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Variation of soil organic matter depends on light-fraction organic matter under long-term monocropping of different crops

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Abstract: Cultivating crops influences soil organic matter (SOM), but the effect of different crops remains unclear, particularly under long-term monocropping. The objective of this study was to identify how different crops influence the content and chemical structures of SOM under long-term monocropping. Here, soils were sampled (0–20 cm) under 27-year soybean and maize monocropping and separated into different physical fractions. The content and chemical structures of SOM in all fractions were determined. SOM contents were higher under soybean than maize in bulk soil and macroaggregates and their light-fractions instead of microaggregates and silt and clay. The difference in SOM chemical structure was observed in aggregates and density fractions rather than bulk soils and supported by the result of principal component analysis. The proportion of O-alkyl C in macro- and microaggregates and all free light fractions and that of aromatic C in mineral-associated fractions were higher, while that of carbonyl C was lower under maize than soybean. These results demonstrated that different crops monocropping influences the content and chemical structures of SOM, and the variations were mainly in the light-fraction SOM and highlight a higher sensitivity of physical fractions than bulk soil to different crops.

Keywords: Mollisol; crop species; chemical stability; farmland; carbon turnover

Soil organic matter (SOM) plays an important role in food security and the global carbon cycle, and a minor change of its content has a tremendous impact on global warming because it is the largest carbon stock in terrestrial ecosystems (Lal 2004). SOM content may be enhanced *via* increasing its stability through the physical protection of aggregates, association with soil minerals, chemical recalcitrance of SOM (Jastrow et al. 2006, Dungait et al. 2012). All these stability mechanisms are closely related to the chemical structure of SOM. However, the chemical structures of SOM and the factors remain unclear due to their diversity and complexity.

The solid ^{13}C NMR (nuclear magnetic resonance) technique is used to investigate SOM chemical structures because it is non-destructive and can examine insoluble SOM and provide comprehensive structural information on the changes of SOM (Baldock et al. 1992, Mao et al. 2008). The major chemical functional carbon (C) of SOM are alky C, O-alkyl C, aromatic C and carbonyl C (Preston 1996) and their proportions are influenced by soil tillages, residue return and fertiliser types (Schulten et al. 1995, Mao et al. 2008, Han et al. 2020). For example, SOM was richer in alkyl C under forest than under grass (Golchin et al. 1997), the proportion of O-alkyl decreased, but

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aromatic groups increased during the decomposition of the straw mixing with soil (Han et al. 2020). These suggest that soil management would change the chemical structures of SOM.

The main resource of SOM is organic inputs from plants, including root exudates and senesced plant residues. Crop species can, directly and indirectly, influence SOM content through various quantities and quality of crop residue inputs and by changing soil physical conditions and microbial properties (Higashi et al. 2014, Tiemann et al. 2015, Dou et al. 2016). A recent study demonstrated that crop species affected SOM turnover rates characterised by natural ^{13}C abundance and suggested SOM chemical structures may be influenced by crop species (Qiao et al. 2015). However, the study focused on the changes of SOM chemical structures under different crop species is rare.

The Mollisol in the Northeast of China is one of the three largest Mollisol areas in the world, with about 73% cultivated (Liu et al. 2003). In this area, continuous soybean and maize monocropping are major cropping systems and have been steadily increased since the 1980s due to huge commodity grain requirements (Chinese Statistical Yearbook 2018). The fertile and productive Mollisol has degraded seriously due to intensive cultivations and tillages (Liu et al. 2010). Therefore, it is urgent to elucidate the effects of the two crops monocropping on both contents and chemical structures of SOM in the Mollisol of the Northeast of China.

The aggregate and density fractionation have been proposed as appropriate physical fractionation to study SOM variations since they are less destructive towards the original properties of SOM relative to chemical fractionation (Christensen 2001). Land-use type has been demonstrated to affect the content and chemical structure of SOM in soil particle size fractions and density fractions by combining SOM physical fractionation and solid-state ^{13}C NMR (Liu et al. 2018, Ji et al. 2020). In this study, the content of SOM in aggregates and density fractions from topsoil (0–20 cm) under soybean and maize monocropping were investigated, and their chemical structures were characterised by using the solid-state ^{13}C CP/TOSS NMR spectroscopy. The objective was to examine the effects of long-term, continuous maize and soybean monocropping on the contents and chemical structures of SOM. We hypothesised that the contents and chemical structures of SOM would be different due to the various quality and quantity of residue inputs, and these differences would vary with physical fractions.

MATERIAL AND METHODS

Site description and sampling. The field experiment is located at the State Key Experimental Station of Agroecology, Chinese Academy of Sciences, Hailun county, Heilongjiang province (47°26'N, 126°38'E). This region has a typical temperate continental monsoon climate with a hot summer and a cold winter. The mean annual temperature is 2.2 °C, with the highest monthly temperature in July (35 °C) and lowest in January (–38 °C). The mean annual rainfall is 550 mm. The soil is Pachi Haploborolls as classified by USDA Taxonomy (Soil Survey Staff 2010). The long-term continuous monocropping experiment was established to evaluate the ecological effects of continuous monocropping with different crop species in 1991. The soil contained soil organic carbon, 29.8 g/kg; total N, 2.2 g/kg; total P, 0.74 g/kg; total K, 20.8 g/kg, and 424 g/kg of clay (< 0.002 mm) and 326 g/kg of silt (0.02–0.002 mm) in the topsoil (0–20 cm). Soil pH in water (1:2.5) was 6.05.

