European Journal of Soil Science

European Journal of Soil Science, January 2017, **68**, 39–47 doi: 10.1111/ejss.12400

Increase in soil organic carbon in a Mollisol following simulated initial development from parent material

M. You[a], N. Li[a], W. Zou[a], X. Han[a] & M. Burger[b]

[a]*Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, 138 Haping Road, Harbin, 150081, China, and* [b]*Department of Land, Air and Water Resources, University of California, One Shields Avenue, Davis, CA 95616, USA*

Summary

Better understanding of the effects of land use and agricultural management on organic carbon (C) sequestration is needed to optimize the restoration of fertility in degraded soil, maintain agricultural sustainability and mitigate emissions of greenhouse gases with C sequestration. Most current studies of C sequestration focus on mature soil, whereas there has been little research on soil development from C-poor parent material. The aim of this study was to assess soil organic carbon (SOC) stocks and C sequestration rates during the early stages of development of a Mollisol from parent material under different types of vegetation, fertilizer application regimes and organic matter inputs, and to compare the results with C sequestration of a mature Mollisol under similar management. Carbon stocks were recorded from 2004 to 2012 in the parent material of a Mollisol under natural fallow (NatF), alfalfa (*Medicago sativa* L.) (Alfa) or soya beans (*Glycine max* (Merrill.) L.) and maize (*Zea mays* L.) (S–M) rotations with and without fertilizer application and crop residues returned or removed at harvest. There was a positive non-linear relation between C inputs and SOC stocks; increases in SOC stocks decreased with larger additions of C. After 8 years of treatments, the SOC stocks at 0–20-cm depth had increased in the order S–M + fertilizer + all residues returned (61%) > Alfa (60%) > S–M + fertilizer + part of residues returned (50%) > NatF (30%) > S–M + fertilizer without residue return (17%) > S–M without fertilizer or residue return (7%). These increases in SOC stocks corresponded to annual C sequestration rates of 0.02–0.83 Mg C ha^{-1} $year^{-1}$. In contrast, SOC stocks in the 0–20-cm layer of the mature Mollisol changed little under similar treatments in a wheat (*Triticum aestivum* L.)–maize–soya bean rotation. Our results have practical implications for how vegetation and agricultural practices could be used to optimize soil restoration and C sequestration in a temperate continental monsoon climate.

Highlights

- What management practices and vegetation lead to carbon sequestration during soil development?
- Use of a subsoil (parent material) to study soil development.
- Carbon stocks increased as much in alfalfa as in soya bean–maize rotations with residue return.
- Perennial legume systems sequester carbon comparable to annual systems with larger carbon inputs.

Introduction

The sequestration of soil organic carbon (SOC) contributes to improvement in soil fertility of agricultural land and mitigation of greenhouse gas emissions (Lal, 2004; Johnson *et al*., 2007; Karlen *et al*., 2013). The Mollisols in northeast China are losing soil in certain areas because of erosion related to continuous cultivation under poor management that causes losses of C, which affects the productivity of agricultural soil (Quinton *et al*., 2010; Liu *et al*., 2011). In addition to the soil's inherent properties (i.e. initial soil organic C content, soil texture and type of clay), land use such as type of vegetation (Bell *et al*., 2012) and agricultural practices such as the application of chemical fertilizers (Cai & Qin, 2006) and crop residues (Qiao *et al*., 2014) also affect SOC stocks. Some research suggests that chemical fertilizers increase soil C content by enhancing C input through rhizodeposition and the amount of

Correspondence: X. Han. E-mail: xzhan@iga.ac.cn
Received 17 September 2015; revised version accepted 13 October 2016

returned residues (Haynes & Naidu, 1998; Purakayastha & Katyal, 1998; Gong *et al*., 2009), whereas other research indicates that organic fertilizers increase C stocks more effectively than the use of mineral fertilizers alone (Srinivasarao *et al*., 2012).

The two main sources of C input to the soil are humified root or shoot material, root exudates and other root-borne organic substances released into the rhizosphere during plant growth (Kuzyakov & Domanski, 2000). Differences in the amounts of C returned to the soil as above- and below-ground biomass might affect C storage. Allmaras *et al*. (2004) reported that root biomass, together with root exudates, mucilages and sloughed off tissue, contributed 1.7–3.5 times more C to soil C than above-ground residues of corn. Rates of humification in soil are also affected by the chemical composition of plant parts. For example, Halvorson *et al*. (2002) reported C sequestration rates of 0.14–0.23 Mg C ha^{-1} $year^{-1}$ in a continuous dryland cropping system in the northern Great Plains of the USA. Sainju & Lenssen (2011), however, attributed a relatively large rate of carbon sequestration of 0.33 Mg C ha^{-1} $year^{-1}$, with a returned biomass that included perennial legume forages of 1.04 Mg C ha^{-1} $year^{-1}$, to a larger rate of humification because of the greater input of C from below-ground biomass. Both the amount and quality of organic matter inputs seem to affect rates of C sequestration.

In northeast China, Mollisols have formed from a parent material of illuvial sediments, which had a larger C content than that of rock; mature Mollisols are well known for their large C content. The Mollisol parent material, however, has a smaller C content than the mature Mollisol, and is therefore an ideal medium to study soil C dynamics during surface soil development. You *et al*. (2014) previously reported significant increases in soil C concentration in the surface soil (0–20 cm) of this parent material under soya bean (*Glycine max* (Merrill.) L.) and maize (*Zea mays* L.) (S–M) rotations with inputs of fertilizers and crop residues over 5 years of cultivation. There were no increases in total C under alfalfa (Alfa) or natural (not seeded) grassland (NatF) over this time frame. Rates of C sequestration appeared to depend mainly on the amount of C inputs. However, the C content increased in the occluded light fraction, determined by density fractionation by mass (including the free and occluded light fractions, and the heavy fraction), for all treatments (S–M rotations, Alfa and NatF). Consequently, the experiment needed to be extended to obtain a better understanding of the effects of land use and management for large-scale soil restoration purposes in this region. We expected that the effects of different agricultural practices on soil C accumulation in the parent material of Mollisols could be demonstrated well by using C contents in the parent material as the baseline.

