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Assessment of long-lived Carbon permanence in agricultural soil: Unearthing 15 years-old biochar from long-term field experiment in vineyard

David Chiaramonti [a,b,*], Giulia Lotti [b], Francesco Primo Vaccari [c], Hamed Sanei [d]

[a] DENERG-Politecnico di Torino and RE-CORD, Corso Duca degli Abruzzi 24, 10129, Torino, Italy
[b] RE-CORD, Viale Kennedy 182, 50038, Scarperia e San Piero, Firenze, Italy
[c] Institute of BioEconomy (IBE), National Research Council (CNR), Via Caproni 8, 50145, Firenze, Italy
[d] Lithospheric Organic Carbon (LOC), Department of Geoscience, Aarhus University, Høegh-Guldbergs gade 2, 8000C, Aarhus, Denmark

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ABSTRACT

Carbon persistence in soil is a key issue in the context of Carbon Dioxide Removal (CDR) policies and regulations: Soil Carbon Accumulation (SCA) is also included in the latest EU regulations on sustainable biofuels, and gaining attention at international level within ICAO and IMO. The long-lived nature of the durable carbon share in biochar can meet the most sever criteria set by relevant and ambitious CDR policies: however, the possibility to quantitatively assess the persistent carbon fraction in biochar has been highly debated in recent years. While lab-scale incubation experiments are intrinsically limited in providing information on long-term permanence, they do not address actual farm-scale persistence under real cultivation management practices. The deployment and combined use of recent analytical techniques allows instead to identify and quantitatively assess the persistence of the durable carbon fractions in biochar, and thus compliance of this carbon removal with the targets of CDR policies. The present work builds on one of the longest, almost unique, biochar experiments in the EU, originally developed for assessing the agronomic performances of biochar amended agricultural soil: for the first time, biochar distributed in a vineyard soil at 22 t/ha scale in 2009 was unearthed in 2024 and collected for full characterization. The agricultural soil was subject to conventional agricultural practices over the 15 years of vineyard cultivation. The scope of this research is to assess the permanence of biochar under these conditions. The present work shows the complexity of unearthing biochar from soil, applying a focused method to recover and clean the material before its characterization, without altering its chemical and physical properties. Both unearthed and original (i.e. before deployment) biochars were washed with water under same condition and procedures, and fully characterized. In addition to analytical practices commonly adopted for biochar characterization, FT-IR, SEM EDX, and Random Reflectance (R_o) techniques were used, quantifying the amount of the inertinite carbon component in biochar. Despite the dilution from the inclusion of exogeneous organic and inorganic matter from soil in the original biochar, the ratio of fixed carbon (Cfix) to total carbon (Ctot) showed minor variations (~8 %). Moreover, the inertinite and semi-inertinite fractions in the washed original and unearthed biochars remained almost unchanged over 15 years of active use in agricultural soil, confirming the permanent nature of the inertinite share of carbon in biochar. This result, together with other recent findings in literature, provides scientific evidence supporting Biochar Carbon Removals (BCRs) as permanent removal in Carbon Dioxide Removal (CDR) regulations.

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* Corresponding author. DENERG-Politecnico di Torino - Corso Duca degli Abruzzi 24, 10129, Torino, Italy.
E-mail addresses: david.chiaramonti@polito.it (D. Chiaramonti), giulia.lotti@re-cord.org (G. Lotti), francesco.vaccari@ibe.cnr.it (F.P. Vaccari), sanei@geo.au.dk (H. Sanei).

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1. Introduction

1.1. *Thermochemical processes for biochar production*

Biochar is produced from thermochemical conversion of biomass, typically lignocellulosic biomass and organic wastes, in absence or reduced oxygen environment. Pyrolysis of lignocellulosic material always delivers three products in different proportions: 1) a solid carbonaceous material (the biochar), and a pyrogas, which can be further separated into 2) a non-condensable and 3) a condensable fraction (in the case of Slow Pyrolysis, composed by an organic liquid - also called biocrude or biooil - and a water phase, containing water soluble organics) (see Table 1). The share of solid, liquid and gaseous products depends on reactor type, operating conditions and feedstock type. The processes typically used for biochar production are Slow Pyrolysis (SP) – which main product is the solid, Fast Pyrolysis (FP) – that targets liquid biocrude, and Gasification – which aims at maximising the gas phase. Torrefaction and HydroThermal Processing (hydrothermal carbonization and hydrothermal liquefaction) are not addressed here, as the characteristics of these solid products significantly differ from biochar.

Slow pyrolysis is a process targeting the production of a solids carbonaceous material as main goal: it is characterised by low Heating Rates (HR), high vapor residence times, as well as longer solid residence times. Typical temperatures for industrial-scale slow pyrolysis are comparable to fast pyrolysis (400–600 °C), even if the upper range of industrial processes can even reach around 700 °C or above.

Gasification, instead, targets the gas phase, and is operated at higher temperatures (from approximately 900 °C up to 1200–1500 °C): tars need to be removed or cracked to make the gas suitable for prime movers as Internal Combustion Engines (ICE) or Gas Turbines (GT) [1], or for lignocellulisic biofuel production. The characteristics of the biochar generated by gasification (in significantly lower amounts, compared to pyrolysis) are also different from slow pyrolysis, given the different operating conditions and reactors'type.

To achieve the high HR requested by fast pyrolysis to maximise biocrude yield, biomass particles need to be reduced to less than 1 mm (or, in case of Flash Pyrolysis, less than 0.2 mm) [2]. On the contrary, slow pyrolysis reactors, with their moderate HR, can be fed with biomass typically grinded from few mm to some cm (e.g. 5–50 mm).

Table 1
Thermochemical conversion processes for lignocellulosic biomass (elaborated from Ref. [2] Pahnila et al., 2023).

Mode	Typical Process Conditions	*Typical Liquid yield*	*Typical Char yield*	*Typical Gas yield*
Fast/Flash Pyrolysis	∼500-550 °C (typical range of 400–600 °C - up to 900–1300 °C for Flash) very short HVRT < 2–5 s (Flash: <0.5 s); short solids RT; HR 10–200 K/s (Flash: ∼10^3–10^4 K/s)	75 %	12 %	13 %
Intermediate Pyrolysis	∼500 °C (typical range of 400–600 °C); short HVRT ∼10–30 s; moderate solids RT	50 %	25 %	25 %
Slow Pyrolysis	∼400–500 °C, up to 700–750 °C; long HVRT >5 s; very long (minutes to days) solids RT; HR ∼ 0.1–2 K/s	35 %	35 %	30 %
Torrefaction	∼200-300 °C; long HVRT; long solids RT	Vapours	85 % solid	15 % vapours
Gasification	∼800-900 °C, up to 1200–1500 °C; short HVRT; short solids RT	<1–5%	<3–5%	95–99 %

HR: Heating Rate; HVRT: Hot Vapor Residence Time; RT: residence Time.

1.2. *Biochar: Soil Carbon Accumulation, Carbon Dioxide Removal, and permanence*

The continuous increase of GHG concentration in the atmosphere hit a new record in 2023, reaching 420 ppm of CO_2, 1934 parts per billion of methane and 336.9 parts per billion (ppb) of nitrous oxide, as documented by the World Meteorological Organization (WMO). United Nations Environment Programme released a strong warning that continuing current policies will bring us to 2.6-3.1 °C temperature increase, off-track to the 2 °C (1.5 °C aimed) target set at COP15 in Paris. Thus, global policies are more and more addressing Carbon Removals, in addition to the necessary shift to renewable energies in place of fossil.

In this framework, biochar, the solid product of thermochemical processing of biomass, gained great attention in recent years as a Carbon Dioxide Removal system (CDR) [3,4] and Negative Emission Technology (NET) [5,6] beyond being a renewable fuel for power and industry (as in the steel sector). Biomass, if not stabilized through carbonization, quickly degrades into CO_2 and minor amount of CH_4: on the contrary, well-carbonized lignocellulosic biomass largely contains long-lived Carbon in soil. The European Commission - General Directorate for Climate Action, is evaluating the inclusion of Biochar in the permanent removal category within the Carbon Removal and Carbon Farming (CRCF) Initiative [7,8].

Voluntary and mandatory carbon removal markets, as the ETS [9], are well established and fast-growing worldwide [10], which can be addressed by NETs. Within the Emission Trading Scheme frameworks, the mandatory EU ETS has been recently updated [11], further challenging obligated stakeholders (i.e. large emitters) with accelerated targets, as well as adding new sectors (such as Maritime) and moving faster towards phasing out of free allowances. Connecting NETS and allowances was investigated in past years also by governmental organisations, such as the International Carbon Action Partnership (ICAP) [12].