The soybean and maize monocropping were chosen in the long-term field experiment in this study. The crop cultivars of maize and soybean were cvs. Haiyu 6 and Heinong 35, respectively. The crop density was 6.0 and 27 seeds/m^2 for maize and soybean, respectively. The crop cultivars, cropping density, chemical fertilisers and crop straw management were kept the same every year. The management was the same as local farming practices. Maize and soybean were sown in May and harvested in October. The soils were shovelled two times and waded three times during the crop growth period, and made rotary tillage ridging with a tillage depth of 20 cm after crop harvest. The basal fertilisers were applied at rates of 65.5 kg N/ha and 30.1 kg P/ha for maize, 27.0 kg N/ha and 30.1 kg P/ha for soybean. The N and P fertilisers were applied as urea and ammonium phosphate. An additional dose of urea was applied at a rate of 65.5 kg N/ha at the booting stage of maize. All crop shoot residues and main roots of maize were removed after harvest. The plots under soybean and maize monocropping included three subplots. In 2014, five soil samples were randomly collected from topsoil (0–20 cm) in each subplot and then mixed as one composite sample. The composite samples from subplots were used as replicates. All the soil samples were taken to the laboratory and manually broken into around 20–40 mm aggregates along the nature failure surfaces of the soil after being air-dried to the plastic limit. Visual plant residues and roots were removed from soil samples.

The fractionation of soil aggregates and density fractions. Aggregates were obtained by the wet-sieving technique (Kemper and Rosenau 1986). In brief, an aliquot of 50 g air-dried soil was pre-soaked in distilled water for 5 min and then passed to 0.25 and 0.05-mm sieve by hand for 50 times over 2 min. The materials remaining on 0.25-mm sieve were macroaggregates (macro, > 0.25 mm). The materials on 0.05-mm sieve and in water were microaggregates (micro, 0.25–0.05 mm) and silts and clays (< 0.05 mm), respectively.

All bulk soils, macroaggregates and microaggregates were separated into three density fractions by using the previous method (Golchin et al. 1997). Aliquots of 20 g air-dried soil samples were mixed with 150-mL sodium iodide (NaI, 1.8 g/cm^3) in a 250-mL centrifugation tube. The mixture was settled overnight after inverting several times by hand. The free light fractions (fLF) in the supernatant was obtained by suction after centrifugation (4 600 rpm, 1 h), and then filtered by using a millipore filter funnel with a glass-fibre paper (0.45 µm) under vacuum, and washed with $CaCl_2$ and distilled water to remove residual NaI. The remaining solution in the tube was adjusted to 80-mL and then sonicated at 300 J/mL for 5 min in an ice bath. The supernatant was collected as occluded light fractions (oLF) with the same procedure above. The remaining sediment was mineral-associated fractions (MF) and washed with distilled water and 95% ethyl alcohol until the solution became colourless. Nine density fractions were obtained, including three from bulk, free light fractions (bulk-fLF), occluded light fractions (bulk-oLF) and mineral-associated fractions (bulk-MF); three from macroaggregates, free light fractions (macro-fLF), occluded light fractions (macro-oLF) and mineral-associated fractions (macro-MF); three from microaggregates, free light fractions (micro-fLF), occluded light fractions (micro-oLF) and mineral-associated fractions (micro-MF). The fractionation scheme to isolate SOM is shown in Figure 1.

