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Pathways of litter C by formation of aggregates and SOM density fractions: Implications from ^{13}C natural abundance

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ABSTRACT

Aggregate formation is a key process of soil development, which promotes carbon (C) stabilization by hindering decomposition of particulate organic matter (POM) and its interactions with mineral particles. C stabilization processes lead to ^{13}C fractionation and consequently to various $\delta^{13}C$ values of soil organic matter (SOM) fractions. Differences in $\delta^{13}C$ within the aggregates and fractions may have two reasons: 1) preferential stabilization of organic compounds with light or heavy $\delta^{13}C$ and/or 2) stabilization of organic materials after passing one or more microbial utilization cycles, leading to heavier $\delta^{13}C$ in remaining C. We hypothesized that: 1) ^{13}C enrichment between the SOM fractions corresponds to successive steps of SOM formation; 2) ^{13}C fractionation (but not the $\delta^{13}C$ signature) depends mainly on the transformation steps and not on the C precursors. Consequently, minimal differences between $\Delta^{13}C$ of SOM fractions between various ecosystems correspond to maximal probability of the SOM formation pathways.

We tested these hypotheses on three soils formed from cover loam during 45 years of growth of coniferous or deciduous forests or arable crops. Organic C pools in large macroaggregates, small macroaggregates, and microaggregates were fractionated sequentially for four density fractions to obtain free POM with $\rho < 1.6$ g cm^{-3}, occluded POM with two densities ($\rho < 1.6$ and 1.6–2.0 g cm^{-3}), and mineral fraction ($\rho > 2.0$ g cm^{-3}).

The density fractions were ^{13}C enriched in the order: free POM < light occluded POM < heavy occluded POM < mineral fraction. This, as well as their C/N ratios confirmed that free POM was close to initial plant material, whereas the mineral fraction was the most microbially processed. To evaluate the successive steps of SOM formation, the $\Delta^{13}C$ values between $\delta^{13}C$ of SOM fractions and $\delta^{13}C$ of bulk SOM were calculated. The $\Delta^{13}C$ indicated that, parallel with biochemical transformations, the physical disintegration strongly contributed to the formation of free and occluded light POM. In contrast, biochemical transformations were more important than physical disintegration for formation of heavy occluded POM from light occluded POM. This was confirmed by review of 70 $\Delta^{13}C$ values from other studies analyzed $\Delta^{13}C$ depending on the density of SOM fractions. Accordingly, the successive steps of SOM formation were: $fLF_{<1.6} = oLF_{<1.6} \rightarrow oDF_{1.6-2.0} = MF_{>2.0}$. The obtained steps of C stabilization were independent on the initial precursors (litter of coniferous forest, deciduous forest or grasses).

To test the second hypothesis, we proposed an extended scheme of C flows between the 3 aggregate size classes and 4 SOM fractions. $\Delta^{13}C$ enrichment of the SOM fractions showed that the main direction of C flows within the aggregates and SOM fractions was from the macroaggregate-free POM to the mineral microaggregate fraction. This confirmed the earlier concept of SOM turnover in aggregates, but for the first time quantified the C flows within the aggregates and SOM density fractions based on $\delta^{13}C$ values. So, the new ^{13}C natural abundance approach is suitable for analysis of C pathways by SOM formation under *steady state* without ^{13}C or ^{14}C labeling.

1. Introduction

Aggregate formation is one of the main soil-forming processes, distinguishing soils from their parent materials. Plant residues and

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root exudates are assumed to be the main drivers of aggregation in the most models of soil structure development (Tisdall and Oades, 1982; Oades, 1984; Six et al., 1999). Nonetheless, the general theoretical principles and hierarchy of aggregate formation were seldom tested experimentally (Angers et al., 1997), mainly because suitable approaches were limited.

Only few methods enable determining the formation of aggregate size classes and the matter exchange between them: microscopic observation of previously disrupted aggregates; labeling of aggregates with radionuclides such as ^{60}Co (Toth and Alderfer, 1960), ^{56}Fe (Wooldridge, 1965) or with the rare earth element oxides La_2O_3, Pr_6O_{11}, Nd_2O_3, Sm_2O_3, and Gd_2O_3 (Zhang et al., 2001); and application of ^{13}C and ^{14}C isotopes (Majumder and Kuzyakov, 2010).

Transmission electron microscopy has been used to analyze the aggregate formation starting from individual particles to macroaggregates (Tisdall and Oades, 1982). Although, this approach distinguishes the particle components and their size, it does not explain mechanisms of aggregate formation or matter exchange between the size classes.

Labeling aggregates with radionuclides, mentioned above, or with ceramic/glass spheres enabled tracing the translocation of particles within and between aggregate size classes (Plante et al., 1999) and to investigate aggregate dynamics (Plante et al., 2002; Plante and McGill, 2002). Accordingly, the mean residence time of macroaggregates ranged from 5 to 33 days (Plante and McGill, 2002). These methods however, are limited for mineral particles and do not allow analyzing the role of aggregates in C stabilization.

