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Can secular stable soil organic carbon be isolated? An assessment of Zimmermann fractionation using a long-term bare fallow

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Abstract. Useful soil organic matter fractionation techniques require fractions with accurate and distinct residence times and must be implementable within reasonable timeframes and with reasonable equipment. Among these techniques, the Zimmermann fractionation is a size- and density-fractionation process which aims at separating soil organic matter into five fractions known to be linked to kinetic compartments of carbon, including one fraction corresponding to the organic carbon in silt and clay fractions resistant to chemical oxidation (rSOC) considered to be stable at the scale of millenia, which is challenging to verify. In this paper, we used a 97-year-old long-term bare fallow experiment located at Versailles (France), to assess the stability of this fraction at the centennial scale. We applied the Zimmermann fractionation to archived soil samples collected in 1929, 1962 and 2021. We observed that the rSOC content was nearly constant over the lifetime of the trial, in contrast to total SOC and to other fractions. However, the rSOC only accounted for a small proportion of total SOC and did not contain all soil carbon persisting at the centennial time scale in this experiment. Our results show that rSOC represents an almost pure but not exhaustive pool of centennially stable SOC and that other methods are needed to obtain a complete estimate of centennially stable SOC.

1 Introduction

Soil organic matter (SOM), which is a critical component of the global carbon cycle, has a very broad chemical composition, comprising molecules with residence times ranging from a few days to several thousand years (Balesdent et al., 1987; Trumbore et al., 1989; Balesdent, 1996). These residence times form a continuum; however, SOC dynamics are commonly represented in models by a limited number of kinetic pools. Depending on the needs, from two to a dozen pools are defined (Clivot et al., 2019; Parton et al., 1987; Coleman & Jenkinson, 1996). Numerous attempts have been made to find chemical or physical fractionation methods capable of isolating fractions corresponding to kinetic compartments, with mixed results. Indeed, finding accurate fractions that can match the conceptual tools – or modifying the pools to match isolable fractions – can largely improve the performance of the models (Dangal et al., 2022). Using isotopic tools, Balesdent (1996) showed that physical methods were actually much more effective than chemical methods for isolating fractions that could be assimilated to the kinetic compartments of the models. This pathed the way for methodological developments, leading toward more efficient physical fractionation techniques. Later on, other techniques, such as thermal analysis, were also used to define SOC

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fractions and build models. Today, many SOC dynamics models exist, but even simplified two compartments representations can differ substantially from one another, for example between particulate organic matter versus mineral-associated organic matter (Cambardella & Elliott, 1992; Lavallee et al., 2020), or active versus stable SOC (Barré et al., 2010; Cécillon et al., 2021). Specifically, the PARTYSOC model based on the use of RockEval pyrolysis (Cécillon et al., 2018; 2021), which separates SOC into centennially active and centennially stable SOC has proven very useful for initializing and improving the AMG model (Andriulo et al., 1999; Clivot et al., 2019). Distinguishing centennially stable SOC has become increasingly important not only for model initialization and evaluation, but also for soil carbon monitoring frameworks and long-term climate change mitigation strategies.

The RothC model (Jenkinson & Coleman, 1999) aims at modelling the decay of topsoil SOC depending on various parameters such as the soil type, temperature, moisture content or vegetation inputs. It consists in five compartments: decomposable (DPM) or resistant plant material (RPM), microbial biomass (BIO), humified organic matter (HUM) and inert organic matter (IOM). Because these compartments are not always easy to quantify, Zimmermann et al. (2007) proposed a physical fractionation approach to estimate them. The Zimmermann fractionation separates SOC into five fractions: dissolved organic carbon (DOC), particulate organic carbon (POC), carbon in sand and aggregates (S+A), carbon in the fine fraction sensitive to oxidation (sSOC) and resistant to oxidation (rSOC). These fractions are then combined with splitting coefficients to correspond to the RothC compartments. Out of these five fractions, the rSOC fraction defined in Zimmermann et al. (2007) is assumed to correspond to the IOM pool of RothC – which is assigned a default residence time of 50,000 years (Jenkinson & Coleman, 1999). In practice, the size of the IOM pool in RothC has often been estimated empirically from total SOC content rather than measured directly, based on radiocarbon datasets and regression approaches (Falloon et al., 1998), further highlighting the conceptual and operational uncertainty associated with this pool. However, the existence and quantification of such an inert SOC pool has long been debated, and resistance to chemical oxidation does not necessarily imply long-term biological persistence (von Lützow et al., 2007; Schmidt et al., 2011).

