



Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison

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ABSTRACT

Fractionation of soil organic carbon (SOC) is crucial for mechanistic understanding and modeling of soil organic matter decomposition and stabilization processes. It is often aimed at separating the bulk SOC into fractions with varying turnover rates, but a comprehensive comparison of methods to achieve this is lacking. In this study, a total of 20 different SOC fractionation methods were tested by participating laboratories for their suitability to isolate fractions with varying turnover rates, using agricultural soils from three experimental sites with vegetation change from C3 to C4 22–36 years ago. Enrichment of C4-derived carbon was traced and used as a proxy for turnover rates in the fractions. Methods that apply a combination of physical (density, size) and chemical (oxidation, extraction) fractionation were identified as most effective in separating SOC into fractions with distinct turnover rates. Coarse light SOC separated by density fractionation was the most C4-carbon enriched fraction, while oxidation-resistant SOC left after extraction with NaOCl was the least C4-carbon enriched fraction. Surprisingly, even after 36 years of C4 crop cultivation in a temperate climate, no method was able to isolate a fraction with more than 76% turnover, which challenges the link to the most active plant-derived

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carbon pools in models. Particles with density $> 2.8 \text{ g cm}^{-3}$ showed similar C4-carbon enrichment as oxidation-resistant SOC, highlighting the importance of sesquioxides for SOC stabilization. The importance of clay and silt-sized particles ($< 50 \mu\text{m}$) for SOC stabilization was also confirmed. Particle size fractionation significantly outperformed aggregate size fractionation, due to the fact that larger aggregates contain smaller aggregates and organic matter particles of various sizes with different turnover rates. An evaluation scheme comprising different criteria was used to identify the most suitable methods for isolating fractions with distinct turnover rates, and potential benefits and trade-offs associated with a specific choice. Our findings can be of great help to select the appropriate method(s) for fractionation of agricultural soils.

1. Introduction

Fractionation of soils to gain a better understanding of element cycling within a ‘black box’ system has a long history. For soil organic carbon (SOC), the techniques applied have evolved according to the current understanding of carbon (C) stabilization and turnover in soils. The traditional view of SOC stabilization was that dead plant material becomes ‘humified’, a process which involves secondary synthesis of ‘humic substances’ that become chemically stabilized against microbial decay (Stevenson, 1994; Burdon, 2001). In this approach, SOC is characterized using alkaline extraction, isolating ‘humic acid’, ‘fulvic acid’ and ‘humin’. The first report of such a procedure dates back to 1786 (Achard, 1786). However, it has been pointed out that this concept may not be completely applicable to C turnover processes in soils, since: i) there is no evidence that synthesized ‘humic substances’ actually exist under natural conditions (Lehmann and Kleber, 2015) and ii) there is evidence that the availability of a substrate for degraders is more important for their persistence in the soil than its chemical recalcitrance (Kögel-Knabner, 2002; Deneff et al., 2009; Dungait et al., 2012). Nonetheless, different views on the fate of organic matter in soils still persist to date (Lützwon et al., 2006; Nebbioso and Piccolo, 2011; Lehmann and Kleber, 2015), owing to: i) the complex nature of SOC and soil organic matter in general, ii) the diversity of potential stabilization mechanisms, and iii) the limited ability to study organic matter molecules in the soil at sufficient temporal and spatial resolution.

