Mitig Adapt Strateg Glob Change (2024) 29:11
https://doi.org/10.1007/s11027-024-10107-7

ORIGINAL ARTICLE

Biochar ageing effects on soil respiration, biochar wettability and gaseous CO_2 adsorption

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Received: 1 March 2023 / Accepted: 24 January 2024 / Published online: 6 February 2024
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Abstract
The CO_2 emission rates have been continuously incremented during the last decades. To mitigate it, a method to store carbon in terrestrial ecosystems is the addition of biochar to soil. After its application to soil, biochar suffers an ageing process, able to deteriorate its functional properties as soil improver. However, at present, it is not clear how to evaluate biochar ageing. The main aim of this study is to evaluate biochar ageing by determination of temporal changes on (a) soil respiration after biochar addition and (b) the relationship between CO_2 adsorption capacity and wettability of biochar as measurable parameters indicating biochar ageing. Results show that 1 month after biochar addition, soil respiration decreased when poplar and pine biochars were applied to bare soils, in the absence of vegetation. One year after biochar addition, this reduction on soil respiration disappeared, evidencing biochar ageing due to decrements on its CO_2 adsorption capacity. Compared with fresh biochar, decreased CO_2 adsorption capacity of biochar corresponded with enhanced biochar wettability for both biochar types. Its means that poplar and pine biochars, while initially hydrophobic, became hydrophilic after 1 year of its application to soil. It is concluded that changes of biochar CO_2 adsorption capacity in time go along with improved wettability as mutually opposed processes. Globally, pine biochar tends to adsorb a higher quantity of CO_2 than poplar biochar. The absence of CO_2 adsorption of soil without biochar demonstrates the remarkable capacity of both biochars to adsorb carbon dioxide and promote carbon storage in soils.

Keywords Biochar · CO_2 adsorption · Soil respiration · Ageing · Contact angle · Carbon sequestration

1 Introduction

Nowadays, scientific and public interest and awareness about climate change and its effects on human life, ecosystem services and biodiversity in general is increasing. In December 2015, the Paris Agreement (194 countries and EU) was signed to enforce the global transformation to a low-carbon and climate-resilient society (Olivier et al. 2015). However, in 2017, more than 60 scientists stated that we had less than 3 years to safeguard our

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climate because, if global CO_2 emission rates continue to rise beyond 2020 or even remain on the present level, the temperature goals set in Paris will become almost unattainable (Figueres et al. 2017). Unfortunately, the forecasts have worsened because recently highest CO_2 emission rates were registered, increasing the CO_2 level from approximately 280 ppm before the industrial revolution period to a maximum of CO_2 concentration of 415 ppm in June 2021 (Zeebe et al. 2016; Tans and Keeling 2021). It is hypothesised that anthropogenic emissions of CO_2 are the main driver to climate change (Friedlingstein et al. 2010) and its concentration in the atmosphere may increase summer temperatures, heat stress and precipitation extremes in many climate zones (Baker et al. 2018). High CO_2 levels are also bringing other unexpected consequences around the world, such as the impoverishment of human nutrition because crop growth under elevated CO_2 level decrease plant protein, iron and zinc concentrations (Smith and Myers 2018). Without any doubt, atmospheric CO_2 emission levels are one of the major concerns today.

Soil respiration, as important source for CO_2 emissions from soils, is composed by autotrophic respiration from plant roots and associated microorganisms and by heterotrophic respiration via microbial decomposition of soil organic matter (SOM) (Vargas et al 2011). Soil CO_2 emissions are regulated by properties such as soil-water contents, local soil-water availability, soil pore size, pH and nutrient status and local meteorological factors such as air temperature, humidity and wind speed, which influence the movement of CO_2 through and out of soil (Orchard and Cook 1983; Raich 1992). There are serious indications that the global increase in temperature is promoting a greater emission of CO_2 from the soils, due to enhanced SOC decomposition in temperate and tropical soils (Bond-Lamberty and Thomson 2010). Consequently, CO_2 capture and storage are crucial to combat excessive atmospheric CO_2 concentrations (Alcalde et al. 2018). Lal (2008) indicated that a strategy to reduce global CO_2 emission rates is C capturing from atmosphere through natural and engineering techniques such as biochar (stable or low degradable carbon obtained from pyrolysed biomass) addition to soil. This strategy to deploy biochar on a large scale would divert a portion of global carbon flux as biomass residues stored in soil lead finally to less carbon emissions back to the atmosphere (Wang et al. 2023). The use of biochar as soil amendment successfully incremented carbon storage and improved soil quality and fertility of different soil types, in different regions and climates (Bolan et al. 2022). Biochar could help to accumulate additional carbon into soils, since its use promotes increments on vegetal biomass (Yu et al. 2019). On the other hand, biochar is considered a material able to increase soil-water contents in soils (Adhikari et al. 2022) and physically and chemically adsorb greenhouse gases (Thomazini et al. 2015). Unfortunately, biochar could contain combustion-driven toxic organic compounds such as polycyclic aromatic hydrocarbons (PAHs), chlorinated hydrocarbons or dioxins (Kookana et al. 2011). Specifically, the release of PAHs resulting from cellulose pyrolysis is a potential drawback regarding biochar usage (Ojeda et al. 2016) because PAHs are considered toxic for organisms due to their carcinogenic and mutagenic properties (Wang et al. 2017). Although biochar was successfully used to reduce CO_2 emissions from SOM decomposition in soils (Li et al. 2018), in some cases, its application either promoted (Smith et al. 2010) or had no influence on soil respiration (Liu et al. 2016). In conclusion, it is important to quantify advantages and disadvantages of biochar use as organic amendment under consideration of soil CO_2 emissions, based on the hypothesis that biochar application to soil could produce both: increase or decrease in soil respiration which determines the long-term soil carbon budget.

However, biochar ageing effects (Mia et al. 2017) after application to soil are currently ignored, especially the consideration of time-dependent alteration effects of physical and chemical soil properties. Biochar ageing processes could be related to changes

on its elemental composition, hydrophilicity and amphotericity (Cheng and Lehmann 2009), caused by biotic (microbial activity) or abiotic (chemisorption of oxygen) oxidation (Cheng et al. 2006). After oxidation, surface hydrophilicity potentially increases on aged biochar surfaces (Joseph et al 2010). More specifically, fresh biochar, initially hydrophobic before its application to soil, could become a highly hydrophilic material (Ojeda et al 2015). Since gas adsorption and liquid adsorption are specific characteristics of porous materials (Shafawi et al. 2021), it is meaningful to establish a relationship between carbon and water adsorption for biochar-enriched soil. However, studies about the biochar ageing process and its impact on soil carbon sequestration dynamics are scarce. Hence, the main objectives of this study were (i) to determine the biochar ageing effects on soil CO_2 emissions by relating soil properties to heterotrophic respiration intensity and (ii) to evaluate the biochar ageing by analysing the relationship between carbon sequestration capacity vs. biochar-water contact angles. Data from Ojeda et al. (2015) and Marks et al. (2014a, 2014b), which are referred to the same experiment, were taken into account to support the observed results in soil respiration and CO_2 adsorption capacity in soils amended with slow-pyrolysed biochars.