Whether or not this rSOC fraction has the same extremely long residence time assigned to the IOM pool remains controversial, as the concept of a SOC pool stable over 50,000 years is itself difficult to reconcile with current understanding of SOC dynamics and to assess empirically. A more realistic and operational question is whether the rSOC fraction is stable at the century scale, which is more relevant for environmental applications and empirically testable. It is important to be able to isolate a fraction comprising only centennially stable SOC, even if it does not include all the centennially stable SOC, because this gives a lower bound for the amount of SOC that is expected persist for at least a century.

To our knowledge, there has been no direct validation so far of rSOC being a fraction with long residence time (> 100 years). This validation would require 14C analyses or long-term field experiments such as C3/C4 chronosequences or long-term bare fallow experiments (LTBF). LTBF are long-term experiments that, because no plant growth is allowed, become progressively depleted in SOC as decomposition proceeds over time and hence become enriched in long mean residence time SOC (Barré et al., 2010; Cécillon et al., 2018; 2021).

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In this study, we used control plots of the "42 plots" long-term bare fallow of Versailles, the oldest LTBF worldwide, sampled in 1929, 1962, and 2021, to test the centennial-scale validity of the Zimmermann fractionation. We hypothesized that (1) the DOC and POC fractions are labile and would strongly decline over the century; (2) the rSOC fraction would remain constant over time, i.e. between 1929 and 2021 and (3) that rSOC would not contain all centennially stable carbon. This last hypothesis is supported by recent estimates of centennially stable SOC obtained using the PARTYSOC model applied to 1892 sites from the French soil quality monitoring network (RMQS) covering the entire French mainland territory leading to an average of 10.47 g C kg-1 soil of centennially stable SOC (Delahaie et al., 2024); a value that largely exceeds the ca. 0.1 g C kg-1 of soil to 2 g C kg-1 soil rSOC values measured on European soils (Poeplau et al., 2013).

2 Material and methods

2.1 Sampling site description

Our study site is a long-term bare fallow (LTBF) called "The 42 plots" (48.80352778° N, 2.08613889° E), located at the INRAE (National Research Institute for Agriculture, Food and Environment) centre of Versailles (France). This LTBF has been installed on a silty loam Luvisol in 1928 to study the impact of prolonged application of fertilizers and amendments on the soil (Burgevin and Hénin 1939). The site has been kept bare ever since, weeded by hand and/or with herbicide, with annual fertilization or amendment and ploughing to 25 cm depth. It consists of 16 different treatments with two repetitions, and ten control plots. In our experiments, we used topsoil samples from five of the control plots (n° 9, 13, 21, 22 and 32), at year 1929 (first sampling), 1962, and 2021 (last sampling to date). Although these plots did not undergo any treatment, the physico-chemical properties varied with time by action of the climatic conditions; in particular the pH dropped from an average value of 6.3 in 1929 to 5.2 in 2008 (Pernes-Debuyser and Tessier 2002; Paradelo et al. 2013).

2.2 Separation

We followed the protocol detailed in Zimmermann et al. (2007) (Fig. 1), with improvements from Poeplau et al. (2013). Poeplau et al. (2013) analyzed the initial protocol and identified the critical steps that were more prone to operational uncertainties, and proposed said improvements to ensure the reproducibility of the method and results. This enhanced protocol is briefly described hereafter.

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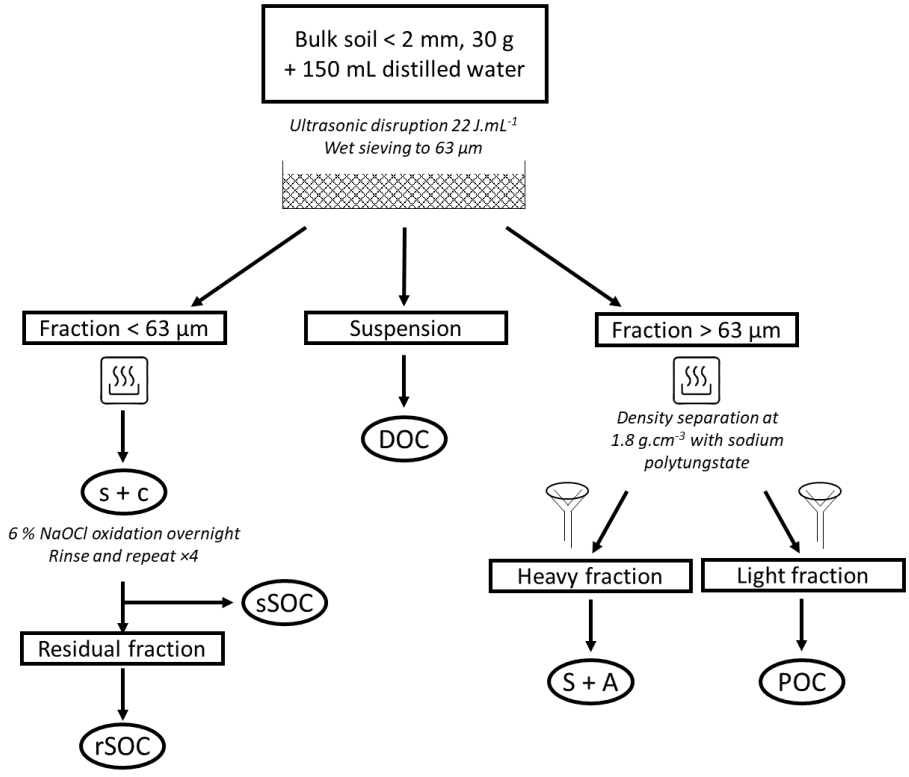

