



Sequential combustion separation of soil organic carbon fractions for AMS measurement of ^{14}C and their application in fixation of carbon

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Abstract

A temperature stepped-combustion method for separating soil organic carbon (SOC) fractions and their ^{14}C ages was developed to investigate SOC fixation and stability in soils. After acid-leaching, SOC was sequentially oxidized, and extracted from three temperature intervals: (1) 25–400 °C, (2) 400–600 °C, and (3) 600–900 °C. The acid-soluble carbon and SOC released below 600 °C are labile components, with relatively younger ^{14}C ages, while the SOC released above 600 °C is stable with older ^{14}C ages. We applied this method in a grassland, maize cropland and forest nursery cropland, to assist in understanding the stability of carbon in soils under different land use conditions.

Keywords ^{14}C ages · Labile-stable SOC · Reclaimed cropland · Temperature stepped-combustion

Introduction

The atmospheric concentration of carbon dioxide (CO_2) has increased globally to 410 ppm [1], contributing to increasing temperatures in recent years [2]. The soil organic carbon (SOC) pool is the largest terrestrial organic carbon pool on

earth, and in the wake of global warming, understanding SOC has become a critical concern. SOC is a major sink for atmospheric CO_2 [3–5]. Increasing the carbon content of the world's soils by just a few parts per thousand (0.4%) annually, could potentially remove the amount of CO_2 emitted every year by European Union fossil-fuels combustion [3]. However, global soils (0–100 cm depth) also represent a huge store (1500 Pg) of organic carbon that could potentially release carbon to the atmosphere, especially as atmospheric temperatures increase [6]. Therefore understanding the sequestration and storage of atmospheric CO_2 as carbon in soils is crucial to predicting atmospheric CO_2 concentrations under our changing climate [1]. Central to this understanding is the role of SOC stability, and the efficiency of different land utilization to facilitate the conversion of atmospheric CO_2 to SOC.

Global warming will likely have a greater effect on SOC fractions than on total SOC [7], and a detailed understanding SOC stability and sustainability under variable environmental conditions is currently lacking. SOC fractions are typically divided into two components: labile and stable [8]. Labile SOC has been shown to be a useful measure of soil quality [9]. Labile SOC is composed primarily of simple alkyl groups, containing carbohydrates, cellulose, and proteins [10–12]. Labile SOC is sensitive to climate change [13], land uses [14, 15], tillage style [16], and soil management practices [17–19]. Stable SOC is the residual carbon

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that remains in soils after the labile component has been extracted. Stable SOC is characterized by relatively high aromaticity (e.g. complex polyphenols, lipids), clay mineral organic carbon [20, 21], stable biochar (e.g. charcoal, soot-graphite and lignin) [2, 12], and other stable organic components. These are resistant to biogeochemical degradation [20] and little-affected by short-term land use changes [21].

Due to heterogeneous SOC sources [12, 22], laboratory separation methods have a variety of limitations. Therefore, a number of studies have focused on optimizing the separation of SOC fractions. These include physical methods (e.g. soil aggregates, density, particle size) [9, 20, 23]; chemical methods (e.g. acid hydrolysis [12, 16], water extraction [24], hydrophobic/hydrophilic carbon, KMnO_4 oxidation [17, 19, 25, 26]); and biological methods (e.g. microbial biomass carbon) [27]. Because SOC accumulates from heterogeneous sources, bioturbation and leaching processes continuously supply younger, suspended, colloidal, and soluble organic carbon, while degradation-resistant organic constituents inherited from parent soils and detrital materials may contribute older organic carbon to the SOC pool [28]. Physical separation techniques suffer from a number of shortcomings. For example, Wang et al. [29] separated clays, sieved to $<64 \mu\text{m}$, into two components that were shown to have different thermal stabilities and turnover times (implying different ^{14}C ages). Benbi et al. [30] conducted SOC aggregate analysis using different sieve sizes. Soils passed through successive sieves required 50 min per sieve, with additional time to be oven-dried. Hence, such physical separations can be very time consuming. The same is true of certain biological approaches, for example where mineralizable carbon must be incubated for several days, and for as long as 30 days. Soil microbial biomass carbon can take more than 10 days to analyze, and can require more than 20 g of soil [15, 16, 30, 31]. Conventional chemical separations usually extract organic carbon with acids and bases, or by wet oxidation with KMnO_4 to isolate labile SOC. Such studies yield mixed results, and the difficulty of using these approaches has led some researchers to develop alternative methods, such as pyrolysis-combustion [28], or temperature-stepped combustion [34]. Here we describe a similar approach that includes the use of chemical extractions to separate SOC fractions.

Disparate ^{14}C ages found for different SOC fractions illustrate the heterogeneous character of organic carbon in bulk soils [32]. The ^{14}C ages of SOC components often increase from low, to high combustion temperatures. Low temperature combustion can minimize the contribution of clay-bound carbon in order to extract labile SOC (soil aliphatics) [33], while stable SOC (soil organic compounds of aromatics) can be extracted at higher temperatures [21]. Temperature stepped-combustion, combined with accelerator mass spectrometry (AMS) ^{14}C age measurements has been shown to be useful to date different carbon fractions in sediments [32, 34], but has yet to be applied to soil ecosystem studies. We adopted a stepped-temperature combustion method with ^{14}C AMS measurements of different components, to characterize labile and stable SOC components in soils. Soil types were chosen to reflect different land use histories (grassland, cropland, forest nursery). Our goal is to understand which land use type is most favorable to carbon sequestration and fixation, as reflected in the ages and proportions of SOC pools in the soils. We are especially interested in intensive management areas, typical of densely populated regions such as the Chinese Loess Plateau.

