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Divergent Responses of Temperature Sensitivity to Rising Incubation Temperature in the Warmed and Un-Warmed Soil from a Subtropical Plantation

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Abstract: The subtropical forest soil plays a pivotal role in terrestrial carbon (C) cycling. Although global warming is expected to influence subtropical soil C cycling, a consensus on its impact on soil C dynamics remains elusive. Given the significant response of soil microbial respiration to temperature in subtropical soils, understanding its behavior under varying temperature regimes is critical for predicting soil organic C (SOC) responses to climate warming. We conducted a short-term laboratory soil warming incubation experiment, sampling both warmed and un-warmed soils from a subtropical plantation in southeastern China, incubating them at 20 °C, 30 °C, and 40 °C. Our aim was to study the SOC mineralization response to increasing temperatures. Our findings revealed that the temperature sensitivity (Q_{10}) of SOC mineralization to short-term experimental warming varied between the warmed soil and the un-warmed soil. The Q_{10} of the un-warmed soil escalated with the temperature treatment (20-30 °C: 1.31, 30-40°C: 1.63). Conversely, the Q_{10} of the warmed soil decreased (20-30 °C:1.57, 30-40 °C:1.41). Increasing temperature treatments enhanced hydrolytic enzyme activity but decreased soil substrate availability in both un-warmed and warmed soil. The Q_{10} of un-warmed soil was positively correlated with the response ratio of DOC. Additionally, soil microbial biomass exhibited a significant decline, leading to a reduced total amount of PLFAs and a decreased abundance of bacteria, fungi, and Gram-negative bacteria (GN). The changes in temperature also considerably altered the composition of the warmed soil microbial community. A drop in the microbial quotient coupled with a rise in the metabolic quotient indicated that warming amplified microbial respiration over microbial growth. The differential Q_{10} of SOC mineralization, in response to temperature across varying soil, can largely be attributed to shifts in soil nutrients, C-degrading enzyme activities, and microbial communities (the ratio of fungal to bacterial PLFAs).

Keywords: warming; soil organic carbon mineralization; temperature sensitivity; substrate availability; enzyme activity; microbial community structure

1. Introduction

Soil plays a central regulatory role in the global carbon (C) cycle [1]. Being the largest terrestrial C pool, soil organic carbon (SOC) contains three times as much C as that stored in the atmosphere and plants [2]. Given this vast pool size, even a slight change in SOC dynamic could significantly affect the global C cycle. While warming experiments in temperate and boreal regions have enriched our understanding of soil C cycling in response to climate warming [3,4], our knowledge regarding the SOC response in tropical and subtropical (sub/tropical) forests remains limited [5,6]. It's noteworthy that sub/tropical forest ecosystems store 46 % and 11 % of the world's living terrestrial C and soil C, respectively [7]. This lack of studies in the region underscores a knowledge gap in our understanding of the responses of sub/tropical forests to climate warming.

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The temperature sensitivity (Q_{10}) is a key metric that gauges the response of SOC decomposition to temperature change. It's been observed that Q_{10} profoundly influenced by biotic factors like soil microbial biomass and community composition, and abiotic ones such as incubation temperature and nutrient availability [8–10]. However, variations in incubation conditions, like different temperatures and durations among experiments, have led to inconsistent predictions about the Q_{10} of SOC mineralization [11]. Q_{10} also mirrors the differences in thermal acclimation of microbial communities between long- and short-term responses to temperature [12]. Such community thermal acclimation can result from direct shifts in the extracellular enzyme pool towards distinct activation energies and temperature optima or indirect alterations in resources composition and availability [13–15]. Furthermore, since Q_{10} is temperature-dependent, the estimation of Q_{10} based on non-site-specific incubation temperatures might not accurately capture in-situ temperature sensitivity [11]. Therefore, analyzing samples from the same site under different warming treatments can provide more accurate insights into the differences in Q_{10} of SOC decomposition due to climate warming, and can also help determine if soils act as sources or sinks.