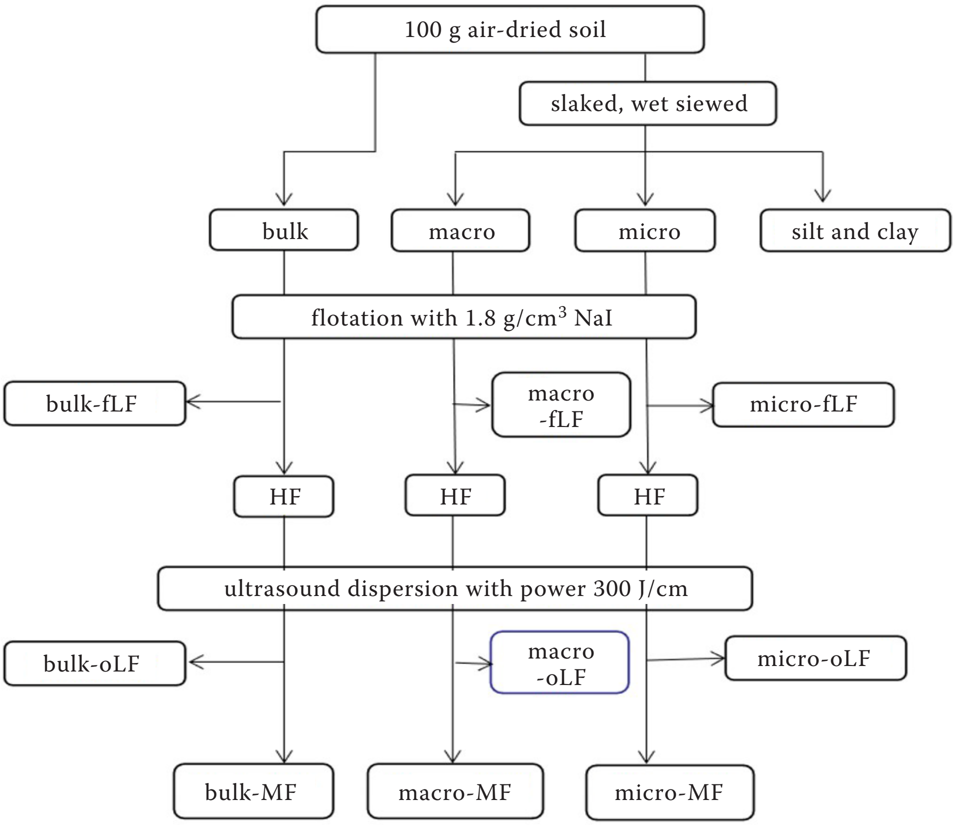

Figure 1. The fractionation scheme to isolate soil organic matter fractions. Bulk-fLF – free light fractions in the bulk soil; macro-fLF – free light fractions in macroaggregates; micro-fLF – free light fractions in microaggregates; bulk-oLF – occluded light fractions in aggregates in bulk soil; macro-oLF – occluded light fractions in aggregates in macroaggregates; micro-oLF – light fractions in aggregates in microaggregates; bulk-MF – mineral fractions in the bulk soil; macro-MF – mineral fractions in macroaggregates; micro-MF – mineral fractions in microaggregates; HF – heavy fractions

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All aggregates and density fractions were transferred into pre-weighed beakers and dried at 50 °C. The carbon concentrations of all fractions were measured by using a VarioEL CHSN elemental analyser (Heraeus Elementar VarioEL, Hanau, Germany). The concentrations of soil organic carbon (g/kg fraction) were converted into its contents (g/kg soil) by multiplying the mass proportion of the fraction in bulk soil (%), and then all soil organic carbon contents were converted into SOM contents by using a factor of 1.724 (Malamoud et al. 2009).

Chemical structures of SOM in bulk soil and physical fractions. The chemical structures of SOM in bulk soils, aggregates and density fractions as well as soybean and maize residues were obtained by using ^{13}C cross polarisation/total sideband suppression (^{13}C CP/TOSS), except for oLF due to small yields of this fraction. Samples were pre-treated with hydrofluoric acid (2%) to remove paramagnetic compounds before NMR measurements (Skjemstad et al. 1994). ^{13}C CP/TOSS was run using a Bruker AVANCE 400 spectrometer at 100 MHz for ^{13}C with 4 mm sample rotors. The NMR experiments were conducted at a spinning speed of 5 kHz and a CP time of 1 ms, with a ^{1}H 90° pulse-length of 4 µs and a recycle delay of 0.8 s. Four-pulse total suppression of sidebands (TOSS) was employed before detection, and two-pulse phase-modulated decoupling was applied for optimum resolution.

The spectra obtained were subdivided into six chemical shift regions, assigned to nonpolar alkyl C (0–44 ppm), N-alkyl/methoxy C (44–64 ppm), O-alkyl C (64–92 ppm), aromatic C (92–145), aromatic C-O (145–161 ppm) and carbonyl C (161–220 ppm) (Cao et al. 2011). The integrals of the peaks were used as the relative proportion of each functional C in the sum of all functional C. The aliphaticity was calculated: A/AR = (alkyl C + N-alkyl C + O-alkyl C)/(aromatic C + aromatic C-O), and indicated simpler chemical structures with higher values.

Data analysis. An independent sample *t*-test using SPSS 16.0 software (SPSS Inc., Chicago, USA) was

