

Stable isotope fractionation provides information on carbon dynamics in soil aggregates subjected to different long-term fertilization practices



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ABSTRACT

Stable carbon (C) isotopes provide valuable information to study C incorporation in soil aggregates. Different soil aggregate fractions have different concentrations of soil organic C (SOC), and changes in the abundance of soil C isotopes are closely linked to aggregate turnover. Soils from rice-wheat rotation fields in a long-term (31 years) experiment established in subtropical Central China were analyzed to improve knowledge of the influence of long-term fertilization (e.g. inorganic fertilizers) on the organic C isotope composition of different aggregate fractions. Aggregate-associated C levels and $\delta^{13}\text{C}$ values (^{13}C to ^{12}C ratios, relative to a standard) ranged from 19.0 to 23.1 g kg⁻¹, and from -24.8 to -23.0‰, respectively, across all treatments. However, SOC concentrations and $\delta^{13}\text{C}$ values were higher under treatments including inorganic fertilization than under the control (fertilizer-free, CK) treatment. In addition, $\delta^{13}\text{C}$ values of micro-aggregates (< 0.25 mm) were higher than those of macro-aggregates (> 0.25 mm), i.e. the micro-aggregates (< 0.25 mm) exhibited ^{13}C enrichment. Analyses of C flows between aggregates of different size classes (modelled in a scheme) indicate that C generally flows from macro-aggregates to micro-aggregates (or remains with them as they become successively smaller), and treatments with mineral fertilizers retarded these flows, relative to rates under the CK treatment. The results show that analyses of the natural abundance of stable C isotopes can provide valuable information on biogeochemical processes related to C transformation in soil aggregates.

1. Introduction

Soil aggregates are the basic units of soil structure. Consisting of primary particles and binding agents, they contain around 90% of the soil organic matter (SOM) in the soil surface layer (Jastrow et al., 1998). The quantity and quality of SOM in soil aggregates both vary since aggregates of different sizes may have different textures and porosities (Cates et al., 2016). There are also complex interactions between aggregate stability and soil carbon (C) cycles (Graf and Frei, 2013). *Inter alia*, aggregates protect SOM but can also retard its mineralization (Rabbi et al., 2015), a process that is sensitive to factors such as climate (Cheng et al., 2011), soil management practices (Alvaro-Fuentes et al., 2008; He et al., 2015), and land use (Soinnie et al., 2016). Hence, due to the importance of aggregates in both soil structure and carbon sequestration there is a growing need to understand the turnover of soil aggregate fractions, and responses of soil organic C (SOC) dynamics to changes in their turnover.

Several methods have been developed to probe SOM dynamics in soil aggregates (Angers et al., 1997; Wang et al., 2015). To link SOM dynamics with the turnover of soil aggregates, radio-C dating has been applied (Angers et al., 1997; Tan et al., 2013), and shown (for example) that the primary formation of macro-aggregates occurs around fresh plant residues (Angers et al., 1997). However, this method is expensive, precluding wide use for measuring SOM stability in soil aggregates. Physical fractionation techniques have also been used, to quantify C distributions in soil aggregates following various soil management practices or land use changes (Hontoria et al., 2016; Puget et al., 2005), which could have profound consequences for SOC sequestration over long timescales. For instance, using this approach, Puget et al. (2005) found that macro-aggregates had higher C concentrations than micro-aggregates because they contained younger organic matter with lower rates of decomposition. However, there is limited information about long-term impacts of fertilization practices on SOM physical fractions (Dou et al., 2016a; He et al., 2015), although they significantly affect

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soil processes at both bulk soil and aggregate levels through inputs of various quantities and qualities of nutrients (Bhattacharyya et al., 2010).

Another approach to assess such impacts is to analyze the natural abundance of stable isotopes, which provide information about sources of elements and the processes affecting them (Busari et al., 2016). Notably, $\delta^{13}\text{C}$ values (^{13}C to ^{12}C ratios, relative to a standard) are often used to study carbon dynamics in terrestrial ecosystems (Dou et al., 2016b; Sun et al., 2016), and coupling SOM fractionation with $\delta^{13}\text{C}$ analyses provides a powerful way of quantifying SOM dynamics in response to changes that alter the relative local dominance of C_4 ($\delta^{13}\text{C}$, -9 to -14‰) and C_3 ($\delta^{13}\text{C}$, -20 to -35‰) plant species (Hyodo et al., 2010). Moreover, in most ecosystems ^{13}C enrichment is greater below ground than at surface level (Werth and Kuzyakov, 2010), partly due to increases in $\delta^{13}\text{C}$ values of SOM during its decomposition (Peri et al., 2012). This is because microbial degradation of organic matter causes preferential losses of light ^{12}C to CO_2 , thereby increasing the relative abundance of ^{13}C in the soil ecosystem (Hogberg, 1998).