Figure 1: Fractionation scheme, based on Zimmermann et al. (2007). POC stands for particulate organic carbon; S+A for the sand and aggregate fraction; DOC for dissolved organic carbon; s+c for the silt and clay fraction; rSOC for the fraction of s+c resistant to oxidation; and sSOC for the fraction of s+c sensitive to oxidation.

2.2.1 Ultrasonic dispersion and sieving at 63 µm

We used dry soil samples previously sieved at 2 mm. 30 g of soil was placed in a glass beaker with 150 mL of distilled water and a magnetic stirrer, and subjected to ca. three minutes of ultrasounds. The precise duration of ultrasonication was determined by calibration of the ultrasonic probe prior to the experiment in order for the sample to receive a volumic energy E_v of 22 J mL-1, as the probe's power P can fluctuate over time, following Eq. (1) and (2):

$$P = \frac{m_w \times C_w \times \Delta T}{t} \quad (1)$$

$$E_v = \frac{P \times t}{V} \quad (2)$$

where P is the power in J s-1, mw the mass of water used, Cw the calorific capacity of water, ΔT the temperature difference measured during calibration in K, t the time during calibration in s, V the volume of water used in mL, and Ev the volumic energy in J mL-1.

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After ultrasonication, the sample was sieved on a 63 µm sieve with at least 2 L of distilled water, and in any case until the rinsing water was clear. The coarse fraction (> 63 µm) contains the sand and aggregate fraction (S+A) and the particulate organic matter quantified here as particulate organic carbon (POC). The fine fraction (s+c) consists in a fraction of SOC resistant to oxidation (rSOC) and a second one sensitive to oxidation (sSOC). The solution contains the dissolved organic carbon (DOC). The solution was separated from the fine fraction by adding 2 g of CaCl2 and centrifugating at 9,000 g for 10 min. Two 20 mL aliquots were taken for analysis. All containers were weighted beforehand. The volume of water used for rinsing was deduced by weighting the container, and used afterwards to determine the concentration of DOC. All fractions were dried in an oven at 40 °C.

2.2.2 Separation of POM and S+A fractions in sodium polytungstate

The coarse fraction was separated into POM and S+A by flotation using sodium polytungstate (SPT). A solution of SPT at 1.8 g mL-1 was prepared by slowly adding 990 g of SPT powder to 810 mL of distilled water. The whole coarse fraction was poured into a centrifugation tube and weighted. 30 mL of SPT solution was added and the sample was mixed for an hour in a rotating agitator at medium speed. The sample was then centrifugated at 9,000 g for 15 min to separate the fractions.

The supernatant (with floating POM) was drained on a previously weighted filter placed on a funnel. 30 mL of SPT solution was added again in the tube and the operation repeated identically (three to four times in total). Then, the filter was rinsed six times with distilled water to remove all SPT from the floating fraction, and the filter was placed in a Petri dish and set to dry in an oven at 40°C.

The heavy fraction (S+A) was retrieved with distilled water on another previously weighted filter on a funnel and rinsed four times with distilled water. The filter was placed in a Petri dish and set to dry in an oven at 40°C.

2.2.3 Oxidation of SOC with NaOCl

Oxidation of the fine fraction was carried out over four days using a 6% NaOCl solution. The first day, 4 g of s+c fraction were weighted in a 250 mL centrifugation bottle with a magnetic agitator. 100 mL of NaOCl solution were added, and the pH was adjusted to 8.0 with a 6 mol L-1 HCl solution. The magnet was retrieved and rinsed with distilled water, the solution was shaken by hand and put to rest 18 hours at 25 °C.