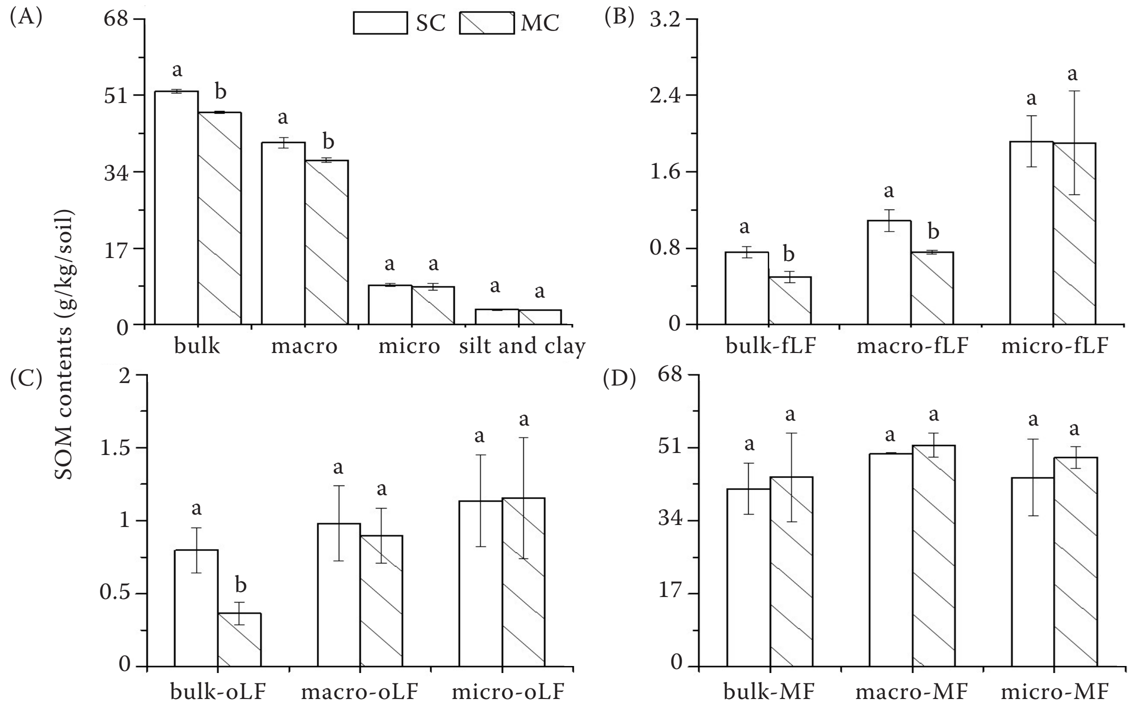

Figure 2. The soil organic matter (SOM) contents in the bulk soils, aggregates and density fractions under soybean and maize monocropping. Vertical bars represent standard deviations of three replicates. The letters indicate the significant differences at $P < 0.05$ between two crops monocropping. bulk-fLF – free light fractions in the bulk soil; macro-fLF – free light fractions in macroaggregates; micro-fLF – free light fractions in microaggregates; bulk-oLF – occluded light fractions in aggregates in bulk soil; macro-oLF – occluded light fractions in aggregates in macroaggregates; micro-oLF – light fractions in aggregates in microaggregates; bulk-MF – mineral fractions in the bulk soil; macro-MF – mineral fractions in macroaggregates; micro-MF – mineral fractions in microaggregates; SC – soybean monocropping; MC – maize monocropping

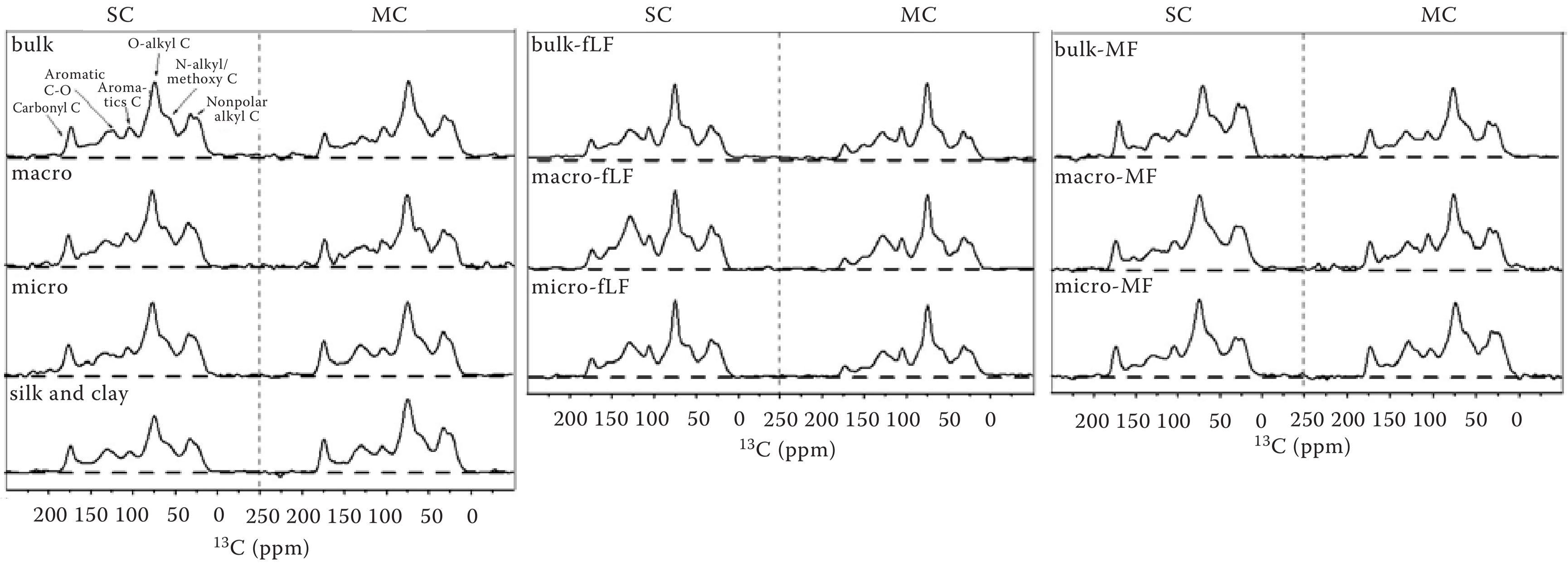

Figure 3. The spectra of soil organic matter (SOM) in the bulk soils, aggregates and density fractions under soybean and maize continuous cropping characterised by ^{13}C CP/TOSS NMR. Bulk-fLF – free light fractions in the bulk soil; macro-fLF – free light fractions in macroaggregates; micro-fLF – free light fractions in microaggregates; bulk-MF – mineral fractions in the bulk soil; macro-MF – mineral fractions in macroaggregates; micro-MF – mineral fractions in microaggregates; SC – soybean monocropping; MC – maize monocropping

