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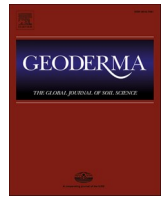
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Evidence of high carbon biodegradability in the subsoil of Mediterranean croplands

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ABSTRACT

Although soil carbon is a key element for soil health and climate change mitigation, our understanding of its dynamics is still incomplete. Deep soil horizons are thought to contain mostly organic carbon stabilised at centennial or even millennia time scales. The present study aimed to investigate the common paradigm of high stability of soil carbon in deep soil horizons in semiarid Mediterranean cultivated environments. It was based on a combined assessment and analysis of soil organic carbon (SOC) contents, stocks, biodegradability, and radiocarbon age of eleven soil profiles in north-eastern Tunisia, in the context of soils developed on marine sedimentary parent material with very low to moderate soil inorganic carbon (SIC) content. SOC content and stocks were found to be typical of cultivated Mediterranean soils, with low SOC content (<2%), decreasing with depth and a predominant stock of SOC in subsoils (>30 cm). Our results first revealed high levels of carbon biodegradability for all the soils investigated, confirming that soil carbon in the Mediterranean context can be rapidly decomposed under optimal temperature and moisture conditions. They also showed that the biodegradability of carbon increased with depth, even in profiles with very low SIC contents, indicating that the organic fraction of subsoil carbon is likely to be less stable than that of topsoil carbon. The significant increase in SOC biodegradability with depth was supported by applying a literature-based correction for the contribution of SIC-derived CO₂ to soil respiration emissions. In addition, SOC biodegradability was strongly positively correlated with its radiocarbon age, implying that SOC stability decreases with increasing mean residence time. We explained these original results by a significant presence of very old and highly biodegradable organic carbon in the subsoil organic carbon pool, probably inherited from a Quaternary paleoenvironment and preserved since then due to the favourable preservation conditions associated with the semi-arid climate. Finally, this study highlights the great vulnerability of the millennia-old organic carbon pool stored in some deep horizons of Mediterranean soils, and the necessity to protect it from reconnection with the atmosphere. More broadly, it demonstrates the need to take greater account of the active role of old organic carbon in carbon cycle studies in these specific environments.

1. Introduction

Soils are the largest carbon pool after oceans and geological reservoirs, containing about 75 % of the terrestrial organic carbon pool (Lal,

2004). Due to its multiple functions, soil organic carbon (SOC) is a key control for food security, soil health and climate change mitigation (Tian et al., 2015; Ramesh et al., 2019; Kopittke et al., 2023; Minasny et al., 2023; Rumpel, 2023).

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Although the number of studies on the drivers of spatial and temporal variability of SOC has greatly increased in recent decades, many knowledge gaps remain. For example, SOC stocks depend on climatic parameters (Zhou et al., 2019), parent material (Wiesmeier et al., 2019), land use and management (Funes et al., 2019), and/or soil properties (Xu et al., 2016). However, the factors and mechanisms explaining the fate of SOC, and in particular their mean residence times, are still not fully understood (Wiesmeier et al., 2019; Basile-Doelsch et al., 2020; Rodrigues et al., 2023). As more than half of the SOC stored in soil profiles is generally located below the topsoil (0–30 cm) horizon (Rumpel and Kögel-Knabner, 2011), the processes leading to its accumulation and persistence are of great interest for the development of management strategies to foster soil organic carbon sequestration.

It is generally recognised that the supply of carbon to (sub)soil is mainly from a biogenic source, as the result of a gradual downward flow from the atmosphere to the soil via net primary production (Basile-Doelsch et al., 2020). According to these authors, carbon predominantly enters the soil through the roots (dead roots or root exudates), in the form of shoot litter and by the return of part of the above-ground biomass, and is progressively transferred to subsoil by physical (e.g. ploughing or through open cracks), biological (i.e. bioturbation) (Don et al., 2008) and chemical processes (Kaiser and Kalbitz, 2012). These pedoturbation mechanisms lead to a mixing process that decreases exponentially with depth (Basile-Doelsch et al., 2020). As a result, deep soil horizons contain low SOC concentrations with high residence times (Balesdent et al., 2018; Basile-Doelsch et al., 2020). This functional description may explain the increasing mean residence time leading to the common paradigm of increasing SOC stability as a function of soil depth (Henneron et al., 2022), and consequently the assumption that SOC in deep soil horizons is composed of highly stable compounds, most probably generated by interactions with the mineral phase (Mikutta et al., 2019). This assumption was supported by the observations of similar microbial functioning of deep soil horizons and topsoil when supplied with fresh material (Sanaullah et al., 2016). Other proposed explanations for higher mean residence time of SOC in subsoils compared to topsoils are related to significantly less favourable conditions for SOC decomposition in subsoils than in topsoils in relation with (i) the lower oxygen supply (Keiluweit et al., 2017); (ii) the lower presence of energy-rich plant material (Fontaine et al., 2007; Henneron et al., 2022); (iii) the lower SOC content that increase the spatial separation of SOC, microorganisms and extracellular enzyme activity (Salomé et al., 2010); and iv) a stronger mineral protection for SOC at depth (Li et al., 2023).

However, the widespread paradigm of increasing SOC stability as a function of soil depth has been challenged in specific environments. For example, it has been suggested that the delivery of SOC in deep soil horizons could be the result of episodic soil burial by volcanic, aeolian, alluvial, colluvial, glacial, and anthropogenic depositional processes (Chaopricha and Marín-Spiotta, 2014; Marín-Spiotta et al., 2014). By examining buried paleosols, these authors described how environmental conditions unfavourable to microbial decomposition, such as low moisture or low oxygen, can lead to the persistence of SOC in buried mineral soils for thousands of years. Some authors have also suggested that the parent material can sometimes be a significant source of SOC, particularly when soils are developed from sedimentary parent materials (Kalks et al., 2021). For example, Copard et al. (2006) highlighted the contribution of geogenic organic carbon to the carbon pool of soils developed from marly bedrock in southern France using rock–eval pyrolysis to track geologic-derived organic carbon. They found that this geologic-derived organic carbon was highly biodegradable, i.e. characterised by a high potential mineralisation rate, and suggested that its flux from the bedrock to the subsoil could be governed by (bio)chemical weathering. Although less frequently mentioned in the literature, these other subsoil carbon supply mechanisms may lead to environments with highly biodegradable carbon stocks at depth. Given its role as a carbon source or sink in the global carbon cycle (IPCC, 2018), particular

attention needs to be paid to the vulnerability of SOC stored in subsoils. It is therefore essential to identify the contexts likely to favour environments with labile SOC in deepsoils.

