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RESEARCH ARTICLE

Manure increases temperature sensitivity of soil organic carbon by increasing soil Alphaproteobacteria, phenols, and pH and decreasing soil esters

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Highlights

- Long-term manure increase Q_{10} and N fertilizer decrease Q_{10}.
- The increased stable organic compounds under manure lead to high Q_{10}.
- Alphaproteobacteria, phenols, and pH promoted Q_{10} and esters inhibited Q_{10}.

Abstract

The temperature sensitivity (Q_{10}) of soil organic carbon (SOC) is a critical parameter in SOC response models concerning climate warming, which governs both the direction and magnitude of soil carbon-climate feedback. However, the relative importance of soil organic compounds in the regulation of the Q_{10} remains unclear, partly due to the relative stability of SOC compounds. Long-term different fertilization could change the quantity and quality of soil organic compounds. Here, a 38-year fertilization experiment combined with pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) was used to identify the effect of key soil organic compounds on the Q_{10}. Five treatments were chosen: no fertilization (CK), nitrogen fertilization (N), N combined with phosphorus and potassium fertilization (NPK), manure (M), and NPK combined with manure (NPKM). The results revealed that the Q_{10} under M and NPKM were 1.59 and 1.66, respectively, which were significantly higher than those under CK (1.35), N (1.29), and NPK (1.36). There was a positive linear relationship between the Q_{10} and SOC (R^2=0.76, P<0.01), whereby manure-enriched SOC is more vulnerable to decomposition under future warming. Among the soil organic compounds, esters and phenols predominated, representing 30.30% and 18.83% of the composition, respectively. Manure increased soil stable organic compounds relative to CK and chemical fertilizer. The increased stable organic compounds under manure led to a high Q_{10}. In addition to the positive effect of soil Alphaproteobacteria and pH on the Q_{10}, manure increased the Q_{10} by increasing phenols and decreasing esters, whereas chemical fertilization did the opposite. These findings first provide substantial evidence that soil organic compounds play an important role in the magnitude and mechanism of SOC response to climate change. Manure-induced SOC, when compared to chemical fertilizers, conferred a heightened sensitivity to climate warming within agroecosystems.

Keywords: soil organic carbon, temperature sensitivity, manure, soil microorganisms, soil organic compounds

1. Introduction

Anthropogenic activities have increased global temperatures by ~0.9°C, which not only increases vegetation productivity but also profoundly improves the carbon cycle of terrestrial ecosystems (IPCC 2014). Soil organic carbon (SOC), as the largest carbon reservoir in terrestrial ecosystems, is three to four times larger than those of the atmosphere and vegetation, where even minor changes can significantly impact the climate on a global scale (Lehmann and Kleber 2015). Climate warming can accelerate the decomposition of

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SOC, which creates a positive feedback loop. The strength of this feedback is largely contingent on the temperature sensitivity of SOC (designated as Q_{10}), which indicates the rate increase in SOC decomposition under a 10°C temperature increase (Davidson and Janssens 2006). The Q_{10} is not only an important indicator of SOC response to climate change, but also an important parameter for modeling the impact of future climate change on SOC. Agricultural soils are more susceptible to human-induced modifications and exhibit a heightened sensitivity in their SOC dynamics compared with their natural counterparts. This amplified sensitivity underscores the importance of investigating Q_{10} in agricultural soils; a topic that has become a research focal point (Todd-Brown *et al.* 2013; Qin *et al.* 2019).

The Q_{10} is theoretically rooted in the Arrhenius and Michaelis-Menten equations (Arrhenius 1889; Michaelis and Menten 1913). The Arrhenius equation delineates the temperature dependence of enzyme-catalyzed reactions, which suggests a positive correlation between activation energy and temperature sensitivity. This relationship infers that complex organic molecules with their higher activation energies are more susceptible to temperature variations, which accelerate their turnover rates. Since this equation is most applicable when the availability of the substrate is not limited, the temperature sensitivity obtained at this time is called the inherent temperature sensitivity (Davidson and Janssens 2006). In contrast, the decomposition of SOC is frequently subject to constraints such as soil moisture and substrate availability. Under conditions where the availability of substrates is limited, this decomposition is governed by the concentrations of both enzymes and substrates, that is, the maximum reaction rate and constant in the Michaelis-Menten kinetics. In such scenarios, the observed temperature sensitivities is called the apparent temperature sensitivity and reflect the combined influences of enzyme kinetics and substrate limitations, typically manifesting at lower values than the inherent Q_{10}, which would be observed under non-limiting conditions (Davidson and Janssens 2006). Thus, the Q_{10} emerges from a complex interplay between intrinsic SOC qualities and the prevailing environmental conditions. Collectively, these factors dictate the apparent Q_{10}, which underscores the necessity to consider both the chemical attributes of SOC and the environmental context in which they exist (Li *et al.* 2020).

SOC is composed of a large number of organic carbon compounds, each endowed with distinct kinetic characteristics (Lehmann and Kleber 2015; He *et al.* 2018; Ren *et al.* 2025). Inputs of exogenous carbon-containing substances, including crop residues like stubble and straw, as well as organic amendments, are an intermediate source of organic compounds (Davidson and Janssens 2006). Exogenous carbon input also undergoes a metamorphosis through microbial action or non-living condensation reactions, leading to the emergence of new aromatic compounds with greater molecular mass and varying solubility, which are noted for their slow breakdown pace, significant activation energy requirements, and a pronounced responsiveness to temperature shifts. Therefore, the sensitivity of SOC to warming is essentially a cumulative reflection of the warming responsiveness of various carbon-containing organic compounds within the soil (Xiong *et al.* 2019). So far, many studies have indicated that the responses of various soil organic compounds to warming vary significantly (Xiong *et al.* 2019; Mao *et al.* 2022). For example, the decomposition rate of glucose, an easily degradable compound, is faster than that of tannins characterized by high recalcitrance. Even with a mere 2-degree Celsius increase in temperature over the year, the decomposition rate of the more recalcitrant tannins would increase by 21% due to their higher temperature sensitivity, compared to a 10% increase for glucose (Imriskova *et al.* 2005). Due to the positive priming effect from enhanced rhizodeposit inputs, the relative abundance of phenolic compounds in soil diminished under warming (Phillips *et al.* 2012). While prior studies established broad relationships between SOM stability and Q_{10} (Davidson and Janssens 2006; Ghosh *et al.* 2019), none have identified the roles of specific organic compound classes (e.g., phenols, esters) or microbial taxa in driving Q_{10} variations under long-term fertilization. Our study bridges this gap by integrating Py-GC/MS-based molecular characterization with microbial community analysis.

The Q_{10} arises from complex interactions between the chemical stability of SOC and a range of soil properties (Margit and Ingrid 2009). In agroecosystems, long-term fertilization not only alters the molecular composition of SOC but also modifies key soil properties (e.g., pH, microbial communities, and enzymes) (Lin *et al.* 2025; Hu *et al.* 2025). Low soil pH can suppress microbial growth and enzymatic activities, thereby slowing SOC decomposition and lowering Q_{10}. Soil microbes play a critical role in the decomposition of SOC through the secretion of extracellular enzymes that can increase Q_{10}, and the generation of binding substances that enhance soil stability, which potentially decreases Q_{10} (Wang Q *et al.* 2019). The composition of microbial communities is closely linked to substrate availability. Bacteria often dominate the initial stages of organic matter decomposition, while fungi become more prevalent in the later stages (Zhang *et al.* 2018). Recent studies highlight manure's dual role in enriching recalcitrant phenols and stimulating Alphaproteobacteria, yet their combined effect on Q_{10} remains untested (Ghosh *et al.* 2019; Wang *et al.* 2021). Moreover, soil nutrients and iron-aluminum oxides are known to influence the structures and diversification of microbial communities, as well as their sensitivity to changes in temperature (Rasmussen *et al.* 2006). Despite these advances, few studies have explicitly linked long-term fertilization-induced changes in recalcitrant compounds (e.g., phenols) and microbial functional groups to Q_{10} dynamics in agricultural soils. The overall contribution of microbial factors to Q_{10} variability remains unclear, with some estimates suggesting that microbial effects explain only 30–50% of observed variation (Hursh *et al.* 2017; Li *et al.* 2020). Therefore, a comprehensive elucidation of Q_{10} requires consideration of both SOC and key soil properties.