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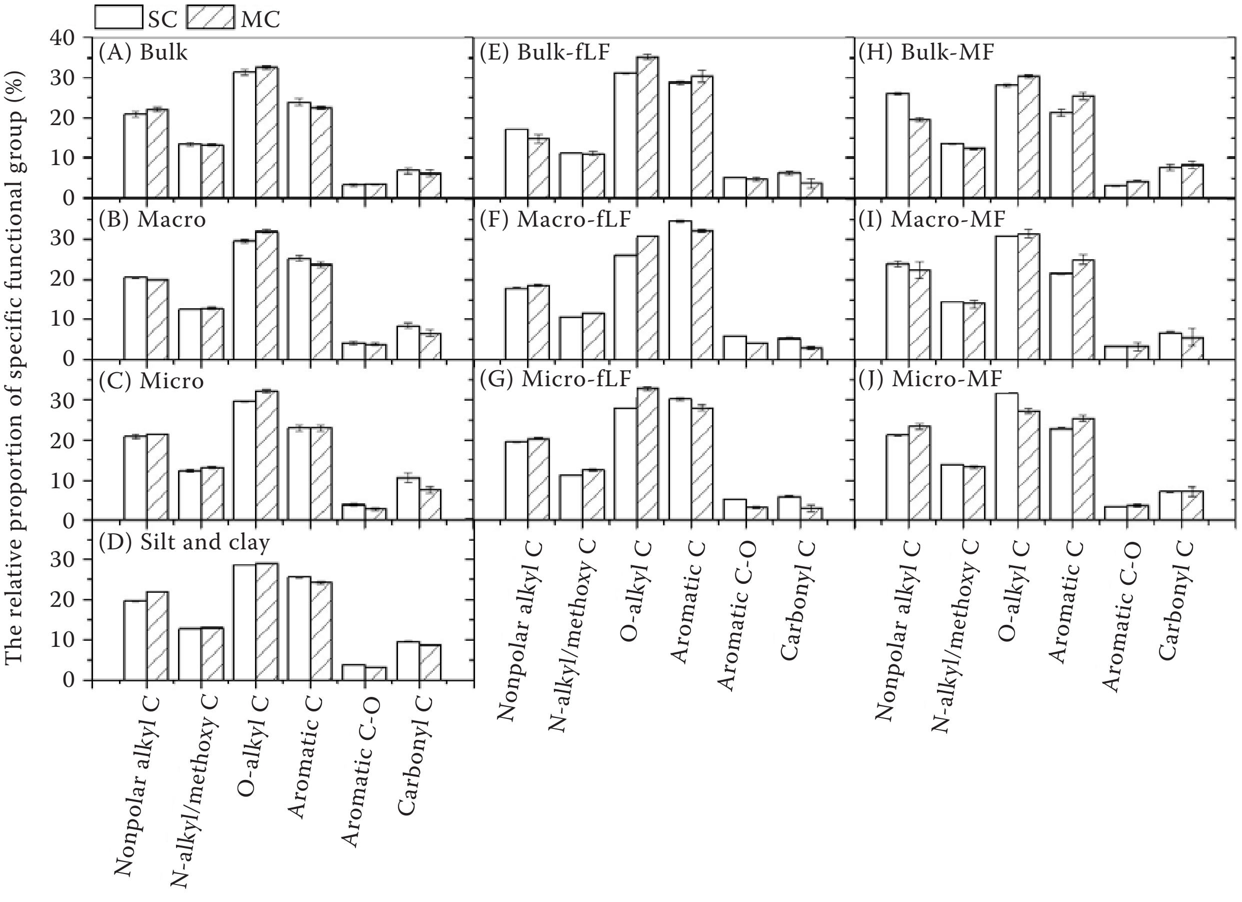

Figure 4. The relative proportion of specific functional carbon (C) of soil organic matter (SOM) in (A) bulk soils; (B–D) aggregates; (E–G) free light fractions, and (H–J) mineral-associated fractions of aggregates and bulk soils. The bars indicate the average noise in the chemical shift region beyond 0–220 ppm. Bulk-fLF – free light fractions in the bulk soil; macro-fLF – free light fractions in macroaggregates; micro-fLF – free light fractions in microaggregates; bulk-MF – mineral fractions in the bulk soil; macro-MF – mineral fractions in macroaggregates; micro-MF – mineral fractions in microaggregates; SC – soybean monocropping; MC – maize monocropping

used to determine the effects of crop species on SOM contents in different aggregates and density fractions. Statistical significance for all tests was set at $P < 0.05$. A principal component analysis (PCA) was performed using six functional C of SOM (in integrations, %) to determine variation in chemical structures of SOM and to specify the loading of individual functional C on the principal components. The average noise of three chemical shift regions sampled from beyond 0–220 ppm in each ^{13}C NMR spectrum was used as an error to determine the significance of spectral differences between soil samples (Zhou et al. 2014).

RESULTS

Soil organic matter contents in the aggregates and density fractions. The SOM contents associated with aggregates decreased in the order of bulk, macroaggregates, microaggregates and silts and clays. The SOM contents in bulk soil and macroaggregates under soybean monocropping were higher than those under maize monocropping, by 9.92% and 10.59%, respectively. The SOM contents in MF accounted for 93.52–96.98% of total SOM, while those in fLF and oLF below 0.12% (Figure 2). The SOM contents in fLF from bulk soil and macroaggregates under soybean monocropping were higher than those under maize monocropping. SOM contents in the microaggregates, silt and clay and all fractions (except for fLF in bulk and macroaggregates) between two crop species were the absence of difference.

Chemical structures of soil organic matter in aggregates and density fractions. The ^{13}C CP/TOSS spectra of SOM in all fractions and the proportions of specific functional C showed that O-alkyl C and aromatic C were dominant and accounted for