2 Materials and methods

2.1 Site description

The experiment was developed in the greenhouses of the Autonomous University of Barcelona (Cerdanyola del Vallès, Spain) (Ojeda et al. 2015; Marks et al. 2014a). The soil samples were collected from the topsoil of a Fluventic Haploxerept (Soil Survey Staff 2010). The sampling site was located in the experimental fields of the Institute of Agro-Food Research and Technology (IRTA) (Marimón Tower, Caldes de Montbui, Catalonia, Spain), and after collection, soil was air-dried for 1 week and sieved < 5 mm (Ojeda et al. 2015). Table 1 shows the main soil physical and chemical properties.

2.2 Biochar characterisation

The soil was amended with two types of biochar, obtained from poplar and pine wood splinters subjected to slow pyrolysis technique. Physical and chemical data are presented in Table 2 (Ojeda et al. 2015, Marks et al. 2014b). With respect to biochar manufacturing, slow pyrolysis of poplar and pine wood splinters was conducted in a low oxygen chamber at 500–550°C for 15 min. Production was conducted at the laboratories of the Chemical and Environmental Engineering Group of the University of León (León, Spain) (Ojeda et al. 2015). A general characterisation of both biochar types is given in Table 2 (data from Ojeda et al. 2015; Marks et al. 2014b).

2.3 Experimental setup

The soil treatments tested were as follows: *C*: control, i.e. soil without biochar; P_oS: soil + slow-pyrolysed poplar wood; and P_iS: soil + slow-pyrolysed pine wood. The biochar doses applied were as follows: 11.6 g of slow-pyrolysed poplar by kg of soil and 10.9 g of slow-pyrolysed pine per kg of soil were added at the beginning of the experiment.

Table 1 Physical and chemical properties of soil without biochar (Ojeda et al. 2015, Marks et al. 2014a, b)

Parameter	Unit	Soil
pH (water, 1:5)		8.3
EC (25°C, 1:5)	dS/m	0.8
Gravel (2–5 mm)	%	5.7
Sand (2–0.05 mm)	%	59.6
Silt (0.05–0.002 mm)	%	23.0
Clay (< 0.002 mm)	%	17.4
Textural class (USDA)	--	Sandy loam
Carbonates (calcimetry)	g kg^{-1}	60
Bulk density (δ_b)	g cm^{-3}	1.3
Organic matter (Walkley-Black)	g kg^{-1}	16
Total N (Kjeldahl)	g kg^{-1}	0.8
Na (ammonium acetate ext.)	mg kg^{-1}	62
K (ammonium acetate ext.)	mg kg^{-1}	159
Ca (ammonium acetate ext.)	g kg^{-1}	5.5
P (Olsen)	mg kg^{-1}	27
Cd (acid ext.)	mg kg^{-1}	0.1
Cr (acid ext.)	mg kg^{-1}	10
Cu (acid ext.)	mg kg^{-1}	17
Hg (acid ext.)	μg kg^{-1}	16
Ni (acid ext.)	mg kg^{-1}	7
Pb (acid ext.)	mg kg^{-1}	25
Zn (acid ext.)	mg kg^{-1}	65

These biochar doses were equivalent to 1% of C, mixing 10 g C of biochar per 1000 g of dry soil. Twelve plastic containers (4 replicates by treatment, 12 samples by sampling time) of 2 L were prepared. The experiment included two sampling dates to evaluate biochar and soil properties: 1 month (S1) and 1 year (S2) after biochar addition to soil. In order to avoid carbon inputs from vegetation, soil respiration was analysed without vegetation cover, simulating fallow scenarios. At each container, soil and a gravel layer at the bottom to facilitate drainage were placed. Soil treatments (with or without slow-pyrolysed biochar) were mechanically homogenised by cement mixer. The material was then placed into the plastic containers, with a layer of gravel at the bottom to facilitate drainage. All plastic containers were placed in a plastic semi-cylindrical walk-in tunnel, partly open (without plastic) laterally during the experiment. All soil samples were irrigated every 3 days (spring, summer) or weekly (autumn, winter) during a year, with a constant water amount equal to 50% of field capacity (FC) (see next section). At each sampling time (destructive sampling), soil samples were air-dried at 25°C and 50% relative air humidity, sieved at 5 mm and stored at 4°C in the dark, before being analysed for soil respiration measurements. From these samples, biochar particles were removed manually, one by one, carefully, using metal tweezers until obtain 0.1 g (in agree with the sample volume required for CO_2 adsorption analysis), 1 month and 1 year after biochar application to soil, in order to analyse changes in biochar wettability and CO_2 adsorption capacity. Weekly, the growing vegetation was manually removed during the entire experimental period (for more details, see Ojeda et al. (2015)).

Table 2 Mean concentration of elements (C, H, N and S by elemental analysis and the remainder soluble in *aqua regia*), doses and physical properties of the different types of biochar (Ojeda et al. 2015; Marks et al. 2014a, b)

Parameter	Unit	P_oS	P_iS
C	g kg^{-1}	811	863
O	g kg^{-1}	140	103
H	g kg^{-1}	21	20
N	g kg^{-1}	4.8	1.2
H:C	--	0.30	0.27
O:C	--	0.13	0.09
S	g kg^{-1}	0.4	0.2
C:N	--	197	839
Ca	g kg^{-1}	9.6	3.8
K	g kg^{-1}	6.6	3.5
Na	g kg^{-1}	1.0	0.3
P	g kg^{-1}	2.0	3.5
Fe	g kg^{-1}	2.0	1.2
Cd	mg kg^{-1}	ND	ND
pH	--	8.3	7.1
Granulometry			
2–1 mm	%	46	41
1–0.5 mm	%	16	20
0.5–0.2 mm	%	14	10
0.2–0.1 mm	%	5	5
0.1–0.05 mm	%	14	10
< 0.05 mm	%	5	14
Dose	g kg^{-1}	11.6	10.9
δ_b	g cm^{-3}	0.138	0.216