Increasing Soil Organic Carbon (SOC) is one of the possible solutions, already considered in some other EU policies in place: the RED-II Implementing Regulation [13] provides a method to account SOC increase in the GHG performances of the derived Sustainable Biofuels. Biochar is here explicitly listed as a sustainable agricultural practice, accounted in a factor named E_{SCA} (Soil Carbon Accumulation, SCA). However, in the context of this EU biofuel regulation, permanence of carbon is currently not considered, as the typical time frame for this regulation is of the order of tens of years. Also, other International Organization in charge of regulation the use of sustainable biofuels, as the International Civil Aviation Organisation (ICAO) and the International Maritime Organization (IMO), are studying possible methods to account for Soil Carbon Accumulation in the GHG performance of Sustainable Aviation Fuel (SAF) and Maritime Fuels.

Instead, in order to include any CDR (Carbon Dioxide Removal) solution under the "permanent" (long-lived) category in policies and regulations, it is requested to demonstrate and assess the duration of the carbon removals.

The current discussion around biochar policies as NET thus focuses today on its permanence [14]: is biochar a permanent removal, analogous to geological storage? Even more relevant: is it possible to estimate the duration of the carbon removal achieved by a specific batch of biochar, given the information on feedstock, reactor type, process conditions and use? Is it possible to quantify the amount of long-lived carbon contained in biochar by analytical techniques?

So far, to answer these questions, most of the research developed incubation experiments: however, it is clearly difficult to use short incubation times (orders of some years, at best), normally carried out at small/lab scale, to derive conclusions on permanencies in the range of hundreds of years or millennia.

A meta-study was recently presented by Azzi [15], and summarised by Bier and Lerchenmüller [16], both in 2024. Azzi collected and examined 134 individual observations and more than 8000 data points:

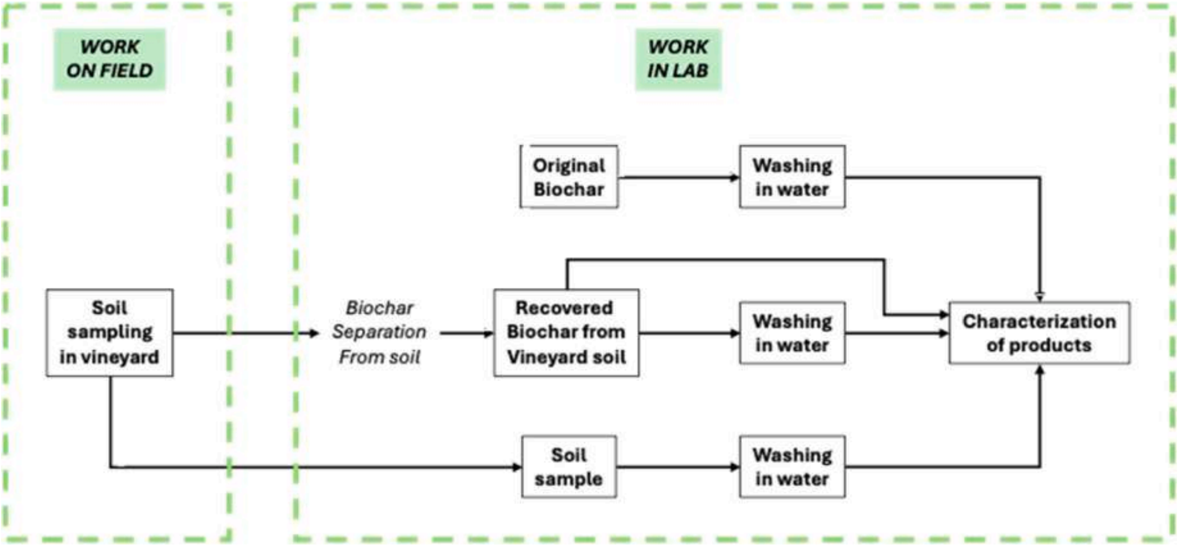


Fig. 1. Procedure adopted for unearthing and separating biochar and soil samples.

after quality assessment of the dataset, 79 individual observations were retained for investigation. This work observed how the incubation experiments and the associated dataset of tested biochars is not representative of industrially and commercially produced biochars, including parameters as pyrolysis temperatures and associated H/C ratio. 10 cases showed H/C ratio <0.4, while 69 samples, i.e. 87 % of the dataset, was above this 0.4 threshold (even above 0.7 in 19 cases). On the contrary, commercial/industrial biochars falls under 0.4, as shown by Sanei et al [17].

Also, the characterization of these products was insufficient in many cases, in particular as regards the quantification of the durable (i.e. long-lived) and the labile (i.e. rapidly degradable) carbon fractions in biochars.

In parallel to this meta-analysis, new techniques have been developed to assess the characteristics of the carbon composing the biochar matrix, with the aims of not only identifying, but also quantifying, the inertinite fraction of carbon in industrially produced biochars.

These works [17,18] considered the composition of biochar as observed though the Random Reflectance (R_o) analysis and focused on quantifying the inertinite (and semi-inertinite) fractions. Inertinite is among the most stable forms of carbon in the earth crust, and the method targets these macerals in biochar.

In his study, Sanei [17] examined 64 biochar samples, extracting those obtained from industrial scale (including industrial demo plants), which left 27 biochars: the work found that 93 % of these products fall in the range below 0.4 H/C ratio. Incubation experiments, instead, examined a large number of biochars samples different from industrial and commercial products. Then, Sanei applied the Random Reflectance (R_o) technique, a well-known method in coal and geology, to biochar: the method allows for direct quantification of the inertinite fraction. Inertinite is the most thermodynamically stable form of organic carbon in the sedimentary rocks, analogous to the stability of carbonates (mineral carbon): it is a second form of carbon storage pathway, named “maceralization”, complementary to “mineralization”, which transform a compound (including CO_2) to a mineral, carbonates. Worth to note, the term mineralization in the CDR and geological science has an opposite meaning than in soil science, where mineralization means the decomposition of organic matter and the release of CO_2 through microbial metabolization, as discussed in Ref. [14].

Random Reflectance (R_o) offers new insights in biochar characterization, quantifying how much of the carbon structure corresponds to Inertinite, and thus can be considered long-lived carbon. The threshold to distinguish inertinite fraction versus other forms of carbon is R_o>2 %, with semi-inertinite fraction (1.2<R_o<2 %) still characterised by very long permanence.

These findings are extremely relevant for modelling biochar permanence in soil, which has been extensively studied in past years [4, 8,19–21], and suggests to reconsider the so-called 2–3 pool models to better address the very slow degradation of the most durable carbon share in biochar.

1.3. Scope of work

As regards incubation experiments, the majority of these studies were carried out at laboratory or at very small scale: only few long-term on-field experiments have been conducted in Europe. Probably, the longest real-field biochar experiment in the EU is the one carried out in Italy, in the South of Tuscany, in the La Braccesca vineyard, where 22 and 44 t/ha of biochar were deployed in 2009 [22,23]. The possibility of unearthing the biochar from this experiment was therefore a unique opportunity to observe the changes in biochar and carbon degradation under real agricultural conditions: however, the recovery of biochar particles after 15 years of use is a new challenge, never explored before.

To our knowledge, an integrated approach combining:

- the recovery of biochar after long-term use in real cultivated agricultural soils,
- the development and use of cleaning and pretreatment techniques to make these biochar particles ready for analytical characterization and
- the application of most innovative techniques to assess the permanency of the different carbon forms in biochar after its long-term use in soil

has never been applied before, representing a unique case and a potentially groundbreaking contribution to the assessment of biochar permanence and carbon removal studies.

The scope of this work was thus to sample soil with biochar from the agricultural fields after 15 years from deployment, separate from soil and properly recover this unearthed biochar, analyze and characterize the aged-in-soil biochar versus the original biochar, with the aim of examining the persistence of the most durable (i.e. harder to degrade, or recalcitrant to degradation) fraction. Agronomic effects of biochar use in the La Braccesca vineyard are instead not the scope in this work, as these have already been extensively discussed in a large set of published literature [22–31], where details of the agricultural practices are also

Fig. 2. Original orchard pruning char sample.

given.

These insights on carbon permanence can provide essential information to policy makers and regulators, supporting with evidences initiatives as the EU CRCF, ICAO, IMO, and IPCC.

2. Materials and methods

The on-field and in-lab procedures for unearthing, collecting, pretreating and characterizing the samples of soil and biochar particles is described in the following sections, with the overall method summarised in Figs. 1 and 4.

