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Temperature sensitivity of respiration in biochar-amended soils with different textures, moisture levels and fertilizer treatments**

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Abstract. Biochar additions change the temperature sensitivity (Q_{10}) of soil respiration. The aim of this study was to assess the effects of biochar on the Q_{10} of CO_2 emissions from fertilized sandy and silty loam soils with different moisture contents. Soil samples fertilized with NPK and NPK+manure (NPK+M) were incubated at temperatures of 5 to 15°C, and 15 to 25°C, under 60 and 100% water holding capacity (WHC). Microbial biomass C, pH and soil organic C concentration were measured in soils with and without biochar. The mean Q_{10} values varied from ~1 to 2 in most cases. The addition of biochar to silty loam affected the Q_{10} in the NPK+M treatment after 24 h, and the Q_{10} value increased from 0.90 to 1.36 at 5/15°C, while it decreased from 1.90 to 1.36 at 15/25°C. A similar effect of biochar was found with a sandy loam soil at 60% WHC. The lower Q_{10} for CO_2 emissions after biochar application were particularly evident under higher temperatures and water saturated conditions. These results indicate that biochar can decrease the temperature sensitivity of native SOC mineralization and potentially enhance C sequestration in soils at higher temperatures.

Keywords: biochar, Q_{10}, soil respiration, fertilization, C sequestration, global warming

1. INTRODUCTION

Soil and biomass carbon (C) stores based on global scale estimations are 373 Petagram (Pg) C and 1086 Pg C, respectively (Lal, 2005), but show significant regional variations due to interactions between climate, topography, parent material, natural vegetation, land use and management, as well as soil properties (Wiesmeier *et al.*, 2019). Grassland and forest soils typically accumulate more C than arable soils and this has important implications for crop production and climate change mitigation actions (Ghorbani *et al.*, 2023a). Among various soil properties, soil texture has the potential to be used as an indicator for soil organic C (SOC) storage given the positive correlation between SOC and clay content since a significant portion of SOC may be absorbed onto the surface of clay minerals (Zhang *et al.*, 2022). Consequently, silty soils often show a greater ability to store nutrients than sandy soils due to their higher clay contents (Tahir and Marschner, 2017).

The release of C from the soil is of particular importance in the context of ongoing global warming. The biogenic sources of CO_2 from the soil are temperature-dependent processes, including root respiration, rhizomicrobial respiration, priming-related effects, and the microbial decomposition of soil organic matter (Kuzyakov, 2006; Upadhyay *et al.*, 2021). Soil microorganisms use C from degraded soil organic matter as an energy source, resulting in the emissions of CO_2 (Plante *et al.*, 2015) and soil respiration is a commonly used parameter for quantifying the total activity of soil microflora and the mineralization of soil organic matter (Zhao *et al.*, 2018). The

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effect of temperature on C storage is partly determined by soil texture, with C storage decreasing with rising temperatures in coarse-textured soil with a greater proportion of unprotected C (Hartley *et al.*, 2021).

The temperature sensitivity of soil respiration, usually expressed as the Q_{10} value, reflects a change in SOC mineralization when temperature increases by 10°C (Kirschbaum, 1995). A lower temperature sensitivity indicates a slower organic C turnover and greater stabilization of C in the soil, potentially reducing the loss of soil C (Chen *et al.*, 2021). The Q_{10} value of soil CO_2 emissions depends on soil type (Fang *et al.*, 2014) and can be altered by soil nutrient enrichment (Tan *et al.*, 2020). The Q_{10} value was shown to increase with fertilization, and both chemical and organic manure + chemical fertilizers had a similar effect on the temperature sensitivity of soil organic C decomposition in an agricultural soil (Wang *et al.*, 2022). The temperature sensitivity of soil respiration was also affected by crop species, fertilizer type and fertilization regime, with the Q_{10} value decreasing in N fertilized soils under maize and wheat (Wang *et al.*, 2016). In another study the Q_{10} varied seasonally and did not change significantly in the growing season but was lower in the non-growing season (Liu *et al.*, 2016). The Q_{10} values were time-dependent and decreased to the same extent in both the N and N + P treatments, revealing that N fertilization could decrease the sensitivity of soil respiration to temperature changes, without a significant impact of P (Wang *et al.*, 2017). The combined addition of biochar and fertilizers did not affect Q_{10}, however it reduced CO_2 emissions which may suggest beneficial synergistic effects (Carpio Espinosa *et al.*, 2024). Other studies have reported that the combined application of biochar and N fertilizer can lead to an increase (Ge *et al.*, 2020) or decrease in Q_{10}, with consequent effects on soil C sequestration (He *et al.*, 2016).

Biochar, as a solid and stable, C-rich material materials, is produced by pyrolysis of waste biomass in the absence or low level of oxygen (Lehmann *et al.*, 2011; Singh *et al.*, 2022). Although the addition of biochar to soils is now being actively considered for waste management, increases in crop yields and/or improvements in several soil physical, chemical, and microbial properties (Xiao *et al.*, 2025), with the aim of increasing C sequestration, its effects are still equivocal (Ghorbani *et al.*, 2023b). While the effects of biochar on soil conditions depend on the type of feedstock used, pyrolysis conditions, and inherent soil properties, it generally improves the chemical, physical, and biological characteristics of soil. It increases soil pH, organic C content, and cation exchange capacity, enhancing nutrient availability. At the same time, it reduces bulk density and increases porosity, which improves aeration and water retention. Biochar can also stimulate soil microbial activity and diversity, contributing to improved soil fertility and productivity (Lehmann *et al.*, 2011; Li *et al.*, 2020; Singh *et al.*, 2022). Freshly added biochar did not significantly affect the temperature sensitivity of CO_2 and N_2O emissions while it decreased the sensitivity of CH_4 uptake from a sandy loam soil (Criscuoli *et al.*, 2019). Conversely, the addition of biochar to a comparable sandy loam soil reduced the temperature sensitivity in the longer term through changes in the soil microbial community composition (Chen *et al.*, 2018). These contrasting findings indicate the lack of consensus on how biochar additions influence the temperature sensitivity of soil respiration and decomposition.

It was previously reported that microbial temperature sensitivity and substrate depletion dictate how warming affects soil C loss via their control over microbial biomass (Walker *et al.*, 2018). Soil microbial biomass C (C_{mic}), consisting mainly of fungi and bacteria, is an important index of soil fertility (Ren *et al.*, 2019). Microbial species differ in their temperature sensitivities, which partly explains differences in respiration from various soils (Wang *et al.*, 2021). In arable soils C_{mic} is generally lower than in unmanaged soils and this may be improved by different soil additives, such as biochar, although this may depend on the dose applied (Evangelou *et al.*, 2021; Li *et al.*, 2020). Fertilization type also affects soil microbiota, and manure addition increases soil microbial biomass compared to soil with inorganic NPK fertilizer additions, although this may depend on the type of manure used (Ren *et al.*, 2019).

To address some of the often-conflicting results associated with the effects of biochar additions on the temperature sensitivity of CO_2 emissions we carried out a laboratory experiment on sandy and silty loam soils with different soil moisture contents and nutrient additions. We hypothesized that biochar in the soil reduces the sensitivity of respiration to temperature and therefore enhances C sequestration, especially under higher soil moisture. These results should contribute to an improved understanding of how soil type and temperature may influence the effect of biochar on C storage.

