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Fallow associated with autumn-plough favors structure stability and storage of soil organic carbon compared to continuous maize cropping in Mollisols

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Abstract
Background and aims: Aggregate formation and stability of soil organic carbon (SOC) differ in different farming systems, probably due to differences in effects of tillage and residue management. This study used a 24-year field experiment to compare the effects of continuous maize cropping and natural fallow on aggregate formation and SOC storage in various aggregate-size classes and density fractions of a Chinese Mollisol.
Methods: Soils collected from the upper 0.2-m layer were wet-sieved into four aggregate-size classes (>2, 0.25-2, 0.053-0.25 and <0.053 mm) which were then fractionated into light, occluded and mineral C fractions. The concentrations of SOC and natural ^{13}C abundance of each fraction in bulk soil and the aggregate classes were determined.
Results: Continuous maize cropping decreased the proportion of macro-aggregates (>0.25 mm) and increased that of micro-aggregates (<0.25 mm) compared to the initial value while the opposite was observed in the natural fallow system. The fallow system generally had greater SOC concentration in the occluded fraction, higher proportion of newly-derived C as % total SOC in the light fraction and greater contribution of total residue C to new C in macro-aggregates and light fractions compared to the continuous maize system. Furthermore, the fallow system resulted in shorter turnover time of SOC than the continuous maize system.
Conclusions: Natural fallow associated with autumn-plough improved soil structural stability and SOC storage while continuous maize cropping with residue removal decreased SOC sequestration and soil aggregate stability.

Keywords: Aggregation; Density fraction; $\delta^{13}C$; Cropping system; Carbon turnover

Introduction

Farmland soil organic carbon (SOC) is an important component of biosphere organic C pool while cropping systems can greatly affect SOC storage and stability (Paustian et al. 1997; Buyanovsky and Wagner, 1998; Six et al. 2002). The storage and stability of SOC not only reflect soil quality, but also closely associate with a sustainable agricultural development. Intensive and continuous cropping has been shown to have positive and negative effects on SOC storage and stability (Six et al. 2002; Kou et al. 2012; Qiao et al. 2015). In Northeast China, continuous maize cropping has been an important cropping system. However, all crop residues are removed from paddocks and harvested for rural fuel, which has resulted in decrease in SOC and soil fertility (Yu et al. 2006). Recent studies have shown that appropriate management strategies can overcome the problem of SOC and soil quality decline (Nyamadzawo et al. 2009; Ando et al. 2014). One of these strategies is the long-term fallow which could minimize the SOC decline through improvement of soil structural stability. However, the information on mechanisms by which fallow affects SOC storage and stability is still limited.

Formation of soil aggregates is one of the main mechanisms contributing to SOC stabilization. It has been shown that SOC in micro-aggregates (<0.25 mm) are older and more stable than that in macro-aggregates (>0.25 mm) (Six et al. 2000; John et al. 2005; Yamashita et al. 2006; McCarthy et al. 2008). Thus, the mean turnover time of SOC increased with decreasing aggregate size. For instance, turnover time of SOC associated with silt/clay fractions (<0.053 mm) was 360-3124 years relative to 14 years in aggregates >0.25 mm (Monreal et al. 1997; Liao et al. 2006; Feng et al. 2016). These studies have also demonstrated that the SOC turnover time varied depending on soil type and depth, climate, SOC content and vegetation (Feng et al. 2016). Long-term fallow has been shown to favor macro-aggregate formation and SOC stability due to lack of disturbance from tillage (Nyamadzawo et al. 2009; Tivet et al. 2013; Xie et al. 2015).

Tillage practice favors breakdown of larger aggregates into smaller aggregates (Álvaro-Fuentes et al. 2008; Kasper et al. 2009). In turn, crop residues provide C to facilitate the formation of macro-aggregates (Six et al. 2000). These opposite processes greatly affect the storage and stability of SOC in various aggregate classes. Moreover, the amount and quality of SOC within aggregate fractions affects the stability and turnover of SOC (Yamashita et al. 2006). The light fraction is relatively labile and assumed to be remaining plant material (von Lützow et al. 2007; Schrumpf et al. 2013; Breulmann et al. 2016). The mineral fraction contains SOC that is adsorbed on clay particles and is more stable, and is regarded as the organo-mineral complex (Yamashita et al. 2006; Zimmermann et al. 2007; Cerli et al. 2012). Gunina and Kuzyakov (2014) indicated the main C flow within aggregates and SOC fractions was from the macro-aggregate-free particulate organic matter to the mineral micro-aggregate fraction. However, the properties and dynamics of SOC in density fractions at the aggregate level and their relations to bulk SOC turnover are poorly understood.

Natural ^{13}C abundance has been used to study the turnover of SOC in aggregates (Millard et al. 2010; Werth and Kuzyakov 2010; Urbanek et al. 2011; Gunina and Kuzyakov 2014). The variation in the natural abundance of ^{13}C in the soil had resulted from the shift of successive C3 versus C4 vegetation or vice versa (Quenea et al. 2009). The present study aimed to compare the long-term effects of cropping and fallow systems on soil aggregation, SOC storage and turnover time of C in various aggregate-size classes and density fractions. Soils were collected from 24-year continuous maize cropping (C4 species) with root and shoot residues removed, and natural fallow (volunteer weeds) under autumn-plough conditions. We hypothesized that natural fallow would improve soil aggregation and macro-aggregate stability, and SOC within aggregates would be more protected physically and more recalcitrant biochemically, in comparison with continuous maize cropping.