The Mediterranean region is one of the environments where the mechanisms by which SOC forms and feeds soils can lead to configurations that differ from the classic one. Indeed, inputs of fresh organic carbon through net primary production (NPP) are generally low, due to low NPP and high uptake by livestock. In addition, major geomorphological changes during the holocene in the Mediterranean basin have led to important infilled areas mainly due to rapid and intense erosion and accumulation processes in relation to human occupation and deforestation (Dotterweich et al., 2013; García-Ruiz et al. 2013; Peñuela et al., 2023). Despite the importance and specificity of Mediterranean soils, there is still too little research into the dynamics of SOC, especially at depth. One reason is that these soils often contain significant levels of inorganic carbon from carbonates, which greatly complicate the study of SOC dynamics. The complication arises from the presence of interactions between the organic and inorganic fractions of carbon (Sun et al., 2023) which make it difficult to identify the origin of the CO₂ released from these soils. However, a few recent studies, such as Cardinael et al. (2019), have assessed the contribution of soil inorganic carbon (SIC) to total CO₂ emissions (%), making it possible to correct for the contribution of SIC to CO₂ emissions and estimate the biodegradability of SOC more accurately.

The aim of the present study was to investigate the distribution and stability of SOC in Mediterranean cultivated soils, which are characterised by low to moderate SIC content, low soil moisture and low inputs of fresh organic carbon via the net primary production (NPP) due to semi-arid climate. We hypothesised that, in such a context, the increase in SOC stability with soil depth might be counteracted by the presence of old and highly unstable carbon preserved in deep soil horizons due to conditions unfavourable to its decomposition. Our study is based on an original approach that combines measurements of carbon contents, stocks, biodegradability, and radiocarbon dating of eleven soil profiles in north-eastern Tunisia, covering soil types typical of semi-arid ecosystems encountered in the Mediterranean region.

2. Material and method

2.1. Study area

The study area consisted of the Kamech catchment (2.63 km²) located in a hilly agricultural region on northern Cape Bon, Tunisia (36.88°N, 10.88° E, Fig. 1A). It is part of the long-term observatory of soil and water resources called OMERE (<https://www.obs-omere.org/>), which was presented in detail by Molénat et al. (2018), and belongs to the OZCAR network (Gaillardet et al., 2018). More than 75 % of the area of this catchment is occupied by annually tilled farmland, mainly cereal crops and, to a lesser extent, leguminous crops. The remaining area consists of degraded rangelands, housing, and a lake at the catchment outlet. Observation of farming practices in the studied area showed that very little organic matter is returned to the soil, whether through crop residues, manure or other soil amendments. The cropping system is rainfed, without tile drainage. The climate is Mediterranean semi-arid with a mean annual precipitation of 620 mm, and a mean annual reference evapotranspiration ET₀ of 1300 mm. The geological material is mainly composed of a marine laminated mudstone between interbedded hard sandstone from the Middle Miocene (10–15 Ma). The long-term processes of differential erosion have determined the current morphology of the landscape, with outcrops of sandstone mainly at the top of hillslope. Soil type and depth depend on the position within the hillslope (Fig. 1B). Calcisols (IUSS Working Group WRB, 2022) are mainly found on the upper part of the hillslope and represent polyphasic soils with a complex history, due to the variation of the degree of weathering over time. Cambisols and Vertisols developed from the laminated mudstone and in relation to the erosion-related redistribution

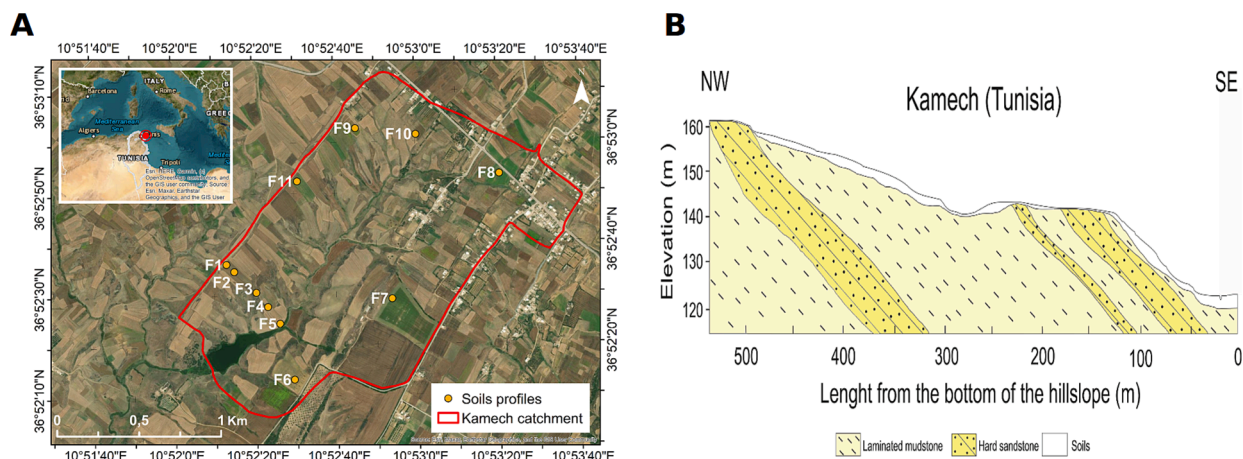


Fig. 1. Study site and soil profiles location (panel A); Typical cross-section representing the lateral distribution of parent material and soil depth in the right bank of the Kamech catchment (approximately a cross-section from F1 to F5, panel B).

of materials along the hillslope. They are more recent than calcisols, and show a typical increase in depth in the lower part of the forms. Overall, soils in the study site area are very clayey (mean and median value to 49 and 53 %, respectively), with a very low fraction of coarse elements (mean and median value to 1.7 and 0.6 %, respectively), with pH ranging from 8 to 9, and very low to moderate SIC content varying from 1 to 30 g.kg^{-1} (see [supplementary material](#)).

2.2. Fieldwork

Eleven soil pits (F1 to F11) were dug across the Kamech catchment. Their location was selected to cover the main diversity of morphopedological characteristics identified within the study area (Fig. 1). Each soil pit was described in terms of a sequence of horizons according to the guidelines for soil description (FAO, 2006). We sampled 3 profiles in the Calcisols, 4 in the Vertisols and 4 in the Cambisols. Based on field soil description, each horizon has been assigned to one of the following master horizon types: Ap (horizon affected by tillage), B (horizon affected by significant pedogenetic processes), C (horizon with the structure of the underlying material with low pedogenic features). The investigation of the underlying material, which was named R in this study, was possible for at least one profile for each soil type. In the study area, this underlying material is more akin to saprolite than bedrock, in that past or current weathering processes have led to geochemical properties that differ from those of deeper, unweathered geological material (Juilleret et al., 2016). Bulk density was measured in each horizon using a core sampling (100 cm^3 , with 3 replicates minimum). A composite soil sample composed of several subsamples in each horizon was brought back to the laboratory for further soil analysis. The description of the eleven profiles and the main soil analyses for each horizon are provided in [supplementary material](#). In the following, we use the term 'topsoil' to refer to the soil in the first 30 cm, and 'subsoil' to refer to the soil from 30 cm down to the saprolite. The analytical methods described below for organic carbon content, organic carbon biodegradability and organic carbon dating were applied in the same way to soil and saprolite samples.

