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Running title: SOM decomposition by earthworms

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Abstract

How and what soil fauna influence the soil organic matter (SOM) decomposition rate (*R*s) and its temperature sensitivity (Q_{10}) have been largely ignored, although this is a crucial matter, especially under the scenario of global change. In this study, a novel approach was adopted with a continuous changing-temperature incubation (daytime, from 7 ℃ to 22 ℃; nighttime, from 22 ℃ to 7 ℃) with rapid and continuous measurement, to examine the effect of soil macrofauna (specifically, earthworms) on *R*s and Q_{10} with three densities (no addition, low density, and high density). According to the results, the earthworms accelerated *R*s. Furthermore, *R*s with earthworm addition had a symmetrical pattern during daytime and nighttime cycles, which is contrary to traditional soil incubation, with only soil microbe as asymmetrical. More importantly, earthworm addition increased Q_{10} markedly, ranging from 48% to 67%. Overall, the findings highlight the pivotal role of earthworms as soil macrofauna that regulating soil carbon release, and their effects should be integrated into process-based ecological models in future.

Keywords: Soil macrofauna; Earthworms; Soil organic matter; Asymmetrical decomposition; Temperature sensitivity

1. Introduction

Soil organisms (including soil fauna and microorganisms), ubiquitous and teeming on Earth, play vital and distinct roles in the process of soil carbon (C) release (Paul and Frey, 2023). As global average temperatures rise steadily, the abundance of these soil dwellers increases and their range expands, thereby exerting a profound influence on soil C dynamics (Phillips et al., 2019). Yet, previous models simulating soil organic matter (SOM) decomposition have largely overlooked the crucial contributions of soil fauna.

Soil fauna exhibit remarkable diversity, with earthworms, celebrated by Darwin as "nature's ploughs," being particularly prominent (Briones and Schmidt, 2017). Earthworms constitute over 60% of the total soil fauna biomass (Jin et al., 2009), with their role in SOM decomposition being particularly critical (Darwin, 1892; Edwards and Bohlen, 1996). Earthworms primarily influence the stability of SOM within soil aggregates through feeding, digestion, and burrowing activities, thereby affecting SOM decomposition. The earthworm addition likely leads to a considerable increase Q_{10}, however, the incremental increase in Q_{10} may not scale with rising earthworm density (Fig. 1; Paul and Frey, 2023). Nevertheless, the extent to which earthworm abundance affects such decomposition processes and how earthworms respond to continuous temperature variations, remain inadequately explored.

The temperature sensitivity of SOM decomposition, defined as Q_{10}, refers to the increase in CO_2 efflux rates in proportion to a 10 °C temperature rise (Davidson and Janssens, 2006). Traditionally, Q_{10} has been considered an intrinsic property that is not influenced by environmental conditions (Davidson and Janssens, 2006). However, recent research has suggested that Q_{10} is influenced by various factors, including environmental conditions (such as diurnal temperature variation), substrate availability, and soil biota (principally microorganisms) (Li et al., 2021; Zhang et al., 2024). Furthermore, regarding the impact of diurnal temperature variation, previous studies have confirmed that, in conditions where only microorganisms are present, the Q_{10} during the nighttime cooling phase is greater than that during the daytime warming phase (Li et al., 2017). We propose that the factors influencing Q_{10} extend beyond environmental variables, with biological factors playing a crucial role as well, particularly the often-overlooked contributions of soil macrofauna. Here, we posed the following scientific questions: (1) How does the presence of soil macrofauna (earthworm) affect SOM decomposition and Q_{10}? (2) Is the Q_{10} of earthworm-mediated SOM decomposition influenced by earthworm density?

2. Materials and methods

2.1 Study area description

The study was conducted at the Maor Mountain Forest Ecosystem Research Station, China (45°20′N, 127°30′E). The area has an average elevation of 400 m and an average slope of 10°–15°. The regional soil was classified as dark brown forest soil. The climate is characterized by continental monsoon, warm and humid in the summer, and cold and dry in the winter. The annual mean temperature is 3.1 °C, with the coldest month being January (average temperature of –18.5 °C), and the warmest month being July (average temperature of 22.0 °C). The area is characterized by vegetation typical of the Changbai Mountain ecosystem, with forests dominated by Korean pine (*Pinus koraiensis*) as the key representative vegetation type.