Materials and Methods

Experimental site and soil sampling

The study site was located within the State Key Agro-ecological Experiment Station, Hailun County, Heilongjiang Province, China (N 47º26′, E 126º38′). Selected physical and chemical properties of the soils are shown in Table 1. The soil did not have carbonate reaction, thus the total C content represents SOC content in further analysis. Details of experimental site and management history have been described elsewhere (Qiao et al. 2015). Briefly, the study site had been cultivated with a rotation of maize-soybean-wheat crops from 1970 to 1989. In 1990, wheat was cultivated without fertilization to homogenize soil chemical fertility across the experimental plots. The 1990 soil samples were collected in triplicate; each from ten locations to form a composite sample, and were used to measure basic physical and chemical properties as shown in Table 1. The triplicate samples were then combined and stored in dark at room temperature. In 1991, the large triplicate plots were subdivided into 77-m^2 plots to establish a long-term cropping system trial which consisted of fallow and continuous cropping of maize, soybean and wheat, and maize-soybean-wheat rotation. Crop varieties, cultivation, fertilizer application and field management were kept the same as local farming practices every year. In this present study, the fallow system and the continuous maize cropping system were selected from the long-term trial. The maize (*Zea mays* L. hybrid Haiyu 6, with $\delta^{13}C$ of -12.5 ‰) plots received basal fertilizers 65.5 kg N ha^{-1} and 30.1 kg P ha^{-1} of urea and ammonium phosphate annually. An additional 65.5 kg N ha^{-1} of urea was applied at the booting stage of maize. In each autumn, the shoots and roots (0-10 cm) of maize were removed from the system and collected to provide rural fuel, as a common practice in Northeast China, before ploughing. The fallow system had been covered with volunteer weeds (mainly *Artemisia annua* L., *Leymus chinensis* (Trin.) Tzvel., <u>*Serratula centauroides*</u> L., *Potentilla tanacetifolia* <u>Willd.</u> ex <u>Schltdl.</u> and *Taraxacum mongolicum* Hand.-Mazz., with $\delta^{13}C$ of -27.6 ‰ from the weed mixture) during growing seasons. All weeds were autumn-ploughed into soil annually. No chemical fertilizers were applied in the fallow system. Annual biomass production of volunteer weeds was around 13-17 t ha^{-1}.

Soils were sampled, from five locations of each plot to make composite samples, from the upper 0.2-m layer of the long-term continuous maize cropping and fallow plots in October 2014, with three field replicates. The soil samples were dried at 25 ºC to the plastic limit (around 40% field capacity), manually broken down into 20-40 mm sizes along natural weak planes, and then air-dried. The same sampling procedure was carried out for the 1990 samples. The samples were archived in dark at room temperature untill analysis of $\delta^{13}C$, aggregates and C-density fractions.

Aggregate size and density fractionation

Fifty grams of air-dried soil (for both 1990 and 2014 samples) were placed in a 500-ml flask, and moisten with 100 ml distilled water drop by drop to remove trapped air. After 10 min, the soil was gently transferred to the top of a set of three sieves with mesh sizes of 2-, 0.25- and 0.053-mm, and kept 30 mm below the water surface (Six et al. 1998; Puget et al. 2000). The set of sieves were then moved up and down 5 times min^{-1} for 10 min. After wet-sieving, the aggregates on each sieve were transferred in to a beaker for freeze-drying. All aggregate fractions were freeze-dried at -20 ºC using a vacuum freeze drier (LGJ-10) to minimize microbial decomposition of SOC, weighed, and stored at -20 ºC untill analysis. The sieving procedure was repeated five times to meet 20 g requirement of each aggregate size for further C-density fractionation. Sub-samples (10 g) of bulk soil and four aggregate classes were ground to pass through a 0.25-mm sieve for SOC and $\delta^{13}C$ analysis.

Sub-samples (around 20 g) from bulk soil and four aggregate sizes were dried at 40 ºC for removal of moisture on the surface induced by the temperature change from -20ºC to room temperature, and subjected to density fractionation into light, occluded and mineral fractions (Golchin et al. 1994; Sohi et al. 2001; Rasmussen et al. 2005; Qiao et al. 2014). Briefly, these samples were placed in centrifuge tubes and then 1.7 g cm^{-3} sodium polytungstate (SPT) was gradually added to 250 ml along the inner wall of the centrifuge tube to remove air from pore spaces of aggregates. The suspensions were turned up and down five times manually, kept in SPT for further 10 min allowing complete infiltration of SPT, and then centrifuged at 4600 rpm for 1 h to produce a stable pellet. The floating part was considered as the light fraction. The pellet part was transferred in a beaker in an ice-water bath and re-suspended in 150 ml of SPT solution. The suspension was sonicated (3000 J g^{-1} soil) for 3 min with the sonicator probe inserted into the solution about 2 cm in depth, centrifuged and then filtered to collect the occluded fraction. The remaining part was rinsed with distilled water for three times to obtain the mineral fraction. All three density fractions were freeze-dried at -20 ºC and stored at -20 ºC prior to $\delta^{13}C$ and SOC analysis.

Analysis of C content and $\delta^{13}C$

Bulk soil, four aggregate-size classes and three C-density fractions from individual aggregate classes were dried at 40 ºC for 60 min to remove the water on the surface induced by the temperature change from -20 ºC to room temperature, weighed, and ground to pass through a 0.25-mm sieve before measuring SOC and total N using an elemental (CHN) analyzer (Heraeus Elementar Vario EL, Germany), and determining $\delta^{13}C$ by GC-IRMS (Eurppa Scientific Ltd., Cheshire, UK). The analytical precision was 0.05% for ^{13}C of all samples.

Calculations and statistics

Mean weight diameter (MWD) (mm) were computed according to Van Bravel (1950) as follows.

$$MWD = \sum_{i=1}^{n} (d)_i \, w_i \qquad (1)$$

where *d* is mean diameter of each aggregate size *i* (mm), *w* is the proportion of sample weight (g) in the size fraction *i*, and *n* is 4, the number of size fractions.