Materials and methods

Sampling and pretreatment

A total of 73 samples from three soil profiles, to 500 cm depth, were collected from an intensively managed ecosystem at Gutun watershed in Yan'an City, Shaanxi Province, China ($36^{\circ}46'39''-37^{\circ}3'34''\text{N}$, $109^{\circ}41'02''-109^{\circ}56'58''\text{E}$) in March 2017. Two cropland profiles were sampled from reclaimed croplands in a gully land, where a forest nursery was cultivated for at least 35 years, and maize had been planted for at least 70 years. These are referred to here as “forest nursery cropland” and “maize cropland”. The third profile is from a grassland. Soil samples were collected from $1 \text{ m} \times 1 \text{ m}$ quadrats (Table 1). In order to fully understand the migration and transformation of SOC in the profile, we refined the sampling depth as much as possible, 10 cm depth intervals in 0–20 cm depth range, and 20 cm intervals in 200–500 cm depth range were sampled by 100 cm^3 cutting

Table 1 Properties and sampling information of soil samples from Gutun watershed

Land use	Texture (%)			SWC (g kg^{-1})	BD (g cm^{-3})
	Clay	Silt	Sand		
Nursery cropland	2.5 ± 0.5	73.9 ± 4.3	23.5 ± 4.5	136.9 ± 29.2	1.20 ± 0.07
Maize cropland	3.5 ± 0.9	78 ± 6.2	18.6 ± 6.8	182.8 ± 25.8	1.62 ± 0.16
Grassland	2.6 ± 0.3	79.9 ± 2.9	17.4 ± 3.1	120.4 ± 17.5	1.32 ± 0.05

SWC, soil water content; BD, soil bulk density

rings, and placed into polyethylene bags (about 500 g) for transport to the laboratory for analysis.

Soil water content (SWC) was determined by the loss of mass after drying at 105 °C [35]. The bulk density (BD) was determined from the volume-mass relationship for each dried core sample [36]. Soil texture was determined by laser diffraction using a Mastersizer3000 (Malvern Instruments, England). The soil samples were air-dried, then sieved through a 2-mm mesh after removing roots and other debris.

Ultra-pure water with a resistivity of 18.2 MΩ from a milli-Q apparatus (Smart2 Pure ultrapure water system integration, TKA, Germany), orthophosphoric acid (purity > 99%, PATINAL, Switzerland), potassium persulfate (purity > 99.99%, Aladdin industrial corporation, China), high purity oxygen (purity > 99.999%), cupric oxide (79.55 MW, Macklin, Shanghai Macklin Biochemical Co., Ltd.), isopropyl alcohol (analytical grade, Guangdong Guanghua Sci-tech Co., Ltd), liquid nitrogen, iron (Sigma Aldrich, USA), zinc free from arsenic (analytical grade) were used in the experiments (Fig. 1).

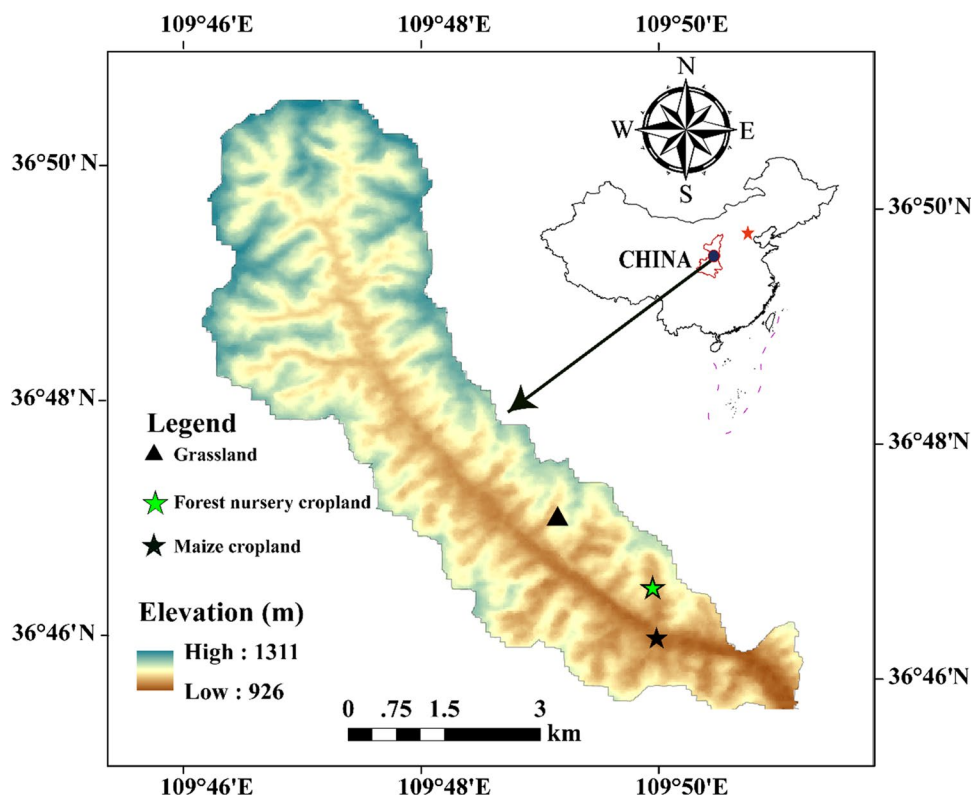
Experimental methods

Separation of soil organic carbon by combustion at different temperatures

A vacuum combustion system with two furnaces (Fig. 2) was established to decompose SOC. The temperatures in the two furnaces were set with a dedicated temperature controller. After every combustion step, the CO₂ released was collected, and converted to graphite for AMS ¹⁴C measurement. Three samples collected from 0 to 10 cm, 280 to 300 cm and 480 to 500 cm depth in a random grassland were used for method development. About 10 g of air-dried soil (< 2 mm) was placed in a 50 ml centrifugation tube, and about 35 ml ultra-pure water was added. The sample mixture was shaken for 30 min at 60 °C to extract water-soluble carbon. The supernatant was separated by centrifuge at 3500 rpm for 15 min. This fraction (usually referred to as water-soluble labile carbon) was excluded from our study because the amount of carbon was insufficient for analysis.