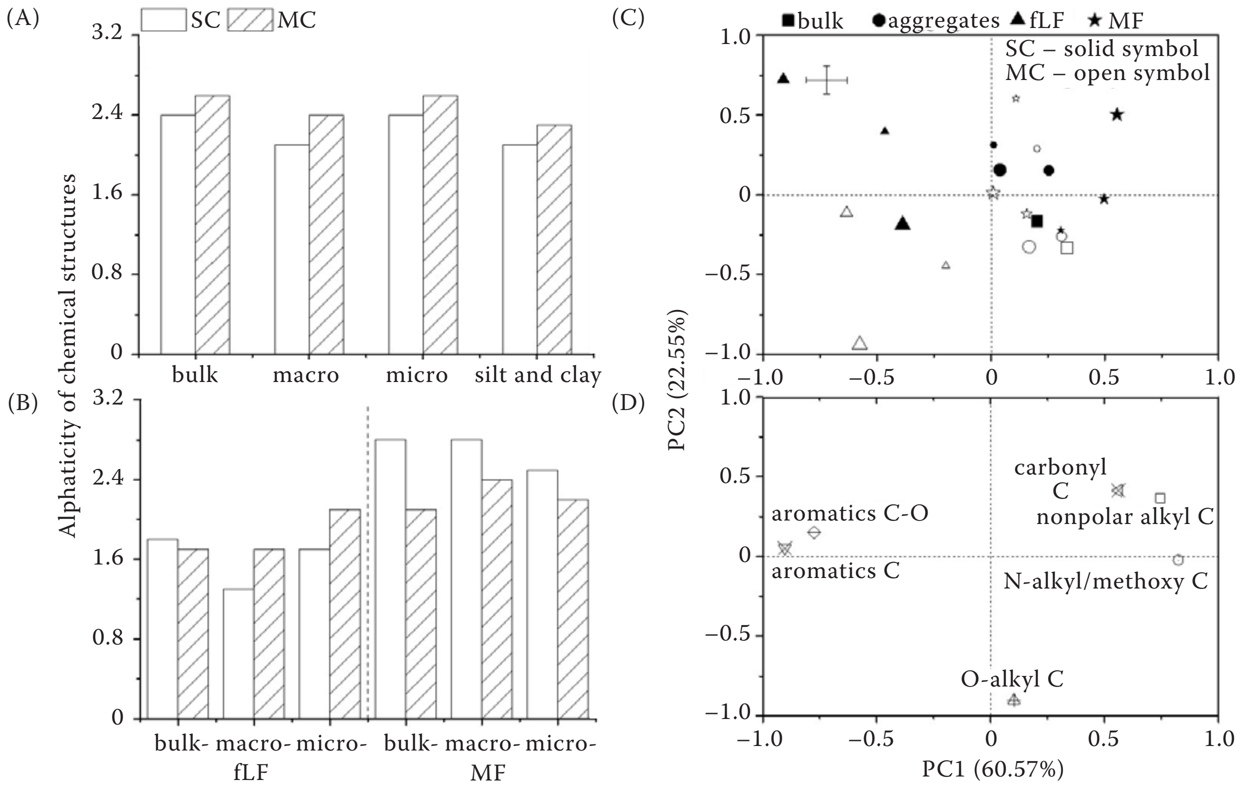

Figure 5. The aliphaticity index of soil organic matter (SOM) in (A) bulk soils and aggregates and (B) density fractions, calculated by the ratio of aliphatic C (alkyl C + N-alkyl C + O-alkyl C) to aromatic C (aromatic C + aromatic C-O). (C) The shifts of soil organic matter chemical structures, and (D) the loadings of individual functional C under soybean and maize monocropping by ^{13}C CP/TOSS NMR. The solid and open symbol represented soybean (SC) and maize monocropping (MC), respectively. The symbol size for two crops monocropping from small to large indicate the fractions from bulk, macroaggregates and microaggregates, respectively. The horizontal and vertical error margins represent a least significant difference among all the measurements on PC1 and PC2, respectively ($P < 0.05$). bulk-fLF – free light fractions in the bulk soil; macro-fLF – free light fractions in macroaggregates; micro-fLF – free light fractions in microaggregates; bulk-MF – mineral fractions in the bulk soil; macro-MF – mineral fractions in macroaggregates; micro-MF – mineral fractions in microaggregates

25.9–35.2% and 21.3–34.5% of all functional C, respectively, and nonpolar alkyl C, N-alkyl/methoxy C and carbonyl C were less abundant, and aromatic C-O were the least, less than 6.0% (Figures 3 and 4).

The difference in SOM chemical structures between two crops monocropping was observed in aggregates rather than the bulk soil. The proportion of O-alkyl C decreased, and that of carbonyl C increased under soybean monocropping compared with the proportions of corresponding functional C of SOM in macroaggregates and microaggregates under maize monocropping. The proportions of other functional C were similar among aggregates and between two crops monocropping.

The differences of SOM chemical structures were also observed among density fractions and between two crops monocropping (Figure 4). The chemical structures of SOM in fLF were different from those in MF. The proportions of nonpolar alkyl C, N-alkyl/methoxy C and carbonyl C were lower, while those of O-alkyl C, aromatic C and aromatic C-O were higher in fLF than MF. The proportion of O-alkyl C was higher in bulk-fLF than bulk-MF, while lower in macro/micro-fLF than macro/micro-MF. The difference of SOM chemical structures between two crops monocropping was larger in fLF than MF. For the proportions of functional C in fLF, aromatic C, aromantic C-O and carbonyl C were higher, while O-alkyl C was lower under soybean monocropping than those under maize monocropping. The carbonyl C in bulk-fLF, macro-fLF and micro-fLF under soybean monocropping were higher than those under maize monocropping, by 75.7, 82.8, and 96.7%, respectively. For the proportion of functional C in MF, aromatic C in the MF-bulk was lower, while that in macro/micro-MF was higher under soybean monocropping than under maize monocropping.