2.3. Standard laboratory soil analysis

Soil and saprolite samples were first air-dried, and crushed and the fraction of coarse elements $> 2 \text{ mm}$ (CE) was determined. Then, 10 g of sieved soil to 2 mm were exposed to hot hydrogen peroxide to eliminate the organic matter and separated into three particle-size fractions according to AFNOR X31107: Clay ($< 2 \mu\text{m}$), Silt (2–50 μm) and Sand (50–2000 μm). Soil pH was determined as described in NF ISO 10390.

Cation exchange capacity (CEC) determination was based on the Metson method as described in NF X 31–130. The total carbon (TC_c) and nitrogen (TN_c) content were measured directly, in a subsample of a few mg of the sieved soil, by dry combustion with an elemental CHNS analyser (thermo Flash2000) on the ECOTROP platform (<https://www.umr-e-cosols.fr/en/eco-sols/ecotrop>). A second subsample of a few mg of the sieved material was analysed after decarbonisation by acid soaking pretreatment to provide the SOC content (SOC_c). The soil inorganic carbon content (SIC_c) was then calculated using equation (1):

$$\text{SIC}_c = \text{TC}_c - \text{SOC}_c \quad (1)$$

where SOC_c is the SOC content expressed in gC per kg of soil (g.kg^{-1}); SIC_c is the SIC content expressed in gC per kg of soil (g.kg^{-1}), and TC_c is the total carbon content expressed in gC per kg of soil (g.kg^{-1}).

The measurement uncertainty related to SOC_c , SIC_c or TC_c with such an analyzer is about 0.1 g.kg^{-1} .

2.4. Soil incubations

Laboratory incubations of soil and saprolite material under controlled environmental conditions have been performed to measure soil respiration (SR), defined as the soil C mineralisation per unit of soil and expressed as mg C-CO₂ per kg of soil. Three 50 g replicates of each soil sample (air-dried and sieved to 2 mm) were incubated for 172 days in the dark at 28 °C, with initial soil water content controlled at 25 %. CO₂ released during incubation was trapped in sodium hydroxide (NaOH, 1 N) pills and quantified by titration of the NaOH solution with 0.25 N HCl in excess of BaCl₂ at the following monitoring dates: 3, 7, 14, 21, 28, 42, 56, 70, 84, 112, 142, and 172 days of incubation. Ten replicates without soils were used as blanks. SR for each soil sample was calculated by subtracting the average cumulative CO₂ emissions in the ten blank experiments from the average cumulative CO₂ emissions in the three replicated incubations.

Soil carbon biodegradability, defined as carbon mineralisation per unit of soil total carbon (STC) was derived from soil incubation experiments using equation (2):

$$\text{STC}_B = 0.1 * \text{SR} / \text{STC}_c \quad (2)$$

where STC_B is the carbon biodegradability expressed in % (equivalent to mg C-CO₂ per 100 mg STC) since the beginning of the incubation; SR is the soil respiration expressed in mg C-CO₂.kg⁻¹ soil since the beginning of the incubation; STC_c is the total carbon content (g.kg^{-1}) and 0.1 is a conversion factor for expressing STC_B in %.

To assess soil organic carbon biodegradability, defined as SOC mineralisation per unit of soil organic carbon (SOC) and expressed in %

(equivalent to mg C-CO₂ per 100 mg SOC), it is necessary to evaluate the fraction of CO₂ emissions originating from the mineralisation/dissolution of inorganic carbon (Sun et al., 2023). Given that the contribution of soil inorganic carbon (SIC) to total CO₂ emission (%), noted SIC_CO₂, was not measured in this study, we used a literature-based relationship (equation (3)) that estimated the CO₂ fraction emitted from an inorganic carbon source from the SIC/STC ratio. This relationship was established by Cardinael et al. (2019). It was derived from fitting an exponential relationship on experimental results from incubations conducted on cultivated Mediterranean carbonate soils, for which the contribution of SIC_CO₂ was performed using a two-source partitioning model and isotopic composition (δ¹³C, ‰).

$$\text{SIC_CO}_2 = 0.00288 * \exp(10.684 * \text{SIC}_c / \text{TC}_c) \quad (3)$$

Given that the results of Cardinael et al. (2019) were obtained from Mediterranean carbonated soils cultivated under sub-humid climate in an environment very similar to this study, we assumed that equation (3) could be applied to evaluate SIC_CO₂ in our incubation experiments. Indeed, it is to our knowledge the most complete study conducted in Mediterranean cultivated soils containing carbonates, i.e. in a context very similar to the study area. SOC_B was therefore estimated using equation (4):

$$\text{SOC_B} = 0.1 * \text{SR} * (1 - 0.01 * \text{SIC_CO}_2) / \text{SOC}_c \quad (4)$$

where SOC_B is the carbon organic biodegradability expressed in % since the beginning of the incubation; SR is the soil respiration expressed in mg C-CO₂.kg⁻¹ soil since the beginning of the incubation; SIC_CO₂ is the contribution of soil inorganic carbon (SIC) to total CO₂ emission (%) as expressed by equation (3); SOC_c is the SOC content (g.kg⁻¹) and 0.1 is a conversion factor for expressing SOC_B in %.

The temporal dynamics of SOC_B were then adjusted using the combined zero and first-order kinetic model (eq. (5) proposed by Bernal et al. (1998):

$$\text{SOC_B}(t) = C_0(1 - \exp(-kt)) + bt \quad (5)$$

where C₀ is the labile proportion (%SOC); k is the labile mineralisation rate constant (day⁻¹), b is the mineralisation coefficient of the resistant compartment (%SOC day⁻¹) and t is time (day).

The three parameters of equation (5) (i.e., C₀, k, and b) were adjusted by minimising the sum of squared residuals of SOC_B using the “optim” function of the “stats” R package and the “L-BFGS-B” method developed by Byrd et al. (1995).

The STC_B value at the end of the 172-day incubation experiment, referred to as STC_B Biodegradability, was used as an overall proxy of (total) carbon biodegradability. The SOC_B value at the end of the 172-day incubation experiment, referred to as SOC_B Biodegradability, was used as an overall proxy of SOC biodegradability, and the three adjusted parameters of equation (5) (i.e., C₀, k, and b) as additional indicators of SOC biodegradability.