On the second day, the solution was centrifugated 15 minutes at 4,000 g. The supernatant was discarded, and 100 mL of distilled water were added. The bottle was shaken by hand, centrifugated again 15 minutes at 4,000 g, the supernatant was discarded again, and the first step was repeated: 100 mL of NaOCl solution were added, and the pH was adjusted to 8.0 with a 6 mol L-1 HCl solution. The magnet was retrieved and rinsed with distilled water, the solution was agitated by hand and put to rest 18 hours at 25 °C. The operations of the second day were repeated identically on the third day.

On the fourth day, the sample was centrifugated 15 minutes at 4,000 g, and the supernatant discarded. 100 mL of distilled water were added, the bottle was shaken by hand and centrifugated 10 minutes at 10,000 g. The supernatant was discarded.

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This operation was repeated again twice (three rinsing steps in total). The sample was retrieved with water in a beaker and put to dry in an oven at 40 °C.

2.3 Elemental analyses and data treatment

The DOC content was measured using a Shimadzu TOC V device, with two standard solutions (Reagecon TOC1025C1 at 10 mg mL-1 and Dutscher TOC100.L5-ACS at 100 mg mL-1, certified ISO 17034 and ISO 17025). Elemental analyses for all the other fractions were conducted on a Vario Isotope Select (Elementar), with tyrosine as a standard, on ground (<250 µm) samples. As the soil contains no carbonates total C concentrations were merged with organic C concentrations.
The data treatment was carried out using the R software.

3 Results

3.1 Mass and C balances

The mass yield of the fractionation, among all samples, ranged from 98.16 to 100.05 %. The carbon yield ranged from 92.45 to 100.10 % for 1929 samples, from 109.94 to 231.22 % for 1962 samples, and from 80.53 to 176.81 % for 2021 samples.

3.2 C content of fractions

The average organic C content of the bulk soil was 18.65 ± 1.09 mg C g^{-1} of soil in 1929, 9.22 ± 0.84 mg C g-1 of soil in 1962, and 6.99 ± 1.62 mg C g-1 of soil in 2021.
The evolution of the different fractions with time is visible hereafter in Fig. 2, Fig. 3, and Table A1.

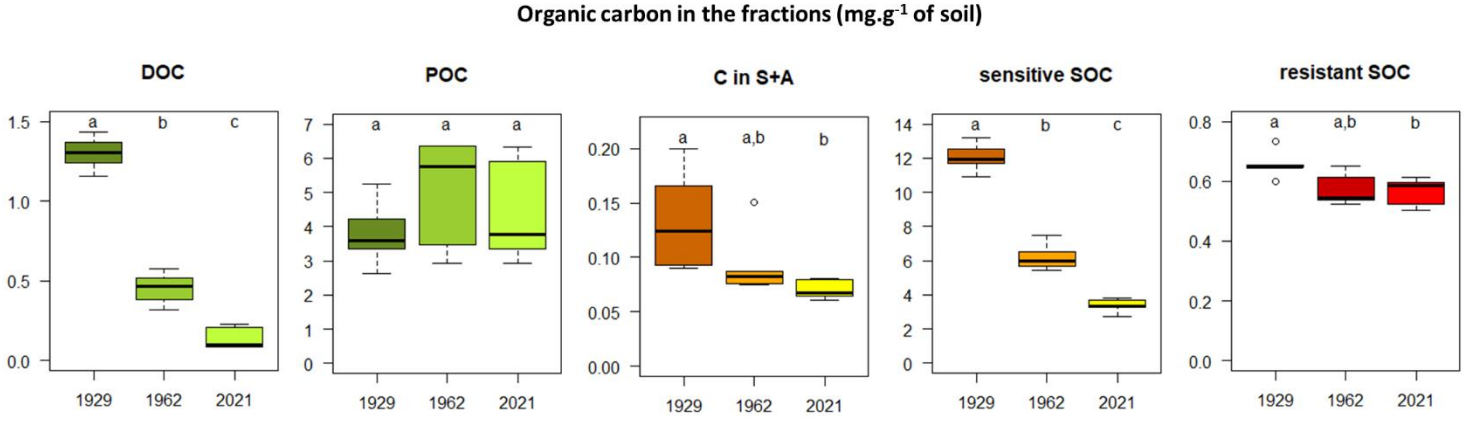

Figure 2: Comparison of the C contents (in mg C g-1 of soil) for each fraction between 1929, 1962, and 2021. The solid black midline in each box is the median (n=5). Different letters indicate significant differences between the means. The values are displayed in Table A1 in Appendix.