The $^{13}C/^{12}C$ isotope ratios were expressed relative to the international PDB limestone standard as $\delta^{13}C$.

$$\delta^{13}C\ (‰) = (\mathrm{Ratio_{sample}} / \mathrm{Ratio_{standard}} - 1) \times 1000 \qquad (2)$$

During the experimental period, ^{13}C derived from plant C had entered into various SOC fractions after plant growth. The $\delta^{13}C$ values of the bulk soil, aggregates and density fractions would change as a function of the amount of heavier isotopic signature of the C4 plant material entering different SOC pools. Plant-derived C and mean apparent SOC turnover time were calculated based on the $\delta^{13}C$ value (Yamashita et al. 2006).

Mean apparent SOC turnover time in years (T) in bulk soil, aggregates and density fractions were calculated according to the following equation (Yamashita et al. 2006):

$$\mathrm{T} = -\frac{t-t_0}{\ln\frac{C_t}{C_0}} \qquad (3)$$

where t is the year of 2014, t_0 is the year of 1990; C_t *and* C_0 are the SOC concentration at t and t_0, respectively.

The proportion of newly-derived C to total SOC in maize and fallow system was calculated using Equation (4) (John et al. 2005).

$$f = (\delta^{13}C_{sample} - \delta^{13}C_{ref}) / (\delta^{13}C_{plant} - \delta^{13}C_{ref}) \qquad (4)$$

where f corresponds to the proportion of newly-derived C to total SOC in the sample; $\delta^{13}C_{sample}$ refers to the measured $\delta^{13}C$ of the soil sample in 2014; $\delta^{13}C_{ref}$ stands for the $\delta^{13}C$ of the 1990 soil sample; $\delta^{13}C_{plant}$ is the $\delta^{13}C$ value of plant roots.

The distribution of new C to total residue C in aggregate-size classes and density fractions was calculated according to the Equation (5).

$$\mathrm{DC}_{fraction}(\%) = \frac{DW_{fraction} \times Ti_{soc} \times f_i}{f \times T_{soc}} \times 100\% \qquad (5)$$

where $DC_{fraction}$ represents the proportion of new C (as % total residue C) in aggregate classes and density fractions; $DW_{fraction}$ is dry yield of the density fraction or aggregate class per kg soil; Ti_{SOC} is the concentration of SOC in the fraction/aggregate class; f, the proportion of newly-derived C to total SOC in bulk soil; f_i is the proportion of newly-derived C to total SOC in density fraction or aggregate class i; T_{soc} is the SOC concentration of bulk soil.

Statistically significant differences were identified by the least significant difference (LSD) at P=0.05 after analysis of variance (ANOVA) using Origin 8 SR4 v8.0951 (B951). All values are the means ± standard error of three replicates. One-way analysis of variance (ANOVA) was conducted to compare the effects of farming system on the aggregate formation, SOC, $\delta^{13}C$, proportion of newly-derived C, distribution of residue-derived C, and turnover time across aggregate sizes and density fractions.

Results

Aggregate size distribution

Aggregate-size distribution differed substantially between the fallow and maize cropping systems. The fallow favored the formation of large (>2 mm) and small (0.25-2 mm) macro-aggregates with the distribution of the large macro-aggregates being 9 fold higher than the continuous maize cropping system (P<0.05). By contrast, the continuous maize cropping had 9 and 1.5 times greater micro-aggregate (0.053-0.25 mm) and silt and clay fractions (<0.053 mm), respectively, than the fallow system (Fig. 1, $P<0.05$). After 24 years, the distribution of the small macro-aggregates decreased by 41% and 51% in the fallow and maize systems, respectively, compared to the initial value in 1990. The MWD, a soil structural stability index, of the soils from the fallow system was 1.9 fold higher than that from the continuous maize system (data no shown).

SOC and ^{13}C abundance

The fallow system had 14% higher SOC concentration of bulk soils than the maize cropping system ($P<0.05$). The greatest difference in SOC concentration between the two systems was found in the occluded fraction of the bulk soil (Fig. 2). In general, the SOC concentration increased with increasing aggregate-size class. Meanwhile, the SOC concentration in small macro-aggregates (0.25-2 mm) was 7% higher in the fallow than the maize system ($P<0.05$). The SOC concentration of the light fraction decreased with increasing aggregate size, and that in large macro-aggregates was 25% higher in the fallow than the maize system ($P<0.05$). The SOC concentration in the occluded fraction of individual aggregate classes was similar between the two systems with the SOC concentration being higher in the 0.053-0.25 and 0.25-2 mm classes. A comparable trend between the two systems was found in the mineral fraction, but the lowest SOC concentration was observed in the <0.053-mm class.

Natural fallow for 24 years enhanced SOC sequestration by 8% while continuous maize cropping resulted in 5% loss of SOC compared to the initial value in 1990. The SOC enrichment under the fallow system was ascribed to the increase in SOC of large macro-aggregate and micro-aggregate classes. Soil C loss in the maize system resulted from decreased SOC concentrations in both micro-aggregates and small macro-aggregate classes (Fig. 2). In general, the maize cropping increased the SOC concentration in the light and mineral fractions of the bulk soil compared with the 1990 values.

The $\delta^{13}C$ value in soil under maize (C4 crop) was 6% higher than that under fallow (C3 plants) (Fig. 3, $P<0.05$). Generally, the $\delta^{13}C$ value increased with increasing aggregate size under the maize system, and reverse was true under fallow. The largest difference between the two systems was observed in the light fraction (17.8%), and the smallest was in the mineral fraction (2.3-4.9%) across various aggregate classes.

Plant C input and SOC turnover

After 24 years, volunteer weeds in the fallow system induced 37% higher proportion of newly-derived C to total SOC than in the maize system with up to 50% increase in large macro-aggregate and up to 62% increase in the light fraction (Fig. 4, $P<0.05$). In general, the proportion of newly-derived C to total SOC increased with increasing aggregate size. Differences in that between the two systems were greater in the light and occluded fractions than the mineral fraction.

