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Influence of organic matter input and temperature change on soil aggregate-associated respiration and microbial carbon use efficiency in alpine agricultural soils

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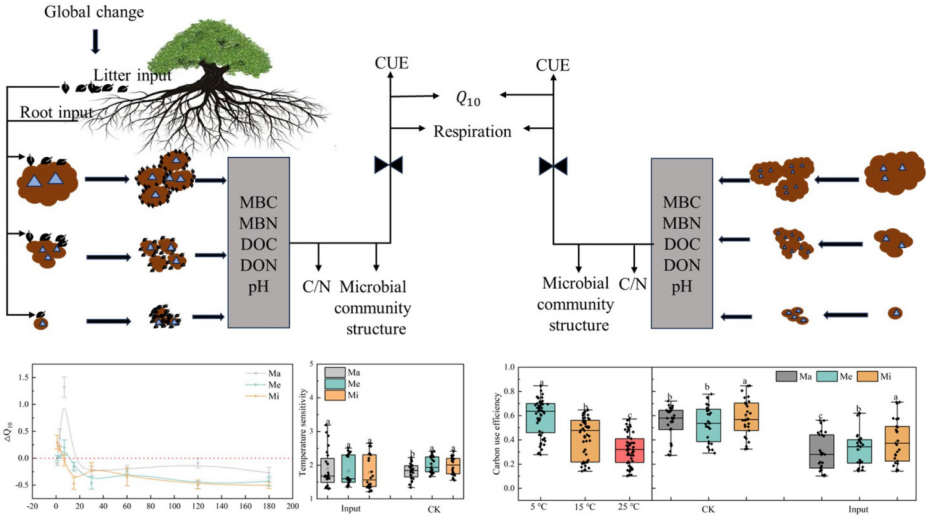
ABSTRACT

- Soil aggregates affect soil respiration and its temperature sensitivity.
- Organic matter input boosts soil respiration, affected by temperature and aggregate size.
- Q_{10} declines with increasing aggregate size, influenced by soil quality index.
- Microbial CUE drops with organic matter input, temperature and aggregate size increase.



Understanding the dynamics of soil respiration, microbial carbon use efficiency (CUE), and temperature sensitivity (Q_{10}) in response to exogenous organic matter (EOM) input, soil aggregate size, and incubation temperature is crucial for predicting soil carbon cycling responses to environmental changes. In this study, these interactions were investigated by 180-day incubation of soil aggregates supplemented with EOM at various temperatures (5°C, 15°C and 25°C). The results reveal an 'L-shaped' trend in soil respiration on the time scale across all treatments, characterized by initial rapid declines followed by stability. EOM input and higher temperatures significantly enhance respiration rates. Notably, the respiratory rates of soil aggregates of different sizes exhibit distinct patterns based on the presence or absence of EOM. Under conditions without the addition of EOM, larger aggregates show relatively lower respiration rates. Conversely, in the presence of EOM, larger aggregates exhibit higher respiratory rates. Furthermore, Q_{10} decreases with increasing aggregate size. The relationship between Q_{10} and the substrate quality index (SQI) supports the carbon quality temperature (CQT) hypothesis, highlighting SQI's influence on Q_{10} values, particularly during later incubation stages. Microbial CUE decreases with EOM input and rising temperatures. Meanwhile, aggregate size plays a role in microbial CUE, with smaller aggregates exhibiting higher CUE due to enhanced nutrient availability. In conclusion, the intricate interplay of EOM input, aggregate size, and temperature significantly shapes soil respiration, microbial CUE, and Q_{10}. These findings underscore the complexity of these interactions and their importance in modeling soil carbon dynamics under changing environmental conditions.

Keywords soil aggregates, soil respiration, temperature sensitivity, Tibetan Plateau

1 Introduction

The soil is the largest carbon reservoir in terrestrial ecosystems and plays a pivotal role in dynamically regulating atmospheric CO_2 concentrations (Bhattacharyya et al., 2019; Zamanian et al., 2021). Soil aggregates, serving as the primary sites for soil organic matter (SOM) storage, occupy a central position in the soil carbon cycle (Ju et al., 2023).

Microbial activity predominantly governs the decomposition of SOM and is highly sensitive to temperature and substrate availability (Chen et al., 2019; Song et al., 2023). In the short-term, responses of SOM decomposition to warming may stem from direct temperature effects on microbial physiology and labile substrates (Song et al., 2023). However, over the long-term, responses may be influenced by microbial thermal adaptation and protection of SOM by soil aggregates (Six et al., 2002; Min et al., 2019; Yu et al., 2022). Yet, the underlying mechanism of soil organic carbon mineralization responses to climate warming remains unclear, since the interaction between soil respiration and the physical protection offered by soil aggregates is still unexplored. Further research in this domain is imperative to advance our comprehension of how soil ecosystems might respond to climate change.

Soil respiration rate is expected to increase due to global warming, triggering a positive feedback loop between higher temperature and soil carbon emission (Crowther et al., 2016). This feedback mechanism depends significantly on the temperature sensitivity (Q_{10}) of soil respiration (Friedlingstein et al., 2006), which gauges the relative proportional change in soil respiration rate for every 10°C rise in temperature. The carbon-quality temperature (CQT) hypothesis posits that the Q_{10} value will rise as the activation energy (Ea) of SOM increases. Consequently, factors such as the molecular weight and complexity of the substrate's molecular structure, along with the stability of chemical bonds, impact the Q_{10} of soil respiration (Davidson and Janssens, 2006a). For instance, Liu et al. (2021) explored Q_{10} during decomposition of organic matter under conditions of low substrate availability, providing empirical evidence in support of the CQT hypothesis. Li et al. (2017) provided further support in their study on the Q_{10} of organic matter decomposition in managed ecosystems. Nonetheless, the CQT hypothesis has yet to attain consensus in the soil research arena, as some studies have indicated that Q_{10} is unrelated to the quality of soil organic carbon (Pang et al., 2015). These conflicting findings may arise from oversight of the protection of soil aggregates as a potential factor influencing SOM dynamics, given that soil respiration is influenced by both the soil microenvironment and the availability of SOM. Hence, assessing the applicability of the CQT hypothesis at the aggregate scale and comprehending the Q_{10} of soil respiration are essential for predicting alterations in the pattern and scale of soil organic carbon storage in ecosystems.