The objectives of the present research were to measure SOC stocks in the 0–20-cm layer of parent material of Mollisols for 8 years under different types of vegetation and in S–M rotations with different fertilizer and residue return management to compare the effects of the following treatments on SOC stocks: (i) perennial legume cultivation and naturally occurring grassland, (ii) S–M rotations with and without fertilizer and (iii) S–M rotations with residue return and fertilizer or fertilizer only. For comparison, we also assessed the effects of similar treatments on SOC stocks in wheat (*Triticum aestivum* L.)–maize–soya bean rotations in the surface soil of a mature Mollisol. In addition, we wanted to quantify the relation between C inputs and SOC stocks in the soil profile of the parent material. The treatments in the parent material included two perennials (alfalfa and natural grassland) and S–M crop rotations with or without chemical fertilizer applications and different amounts of above-ground residues incorporated into the soil. We hypothesized that more C would be sequestered under perennial legumes than non-legumes, and that both fertilizer application and residue return management would increase SOC stocks in S–M rotations.

Materials and methods

Site description and soil sampling

The long-term restoration experiment was in the centre of the area of Mollisols in northeast China at the State Key Experimental Station of Agroecology, Chinese Academy of Sciences, Hailun, Heilongjiang province (47°26′N, 126°38′E). The Mollisols have formed from sedimentary materials of loamy loess parent material (Xiong & Li, 1987). Typical soil profiles are A–AB–BC (A, 0–0.6 m depth; AB, 0.6–1.15 m; BC, 1.15–1.5 m; C, > 1.7 m). The depth to bedrock is > 20 m. The soil type is classified as Pachic Haploborolls according to the USDA Soil Taxonomy (Soil Survey Staff, 2010). The climate in the region is typical temperate continental monsoon, characterized by cold winters and hot summers; the mean annual temperature is 2.2°C. Total annual precipitation averages 550 mm, and about 358 mm occurs from June to August.

The long-term restoration experiment started in June 2004 with the construction of 24 enclosed plots (1.4-m long, 1.0-m wide and 0.8-m deep). The enclosures consisted of cement strips (0.2-m wide and 0.8-m depth) that protruded 0.1 m above the ground. These enclosures were refilled with parent material taken from below 2-m depth (C horizon) at a location 10 m from the experimental plots. The parent material had a semi-blocky structure. A piece of nylon mesh with 0.075-mm openings was placed on the parent material at 0.8-m depth to prevent root penetration, but it allowed water and nutrient exchange. The parent material contained 4.79 g C and 0.41 g N kg^{-1} soil, and had a pH of 6.88 (soil:deionized water 1:2.5), a soil bulk density (SBD) of 1.35 Mg m^{-3}, 420 g kg^{-1} clay (<0.002 mm) and 356 g kg^{-1} silt (0.02 – 0.002 mm). The dominant clay minerals were vermiculite, chlorite and illite.

In Experiment I, the following six treatments were assigned to the 24 plots with the parent material as a randomized block design with four replicates: natural fallow without weed control (vegetation consisted mainly of *Leymus chinensi*, *Poa annua* L. and *Equisetum tataricus*) (NatF), N-fixing alfalfa (*Medicago sativa* L.) (Alfa) and four S–M rotations: (i) S – M without mineral fertilizer and biomass removal (F0C0), (ii) S–M with mineral fertilizer and biomass removal (F1C0), (iii) S–M with mineral fertilizer and partial return of residue as a soya bean powder-maize straw mixture (F1C1) and (iv) S–M with mineral fertilizer and all biomass incorporated (F1C2) (Table 1). The cut biomass in each plot of NatF and Alfa treatments was left in the fields as mulch. In F1C1, 2250 kg ha^{-1} ground soya bean seeds that had been baked at 200°C and 4500 kg ha^{-1} chopped (20–50-mm long pieces) maize straw were incorporated into the soil at the same time as the other residue return treatments. The C content in soya bean powder and maize straw was equivalent to the C content of a typical organic manure application and return of maize straw in this region (Qiao *et al*., 2014). In the F1C2 treatment, the entire biomass of each year's crop was incorporated, but the maize grain and soya beans were pulverized as above. The fertilizer sources were diammonium phosphate and potassium sulphate, applied at 54, 60 and 54 kg N, P and K ha^{-1} year^{-1}, respectively, for soya beans and maize. An additional 140 kg N ha^{-1-}year^{-1} was applied as urea at the V8 stage of maize growth. The parent material was tilled to 20-cm depth with a spade to incorporate the chopped crop residue, and ridges (0.7-m wide and 0.25-m high) were made after applying the organic amendments. The first crop of the rotation was soya beans. The crop densities were 70 000 and 270 000 plants ha^{-1} for maize and soya beans, respectively. The crops were sown in rows in May and harvested in October. At harvest, the above-ground biomass in each plot was cut and weighed, and a subsample was oven-dried at 60°C to determine the dry weight of grain and non-grain biomass.

Experiment II was carried out in a mature Mollisol soil in a long-term field experiment (established in 1990) of a wheat (*Triticum aestivum* L.)–maize–soya bean rotation in a randomized block design with three treatments, each with three replicates: (i) no fertilizer and without residues returned (M0C0), (ii) chemical fertilizer made up of N, P and K (M1C0) and without residues returned and (iii) application of chemical fertilizers (N, P and K) and crop residues (M1C1). Each replicate covered a surface area of 12 m × 5.6 m and was separated from other replicates by a 0.7-m buffer strip. The fertilizer and residue inputs were as follows: (i) for maize, 120 kg N ha^{-1}, 36 kg P ha^{-1}, 30 kg K ha^{-1} and 1.8 Mg C-derived maize residue ha^{-1}, (ii) for soya beans, 20.25 kg N ha^{-1}, 36 kg P ha^{-1}, 30 kg K ha^{-1} and 1.9 Mg C-derived soybean residue ha^{-1} and (iii) for wheat, 75 kg N ha^{-1}, 36 kg P ha^{-1}, 30 kg K ha^{-1} and 1.7 Mg C-derived wheat residue ha^{-1}. The residues were chopped into 5-cm long pieces and air-dried before they were returned to the soil. Urea was split into two applications for the treatments that received N fertilizer, the basal and supplementary fertilizers, applied at the ratio of 1:2 for maize. In all plots, the soil was rotary tilled to a depth of 20 cm and ridges were established after the crop harvest in autumn.

At each experimental site (Experiments I and II), 20 g soil were sampled from the surface layer (0–20 cm) of each replicate in October after the autumn harvest each year from 2004 to 2012. Five cores of soil were taken with a 50-mm diameter auger and mixed to form a composite sample. After the visible roots, fauna and organic debris had been removed by hand, the soil samples were sieved (< 2 mm) and air-dried. A portion of the air-dried samples was ground (< 0.25 mm) before the organic C analyses.

