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

Yi Liu[a,b], Cheng Hu[c], Wei Hu[d], Li Wang[a], Zhiguo Li[a,*], Junfeng Pan[a], Fang Chen[a,*]

[a] Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China
[b] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resource, Yangling 712100, China
[c] Institute of Plant Protection and Soil Fertilizer, Hubei Academy of Agricultural Sciences, Wuhan 430064, China
[d] New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch 8140, New Zealand

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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 $\delta^{13}C$ 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 $\delta^{13}C$ values were higher under treatments including inorganic fertilization than under the control (fertilizer-free, CK) treatment. In addition, $\delta^{13}C$ 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 (Soinne 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

* Corresponding authors.
*E-mail addresses:* lyle3521@126.com (Y. Liu), lzg360@wbgcas.cn (Z. Li), cf87510433@163.com (F. Chen).

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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, $\delta^{13}C$ 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 $\delta^{13}C$ analyses provides a powerful way of quantifying SOM dynamics in response to changes that alter the relative local dominance of $C_4$ ($\delta^{13}C$, −9 to −14‰) and $C_3$ ($\delta^{13}C$, −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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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

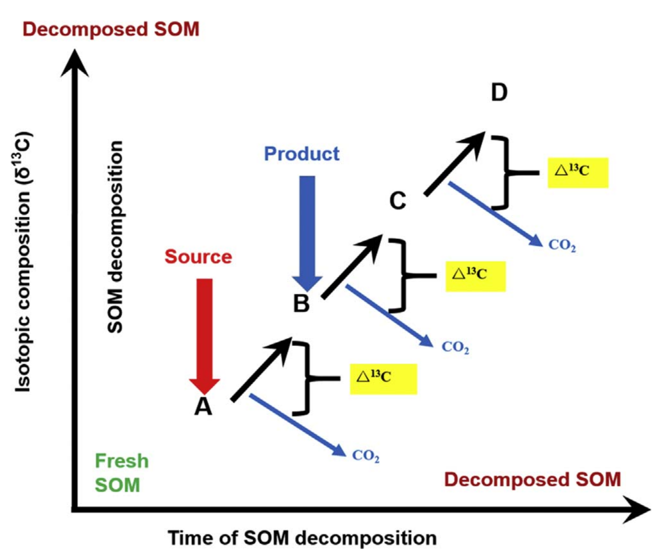


**Fig. 1.** $^{13}C$ enrichment reflects the formation steps of SOM pools.

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°28′N, 114°25′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 m × 5 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 kg N $ha^{-1}$ $yr^{-1}$, 75 kg $P_2O_5$ $ha^{-1}$ $yr^{-1}$, 150 kg $K_2O$ $ha^{-1}$ $yr^{-1}$, and 22,500 kg manure $ha^{-1}$ $yr^{-1}$, 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 mm, 1–5 mm, 0.25–1 mm, and < 0.25 mm. After the final sieving cycle, sub-samples of the four