2. MATERIALS AND METHODS

2.1. Tested soils and experimental design

The research was conducted on long-term fertilized (>10 years) silty loam and sandy loam soils under maize (preceded by wheat in the previous year) and spring wheat (preceded by winter wheat in the previous year) cultivations, respectively. The fields have a long history of cultivation, and the crops are rotated annually. The fields were in the Lublin Upland, Poland (51°21' N, 22°51' E; 168.5 m asl) and the agricultural practices used were typical of mixed farms in this region. Maize was sown on 6th May 2022. The fertilizers applied, their rates and dates of application are given in Table S1. Based on the optimal recommended amounts of fertilizers that should be applied (Pott *et al.*, 2021; Sharma *et al.*, 2020), the wheat field was fertilized at a rate of 200 kg NPK ha^{-1} in September 2021 (before sowing) and 140 kg NPK ha^{-1} in Spring 2022, whilst the maize field was fertilized at a rate of 100 kg NPK ha^{-1} in May 2022 during sowing. The cattle manure treatments were applied before sowing at 30 t ha^{-1} to the wheat and maize fields. Soil samples (0-20 cm depth) were collected in May 2022 at six randomly selected sites in each field, covering all the treatments, then mixed, and thoroughly homogenized before sieving through a 2 mm mesh and stored for approximately four months in the dark at 4ºC prior to chemical analysis. The initial organic C concentration was 10.2 and 19.1 g kg^{-1}, while the N concentrations were 0.10 and 1.3 g kg^{-1} in the field under maize and wheat, respectively.

The biochar used in the experiment was produced from maize grown in 2016 by pyrolysis at 400ºC and had a C concentration of 28.9%, H of 1.06%, N of 0.68%, H/C ratio of 0.44, C/N ratio of 42.5 and a pH of 9.2. The use of aged (>6 years) biochar is known to reduce potentially confounding time-dependent effects (Wang *et al.*, 2020). The porosity of biochar used is shown in Fig. 1.

The samples were split for laboratory incubation studies and the analysis of soil parameters (Table 1). The following fertilization treatments were included in the short-term laboratory soil incubations: samples collected from i) controls (unfertilized soil taken from adjacent plots), ii) soils with mineral fertilizers (NPK), and iii) soil with mineral fertilizers and manure (NPK+M). For each fertilization treatment 10 g of soil in 120 cm^3 glass vessels (n = 3) were prepared with and without the addition of biochar at a rate corresponding to 30 Mg ha^{-1}. To acclimate the soil microorganisms to the experimental conditions, the soil was preincubated in the dark at room temperature (20 ± 1°C) at the initial moisture content (corresponding to the field moisture content at the time of soil sampling) for approximately one month after the addition of biochar. After preincubation, the soil moisture content was adjusted to 60 and 100% water holding capacity (WHC) and the samples left for another 3 days to stabilize to the new moisture conditions, which has been recommended for small soil samples in Q_{10} measurements (Chen *et al.*, 2010). Then, the vessels were sealed and the samples incubated for subsequent determinations of soil microbial biomass (C_{mic}) and assessments of the temperature sensitivity of soil respiration (Q_{10}).

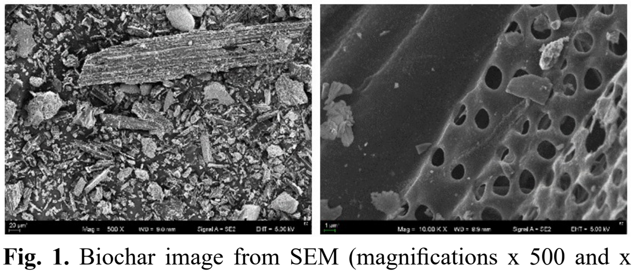


Fig. 1. Biochar image from SEM (magnifications x 500 and x 10 000).

For the calculations of Q_{10}, samples were incubated in darkness at temperature ranges of: 1) 5 to 15°C, and 2) 15 to 25°C. A separate set was prepared for each temperature. Headspace CO_2 was taken using a syringe (injection volume – 150 µL) and measured after 2 and 24 h of incubation by the gas chromatography method (Shimadzu GC-2014, Japan). We used a short-term incubation approach to minimize shifts in C pool size (Meyer *et al.*, 2018).

Soil microbial biomass was determined at 25°C (regarded as optimal for many soil microorganisms) from each fertilization treatment, with and without biochar using the substrate-induced respiration (SIR) method with glucose amendments as an easily available source of C and energy (Anderson and Domsch, 1978). The soil samples were enriched with the glucose solution (10 mg per gram of soil) and incubated with shaking at 25°C in a water bath. After 2 h, the CO_2 produced was measured using gas chromatography (Shimadzu GC-2014, Japan).

The soil pH and SOC concentration were also measured in soils with and without biochar.

2.2. Measurements of soil physical, chemical and microbiological characteristics

Soil organic carbon (SOC) and dissolved organic carbon (DOC) concentrations were measured with a TOC-VCPH analyzer (Shimadzu Corp., Kyoto, Japan). The soil texture was classified using Ferret's Triangle based on the sand, silt, and clay fractions determined with the laser diffraction method using a Mastersizer 2000 (Malvern, UK) with HydroG dispersion (Polakowski *et al.*, 2021). The continuous-flow analysis (CFA) method with spectrophotometric detection was used to determine the concentration of mineral N forms (NH_4^+ and NO_3^-). Dehydrogenase

Table 1. Chemical characteristics and dehydrogenase activity of the soils collected from control plots (without fertilization), and plots with either NPK (mineral fertilization), or NPK+M (mineral fertilization + manure) treatments (mean value ± standard deviation, n = 3)

Crop/Soil texture	Maize/Silty loam			Wheat/Sandy loam		
Fertilization variants	Control	NPK	NPK+M	Control	NPK	NPK+M
pH (H_2O)	6.23 ± 0.06	5.80 ± 0.02	6.11 ± 0.09	6.93 ± 0.03	7.14 ± 0.04	7.19 ± 0.06
NH_4^+-N (mg kg^{-1})	5.12 ± 0.03	36.9 ± 1.12	16.9 ± 0.29	4.08 ± 0.02	1.57 ± 0.01	2.67 ± 0.04
NO_3^--N (mg kg^{-1})	238.1 ± 8.27	151.4 ± 1.35	168.9 ± 5.06	113.0 ± 3.13	20.7 ± 1.28	25.1 ± 1.02
DOC (mg kg^{-1})	159.7 ± 23.4	132.3 ± 32.9	126.3 ± 5.41	204.5 ± 76.6	205.8 ± 98	137.6 ± 1.6
DHA (µg TPF g^{-1} 20 h^{-1})	0.99 ± 0.22	1.03 ± 0.13	0.67 ± 0.05	9.91 ± 0.95	4.12 ± 0.33	3.82 ± 0.53

DOC – dissolved organic carbon, DHA – dehydrogenase activity.

activity (DHA) was determined using the triphenyl tetrazolium chloride (TTC) method (Casida *et al.*, 1964) based on the amount of triphenyl formazan (TPF) produced after a 20 h incubation of the soil samples at 30ºC. DHA was measured spectrophotometrically based on the absorbance measured at 485 nm (UV-1601PC, Shimadzu Corp., Kyoto, Japan) with the reduction of the activity of the blank control without TTC addition. Soil, biochar, and manure pH were measured in a ratio of 1:2.5 soil: water using an HQ40D portable multimeter analyzer (Hach Lange). The concentrations of CHN in biochar were determined using an elemental analyzer (Perkin Elmer CHN 2400).