All aliphaticity of SOM were lower in bulk soil, aggregates and fLF, while higher in all MF under soybean monocropping than under maize monocropping (Figure 5A, B). The proportions of six functional C of SOM were subjected to principal component analyses to determine the effects of different crops monocropping on the chemical structures of SOM in the aggregates and density fractions (Figure 5C, D). PC1 and PC2 accounted for 60.57% and 22.55% of total varia, respectively. The chemical structures of SOM in fLF were separated from those in MF *via* PC1, and the separation was driven by the association of nonpolar alkyl C, N-alkyl/methoxy C, carbonyl C with MF and the association of aromatic C, aromatic C-O with fLF. The chemical structures of SOM under maize monocropping were separated from those under soybean monocropping by PC2, and

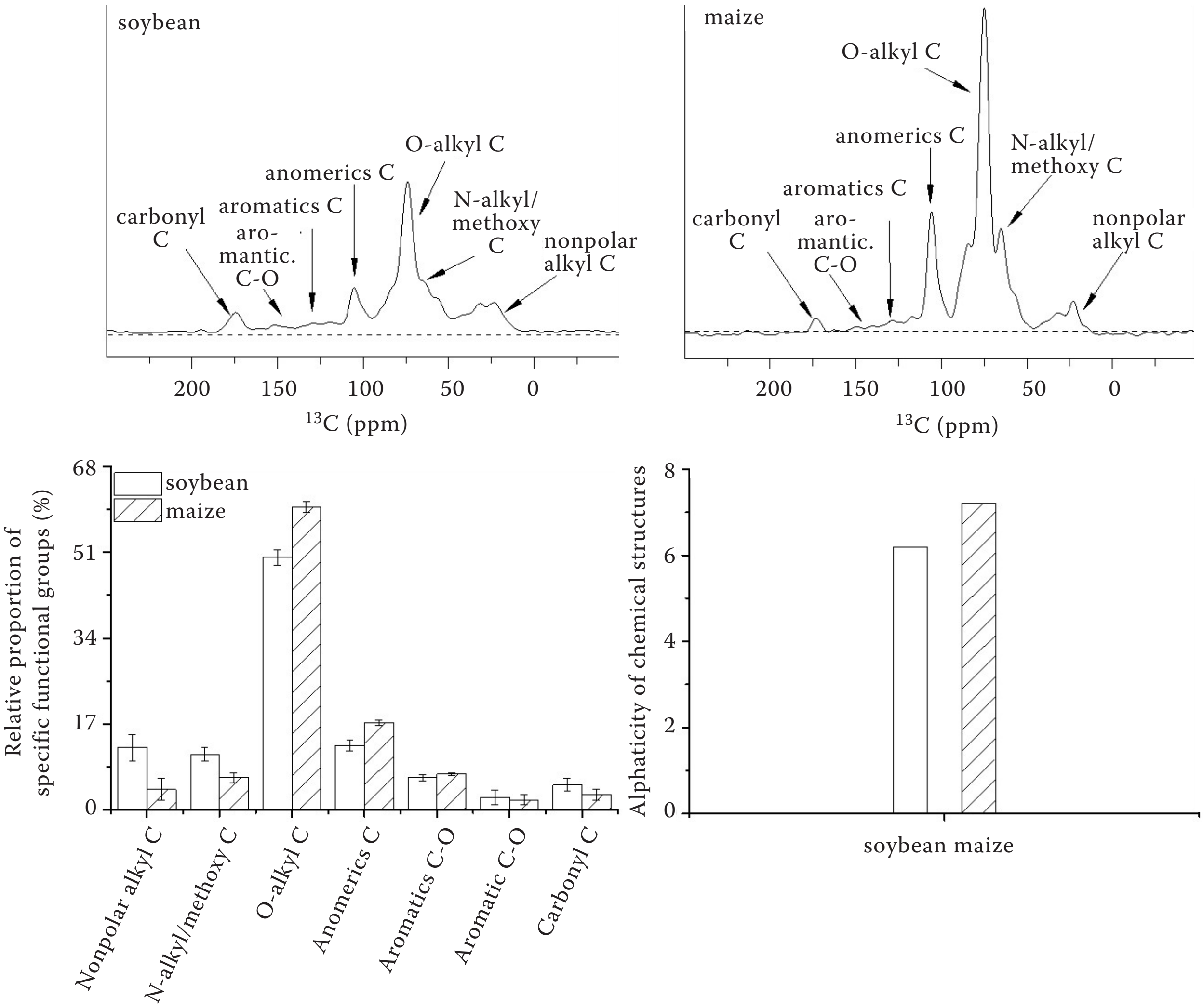

Figure 6. The aliphaticity index of soil organic matter (SOM) in bulk soils and (A) aggregates and (B) density fractions, calculated by the ratio of aliphatic C (alkyl C + N-alkyl C + O-alkyl C) to aromatic C (aromatic C + aromatic C-O). (C) The shifts of soil organic matter chemical structures, and (D) the loadings of individual functional C under soybean and maize monocropping by ^{13}C CP/TOSS NMR. The solid and open symbols represented soybean (SC) and maize monocropping (MC), respectively. The symbol size for two crops monocropping from small to large indicated the fractions from bulk, macroaggregates and microaggregates, respectively. The horizontal and vertical error margins represent the least significant difference among all the measurements on PC1 and PC2, respectively ($P < 0.05$)

this separation was driven by O-alkyl C associated with maize monocropping and carbonyl C associated with soybean monocropping. The separation was larger between fLF and MF than that between two crops monocropping.

DISCUSSION

The effect of two crops monocropping on SOM contents in aggregates and density fractions. The SOM content decreased with smaller aggregates sizes and was much higher in the mineral-associated fractions than in the free and occluded light fractions under both soybean and maize monocropping (Figure 2), consistent with the results in previous literature (John et al. 2005, Guan et al. 2018, Liu et al. 2018). Macroaggregates accounted for 70–80% of all aggregates (data not shown) and were formed by binding particle organic materials, while microaggregates were formed in macroaggregates (Six et al. 2000). Mineral-associated fractions account for above 90% of soil dry weight, and minerals can associate steadily with organic carbon (Torn et al. 1997). Thus, macroaggregates and mineral-associated fractions contained the most SOM contents among aggregates and density fractions, respectively.