Newly-derived C distributed in large macro-aggregates was 74% greater under the fallow than under maize cropping (Fig. 5, $P<0.05$). However, the proportion of new C as % total residue-derived C in micro-aggregates and silt/clay (<0.053 mm) fractions was 6.6 and 3.9 times greater in the maize system than the fallow system, respectively. When considering the distribution of C in various density fractions, the largest distribution was in the mineral fraction, which was 15% greater in the maize than the fallow system. The new C distribution was lowest in the light fraction and this distribution was 46% greater under the fallow than the maize system (Fig. 5, P<0.05).

In general, the turnover time of SOC was greater in the fallow than the maize cropping system (Fig. 6). It increased with decreasing aggregate size, and was greater in the mineral fraction than in the light and occluded fractions. The mean SOC turnover time in the bulk soil, large macro-aggregates, and silt/clay fraction was 40%, 58% and 61% greater under the maize cropping than the fallow, respectively (P<0.05). Furthermore, the turnover time was 80% and 51% higher in the light and mineral fractions under the maize cropping (P<0.05).

Discussion

Two systems differ in soil structure formation

This study showed that the two farming systems had contrasting effects on soil structure formation. The 24-year fallow favored the formation of macro-aggregates, while the continuous maize cropping increased the distribution of micro-aggregates. Our results were in line with John et al. (2005) who reported that the percentage of macro-aggregates was higher in grassland than arable soils. In another study, macro-aggregates were increased by a natural fallow system compared with the continuous maize system in Alfisols (Nyamadzawo et al. 2009). Similarly, Song et al. (2015) reported that the dominant fraction was macro-aggregates in the fallow treatment, but micro-aggregates in continuous maize.

The greater distribution of macro-aggregates in the fallow than the continuous maize cropping system might be ascribed to the following reasons. First, the amount of primary inputs differed between the two systems. In the fallow, it was estimated about 13-17 t ha^{-1} of biomass production of volunteer weeds annually. All biomass had been incorporated into the topsoil by autumn-plough annually. In comparison, in the continuous maize system, the above-ground biomass and roots were removed from the paddock, which is common farming practice in the maize belt in northeast China (Yang 2000). Thus, the annual addition of plant residues had been greater in the fallow system than continuous maize system. These residues are the important binding materials for macro-aggregates (Majumder and Kuzyakov 2010). Second, there was a morphological and architectural difference in root system between the two systems. Volunteer weeds were shallow rooted while maize had deep root systems up to 1.0 meter in this soil (Qiao et al. 2015). The shallow root system of volunteer weeds is extensive and mostly distributed in the upper layer of the soil (Barley 1970). The denser superficial root network and associated fungal hyphae were two main factors to hold water-stable macro-aggregates (>2 mm) together in soil (Tisdall and Oades 1982; Lichter et al. 2008). Third, the greater residue inputs and more diversified plant species in the fallow systems could have had higher hyphal densities and thereby favored macro-aggregates formation (Burrows and Pfleger 2002; Oehl et al. 2010; Soka et al. 2015). Finally, annual application of chemical fertilizers in the continuous maize cropping would probably have exerted negative impacts on soil macro-aggregation (Xie et al. 2015) compared to the natural fallow system. It was suggested that the negative impact of NH_4^+-based fertilizers on soil aggregation was associated with dispersion of soil colloids when the monovalent NH_4^+ accumulates (Haynes and Naidu 1998).

Effect of cropping system on carbon storage

The natural fallow system increased SOC content whereas the continuous maize cropping decreased it over the 24 years. The overall lower SOC in the maize system than the fallow system could be explained by the lower C input due to the removal of maize residues as stated above. The lower SOC in the maize system was ascribed to the decreased SOC concentrations in various aggregate classes although only the SOC concentration in small macro-aggregates was significantly lower than that under fallow. The results suggest that long-term fallow facilitates SOC storage, which result from the increased C input and improved structure formation. Our findings are also supported by previous studies (Carter 2002; Kou et al. 2012). He et al. (2016) showed that SOC storage was enhanced by grass restoration after transferring cropland to natural grassland, and explained that natural grasses represented a range of life forms with various ecological adaptabilities, that favor SOC accumulation (McLauchland et al. 2006).

Generally, the SOC in the light and mineral fraction had increased, and that in the occluded fraction decreased after the 24-year period in this present study. Only the SOC concentration in the occluded fraction was significantly lower under the maize system than under the fallow, although there was the same trend in the light C fraction from large macro-aggregates. The lower SOC under the maize system might be owned to the same reason as above, and from the decrease in various density fractions. These results indicate that the increased SOC storage in the fallow depends not only on the SOC in the occluded fraction, but also on the percentage of large macro-aggregates. The results were in line with John et al. (2005) who reported a higher percentage of macro-aggregates and relative higher SOC storage in the occluded fraction of these macro-aggregates in 41-year grassland than continuous maize. The benefit of grassland was ascribed to the fact that plant residues were not removed from the site, and the beneficial root systems of grasses to macro-aggregation and the key role of SOC in the occluded fraction, as a binding agent between micro-aggregates (Golchin et al. 1997). The SOC in the occluded fraction is also known as a significant C pool (Rasmussen et al. 2005; Wagai et al. 2008) which play a vital role in total SOC storage.

The considerably greater amount of SOC storage in the light fraction of macro-aggregates in the fallow than the maize might be explained by the 7% higher proportion of newly-derived C to total C in the former than the latter. The greater plant biomass C input in the fallow system would have enhanced the proportion of the light C fraction which mainly consists of undecomposed plant residues (Shang et al. 2014) associated with relatively labile C pool. This indicates that the significant difference in SOC of light fraction in large macro-aggregates might not be the major factor governing long-term SOC storage. On the other hand, SOC concentration of the mineral fraction did not differ between the two systems and this might be explained by more stable C pool of this fraction which interacts strongly with the mineral phase (Yamashita et al. 2006), therefore it was not affected by the 42 years of cropping system treatment.