2.5. Organic carbon stocks

SOC stocks (SOCs, expressed in kg.m⁻²) have been evaluated for each soil profile using equation (6):

$$\text{SOCs} = \sum_{i=1}^n \left(\text{BD}_i * \text{SOC}_{ci} * (100 - \text{CE}_i) * \frac{e_i}{100} \right) \quad (6)$$

Where i is the considered horizon, n is the number of horizon increments, SOC_c is the soil organic carbon content (g.kg⁻¹); BD is the soil bulk density (g.cm⁻³); CE is the fraction in % of a coarse element; and e is the horizon thickness (m).

It was assessed for the entire soil profile, for topsoils (0–30 cm) and subsoils (>30 cm, but excluding R horizons). An assessment was also carried out for a depth of 1 m, to enable comparison between soil types

and with other studies.

2.6. SOC dating by radiocarbon analysis

SOC radiocarbon dating (14C_{age}) resulting from 14C activity values is known to be a good proxy of SOC mean time residence (Rumpel et al., 2002). In this study, the radiocarbon dating of SOC (14C_{age}) was used to investigate the relationships between SOC_B and depth, or between SOC_B and SOC mean time residence. For reasons of analytical cost, radiocarbon analysis was performed on a subset of 11 horizon samples (10 in soils and 1 in saprolite) selected with the emphasis on dating near complete profiles covering very low levels of SIC content (i.e. vertisol profile F11) on the one hand and relatively high levels of SIC content (i.e. calcisol profile F1) on the other. These two soil profiles were selected because they correspond to the conditions under which the impact of correcting for the SIC's contribution to CO₂ emission during the incubation experiment on the calculation of SOC_B is the strongest and weakest. Analyses were made at the Artemis LMC14 AMS-French National facility (Moreau et al., 2020). Bulk soil samples were first decarbonated and converted to graphite as described by Dumoulin et al. (2017). The ¹⁴C measurements were corrected for the isotopic fractionation according to the δ¹³C values measured on the AMS, following international recommendations (Mook and Van Der Plicht, 1999). SOC radiocarbon dating (14C_{age}) is expressed in calendar years (years Cal BP).

2.7. Data analysis

The variability of SOC content, SOC stock, carbon biodegradability and SOC biodegradability was analysed by grouping results by horizon or soil type, and comparing mean or median values between groups. The significance of difference between groups was tested using the Student's t-test or the non-parametric Wilcoxon test, also known as ‘Mann-Whitney’ test, with a p-value of 0.05. The vertical distribution of SOC radiocarbon age and its relationship with SOC biodegradability were both investigated. All the statistical treatments have been performed using R software (R core team, 2021) and a set of additional packages including ggplot2, Hmisc, corrplot, and cowplot.

3. Results

3.1. Distribution of soil organic carbon content

The vertical distribution of SOC content is illustrated in Fig. 2A for the eleven profiles. The SOC content varied from 1.8 to 13.2 g.kg⁻¹ with an average value of 6.0 g.kg⁻¹ and showed a general decreasing trend with depth for all profiles. SOC content was significantly higher in the Ap horizons than in the B horizons, the latter being significantly higher than in the C or R horizons (Fig. 2B). Vertisol profiles show slightly higher SOC content values compared to Calcisol and Cambisol profiles (Fig. 2C), but without significant differences of the average or median value when considering the whole profile. SOC content in saprolite showed low but non-negligible values with SOC content ranging from 0.9 to 3.1 g.kg⁻¹, with an average value of 2.3 g.kg⁻¹. Finally, a significant (r = 0.55, p = 0.002) or non-significant (r = 0.21, p = 0.15) correlation between SOC content and clay content was found when respectively excluding or including the Ap horizons (Fig. S1A), as well as a significantly higher clay content in Vertisols than in Cambisols or Calcisols (Fig. S1B).

3.2. Soil carbon biodegradability

Our results showed a general increase in carbon biodegradability as a function of soil depth, as illustrated for the biodegradability of total and organic carbon after 172 days (Fig. 3A and 3D). The biodegradability of total carbon (i.e. STC_B Biodegradability) ranged from 4.5 % to 13.3 % in

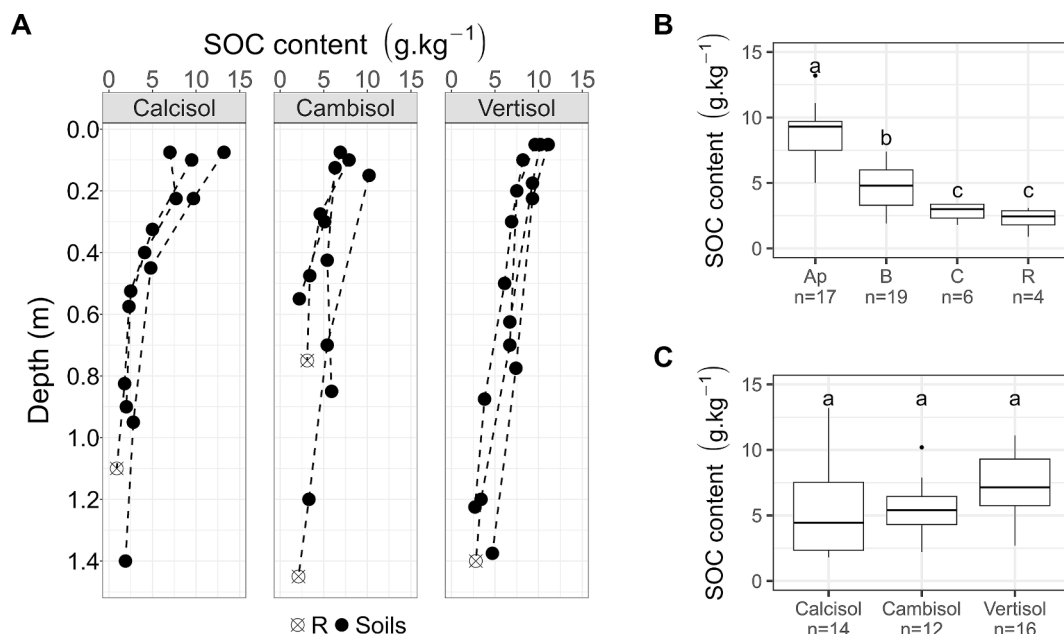


Fig. 2. Variability of SOC content in the eleven profiles studied in the Kamech catchment: vertical distribution grouped by soil types (panel A); boxplot for the different master horizon types (panel B) and boxplot for the different soil types considering only horizons of the first metre (panel C). Note that SOC content values measured in saprolite layers (i.e., in the R horizon) have been added with a different symbol in Fig. 2A, they are explicitly described in Fig. 2B, but they have not been included in Fig. 2C as they do not strictly belong to soil layers.