Microbial carbon use efficiency (CUE) is a crucial indicator that balances microbial catabolism and anabolism. Various biotic factors (e.g., extracellular enzyme activity, microbial composition, and diversity) and abiotic factors (including climate change, soil properties, and substrate quality) have been shown to impact microbial CUE to varying extents (Manzoni et al., 2012; Frey et al., 2013; McGee et al., 2019; Cui et al., 2022). Among these, the presence of carbon (C) and nitrogen (N) in the soil significantly influences the growth and respiration of microorganisms, governing their CUE, as a balanced C:N ratio is necessary for their cellular functions (Ma et al., 2023). The continuous influx of external labile carbon into the soil through root exudates or litter alters soil stoichiometry, subsequently impacting microbial CUE. When microorganisms utilize high C:N ratio substrates, overflow respiration due to nutrient limitation results in excessive carbon loss and decreased carbon uptake by microbial decomposers (Manzoni et al., 2012; Qiu et al., 2023). Recent observations suggest that microbial CUE tends to be initially high and then decreases during residue decomposition (Fang et al., 2019). Despite ongoing progress in investigating the mechanisms of microbial CUE, the specific effects of EOM inputs on CUE at the soil aggregate scale remain elusive. Acquiring such insights is essential for a deeper understanding of how changes in aggregate structure influence soil biological functions, particularly carbon metabolism.

Soil aggregates constitute the material foundation of soil structure, which is an important factor in fertility (Ju et al., 2023). The interaction between SOM and soil aggregate structure determines the quantity and quality of the soil organic carbon pool. An increase in the amount of soil organic carbon will inevitably reduce the concentration of CO_2 in the atmosphere, and will promote the formation of soil aggregates and improve their stability. Conversely, soil aggregates also have a critical role in regulating the content of soil organic carbon (Liu et al., 2022). Microaggregates, held together by microbial-derived humic substances and clay particles, primarily rely on chemical protection to preserve organic carbon. On the other hand, macroaggregates mainly depend on physical protection (Six et al., 2000). The varying sizes of soil aggregates, combined with varying mineral content, result in significant variability in the response of soil organic carbon to temperature. These differences substantially influence the decomposition of soil organic carbon, and consequently impact the capacity for soil carbon sequestration (Six et al., 2000; Six et al., 2002). Prior studies have revealed substantial uncertainty in soil respiration and Q_{10} at the aggregate scale (Ghosh et al., 2016; Li et al., 2017; Bhattacharyya et al., 2019; Liu et al., 2022). These conflicting findings can be attributed to the combined influence of physicochemical properties of aggregates, microbial activities, quality of SOM, soil moisture, and substrate availability (Davidson et al., 2006b; Wei et al., 2016; Fu et al., 2023). Considering the inevitable input of EOM into the soil in the form of residues, it remains to be determined how the temperature sensitivity of soil aggregates

will change during this process.

The Tibetan Plateau is one of the regions most dramatically affected by global warming. For a long time, the region has been exposed by low temperature and soil moisture, which has led to the severe inhibition of the soil biological activities and decomposition enzyme systems. As a result, the SOM in this region decomposes slowly and accumulates in large quantities, with soil carbon density as high as 7.2 kg m^{-2}, underscoring its substantial potential for carbon release. In the face of climate warming, the low-temperature limiting effect of soil ecological processes will be weakened or eliminated, thereby affecting the soil carbon cycling in the regional ecosystem (Wang et al., 2020; Liu et al., 2023). Against the backdrop of global warming, several studies have probed the impact of temperature on the decomposition rate of the soil carbon pool in this region (Ding et al., 2016; Li et al., 2019; Chen et al., 2020). For instance, Ding et al. (2016) examined Q_{10} patterns and determinants across a wide geographic range by incubating surface soils (0−10 cm) from 156 sites in the Tibetan alpine steppe. They proposed that both carbon quality and environmental variables regulate Q_{10} in alpine ecosystems. However, the effects of unstable carbon inputs and aggregate size on Q_{10} were not taken into account. Additionally, the relationship between microbial CUE and EOM inputs in high-altitude regions remains unclear.

In this study, we hypothesize that Q_{10} and microbial CUE are influenced by aggregate size, with smaller aggregates displaying larger Q_{10} and higher CUE. To test this hypothesis, we employed corn straw as an analog for EOM input and conducted a 180-day laboratory incubation experiment using aggregates of varying sizes within a temperature range of 5°C to 25°C. This approach enabled us to examine soil respiration temperature sensitivity and CUE at the soil aggregate scale. Our objectives were as follows: 1) to examine the influence of unstable substrate inputs on Q_{10} at different aggregate sizes; 2) to observe the temporal variation of the effects of unstable substrates on Q_{10} and microbial CUE; 3) to understand how microbial CUE responds to EOM input at the aggregate scale in alpine ecosystems.

2 Materials and methods

2.1 Soil sampling and aggregate separation

The soil samples were collected from typical farmland soils on the Qinghai-Tibet Plateau where highland barley (*Hordeum vulgare* Linn. var. nudum Hook. F) has been grown for 20 consecutive years. The region is located at E: 96°29′ and N: 30°53′, with an altitude of 4 180 m, and the soil type is classified as Cambosols according to the US Soil Taxonomy. The region experiences a plateau temperate semi-arid climate, with an average annual precipitation of 423.7 mm, an average annual evaporation of 2026 mm, an average annual temperature of 5.5°C, a maximum temperature of 30.6°C, and a minimum temperature of −22.1°C. The topsoil, 0−20 cm deep, was excavated using a shovel, and the collected soil samples were promptly transported to the laboratory. After removing visible plant residues, the soil samples were dried and subjected to sieving for soil aggregates.