Dilute H₃PO₄ was applied to remove carbonate and extract acid-soluble organic carbon from the soil. The concentration of the H₃PO₄ solution was chosen as 0.35 mol L⁻¹ to insure complete carbonate dissolution. Although dilute H₂SO₄ has been suggested for this purpose [37], we used H₃PO₄ because H₂SO₄ (a strong acid) might oxidize a portion of the organics. The soil samples were leached in H₃PO₄

Fig. 1 Location of the study area and sampling points under maize cropland, forest nursery cropland and grassland in the Gutun watershed



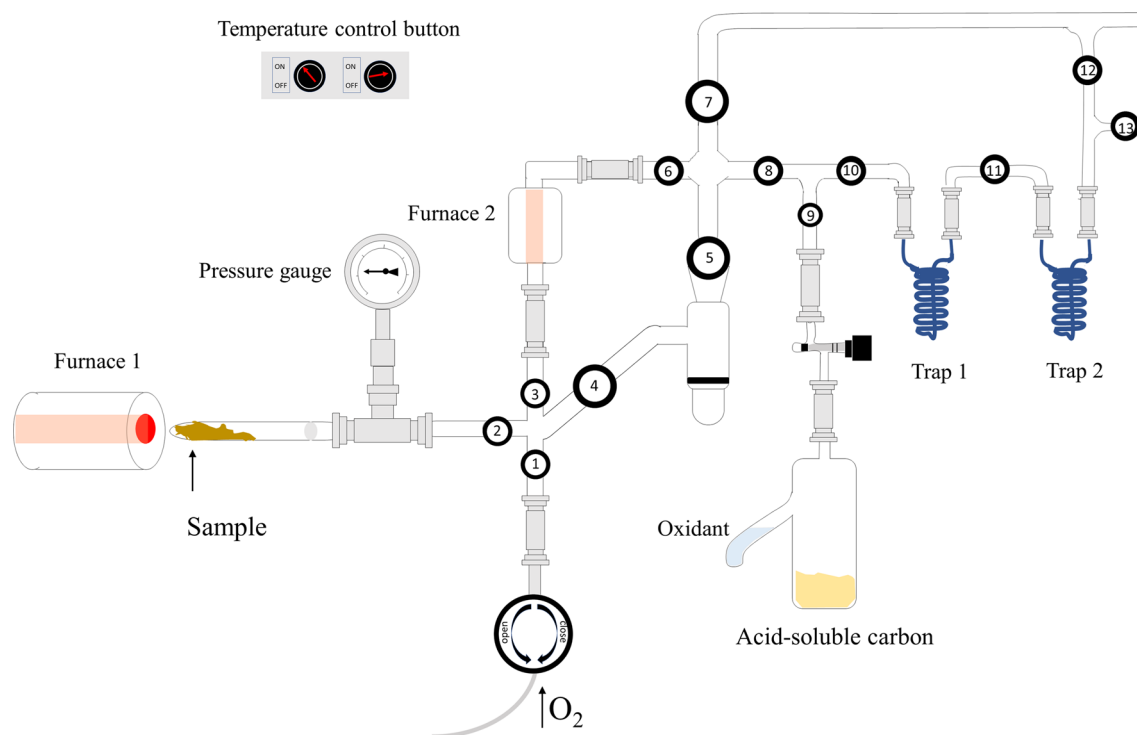


Fig. 2 Schematic diagram of the experiment setup for separation of different species of carbon in soil by sequential combustion

at 60 °C for 2 h, and agitated to facilitate carbonate dissolution [34, 38, 39]. The pH of the leachate was measured using pH paper to ensure that sufficient acid had been added (pH < 5) to completely dissolve the carbonate. The supernatant was separated by centrifugation (15 min, 3500 rpm) and used to measure the amount of acid-soluble SOC. The residue was rinsed with 10–20 ml of ultra-pure water and the rinse water was collected and combined with the supernatant. We transferred the supernatant to a quartz “Y”-shaped reaction flask (Fig. 2) for the separation of the acid-soluble SOC fraction. The acid-soluble SOC fraction was oxidized with 0.15 M potassium persulfate. A volume ratio of 2:3 (persulfate:SOC) was found to be sufficient to decompose all of the acid-soluble SOC. The oxidant was placed in the “Y”-shaped vessel of the reaction flask with the acid-soluble SOC as shown in Fig. 2. The sample was evacuated to $< 10^{-2}$ bar, and the reaction vessel was closed. Then the oxidant was introduced into the reaction flask. The reaction flask was heated to 95 °C for 30 min, after which the CO₂ produced was passed through an isopropyl alcohol-dry ice cold trap to remove water, and the purified gas was collected in a liquid nitrogen trap. We refer to the CO₂ collected in this step as the acid-extractable, or the F1 SOC fraction.

The remaining soil was rinsed until neutral using ultra-pure water, and dried at 60 °C in an oven. It was estimated that at least 0.5 mg carbon in each SOC fraction was sufficient for a ¹⁴C AMS age measurement, which was available

from about 1.5 g dried soil. Each pretreated soil sample was placed in a 9 mm vycor combustion vessel, and evacuated to 10^{-2} bar. Ultra-pure O₂ was introduced into the system to a pressure of 0.3 bar and the sample was heated to the first temperature step (200 °C) for 30 min. The resulting gas, was first passed through a CuO trap to convert any CO to CO₂, then through an isopropyl alcohol-dry ice trap to remove water, and collected in a liquid nitrogen trap (Fig. 2). The CO₂ produced in this step was used to measure the volume of carbon produced. After evacuating the vacuum line and reaction tube, a fresh supply of ultra-pure oxygen was added, and the reaction tube was isolated and heated to the next temperature (400 °C) and held there for 30 min. This time was chosen to completely oxidize the carbon and agrees with previous reports for the oxidation of loess and sediment samples [38]. We made repeated trials with temperature intervals of 25–200 °C, 200–400 °C, 400–600 °C, 600–800 °C, and 800–900 °C and found that negligible carbon was oxidized below 200 °C, and between 800 and 900 °C, as discussed later.

