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Research paper

Impact of organic amendments on carbon stability and carbon use efficiency in acidic and alkaline soils

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ABSTRACT

Soils represent the largest reservoir of organic carbon in terrestrial ecosystems, yet the mechanisms controlling its stabilization and turnover are still not fully understood, limiting our ability to anticipate their response to climate change. Microbial processes are central to the formation, preservation, and loss of soil organic carbon (SOC), with microbial carbon use efficiency (CUE)—the fraction of assimilated carbon allocated to growth versus respiration—emerging as a key integrative parameter of microbial functioning. While CUE has been proposed as a predictor of SOC persistence, its contribution remains debated. In parallel, CUE is gaining attention in the context of carbon farming policies, as it links microbial functioning with soil carbon sequestration. Among the management practices aimed at enhancing SOC, organic amendments such as compost and biochar stand out for their capacity to influence CUE and improve soil functioning. In this study, we assessed how different organic amendments affect SOC stability and sequestration in two contrasting soils from the Iberian Peninsula: acidic grasslands and alkaline rain-fed soils. The amendments included four biochars, two cattle digestates, a green compost, and a biochar–compost mixture. Over 100 days, soil respiration (CO_2 emissions), microbial biomass, and soil properties were monitored using an automatic respirometer. Microbial CUE and microbial activity largely determined carbon (C) retention in the studied soils. Cow digestate increased microbial activity but reduced microbial CUE in both soils, leading to higher C losses through respiration and lower C retention. In contrast, biochars—particularly those produced from white poplar wood, olive pomace and rice husk—enhanced carbon recalcitrance, extending the residence time of the stable C pool by six to nine times compared with unamended soils. Microbial analyses showed that bacterial loads were 2–3 orders of magnitude higher than fungal loads. Compared with acidic grassland soils, alkaline soils generally showed higher microbial CUE values, reflecting a greater potential for C sequestration. These findings also indicate that microbial CUE exhibited clear soil-specific behavior, being consistently higher in the AS than in the acidic GS. This pattern suggests that differences in microbial community dominance—particularly the relative contribution of bacteria and fungi—may underlie the contrasting CUE responses observed between soils, a topic that warrants further investigation in future studies. In the alkaline soils, digestate amendments resulted in the highest bacterial abundance, whereas rice husk biochar favored fungal growth. Additionally, the high Cu and Zn content of cow manure digestate posed risks in acidic soils. This study also emphasizes that amendment strategies should be tailored to soil type to optimize carbon sequestration. Moreover, a novel thermal–respirometry correlation model was also developed, providing a practical tool for assessing soil carbon dynamics and C stability.

1. Introduction

Soil organic carbon (SOC) plays a pivotal role in the functioning of terrestrial ecosystems, influencing nutrient availability, soil structure, and overall soil health (Bauer and Black, 1994; Sáez-Sandino et al., 2024). Over the past millennia—and especially in the last 200

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years—land conversion to agriculture has led to significant losses of soil organic matter (SOM) by accelerating mineralization and erosion beyond natural inputs (Cotrufo and Lavallee, 2022). Soils represent the largest carbon reservoir in terrestrial ecosystems (Lal, 2004), and SOC is typically conceptualized as two contrasting pools: a labile fraction that decomposes rapidly within days to months, and a recalcitrant fraction that persists for years (De la Rosa et al., 2008; Zou et al., 2005). This two-pool model provides a more accurate framework for evaluating carbon residence time than the simpler one-exponential approach (Zimmerman et al., 2011; Leng et al., 2019). The two-pool model remains a simplification of highly interrelated processes, as the mechanisms controlling the magnitude of global SOC storage and its spatial distribution are still poorly understood, constraining our ability to make robust predictions of terrestrial feedbacks to climate change. Increasing evidence identifies soil microorganisms as pivotal agents, not only mediating carbon losses through decomposition but also fostering SOC accumulation and stabilization, as suggested by the strong linkages between microbial biomass, necromass, and SOC stocks. Although microorganisms influence soil organic matter dynamics through multiple pathways, microbial carbon use efficiency (CUE_{micro}) provides an integrative measure of their overall effect (Cotrufo et al., 2013). It is defined as the proportion of assimilated carbon that is invested in anabolic processes relative to catabolic losses, and it represents a fundamental control over the balance between SOC stabilization and mineralization (Liang et al., 2017). High CUE tends to favor SOC stabilization through microbial biomass and necromass inputs, whereas low CUE increases carbon release as CO_2. This trait is inherently scale-dependent, with distinct expressions measurable at the population, community, and ecosystem levels (Geyer et al., 2016; He et al., 2024). Yet the influence of CUE on SOC persistence is context-dependent, as environmental and substrate factors can override microbial allocation patterns (Allison et al., 2010). Even small shifts in CUE can strongly affect long-term carbon storage and climate mitigation potential, and global syntheses identify CUE as a stronger determinant of SOC stocks than carbon inputs or decomposition rates (Tao et al., 2023). In this study, we focus on the community-level expression of this trait, hereafter referred to as CUE_{micro}, to distinguish it from broader conceptual or ecosystem-scale definitions of CUE.

Among the strategies to enhance soil fertility and productivity, organic amendments are widely promoted (Luo et al., 2018), due to their potential for carbon sequestration (Lehmann and Joseph, 2009; Spokas and Reicosky, 2009). Such practices are embedded in policies like the EU long-term climate neutrality strategy, which emphasizes soils in achieving net-zero emissions by 2050 (European Commission, Energy, climate change, Environment, n.d). Yet, as Don et al. (2023) caution, the term “carbon sequestration” is often misused, leading to inflated expectations regarding its role in climate change mitigation. Many soils, particularly those depleted in organic matter, could act as carbon sources rather than sinks. The stability of applied amendments is therefore critical, since their decomposition may stimulate microbial metabolism and trigger CO_2 fluxes via priming effects (Fontaine et al., 2007; Kuzyakov et al., 2009). A recent meta-analysis of 9296 paired observations from 363 studies reported that fresh carbon inputs triggered positive priming effects in 97 % of cases, with an average increase of 37 %. Labile compounds accounted for most of this response, contributing to a 73 % increase (Xu et al., 2024a). If such effects are widespread globally, largely driven by labile organic matter, then the use of recalcitrant amendments becomes essential to counteract priming-induced acceleration of soil organic carbon turnover. Comparative evidence indicates that amendments such as biochar, compost, and digestates also regulate SOC accumulation by altering microbial CUE and respiratory losses (Blanco-Canqui et al., 2013; Giagnoni and Renella, 2022; Su et al., 2025). It is demonstrated that small shifts in soil CUE_{micro} can substantially influence carbon storage and gas fluxes (Tao et al., 2023). Indeed, high CUE tends to promote SOC stabilization through microbial biomass and necromass formation, whereas lower CUE increases the proportion of carbon lost as CO_2 (Tao et al., 2023; Sokol et al., 2022). However, in certain contexts, CUE either exerts no discernible effect on SOC or is even negatively correlated with its persistence.

Among the organic amendments of greatest interest for their potential to recycle agricultural, livestock, and forestry residues are the well-known green composts, as well as anaerobic digestates and biochars. Biochar is the carbonaceous aromatic material produced through the pyrolysis of biomass. It has been shown to enhance soil carbon stability by providing a recalcitrant carbon pool resilient to microbial decomposition (Lehmann et al., 2006; Jeffery et al., 2011; De la Rosa et al., 2016). Furthermore, the concept of ‘green compost’ refers to compost derived solely from plant residues (Toledo et al., 2018), representing as well a promising organic amendment due to its ability to enhance SOC content, to stimulate microbial activity, and to improve soil structure (Vivas et al., 2009; Powlson et al., 2011; Bernal et al., 2017). Lastly, the use of manure digestates, resulting from the anaerobic digestion of cattle waste, offers a nutrient-rich source that can influence soil carbon dynamics (Coelho et al., 2019; van Midden et al., 2023). Additionally, digestates contribute to the formation of stable organic-mineral associations, which could potentially increase the average residence time of carbon in the soil (Kallenbach et al., 2010).

In principle, biochars are expected to enhance CUE_{micro} by promoting necromass stabilization and reducing respiratory losses, whereas compost and digestates, being richer in labile organic fractions, may stimulate respiration and lower CUE, thereby increasing CO_2 fluxes. Yet such patterns remain uncertain, as both amendment composition and soil properties strongly modulate outcomes. The interaction between amendment pH and soil pH is particularly relevant: biochar, typically alkaline (pH 8–10), may enhance CUE in acidic soils by alleviating acidity but exert neutral or negative effects in alkaline soils (Lehmann and Joseph, 2009; Glanville et al., 2016). Compost, with near-neutral pH, can buffer soil conditions and moderately improve CUE across systems (Cesarano et al., 2017), while manure-based inputs, usually near-neutral to slightly alkaline (pH 7–8), supply labile carbon that stimulates microbial activity but often reduce CUE by increasing CO_2 losses, especially in alkaline soils. These contrasting responses suggest that biochar could be more effective in acidic soils, compost may provide intermediate buffering effects, and manure-derived amendments might decrease CUE_{micro} in alkaline soils while potentially improving it in acidic contexts. Nevertheless, major uncertainties remain regarding the long-term stability of added carbon, soil-specific responses, and the net contribution of organic amendments to SOC sequestration under field conditions (Tao et al., 2023; Sokol et al., 2022).