2.2 Soil sampling

Soil samples were collected in June 2023 from a typical *P. koraiensis* forest. Four 30 m × 30 m plots were randomly selected, with plots spaced approximately 100 m apart (Liu et al., 2023). Within each plot, five sampling sites were randomly selected to collect soil samples, which were then thoroughly mixed to form a single composite sample for each plot. Before collecting the samples, the surface litter was removed. Soil samples were obtained at depths of 0–20 cm. The samples were then sieved through a 2-mm mesh to remove roots and other visible debris. The composite soil samples from each plot placed in labeled polyethylene bags and immediately stored in portable iceboxes at 4 °C. After all the samples were collected, the soil was promptly transported to the laboratory and stored in a refrigerator at 4 °C for subsequent soil incubation experiments.

2.3 Laboratory incubation and analysis

To simulate diurnal temperature variations, a continuous automatic temperature variation system combined with a high-frequency automated SOM decomposition measurement system was utilized (Fig. S1). In natural environments, there are periodic daytime warming and nighttime cooling cycles. Simulating nighttime SOM decomposition is essential for improving the accuracy of SOM decomposition rate estimates and overcoming the limitations of temperature dependence in traditional incubation experiments (Liu et al., 2017). Based on field temperature variations and the temperature adaptation of earthworms, a temperature gradient was established to simulate the processes of daytime warming (from 7 to 22 °C) and nighttime cooling (from 22 to 7 °C) (He and Yu, 2016).

2.4 Soil incubation experiment

In this study, *Eisenia fetida*, a typical earthworm species found in the forest surface layers of northeastern China, was selected as the model soil fauna. Incubation experiments were divided into three stages. In the first stage (earthworm breeding), the earthworms were acclimated in the laboratory at 15 °C for seven days to ensure healthy growth. During the first stage, SOM (animal manure) was added to the soil to ensure adequate food supply for the earthworms (Liao et al., 2024). Distilled water was added daily to maintain suitable soil moisture levels for the earthworms. To ensure accurate weight measurements, the earthworms were allowed to empty their gut contents in empty dishes before weighing. Earthworms of similar weight (0.55 ± 0.05 g, Table S1) were selected for the experiment to avoid the impact of earthworm size and weight on SOM decomposition.

In the second stage (pre-incubation soil), the experiment was conducted as follows: 50 g of fresh soil was thoroughly mixed and placed into incubation bottles. Distilled water was added to adjust the soil to 60% of its field water holding capacity to ensure optimal soil microbial activity. During the incubation process, incubation bottles containing soil and earthworms were covered with plastic film to prevent earthworm escape and minimize moisture loss. Ventilation holes were evenly spaced in the film to ensure sufficient oxygen exchange, thereby preventing the development of anaerobic conditions. Earthworms were then added to the incubation bottles at a density of 150 individuals/m², considering the bottle's base diameter, based on a previous study (Lubbers et al., 2017; Phillips et al., 2019). A total of three density gradients were established: no earthworms (CK), low-density earthworms (Low), and high-density earthworms (High), with four replicates for each treatment. After adding the earthworms, all incubation bottles were pre-incubated at 15 °C for seven days to allow the soil biota (earthworms and microorganisms) to stabilize.

In the third stage (SOM decomposition measurement), SOM decomposition rates were measured using a modified automatic temperature control soil flux system (PRI-8800; PRE-ECO, Beijing, China) developed by He et al. (2016). Briefly, four replicates were placed in an electric water bath. The water bath with an automatic temperature regulator (Julabo FP50; Seelbach, Ortenau, Germany) was connected to a CO_2 analyzer (G2131-I; PICARRO Inc., Sunnyvale, California, USA), which recorded the CO_2 concentrations every second. The temperature in the first phase of incubation ranged from 7 to 22 °C (simulation of the daytime warming process, 12 h), and in the second phase, from 22 to 7 °C (simulation of the nighttime cooling process, 12 h). The SOM decomposition rate and soil temperature were measured simultaneously and continuously throughout a 24-hour period at varying temperatures, as per the program. For each sample, the dynamics of SOM decomposition were recorded at 120-second intervals, with each sample measured 39 times over the 24-hour period. During the measurements, soil temperature changes were monitored in real-time on a minute-by-minute scale, using button thermometers (Fig. S2; DS