Hence, SOM decomposition leads to potentially informative variations in $\delta^{13}\text{C}$ values of SOM fractions (Cheng et al., 2013; Dou et al., 2013), as the C remaining in soil becomes successively more enriched in ^{13}C with each step of decomposition (Werth and Kuzyakov, 2010) (Fig. 1). Aggregate turnover (formation and breakdown rates) plays important roles in the kinetics of SOM decomposition and changes in $\delta^{13}\text{C}$ fractions, as SOM is stabilized by processes that hinder decomposition of particulate organic matter and its interactions with mineral particles (Dungait et al., 2012; Six and Paustian, 2014). Moreover, aggregates with high $\delta^{13}\text{C}$ values can be regarded as products of less ^{13}C -enriched aggregates, and there is a direct link between soil aggregation and C cycling. Thus, Gunina and Kuzyakov (2014) developed an approach to quantify C flows between aggregate size classes based on isotopic fractionation that is suitable for analyzing C pathways in aggregates.

The approach presented by Gunina and Kuzyakov (2014) provides a means to address the gap in knowledge, mentioned above, of long-term impacts of fertilization practices on SOM physical fractions (and the associated phenomena discussed above). Long-term field experiments are essential sources of information for such analyses of effects of management practices on soil and the sustainability of agro-ecosystems (Dou et al., 2016a; He et al., 2015). Therefore, in the study presented here we examined the C isotope fractionation that occurs during soil aggregate turnover in plots under rice/wheat rotations with various inorganic fertilizer treatments (and a fertilizer-free control treatment) in a long-term experiment in China. The objectives were to evaluate

effects of long-term fertilization on aggregate-associated stable C isotope compositions, and develop an extended scheme of C flows between aggregates of different size classes.

2. Materials and methods

2.1. The long-term fertilization trial

The long-term trial is located at Nanhu Agricultural Research Station ($30^{\circ}28'\text{N}$, $114^{\circ}25'\text{E}$, altitude 20 m) of Hubei Academy of Agricultural Sciences in Wuhan city, Hubei province, China. The site's soil is classified as a hydromorphic paddy soil that developed from yellow-brown soil. It is located in a typical area of China's Yangtze River valley, which has a humid mid-subtropical monsoon climate. According to data recorded *in situ* between 1981 and 2012, the mean annual temperature at the experimental site is 17°C , the cumulative temperature above 10°C is 5190°C , the average annual frost-free period is 276 d, and average annual precipitation (most of which occurs between April and August) is 1300 mm.

A long-term fertilization experiment was initiated in 1981 at the site to test effects of various long-term fertilization treatments of plots under a rice-wheat cropping system. Rice (*Oryza sativa* L.) was planted from June to October, while winter wheat (*Triticum aestivum* L.) was planted from November to May. At the beginning of the experiment, the soil had a pH of 7.8, SOM concentration of 1.1%, 1.8 g kg^{-1} of total N, 5.0 mg kg^{-1} of available P, and 87.0 mg kg^{-1} of K. Eight treatments with three replicates were implemented in 24 plots ($8\text{ m} \times 5\text{ m}$ each). The treatments were: no fertilizer application (control/CK), N application (N), N and P application (NP), N, P and K application (NPK), organic manure application (M), N and organic manure application (N + M), N, P and organic manure application (NP + M), and N, P, K and organic manure application (NPK + M). The average fertilization rates were $150\text{ kg N ha}^{-1}\text{ yr}^{-1}$, $75\text{ kg P}_2\text{O}_5\text{ ha}^{-1}\text{ yr}^{-1}$, $150\text{ kg K}_2\text{O ha}^{-1}\text{ yr}^{-1}$, and $22,500\text{ kg manure ha}^{-1}\text{ yr}^{-1}$, respectively, from 1981 to 2012 in case corresponding fertilization was required. Only the CK treatment and treatments with applications of inorganic fertilizers (N, NP and NPK) were considered in this study as organic fertilizer was expected to significantly affect the natural soil C isotope fractionation processes. The N fertilizer was applied in three splits in a 2:2:1 ratio at the basal, tillering, and panicle initiation stages, respectively, for rice cultivation, and a 2:1:1 ratio at the basal, overwintering, and jointing stages, respectively, for wheat cultivation. The P and K fertilizers were applied once as basal dressing during the wheat and rice seasons, respectively. These fertilizers were evenly broadcasted onto the soil surface and immediately incorporated into the plowed soil (at a depth of 0–20 cm) by tillage before sowing.

2.2. Soil sampling and aggregate separation

Immediately after the rice was harvest on 28 September 2012, soil samples were taken from the 0 to 20 cm soil layer at 5 randomly selected positions in each plot using a 5 cm diameter stainless steel soil sampler. All samples from each plot were carefully mixed to form a composite sample, which was then air-dried in the laboratory. A part of each dried composite sample was gently crushed and then processed to remove stones and large fragments of plant residues.