topsoils with an average value of 9.0 %, from 2.6 % to 19.4 % in subsoils with an average value of 10.0 %, and from 10.1 % to 19.3 % in the saprolite parent material with an average value of 15.3 %. The biodegradability of organic carbon (i.e. *SOC_Biodegradability*) ranged from 10.8 % to 25.3 % in topsoils with an average value of 14.1 %, from 13.2 % to 53.8 % in subsoils with an average value of 25.7 %, and from 27.1 % to 57.0 % in the saprolite with an average value of 39.7 %. *STC_Biodegradability* was significantly lower in the Ap or B horizons than in the R horizons, with intermediate value in the C horizons (Fig. 3B). The difference in *SOC_Biodegradability* according to the master horizon types was even more marked, with *SOC_Biodegradability* significantly lower in the Ap horizons than in the B horizons, the latter being significantly lower than in the C or R horizons (Fig. 3E). A significantly lower value of *STC_Biodegradability* was found in Calcisol compared to Vertisol and Cambisol (Fig. 3C), whereas no significant difference in *SOC_Biodegradability* between the different soil types was found (Fig. 3F).

Our results also indicated that the increasing carbon biodegradability with depth is the result of a combined increase in the labile organic carbon fraction and mineralisation coefficient of the resistant compartment, i.e. the C_0 and b coefficients as illustrated in supplementary Fig. S2.

3.3. Distribution of organic carbon stocks

The SOC stock for the eleven profiles ranged from 4.4 to 17.9 kg.m⁻², with an average of 10.0 kg.m⁻² (Fig. 4A) when considering the entire soil profile, with levels from 9.7 to 17.9 kg.m⁻² for Vertisols, from 4.4 to 12.8 kg.m⁻² for Cambisols, and from 6.3 to 11.8 kg.m⁻² for Calcisols. When considering the first top metre only, the SOC stock ranged from 4.4 to 12.7 kg.m⁻², with an average of 8.6 kg.m⁻², with levels from 7.1 to 12.7 kg.m⁻² for Vertisols, from 4.4 to 10.9 kg.m⁻² for Cambisols, and from 6.3 to 9.6 kg.m⁻² for Calcisols. The mean and median SOC stocks for Vertisol profiles were higher than the mean and median SOC stocks for Cambisol or Calcisol profiles, but with no significant difference between soil types (Fig. 4B). A moderate variation in SOC stock was observed in topsoils (i.e., [0–30 cm]), with values ranging from 2.5 to

5.3 kg.m⁻² and little difference between soil types (Fig. 4A and Fig. 4C). A higher variation in SOC stock was observed in subsoils ([>30 cm]), with values ranging from 1.5 to 14.0 kg.m⁻² and higher values for vertisols compared to other soil types (Fig. 4A and Fig. 4D). Higher SOC stocks in subsoils compared to topsoils were found in nine of the eleven profiles studied. Therefore, our study confirms that subsoil horizons can contribute to more than half of total SOC stock, as already evidenced in other Mediterranean (Batjes, 2014; Funes et al., 2019) or non-Mediterranean (Lacoste et al., 2014; Amirian Chakan et al., 2017) regions.

3.4. Radiocarbon dating

Radiocarbon ages in the soil profiles ranged between 600 (+/- 30) and 17,780 (+/- 110) years BP. As expected, there is an increase in the SOC radiocarbon age from the Ap horizons in the topsoil to the C horizons (Fig. 5A), illustrating an increase in SOC radiocarbon age with depth. More interestingly, we found a high correlation between the SOC biodegradability (*SOC_Biodegradability*) and the SOC radiocarbon age (¹⁴C age), considering whether or not the age of organic carbon in the R horizon ($r = 0.9$, p -value = 0.0003 and $r = 0.8$, p -value = 0.0056, respectively, Fig. 5B).

4. Discussion

4.1. Lateral and vertical distribution of SOC content and SOC stock in three mediterranean soil types under cropland

SOC stocks in the first top metre show an average of 8.6 kg/m² (0–1 m, Fig. 4A). This value is comparable to the global average observed for croplands (Jobbágy and Jackson, 2000) and aligns well with several estimates of SOC stocks in cultivated Mediterranean soils found in Spain (Albaladejo et al., 2013; Funes et al., 2019) and Tunisia (Brahim et al., 2014). Our results show that the average SOC stock in the Kamech subsoils (below 30 cm) is 6.5 kg/m², closely aligning with the 5.9 kg/m² reported by Brahim et al. (2014) for subsoils in northeastern Tunisia. For topsoils, the average SOC stock is 3.5 kg/m², which is slightly lower than