1922; Maxim Integrated, Dallas, TX, USA). Based on previous research indicating that earthworms are more sensitive to SOM decomposition on a time scale of days, a short-term experiment was designed in this study (Vion-Guibert et al., 2024). The SOM decomposition rates were measured on the first and second day following pre-incubation, to verify the repeatability of the impact of soil fauna on SOM decomposition. Given that earthworms exhibit negative phototaxis and are particularly sensitive to strong light, a 3 m × 3 m black cloth was used to cover the experimental setup, ensuring that the experiment was conducted under low-light conditions. Additionally, the weight of earthworms remained relatively stable throughout the experiment and their survival rate was 100% at the end of the experiment (Table S2).

Calculation of *R*s and *Q*10

The SOM decomposition rate (*R*s) was calculated using the following equation:

$$Rs=\frac{C\times V\times \alpha\times \beta}{m} \quad (1)$$

where *R*s represents the SOM decomposition rate (μg C g^{-1} $soil^{-1}$), *C* is the slope of the change in soil CO_2 concentration over 120-second for each sample, *V* is the volume of the experimental bottle, *α* is the atomic mass of C, *β* is the time conversion factor, and *m* is the dry weight of the soil sample.

The temperature sensitivity of SOM decomposition (Q_{10}) was calculated as follows:

First, an exponential equation was used to describe the relationship between *R*s and temperature:

$$Rs = ae^{bT} \quad (2)$$

Using the temperature sensitivity coefficient (*b*) obtained from the exponential equation, the temperature sensitivity (Q_{10}) was calculated as follows:

$$Q_{10} = e^{10b} \quad (3)$$

where *R*s represents the SOM decomposition rate (μg C g^{-1} $soil^{-1}$), *a* is the fitting parameter of the equation, *b* is the temperature sensitivity coefficient, *T* is the test temperature, and Q_{10} indicates the temperature sensitivity of SOM decomposition.

2.5 Statistical analysis

Statistical analyses were performed and plots were constructed using R v.4.2.1 (R Development Core Team), with significance set at $P < 0.05$. Linear mixed effect models (LMMs) were employed to evaluate the effects of earthworm density on *R*s and Q_{10}, with earthworm density as the fixed effect, and plot and site as the random effect. LMMs were implemented using the *lmer* function from the '*lme4*' package (Bates et al., 2015).

3. Results and Discussion

Earthworm addition significantly accelerated the SOM decomposition rate (*R*s, Table S3, Fig. 2). A comparison revealed that the cumulative *R*s exhibited an increasing trend with rising earthworm density (Fig. 2). This substantial enhancement in *R*s could be attributed to the multifaceted influence of earthworms on the soil environment and C substrate availability. One such influence is the augmentation of soil porosity and microstructural cast through earthworm activity, which improves soil permeability, and facilitates CO_2 release (Le Mer et al., 2022). Earthworms may also fragment soil aggregates through their feeding activities, making the C within these aggregates more readily decomposable and increasing the substrate availability (Paul and Frey, 2023). Furthermore, earthworms influence the priming effect of recalcitrant C in the soil by burrowing to reduce SOM stability, thereby accelerating SOM decomposition (Fox et al., 2006).

Our findings revealed that the rate of cumulative *R*s increase from $R\mathrm{s}\text{-}_{\mathrm{CK}}$ to $R\mathrm{s}\text{-}_{\mathrm{Low}}$ was higher than that from $R\mathrm{s}\text{-}_{\mathrm{Low}}$ to $R\mathrm{s}\text{-}_{\mathrm{High}}$ (Fig. 2A). The discrepancy in increase rates may be attributed to the non-equivalence effect of earthworm density on *R*s (Li et al., 2023). The non-equivalence effect is characterized by an increment in SOM decomposition mediated by earthworms, which does not rise continuously with increasing earthworm density. This also supports previous research suggesting that earthworm density may impact the ecological functions in soil (Paul and Frey, 2023). It is speculated that there is a threshold for the effect of earthworm density on *R*s. This may be because the soil substrate became insufficient as earthworm density increased, thereby reducing SOM decomposition. Consequently, *R*s did not increase proportionally with an increase in earthworm density. In other words, an increase in earthworm density had a non-equivalent effect on SOM decomposition.

