



# Is soil sampling appropriate for quantitative carbon accounting for biochar? An experimental investigation to assess soil carbon accumulation

David Chiaramonti<sup>a,b,\*</sup>, Giulia Lotti<sup>b</sup>, Francesca Tozzi<sup>b</sup>, David Casini<sup>b</sup>,  
Francesco Primo Vaccari<sup>c</sup>, Hamed Sanei<sup>d</sup>, Michaela Luconi<sup>e</sup>, Marco Buffi<sup>f</sup>

<sup>a</sup> DENERG-Politecnico di Torino and RE-CORD, Corso Duca degli Abruzzi 24, Torino, 10129, Italy

<sup>b</sup> RE-CORD, Viale Kennedy 182, Scarperia e San Piero, Firenze, 50038, Italy

<sup>c</sup> CNR-IBE National Research Council Institute of BioEconomy, Via Caproni, 8, Firenze, 50145, Italy

<sup>d</sup> Lithospheric Organic Carbon (LOC), Department of Geoscience, Aarhus University, Høegh-Guldbergs gade 2, 8000C, Aarhus, Denmark

<sup>e</sup> Department of Experimental & Clinical Biomedical Sciences, University of Florence, Florence, Italy

<sup>f</sup> European Commission, Joint Research Center (JRC), Ispra, Italy

## ABSTRACT

Biochar, a major CDR method with significant co-benefits to agriculture, is listed as a sustainable agricultural method for SCA in sustainable biofuel regulations. In Europe, this is accounted via the  $e_{sca}$  factor (REDII-IR), while at international level this is considered through the  $F^{sca}$  factor.  $F^{sca}$  is analogous to  $e_{sca}$  in REDII, with similar, even if not identical, requirements (ICAO, for SAF). RED-II requires soil sampling to quantitatively assess the SCA from biochar addition: instead, ICAO CORSIA, as well as the draft incoming EU-CRCF (for voluntary carbon removals), require full characterization of biochar, incorporation in soil and third-party auditing during deployment (ICAO), but not necessarily soil sampling. This study presents experimental evidence evaluating the adequacy of current soil sampling protocols for the quantitative accounting of carbon saving/removals from biochar application to soil. The findings demonstrate that these protocols have intrinsic limitations, even when applied within a narrowly defined (75 m<sup>2</sup>), homogeneous, and controlled area. Key issues include the arbitrary selection of sampling locations, the limited quantity of material analysed by standard laboratory instrumentation, and the statistically insignificant variation observed in SOC and BD measurements. Measured SOC figures were inconsistent with the amount of carbon introduced through biochar amendment: the SOC content of the biochar-amended soil plot was larger than the one actually introduced and thus expected to be retrieved *via analytics*. This observation is attributed to the spatial heterogeneity of soil characteristic, and statistical significance of measured samples, in addition to the physical challenge of blending homogeneously a solid amendment (biochar) in a the solid soil phase, a limitation that cannot be entirely overcome even when employing conventional and appropriate tillage methods.

These results also raise broader concerns regarding the use of conventional soil sampling protocols for establishing SOC baselines in other (i.e. non biochar-based) carbon farming approaches. The observed high variability in carbon stock measurements hardly matches the precision required for assigning economic value. To address these shortcomings, an integrated approach combining rigorous experimental design with validated modelling frameworks is necessary to ensure scientifically robust and quantitatively defensible allocation of greenhouse gas (GHG) mitigation benefits and carbon savings/credits.

## 1. Introduction

The attention to the key role of biochar as Carbon Dioxide Removal (CDR) is rapidly growing worldwide. As the first deadlines (2030) in European Union (EU) and Global Climate targets are approaching, the need for sustainable offsetting is growing. In this context, the acronym BCR (Biochar Carbon Removal) was *ad hoc* coined to indicate the action of removing Carbon Dioxide (CO<sub>2</sub>) from the atmosphere in the form of solid organic carbon. This happens thanks to the combination of photosynthesis [1] and biomass pyrolysis (which converts the organic carbon into a more stable and durable form). BCR is therefore considered as a CDR (Carbon Dioxide Removal) technology to offset, i.e.

compensate, fossil GHG (GreenHouse Gas) emissions.

Several policies and regulations have been and are being developed to incorporate the case of biochar into actual applications. Beyond IPCC (as explained later in this paper), the Renewable Energy Directive (EU) 2001/2018 [2] (also called REDII, now amended as Directive 2023/2413, also known as REDIII [3]), as well as the United Nation International Civil Aviation (UN ICAO) methodology for CORSIA Eligible Fuel (CEF), specifically regarding bio-based Sustainable Aviation Fuels (SAF) [4] can be mentioned. All these regulations acknowledge Soil Carbon Accumulation (SCA) as carbon component to discount GHG emissions associated to biofuels produced from lands where such practices are adopted.

Biochar is considered a sustainable agricultural practice which can

\* Corresponding author. DENERG-Politecnico di Torino and RE-CORD, Corso Duca degli Abruzzi 24, 10129, Torino, Italy.

E-mail address: [david.chiaramonti@polito.it](mailto:david.chiaramonti@polito.it) (D. Chiaramonti).

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## Glossary

AT	After Tillage
BE	Biochar Europe
BCR	Biochar Carbon Removal
BT	Before Tillage
CAPE	Committee on Aviation Environmental Protection of ICAO
CCS	Carbon Capture and Sequestration
CCU	Carbon Capture and Utilization
CDR	Carbon Dioxide Removal
CEF	Corsia Eligible Fuel
CORSIA	Carbon Offsetting and Reduction Scheme for International Aviation
CRCF	Carbon Removal and Carbon Farming

DACS	Direct Air Capture and Sequestration
FB	Biochar applied on the field
GHG	GreenHouse Gas
ICAO	International Civil Aviation Organization
ILUC	Indirect Land Use Change
LB	Biochar applied to soil samples in laboratory
MRV	Monitoring, Reporting and Verification
RED	Renewable Energy Directive
SAF	Sustainable Aviation Fuels
SCA	Soil Carbon Accumulation
SOC	Soil Organic Carbon
UN	United Nations
WG	Working Group

generate GHG emissions savings and thus reduce the carbon intensity of the biofuels. As regards the ICAO-CORSIA initiative, the new CAEP 14 WG5 cycle will thus address the Implementation of the methodology developed during CAEP 13 cycle. It refers to land that was agricultural land in 2018, and feedstocks produced in unused land, including degraded land. Currently, WG5 is extending the approved methodology to post-2018 methodology, so to include ILUC/DLUC components as prescribed by CORSIA. At EU level, biochar is already recognized as an option to generate carbon savings, such as substituting fossil carbon in steel making (EU ETS mandated carbon market). It is also worth mentioning the significant effort being carried out by the European Commission DG Clima, through the Carbon Removal and Carbon Farming (CRCF) [5,6] initiative.

As regards REDII, SCA is computed in the GHG performances of the biofuel through the parameter  $e_{sca}$ , which accounts for the net emissions savings achieved by accumulating carbon in soil. Initially, REDII did not include biochar among the list of the sustainable agricultural practices: this SCA method was introduced in the following REDII-Implementing Regulation in 2022 [7]. Today, the latest revision of the Renewable Energy Directive, REDIII, entering into force in May 2025, makes the GHG performances of the biofuel even more important. In fact, in REDIII EU Member States can choose either the energy target of 29 % of renewable energy in transport, or a target based on the GHG intensity reduction (set at 14.5 %) for the transport sector. This second option, based on GHG CI reduction, will reward the most performing sustainable alternative fuels [2,3].

Recently, the ICAO-CORSIA programme, through the Fuel Transport Group (FTG) activity during the last cycle of the Committee on Aviation Environmental Protection (CAEP/13, 2022–2025), elaborated and approved a methodology for SCA. Here, biochar is included among the sustainable agricultural practices and can be accounted in the SAF GHG performance through a parameter named  $F^{sca}$ .