The quality and quantity of substrates influence microbial use of organic substances and the Q_{10} values of SOC decomposition [16,17]. For instance, as the temperature rises, the increase of dissolved organic carbon content and soil bacterial abundance can enhance SOC mineralization [18]. Regardless of warming, a richer soil C boosts the extracellular enzymatic pool and its temperature sensitivity [12]. Contrarily, Jiang et al. [19] found that Q_{10} had a positively correlation with the changes in soil dissolved organic carbon, soil ammonium nitrogen contents, and β-glucosidase activities, but a negatively one with fungi: bacteria and urease activities post a 5°C of warming. Clearly, soil substrate quality and quantity play pivotal roles in SOC decomposition by microbes [20]. Temperature-induced changes in nutrient demand and supply can impact the available C to microbes [21,22]. Additionally, microbial biomass and community composition have a pronounced effect Q_{10} values [23,24]. For example, temperature can accelerate SOC decomposition by altering soil enzymes activity and SOC turnover rate [25]. On the other hand, soil microorganisms can adapt to sustained temperature increments by modifying their community composition [26]. However, not all substrates are created equal in fulfilling microbial demands. Factors such as enzyme investment, activation energy for decomposition, and energetic richness of compounds determine their usability by microbes [27–29]. Moreover, the various components of soil organic matter (SOM) differ in their decomposition temperature sensitivity, leading to significant shifts in SOM chemistry with chronic warming [21,30]. This means that long-term warming-induced changes in SOM chemistry could alter the potential resource pool available for organisms when they encounter short-term temperature fluctuations [31]. Given this complex interplay, there's a pressing need for further research on the effects of soil microbial communities and their relationship with soil substrate on SOC mineralization.

This study was conducted on a field soil warming experiment in a subtropical plantation in southeast China. Previous studies on this experiment revealed that warming can reshape microbial community structure and enzyme activity, leading to a significant imbalance between soil N and C decomposition, and suggesting that heterotrophic respiration could be more sensitive to climate warming [32,33]. Therefore, we designed a laboratory-based soil incubation experiment that investigates the changes in Q_{10} of SOC mineralization between un-warmed and warmed soils across three temperature regimes (20, 30, and 40°C). This investigation is rooted in a field soil warming experiment conducted in a subtropical plantation in southeastern China. In this study, we hypothesis that the difference in Q_{10} of SOC mineralization in warmed and un-warmed soils to warming hinges on alterations in microbial and enzyme activities.

2. Materials and Methods

2.1. Study Site and Soil Sampling

The experiment was located at the Chenda research site (300 m above sea level) of Sanming Forest Ecosystem and Global Change National Observation and Research Station, Fujian Province (26°19N, 117°36E), southeastern China. This region is characterized by a subtropical monsoonal climate with a mean annual precipitation of 1670 mm (from 1959-2015), with 77% occurring from March to August, and a mean annual temperature of 19.1 °C [33].

In October 2013, ten experimental plots were established, following a randomized block design. Each block consisted of a warmed and un-warmed plot, with each plot measuring 2 m × 2 m. Heating cables (TXLP/1, Nexans, Norway) were buried in both un-warmed and warmed plots at a depth of 10 cm with a horizontal interval of 20 cm. However, the cables in the un-warmed plots remained unheated. In the warming plots, soil temperature was continuously maintained at 5 °C above that in the un-warmed plots. The warming experiment was carried out in a young Chinese fir plantation established in 2013. In June 2016, nine 0-10 cm depth soil cores were collected each of 5 plots per treatment using a 3.5 cm diameter soil corer. These samples were immediately transported to the laboratory, stored at 4 °C before analyses and subsequently incubated. For determining basic soil properties (Table 1), fresh soil was sieved through a 2 mm mesh and soil moisture was measured by drying the soil (2.0 g) at 105 °C for 24 h.