In this study, we employed the dry sieving method to categorize the soil into three aggregate size fractions: macroaggregates (> 2 mm, Ma), mesoaggregates (0.25−2 mm, Me), and microaggregates (< 0.25 mm, Mi). The air-dried soil samples were placed on a double-layer sieve with two sets of screens having apertures of 2 mm and 0.25 mm, respectively. A collection pan was positioned at the bottom, and the samples were manually shaken for 10 min. Soil aggregate fractions retained on the screens were collected: Ma from the 2 mm screen, Me from the 0.25 mm screen, and Mi from the collection pan at the bottom. The weight of each aggregate size fraction was measured to determine the soil aggregate composition.

2.2 Soil incubation and index analysis

This study involved three input (independent) factors: soil aggregate size, incubation temperature, and the presence of EOM. Soil aggregate size had three levels: Ma, Me, and Mi. Incubation temperatures were set at three gradients: 5°C, 15°C, and 25°C. EOM presence had two levels: addition and non-addition of corn straw. A complete $3 \times 3 \times 2$ factorial design resulted in 18 treatment combinations (Fig. 1).

To prepare for incubation, 100 g (dry weight) of sieved soil aggregates were placed into 500 mL jars, and the soil moisture was adjusted to 60% of the field water holding capacity. A pre-incubation process was carried out at 25°C for one week. Subsequently, for the treatment with EOM, 1 g of corn straw (total C: 35.88%; total N: 1.06%; C:N = 34.02) was added as 1% of the soil weight, and the straw was thoroughly mixed with the soil aggregates. Small beakers containing 20 mL of 1 M NaOH solution were placed inside the jars to capture CO_2. Once the jars were sealed, the formal incubation began at three temperatures: 5°C, 15°C, and 25°C. The NaOH solution was replaced at the intervals of 1, 3, 7, 15, 30, 60, 120, and 180 days after the start of incubation. To ensure sufficient oxygen levels, the jars were opened for 30 min after each NaOH solution replacement. Additionally, three control jars containing only NaOH solution were used as blanks to calculate the amount of captured CO_2 in the air inside the jars. For soil respiration determination, 20 mL of 1 M $BaCl_2$ was added to the collected NaOH solution, and the captured CO_2 was measured by titration with 0.1 M HCl.

The soil's basic physicochemical properties, both for the

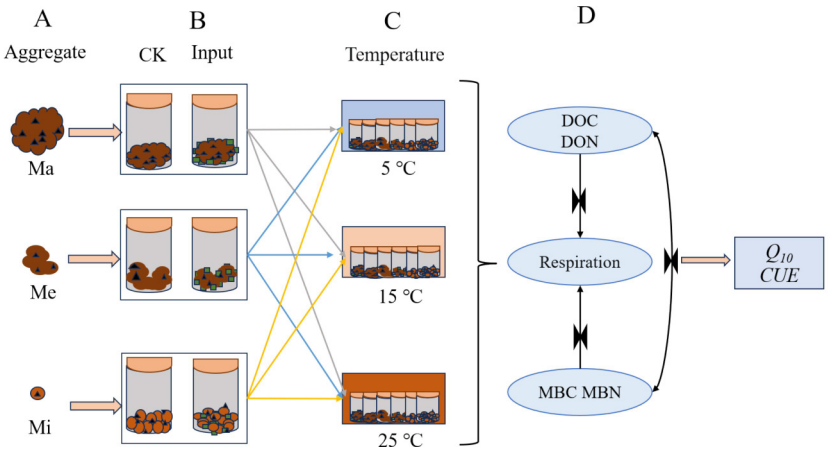


Fig. 1 Research ideas and treatments. This study encompassed three factors: soil aggregate size, incubation temperature, and the presence of exogenous organic matter. Soil aggregate size had three levels: Macroaggregates (Ma), Mesoaggregates (Me), and Microaggregates (Mi). Incubation temperatures were set at three gradients: 5°C, 15°C, and 25°C. Exogenous organic matter presence had two levels: CK and exogenous organic matter input (Input).

unsieved soil and the sieved aggregates, were determined using standard analytical methods. The soil pH was measured by analyzing a soil-water extract at a ratio of 1:2.5 using a pH meter. SOM content was quantified through the potassium dichromate oxidation method. Soil total carbon (TC) and total nitrogen (TN) contents were measured using an elemental analyzer (Elementar Vario Max, Germany), and the C/N ratio was subsequently calculated. Microbial biomass carbon (MBC) was determined after fumigating the soil with chloroform and extracting it with 0.5 M K_2SO_4. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined using a total organic C and N meter, with a soil-to-water ratio of 5:1.

2.3 Data calculation and statistical analysis

To describe how soil respiration is related to temperature, we calculated Q_{10} for control and EOM addition treatments using the following equations:

$$R = A \times e^{kT}$$

$$Q_{10} = e^{10k}$$

where R is soil microbial respiration rate (mg CO_2 kg^{-1} soil h^{-1}) at a given temperature T (°C); A and k are exponential fitting parameters. Parameter A represents the basal soil respiration rate at 0°C and is used as the overall Substrate Quality Index (SQI) available to microorganisms at a specific point in time. Parameter k represents the temperature response coefficient (Fierer et al., 2005; Ding et al., 2016).

ΔQ_{10}, defined as the difference between $Q_{10input}$ under EOM input and Q_{10CK} (control check) under control conditions, was used to characterize the effect of EOM on Q_{10}:

$$\Delta Q_{10} = Q_{10input} - Q_{10CK}$$

CUE was determined as follows (Maynard et al., 2017; Wang et al., 2020.):

$$CUE = \frac{B}{B + CR}$$

where B is the content of biomass C (μg C g^{-1} soil); CR is the content of respiration C (μg C g^{-1} soil). The value of CUE ranges from 0 to 1.