The two regulations, despite the many similarities, contain some key differences, the most relevant probably being the following:

- REDII-IR prescribes that biochar (which must be sustainably sourced) can be considered up to a maximum of 45  $g_{CO_2e}/MJ$  in the carbon intensity accounting of the biofuels. This figure is the highest among all the sustainable agricultural practices.
- In ICAO, on the contrary, no threshold (i.e. limit) is set.
- The ICAO methodology, however, requires that the biomass used to produce the biochar must come from the same field where the feedstock to produce the sustainable biofuels originates.
- While in RED the ILUC component is not included in the GHG calculation of the biofuel, ILUC is instead part of the ICAO CoreLCA methodology.
- In the currently approved version of the ICAO Methodology, the SCA component ( $F^{sca}$ ) can be only considered for “fields that were already

cultivated before January 1, 2008”, or “feedstocks produced in unused land, including degraded land”. This because no Indirect Land Use Change-ILUC applies to these cases, according to ICAO rules.

- The new CAEP 14 cycle will address the post-2008, as well as other aspects related to modelling, in the newly set WG5.
- As specifically regards biochar long-term durability, verification on Carbon permanence is not requested by the REDII-IR, while in ICAO it must be accounted for via analytical techniques as inertinite benchmarking (IBR<sub>02</sub>) [8] or estimated by using a factor with an empirical equation ( $F^{perm}$  factor) [9,10].
- ICAO, in a conservative approach, includes a 15 % CCF (Conservative Correction Factor), which proportionally reduces the effect of the SCA component in the GHG emissions calculation formula for sustainable agricultural practices. However, for the specific case of biochar only, given the assessment done via RR analysis or  $F^{perm}$  calculation, this CCF reduction does not apply. Instead, this CCF element is not present in REDII-IR.
- REDII-IR requires soil sampling to compute the  $e_{sca}$  component also in the case of biochar, despite the scientific inadequateness of soil sampling in quantitative biochar-in-soil accounting (the scope of the present work is to provide evidence of this). On the contrary, ICAO is not requiring soil sampling to quantify the SCA effect and therefore compute the  $F^{sca}$  factor, but requires that the biochar is fully characterized and deployment is audited (in order to verify the actual amount that is deployed).

A relevant common point to both regulations lies in the fact that baseline calculation is not requested for the biochar case. In fact, to quantitatively assess the carbon accumulation effect obtained through sustainable agricultural practices, a reference carbon stock baseline must be defined. In case of biochar, instead, being this exogenous carbon added to the topsoil, all this carbon can be considered additional, thus setting a reference SOC baseline becomes unnecessary. This is another significant cost-saving advantage offered by biochar to economic operators and stakeholders.

The potential impact of the SCA component ( $e_{sca}$  and  $F^{sca}$ ) from biochar can be groundbreaking in the biofuel sector, since in many cases there is more carbon in the agricultural co-products rather than in the main product destined to biofuel production (such as lipids or sugars). When this carbon is considered in the formula calculating the GHG balance of the biofuel, as in the case of REDII-IR or ICAO methodologies, the impact can be large, even to making the value chain as carbon negative. In such a case, the production of sustainable biofuels removes CO<sub>2</sub> from the atmosphere through the photosynthesis process, acting as a sort of natural DACS (Direct Air Capture and Sequestration) or bio-CCS (Carbon Capture and Storage). Biochar, when produced from sustainable biomass, thus allows to deploy large amounts of carbon in soil in a safe and very controlled mode, and can offer a very significant

contribution to cost-effective, environmentally friendly carbon removals.

A new relevant EU regulation, already mentioned, is the EU CRCF, under development by EC DG Clima, where Biochar Carbon Removal (BCR) should be included in the category of Permanent Carbon Removals. However, only the long-term durable carbon fraction can be accounted as permanent CDR (a condition not yet requested in the REDII-IR).

This fraction can be measured through the inertinite benchmarking method (IBR<sub>02</sub>) developed by Sanei [8], also experimentally assessed by investigating 15-years old biochar unearthed from soil [11].

As an alternative to the inertinite benchmarking (IBR<sub>02</sub>), the IPCC method of biochar decay as defined by Woolf et al., 2021 [12] can be used to estimate the permanent fraction. However, evidence exists today that the model, in a conservative approach, underestimates the duration of this carbon share in the biochar [10]: thus, an update of the IPCC model can be expected in the future.

It is very important to remark that IPCC since 2019 addressed biochar as a CDR method, also providing a formula cited above. There is thus scientific consensus on the role BCR can play in the climate context [37].

Also, there is scientific consensus about the long-term duration of biochar in soil among different scientific disciplines [38]. The EU Scientific Advisory Board and CESifo, the international platform of Ludwigs-Maximilians University's Center for Economic Studies and the ifo Institute, have included biochar in the list of long-term carbon removal techniques [39,40].

In the European regulation, biochar was added to the REDII-Implementing Regulation in 2022 [7], setting the threshold of 45 g<sub>CO2</sub>/MJ, higher than any other agricultural sustainable management practice (for which threshold is established at 25 g<sub>CO2</sub>/MJ). However, since biochar was added to REDII-IR only at a late stage, when the regulatory scheme was already well defined, this implementing regulation still requests to quantify the SCA from biochar via soil sampling, applying the same method as for all the other sustainable agricultural practices. These other types of SCA, however, refer to a distributed form of organic carbon in soil, while biochar is instead a concentrated type of carbon added to soil, thus, exogenous to soil and in particle form. Quantitative accounting via this method is not possible for the scope of allocating precise amounts of carbon savings/credits, as we demonstrate in this paper.

A further element to consider is the typical amount of biochar weight deployed in the 30 cm topsoil versus the soil weight per ha, so to have a clear understanding of the order of magnitude of these masses. Even when biochar is added in rather large quantities (e.g. up to 40–50 t/ha), it still represents a very minor (if not negligible) quantity compared to the typical weight of 30 cm topsoil over 1 ha. Estimating this weight between 3000 and 4000 t/ha (which can vary depending on the soil's bulk density and soil type), biochar will represent a weight fraction ranging from 0.5–0.67 to 1.0–1.3 % w/w, assuming for instance 20 to 40 t/ha amount of distributed biochar. Moreover, as said, it is a concentrated form of carbon, not uniformly distributed in the topsoil (differently from two liquids, for instance, which can perfectly mix). Therefore, soil sampling can, or cannot, find the biochar in the sampled amount depending on the exact point where the sample is actually taken, and the specific point where the very small micro sample is taken from the soil sample. This is what actually goes into the analytical instrument used for the determination of the carbon content, as discussed in this research work. Results thus depend on the specific location where the sample is taken from both the soil and the soil carrot (two unavoidable biases), and thus how the analytics are carried out.

Both ICAO SCA methodology and, so far, the draft CRCF methodology (developed over the 2024–2025 period), contrary to REDII-IR (released in 2022), benefited instead from latest research finding on the subject, and for this, they specifically treat the biochar case: if biochar is properly characterized and certified, as well as the deployment

(in soil and not above soil) is third-party audited, this is sufficient towards Carbon accounting, and soil sampling is not required. This recognition acknowledges the impossibility to quantify the amount of biochar deployed in soil by sampling. However, this scientific inconsistency is still present in the current version of the REDII-IR, which would indeed require reconsideration to be consistent with science and aligned to other works on biochar carbon accounting within the RED methodology [36].

Despite this scientific methodological evidence about the impossibility to quantitatively account for biochar content by soil sampling for carbon saving/credits, several research works in soil science and biochar investigated the evolution of carbon stock from various sustainable agricultural practices, including biochar addition, and derived consideration on carbon permanence by soil sampling. In the case of biochar, however, this brought to estimating decay rates inconsistent with the physical and chemical structure of this carbonaceous product. The problem was related to the randomness in retrieving the biochar particles in the soil sample, depending on the exact point where the sample was taken, as well as in the (dynamic) variation of the reference SOC baseline over time.