Although CUE_{micro} generally converges around 0.30 across field conditions (Sinsabaugh et al., 2013), it is also shaped by multiple environmental and biological drivers (Xu et al., 2024b), which complicates predictions of amendment effects on SOC persistence. To investigate these effects under controlled conditions, soil incubations are widely employed, with CO_2 evolution serving as a key indicator of microbial activity. In this study, we applied an innovative CUE_{micro} calculation that avoids the limitations of conventional $^{13}C/^{14}C$ labeling and chloroform-fumigation-extraction methods, which often yield inaccurate estimates (Sinsabaugh et al., 2013). We examined two representative Spanish soils amended with cow manure digestate, anaerobic digestate from cattle manure and straw, white poplar wood biochar, olive pomace biochar, and wastewater sludge char. A 100-day incubation was conducted using an automatic respirometer, a reliable and cost-effective tool for monitoring soil respiration (San-Emeterio et al., 2023; Hilscher and Knicker, 2011). While isotopic approaches such as $\delta^{13}C$ analysis or ^{14}C labeling offer high precision, they are limited by cost, practicality, and overlapping isotopic signatures (Zimmerman et al., 2011). In contrast, the respirometer effectively captured treatment-induced differences, and CUEmicro—derived from qPCR-based microbial DNA and cumulative CO_2 release—provided insights into carbon turnover. This integrative approach combined physical, elemental, thermal, spectroscopic, and microbial analyses (Stone and Plante, 2015). Furthermore, respiration data were linked with thermal analysis to differentiate labile and recalcitrant fractions by their thermal stability (de la Rosa et al., 2008; De la Rosa et al., 2016; Prats et al., 2020; Siles et al., 2024), thereby clarifying the mechanisms of SOC persistence and offering insights for sustainable soil management and climate change mitigation.

2. Materials and methods

2.1. Description of soils

The main properties of the soils and organic amendments used in the experiment are described in Table 1. For this study, two distinct Spanish soils, emblematic of contrasting environmental conditions and agricultural practices, were sampled from the upper 10 cm across a 100 m^2 area at nine sampling points using a sterilized aluminum trowel, combined into a composite sample, then homogenized, dried at 25 °C for 24 h, sieved to 2 mm and stored at 4 °C until their use.

Table 1
Achronym and brief description of soils and organic amendments.

Short name	Code	Sort	Description
Grassland soil	GS	Soil	*Distric Cambisol* (WRB; 2015), loamy silt texture; pH 5.2; Originates from a farm dedicated to extensive grass production for livestock located in Meira (Lugo province; Spain 43° 14′ 14.3" N, 7° 18′ 16.0" W).
Alkaline soil	AS	Soil	*Xerochrept* alkaline soil, sandy loam texture; pH 8.1; Originates from *La Hampa* experimental farm mainly dedicated to the cultivation of olive groves and rain-fed cereals. (Seville, Spain; 37° 21.32′ N, 6° 4.07′ W).
Olive pomace biochar	OB	Biochar	Biochar from washed olive pomace prepared in a rotary pyrolysis reactor at 500 °C; with 20 min of residence time by Carboliva S.L. (Puente del Obispo, Jaén, Spain).
White poplar wood biochar	WB	Biochar	Biochar from white poplar wood chips produced at 500 °C at a low-tech pyrolysis reactor consisting of a closed chamber where the biomass is stacked during 2 h.
Wastewater sludge char	WSB	Biochar	Pyrogenic material produced through the pyrolysis of 80 % sewage sludge +20 % chipped waste wood from Helsinki region pyrolyzed during 75 min at 565 °C (Helsinki Environmental Services HSY; Finland).
Rice husk biochar	RB	Biochar	Biochar from rice husk variety Argilla (*Oryza sativa*) produced at a cilindric fixed bed reactor at 500 °C during 3 h (IRNAS-CSIC, Spain).
Cattle manure and straw digestate	CSD	Digestate	Digestate from the anaerobic co-digestion of 84 % cattle manure 16 % and wheat straw produced at a Batch-type solid-state anaerobic digester 2 × 1 m^3 leach-bed reactor in mesophilic conditions during 139 days.
Cow manure digestate	CD	Digestate	Digestate from the anaerobic mesophylic digestion of cow slurry stabilised with pruning waste at a 3 m^3 reactor by SOLOGAS S.A. (As Somozas, Spain).
Green compost	GC	Compost	Commercial compost made from 100 % pruning vegetable waste, purchased from Carrefour Spain.
Olive pomace biochar + green compost	OB + GC	Biochar+ Compost	Mixture of olive pomace biochar and green compost (1:1).

The first soil is a *Distric Cambisol* (WRB; 2015) from an elevated precipitation area in the Galician grasslands (hereafter, 'GS'), characterized by a temperate oceanic climate (Cfb, Köppen–Geiger), and is used primarily for extensive grass production for livestock in Meira. (Lugo province; Spain; 43° 14′ 14.3" N 7° 18′ 16.0" W). This soil was previously described in Ibáñez et al. (2013), it is characterized by its acidity (average pH value of 5.2), low phosphorus content and a loamy silt texture. The GS soil has a total organic C and N contents of 24 ± 1, and 2.0 ± 0.1 g kg^{-1}, respectively.

The second soil, is a sandy loam alkaline *Xerochrept* (hereafter, 'AS'), commonly employed in olive groves (García-Orenes et al., 2016). AS was sampled at *La Hampa* experimental farm, which is located about 20 km west of Seville city, (SW Spain; 37°17′N, 6°3′W), with a Csa climate (hot-summer Mediterranean climate). The soil has high carbonate content (32 %), alkaline pH (average value of 8.1) and total C, organic C and N contents of 29 ± 1.0, 9.0 ± 2 and 1.0 ± 0.3 g kg^{-1}, respectively. The concentrations of the main soil nutrient were as follows: P, 37 mg kg^{-1}; Ca, 12 g kg^{-1}; K, 21 g kg^{-1}; and Mg, 193 mg kg^{-1}. Further details are provided in Madejón et al. (2023).

2.2. Characterization of organic amendments

Eight contrasting organic amendments were included in this study: Olive pomace biochar (OB), White poplar wood biochar (WB), Wastewater sludge char (WSB), Rice husk biochar (RB), Cattle manure and straw digestate (CSD), Cow manure digestate (CD), Green compost (GC) and a mixture of common biochar and compost types in SW Spain, Olive pomace biochar + green compost (OB + GC). Table 1 describes the methodology used to prepare each of the organic amendments and its origin.

2.3. Soil incubation

A Respicond IV conductimetric automated respirometer (Nordgren Innovations, Sweden) was used to monitor CO_2 release every 6 h during an accelerated aging process of amended soil samples lasting 100 days. Prior to incubation, the water content of each soil sample was adjusted to 60 % of its maximum water holding capacity (WHC). The controlled microbial degradation experiment involved randomly distributing 100 mL glass beakers ($n = 4$) at the respirometer, each containing 9 g of soil and 1 g of each amendment, alongside the untreated control soils. The amendment dose applied in this study is intentionally higher than those typically used under field conditions. This strategy aligns with previous research employing the same automatic respirometry system (e.g., Knicker et al., 2013; Campos et al., 2021a, 2021b), and is designed to enhance the detection of treatment effects during the 100-day incubation period. By amplifying the microbial response, this approach allows for a clearer differentiation between amendments under controlled conditions. Moreover, the high-frequency monitoring of CO_2 evolution—conducted four times daily—provides detailed insight into the temporal dynamics of soil respiration, thereby increasing the robustness and resolution of the dataset. Each glass beaker received 2 mL of a microbial suspension previously extracted of the corresponding unamended fresh soils with water (100 g of fresh soils in 500 mL of deionized water) and subsequent filtered (5 μm pore size). Additionally, four vessels were prepared as blanks without soil to monitor potential alterations during the experiment.

The glass beakers were placed into closed 250 mL vessels and CO_2 produced during incubation was estimated as in Knicker et al. (2013), by measuring conductivity shifts in a 0.6 M KOH solution enclosed in small vials attached to the lid of the 250 mL jars. All vessels were maintained at 25 °C throughout the whole experiment by immersing the samples in a thermostated water bath. This monitoring system has the advantage of maintaining a constant temperature, thereby eliminating one of the most influential factors affecting microbial development and reducing uncertainty related to the temperature sensitivity of CUE_{micro}. The CO_2 released and captured by the KOH solution was quantified by normalizing production to the carbon content of each sample, utilizing a calibration constant provided by the instrument manufacturer. The constant facilitated the conversion of the decrease in electrical conductance to accumulated CO_2 at a constant temperature (25 °C). Figure SF1 of the electronic annex shows the respirometer instrument and a schematic diagram illustrating its operation.

The remaining calculated carbon was plotted against incubation time, and a double exponential decay model was used to assess both, fast (labile) and slow (recalcitrant) soil organic carbon (SOC) fractions, as defined in Knicker et al. (2013) and San-Emeterio et al. (2023), and depicted in Eq. (1):

$$C(t) = A_{1fast} x\ e^{-k_1\_fast\ x\ t} + A_{2slow} x\ e^{-k_2\_slow\ x\ t} \tag{1}$$

where C(t) is the remaining C as a % of the total carbon; A_{1fast} and A_{2slow} are the amount of C relatively labile and more stable against mineralization as a % of the total C, respectively; k_1_fast and k_2_slow are the degradation constants (curve slopes) corresponding to the labile and the more stable pools in years^{-1}, respectively; and t is the incubation time. Then, mean residence times (MRT) for the labile and more stable pools, MRT_1 and MRT_2, were calculated according to Eq. (2):

$$MRT_n = 1/k_n \tag{2}$$

2.4. Proximate analyses

Total carbon (TC), total organic carbon (TOC) and total nitrogen (TN) contents of soils and amendments were determined by dry combustion at 1020 °C, using a Flash 2000 HT elemental micro-analyzer (Thermo Instruments, Bremen, Germany) equipped with a thermal conductivity detector (TCD).