The composition of soil organic compounds is relatively stable without human disturbance. Long-term different fertilization experiments can provide a good platform for constructing the relationship between different soil

organic compounds and Q_{10} under the same climate and parent material conditions. It has been proved that long-term different fertilization treatments significantly changed the chemical composition and molecular structure of SOC (He *et al.* 2018). Therefore, our specific objectives were: (1) to systematically quantify the differences in Q_{10} under various fertilization treatments; (2) to identify the key soil organic compounds and soil properties that drive the Q_{10}. We hypothesized that manure increases Q_{10} by enhancing recalcitrant compounds (e.g., phenols) and Alphaproteobacteria activity. By elucidating the variability and driving factors of Q_{10} under different fertilization treatments, this research provides a theoretical basis for understanding critical processes in agricultural carbon cycling, supporting the sustainable management of SOC, and enabling precise modeling under various climate change scenarios.

2. Materials and methods

2.1. Description of experimental site

This study is based on a long-term fertilization experiment on red soil developed from Quaternary Red Clay in southern China. The experimental station was established in 1986 and is located in Jinxian County, Jiangxi Province, China (116°20′24′′E, 28°15′30′′N). This area is home to a subtropical climate with an average annual temperature of 18.1°C, cumulative temperatures ≥10°C of 6,480°C, annual precipitation of 1,537 mm, annual evaporation of 1,150 mm, frost-free period of 289 d, and annual sunlight duration of 1,950 h. The soil is classified as Red Soil according to the Chinese soil genesis classification, which corresponds to Acrisol in the FAO World Reference Base. The initial basic physicochemical properties of the surface soil samples (0–20 cm) were: SOC 9.39 g kg^{-1}, total nitrogen (TN) 0.89 g kg^{-1}, total phosphorus (TP) 0.62 g kg^{-1}, total potassium (TK) 11.36 g kg^{-1}, alkaline hydrolyzable nitrogen 60.3 mg kg^{-1}, available phosphorus (AP) 12.9 mg kg^{-1}, available potassium (AK) 102 mg kg^{-1}, and soil pH 6.0.

The experiment employed a randomized design with five treatments: no fertilization (CK), nitrogen fertilization (N), N combined with phosphorus and potassium fertilization (NPK), manure (M), and NPK combined with manure (NPKM). Each treatment had three replicate plots, with each plot measuring 22.2 m² (5.5 m×4.0 m). The applied chemical fertilizers included urea (46% N), calcium magnesium phosphate (12% P_2O_5), and potassium chloride (60% K_2O) at rates of 120 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1} yr^{-1}, and 120 kg K_2O ha^{-1} yr^{-1}, respectively. P and K fertilizers were applied as a basal application, with N applied two-thirds as basal and one-third as topdressing at the seedling stage. The organic fertilizer was fresh pig manure (70% water content), with dried pig manure containing 413.5 g kg^{-1} C, 4.5 g kg^{-1} N, 3.5 g kg^{-1} P, and 5.0 g kg^{-1} K, applied entirely as a basal fertilizer at 15,000 kg ha^{-1} yr^{-1} (wet weight). Crop residues were removed after harvest.

The cropping system at the experimental station followed a spring maize–autumn maize–winter fallow rotation. For the first three years before the experiment, the soil was thoroughly mixed to ensure consistent physicochemical properties. The maize variety was Yedan 13, sown in early April and harvested in mid-July for early maize, and sown in late July and harvested in early November for late maize, with an average planting density of 60,000 plants ha^{-1} and a row spacing of 50 cm×25 cm. Due to high early-year rainfall, the early maize was not irrigated, while the late maize was irrigated at the seedling and tasseling stages with furrow irrigation at 300 m^3. For the control of corn borer, the pesticides abamectin and chlorpyrifos were used during the seedling and boot stages. Glyphosate herbicide was used post-harvest for weed control. The maize was harvested manually with the stubble height at 3–5 cm, leaving the roots in the soil.

2.2. Soil sampling and analysis

Surface soil samples (0–20 cm) were collected following the late maize harvest in September 2021. To minimize experimental errors due to field sampling, the soil samples were collected from each plot using a 5 cm soil auger drill following a five-point “S” shape sampling method. The soil samples from each plot were thoroughly mixed to create a composite sample, which was divided into two portions for subsequent analysis and incubation experiments. One portion was air-dried, cleaned of plant debris and stones, and passed through a 0.25 mm sieve. Composite samples (0–20 cm depth) were homogenized to minimize spatial variability, ensuring representative analysis of topsoil properties. The SOC content was determined using the potassium dichromate oxidation method (Page *et al.* 1982). The soil available nitrogen (AN) was measured following Lu (2000), AP using the Olsen-P method (Olsen 1954), and soil pH with a pH meter at a 1:2.5 soil-to-water ratio. Free iron and aluminum oxides (Fe_d and Al_d) were extracted using sodium dithionite-citrate-bicarbonate; amorphous iron and aluminum oxides (Fe_o and Al_o) were extracted with acid ammonium oxalate; and complexed iron and aluminum oxides (Fe_p and Al_p) were extracted using sodium pyrophosphate (Karine *et al.* 2012; Keiluweit *et al.* 2015). The values of iron and aluminum oxides in the soil were expressed as Al+0.5Fe (Wagai *et al.* 2020).

The soil dissolved organic carbon (DOC) and microbial biomass carbon (MBC) were determined as follows: two 10 g soil samples were placed in 50 mL beakers. One sample was placed in a desiccator with ethanol-free chloroform vapor for 24 h, while the other sample was placed in a desiccator without chloroform under the same conditions. After fumigation, the soil was transferred to plastic bottles, extracted with 40 mL of a 0.5 mol L^{-1} K_2SO_4 solution, shaken for 30 min, and filtered. The carbon content in the extracts was measured using a TOC analyzer (Multi N/C 3100). The difference in organic carbon content between the fumigated and non-fumigated extracts (ΔEC) was employed to calculate MBC: MBC=ΔEC/kC, with kC=0.45.

The soil enzyme activities involved in carbon cycling (α-glucosidase, β-glucosidase, β-cellobiohydrolase, β-xylosidase, and sucrase) were quantified using a modified microplate fluorescence method (Deforest 2009). Briefly, 1,000× g of fresh soil was added to 100 mL centrifuge tubes with 50 mL ultrapure water, vortexed, shaken at 180 r min^{-1}

for 30 min, after which the suspension was transferred to a 500 mL beaker. The remaining suspension was rinsed with 50 mL of ultrapure water into the beaker. Samples, buffers, and substrates were added to a 96-well black microplate with eight replicate wells per sample. After adding the substrates, the microplates were incubated at 25°C in the dark for 4 h, followed by the addition of 10 μL of 1.0 mol L^{-1} NaOH to stop the reaction. The fluorescence was measured using a microplate reader (Synergy H1M, BioTek Instruments) at a 365 nm excitation and 450 nm emission.

The soil microbial diversity, including fungi and bacteria, was assessed *via* high-throughput sequencing, which involved three main steps. First, the soil DNA was extracted using the E.Z.N.A.® Soil DNA Kit from Omega Bio-tek, USA. Each sample used 0.5 g of fresh soil, and the DNA quality was verified using 1% agarose gel electrophoresis, while the DNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The extracted DNA was stored at –20°C for future use. Second, PCR amplification and gene sequencing were performed. The extracted soil DNA served as the template, and PCR amplification targeted the 16S rRNA V3–V4 regions with primers 338F/806R (5′-ACTCCTACGGGAGGCAGCA/GGACTACHVGGGTWTCTAAT-3′) for bacteria and ITS1F/ITS2R (5′-CTTGGTCATTTAGAGAGTAA/GCTGCGTTCTTCATCGATGC-3′) for fungi. The PCR program included initial denaturation at 95°C for 3 min, followed by 27 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min, and storage at 4°C (ABI GeneAmp® 9700, Thermo Fisher Scientific, USA). Each sample was amplified in triplicate, and the PCR products were pooled and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). The purified products were quantified using a Quantus™ Fluorometer (Promega, USA), and library development was completed using the NEXTFLEX® Rapid DNA-Seq Kit (Bioo Scientific, USA). Sequencing was outsourced to Shanghai Majorbio Bio-pharm Technology Co., Ltd., China. Third, data analysis involved the quality control of the raw sequences using Fastp (https://github.com/OpenGene/fastp, version 0.19.6), and sequence assembly using FLASH (http://www.cbcb.umd.edu/software/flash, version 1.2.11). Operational taxonomic units (OTUs) were clustered using UPARSE (http://drive5.com/uparse/, version 7.1) and chimeras were removed. The sequences were rarefied to 20,000 reads per sample for further analysis. OTU taxonomic classification was performed, and community composition was analyzed at different taxonomic levels. Diversity indices (e.g., Shannon and Simpson) were calculated to assess community evenness.