Mertens et al. [13] in 2016 studied extensive pomiculture marginal soils, through a field experiment lasting 16 months. The scope of the work was to investigate the effect of using biochar, clay substrate and goat manure as soil conditioners on soil physical parameters of this sandy Brazilian soil and on seedling performance of *Spondias tuberosa* Arruda: the assessment of biochar permanence was thus not the main objective of this work. Biochar from a local charcoal kiln was added at 5 % volume level to some of the studied treatments: pyrolysis temperature and residence time were not recorded. Sixteen months after the planting holes had been refilled, additional soil samples were taken. Among other parameters, carbon I stock was measured via gas chromatographic technique, and the C stocks variation assessed. The work observed a loss of 51.4 % of C stock (all treatments determined a loss of C stock during the complete cycle of dry and rainy season) after only 16 months. However, this result cannot be attributed to carbon degradation in biochar, as no evidence was provided, nor the ratio among labile organic Carbon fraction in biochar and in soil (and the evolution during the 16 months experiment) and the most durable Carbon share added with biochar: also, no characterisation of carbon forms in biochar itself was carried out, not being this the scope of the work. Therefore, no conclusion on the origin and type of the lost Carbon can be derived, nor it can be assigned arbitrarily to biochar: moreover, any scientific literature on biochar permanence reports such extremely fast degradation [14–19], even for very low quality biochars. Specifically on the durable carbon fraction in biochar, there is scientific evidence of the long term permanence of this carbon share, and the double first-order model to predict the carbon decay in biochar, which has been and is still used to date, is now examined in literature [10,11,20,21] as it is underestimating the permanence of the most durable carbon shares. In addition, when investigating SOC variations (i.e. beyond the added biochar), it is not unusual to observe changes that are large and/or non-statistically significant, or SOC that during the first phase of the implementation of the new sustainable agricultural practice initially decreases, to then increase again in the following years (thus, SCA takes place on a medium term) [22,23]. For this reason, the ICAO methodology prescribes assessments of the impact of non-biochar sustainable agricultural practices on SCA at regular intervals not greater than 5 years from the start of the practice.

Beush et al. [24] in 2018 investigated nutrient retention potential in the same sandy soil of Mertens et al. [13], a semi-arid seasonally dry tropical forest, by combining locally produced biochar and clay sediments and low-cost planting techniques. However, differently from Martens, Brush also stated that the objective of adding biochar in this work was to increase the C-content of the Arenosol under. This article reports again the 51.4 % loss of C stock, but it arbitrarily allocates this loss to a rapid decomposition of the biochar: however, the work does not

provide any evidence to confirm that this extremely high loss is due to biochar decomposition. Biochar was not assessed against the forms of carbon shares, nor the biochar was retrieved from soil after 16 months and compared to the initial material, as done after 15 years of permanence in soil in a cultivated vineyard by Chiaramonti et al. [11]. The pyrolysis temperature of 450 °C was not measured, but only estimated based on C, H, O, VM, ash, O:C and H:C. Volatile matter content of biochar was 19.1 %, molar H:C ratio equal to 0.46, indicating a low-quality product, obtained from low to medium temperature pyrolysis. Noteworthy, Beush reported the dimensions of the planting holes, to which biochar was added at 5 % in volume: 0,155 m<sup>3</sup> (0.6 diameter, 0.55 m depth). This aspect is particularly relevant for the analytical procedure of SOC determination, as explained later in this work and illustrated in Fig. 8.

Similarly, Singh et al. [25], which carried out a 5-years incubation experiment in vertisil, assumed the labile biochar fraction by fitting the two-pool exponential model, which is known to underestimate the permanence of most the durable Carbon fractions [10,11,20]. Authors themselves already commented, in their 2012 paper [25], that their estimates “represent MRT of relatively labile and intermediate-stability biochar C components”: and, in fact, their analysis showed a mineralization rate (as defined in soil science, i.e. conversion of C to CO<sub>2</sub>) almost equal to zero after 260 days of incubation in average, which is consistent with a low quality – low temperature biochar).

More recently, Gross et al., 2024 [26] carried out a research work on two long-term field experiments in Germany, where biochar was applied 12 and 14 years ago in the rather large amount of 31.5 t/ha (loamy soils) and 40 t/ha (sandy soils) respectively. Biochar (produced at 550 °C and 540 °C, H/C = 0.11 and 0.1–0.2 for the two sites) was used together with compost in loamy soils, digestates, compost or synthetic fertilisers on sandy soils. The study is a very accurate systematic investigation, providing data. However, as for the previously mentioned studies, biochar was not characterized as regards the type of carbon fractions contained. The assessment of Carbon stock (SOC) and from this the estimates on carbon permanence in biochar were done by soil sampling (a single composite sample), bringing in the analysis the already mentioned limitations and bias intrinsic to the use of soil sampling for quantitative and not qualitative biochar-derived SCA assessment. Also in this case, biochar was not retrieved from soil and compared to the original product, in particular as regards the carbon forms contained in it.

Overall, the methodology adopted in these works, i.e. soil sampling, is not adequate to provide quantitative and statistically representative assessment of the amount of organic carbon in soil when biochar is used as soil amendment, for the reasons that will be demonstrated hereafter in this experimental work: conclusions on biochar mass loss by soil sampling should be re-examined.

The scope of this work is to provide systematic experimental evidence and demonstrate why soil sampling (as well as open field experiments) is not suitable for quantitative assessments of BCR (while it is used to qualitatively report averaged regional SOC variations), identifying the barriers on using this method for assessing carbon saving and credits, as CDR. This work also shows how this requirement from the EU REDII-IR, the current EU regulation in force, might hamper investments.

Finally, this study provides original and systemic data analysis on soil sampling and carbon analysis (before the addition of any biochar) with a higher level of detail than requested by REDII-IR and the draft CRCF, showing the difficulty in defining statistically significant baseline figures on SOC, suitable for the following accounting of carbon credits based on sustainable agricultural practices. A well-designed mix of experimental and validated modelling is probably the only feasible solution to address this issue.

To the authors' knowledge, this is the first time such issue on biochar quantitative assessment versus soil sampling is systematically addressed towards the EU legislation, providing experimental evidence why this method in open field is unsuited for the scope, supporting the

methodologies adopted in ICAO and the draft CRCF. This work therefore identifies real, operational barriers for biochar introduced by the current methodology required by the EU REDII-IR legislation.

## 2. Materials and methods

### 2.1. Biochar production and characterisation

The feedstock selected for biochar production was alder (*Alnus* Mill.) wood chips, sourced locally and supplied by AgriAmbiente Mugello. Alder was specifically chosen because it is a typical fast-growing hardwood, representing a major category of woody biomass, and for its easy availability within the Tuscany region. The raw feedstock was processed using an industrial chipper (LAIMET 400, that processes logs up to 400 mm of diameter) and, subsequently, chips were dried to a target moisture content of about 15 %, to be fed to CarbOn. Prior to conversion, the feedstock was analysed and the main observed characteristics were as follows: ash content of 0.6 % (w/w db) at 550°C, 81.3 % (w/w db) volatile matter, a bulk density of 152 kg/m<sup>3</sup>, and an elemental composition of 49.6 % (w/w db) C, 5.9 % (w/w db) H, 0.1 % (w/w db) N, and 0.09 % (w/w db) S. The content of elements and metals was also analysed, and no relevant heavy metal contamination was observed.

The CarbOn unit is a small-scale oxidative fixed-bed prototype carbonizer that was designed, developed, and operated by RE-CORD. Over time, the system has been optimized for deployment in forestry operations, thanks to its innovative nature (oxidative configuration). For this end-use, the system is skid-mounted and can be transported on site. CarbOn utilizes fixed-bed, open-top, downdraft technology to perform the oxidative slow pyrolysis process. The externally insulated reactor is a cylindrical chamber where biomass conversion occurs in a controlled oxidative environment; operating in autothermal slow pyrolysis mode, the plant can achieve a peak temperature of approximately 650 °C. The solid residence time within the reactor is about 3 h, followed by 2 h in the cooled discharge section. A detailed description of the process and the facility is already documented in the scientific literature [27–29]. Based on the quality of the woody feedstock fed to the reactor, and the numerous experimental campaigns, CarbOn has demonstrated its ability to consistently deliver high-quality biochar, compliant with the major international quality standards and EU Regulation 1009/2029. Biochar was characterised chemically, physically and toxicologically to verify its agronomic suitability, in accordance with the Italian Legislative Decree n.75/2010 (Annex II, category 16) [30] and with the European Regulation (EU) 2019/1009 [31] setting limits for the use of biochar as a soil organic amendment. Full details of the biochar characterisation are reported in Table 1S of the Supplementary Materials, while the main parameters are presented in Table 2.