2.1. *"La Braccesca" vineyard biochar experiment and the soil sampling procedure*

Biochar produced from orchard pruning and commercially available in 2009 from "Romagna Carbone snc" was used in the original experiment, which was carried out at the "Marchesi Antinori – La Braccesca Estate" farm (Lat. 43°10′15''N; Long. 11°57′ 43''E; 290 m a.s.l.).

The original biochar (i.e. before being deployed in the farm soil) is shown in the following Fig. 2: as regards the size of biochar pieces at the moment of deployment, this material was crushed below 5 mm before being deployed. The biochar was produced at low temperature (500 °C) by slow pyrolysis: the initial (2009) laboratory characterization of biochar reported 77.8 % C, 0.91 % N, 101 cmolc kg^{-1} CEC, 25 % water content, 2722 mm^3 g^{-1} porosity, 410 m^2 g^{-1} BET, and 9.8 pH (as given in Ref. [22] Baronti et al., 2014).

Samples of the original biochar were however newly and extensively characterised also in the present work, as explained in the following section on Results.

The vineyard was planted in 1996: 22 t/ha and 44 t/h of biochar were deployed in the soil in 2009 through mechanical mixing by a chisel plough tiller up to 30 cm depth. Grapes were then cultivated as usual management practices under rainfed conditions following a three-year alternate management practice (as reported in the already referenced literature). Each year, the farm works on one specific inter-row, using a rototiller and ploughing to 0–20 cm. Meanwhile, the two adjacent inter-rows are left uncultivated and covered with volunteer grass, which is mowed twice a year. Vineyard rows are shown in Fig. 3.

The soil at La Braccesca is acidic (pH 5.37), with a sandy-clay-loam texture (35 % clay, 20 % silt, 45 % sand), and highly compacted below 0.4 m depth. More details can be found in Refs. [22,23].

The present work considered the portion of vineyard where the 22 t/ha were deployed in 2009.

In January 2024 soil sampling was carried out on the farm, and so biochar was unearthed together with the soil. As shown in Fig. 3, biochar at the time of deployment was not uniformly distributed, but it was concentrated among the rows: thus, in this specific inter-row region the actual amount of biochar deployed in soil was expected to be even higher than the average of 22 t/ha.

As described in the following Fig. 4, soil sampling was performed by means of a soil auger: 3 different sub-samples (SS1, SS2, SS3) were collected at 0–40 cm for each sampling location. These subsamples were then mixed into a single sample (S): a total of 4 different samples S were collected, and biochar was recovered from each of them (BC). The recovered biochar BC1, BC2, BC3 and BC4 were then mixed into a one Final Biochar Sample (FBCS) to average the results, then delivered to the laboratory for characterization.

As shown in Fig. 5, biochar particles are hardly visible after 15 years in vineyard soil: biochar also appears highly fragmented and comminuted, compared to its original size shown in Fig. 2.

Fig. 3. La Braccesca farm vineyard rows (left) and biochar distribution in 2009 (right, reprinted from Baronti et al., 2014).

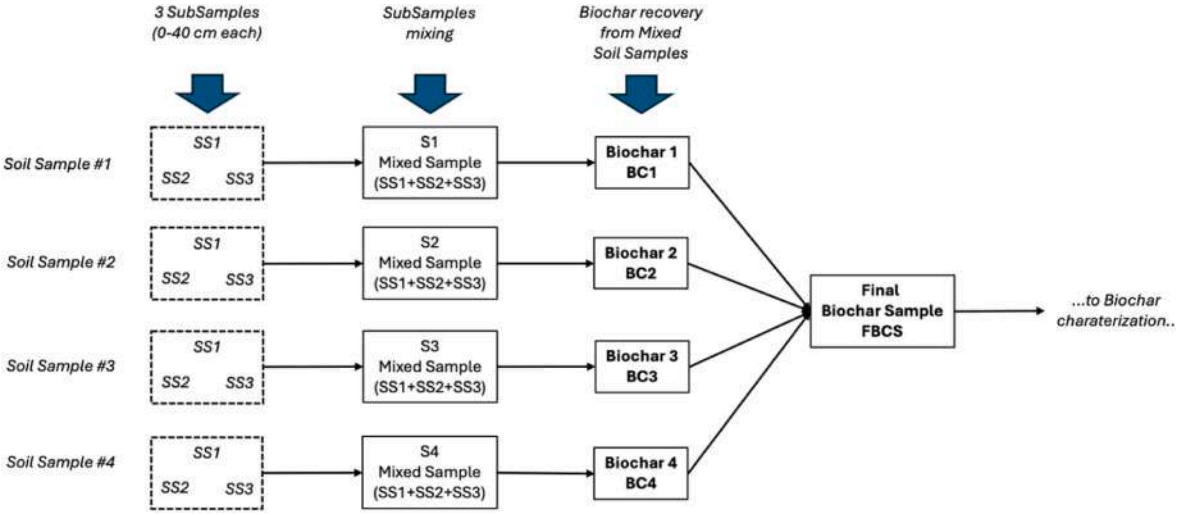


Fig. 4. Soil and Biochar sampling procedure.

Fig. 5. Auger soil sampling at La Braccesca farm (left) and image of sampled soil with biochar (right).

Fig. 6. Biochar-containing soil sample (left - visible biochar particles in red circles) and weight of one soil sample (right). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Separation of biochar and soil in the laboratory

The aged biochar enriched soil samples were spread on filter paper sheets forming a layer of few centimeters, and then air dried at room temperature for three days, following the current Italian decree D.M. 13/09/99 specifications on sample preparation. The soil sample is shown in Fig. 5, which provides visual evidence on the very small quantity of biochar which is actually contained in the sample (biochar particles indicated by red circles): this is fully consistent with the biochar weight relative to soil.

As regards soil bulk density [22], Baronti et al., 2014 measured 1450 kg/m^3 dry weight at the time of deployment, while after biochar addition and 15 years the measured density was 894–1089 kg/m^2, with a significant reduction. Considering the bulk density observed in 2009 by

Fig. 7. Soil sample manipulation.

Ref. [22], the average soil weight can be calculated as 5800 t/ha up to 40 cm depth: as the amount of biochar deployed in soil was 22 t/ha, biochar merely represents 0.38 % of soil weight in 1 ha, considering the 40 cm depth. The amount of biochar in the sample thus approximately corresponds to 6.19 g on the weight of 1630 g of the 0–40 cm soil sample (Fig. 6).

Preparing the soil for subsequent physical and chemical characterization requires other relevant steps, as soil aggregates (and some stones, as shown in Fig. 7) are mostly composing the sample. Thus, these aggregates - formed also during the air-drying process – were first manually broken: when manual manipulation was not sufficient to break the aggregates, a mortar was used to destroy the hardest ones.

Biochar particles (Fig. 8) were then collected manually by forceps until no visible biochar particles were found in the soil sample [32,33]. This work delivered 23 g of separated unwashed biochar.

2.3. *Removing soil in char: biochar washing procedure and preparation for characterization*

Collected biochar samples however still contain soil and other organic matter exogenous to biochar, which "dilute" the quantitative assessment of carbon content. It is therefore necessary to separate the biochar from this exogenous organic material.

The collected biochar particles were repeatedly washed with distilled water at ambient temperature to remove as much as possible the soil. Then, biochar was suspended in distilled water (1:10 w/v) under agitation for 1 h and then filtered at 500 μm according to the procedure reported in Ref. [32]. The recovered solids were washed again in water under agitation. The operation was repeated four times, and the final collected product was dried at 60 °C. The weight of the collected biochar therefore decreased further, from the initial 23 g to approximately 5 g.