Slow pyrolysis: temperature range of 500–550°C; duration: 15 min; production efficiency: kilogram of biochar/kilogram of biomass, 0.29 (poplar) and 0.27 (pine). Dose: gram of biochar kilogram of $soil^{-1}$. δ_b: bulk density. CAc: soil water contact angle by sessile drop method on crushed samples. CAuc: soil water contact angle by sessile drop method on uncrushed samples. P_oS: soil + slow pyrolysed poplar. P_iS: soil + slow pyrolysed pine

2.4 *Soil-water retention and soil* CO_2 emissions or soil respiration

Soil-water contents at field capacity of subsamples with and without biochar were estimated gravimetrically weighing the PVC soil cores (height: 3.4 cm, inner diameter: 1.7 cm) using an electronic balance (0.001 g precision). After 24 h of soil saturation, followed by 24 h of free drainage, in a room at 20°C, the drained soil cores were placed over a sand box (Eijkelkamp®) at a soil suction of − 0.03 MPa to establish field capacity conditions. Soil core weight was recorded until equilibrium (usually during 5 days) (Ojeda et al. 2015). With respect to soil respiration, soil CO_2 emissions of fresh soil subsamples (with and without biochar) taken from containers were adjusted to a soil-water content equal to 50% field capacity and incubated at 20°C during 5 days. The measurement of carbon mineralised to CO_2, trapped in 1 M NaOH, was performed by titration against 0.5 M HCl (Anderson 1982). After incubation, the CO_2 trapped in NaOH were compared to those observed

in containers without soil (blanks). All sodium hydroxide lost during the sample incubation time was finally attributed to the CO_2 emitted from soil samples during soil organic matter (SOM) decomposition.

2.5 CO_2 adsorption in soils and biochar

The CO_2 adsorption capacity of soil aggregates (< 2mm, 2g) and particles of fresh biochar (not applied to soil, categorised as S0, < 2mm, 0.1g) and particles of aged biochar particles (removed from soil subsamples manually after 1 month (S1) and 1 year (S2) after its application, < 2mm, 0.1g) was evaluated by an in-house built volumetric Sievert system (Silva et al. 2013). Two volumes—reference volume (27.2 cm^3) and sample chamber volume (3.2 cm^3)—were measured with high precision before the CO_2 adsorption measurements. Initially, the sample chamber was evacuated, and the reference volume was filled with a defined amount of CO_2. Then, a valve was opened to let the gas expand to the combined volume of the two chambers. The final pressure value after its stabilisation (10 min, approximately), under constant temperature (20°C), was recorded. Consequently, the present experiment evaluates the CO_2 adsorption capacity of biochar during 10 min. The difference between the initial and final volume and pressure values defined the variation of the number of moles adsorbed by porous materials (soil, biochar), calculated using Benedict-Webb-Rubin (BWR) equation of state by the software GS2013 (Domingos 2013). Six successive expansions gave the total quantity of gas adsorbed at each equilibrium, obtaining a curve between 0 and 5 bars calculated from a CO_2 adsorption rates vs. pressure values. This procedure included calibration corrections of the pressure transducer. At each sample, the CO_2 adsorption capacity at atmospheric pressure was estimated from plots of CO_2 adsorption vs. pressure values. All the CO_2 adsorption experiments carried out were preceded by the determination of the void volume of the respective sample chambers containing the soil or biochar samples, respectively, by using helium gas as testing agent.

2.6 Biochar wettability

To assess the ageing process of biochar particles applied to soil, biochar-water contact angles were determined by the sessile drop method to indicate modifications in wettability. An optical contact angle measuring and contour analysis system (OCA 15, DataPhysics, Filderstadt, Germany) was used to determine soil-water contact angles on crushed and uncrushed biochar particles, previously removed from soil subsamples. A double-sided adhesive tape was fixed to a flexible tissue, and then, biochar particles (< 2 mm) were adhered, obtaining a homogeneous grain cover on the tape surface. After the tissue with biochar particles was placed under a syringe with distilled water (fixed vertically at OCA 15), a drop of 1 μg of water was placed on the surface of several biochar particles and the formation of the water drop contour line was recorded with a video camera, by a CCD-equipped contact angle microscope (OCA 15, DataPhysics, Filderstadt, Germany). The direct measurement of contact angles at the solid-liquid interphase as provided by the sessile drop method could be considered the best option to evaluate biochar wettability and respective modifications compared to other tests such as the indirect capillary rise method (Bachmann et al. 2003), where contact angles are estimated by an equation that compares the adsorption of two different liquids (e.g. water and hexane).

The mean solid-liquid contact angle (CA) value measured between biochar surface and the water drop was calculated after 30 ms, after drop placement by the SCA 20 programme

(DataPhysics, Filderstadt, Germany) (Bachmann et al. 2013). The measurements were taken with fresh biochar particles (initial condition (S0)—not exposed to field conditions) and with biochar particles exposed to environmental conditions, after 1 month (S1) and after 1 year of its application (S2) A general description of biochar granulometry is presented in Table 2 (data from Ojeda et al. 2015).

2.7 Soil organic carbon

Total organic carbon (TOC) contents were estimated by a dichromate acid oxidation at 150°C in strong acid media (Nelson and Sommers 1982) in soil fraction < 2 mm.

2.8 Polycyclic aromatic hydrocarbons (PAHs)

The concentration of 13 different types of PAHs were determined in pine and poplar slow-pyrolysed biochars, after its extraction and purification by gas chromatography (Agilent GC system 7890A, Paris, France) coupled with a mass spectrometer (Agilent 5975C inert XL MSD, Paris, France) (Gateuille et al. 2014): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h) anthracene, indeno(c,d)pyrene and benzo(g,h,i)perylene. Approximately 1 g of biochar was extracted by ASE 350TM (Thermo Scientific). The extraction consisted of 3 cycles of 5 min at 100°C with a dichloromethane: acetone mixture (50/50), followed by 3 cycles of 5 min at 100°C with a hexane/acetone mixture (50/50). The two extracts were then combined and concentrated by an EZ-2 evaporator, a rotary evaporator, or under nitrogen flow. The extracts were purified with copper (one night) and then on a Florisil Superclean column (MgO-SiOH) with two mixtures of solvents (hexane/dichloromethane 50/50 and hexane/acetone 50/50) according to the method developed by Sánchez-Avila et al. (2011). After a final concentration step, the samples were analysed by gas chromatography coupled with double mass spectrometry (GC/MS-MS) (Alliot et al. 2014).