### 2.2. Rationale and description of the experimental methodology

The experimental campaign was conducted at the Azienda Agricola Villa Montepaldi, an experimental farm owned by the University of Florence, located in the Chianti Region (San Casciano Val di Pesa – Florence, Italy). The selected field (Lat: 43°39'42.6"N; Long: 11°08'31.2"E) was situated in the flat area of the property next to the Val di Pesa River and it has remained uncultivated for over 20 years. The activity performed on the land a few times each year involved the cutting of weeds; therefore, no fertilization or addition of organic amendments has occurred during this period. The soil was classified as a loamy loam texture (34 % sand, 43 % silt, 23 % clay), with a high skeleton content (>16 %) and limestone (>30 %) and a neutral pH of 6.9.

A homogenous area in terms of pedological conditions and light exposure was selected for the experiment: it was then divided into 3 experimental plots of 25 m<sup>2</sup> (5 × 5 m) each, again with uniform soil properties, soil slope and exposure. A space of 5 m was left between the plots to minimize border effects from the treatments. The 3 experimental plots were designated as follows: Plot 1) Control treatment (CK



treatment), where biochar has not been added; Plot 2) Lab Biochar treatment (LB), where the application of the biochar occurred in the laboratory on the soil samples collected after the tillage; iii) Field Biochar treatment (FB), where biochar was into the soil with the tillage operation (Fig. 1). Both LB and FB treatments were treated with biochar at a rate equivalent to 10 t/ha on a dry basis (db).

Soil sampling was realized in each plot in two different steps, but during the same 22–25/7/2024 week: therefore, no priming effect needs to be taken into account for this work. The first sampling occurred on the undisturbed soil, before tillage. The second sampling was realized immediately after the tillage operations on tilled soil. The tillage was realized using a ripper equipped with a spiked roller, which tilled the soil to a depth of 30 cm. Since tillage changes soil bulk density, all the plots were tilled the same way, to maintain methodological consistency. Soil sampling before and after tillage was realized following the same methodology.

The procedure to collect soil samples consisted in recovering 5 independent samples for each plot within the first 30 cm depth (Fig. 2). The soil samples harvested before the tillage (BT) were named as follows: CK-SO-BT, CK-LB-BT, CK-FB-BT, collected from each plot. Samples collected after the tillage (AT) were: CK-SO-AT, BC-LB-AT, BC-FB-AT, collected from each plot. Therefore, 15 soil samples of undisturbed soil were collected before tillage, and 15 disturbed soil samples were harvested after the tillage (see Figs. 1 and 2).

Considering the methodology prescribed in the EU REDII-IR, a minimum of 15 “Sub-samples” must be collected over 5 ha of homogeneous area, still considering 30 cm of topsoil. With the term “sample”, REDII-IR means the set of 15 Sub-samples, then combined and mixed all together in a single “Sample”. In this experiment, instead, with the term “Sample” we identify what is defined as “Sub-sample” in RED. In order to maintain a higher degree of accuracy of the analysis, the 15 samples have been kept separated and independently investigated and characterized, without mixing these to obtain a single sample. Thus, while in REDII-IR one single sample refers to 15 subsamples mixed and obtained from a homogeneous area of 15 ha, here we have kept separated the 15 samples, over a much smaller and significantly more homogeneous area of 75 m<sup>2</sup> only. The level of the accuracy used in this work is thus considerably higher than what prescribed in the REDII Implementing regulation [7]. The density of soil sampling in our experiment is in fact orders of magnitude higher than what prescribed by REDII-IR: sampling 15 points over an area of 75 m<sup>2</sup> is theoretically equivalent to sampling 10.000 points on a 5 ha area.

A summary comparing REDII-IR prescription and our work is given in the next Table 1:

In this experiment, the first sampling point was taken at the centre of each plot, while the remaining four were collected 2 m away from the

central point, along the cardinal directions (North, East, South, West) (Fig. 1). Prior to performing the first sampling, vegetation was removed using scissors [32]. Soil sampling followed the undisturbed (intact) core method, which involves collecting soil using a metal ring pressed into the ground according to indications provided by the EU Joint Research Centre (JRC) [32].

As explained before, a key characteristic of the experiment lies in the fact that all activities were conducted during the same day: from BT soil sampling, to tillage, to biochar incorporation, to BT soil sampling. In addition, no other amendments were used beyond biochar, or other agronomic interventions. Only biochar can thus be responsible for observed change of carbon stock, or the variability of SOC itself before the biochar addition (i.e. independently from the exogenous carbon added via biochar). Moreover, any degradation of biochar is not possible in such a short time.

### 2.3. Soil sample preparation and biochar addition

Soil sampling was carried out by applying the undisturbed (intact) core method, through a metal ring pressed in the ground, according to JRC 2018 indications as shown in Fig. 3 [32]. Three consecutive sub-samples were collected at the same sampling point at the following depths: 0–10 cm, 10–20 cm, and 20–30 cm. More in detail, once the ring was inserted into the soil using a hammer, the surrounding soil was carefully removed with a spade to facilitate the core extraction. The metal ring was then lifted, and its intact contents were stored in a plastic bag. The excess soil at the bottom of the ring was manually trimmed using a knife. Given the ring height of 10 cm, three consecutive cores were collected from the same sampling point, representing depths of 0–10 cm, 10–20 cm, and 20–30 cm. Thus, from the same sampling point, three sub-samples were taken and placed together in the same labelled plastic bag in order to represent a volume of soil taken over the first 30 cm of soil.

Bulk density was determined on the raw soil material as collected in the field (ISO 11272:2017). Soil volume was equal to the sum of the 3-cylinder volumes, while soil weight (including stones and particles larger than 2 mm) was taken after drying the soil sample.

The sample preparation was realized according to the ISO 11464:2006, where samples were weighed to determine the fresh weight and then they were left air dried for 3 days. After, samples were weighed again to determine the dry weight for the bulk density calculations.

Stones and soil exogenous materials (i.e. glass or rubbish) were manually separated from the dried soil samples, while soil aggregates were crushed using a mortar [33] and sieved at 2 mm. Concerning the samples containing biochar, the crushing involved also the biochar

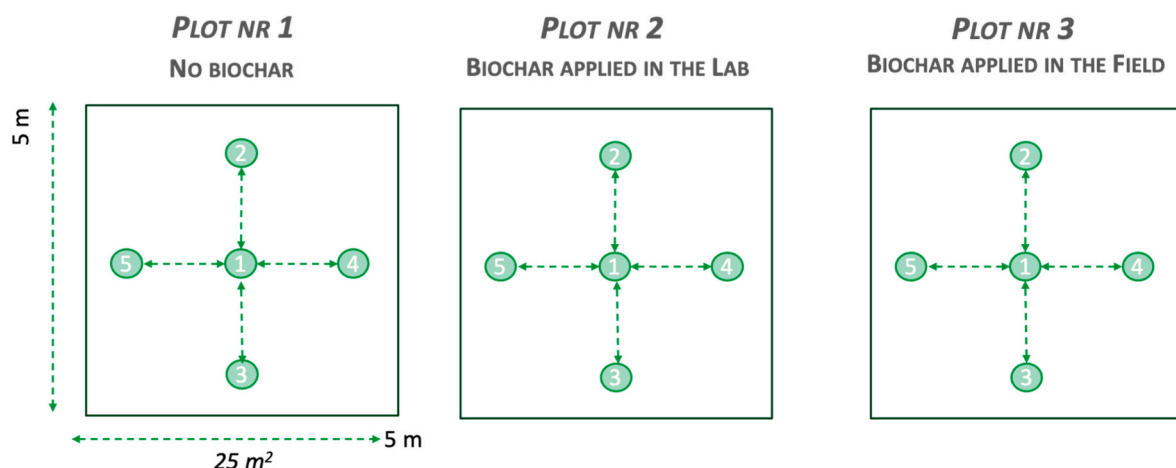


Fig. 1. Description of the three plots and the five independent samples for each experimental plot.