Effect of farming system on SOC stability

Land-use change had a remarkable effect on turnover time and stability of SOC. Although methodological inconsistency is a concern in determining SOC turnover time, the value in our study was within the range of the turnover time of 197 years estimated by Feng et al. (2016). The shorter SOC turnover time in the fallow than the maize system could be explained by the substantially higher fresh C input in the fallow system. The turnover time of SOC in all aggregate classes was also shorter in the fallow system, of which the most significant difference showed in large macro-aggregates and the silt/clay fraction of the bulk soil. The results suggest that long-term fallow promotes SOC turnover rates, which could have resulted from the greater amount of newly-derived C input, particularly in the large macro-aggregates. The results are consistent with the previous findings (Fu and Cheng 2002; Johnston et al. 2009; Fuentes et al. 2010). The SOC turnover time has been reported to increase with decreasing aggregate sizes (Yamashita et al. 2006; Dorodnikov et al. 2011; Qiao et al. 2015). This could be associated with more labile C within macro-aggregates (Mandiola et al. 2011; Tisdall and Oades 1982) which had faster decomposition rate compared to the C in micro-aggregates (Jastrow 1996). The shorter SOC turnover time in the fallow than the maize system could be attributed to the noticeably greater proportion of macro-aggregates and the greater amount of newly-derived C entered these aggregates in the fallow system. The continuous supply of new C to macro-aggregates from greater plant biomass production in the fallow system might have been able to compensate for the faster turnover rates of SOC, which led to net C sequestration.

The shorter SOC turnover time in density fractions in the fallow system indicated again the lower C stability in the fallow than in the maize system. Except for above-mentioned reasons, the addition of new C to the soil might enhance native SOC decomposition via priming effect (Kuzyakov 2010). Thus, the shorter turnover time of SOC in the fallow system could possibly be explained by the faster turnover rate of these SOC and replenished with new C. The results highlight that the faster SOC turnover rate in the fallow system mainly contributed to the higher proportion of newly-derived C as % of total SOC. In turn, larger amounts of new C input enhance aggregate stability. The combined effects of the two processes had contributed to larger SOC sequence in the fallow than the maize system.

The longest SOC turnover time observed in the mineral fraction across three density fractions benefited SOC sequestration due to physical protection pathways (Cotrufo et al. 2015). These results were supported by greater SOC contents in the mineral than in the other fractions (Yamashita et al. 2006), and 86-91% of total SOC was stored in the mineral fraction (John et al. 2005). The important role of the mineral-associated C fraction in SOC sequestration could be explained by the fact that the C in the mineral fraction is more resistant to decompose than the C in the light and occluded fractions (Six et al. 2002; John et al. 2005). This is because the chemical protection of the SOC in the mineral fraction via adsorption by minerals against decomposition (Puget et al. 2000; Six and Jastrow 2002; Yamashita et al. 2006).

Conclusions

This study demonstrated that 24 years of continuous maize cropping with removal of most residues decreased SOC storage and aggregate stability compared to the fallow system. The lower SOC storage under the maize cropping system had resulted from the decreased SOC in the micro-aggregate and occluded fraction. These suggest that physical protection via soil structure and plant-derived residue distribution were two main factors controlling overall SOC storage. This study has an important implication. The removal of maize residues from farmland is not a good practice for SOC sequestration and should be minimized. Further studies are warranted to explore the mechanisms that drive SOC dynamics under different farming systems, and to develop integrated management strategies to protect SOC and soil structure under cropping.

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Table 1. The pH, total nitrogen, phosphorus and potassium, and texture of the soil (0-20 cm) collected in 1990 before and in 2014 after 24 years of natural fallow and continuous maize management. Values represent mean of three replications ± standard error

Sampling time	Crop	Soil pH (1:2.5 water)	Total (g kg^{-1}) N	P	K	Soil texture (g kg^{-1}) Clay	Silt
1990	Wheat	6.19±0.03	2.2±0.03	0.74±0.01	20.8±0.69	424±17	326±12
2014	Fallow	6.28±0.04	2.2±0.02	0.80±0.02	20.9±0.73		
	Maize	6.11±0.04	2.3±0.03	0.84±0.03	21.1±0.71		

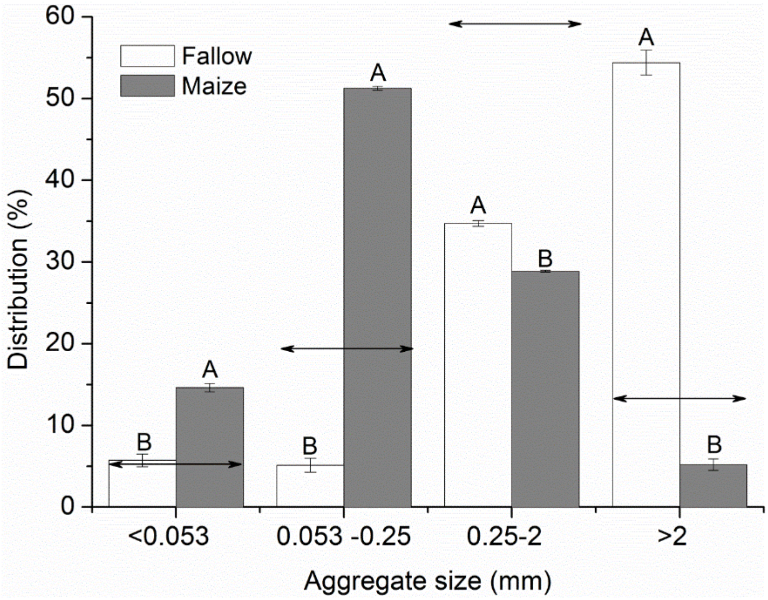

Figure 1. Distribution of aggregate classes after 24-year natural fallow and continuous maize cropping systems. Values represent mean of three replications ± standard error. Arrow lines represent the initial value before the study (1990). Different letters indicate significant difference between natural fallow and maize systems (*P*<0.05).