At the end of Experiment I in 2012, four soil cores (100 cm^3, 5-cm width) per layer and replicate were taken to measure soil bulk density (SBD) after drying the soil cores at 105°C for 48 hours. Soil from each of the 0–20-, 20–30- and 30–40-cm depth intervals of three replicates of Experiment I was excavated and mixed thoroughly to form homogeneous composite samples, from each of which a subsample was taken. The fourth replicates were kept for other observations.

Total soil organic C content and total N were analysed with a VarioEL CHN elemental analyser (Heraeus Elementar Vario EL, Hanau, Germany) in finely ground subsamples of the air-dried field samples. The organic C content was estimated after accounting for $CaCO_3$, which was determined separately as described by Santi *et al*. (2006).

Calculations and statistical analyses

Soil organic C stocks in the top 0–40 cm for each treatment were calculated as:

$$SOC_{stock} = C \times SBD \times T,$$

where C is the organic C concentration (kg Mg^{-1}), SBD is soil bulk density (Mg m^{-3}) and T is the thickness of the soil layer (m).

Estimates of cumulative C inputs for the six treatments of Experiment I were derived from 8 years (2004–2011) of yield data. Carbon inputs included above-ground biomass C, root biomass C and external C sources, if applicable (Table 1). The average C concentrations in above-ground biomass and roots, measured every year in the harvested biomass after oven-drying at 60°C, were 430, 440, 440 and 430 g C kg^{-1} dry biomass for grasses, alfalfa, maize and soya beans, respectively. The root biomass, which was considered as a C input in all the treatments, was derived from shoot biomass and published root:shoot ratios observed in other studies. The ratio of root to above-ground biomass for grasses and alfalfa was assumed to be 2.0 and 0.4 (Mokany *et al*., 2006), respectively, and for maize and soya beans, 0.19 and 0.13, respectively (Zhang & Zhu, 1990).

Carbon stocks were calculated from measurements taken year after year in the same plots. Therefore, a residual maximum likelihood (REML) model with an ante-dependence covariance structure, which takes autocorrelation and heteroscedasticity among samples within treatment plots over time into account, was used (Webster & Payne, 2002) to assess C sequestration in the 0–20-cm layer of the six treatments imposed in the subsoil plots (Experiment I) and in the three treatments in the mature Mollisol soil (Experiment II).

In addition, an exponential regression model was fitted to the cumulative C input and C stocks at 0–20-cm depth of Experiment I. The F0C0 treatment was used as the baseline treatment to calculate the cumulative C inputs and C sequestration rates in the S–M treatments. At the end of Experiment I in 2012, SOC stocks for the different treatments were measured in three layers (0–20, 20–30 and 30–40 cm) and evaluated by a mixed effects model with an

Table 1 Experimental treatments (i.e. crop rotations, inputs of plant material and mineral fertilizer, tillage and sample layers) in the two 8-year experiments

Experiment	Treatments	Land use	Organic inputs[a]	Mineral fertilizer input[b]	Tillage[c]	Sample layers[d] 2004–2012 Depth/cm	2012 Depth/cm
Experiment I, four replicates	NatF	Natural fallow	Litter and roots	None	No	0–20	20–30
	Alfa	Alfalfa	Litter and roots	None	No		30–40
	F0C0	Soya beans or maize	Roots only	None	Yes		
	F1C0	Soya beans or maize	Roots only	NPK	Yes		
	F1C1	Soya beans or maize	Soya bean seeds, maize straw, roots	NPK	Yes		
	F1C2	Soya beans or maize	Crop straw, seeds and roots only	NPK	Yes		
Experiment II, three replicates	M0C0	Wheat, soya beans or maize	Roots only	None	Yes	0–20	
	M1C0	Wheat, soya beans or maize	Roots only	NPK	Yes		
	M1C1	Wheat, soya beans or maize	Crop straw and roots	NPK	Yes		

[a]The amendment rates in F1C1 were 2250 kg ha^{-1} of baked soya bean powder and 4500 kg ha^{-1} of maize straw mixed homogeneously.
[b]Diammonium phosphate was the source of N and P, applied at a rate of 300 kg ha^{-1} year^{-1}; potassium sulphate (K) was applied at a rate of 120 kg ha^{-1} year^{-1}.
[c]Tillage to 20-cm depth was carried out manually in both experiments, with ridges made in October after harvest.
[d]Soil samples were collected from the 0–20-cm depth every October after harvest from 2004 to 2012. A soil profile (0–20, 20–30 and 30–40 cm) was sampled in 2012 only.
NatF, natural fallow (grasses); Alfa, alfalfa; F0C0, unfertilized soya bean-maize (S–M) F1C0, chemical fertilizer S–M; F1C1, organic amendments plus chemical fertilizer S–M; F1C2, chemical fertilizer and above-ground crop biomass returned S–M; M0C0, mature surface Mollisol without fertilizer S–M; M1C0, mature surface Mollisol with chemical fertilizer S–M; M1C1, mature surface Mollisol with chemical fertilizer plus crop residues S–M.

ante-dependence covariance structure to account for autocorrelation and heteroscedasticity within treatment plots. Differences in SOC stocks at 0–40-cm depth of the treatments NatF against Alfa, F0C0 against F1C0, and F1C0 and F1C2 were assessed by contrasts with Fisher's least significant difference (LSD), with $P < 0.05$ as the criterion of significance. The response variables of all three datasets were transformed to natural logarithms (ln) for the analyses to improve the distribution of the residual errors, which were assessed by Shapiro–Wilk tests. All analyses were carried out with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Soil bulk density

In Experiment I, the soil bulk density in the surface layer (0–20-cm depth) decreased from 1.35 Mg m^{-3} to between 0.90 (F1C2 and F1C1) and 1.13 Mg m^{-3} (NatF) during 8 years of soil development (Figure 1). The repeated measures analysis suggested that there were significant effects from treatment and for time on bulk density (statistical results not shown); the decrease in bulk density for the treatments with perennial vegetation was less than that for S–M rotations with crop residues applied. Bulk density in the mature Mollisol (Experiment II) ranged from 0.96 to 1.05 Mg m^{-3}. For the mature Mollisol, the decrease in soil bulk density in the surface layer (0–20-cm depth) ranged from 0.01 to 0.04 Mg m^{-3} for the three treatments, and almost no change occurred over the 8 years of the experiment.