To link the aggregate dynamics with the organic substances, ^{13}C and ^{14}C isotopes have been applied. For example, the primary formation of macroaggregates around fresh plant residues was proved using ^{13}C labeled wheat straw (Angers et al., 1997). Moreover, during long-term incubation of ^{13}C labeled plant residues in soil, the ^{13}C enrichment of macroaggregates decreased, whereas microaggregates increased (Angers et al., 1997). These results, however, focused on the transformation of organic substances during aggregate formation and do not explain aggregate formation *per se*. Thus, even though information on the sequence of aggregate formation and aggregate turnover exists, no clear evidence is available about what the sources are and what the products of organic materials in various aggregate size classes are.

Additional difficulties in studying aggregate formation and its role in C stabilization arise due to the heterogeneity of soil organic matter (SOM) in aggregate size classes (Besnard et al., 1996; Six et al., 1998; Yamashita et al., 2006). Free particulate organic matter (fPOM), light and heavy occluded POM (oPOM), and mineral-associated organic matter (OM) have been distinguished from the aggregates by physical (Yamashita et al., 2006) and chemical fractionation methods (Stewart et al., 2008). This means that aggregates can include SOM of various origin, composition and degree of microbial degradation, i.e. as "products" or as "sources". The quality of SOM fractions has been analyzed based on the C/N ratios and NMR spectroscopy. These approaches have shown the following order of SOM fraction formation: free POM → heavy occluded POM → light occluded POM → mineral fraction (Golchin et al., 1998). Based on δ^{13}C analysis, however, the following formation sequence has been proposed: free POM → light occluded POM → heavy occluded POM → mineral fraction (Baisden and Amundson, 2002). Moreover, the simultaneous presence of light occluded and heavy occluded POM as one pool has been noted based on ^{14}C dating (Baisden and Amundson, 2002). There is therefore no unitary concept of SOM fraction formation and especially of the C flows between the SOM fractions separated from the aggregate size classes.

A promising approach to studying the sources of organic materials and C flows within and between the aggregates is using natural differences in the stable isotope composition of aggregates and SOM fractions. The differences in stable C isotope composition (^{13}C/^{12}C) may have two reasons (Werth and Kuzyakov, 2010): 1) preferential stabilization of substrates with light or heavy δ^{13}C and/or 2) stabilization of organic materials after passing one or more microbial utilization cycles, leading to heavier δ^{13}C in remaining organic matter (because of release of CO_2 with light δ^{13}C). The first mechanism – stabilization of preferential substrates with light or heavy δ^{13}C – would occur mainly by chemical sorption of specific groups of organic substances, e.g. with light (lipids, phenols, lignins) or heavy δ^{13}C (cellulose, amino acids, hemicellulose) (Sollins et al., 2006; von Luetzow et al., 2006). This would lead to divergent δ^{13}C signatures in various aggregate size classes and density fractions and would not be connected with steps of organic C utilization by microorganisms. Importantly, however, microbial uptake and utilization of organic materials outcompete all physicochemical processes in soil (Fischer et al., 2010). Therefore, we assume the dominance of the second mechanism – stabilization of organic materials after passing one or more microbial utilization cycles. In contrast to the first mechanism, the second one generally leads to heavier δ^{13}C in remaining organic matter in soil. This is connected with the preferential decomposition of light C to CO_2 and remaining heavier C in microorganisms and their residues (Werth and Kuzyakov, 2010) that are a key SOM source (Kindler et al., 2006; Miltner et al., 2012). The δ^{13}C increase by this mechanism is proportional to the number of cycles in which C is passed through the microorganisms before being stabilized in SOM.

We combined aggregate's size and density fractionations with natural differences in stable C isotope composition to investigate possible flows of C between and within the aggregate size classes. Using the δ^{13}C approach, we hypothesized: 1) During SOM formation, the C remaining in soil by litter decomposition will be enriched by ^{13}C (as the light C will be evolved as CO_2). This leads to a δ^{13}C increase both: of total SOM compared to plant residues, as well as of SOM fractions formed within individual steps of SOM formation. Consequently, the ^{13}C enrichment (=δ^{13}C less negative) of one SOM fraction compared to the other shows that the more enriched is the product of the less enriched one. Based on these ^{13}C enrichments between the fractions, it is possible to predict the formation steps of SOM fractions. 2) ^{13}C fractionation within individual steps of the formation of SOM pools and fractions is relatively constant, independent of the ecosystem type. Thus, the ^{13}C fractionation *per se* (Δ^{13}C, but not the δ^{13}C of the SOM fraction) depends mainly on the transformation steps of the fraction but not on the δ^{13}C of the precursors. This is true for continuous C input. Consequently, minimal differences between Δ^{13}C of SOM fractions in various ecosystems correspond to the maximal probability of the SOM formation pathways.

2. Materials and methods

2.1. Experimental set-up and soil sampling

Soils from large open lysimeters located within the Moscow State Lomonosov-University area were used. The installation of the lysimeters has been described in detail elsewhere (Vinnik and Bolyshev, 1972). Briefly, the large lysimeters were installed in 1965 and included 20 plots of 3 × 3 m (area 9 m^2) and depth of 1.5 m. Each lysimeter was filled with carbonate-free loam, which originated from the cover loams of Valday (corresponds to Würm/Weichsel/Wisconsin) glaciation, collected in the north of the Moscow region. The texture of this parent material was silty loam with the following particle distribution: 3.65% sand, 65.3% silt, and 28.6% clay (Vinnik and Bolyshev, 1972). The predominant minerals of the clay fraction were smectites (56.1%), illites (34.0%), and