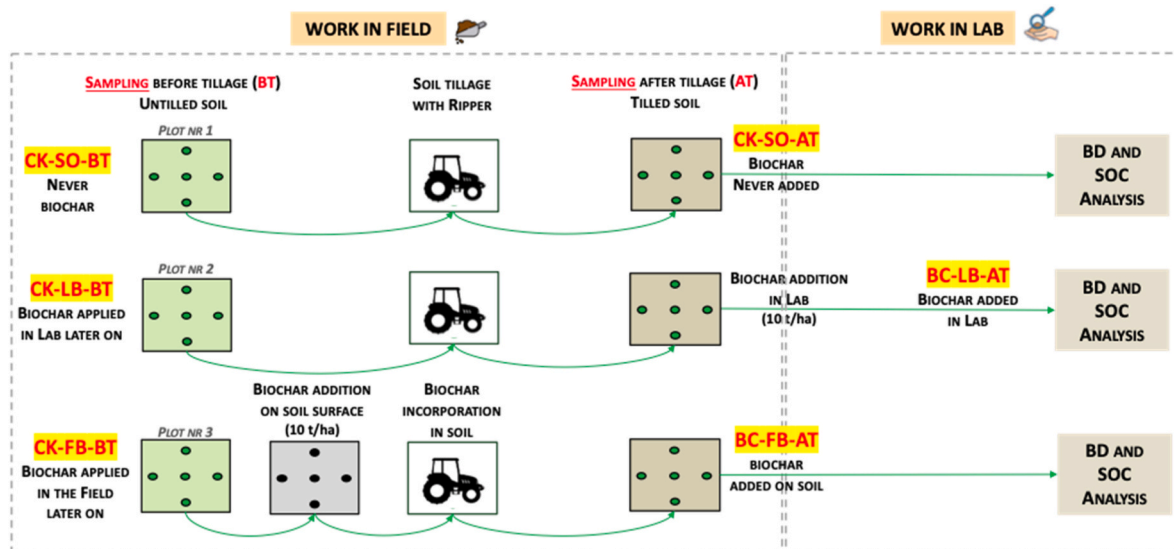


Fig. 2. Logical framework of the field and lab of activities of the present work and sample coding.

**Table 1**  
Summary of soil sampling REDII-IR prescriptions versus this experiment.

REDII Implementing Regulation prescriptions	This experiment
<ul style="list-style-type: none"> <li>15 subsamples must be collected every 5 ha (50.000 m<sup>2</sup>), ensuring that these are homogeneous in terms of soil quality</li> <li>The 15 subsamples must be mixed in a single larger sample, from which the microsample to be sent to the carbon analyzer is extracted.</li> </ul>	<ul style="list-style-type: none"> <li>The 15 samples are taken by a very homogeneous area of 25 x 3 = 75 m<sup>2</sup>.</li> <li>The 15 samples (named "subsamples" in the REDII-IR) are not mixed: these are instead kept separated, and each one analysed independently from the others</li> <li>Averages, standard deviations, and statistics are elaborated on the data obtained by each specific measurement</li> </ul>

**Table 2**  
Alder biochar characterization of the main chemical physical parameters.

Parameter	Method	UoM	Alder biochar
Moisture	UNI EN ISO 18134-2: 2017	% w/w ar	4.9
Ashes 550 °C	UNI EN 13039: 2012	% w/w db	5.5
Volatiles	UNI EN 18123: 2023	% w/w db	13.0
Fixed C	UNI EN 18123: 2023	% w/w db	81.5
C	UNI EN ISO 16948: 2015	% w/w db	89.4
H	UNI EN ISO 16948: 2015	% w/w db	1.6
Inorganic C	Italian D.M. 13/09/99 Met. V.1	% w/w db	0.6
Organic C	UNI EN ISO 16948: 2015 + D.M. 13/09/99 Met. V.1	% w/w db	88.8
H:Core molar ratio	UNI EN ISO 16948: 2015 + D.M. 13/09/99 Met. V.1	molar ratio	0.22
pH	UNI EN ISO 10390: 2022	–	8.3
Bulk density	UNI EN ISO 17828: 2016	t/m <sup>3</sup> db	0.124

material to allow the homogenous mixing of the biochar with soil. The removed stones were weighted to calculate the soil skeletal coarse fraction at >2 mm. All the soil materials (with or without biochar) retained in the 2 mm sieve were crushed and sieved again until all samples passed the 2 mm sieve. The 2 mm fine soil samples were stored

in clean, dry, hermetically sealed and clearly identified containers. Before weighing for each analytical determination, the sample was thoroughly homogenized.

As regards plot nr 2, the experiment required adding the biochar in the lab, to the collected soil sample after tillage. Biochar was thus manually added, at a dose equivalent to 10 t/ha (db) solely to the five soil samples collected after tillage from Plot nr 2, and labelled BC-LB-AT. The biochar was applied to the soil samples as they were collected from the field, in order to simulate the field application of biochar on a disturbed soil containing skeletal material and organic residues of biomass and roots (Fig. 4).

#### 2.4. Soil bulk density and organic carbon determination

The air-dried moisture was calculated according to ISO 11465:1993, while the residual moisture was determined after oven-drying the samples at 105 °C according to ISO 11465:1993. Bulk density was calculated on the dried soil material according to ISO 11272:2017. The total organic carbon was calculated indirectly as the difference between total carbon and inorganic carbon according to ISO 10694:1995. More into details, the total carbon was analysed by a CHN analyser LECO Truspec CHN, about 200 mg of sample material were combusted at high temperature (950 °C) and converted by catalysts to carbon dioxide, water vapor and elemental nitrogen and read as weight percentages after a 5 points calibration with a soil standard. The inorganic carbon was measured using a Dietrich-Fruhling calcimeter according to UNI EN ISO 10693:2014. An appropriate amount of sample (about 1–20 gr according to the expected carbonate content) was weighed and then treated with hydrochloric acid (4M) and the CO<sub>2</sub> produced by the carbonates was measured through the calcimeter. The carbon dioxide volume was mathematically converted to the soil inorganic carbon content through equations.

#### 2.5. Carbon stock calculation

Carbon stock was calculated using Equation (1), from FAO 2019 method "Measuring and modelling soil carbon stocks and stock changes in livestock production system" [34], using the bulk density of the whole soil.

$$\text{Carbon stock} = (\text{SOC} \times \text{BD} \times T (1 - (F))) / 10 \quad \text{Equation 1}$$

where carbon stock was the soil organic carbon expressed as tonnes of



Fig. 3. Metal ring method used in sampling 30 cm topsoil.



Fig. 4. Manual addition of biochar on BC-LB-AT \_1 sample (left); BC-LB-AT \_1 after biochar addition (right).

Carbon per ha on a dry base; SOC is the soil organic carbon content expressed as mg/g db; BD is the soil bulk density expressed as g/cm<sup>3</sup>; T is the thickness (depth, cm) of 30 cm; F is the coarse mineral fraction expressed by mass (g/g).

Considering that the AT samples were subjected to tillage operations a bulk density adjustment was performed on these samples for the C stock calculation to better compare the C stock results before and after tillage. In particular, the C stock AT data were multiplied by a factor calculated according to Equation (2).

$$C \text{ stock adjustment factor} = BD_{BT} / BD_{AT} \quad \text{Equation 2}$$

where  $BD_{BT}$  corresponds to average bulk density of the plot before tillage and  $BD_{AT}$  corresponds to average bulk density of the plot after tillage.

## 2.6. Statistical analysis

Statistical analysis was performed using the Excel program. Variables as normally distributed were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed by one way ANOVA for comparison between more than 2 groups or by Student *t*-test for comparison between 2 groups. A *p* value < 0.05 was considered as statistically significant.

## 2.7. Soil samples coding

Each plot, (SO) (control without biochar), LB (lab added biochar)

and FB (field added biochar), were sampled 5 times before tillage BT (1–5), and 5 times after tillage AT (1–5) considering the presence (BC) or absence (control CK) of biochar. On each of the AT and BT samples 3 measures of the Organic Carbon were performed. The sample coding is further explained in Table 2S of the Supplementary file.