Radiocarbon measurements

Soils from 0 to 10 cm, 20 to 40 cm, 80 to 100 cm, 180 to 200 cm, 280 to 300 cm, 380 to 400 cm and 480 to 500 cm depth were analyzed using the method described above. The SOC content of each temperature fraction was determined

from the CO₂ volume collected during each oxidation step. Each of the CO₂ samples was converted to graphite in the presence of ZnO at 425 °C, using an Fe catalyst at 575 °C [38, 40]. The graphite samples were pressed into aluminum target holders and analyzed with a 3 MV AMS machine at the Xi'an AMS center. The measurement uncertainty (¹⁴C/¹²C) in this instrument is better than 0.3% in a modern sample, with 250,000–300,000 ¹⁴C counts collected. Online δ¹³C measurements were made by the AMS, to correct for machine-related isotopic fractionation. A_{SN} is the measured ¹⁴C/¹²C atomic ratio in the sample normalized to a δ¹³C of –25‰. In our study, the correction of radioactive decay is negligible because of the slight difference between the dates of sample collection and instrument analysis. The measurements were determined relative to the NBS Oxalic acid II (SRM-4990C) modern standard ratio at AD 1950, also normalized to δ¹³C = –25‰ (A_{ON}) [29, 40].

Calculation of ¹⁴C ages and F¹⁴C

The ¹⁴C age of a sample, *t*, is calculated by the normalized sample ¹⁴C activity and normalized oxalic acid activity [41].

$$t = -\tau \ln \left[\frac{(^{14}\text{C}/^{12}\text{C})_{\text{SN}}}{(^{14}\text{C}/^{12}\text{C})_{\text{ON}}} \right] \quad (1)$$

here τ is the mean life of ¹⁴C, 8033 yrs; (¹⁴C/¹²C)_{SN} is the sample normalized activity ratio, (¹⁴C/¹²C)_{ON} is standard normalized activity ratio. The activity (¹⁴C/¹²C)_{ON} depends on the year of measurement (yrs) and is corrected for decay between 1950 and year (yrs) of actual counting date.

$$\text{PMC} = \frac{(^{14}\text{C}/^{12}\text{C})_{\text{SN}}}{(^{14}\text{C}/^{12}\text{C})_{\text{ON}} e^{\lambda(y-1950)}} \times 100\% \quad (2)$$

$$\text{F}^{14}\text{C} = \text{PMC}/100 \quad (3)$$

PMC, percent of modern carbon, Eq. (2), where *y* is the year of oxalic measurement, and $\lambda = 1/8267 \text{ yrs}^{-1}$ is based on the 5730 yrs half-life. F¹⁴C means fraction of modern carbon [11], Eq. (3).

Results and discussion

Soil organic carbon fractions

The acid-soluble SOC content for all samples averaged 0.24 g kg⁻¹ and was the smallest SOC fraction in nearly every instance (0.32, 0.26, and 0.18 times the average values of F2, F3, and F4, respectively), with values as low as 0.01 g kg⁻¹ (Table 2). For these soils, the acid-soluble SOC fraction was estimated to be 3.2–11.9% of the total

SOC from 0 to 500 cm depth. Therefore, although it is a minor fraction, it cannot be ignored.

The percentage of SOC fractions released during each combustion step are shown in Fig. 3. A minor amount of SOC (1–7%) was released between 25 and 200 °C, and between 800 and 900 °C. About half (44–49%) of the SOC was released between 200 and 400 °C. Another 9–31% was released between 400 and 600 °C, and 14 to 40% of SOC was released between 600 and 800 °C. We assume that SOC stability increased with combustion temperature, as this is a measure of thermal stability. Cheng et al. [38] reported that relatively young carbon is normally decomposed at low temperature during combustion. Labile SOC is characterized as a simple alkyl group derived mainly from materials containing carbohydrates and proteins [11]. The exothermic decomposition of labile aliphatic and carboxyl groups can occur at around 300 °C [42]. In contrast, old carbon was released at high temperature, and this SOC fraction is expected to contain complicated poly-phenol group aromatic carbon, as more refractory aromatic carbon is decomposed at higher temperature (450 °C) [42]. To capture these differences in SOC fractions, we chose to focus on three temperature steps: 25–400 °C (F2), 400–600 °C (F3) and 600–900 °C (F4).

The ¹⁴C content of each of these SOC fractions assists in understanding their stability. Hence, we measured ¹⁴C ages of each fraction, and calculated their respective PMC and F¹⁴C values. The ¹⁴C ages measured in the four fractions (F1, F2, F3 and F4), in three depth layers (0–10 cm, 280–300 cm and 480–500 cm), and from the three land use types (forest nursery cropland, maize cropland and grassland) are presented in Fig. 4. The ¹⁴C ages of four fractions in forest nursery cropland, maize cropland and grassland were in the ranges of 1060 ± 23 to 19,195 ± 75 yrs (forest nursery cropland), 346 ± 23 to 49,222 ± 643 yrs (maize cropland), and 945 ± 27 to 23,342 ± 144 yrs (grassland). The ¹⁴C ages of F1, F2 and F3 were distinctly younger than the ages observed for F4 in the same soils, and the F4 ¹⁴C ages were uniformly older than the other fractions (Fig. 4). This is consistent with our assumption that F4 is composed of stable SOC, and we see that the F1, F2 and F3 fractions are more labile than F4.