The main organic compounds in the soil were determined using pyrolysis gas chromatography-mass spectrometry (Py-GC/MS), following the procedure of Vancampenhout *et al.* (2009). Soil samples were ground into fine powder and air-dried; where after 10 mg of soil was placed in a quartz boat, wetted with 1 mL of 25% tetramethylammonium hydroxide (TMAH) in methanol, and dried under nitrogen gas for 10 min. The sample was then heated in a Pyrex tube reactor (50 cm×3.5 cm i.d.) to 400°C at a rate of 20°C min^{-1} and held for 5 min. The thermal decomposition products were transferred by helium gas to chloroform (50 mL) in ice baths. The chloroform solution was combined, concentrated by rotary evaporation, and dissolved in 0.2 mL of chloroform for GC-MS analysis using a GCMS-QP2010 Ultra (Shimadzu, Japan). Chromatographic separation was achieved using the following temperature program: 40°C (1 min), increased to 80°C at 5°C min^{-1}, then to 300°C at 15°C min^{-1} and held for 5 min, with a total run time of 40 min. Helium was used as the carrier gas (1.90 mL min^{-1}), with an injection port temperature of 320°C, a split ratio of 30, and a column flow rate of 1 mL min^{-1}. Mass spectra were obtained in EI mode (70 eV) with an ion source temperature of 230°C, a transfer line temperature of 300°C, and a scan range of m/z 40–550 in full scan mode. Peaks obtained from Py-GC/MS were analyzed and identified to determine the composition of soil organic compounds using the Automatic Mass Spectral Deconvolution and Identification System (AMDIS) and the National Institute of Standards and Technology (NIST) mass spectral library.

2.3. Experimental incubation design

Traditional Q_{10} studies often employ constant temperature incubation, which has the drawback of allowing soil microbes to adapt to specific temperatures. This can also lead to inconsistent substrate consumption across different sample treatments. To address these limitations, a gradual temperature variation incubation method has recently been developed. This approach involves progressively increasing and then decreasing the temperature of the same batch of soil samples, accompanied by intermittent low-frequency measurements of soil organic matter decomposition rates (Liu *et al.* 2017).

Fifty grams of fresh dry weight soil was collected from each soil sample and adjusted to 60% of field capacity. Each sample was divided into two aliquots and placed into 250 mL glass jars, then pre-incubated at 18.5°C for 15 days to activate the microbes, while minimizing external interference. After preincubation, the soil samples were placed in two incubators: Incubator 1 (18.5°C, the 30-year average temperature of the region) and Incubator 2 (28.5°C). Incubator 1 cycled daily from 13.5°C to 23.5°C (±5°C around a mean of 18.5°C), and Incubator 2 from 23.5°C to 33.5°C (±5°C around 28.5°C). One aliquot of the soil sample underwent short-term incubation, with CO_2 emissions collected on days 1, 3, 5, 7, 14, and 28. The detailed process is shown in Fig. 1.

For gas sample collection, we conduct our experiments during 24-h basis. In brief, before initiating the culture, we replace the air inside the culture flask with synthetic air to ensure that there is no CO_2 present while maintaining the same atmospheric pressure as the ambient air. After the replacement, an EGM-5 CO_2 analyzer was used to test the gas within the culture flask, confirming the absence of CO_2. Then, the culture flask was introduced into an incubator. Upon completion of 24 h, the culture flask was retrieved. Initially, a syringe was employed to thoroughly mix the gases within the culture flask. Subsequently, a 40 mL gas sample

was extracted using the syringe and promptly transferred into a vacuum vial. Ultimately, the CO_2 concentrations in vacuum vials were measured using gas chromatography to calculate the soil respiration rates (Fig. 1).

The Q_{10} was calculated based on the experimental temperature gradient using the following equation:

$$Q_{10}=\left(\frac{R_W}{R_C}\right)^{\left(\frac{10}{R_W-T_C}\right)} \quad (1)$$

where Tc and Tw represent the 30-year average annual temperature (18.5°C) and the temperature increased by 10°C (28.5°C), respectively. Rc and Rw are the soil organic carbon decomposition rates at Tc and Tw (mg CO_2-C g^{-1} d^{-1}) (Qin *et al.* 2019).

2.4. Statistical analysis

Fisher's LSD test was used to compare differences in the Q_{10}, soil chemical properties, organic compounds, and microbes under different fertilization treatments. To explore how environmental variables affected Q_{10} under different fertilization regimes, a series of correlation analyses were conducted based on theoretical knowledge to avoid autocorrelation in subsequent statistical analyses. Firstly, environmental variables were categorized into soil chemical properties (SOC, DOC, AN, AP and soil iron-aluminum oxides), soil organic compounds (15 types), and soil microbes (biomass, function, and diversity of fungi and bacteria). Stepwise regression was employed to identify the main controlling factors of Q_{10} for each category of environmental variables to eliminate autocorrelation. Thirteen factors were identified as driving factors: soil chemical properties included soil pH and soil iron-aluminum oxides (Al_o+0.5Fe_o, sum of oxalate-extractable Fe/Al oxides); soil organic compounds included esters, phenols, and heterocyclic. Soil microbiology included the Shannon of bacteria, Acidobacteriia, Alphaproteobacteria, Ktedenobacteria, Eurotiomycetes, and Sordariomycetes; and soil enzymes included β-xylosidase and sucrase. Linear correlations between these factors and Q_{10} were discovered (Fig. 2). Variance decomposition analysis was utilized to quantify the influence and relative contributions on Q_{10} of the selected environmental factors.

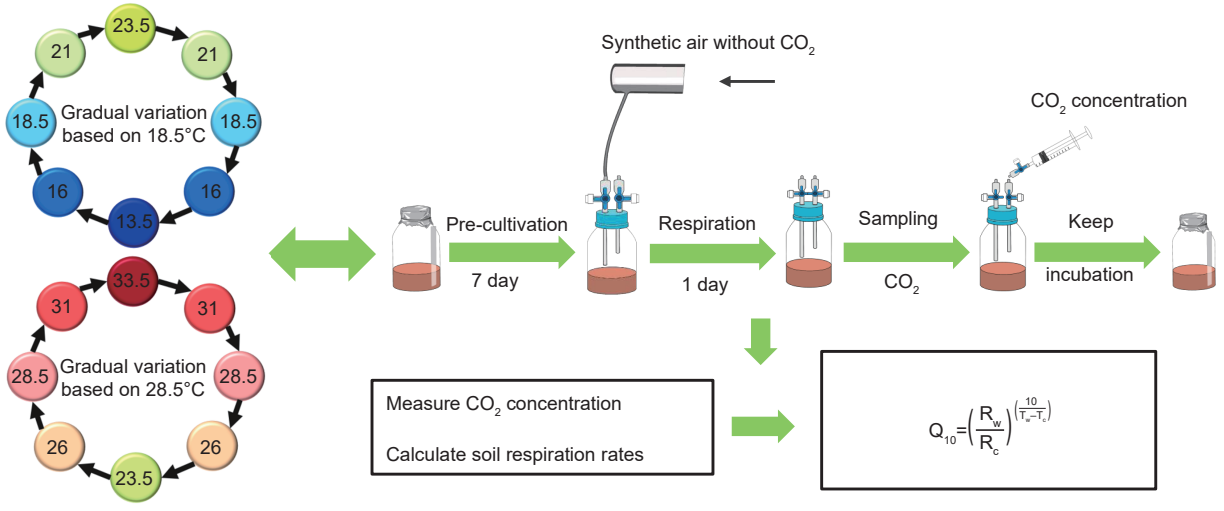


Fig. 1 A conceptual diagram illustrating the whole process of the culture experiment.