Air-dried soil samples (100 g) were wet-sieved using a modified version of the procedure described by Elliott (1986). Each sample was placed on a 5 mm sieve and shaken by submersion in deionized water for 5 min. The sieve was then gently moved up and down by hand for 4 min. The material remaining on the sieve was rinsed into containers, while material passing through the 5 mm sieve mesh was further fractionated using the same protocol with a 1 mm sieve and then with a 0.25 mm sieve. This ultimately yielded four aggregate fractions comprising soil particles with diameters of $> 5\text{ mm}$, $1\text{--}5\text{ mm}$, $0.25\text{--}1\text{ mm}$, and $< 0.25\text{ mm}$. After the final sieving cycle, sub-samples of the four

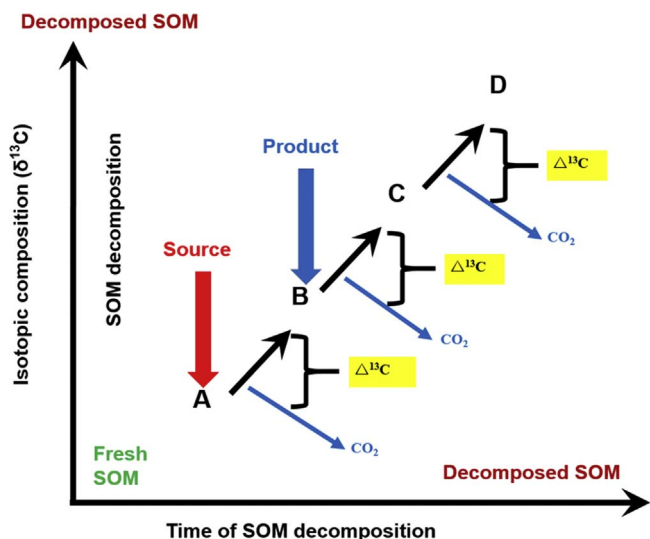


Fig. 1. ^{13}C enrichment reflects the formation steps of SOM pools.

aggregate fractions were transferred to aluminum pans and dried in an oven at 60 °C to determine the proportion of total soil weight in each fraction.

2.3. Carbon isotope analyses

SOC concentrations of the samples were assayed by the dichromate oxidation method. Subsamples were treated with 1 mol L⁻¹ HCL to remove soil carbonates, and C isotope ratios were determined using a Thermo Delta V Advantage Continuous Flow mass spectrometer coupled to a thermal combustion elemental analyzer and both liquid and solid zero blank auto-samplers (CF-TC/EA IRMS) (Thermo Fisher, USA). The ratio of ¹³C to ¹²C isotopes in the sample material (R_{sample}) was measured by mass spectrometry as the deviation from the isotopic ratio of a standard (R_{std}), here Vienna Pee Dee Belemnite. The ratio was then expressed in δ notation as:

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}}/R_{\text{std}} - 1) \times 1000$$

High $\delta^{13}\text{C}$ values indicate that the sample is enriched in ¹³C relative to the standard, while low values indicate depletion of ¹³C in the sample.

2.4. Analysis of C flows in aggregates

Flow of organic C flows within and between SOM fractions (as illustrated in Fig. 1) were analyzed, following (Gunina and Kuzyakov, 2014), by measuring $\delta^{13}\text{C}$ values of the soil aggregate fractions generated by wet sieving as described above. The method is described in detail by the cited authors. Briefly, differences ($\Delta^{13}\text{C}$) between $\delta^{13}\text{C}$ values of the fractions and the bulk soil were calculated to analyze changes in C isotopic composition of the soil aggregates. We then arranged the four aggregate size classes in a graphical scheme, and drew arrows between them, with their heads pointing in the direction of increasing $\Delta^{13}\text{C}$ values. As $\delta^{13}\text{C}$ values increase during SOM decomposition, the aggregate fractions at the start and end of an arrow can be respectively regarded as the 'source' and 'product' (Fig. 1). The product's $\Delta^{13}\text{C}$ value was then subtracted from that of the 'source' for each pair of aggregate fractions. In principle, the smaller this difference, the greater the probability of a C flow between the two fractions (or the source fraction being converted into the product fraction through SOM decomposition and/or other processes).

2.5. Statistical analyses

The experimental data were statistically analyzed using SPSS 16.0. One-way analysis of variance (ANOVA) was applied to assess the significance of observed differences in measured parameters between samples from plots subjected to different treatments, and between different aggregate fractions. The least significant difference (LSD) test with a probability threshold of $P = 0.05$ was used to identify significant differences between the experimental treatments.

