Soil Biology and Biochemistry 203 (2025) 109733

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Comparison of different methods for estimating microbial biomass in biochar-amended soils

Sara Paliaga [a,*], Vito Armando Laudicina [a,c], Sofia Maria Muscarella [a], Daniel Said-Pullicino [b], Luigi Badalucco [a]

[a] Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy
[b] Department of Agricultural, Forest and Food Sciences, University of Torino, Grugliasco, Italy
[c] NBFC, National Biodiversity Future Center, Palermo, Italy

A R T I C L E I N F O

Keywords:
Microbial biomass C and N assessment
Biochar
$CHCl_3$ fumigation-incubation
$CHCl_3$ fumigation-extraction
CO_2-high pressurization

A B S T R A C T

Biochar use as a soil amendment can improve soil functions, enhances microbial activity, and increases crop production. However, due to its high sorptive capacity, it may interfere with traditional methods for determining soil microbial biomass, specifically chloroform fumigation-incubation (FI) and fumigation-extraction (FE). This study aimed to assess the impact of biochar on microbial biomass determination using traditional methods and a new CO_2 high pressurization (CO2HP) technique. Five treatments were set up: unamended soil (control), and soil amended with two types of biochar, produced at 440 °C (B440) or 880 °C (B880), at two application rates (20 or 40 t ha^{-1}). Following cell lysis by fumigation or CO2HP, released microbial C (ΔC_{mic}) was estimated by determining microbial respiration over a 10-d incubation (FI and CO2HP-I methods, respectively) or by extracting soluble organic C (FE, and CO2HP-E, respectively), while released microbial N (ΔN_{mic}) was estimated by determining extractable total N exclusively by FE and CO2HP-E methods.

Without biochar, ΔC_{mic} estimates were similar across FE, FI, and CO2HP-E methods. Contrarily, CO2HP-I method greatly overestimated ΔC_{mic} compared to the other three methods, particularly at the higher biochar rate, suggesting that the adsorption of CO_2 within biochar pores during CO2HP treatment and subsequent slow release during incubation could have produced artifacts. The presence of B880 resulted in a decrease in ΔC_{mic} values, which might have been caused by an acclimation of microbial biomass to new habitat. Contrarily, the addition of B440, increased ΔC_{mic} when determined by the FE method, compared to FI and CO2HP-E methods. This suggested an overestimation of extractable C after fumigation, possibly due to adsorption of $CHCl_3$ by the B440 biochar, rich in functional groups, that might have bound $CHCl_3$. We concluded that biochar interfered with the determination of $\Delta C_{mic}$and ΔN_{mic} as a function of both type and amount added.

1. Introduction

Biochar, a material resulting from the thermochemical decomposition of different biomasses under controlled temperature and oxygen conditions, in recent decades has gained significant attention for its many applications in various fields, including soil fertility, crop productivity, environmental remediation, nutrient recovery, catalysis, animal feed, and odor adsorption (Brassard et al., 2019; Conte et al., 2021; Marcinczyk et al., 2022).

Indeed, biochar amendment has been reported to influence soil properties, such as porosity, structure, surface area, cation exchange capacity, organic carbon content, and microbial biomass, but these effects can vary widely depending on soil type, environmental conditions, and the characteristics of the biochar itself (Hardie et al., 2014; Brassard et al., 2019; Wang et al., 2022). For example, Wang et al. (2022) observed an increase of 41% and 32% of soil microbial biomass C and N, respectively, after a 3% biochar amendment (Wang et al., 2022). Moreover, Chen et al. (2018) have previously shown that the addition of biochar increased the ratio of Gram-positive to Gram-negative bacteria and decreased that of fungi to total bacteria in soil, indicating an effect on microbial community composition. Steiner et al. (2008) also noted that the basal respiration of biochar amended soil increased about by 30% in the 35 h after substrate addition. However, biochar sorption properties may clearly interfere with standard extraction procedures for

* Corresponding author.
E-mail address: sara.paliaga@unipa.it (S. Paliaga).

https://doi.org/10.1016/j.soilbio.2025.109733
Received 20 June 2024; Received in revised form 22 January 2025; Accepted 24 January 2025
Available online 30 January 2025

the quantification of soil microbial biomass or enzyme activities (Lehmann et al., 2011).

Soil microbial biomass, a crucial biological indicator for assessing soil potential activities and fertility, plays a key role in energy transfer and nutrient transformations within the soil ecosystem. Accurate and reliable methods for measuring soil microbial biomass are mandatory in soil science research. The chloroform fumigation-incubation (FI) (Jenkinson and Powlson, 1976) and chloroform fumigation-extraction (FE) (Vance et al., 1987a) methods have been widely used over the last decades for this purpose. They rely on using chloroform ($CHCl_3$) vapors to fumigate the soil, causing the lysis of microbial cells and the release of their contents. However, many concerns have been raised regarding the use of $CHCl_3$ due to its toxicity to humans and the environment, as well as its effectiveness in lysing soil microbial cells (Toyota et al., 1996; Badalucco et al., 1997; Alessi et al., 2011; Rotbart et al., 2020). Recently, Paliaga et al. (2023) have proposed a less harmful and likely more efficient approach for lysing soil microbial cells, which is essentially based on the high pressurization of soil with CO_2 (CO2HP).

The ability of $CHCl_3$ to lyse soil microbial cells with adequate efficiency could be reduced when applied to soils amended with biochar. As evidenced by Lehmann et al. (2011), biochar poses analytical problems due to its capacity to sorb lysed cells, cellular contents, and microbial exoenzymes, particularly evident during DNA extraction, determination of microbial biomass by FE method and measurement of enzyme activity by fluorometric methods. This increased adsorption of lysed cellular components on biochar required the application of correction factors in fumigation procedures. Liang et al. (2010) established an extraction correction factor through the recovery of ^{13}C-labeled microbial biomass added to the soil, revealing a 21–41% lower recovery of microbial biomass C in biochar-rich Terra Preta soils than in adjacent biochar-poor soils. Moreover, the adsorption capacity of biochar for volatile organic compounds, such as $CHCl_3$, needs to be considered when employing $CHCl_3$ fumigation methods in amended soils. The specific properties of the biochar, such as its surface chemistry, specific surface area and average pore size, can favor the adsorption and desorption of various compounds, thereby influencing the availability and interaction of $CHCl_3$ with the soil microbial community (Kumar et al., 2020). These adsorption phenomena can lead to reduced concentrations of $CHCl_3$ within the soil pores and solution during fumigation, thus potentially reducing the efficacy of $CHCl_3$ in lysing microbial cells and altering the accurate assessment of soil microbial biomass. Alternatively, the adsorption of $CHCl_3$ by biochar could cause an overestimation of the MBC, recovering part of the desorbed non-biomass $CHCl_3$–C in the soil extract after fumigation. Previous studies have demonstrated that biochar exhibits significant adsorption potential for $CHCl_3$. For instance, Xiang et al. (2020) have shown that some types of ball-milled biochar can adsorb more than 80 mg $CHCl_3$ g^{-1}.

The physical and chemical properties of biochar depend highly on pyrolysis temperature and process parameters, such as residence time as well as on the feedstock type (Bruun et al., 2011). Thus, biochar derived from different feedstocks or produced under varying pyrolysis conditions may be structurally different and exhibit variable adsorption capacities for $CHCl_3$ (Kumar et al., 2020). Therefore, when biochar is incorporated into soil, even at different rates, its unique characteristics should be considered to ensure accurate assessments of soil microbial biomass when using methods based on fumigation with $CHCl_3$.

Hence, the aim of this study was to evaluate whether the presence of biochar in soil can, and to what extent, influence the determination of soil microbial biomass by both classical $CHCl_3$ fumigation-extraction (FE) and fumigation-incubation (FI) methods. In parallel, the FE and FI methods were compared with the recently proposed CO2HP alternative technique (Paliaga et al., 2023). This new approach causes the lysis of soil microbial cells by high CO_2 pressurization and subsequent rapid decompression. We hypothesized that the CO2HP method, being based on a physical approach, should overcome the limitations of the classical FI and FE methods linked with the adsorption of $CHCl_3$ on biochar during the assessment of soil microbial biomass.