The SOM contents were higher under soybean monocropping than those under maize monocropping in bulk soil, macroaggregates, and the fLF and oLF of macroaggregates (Figure 2). SOM contents depend on the balance between formation and decomposition, and its variation may arise from multiple and complex processes due to SOM complexity and chain reactions (Post and Kwon 2000, Liu et al. 2020). The most important resource of SOM formation is plant residues (Kögel-Knabner 2002). Our previous study showed that the amount of residues input was higher under soybean monocropping than under maize monocropping in the soil plough layer (Qiao et al. 2015). Exogenous organic inputs into the soil do not only promote the formation of SOM but also prime the decomposition of native SOC (Kuzyakov et al. 2000). Soybean residues are more available to microorganisms than maize residues due to containing more nitrogen and less lignin (Huggins et al. 2007). Thus, SOM may be decomposed less under soybean monocropping than under maize monocropping due to microbial preferential substrate utilisation (Lyu et al. 2018). Therefore, more formation but less decomposition of SOM led to a higher SOM content under soybean monocropping than under maize monocropping.

Moreover, the difference of SOM content between two crops monocropping was observed in the SOM associated with macroaggregates, fLF and oLF. The SOM in fLF and oLF is mainly composed of plant residues with various extents of decomposition (Helfrich et al. 2006), and more fresh organic input was occluded into macroaggregates than other aggregates (Six et al. 2000). Tillages and other land management strategies mainly accelerate the turnover of macroaggregates and then influence the organic matter associated with macroaggregates (Six and Paustian 2014). Crop species were reported to affect SOM contents before (Bhattacharyya et al. 2009, Higashi et al. 2014, Gu et al. 2019), but the time of cropping was short-term. In the present study, we demonstrated crop species influenced SOM contents, especially SOM in the light fractions, by using the soil under long-term (24 years) monocropping.

The effect of two crops monocropping on the chemical structures of SOM in aggregates and density fractions. O-alkyl C and aromatic C were dominant in SOM chemical structures in all the aggregates and density fractions in the present study (Figure 3), consistent with previous results (Baldock et al. 1992, Yao et al. 2019). Chemical structures of SOM were the absence of difference among aggregates of different sizes (Figures 3 and 4). The proportion of aromatic C in microaggregates tended to be higher relative to that in macroaggregates under pasture and woodland (Rabbi et al. 2014). The aliphaticity of SOM increased with decreased aggregate size (Guo et al. 2013). The different shifts of chemical structures of SOM among aggregates compared with previous studies could be explained by plant systems, fertilisation and management strategies in different soil types. Therefore, more studies are needed to explore the response of the chemical structures of SOM associated with aggregates to soil managements in the future.

In bulk soil, SOM in fLF contained less nonpolar alkyl C and carbonyl C, but more O-alkyl C, aromantic C and aromantic C-O than SOM in MF (Figures 3 and 4). The SOM in fLF contains more labile components than that in MF (Helfrich et al. 2006, Rabbi et al. 2014) because fLF is mainly from residues and MF is mainly composed of microbial residues and products associated with soil minerals (Rabbi et al. 2014, Shahbaz et al. 2017). In macro- and microaggregates, the difference of SOM chemical structures between in fLF and MF changed to different extent relative to these in bulk soil (Figures 3 and 4) because the crop residues occluded into aggregates and transformed into SOM have been subject to the decomposition of the different extent (Six et al. 2001).

In macro- and microaggregates and fLF, the largest difference of SOM chemical structures between two crops monocropping was in the proportion of O-alkyl C (Figures 4–6). Principal component analysis showed the separation of SOM chemical structures under soybean monocropping from those under maize monocropping was driven by the O-alkyl C and carbonyl C. The proportion of O-alkyl C was the most among functional groups of SOM and showed the largest difference between soybean and maize residues (Figure 6). These findings indicated that the chemical structures of SOM were related to inputted crop residues. The aliphaticity of SOM in aggregates and fLF was higher, while that was lower in MF under soybean monocropping than maize monocropping (Figure 5). These results supported that the SOM in aggregates and fLF were mainly transformed by plant residues through selective retention (Schmidt et al. 2011) and suggested that the SOM in MF was associated with soil minerals before or after the decomposition of microbes to a less extent.

Implications and perspectives for future research. Increased sequestration of SOM has a significant impact on improving soil fertility and ensuring food security, and mitigating global warming (Lal 2004). Intensive tillages such as increasing soybean and maize monocropping resulted in the degradation of fertile and productive Mollisol in the Northeast of China (Liu et al. 2003). Mitigating the degradation of this Mollisol or increasing its quality is highly challenging at this stage because of our limited understanding of the factors controlling SOM stability. In this study, we demonstrated that different crop monocropping affected the contents and chemical structures of SOM and the chemical structures of SOM associated with aggregates and density fractions were more sensitive, especially those in the light fractions. These findings have important implications for improving the understanding of the factors controlling SOM stability and provide a theoretical base for cropping systems. However, only two crops of monocropping were chosen, and the contents and chemical structures of SOM were measured in the plough layer in this study. Future studies should further investigate the shift of SOM in profile under long-term monocropping of more crop species. Moreover, to keep the same management with local farming practices, the crop density and fertilisation were different between maize and soybean monocropping. This study demonstrated the crop species influenced the content and chemical structures of SOM based on the inputted amount and chemical structures of crop residues. Further study should set the same fertiliser use and crop density under different crop monocropping to further verify the effect of crop species.

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