To analyze TOC, bulk AS samples were first treated with 1 M HCl for 24 h at room temperature to eliminate carbonates. No carbonates were detected at the GS samples. After decarbonation, the samples were rinsed with distilled water until reaching a pH of 7, ensuring the removal of acid to prevent instrument damage and interference in subsequent organic carbon content determination (Verardo et al., 1990). The pH and the electrical conductivity (EC) were measured by the procedure described by Campos et al. (2021a, 2021b) in a 1:5 (*w*/w) soil: distilled water mixture, whereas for pure organic amendments a 1:10 (w/w) mixture was used. The WHC of bulk soils and pure amendments was determined by following the procedure described in De la Rosa et al. (2014).

2.5. Determination of trace elements and nutrients

The total content of trace elements (As, Ba, Cd, Cu, Ni, Pb, Sr, and Zn) in soils and pure amendments was conducted in triplicate following extraction and digestion with aqua regia (1:3 *v*/v conc. HNO_3/HCl). This process was carried out in a DigiPREP Jr. Block Digestion System (SPS Science, Quebec, Canada) at 110 °C for 2 h (Madejón et al., 2017). Post-digestion, the extracts were analyzed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) in a Varian ICP 720-ES instrument (Varian, Santa Clara, CA, USA). The method's accuracy was validated by measuring trace elements in a certified reference material (soil sample: ERM-CC141; Joint Research Centre). Recovery rates ranged from 85 to 110 %.

2.6. Thermal analysis

Thermal analyses, including Thermogravimetry (Tg), derivative TG, and Differential Scanning Calorimetry (DSC), of dried samples (40 °C) were conducted using a Discovery series SDT 650 simultaneous DSC/TGA instrument (T.A Instruments Inc., Delaware, USA) under a N_2 flow rate of 50 mL min^{-1}. Fine powdered 5 mg of each sample were placed in uncovered Alumina cups and heated from 50 to 850 °C at a rate of 20 °C min^{-1}.TG, dTG curves, mass loss, and calorimetry data were acquired using TRIOS software (T.A. Instruments, Delaware, USA). Three analyses with a reproducibility error $\leq$ 0.2 % were performed for each soil type (n = 3). The weight loss of decomposed materials was categorized into four fractions: W_1 (50–180 °C), W_2 (180–380 °C), W_3 (380–580 °C), and W_4 (580–800 °C) corresponding to losses typically attributed to water and labile organic matter (W_1), organic matter of intermediate stability (W_2), recalcitrant organic matter (W_3), and stable fraction + mineral fraction (W_4), respectively.

2.7. Fourier transform infra-red spectroscopy (FT-IR)

The FT-IR analysis of soils was conducted using a BRUKER spectrometer (Invenio-X, Bruker Corporation, Billerica, USA) in ATR mode (Attenuated Total Reflection, direct sample analysis). Sixty scans were acquired for each dry, ground sample, within a range of 4000 cm^{-1} to 400 cm^{-1} in absorbance with a resolution of 2 cm^{-1} with subtraction of the blank. Subsequently, the baseline was manually corrected, and the spectra were normalized using the OPUS software (Bruker Corporation, Billerica, United States).

2.8. Soil DNA isolation and quantification of microbial biomass by qPCR

Total genomic DNA from soil samples collected after the respiration assay was extracted from 250 mg of each sample using the DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA integrity and purity were assessed on 1 μL of DNA extract by 1.5 % agarose gel electrophoresis. The DNA concentrations were measured using a Qubit 4.0 fluorometer through a Qubit 1× dsDNA BR assay kit (Invitrogen,Thermo Fisher Scientific, Oregon, USA). Subsequently, the microbial load of the soil samples was quantified by qPCR using universal bacterial (27f 5'-AGAGTTTGATCMTGGCTCAG-3′ and 338Rr 5'-GCTGCCTCCCGTAGGAGT-3′ targeting the 16S rRNA gene; Oldham and Duncan, 2012) and fungal (NL1f 5'-ATATCAATAAGCGGAGGAAAAG-3′ and LS2r 5'-ATTCCCAAACAACTCGACTC-3′ targeting the 28S rRNA gene; Bates and Garcia-Pichel, 2009) primers. Standard curves were constructed using six serial decimal dilutions of equimolar pools of bacterial (*Streptomyces* sp. and *Bacillus* sp.) and fungal (*Trichoderma* sp. and *Alternaria* sp.) DNA extracts in triplicate, ranging from 2 ng to 0.02 pg (Supplementary Figure SF2). qPCR assays were performed as described in previous studies (Martin-Sanchez et al., 2018) with slight modifications, including four reactions for each soil sample (two dilutions of the DNA extract in duplicate), two positive standards in duplicate and two negative controls without DNA. The 10-μL qPCR reactions, including 5 μL of Sso advanced SYBR Green Supermix (BioRad, Hercules, CA, USA), 0.3 μL of each 10 μM primer, 2 μL of template DNA and 2.4 μL sterile ultrapure water, were run in a CFX Connect Real-Time PCR Detection System (BioRad) with the following cycling parameters: 95 °C for 5 min followed by 40 cycles consisting of 5 s at 95 °C, 1 min at 60 °C and subsequent reading of fluorescence at 520 nm. To construct the melting curve, 60 steps of 5 s at increasing temperature by 0.5 °C (from 65 to 95 °C) and reading the fluorescence at 520 nm were added. Data analyses were conducted using the CFX Maestro software (BioRad). The resulting microbial (bacterial and fungal) loads were expressed as amount of microbial DNA in soil samples (mg of DNA g^{-1} of soil).

2.9. Determination of microbial carbon use efficiency (CUE_{micro})

A method has been developed to assess the bacterial and fungal CUE_{micro} in soils. The main innovation of this method lies in extracting DNA from the soil and estimating the carbon content of the main soil microorganisms (i.e. bacteria and fungi) based on a qPCR-based DNA quantification of these fractions, while CO_2 is measured automatically in a respirometer over the 100-day incubation period. The concept is similar to other metabolic CUE calculations (e.g. Tao et al., 2023; Geyer et al., 2016), where higher CUE indicates more efficient carbon use by microorganisms. The equation used was as follows:

$$CUE_{micro} = C_{micro} / \left[C_{micro} + C_{respired}\right] \tag{3}$$

where: C_{micro} refers to the carbon content of soil microorganisms, which has been calculated based on the measured DNA content of fungi and bacteria per gram of soil. These carbon contents referencing highly abundant representative soil bacteria (genera *Streptomyces* and *Bacillus*) and fungi (genera *Trichoderma* and *Alternaria*) that were used as standards in the qPCR assays. The calculation accounted for the average base pairs per bacterial/fungal cell and the average DNA mass per cell of each type (Li et al., 2014; Ni et al., 2021). The electronic annex includes detailed information on the estimations and formulas used for calculating C_{micro} for fungi and bacteria. $C_{respired}$ represents the carbon content respired per gram of soil over 100 days of incubation, determined from the amount of CO_2 respired in the respirometer by each vessel. The calculation considered the weight of carbon in a mole of CO_2.

2.10. Statistical analysis

Data analysis before and after incubation was conducted using IBM SPSS Statistics 26.0 (SPSS, Chicago, USA). Normality and homoscedasticity were assessed using the Shapiro-Wilk and Levene tests, respectively. To compare control and amended soils, as well as to evaluate the impact of different amendments in the incubation experiment, we utilized Multivariate Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test. In cases where the data did not meet normality and homoscedasticity criteria, we employed the Kruskal Wallis test, followed by the Mann-Whitney *U* test as an alternative method. Significance for all statistical tests was determined at a *p*-value <0.05.

The curve fitting of remaining carbon versus incubation time was performed using SigmaPlot 14 software (Systat Software Inc.), following Eq. (1). The analysis yielded high coefficients of determination, with R^2 values >0.994 for all derived curves.

To conduct the comparative heat map of the thermogravimetric and respirometry analysis results, Python software (v3.x) was used with the following key libraries: 'Pandas' for data manipulation and cleaning, 'Seaborn' for creating correlation heatmaps and 'Matplotlib' for additional visualization support. The heatmaps were generated using the 'seaborn.heatmap' function, which displays correlations between all numerical parameters. The correlation matrix was computed using the Pearson correlation method, and *p*-values were calculated to assess the statistical significance of the correlations.

3. Results

3.1. Composition and properties of bulk soils and organic amendments

The elemental composition and physical properties of the soils and pure amendments are summarized in Table 2. All amendments exhibited alkaline pH (above 8), except wastewater sludge char (pH = 7.5), cow manure digestate (pH = 7.5), and green compost (pH = 6.3). EC values were appropriate, except for the high values of cow manure digestate (8940 μS cm^{-1}) and cattle manure + straw digestate (2135 μS cm^{-1}).

Table 2
Physical properties and elemental composition of bulk soils and amendments.