Without the earthworm addition, *R*s was higher during the warming (daytime) process than during the cooling (nighttime) process (Fig. 3). Earthworm addition increased *R*s significantly, with an approximate 4–6 times increase during the daytime and 5–7 times increase during the nighttime. Moreover, following the earthworm addition, *R*s exhibited a symmetric pattern during the warming and cooling processes, which is contrary to the case in traditional soil incubation, with only soil microbe as asymmetrical (Fig. 3). This change was attributed to the earthworms' enhancement of *R*s, particularly during the cooling phase (Fig. 3). This phenomenon may be due to earthworm activities, such as burrowing and feeding, which reduce the proportion of large soil aggregates and increase substrate availability in the soil. These processes provide more labile C for the microbial community during cooling, in contrast to soils devoid of earthworms (Curry and Schmidt, 2007). The primary underlying mechanism may lie in the fact that, under low temperature stress, earthworms generate glucose and other carbohydrate compounds to boost their own activity, thus providing microorganisms with a wealth of available substrates, ultimately accelerating *R*s during the cooling phase (Shekhovtsov et al., 2023). Furthermore, earthworms help sustain microbial activity through the production of excretions and buffering of the soil pH during the cooling phase (Gong et al., 2019). In this way, the metabolic changes in earthworms may indirectly enhance *R*s by shaping both the soil substrate and the soil environment.

Earthworm addition considerably increased the Q_{10}, a trend observable both during the warming and cooling phases, as well as throughout the entire temperature variation process (Fig. 4). This increase in Q_{10} can be attributed to enhanced substrate availability resulting from earthworm addition, and high substrate availability has been shown to significantly enhance Q_{10} (Liu et al., 2023). Additionally, through their burrowing activities, earthworms augment the proportion of macroaggregates in the soil (Gong et al., 2019). Considering that physical protection of SOM by soil aggregates is reinforced as the aggregate size decreases, the Q_{10} of macroaggregates is higher than that of microaggregates (Kan et al., 2022).

Our study found that earthworm density had no significant effect on Q_{10} throughout the temperature variation process. However, earthworm density exerted a notable influence on Q_{10} during the cooling phase (Fig. 4B and E). Previous research has suggested that the cooling process can hamper microbial SOM decomposition (Li et al., 2017), whereas earthworms can enhance substrate availability through their secretions and excretions (Ferlian et al., 2017). This process provides resources for microbes, thereby mitigating substrate limitations and significantly elevating Q_{10}. Conversely, during the warming phase, microbial activity increased progressively, weakening the impact of varying earthworm densities on Q_{10}.

4. Conclusions

Overall, this study provides direct evidence that the addition of a model soil macrofauna, specifically earthworms, promotes SOM decomposition. Furthermore, the introduction of earthworms altered the asymmetric pattern of SOM decomposition during the warming (daytime) and cooling (nighttime) processes. Notably, the pronounced effect of earthworm density on Q_{10} was observed exclusively during the simulated cooling (nighttime) process. In the context of future climate warming, increased soil fauna diversity will greatly enhance C emissions and Q_{10}. Therefore, it is crucial to consider the role of soil fauna in future global C cycle models, particularly regarding temperature changes at night.

Acknowledgements

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Author contribution

N.P.H, J.L, S.L.Z, and H.C designed the study. S.L.Z analyzed data and wrote the manuscript. All authors contributed to the revision of the manuscript.