## 3. Results and discussion

### 3.1. Biochar characterization

Alder, a fast-growing hardwood, was selected as feedstock for carbonization given its high availability and rapid growth characteristics. It can thus be considered representative of the local hard woody biomass category. This low-ash material exhibited optimal conversion. The alder biochar showed 5.5 % w/w d.b. ash content and 13.0 % w/w d.b. volatile matter, leading to a fixed carbon content of 81.5 % w/w d.b. This biochar presented a high carbon content, equal to 89.4 % w/w d.b., the majority of which is organic carbon (88.8 % w/w d.b.), and a hydrogen content of 1.6. The corresponding H:C<sub>org</sub> molar ratio is 0.22 index of a good stability of the material suggesting a stable and aromatic biochar. According to EBC guidelines [35] “The molar H/C<sub>org</sub> ratio is an indicator of the degree of carbonization and therefore of the biochar stability. The ratio is one of the most important characterising features of biochar and is indispensable for the determination of the C-sink value. Values fluctuate depending on the biomass and process used. Values exceeding 0.7 are an indicator of non-pyrolytic chars or pyrolysis deficiencies”. However, recent



works also show that  $H/C_{org}$  does not necessarily always correspond to high inertinite content and thus highly recalcitrant carbon in biochar [41]. pH was slightly alkaline (8.3) and with a bulk density of  $0.124 \text{ t/m}^3$  d b. in line with the characteristics of most lignocellulosic biochar.

### 3.2. Comparison of soil bulk densities before and after the tillage

Table 3 shows the bulk densities reported in each of the 5-sampling points for Plot nr. 1, 2 and 3 taken before and after the tillage. The bulk densities determined on soil samples collected before tillage BT ranged from  $0.70$  to  $1.19 \text{ t/m}^3$ , like those observed in tilled samples, AT ( $0.73$ – $1.15 \text{ t/m}^3$ ) (Table 3). Considering the average of the bulk density for each plot, the range of these figures for untilled samples was  $0.97$ – $1.13 \text{ t/m}^3$  whereas for tilled samples it was  $0.83$ – $1.10 \text{ t/m}^3$ . A slight decrease in bulk density is thus observed for tilled soils, as expected and consistent with literature. In fact, among others, Pittarello et al. [42] observed a bulk density reduction due to soil tillage, while Polizio et al. [43] reported a lower bulk density for soil subject to minimum tillage, compared to no tilled soils.

Considering samples collected in Plot nr. 1 (CK-SO-BT), 2 (CK-LB-BT) and 3 (CK-FB-BT) before tillage, no statistically significant difference was observed (as shown in Fig. 5A), indicating that bulk density before tillage was similar across the three plots.

If the entire number of samples collected in all BT plots is considered, i.e. 15 samples (in triplicates, for the analytics) over the entire  $75 \text{ m}^2$  area (sum of three plots) for a total of 45 data (as given in the Supplementary material), the average BT bulk density is  $1.05 \text{ t/m}^3$  and the standard deviation equal  $0.14 \text{ t/m}^3$ .

The differences between all BT cases are non-significant (Fig. 5A): however, a similar trend is noted among the BT and AT cases (Fig. 5B) for the three plots. In all cases Plot nr 1 and Plot nr 3 show the min and max average figures.

Worth to remark, the BC-FB-AT is the only case where biochar was already present in the soil at the moment of soil sampling and therefore included in the measurement of the BD. Thus, the presence of biochar in soil in the amount of  $10 \text{ t/ha}$  is not relevant to modify the trend of Bulk Densities. This is reasonable, given that the weight of  $1 \text{ ha}$  of  $30 \text{ cm}$  of soil correspond to an average of  $3150 \text{ t/ha}$  Before Tillage and  $2910 \text{ t/ha}$  After Tillage. The influence of  $10 \text{ t}$  becomes almost irrelevant, being two orders of magnitudes lower than soil weight.

### 3.3. Organic carbon content and C stock calculation

Table 4 and Fig. 6 show the average values for measured soil organic carbon (SOC), and carbon stock within the first  $30 \text{ cm}$  of topsoil, determined in the three plots before and after the tillage, based on the 5 different soil samples taken at each plot.

Soil samples showed no statistically significant difference of SOC and carbon stock before tillage, BT (Table 4).

However, from a GHG accounting and carbon crediting perspective, despite this absence of statistically significant difference regarding C stock before tillage (BT), in absolute terms the difference between the max and min measured values ( $57.1$  and  $49.7 \text{ t/ha}$ , respectively, i.e.  $7.4 \text{ t/ha}$  difference), as well as the max standard deviation (up to  $15.2 \text{ t/ha}$ ) looks considerable, when the scope is to allocate an economic value (as a carbon credit) based on soil measurement. The assessment of a carbon stock baseline for the soil thus looks very variable, even in such a constrained, narrow and homogeneous piece of land. At an average price of  $100 \text{ €/tCO}_2$ , for instance, this would mean a spread of  $740 \text{ €}$  of carbon value in the stock, with a high economic variation also allocated to the standard deviation. All this would reflect on the assessment of the SCA component, which compares the ex-post situation to the ex-ante case, i.e. the baseline.

This consideration becomes even more relevant if we consider that the carbon stock baseline is dynamic and not static: this will increase the spread between measurements, due to the combined effect of intrinsic

**Table 3**

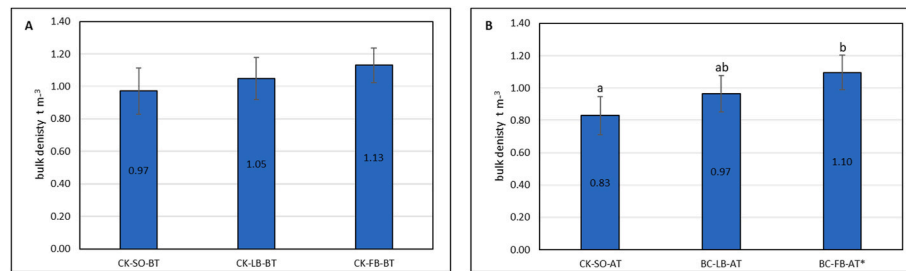
Soil bulk density in the five sampling points for each plot before (BT) and after (AT) tillage. Standard deviations are indicated in brackets.

Sample name	Samples number #	Bulk density [t/m <sup>3</sup> ]	Mean bulk density (standard deviation) [t/m <sup>3</sup> ]
<b>Before tillage</b>			
CK-SO-BT_1	1	1.09	0.97 (0.15)
CK-SO-BT_2	2	0.70	
CK-SO-BT_3	3	1.04	
CK-SO-BT_4	4	1.01	
CK-SO-BT_5	5	1.01	
CK-LB-BT_1	1	1.05	1.05 (0.14)
CK-LB-BT_2	2	1.11	
CK-LB-BT_3	3	0.82	
CK-LB-BT_4	4	1.19	
CK-LB-BT_5	5	1.08	
CK-FB-BT_1	1	0.97	1.13 (0.11)
CK-FB-BT_2	2	1.11	
CK-FB-BT_3	3	1.15	
CK-FB-BT_4	4	1.11	
CK-FB-BT_5	5	1.29	
<b>After tillage</b>			
CK-SO-AT_1	1	0.73	0.83 (0.13)
CK-SO-AT_2	2	0.77	
CK-SO-AT_3	3	0.79	
CK-SO-AT_4	4	1.05	
CK-SO-AT_5	5	0.80	
BC-LB-AT_1	1	1.15	0.97 (0.12)
BC-LB-AT_2	2	0.86	
BC-LB-AT_3	3	0.92	
BC-LB-AT_4	4	1.03	
BC-LB-AT_5	5	0.88	
BC-FB-AT_1	1	1.11	1.10 (0.11)
BC-FB-AT_2	2	0.90	
BC-FB-AT_3	3	1.19	
BC-FB-AT_4	4	1.13	
BC-FB-AT_5	5	1.15	

variability of the SOC measures and the time evolution of the carbon stock. Some authors in literature [44,45] already explicitly cited the problems associated with soil sampling to catch the intrinsic variability of SOC in a given area.