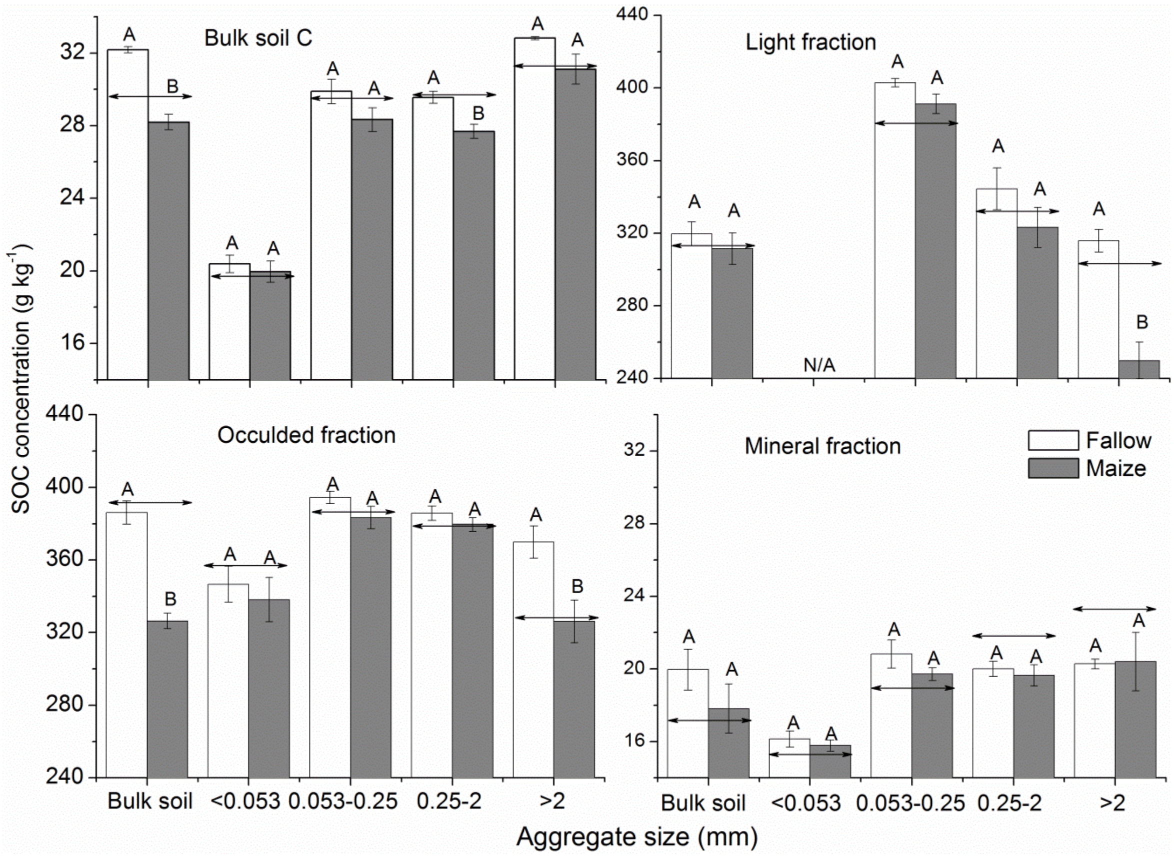

Figure 2. Soil organic C (SOC) of bulk soil and aggregate size classes, and of density fractions (light, occluded and mineral fractions) after 24-year natural fallow and continuous maize cropping. Arrow lines represent the initial SOC values of bulk soil and various fractions before the study (1990). Values represent mean of three replications ± standard error. Different letters indicate significant difference between natural fallow and maize systems (P<0.05). N/A, not analysed due to insufficient samples.