2.3. Q_{10} calculation and data analysis

The temperature sensitivity of soil CO_2 emissions was calculated according to the formula of Zhang *et al.* (2020):

$$Q_{10} = \frac{R_{T+10}}{R_T},$$

where: R_T and R_{T+10} are the CO_2 emissions (mg C kg^{-1}) at temperatures T and T+10, calculated separately at 5/15°C and 15/25°C.

The experiment was arranged in a split plot design with three replicates, and a one-way analysis of variance (ANOVA) was used to examine the effects of the different treatments and soil characteristics. Lowercase letters in the figures indicate statistically significant differences after the Tukey test at $p < 0.05$ and SPSS 26.0 used to analyze the data. A multi-factor analysis of variance (MANOVA) was used to evaluate the significance of four factors (soil texture, fertilization type, biochar application and WHC, and their interactions) on C_{mic} and Q_{10}-value. OriginPro 2020 and Excel 2020 were used to extract the figures.

3. RESULTS

3.1. Changes in soil microbial biomass (C_{mic}), pH and SOC

The effects of the different treatments on soil microbial biomass (C_{mic}) with and without biochar for the two soil types are presented in Fig. 2.

The addition of biochar to a silty loam soil at 60% WHC significantly increased C_{mic} in the treatments with mineral fertilization and manure. In contrast, for the same moisture content, the effect of biochar with the sandy loam soil significantly decreased C_{mic} in the control and mineral + manure (NPK+M) treatments. Under water saturated conditions, the addition of biochar only significantly reduced C_{mic} in the control silty loam soil. Where there was an effect of fertilization on soil C_{mic} this depended on soil moisture content. In the silty loam soil at 60% WHC, C_{mic} was the lowest in the NPK+M treatment, while under water saturated conditions it decreased in the order: control > NPK > NPK+M. In the sandy loam soil fertilization reduced C_{mic}, although manure addition did not have a significant effect on C_{mic} and the different fertilization treatments had similar values, regardless of soil moisture content.

The effect of biochar on soil pH and SOC concentrations is shown in Table 2. The silty loam soil had a lower pH and contained less SOC than the sandy loam soil. Fertilization decreased the pH of the silty loam soil, and this was significantly increased after the addition of biochar, regardless of moisture content. In the sandy loam soil, fertilization did not change the soil pH, while it significantly increased the pH after biochar application at 60% WHC. The addition of biochar (B) to the silty loam soil increased the SOC content in the different treatments by 25% (control + B), 36% (NPK+B

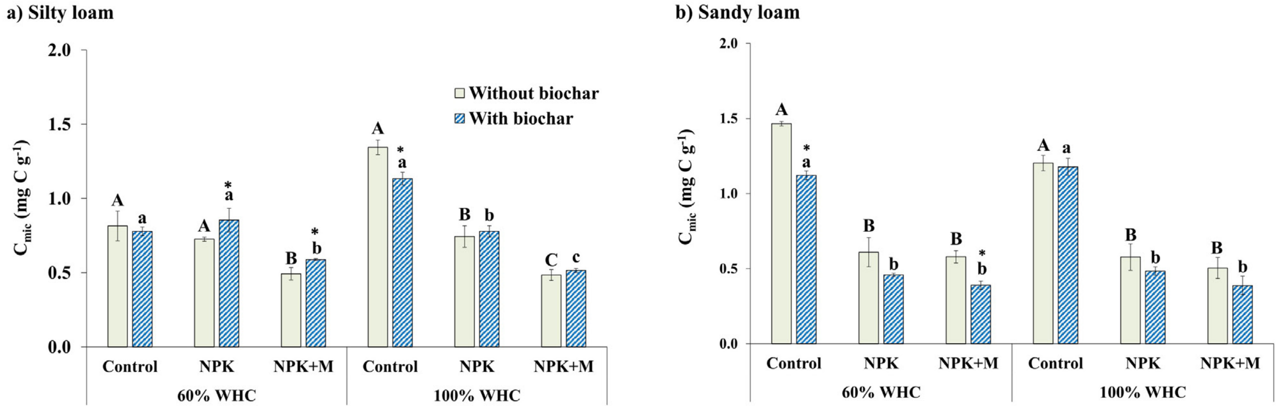


Fig. 2. Microbial biomass C (C_{mic}) in the: a) silty loam and b) sandy loam soils incubated at 25°C with and without biochar under two moisture levels (60 and 100% WHC), including control (without fertilization), NPK (mineral fertilization), and NPK+M (mineral fertilization + manure) treatments. Different upper-case letters indicate statistically significant differences between fertilization treatments without biochar and different lower-case letters indicate statistically significant differences between fertilization treatments with biochar. Asterisks indicate the significant effects of biochar application ($p < 0.05$) and vertical lines indicate standard deviations.

Table 2. Soil pH and organic carbon (SOC) concentrations in samples with and without biochar for the control (without fertilization), and for different NPK (mineral fertilization), and NPK+M (mineral fertilization + manure) treatments

Crop/Soil texture	Maize/Silty loam					
Fertilization variants	Control	Control + biochar	NPK	NPK + biochar	NPK+M	NPK+M + biochar
pH (60% WHC)	5.85 ± 0.06	6.18 ± 0.04*	4.57 ± 0.06*	5.09 ± 0.07*	4.87 ± 0.19	5.65 ± 0.03*
pH (100% WHC)	5.66 ± 0.03	5.97 ± 0.02*	4.83 ± 0.06	5.44 ± 0.07*	5.29 ± 0.06	5.76 ± 0.11*
SOC (g kg^{-1})	10.5 ± 0.86	13.1 ± 0.17*	8.85 ± 0.16	12.1 ± 0.61*	8.48 ± 0.08	12.1 ± 0.65*
	Wheat/Sandy loam					
pH (60% WHC)	6.64 ± 0.10	7.03 ± 0.05*	6.67 ± 0.10	7.07 ± 0.07*	6.64 ± 0.11	7.07 ± 0.13*
pH (100% WHC)	6.68 ± 0.06	6.85 ± 0.09	6.79 ± 0.06	6.93 ± 0.08	6.85 ± 0.06	6.93 ± 0.05
SOC (g kg^{-1})	20.0 ± 0.20	22.3 ± 0.90*	16.3 ± 0.54	17.7 ± 0.01*	16.5 ± 0.75	18.4 ± 0.49*

Mean value ± standard deviation (n=3), *indicate significant effects of biochar addition.

treatment), and 42% (NPK+M+B treatment). Enrichment of the sandy loam soil with biochar increased the SOC concentration to a lesser extent (8-12%, depending on treatment).

3.2. Temperature sensitivity of respiration (Q_{10})

The results of the Q_{10} analysis for the two types of soils as influenced by biochar, moisture content and temperature, are presented in Fig. 3.