	Soils		Amendments							
	GS	AS	OB	WB	WSB	RB	CSD	CD	GC	OB + GC
pH (H_2O)	5.2 ± 0.0	8.1 ± 0.0	9.9 ± 0.0	9.1 ± 0.1	7.5 ± 0.2	10.9 ± 0.1	8.7 ± 0.0	7.5 ± 0.0	6.3 ± 0.2	8.1 ± 0.1
EC (μS cm^{-1})	136.0 ± 16	180 ± 12	13,700 ± 389	224 ± 3	618 ± 26	1153 ± 46	2135 ± 64	8940 ± 594	440 ± 8	7070 ± 199
WHC (%)	92.0 ± 37	72 ± 41	78 ± 15	159 ± 19	99 ± 1	342 ± 22	369 ± 27	liquid	315 ± 61	197 ± 38
Density (g cm^{-3})	0.9 ± 0.0	1.2 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	liquid	0.3 ± 0.0	0.4 ± 0.0
Moisture (%)	13.2 ± 0.0	6.2 ± 0.1	10.9 ± 0.2	5.8 ± 0.1	8.4 ± 0.0	10.3 ± 0.4	0.8 ± 0.6	89.0 ± 0.1	60.7 ± 0.9	35.8 ± 0.6
C (g kg^{-1})	24.0 ± 1.0	29.0 ± 1.0	594.0 ± 4.0	834.0 ± 2.0	271.0 ± 2.0	523.0 ± 2.0	410.0 ± 2.0	316.0 ± 7.0	429.0 ± 3.0	511.5 ± 3.5
H (g kg^{-1})	–	–	25.0 ± 4.0	27.0 ± 0.0	10.0 ± 1.0	15.8 ± 1.0	51.0 ± 2.0	40.0 ± 1.0	50.0 ± 0.0	37.5 ± 2.0
N (g kg^{-1})	2.0 ± 0.1	1.0 ± 0.3	14.0 ± 1.0	2.0 ± 0.0	20.0 ± 1.0	12.6 ± 0.0	15.0 ± 1.0	32.0 ± 0.0	12.0 ± 1.0	13.0 ± 1.0
P (g kg^{-1})	0.8 ± 0.0	0.5 ± 0.0	5.7 ± 0.1	0.2 ± 0.0	53.8 ± 0.6	3.2 ± 0.0	2.6 ± 0.0	9.7 ± 0.0	1.2 ± 0.0	3.2 ± 0.0
K (g kg^{-1})	6.9 ± 0.2	2.4 ± 0.1	78.0 ± 0.1	2.2 ± 0.0	2.4 ± 0.0	12.5 ± 0.3	19.8 ± 0.4	76.0 ± 2.5	2.8 ± 0.0	31.6 ± 0.3
S (g kg^{-1})	0.2 ± 0.0	0.2 ± 0.0	1.1 ± 0.1	0.1 ± 0.0	12.8 ± 0.1	0.3 ± 0.0	2.2 ± 0.1	7.4 ± 0.1	3.2 ± 0.0	2.1 ± 0.0
O (g kg^{-1})	–	–	365.9 ± 9.0	136.9 ± 2.0	686.2 ± 4.1	686.2 ± 4.1	521.8 ± 5.1	604.6 ± 8.1	505.8 ± 4.0	435.9 ± 6.5
Ca	863 ± 7	45,914 ± 603	16,066 ± 343	11,330 ± 681	26,201 ± 209	2001 ± 1	6189 ± 0	46,478 ± 573	19,782 ± 1007	18,022 ± 496
Fe	36,195 ± 888	10,751 ± 324	589 ± 5	576 ± 14	202,627 ± 1787	166 ± 10	1524 ± 1	3378 ± 32	9461 ± 63	3162 ± 9
Mg	9668 ± 289	2502 ± 57	4978 ± 11	580 ± 3	3125 ± 19	1831 ± 32	4026 ± 239	13,995 ± 239	1643 ± 40	3032 ± 10
Na	123 ± 4	137 ± 6	1072 ± 4	77 ± 2	1126 ± 21	1570 ± 38	1658 ± 16	22,544 ± 170	366 ± 39	680 ± 8
S	200 ± 7	168 ± 2	1135 ± 18	121 ± 1	12,754 ± 126	269 ± 10	2177 ± 116	7364 ± 66	3159 ± 10	2129 ± 48
As	22.2 ± 0.2	2.2 ± 0.3	b.d.l	b.d.l	4.1 ± 0.4	b.d.l	b.d.l	2.4 ± 0.3	1.9 ± 0.7	b.d.l
Cd	b.d.l	b.d.l	b.d.l	b.d.l	b.d.l	b.d.l	b.d.l	b.d.l	b.d.l	b.d.l
Cr	60.5 ± 1.4	14.1 ± 0.6	11.6 ± 0.3	1.0 ± 0.0	34.9 ± 0.0	1.3 ± 0.7	5.8 ± 0.9	71.7 ± 1.0	17.4 ± 4.0	16.5 ± 7.1
Cu	43.9 ± 1.7	21.3 ± 0.2	42.2 ± 0.4	2.5 ± 0.0	514.5 ± 12.9	3.2 ± 0.1	24.1 ± 0.1	155.3 ± 2.3	17.1 ± 0.5	26.6 ± 0.2
Ni	35.2 ± 0.6	9.0 ± 0.3	12.0 ± 0.4	0.8 ± 0.4	30.7 ± 0.6	2.0 ± 1.0	5.1 ± 0.2	60.7 ± 3.8	4.9 ± 0.3	9.1 ± 1.1
Pb	19.5 ± 0.2	13.3 ± 0.3	0.8 ± 0.6	0.4 ± 0.0	24.1 ± 0.1	0.4 ± 0.2	2.4 ± 0.2	1.1 ± 0.1	23.0 ± 0.0	10.6 ± 0.2
Zn	88.8 ± 0.0	33.1 ± 0.2	49.2 ± 0.1	6.5 ± 0.2	989.7 ± 11.5	25.1 ± 0.1	190.1 ± 13.0	456.8 ± 3.9	64.5 ± 1.1	54.1 ± 0.4

b.d.l.: below detection limit; The abundance of nutrients and micronutrients is expressed in mg kg^{-1} (dry weight basis) except for those indicated;
Given error is standard error ($n = 3$).

Regarding WHC, rice husk biochar, cow manure digestate, and green compost exceeded 300 %.

Biochar samples showed the greatest C contents, particularly poplar wood biochar (834 g kg^{-1}), consistent with biochar as a carbon-rich material (Lehmann et al., 2006). Wastewater sludge char, however, presented unusually low C content (271 g kg^{-1}; Table 2). Macronutrient analyses revealed high N in cow manure + straw digestate, P in wastewater sludge char and cow manure digestate, and K in olive pomace biochar, cow manure digestate, and olive pomace biochar + green compost. Wastewater sludge char also showed elevated S and Fe, while cattle manure + straw digestate was rich in Ca and Mg (Havlin et al., 2013).

Wastewater sludge char and cow manure digestate exceeded Cu and Zn limits set by Spanish fertilizer regulations (Ministerio de Agricultura, Pesca y Alimentación, 2020; Spanish Organic Fertilizer Legislation, Royal Decree 506/2013), restricting their direct use.

3.2. Soil organic matter composition

3.2.1. FT-IR spectroscopy

FT-IR spectra of GS and AS (Supplementary Figure SF3) showed mineral and organic functional groups as follows. In AS, bands at 835 and 875 cm^{-1} indicate silicates (Si—O bending) and carbonates; both soils exhibited 920–930 cm^{-1} (silicates). A strong 995–1005 cm^{-1} signal (C—O stretching in carbohydrates/polysaccharides, with possible silicate contribution) appeared in both soils. AS alone showed bands at 1120 and 1270 cm^{-1} (esters/ethers, carboxylic acids, and clay minerals; Jiang et al., 2019), and at 1350 cm^{-1} (aliphatic C—H bending and carbonates; Liu et al., 2022). The 1425 cm^{-1} band corresponds to carboxylate ions (COO^-) and/or carbonates (Jiang et al., 2019). The 1580 cm^{-1} band, present in both soils, reflects asymmetric COO^- stretching and aromatic C═C (Campos et al., 2021a, 2021b). GS also showed a small band at 1630 cm^{-1} (protein/peptide C═O and/or conjugated C═C).

Amendments produced few spectral changes in GS soils (SF3). After 100 days, the main difference was reduced intensity at 995 cm^{-1}. In AS soils, biochar increased the relative intensity at 1425 cm^{-1}. Mineral-related peaks in AS were retained across treatments.

3.2.2. Thermal analyses

Thermogravimetry (Tg) and derivative thermogravimetry (dTg) resolved moisture loss (50–180 °C), decomposition of labile/intermediate OM (180–380 °C), and recalcitrant OM (380–580 °C); AS additionally exhibited carbonate decomposition at 640–750 °C (Fig. 1; SF4a, b). Amended soils showed greater total mass loss than controls, indicating higher OM contents, especially within the intermediate (W_3) and recalcitrant (W_4) fractions (ST1; Fig. 1). In GS, wastewater sludge char—followed by green compost and cow manure digestate—increased the labile/intermediate fraction at t_{100} (W_1), whereas most biochars increased the most stable fraction (W_4) and poplar wood biochar exhibited high stability above 600 °C.

After 100 days, stable fractions increased at the expense of labile/intermediate fractions in both soils (Fig. 2). In AS, cow manure digestate markedly reduced the intermediate-OM signal in dTg; in GS, dTg curves shifted to higher temperatures within the intermediate/recalcitrant domains (SF4a). Olive pomace and wastewater sludge chars maintained elevated relative thermal stability (RTS) at t_{100}.

3.3. Effects of organic amendments on soil carbon stability

The control soil and those amended with cow manure digestate exhibited the highest relative losses of organic carbon during soil incubation (Fig. 3). In contrast, soils amended with poplar wood biochar followed by olive pomace and rice husk biochars, showed the lowest SOC losses.

The mean residence times (MRT) of fast and stable soil C pools (MRT_1 and MRT_2, respectively), indicate in both soils the least abundant fraction is the labile one (Table 3), and only minor variations were observed in the mineralization rates constants (k_1) of the rapidly decomposing SOC pool (fast pool). The k_1 values ranged between 18 and 55 and 3 to 44 days for GS and AS, respectively, accounting for average MRT_1 of around 20 days (Table 3). Such decomposition rate constants are typically found for easily decomposable OM and fresh plant residues (Paul and Clark, 1996). In the case of GS, the addition of cow manure (with and without straw) digestate significantly increased the abundance of the labile fraction compared to the control soil (Table 3 and Fig. 1a). The slow turning carbon pool has an average MRT_2 of approximately 7 years for both soils. The application of the amendments caused a significant reduction of the degradation rate constants k_2 of the total C in comparison to the respective control soil and consequently significantly increased $MRT_{2,}$ meaning a slower decomposition of the stable SOC pool of the amended soils (Table 3). Furthermore, the application of poplar wood, rice husk and olive pomace biochars, in that order, substantially increased MRT_2 compared to the control soil by 5–6 times for GS and 22−49 times for AS; (Table 3). In contrast, the application of cow manure digestate did not alter the MRT_2 of GS and caused the smallest increase of MRT_2 for AS.