2. Materials and methods

2.1. Soil sampling and characterization

Approximately 30 kg of fresh soil was collected from an agricultural field at a depth of 0–20 cm within the Department of Agricultural, Food, and Forest Sciences, University of Palermo (*38°10′66″N, 13°35′03″E*). The collected soil was air-dried and sieved at < 2 mm. The main chemical and physical characteristics of the soil were: clay 27%, sand 51%, silt 22%, pH and electrical conductivity in water extract (1:2.5, *w/v*) 7.9 and 0.48 dS m^{-1}, respectively, total organic carbon 48.5 g kg^{-1}, total nitrogen 3.5 g kg^{-1}, and total carbonates 170 g kg^{-1}.

2.2. Biochar preparation and characterization

Two types of biochar were used, both produced from wood residues consisting of 50% from eucalyptus and 50% from pine (*w/w*). The pyrolysis process was conducted using two different temperatures and methods: pyro-gasification at 880 °C (referred as B880) and pyrolysis at 440 °C (referred as B440). The main characteristics of the two biochars, including physical and chemical properties, are provided in Table 1.

To qualitatively assess the possible adsorption of $CHCl_3$ by the biochar, four 10 g aliquots of both B440 and B880 were fumigated similarly to soil samples, as described later. After the fumigation procedure, biochar samples were placed in the dark at 22 °C in 200 mL glass jars sealed with rubber stoppers containing silicone septa. Simultaneously, four jars, each containing 10 mL of $CHCl_3$, were prepared and used as a standard.

The presence of $CHCl_3$ in the headspace of all the glass jars, containing biochar or not, was assessed after 24 h, to allow the $CHCl_3$ desorption. Then, 1 mL of air from the headspace of each jar was injected into a gas-chromatograph equipped with an electron capture detector (TRACE-GC-ECD, Thermo Scientific, Milan, Italy), and $CHCl_3$ peaks from the biochar samples were compared with those from standard jars with only $CHCl_3$.

A PerkinElmer Spectrum Two FTIR spectrometer equipped with an attenuated total reflectance (ATR) device was used for acquiring the Fourier Transform Infrared-Attenuated Total Reflectance (ATR-FTIR) spectra (i.e., absorbance vs. wavenumber) of the two biochar types. Such spectra were acquired to assess the main functional groups of tested biochar. A few milligrams of each biochar sample were used to acquire the spectra in the wavenumber range 4000–400 cm^{-1}, with a resolution of 4 cm^{-1} and 64 scans, as recommended by Janu et al. (2021) for the

Table 1
Physical and chemical characteristics of the two biochars tested. Biochars were produced at two different pyrolysis temperatures: 440 °C (B440) and 880 °C (B880). Values are arithmetic means (n = 3) ± standard deviation.

Parameters	Unit of measure	B880	B440
Bulk density	g L^{-1}	126 ± 12	180 ± 17
Surface area	m^2 g^{-1}	227 ± 21	164 ± 11
Total pore volume	cm^3 g^{-1}	51 ± 7	38 ± 4
Maximum water retention	%	400 ± 7	62 ± 5
pH		10.0 ± 0.3	9.1 ± 0.2
Electrical conductivity	dS m^{-1}	2.0 ± 0.2	1.3 ± 0.2
Moisture	%	6.7 ± 1.2	3.1 ± 1.1
Total carbonates	%	2.7 ± 0.3	5.0 ± 0.7
Total carbon	%	72 ± 3	65 ± 3
Ashes to 550 °C	%	6.4 ± 0.6	3.4 ± 0.9
C/H Molar ratio		5.0 ± 0.6	1.4 ± 0.3
Total nitrogen (N)	%	0.3 ± 0.1	0.3 ± 0.1
Total phosphorus (P_2O_5)	g kg^{-1}	0.2 ± 0.1	0.5 ± 0.2
Total potassium (K_2O)	g kg^{-1}	4.0 ± 1.1	9.0 ± 1.4
Total calcium (CaO)	g kg^{-1}	11.0 ± 0.5	4.0 ± 0.3
Total magnesium (MgO)	g kg^{-1}	2.0 ± 0.3	1.0 ± 0.2
Total sodium (Na_2O)	g kg^{-1}	0.11 ± 0.04	0.05 ± 0.02

characterization of the structural features of biochar.

2.3. Experimental setup

An air-dry soil aliquot weighing one kg was prepared for each of the following five treatments (Table 2): control soil without biochar, soil amended with 16 g of B880 or B440 biochar per kg of soil (corresponding to 20 t ha^{-1}) and soil amended with 32 g of B880 or B440 biochar (corresponding to 40 t ha^{-1}). Each treatment consisted of 4 replicates. All five treatments were pre-incubated at 25 °C and at 50% of the water holding capacity (WHC) for 10 days, in order to restore and stabilize the microbial biomass and biological activity, before the lysis of microbial cells by $CHCl_3$-fumigation or CO2HP methods (Fig. 1).

2.4. Soil microbial cell lysis by $CHCl_3$ fumigation and CO_2 high pressurization (CO2HP) methods

The potential role of biochar in affecting the lysis of soil microbial cells, for microbial biomass determination, was evaluated by comparing two different approaches: $CHCl_3$ fumigation and CO_2 high-pressurization (CO2HP). The soil $CHCl_3$ fumigation was performed according to the method first proposed by Jenkinson and Powlson (1976) but with slight modification, i.e., without fresh unfumigated soil inoculation. In fact, as reported by Vance et al. (1987b), provided that the soil pH is above 5.0, like here, it is irrelevant whether the soil is inoculated or not. Eight soil sub-aliquots (each 15 g, four later used for FI method and four for FE method), per each of the five above treatments, were put inside glass beakers and fumigated with $CHCl_3$ (stabilized with amylene) in a sealed desiccator (40 cm diameter) for 24 h at 22 °C in the dark. Then, the fumigant was removed by a vacuum pump by ten cycles of alternate suction (2 min each) and air re-introduction (1 min each). The CO2HP approach for lysing microbial cells was conducted according to Paliaga et al. (2023) using the Cells Disruption Bomb equipment (Parr Instrument Company, USA). Briefly, four soil sub-aliquots of 15 g for each of the five above treatments were put into 50 mL plastic Falcon tubes without caps, placed in the steel vessel inside a thermostatic water bath at 40 °C and CO_2 pressurized for 24 h at 600 psi. At the end of the pressurization procedure, the outlet valve of the vessel was immediately opened (Fig. S1).

2.5. Cumulative CO_2–C and extractable C and N determination

After soil microbial cell lysis by either $CHCl_3$ fumigation or CO2HP, the sub-aliquots were treated similarly to either the FI method or the FE method (Jenkinson and Powlson, 1976; Vance et al., 1987a,b), in order to determine the carbon or nitrogen released and ascribable to microbial biomass (ΔC_{mic} and ΔN_{mic}, respectively).

More precisely, following FI method, half of $CHCl_3$ fumigated and CO2HP pressurized soil sub-aliquots were incubated in 250 mL glass jars sealed with rubber stoppers containing silicone septa and placed in the dark at 22 °C for 10 days. For all the 5 treatments, further four untreated soil sub-aliquots, neither fumigated nor pressurized, were similarly incubated as controls (non-fumigated). The CO_2 accumulated after 1, 3, 7 and 10 days of incubation in the headspace of the glass jars was determined by injecting 1 mL of air from each jar into a gas chromatograph (TraceGC, Thermo Fisher Scientific, Milan, Italy) equipped with a thermal conductivity detector and a Poropak Q column, using He as the carrier (oven 50 °C, injector at 225 °C, column flow 40 mL/min, split mode 10:1). The cumulative CO_2–C in all sub-aliquots after 10 days of incubation was called C_{flux}.