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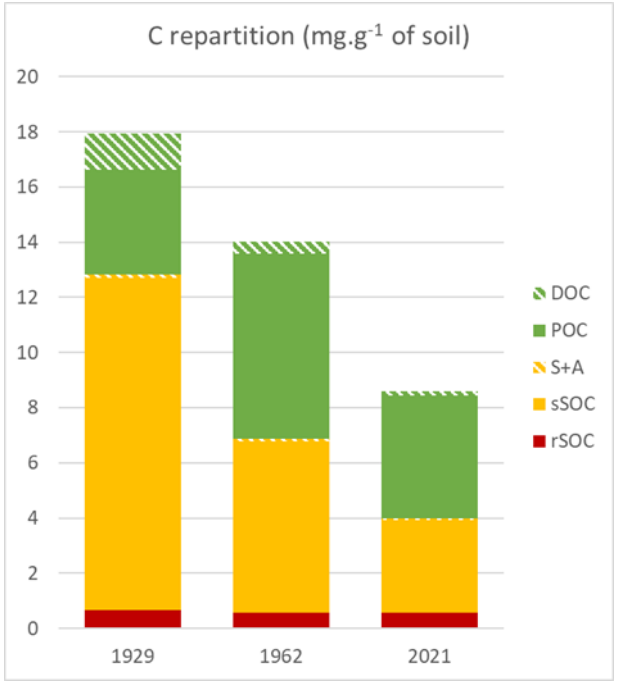

Figure 3: Carbon repartition though time, in mg C g-1 of soil.

The two expected labile fractions showed contrasting behaviours. The DOC fraction displayed a sharp decrease with time, from a mean of 1.30 ± 0.11 mg C g^{-1} of soil in 1929 to 0.14 ± 0.07 mg C g^{-1} of soil in 2021 (that is, a reduction of nearly 90 %). Surprisingly, the POC fraction strongly increased from 3.80 ± 0.98 mg C g^{-1} of soil in 1929 to 6.68 ± 4.87 mg C g^{-1} of soil in 1962, before decreasing without falling below its initial value at 4.45 ± 1.55 mg C g^{-1} of soil in 2021.

The two expected slow fractions both exhibited a substantial decrease, going from 0.13 ± 0.05 mg C g^{-1} of soil in 1929 to 0.07 ± 0.01 mg C g^{-1} of soil in 2021 for the S+A pool (a reduction of 46 %), and from 12.04 ± 0.87 mg C g^{-1} of soil in 1929 to 3.35 ± 0.44 mg C g^{-1} of soil in 2021 for the sSOC (a reduction of 72 %).

In contrast to other fractions, the expected stable fraction, rSOC, exhibited only a slight decrease, from 0.66 ± 0.05 mg C g^{-1} of soil in 1929 to 0.57 ± 0.06 mg C g^{-1} of soil in 1962 and 0.55 ± 0.05 mg C g^{-1} of soil in 2021, that is, a reduction of 15 %.

3.3 A focus on the particulate organic matter (POM) fraction

The POM fraction displayed an unplanned evolution in both quantity and quality. Through time, the mass of this fraction went from 7.95 ± 2.26 g kg^{-1} of soil in 1929, to 9.17 ± 6.26 g kg^{-1} of soil in 1962, to 6.44 ± 2.18 g kg^{-1} of soil in 2021. However, the POM fraction had a higher C content in 2021 than in 1929, meaning more POC in 2021 than in 1929 (690.7 ± 40.1 mg C g^{-1} of fraction in 2021 vs 483.4 ± 34.5 mg C g^{-1} of fraction in 1929; and peaking at 717.4 ± 28.4 mg C g^{-1} of fraction in 1962), indicating a change in the nature of the POM. The C/N ratio of the POM also increased from 32.4 ± 3.4 in 1929 to 64.2 ± 4.1

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in 2021 (and 57.9 ± 5.8 in 1962), hinting that an important part of the POM was pyrogenic carbon, which was confirmed visually (Table A2).

At least two different kinds of coal particles were identified in the samples and manually separated with tweezers (Fig. 4): mat, smooth-edged pieces, and shiny, sharp-edged pieces. Some presented conchoidal fractures. The POM fraction visibly differed between the years, with a mixed composition of plant debris and coal pieces in 1929, versus a dark, highly coal-rich fraction with almost no plant debris in 2021. Coal particles were found in all three years but in largely varying quantities.