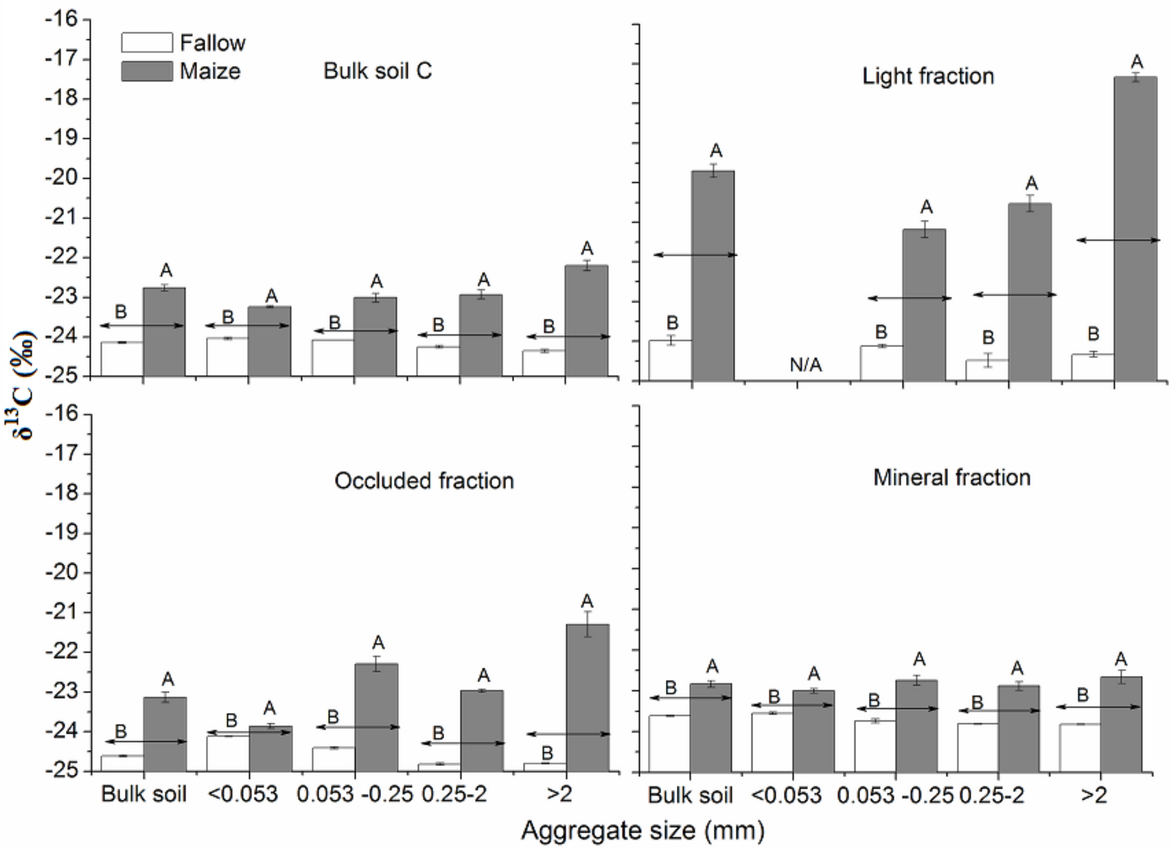

Figure 3. $\delta^{13}C$ values of bulk soil and aggregate size classes, and of density fractions (light, occluded and mineral fractions) after 24-year natural fallow and continuous maize cropping. Arrow lines represent the initial $\delta^{13}C$ values of bulk soil and various fractions before the study (1990). Values represent mean of three replications ± standard error. Different letters indicate significant difference between natural fallow and maize systems ($P<0.05$). N/A, not analysed due to insufficient samples.

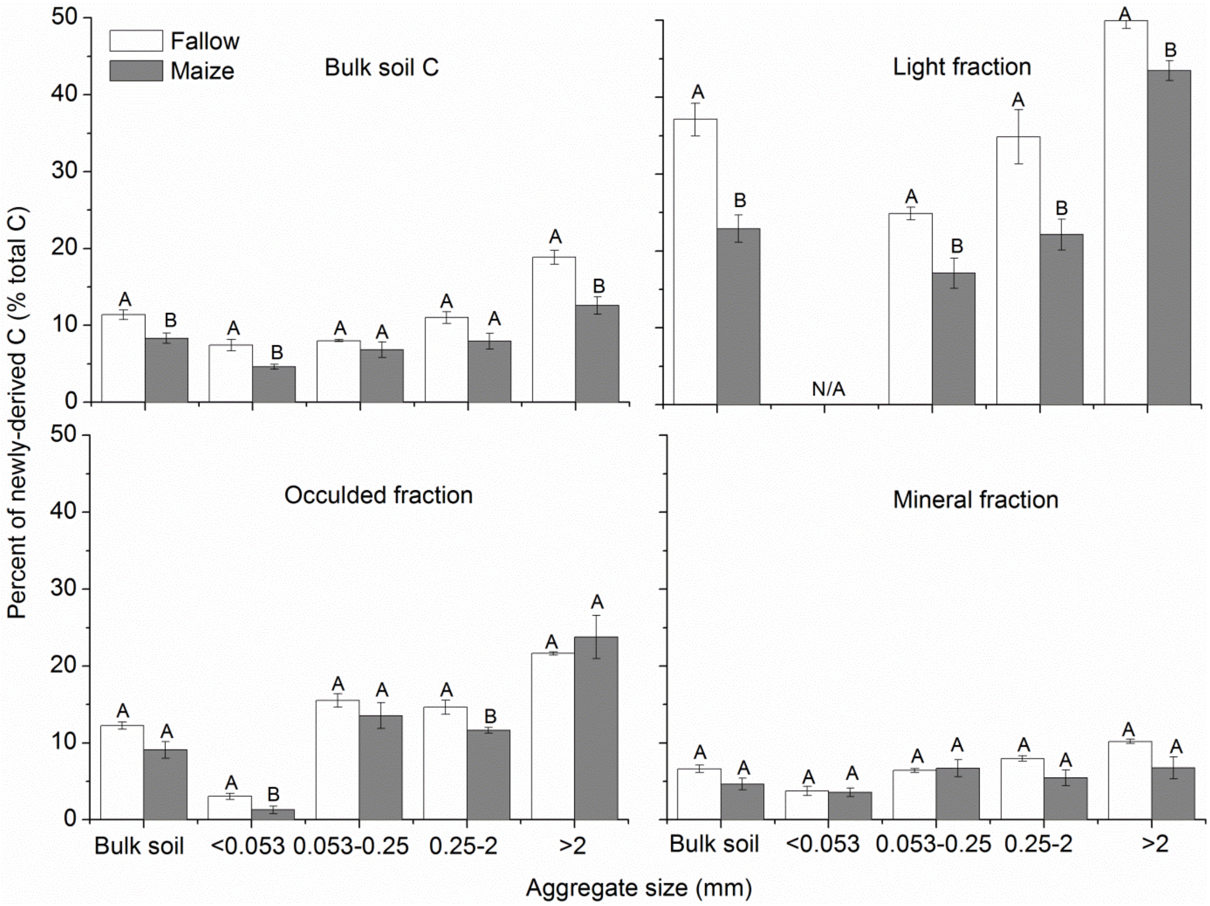

Figure 4. Percent of newly-derived C (as % total C) in bulk soil and aggregate size classes, and in density fractions (light, occluded and mineral fractions) after 24-year natural fallow and continuous maize cropping. Values represent mean of three replications ± standard error. Different letters indicate significant difference between natural fallow and maize systems (P<0.05). N/A, not analysed due to insufficient samples.