3.4. Thermal–respirometric correlations

To test whether thermal fractions of the soils determined by thermogravimetry reflect relevant microbiological stability, we compared data from independent thermal metrics (labile W_2, 180–380 °C; stable W4, ≥580 °C) with independent respiration-derived metrics (cumulative CO_2 and the mean residence time of the stable pool, MRT_2) measured on the same samples (Fig. 4). This within-system provides a novel, operational cross-check between physical–chemical and microbial indices of stability. Across both soils (AS and GS), cumulative CO_2 was inversely related to the relative abundance of the thermally stable fraction (W_4) and positively related to the labile fraction (W_2). The negative association with W_4 was stronger in AS ($r = -0.75$) than in GS ($r = -0.67$), whereas the positive association with W_2 was also stronger in AS ($r = 0.87$) than in GS ($r = 0.63$) (Figs. 4a–b). MRT_2 displayed the complementary pattern: it correlated positively with W_4—again more strongly in AS ($r = 0.85$) than in GS ($r = 0.42$)—and negatively with W_2, with the inverse association being more pronounced in GS (r = −0.67) than in AS ($r = -0.41$). Taken together, these significant correlations delineate a coherent cross-metric structure in which higher thermal stability (greater W_4, lower W_2) coincides with lower cumulative CO_2 release and longer MRT_2 across both soils (Fig. 4).

3.5. Effects of the amendments on the microbial development and CUE_{micro}

The amount of bacterial DNA in all the soil samples were 2 to 3 magnitude orders greater than fungal DNA (Table 4). The highest bacterial load was found in the AS amended with cattle manure + straw digestate, whilst the same soil amended with rice husk biochar resulted on the highest fungal load. Samples from all the biochar amended acidic soil shows lower bacterial and fungal loads compared to the un-amended GS, resulting in reduced total DNA relative to the other samples. Nevertheless, the application of olive pomace biochar to the GS led to an increase in bacterial load and a decrease in fungal load. Another remarkable difference was the reduction in fungal load due to the amendment with cow manure digestate, which resulted in very high bacteria/fungi ratios for both soil types with this amendment.

For the calculation of CUE_{micro}, both the microbial carbon (C_{micro}) content of each soil and the CO_2 respired over 100 days were considered. The CUE_{micro} of the samples from all the alkaline soils was higher than of the grassland acidis samples, with values of 0.20 for GS control soils and 0.28 for AS control soils, respectively.

The application of cow manure digestate, and to a lesser extent cattle

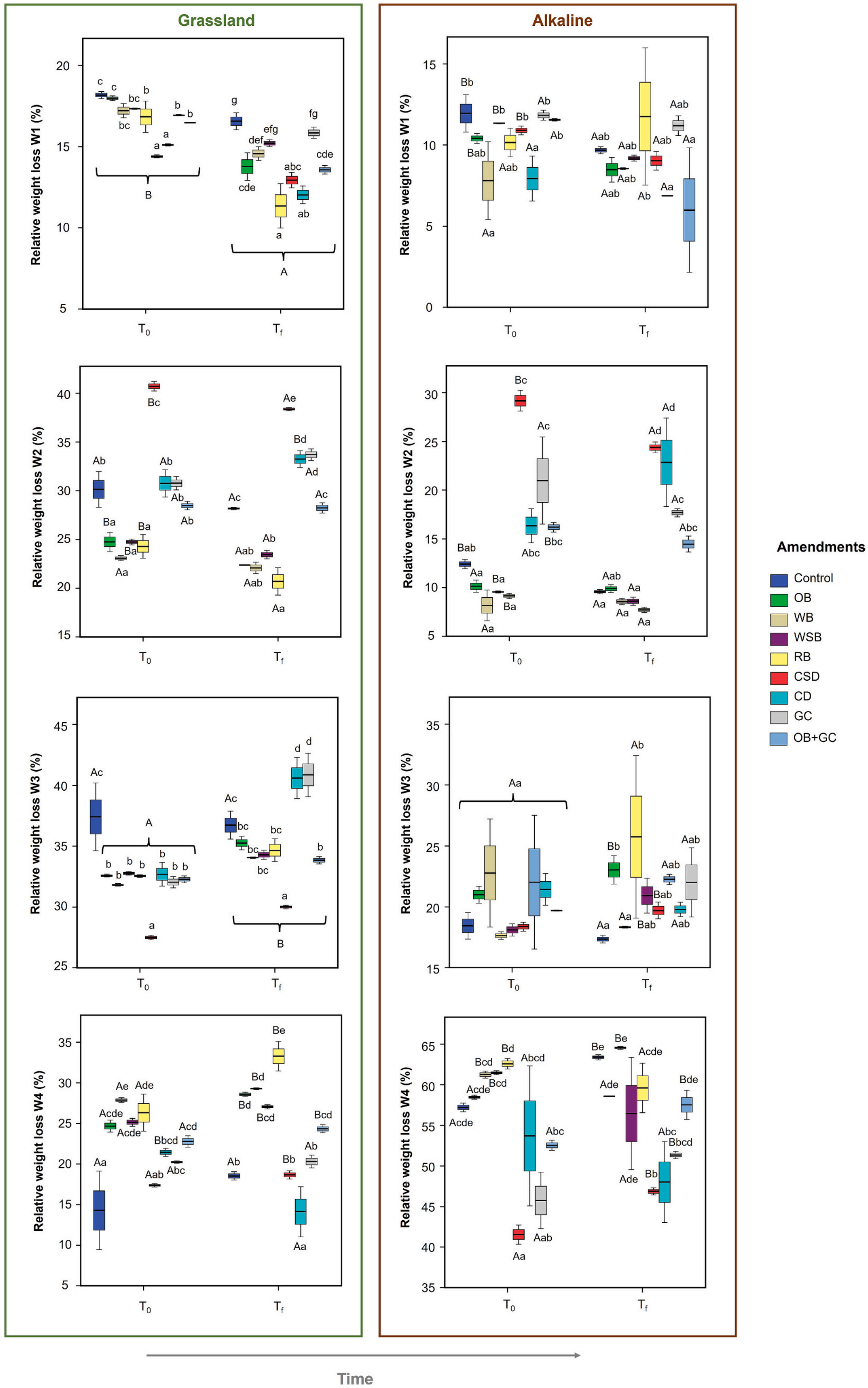

Fig. 1. Relative weight losses W_1, W_2, W_3 and W_4 (%) corresponding to the thermogravimetric analysis of a) grassland and b) alkaline soils at t_0 and t_{100}. Different letters indicate significance. Capital letters are used to compare the same treatment between t_0 and t_{100}. Lowercase letters are used to compare treatments for the same soil type and time. All significance levels at $p < 0.05$.

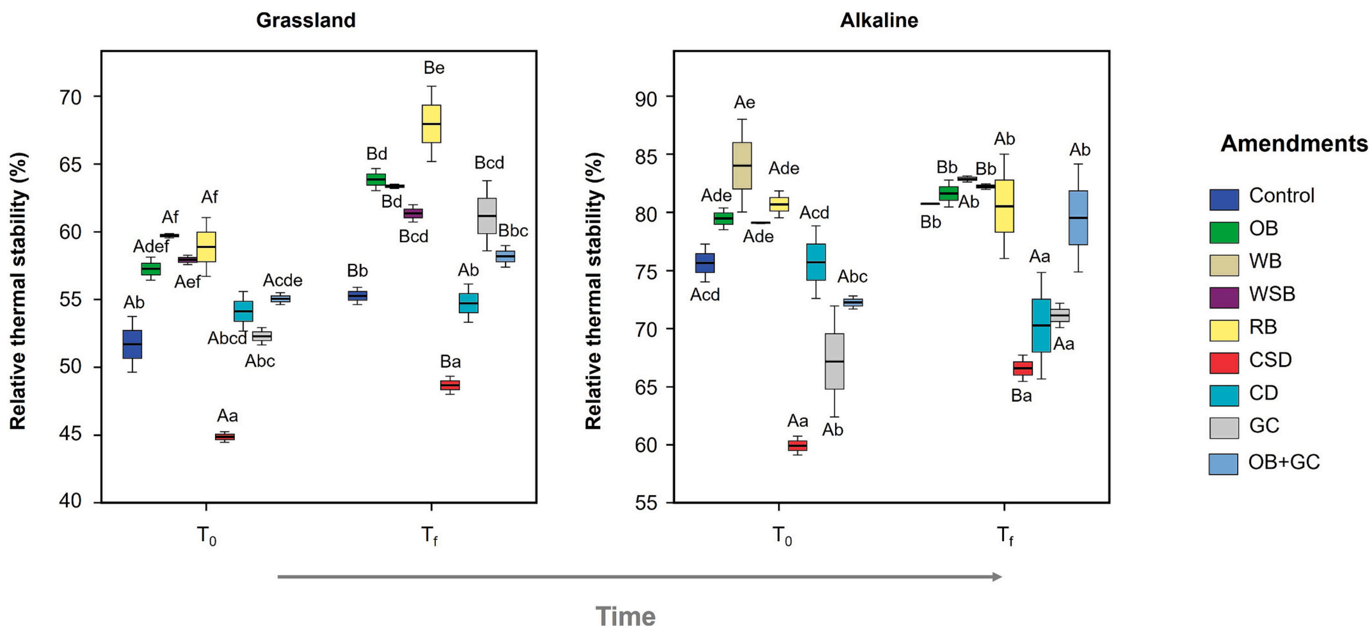

Fig. 2. Relative thermal stability (%) analysis of a) grassland and b) alkaline soils at t_0 and t_{100}. Different letters indicate significance. Capital letters are used to compare the same treatment between t_0 and t_{100}. Lowercase letters are used to compare treatments for the same soil type and time. All significance levels at $p < 0.05$.