In general, Q_{10} had mean values between 1 and 2, although after a shorter incubation time (2 h) Q_{10} was sometimes below 1, especially at the lower soil temperature. The addition of biochar to the silty loam soil significantly affected Q_{10} in treatments with mineral fertilization and manure after 24 h of incubation, and the Q_{10} value increased at temperatures of 5/15°C (60% and 100% WHC) while it decreased at 15/25°C (60% WHC) after 24 h (Fig. 3a-d). Biochar-amended sandy loam soil with mineral fertilization + manure had a higher Q_{10} after 2 h of incubation at 5/15°C (60% WHC), while the opposite effect was observed at 15/25°C, regardless of the incubation period (Fig. 3e and f). Under water saturated conditions, biochar decreased Q_{10} in the sandy soil with the mineral fertilizer at 15/25°C regardless of the incubation period (Fig. 3h).

Statistical analysis showed that the type of fertilization, biochar addition, soil moisture, and most interactions significantly influenced soil microbial biomass C (Table 3). Based on incubation time, soil temperature, and moisture, the interactions among temperature and fertilization type, soil temperature and moisture, and the interactions of soil texture, soil temperature, and moisture all significantly affected the Q_{10} value (Table 4).

4. DISCUSSION

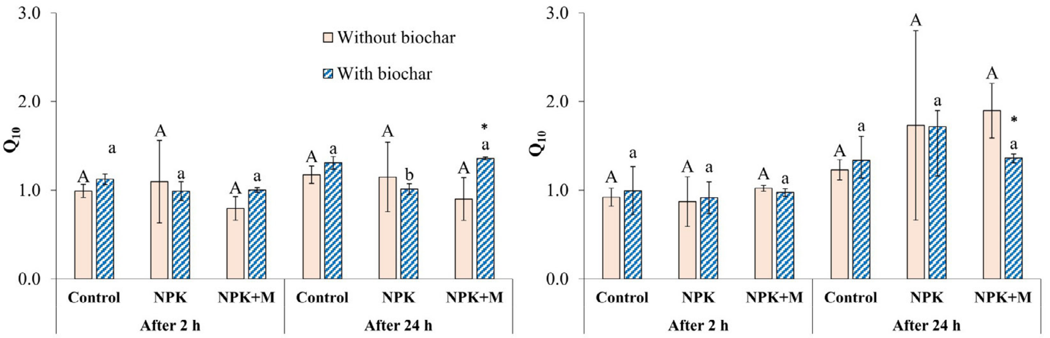
4.1. Response to soil fertilization and enrichment with biochar

Fertilization, soil moisture, biochar addition, and most interactions significantly impacted on soil microbial biomass C. Fertilization decreased dehydrogenase activity (with the exception of the NPK treatment in silty soils) and soil microbial biomass, in line with a number of reports, although the opposite effects have been observed (Guan *et al.*, 2022; Moeskops *et al.*, 2010; Ren *et al.*, 2019). Based on long-term field studies, the effect of fertilization on C_{mic} was determined by soil pH, and fertilization slightly reduced C_{mic} in soils with a pH < 5, while C_{mic} increased at higher soil pH values (Geisseler and Scow, 2014). This has not been confirmed in the present study and C_{mic} was reduced by fertilization despite differences in pH. Considering fertilization type, the addition of NPK+M resulted in a greater reduction in C_{mic} than NPK alone in silty soil, while in the sandy loam fertilization had no effect and both treatments reduced C_{mic} to a similar extent. It has recently been reported that organic fertilizers can have a negative effect on soil microbial biomass C (Wang *et al.*, 2023a). In contrast, in other studies the application of organic fertilizers increased C_{mic} more than NPK additions (Francioli *et al.*, 2016; Lori *et al.*, 2017; Ren *et al.*, 2019). Beside fertilization, C_{mic} may also be affected by planting pattern, and wheat continuous cropping (without fertilization, with NP fertilizer, and with NP and organic fertilizer) significantly increased C_{mic}, whereas a wheat and corn rotation (with NP and organic fertilizers), and a wheat, millet and pea rotation (with NP and organic fertilizer) decreased C_{mic} (Wang *et al.*, 2023a). Given the different cropping systems associated with the soils used in the present study (a maize crop was preceded by winter wheat, and winter wheat was preceded by spring wheat) some of the effects on C_{mic} may have been influenced by cropping history.

It has been reported that, in addition to pH, soil texture can influence microbial community structure and composition, and various microorganisms are correlated with the sand, silt and clay contents (Xia *et al.*, 2020). Thus, the presence of different microbe populations in the soils used in the present study may explain the different effects on C_{mic}. Moreover, a higher C_{mic} in silty compared to sandy fertilized soils may result from differences in the tolerance of microorganisms to variable water availability given their differences

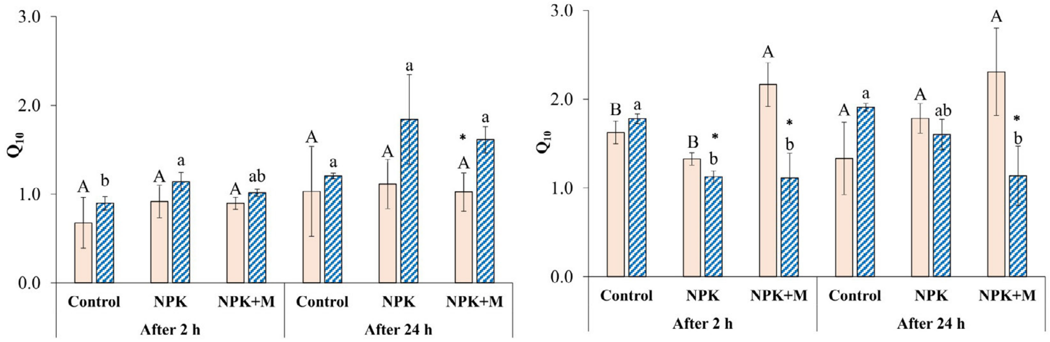
a) Silty loam at 60% WHC, Temp. 5/15 °C

b) Silty loam at 60% WHC, Temp. 15/25 °C



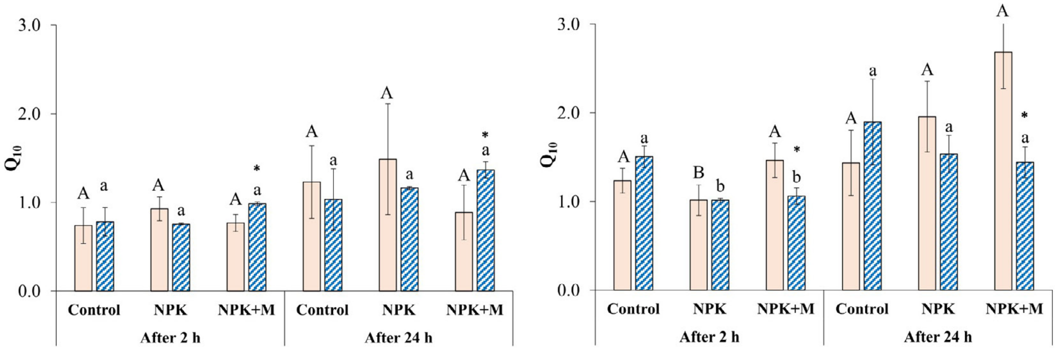
c) Silty loam at 100% WHC, Temp. 5/15 °C