The ¹⁴C ages of the three fractions released from by temperature stepped-combustion increased with increasing temperature (Fig. 4). The ¹⁴C ages of F1, F2 and F3 are similar, both in the maize cropland and forest nursery cropland, indicating carbon exchange within this layer, between these three SOC fractions. The ¹⁴C in F1, F2 and F3 in the grassland surface soils contained modern (post-bomb) carbon, pointing to a rapid turnover time, typical of thermally labile carbon [42]. Therefore, we consider the F1, F2, and F3 SOC fractions to be labile, and the F4 SOC fraction to be stable.

Table 2 The content of labile and recalcitrant soil organic carbon and their responding $F^{14}C$ values of the dried soils

Sites	Depth (cm)	F1 (g kg ⁻¹)	F2 (g kg ⁻¹)	F3 (g kg ⁻¹)	F4 (g kg ⁻¹)	Labile SOC (g kg ⁻¹)	$F^{14}C_{\text{labile}}$	Stable SOC (g kg ⁻¹)	$F^{14}C_{\text{stable}}$
Forest nursery cropland	0–10	0.19	1.23	1.27	0.46	2.69	0.8116 ± 0.0027	0.46	0.3731 ± 0.0020
	20–40	0.28	1.32	1.79	0.33	3.39		0.33	
	80–100	0.35	0.47	0.61	0.25	1.44		0.25	
	180–200	0.24	0.64	0.57	0.23	1.45		0.23	
	280–300	0.17	0.60	0.37	0.44	1.14	0.4638 ± 0.0021	0.44	0.0917 ± 0.0009
	380–400	0.30	0.21	0.25	1.53	1.54		0.77	
Maize cropland	480–500	0.12	0.80	0.44	0.46	1.36	0.4519 ± 0.0023	0.46	0.2038 ± 0.0013
	0–10	0.05	1.15	1.47	1.86	2.66	0.8519 ± 0.0025	1.86	0.0426 ± 0.0007
	20–40	0.03	1.07	0.93	2.09	2.02		2.09	
	80–100	0.02	0.15	0.38	3.03	0.55		3.03	
	180–200	0.02	0.12	0.66	3.30	0.80		3.30	
	280–300	0.01	0.21	0.20	2.30	0.42	0.2056 ± 0.0023	2.30	0.0022 ± 0.0002
Grassland	380–400	0.01	0.29	0.29	2.58	0.59	0.1638 ± 0.0014	2.58	0.0026 ± 0.0001
	0–10	1.45	3.07	3.79	4.22	8.31	1.0360 ± 0.0033	4.22	0.8890 ± 0.0030
	20–40	0.38	1.35	1.76	1.21	3.49		1.21	
	80–100	0.27	0.30	0.65	0.37	1.22		0.37	
	180–200	0.10	0.45	1.02	0.19	1.56		0.19	
	280–300	0.37	0.56	0.48	0.37	1.41	0.1887 ± 0.0023	0.37	0.0547 ± 0.0010
	380–400	0.17	0.46	0.87	0.31	1.50		0.31	
	480–500	0.24	0.43	0.26	0.71	0.93	0.2161 ± 0.0015	0.71	0.0628 ± 0.0008

F1 is the SOC fraction from acid-soluble, F2, F3 and F4 are the SOC fractions released from the temperature intervals of 25–400 °C, 400–600 °C and 600–900 °C, respectively. Labile SOC is the plus of F1, F2 and F3, and the stable SOC is F4

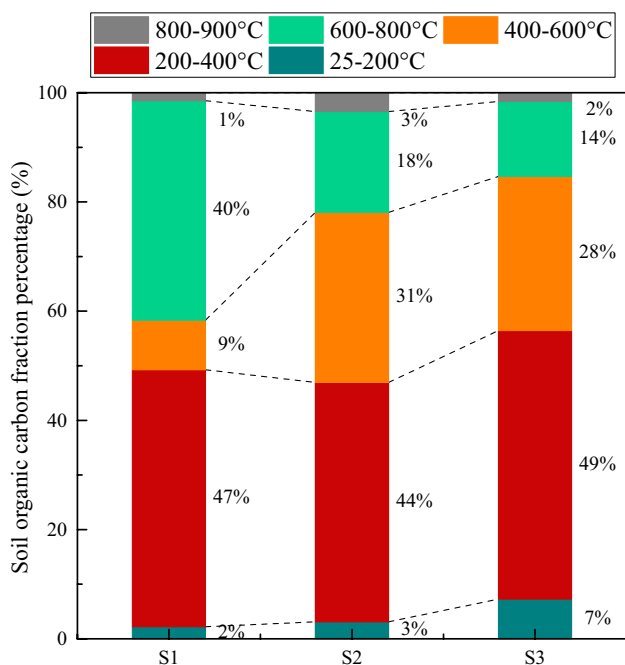


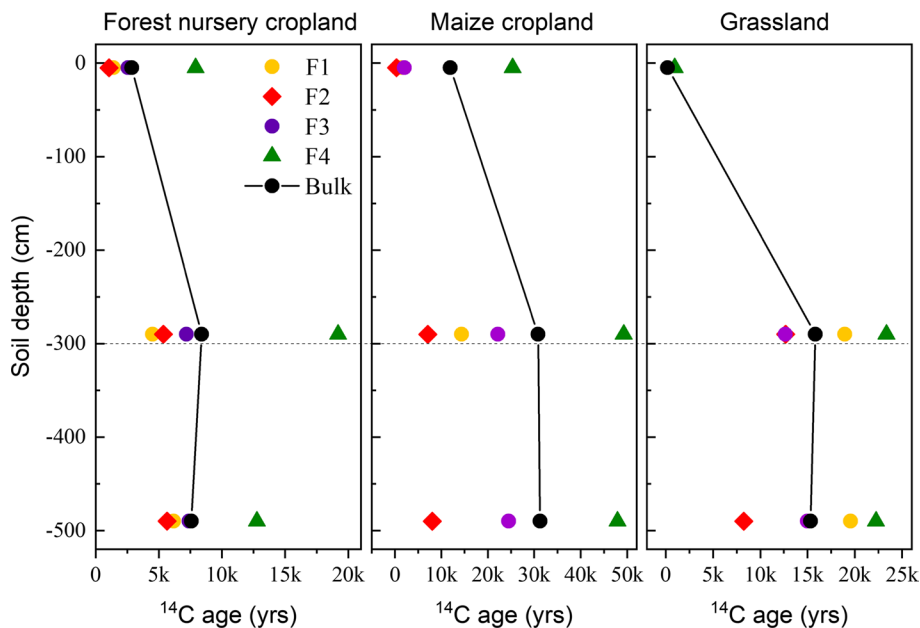
Fig. 3 Soil organic carbon fractions in three soil samples by sequential combustion. S1: grassland soil in 0–10 cm, S2: grassland soil in 280–300 cm, S3: grassland soil in 480–500 cm. (Color figure online)