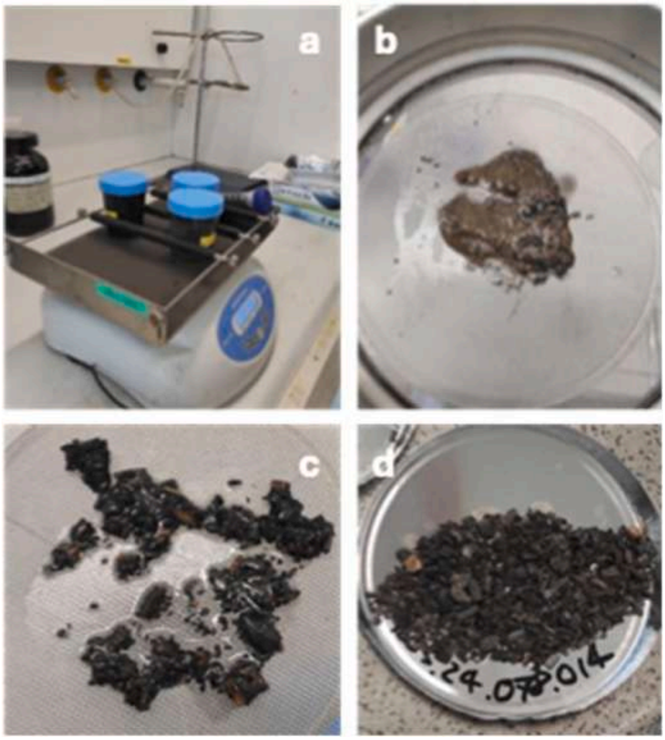


Fig. 9. Biochar washing: a) agitation step in water, b,c) biochar filtration at 500 μm on sieve d) dried recovered char after washing steps.

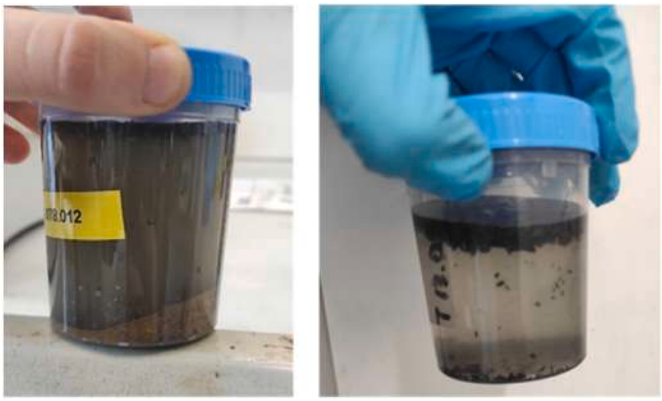


Fig. 10. Soil collected biochar in water after two sequential washing steps. The amount of soil sediments on the bottom diminished during the sequential washing steps while biochar appears as supernatant.

Fig. 8. Biochar manual recovery from soil samples.

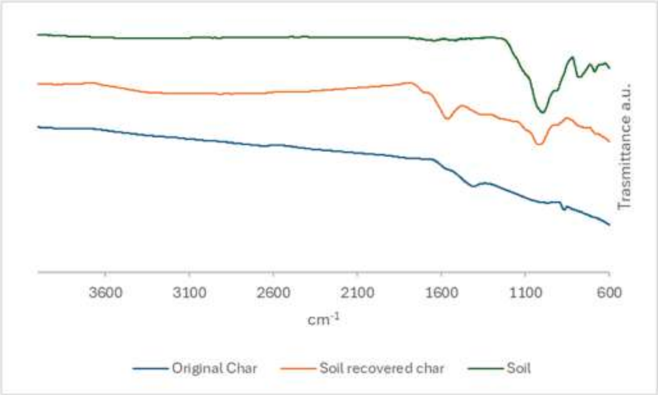


Fig. 11. FT IR spectra of the original char, the soil recovered char and soil.

The original biochar (i.e. biochar that was not used in the farm experiment) was also treated the same way (i.e. with same procedure), so to have similar conditions for all samples, allowing for proper comparison.

In addition, part of the biochar sample was also separated and collected before the washing procedure, in order to provide the possibility to compare chemical and physical characteristics with the washed sample, such as surface area (BET) and SEM analysis.

During the washing step the sedimentation of the soil part occurred while the biochar moistly floated in the water. The biochar then remained in the sieve during the filtration (Fig. 9), while the soil particles passed easily in the sieve and were removed in every washing step. The soil sediments on the bottom diminished during the sequential washing steps, confirming their increasing removal.

Being biochar mostly in the supernatant from water washing and separation, as seen in Fig. 10, also samples from the undernatant (soil) were collected for characterization, together with some unwashed biochar.

Finally, in order to apply the same procedure to all samples being analytically characterized, also straight soil sample without biochar was washed using the same procedure, and then recovered for characterization.

2.4. Analytical characterization of biochar

Proximate analysis was determined in a TGA apparatus LECO TGA701; moisture was analysed at 105 °C according EN 13040, while volatiles were determined at 900 °C according EN 18123, and ash content at 550 °C according EN 18122. Fixed carbon was calculated by

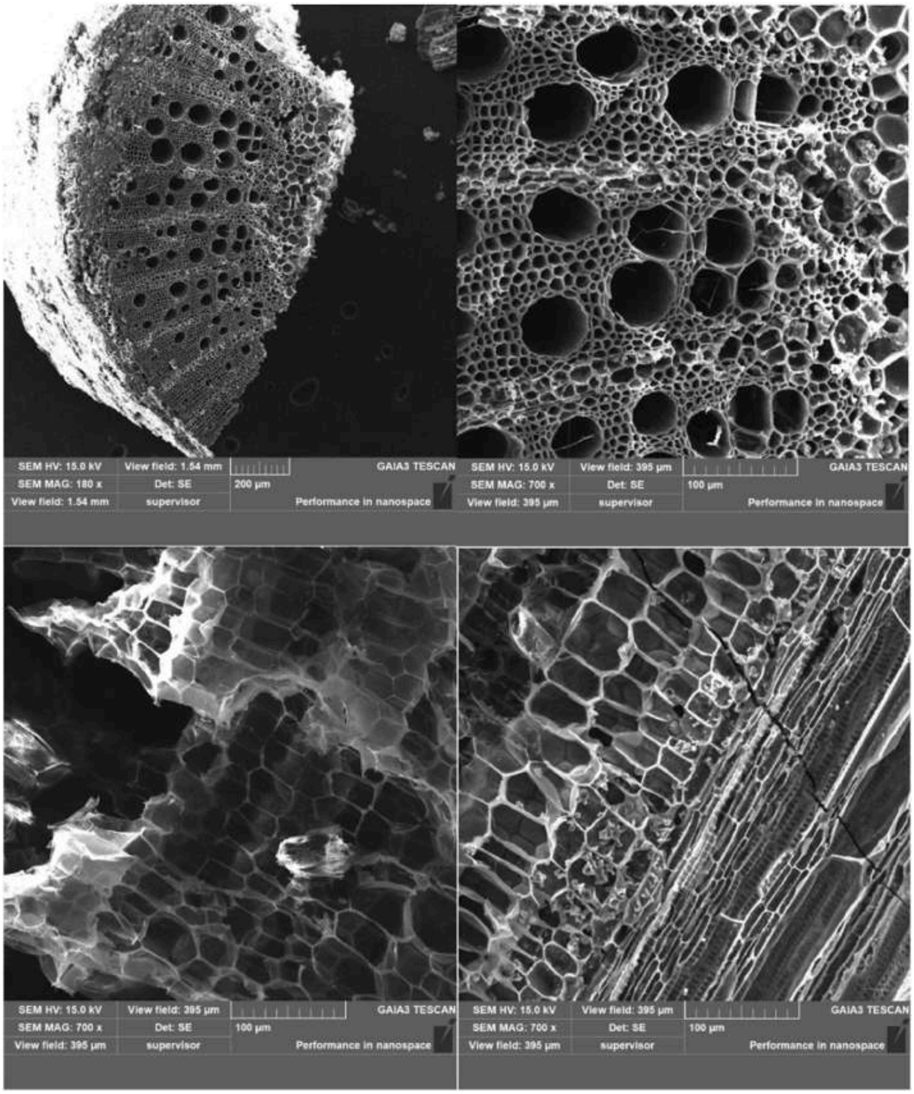
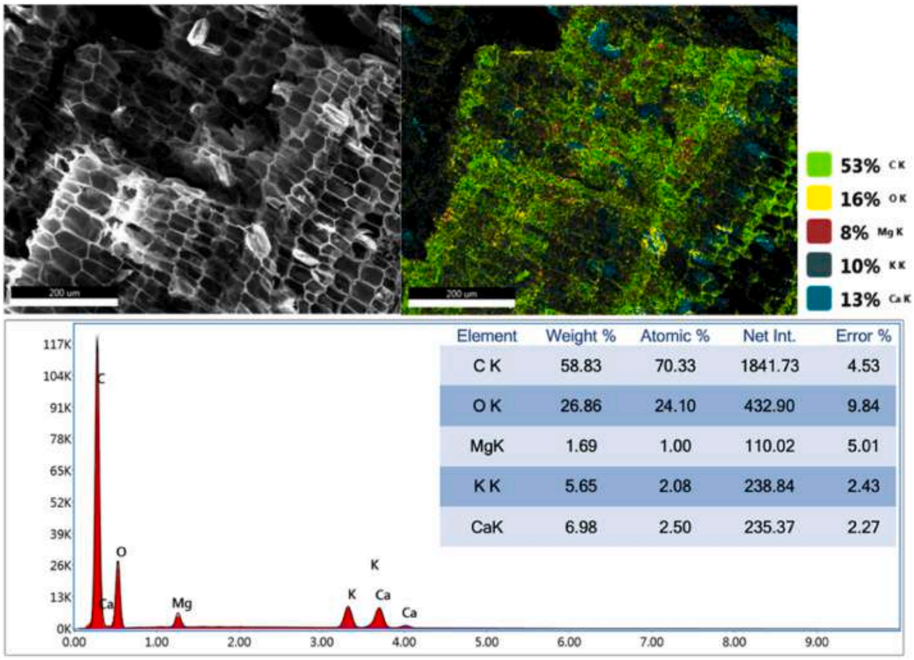


Fig. 12. SEM image of original biochar.