Carbon content and C stocks

During the 8 years of soil development, the increase in SOC stocks at 0–20-cm depth for Experiment I ranged from 1.61 to 7.84 Mg C ha^{-1} in the six treatments (Figure 2a). The SOC stocks at 0–20-cm depth in NatF and Alfa increased by 30 and 60%, respectively, in the fertilized S–M systems with residue return it increased by 50 (F1C1) and 61% (F1C2), respectively, and in the S–M system without residue return and fertilizer application by 7% and by 17% with chemical fertilizer application. The carbon content at 0–20-cm depth increased from 4.8 g C kg^{-1} in the parent material to 7.2–12.3 g C kg^{-1} in the six treatments (Figure 2b). Because bulk density decreased in general, the increases in SOC stocks were smaller than those of carbon content. The largest increase in SOC stocks was for the treatment with the largest C residue inputs (F1C2), with cumulative inputs of 33.27 Mg dry matter ha^{-1}, and in the Alfa treatment, with C inputs of 21.19 Mg dry matter ha^{-1}. In F1C2, the SOC stock after 8 years of restoration was equivalent to 37% of that of a mature Mollisol (M1C0).

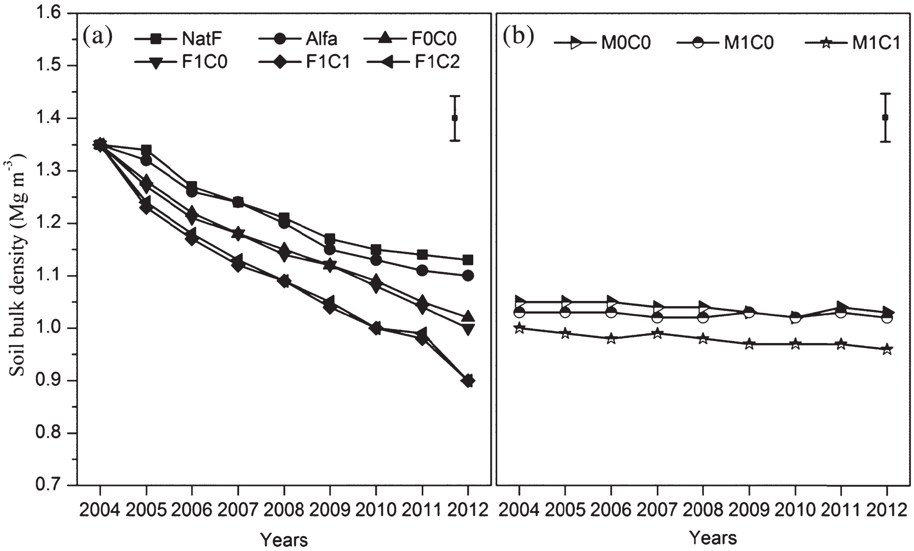

Figure 1 Soil bulk density 2004–2012: (a) parent material under treatment and (b) mature Mollisol. NatF, natural fallow (grasses); Alfa, alfalfa; F0C0, unfertilized soya bean–maize (S–M) control; F1C0, chemical fertilizer S–M; F1C1, organic amendments plus chemical fertilizer S–M; F1C2, chemical fertilizer and above-ground crop biomass returned S–M; M0C0, mature surface Mollisol without fertilizer S–M; M1C0, mature surface Mollisol with chemical fertilizer S–M; M1C1, mature surface Mollisol with chemical fertilizer plus crop residues S–M. The standard error is shown in the upper right of each figure.

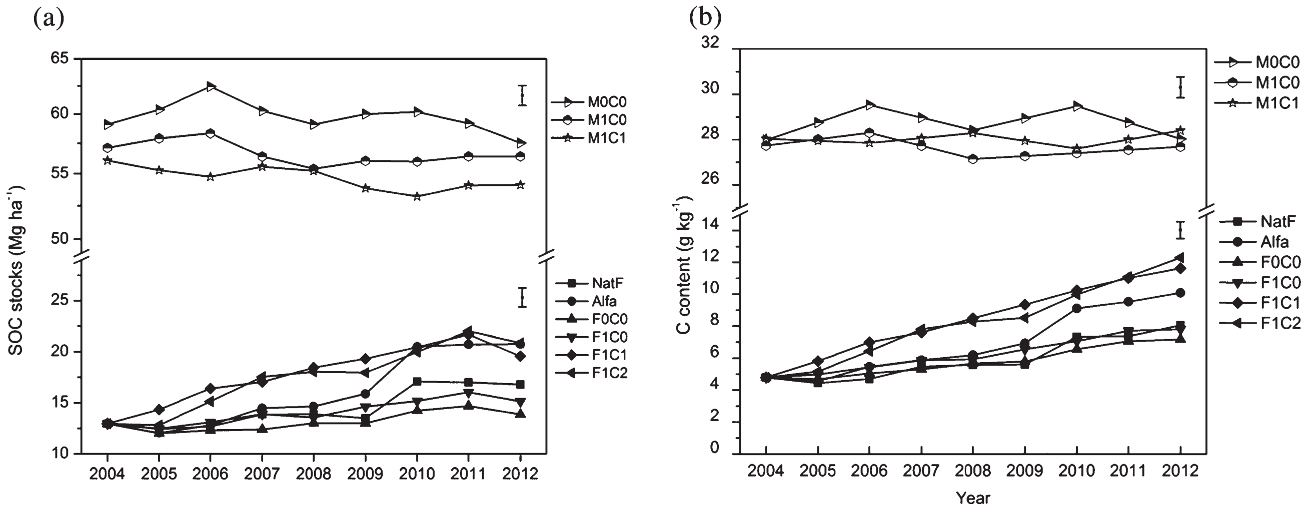

Figure 2 Dynamics of (a) soil organic carbon stocks (SOC stocks) and (b) C content in parent material and mature Mollisol soil 2004–2012. NatF, natural fallow (grasses); Alfa, alfalfa; F0C0, unfertilized soya bean–maize (S–M) control; F1C0, chemical fertilizer S–M; F1C1, organic amendments plus chemical fertilizer S–M; F1C2, chemical fertilizer and above-ground crop biomass returned S–M; M0C0, mature surface Mollisol without fertilizer S–M; M1C0, mature surface Mollisol with chemical fertilizer S–M; M1C1, mature surface Mollisol with chemical fertilizer plus crop residues S–M. The standard error is shown in the upper right of each figure.

In Experiment I, the treatment effects on SOC stocks at 0–20-cm depth were significant (Table 2). After 8 years, the SOC stocks at 0–40-cm depth were significantly larger in F1C2 than for the fertilized S–M treatment without residue return. Soil organic carbon stocks were not significantly different for F1C0 and F0C0 and the perennials, which suggested that fertilizer application did not increase SOC stocks at 0–40-cm depth. Furthermore, at this depth, SOC stocks were greater for Alfa than for NatF (Table 3).