d) Silty loam at 100% WHC, Temp. 15/25 °C



e) Sandy loam at 60% WHC, Temp. 5/15 °C

f) Sandy loam at 60% WHC, Temp. 15/25 °C



g) Sandy loam at 100% WHC, Temp. 5/15 °C

h) Sandy loam at 100% WHC, Temp. 15/25 °C

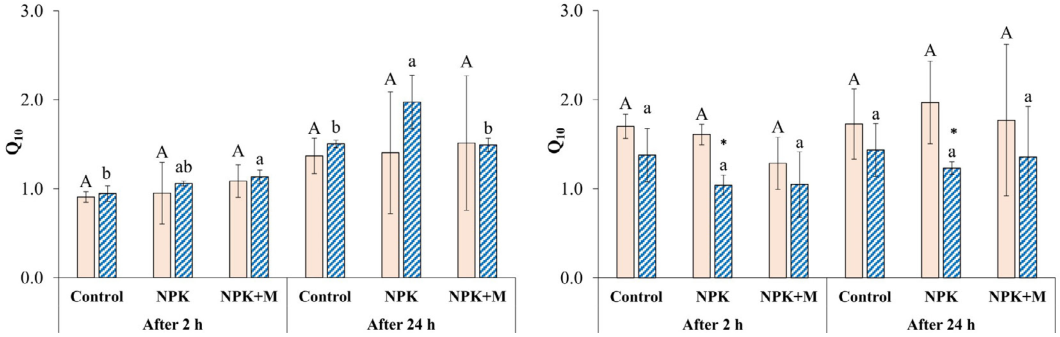


Fig. 3. Temperature sensitivity (Q_{10}) of CO_2 emissions for the silty loam and sandy loam soils incubated at 25°C with and without biochar and subjected to two moisture levels (60 and 100% WHC), after 2 and 24 h. Control (without fertilization), NPK (mineral fertilization), and NPK+M (mineral fertilization + manure) treatments. Different upper-case letters indicate statistically significant differences between fertilization treatments without biochar, while different lower-case letters indicate statistically significant differences between fertilization treatments with biochar. Asterisks indicate the significant effects of biochar applications ($p < 0.05$) and vertical lines indicate standard deviations.

Table 3. Results of a multifactor analysis of variance (MANOVA) for soil microbial biomass (C_{mic}) with F-ratios and p-values for the factors, soil texture (silty loam and sandy loam), fertilization (Control, NPK, and NPK+M), biochar application (with and without biochar), soil WHC (60 and 100%), and their interactions

Main effect on C_{mic}	F-ratio	p-value
Soil texture (ST)	ns.	ns.
Fertilization type (FT)	936.29	< 0.0001
Biochar application (BA)	34.04	< 0.0001
Soil water holding capacity (WHC)	9.29	< 0.01
ST * FT	117.82	< 0.0001
ST * BA	41.03	< 0.0001
ST * WHC	47.57	< 0.0001
FT * BA	10.79	< 0.001
FT * WHC	28.03	< 0.0001
BA * WHC	ns.	ns.
ST * FT * BA	4.01	< 0.05
ST * FT * WHC	55.49	< 0.0001
ST * BA * WHC	27.06	< 0.0001
FT * BA * WHC	ns.	ns.
ST * FT * BA * WHC	5.41	< 0.05

*Indicates interactions between parameters, ns. – not significant.

Table 4. Results of a multifactor analysis of variance (MANOVA) of the Q-values (Q10) with F-ratios and p-values for the factors, soil texture (silty loam and sandy loam), fertilization (control, NPK, and NPK+M), biochar application (with and without biochar), incubation temperature (5/15 and 15/25°C), soil WHC (60 and 100%), and their interactions

Main effects on Q_{10}	Q_{10} value after	
	2 h	24 h
	F-ratio / p-value	
Soil texture (ST)	ns.	ns.
Fertilization type (FT)	ns.	ns.
Biochar application (BA)	ns.	ns.
Temperature of incubation (TI)	69.51 / <0.0001	19.94 / <0.0001
Soil water holding capacity (WHC)	28.51 / <0.0001	ns.
FT * TI	8.08 / <0.001	ns.
TI * WHC	14.99 / <0.001	ns.
ST * TI * WHC	21.77 / <0.0001	ns.

Explanations as in Table 3.

in texture, with sandy soils being more freely draining than silty soils, with a lower ability to retain moisture. Therefore, the microbes present in sandy soils may be adapted to significant moisture fluctuations (Patel *et al.*, 2021). Soil texture-related variations in water retention and oxygenation may also contribute to differences in the microbial populations (Xia *et al.*, 2020). In a laboratory study a short-term change in moisture contents in both soils did not change C_{mic}, although reductions in C_{mic} through a longer lasting O_2 deficiency may result in major reductions in microbial activity (Uhlířová *et al.*, 2005).

The increasing degradation of arable soils and the need to increase soil C storage whilst maintaining agricultural productivity has led to several investigations involving biochar additions (summarized by Schmidt *et al.*, 2021) and how these can result in modifications in soil physical and chemical properties and enhance crop yields. Biochar has also been shown to improve the activity and diversity of soil microorganisms by increasing soil pH, WHC, nutrient availability and porosity (Lehmann *et al.*, 2011; Pokharel *et al.*, 2020; Xu *et al.*, 2023), especially in sandy soils (De Sousa Lima *et al.*, 2018). Although the effects of biochar on soil properties depend on pyrolysis conditions and soil type, several consistent general trends have been observed across studies. For example, meta-analysis by Singh *et al.*, (2022) reported that soils amended with biochar prepared at both high (> 500ºC) and low (< 500ºC) pyrolysis temperatures exhibited significant increases in porosity, indicating that improved soil pore architecture is not strictly limited to a narrow temperature range. Biochar's beneficial effects on soil oxygenation and porosity derive not solely from pyrolysis temperature but from its intrinsic porous nature and effects on soil physical structure. However, high pyrolysis temperatures (> 500°C) lead to increased pore volume in biochar compared to lower temperatures (Li *et al.*, 2022).

In the current study significant increases in C_{mic} were associated with the addition of biochar to a fertilized silty loam soil at a 60% moisture content, while the opposite effect was found with the sandy loam soil Our observations are, however, in line with the meta-analysis conducted by Pokharel *et al.* (2020) which reported that biochar increased microbial biomass (and enzymatic activity) in fine textured soils with a lower pH, total C and N, but not in neutral, alkaline or coarse-textured soils. Soil pH was lower in the silty soil (in the range 4.61-5.85 without biochar, and 5.10-6.18 after biochar addition) than in the sandy soil (in the range 6.64-6.85 without biochar, and 6.85-7.07 after biochar addition) in the current study. Our results also generally showed a positive relationship between soil pH and C_{mic} in the silty loam soil while a negative relationship was found with the sandy loam soil, although the correlations were not always significant (Table 5).