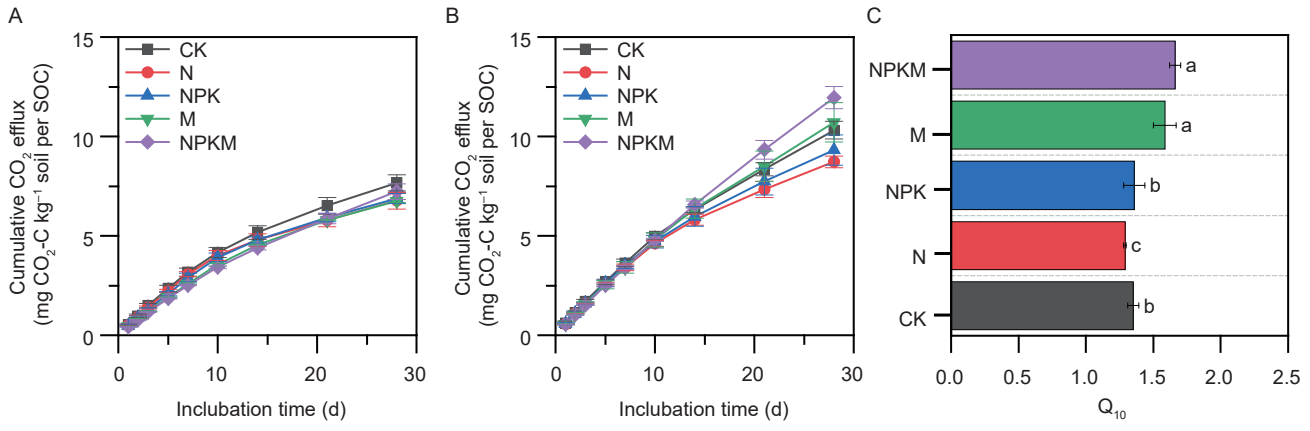


Fig. 2 Cumulative CO_2 efflux during a 28-day incubation at 18.5°C (A) and 28.5°C (B) and temperature sensitivity (Q_{10}) of soil organic carbon decomposition (C) under different fertilization treatments. Error bars indicate standard deviation. Various lowercase letters indicate significant differences in Q_{10} between fertilization treatments at P<0.05 (Fisher's LSD test). Fertilization treatments include no fertilizer (CK), chemical nitrogen (N), chemical N, phosphorus, and potassium (NPK), manure (M), and M combined with NPK (NPKM).

All model subsets were ranked by AIC values using MuMIn, after which an optimal model was selected. The relative importance of variables was analyzed using the "rdacca.hp" package. Finally, partial correlation analyses were performed to examine whether the interactions between these variables were interdependent. Variance decomposition and partial correlation analyses were conducted using R 3.3.3 software.

3. Results

3.1. The Q_{10} under long-term fertilization

The cumulative CO_2 emissions from the soil under various long-term fertilization treatments increased exponentially over the 28-d incubation period (Fig. 2-A and B). The cumulative CO_2 emissions were significantly higher under the M (85.36 mg CO_2-C kg^{-1} soil) and NPKM (98.95 mg CO_2-C kg^{-1} soil) compared with CK (74.55 mg CO_2-C kg^{-1} soil), N (66.37 mg CO_2-C kg^{-1} soil), and NPK (72.42 mg CO_2-C kg^{-1} soil) at an incubation temperature of 18.5°C. The N resulted in significantly lower cumulative CO_2 emissions in contrast to the other treatments. Increasing the incubation temperature by 10°C did not alter the trend of cumulative CO_2 emissions between the different fertilization treatments but did increase the overall emission quantities. Treatments with higher cumulative CO_2 emissions (M and NPKM) also exhibited the highest Q_{10} values. The Q_{10} was 1.59 and 1.66 for the M and NPKM, respectively, which were significantly higher than CK (1.35), N (1.29), and NPK (1.36). The Q_{10} under N was significantly lower than that under the other treatments.

3.2. Soil properties under long-term fertilization

For soil chemical properties, M and NPKM significantly increased SOC, DOC, pH, AN, AP, Fe_o, and Al_o compared to CK (Table 1). N significantly decreased the soil pH from 5.13 (CK) to 4.34 (N). For soil microbial properties, the β-xylosidase was the lowest under M and NPKM, at 13.04 and 33.88 nmol h^{-1} g^{-1}, respectively (Appendix A). M and NPKM resulted in a significantly higher MBC than CK and N. The bacterial Shannon index was significantly higher under M and NPKM in contrast to CK, while it was significantly lower under N compared with CK (Appendix B). Acidobacteria was highest under N at 11.33% (Appendix C). Manure resulted in significantly lower relative abundance of Acidobacteria (2.30% of 16S rRNA gene reads) compared to other treatments (Appendix C), suggesting strong suppression of this bacterial class.

For soil organic compounds in 1986, esters (30.30%) and phenols (18.83%) were the dominant compound classes (Table 2). The relative percentage of alkanes/alkenes/alkynes, amines, heterocyclic and organic sulfur compounds was more than 5%. Compared with the initial soil organic compounds, CK and N increased the ester content and decreased phenol content, while M and NPKM showed the opposite trend. N raised esters from 30.30% (initially) to 42.38%, while M increased phenols from 18.83% (initially) to 21.17%. In the 2021 year, NPKM significantly decreased the alcohol compounds to 3.40% compared with other treatments. The relative percentage of ester, heterocyclic, and organic sulfur compounds under NPKM was 25.91, 2.17, and 1.77%, respectively, and significantly low than that of CK. The relative percentage of phenol compounds under NPKM (24.59%) significantly increased than that of CK (18.83%). The relative percentage of organic sulfur and phenol compounds under N significantly increased than that of CK.

3.3. Drivers of the Q_{10}

A significantly positive linear correlation was observed between the Q_{10} and SOC (Fig. 3). Further, there was a significant positive linear relationship between the Q_{10} and soil phenols compounds (Fig. 4). The Q_{10} presents a linear decreasing trend with the increase of soil alcohols, esters, heterocyclic, and organic sulfur compounds. Stepwise regression revealed that the soil chemical properties (soil pH, Fe_o, and Al_o), soil organic compounds (esters, phenols, and heterocyclic), and soil microbes (Shannon index, Alphaproteobacteria, Sordariomycetes, Acidobacteriia, Eurotiomycetes, and Ktedonobacteria) jointly regulated the Q_{10} (Figs. 3–6). Besides soil organic compounds, the Q_{10} exhibited a positive linear correlation with the soil pH, soil Fe_o and Al_o, Shannon index, Alphaproteobacteria, Sordariomycetes, and sucrase (Figs. 5 and 6). Conversely, the Q_{10} demonstrated a negative linear correlation with Acidobacteriia, Eurotiomycetes, Ktedonobacteria, and β-xylosidase. The combined effects of

Table 1 Soil chemical properties after 35 years of various fertilization treatments

Index[1)]	Treatments[2)]				
	CK	N	NPK	M	NPKM
SOC (g kg^{-1})	9.73±0.33 d	9.80±0.22 d	10.53±0.12 c	12.63±0.38 b	13.70±0.29 a
DOC (mg kg^{-1})	27.96±1.77 c	34.44±1.33 c	39.60±1.31 c	84.10±5.72 b	100.76±10.12a
Soil pH	5.13±0.08 c	4.34±0.13 d	4.95±0.10 c	6.39±0.03 a	5.89±0.18 b
Available N (mg kg^{-1})	48.67±0.49 c	47.47±1.01 c	57.20±3.02 b	62.13±1.32 b	71.17±7.40 a
Available P (mg kg^{-1})	16.33±0.54 d	18.20±0.71 d	30.73±0.46 c	208.33±9.10b	286.67±5.25 a
Al_d+0.5Fe_d (mg kg^{-1})	23.63±0.88 a	11.62±1.66 d	12.49±0.94 bc	14.26±0.71 b	24.80±0.40 a
Al_p+0.5Fe_p (mg kg^{-1})	2.07±0.10 bc	5.65±0.84 a	5.90±0.69 a	1.74±0.17 d	3.09±0.61 b
Al_o+0.5Fe_o (mg kg^{-1})	5.40±0.28 c	6.79±0.19 b	6.65±0.57 bc	7.66±1.00 b	10.73±0.38 a

[1)] SOC, soil organic carbon (g kg^{-1}); DOC, soil dissolved organic carbon (mg kg^{-1}); Available N, soil available nitrogen (mg kg^{-1}); Available P, soil available phosphorus (mg kg^{-1}); Ald+0.5Fed, sum of dithionite-citrate-bicarbonate-extractable Fe/Al oxides; Alp+0.5Fep, sum of pyrophosphate-extractable Fe/Al oxides; Alo+0.5Feo, sum of oxalate-extractable Fe/Al oxides.
[2)] CK, no fertilizer; N, nitrogen; NPK, N, phosphorus, and potassium; M, pig manure; NPKM, NPK and M.
Various lowercase letters indicate significant differences between various fertilization treatments for the same index of soil nutrients and mineral protection at P<0.05 (Fisher's LSD test).