We conducted one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$ to compare the effects of EOM input levels and aggregate sizes on soil respiration, Q_{10}, and microbial CUE. Two-way or three-way ANOVA was used to examine significant differences between EOM addition, soil aggregates, temperature, and their interactive effects on soil respiration, CUE, and Q_{10}. Prior to analysis, we assessed the normality and homogeneity of variance of the data. To determine the relative importance of soil properties and carbon quality index in relation to Q_{10} and CUE, we performed regression Random Forest analysis. The relationship between Q_{10} and SQI was explored using regression analysis. Curve fitting was carried out using Origin 2020, and statistical analysis was conducted using SPSS 19.0.

3 Results

3.1 Soil aggregate-associated respiration (R)

This study conducted a 180-day incubation experiment using soil aggregates of varying sizes, considering the addition of EOM (with addition and without addition) and different temperatures (5°C, 15°C, and 25°C). The dynamics of organic carbon mineralization and cumulative mineralization for each treatment are shown in Fig. 2. All treatments exhibited an ‘L-shaped’ trend on the time scale, characterized by a rapid decline in respiration rates during the initial stage of incubation, followed by a relatively stable respiration rate after 3 weeks. Throughout the incubation period, the addition of EOM and elevated temperatures significantly enhanced

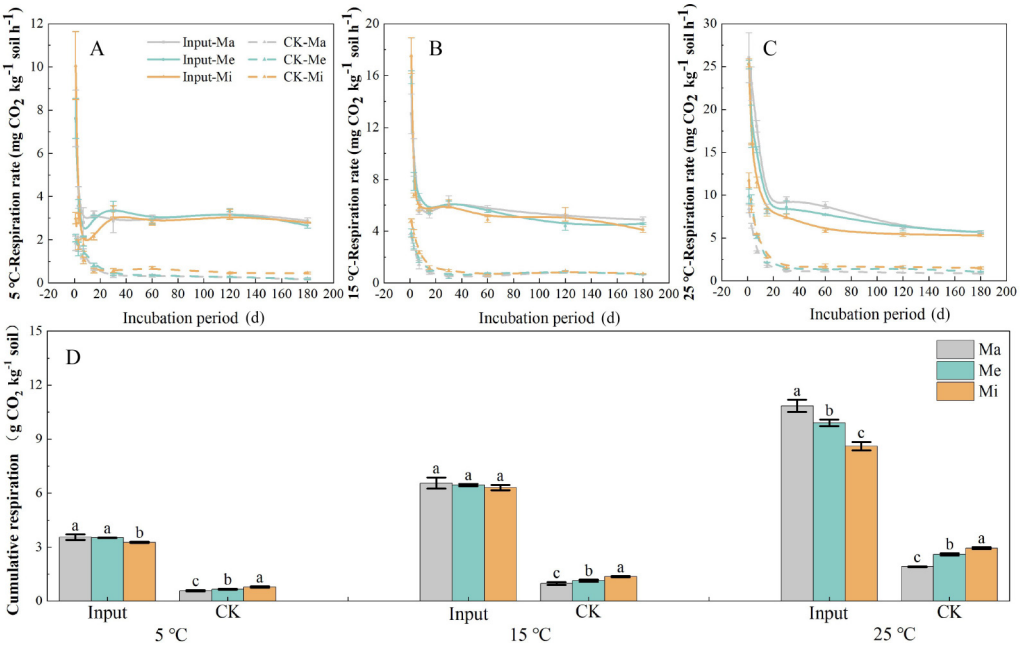


Fig. 2 Effects of exogenous organic matter input on soil aggregate respiration. The data is presented as Mean ± SE (standard error) with n=3 replicates. Error bars represent the standard deviations of the mean. Different letters indicate significant differences in cumulative respiration among soil aggregates ($P \leqslant 0.05$). Input refers to exogenous organic matter input, while CK represents the control group. Soil aggregates are categorized into macroaggregates (Ma), mesoaggregates (Me), and microaggregates (Mi).

soil respiration rates. EOM, temperature, aggregate size class and their interactions significantly affected cumulative respiration (See Supplementary Table S1). Concerning cumulative mineralization, in the control group without EOM input, the cumulative organic carbon respiration sequence was R_{Mi} > R_{Me} > R_{Ma} for the various particle-sized soil aggregates, and all differences between treatments were statistically significant. However, with the input of EOM, the cumulative respiration among aggregates showed the sequence R_{Mi} < R_{Me} < R_{Ma}. This implies that the EOM input altered the carbon source in soil aggregate respiration, consequently influencing the respiration rates of soil aggregates.

3.2 Soil aggregate-associated respiration temperature sensitivity (Q_{10})

EOM and aggregate size class had significant effects on Q_{10}, but their interaction had no significant effect on Q_{10} (See Supplementary Table S2). Figure 3 illustrates the influence of EOM input on the Q_{10} of soil respiration at the soil aggregate scale. As in the case of the patterns observed for soil respiration rates, the Q_{10} values for all treatments displayed an 'L-shaped' pattern throughout the 180-day incubation period. During the initial phase of the incubation experiment (0−15 days), there was a noticeable decline in Q_{10} values. Moreover, the treatment group that received EOM input exhibited significantly higher Q_{10} values than the control group. However, as the incubation period progressed, the input of EOM led to a notable reduction in the Q_{10} of soil respiration. This reduction was evident not only in the overall Q_{10} values, but also in the transition of the ΔQ_{10} (difference between Q_{10} with EOM input and Q_{10} in the control) from positive to negative values throughout the incubation process. When examining various particle-sized soil aggregates under conditions without EOM addition, the average Q_{10} values for different aggregate sizes followed the order Mi > Me > Ma. However, with the introduction of EOM, the distinctions in average Q_{10} values among Ma, Me and Mi were not statistically significant. This observation suggests that the input of EOM mitigated the differences in Q_{10} of soil aggregates respiration.