The diversity of mechanistic theories regarding turnover, stabilization, and formation of SOC and different goals in measuring SOC and its pools are reflected in the wide range of fractionation methods currently applied (von Lützwon et al., 2007). While some methods are designed purely to assess turnover, others might reveal mechanistic details of how SOC is formed and interacts with the soil matrix. Each method has its own rationale and has a more or less extensive community of users and supporters. The majority of the more recently developed SOC fractionation methods use physical fractionation approaches, such as separation of particles by density and/or size, with or without previous dispersion to break aggregate structures (Golchin et al., 1994b; Six et al., 2002a; Sollins et al., 2006). This approach emphasizes the importance of the fundamental interactions between organic and inorganic soil components in the turnover of organic matter (Christensen, 2001). Physical protection by aggregates and by organo-mineral complexes (especially in the silt and clay-sized fractions) is acknowledged to be crucial for SOC stabilization (Six et al., 2002b; Eusterhues et al., 2003; Kaiser and Guggenberger, 2003). Chemical fractionation, which is usually done with (hot) water, alkali, acid, or organic solvents (Hayes and Clapp, 2001) can be roughly divided into extraction and hydrolysis, chemical destruction of the mineral phase as well as oxidative degradation of organic matter. Extraction is done to isolate specific compounds of varying chemical recalcitrance, which is based on the concept that chemical recalcitrance is of major importance for organic matter stability (Olk and Gregorich, 2006). Extraction with water is applied to isolate dissolved organic carbon (DOC), a highly mobile C fraction (Michalzik et al., 2003). Chemical destruction of the mineral phase or sesquioxides (Mikutta et al., 2005a) is done to release and subsequently characterize or quantify complexed organic matter, which is generally found to have a much higher turnover time as compared to

uncomplexed organic matter (Torn et al., 1997). Chemical oxidation is performed to mimic strong enzymatic decay (Helfrich et al., 2007; von Lützwon et al., 2007). The oxidation-resistant fraction is then subsequently linked to certain soil properties that might be responsible for biological stability of organic matter, such as the content of Al-/Fe-oxides (Mikutta et al., 2005b). Since both approaches, physical and chemical fractionation, may have their shortcomings regarding the isolation of meaningful, distinct functional pools, combined approaches of chemical and physical fractionation have emerged (Plante et al., 2006; Zimmermann et al., 2007b). In these, size or density separation is often used to isolate mineral-associated SOC, which is then chemically treated to separate an oxidation-resistant fraction. Frequently used oxidation agents are hydrogen peroxide (H_2O_2) (Eusterhues et al., 2005), sodium hypochlorite (NaOCl) (Kaiser and Guggenberger, 2003), and sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$) (Eusterhues et al., 2005; Helfrich et al., 2007). However, critics point out that chemical and biological oxidation are not the same and are thus driven by different SOC properties (Leifeld and von Lützwon, 2014; Lutfalla et al., 2014). Similar criticisms have been made of thermal oxidation methods, which are believed to derive fractions differing in biological stability via stepwise thermal oxidation (Helfrich et al., 2010; Schiedung et al., 2017). An alternative to chemical treatment of size fractions is the use of spectral methods (e.g., nuclear magnetic resonance) to estimate the resistant carbon within size fractions (Guggenberger et al., 1994; Six et al., 2001; Baldock et al., 2013). However, this method does not allow for separation and isolation of the final fraction components.

The existing SOC fractionation methods have been developed for different ecosystems and soils and to answer different research questions. However, they are frequently used for one single purpose, which is to isolate SOC pools that are as homogeneous and distinct in their turnover rates as possible (Trumbore and Zheng, 2016). This is challenging, since SOC comprises a wide range of different components with ages ranging from hours to millennia (Trumbore et al., 1989; Paul et al., 1997). Fraction-derived C pools are used to develop, initialize, and validate mechanistic models of SOC turnover (Segoli et al., 2013), and to characterize SOC regarding its formation and stability (Baldock et al., 2013; Cotrufo et al., 2015), in undisturbed conditions or following environmental perturbation. Several studies have been able to link empirically isolated fractions to the theoretical, kinetically delineated components of SOC (i.e., pools) of the RothC model (Balesdent, 1996; Skjemstad et al., 2004; Zimmermann et al., 2007b). However, an empirical link, i.e. comparable distribution of carbon in fractions and pools, does not necessarily mean a functional link, i.e. that isolated fractions or fraction combinations and model pools have a similar turnover or respond to changes in a similar way (Poeplau and Don, 2014a). To evaluate the mean residence time of a certain fraction or to have a proxy for its turnover rate, either ^{14}C measurements (Marzaioli et al., 2010) or environmental changes (land use, land management, soil temperature) ideally creating a shift in ^{13}C abundance or other biomarker are necessary (Del Galdo et al., 2003; Dondini et al., 2009). However, such an evaluation has not previously been broadly applied across commonly used fractionation methods.

The diversity and large number of fractionation methods hamper quantitative comparisons between studies and model initialization across studied soils. Ideally, all scientists with a common goal would

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