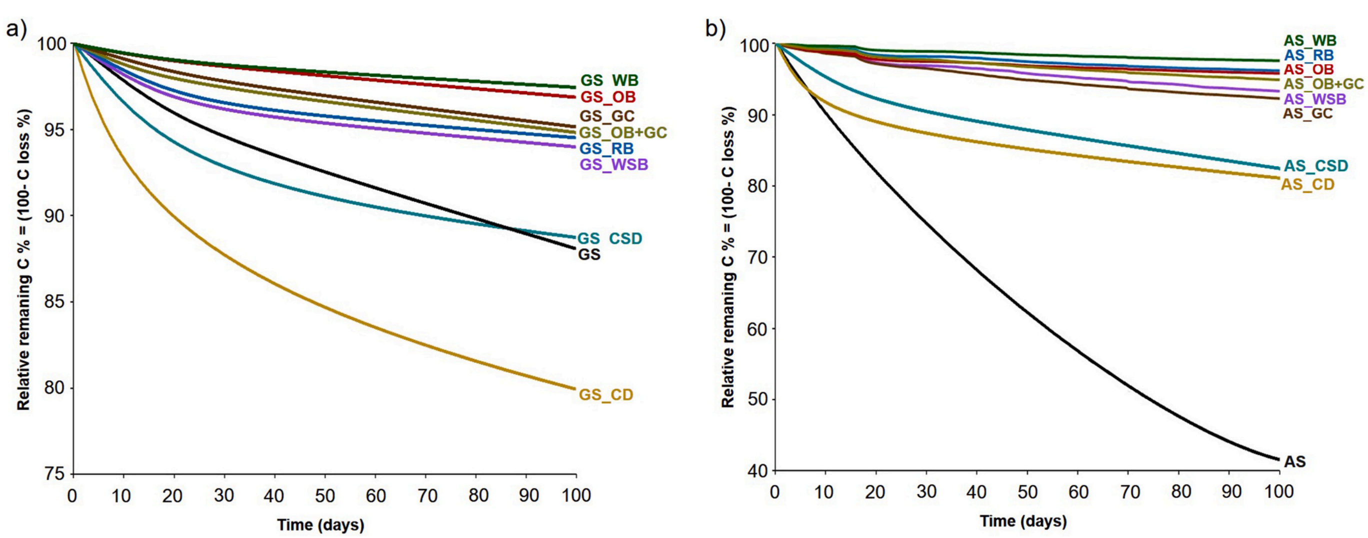

Fig. 3. Relative remaining carbon (%) during the respiration experiment (%, Oct_0-CO_2 respired for a) GS and b) AS soils.

manure + straw digestate, green compost, and the mixture of olive pomace biochar + green compost, significantly increased the amount of CO_2 released in both soils compared to the unamended soils, suggesting that these amendments are less suitable if we intend to drastically reduce CO_2 emissions into the atmosphere. The GS amended with olive pomace biochar showed a high CUE_{micro} (0.38), and the majority of the detected DNA derived from microorganisms (MicroDNA/total DNA = 0.9). Both parameters pointed that in this case a significant fraction of the C was not lost through respiration but instead converted into bacterial biomass, increasing the SOC and enhancing carbon sequestration in the soil (as indicated by the low amount of CO_2 respired compared to the control). On the contrary, the application of rice husk biochar amendment at GS decreased bacterial and fungal biomass, but increased the amount of respired C (3.8 mg g^{-1} soil) and resulted in a low CUE_{micro}. In the AS, with the exception of the mixture of biochar and compost and particularly cow manure digestate, which significantly reduced CUE_{micro} compared to the control, the other amendments enhanced the potential for C sequestration by increasing CUE_{micro}. However, taking into account the net CO_2 emissions, the application of both cattle manure digestates would be ruled out for both soils, as well as rice husk biochar for GS. Likewise, the use of olive pomace biochar as organic amendments for the GS soil and the rest of char amendments for the AS would be recommend.

4. Discussion

4.1. Composition and physical properties

Across amendments, the chemical composition and physical properties provide an informative context for the spectroscopic and thermogravimetric responses: most of amendments were alkaline; cow-manure digestate and cattle manure+straw digestate showed the highest EC; biochars—particularly white poplar wood—were carbon-rich, whereas wastewater sludge char was anomalously carbon-poor; and

Table 3
Parameters calculated by the incubation-respiration experiment. Relative percentages of CO_2 released by the fast (A_1) and slow (A_2) organic C pools, degradation rate constants (k_1 and k_2), their respective mean residence times (MRT_1, MRT_2), and relationships between them for each amendment-soil mixture and for the unamended soils (control).

Sample identification		Fast (labile) C pool			Slow (recalcitrant) C pool			MRT_2 / MRT_1	MRT_{1sampl} / MRT_{1contr}	MRT_{2sampl} / MRT_{2contr}
		A_1	k_1	MRT_1	A_2	k_2	MRT_2			
Soil	Amendment	(% OC)	(y^{-1})	(days)	(% OC)	(y^{-1})	(years)			
Grassland soil (GS)	Control	2.5 ± 0.0 [a]	20.1 ± 4.0 [a]	19.8 ± 4.2 [c]	96.6 ± 0.7 [c]	0.44 ± 0.03 [e]	2.3 ± 0.2 [a]	43	–	–
	OB	0.60 ± 0.02 [a]	55.2 ± 3.5 [b]	6.6 ± 0.4 [a]	99.4 ± 0.0 [d]	0.09 ± 0.00 [ab]	11.8 ± 0.6 [c]	644	0.3	5.1
	WB	0.79 ± 0.01 [a]	20.4 ± 5.0 [a]	21.2 ± 6.8 [c]	99.1 ± 0.0 [d]	0.07 ± 0.00 [a]	14.6 ± 0.8 [d]	252	1.1	6.3
	WSB	2.0 ± 0.3 [a]	18.4 ± 2.2 [a]	20.3 ± 2.2 [c]	97.9 ± 0.3 [cd]	0.14 ± 0.01 [abc]	7.0 ± 0.4 [b]	126	1.0	3.0
	RB	2.5 ± 0.9 [a]	26.3 ± 3.5 [a]	14.5 ± 2.0 [bc]	97.7 ± 0.7 [cd]	0.09 ± 0.01 [ab]	11.8 ± 1.0 [c]	300	0.7	5.1
	CSD	6.0 ± 0.7 [b]	20.1 ± 1.3 [a]	18.4 ± 1.2 [bc]	93.9 ± 0.5 [b]	0.18 ± 0.00 [c]	5.6 ± 0.1 [b]	112	0.9	2.4
	CD	9.5 ± 0.3 [b]	28.9 ± 1.0 [a]	12.8 ± 0.4 [b]	89.9 ± 0.4 [a]	0.36 ± 0.03 [d]	2.8 ± 0.3 [a]	82	0.6	1.2
	GC	1.9 ± 0.6 [a]	21.6 ± 5.5 [a]	19.0 ± 4.2 [c]	98.1 ± 0.6 [cd]	0.13 ± 0.00 [abc]	7.5 ± 0.2 [b]	144	1.0	3.2
	OB + GC	1.4 ± 0.0 [a]	30.7 ± 0.5 [a]	12.0 ± 0.2 [b]	98.4 ± 0.0 [cd]	0.15 ± 0.00 [bc]	6.8 ± 0.1 [b]	208	0.6	2.9
Alkaline soil (AS)	Control	13.3 ± 0.4 [c]	17.1 ± 0.3 [ab]	21.4 ± 0.3 [ab]	88.2 ± 0.1 [a]	2.6 ± 0.0 [c]	0.38 ± 0.00 [a]	6	0.0	0.0
	OB	1.8 ± 0.2 [a]	40.3 ± 26.7 [ab]	19.2 ± 7.6 [ab]	98.2 ± 0.2 [bc]	0.16 ± 0.04 [a]	7.2 ± 1.5 [bcd]	118	1.2	22.4
	WB	0.83 ± 0.17 [a]	19.7 ± 4.8 [ab]	21.3 ± 5.6 [ab]	99.2 ± 0.2 [c]	0.05 ± 0.00 [a]	18.6 ± 1.0 [e]	320	1.0	48.9
	WSB	11.0 ± 0.3 [bc]	3.1 ± 0.3 [a]	120.7 ± 10.2 [c]	92.0 ± 3.0 [ab]	0.20 ± 0.02 [a]	5.2 ± 0.5 [abc]	16	5.6	13.7
	RB	1.8 ± 0.1 [a]	17.5 ± 2.5 [ab]	21.8 ± 3.2 [ab]	98.3 ± 0.1 [bc]	0.08 ± 0.00 [a]	12.4 ± 0.4 [d]	208	1.0	32.6
	CSD	9.0 ± 1.1 [b]	32.9 ± 2.3 [ab]	11.2 ± 0.9 [ab]	93.0 ± 2.3 [abc]	0.25 ± 0.11 [ab]	5.7 ± 2.3 [abc]	185	0.5	14.9
	CD	8.2 ± 1.1 [b]	72.7 ± 28.8 [b]	6.6 ± 2.0 [a]	91.7 ± 1.5 [ab]	0.46 ± 0.05 [b]	2.2 ± 0.3 [ab]	102	0.4	6.2
	GC	3.3 ± 0.6 [a]	12.0 ± 1.3 [ab]	31.3 ± 3.5[b]	96.8 ± 0.7 [bc]	0.16 ± 0.01 [a]	6.3 ± 0.5 [bc]	74	1.5	16.7
	OB + GC	2.4 ± 0.0 [a]	13.9 ± 0.7 [ab]	26.5 ± 1.3 [ab]	99.3 ± 1.0 [c]	0.12 ± 0.02 [a]	8.5 ± 1.3 [cd]	136	0.9	18.9