Element	Weight %	Atomic %	Net Int.	Error %
C K	58.83	70.33	1841.73	4.53
O K	26.86	24.10	432.90	9.84
MgK	1.69	1.00	110.02	5.01
K K	5.65	2.08	238.84	2.43
CaK	6.98	2.50	235.37	2.27

Fig. 13. EDX of original biochar.

difference from volatiles and ashes according EN 1860-2.

Elemental analysis CHN was performed in a CHN apparatus LECO Truspec CHN according EN ISO 16948 while sulphur content was determined in a LECO Truspec S according to ASTM D4239-18e. Oxygen Content was calculated by difference from CHN-S and ashes content.

Inorganic carbon was measured in a Dietrich-Fruhling calcimeter according to the current Italian decree D.M. 13/09/99 Met. V.1, and the organic carbon was calculated as difference from the total carbon analysed by CHN.

The specific surface area of biochar was determined by N_2 adsorption isotherms with Bruneuer-Emmett-Teller method (BET) in a Quantachrome NOVA 2200E instrument. Experiments were performed on 60 mg of samples preliminarily dried at 200 °C for 48 h. All measurements were performed after degassing (200 °C for 24 h) and following ASTM D6556-21.

FT-IR spectra were recorded from 4000 to 600 cm^{-1}, with a 4 cm^{-1} resolution, using a Shimadzu IRTracer-100 equipped with QATR™ 10 Single-Reflection ATR and a diamond crystal.

Scanning electron microscopy, SEM, along with Energy Dispersive X-ray Analysis, EDX, was conducted directly on the uncoated biochar samples using a TESCAN GAIA3 electron microscope operating at 15 kV and the module EDAX Octane Elect.

For Random Reflectance (R_o) analysis, the samples were first ground into a fine powder and dried in an oven at 60 °C. A portion of the samples was finely crushed to prepare them for further analysis. These particles were embedded in a cold-setting epoxy resin, forming cylindrical pellets approximately one inch in diameter. The base of each pellet was ground and polished to reveal random cross-sections of the sample fragments, preparing smooth, scratch-free surface, which is essential for accurate reflectance measurements.

Microscopic analysis was performed using both white-light and fluorescence incident-light microscopy on a Zeiss Axio Imager II microscope equipped with the Discus-Fossil system (Hilgers Technisches Buero, Königswinter, Germany) and a high-contrast 50x oil immersion objective lens. Reflectance measurements were calibrated with KB N-LASF with a R_o value of 1.317 % and Cubic Zirconia with an R_o value of 3.11 %. The random reflectance (R_o) of organic carbon fragments was determined by measuring the reflected white light from an area of approximately 0.3 μm under the microscope. To avoid distortions, reflectance was measured on highly polished surfaces. For each sample, 500 individual R_o measurements were taken across various biochar fragments to ensure statistical robustness. These results were displayed in a frequency distribution histogram, representing the range of R_o values found in the sample. The mean R_o value, calculated from the 500 measurements, served as the representative R_o for the sample. A larger standard deviation in these measurements indicated a wider distribution of R_o values and a lower reliability of the mean R_o value.

Along with point measurements, a volumetric R_o analysis was carried out by measuring R_o at every pixel across the sample surface, generating millions of data points. This comprehensive approach provided a quantitative distribution of carbon content. The frequency distribution of R_o values showed the volumetric proportions of different R_o populations in the sample. R_o values were categorized as follows: $R_o <$ 1.2 % for plant tissues, huminite/liptinite, 1.2 % $< R_o <$ 2 % for semi-inertinite, and $R_o >$ 2 % for inertinite. These classifications covered the entire solid carbon fraction in the biochar. To achieve a full characterization of the biochar composition, the reactive carbon fraction, determined through thermochemical methods, was added to the solid carbon fraction, summing to a total of 100 %.

Around 10 mg of each sample also underwent a re-pyrolysis carbon analysis using Hawk Analyzer (Wildcat Technologies, Houston, US). This process started at 300 °C for 3 min, with the temperature then increased at a rate of 25 °C per minute until reaching 650 °C. After this stage, the samples were moved to a combustion furnace where they were flushed with oxygen. The furnace temperature was raised from 150 °C to 850 °C at a consistent 25 °C per minute. During pyrolysis, the carbon emitted in the form of hydrocarbons, CO, and CO_2 was identified as the reactive carbon, expressed as a percentage of air-dried weight. What remained was considered the residual carbon fraction. The residual carbon, also expressed as a percentage of air-dried weight, was quantified during the oxidation phase, with real-time measurements of CO_2 and CO emissions used to determine the amount of residual carbon. Since the samples had low ash content, it was assumed that the contribution of mineral carbon was minimal, and inorganic carbon was not removed.

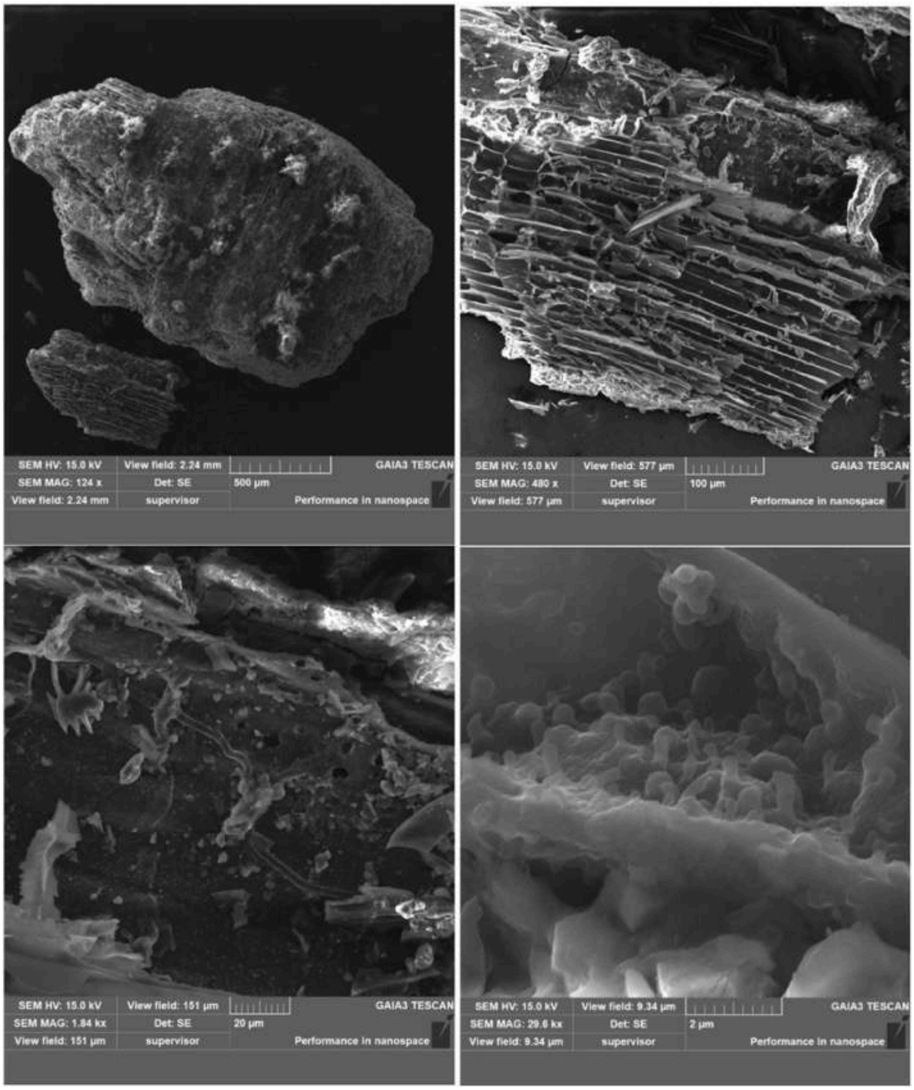


Fig. 14. SEM image of unwashed unearthed biochar.