Furthermore, we conducted an analysis to explore the relationship between the Q_{10} of soil respiration and the SQI, as depicted in Fig. 4. Notably, we observed significant differences between the treatment groups with and without EOM input. In the control group without EOM addition, Q_{10} exhibited a pronounced negative correlation with SQI during both the early stage (0−15 days) and the later stage (15−180 days) of incubation. The correlation coefficients (R^2) were 0.51 and 0.42 for the early stage and the later stage of incubation, respectively, and both were statistically significant (P < 0.05). Conversely, for the treatment group with EOM input, this negative correlation between Q_{10} and SQI was evident only during the later stage of incubation (R^2 = 0.17, P < 0.05), and no significant correlation was observed during the

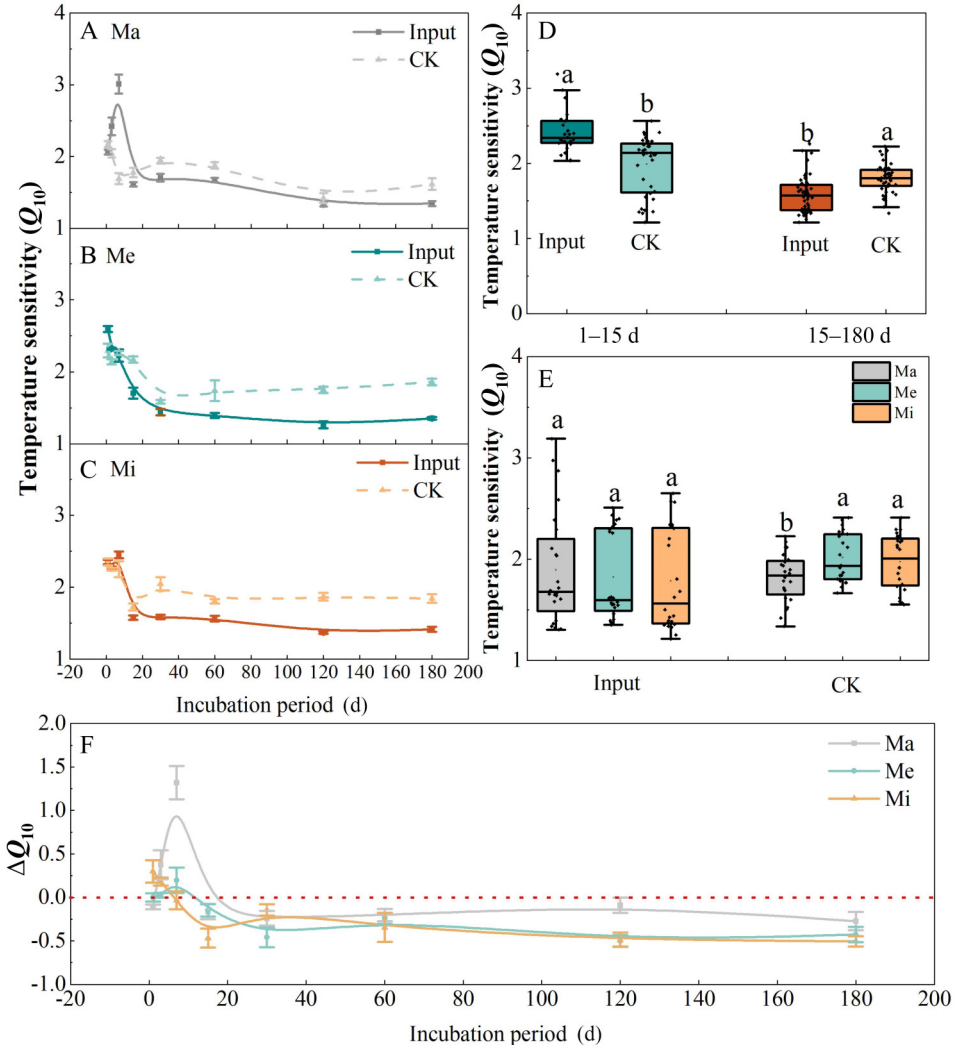


Fig. 3 Impact of exogenous organic matter input and aggregate sizes on Q_{10} and ΔQ_{10}. This figure presents the changes in Q_{10} at different time scales (A–C), the effect of exogenous organic matter input (D), variations across different soil aggregates (E), and the difference in ΔQ_{10} (F). Different letters denote significant differences in cumulative respiration among soil aggregates ($P \leqslant 0.05$). Input signifies exogenous organic matter input, and CK represents the control group. Soil aggregates are categorized into macroaggregates (Ma), mesoaggregates (Me), and microaggregates (Mi).

initial phase. These findings underscore the observation that the input of EOM modified the relationship between Q_{10} and SQI, particularly in the early phases of EOM input.

3.3 Soil aggregate-associated microbial CUE

Soil microorganisms play a central role in the carbon cycling process within soils, with their CUE serving as a crucial indicator of soil carbon sequestration. The magnitude of microbial CUE is significantly influenced by various factors, including soil aggregate size, EOM input, incubation temperature, and their complex interactions (See Supplementary Table S3). Here we showed that the microbial CUE tends to decrease as temperature rises, and these differences across the temperature treatments (5°C, 15°C, and 25°C) are statistically significant. Notably, in this study, the introduction of EOM led to a substantial reduction in CUE. Specifically, in comparison to the control group, the microbial CUE in the organic matter input group experienced a marked decline of approximately 40% (Fig. 5). Moreover, over different stages of incubation, microbial CUE displayed discernible variations, gradually diminishing as the incubation period extended. When considering distinct particle-sized aggregates, regardless of the presence of added organic matter and the variations in incubation temperatures, the microbial CUE exhibited a consistent pattern: Mi > Me > Ma. These differences in microbial CUE among particle sizes were statistically significant. In summation, the decrease in microbial CUE attributed to EOM input, alongside the disparities in CUE observed among diverse soil aggregate sizes, underscore the intricate and essential role of microorganisms in governing soil carbon cycling dynamics.