Table 1. Mean soil organic carbon (SOC), total N (TN), post-incubation soil ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), dissolved organic N (DON), dissolved organic C (DOC), microbial biomass carbon (MBC), nitrogen (MBN), microbial quotient, and metabolic quotient at different incubation temperatures in un-warmed and warmed soil. Values are expressed as (mean ± standard deviation; n = 3). Different capital letters denote significant difference between situ un-warmed soil and warmed soil ($p < 0.05$).

Treatment	SOC (mg·g^{-1})	TN (mg·g^{-1})	NH_4^+-N (mg·kg^{-1})	NO_3^--N (mg·kg^{-1})	DON (mg·kg^{-1})	DOC (mg·kg^{-1})	MBC (mg·kg^{-1})	MBN (mg·kg^{-1})
un-warmed soil	13.02±1.14A	1.12±0.08A	4.48±0.60A	2.04±0.54A	1.66±0.21A	13.58±1.46A	285.50±20.74A	24.60±2.20A
warmed soil	11.60±1.38A	0.98±0.09A	4.08±1.38A	2.32±0.45A	1.17±0.25A	9.86±2.24B	203.55±28.75B	19.07±2.43B

2.2. Laboratory Incubation Experiment

The incubation experiment encompassed three temperature levels (20, 30, and 40 °C) and two types soil (warmed soil and un-warmed soil). In total, 18 soil samples were placed into a 500-mL incubation jar, maintaining 60% of the field capacity moisture content. All samples were pre-incubated at 20 °C for two weeks, following the procedure by Hamdi et al. [34], to mitigate the burst of respiration due to wetting the dry soils. Throughout the incubation, three samples of each treatment were used for the determination of soil CO_2 concentration, The CO_2 concentration determination was carried out on days 1, 7, 14, 24, 34, 49, and 63. During each gas sampling, compressed air was used to flush the headspace for 60 s to standardize the starting atmospheric CO_2 concentration of each incubation jar [35]. Two hours later, the gas sample of each jar was collected again. The gas sample was injected into an evacuated 20 mL glass vial with a syringe to measure its CO_2 concentration using a thermal conductivity detector at 400 °C on gas chromatography (GC-2014, Shimadzu, Kyoto, Japan) within 24 h. Three blank jars without soil were used to determine the background CO_2 concentrations. To minimize the effect of different levels of SOC on the amount of CO_2 produced, the cumulative CO_2-C was expressed as a proportion of the SOC (µg CO_2-C g^{-1} SOC h^{-1}).

2.3. Soil Chemical Analyses

Total soil nitrogen (TN) and SOC were determined using a Vario MAX CN elemental analyzer (Elementar Vario EL III, Germany). The Dissolved organic carbon (DOC) and nitrogen (DON) were extracted from 10 g of incubation soil with 40 mL Milli Q water at 20 °C and shaking for 30 min [36]. The mixture solution was then centrifuged at $11.5 \times g$ for 20 min and then filtered through a 0.45-μm glass fabric filter paper. The DOC concentration was determined using a TOC analyzer (Shimadzu Corporation, Japan), the DON concentration was determined using a Continuous Flow Analyzer (SKALAR San++, Holland). Soil ammonium and nitrate determinations, 5 g of freshly collected soil was extracted with a 2 mol L^{-1} KCl solution [37]. The solution was shaken for 20 min and then filtered, and the concentrations of soil ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) in the supernatant were determined using a Continuous Flow Analyzer (SKALAR San++, Holland).

2.4. Microbial Biomass C and N

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the chloroform ($CHCl_3$) fumigation and potassium sulfate (K_2SO_4) extraction techniques as described by Vance et al. [38] and Xu et al. [39]. In summary, $CHCl_3$ was used to fumigate 5-g fresh soil for 24 h in dark plastic bags paired with unfumigated ambient. A 20-mL solution of 0.5 mol L^{-1} K_2SO_4 was used and the samples were fumigated and then shaken for 30 min at a rate of 250 r min^{-1}. Samples were centrifuged at $13.1 \times g$ for 10 min and then passed through a 0.45-μm glass fabric filter paper. The filtrates were analyzed for organic C using a TOC analyzer (Shimadzu VCPH/TNM-1, Japan). The difference in organic C in fumigated and unfumigated samples was assumed to originate from MBC. MBC concentrations were corrected for unrecovered biomass using a conversion factor of 0.45. The difference in total N between fumigated and non-fumigated samples was assumed to originate from MBN. The filtrates were used to determine total N (TN) using a continuous flow analytic system analyzer (Skalar San++, Holland. Fitted with a TN unit), and TN was converted to MBN using a factor of 0.54. The ratio of MBC to SOC represents the microbial quotient (qMBC), and the ratio of basal respiration to total MBC represents the metabolic quotient (qCO_2) [40].