Using FE method, half of $CHCl_3$ fumigated and CO2HP pressurized soil sub-aliquots (CO2HP-E) were extracted with 0.5 M K_2SO_4 at a weight:extractant volume (g mL^{-1}) ratio of 1:4. As above, for each of the 5 treatments, further four untreated soil sub-aliquots, neither fumigated nor pressurized, were similarly extracted and used as controls (non-fumigated). All soil suspensions were shaken horizontally for 45 min at 75 rpm. At the end of shaking, the suspensions were filtered with Whatman No. 42 paper and then K_2SO_4 extractable organic C was determined using Pt-catalyzed, high-temperature combustion (680 °C) followed by infrared detection of CO_2 (VarioTOC, Elementar, Hanau, Germany), while K_2SO_4 extractable total N was determined by the micro-Kjeldahl method. The extracted organic C in all sub-aliquots was called C_{extr}, while extractable total N as N_{extr}.

The ΔC_{mic} was calculated as the difference between C_{extr}, or C_{flux} from soil fumigated or pressurized with CO2HP minus C_{extr} or C_{flux} extracted or cumulated from control soil (non-fumigated), i.e., by FE, CO2HP-E, FI and CO2HP-I methods, respectively. Similarly, the ΔN_{mic} was calculated as the difference between N_{extr} from soil fumigated or pressurized with CO2HP minus N_{extr} from non-fumigated soil, i.e., by FE and CO2HP-E methods, respectively.

2.6. Statistical analysis

The reported results are the arithmetic means of four replicates ($n = 4$) and are expressed on dry soil mass basis at 105 °C. Before performing parametric statistical analyses, normal distribution and variance homogeneity of the data were checked by Kolmogorov–Smirnoff goodness-of-fit and Levene's tests, respectively. Significant statistical differences ($p < 0.05$) within the same approach or method (non-fumigated, FE, FI, CO2HP-E, CO2HP-I), among the five biochar treatments (Control, B880 20 and 40 t ha^{-1}, B440 20 and 40 t ha^{-1}), and within the same biochar treatment, among the different approaches or methods, were assessed by the Least Significant Difference test (LSD). In order to point out possible interactions between biochar treatments and approaches or methods, as main factors, a two-way analysis of variance (ANOVA) with replicates of the measured variables was performed (Table S1). All statistical analyses were carried out using SPSS 13.0 (IBM, USA).

3. Results

3.1. FTIR and GC-ECD analysis of biochar

The FTIR spectra of B440 and B880 are shown in Fig. 2. The FTIR spectrum of B440 displayed absorption bands around 1620 cm^{-1}. These bands are indicative of O–H stretching vibrations in groups bound by hydrogen bonds and water molecules within the inner layer, as reported by Jung et al. (2015). Additionally, the peaks at 1260 and 1020 cm^{-1} suggested the presence of C–O stretching in aromatic components, C=O stretching in conjugated ketones and quinones, and symmetric C–O–C stretching in ester groups found in cellulose and hemicellulose (Lucas et al., 2018). These components are integral to the aromatic structure of lignin (Liu et al., 2015; Tomin et al., 2022). Another peak identified around 800 cm^{-1} is generally associated with bending vibrations of = C–H bonds in aromatic rings. The spectra of B880 resembled those of wood matrix biochar pyrolyzed at temperatures above 800 °C, as described by Liu et al. (2015). The absence of peaks in the B880 spectra can be attributed to the high pyrolysis temperature since, as it increases, biochar assumes characteristics similar to pure graphite (Cai et al., 2018).

Qualitative GC-ECD analysis of the gas accumulated in the headspace

Table 2
Experimental setup. Five treatments were set up: unamended soil (Control), and soils amended with two types of biochar, produced at 440 °C (B440) or 880 °C (B880), at two application rates (20 or 40 t ha^{-1}).

Name	Biochar	g biochar kg^{-1} dry soil	Corresponding amount (t ha^{-1})
Control	–	0	0
B880 20 t ha^{-1}	B880	16	20
B880 40 t ha^{-1}	B880	32	40
B440 20 t ha^{-1}	B440	16	20
B440 40 t ha^{-1}	B440	32	40

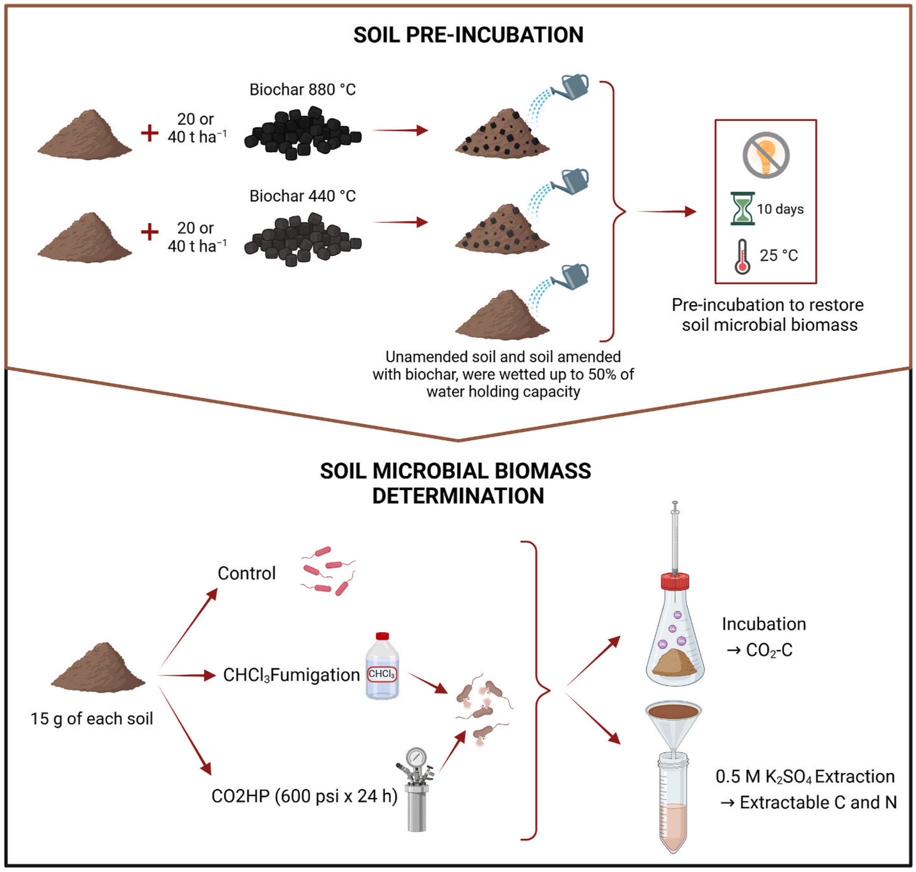


Fig. 1. Materials and methods scheme. One soil was amended with two biochars produced at 440 and 880 °C (B440 and B880, respectively). After a pre-incubation period, carbon or nitrogen released and ascribable to microbial biomass were determined by fumigation-incubation (Jenkinson and Powlson, 1976), fumigation-extraction (Vance et al., 1987), CO2HP-incubation and CO2HP-extraction methods (Paliaga et al., 2023). CO2HP; high pressurization with CO_2.

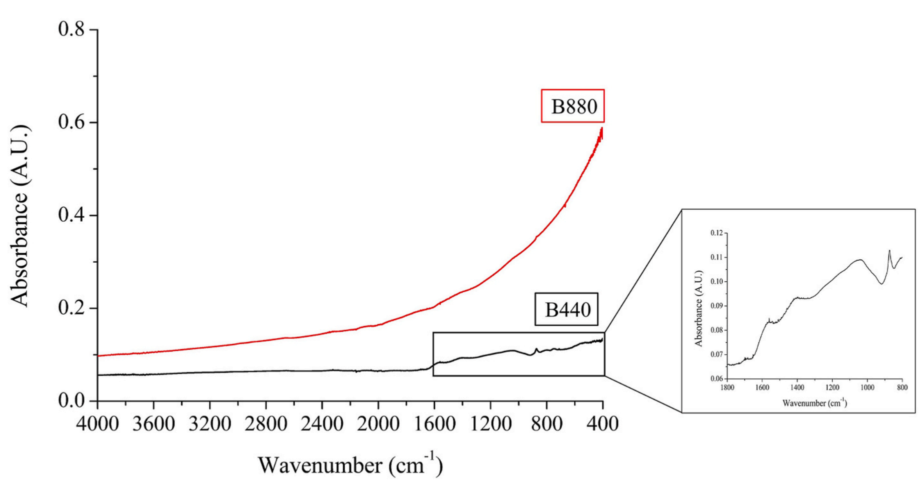


Fig. 2. ATR-FTIR spectra, within the 4000-400 cm^{-1} wavenumber, of biochars produced at two different pyrolysis temperatures: 440 °C (B440) and 880 °C (B880).

of bottles incubated with $CHCl_3$-fumigated biochar samples showed the presence of $CHCl_3$ in the fumigated B440 samples, while it was not detected in the B880 samples (data not shown).