Table 2 Relative abundance of compound classes (%) under initial soil and after 35 years under various fertilization treatments

Classes[1)]	Initial soil	Treatments[2)]				
		CK	N	NPK	M	NPKM
AC	4.47	5.16±0.11 a	5.37±0.36 a	5.14±0.34 a	5.56±0.75 a	3.4±0.07 b
AH	1.64	2.25±0.45 a	1.71±0.38 ab	1.04±0.16 b	1.56±0.32 ab	1.65±0.22 ab
AL	6.18	5.56±0.57 a	4.28±1.55 ab	4.99±0.38 ab	5.38±0.70 a	3.19±0.48 b
AM	7.74	8.29±0.69 c	8.89±1.09 c	15.53±1.04 a	13.81±1.40 a	11.38±0.53 b
AR	3.70	0.73±0.15 b	1.69±0.34 a	1.65±0.08 a	1.52±0.39 a	1.1±0.18 ab
CA	0.93	0.59±0.01 b	1.34±0.2 a	0.98±0.19 ab	1.00±0.14 ab	0.82±0.32 b
ES	30.30	36.54±0.89 b	42.38±0.89 a	30.6±1.90 c	27.41±1.40 c	25.91±1.04 d
ET	3.24	1.54±0.23 ab	1.44±0.33 ab	1.18±0.03 b	1.63±0.13 ab	1.87±0.19 a
HE	6.43	7.52±0.55 ab	9.34±1.2 a	7.55±0.75 ab	6.43±0.95 b	2.17±0.6 c
HL	1.36	3.68±0.67 a	1.78±0.54 b	1.25±0.17 bc	0.38±0.09 c	3.49±0.93 a
KE	1.41	0.76±0.16 a	0.63±0.16 a	0.98±0.17 a	0.97±0.26 a	0.86±0.09 a
NI	1.65	1.53±0.30 c	3.77±0.37 b	5.52±0.84 a	5.15±0.49 a	4.59±0.74 ab
OS	6.82	9.97±0.72 a	5.76±0.29 c	6.94±0.45 b	5.03±0.43 c	1.77±0.42d
PH	18.83	13.02±1.06 c	7.44±1.13 d	7.59±0.42 d	21.17±2.19 b	24.59±1.21 a
SI	3.47	0.39±0.21 c	0.34±0.07 c	9.5±0.57 a	1.17±0.09 c	5.88±0.55 b

[1)] AC, alcohols; AH, alcohols; AL, alkanes/alkenes/alkynes; AM, amines; AR, Aromatic hydrocarbon; CA, carboxylic acids; ES, esters; ET, ethers; HE, heterocyclic; HL, halides; KE, ketones; NI, nitriles; OS, organic sulfur; PH, phenols; SI, silane.
[2)] Fertilization treatments include no fertilizer (CK), chemical nitrogen (N), chemical N, phosphorus, and potassium (NPK), manure (M), and M combined with NPK (NPKM).
Various lowercase letters indicate significant differences between different fertilization treatments for the same index of soil nutrients and mineral protection at $P<0.05$ (Fisher's LSD test).

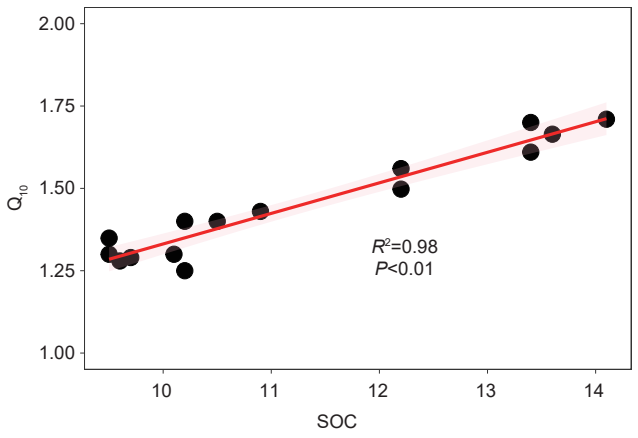


Fig. 3 Relationships between soil organic carbon (SOC) and temperature (Q_{10}) sensitivity of SOC decomposition. Red dashed lines denote the regression fits, and shaded bands show the 95% confidence intervals.

these 13 factors accounted for 89% of the variations in the Q_{10}, with the soil chemical properties, organic compounds, and microbes contributing 17, 29, and 44%, respectively, of the variations (Fig. 6). Partial correlation analysis revealed that the significant correlation between alphaproteobacteria and the Q_{10} persisted even when other variables were excluded (Fig. 7). Furthermore, removing alphaproteobacteria retained the significant correlations between soil pH, esters, and phenols with Q_{10}. Overall, the Q_{10} increase under manure was mainly due to a 63% increase in phenols and a 47% rise in alphaproteobacteria, whereas the Q_{10} decline under N fertilizer was due to a 43% drop in phenols and a 38% decrease in Alphaproteobacteria (Fig. 8).

4. Discussion

4.1. Temperature sensitivity of SOC under different fertilization treatments

Our study elucidated the relationship between the Q_{10} and agricultural management practices. The chosen temperature range (18.5°C to 28.5°C) aligns with projected subtropical warming under intermediate climate scenarios (IPCC 2021), ensuring relevance to future climate conditions. Higher CO_2 emissions under manure (Fig. 2-A and B) reflect greater SOC availability, while elevated Q_{10} (Fig. 2-C) indicates stronger temperature dependence of decomposition. The variation range of Q_{10} under long-term different fertilization treatments was 1.35–1.66, which was lower than the parameters in the previous models (Fig. 2). Traditionally, terrestrial ecosystem models set the Q_{10} at the same magnitude (constant 2) to forecast the response of soil organic carbon to climate change (Yang *et al.* 2022). This discrepancy may stem from the simplification of soil carbon cycle complexities in models and the insufficient consideration of diversity in management practices. Applying a uniform Q_{10} parameter of 2 in models, as done for other ecosystems, proves inadequate for agroecosystems. With global agroecosystems constituting 38.5% of the Earth's terrestrial ecosystems, it becomes imperative to integrate unique Q_{10} into models. This adjustment is essential for diminishing uncertainties in the projections of global SOC-climate interactions.