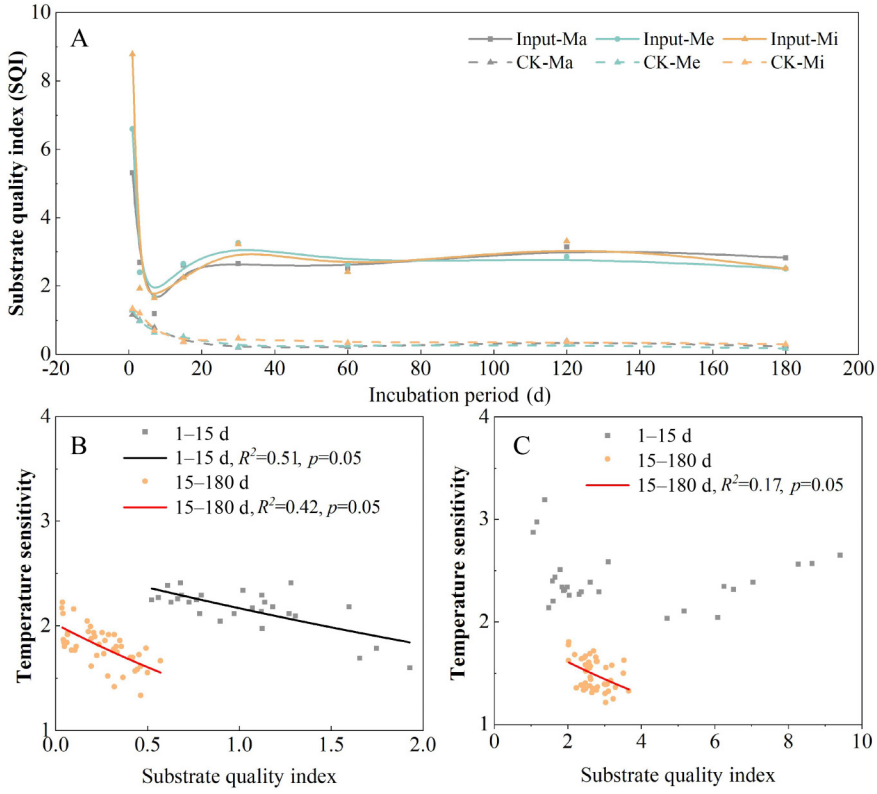


Fig. 4 Dynamics of SQI over the 180-d incubation period (A), the relationship between carbon quality index (SQI) and Q_{10} in the control (B), and exogenous organic matter input (C) treatments.

3.4 Dominant factors regulating Q_{10} and Microbial CUE

In this study, a Random Forest model was employed to dissect the primary drivers underpinning the regulation of both Q_{10} and microbial CUE. The insights gleaned from this model offer a comprehensive understanding of the main determinants of these critical aspects of soil carbon cycling. The results of the Random Forest analysis explained 40.6% of the variation in Q_{10}. The outcomes of the analysis indicated that EOM input and SQI were the most prominent controllers of Q_{10} (Fig. 6A). Moreover, other variables such as SOM, C:N, TN, and macroaggregates also exerted significant influences on regulating Q_{10}. Turning to the determinants influencing Microbial CUE, it was observed that all the factors considered in this study held notable regulatory effects. This collective influence underscores the conclusion that microbial CUE is a consequence of intricate multifactorial regulation (Fig. 6B).

4 Discussion

4.1 Effects of soil aggregate sizes on soil respiration

The size of soil aggregates plays a crucial role in influencing the composition of microbial communities, pore structure, and organic matter content within these aggregates, ultimately leading to changes in aggregate respiration rates (Six et al., 2000; Luo et al., 2018). The results of this study indicate that under conditions without the addition of EOM, the respiration rates of soil aggregates decrease with increasing particle size. The physical barrier effect of soil aggregates controls the entire process of soil respiration, especially in Ma (Ju et al., 2023). The organic matter enclosed within Ma is less accessible to highly active external microorganisms (Six et al., 2000). However, as aggregate particle size decreases, the specific surface area increases (Zhu et al., 2016). This exposes more organic matter and microorganisms to the external environment during incubation. As a result, smaller-sized aggregates exhibited higher respiration rates in this study.

However, EOM inputs altered the original respiration pattern at the soil aggregate scale, resulting in greater respiration rates and cumulative respiration for Ma than for Mi (Fig. 2). This might be linked to the association between EOM input and aggregate state. In this study, EOM often exists as free particles in larger pores between Ma. However, Mi and EOM input gradually formed larger aggregates, leading to the encapsulation of EOM within soil aggregates and reducing its accessibility (Six et al., 2000). These observations indicated that the impact of EOM input on respiration was more pronounced in larger aggregates.