2.5. Enzyme Analysis

The Enzyme analysis was conducted following a procedure described in Saiya-Cork et al. [41] and Sinsabaugh et al. [42]. Enzymes assayed in this study included β-1, 4-glucosidase (βG); Cellobiohydrolase (CBH); β-1,4-N-acetylglucosaminidase (NAG); Acid phosphatase (AP); Phenol Oxidase (PHO); Peroxidase (PEO). Suspensions of 1 g soil to 125 mL of acetate buffer at a concentration of 50 mol L^{-1} were prepared for each sample and agitated for 1 min using a Brinkmann Polytron PT 3000 homogenizer. The sample suspensions were continuously mixed with a magnetic stir plate during which 200 mL of the suspensions was portioned into 96-well microplates at 16 replicate wells per sample per assay. Fluorimetric assays were conducted for all enzymes except phenol oxidase and peroxidase. Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filter. After a correction for quenching and negative ambients, all activity units were presented as nmol h^{-1} g^{-1}, and 4-dihydroxyphenylalanine (DOPA) was used as the substrate for spectrophotometrical analysis for phenol oxidase and peroxidase activity. Each sample well had the 50-μL solution of 25 mM DOPA and peroxidase assays had 10-μL of 0.3 % H_2O_2. Blank wells had 200-μL of the sample suspension and 50 μL of acetate buffer. A 50-μL solution of DOPA and 200 μL of acetate buffer were added to negative ambient wells for phenol oxidase. Negative ambient and blank wells for peroxidase had 10 μL of H_2O_2. This produced eight replicate wells for blanks and ambients, and 16 replicate sample wells for each enzyme assay. Microplates were incubated at 20 °C for 18 h in the dark. The activity was considered as a measure of absorbance at 450 nm by using a microplate spectrophotometer.

2.6. Phospholipid Fatty Acids (PLFAs) Analysis

At the end of the incubation, the microbial community structure was determined using the phospholipid fatty acids (PLFAs) analysis as described by Wan et al. [43]. In summary, an extracted mixture of chloroform, methanol, and citrate buffer with a volume ratio of 1:2:0.8 from a 10-g dry sieved soil was used for PLFA analysis. The extraction process included two phases: the chloroform phase and the citrate buffer phase. The lipid materials were recovered and evaporated under nitrogen gas during the chloroform phase. These lipids were then resuspended in chloroform and then fractionated on silicic chromatography acid columns (CNWBOND, 500 mg, 3 mL). Neutral lipids, glycolipids, and phospholipids were eluted with 5.0 mL of chloroform, acetone, and methanol, respectively. Nitrogen gas was used to dry the phospholipid fraction before a mild alkaline methanolysis was conducted to prepare for fatty acid methyl esters. For alkaline methanolysis, one mL of methyl alcohol and methylbenzene with a ratio of 1:1 in volume and then 1.0 mL of methanolic KOH were added to each sample. After swirling to mix, the samples were sealed and placed in a bath at 37 °C for 30 min. Afterward, 2.0 mL of hexane was added to each sample and after swirling 0.2 mL of 1.0 M acetic acid was added to each sample. 2.0 mL of deionized H_2O was added to each sample to break phase and vortex samples for 30 s and then the samples were centrifuged for 2 min. The top phase was transferred to clean labeled 10-mL vials with a short Pasteur pipette. Afterward, 2.0 mL of hexane was added to each sample and then swirled. Nitrogen gas was also used to evaporate these methyl esters and stored at -20 °C until running gas chromatography analysis (GC). For the CG process, 200-µL HPLC-grade ethyl acetate was used to dissolve and separate the individual methyl esters. They were then run on GC with SGE 25QC3 BP-5 25 m × 0.32 µm film in thickness. A chromatographic retention time comparison to bacterial methyl esters was used to identify and quantify the separated fatty acids (Supeloc Bacterial Acid Methyl Esters CP Mix 47080-U). The abundance of individual fatty acids was expressed as nanomoles per gram of dry soil and using standard nomenclature. The following PLFAs were used as biomarkers: i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0 for Gram-positive bacteria (GP), 16:1ω7c, cy17:0, 18:1ω7c, 18:1ω5c and cy19:0 for Gram-negative bacteria (GN), 18:1ω6c and 18:2ω9c for fungi, 10Me16:0, 10Me17:0 and 10Me18:0 for actinomycetes (ACT), and 16:1ω5c for arbuscular mycorrhizal fungi (AMF) [44,45]. Total bacterial PLFAs were calculated as the sum of Gram-negative and Gram-positive bacteria. The ratio of fungal to bacterial PLFAs (F:B) was used to estimate the relative importance of the bacterial and fungal metabolic presence in the community.