The debate on different fertilizer effects on the Q_{10} is significant. Manure is thought to increase microbial activity, which may raise soil organic matter and the Q_{10} by speeding up organic carbon decomposition (Ghosh *et al.* 2019). In contrast, the effects of chemical fertilization on the Q_{10} are still inconsistent. While some studies suggest that chemical fertilizers reduce Q_{10} by decreasing microbial biomass and

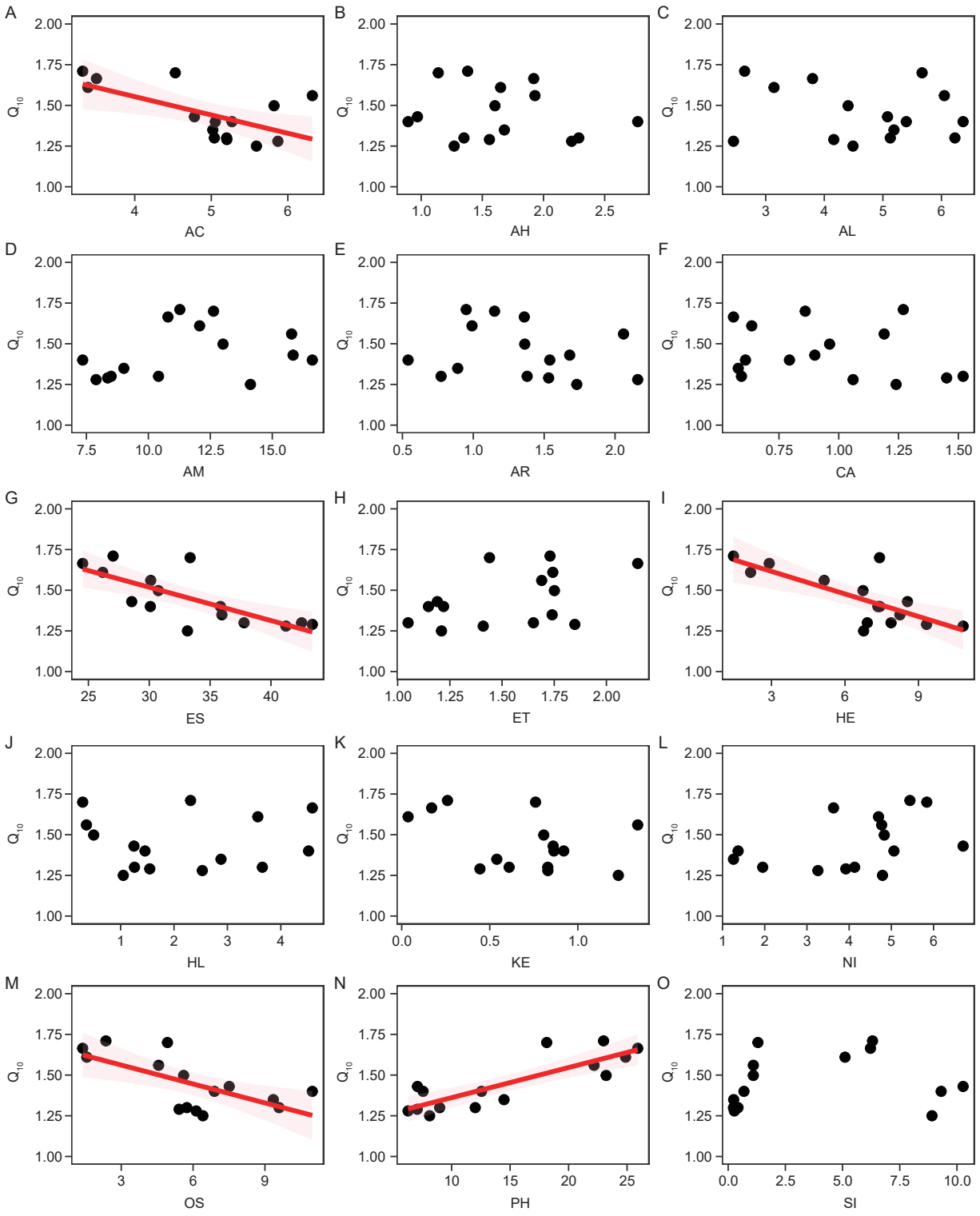


Fig. 4 Relationships between temperature sensitivity (Q_{10}) of soil organic carbon decomposition and soil organic compounds. AC, alcohols; AH, alcohols; AL, alkanes/alkenes/alkynes; AM, amines; AR, Aromatic hydrocarbon; CA, carboxylic acids; ES, esters; ET, ethers; HE, heterocyclic; HL, halides; KE, ketones; NI, nitriles; OS, organic sulfur; PH, phenols; SI, silane. All soil organic compound relative contents (*x*-axis) are expressed as percentages (%).

diversity, which slows the SOC decomposition rate (Wang R *et al*. 2019), other studies report no significant effect (Ghosh *et al.* 2019). Our results indicated that the Q_{10} under M and MNPK was significantly higher than under CK, N, and NPK, with the lowest Q_{10} observed under N (Fig. 2). This has been attributed to several factors. Firstly, manure could directly increase soil MBC (Appendix A), which led to the release of significant amounts of CO_2 by accelerating SOC decomposition. Secondly, manure can promote the number and variety of microbial communities associated with

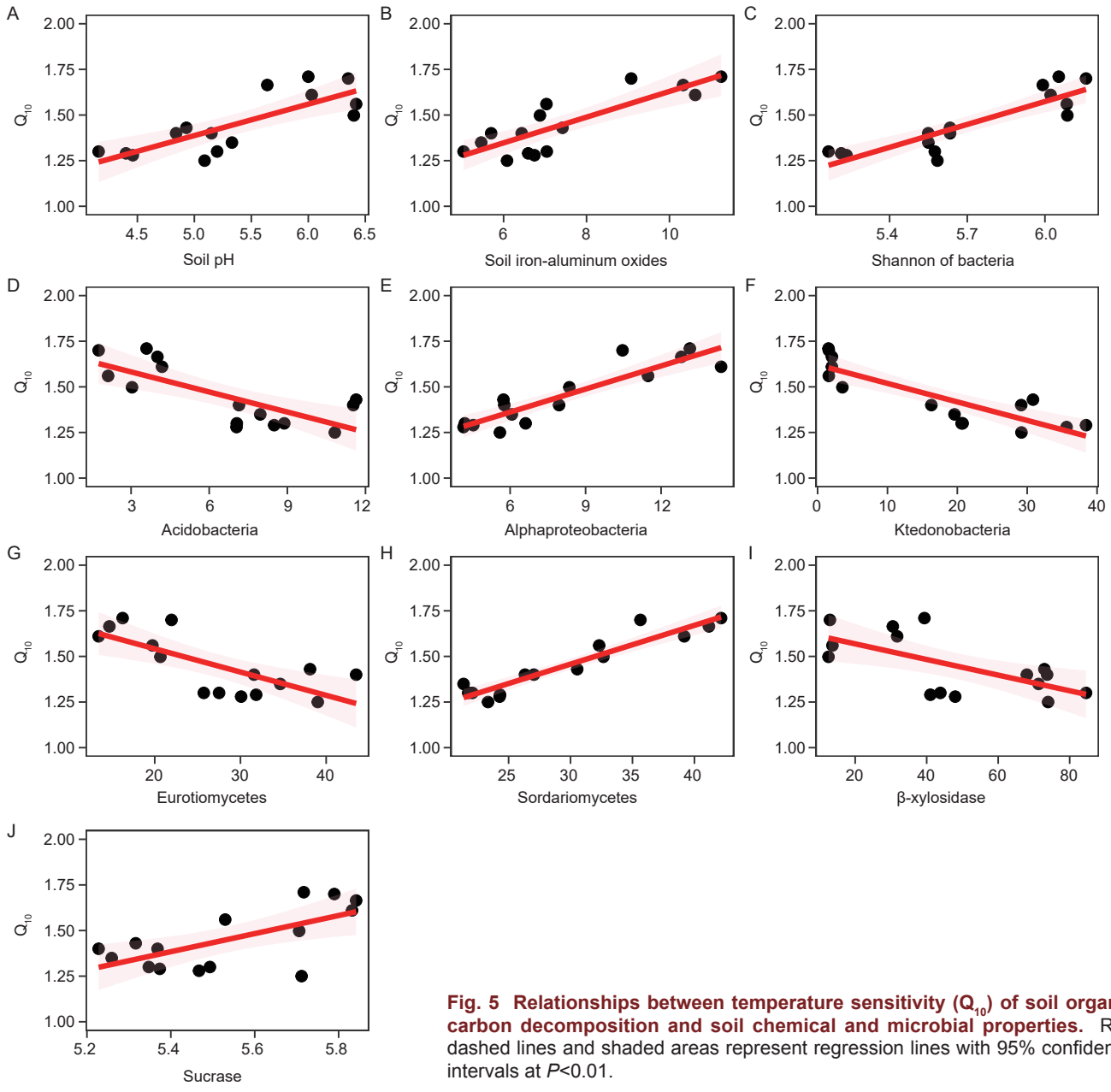


Fig. 5 Relationships between temperature sensitivity (Q_{10}) of soil organic carbon decomposition and soil chemical and microbial properties. Red dashed lines and shaded areas represent regression lines with 95% confidence intervals at P<0.01.

increased rates of decomposition of SOC, which increases the Q_{10}. Conversely, long-term chemical fertilization led to a low soil pH (Table 1). Acidic conditions elevate the energy costs of microbial growth and metabolism, which limits the decomposition of SOC. While manure enhances SOC stocks, the higher Q_{10} implies greater vulnerability to warming-induced decomposition. This trade-off underscores the need for balanced management — optimizing carbon sequestration while mitigating climate feedbacks through practices like intermittent manure application or crop residue retention. In summary, the higher Q_{10} under manure suggests a positive climate feedback, as warming could accelerate decomposition of this carbon pool, potentially offsetting sequestration benefits. These findings provide empirical data to refine climate-carbon cycle models by incorporating management-specific Q_{10} values.