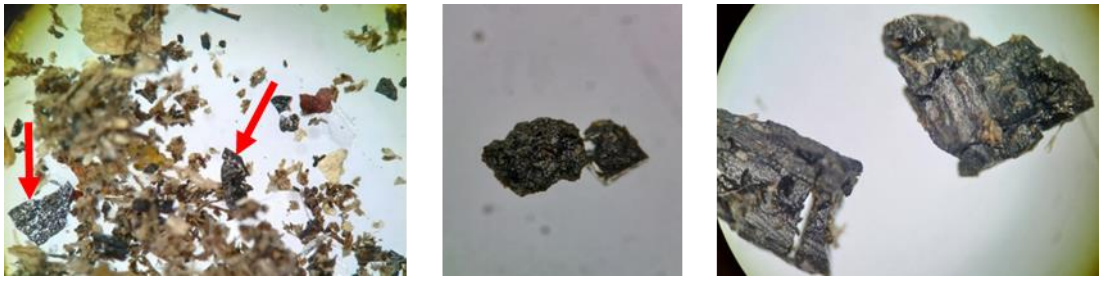

Figure 4: Observation of coal particles found in the samples, visible under a binocular microscope. Credits: Amicie A. Delahaie.

4 Discussion

4.1 An almost pure but non-exhaustive centennially stable SOC fraction

The rSOC fraction isolated using the Zimmermann fractionation exhibited very limited variation over the 92 years of the long-term bare fallow experiment (-15%), in contrast to the strong decline observed for total SOC. This temporal, stability indicates that this fraction predominantly consists of carbon with long residence times, at least at the centennial scale. However, the rSOC fraction does not contain all the centennially stable SOC. If rSOC was to comprise all centennially stable SOC its amount in 2021 should be close to total SOC at the same date, since the 2021 samples have experienced nearly a century of biodegradation.

Lutfalla et al. (2014) already highlighted the difficulty to isolate exactly a centennially stable fraction. They realised chemical oxidations on bulk soil samples from the 42 plots LTBF from the beginning of the experiment and after 79 years, with two different oxidizing agents (NaOCl and H2O2). They expected to find an oxidized fraction identical between both dates and nearly equating the total SOC content in the 79 years old samples. However, the oxidized fraction was, with both oxidizing agents, largely smaller than expected, suggesting that chemical oxidation removes far more carbon than long-term biodegradation, especially with NaOCl as the oxidizing agent. However, the protocol used in Lutfalla et al. (2014) differs from ours, as they applied the oxidation to the bulk soil while we only oxidized the fine fraction (s+c), which should lead to less losses as the DOC, POC and S+A fractions were left intact.

While rSOC content remained low and stable over time, the sSOC fraction, although strongly depleted, still represented a substantial carbon pool in 2021, and POC also persisted in significant amounts. This suggests that rest of the centennially

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stable carbon lies in the sSOC fraction, that is, the part of the fine fraction sensitive to the oxidation, and in the POC fraction, which is consistent with the known persistence of coal.

An important open question is whether the rSOC fraction, shown here to be stable at the centennial scale, could also correspond to SOC with millennial residence times. We cannot directly address this question, as the duration of the long-term bare fallow experiment remains far shorter than millennial timescales. The centennial-scale stability demonstrated here should therefore be viewed as a necessary, but not sufficient, condition for millennial persistence.

Taken together, these results indicate that the rSOC fraction can be considered an almost pure but not exhaustive pool of centennially stable SOC, providing a conservative lower bound for the amount of SOC persisting over centennial timescales. This further highlights the challenge of finding efficient fractionation techniques, as even an efficient protocol such as Zimmermann's does not encompass the whole targeted fraction.

4.2 Coal and fractionation yields

The persistence of substantial amounts of SOC outside the rSOC fraction can, at least partly, be explained by the presence of coal-derived carbon in the soil. The presence of coal particles in this soil was attested by previous studies, which revealed the presence of both charcoal and geological coal (Chassé et al., 2021; Lutfalla et al., 2017). Their presence at the beginning of the experiment is likely due to historical domestic activities in the research centre situated in the park of the Versailles palace, where the LTBF is located.