2.9 Statistical analysis

To evaluate the significance of temporal changes on soil and biochar properties, a two-way ANOVA was applied among treatments (C: control, P_oS: slow-pyrolysed poplar, P_iS: slow-pyrolysed pine) for each sampling time (S0: fresh biochar not applied to soil, S1: after 1 month, and S2: after 1 year of biochar application to soil). When soil and biochar properties showed significant interaction among treatments and sampling time, a one-way ANOVA was conducted at each sampling, followed by Tukey's post hoc tests to permit pairwise comparisons of means ($p < 0.05$). When data normality (Shapiro-Wilk test) or the equality of error variances (Levene test) was not confirmed per dataset, nonparametric test (Kruskal-Wallis test) were used, followed by a Dunn test to determine significant differences between treatments or sampling times. In terms of simple linear regressions, its statistical validity was verified by several tests available from R software[42]: (a) ANOVA of each model tested, (b) a mean of residues close to zero, (c) normality of unstandardised residues values ($p > 0.05$) by Shapiro-Wilk test, (d) the existence of potential outliers by Cook's distance higher than 1 and (e) homoscedasticity by studentised Breusch-Pagan test.

For all tests required to compare treatment-time interaction and to obtain simple regressions, the R software was used (R Core Team 2013).

3 Results

3.1 Soil CO_2 emissions

In terms of CO_2 emitted during SOM decomposition (soil respiration (SR)), a significant interaction among treatments and sampling times was observed (Table 3). The SR values were reduced by slow-pyrolysed poplar and pine compared to the control treatment without biochar (Fig. 1a) by 11.1% and 13.4% respectively, 1 month after the starting of the experiment (S1). In contrast, the capacity of biochar to reduce CO_2 emissions from soils disappeared 1 year after its addition to soils (S2) (Fig. 1a). Globally, regardless of the treatment type, the SR values decreased between sampling times S1 and S2 by 18.9%. In addition, SR values at S1 were higher than those observed at S2 in control and P_oS treatments, while in the case of P_iS treatment, SR values at S2 were higher than those observed at S1 (Fig. 1a).

3.2 Soil and biochar CO_2adsorption capacity

Significant differences of CO_2 adsorption among soil samples and poplar and pine biochar particles were observed (Table 3). Pieces of slow-pyrolysed pine and slow-pyrolysed poplar showed a remarkably high CO_2 adsorption capacity, in comparison with the low CO_2

Table 3 Summary of two-way variance analysis of soil properties between treatments and sampling times

Parameters	*F* and $Chi2^{\xi}$ values		
	Treatment (*T*)	Sampling time (*S*)	$T \times S$
SR	7.1**	36.3***	27.2***
$CO_{2\ ads}{}^{\xi}$	15.7***	4.0*	NA
TOC	31.3***	11.1***	NS
$CO_{2\ ads\text{-}b}$	12.6**	26.7***	7.4**
$CA_c{}^{\xi}$	3.9*	15.7***	NA
$CA_{uc}{}^{\xi}$	NS	15.0***	NA

SR: soil respiration (mg CO_2-C kg^{-1} h^{-1}). TOC: total organic carbon (%). $CO_{2\ ads}$: comparison of CO_2 adsorption rates between control treatment (soil without biochar) and aged slow-pyrolysed biochars. $CO_{2\ ads\text{-}b}$: comparison of CO_2 adsorption rates between for fresh biochar at S0 (sampling 0—initial conditions of fresh biochar), S1 (sampling 1—1 month after its application) and S2 (sampling 2—1 year after its application) (mmol CO_2 g^{-1}). CAuc: biochar-water contact angles over uncrushed biochars (°). CAc: biochar-water contact angles over crushed biochars (°). Differences were significant at $p < 0.05$ (*), 0.01 (**) or 0.001 (***) for ANOVA (*F* value) or Kruskal-Wallis tests (Chi2 value$^{\xi}$). NS: not significant. TOC, CA_c and CA_{uc} values from Ojeda et al. (2015). For SR, $CO_{2\ ads}$ and TOC, two sampling times (S1, S2) were analysed, while for $CO_{2\ ads\text{-}b}$, CAc and CAuc, three sampling times (S0, S1, S2) were analysed. NA: not applicable

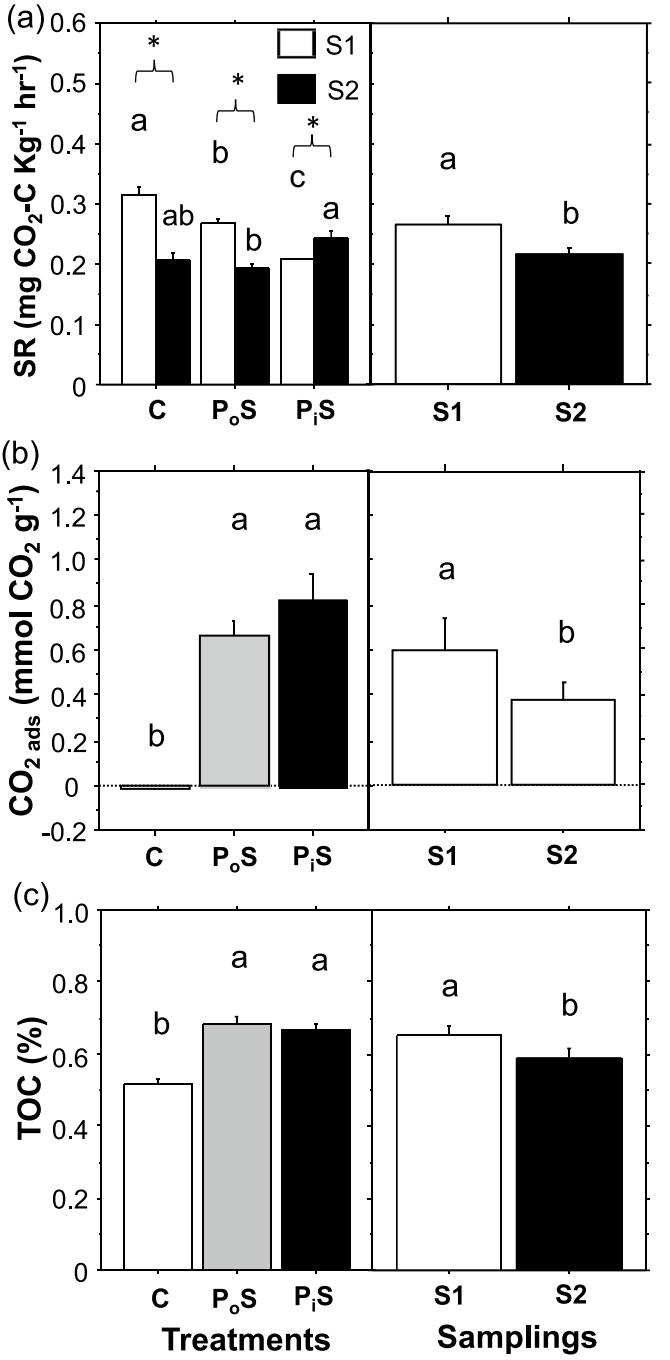

Fig. 1 Mean values of **a** soil respiration (SR) rates, **b** CO_2 adsorption capacity ($CO_{2\ ads}$) and **c** total organic carbon (TOC). C: control treatment without biochar, P_iS: slow-pyrolysed pine biochar, PoS: slow-pyrolysed poplar biochar. S1: sampling 1 month after the start of the experiment. S2: sampling 1 year after the start of the experiment. Different lowercase letters mean significant differences between treatments ($p < 0.05$)