In Experiment II with the mature Mollisol soil, SOC stocks and C content at 0–20-cm depth in the treatments did not change between 2004 and 2012 (Figure 2, Table 4). Carbon stocks ranged from 54.11 to 57.52 Mg C ha^{-1} in the three treatments. For each treatment in the mature Mollisol, the C concentration and C stocks in 2012 were almost equal to those of 2004.

Carbon inputs and SOC accumulation

In Experiment I, there was a positive non-linear relation between cumulative SOC stocks (SOC_{stock}) at 0–20-cm depth and C inputs ($R^2 = 0.74$) according to the following exponential equation:

$$SOC_{stock} = 12.62 + 11.03 \left\{ 1 - \exp\left(-\frac{C_{input}}{14.5} \right) \right\},$$

where C_{input} is the annual biomass C input (Figure 3).

In Experiment I, cumulative C inputs as shoots and roots for the different vegetation and agricultural practices during the 8 years of the experiment ranged from 6.79 to 33.27 Mg C ha^{-1} and were in the order Alfa > NatF > F0C0 (Figure 4a) and F1C2 > F1C1 > F1C0 (Figure 4b). The rate of increase in SOC stocks at 0–20-cm depth

Table 2 Wald chi-squared (χ^2) and approximate F statistics for soil organic C stocks transformed to natural logarithms in the subsoil of a Mollisol soil (Experiment I), determined by a residual maximum likelihood (REML) model with an ante-dependence covariance structure

Fixed effects	DF	χ^2	F value	χ^2 probability	Probability > F
Block	3	2.7	0.9	0.4387	0.4627
Treatment	5	725.7	145.1	<0.0001	<0.0001
Time	7	1232.0	176.0	<0.0001	<0.0001
Time × treatment	35	237.9	6.8	<0.0001	<0.0001

DF, degrees of freedom.

Table 3 Mean soil carbon (SOC) stocks and standard error (SE) at 0–40-cm depth after 8 years of treatments in the subsoil of a Mollisol soil

Treatments	SOC stocks 0–40 cm / Mg ha^{-1}
F1C2	48.20
F1C0	39.93
F0C0	37.61
Alfa	46.87
NatF	41.71
SE	1.35
LSD	2.46

The treatments were fertilized soya bean–maize (S–M) rotation with (F1C2) and without residue return (F1C0), S–M without fertilizer and residue return (F0C0), alfalfa (Alfa) and natural fallow (NatF). The least significant difference (LSD) among these treatments ($P < 0.05$) and the standard error (SE) are also given, $n = 3$.

Table 4 Wald chi-squared (χ^2) and approximate F statistics for soil organic carbon stocks data transformed to natural logarithms at 0–20-cm depth in a mature Mollisol soil (Experiment II), analysed by a residual maximum likelihood (REML) model with an ante-dependence covariance structure

Fixed effects	DF	χ^2	F value	χ^2 probability	Probability > F
Block	2	5.58	2.79	0.0613	0.1742
Treatment	2	26.22	13.11	<0.0001	0.0175
Time	8	8.97	1.12	<0.3444	0.3659
Time × treatment	16	11.38	0.71	<0.7852	0.7685

DF, degrees of freedom.

from 2004 to 2012 under different land use (natural fallow, alfalfa and unfertilized S–M without residue return) varied from 0.02 to 0.83 Mg C ha^{-1} year^{-1} (Figure 4c). The C sequestration rate among the fertilized S–M treatments at 0–20-cm depth was between 0.09 and 0.80 Mg C ha^{-1} year^{-1} (Figure 4d).

Distribution of SOC after 8 years of soil development

In Experiment I, the contribution of the different soil layers to SOC stocks in the top 40 cm, assessed in 2012 after 8 years of soil development, was 37–45% for the 0–20-cm depth, 27–30% for the 20–30-cm depth and 27–33% for the 30–40-cm depth (Figure 5a). Both treatments and depth had significant effects on SOC stocks (Table 5). Among the S–M systems, fertilizer

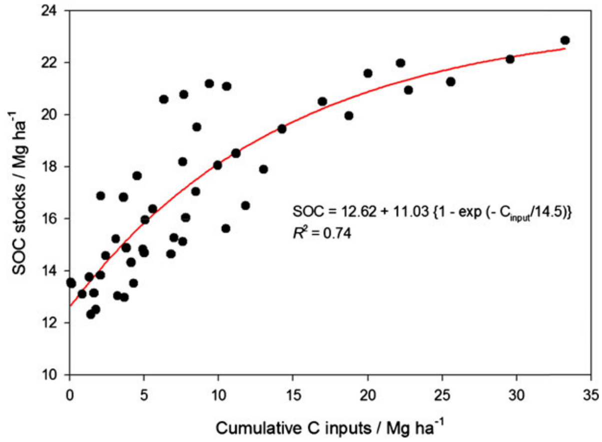

Figure 3 Relation between cumulative C inputs and soil C stocks at 0–20-cm depth in the parent material under development.

application increased SOC stocks at 20–30-cm depth by 8% (F1C0 compared with F0C0), and residue return increased SOC stocks by 11% (F1C2 compared with F1C0). At 30–40-cm depth residue return increased SOC stocks by 7% (F1C2 compared with F1C0). The soil C content showed the same trend as SOC stocks for the different treatments (Figure 5b).

Discussion

The present study compared SOC stocks of a young soil (i.e. parent material under development) under different agricultural practices and types of vegetation with those of their initial parent material (i.e. mature Mollisol) during 8 years. The SOC stocks in the parent material under development increased steadily, and after 8 years of treatments both the soil that had the largest C inputs and the one in the Alfa treatment had SOC stocks at 0–20-cm depth equivalent to 37% of the SOC stock of mature surface Mollisols. Over the same period, bulk density decreased for all the treatments. Both tillage and additions of organic matter appeared to decrease bulk density; this decrease was less for the perennial treatments.