Distribution of labile and stable SOC

Table 2 lists the profile distribution of labile and stable SOC from all of the study sites. A uniform decrease in labile SOC was observed for all of the sites, to a depth of about 100 cm, then remained constant. The stable SOC profiles were less consistent, showing both increases and decreases with depth (Table 2). The highest labile SOC observed (8.31 g kg⁻¹) and highest stable SOC (4.22 g kg⁻¹) were found in the grassland surface soil (0–10 cm). This may be attributed to carbon derived from grass detritus from a previous season. Such enhanced SOC is observed where straw mulching is practiced, especially for labile organic carbon [19, 43, 44].

The ^{14}C ages in the SOC fractions increase with depth from the surface (Fig. 4), which implies an increase in mean residence time with depth. The bulk soil values show that active exchange with young carbon from the surface (with relatively young ^{14}C ages) is largely restricted to the upper 300 cm (Fig. 4). This trend is consistent from site to site, despite their very different absolute age-depth characteristics. This suggests that they share common SOC exchange characteristics. Below 300 cm, the ^{14}C ages are constant, indicating limited exchange with fresh carbon from the surface. Deep soils cannot sustain active microbial populations to decompose SOC, and promote carbon exchange [45] and

Fig. 4 The ^{14}C ages of four fractions of carbon, i.e. F1 (yellow circles), F2 (red rhombus), F3 (purple circles), F4 (green triangle) and calculated bulk soils (black circles) at the depth of 0–10 cm, 280–300 cm and 480–500 cm in three types of soil. (Color figure online)



the thermal stability of deep soil SOC imply reduced biological degradability [21].

The maize cropland contained a relatively small amount of labile SOC, and large amount of stable SOC, especially below 20 cm. The stable SOC content in maize cropland was approximately 3.5–14.2 times greater than the forest nursery cropland, and 1.8–17.7 times greater than the grassland. In addition, $F^{14}\text{C}$ values in maize cropland were also much smaller than the other sites in corresponding layers (Table 2). This could be related to the high BD in the maize cropland soil (Table 1) with a higher fraction of older stable SOC [46]. Moreover, maize has short roots and with no regular fertilization management, limits the supply of fresh C to the deep soils [45].

^{14}C signal from above-ground nuclear weapons tests

A large amount of ^{14}C was released by atmospheric nuclear weapons testing in 1945–1980 [47–49]. Positive $\Delta^{14}\text{C}$ values in SOC show the presence of “bomb carbon” [50, 51], where $F^{14}\text{C} > 1$. In the grassland surface soil, the $F^{14}\text{C}$ values of F1, F2 and F3 were between 1.02 and 1.06, with a $F^{14}\text{C}_{\text{labile}}$ value of 1.0360 ± 0.0033 (Table 2), reflecting nuclear fallout in all of these labile SOC fractions. Hence, our temperature-stepped combustion technique allows us to identify the sequestration of bomb carbon in the SOC fractions.

There was no significant difference in the labile and stable SOC contents between the forest nursery cropland and natural grassland at depths of 20–500 cm. The $F^{14}\text{C}$ values of the forest nursery cropland at 280–300 cm and 480–500 cm were 0.4638 ± 0.0021 and 0.4519 ± 0.0023 , respectively, which were significantly larger than that in the grassland at

corresponding depths (Table 2). Since the sampling sites are very close to one another (1–4 km), we can assume that their ^{14}C fallout histories are the same. We attribute the difference in the soil carbon to the relative SOC fixation efficiencies between the two sites. The forest nursery cropland soil was able to fix a greater proportion of bomb carbon in the deep soil, as compared to the grassland soil. This idea could be confirmed with additional analysis of soil profiles.

Conclusions

In this study, we introduce a combined chemical-physical approach to the analysis of SOC fractions, using acid and base extractions, wet oxidation, and temperature stepped-combustion. We complement this protocol with AMS ^{14}C analysis. The SOC is separated into four components: (1) acid-soluble SOC, and (2) SOC oxidized in the temperature interval 25 to 400 °C, (3) 400 to 600 °C, and (4) 600 to 900 °C. We associate the three lowest temperature fractions with labile SOC. This is apparent in their relatively younger and tightly clustered ^{14}C ages ($F^{14}\text{C}$ in the range 0.73–1.05). The high temperature fraction is characteristic of stable SOC, with generally older ^{14}C ages ($F^{14}\text{C}$ in the range 0.04–0.889). We applied this method to three soil types: forest nursery cropland, maize cropland, and grassland. We show that most of the carbon exchange in all three sites occurs in the upper 300 cm of these soils. The forest nursery cropland was the most efficient of the three in sequestering fresh carbon from the surface into deep soils. The grassland surface soils contained the highest amounts of labile and stable SOC, and labile SOC in the surface soils

