



## Methodological uncertainty in estimating carbon turnover times of soil fractions



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### ABSTRACT

Improving predictions of soil organic carbon (SOC) dynamics by multi-compartment models requires validation of turnover times of different SOC pools. Techniques such as laboratory incubation and isotope analysis have been adopted to estimate C turnover times, yet no studies have systematically compared these techniques and assessed the uncertainties associated with them. Here, we tested whether C turnover times of soil fractions were biased by methodology, and how this changed across soil particle sizes and ecosystems. We identified 52 studies that quantified C turnover times in different soil particles fractionated either according to aggregate size (e.g., macro- versus micro-aggregates) or according to soil texture (e.g., sand versus silt versus clay). C turnover times of these soil fractions were estimated by one of three methods: laboratory incubation (16 studies),  $\delta^{13}\text{C}$  shift due to C<sub>3</sub>–C<sub>4</sub> vegetation change (25 studies), and <sup>14</sup>C dating (19 studies). All methods showed that C turnover times of soil fractions generally increase with decreasing soil particle size. However, estimates of C turnover times within soil fractions differed significantly among methods, with incubation estimating the shortest turnover times and <sup>14</sup>C the longest. The short C turnover times estimated by incubation are likely due to optimal environmental conditions for microbial decomposition existing in these studies, which is often a poor representation of field conditions. The <sup>13</sup>C method can only be used when documenting a successive C<sub>3</sub> versus C<sub>4</sub> vegetation shift. C turnover times estimated by <sup>14</sup>C were systematically higher than those estimated by <sup>13</sup>C, especially for fine soil fractions (i.e., silt and clay). Overall, our findings highlight methodological uncertainties in estimating C turnover times of soil fractions, and correction factors should be explored to account for methodological bias when C turnover times estimated from different methods are used to parameterize soil C models.

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## 1. Introduction

Uncertainty in predicting carbon–climate feedbacks largely stems from poor representation of soil organic carbon (SOC) pools. This is an important consideration as SOC is the largest C pool in terrestrial ecosystems and perturbation of it strongly modulates climate change (Todd-Brown et al., 2013; Koven et al., 2015; Luo et al., 2016). SOC is heterogeneous in terms of composition,

structure, location, and stabilization mechanism (Stevenson, 1994; Sollins et al., 1996; Schmidt et al., 2011; Lehmann and Kleber 2015). Conventional soil C models classify SOC into multiple conceptual pools with different turnover times based on their resistance to microbial decomposition (Jenkinson and Rayner, 1977; Parton et al., 1987). A growing body of research calls for mechanistic representations of SOC processes in Earth System Models, such as protection by physical isolation and mineral sorption (Sulman et al., 2014; Wieder et al., 2014; Tang and Riley, 2015). Therefore, attention should be paid to physically fractionated SOC fractions which are measurable and could represent soil organic matter (SOM) protection mechanisms (Christensen, 1996; von Lütow et al., 2007; Schmidt et al., 2011). Quantifying C turnover times of these soil

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fractions is important for models which integrate explicit mineral protection processes. Until now there has been no consensus on the turnover times of various measurable SOC fractions, due to various methodologies being used to estimate C turnover times.

There are three commonly used methods for assessing SOC turnover times: the laboratory incubation (Christensen, 1987), shifts in natural  $^{13}\text{C}$  abundance after  $\text{C}_3$ – $\text{C}_4$  vegetation change (Balesdent et al., 1987), and  $^{14}\text{C}$  dating (O'Brien and Stout, 1978; Trumbore, 2000). The laboratory incubation directly quantifies biological decomposition of isolated soil fractions under controlled optimal conditions. This method is easy to conduct and has been widely used. In contrast, the  $^{13}\text{C}$  and  $^{14}\text{C}$  methods trace C isotopes during decomposition and stabilization processes to estimate C turnover times (O'Brien and Stout, 1978; Balesdent et al., 1990). The  $^{13}\text{C}$  method can only be used in studies where there are  $\delta^{13}\text{C}$  shifts after years of successive  $\text{C}_3$ – $\text{C}_4$  vegetation change and requires careful C inventory measurements of disturbed and undisturbed soils (Balesdent et al., 1987; Zhang et al., 2015). The  $^{14}\text{C}$  dating method assumes that SOC fractions are at equilibrium between input and decay, and that all the C inputs to soils enter the system at the same time or are constant (Trumbore, 1993; Bruun et al., 2005). These assumptions are often not met in reality and soil  $^{14}\text{C}$  is expensive to measure. Due to these differences in methodology, the three methods likely generate different estimates of SOC turnover times. For instance, the turnover times of mineral associated organic matter (MOM) at 0–10 cm depth has been reported to be 8–43 years using the laboratory incubation method (Rabbi et al., 2014), 53–63 years using the  $^{13}\text{C}$  abundance after  $\text{C}_3$ – $\text{C}_4$  vegetation change (Dalal et al., 2013; Liang et al., 2014), and 52–381 years when using  $^{14}\text{C}$  dating (Budge et al., 2011).