desorption rates (negative values) observed in soil without biochar, i.e. 73.8- and 91.1-fold the soil desorption rate, respectively (Fig. 1b). In addition, irrespective of the evaluated material (soil, biochars), the CO_2 adsorption capacities were higher at S1 than those at S2 (Fig. 1b).

3.3 Soil organic carbon

The application of slow-pyrolysed biochars made it possible to permanently modify soil carbon stock (Fig. 1c, data from Ojeda et al. (2015)) over the entire length of the experiment, as shown by the absence of significant interaction among treatments and sampling times (Table 3). The addition of biochar to soils increased the total organic carbon (TOC) contents around 32.4% by slow-pyrolysed poplar and around 29.1% by slow-pyrolysed pine. In total, without taking into account the type of treatment, TOC values at S1 were higher than those at S2 (Fig. 1c).

3.4 Biochar ageing influence on CO_2adsorption and wettability

Comparing biochar particles before and after its application to soil, it was possible to observe that biochar capacity to adsorb CO_2 ($CO_{2\ ads\text{-}b}$) changed significantly with time, with a significant interaction between treatments and sampling times (Table 3). The $CO_{2\ ads\text{-}b}$ values of slow-pyrolysed poplar decreased 34.1% after 1 year (S2) of application to soil with respect to biochar particles which were not applied to soils (S0) as shown by Fig. 2a. Similarly, the $CO_{2\ ads\text{-}b}$ values of slow-pyrolysed pine decreased by 61.9% 1 year (S2) after application regarding biochar particles that were not added to soil (S0). In contrast, the CO_2 adsorption capacity of biochars ($CO_{2ads\text{-}b}$) was higher in slow-pyrolysed pine than that in slow-pyrolysed poplar biochar particles (Fig. 2a), at S0 (28.5%) and at S1 (38.3%) (Fig. 2a). Interestingly, at sampling time S2, these differences of $CO_{2ads\text{-}b}$ values between biochar types disappeared (Fig. 2a). In summary, irrespective of the treatment method, CO_2 adsorption capacity of slow-pyrolysed pine biochar was higher than that in slow-pyrolysed poplar biochar, and CO_2 adsorption capacity at S0 and S1 was higher than that observed at S2 (Fig. 2a).

On the other hand, the internal (crushed biochar particles) wettability and superficial (uncrushed biochar particles) wettability of poplar and pine biochars were evaluated by the measurement of biochar-water contact angles on crushed (CAc) and uncrushed (CAuc) biochar particles (Fig. 2b, c, data from Ojeda et al. 2015). Globally, regardless of the sampling times, two observations were made: on one hand, the mean CAc values of slow-pyrolysed poplar biochar were higher than those observed at slow-pyrolysed pine biochar, 8.4% (Fig. 2b). On the other hand, the mean CAc values at samplings S0 and S1 were 32.8% higher than those observed at sampling S2 1 year after biochar application to soil (Fig. 2b).

Finally, superficial wettability of biochar particles was evaluated by the measurement of biochar-water contact angles on uncrushed biochar particles (CAuc) (Fig. 2c, data from Ojeda et al. (2015)). Without taking into account the sampling times, CAuc values of both biochars were similar (Fig. 2c). However, examining the temporal variation of superficial wettability of biochars, it was observed that CAuc values at samplings S0 and S1 were higher than those observed at S2, 76.3% (Fig. 2c).

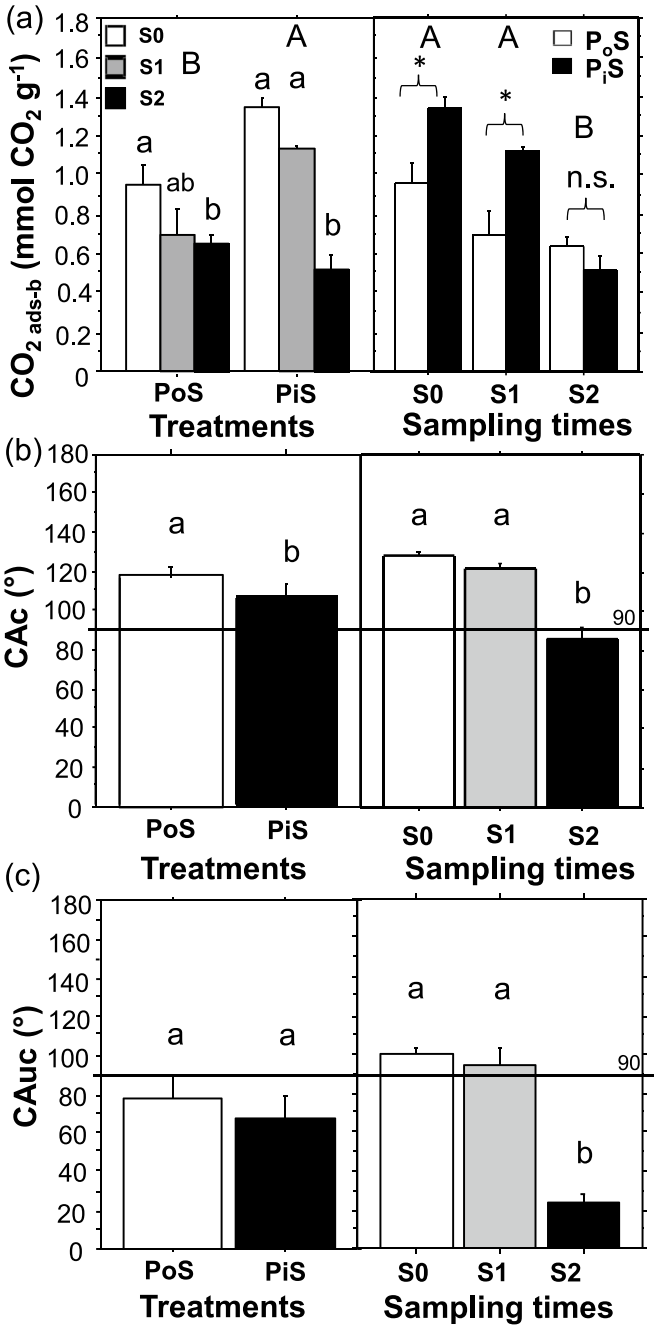

Fig. 2 Mean values of **a** CO_2 adsorption capacity of biochars ($CO_{2\ ads-b}$), **b** contact angles of crushed biochar (CAc) and **c** contact angles of uncrushed biochar (CAuc). PiS: slow-pyrolysed pine biochar, PoS: slow-pyrolysed poplar biochar. S0: initial sampling (biochar before its application to soil). S1: sampling 1 month after the start of the experiment. S2: sampling 1 year after the start of the experiment. Different lowercase letters mean significant differences between treatments, while different capital letters mean significant differences between sampling times ($p < 0.05$)