The SOC stocks at 0–20-cm depth of the parent material under treatment showed a strong non-linear relation with C inputs (Figure 3), which indicated that the parent material had great potential to sequester added C. The mineral composition of the parent material, in addition to the saturation deficit of the parent material, and the amount of C inputs explain the rapid increase in SOC stock of the topsoil of Experiment I. In northeast China, 2:1 clays are dominant in the Mollisols; they have large montmorillonite and illite contents. The 2:1 clay minerals with a large CEC and large specific surface area have a larger binding potential to stabilize C than the 1:1 clay minerals (Greenland, 1965). The relation between C inputs and soil C sequestration during 8 years of development suggested a maximum SOC stock of 23.65 Mg ha^{-1} for this subsoil (Figure 3). Approximately 95% of the increase in SOC stocks would be reached with cumulative C inputs of 43.5 Mg ha^{-1}, and additional inputs would increase SOC stocks only slightly. However, mature Mollisols have C stocks of > 54 Mg ha^{-1} (Figure 2a). Consequently,

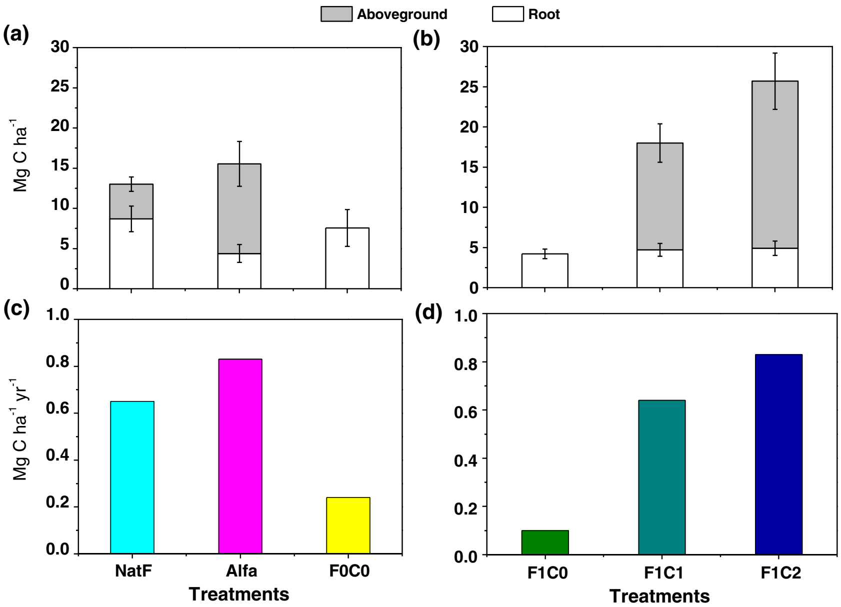

Figure 4 (a) Cumulative inputs of carbon in natural fallow (NatF), alfalfa (Alfa) and unfertilized soya bean–maize (S–M) rotation, (b) cumulative C inputs in excess of the unfertilized S–M rotation without residue return in fertilized S–M without residue return (F1C0), fertilized S–M with organic amendments (F1C1) and fertilized S–M with residue return (F1C2), (c) annual C sequestration rate at the 0–20-cm depth for NatF, Alfa and F0C0 and (d) F1C0, F1C1 and F1C2 in relation to the rate of C sequestration in F0C0. Error bars represent the standard error.

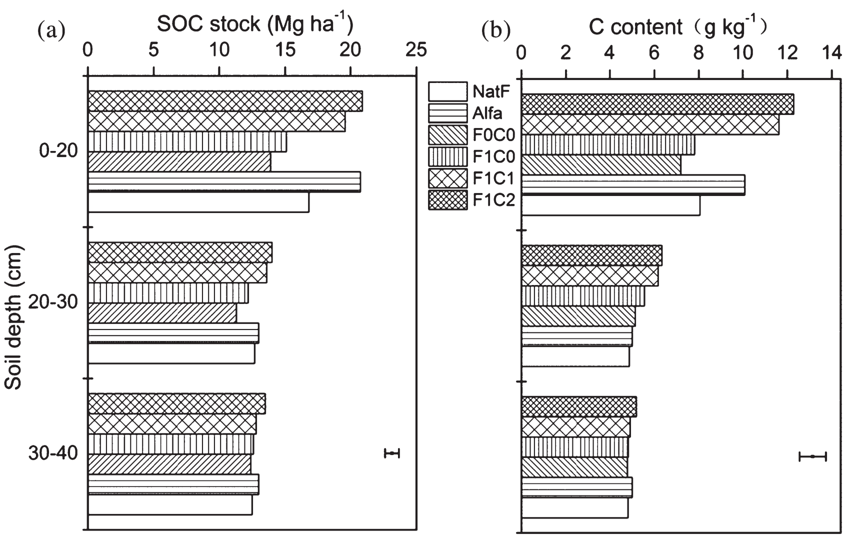

Figure 5 Distribution of soil (a) organic carbon stocks (SOC stock) and (b) C content at 0–40-cm depth in the profile for the six treatments in 2012. The standard error is shown in the lower right of the figures.

the relation between cumulative C inputs and C stocks that developed based on this experiment is valid only for this relatively short initial period of soil formation from parent material.

In contrast to the parent material under development, Experiment II showed that similar organic C inputs during the same period did not increase SOC stocks or C content in the surface soil of the mature Mollisol at the same site. Similarly, Ding *et al*. (2012) found no effect on SOC stocks by varying C inputs in a soil with a large organic matter content in northeast China, probably because that soil was at equilibrium (Soon, 1998) and saturated with C (Six *et al*., 2002). Physiochemical processes place upper limits on SOC stocks; mature Mollisols are almost saturated with C. The saturation value of C content for Mollisols has been estimated to be between 27 and 30 g C kg^{-1} (Qiao *et al*., 2014).

In the parent material under treatment, the F0C0 had the smallest and F1C2 the largest rate of C sequestration. Parent material that received organic amendments as residues in addition to chemical fertilizer had rapid increases in SOC stocks, which emphasizes the importance of residue return for C sequestration. For the treatments that represented agricultural practices, which included removal of all the above-ground biomass at harvest (F0C0 and F1C0), the increases in SOC stocks were relatively modest. Contrary to our hypothesis, fertilizer application alone did not increase carbon stocks (Table 3).