On top of these domestic activities, aerial archive photographs from the Second World War (Fig. A1) showed that at least once during the war, bombing affected the study site, with two bombs falling less than 30 m from the experiment. Even though the 42 plots were continually maintained throughout the war, debris and dust fallouts may have impacted the soils, leading to the incorporation of burnt particles and coal pieces. Due to the centimetric size of some coal pieces found in the LTBF plots we even suspect that the coal reserve may have been spread over the experiment area during the war. This could explain the increase of POM quantity between 1929 and 1962, with an increase in coal content that we witnessed visually, as well as the changes in POM nature (C content and C/N ratio). In a previous study (Vasilyeva et al., in preparation), char particles were hand-picked and analysed, and presented an average concentration of 764 mg C g-1 of fraction. This confirms that most of POM fraction isolated in the present study was indeed coal. The same study also noticed very little to no plant debris in the plots after 1939, which we also observed in our samples.

No other source of coal, especially in such quantities, has been identified: no fires were recorded in the area across the past century; the railway line – where steam trains used to run – is more than 500 meters away. The nature of the POM in 2021 could contribute to explain the unsatisfactory C yields obtained with 2021 samples, while the mass yields remained very satisfactory: with such high C concentrations, even a very slight experimental error (loss of matter or weight uncertainty) could lead to significant impacts on the yields.

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4.3 Dissolved organic carbon: an ambiguous fraction

The DOC proportion in our 1929 samples (ca. 6.9 %) was strongly higher than what is usually observed in either agricultural soils (0.05 to 0.4 %) or forest soils (0.25 to 2 %) (Von Lützow et al., 2007). DOC is generally considered a labile SOC fraction. However, in the present study, the DOC proportion decreased from ca. 6.9 % in 1929 to 3.2 % in 1962 and 2 % in 2021, showing that part of DOC was rather persistent, or was continuously supplied by the biodegradation of other SOC fractions. The first explanation concurs with the observation that DOC dynamics are actually more complex, as the fraction is very heterogeneous, with turnover times that can range from days to decades, even centuries (Kalbitz & Kaiser, 2008), for example in the case of pyrogenic C-containing soils (Von Lützow et al., 2007).

5 Conclusions

Using a 92-year long-term bare fallow experiment, we tested the centennial scale validity of the rSOC fraction isolated with the Zimmermann fractionation. The absence of clear temporal change in rSOC content, that remains almost stable between 1929 and 2021, supports the understanding of this fraction as highly persistent SOC at the century scale. This result provides rare empirical validation of rSOC temporal stability and thereby of a proposed fractionation procedure. However, we also observed that rSOC did not encompass all the centennially stable SOC. rSOC provides a conservative lower bound for the amount of SOC persisting over centennial time scales, rather than a complete separation of such a kinetic pool.

Substantial amounts of centennially stable SOC remained outside of the rSOC fraction, presumably in the sSOC and POC fractions, which is consistent with the intrinsic chemical and biological stability of coal. It is also possible that the drastic reduction in the amount of labile DOC led to a sharp decline in biological activity – labile DOC being an important source of energy for microorganisms (Marschner & Kalbitz, 2003) – which may have contributed to preserving carbon compounds in other fractions. More generally, chemical oxidation does not seem to mimic natural biodegradation at the century scale.

Long-term experiments such as bare fallows remain essential benchmarks for evaluating the residence time of SOC fractions and for improving the conceptual and empirical foundations of soil carbon models kinetic pools.

6 Appendix A

C (mg g^{-1} of soil)	1929	1962	2021
total	18.65 ± 1.09	9.22 ± 0.84	6.99 ± 1.62
rSOC	0.66 ± 0.05	0.57 ± 0.06	0.55 ± 0.05
sSOC	12.04 ± 0.87	6.22 ± 0.80	3.35 ± 0.44
S+A	0.13 ± 0.05	0.09 ± 0.03	0.07 ± 0.01
POC	3.80 ± 0.98	6.68 ± 4.87	4.45 ± 1.55
DOC	1.30 ± 0.11	0.45 ± 0.10	0.14 ± 0.07

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Table A1: C repartition in the different fractions for the three sampling years, in mg g-1 of bulk soil. The total derives from the analysis of bulk soil. Due to the varying yields, the sum of C in the fractions does not equate the total.

C/N (no unit)	1929	1962	2021
bulk	10.01 ± 0.45	9.65 ± 0.31	11.85 ± 1.87
rSOC	6.89 ± 0.64	6.00 ± 0.90	6.89 ± 0.67
s+c	9.26 ± 0.31	8.21 ± 0.35	8.81 ± 0.49
S+A	11.26 ± 1.74	7.85 ± 1.85	8.42 ± 1.49
POC	32.39 ± 3.38	57.92 ± 5.80	64.21 ± 4.01

Table A2: C/N ratio of the different solid fractions for the three sampling years (no unit).

