



Prediction of successive steps of SOM formation in aggregates and density fractions based on the ^{13}C natural abundance

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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 $\delta^{13}\text{C}$ values of soil organic matter (SOM) fractions. Differences in $\delta^{13}\text{C}$ within the aggregates and fractions may have two reasons: 1) preferential stabilization of organic compounds with light or heavy $\delta^{13}\text{C}$ and/or 2) stabilization of organic materials after passing one or more microbial utilization cycles, leading to heavier $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ signature) depends mainly on the transformation steps and not on the C precursors. Consequently, minimal differences between $\Delta^{13}\text{C}$ 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 $\rho < 1.6 \text{ g cm}^{-3}$, occluded POM with two densities ($\rho < 1.6$ and $1.6\text{-}2.0 \text{ g cm}^{-3}$), and mineral fraction ($\rho > 2.0 \text{ g cm}^{-3}$).

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 $\Delta^{13}\text{C}$ values between $\delta^{13}\text{C}$ of SOM fractions and $\delta^{13}\text{C}$ of bulk SOM were calculated. The $\Delta^{13}\text{C}$ 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 $\Delta^{13}\text{C}$ values from other studies analyzed $\Delta^{13}\text{C}$ depending on the density of SOM fractions. Accordingly, the successive steps of SOM formation were: $\text{fLF}_{<1.6} = \text{oLF}_{<1.6} \rightarrow \text{oDF}_{1.6-2.0} = \text{MF}_{>2.0}$. 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. $\Delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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.