The SOC stocks under alfalfa after 8 years of continuous cropping were almost equal to those of the F1C2 treatment (Figure 2). Continuous alfalfa has been shown to have a positive effect on the accumulation of SOC and improvement in soil fertility (Kuo *et al*., 1997). In our study, the SOC stocks after 8 years under alfalfa were greater by 44 and 27% than those of the F0C0 and NatF treatments, respectively (Figure 2, Table 3). The C sequestration rate we observed (0.83 Mg C ha^{-1} year^{-1}) was considerably larger than the

Table 5 Wald chi-squared (χ^2) and approximate F statistics for soil organic carbon stocks at 0–20-, 20–30- and 30–40-cm depth of the subsoil after 8 years of treatments, analysed by a residual maximum likelihood (REML) model with an ante-dependence covariance structure

Fixed effects	DF	χ^2	F value	χ^2 probability	Probability > F
Block	2	7.2	3.6	0.0268	0.0658
Treatment	5	170.6	34.1	<0.0001	<0.0001
Depth	2	516.1	258.1	<0.0001	<0.0001
Depth × treatment	10	104.7	10.5	<0.0001	<0.0001

DF, degrees of freedom.

one reported by Syswerda *et al.* (2011) of 0.33 Mg C ha^{-1} $year^{-1}$ for the surface layer (0–21 cm) under alfalfa in the northern portion of the United States corn belt. Zhang *et al.* (2009), on the other hand, reported a similar increase in SOC of 0.77 Mg C ha^{-1} $year^{-1}$ at 0–15-cm depth over 10 years after the conversion of grassland to alfalfa in northwestern China.

Among the factors that might explain the relatively large rate of C sequestration for the alfalfa treatment of the present study are greater root biomass of perennial vegetation and litter quality. The amount of root C and rates of SOC sequestration have been shown to be positively correlated (Rasse *et al.*, 2005; Baker *et al.*, 2007). Both shoots and roots are sources of C that can help to improve soil fertility, but perennials allocate relatively more C to roots than shoots compared with annual crops (Bolinder *et al.*, 2002). In other experiments, the relative contribution of alfalfa roots to SOC was 2.7 times more than that of the shoots (Puget & Drinkwater, 2001) and 1.8 times more than that of corn shoots (Molina *et al.*, 2001), which suggests that root-derived C persists for longer in soil than shoot-derived C. The length of time over which net C assimilation occurs, which is longer for perennial vegetation than annual crops, is also an important factor that affects rates of C sequestration (Baker *et al.*, 2007). In our study, the greater length of time of CO_2 fixation shown by the perennial plants might have affected C sequestration rates. High quality litter (e.g. legume biomass and fine roots), such as alfalfa in our study, is thought to lead to greater efficiency in microbial carbon use, and therefore more microbial products and greater rates of C sequestration compared with lower quality litter (Cotrufo *et al.*, 2013), such as corn residue in the present study. The decrease in the rate of increase in SOC stocks with increased rates of C additions in the S–M rotations (Figure 3) might have resulted partly from the poorer quality of the litter leading to less efficient C sequestration in the S–M rotations.

Other factors such as physical protection of organic matter against decomposition might have affected C sequestration (Ding & Han, 2014). For example, soil biota improve soil structure and enhance aggregation, which provides better physical protection of organic matter (Six *et al.*, 2002). In the soil of the present experiment, Li *et al.* (2014) found that the biomass of total bacteria, Gram-positive bacteria and fungal phospholipid fatty acids (PLFAs) was largest and the biomass of eukaryotic PLFAs second largest in alfalfa. This and the lack of tillage might have led to favourable conditions for C sequestration in this treatment.

Although the establishment of plant communities is usually a much faster process than soil development, the vegetation of the natural fallow treatment did not reach its full growth potential until the third year. This was probably because the fertility of the parent material was poor and unable to provide enough nutrients for plant growth. Forage alfalfa was established more rapidly than vegetation in natural fallow. Alfalfa, a perennial legume, is tolerant of poor conditions (Jiang *et al.*, 2007). Nevertheless, it is nutritious as a livestock feed because of the large N content in its tissues, which also improves soil quality (Yang & Kay, 2001). The C inputs of the two perennial crops in the present experiment were greater for alfalfa than natural fallow, probably because alfalfa can derive N from symbiotic fixation.

Experiment I also provided an opportunity to study C dynamics within the soil profile. We observed stratification of SOC stocks with soil depth. In all treatments, SOC stocks decreased with an increase in depth. Carbon accumulation occurred in the topsoil first; at 20–30-cm depth there were some changes in SOC stocks, but below 30 cm the C content was mostly unchanged.

Conclusions

During the first 8 years of soil development from parent material, SOC stocks increased with all treatments in this study, including no fertilizer, chemical fertilizer and fertilizer plus organic residue amendments. Our study corroborated the results of studies that found strong relations between changes in SOC stocks and C input, but our data also showed that the perennial crops, alfalfa and natural fallow (weeds), sequestered carbon at similar rates to annual cropping with the return of residues. This might have occurred because of the size of the root biomass, the longer period of net carbon assimilation of perennial than annual systems and litter quality.

Acknowledgements

The study was funded by the National Natural Science Foundation of China (41371296, 41571219) and the Youth Innovation Promotion Association CAS (2016211). The authors thank Guidan Sun and Yang Bian for laboratory assistance, an editor and Neil Willits for advice on the statistical analysis, and Timothy A. Doane for constructive comments on the manuscript.

References

Allmaras, R.R., Linden, D.R. & Clapp, C.E. 2004. Corn-residue transformations into root and soil carbon as related to nitrogen, tillage, and stover management. *Soil Science Society of America Journal*, **68**, 1366–1375.

Baker, J.M., Ochsner, T.E., Venterea, R.T. & Griffis, T.J. 2007. Tillage and soil carbon sequestration – What do we really know? *Agriculture, Ecosystems & Environment*, **118**, 1–5.

Bell, L.W., Sparling, B., Tenuta, M. & Entz, M.H. 2012. Soil profile carbon and nutrient stocks under long-term conventional and organic crop and Alfalfa-crop rotations and re-established grassland. *Agriculture, Ecosystems and Environment*, **158**, 156–163.

Bolinder, M., Angers, D., Bélanger, G., Michaud, R. & Laverdière, M. 2002. Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. *Canadian Journal of Plant Science*, **82**, 731–737.

Cai, Z.C. & Qin, S.W. 2006. Dynamics of crop yields and soil organic carbon in a long-term fertilization experiment in the Huang-Huai-Hai Plain of China. *Geoderma*, **136**, 708–715.

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K. & Paul, E. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology*, **19**, 988–995.

Ding, X.L. & Han, X.Z. 2014. Effects of long-term fertilization on contents and distribution of microbial residues within aggregate structures of a clay soil. *Biology and Fertility of Soils*, **50**, 549–554.

Ding, X.L., Han, X.Z., Liang, Y., Qiao, Y.F., Li, L.J. & Li, N. 2012. Changes in soil organic carbon pools after 10 years of continuous manuring combined with chemical fertilizer in a Mollisol in China. *Soil & Tillage Research*, **12**, 236–241.