3.5 Interaction between respiration and CO_2adsorption capacity of biochars

In terms of the relationship between soil respiration rates and CO_2 adsorption capacity of the biochar particles, it was observed that increased CO_2 adsorption capacity of biochar corresponded to decreased soil respiration rates, 1 month after biochar application to soil (S1) (Fig. 3a). In contrast, no significant relationship between soil respiration rates and CO_2 adsorption capacity of biochars was observed, 1 year after biochar application to soil (S2) (Fig. 3b).

Biochar wettability was now related to changes in the CO_2 adsorption capacity of biochars as a function of time; i.e. two relationships were evaluated (Fig. 4): (a) CO_{2ads} vs. CAc and (b) CO_{2ads} vs. CAuc. It was observed that increased biochar-water contact angles (CAc and CAuc), equivalent to reduced biochar wettability, were related to increased CO_2 adsorption capacity of slow-pyrolysed poplar and also for pine biochars (Fig. 4a, b). Both relationships explained approximately a 30% of observed variability.

3.6 Polycyclic aromatic hydrocarbon (PAH) and metal contents in biochars

In terms of to the contaminants associated with biochars (EBC 2023), the slow-pyrolysed poplar and pine biochars may have the potential to elevate PAHs above permissible levels (Table 4). In terms of metal contents, slow-pyrolysed poplar and pine biochars presented Cr, Cu and Ni contents higher than the permissible limit, while Pb and Zn contents were lower than the mentioned limits (Table 4).

4 Discussion

4.1 Biochar effects on soil respiration

In general, soil respiration or CO_2 emissions from soils occur when environmental conditions are able to promote SOM decomposition by microbial activity (Wang et al 2014). During this process, soil organic matter is partially transformed to CO_2, which is released to the atmosphere reaching around 30 Pg C yr^{-1} of SOM-derived CO_2 from tropical forest soils (Nottingham et al. 2022). The addition of biochar to soil is a practice addressed to replace easily-mineralised organic matter or fertilisers with a material able to improve soil fertility and resistant to the decomposition processes promoted by microbial activity (Tang et al. 2022), promoting carbon storage in soils and helping to mitigate climate change (Keith et al. 2015). The viability of this idea rose from the discovery of Terra Preta soils in Brazil (Sombroek 1966), where patches of high fertile soils were found surrounded by unfertile soils (Lal 2016), suggesting that the addition of stable carbon to soil improves significantly soil quality (Lorenz and Lal 2014). The benefits of biochar used as soil amendment include (a) increasing the number of beneficial bacteria on soil contaminated by microplastics (Ran et al. 2023), (b) decreasing cadmium contamination in crops (Wang et al. 2024), (c) improvement of crop adaptation to ambient conditions such as nutrient deficiency, aridity and water stress conditions (Zhang et al. 2024) and (d) reductions of pesticide residues (Sarker et al. 2023). With respect to the soil respiration (SR) rates observed in soil with and without biochar, two clear processes were observed: (a) a reduction of CO_2 emissions from soil incubated at 50% of field capacity after 1 month of

biochar addition (Fig. 1a) and (b) the disappearance of this effect of slow-pyrolysed poplar and pine biochars on soil respiration, after 1 year of biochar addition to soil (Fig. 1a). In the absence of a cover vegetation, a similar scenario to fallow lands without carbon inputs from plants, these consecutive events indicate that the effect of biochar on soil microbial activity is temporal, not permanent. The reduction of SR rates, in terms of soil microbial activity, could be due to (a) SOM encapsulation on biochar pores, physically protecting SOM to access of microbial activity (Zimmerman et al. 2011), (b) biochar-induced increments on soil-water storage capacity (Wong et al. 2022), able to decrease oxygen availability on soils (Or et al. 2007), (c) reduction in SOM decomposition due to an inhibition of carbohydrate catabolism by increments in bacterial and fungal diversity promoted after biochar application (Chen et al. 2019), (d) toxic effects of the biomass combustion products contained in biochars, such as polycyclic aromatic hydrocarbon (PAH) (Godlewska et al. 2021), and (e) physical adsorption of CO_2 by biochar (Zhang et al. 2019) (Figs. 1b, 2a, and 3a). In addition, decrements in SR values after 1 year of continuous addition of water in soils without biochar and in soils with slow-pyrolysed poplar biochar probably indicated that the quantity of easily decomposable SOM was depleted after 1 year (Fig. 1a), in the absence of carbon inputs from vegetation cover (Fig. 1c).

As a consequence, the temporal influence of biochar on the reduction of SR rates (Fig. 1a) could suggest that it is necessary to repeatedly add biochar to soil to maintain the observed initial benefits of biochar application, i.e. in terms of the adsorption of CO_2 emitted from soils. The CO_2 adsorption capacity of biochar disappeared after 1 year of biochar application to soil (Fig. 3) probably due to biotic or abiotic oxidation processes able to transform biochar initially water-repellent biochar into hydrophilic biochar (Zimmerman 2010), especially on its surface (Fig. 4). However, it is also necessary to take into account that PAHs contained in slow-pyrolysed poplar and pine biochars (Table 4) are part of the 16 PAHs from the US EPA priority pollutant list (EBC 2023). Then, repeated applications of biochar to soil could include the risk of accumulating PAHs or other contaminants over advisable limits (Table 4) for human health (Wang et al. 2019), since biochar is able to adsorb and store a wide range of contaminants (e.g. PAHs and metals) (Abbas et al. 2018), with undesirable consequences. Specifically, PAH contents of both biochars could reduce soil microbial activity due to high contents of naphthalene (Table 4), considered the most abundant PAH in biochars, which may have the potential to reduce nitrogen transformations in soils amended with biochar (Chang et al. 2002), although its residence time on soil could be low due its volatility. Odinga et al. (2021) made several recommendations to ensure a safe application of biochar to soils, including (a) analytical studies of PAHs, polychlorinated biphenyls (PCBs), volatile organic compounds (VOCs), environmentally persistent free radicals (EPFRs) and metal contents, (b) optimal manufacturing temperatures to obtain biochars, able to avoid or reduce the precursor substances in biochars, and (c) ecotoxicological evaluation of biochar doses applied to soils. However, the technical challenge to obtain biochar with these quality requirements is still a goal worth to be achieved.