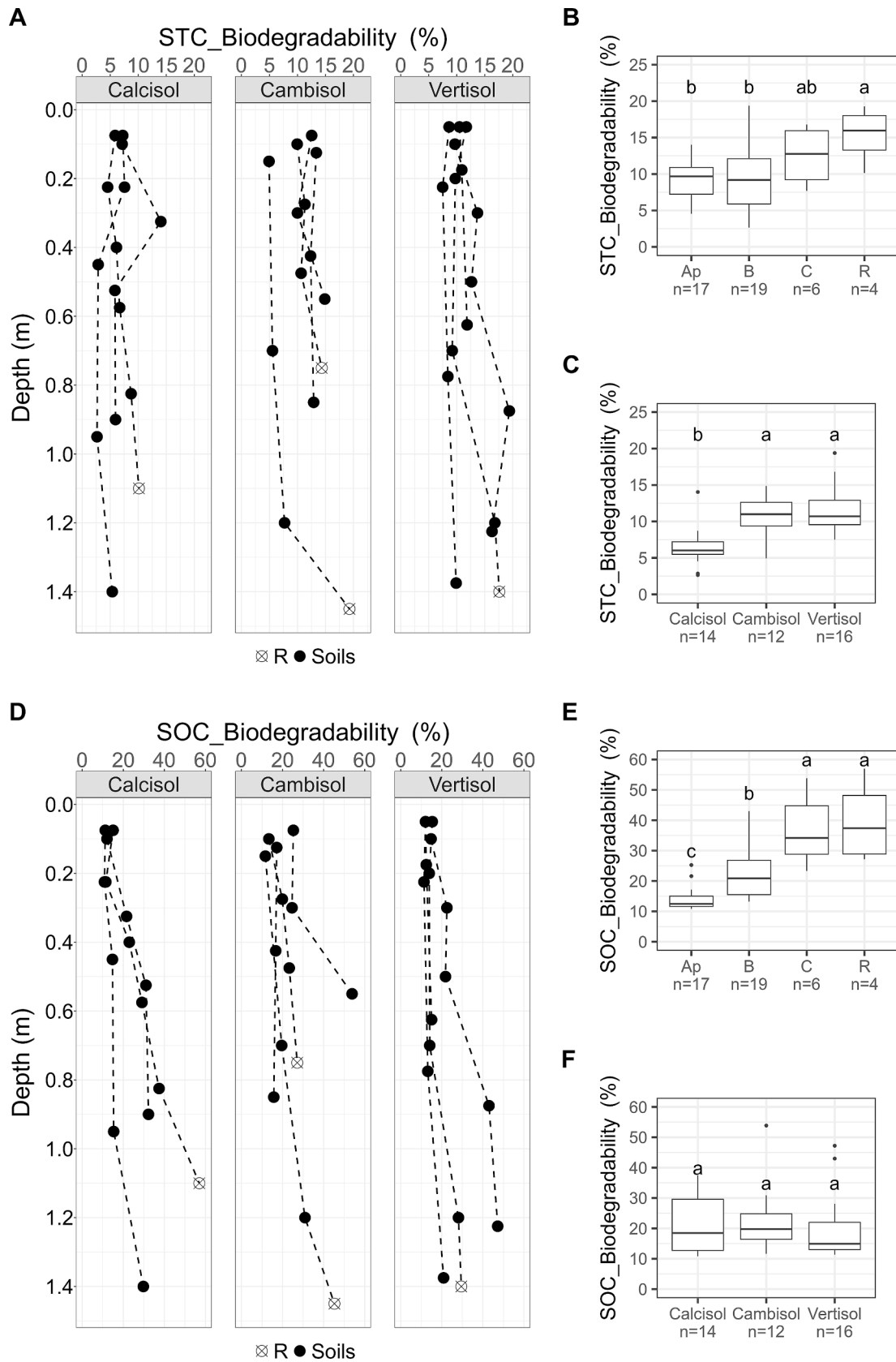


Fig. 3. Variability of biodegradability in the eleven profiles studied in the Kamech catchment: vertical distribution of STC_Biodegradability grouped by soil types (panel A); boxplot of STC_Biodegradability for the different master horizon types (panel B) and soil types (panel C); vertical distribution of SOC_Biodegradability grouped by soil types (panel D); boxplot of SOC_Biodegradability for the different master horizon types (panel E) and soil types (panel F).

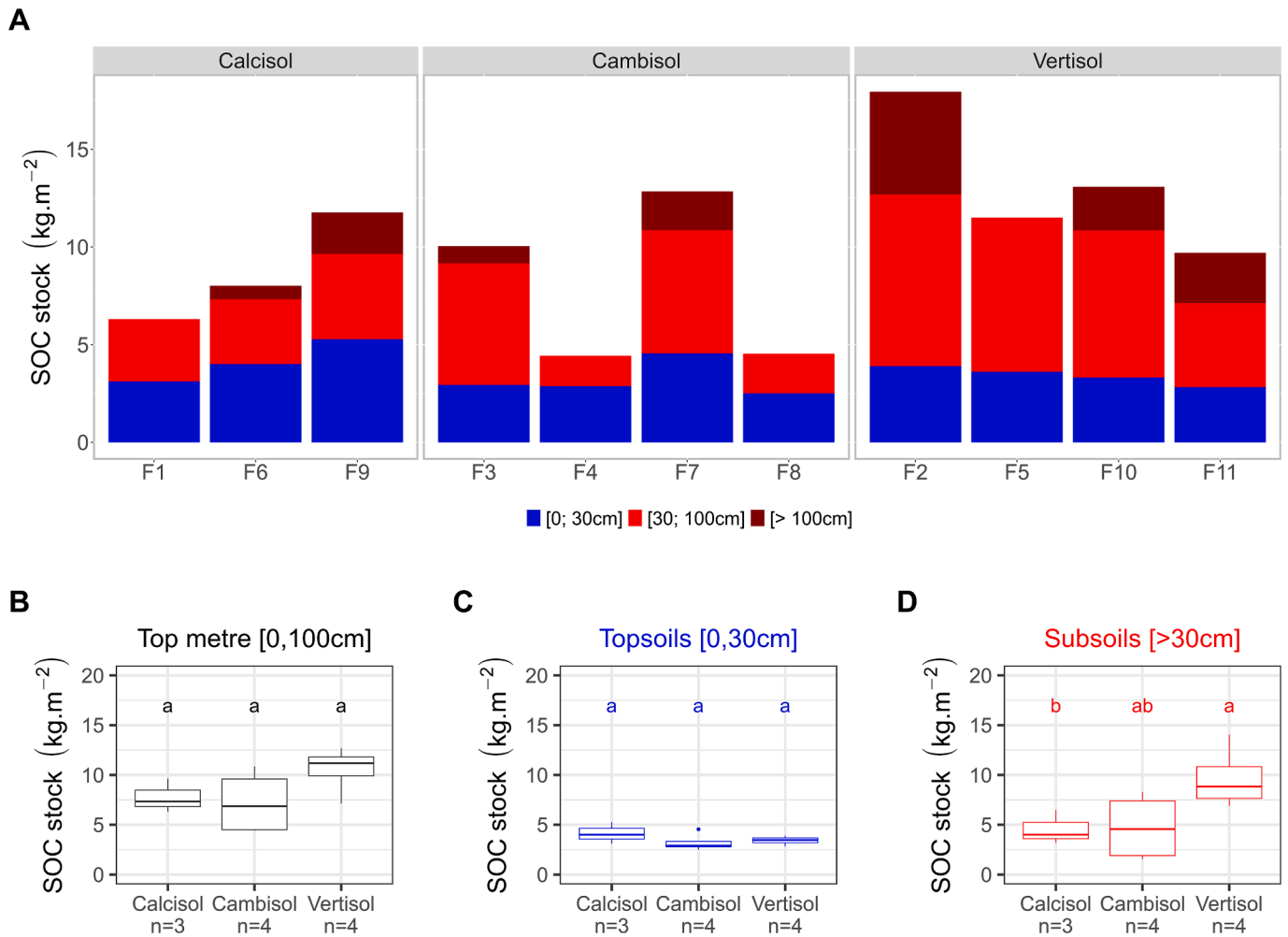


Fig. 4. Vertical SOC stock distribution among the different soil profiles (panel A); SOC stock variability between soil types considering the first top metre (panel B), topsoils (panel C) and subsoils (panel D).