Biochar has been shown to be more effective in enhancing C_{mic} in acidic (with pH < 6.5) compared to alkaline soils through an increase in pH, resulting in conditions

Table 5. Correlations (R value) between soil microbial biomass C (C_{mic}), pH and SOC in soils with and without biochar, for each fertilization treatment

Correlation	Crop/Soil texture	Maize/Silty loam			Wheat/Sandy loam		
	Fertilization variants	Control	NPK	NPK+M	Control	NPK	NPK+M
Soil C_{mic} x pH	60% WHC	0.14	0.71*	0.71*	-0.86*	-0.54	-0.74*
	100% WHC	-0.84*	0.04	0.29	-0.021	-0.21	-0.51
Soil C_{mic} x SOC	–	-0.22	0.78*	0.72*	-0.89*	-0.25	-0.70*

*Indicate significant correlations ($p < 0.05$).

more favourable to microorganisms (Lehmann *et al.*, 2011; Pokharel *et al.*, 2020), and this is supported by the results obtained with the silty loam soil in our experiments. Long-term studies on forest soils also reported a higher microbial biomass at low soil pH (~ 4.5), while C_{mic} decreased at higher pH values, especially in calcium-treated soils (Ontman *et al.*, 2023), as in our sandy loam, although this had a much higher pH. Although not common, another study on coarse textured soil also showed a decrease in soil microbial biomass after biochar addition (Dempster *et al.*, 2012). However, when analyzing the response of soil microbes to the addition of biochar, it is important to consider that this may be determined by several factors, including soil properties, pyrolysis conditions and biochar parameters, as well as the experimental duration and the method of application/incorporation (Dempster *et al.*, 2012; Pokharel *et al.*, 2020).

In addition to pH, the soils differed in SOC concentration, which increased after biochar addition. The silty loam had a lower SOC concentration than the sandy loam soil, although the reverse relationship, and a higher capacity to retain SOC, might have been expected due to the SOC being protected against microbial mineralization (Six *et al.*, 2002). Nevertheless, many silty loam soils in Western Europe have lower SOC concentrations than coarser textured soils (Li *et al.*, 2022). The SOC concentrations were higher after biochar application to the soils in the current study and increased by 25-43 and 8-12% in the silty and sandy loam soils, respectively. The increase in SOC was also consistent with previous laboratory experiments on agricultural soils with added biochar (Kubaczyński *et al.*, 2023). Since SOC is a key factor in increasing soil C_{mic} (Geisseler and Scow, 2014), this may explain the higher C_{mic} in the silty loam soil since additional soil C sources may be used by bacteria after the application of biochar (Xu *et al.*, 2023). This is supported by the positive and significant relationships between these parameters in the fertilized silty loam soil (Table 5). In contrast, in the sandy loam soil correlations between C_{mic} and SOC were negative and the reason(s) for this requires further research.

4.2. Temperature sensitivity of soil respiration

In the present study the mean Q_{10} values varied from approximately 1 to 2 in most cases (Fig. 4), indicating that a 10°C increase in temperature can result in an up to 2-fold increase in soil respiration.

For the 5/15°C treatment and the shorter incubation time (2 h), Q_{10} was sometimes below 1, suggesting a lower activity as temperature rises, which may reflect the time needed for the soil microbes to acclimate to the new conditions (Chen and Tian, 2005). Although Q_{10} values below 1 have not commonly been reported, a study on meadow soil also showed a Q_{10} of 0.67 in the temperature range of 5/11°C while this was above 1 at higher temperatures (Liang *et al.*, 2019). In forest soils the Q_{10} values have been shown to range from 0.3 to 5.4, revealing strong seasonal variation (Han and Jin, 2018). However, most of the Q_{10} values found in the current study were comparable to those previously reported, *e.g.* 1.2-2.8, with a temperature range of 5-25°C at 30-75% WHC (Meyer *et al.*, 2018), 1.35-1.68 with a temperature range of 5-25°C and a WHC of 60% (Zhang *et al.*, 2020), and 1.8-2.8 with a temperature range of 15-35°C at 60% WHC (Wang *et al.*, 2022). Inclusion of soils sampled at different times of the year, showed even higher Q_{10} values, reaching approximately 2 and 4, although most often between 2 and 3 with site-dependent seasonality (Yang *et al.*, 2022). The temperature sensitivity of soil respiration is regulated by many factors including the experimental conditions (field or laboratory-based assessments, incubation time and temperature), crop type, soil depth, ecosystem type, pH, C/N, moisture content and microbial biomass (Meyer *et al.*, 2018; Wang *et al.*, 2022; Zhang *et al.*, 2020). The short-term incubations applied in our study aims to minimize shifts in C pool size (Meyer *et al.*, 2018). As previously reported, prolonged incubation can result in lower Q_{10} estimates because SOC declines with increasing incubation duration (Hamdi *et al.*, 2013; Kirschbaum, 2006) which could make it difficult to assess the impact of biochar in our study. A short incubation time was also recommended and applied in previous studies (Koch *et al.*, 2007; Meyer *et al.*, 2018; Vanhala *et al.*, 2008). Laboratory experiments with controlled moisture and temperature allow for a better assessment of the effects of biochar than uncontrolled field conditions, although some field studies also confirm a reduction in Q_{10} in soil with biochar (He *et al.*, 2016).

In the present study the Q_{10} values for CO_2 emissions were usually not significantly altered by fertilization alone, while the effect of interactions with temperature and fertilization type was significant. Other incubations with soils