3.2. CO_2 evolution with FI vs. CO2HP procedures

The cumulative CO_2–C evolved during 10 days (C_{flux}) of incubation was higher in fumigated and pressurized soils compared to soil not fumigated or not pressurized, regardless of biochar content. Notably, the C_{flux} from pressurized soils was much higher than that from fumigated soils (Fig. 3). After three days of incubation, the CO_2 flux from the non-fumigated soils tended to be the lowest in the absence of biochar, in the opposite way to what happened in fumigated soils, where the highest C_{flux} occurred without biochar. Moreover, among non-fumigated soils the 10 days cumulative C_{flux} was significantly ($p < 0.05$) higher with higher biochar added, regardless of its type. Considering only the soil fumigated treatments, no significant ($p < 0.05$) difference occurred between soil amended with biochar and not (Table 3).

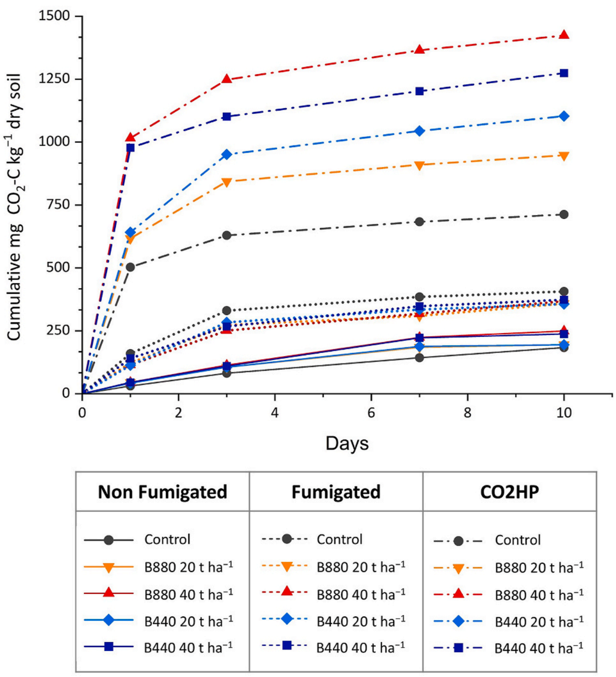


Fig. 3. Arithmetic means (n = 4) of cumulated CO_2–C emitted from soil after addition of biochar at 20 and 40 t ha^{-1} during 10 days incubation at 25 °C and 50% of water holding capacity (WHC). Graph shows the comparison between non-fumigated, fumigated and pressurized (CO2HP) soils with CO_2 at 600 psi for 24 h.

Table 3
Cumulative CO_2–C after 10 day incubation. Values are arithmetic means ($n = 4$). Tested experimental factors were treatments (Control, B880 20 t ha^{-1}; B880 40 t ha^{-1}; B440 20 t ha^{-1}; B440 40 t ha^{-1}) and approach (non-fumigated, fumigated and pressurized with CO_2, CO2HP). Along columns, different lowercase letters indicate significant differences ($p < 0.05$) among treatments within the same approach; along rows, capital letters indicate significant differences ($p < 0.05$) among approaches within the same treatment.

	Non-fumigated	Fumigated	CO2HP	LSD ($p < 0.05$)
	mg CO_2–C kg^{-1} dry soil			
Control	183.3 Cb	406.5 Ba	712.3 Ad	155.5
B880 20 t ha^{-1}	194.7 Cb	358.6 Ba	947.9 Ac	102.0
B880 40 t ha^{-1}	249.0 Ca	367.7 Ba	1423.4 Aa	34.0
B440 20 t ha^{-1}	194.5 Cb	356.5 Ba	1103.5 Ab	89.1
B440 40 t ha^{-1}	239.4 Ca	373.6 Ba	1274.0 Aa	114.7
LSD ($p < 0.05$)	55.5	68.4	155.4	

On the other hand, the C_{flux} evolved from soils pressurized with CO2HP method, was always higher than that of fumigated soils, regardless of biochar addition (Fig. 3). This C_{flux} increase occurred even after one day of incubation and was particularly high with the two higher biochar amounts, being maximal for the soil amended with B880. However, with the lower biochar amount, the C_{flux} increase was maximal with B440. More precisely, by comparing the soil CO2HP pressurized with the $CHCl_3$ fumigated one, after 10-d incubation the maximum increase in C_{flux} was recorded in soil amended with higher biochar concentrations, on average by 264%, while the soil amended with lower quantity of biochar and the not-amended one (Control) showed, on average, a C_{flux} increase of 187% and 75%, respectively (Fig. 3; Table 3).

3.3. *Extractable organic C and total N with FE vs. CO2HP procedures*

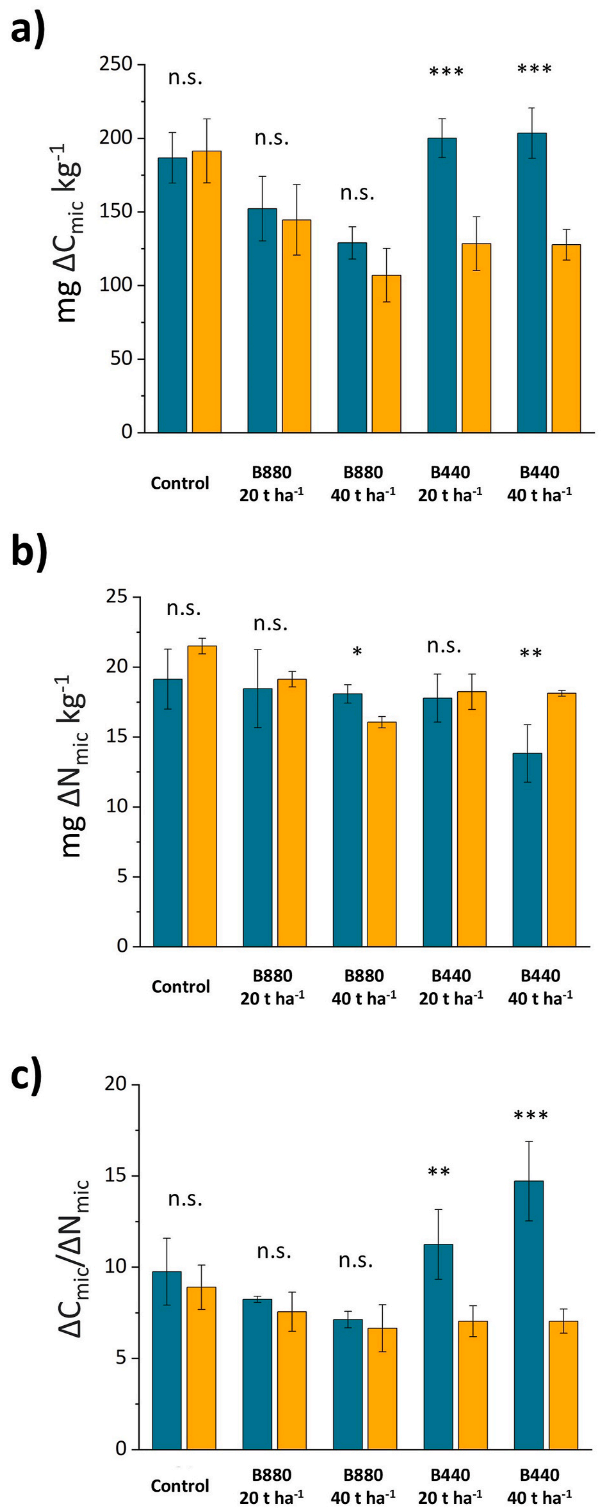
Extractable organic C (C_{extr}) and total N (N_{extr}) determined in non-fumigated soil were affected by the addition of biochar; indeed, C_{extr} decreased by about 9% with B880, regardless of addition rate, whereas with B440 decreased only at higher rate by 16%. Instead, N_{extr} was not affected by B880 at any rate and, similarly to C_{extr}, decreased by 11% with B440 at higher rate (Table 4). As expected, C_{extr} and N_{extr} in fumigated (F) and pressurized (CO2HP) soils were higher than in non-fumigated soils, regardless of any biochar treatment. However, when comparing C_{extr} among the five biochar treatments within the fumigation and CO2HP approaches, some specific trends occurred. More precisely, for CO2HP, C_{extr}, as well as N_{extr}, decreased as biochar concentration increased. On the other hand, for the fumigation approach there was an interaction between the type and amount of biochar added. In fact, although C_{extr} in soil amended with B880 followed the same trend as the CO2HP approach, this was not the case with B440. In fact, C_{extr} at both biochar rates showed no significant difference from the soil without biochar. In contrast, the N_{extr} of soil amended with B440 at the highest concentration, and fumigated, showed the lowest value. No difference was found among the other four biochar treatments (Table 4).