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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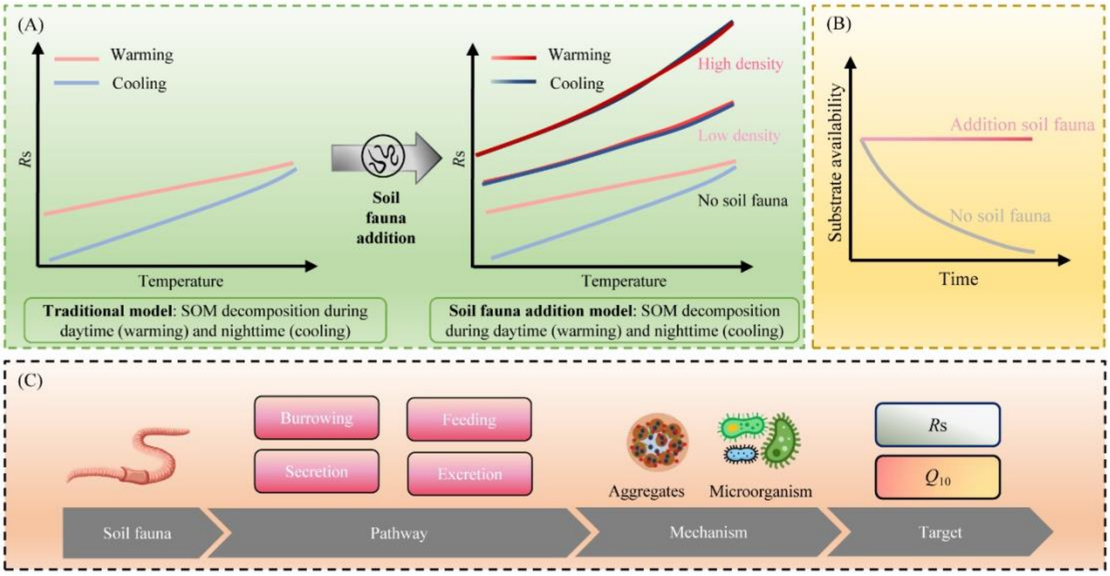
384 

385 **Fig. 1.** Hypothesis diagram of soil fauna affecting soil organic matter (SOM)
386 decomposition. (A) Traditional model *vs.* soil fauna addition model of SOM
387 decomposition dynamic change with temperature during the warming (red line) and
388 cooling (blue line) processes; (B) The colored lines represent the expected changes in
389 substrate availability with increasing incubation time; (C) Impact pathways of soil
390 fauna on SOM decomposition.

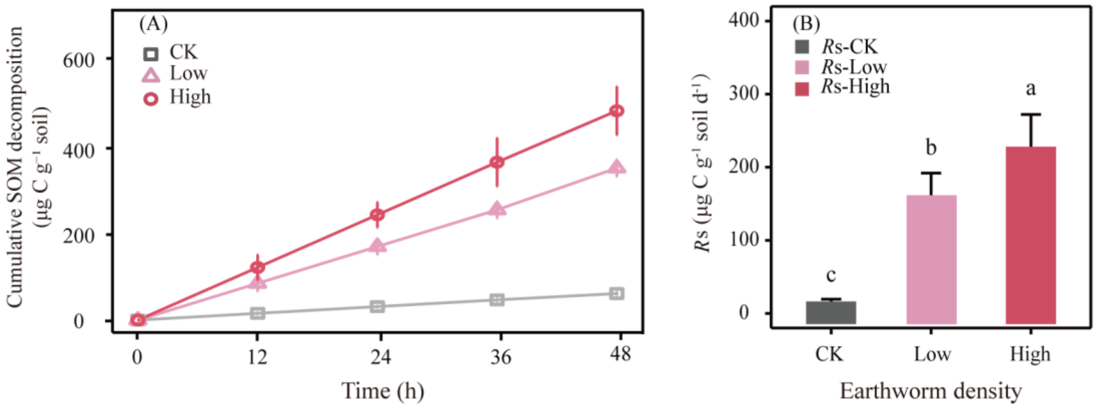


Fig. 2. SOM decomposition rate based on earthworm density. (A) Cumulative SOM decomposition under different earthworm densities (CK, no addition; Low, low density, and High, high density); (B) Impact of earthworm density on mean SOM decomposition rate at a temperature of 20 °C. Different lowercase letters indicate significant differences among the levels of earthworm density (LMMs; $P < 0.05$, n = 4, mean ± SE).