3. Results

3.1. Treatment- and aggregate fraction-associated carbon levels

SOC levels under the compared treatments ranged from 19.0 to 23.1 g kg⁻¹ across all aggregate size fractions (Fig. 2). SOC concentrations of the aggregate fractions generally increased with increases in size class under all fertilization treatments, except that the > 5 mm aggregates had slightly lower SOC concentrations than the 1–5 mm aggregates under the CK treatment. There were significant differences ($P < 0.05$) in SOC concentrations between > 1 mm aggregates (1–5 mm, and > 5 mm) and < 0.25 mm aggregates. The SOC levels were also affected by the long-term fertilization practices, increasing in

the order CK, N, NP, and NPK, and all the plots treated with inorganic fertilizers (N, NP and NPK) had significantly higher ($P < 0.05$) SOC levels than the fertilizer-free (CK) plots.

3.2. Treatment- and aggregate-associated $\delta^{13}\text{C}$ values

Soil $\delta^{13}\text{C}$ values under the compared treatments ranged from -24.8 to -23.0‰ across all aggregate size fractions (Fig. 3). Generally, $\delta^{13}\text{C}$ values were lowest in bulk soil and aggregate samples from CK plots, and highest in samples from NPK plots. Moreover, the CK treatment resulted in slightly lower $\delta^{13}\text{C}$ values in all aggregate size fractions than the treatments involving fertilization. In addition, $\delta^{13}\text{C}$ values of aggregates slightly decreased with increases in size class, and < 0.25 mm aggregates always had the highest $\delta^{13}\text{C}$ values, but the pattern was strongest in samples from N and NPK plots.

As shown in Fig. 4, plots of $\Delta^{13}\text{C}$ values (differences in $\delta^{13}\text{C}$ values from that of the bulk soil) of aggregate fractions under each treatment were plotted against their sizes demonstrated that ¹³C enrichment increased with reductions in aggregate size. For example, ¹³C was depleted in the > 5 mm and 1–5 mm fractions, but enriched in the < 0.25 mm fraction under all treatments (Fig. 4). Thus, SOM decomposition processes in aggregates lead to ¹³C fractionation, and different aggregate-associated SOM fractions have different $\delta^{13}\text{C}$ values. Various processes stabilize SOM more in micro-aggregates than in macro-aggregates, and/or aggregates become progressively smaller as SOM decomposes.

3.3. C flows in the aggregates system

A schematic diagram of C flows in the system of aggregates under each of the long-term treatments considered here is shown in Fig. 5, where each node is labeled (in red) with the $\Delta^{13}\text{C}$ value for the corresponding aggregate size fraction. The arrows show the direction of C flows according to observed differences in the aggregate fractions' $\Delta^{13}\text{C}$ values, and the arrows' sizes are inversely proportional to the sizes of these differences, so larger arrows reflect a greater probability of C flux between the connected aggregate fractions.

The C flows among aggregates of different sizes were similar under all treatments. Generally, most of the flows were from large aggregates towards progressively smaller aggregates, as shown by the blue arrows, but there were some exceptions, shown by the yellow arrows. In addition, under all treatments C flux occurred from macro-aggregates (> 0.25 mm) to micro-aggregates (< 0.25 mm). The C flows in the aggregates system were also affected by the treatments. The apparent 'source' of the C flow (i.e. the fraction with the most negative $\Delta^{13}\text{C}$ value) was the 0.25–1 mm size class under the CK treatment, and the > 5 mm size class under all the chemical fertilization treatments (N, NP and NPK) (Fig. 5). Moreover, as shown by the widths of the arrows, the C flow between the size classes was considerably greater under the CK than under the N, NP and NPK treatments. C flux among the aggregate classes was more likely under the CK treatment, and the calculated differences between their $\Delta^{13}\text{C}$ values were less than 0.60, than under the N, NP and NPK treatments. C flow from the > 5 mm aggregates to the < 0.25 mm aggregates was particularly unlikely under the N and NPK treatments, because the differences between their $\Delta^{13}\text{C}$ values exceeded 1.00.

4. Discussion

Applications of inorganic fertilizer have variable effects on soil aggregation and SOC sequestration (Benbi et al., 2016; Ogunwole, 2008). In the experimental plots we examined, the treatments including N inputs resulted in higher SOC concentrations in both bulk soil and all the aggregate fractions than the CK treatment. The results are consistent with findings by Bhattacharyya et al. (2010) and Manna et al. (2007), that treatment with mineral fertilizers resulted in accumulation of SOM

