M3 treatments accounted for 56% of the C input (sum of root and organic manure input C), while this value was 74, 65, and 77% in NPK1M3, NPK2M2, NPK3M1 treatments, respectively. The results indicate that integrated fertilization treatments are, at least, losing less soil C than the organic manure application treatment. Some of this input C may be sequestered for prolonged periods, while the rest may be respired through microbial metabolism or leaching to deeper soil horizons to determine whether there is net C storage or loss (Bagchi and Ritchie 2010). So integrated fertilization could avoid a relative loss of at least 9% of input C compared with only organic manure application. There may be several potential mechanisms contributing to this phenomenon, including (1) balanced fertilization can increase the input C through increasing crop belowground biomass; and (2) different fertilization may affect the soil microbial metabolism and then C respiration.

Although the N accumulation rate had an increasing trend with increasing N input, the linear relationship between them was not significant (Fig. 2). Different from soil C, the crop N uptake had a large influence on soil N accumulation

(Yang *et al.* 2006). However, the linear regression between output N and N accumulation rate was still not significant. In sandy soil texture, the inorganic N leaching rate was also high (Di *et al.* 2002). Therefore, the more likely explanation for this phenomenon is that large N loss through leaching in the sandy soil has a significant influence on soil N balance, and the different N losses from different fertilization treatments weakened the influence of N input and output on soil N accumulation.

Because the soil N accumulation rate had no significant relationship with N input and output, the treatment differences in the soil N accumulation rate must have been caused by other factors. Yang *et al.* (2011) reported that organic manure combined with chemical fertilizer application can reduce soil BD, increase total porosity, improve soil water retention, and decrease unsaturated hydraulic compared with only chemical fertilizer application. In this paper, our study confirmed that organic manure can decrease BD but increase FC and  $\theta_s$ . These results indicated that organic manure application has positive influence on improving soil structure. Other studies (Rochon *et al.* 2004; Holtham *et al.* 2007) reported that soil structure and soil aggregation improvement have positive influence on the physical protection of C and N in the soil, which has a positive influence on soil C and N accumulation. This conclusion was confirmed by the relationships of the soil C and N to soil physical prop-

erties in our study. The findings indicate that the changes in some soil properties in different treatments could be a driving factor behind soil C and N accumulation.

## 4. Conclusion

Our results showed that in newly cultivated farmland of the Hexi Corridor Oasis, organic manure and integrated fertilization can yield significant benefits for soil C and N accumulation. The increasing C input may be the main factor in soil C accumulation. Soil structure improvement, which promotes the physical protection of soil N, can contribute to the increasing of soil N accumulation, also has benefit for soil C accumulation.

## 5. Materials and methods

### 5.1. Study site and experimental design

The field experiment was established in 2005 at the Linze Inland River Basin Research Station (39°21'N, 100°07'E, elevation 1367 m) in Gansu Province, northwest China. The site has a sandy loamy soil, and the pre-experimental soil at the 0–20 cm depth contained 658 g kg<sup>-1</sup> sand, 201 g kg<sup>-1</sup> silt, 141 g kg<sup>-1</sup> clay with a pH value of 8.9. The climate is strongly continental, with long cold winters and dry hot summers (Su *et al.* 2007). The 7-year (2006–2012) average precipitation and daily temperature during the April through September growing season are 110.6 mm and 19.3°C, respectively.

The experiment consisted of nine fertilization treatments, established on 27 plots (3.7 m×3.7 m) in a randomized block design with three replicates. Crops were planted in a rotation sequence of maize (*Zea mays* L.)-maize-soybean (*Glycine max* L. Merr.) with various fertilization treatments. The fertilization treatments were: (1) M3, high organic manure level; (2) NP3, high chemical N and phosphorus (P) levels (CK, which represented the local fertilization practice); (3) NPK1, low chemical N, P, and potassium (K) levels; (4) NPK2, medium chemical N, P, K levels; (5) NPK3, high chemical N, P, K levels; (6) NP1M3, low chemical N, P levels combined with high organic manure level; (7) NPK1M3, low chemical N, P, K levels combined with high organic manure level; (8) NPK2M2, medium chemical N, P, K levels combined with medium organic manure level; (9) NPK3M1, high chemical N, P, K levels combined with low organic manure level. Chemical N, P, and K fertilizer were applied at rate of 300–225–150 (high–medium–low) kg N ha<sup>-1</sup>, 225–135–90 (high–medium–low) kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 225–135–90 (high–medium–low) kg K<sub>2</sub>O ha<sup>-1</sup> for maize, and at rate of 105–75–45 (high–medium–low) kg N ha<sup>-1</sup>, 90–60–30 (high–medium–low) kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 90–60–30

(high–medium–low) kg K<sub>2</sub>O ha<sup>-1</sup> for soybean. Organic manure was applied at rate of 30–22.5–15 (high–medium–low) Mg M ha<sup>-1</sup> for both maize and soybean.