4.2. Key drivers of the temperature sensitivity of SOC

Long-term fertilization alters the soil esters and phenols compounds, soil microbial communities, and soil physicochemical properties, all of which play critical roles in regulating the formation and decomposition of SOC (Moinet *et al.* 2018; Qin *et al.* 2019; Chen *et al.* 2022).

In our study, N-fertilized soils showed the highest ester content (42.38%) and the lowest Q_{10} (1.29), consistent with this mechanism of rapid ester turnover leading to less temperature sensitivity. Soil esters and phenols compounds were the predominant compounds in soil samples, accounting for about 50% (Table 1). The high proportion of soil esters and phenol compounds is primarily attributed to their abundant presence in plant residues and their generation during microbial metabolism. Chemically, esters contain a carbonyl group (C=O) bonded to an alkoxy group (–OR), making them relatively labile and easily hydrolyzed by microbial enzymes.

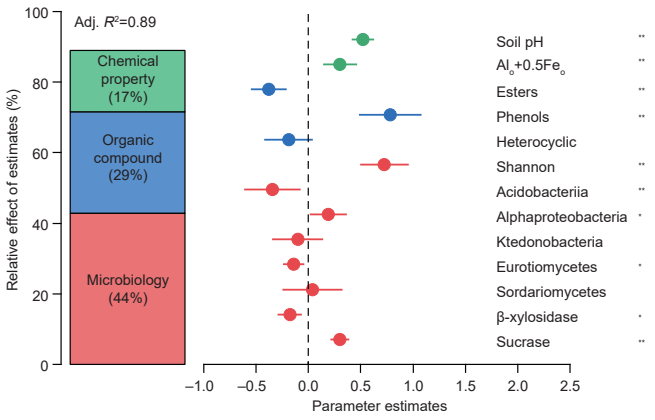


Fig. 6 Effects of soil chemical properties, soil organic compounds, soil microbiology, and soil enzymes on temperature sensitivity of soil organic carbon decomposition. Soil chemical properties include soil pH and soil iron-aluminum oxides (Al_o+0.5Fe_o, sum of oxalate-extractable Fe/Al oxides). Soil organic compounds include esters, phenols, and heterocyclic. Soil microbiology includes Shannon of bacteria, Acidobacteriia, Alphaproteobacteria, Ktedenobacteria, Eurotiomycetes, and Sordariomycetes. Soil enzymes include β-xylosidase and sucrase. Average parameter estimates (standardized regression coefficients) of model predictors, associated 95% confidence intervals, and relative importance of each factor are expressed as the percentage of explained variance. The adjusted (adj.) R^2 of the averaged model and the *P*-value of each predictor are given as: *, P<0.05; **, P<0.01.

Phenolic compounds, in contrast, possess an aromatic ring with one or more hydroxyl groups (–OH), forming structurally complex and often recalcitrant molecules such as lignin derivatives. These fundamental differences in chemical structure partly explain why esters decompose rapidly with lower temperature sensitivity, whereas phenolics degrade more slowly but are more sensitive to warming. Esters and phenols synthesized by plants during growth, such as waxes, resins, and lignin derivatives, enter the soil in large quantities after plant decomposition (Pratyusha 2022). Microbial metabolism not only produces these compounds but also secretes enzymes capable of breaking down complex organic matter, releasing esters and phenols that subsequently participate in the soil organic matter cycle (Barré *et al.* 2018). Chemical fertilization increased the presence of ester organic compounds (Table 1). Although organic fertilizers are rich in fatty acids and fatty alcohols, their application stimulates soil microbial activities and biodiversity, which accelerates the decomposition of lipid organic compounds in the soil. Consequently, the use of organic fertilizers significantly reduced the content of ester organic compounds in contrast to CK and chemical fertilization (Zhou *et al.* 2022). Manure application significantly increased phenolic compounds, particularly mono- and polyphenols, likely due to the inherent phenolic content of manure (Mary and Nithiya 2015).

The Q_{10} had a significantly negative correlation with the relative content of soil ester organic compounds, and a significantly positive correlation with the relative content of soil phenol organic compounds (Fig. 4). High soil ester organic compounds indicate that the soil organic matter has a rapid decomposition rate (Xiong *et al.* 2019). Soil ester compounds can be quickly broken down by microorganisms through secreted enzymes into nutrients, which can be utilized by microbes (Neemisha and Sharma 2022). This efficient metabolic activity of the microorganisms is relatively less dependent on temperature changes. Under high microbial activity, further temperature increases do not significantly enhance the decomposition rate of esters because the microbes are already operating near their maximum metabolic rate (Davidson and Janssens 2006). Furthermore, the rapid decomposition of esters depletes the available carbon sources in soil, which narrows the range of variability in the decomposition rate of SOC due to temperature. Microorganisms quickly utilize readily decomposable carbon

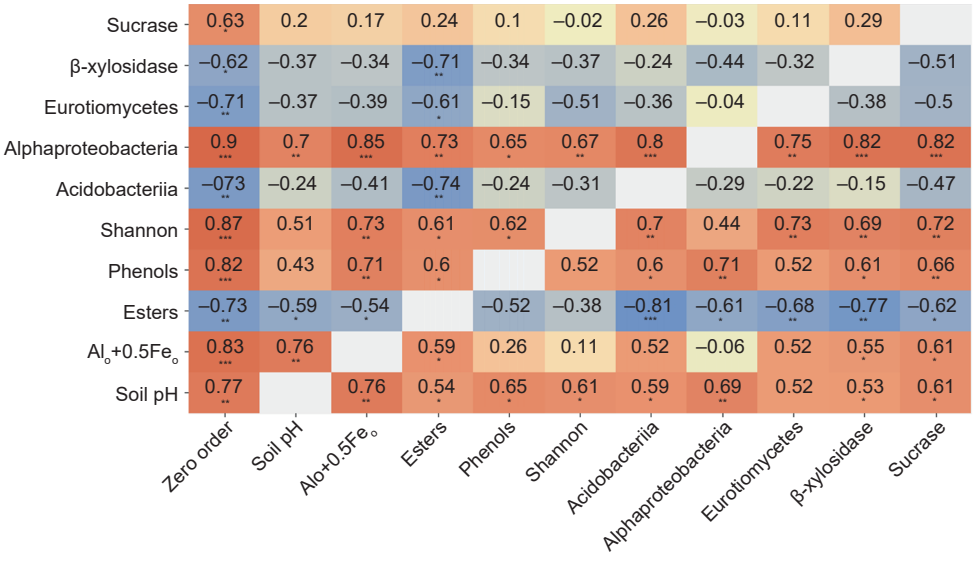


Fig. 7 Partial correlations between the temperature sensitivity of soil organic carbon decomposition (Q_{10}) and driving factors. Differences between zero-order and partial correlations indicate the level of dependency of the correlation between a given variable and Q_{10}. Asterisks indicate statistical significance levels: *, P<0.05, **, P<0.01, and ***, P<0.001.