contained bomb carbon, showing that the grassland had not been disturbed since at least 1963. The maize cropland had lower labile SOC and the highest stock of stable SOC, which is not conducive to maize growth. Our results illustrate the efficacy of the combined chemical and temperature-stepped combustion approach, for the study of SOC, and such results can be helpful in the management of reclaimed croplands. Additional studies of this nature will both improve the method and increase our understanding of SOC dynamics in reclaimed croplands.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Singh M, Sarkar B, Sarkar S, Churchman J, Bolan N, Mandal S, Menon M, Purakayastha T, Beerling DJ (2017) Stabilization of soil organic carbon as influenced by clay mineralogy. *Adv Agron* 148:33–84
- Dungait JAJ, Hopkins DW, Gregory AS, Whitmore AP (2012) Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biol* 18:1781–1796
- Rumpel C, Amiraslani F, Koutika LS, Smith P, Whitehead D, Wollenberg E (2018) Put more carbon in soils to meet Paris climate pledges. *Nature* 564:32–34
- Fromm SF, Schwab VF, Trumbore SE, Tichomirowa M (2019) Parent material and depth effects on the age of radiocarbon in chemical fractions for Central German soils. *Geophysical Research Abstracts* 21
- Leinweber P, Schulten HR (1995) Composition, stability and turnover of soil organic-matter-investigations by off-line pyrolysis and direct pyrolysis mass-spectrometry. *J Anal Appl Pyrol* 32:91–110
- Lin ZB, Zhang RD (2012) Dynamics of soil organic carbon under uncertain climate change and elevated atmospheric CO₂. *Pedosphere* 22:489–496
- Zhou XQ, Chen CR, Wang YF, Smaill S, Clinton P (2013) Warming rather than increased precipitation increases soil recalcitrant organic carbon in a semiarid grassland after 6 years of treatments. *PLoS ONE* 8:e53761
- Parton WJ, Schimel DS, Cole CV, Ojima DS (1987) Analysis of factors controlling soil organic-matter levels in great-plains grasslands. *Soil Sci Soc Am J* 51:1173–1179
- Stavi I, Gusarov Y, Halbac-Cotoara-Zamfir R (2019) Collapse and failure of ancient agricultural stone terraces: on-site geomorphic processes, pedogenic mechanisms, and soil quality. *Geoderma* 344:144–152
- Mueller CW, Rethemeyer J, Kao-Kniffin J, Loppmann S, Hinkel KM, Bockheim J (2015) Large amounts of labile organic carbon in permafrost soils of northern Alaska. *Global Change Biol* 21:2804–2817
- Wang H, Stumpf AJ, Kumar P (2018) Radiocarbon and stable carbon isotopes of labile and inert organic carbon in the critical zone observatory in Illinois, USA. *Radiocarbon* 60:989–999
- Rovira P, Vallejo VR (2007) Labile, recalcitrant, and inert organic matter in Mediterranean forest soils. *Soil Biol Biochem* 39:202–215
- Bu XL, Gu XY, Zhou XQ, Zhang MY, Guo ZY, Zhang J, Zhou XH, Chen XY, Wang XH (2018) Extreme drought slightly decreased soil labile organic C and N contents and altered microbial community structure in a subtropical evergreen forest. *For Ecol Manag* 429:18–27
- Yao SH, Zhang YL, Han Y, Han XZ, Mao JD, Zhang B (2019) Labile and recalcitrant components of organic matter of a Mollisol changed with land use and plant litter management: an advanced C-13 NMR study. *Sci Total Environ* 660:1–10
- Xie WH, Jia GM, Chen FQ (2013) The soil labile organic carbon fractions at the different ages of tea in three gorges reservoir area. *Adv Mater Res* 864–867:2645–2648
- Dou F, Wright AL, Hons FM (2008) Sensitivity of labile soil organic carbon to tillage in wheat-based cropping systems. *Soil Sci Soc Am J* 72:1445–1453
- Li J, Wen YC, Li XH, Li YT, Yang XD, Lin Z, Song ZZ, Cooper JM, Zhao BQ (2018) Soil labile organic carbon fractions and soil organic carbon stocks as affected by long-term organic and mineral fertilization regimes in the North China Plain. *Soil Till Res* 175:281–290
- Figueiredo CCd, Farias WM, Melo BAd, Chagas JKM, Vale AT, Coser TR (2019) Labile and stable pools of organic matter in soil amended with sewage sludge biochar. *Arch Agron Soil Sci* 65:770–781
- Li S, Zhang SR, Pu YL, Li T, Xu XX, Jia YX, Deng OP, Gong GS (2016) Dynamics of soil labile organic carbon fractions and C-cycle enzyme activities under straw mulch in Chengdu Plain. *Soil Till Res* 155:289–297
- Sun HM, Jiang J, Cui L, Feng WT, Wang YG, Zhang JC (2019) Soil organic carbon stabilization mechanisms in a subtropical mangrove and salt marsh ecosystems. *Sci Total Environ* 673:502–510
- Helfrich M, Flessa H, Dreves A, Ludwig B (2010) Is thermal oxidation at different temperatures suitable to isolate soil organic carbon fractions with different turnover? *J Plant Nutr Soil Sci* 173:61–66
- Sutton R, Sposito G (2005) Molecular structure in soil humic substances: the new view. *Environ Sci Technol* 39:9009–9015
- Cambardella CA, Elliott ET (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci Soc Am J* 56:777–783
- Yang YS, Guo JF, Chen GS, Yin YF, Gao R, Lin CF (2009) Effects of forest conversion on soil labile organic carbon fractions and aggregate stability in subtropical China. *Plant Soil* 323:153–162
- Dias FPM, Hubner R, Nunes FD, Leandro WM, Xavier FAD (2019) Effects of land-use change on chemical attributes of a ferralsol in Brazilian Cerrado. *CATENA* 177:180–188