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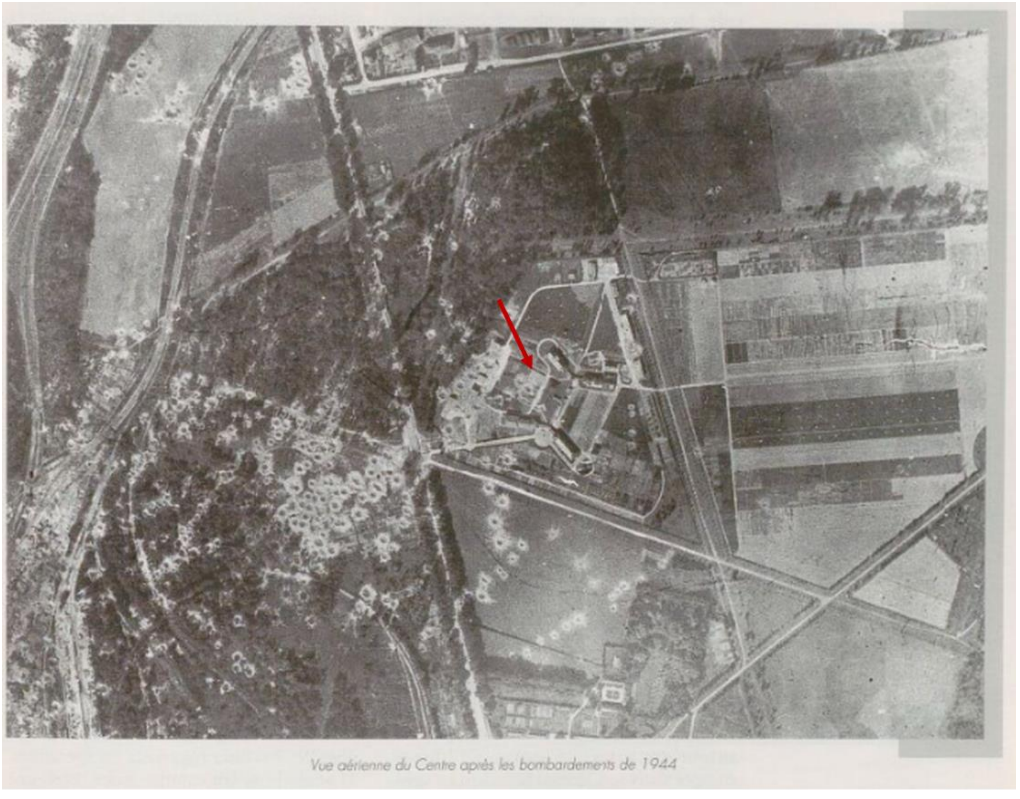

Vue aérienne du Centre après les bombardements de 1944

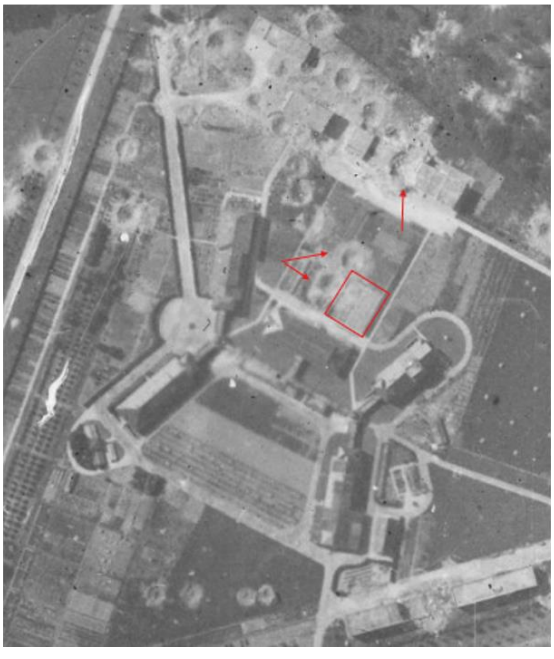

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Figure A1: Aerial photo of the site after the 1944 bombing. The large red arrow touches the upper right corner of the 42 plots experiment; smaller arrows indicate two bomb impacts a few meters away from the plots, and a destroyed building about 50 meters away. Credits: Source IGN, Photothèque Nationale, 1944-08-12.

Data availability

The data used in this paper will be made available on the French FAIR repository Recherche Data Gouv at https://doi.org/10.57745/I92WTM.

Author contributions

CC designed the experiments and acquired the funding. AAD carried out the experiment, with help from VP and CP. AAD analysed the data and prepared the manuscript with contribution from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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