The ΔC_{mic} and ΔN_{mic}, as well as the relative carbon/nitrogen ratios ($\Delta C_{mic}/\Delta N_{mic}$) for both FE and CO2HP-E methods are shown in Fig. 4. In control soil, neither ΔC_{mic} or ΔN_{mic} differed between the two methods. The ΔC_{mic} by the FE method in the soil amended with B880 at low rate was slightly higher, although not significantly, than by CO2HP-E method. In comparison with the control soil, ΔC_{mic} in the soil with B880 at low rate was significantly lower by both FE and CO2HP-E methods, 18% and 25%, respectively. Again, the ΔC_{mic} of soil with B880 at high rate was not significantly different between FE and CO2HP-E methods, but it was lower by 31% and 44% when compared to the control, respectively. The ΔC_{mic} determined by the FE method in soils amended with B440, regardless of the rate, was on average 36% higher than by the CO2HP-E method. Moreover, compared with the control soil, the ΔC_{mic} in soil amended with B440 by the FE method was not significantly different, regardless of the biochar rate.

With regard to ΔN_{mic}, its trend was quite different compared to ΔC_{mic}. In particular, with FE method ΔN_{mic} did not differ among biochar treatments, except for B440 at high rate, when it was, on average, by about 29% lower than the other four treatments. Otherwise, soil ΔN_{mic} determined by CO2HP-E decreased in the presence of B880 compared to the control, with a reduction of 11% at lower biochar rate and of 27% at higher rate. Moreover, soil ΔN_{mic} did not differ between FE and CO2HP-E methods for the control and B880 at the lower rate, while with CO2HP-E it decreased by about 12% for B880 at higher rate. On the other hand, with CO2HP-E compared to FE, ΔN_{mic} with B440 at the higher rate increased by about 22% instead of decreasing, while at the lower rate it

Table 4
Extractable organic C (C_{extr}) and extractable total N (N_{extr}) of non-fumigated, fumigated and pressurized (CO2HP) soils, with and without biochar addition. Values are arithmetic means ($n = 4$). Along columns, different lowercase letters indicate significant differences ($p < 0.05$) among treatments within the same approach; along rows, capital letters indicate significant differences ($p < 0.05$) among approach within the same treatment.

C_{EXTR}	Non-fumigated	Fumigated	CO2HP	LSD ($p < 0.05$)
	mg C kg^{-1} dry soil			
Control	249.6 Ba	436.4 Aab	441.1 Aa	30.9
B880 20 t ha^{-1}	230.0 Bb	382.1 Ac	374.5 Ab	45.0
B880 40 t ha^{-1}	227.2 Bbc	356.1 Ac	334.2 Ac	22.1
B440 20 t ha^{-1}	244.7 Cab	444.8 Aa	373.1 Bb	24.0
B440 40 t ha^{-1}	208.6 Cc	412.1 Ab	336.3 Bc	33.9
LSD ($p < 0.05$)	19.2	26.4	24.4	
N_{EXTR}	mg N kg^{-1} dry soil			
Control	36.1 Bab	55.2 Aa	57.6 Aa	4.8
B880 20 t ha^{-1}	37.9 Ba	56.4 Aa	57.0 Aa	6.4
B880 40 t ha^{-1}	34.4 Bbc	52.5 Aa	50.5 Ab	5.2
B440 20 t ha^{-1}	37.4 Ba	55.2 Aa	55.6 Aa	5.2
B440 40 t ha^{-1}	32.2 Bc	46.0 Ab	50.3 Ab	6.1
LSD	2.9	4.7	4.3	



Method	LSD (p < 0.05)
Fumigation	20.3
CO2HP	23.5

Method	LSD (p < 0.05)
Fumigation	2.5
CO2HP	0.9

Method	LSD (p < 0.05)
Fumigation	1.9
CO2HP	1.2

Fig. 4. a) C and b) N ascribable to microbial biomass, and c) their ratio (ΔC_{mic}, ΔN_{mic}, $\Delta C_{mic}/\Delta N_{mic}$) determined by fumigation-extraction (FE) and CO2HP-extraction (CO2HP-E) methods. Values represent means ± SD ($n = 4$). For each of the four biochar treatments and control, the level of significant difference between FE and CO2HP-E methods is indicated: n.s., not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. The least significant difference (LSD, $p < 0.05$) among the treatments for each of the two methods is indicated in boxes on the right. CO2HP; high pressurization with CO_2.

did not differ between the two methods, being also equal to the control soil.

The ratios $\Delta C_{mic}/\Delta N_{mic}$, decreased progressively from control soil to B880 at low rate and then to B880 at high rate, with both FE and CO2HP-E methods, showing no significant difference between them. Among these three treatments, if any, significant differences were minimal, with the maximal $\Delta C_{mic}/\Delta N_{mic}$ ratio of 9.8 (control soil with FE method) and the minimum value of 6.7 (B880 soil at the low rate with the CO2HP-E method). On the other hand, the trend of $\Delta C_{mic}/\Delta N_{mic}$ ratio in the presence of B440 was totally different compared to B880. With FE method, this ratio more than doubled at high biochar rate compared to CO2HP-E and was by about 70% higher with the low biochar rate.

However, $\Delta C_{mic}/\Delta N_{mic}$ ratio by CO2HP-E did not differ between the two biochar rates and was on average 7.4 (Fig. 4).