The proportion of newly derived C = $\frac{C_{new-C}}{Total\ soil\ organioc\ C}$

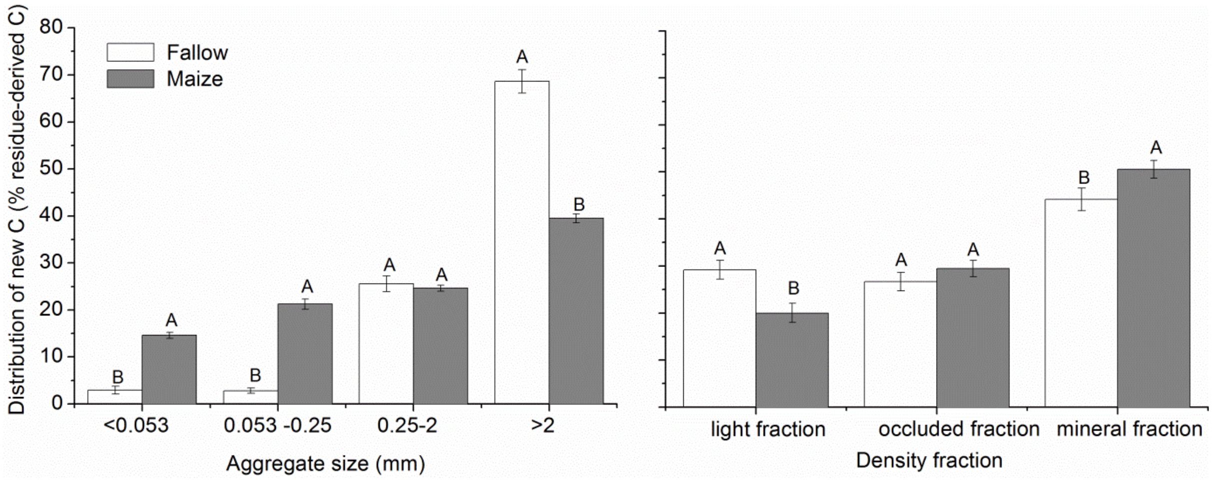

Figure 5. Distribution of new C (as % residue derived C) in aggregates and density fractions (light, occluded and mineral fractions) after 24-year natural fallow and continuous maize cropping. Values represent mean of three replications ± standard error. Different letters indicate significant difference between natural fallow and maize systems (P<0.05). Total amounts of new C in various aggregate sizes or in various density fractions were 100%.

Distribution of new C = $\frac{New\ C\ of\ each\ fraction\ or\ aggregate\ class}{\Sigma\ New\ C\ in\ individual\ fractions\ or\ aggregate\ classes} \times 100$

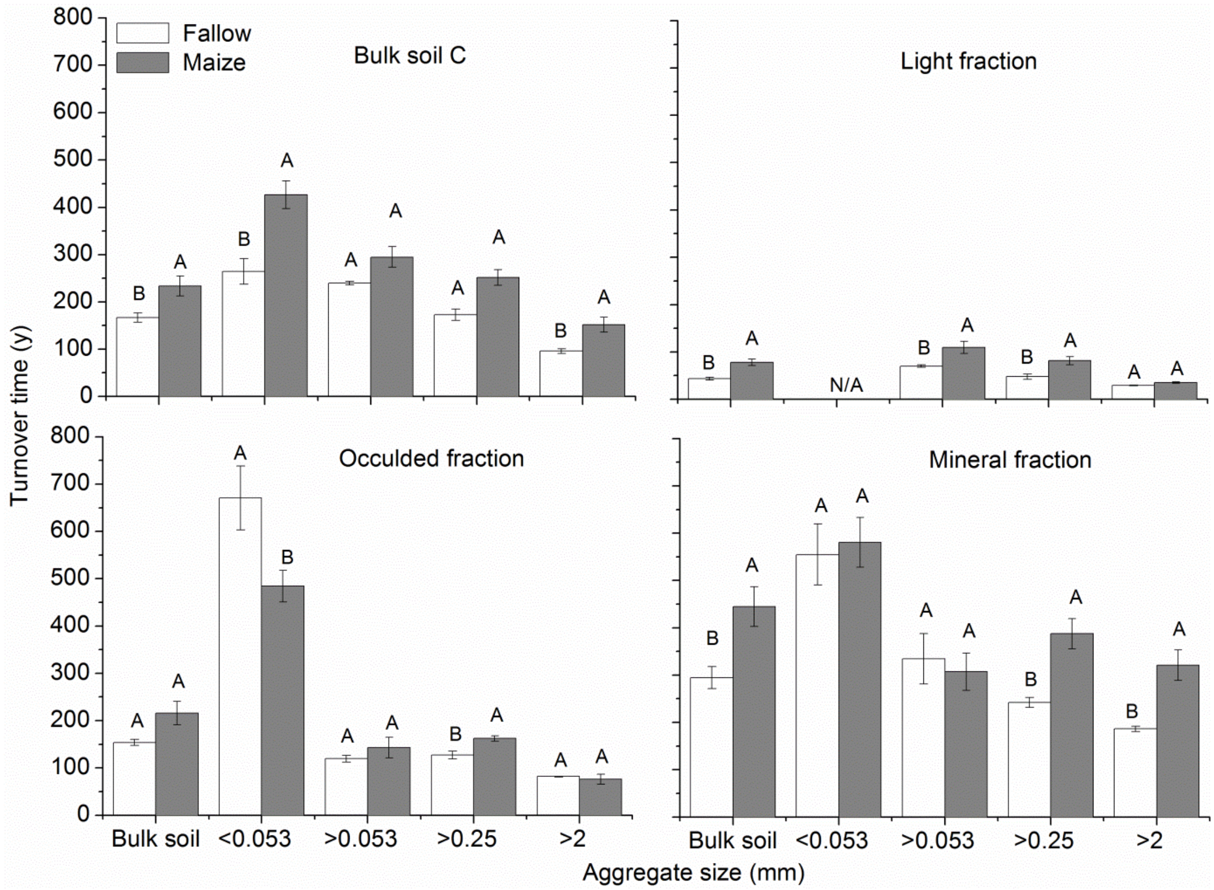

Figure 6. Turnover time of soil organic C in bulk soil, various aggregate classes, and various density fractions (light, occluded and mineral fractions) after 24-year natural fallow and continuous maize cropping. Values represent mean of three replications ± standard error. Different letters indicate significant difference between natural fallow and maize systems (P<0.05). N/A, not analysed due to insufficient samples.