4.2 Biochar ageing effects on CO_2 adsorption andwettability

Biochar ageing could be caused by interactions with soil mineral colloids (Ren et al. 2018), soil wetting-drying cycles, temperature changes and soil biological activity, whereby the latter is considered the most important factor in comparison to abiotic processes (Quan et al. 2020). In this study, biochar wettability and CO_2 adsorption capacity were selected as

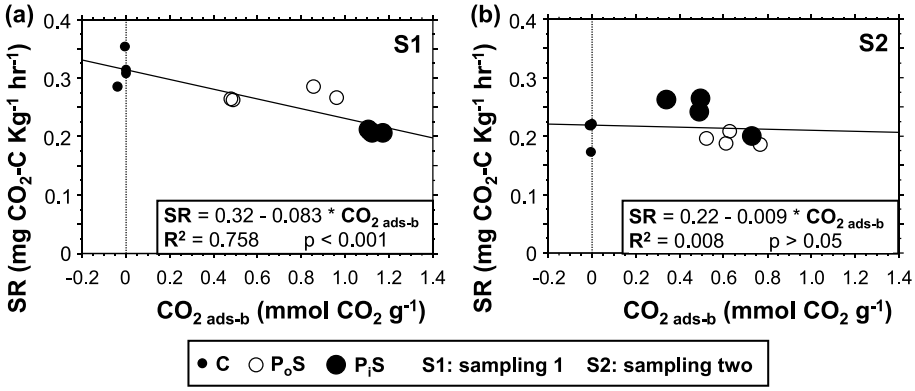

Fig. 3 Relationship between soil respiration (SR) rates and CO_2 adsorption capacity of biochars ($CO_{2\ ads\text{-}b}$). **a** S1: sampling 1 month after the start of the experiment. **b** S2: sampling 1 year after the start of the experiment

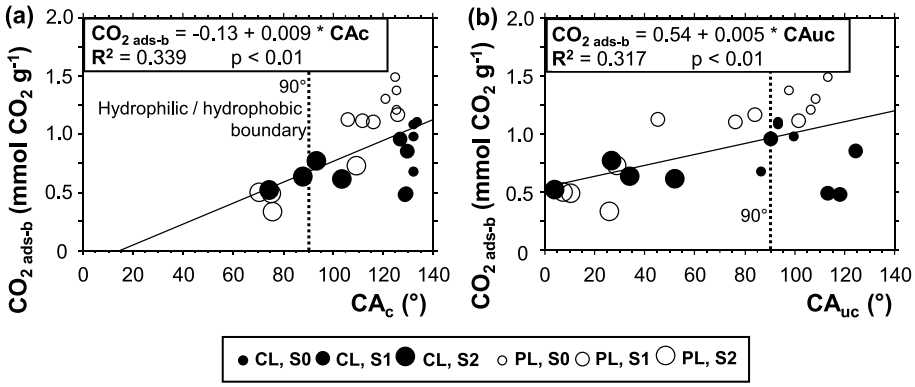

Fig. 4 CO_2 adsorption capacity of biochars ($CO_{2\ ads\text{-}b}$) and CO_2 adsorption capacity of biochars ($CO_{2\ ads\text{-}b}$). **a** Contact angles of crushed biochar (CAc). **b** Contact angles of uncrushed biochar (CAuc)

analytical and easily accessible key properties to evaluate biochar surface ageing. A powerful tool to evaluate changes of wettability in solid and porous materials is the measurement of contact angles at the solid-liquid interphase (Woche et al. 2017). When biochar is applied to soil, probably a part of CO_2 emissions produced by SOM decomposition can be adsorbed by biochar (Figs. 1a, b and 2a). The remarkable CO_2 adsorption capacity of slow-pyrolysed biochars compared to CO_2 desorption rates observed in soil (Fig. 1b) could be caused by (i) biochar aromaticity as observed by Marks et al. (2014b) on the same biochars used in this study, able to increase the van der Waals forces during biochar matrix and CO_2 contact (Igalavithana et al. 2020), and (ii) high degree of hydrophobicity because water repellency pushes the competition between water and gas adsorption towards the gas phase and may also improve accessibility of small biochar pores for CO_2 adsorption (Guo et al. 2022) (Figs. 2b, c and 4a, b). The main evidence that slow-pyrolysed biochars could suffer an ageing process was reflected by the fact that biochars increased its wettability, favouring water adsorption before CO_2 in time (Fig. 2b, c). In contrast, the CO_2 adsorption capacity of biochars decreased continuously with time (Figs. 2a and 4a, 4b), probably due

Table 4 Polycyclic aromatic hydrocarbons (PAHs) and metals of poplar (P_oS) and pine (P_iS) biochars, together with the limit values of PAH and metals allowed in biochars

Element/compound	P_oS (mg kg^{-1})	P_iS (mg kg^{-1})	Limit values (mg kg^{-1})
Naphthalene	57.4	42.4	-
Acenaphthylene	0.173	0.145	-
Acenaphthene	0.038	0.029	-
Fluorene	0.107	0.055	-
Phenanthrene	0.590	0.523	-
Anthracene	0.067	0.074	-
Fluoranthene	0.203	0.257	-
Pyrene	0.427	0.517	-
Benzo(a)anthracene	0.030	0.085	-
Chrysene	0.089	0.175	-
Benzo(b)fluoranthene	0.017	0.060	-
Benzo(k)fluoranthene	0.005	0.019	-
Benzo(a)pyrene	0.003	0.018	-
Indeno(c.d)pyrene	0.001	0.008	-
Dibenzo(a.h)anthracene	< udl	0.004	-
Benzo(g.h.i)perylene	< udl	0.011	-
Total PAHs	59.1	44.3	6–300***
Cr	213*	83*	93–1200**
Cu	95–728*[£]	35–446*[£]	143–6000**
Ni	253*	97*	47–420**
Pb	75*	16*	121–300**
Zn	140*	92-318*[£]	416–7400**

*Data of metals contained in biochars studied from Ojeda et al. (2015) and Marks et al. (2014b). **Limit values of metals in biochars (mg kg^{-1}) (EBC, 2012). ***Limit values of PAHs in biochars (mg kg^{-1}) (EBC, 2012[1]). [£]Element not homogeneously distributed in the sample

[1]EBC. (2012). European Biochar Certificate - Guidelines for a Sustainable Production of Biochar. European Biochar Foundation (EBC), Arbaz, Switzerland. (https://biochar-international.org/wp-content/uploads/2020/06/IBI_Biochar_Standards_V2.1_Final2.pdf). Version 9.1E of 25th September 2020

to oxidation or leaching processes of biochar particles (Thomas 2021). Another possibility is that internal pores in biochar particles can be blocked at the particle surface through biofilm formation, hence reducing overall the capacity for physical and chemical adsorption of CO_2 molecules (Amer et al. 2022).