Consistent with previous research, this study found that

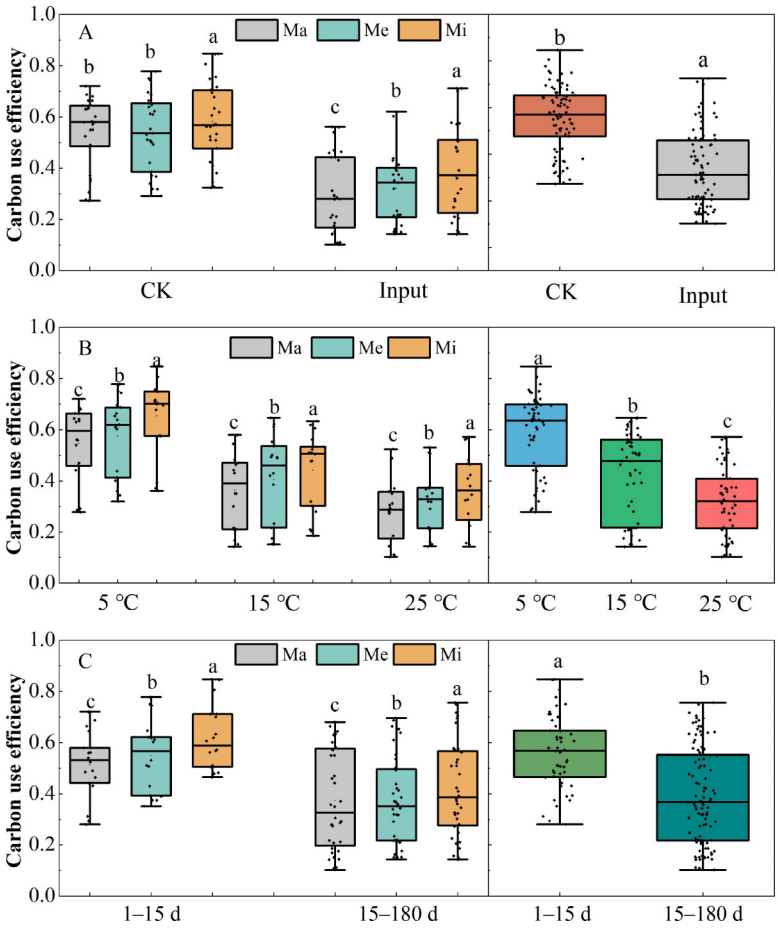


Fig. 5 Effects of exogenous organic matter input (A), incubation temperature (B), and incubation time (C) on microbial carbon use efficiency (CUE) at the soil aggregate scale. Different letters denote significant differences in cumulative respiration among soil aggregates ($P \leqslant 0.05$). Input signifies exogenous organic matter input, and CK represents the control group. Soil aggregates are categorized into macroaggregates (Ma), mesoaggregates (Me), and microaggregates (Mi).

EOM input and elevated temperature significantly stimulated the respiration rates for the various particle-sized aggregates (Liu et al., 2022). This phenomenon has previously been attributed to the increase in respiratory substrates and activation of microbial vitality (Panettieri et al., 2020). EOM input not only introduces a substantial carbon source, providing ample energy for soil microorganisms, but may also induce a priming effect, intensifying soil respiration and CO_2 release (Cui et al., 2022). Elevated temperature increases the rate of microbial reproduction and metabolism, producing more enzymes to participate in the carbon and nitrogen cycle, thereby increasing the respiration rate and cumulative mineralization of soil aggregates.

4.2 Effects of EOM input on soil respiration temperature sensitivity at the soil aggregate scale

In the early stage of incubation (1−15 days), the introduction of EOM led to an increase in Q_{10} (Fig. 3A−D), aligning with previous research findings (Liu et al., 2021). Drawing on the Michaelis-Menten kinetic interpretation, the infusion of EOM into the soil elevates the presence of available substrates, particularly those that are easily decomposable, thus augmenting the saturation concentration (C > Km). Consequently, the influence of Km on the Q_{10} value becomes negligible (Davidson et al., 2006b). Furthermore, microorganisms react to higher or lower temperatures by engaging a series of adaptation mechanisms, such as altering lipid composition, synthesizing new proteins, and reallocating resources for growth mechanisms or survival strategies according to circumstances. This adaptive process ultimately modulates the rate of substrate decomposition following microbial respiration adjustments to temperature changes, leading to the observed decline in the Q_{10} value (Min et al., 2019).

Drawing from previous research (Li et al., 2017), the proportional representation of unstable and refractory carbon pools experiences shifts throughout the incubation process. Upon the introduction of EOM, its decomposition progressively replenishes the depleted unstable carbon pool

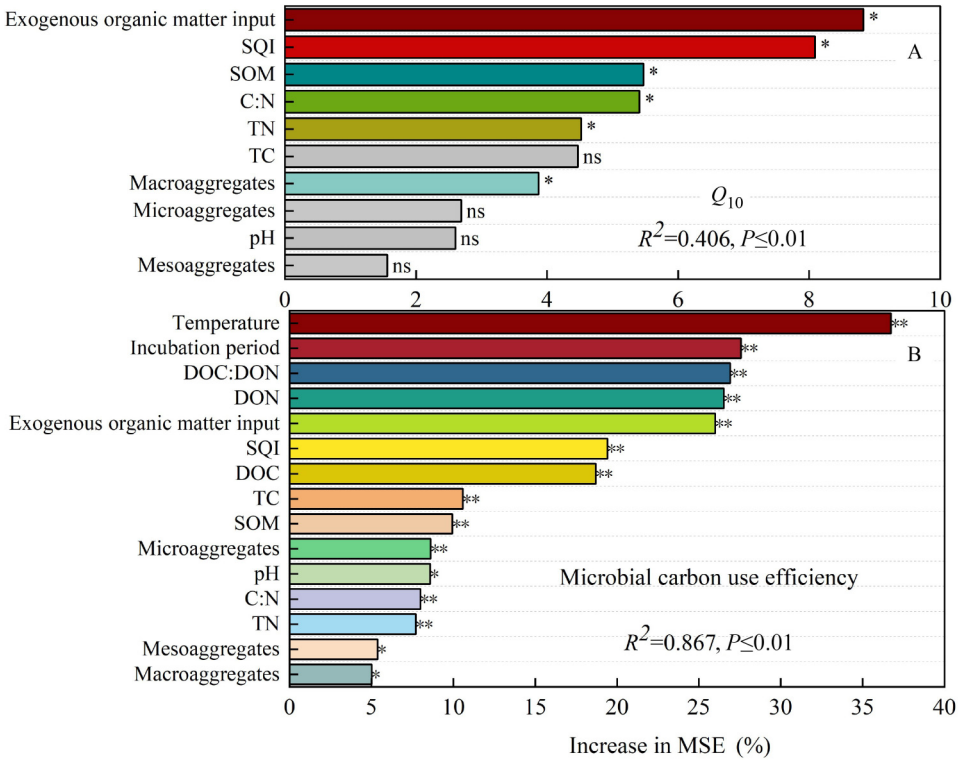


Fig. 6 Importance of key factors influencing Q_{10} (A) and microbial carbon use efficiency (CUE) (B) based on random forests model analysis. SOM: soil organic matter; TC: total carbon; TN: total nitrogen; DOC: dissolved organic carbon; DON: dissolved organic nitrogen; SQI: carbon quality index; C:N: ratio of carbon to nitrogen. * represents P < 0.05; ** represents P < 0.01; ns represents not significant.