3.4. Comparison of ΔC_{mic} assessment methods

The differences between ΔC_{mic} determined by both cell lysis procedures, i.e., the methods of fumigation with $CHCl_3$ and pressurization with CO_2, and followed by both analytical methods, i.e., after extraction or after incubation (FE, CO2HP-E, FI, CO2HP-I, respectively, Table 5) were compared. The ΔC_{mic} determined by the CO2HP-I method was always higher than the other three methods, regardless of biochar treatment. On the contrary, among the other three assessed methods (FE, CO2HP-E and FI) no significant differences were observed in the determined ΔC_{mic} for control soil and both B880 treated soils. Instead, soil amended with B440, at both rates, showed a peculiar behavior, whereby the FE method determined a higher ΔC_{mic} content than CO2HP-E and FI methods. Also, when comparing the effects of biochar within the same ΔC_{mic} assessment method, with CO2HP-I method both biochar types, compared to control, greatly increased the ΔC_{mic} value, but with minimal and maximal increase for B880 at low and high rate, respectively, while in the presence of B440 the ΔC_{mic} increase was intermediate and not significantly different between the two biochar rates. On the contrary, with the other three methods (FE, FI and CO2HP-E) generally biochar always decreased ΔC_{mic} content, compared to control soil, although biochar effect changed among methods; moreover, it depended on both biochar type and addition rate. More precisely, with FI method, the addition of biochar induced the highest variation range in ΔC_{mic} content (LSD among the five biochar treatments at $p < 0.05$ was 97.0), i.e. FI method suffered the highest disturbance in ΔC_{mic} assessment. Indeed, the addition of B880 at high rate, halved ΔC_{mic} compared to control soil. The CO2HP-E method behaved similarly to the FI one, since the lower ΔC_{mic} decrease, compared to the control soil, occurred again with B880 at the high rate. Also, the other three biochar added soils did not differ among them and intermediate values between the previous two occurred. Notably, the CO2HP-E method was more reliable than FI one, since the LSD ($p < 0.05$) among biochar treatments was only 29% that of FI. Finally, within the FE method, the B440 addition to soil did not cause any change in ΔC_{mic} compared to the control soil. On the other hand, B880 decreased the ΔC_{mic} content significantly, and the higher the biochar rate, the greater the ΔC_{mic} reduction (Table 5).

Table 5
Carbon derived from microbial biomass (ΔC_{mic}) determined by four different methods: fumigation-extraction (FE); CO2HP-extraction (CO2HP-E); fumigation-incubation (FI); CO2HP-incubation (CO2HP-I). Tested experimental factors were treatments (Control, B880 20 t ha^{-1}; B880 40 t ha^{-1}; B440 20 t ha^{-1}; B440 40 t ha^{-1}). Values are arithmetic means (n = 4). Along columns, different lowercase letters indicate significant differences (p < 0.05) among treatments within the same method; along rows, capital letters indicate significant differences ($p < 0.05$) among methods within the same treatment.

Method	FE	CO2HP-E	FI	CO2HP-I	LSD (p < 0.05)
	ΔC_{mic} (mg C kg^{-1} dry soil)				
Control	186.8 Ba	191.4 Ba	223.2 Ba	469.9 Ad	80.6
B880 20 t ha^{-1}	152.2 Bb	144.6 Bb	163.9 Bab	753.2 Ac	184.6
B880 40 t ha^{-1}	129.9 Bc	107.0 Bc	118.7 Bb	1174.4 Ab	70.1
B440 20 t ha^{-1}	200.1 Ba	128.4 Cbc	162.0 Cab	909.0 Aa	53.9
B440 40 t ha^{-1}	203.5 Ba	127.7 Cbc	136.0 Cab	1036.6 Aab	97.2
LSD (p < 0.05)	20.3	23.2	97.0	154.0	

4. Discussion

The addition of the two types of biochar at different rates demonstrated that the estimation of microbial biomass, both through classical chloroform fumigation methods, but also by the recently proposed one based on high CO_2 pressurization (Paliaga et al., 2023), was impaired when compared to the control soil without biochar. Furthermore, the extent of the alteration was variable and depended on the approach for microbial determination, as well as on the type and quantity of biochar added to the soil.

The increases of C_{flux} in both fumigated (FI) and pressurized (CO2HP-I) soils may be due to, at least partially, the microorganisms surviving the cell lysis disruption and which respired the cytoplasmic materials released from lysed microbial cells during the subsequent 10-days incubation (Fig. 3., Jenkinson and Powlson, 1976; Paliaga et al., 2023). The increases of C_{flux} even among non-fumigated soils after biochar addition suggested that soil respiration was correlated with the high rate only of both biochar applied, while the low rate had no significant effect compared to the absence of biochar (Table 3). This result was consistent with the findings of Tasneem and Zahir (2017), who noted that biochar application increased CO_2 production during incubation and enhanced microbial activity when compared to unamended soil. Indeed, during 10 days of incubation period, increases of CO_2 production ranged from 15 to 30%, proportionally to the concentration of biochar added. The rise of soil respiration could be attributed to the addition of labile C derived from biochar, which provides readily available substrate for soil microorganisms. This short-term effect was also highlighted by Smith et al. (2010) who, by analyzing the $\delta^{13}C$ signature of biochar, showed that biochar contributed to the CO_2 flux predominantly during the initial days of incubation. However, the high respiration rate in soil amended with biochar could be ascribed not only to the mineralization of readily available biochar-C (Laudicina et al., 2012) but also to improved soil aeration by biochar (Case et al., 2012). Considering that C_{extr} decreased following the addition of biochar (Table 4), this decrease could be ascribed to both the mineralization of extracted labile biochar-C and improved aeration supplying more O_2 by added biochar (Zimmerman et al., 2011).

Interestingly, in soil fumigated with chloroform, the addition of biochar, regardless of both type and rate, decreased the C_{flux} during the 10 day incubation in comparison with control soil (no biochar added), although not significantly, suggesting that the presence of biochar could have decreased the chloroform lysing efficiency and/or trapped the CO_2 deriving from the cytoplasm mineralization by alkalinization (Table 3; Fig. 3). However, the increase of pH following the addition of biochar was unlikely at least for two reasons: 1) the pH of the two used biochar ranged between 9 and 10 (Table 1), while their amounts in soil never exceeded 3.3 %; 2) on the other hand, the soil native organic C content was higher than that of added biochar, i.e., its buffering capacity was likely enough to counterbalance the possible alkalinizing effect of added biochar. This result suggested that biochar, due to its high porosity and specific surface area, can retain CO_2 and/or adsorb chloroform, thus lowering its lysing ability.

Similarly, the increase of C_{flux}, and consequently of ΔC_{mic}, observed in soil pressurized with the CO2HP approach could be attributed to the high retention of abiotic CO_2 by the biochar and, likely, also by soil (Fig. 3; Tables 3 and 5). The large amount of CO_2, used for high pressurization and entrapped within biochar and/or soil, may have been gradually released during the 10 day incubation, overestimating ΔC_{mic} content (Table 5). The increase of C_{flux} in pressurized soils could be mediated by the solubilization increase of CO_2 in soil pore water as CO_2 pressure increased for the operating conditions of the CO2HP-I method, i.e., 600 psi (Paliaga et al., 2023). Indeed, the CO_2 solubility in water at this pressure can be estimated to be around 3 g of CO_2 per 100 g of H_2O (Perkins and Innovates, 2003). Following depressurization and the return to atmospheric pressure conditions, the CO_2 dissolved in water could revert to its gaseous state and be gradually released during the incubation period. However, it is important to know that the increase in C_{flux} was proportional to the rate of both biochar added, with the highest effect with B880 at high rate (Fig. 3; Tables 3 and 5). Biochar has remarkable CO_2 adsorption capacities, primarily due to its porous structure and large surface area (Tan et al., 2017; Guo et al., 2022). Additionally, as the gas pressure increases, the adsorption capacity of biochar for CO_2 also increases. Indeed, increased pressure results in higher gas density, which implies much more CO_2 molecules per volume unit. Therefore, the increased gas molecular concentration enhances the probability of CO_2 adsorption by biochar, as reported by Jung et al. (2019). This biochar behavior suggested that the CO2HP-I procedure may have caused the observed large overestimation of the microbial biomass (Table 5), contrarily to CO2HP-extraction procedure, as discussed later.