To standardise the function of biochar in soil, categorisation of biochar wettability could follow the same standards as for soil; i.e. (i) a solid-liquid contact angles higher than 90° indicate hydrophobic material, (ii) solid-liquid contact angles less than 90° are indicators of still wettable but slightly to moderately water-repellent material and (iii) solid-liquid contact angles equal to zero indicate completely wettable material, as was observed for soil particles. Taking 90° as limit that differentiates hydrophobic materials from wettable materials, it was observed that the oxidation process of our biochars is more intensive on surfaces than inside the biochar particles (Fig. 2b, c). This is clear because the reduction of biochar-water contact angles was greater in uncrushed than in crushed biochar particles (Fig. 2b, c). This increment in biochar wettability could be due to (i) oxidation processes promoted by low molecular weight organic acids (LMWOAs) derived from microbial secretions and organic

matter decomposition (Sun et al. 2016), (ii) the loss of organic surface coating of fresh biochar during leaching, exposing underlying micropores (He et al. 2019), and (iii) changes in bacterial cell surface properties (Karagulyan et al. 2022). In this study, around 30% of reduction of biochar CO_2 adsorption capacity was explained by reduction in biochar-water contact angles (Fig. 4). It is possible that slow-pyrolysed biochars that initially promote CO_2 adsorption turn after their transformation under ambient soil conditions to the hydrophilic state (Fig. 2b, c) which goes along with a reduction of physically adsorbed gaseous CO_2. In addition, pore clogging due to clay contents and/or calcium carbonate leaching in soils with high carbonate contents (Table 1) could be another reason to explain the reductions on CO_2 adsorption capacity of biochar in time (Sun et al. 2016).

As an outcome of the present investigation, the recent technological advances, addressed to improve biochar properties such as biochar activation (Sakhiya et al. 2020) or artificial pre-oxidation or post-oxidation methods (Nidheesh et al. 2021), need to take into account that probably CO_2 adsorption and water storage in biochars change with time after application which should be subject of further investigations.

4.3 Limitations and recommendations

The present study was generally successful to demonstrate if and to what extend biochar ageing is able to reduce its capacity to adsorb CO_2 in conjunction with increased wettability. However, some experimental limitations were observed during this study which could be improved: (a) CO_2 adsorption rates on soil + biochar mixtures were not measured on the long term, (b) the manual removal of biochar is not efficient to remove the finest biochar particles and therefore one biochar fraction remains in soil not accessible for direct analysis, (c) the CO_2 adsorption process was evaluated under limited conditions, i.e. during 10 min in a closed chamber, and (d) the measurement of soil organic carbon by a dichromate acid oxidation probably is not the best method to quantify biochar carbon because it could underestimate some of its components (Hammes et al. 2007). This was eventually observed in Fig. 1c, where the increment of 1% of soil carbon due to biochar addition was not reflected by this method. To overcome these limitations, it is necessary to evaluate CO_2 adsorption rates on soils amended with biochar, using higher volumes of sample, at best also under field conditions for comparison. To improve the determination of biochar carbon on soils, the measurement of total carbon by combustion methods is recommended. With respect to the future use of biochar amendment, it is necessary to analyse the biochar CO_2 adsorption capacity of different types of biochar considering the production mode as well as the origin material for biochar production. Finally, it is recommended to find a sustainable biochar dose, taking into account not only its potential benefits for carbon storage in soils but also its ecotoxicological effects on soil biota, evaluated by standard methods for micro-, meso- and microorganisms.

5 Conclusions

Biochar ageing is an important challenge in terms of the use of biochar as organic amendment. The measurement of CO_2 adsorption capacity and wettability in biochar particles could be considered useful to evaluate biochar ageing. The observed relationship between

biochar contact angles and CO_2 adsorption capacity could explain partially the role of temporal changes of biochar wettability on soil organic matter decomposition process and, consequently, on the persistence of carbon storage in the soil. In general, slow-pyrolysed biochars could be considered an interesting aspect to mitigate greenhouse gas emissions, at least temporarily, due to an unexpected great capacity to adsorb CO_2, at short term in soil. The future improvements on biochar technology could be addressed to obtain biochars that are more resistant to natural oxidation processes, in terms of preserving its CO_2 adsorption capacity and its impact on reduced soil respiration along with low contents of contaminants produced during biomass pyrolysis. It is also very important to determine what percentage of the yearly soil respiration rate can be adsorbed by biochar and for how long this material could maintain its maximum adsorption capacity, i.e. to keep CO_2 in soil for further transformation processes such as carbonate formation (Guo et al. 2022), promoting long-term stability of CO_2 adsorption process at large scale. On the other hand, it is recommended to determine if hydrophobicity has further positive attributes or if negative effects on carbon storage in soils occur with time.

Author contribution Gerardo Ojeda: methodology, investigation, statistical analysis, writing—original draft. João M. Gil: methodology and resources (CO_2 adsorption). Stefania Mattana: methodology and resources (soil respiration) and review. Jörg Bachmann: methodology and resources (biochar-water contact angles). Katell Quenea: methodology and resources (PAH analysis). Abílio Sobral: formal analysis, review and editing

Funding This study was associated to the Colombian project PS-35-2020 (Universidad Nacional Abierta y a Distancia - UNAD). The authors thank also the Centro de Quimica de Coimbra supported by Fundação para a Ciencia e Tecnologia - FCT (Portugal). The work of CO_2 adsorption performed at the CFisUC was supported by the Portuguese Science and Technology Foundation (FCT - Fundação para a Ciência e Tecnologia, I. P., Portugal) through projects UIDB/04564/2020 and UIDP/04564/2020. We want to thank all institutions involved in this study, for facilitating the access to their laboratories and giving support with the corresponding methodologies, equipment and funding resources. Biochar and soil respiration samples were provided from the SOCARRAT project (contract AGL2009-12343 of the Spanish Ministry of Science and Innovation, Spain).

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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