26. Tirol-Padre A, Ladha JK (2004) Assessing the reliability of permanganate-oxidizable carbon as an index of soil labile carbon. *Soil Sci Soc Am J* 68:969–978
27. Jiang JY, Li ZW, Xiao HB, Wang DY, Liu C, Zhang XQ, Peng H, Zeng GM (2018) Labile organic matter plays a more important role than the autotrophic bacterial community in regulating microbial CO₂ fixation in an eroded watershed. *Land Degrad Dev* 29:4415–4423
28. Wang H, Hackley KC, Panno SV, Coleman DD, Liu CL, Brown J (2003) Pyrolysis-combustion ¹⁴C dating of soil organic matter. *Quat Res* 60:348–355
29. Wang SL, Burr GS, Wang PL, Lin LH, Nguyen VJQG (2016) Tracing the sources of carbon in clay minerals: an example from western Taiwan. *Radiocarbon* 34:24–32
30. Benbi DK, Brar K, Toor AS, Singh P (2015) Total and labile pools of soil organic carbon in cultivated and undisturbed soils in northern India. *Geoderma* 237–238:149–158
31. Lefevre R, Barre P, Moyano FE, Christensen BT, Bardoux G, Eglin T, Girardin C, Houot S, Katterer T, van Oort F, Chenu C (2014) Higher temperature sensitivity for stable than for labile soil organic carbon—evidence from incubations of long-term bare fallow soils. *Global Change Biol* 20:633–640
32. Wang S-L, Burr GS, Chen Y-G, Lin Y, Wu T-S (2013) Low-temperature and temperature stepped-combustion of terrace sediments from Nanfu, Taiwan. *Radiocarbon* 55:553–562
33. McGeehin J, Burr GS, Jull AJT, Reines D, Gosse J, Davis PT, Muhs D, Southon JR (2016) Stepped-combustion ¹⁴C dating of sediment: a comparison with established techniques. *Radiocarbon* 43:255–261
34. McGeehin J, Burr GS, Hodgins G, Bennett SJ, Robbins JA, Morehead N, Markewich HJR (2004) Stepped-combustion ¹⁴C dating of bomb carbon in Lake sediment. *Radiocarbon* 46:893–900
35. Wang YQ, Han XW, Jin Z, Zhang CC, Fang LC (2016) Soil organic carbon stocks in deep soils at a watershed scale on the Chinese loess plateau. *Soil Sci Soc Am J* 80:157–167
36. Wang YQ, Shao MA, Liu ZP (2013) Vertical distribution and influencing factors of soil water content within 21-m profile on the Chinese Loess Plateau. *Geoderma* 193:300–310
37. Duval ME, Galantini JA, Martinez JM, Limbozzi F (2018) Labile soil organic carbon for assessing soil quality: influence of management practices and edaphic conditions. *CATENA* 171:316–326
38. Cheng P, Zhou WJ, Wang H, Lu XF, Du H (2013) ¹⁴C dating of soil organic carbon (SOC) in Loess-Paleosol using sequential pyrolysis and accelerator mass spectrometry (AMS). *Radiocarbon* 2–3:563–570
39. Purdy CB, Burr GS, Meyer R, Helz GR, Mignerey ACJR (2006) Dissolved organic and inorganic ¹⁴C concentrations and ages for coastal plain aquifers in southern Maryland. *Radiocarbon* 34:654–663
40. Xiong XH, Zhou WJ, Cheng P, Wu SG, Niu ZC, Du H, Lu XF, Fu YC, Burr GS (2017) Δ¹⁴CO₂ from dark respiration in plants and its impact on the estimation of atmospheric fossil fuel CO₂. *J Environ Radioact* 169–170:79–84
41. Stuiver M, Polach AH (2006) Reporting of C-14 data—discussion. *Radiocarbon* 19:355–363
42. Kuzyakov Y, Mitusov A, Schneckenberger K (2006) Effect of C3–C4 vegetation change on δ¹³C and δ¹⁵N values of soil organic matter fractions separated by thermal stability. *Plant Soil* 283:229–238
43. Zhou ZJ, Zeng XZ, Chen K, Li Z, Guo S, Shangguan YX, Yu H, Tu SH, Qin YS (2019) Long-term straw mulch effects on crop yields and soil organic carbon fractions at different depths under a no-till system on the Chengdu Plain, China. *J Soil Sediment* 19:2143–2152
44. Huo L, Pang HC, Zhao YG, Wang J, Lu C, Li YY (2017) Buried straw layer plus plastic mulching improves soil organic carbon fractions in an arid saline soil from Northwest China. *Soil Till Res* 165:286–293
45. Fontaine S, Barot S, Barre P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450:277–U210
46. Sanderman J, Baisden WT, Fallon S (2016) Redefining the inert organic carbon pool. *Soil Biol Biochem* 92:149–152
47. Goh KM, Rafter TA, Stout JD, Walker TW (1976) The accumulation of soil organic matter and its carbon isotope content in a chronosequence of soils developed on aeolian sand in New Zealand. *J Soil Sci* 27:89–100
48. Reimer PJ, Brown TA, Reimer RW (2004) Discussion: reporting and calibration of post-bomb ¹⁴C data. *Radiocarbon* 46:1299–1304
49. Naegler T, Levin I (2006) Closing the global radiocarbon budget 1945–2005. *J Geophys Res Atmos* 111:D12311
50. Angst G, Mueller KE, Eissenstat DM, Trumbore S, Freeman KH, Hobbie SE, Chorover J, Oleksyn J, Reich PB, Mueller CW (2019) Soil organic carbon stability in forests: distinct effects of tree species identity and traits. *Global Change Biol* 25:1529–1546
51. Torn MS, Trumbore SE, Chadwick OA, Vitousek PM, Hendricks DM (1997) Mineral control of soil organic carbon storage and turnover. *Nature* 389:170–173

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