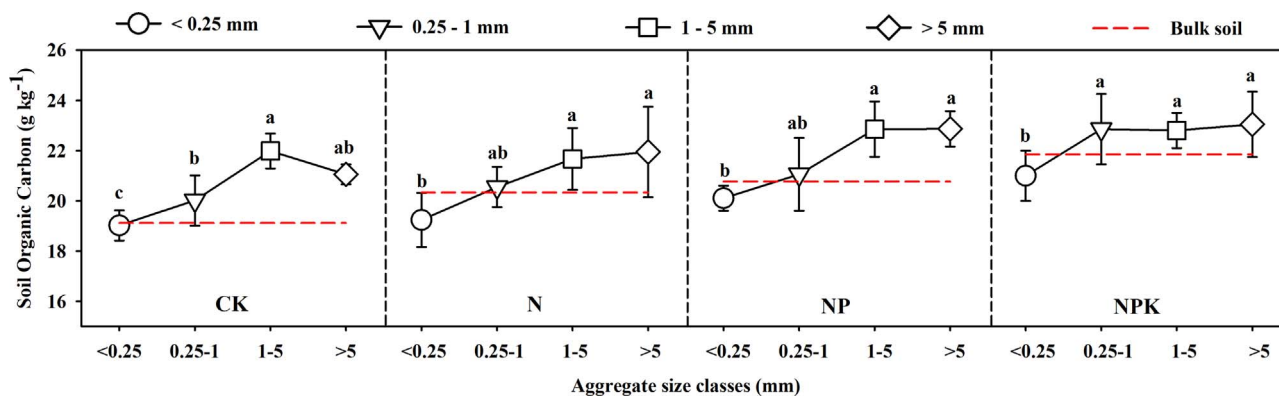


Fig. 2. Aggregate-associated SOC levels under indicated long-term fertilization and control (CK) treatments.

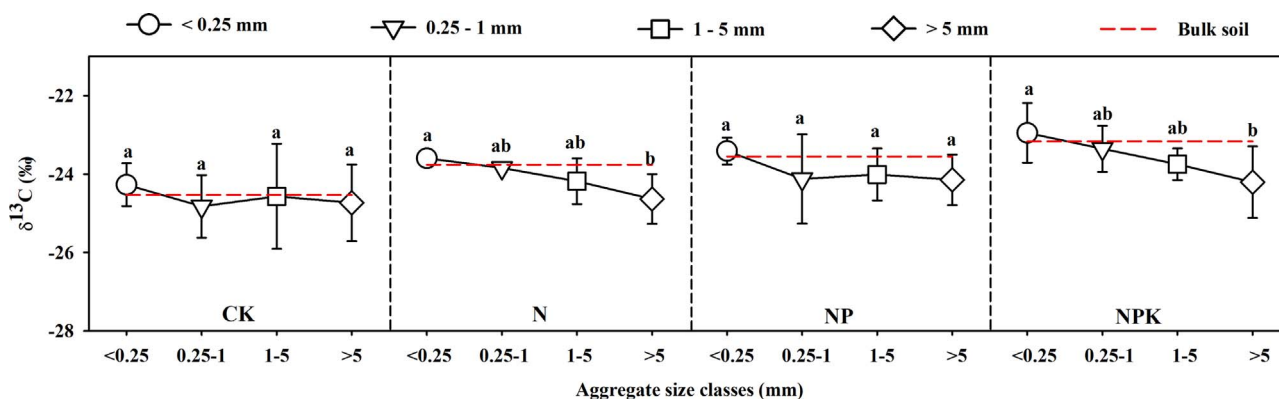


Fig. 3. Aggregate-associated δ¹³C values indicated long-term fertilization and control (CK) treatments.

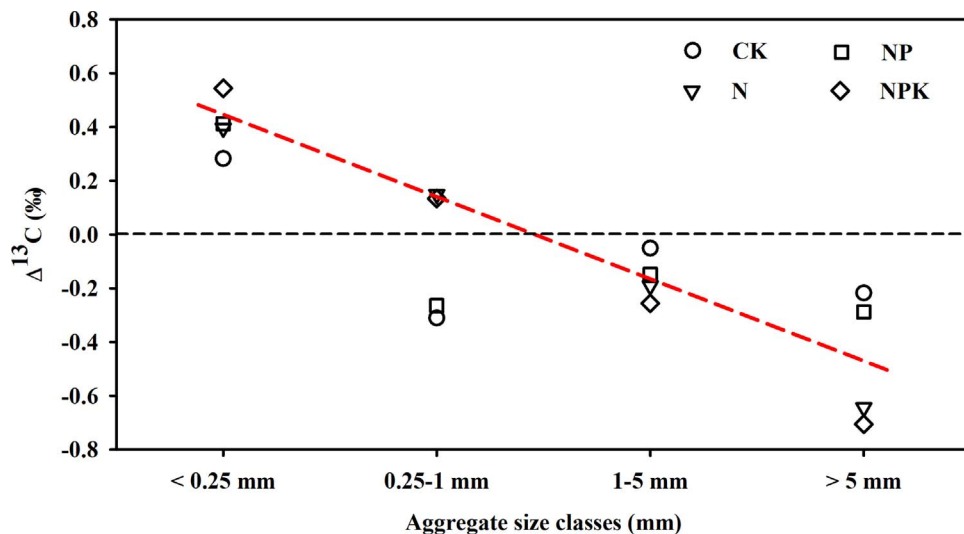


Fig. 4. Changes in the isotopic composition of soil aggregates expressed using Δ¹³C values, calculated relative to the δ¹³C values of bulk soil.

due to increases in plant biomass. However, Su et al. (2006) and Yan et al. (2007) found that inorganic fertilizer application had at most small effects on SOM concentrations, although it increased crop yields, and (Qiu et al., 2016) even found that it promoted the decomposition of SOC.