Bulk soil can be separated into soil fractions using the physical, chemical, density, and combined fractionation methods, among which the physical fractionation is able to generate soil fractions with distinct C turnover times (Christensen, 2001; Mikutta et al., 2006; von Lützow et al., 2007). Variation in C turnover times results from different SOC protection mechanisms associated with soil particles as well as inconsistent methods used to estimate C turnover times (Bird et al., 2002; Tan et al., 2013; Yonekura et al., 2013; Beniston et al., 2014). Physically fractionated soil particles are often obtained according to soil aggregate size or soil texture. According to soil aggregates size, C in macro-aggregates (*i.e.*, coarse organic matter, COM) turns over fast, while C in the micro-aggregates (*i.e.*, fine organic matter, FOM) and MOM is supposed to represent C that is primarily protected by physical isolation and mineral matrix, respectively (Six et al., 1998; Baldock and Skjemstad, 2000; von Lützow et al., 2007). According to soil texture, C in the sand fraction has a short turnover time and C associated with the silt and clay fractions is considered as mineral associated OM in models (Parton et al., 1987; Beniston et al., 2014; Tang and Riley, 2015; Wieder et al., 2014). However, we still do not know whether different classifications to separate soil fractions can differentiate their C turnover times.

By synthesizing published studies, we compared C turnover times of physically fractionated soil particles (*i.e.*, COM – FOM – MOM or sand – silt – clay) across ecosystems. We aimed to test whether C turnover times of soil fractions estimated using the laboratory incubation,  $^{13}\text{C}$ , and  $^{14}\text{C}$  were different, and how this changed with soil particle size and ecosystems. We predicted that C turnover times estimated using the laboratory incubation would be shorter than those using the C isotope methods, and that C turnover times based on soil fractions would increase with decreasing particle size.

## 2. Material & methods

### 2.1. Data sources

We searched the literature to find information that included: (1) at least one of the following physically fractionated soil particles as study materials: macro-aggregates (coarse organic matter, COM, 250–2000  $\mu\text{m}$ ), micro-aggregates (fine organic matter, FOM, 20/53/63–250  $\mu\text{m}$ ), MOM (<20/53/63  $\mu\text{m}$ ), sand (20/53/63–2000  $\mu\text{m}$ ), silt (2–20/53/63  $\mu\text{m}$ ), and clay (<2  $\mu\text{m}$ ), and (2)  $\text{CO}_2$  flux measured multiple times over the time course of laboratory incubations, or C turnover rates or times assessed based on the  $\delta^{13}\text{C}$  difference after years of successive  $\text{C}_3$ – $\text{C}_4$  vegetation change, or mean residence times estimated based on  $\Delta^{14}\text{C}$  activity. Detailed information of the selected studies can be found in Table 1 and the supplementary materials (Supplementary Material Table S1). We extracted information on 537 soil fractions from 52 studies around the world (Fig. 1). For all the studies identified, we also gathered the information regarding soil fraction classification used, the coordinates, climate, soil depth, soil type, vegetation at soil sampling sites, and the mass proportion and organic C concentration or content of each soil fraction (Supplementary Material Table S1).

### 2.2. Carbon turnover estimate

For the studies using laboratory incubations to estimate C turnover time, we generated a sub-dataset that included the following data for each soil fraction: the date of measurement, initial organic C concentration or content, and  $\text{CO}_2$  respiration rate or cumulative  $\text{CO}_2$  respiration at each time point. We used the two-pool rather than one-pool exponential decomposition model to estimate C turnover times of soil fractions, because C in soil fractions is not homogeneous and so the two-pool model could more accurately describe decomposition than the one-pool model (Derrien and Amelung, 2011). For comparison, we converted values of cumulative  $\text{CO}_2$  respiration from the original unit ( $\text{mg CO}_2\text{-C g}^{-1}$  sample) to  $\text{mg CO}_2\text{-C per gram of initial organic C concentration of a sample}$ .

$$C_t = f_l \times (1 - e^{-k_l \times t}) + (1 - f_l) \times (1 - e^{-k_s \times t}) \quad (1)$$

was used to estimate C turnover times of soil fractions, where  $C_t$  is the cumulative  $\text{CO}_2$  respired,  $f_l$  is the proportion of labile SOC pool, and  $k_l$  and  $k_s$  are the decomposition constants of labile and stable SOC pools. The turnover times of labile ( $\tau_l$ ) and stable ( $\tau_s$ ) SOC are the reciprocal of  $k_l$  and  $k_s$ , respectively. Given that stable SOC accounts for a large proportion of total SOC and  $\tau_l$  is similar for the studied soil fractions from a variety of ecosystems, using  $\tau_s$  instead of  $\tau_l$  is much more representative to characterize C turnover of the entire SOC. Therefore,  $\tau_s$  values of soil fractions were used to compare whether the three methods provide different C turnover times values. Parameters in the two-pool model were estimated using probabilistic inversion approach (Xu et al., 2006; Weng and Luo, 2011), which was performed using the Metropolis-Hastings (M-H) algorithm – a Markov Chain Monte Carlo (MCMC) technique (Metropolis et al., 1953; Hastings, 1970). Rationale and details about this technique can be seen in Schädel et al. (2013).

For the studies using  $\delta^{13}\text{C}$  after  $\text{C}_3$ – $\text{C}_4$  vegetation change to estimate C turnover time, we collected the data of turnover time ( $\tau$ , year) or decomposition constant ( $k$ ,  $\text{year}^{-1}$ ) for all of the six soil fractions (*i.e.*, COM-FOM-MOM and sand-silt-clay). In the studies where neither  $k$  nor  $\tau$  were reported, we calculated  $k$  using Equation (2) or (3) according to the data available in selected studies.

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