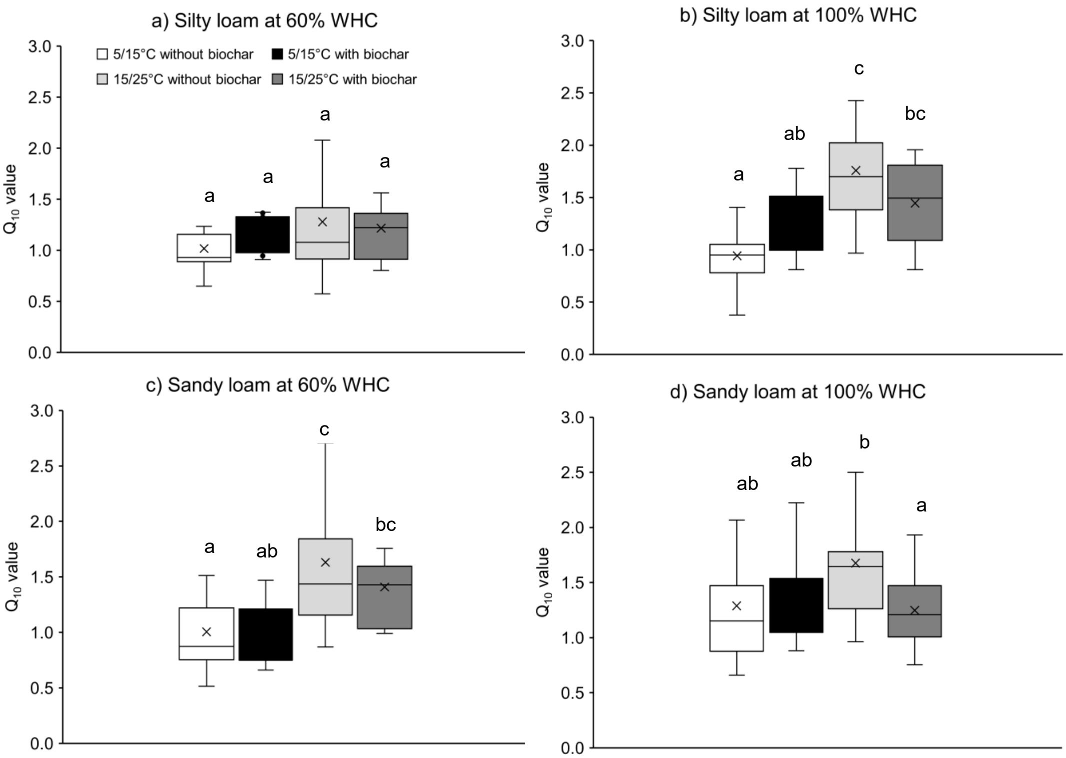


Fig. 4. Range of Q_{10} values for the silty and sandy loam soils incubated at 60 and 100% WHC with and without biochar. Distribution of data in quartiles with the average Q_{10} value shown as an x symbol. Different letters indicate statistically significant differences between soils with and without biochar incubated at 5/15 and 15/25°C ($p < 0.05$).

from various ecosystems enriched with N and P, showed that the Q_{10} value was not significantly changed by the short-term addition of nutrients, but soil microbial respiration increased with increasing temperature (Qian *et al.*, 2016). In the present study the exceptions were the silty loam soil, where NPK+M fertilization significantly increased the Q_{10} value (at 100% WHC after 2 h) and the sandy loam soil, where NPK fertilization significantly decreased the Q_{10} value (at 60% WHC after 2 h). This indicated that both mineral and organic fertilizers enhanced SOC decomposition in silty soil under water saturated conditions, while the effect was opposite in sandy soils with mineral fertilization, when they were below their maximum water holding capacity. Higher Q_{10} values for SOC mineralization after fertilization (at 15, 25, and 35°C and under 60% WHC) were also reported by Wang *et al.* (2022), although in this case it was not influenced by the type of fertilization (inorganic or organic + inorganic). Increases in the temperature sensitivity of SOC mineralization has been explained by higher C stocks and enhanced C mineralization by microorganisms after mineral and organic fertilization (Gross and Glaser, 2021; Wang *et al.*, 2022), which was not apparent in our study. Based on *in situ* measurements a decrease in Q_{10} after N fertilization has been explained through the higher availability of substrate, changes in substrate C quality, a lower activation energy, a lower root biomass, a lower soil C/N ratio or a lower microbial activity (Huang *et al.*, 2022; Wang *et al.*, 2016; 2019).

Our study revealed that incubation temperature, soil moisture content, the interactions among temperature and fertilization type, soil temperature and moisture, and interactions among soil texture, soil temperature, and moisture can significantly affect the Q_{10} value while the effect of incubation temperature can also be significant. Microbial activity and, consequently soil CO_2 emission, are strongly determined by soil temperature and moisture content, factors that are particularly relevant to projected climate change related warming and modifications in precipitation. Both temperature and soil moisture can directly or indirectly affect soil microbes since temperature regulates enzyme activity, influences water availability and the diffusion of gases, while soil moisture content can impact directly on microbial metabolism while also being a transport medium, that is essential for nutrient cycling and influences gas diffusion (Cruz-Paredes *et al.*, 2021; Yan *et al.*, 2015). In the present study we created conditions that are considered near optimal for soil microorganisms (60% WHC and a temperature of 15/25°C), as well as less favourable conditions for microbial growth and activity (100% WHC and a temperature of 5/15°C). Generally, higher Q_{10} values were found under higher, potentially less favourable, soil moisture contents and after incubation at a higher temperature than at a lower temperature. Like our results, a study on lowland grassland soils revealed a higher Q_{10} value after incubation at 75% than at 30% WHC (Craine and Gelderman, 2011). However, in the forest soil this was dependent on incubation time, but on average the Q_{10} values were higher at 80 and 100% WHC compared to those at 20 – 60% WHC and attributed to substrate limitation caused by the limited diffusion of solutes (Zhou *et al.*, 2014). Different responses of the temperature sensitivity to moisture and temperature that have been reported previously, may be due to the use of contrasting experimental approaches and different soil temperature and moisture values. For example, a laboratory study with lower soil moisture contents (10 – 50% WHC) revealed that the Q_{10} for microbial growth and respiration was not affected by changes in moisture (Cruz-Paredes *et al.*, 2021). However, information based on field measurements at different soil depths showed that a 1°C increase in soil temperature at 10 cm depth will reduce the Q_{10} by 0.084, while a 1% decrease in soil moisture will reduce the Q_{10} value by 0.08 (Qi *et al.*, 2002). In contrast to our study, the Q_{10} value decreased as soil moisture decreased (30, 60 and 90% WHC) presumably due to the reduced O_2 supply at higher moisture levels (Banerjee *et al.*, 2016).

In the present study contrasting effects of biochar application on the Q_{10} values for CO_2 emissions were found. In many cases, the effects were not significant, with evidence of both increases after incubation at 5/15°C (silty loam soils fertilized with NPK+M, regardless of moisture content, and at 60% WHC for the sandy loam soil), and decreases at 15/25°C (for both soils fertilized with NPK or NPK+M, regardless of moisture content). Analysis of the range of Q_{10} values observed (Fig. 4) revealed the largely positive effects of biochar, which was visible as a reduction in temperature sensitivity, especially after incubation at 15/25°C and under 100% WHC. At higher temperatures soil microorganisms are more active than under lower temperatures, and biochar application may introduce more C for microbial metabolism (Huang *et al.*, 2022). Reports in the literature on the temperature sensitivity after biochar enrichment of soils vary, including no effect (Bamminger *et al.*, 2018; Fang *et al.*, 2014), an increase (Fang *et al.*, 2017; Sun *et al.*, 2016) or a decrease (Fang *et al.*, 2014; He *et al.*, 2016), with significant temporal variations in the Q_{10} values (Wang *et al.*, 2019), although this was mainly based on field studies. Similar to our results, a study on agricultural sandy loam soils in China showed temperature-dependent values for Q_{10} in response to biochar addition. The values were lower at 15-20 and 15-25°C, while no effect was observed in the 20-25°C range (Chen *et al.*, 2018). Contrasting results in the literature have been explained by a dependence of the Q_{10} value, for CO_2 emissions, on many factors, such as the experimental conditions, soil type, temperature regime, soil properties and the amount of biochar used (Chen *et al.*, 2018; Fang *et al.*, 2014). Differences in the age of the biochar used may also have influenced some previous results. The enrichment of soils with biochar that was several years old (rather than fresh material) in the current study was aimed at minimizing the impact of aging-related effects (Wang *et al.*, 2020). Despite the use of a one-month pre-incubation of the soil with the addition of biochar, it cannot be ruled out that the results may have been influenced by the inability of some soil microorganisms to acclimate to the new conditions, which is also likely to be dependent on the incubation time or even result in some cell death. Since biochar can significantly affect several soil characteristics as well as microbial activity, several mechanisms have been proposed to explain the different temperature responses to biochar presence in the soil. The lack of an effect of biochar on Q_{10} has been explained by its high stability and its limited influence on microbial C cycling (Bamminger *et al.*, 2018). This was also confirmed by the H/C ratio of 0.44 obtained and C/N ratio of 42 in our study. An H/C ratio below 0.7 and high C/N ratio indicate the presence of aromatic structures with low microbial C availability (Jiang *et al.*, 2020), and H/C ratio values below 0.4 reflect a higher degree of aromaticity that enhances C stability and increases its long-term sequestration potential and reduced decomposition rates (Li and Tasnady, 2023). Higher Q_{10} values after biochar applications to soil have been explained by instantaneous decreases in C availability due to the higher recalcitrant C fraction in biochar compared to soil and the higher nutrient contents led to enhanced microbial biomass and activity, and the reduced degradability of resistant C (Huang *et al.*, 2022; Lehmann *et al.*, 2011; Wang *et al.*, 2019). Lower Q_{10} values in soils with biochar have been explained by the consumption or limitation of labile dissolvable C by entrapment in biochar pores, water limitation due to its absorption by biochar, stabilization of soil organic C, formation of organic-mineral interactions and the lower activity of soil microorganisms, as well as the altered structure of the microbial community or their colocation to the biochar pores (He *et al.*, 2016; Ma *et al.*, 2023; Wang *et al.*, 2019; Yang *et al.*, 2021). However, the limitations of the research is the lack of specific surface area and pore size and distribution. The monthly preincubation of soils with biochar may have facilitated the microbial colonization of its pore structure (Fig. 1), and caused a depletion in labile C, leading to the greater contribution of more resistant carbon to CO_2 emissions (Wang *et al.*, 2020), potentially resulting in higher Q_{10} values. It is also known that soil minerals interact with soil organic matter to form organo-mineral complexes or organo-biochar-mineral complexes, thus reducing C release from soil organic matter and dissolvable biochar (Yang *et al.*, 2021), which may explain why the smallest Q_{10} was found with the biochar-amended silty loam soil.