Moreover, different aggregate size fractions have different SOM concentrations and turnover times (Hontoria et al., 2016). Macro-aggregates generally contain more SOC and more labile young C than micro-aggregates, hence rates of C turnover are generally higher in macro-aggregates (Bimuller et al., 2016). Accordingly, we found that macro-aggregates (> 0.25 mm) contained significantly more SOC than micro-aggregates (< 0.25 mm) (Fig. 2). Puget et al. (2005) also found

that macro-aggregates had higher C concentrations because they contained younger SOM with lower rates of decomposition, and only macro-aggregates (> 0.25 mm) were C-rich. The tendency for SOM to be most abundant in macro-aggregates may be partly due to the ability of microorganisms and fungal hyphae to bind soil particles and organic residues (Six et al., 2000).

The input of organic matter (primary production) to the soil ecosystem is an important factor influencing the C isotope composition of SOM (Peri et al., 2012). Both crops in rice-wheat rotations are typical C₃ plants, which have an average δ¹³C value of -27‰. The soil δ¹³C values we obtained (-24.82 to -22.95‰; Fig. 2) are generally similar to those previously reported for systems dominated by C₃ plants, and

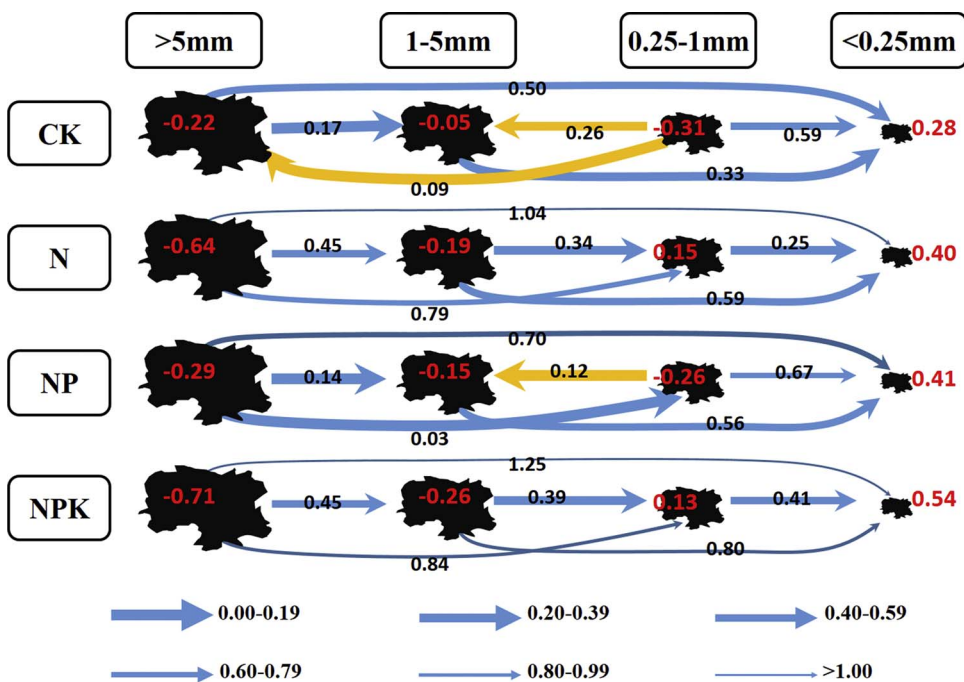


Fig. 5. Schematic diagram of C flows in the system of aggregates under indicated long-term fertilization and control (CK) treatments. The values, superimposed on the images representing the aggregate fractions, are $\Delta^{13}\text{C}$ values indicating the difference between the $\delta^{13}\text{C}$ of the relevant fraction and that of the bulk soil. The arrows show the directions of C flows based on the differences between the aggregate fractions' $\Delta^{13}\text{C}$ values; their widths reflect the relative probability of C flow between the aggregate fractions connected by the corresponding arrows, and the numbers along the arrows show the relative probability of a C flow between the connected aggregate fractions in the studied soils. Smaller $\Delta^{13}\text{C}$ differences correspond to higher probabilities of C flux. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