3. Results

3.1. FT IR

The FT IR spectra of the original unused biochar, the recovered biochar and the soil were acquired and compared, as shown in Fig. 11: all samples were washed the same way according to the previously described procedure. The original biochar presented signals around 1560 and 1400 cm^{-1} related to aromatic C=C ring stretch or aliphatic C-H bending [34–36] suggesting a mainly aromatic structure not presenting particular surface functional groups. The soil sample shows evident silicate Si-O signals in the region 800-1100 cm^{-1}, which are typical of both clay and sandy soils [37]. The soil recovered biochar presents both the aromatics signals of the original char and the silicate soil signals, indicating that some soil interaction is still present in the biochar as the spectrum resulted as a sum of the original biochar, and the soil silicates. At around 1700 cm^{-1} a low signal was also recorded, probably related to C=O stretch which could be related to the presence of new functional oxygenated group on the char surfaces derived from a slight oxidation of the char or to exogenous organic material incorporated from the soil.

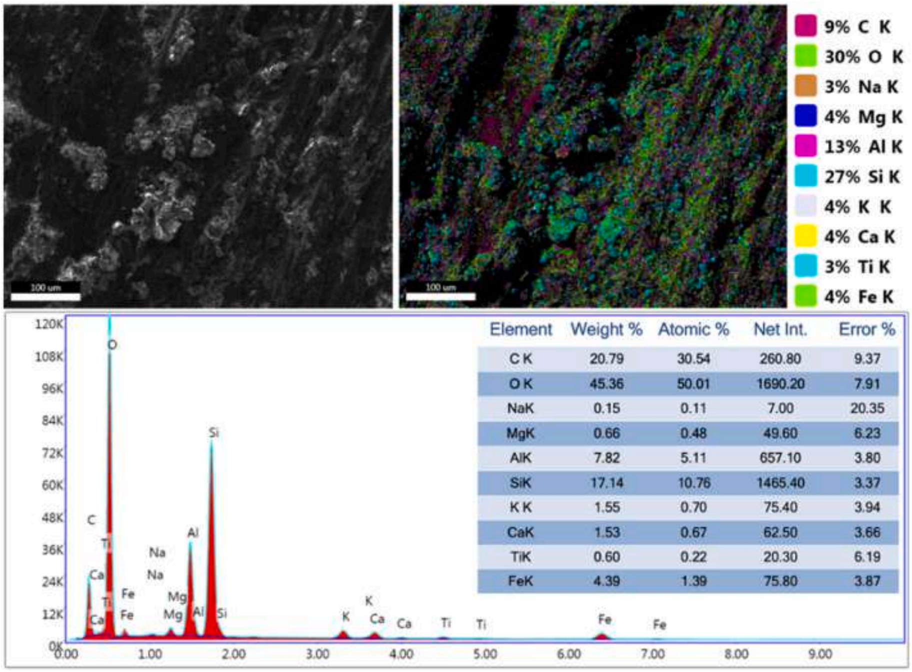
3.2. SEM-EDX (Scanning electron microscope - Energy Dispersive X-ray) analysis

SEM-EDX allows to observe the structure of the biochar down to the scale of tens of nanometers: the scope of SEM-EDX analysis in this context is to provide insights on comparing the surfaces of the original biochar, the unwashed unearthed biochar and the unearthed washed biochar. Worth to remind, the biochar that remained in soil for 15 years was subject to conventional agronomic treatments [22].

The following Figs. 12–13-14-15-16-17-18 respectively show SEM and EDX analysis for the original biochar before use, the unearthed unwashed biochar, and the unearthed washed biochar.

SEM images (Fig. 13) show the porous structure of the original washed biochar, which seems mainly unaltered compared to wood [32] as regards the pores' amplitude and disposition. Unwashed biochar images (Fig. 14) instead show pores blocked by particles and exogenous material, and larger presence of O and Si.

The washed and soil recovered biochar presented soil granules inside larger pores or fractures, while the remaining porosities appear free from soil, unaltered and consistent with the original char structures. EDX analysis on the original char shows the presence of Ca, Mg and K mainly bonded with O in forming ashes particles on the carbon structure, while in the unearthed biochar the presence of soil elements (Si, Al and O) was observed in the larger granules trapped in biochar fractures,



Element	Weight %	Atomic %	Net Int.	Error %
C K	20.79	30.54	260.80	9.37
O K	45.36	50.01	1690.20	7.91
NaK	0.15	0.11	7.00	20.35
MgK	0.66	0.48	49.60	6.23
AlK	7.82	5.11	657.10	3.80
SiK	17.14	10.76	1465.40	3.37
K K	1.55	0.70	75.40	3.94
CaK	1.53	0.67	62.50	3.66
TiK	0.60	0.22	20.30	6.19
FeK	4.39	1.39	75.80	3.87

Fig. 15. EDX of unwashed unearthed biochar.

as soil exogenous particles.

3.3. Chemical composition of investigated biochar samples and soil

The samples of original biochar, unearthed biochar and soil were subject to investigation according to the described procedure.

The analysis was carried out also on soil and undernatant obtained after washing the soil, to cross-check the carbon content. This was below 1.4 % wt in both unwashed soil and undernatant soil, thus n allowing to conclude that the undernatant is almost entirely composed by soil.

Two of the most evident differences observed in comparing the original and the unearthed biochar relate to the measured volatile and ash contents.

After such long time in topsoil, biochar showed 74 % and 75 % weight increase in volatiles and ash, respectively. This is a clear indication that - despite the intense washing procedure, and the 82 % weight reduction from unwashed to washed biochar particles - there is still a significant amount of soil and organic matter contained as exogenous material in the unearthed biochar particles, which dilutes the results of the chemical characterization.

This additional organic fraction mostly contains volatile carbon, as no biogenic carbon stabilization can occur in such a short time in the topsoil. The increased amount of labile carbon is consistent with the outcome of the SEM-EDX investigation, when observing the unwashed unearthed biochar: SEM analysis however cannot provide a view of the materials contained below the biochar surface, within the core of the biochar particle.

Measured carbon content in the original sample slightly differs from the analysis in previous literature [22], being 82.85 % instead of 77.81 %: this could be simply due to the fact that different samples were analysed in 2024 and the char results of this paper are referred to the washed sample while in Baronti 2014 was not washed before the analysis. Indeed, the referenced literature did not provide a complete ultimate and proximate analysis of the analysed biochar, thus making impossible a more extended verification. A difference in BET surface was also found (271 m^2/g for the washed original biochar, versus 410 g/m^2 of the unwashed biochar reported by Baronti in literature), which could be likely due to the disomogeneity of the two samples.

When comparing the original and the unearthed washed biochar samples, total carbon reduces from 82.85 % to 63.81 %, for the previously mentioned reasons, while also fixed carbon decreased from 71,88 %–50.92 %, reflecting the largely increased amount of volatile exogeneous material absorbed in the unearthed biochar.

Fixed carbon is a widely adopted indicator for the non-volatile carbon fraction in biochar, but as explained above, it can only provide an indication of what has happened (or not happened, i.e. low or zero devolatilization) in the biochar during 15 years of use in soil.

As a first approximation to derive information about the recalcitrant (i.e. harder to degrade) fraction of carbon contained in biochar before and after use in soil, purging from the effects of the new biogenic fraction incorporated in the biochar over the year, the ratio between fixed carbon and total carbon has been calculated, as well as the ratio removing the inorganic carbon from the calculated fixed carbon.

This parameter decreased from 0.87 to 0.80, i.e. only 7 % variation in 15 years.

Even if fixed carbon is not able to provide a quantitative assessment of the durable Carbon fraction in biochar after 15 years and separate the dilution effect of soil and exogeneous materials (which penetrated the pores beyond the external surface, as shown by the SEM investigation) from the original carbon material, this result is however a strong indication of the stability of the carbonaceous material.

More and decisive final insights on the carbon type retained in biochar can be obtained through R_o analysis. The R_o Investigation showed the following distribution, as share of carbon forms respect to total carbon content:

The detected mean standard deviation error on R_o was 3.42 % for the original biochar, and 2.39 % for the unearthed one.