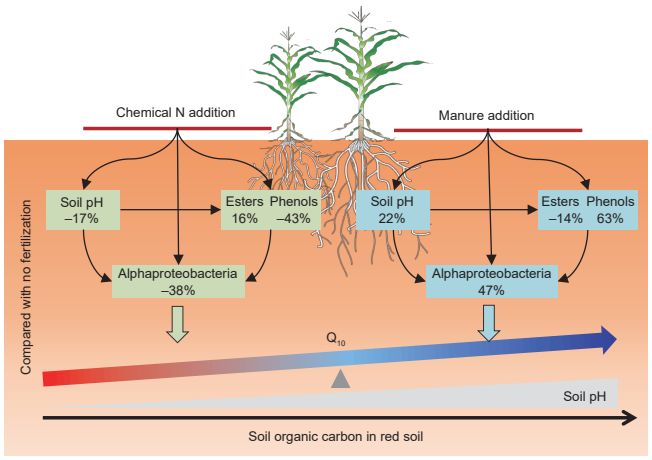


Fig. 8 Conceptual diagram showing regulators and mechanisms of the temperature sensitivity of soil organic carbon decomposition (Q_{10}) under long-term chemical nitrogen and manure application. Arrows indicate that the value of a variable gradually increases. All values are derived from comparison with no fertilization.

from esters, making further temperature increases ineffective in boosting SOC decomposition. This leads to a lower Q_{10} value in soils rich in esters. Conversely, elevated levels of phenolic organic compounds in the soil indicate a higher concentration of recalcitrant organic matter. With intricate structures featuring numerous phenolic hydroxyl groups and extended carbon chains, phenolic compounds, notably polyphenols, decompose slowly in the soil environment. The breakdown of these complex molecules is typically dependent on specific microbial communities, whose metabolic activities and proliferation is often temperature-sensitive (Garcia-Palacios *et al.* 2016). At cooler temperatures, microbial decomposition of these phenolic compounds is constrained, leading to a slower rate of decomposition. Reduced β-xylosidase under manure (Appendix A) suggests slower hemicellulose degradation, aligning with higher phenol content and slower SOC turnover. In contrast, an increase in temperature can invigorate microbial metabolic processes, enabling a more efficient breakdown of these resistant phenolic compounds and the release of additional nutrients and energy (Derocles *et al.* 2018). Consequently, an increase in soil phenolic corresponds with the heightened sensitivity of SOC decomposition to temperature fluctuations, which is reflected in a higher Q_{10}.

The strong correspondence between elevated CO_2 emissions and higher Q_{10} values under manure treatments indicates that intensified SOC mineralization is closely linked to an increased temperature sensitivity of decomposition. A major driver of this relationship appears to be the proliferation of Alphaproteobacteria. These bacteria, which reached their highest abundance under NPKM and the lowest under N treatments (Appendices B and C), are known producers of oxidative enzymes such as laccases and peroxidases, along with diverse hydrolases that can degrade complex, recalcitrant organic matter (Wang *et al.* 2021). Previous studies have shown that long-term nitrogen fertilization suppresses Alphaproteobacteria diversity and abundance, whereas organic amendments stimulate their proliferation and enhance SOC transformation(Chen *et al.* 2021). Alphaproteobacteria facilitate SOC decomposition by oxidizing phenolic and lignin-derived compounds, breaking them into simpler substrates that can be further mineralized or incorporated into more stable lipid- and protein-bound carbon pools (Salgado *et al.* 2011). Their metabolic activity is strongly temperature-dependent: moderate warming accelerates microbial growth and enzyme secretion, increasing decomposition rates and favoring gaseous carbon losses over acidic metabolites. However, extreme temperature deviations can impair Alphaproteobacteria activity, reducing their ability to transform SOC (Wang *et al.* 2021). While other microbial groups, such as Eurotiomycetes and Acidobacteriia, also correlated with Q_{10}, partial correlation analyses revealed that their effects were largely mediated through Alphaproteobacteria (Fig. 7). This finding underscores Alphaproteobacteria as a key microbial indicator of SOC temperature sensitivity. Collectively, these results highlight an enzyme-driven microbial mechanism through which long-term manure application enhances both SOC mineralization and its responsiveness to warming. Future management strategies could explore balancing organic fertilization with targeted microbial regulation to improve SOC stability under climate change.

Regarding soil physicochemical properties, soil pH can strongly influence the Q_{10} through its effects on both soil microorganisms and organic compounds. Changes in soil pH directly regulate microbial activities and growth, thereby impacting the decomposition rate of organic matter and the generation of metabolites, which in turn modifies the temperature sensitivity of SOC. Acidic conditions induced by long-term nitrogen fertilization can suppress microbial biomass and reduce extracellular enzyme activities, leading to slower carbon mineralization and a lower Q_{10}. In contrast, manure application increased soil pH toward neutral levels, creating a more favorable environment for microbial proliferation and enzymatic function. For instance, Alphaproteobacteria preferentially thrive in soils with near-neutral or slightly acidic pH. These conditions enable the release of essential nutrients and facilitate their avoidance of the adverse effects of excessively high or low pH on their survival and metabolic product generation (Wang *et al.* 2015). When soil pH is slightly acidic to neutral, both the abundance and activity of microorganisms increase, enhancing the decomposition and transformation of organic matter. This, in turn, promotes the formation of more stable compounds such as lipids and accelerates the breakdown of complex SOC pools, ultimately contributing to the observed higher Q_{10} under manure treatments (Ding *et al.* 2015). In addition to pH effects, iron and aluminum oxides also play a critical role in controlling SOC decomposition and its temperature sensitivity. These metal oxides can strongly bind with organic matter *via* ligand exchange, surface adsorption, and co-precipitation, leading to the formation of mineral-associated organic matter. Such associations can physically protect organic compounds from microbial attack and enzymatic degradation (Karine *et al.* 2012; Wagai *et al.* 2020). The presence of

more Fe/Al oxides can therefore enhance the stabilization of SOC and potentially lower its decomposition rate under warming conditions. While our findings are robust for clay-rich subtropical soils, their generalizability to other systems (e.g., sandy or alkaline soils) may be limited by differences in mineralogy or microbial communities. Validations across pedoclimatic gradients are warranted.

4.3. Potential uncertainties and future research

The gradual temperature variation incubation method offers a more natural simulation of temperature fluctuations and microbial acclimation, which can provide insights into dynamic microbial responses. However, this method expresses the influence of unit temperature change on soil organic carbon, which is finally modified to Q_{10} by the formula. In contrast, the constant temperature incubation method provides a controlled measure of the Q_{10}, reflecting the impact of a 10°C change. Yet, it might not capture the substrate depletion over time and may not accurately represent the real-world temperature variability and microbial adaptation. Combining gradual temperature variation with constant temperature incubation methods offers a comprehensive approach to studying soil organic carbon decomposition, capturing both immediate and acclimated microbial responses to temperature changes. However, this combined approach may introduce uncertainties in data integration and interpretation due to the contrasting conditions each method creates.

Future research on the Q_{10} under various fertilization practices should concentrate on two points. First, it will be essential to study how aggregate structure influences the microenvironment and consequently, the Q_{10}, taking into account how different fertilizers impact aggregate formation and stability. For example, future studies could examine: (i) How do micro- and macroaggregate fractions differ in their contributions to Q_{10} under long-term organic *vs*. chemical fertilization? (ii) What are the specific roles of intra-aggregate oxygen diffusion and mineral-organic associations in stabilizing SOC decomposition? Second, considering the long-term impacts of climate change, research should evaluate how projected climate scenarios, including temperature increases and altered precipitation, will affect the decomposition rates of SOC and its Q_{10} responsiveness. This could be achieved by conducting factorial field warming — precipitation experiments combined with microbial functional gene analysis to predict SOC responses under future climate scenarios, and integrating these experimental results into process-based models to improve predictions of SOC–climate feedbacks in agricultural ecosystems.

5. Conclusion

Long-term application of manure significantly increased both the Q_{10} and SOC, whereas nitrogen fertilizer had the opposite effect. There was a positive linear relationship between Q_{10} and SOC, indicating that future climate warming could intensify the decomposition of SOC derived from manure. N increased the ester and decreased phenol, while manure showed the opposite trend compared with the initial soil organic compounds. Soil chemical properties, soil organic compounds, and microbial communities jointly influence the variation of Q_{10}. Alphaproteobacteria, as a key influencer of Q_{10}, underscores the significant role in modulating soil organic carbon cycling under climate warming. Furthermore, removing Alphaproteobacteria retained the significant correlations between soil pH, esters, and phenols with Q_{10}. The positive linear relationship between Q_{10} and soil phenols compound further substantiates the notion that complex organic compounds are more sensitive to temperature changes. Overall, these findings enhance our estimating the contribution of long-term manure to SOC sequestration under climate warming and highlight the role of soil organic compounds in the soil carbon cycle.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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