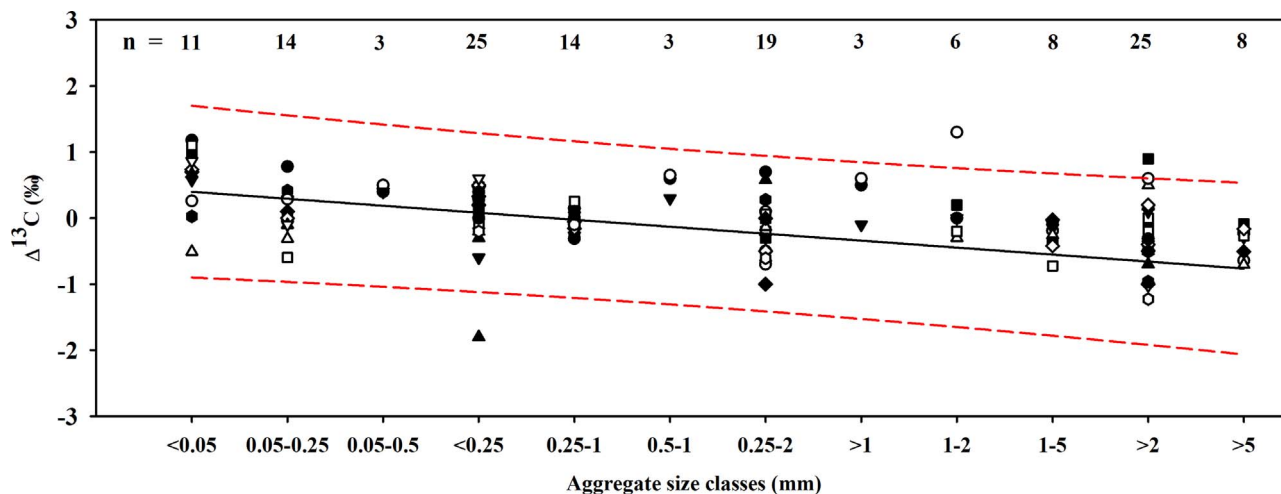


Fig. 6. $\Delta^{13}\text{C}$ values of soil aggregates (relative to $\delta^{13}\text{C}$ values of bulk soil) vs. aggregate size classes for various soils. According to data from Cheng et al. (2011), Dou et al., (2013, 2016a, 2016b), Fan et al., (2010), Gunina and Kuzyakov, (2014), John et al., (2005), Tan et al., (2013), Wang et al., (2015), Yamashita et al., (2006).

appreciably higher than average reported values for rice and wheat (-26.34 and -28.50‰, respectively), especially under the inorganic fertilization treatments. Similarly, Cheng et al. (2013) determined soil $\delta^{13}\text{C}$ values of -20.54‰ and -20.17‰ at depths of 0–10 and 10–30 cm, respectively. Both of these values are higher than the $\delta^{13}\text{C}$ values (average of -26.18‰) for the crops planted on these soils. This was presumably at least partly because microbial organic matter decomposition causes heavy isotope (^{13}C) enrichment in the soil as microbes preferentially utilize the lighter ^{12}C in metabolic and respiratory processes, and thus preferentially release ^{12}C (Hyodo et al., 2010; Werth and Kuzyakov, 2010).

Furthermore, the $\delta^{13}\text{C}$ signatures of SOM can change substantially in response to changes in the availability of soil nutrients (Dou et al., 2016a). For example, our plots subjected to inorganic fertilization treatments had significantly higher soil $\delta^{13}\text{C}$ values than CK plots (Fig. 1). A growing body of evidence suggests that such enrichment is due to the incorporation of nitrogen into SOM, because nitrogen addition promotes the turnover of SOM (Khan et al., 2007), and any

mechanism that increases numbers of C turnover cycles will increase losses of the lighter ^{12}C , leaving higher proportions of ^{13}C behind (Werth and Kuzyakov, 2010). Thus, increases in a soil's ^{13}C -enrichment (reflected in increases in $\delta^{13}\text{C}$ values) indicate increases in rates of C-cycling and subsequent C losses (De Clercq et al., 2015).

Differences in $\delta^{13}\text{C}$ values in aggregates also likely reflect preferential losses of ^{12}C during decomposition of SOM, leaving higher concentrations of ^{13}C in microorganisms and their residues (Dou et al., 2013; Werth and Kuzyakov, 2010). Therefore, for a given ecosystem, lower $\delta^{13}\text{C}$ values have been linked to more recent litter inputs, and higher $\delta^{13}\text{C}$ values to older organic matter and increasing SOM humification (Cheng et al., 2013; Wang et al., 2015) (Werth and Kuzyakov, 2010). Our stable isotopic analysis showed that micro-aggregates (< 0.25 mm) had higher $\delta^{13}\text{C}$ values than macro-aggregates (> 0.25 mm) (Fig. 3), corroborating a hypothesis that the C in micro-aggregates has generally persisted longer (or at least been subjected to more decomposition) than the C in macro-aggregates (Alvaro-Fuentes et al., 2008; Six et al., 2000).