Gong, W., Yan, X.Y., Wang, J.Y., Hu, T.X. & Gong, Y.B. 2009. Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat–maize cropping system in northern China. *Geoderma*, **149**, 318–324.

Greenland, D.J. 1965. Interaction between clays and organic compounds in soils. Part I. Mechanisms of interaction between clays and defined organic compounds. *Soils and Fertilizer*, **28**, 415–532.

Halvorson, A.D., Wienhold, B.J. & Black, A.L. 2002. Tillage, nitrogen, and cropping system effects on soil carbon sequestration. *Soil Science Society of America Journal*, **66**, 906–912.

Haynes, R. & Naidu, R. 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutrient Cycling in Agroecosystems*, **51**, 123–137.

Jiang, J.P., Xiong, Y.C., Jia, Y., Li, F.M., Xu, J.Z. & Jiang, H.M. 2007. Soil quality dynamics under successional alfalfa field in the semi-arid Loess Plateau of northwestern China. *Arid Land Research and Management*, **21**, 287–303.

Johnson, J.M.F., Franzluebbers, A.J., Weyers, S.L. & Reicosky, D.C. 2007. Agricultural opportunities to mitigate greenhouse gas emissions. *Environmental Pollution*, **150**, 107–124.

Karlen, D.L., Cambardella, C.A., Kovar, J.L. & Colvin, T.S. 2013. Soil quality response to long-term tillage and crop rotation practices. *Soil & Tillage Research*, **13**, 354–364.

Kuo, S., Sainju, U. & Jellum, E. 1997. Winter cover crop effects on soil organic carbon and carbohydrate in soil. *Soil Science Society of America Journal*, **61**, 145–152.

Kuzyakov, Y. & Domanski, G. 2000. Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science*, **163**, 421–431.

Lal, R. 2004. Soil carbon sequestration impacts on global climate change and food security. *Science*, **304**, 1623–1627.

Li, N., Yao, S.H., You, M.Y., Zhang, Y.L., Qiao, Y.F., Zou, W.X. *et al.* 2014. Contrasting development of soil microbial community structure under no-tilled perennial and tilled cropping during early pedogenesis of a Mollisol. *Soil Biology & Biochemistry*, **77**, 221–232.

Liu, X., Zhang, S., Zhang, X., Ding, G. & Cruse, R. 2011. Soil erosion control practices in Northeast China: a mini-review. *Soil & Tillage Research*, **11**, 744–748.

Mokany, K., Raison, R.J. & Prokushkin, A.S. 2006. Critical analysis of root: shoot ratios in terrestrial biomes. *Global Change Biology*, **12**, 84–96.

Molina, J., Clapp, C., Linden, D., Allmaras, R., Layese, M., Dowdy, R. *et al.* 2001. Modeling the incorporation of corn (*Zea mays* L.) carbon from roots and rhizodeposition into soil organic matter. *Soil Biology & Biochemistry*, **33**, 83–92.

Puget, P. & Drinkwater, L. 2001. Short-term dynamics of root- and shoot-derived carbon from a leguminous green manure. *Soil Science Society of America Journal*, **65**, 771–779.

Purakayastha, T. & Katyal, J. 1998. Evaluation of compacted urea fertilizers prepared with acid and non-acid producing chemical additives in three soils varying in pH and cation exchange capacity; I. NH3 volatilization. *Nutrient Cycling in Agroecosystems*, **51**, 107–115.

Qiao, Y.F., Miao, S.J., Han, X.Z., You, M.Y., Zhu, X. & Horwath, W.R. 2014. The effect of fertilizer practices on N balance and global warming potential of maize–soybean–wheat rotations in Northeastern China. *Field Crops Research*, **161**, 98–106.

Quinton, J.N., Govers, G., van Oost, K. & Bardgett, R.D. 2010. The impact of agricultural soil erosion on biogeochemical cycling. *Nature Geoscience*, **3**, 311–314.

Rasse, D.P., Rumpel, C. & Dignac, M.F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, **269**, 341–356.

Sainju, U.M. & Lenssen, A.W. 2011. Dryland soil carbon dynamics under alfalfa and durum-forage cropping sequences. *Soil & Tillage Research*, **113**, 30–37.

Santi, C., Certini, G. & D'Acqui, L.P. 2006. Direct determination of organic carbon by dry combustion in soils with carbonates. *Communications in Soil Science and Plant Analysis*, **37**, 155–162.

Six, J., Conant, R., Paul, E. & Paustian, K. 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant and Soil*, **241**, 155–176.

Soil Survey Staff 2010. *Keys to Soil Taxonomy*. United States Department of Agriculture, Natural Resources Conservation Service, Washington.

Soon, Y. 1998. Crop residue & fertilizer management effects on some biological and chemical properties of a Dark Grey Solod. *Canadian Journal of Plant Science*, **78**, 707–713.

Srinivasarao, C., Venkateswarlu, B., Lal, R., Singh, A.K., Vittal, K.P.R., Kundu, S. *et al.* 2012. Long-term effects of soil fertility management on carbon sequestration in a rice-lentil cropping system of the Indo-Gangetic plains. *Soil Science Society of America Journal*, **76**, 168–178.

Syswerda, S., Corbin, A., Mokma, D., Kravchenko, A. & Robertson, G. 2011. Agricultural management and soil carbon storage in surface vs. deep layers. *Soil Science Society of America Journal*, **75**, 92–101.

Webster, R. & Payne, R.W. 2002. Analysing repeated measurements in soil monitoring and experimentation. *European Journal of Soil Science*, **53**, 1–13.

Xiong, Y. & Li, Q.K. 1987. *Soils in China*. Science Press, Beijing(in Chinese).

Yang, X. & Kay, B. 2001. Rotation and tillage effects on soil organic carbon sequestration in a typic Hapludalf in Southern Ontario. *Soil & Tillage Research*, **59**, 107–114.

You, M.Y., Burger, M., Li, L.J., Zou, W.X., Li, N., Qiao, Y.F. *et al.* 2014. Changes in soil organic carbon and carbon fractions under different land use and management practices after development from parent material of Mollisols. *Soil Science*, **179**, 205–210.

Zhang, F.C. & Zhu, Z.H. 1990. Harvest index for various crops in China. *Scientia Agricultura Sinica*, **23**, 83–87 (in Chinese).

Zhang, T.J., Wang, Y.W., Wang, X.G., Wang, Q.Z. & Han, J.G. 2009. Organic carbon and nitrogen stocks in reed meadow soils converted to alfalfa fields. *Soil & Tillage Research*, **105**, 143–148.