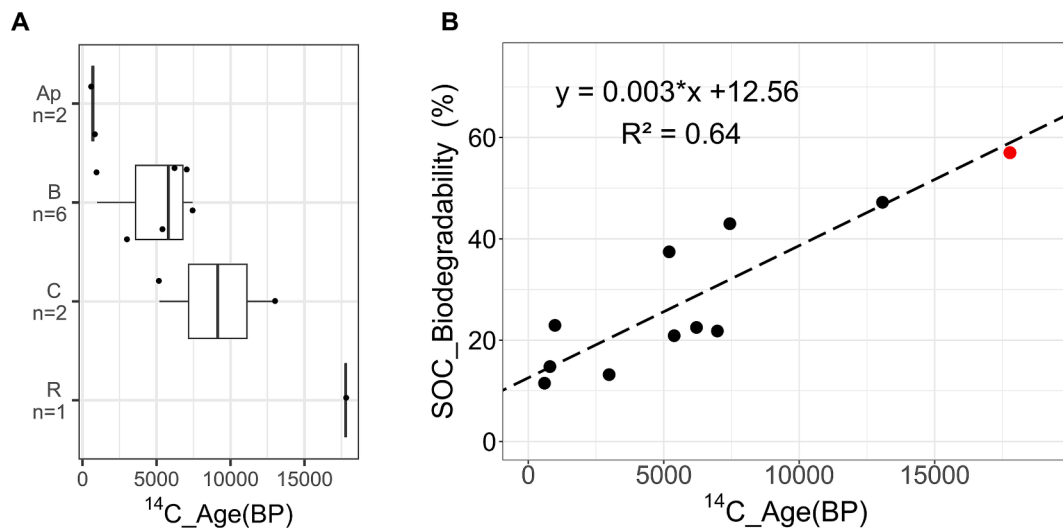


Fig. 5. Distribution of SOC radiocarbon age with the different horizon types (panel A) and variability of SOC biodegradability after 172 days with the SOC radiocarbon age (panel B). In Fig. 5B, the dashed black line is adjusted only on dark points, which refer to soil horizons whereas the red point refers to the R horizon, i.e. the saprolite. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the 4.6 kg/m² reported for northern Tunisia by Bahri et al. (2022) and the 4.5 kg/m² observed in rainfed croplands in Morocco and Spain by Sabir et al. (2020) and Rodríguez Martín et al. (2016), respectively.

The soils considered in this study thus showed SOC contents and distributions (Fig. 2A) typical of Mediterranean cultivated soils, with low SOC contents (<2%) in topsoils, decreasing with depth. The low SOC contents, even for tilled horizons, can be explained by very low inputs of fresh carbon due to the combination of low inputs in form of organic amendments or crop residues, and limited net primary productivity (Rashid and Ryan, 2004; Torrent, 2005). Furthermore, the decrease in SOC content with depth (Fig. 2A) is in line with studies in many global regions (Juarez et al., 2013; Li et al., 2019; Qin et al., 2022; Choudhury, 2023).

No significant differences in mean SOC content or mean SOC stock were found between the three soil types studied when the whole profile was considered (Fig. 2C and Fig. 4B). The fact that they are quite close to each other, and all developed from the same parental material, may partly explain this result. Another important reason may be that the influence of depth on SOC content or SOC stock exceeds that of soil types in our context. More importantly, our results also showed significant differences in SOC stocks among subsoils (Fig. 4D), but not among topsoils (Fig. 4C), underlining once again the importance of considering SOC in deep soil horizons. In this study, a significant correlation between clay and SOC contents was obtained for the soils of Kamech only when excluding the Ap horizons (Fig. S1A). This result is perfectly in line with literature since the SOC content in subsoils is known to be highly controlled by the particle size distribution (Barré et al., 2017; Wiesmeier et al., 2019), which is not the case in topsoils where SOC is largely affected by vegetation and agricultural practices (Gray et al., 2016; Choudhury, 2023). The significantly higher clay content in Vertisols compared to Cambisols and Calcisols (Fig. S1B) can therefore explain, at least partially, the slightly higher SOC contents and therefore the slightly higher SOC stock found in Vertisols compared to the two other soil types, as already suggested by Segalen et al. (1970). For Cambisols, lower SOC stocks compared to Vertisols can also be explained by shallower soils.

4.2. Assessment of biodegradability of soil organic carbon across depths in the three investigated soil types

The studied soils showed high levels of carbon biodegradability, with mineralisation rates after 172 days under optimal conditions of temperature and soil moistures ranging from 2 to 20 % for total carbon and from 10 to more than 50 % for organic carbon. These levels are in a similar range as previous rates observed for tunisian alkaline soils (Bouajila et al., 2021). The mineralisation rates recorded in this study were also consistent with the values obtained for North tunisian cropland topsoils by Annabi et al. (2009) with a potential SOC mineralisation after 28 days ranging from 1 to 8 % or by Hamdi et al. (2011 and 2012) with a potential SOC mineralisation after 56 days of 4 %. They are also very similar to values obtained by Rovira et al. (2010) for spanish carbonate topsoils under forest, who found a SOC biodegradability ranging from 3 to 6 % after 45 days, and about twice higher than those estimated by Bounouara et al. (2017) using algerian non-carbonate topsoils cultivated under tree crops or those estimated by Lorente and Turrión (2010) in Spanish carbonate topsoil cultivated with crops. However, they are significantly higher than values observed in other Mediterranean soils containing carbonates, such as those studied by Cardinael et al. (2019) in South France (about 10 times higher) under sub-humid climate.

The main limitations of the correction for C from carbonate applied in this study (Cardinael et al., 2019) are that (1) dissolved inorganic carbon may be retained in soil solution and (2) the isotopic fractionation between SIC and SIC-derived CO₂ and between SOC and SOC-derived CO₂ were neglected. The SOC biodegradability rates proposed in this study could consequently be lower or higher. However, the following

considerations lead us to believe that the relationship used, even if imperfect, does not call into question the conclusions of this study, and in particular the general trend towards an increase in SOC biodegradability with depth. Indeed, the increase in SOC biodegradability with depth was observed for all soil types, even in soils with very low SIC contents throughout the profile (SIC/STC < 0.5), such as in vertisols (supplementary Fig. S3A). In addition, the correction factor derived from Cardinael et al. (2019) was overall very low for Vertisols and Cambisols, with little depth dependence as illustrated in supplementary Fig. S3B.

Finally, while the biodegradability of total carbon was found to be significantly lower in Calcisols than in other soil types, no significant difference in biodegradability according to soil type was found when examining the biodegradability of organic carbon. Given that soil types are spatially organised within the catchment area as described in section 2.1, this result suggests that soil organic carbon probably comes from the same origin and has undergone more or less the same transformation processes throughout the catchment.