As expected, biochar addition resulted in the higher measured levels of SOC, which were statistically significant only in the lab-added plot





**Fig. 5.** A, left) Mean bulk density before tillage in Plot nr. 1 (CK-SO-BT), nr. 2 (CK-LB-BT) and nr. 3 (CK-FB-BT); the data differences from ANOVA test resulted as not significant; B, right). Mean bulk density after tillage in Plot nr. 1 (CK-SO-AT), nr. 2 (BC-LB-AT) and nr. 3 (BC-FB-AT); \*this is the only case where biochar was added in the field, thus included in the measure of the BD. Error bars represent the standard deviations; ANOVA tests result a = 0.051 ab = 0.054 b = 0.056, not statically significant differences between plots if  $p < 0.05$ .

**Table 4**

Mean SOC and carbon stock values (from sampling  $n = 5$ ) determined in the three plots before (BT) and after the tillage (AT). Standard deviation is indicated in brackets; p values and increasing levels of significance (\*, \*\*) are indicated as obtained by one way ANOVA (among BT groups) or by Student *t*-test; ns = not significant.

Sample code	Mean SOC (standard deviation) [% db]	p value	Mean C stock (standard deviation) [t/ha]	p value
CK-SO-BT	2.10 (0.44)	ns among BT	51.9 (4.9)	ns among BT
CK-LB-BT	2.14 (0.35)		57.1 (15.2)	
CK-FB-BT	1.88 (0.27)		49.7 (5.7)	
CK-SO-AT	2.28 (0.28)*	0.03 vs BT together	51.1 (15.1)	ns vs BT together ns vs CK-SO-BT
BC-LB-AT	2.62 (0.52)*	0.03 vs CK-SO-AT	62.0 (9.9)*	0.03 vs CK-SO-AT
BC-FB-AT	2.40 (0.92)	ns vs CK-SO-AT	66.9 (11.9)**	0.0036 vs CK-SO-AT ns vs BC-LB-AT

but not for field-added biochar plot (BC-FB-AT, probably due to the high standard deviation) compared to non-added controls after tillage (Table 4).

The highest C stock figures were however observed after tillage in

biochar-added samples (respectively in BC-FB-AT, 66.9 t/ha, and BC-LB-AT, 62.0 t/ha), whereas the lowest value was observed in CK-SO-AT (51.1 t/ha), with a statistically significant lower level compared to both CK-SO-BT and to all the three samples before tillage. Interestingly, while AT bulk density decreases irrespective of adding biochar, SOC increases.

Adding biochar anyway resulted in a statistically significant increase in the measurable C stock AT both for FB and LB cases. Standard deviations are however high and then, from an economic point of view, the impact of the actual SCA measurement on the economic aspect is too large for being acceptable by stakeholders and Institutions.

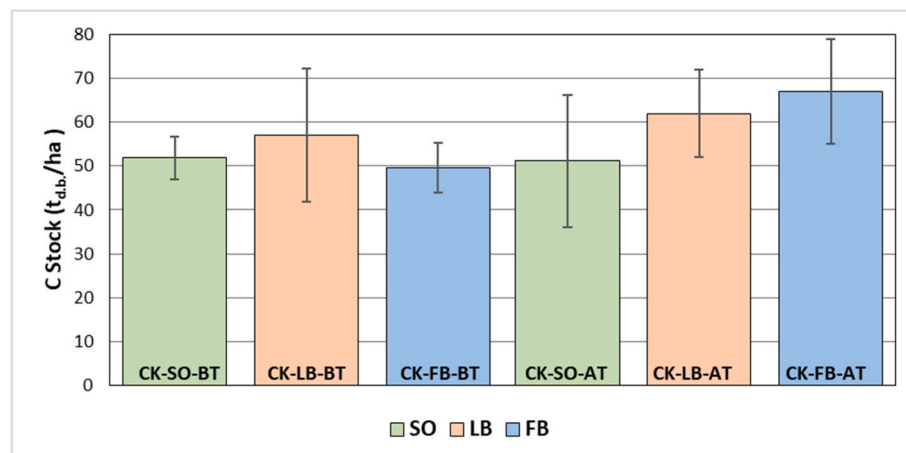
#### 3.4. Expected C stock from biochar addition versus actual measurements

Given the results from the experimental activity, it is now possible to address the main research question and the scope of this work: is soil sampling suited for quantitative assessment of SCA? Based on the given physico-chemical characterization of the biochar, the addition of 10 t/ha of the selected and characterised type of biochar was equivalent to adding 8.9 t<sub>db</sub>/ha of organic carbon (for both cases of field and laboratory): it would thus be reasonable to expect to observe approximately this increase in both the LB-AT and FB-AT samples, given that the soil sampling was done immediately, and no priming effect was possible.

Adding 8.9 t<sub>db</sub>/ha of organic carbon to the reference control of CK-SO-AT (51.1 ± 15.1 t<sub>db</sub>/ha) should bring to a final average value of approximately 60.0 t<sub>db</sub>/ha.

Instead, the average C stock measured in LB and FB samples exceeded this expectation respectively by 2 and 7 t<sub>db</sub>/ha respectively, being 62 and 66.9 t<sub>db</sub>/ha.

The two biochar-added plots therefore led to different C stocks, both higher than what theoretically expected with reference to CK-SO-AT



**Fig. 6.** Mean C Stock of AT and BT plots [t<sub>d.b.</sub>/ha].

(Table 5, Fig. 7).

Also, the higher deviation was registered for the field biochar addition with respect to laboratory addition. This interesting result is consistent with the fact that biochar addition in lab was more controlled and precise than in the field.

Nevertheless, in both cases the measured C stock was well above the expected figures: this was due to the intrinsic variability of the C stock baseline in soil, independent from biochar addition.

Finally, to better explain why this inevitable variability of soil carbon measurement is observed due to the adopted methodology, the overall mass flows of soil and biochar through the entire experimental and analytical procedure is shown in the following Fig. 8.

Based on this procedure, it is not surprising that observed results differ from expectations, as the methodology includes unavoidable choices by the operator while carrying out the soil sampling and SOC analytical measurements. Soil and biochar cannot be considered as two perfectly mixable product, like instead it happens with liquids. Results are thus due to the intrinsic and unavoidable bias of the procedure:

- in choosing the sample points in the given area (even if, in our experiment, concentrated in a very small and homogeneous region of only 25 m<sup>2</sup> per plot)
- in selecting the sample (200 mg) from the whole solid sample (approximately 1500 g at beginning) to be injected in the analytical equipment (CHN analyser)
- in the fact that biochar is a concentrated form of carbon, unevenly distributed in soil since the initial deployment stage: biochar will likely concentrate in some parts of topsoil rather than others, which makes the point of samples a truly random exerciser.

Also, the already shown non statistically significant variability (Fig. 5) of the bulk density adds another significant uncertainty to the results, as carbon stock data must be adjusted to consider the change in bulk density.

Similar results are being obtained in other research activities, as those carried out in the EU TULIPS project, where biochar was added to soil of airports of Schiphol (The Netherlands), Turin (Italy) and Larnaka (Cyprus). In some of these cases the observed carbon stock after biochar addition was lower than expected, further confirming the random nature and intrinsic bias of the soil sampling procedure in the case of biochar.

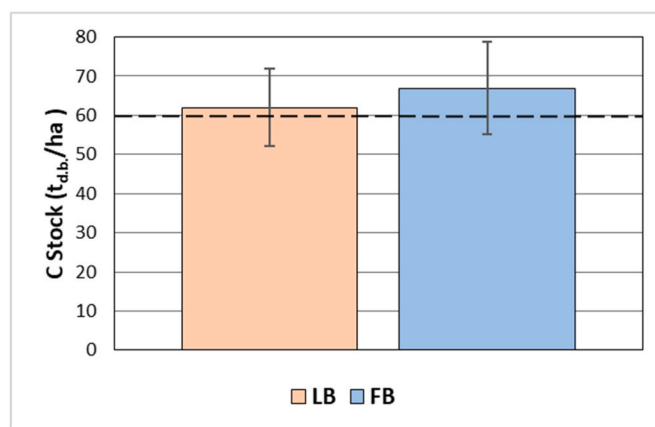
Another notable result of this work lies in the observed values of SOC before adding the biochar, and thus in determining the baseline for SOC. This baseline, not relevant when exogenous carbon is added as biochar, becomes essential for all other sustainable agronomic practices. The difficulty in assessing a reliable SOC baseline, over which the SCA can be accounted for, is such that probably only a combination of experimental effort and modelling can generate carbon stock figures sufficiently solid to be used in GHG assessment or carbon markets.