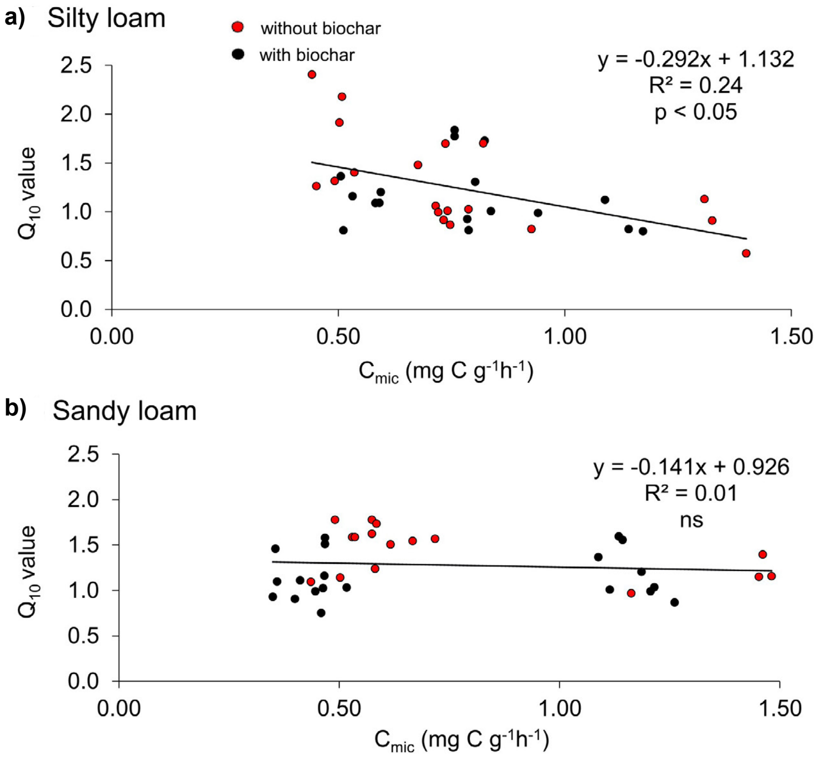


Fig. 5. Relationships between the Q_{10} values and soil microbial biomass C (C_{mic}) for the: a) silty and b) sandy loam soil.

In our study we also show a negative relationship between Q_{10} values and soil microbial biomass C in the silty loam soil (Fig. 5), which might be because these are dominated by bacteria whilst fungi may be more important in sandier soils (Sessitsch *et al.*, 2001).

Changes in the soil microbial community, due to the high porosity (Fig. 1) and increased surface area of biochar, may be more beneficial for bacteria than for fungi (Chen *et al.*, 2013), although positive effects on both groups have been demonstrated (Chen *et al.*, 2018) probably because the porous structure of biochar creates additional niches for colonization by a range of microbes. Although we did not conduct microbial community analyses in this study, numerous publications reported that biochar addition to soil more frequently stimulates bacterial communities relative to fungal communities (Iturbe-Espinoza *et al.*, 2025; Wang *et al.*, 2023b). Based on this evidence it is likely that the main source of CO_2 in the biochar amended silty loam soil examined in this study was of bacterial origin, but this would need to be confirmed by microbial analysis. The absence of a correlation between microbial biomass and Q_{10} for the sandy loam soil suggests that respiration is controlled more by abiotic factors, including moisture or substrate availability, in finer textured soils (Allison and Treseder, 2008; Kirschbaum, 2006) (Fig. 5). Based on the distribution of points, biochar reduces and stabilizes the temperature sensitivity of microbiological processes in silty loam soil, while in sandy loam it has no significant effect, which confirms that the effect of biochar is strongly dependent on the physicochemical properties of the soil.

5. CONCLUSIONS

To assess the interaction between microbial biomass and the temperature sensitivity of soil CO_2 emissions and C stability, laboratory research was conducted considering the effect of different soil textures, types of fertilization, soil moisture, and the presence of biochar. The effect on C_{mic} and Q_{10} values in response to biochar addition depended on soil physicochemical and microbial parameters. Due to improved organic-mineral interactions, biochar amendments may increase the C consumption rate of microorganisms and stabilize soil organic carbon by reducing soil respiration, however variable effects on the Q_{10} value were found, which depended on the incubation temperature and soil characteristics. The lower temperature sensitivity of CO_2 emissions after biochar application was particularly evident under 100% WHC suggesting that biochar addition appears particularly beneficial in soils prone to high moisture conditions (*e.g.*, fine-textured or periodically waterlogged soils). Biochar can decrease the temperature sensitivity of native SOC mineralization and potentially enhance C sequestration in soil at higher temperatures, which is an important factor to consider in the context of global warming. Since biochar may help stabilize SOC under conditions of high water availability and warming, from an agricultural perspective, its application may therefore be most suitable in soils with high moisture retention or poor drainage, fine-textured (clay-rich) soils, and systems vulnerable to warming-induced increases in SOC mineralization. Biochar could cause a shift in microbial community functioning towards slower organic C turnover and the greater stability of SOC stocks, which would need to be confirmed by detailed microbiological analyses, as well as the inclusion of biochar from different pyrolysis conditions and applied to different types of soil.

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

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Conflict of interest: The authors declare that they have no conflict of interest.

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