4.3. Old SOC does not mean high SOC stability

Here we found a high positive correlation between SOC biodegradability and the SOC radiocarbon age (Fig. 5B), whether saprolite material was under consideration or not. This counter-intuitive result proved that old SOC with low turnover is not synonymous with high SOC stability, as widely accepted in literature (Eusterhues et al., 2003; Krull and Skjem, 2003; Schrumpp et al., 2013; Li et al., 2023). It also proved that the increase in SOC biodegradability with depth cannot be explained by a significant input of young fast-cycling biogenic carbon, for example through root litter, as sometimes evoked in previous studies (Wordell-Dietrich et al., 2017) for explaining lower SOC stability in deep soil horizons compared to topsoils. The widespread increase in SOC biodegradability with depth found over Kamech catchment soils (Fig. 3) corroborates Rovira et al.'s (2010) presumption that the increase in SOC stability with depth may be less universal than previously thought.

The presence of very old and highly biodegradable organic carbon in deep soil horizons is in line with the findings of Copard et al. (2006), who identified a significant contribution of geogenic organic carbon in C and R horizons derived from the (bio)chemical weathering of the parent material, and characterised by a high level of biodegradability. A first plausible origin of the very old and highly biodegradable organic carbon in the deep soil horizons of the study area would therefore be geological, SOC in deep soil horizons being a mixture of geogenic and quite recent biogenic OC. The identification of very old carbon in deep soil horizons is also consistent with the findings of Kalks et al. (2021) who found that bulk sedimentary OC in terrestrial sediments was composed of a mixture of geogenic and biogenic components, the former being quite stable and the latter being relating to OC that has entered the sediments from plant sources since the onset of soil development. By analogy with finding of Kalks et al. (2021), a second plausible origin of the very old and highly biodegradable organic carbon in the deep soil horizons of the study area would be the presence of biogenic OC inherited from paleo-vegetation. This explanation is consistent with the succession of paleo-environments in the study area during the Quaternary period as described by Rognon (1987) and Zielhofer et al. (2002). According to these authors, after the late Pleistocene (15000–10000 yr B.P.), the region was characterised by vegetation composed mainly of trees (*quercus ilex* and *canariensis*) with large rooting system and deep soils due to a long colder and wet period. Vegetation changed radically during the Holocene (around 8000–5000 BP) in response to the transition from a humid to a semi-arid climate (e. g. long periods of drought, short heavy rains), shrubs were gradually replacing the forest and the landscape was changing with the incision of the wadis. The highly biodegradable biogenic OC inherited from ancient dense vegetation would have been preserved at depth thanks to the very favourable conservation conditions associated with the semi-arid climate, in particular soil moisture and oxygen limitation, while the

greater input of modern biogenic OC and the increasingly favourable decomposition conditions closer to the soil surface would explain the observed gradient in SOC biodegradability with depth.

4.4. Implications

We showed that the SOC pool in Mediterranean subsoils can contain a large fraction of highly biodegradable millennia-old organic carbon. A first practical implication is that the use of radiocarbon age to estimate the SOC stability is not adapted to all soil environments. Because of their similar context in terms of NPP, paleo-environment and parent material, we can assume that this will be the case in a large proportion of Mediterranean soils. It is probably for this reason that the decrease in SOC stability with depth has mostly been identified in Mediterranean environments. However, even if old carbon is not the dominant SOC fraction in many other modern soil environments, it may have a significant impact on soil SOC dynamics. We therefore suggest that old carbon, either geogenic or biogenic-derived, should be tracked and more widely considered in research on soil carbon dynamics in particular when subsoils are considered. New developments such as those proposed by Zethof et al. (2019) or Zondervan et al. (2023) could help to quantify the fraction of geological-derived organic carbon in SOC.

Even if the origin of this organic carbon in the study area is still open to question, the high contribution of unstable old carbon makes these soils very fragile and vulnerable to global changes, highlighting the need to study and preserve them more effectively. In terms of management it is therefore essential to prevent soil erosion in these areas, to avoid the accelerated thinning of topsoil horizons that would bring this old organic carbon to the surface. For example, the rate of soil water erosion in the catchment has been estimated at around $15 \text{ t.ha}^{-1}.\text{yr}^{-1}$ (Inoubli et al., 2017; Ben Slimane et al., 2018), with a predominance of interrill and rill processes (Ben Slimane et al., 2013). In the absence of measures to control soil erosion, the highly biodegradable organic carbon in the underlying horizons is likely to find itself in conditions unfavourable to its preservation, resulting in soils with even less organic carbon and thus promoting erosion in a negative feedback loop.

A final point of vigilance relates to the future of this deep carbon in the context of global warming. Indeed, it has been shown that warmer conditions could threaten subsoil SOC in temperate environments (Soong et al., 2021). However, in semi-arid environments, global warming will favour even drier soil conditions, which means conditions that are even more unfavourable to the rapid degradation of carbon throughout the profile. We therefore believe that soil erosion may threaten subsoil SOC more than global warming, and that conservation practices that preserve both water and soil resources while ensuring agricultural production should be promoted in these semi-arid Mediterranean areas, in line with what Raclot et al. (2018) have already suggested.

5. Conclusion

This study showed that SOC in deep soil horizons of Mediterranean cultivated soils can be both very old and very unstable. It also showed that SOC stability does not always increase with depth, and that old SOC with low turnover times does not mean high SOC stability. These original results are contradictory to the paradigm of increased stability with the age of the SOC. Among the possible explanations for the contribution of a large fraction of millennia-old highly biodegradable organic carbon in these subsoils, we identified the legacy of paleo-environments based on a lush wooded vegetation, highly favourable to delivery of SOC content in subsoil by the root systems, and/or the delivery of geogenic organic carbon which had been progressively altered and modified during the quaternary because of weathering processes. The shift to a semi-arid climate has led to conditions of low soil moisture and oxygen supply favourable to the persistence of this old carbon, as well as a reduction in the supply of modern carbon due to the very sharp drop in

NPP. The high proportion of unstable carbon in deep horizons makes these soils very fragile and vulnerable to global change, highlighting the need to study and preserve them more effectively. Ultimately, our results reopen the debate on the inclusion of old organic carbon in studies of the carbon cycle in modern soil environments.

CRediT authorship contribution statement

W. Khairallah: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **D. Raclot:** Writing – original draft, Visualization, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **M. Annabi:** Writing – review & editing, Supervision, Resources, Conceptualization. **G. Coulouma:** Writing – review & editing, Resources, Methodology, Conceptualization. **B. Guenet:** Writing – review & editing. **C. Rumpel:** Writing – review & editing, Conceptualization. **H. Bahri:** Writing – original draft, Supervision, Resources, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geoderma.2025.117212>.

Data availability

Data will be made available on request.

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