(Li et al., 2021). However, within the control group, the absence of EOM prevents the replenishment of the unstable carbon pool, resulting in its depletion over extended incubation durations. Consequently, the refractory carbon pool assumes prominence as the principal contributor to soil respiration during the later incubation period. In line with the CQT hypothesis, the Q_{10} value is anticipated to be higher in the control group period in comparison to the treatment groups benefiting from EOM input (Lin et al., 2022).

In addition to individual treatment effects, there was a noticeable decrease in the Q_{10} value as aggregate size increased (Fig. 3E). This trend aligns with observations made by Bhattacharyya et al. (2019) and Ghosh et al. (2017). As proposed by Qin et al. (2019), the physical protection of SOM offered by aggregates could impede the temperature-driven enhancement of soil respiration. This observed pattern may be attributed to variations in microbial communities across different aggregate sizes (Lu et al., 2021). Nevertheless, the validity of this speculation requires further substantiation through more comprehensive investigations in the future.

In this study, we demonstrated that the Q_{10} value exhibited a decrease with an elevation in the soil SQI, underscoring the applicability of the CQT hypothesis. However, this relationship was only evident in the later phases of aggregate incubation. This could be attributed to the initial addition of organic matter, which provided ample readily decomposable substrates to support microorganisms. As a result, the influence of substrate mass on the Q_{10} value might have been concealed due to the availability of unstable substrates (Davidson et al., 2012).

4.3 Effects of EOM input on microbial CUE at the soil aggregate scale

The addition of EOM modifies the substrate landscape and microbial community dynamics, leading to reduced CUE (Fang et al., 2019). This phenomenon is shaped by nutrient limitation effects, the availability of easily decomposable carbon sources, and pH-induced microbial community changes (Liu et al., 2021a). Specifically, the incorporation of a substantial quantity of high carbon-to-nitrogen (C:N) maize straw elevated the soil's C:N ratio. The onset of nutrient limitation, often evidenced by elevated C:N or C:P ratios, prompts the microbial community to prioritize resource acquisition traits at the expense of growth yield. This trade-off mechanism results in a reduction in CUE (Öquist et al., 2016; Cui et al., 2022; Chen et al., 2023). During the early incubation stage, a nutritionally balanced soil microbial community finds it easier to access precursor molecules, so

that utilization of carbon is effective (Malik et al., 2019). Nonetheless, as the incubation period extends, easily decomposable carbon sources are gradually exhausted, leaving behind complex molecular substances that are challenging for microorganisms to absorb through the microbial biofilm. Consequently, the breakdown of these substantial molecular substances must be degraded by extracellular enzymes. This enzymatic degradation process demands substantial energy expenditure from microorganisms, to identify and break down the substrates, ultimately contributing to diminished CUE during later stages (Fang et al., 2019). Furthermore, the decomposition of EOM in the soil can moderately influence the soil pH (Silva-Sánchez et al., 2019), thereby altering the dominance between bacterial and fungal populations and subsequently affecting microbial growth and respiration dynamics. These changes culminate in notable shifts in microbial CUE (Jones et al., 2019).

As mentioned earlier, there was a decrease in soil microbial CUE with increasing temperature across all soil aggregate levels (Fig. 5B). The adaptive capacity of soil microbial communities allows them to fine-tune their carbon uptake rates and allocation efficiencies within the constraints of a specific finite respiration rate (Liu et al., 2021a). This observation aligns with the negative effects of elevated temperatures on energy maintenance observed in experiments focusing on heterotrophic soil microbes (Frey et al., 2013; Crowther and Bradford, 2013). Given maize straw's intricate composition, preliminary extracellular enzyme-driven decomposition is necessary before it becomes accessible to the microbial community. Consequently, as temperatures rise, it is anticipated that respiration linked to enzyme production and secretion will intensify (Yu et al., 2022). Consequently, whether in field or laboratory settings, soil warming often entails a higher energy expenditure to sustain microbial biomass and results in energy surplus (metabolic waste), thereby constraining overall microbial metabolic activity (Frey et al., 2013; Bradford, 2013).

Furthermore, our study revealed varying sensitivities of microbial CUE to EOM additions across different aggregates, with smaller aggregates exhibiting more elevated microbial CUE than was the case for larger aggregates (Fig. 5A−C). This difference could potentially be attributed to inherent variability and differences in biological activity among the distinct aggregate sizes (Liu et al., 2021a; Ju et al., 2023). When compared to Ma, the smaller-sized aggregates boast a larger specific surface area, facilitating more extensive interaction between soil aggregates and EOM (Zhu et al., 2016). This enhanced contact promotes microbial nutrient uptake, consequently amplifying CUE (Crowther and Bradford, 2013). Additionally, the heightened presence of active organic carbon substrates in smaller aggregates further contributes to their elevated microbial CUE.

5 Conclusion

This study focused on the impact of EOM addition, soil aggregate size, and incubation temperature on soil respiration and microbial CUE. The results indicated that EOM input elevated respiration rates and cumulative respiration across aggregates, altering the respiration rate patterns for the different aggregate sizes. Early-stage incubation showed heightened Q_{10} due to EOM, which later decreased. Microbial CUE exhibited negative correlations with EOM input, incubation factors, and aggregate size. The relationship between Q_{10} and the SQI supports the CQT hypothesis, highlighting SQI's influence on Q_{10} values, particularly during later incubation stages. Caution in using this model to predict soil carbon dynamics is advised due to variations. The significance of accounting for EOM in soil carbon cycles and considering different aggregate sizes in modeling is emphasized.

Declaration of competing interest

The authors report no declarations of competing interest.

Data availability

Data will be made available on request.

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Electronic supplementary material

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