The pie chart given in Fig. 19 clearly shows the amount of exogeneous organic matter that was incorporated in the biochar during 15 years in soil, and that was not possible to remove even after the intense washing that was applied to biochar particles before characterization. This exogeneous matter is diluting the carbon share in biochar: therefore, it must be excluded from quantitative accounting of carbon evolution in time for the original material.

The result (Table 3) shows that the original biochar was almost entirely composed by Inertinite fraction (94 %), small fraction of semi-inertinite (5.2 %), and negligible fraction of reactive Organic Matter (0.8 %) (Fig. 19). The unearthed biochar shows the emergence of

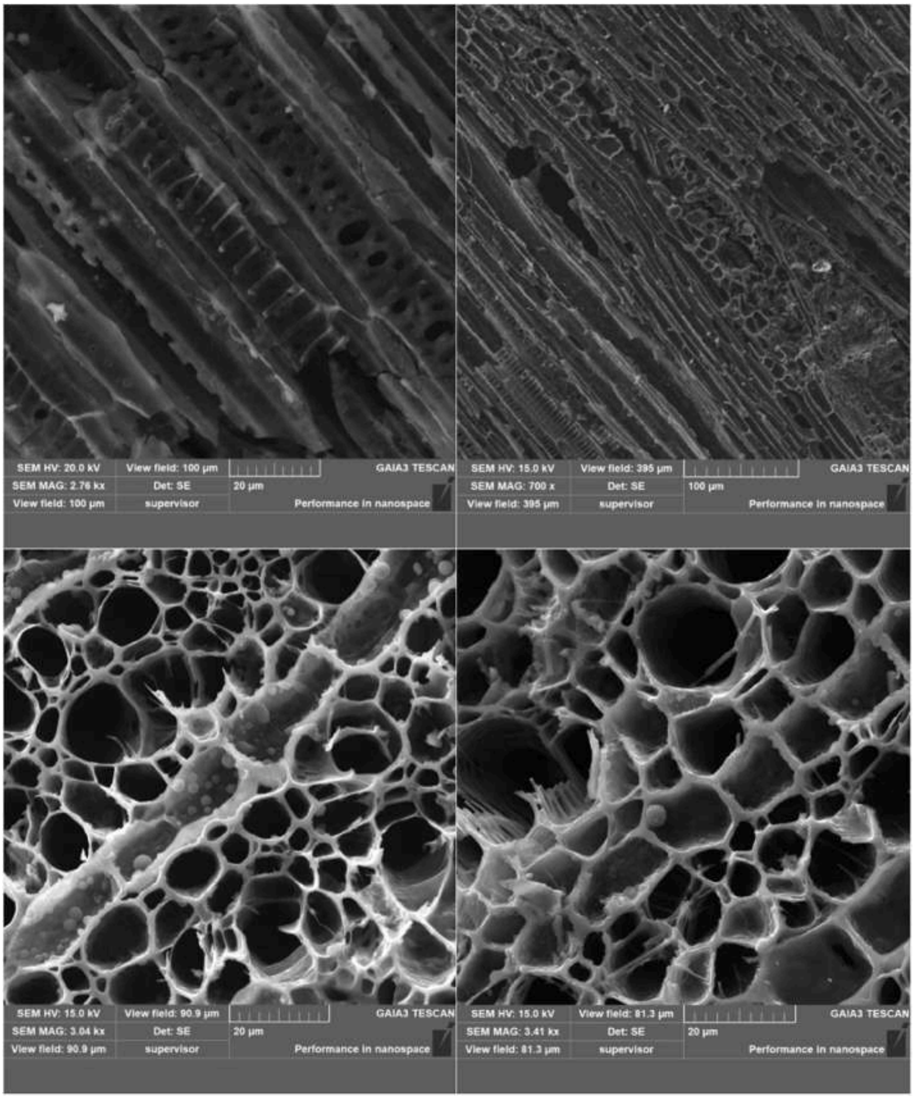


Fig. 16. SEM image of washed unearthed biochar.

huminite/liptinite (20 %) and a notable increase in the reactive organic matter fraction (3.6 %). The presence of these carbon fractions is attributed to the incorporation of soil organic matter into the extensive pore network of the biochar, suggesting they are likely exogenous in origin, and in agreement with the previously reported characterization (Table 2). This soil organic matter represents material that has been integrated over the duration of the biochar's residence in agricultural soil and is not considered part of the original biochar composition. (Fig. 19).

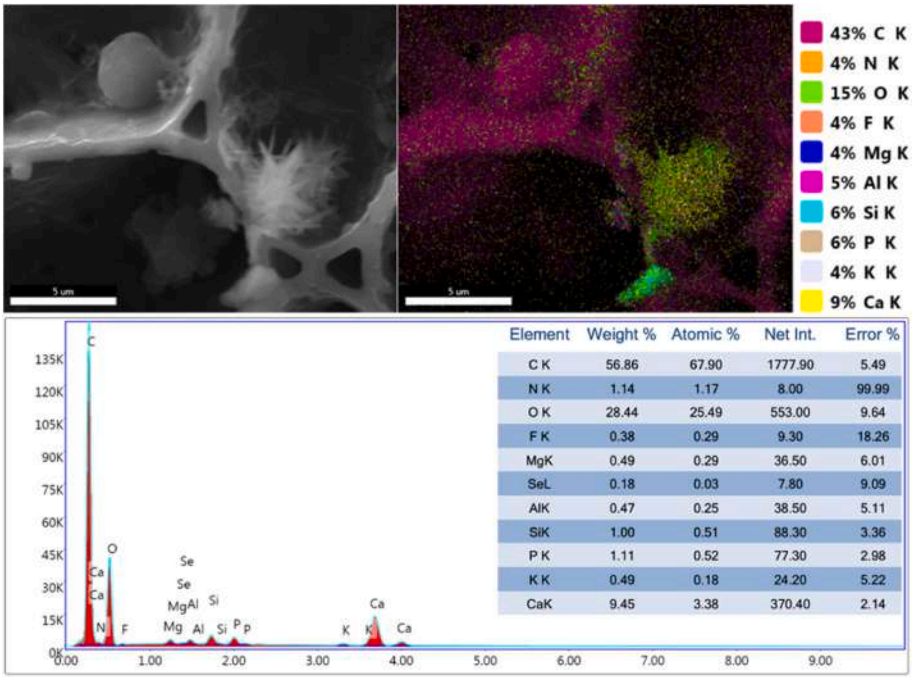
To assess potential degradation, only the relative distribution of semi-inertinite fraction to inertinite fraction should be considered, when comparing the original and unearthed biochar samples. By adjusting the carbon distribution to exclude the reactive organic matter and huminite/liptinite fractions, the results indicate that the relative distribution of semi-inertinite to inertinite has not changed significantly between the original and unearthed biochar. Specifically, there is only a slight increase in semi-inertinite (1.3 %) in the unearthed biochar compared to the original. This difference likely falls within the range of analytical error, but even if valid, it is contrary to expectations. Given that semi-inertinite represents a less stable carbon fraction, one would expect preferential degradation, leading to a relative decrease in the semi-inertinite fraction compared to inertinite. However, the observed trend suggests the opposite, reinforcing the conclusion that the semi-inertinite and inertinite fractions, and their relative distribution, have remained largely unchanged from the original biochar to the unearthed biochar, within the limits of analytical error.

Worth to remark, these figures inevitably refer to two different samples, which have been taken and treated in the same way, to make the results as much as possible comparable, but that cannot be identical by definition. Nevertheless, despite this intrinsic bias, the results are almost the same.

This analysis clearly shows how the content of inertinite fraction share did not change during the 15 years in which biochar was active in soil, a soil which was subject to conventional farming practices, i.e. tillage, fertilisation, and all the typical farming operations carried out in vineyards. It confirms the permanence of such inertinite fraction under actual agricultural conditions even over long-term cultivation.

4. Conclusions

Permanence of carbon in biochar over very long periods is subject to investigation worldwide, as a sustainable and effective method for Carbon Dioxide Removal. The decay of most durable carbon shares in biochar can be estimated by applying models, or otherwise the inertinite content can be measured by Random Reflectance. There is however lack of real field long-term experiments, assessing the permanence of the



Element	Weight %	Atomic %	Net Int.	Error %
C K	56.86	67.90	1777.90	5.49
N K	1.14	1.17	8.00	99.99
O K	28.44	25.49	553.00	9.64
F K	0.38	0.29	9.30	18.26
MgK	0.49	0.29	36.50	6.01
SeL	0.18	0.03	7.80	9.09
AlK	0.47	0.25	38.50	5.11
SiK	1.00	0.51	88.30	3.36
P K	1.11	0.52	77.30	2.98
K K	0.49	0.18	24.20	5.22
CaK	9.45	3.38	370.40	2.14

Fig. 17. EDX of washed unearthed biochar.