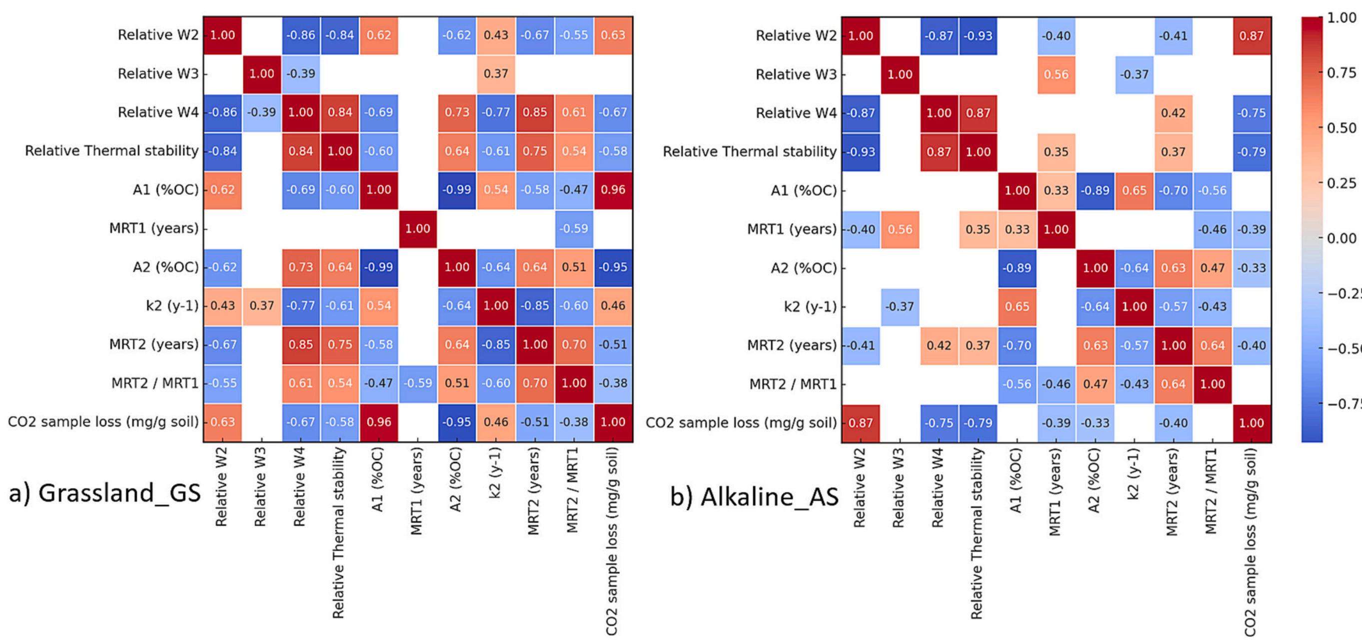

Fig. 4. Heatmap of statistically significant correlations ($p < 0.05$) between soil fractions by thermal analysis (Tg) and soil Carbon dynamic parameters determined by respirometer for a) Grassland and b) Alkaline soils.

both wastewater sludge char and cow digestate exceeded Cu and Zn thresholds under Spanish fertilizer regulations (Table 2). High C and aromaticity in wood/rice-husk/olive-pomace biochars are consistent with the known condensed character and persistence of biochar (Lehmann et al., 2006), while the low C in wastewater sludge char accords with high inorganic loading and partial carbonization (Paneque et al., 2017). Elevated EC in digestates falls within ranges that could impair germination and root growth (Rengasamy, 2010), and metal exceedances constrain field use despite potential nutrient benefits (Royal Decree 506/2013; Ministerio de Agricultura, Pesca y Alimentación, 2020). Together with the very high WHC of rice-husk biochar, cow digestate and green compost (>300 %), which implies enhanced water retention, these properties frame the likely soil functional responses (De la Rosa et al., 2022; Qian et al., 2023).

Changes due to the application of different amendments on the FT-IR spectra were modest but diagnostic (SF3). GS spectra showed a slight attenuation at 995–1005 cm^{-1} after incubation, which is consistent with depletion of carbohydrate-rich moieties. FT-IR spectra of biochars amended AS samples increased the 1425 cm^{-1} band (carboxylates/carbonates) while mineral peaks were retained. The 1425 cm^{-1} increase in an alkaline matrix is congruent with carboxylate enrichment and/or carbonate dissolution reported for biochar-amended soils (Yadav et al., 2019) and suggest functionalization that can promote sorption and organo-mineral association (Jiang et al., 2019; Liu et al., 2022; Campos et al., 2021a, 2021b).

Table 4
Amount of CO_2 respired after 100 days, total DNA content, microbial DNA content (bacterial and fungal) by Q-PCR, and estimations of C_{micro} and CUE_{micro}, per gram of dry soil for all the treatments.

Sample identification		Respired CO_2 (mg C g_{soil}^{-1})	Microbial DNA by q PCR (mg g_{soil}^{-1})		Total DNA (mg g_{soil}^{-1})	Micro DNA / Total DNA	Ratio Bacterial / Fungal	C_{micro} (mg g_{soil}^{-1})	Respired C (mg g_{soil}^{-1})	CUE_{micro}
Soil	Amendment		Bacterial	Fungal						
Grassland soil (GS)	Control	10.9 ± 1.0 [a]	0.0046 ± 0.0011	8E-05 ± 0.0	0.0094	0.5	58	0.7	3.0	0.20
	OB	9.7 ± 0.4 [a]	0.0106 ± 0.0025	4E-05 ± 0.0	0.0123	0.9	271	1.6	2.6	0.38
	WB	10.3 ± 0.4 [a]	0.0033 ± 0.0002	4E-05 ± 0.0	0.0073	0.5	78	0.5	2.8	0.15
	WSB	10.3 ± 0.2 [a]	0.0039 ± 0.0008	3E-05 ± 0.0	0.0068	0.6	122	0.6	2.8	0.18
	RB	14.1 ± 0.4 [a]	0.0027 ± 0.0006	5E-05 ± 0.0	0.0043	0.6	59	0.4	3.8	0.10
	CSD	24.0 ± 0.8 [b]	0.0099 ± 0.0024	8E-05 ± 0.0	0.0132	0.8	127	1.5	6.5	0.19
	CD	35.4 ± 2.0 [c]	0.0148 ± 0.0013	4E-06 ± 0.0	0.0145	1.0	3925	2.2	9.6	0.19
	GC	12.5 ± 1.4 [a]	0.0066 ± 0.0007	9E-05 ± 0.0	0.0107	0.6	69	1.0	3.4	0.23
	OB + GC	14.0 ± 0.2 [a]	0.0068 ± 0.0010	6E-05 ± 0.0	0.0085	0.8	119	1.0	3.8	0.22
Alkaline soil (AS)	Control	8.5 ± 0.2 [a]	0.0058 ± 0.0012	5E-05 ± 0.0	0.0133	0.4	125	0.9	2.3	0.28
	OB	9.5 ± 0.6 [a]	0.0083 ± 0.0029	3E-05 ± 0.0	0.0125	0.7	249	1.3	2.8	0.31
	WB	7.6 ± 0.4 [a]	0.0076 ± 0.0019	4E-05 ± 0.0	0.0140	0.5	188	1.2	2.1	0.36
	WSB	7.1 ± 0.3 [a]	0.0072 ± 0.0016	3E-05 ± 0.0	0.0126	0.6	224	1.1	1.9	0.36
	RB	7.9 ± 0.1 [a]	0.0096 ± 0.0012	1E-04 ± 0.0	0.0159	0.6	69	1.5	2.2	0.41
	CSD	21.4 ± 4.5 [b]	0.0242 ± 0.0086	8E-05 ± 0.0	0.0278	0.9	290	3.7	5.8	0.39
	CD	24.7 ± 1.0 [b]	0.0035 ± 0.0002	3E-06 ± 0.0	0.0061	0.6	1326	0.5	6.7	0.07
	GC	12.4 ± 0.5 [a]	0.0130 ± 0.0030	5E-05 ± 0.0	0.0213	0.6	249	2.0	3.4	0.37
	OB + GC	10.1 ± 0.4 [a]	0.0039 ± 0.0009	4E-05 ± 0.0	0.0088	0.4	98	0.6	2.6	0.19