The addition of biochar B440 at high rate substantially decreased both extractable C and N in non-fumigated soils and CO_2-higly pressurized (CO2HP) soils, i.e., in the absence of $CHCl_3$, while in chloroform fumigated soils the decrease of extractable C was higher with B880 (Table 4). This can be attributed to the adsorption of soil extracted organic molecules by biochar, as described by Feng et al. (2021). Applying four different types of biochar, they showed that biochar obtained by pyrolysis at 700 °C adsorbed more dissolved organic matter than those obtained at 300 °C, which seems partially to disagree with our results, where a significant decrease of extractable C and N was only found without $CHCl_3$ in soil amended with B440 at high rate. However, notably, due to the very high pyrolysis temperature, B880 showed few no functional groups able to act as binding sites for organic matter (Fig. 2). Indeed, the FTIR spectra indicated that –OH and carboxyl (–COOH) groups were more abundant in biochar produced at the lower pyrolysis temperature (440 °C), explaining the greater adsorption for B440 than B880 (Fig. 2). Hence, the adsorptive capacity of B440 likely interfered, especially at high addition rate, with both C_{extr} and N_{extr} determined before and after CO_2 pressurization. Also, our data do not allow us to explain the peculiar behavior of extractable C with B880 in fumigated soils. However, on the above premises, it was expected that the respective ΔC_{mic}, ΔN_{mic}, and $\Delta C_{mic}/\Delta N_{mic}$ values assessed by FE and CO2HP-E methods could differ between them, as was observed (Table 4; Fig. 4).

The decrease of ΔC_{mic} in soils amended with B880, assessed by both FE and CO2HP-E methods (Fig. 4), is in agreement with Dempster et al. (2012), who reported a 25% decrease in ΔC_{mic} ten weeks after the application of biochar produced from eucalyptus. These results could be attributed to several factors: a) the biochar, due to the high surface area and porosity, can adsorb soil nutrients and lead to their temporary sequestration, making them less accessible to the soil microbial community; b) on the other hand, the decrease of ΔC_{mic} could have been caused by the adsorption of dissolved and extracted carbon after microorganisms lysis, as described by Durenkamp et al. (2010). Indeed, the addition of activated charcoal to different soils induced a decrease from 30 to 56 % of microbial biomass C; c) additionally, the biochar itself may have exerted a biofumigant effect or displayed toxic properties, which could have negatively impacted microbial biomass (Dempster et al., 2012).

Moreover, the lower values of ΔC_{mic} and ΔN_{mic} determined by CO2HP-E compared to FE method in soil amended with 40 t of B880 ha^{-1} (Fig. 4; Table 5) suggested that the new approach to lyse the soil microbial cells, in the presence of substantial amounts of biochar, it is not as effective as the FE method. Further tests are needed to improve the CO2HP-E method under unusual conditions, such as the presence of biochar.

On the other hand, the high C_{extr} and ΔC_{mic} values, and $\Delta C_{mic}/\Delta N_{mic}$ ratio, determined by FE method in soils amended with B440 (Table 4; Fig. 4), suggest an overestimation of organic C. This overestimation can be ascribed to at least two reasons: the first is that organic C is mainly derived from $CHCl_3$–C adsorbed by biochar and then released during extraction, and the second is that it is derived from biochar-C solubilized by chloroform. The adsorption of $CHCl_3$ by B440 was demonstrated by GC-ECD analysis. Indeed, B440, unlike B880, is characterized by the presence of several functional groups, as revealed by FT-IR analyses (Fig. 2). These functional groups may give B440 high capacity to bind and adsorb chloroform. This agrees with Xiang et al. (2020), who have shown that biochar derived from Hickory wood and pyrolyzed at 450 °C was able to adsorb more than 15 mg $CHCl_3$ g^{-1} biochar. Thus, this adsorption resulted in an overestimation of extractable organic C in fumigated soils. On the other hand, proposing the standardization of further degassing periods to nullify $CHCl_3$ adsorption on biochar, when determining ΔC_{mic} by the FE method, is unreliable due to extremely variable chemico-physical properties of different biochar.

Further evidence that the FE approach could overestimate microbial C in B440-amended soils was provided by the lack of ΔC_{mic} differences between the FI and CO2HP-E methods, regardless of biochar treatment (Table 5). Indeed, values determined by either the FI or CO2HP-E methods, i.e., either by extra fluxes of CO_2 or without chloroform fumigation, respectively, were significantly lower than those obtained by the FE method, i.e., that one based on both extracted organic C and chloroform fumigation (Table 5). Furthermore, the higher $\Delta C_{mic}/\Delta N_{mic}$ ratio in B440 amended soils by FE method, compared to CO2HP-E approach, further supported this organic C overestimation hypothesis. Moreover, the ΔN_{mic} values in soils amended with B440 were in contrast. The addition at the low rate causes no significant differences between FE and CO2HP-E methods; however, the high B440 rate decreased the ΔN_{mic} determined by FE method, compared to both CO2HP-E method and low rate of B440. These results are probably due to low lysis efficiency by chloroform. As previously observed, B440 possesses an ability to adsorb $CHCl_3$ that is not recorded in B880. This adsorption phenomenon may lead to reduced concentrations of $CHCl_3$ within soil pores during fumigation, consequently reducing the effectiveness of $CHCl_3$ in lysing microbial cells and then accurately estimating soil microbial biomass.

In conclusion, the comparison of the two approaches used to determine soil microbial biomass, i.e., chloroform fumigation and CO2HP, revealed a significant influence of different biochars, especially biochar obtained with pyrolysis at 440 °C (B440). In fact, in unamended soil, no significant differences were recorded between the different methods except for incubation with CO2HP. This suggested that the new approach for lysis of microbial cells, when combined with the extraction process, is equivalently effective in the absence of biochar. In general, our results suggested that while classical FE and FI methods were affected by $CHCl_3$ and CO_2 adsorption by biochar, respectively, the CO2HP-incubation method was more influenced by the gradual release of CO_2 used for pressurization. Therefore, the CO2HP-extraction method was a more reliable approach, showing the least interference in microbial biomass determination.

CRediT authorship contribution statement

Sara Paliaga: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Vito Armando Laudicina:** Writing – review & editing, Validation, Supervision, Data curation. **Sofia Maria Muscarella:** Investigation, Formal analysis, Data curation. **Daniel Said-Pullicino:** Writing – review & editing, Formal analysis. **Luigi Badalucco:** Writing – review & editing, Validation, Supervision, Investigation, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2025.109733.

References

Alessi, D.S., Walsh, D.M., Fein, J.B., 2011. Uncertainties in determining microbial biomass C using the chloroform fumigation–extraction method. Chemical Geology 280, 58–64. https://doi.org/10.1016/j.chemgeo.2010.10.014.

Badalucco, L., De Cesare, F., Grego, S., Landi, L., Nannipieri, P., 1997. Do physical properties of soil affect chloroform efficiency in lysing microbial biomass? Soil Biology and Biochemistry 29, 1135–1142. https://doi.org/10.1016/S0038-0717(96)00307-0.

Brassard, P., Godbout, S., Lévesque, V., Palacios, J.H., Raghavan, V., Ahmed, A., Hogue, R., Jeanne, T., Verma, M., 2019. Chapter 4 - biochar for soil amendment. In: Jeguirim, M., Limousy, L. (Eds.), Char and Carbon Materials Derived from Biomass. Elsevier, pp. 109–146.

Bruun, E.W., Hauggaard-Nielsen, H., Ibrahim, N., Egsgaard, H., Ambus, P., Jensen, P.A., Dam-Johansen, K., 2011. Influence of fast pyrolysis temperature on biochar labile fraction and short-term carbon loss in a loamy soil. Biomass and Bioenergy 35, 1182–1189. https://doi.org/10.1016/j.biombioe.2010.12.008.

Cai, Z., Jiang, C., Xiao, X.F., Zhang, Y.S., Liang, L., 2018. Lignin-based biochar/graphene oxide composites as supercapacitor electrode materials. In: IOP conference series: materials science and engineering, 359. IOP Publishing, p. 012046. https://doi.org/10.1088/1757-899X/359/1/012046.

Case, S.D., McNamara, N.P., Reay, D.S., Whitaker, J., 2012. The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil–the role of soil aeration. Soil Biology and Biochemistry 51, 125–134. https://doi.org/10.1016/j.soilbio.2012.03.017.

Chen, J., Chen, D., Xu, Q., Fuhrmann, J.J., Li, L., Pan, G., Li, Y., Qin, H., Liang, C., Sun, X., 2018. Organic carbon quality, composition of main microbial groups, enzyme activities, and temperature sensitivity of soil respiration of an acid paddy soil treated with biochar. Biology and Fertility of Soils 55 (2), 185–197. https://doi.org/10.1007/s00374-018-1333-2.