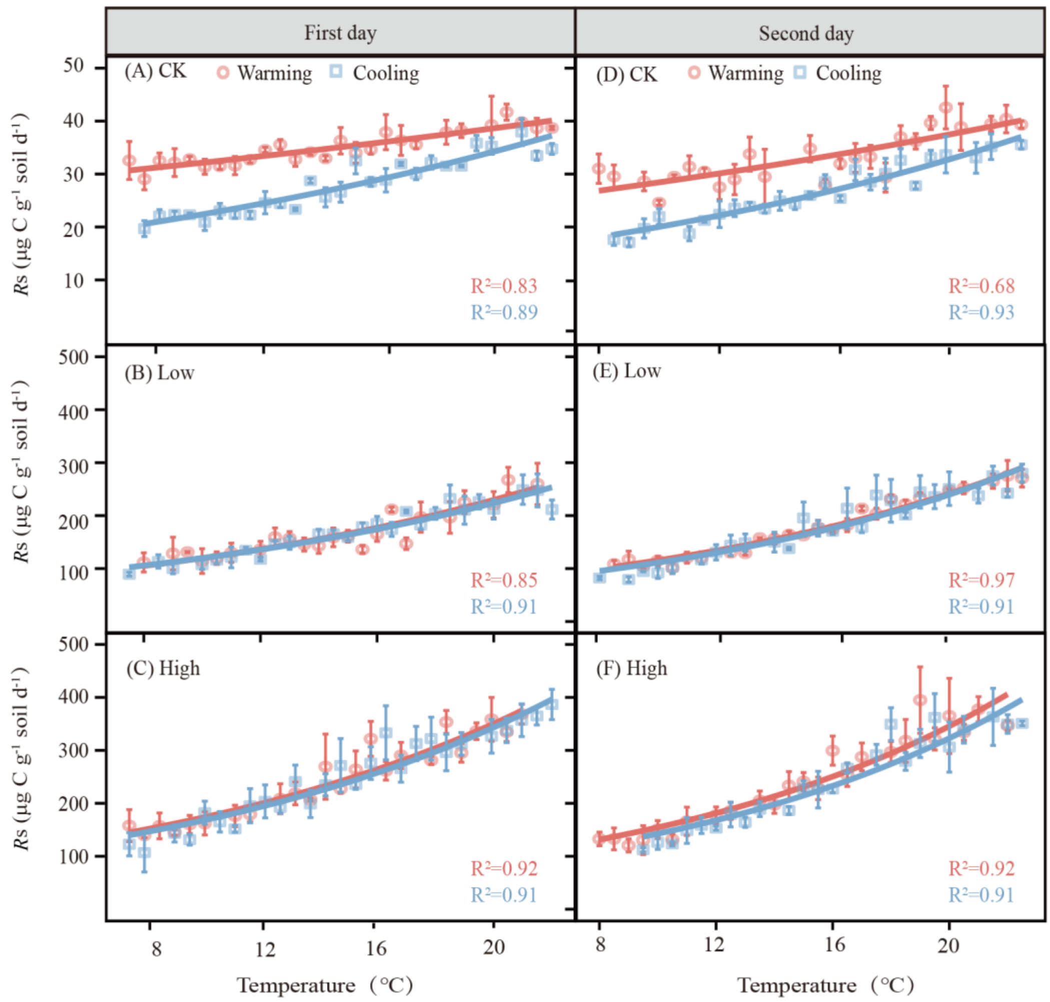


Fig. 3. Dynamic changes in SOM decomposition rate with temperature during the warming (daytime, red circles) and cooling (nighttime, blue squares) processes. (A–C) SOM decomposition trends under varying earthworm densities on the first day during the warming and cooling processes; (D–F) SOM decomposition trends under varying earthworm densities on the second day during the warming and cooling processes. The different colored lines indicate the fitted curves of the corresponding parameters versus temperature, with R^2 denoting the goodness of fit (n = 4, mean ± SE).