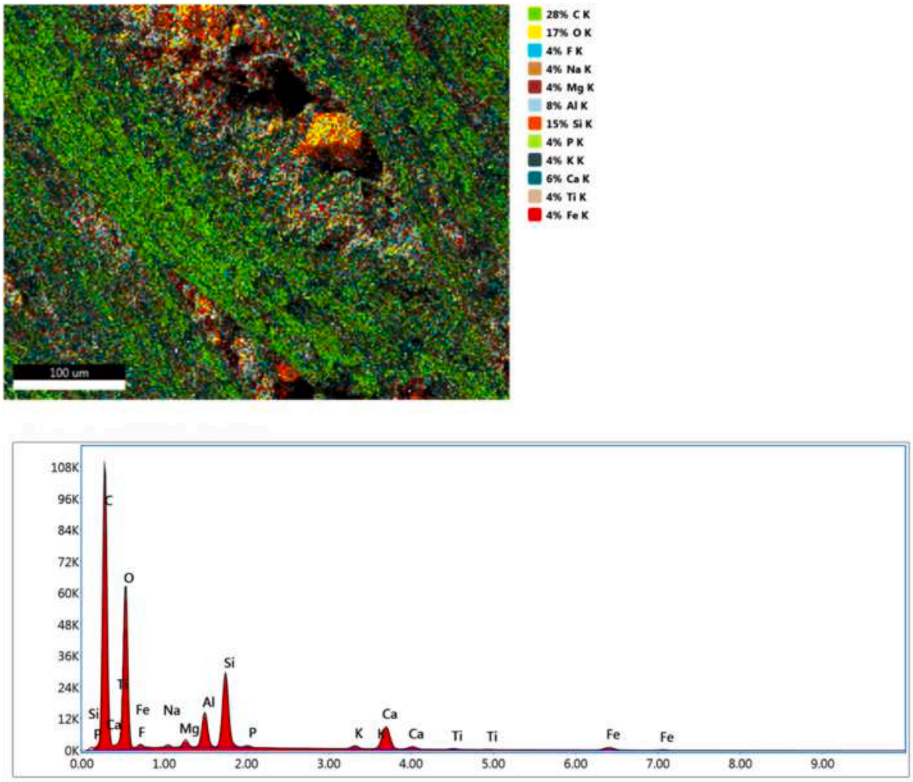


Fig. 18. EDX image and spectra in granules trapped in biochar fractures.

different fractions of carbon in biochar: lab scale incubation experiments have intrinsic limitations, not representing real field conditions.

The present work aimed at covering this gap of knowledge, applying the most recent and complete analytical techniques on biochar retrieved after long-term use in real agricultural soil, with the goal of quantifying carbon permanence.

The study considered the longest field experiment in Italy, where 22 t/ha of biochar was deployed in a vineyard which was then cultivated with conventional agricultural practices. Biochar remained in soil for 15 years: during this time, exogeneous organic and inorganic matter was incorporated in the material, penetrating the biochar structure and so diluting the carbon content in the analysed biochar particles.

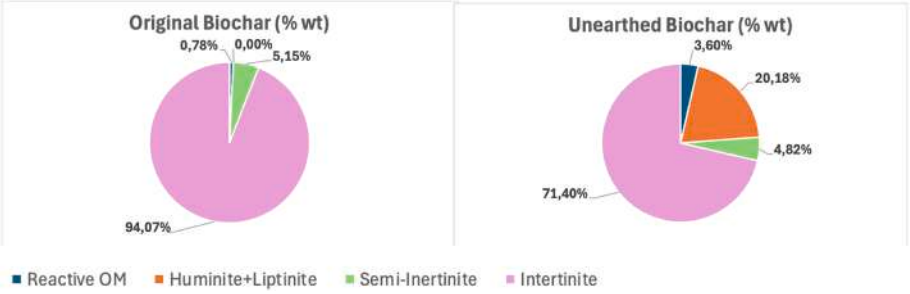


Fig. 19. Distribution of different carbon forms on total carbon.

Table 2
Analytical characterization of washed original, unearthed, unwashed and undernatant soil.

Parameter	Unit	Original biochar (washed)	Unearthed biochar (washed)	Unwashed Braccesca soil	Undernatant Braccesca soil after washing
Moisture	*wt% wb*	7.73	9.10	1.54	2
Volatile matter	*wt% db*	15.52	27.03	4.75	6.05
Ash content 550 °C	*wt% db*	12.60	22.05	95.45	94.15
C	*wt% db*	82.85	63.81	1.40	1.40
H	*wt% db*	1.15	1.46	–	–
N	*wt% db*	0.94	0.67	0.12	0.13
S	*wt% db*	0.11	–	0.02	0.10
O	*wt% db*	2.35	12.01	3.01	4.22
Inorganic C	*wt% db*	0.79	0.02	–	0.04
Corg	*wt% db*	82.05	63.79	–	1.36
BET area	$m^2\ g^{-1}$	276	91	–	–
C fix	*wt% db*	71.88	50.92	–	–
(C fix/ Ctot) ratio	-	0.87	0.80	–	–

wb = wet basis; db = dry basis.

Table 3
Semi-Inertinite vs Inertinite % fractions on Original and Unearthed biochar.

Sample	Semi-inertinite% vs Inertinite % fractions *(Corrected by removing reactive OM and Huminite/Liptinite fractions)*
Original biochar (2009)	5.5 %: 94.8 %
Unearthed biochar (2024)	6.8 %: 93.7 %

Following unearthing by soil sampling, biochar was pretreated by washing with distilled water, and analysed by physical-chemical characterization, SEM EDX, FT-IR and Random Reflectance (R_o). The characterized unearthed biochar was compared to original samples of unused biochar.

FT-IR spectra were investigated for both original and unearthed biochar, revealing exogenous matter inclusion from soil. Original biochar showed a mainly aromatic structure without functional groups, while unearthed biochar showed clear signals indicating the presence of silicates, typical of both clay and sandy soils. New functional oxygenated groups were also found on biochar surface, probably due to slight oxidation, or again to the exogenous organic material absorbed from soil.

SEM images of biochar surface showed consistent structure for both the unearthed biochar and the original biochar sample, but also showed the presence of exogeneous matter as large soil granules, still present even after repeated washing. EDX analysis showed the presence of Ca, Mg and K, mainly bonded with O, in the original char, while unearthed biochar showed also soil elements (Si, Al and O), contained in the granules present in biochar fractures.

Chemical analysis confirmed the inclusions of soil and organic matter in the original biochar structure, as both ash content and volatile matters increased (+9.45 % and +11.51 % respectively for unearthed biochar compared to original biochar), as well as oxygen (+9.02 %).

However, the ratio among fixed carbon and total carbon showed only a modest 7 % difference (87 % in original biochar versus 80 % in unearthed biochar): this is already a strong indication of the persistence of most permanent carbon share, despite the inclusion of exogeneous matter (which cannot be completely removed for chemical analysis), and the intrinsic bias given by the unavoidable fact of comparing different biochar samples.

Random Reflectance (R_o) analysis was then applied to cover the missing information: it succeeded to provide evidence of the long-term stability of the most resilient carbon fraction, i.e. the inertinite share. Comparing the original biochar with unearthed samples provided further evidence of the incorporation of exogenous carbon: in fact, huminite/liptinite fractions and minor amounts of reactive organic matter (OM) appeared in the unearthed samples. In contrast, the original biochar was almost exclusively composed by inertinite and semi-inertinite.

Thus, after removing from carbon accounting this exogenous material (i.e. not originally contained in the biochar before deployment in soil), the R_o analysis retrieved the same amount of inertinite and semi-inertinite shares before and after 15 years.

This result confirms the long-term persistence of the inertinite fraction, even under multi-year and real agricultural conditions. Consequently, the measurable inertinite component of biochar can be reliably classified within the permanent carbon removal category under Carbon Dioxide Removal (CDR) regulations.

CRediT authorship contribution statement

David Chiaramonti: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Giulia Lotti:** Writing – review & editing, Methodology, Investigation, Data curation. **Francesco Primo Vaccari:** Writing – review & editing, Validation. **Hamed Sanei:** Writing – review & editing, Investigation, Data curation.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Data availability

No data was used for the research described in the article.

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