Diammonium phosphate (46% P<sub>2</sub>O<sub>5</sub>, 18% N), muriate of potash (60% K<sub>2</sub>O), and urea (46% N) were applied as chemical fertilizer. Organic manure was collected from composted domestic animal waste (sheep or cattle), which contained 20% moisture, 111.5 g kg<sup>-1</sup> organic carbon (OC), 6.2 g kg<sup>-1</sup> N in 2006–2007; 20% moisture, 90.5 g kg<sup>-1</sup> OC, 5.4 g kg<sup>-1</sup> N in 2008–2010; 25% moisture, 109.8 g kg<sup>-1</sup> OC, 5.6 g kg<sup>-1</sup> N in 2011–2012. All chemical P, K fertilizers, organic manure and one-third of chemical N fertilizer were applied as basal fertilizers, which were evenly broadcasted onto the soil surface and immediately ploughed into the soil (0–20 cm depth) before sowing. Supplementary N fertilizers were manually applied before irrigation in the jointing and booting stages for maize and in jointing and anthesis stages for soybean. A total of 8500 to 11500 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> of irrigation water was applied in whole growth stage in each of the treatments. Identical irrigation, herbicides and other field management strategies were adopted for all treatments.

### 5.2. Data collection

In each of the plots, the dry biomass of grain, stems, and leaves were weighted during crop harvest period (late September) for seven consecutive growing seasons (2006–2012). Root dry biomass was estimated using a proportion of above-ground and under-ground biomass, which came from the long-term (2004–2012) monitoring data of the Chinese Ecosystem Research Network in the Linze Inland River Basin Research Station. The C and N contents in grains, stems, leaves, and roots were measured using an elemental analysis instrument (Elementar Vario MICRO cube, Germany).

After crop harvest, one disturbed soil (mixed from six soil cores which were taken randomly by a 7-cm-diameter soil column cylinder auger) and two undisturbed core samples (100 cm<sup>3</sup>) were collected from the 0–20 cm soil layer in each of the experimental plots. A total of 27 disturbed samples and 54 undisturbed core samples were collected from 27 plots. Disturbed samples were air-dried at room temperature and separated into two sub-samples. One sub-sample was ground to pass through a 2-mm mesh and was subjected to particle-size analysis by pipette method in a sedimentation cylinder using sodium hexametaphosphate as the dispersing agent (Gee and Bauder 1986). The other sub-sample was passed through a 0.25-mm mesh for analysis of soil C content (g kg<sup>-1</sup>) by the dichromate oxidation of Walkley-Black (Nelson and Sommers 1982) and soil N content (g kg<sup>-1</sup>) was measured by the micro-Kjeldahl procedure (Bremner and Mulvaney 1982). Undisturbed soil samples were used to

determine soil bulk density (BD, g cm<sup>-3</sup>), field capacity (FC, %), and saturated soil water content ( $\theta_s$ , %) by the Wilcox method (Cassel and Nielsen 1986).

### 5.3. Calculations of C and N input, and accumulation

The C and N inputs by crop residues were estimated by the dry weights and the C and N content of crop residues. The C and N inputs by fertilization were calculated as follows:

$$E_{in} = \sum_{i=1}^n [Q_{om_i} \times E\text{-content}_{om_i} \times (1 - W_{om_i}) + E_{fer_i}] / n \quad (1)$$

Where,  $E_{in}$  is the C or N input by fertilization (kg ha<sup>-1</sup> yr<sup>-1</sup>),  $i$  is the  $i$ th experiment year,  $Q_{om}$  is the amount of organic manure (kg ha<sup>-1</sup> yr<sup>-1</sup>),  $E\text{-content}_{om}$  is the C or N content in organic manure (g kg<sup>-1</sup>),  $W_{om}$  is the organic manure moisture (%), and  $E_{fer}$  is the C or N content of chemical fertilizer (kg ha<sup>-1</sup>),  $n$  is the experimental years.

Soil C and N stocks can be calculated as follows:

$$E\text{-stock} = BD \times d \times E\text{-content} \times 0.1 \quad (2)$$

Where,  $E\text{-stock}$  is C or N stock (t ha<sup>-1</sup>),  $BD$  is the soil bulk density (g cm<sup>-3</sup>),  $d$  is the soil depth (20 cm), and  $E\text{-content}$  is C or N content in soil (g kg<sup>-1</sup>). By using stock-difference method, soil C and N accumulation rates were calculated as follows:

$$\Delta E = \frac{(E_{t_2} - E_{t_1})}{t_2 - t_1} \quad (3)$$

Where,  $\Delta E$  is the soil C or N accumulation rates (t ha<sup>-1</sup> yr<sup>-1</sup>),  $E_{t_2}$  is the soil C or N stocks in  $t_2$  (t ha<sup>-1</sup>),  $E_{t_1}$  is the soil C or N stocks in  $t_1$ , and  $t$  is the time.

### 5.4. Statistical analysis

Analyses of variance (ANOVAs) were performed using SPSS 19.0 for Windows statistical software (Arbuckle 2010) to determine treatment effects on soil properties. When treatment effects were significant ( $P < 0.05$ ), treatment means were compared using Duncan's multiple range test at 5% significance level. The linear regressions were used to examine the relationships of BD, FC, soil sandy and silt content, C and N input with soil C and N. Data are reported as means  $\pm$  SE.

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