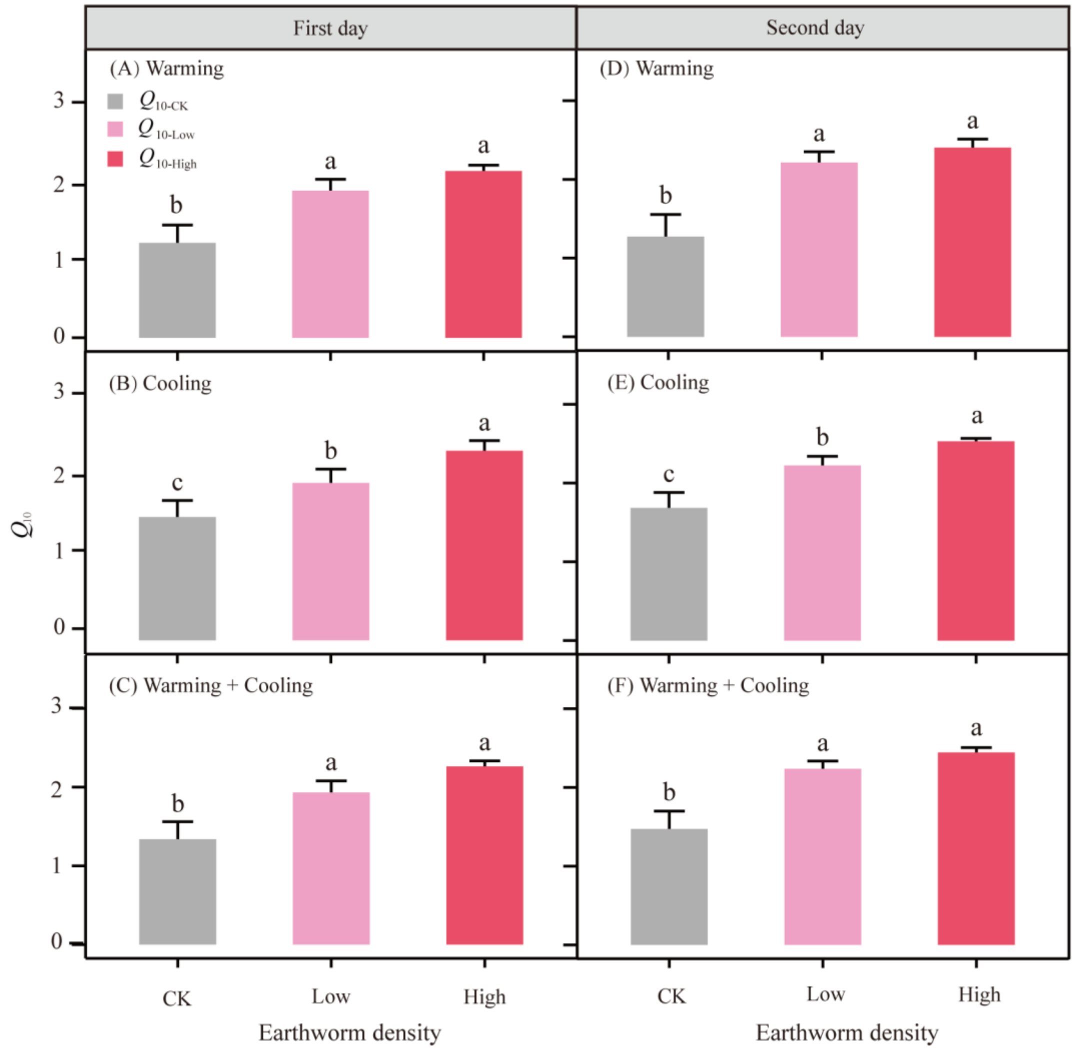


Fig. 4. Temperature sensitivity (Q_{10}) changes in SOM decomposition from earthworm density during all temperature change processes. (A–C) Comparison of Q_{10} ($Q_{10\text{-CK}}$, $Q_{10\text{-Low}}$, $Q_{10\text{-High}}$) across three temperature modes with varying earthworm densities on the first day; (D–F) Comparison of Q_{10} ($Q_{10\text{-CK}}$, $Q_{10\text{-Low}}$, $Q_{10\text{-High}}$) across three temperature modes with varying earthworm densities on the second day. Different lowercase letters indicate significant differences between earthworm densities ($P < 0.05$, n = 4, mean ± SE).