Conte, P., Bertani, R., Sgarbossa, P., Bambina, P., Schmidt, H.P., Raga, R., Lo Papa, G., Chillura Martino, D.F., Lo Meo, P., 2021. Recent developments in understanding biochar's physical–chemistry. Agronomy 11, 615. https://doi.org/10.3390/agronomy11040615.

Dempster, D.N., Gleeson, D.B., Solaiman, Z.I., Jones, D.L., Murphy, D.V., 2012. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. Plant and Soil 354, 311–324. https://doi.org/10.1007/s11104-011-1067-5.

Durenkamp, M., Luo, Y., Brookes, P.C., 2010. Impact of black carbon addition to soil on the determination of soil microbial biomass by fumigation extraction. Soil Biology and Biochemistry 42, 2026–2029. https://doi.org/10.1016/j.soilbio.2010.07.016.

Feng, Z., Fan, Z., Song, H., Li, K., Lu, H., Liu, Y., Cheng, F., 2021. Biochar induced changes of soil dissolved organic matter: the release and adsorption of dissolved organic matter by biochar and soil. Science of the Total Environment 783, 147091. https://doi.org/10.1016/j.scitotenv.2021.147091.

Guo, S., Li, Y., Wang, Y., Wang, L., Sun, Y., Liu, L., 2022. Recent advances in biochar-based adsorbents for CO_2 capture. Carbon Capture Science & Technology 4, 100059. https://doi.org/10.1016/j.ccst.2022.100059.

Hardie, M., Clothier, B., Bound, S., Oliver, G., Close, D., 2014. Does biochar influence soil physical properties and soil water availability? Plant and Soil 376, 347–361. https://doi.org/10.1007/s11104-013-1980-x.

Janu, R., Mrlik, V., Ribitsch, D., Hofman, J., Sedlacek, P., Bielská, L., Soja, G., 2021. Biochar surface functional groups as affected by biomass feedstock, biochar composition and pyrolysis temperature. Carbon Resources Conversion 4, 36–46. https://doi.org/10.1016/j.crcon.2021.01.003.

Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biology and Biochemistry 8, 209–2013. https://doi.org/10.1016/0038-0717(76)90001-8.

Jung, K.W., Jeong, T.U., Hwang, M.J., Kim, K., Ahn, K.H., 2015. Phosphate adsorption ability of biochar/Mg–Al assembled nanocomposites prepared by aluminum-electrode based electro-assisted modification method with $MgCl_2$ as electrolyte. Bioresources Technologies 198, 603–610. https://doi.org/10.1016/j.biortech.2015.09.068.

Jung, S., Park, Y., Kwon, E.E., 2019. Strategic use of biochar for CO_2 capture and sequestration. Journal of CO_2 Utilization 32, 128–139. https://doi.org/10.1016/j.jcou.2019.04.012.

Kumar, A., Singh, E., Khapre, A., Bordoloi, N., Kumar, S., 2020. Sorption of volatile organic compounds on non-activated biochar. Bioresource Technology 297, 122469. https://doi.org/10.1016/j.biortech.2019.122469.

Laudicina, V.A., Dennis, P.G., Palazzolo, E., Badalucco, L., 2012. In: Malik, A., Grohmann, E. (Eds.), Key Biochemical Attributes to Assess Soil Ecosystem Sustainability. Environmental Protection Strategies for Sustainable Development. Springer, Dordrecht, pp. 193–227. https://doi.org/10.1007/978-94-007-1591-2.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – a review. Soil Biology and Biochemistry 43, 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022.

Liang, B., Lehmann, J., Sohi, S.P., Thies, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G., Luizão, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Organic Geochemistry 41, 206–213. https://doi.org/10.1016/j.orggeochem.2009.09.007.

Liu, Y., He, Z., Uchimiya, M., 2015. Comparison of biochar formation from various agricultural by-products using FTIR spectroscopy. Modern Applied Science 9 (4), 246–253. https://doi.org/10.5539/mas.v9n4p246.

Lucas, E.G., Izquierdo, C.G., Fernández, M.T.H., 2018. Changes in humic fraction characteristics and humus-enzyme complexes formation in semiarid degraded soils restored with fresh and composted urban wastes. A 5-year field experiment. Journal of Soils and Sediments 18, 1376–1388. https://doi.org/10.1007/s11368-016-1537-8.

Marcinczyk, M., Ok, Y.S., Oleszczuk, P., 2022. From waste to fertilizer: nutrient recovery from wastewater by pristine and engineered biochars. Chemosphere 306, 135310. https://doi.org/10.1016/j.chemosphere.2022.135310.

Paliaga, S., Laudicina, V.A., Badalucco, L., 2023. Lysis of soil microbial cells by CO_2 or N_2 high pressurization compared with chloroform fumigation. Biology and Fertility of Soils 59, 609–618. https://doi.org/10.1007/s00374-023-01725-5.

Perkins, E., Innovates, A., 2003. Fundamental geochemical processes between CO_2, water and minerals. Alberta Innovates–Technology Futures. In: https://www.alberta.ca/system/files/custom_downloaded_images/energy-fundamental-geochemical-processes-co2-water-minerals.pdf.

Rotbart, N., Borisover, M., Bukhanovsky, N., Beriozkin, A., Eshel, G., Bar-Tal, A., Oren, A., 2020. The assessment of microbial biomass C in subsoil samples using fumigation-extraction is negligibly affected by residual chloroform. Arid Land Research and Management 34, 474–482. https://doi.org/10.1080/15324982.2020.1767226.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biology and Biochemistry 42, 2345–2347.

Steiner, C., 2008. Biochar Carbon Sequestration. University of Georgia, Biorefining and Carbon Cycling Program, Athens, GA, 30602. https://api.semanticscholar.org/CorpusID:195253521.

Tan, X., Liu, S., Liu, Y., Gu, Y., Zeng, G., Hu, X., Wang, X., Liu, S., Jiang, L., 2017. Biochar as potential sustainable precursors for activated carbon production: multiple applications in environmental protection and energy storage. Bioresource Technology 227, 359–372. https://doi.org/10.1016/j.biortech.2016.12.083.

Tasneem, S., Zahir, S., 2017. Soil respiration, pH and EC as influenced by biochar. Soil & Environment 36, 77–83. https://doi.org/10.25252/SE/17/51184.

Tomin, O., Yazdani, M.R., 2022. Production and characterization of porous magnetic biochar: before and after phosphate adsorption insights. Journal of Porous Materials 29 (3), 849–859. https://doi.org/10.1007/s10934-022-01217-1.

Toyota, K., Ritz, K., Young, I.M., 1996. Survival of bacterial and fungal populations following chloroform-fumigation: effects of soil matric potential and bulk density. Soil Biology and Biochemistry 28, 1545–1547. https://doi.org/10.1016/S0038-0717(96)00162-9.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987a. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987b. Microbial biomass measurements in forest soils: determination of kc values and tests of hypotheses to explain the failure of the chloroform fumigations-incubation method in acid soil. Soil Biology and Biochemistry 19, 696–986. https://doi.org/10.1016/0038-0717(87)90050-2.

Wang, H., Zhang, R., Zhao, Y., Shi, H., Liu, G., 2022. Effect of biochar on rhizosphere soil microbial diversity and metabolism in tobacco-growing soil. Ecologies 3, 539–556. https://doi.org/10.3390/ecologies3040040.

Xiang, W., Zhang, X., Chen, K., Fang, J., He, F., Hu, X., Tsang, D.C.W., Ok, Y.S., Gao, B., 2020. Enhanced adsorption performance and governing mechanisms of ball-milled biochar for the removal of volatile organic compounds (VOCs). Chemical Engineering Journal 385, 123842. https://doi.org/10.1016/j.cej.2019.123842.

Zimmerman, A.R., Gao, B., Ahn, M.Y., 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biology and Biochemistry 43, 1169–1179. https://doi.org/10.1016/j.soilbio.2011.02.005.