Under this light, the use of biochar as CDR emerges as one of the most reliable, solid and verifiable options to account and allocate GHG reduction and/or carbon credits in farming, not requiring the assessment of a baseline (all carbon from biochar is additional), and allowing a full characterisation of the carbon amount and type in biochar before being deployed in soil.

**Table 5**

C stock comparison between biochar added plots BC-LB-AT and BC-FB-AT and the expected C stock value.

Treatment	Measured C stock in soil sample [ $t_{db}/ha$ ]	C stock from given biochar addition [ $t_{db}/ha$ ]	Expected total C stock [ $t_{db}/ha$ ]	Deviation from expected [ $t_{db}/ha$ ]
BC-LB-AT	62.0	8.9	60.0	2.0
BC-FB-AT	66.9	8.9	60.0	6.9



**Fig. 7.** Comparison of BC-LB-AT ( $62.0 \pm 9.9 t_{db}/ha$ ) and BC-FB-AT ( $66.9 \pm 11.9 t_{db}/ha$ ) mean C Stock in added soil. The dotted line represents the expected C stock ( $60.0 t_{db}/ha$ ) on the base of added biochar and with reference to CK-SO-AT.

#### 4. Conclusions

The present work investigated the possibility to use soil sampling techniques to quantitatively assess the amount of permanent carbon added via biochar incorporated into the soil. The research question was about the appropriateness of this methodology in view of allocating carbon savings and credits.

The investigation was carried out in a very small and homogeneous area in a farm nearby Florence, Tuscany, Italy. Three plots of 25 m<sup>2</sup> each, located one next to the other and very homogeneous in terms of soil characteristics and agroclimatic conditions, were selected in a flat area of the farm, totalling 75 m<sup>2</sup> of test area.

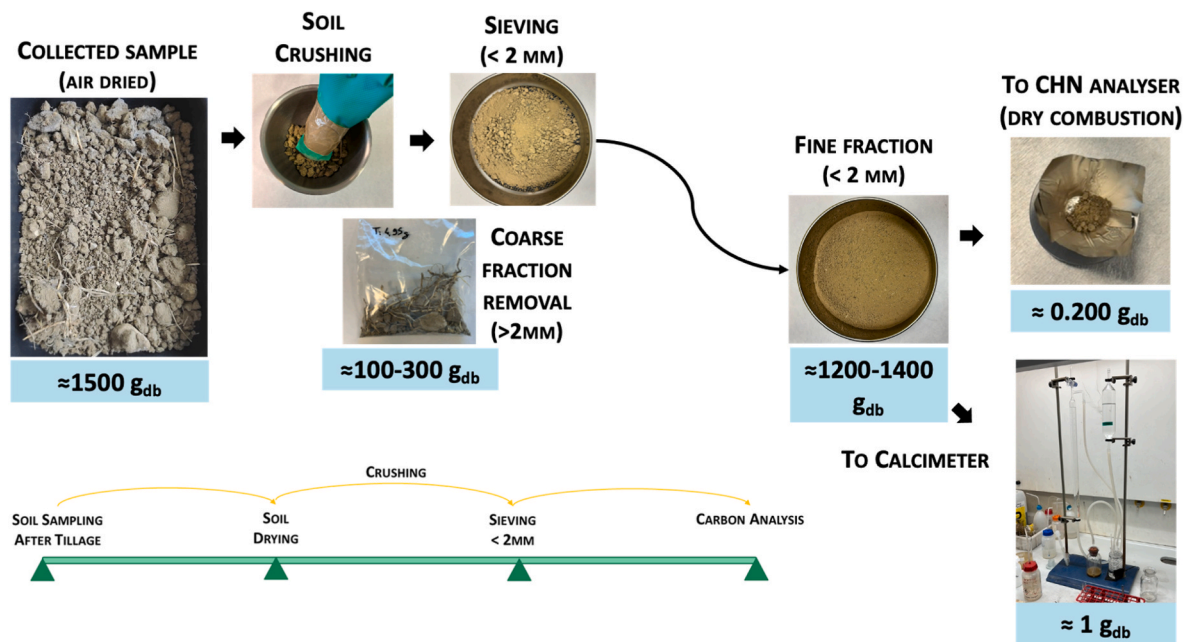
Biochar was produced in a demo scale unit and fully characterised. The experimental investigation on biochar deployment and soil sampling was carried out during the same day, to avoid any priming effect in soil or degradation (devolatilization) of biochar.

The research work provided the evidence that soil sampling is unfit to provide quantitative assessment of the C stock in soil from biochar addition (in the case of this specific experiment, overpredicting the amount, with statistical significance vs the control). The deviation of C stock in BC-FB-AT was about 6.9 t/ha, while the BC-LB-AT case showed a deviation of approximately 2.0 t/ha, both in excess to expectations. The amount retrieved in the samples was thus greater than the actual carbon addition. The reasons for such offsets are due to

- the intrinsic variability and statistical significance of soil carbon stock measurements, even in such very controlled conditions,
- the weight of the specific microsample that is fed to the analytical instrument versus the weight of soil collected through sampling. Typically, 0.2 g, while the weight of a single subsample weight is approx. 1.5 kg, and the collective weight of the sample composed by 15 soil subsamples prescribed by REDII-IR is 22.5 kg.
- the fact that biochar is a concentrated form of carbon, unevenly distributed in soil and in the collected samples.

Our findings confirm that the certification requirements for biochar use under the draft EU CRCF (under development) and ICAO CORSIA regulations are well-designed for certifying CDR and SCA, without prescribing soil sampling but rather requiring full product certification and third-party auditing during deployment. This research work provided experimental evidence to this. Soil sampling is also a significant cost for the biochar operators, not justified due to the inadequateness of this technique for the scope.

In addition, as regards carbon savings and credits associated to biochar, given the possibility to fully characterise the biochar, not only



**Fig. 8.** Mass flows of soil and biochar along the experimental procedure utilised to assess SOC and determine organic and inorganic carbon (Organic C = Total C – Inorganic C).

in terms of elemental chemical compositions but also as labile and durable carbon fractions via the inertinite benchmarking method, the actual long-lasting nature of the durable carbon share in biochar can be assessed and not only modelled. This makes the soil sampling even more unnecessary, when dealing with permanence and allocation of credits.

Consequently, REDII-IR should align with scientific evidence and EU CRCF and ICAO CORSIA regulations.

In conclusion, for the scope of quantitative assessment of Soil Carbon Accumulation and the specific case of BCR, soil sampling is not a method adequate to monitor, report and verify (MRV): biochar must be assessed upstream by full characterization of the product as well as control during the incorporation in soil.

Finally, the work done here on SOC before and after tillage, independently from the addition of biochar, opens another significant research question for all the other sustainable agricultural practices: assessing a solid baseline, necessary to calculate SCA and thus deliver carbon savings and credits, appears as a very critical element. The observed variations in SOC figures, even in a very controlled and spatially limited experiment, makes it very difficult to link carbon farming practices to solid economic figures associated with well accounted carbon removals. The combination of state-of-the-art validated modelling and soil sampling will likely allow to narrow the uncertainties and achieve sufficient confidence in quantitative carbon accounting for SOC.

#### CRediT authorship contribution statement

**David Chiaramonti:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Giulia Lotti:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Francesca Tozzi:** Writing – review & editing, Methodology, Investigation. **David Casini:** Writing – review & editing, Investigation. **Francesco Primo Vaccari:** Writing – review & editing. **Hamed Sanei:** Writing – review & editing. **Michaela Luconi:** Writing – review & editing, Data curation. **Marco Buffi:** Writing – review & editing.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biombioe.2025.108537>.

#### Data availability

Experimental data are made fully available as Supplementary material.

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