Soil aggregate turnover processes are thus likely to be at least partly responsible for the higher $\delta^{13}\text{C}$ values of micro-aggregates (De Clercq et al., 2015; Dou et al., 2016a). To test this hypothesis, we calculated differences between $\delta^{13}\text{C}$ values for soil aggregates and bulk soil ($\Delta^{13}\text{C}$) reported in other studies and related them to the aggregate size classes (Fig. 6). Across a broad range of soil types, $\Delta^{13}\text{C}$ values tended to increase (albeit not always significantly) from large to small aggregates. This heavy isotope enrichment is consistent with isotopic fractionation by the progressive degradation of organic matter and preferential consumption of light isotopes (^{12}C) by microbes (Hyodo et al., 2010), and the aggregate hierarchy hypothesis that macro-aggregates form (in some soils) from the binding of C-rich micro-aggregates (Six et al., 2000).

To predict the steps whereby SOM is sequestered in soil aggregates, we created the scheme shown in Fig. 5. This is based on indications of the ‘sources’ and ‘products’ of organic matter in soil aggregates provided by our measured $\delta^{13}\text{C}$ values, and two assumptions: C isotope fractionation is a function of soil aggregate turnover (Figs. 1 and 6), and C flows are more likely between soil aggregate fractions with similar $\Delta^{13}\text{C}$ values. Generally, the $\Delta^{13}\text{C}$ values increased with decreasing aggregate size (Fig. 4). Macro-aggregates (> 0.25 mm, which had the lowest $\Delta^{13}\text{C}$ values) appeared to be the primary ‘sources’ of the C flows in the aggregate system, while micro-aggregates (< 0.25 mm, which had higher $\Delta^{13}\text{C}$ values) appeared to be the final products. The C flows presented in our scheme are consistent with the embryonic development model proposed by Six and Paustian (2014) as the dominant mechanism for the formation of soil aggregates; both predict that macro-aggregates are formed around fresh residues which then become intra-aggregate particulate organic matter. Thus, macro-aggregates (> 0.25 mm) represent the point at which organic matter enters the system of aggregates, and micro-aggregates (< 0.25 mm) the final stage in the organic matter’s transformation. Studies on the distribution of litter across aggregate size classes have also shown that plant residues are first associated with macro-aggregates and then redistributed to micro-aggregates, explaining the greater degree of SOM decomposition in the latter (Angers et al., 1997).

Our approach builds on these efforts by showing the complexity of C transformation processes, including the occurrence of multidirectional fluxes of C between differently sized aggregates. The N, NP and NPK treatments have shown a low incorporation of C within the aggregate classes compared to CK treatment. The cause of this phenomenon may be associated with the nitrogen addition of the fertilizer application that leads to alter SOM C/N ratio (Khan et al., 2007). From a soil science perspective, the SOM C/N ratio is one important factor related to C isotope discrimination in microbial processes (including SOM decomposition and respiration) (Bowling et al., 2008). Higher inorganic N levels may favour soil-labile SOM decomposition (Huang et al., 2009), in various ways, but particularly by promoting increases in soil respiration (Liu et al., 2016). Thus, treatment with chemical fertilizers (N, NP and NPK) should theoretically raise proportions of recalcitrant compounds, which (like ^{13}C enrichment) may retard C flows in the system of aggregates (Gunina and Kuzyakov, 2014).

5. Conclusions

We found that aggregate-associated SOC concentrations and $\delta^{13}\text{C}$ values ranged from 19.0 to 23.1 g kg⁻¹, and from -24.8 to -23.0‰, respectively, in soils under rice-wheat rotations subjected to various fertilization treatments and a fertilizer-free control (CK) treatment. Compared to the CK treatment, applications of inorganic fertilizers resulted in higher soil SOC concentrations and $\delta^{13}\text{C}$ values in all aggregate size fractions. Moreover, micro-aggregates (< 0.25 mm) had higher $\delta^{13}\text{C}$ values than macro-aggregates (> 0.25 mm). Hence, micro-aggregates (< 0.25 mm) exhibited ^{13}C enrichment. In addition, $\delta^{13}\text{C}$ values of the soil aggregate fractions and fractionation of ^{13}C between the different aggregate size classes clearly demonstrated the occurrence

of ongoing C fluxes from macro (> 0.25 mm) to micro-aggregates (< 0.25 mm). However, the treatments with mineral fertilizers appeared to retard C fluxes among the aggregate classes, relative to those observed under the CK treatment. Stable C isotope ratios may thus provide a measure of the relative openness of C cycling in ecosystems.

Acknowledgments

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