4.2. Carbon stability and microbial carbon use efficiency (CUE_{micro})

Thermogravimetry provides information concerning the stabilization mechanism with higher specificity. Amended soils exhibited greater total mass loss, particularly within W_3–W_4, relative to controls. In GS, wastewater sludge char, followed by green compost and cow digestate, increased the labile/intermediate fraction (W_1), typically associated with cellulose, proteins, and selected aliphatics (De la Rosa et al., 2022). In contrast, biochar amendments shifted mass toward the most stable fraction (W_4), which reflects an enhanced contribution of condensed aromatic structures (De la Rosa et al., 2016). Poplar-wood biochar, in particular, retained a substantial proportion of mass above 600 °C, indicative of highly condensed pyrogenic carbon. Temporal trajectories reinforced this trend. Over 100 days, both soils gained relative thermal stability at the expenses of labile/intermediate fractions; in AS, cow digestate depressed the intermediate-OM signal as shown at the dTg lines, and in GS the dTg peaks shifted to higher temperatures within intermediate/recalcitrant domains. Under plant- and fauna-free incubation conditions, as used here, these trends are consistent with microbial depletion of polysaccharides and progressive concentration of lignin-like and pyrogenic components; notably, olive-pomace and sludge chars sustained elevated RTS, and Tg sensitively detected pyrogenic signatures (Plante et al., 2009; San-Emeterio et al., 2023; Turmel et al., 2015; Giannetta et al., 2023). The observed short flush of CO_2 release during the first 20 days at the GS samples (Fig. 3) has been also previously reported by Knicker et al. (2013) and attributed to the metabolization of remaining microbial necromass added to SOM liberated and activated due to rewetting and mixing during sample preparation rather than amendment instability. Carbon-loss patterns displayed in Fig. 3 also distinguished labile from recalcitrant amendments. The application of cow manure digestate exhibited the greatest SOC losses, whereas biochars (wood > olive-pomace ≥ rice-husk) minimized them. That contrasting behavior indicates that whereas cow digestate supplied labile C that stimulated microbial activity (positive priming; Kok et al., 2022), biochars applied provided a source of organic carbon that is more recalcitrant than the native SOC. Kinetic fits shown in Table 3 corroborated this hierarchy. The fast pool (MRT_1) averaged ~20 days with modest variation in k_1; biochars markedly increased MRT_2 (~5–6× in GS; 22–49× in AS), whereas cow digestate left MRT_2 unchanged in GS and produced the smallest increase in AS. Consequently, biochar reallocates carbon to slow pools, while labile inputs do not. Similar MRT_2 to the biochar amended soils were previously obtained for agricultural soils from North America (Paul et al., 2001), fire affected alkaline Cambisols from SW Spain (Knicker et al., 2013), and charred grass during short term laboratory degradation studies (Hilscher et al., 2009). MTR_2 magnitudes of the cow manure amended soils agree with values for fresh plant residues (Paul and Clark, 1996; Paul et al., 2001). The latter confirms its instability as indicated by thermal analyses and the high CO_2 emissions associated with this amendment. Considering that the conditions in the Respicond instrument (humidity and temperature) promote microbial development and SOM degradation, the actual MRT_2 values in these amended soils under field conditions could be significantly higher. In fact, Knicker et al. (2013), using the same equipment, estimated that the Respicond could accelerate decomposition by up to ten times. Thus, these results are highly valuable for certifying carbon stability when using organic amendments in agricultural soils. In any case, we have to consider that the MRT_2 values obtained from the soils are not comparable to those measured in the decomposition of pure amendments, without soil.

Thermal–respirometric comparisons show that thermally defined SOM stability aligns with microbial degradability (Fig. 4), supporting the hypothesis that the thermal stability/lability of soil organic fractions is directly related to their microbial degradability (Gregorich et al., 2015). Across both soils, cumulative respired CO_2 correlated negatively with the stable fraction W_4 and positively with the labile fraction W_2 (180–380 °C), while MRT_2 exhibited the complementary pattern ($W_4\uparrow \rightarrow MRT_2\uparrow$; $W_2\uparrow \rightarrow MRT_2\downarrow$), with somewhat stronger associations in AS—consistent with established understandings of soil carbon dynamics, the preferential mineralization of labile pools and persistence of recalcitrant carbon (Lehmann and Kleber, 2015; Schmidt et al., 2011; Plante et al., 2009, 2011; de la Rosa et al., 2008). These concordant relationships validate the suitability of thermal analysis as a proxy for estimating labile and stable soil organic fractions in soils. This approach opens up the possibility of developing predictive models for soil carbon dynamics, integrating both CO_2 emission measurements and the thermal stability of organic fractions.

Microbial determinations shown in Table 4 align with previously reported carbon dynamics. Bacterial DNA exceeded fungal DNA by 2–3 orders of magnitude. CUE_{micro} was higher in AS than GS and, excluding digestate outliers, covaried positively with the bacteria/fungi ratio. Higher CUE_{micro} means that more carbon is converted into microbial biomass rather than being lost as CO_2 through respiration (Liang and Balser, 2011). Thus, this result indicates a greater C sequestration efficiency in AS than GS soils. In a global soil meta-analysis, Sinsabaugh et al. (2016) identified a pronounced minimum in CUE at pH 5.4, which they linked to shifts in the bacterial-to-fungal ratio. Pei et al. (2021) further observed that CUE exhibited soil-specific variability, with notable increases correlated with higher soil pH and a transition from clay-rich textures to coarser, sand-dominated ones. Fang et al. (2018) determined CUE_{micro} values for Cambisols in New South Wales (Australia), ranging from 0.20 to 0.28, with lower values attributed to soils with finer textures. These observations confirm the pH-linked optima and soil-specific variability in CUE. It is also important to consider the high Ca content of the AS soil, as Ca contributes to SOC stabilization by promoting organo-mineral associations via cation bridging and facilitating co-precipitation with carbonates. These processes restrict microbial access to labile fractions, thereby enhancing organic matter persistence and likely reinforcing amendment-induced effects on microbial CUE and SOC dynamics (Rowley et al., 2018).

Regarding the amendments, recent findings indicate that biochar generally favors bacterial growth over fungal development, potentially altering microbial community composition and influencing carbon cycling dynamics (Wang et al., 2023; Manirakiza et al., 2024). This bacterial predominance may result from their higher mobility and metabolic versatility, which facilitate colonization of biochar pores and access to diverse nutrient sources. The resulting shifts in microbial community structure are critical to understand long-term implications for soil carbon sequestration. The observed reduction in fungal load following cow manure digestate application underscores how amendments strongly modulate bacteria-to-fungi ratios, with consequences for microbial efficiency in carbon use. In this study, CUE_{micro} was positively correlated with bacterial dominance ($r^2 = 0.8116$; $p < 0.05$), supporting the hypothesis that higher bacteria/fungi ratios can enhance microbial efficiency and thus soil carbon stabilization (Liang and Balser, 2011; Soares and Rousk, 2019). This hypothesis supports that during the stages of soil organic matter degradation dominated by fungi, lower CUE_{micro} would be anticipated compared to stages dominated by bacteria. However, this paradigm remains under debate and is yet far to be confirmed (Bölscher et al., 2016; Manzoni et al., 2018). Nevertheless, the 5 μm filtration used to prepare the inoculum may under-represent fungal propagules, since a relevant fraction of fungal spores and hyphal fragments are larger than the selected pore diameter, which should be considered when relating community structure to CUE_{micro}.

The results show that soil microbiological parameters—including total DNA, bacterial biomass, and fungal biomass—are shaped by both soil type and the amendment applied. It is important to note that microbial CUE was soil specific, with higher values in the alkaline soil than in the acidic soil. While bacterial dominance appeared linked to higher CUE, further work combining community profiling and functional analyses is needed to determine which microbial groups drive these differences, which also emerged between broad amendment categories, such as biochar and digestate, and within specific amendment types. This distinction is critical, as soil microbes regulate the fate of organic carbon, either releasing it as CO_2 through respiration or incorporating it into microbial biomass, thereby contributing to carbon stabilization (Fisk et al., 2015). Furthermore, the contrasting effects of amendments highlight the need to balance CO_2 emissions with microbial efficiency. For instance, digestates increased respiration and lowered CUE_{micro}, suggesting reduced potential for carbon retention. Conversely, olive pomace biochar in GS soils enhanced CUE_{micro} and microbial DNA yields, pointing to improved microbial carbon allocation and sequestration capacity. These outcomes align with global evidence that labile organic inputs often trigger strong positive priming effects (Xu et al., 2024b), emphasizing the importance of recalcitrant amendments such as biochars to mitigate accelerated turnover.

5. Conclusions

This study demonstrates that organic amendment type is a key determinant of soil carbon stabilization and microbial carbon use efficiency (CUE_{micro}). Biochars from woody and agro-industrial residues enhanced long-term carbon persistence by reallocating organic matter into recalcitrant pools and supporting higher CUE_{micro}, whereas cow manure digestate accelerated decomposition of labile fractions, leading to elevated CO_2 losses and reduced sequestration potential. These contrasting behaviors highlight the need to match amendment properties with soil conditions to maximize benefits.

The combined use of thermal analysis and automatic respirometry proved to be an effective and affordable approach to distinguish labile from stable fractions and to predict carbon turnover. Our results demonstrate that microbial CUE was soil dependent, showing higher values in the alkaline soil than in the acidic counterpart. This finding highlights the influence of microbial community composition on carbon allocation and underscores the need for future studies to disentangle the respective contributions of bacterial and fungal groups to soil-specific mechanisms of carbon stabilization. Nevertheless, CUE_{micro} provided a complementary metric of microbial efficiency, though its dependence on conversion factors and underrepresentation of fungal contributions underscores the importance of validation through isotopic and community-level analyses.

Overall, biochar amendments stand out as promising tools for carbon farming, particularly in Mediterranean and semi-arid regions where soil carbon retention is crucial for climate resilience. Incorporating low-cost proxies such as thermal stability and CUE_{micro} into monitoring frameworks can strengthen soil carbon assessments, while advancing interdisciplinary approaches will be key to scaling these strategies for sustainable agriculture and climate change mitigation.

CRediT authorship contribution statement

José M. de la Rosa: Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Data curation, Conceptualization. **Sara M. Pérez-Dalí:** Writing – review & editing, Visualization, Investigation. **Águeda Sánchez-Martín:** Writing – review & editing, Visualization, Investigation. **Jorge Márquez-Moreno:** Writing – review & editing, Investigation, Formal analysis. **Pedro**

M. Martin-Sanchez: Writing – review & editing, Formal analysis. **Layla M. San Emeterio:** Writing – review & editing, Investigation, Formal analysis. **Sara Gutiérrez-Patricio:** Writing – review & editing, Formal analysis. **Beatriz Cubero:** Writing – review & editing. **Heike Knicker:** Writing – review & editing, Resources. **Paloma Campos:** Writing – review & editing, Visualization, Formal analysis. **José A. González-Pérez:** Writing – review & editing, Supervision.

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Declaration of competing interest

The authors declare that there are no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

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Appendix A. Supplementary data

The electronic annex includes: A detailed description of the C_{micro} calculation, an image of the automatic respirometer along with a diagram of its operation (SF1), standard curves for qPCR-based quantification (SF2), FT-IR spectra (SF3), thermal analysis profile of soils (SF4), and ST1 containing all the results of thermogravimetric analyses. Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106577.

Data availability

Data will be made available on request.

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