2.7. Calculation of Rate, Cumulative Mineralization, and Temperature Sensitivity

The CO_2-C mineralization rate was determined using the following equation:

$$R = k * \frac{\frac{v}{m}}{C} * \frac{\Delta C}{\Delta t} * \frac{273}{(273 + T)} * \frac{12}{44} * 1000 \tag{1}$$

where R is mineralization rate (µg CO_2-C g^{-1} SOC h^{-1}), k is the coefficient of conversion to standard units of CO_2 (1.964 g m^{-3}), v is the jar volume (m^3), m is the soil weight (g) and C is the SOC content (g kg^{-1}), $\Delta c/\Delta t$ is the concentration of CO_2 per unit time (mg kg^{-1} h^{-1}), and T is incubation temperature (°C) [46].

The cumulative CO_2-C mineralization was determined using the following equation:

$$C_m = \sum \left(R * (T_{i+1} - T_i) \right) * 24 \tag{2}$$

where C_m is cumulative mineralization (mg CO_2-C g^{-1} SOC), R is the average mineralization rate between T_i and T_{i+1} (µg CO_2- C g^{-1} SOC h^{-1}), T is incubation time between T_{i+1} and T_i (Day), i is gas sampling time.

The short-term temperature sensitivity (i.e., based on differences in respiration between warmed and un-warmed soils) of soil decomposition for each incubation temperature was determined using the following equation:

$$Q_{10} = \left(C_{high}/C_{low}\right)^{(10/[T_{high}-T_{low}])} \qquad (3)$$

where C is cumulative respiration, T is the temperature (°C), and the subscripts low and high indicate incubation at 20, 30, and 40 °C, respectively [47,48].

2.8. Statistical Analyses

Student's-t test was used to analyze the differences in the measured items (i.e., cumulative mineralization, NH_4^+-N, NO_3^--N, DON, DOC, MBC, MBN, qMBC, qCO_2, soil enzyme activity, and microbial community) between incubation for the different temperature treatments in un-warmed and warmed soil. Two-way ANOVA was performed to examine the effects of soil types and incubation temperatures on the cumulative SOC mineralization. The concentrations of the individual PLFAs (expressed as mol %) were subjected to principal component analysis (PCA) to determine the differences in soil microbial community composition between treatments.

We used a structural equation modeling (SEM) approach to examine the relationships among temperature, soil property, enzyme activity and microbial community and the Q_{10} of warmed and un-warmed SOC mineralization. All the data in the SEMs were scaling by one standard deviation [49]. We used composite variables to explain the collective effects of temperature (20, 30, and 40 °C), soil property (NH_4^+-N, NO_3^--N, DON, DOC, MBC, MBN), enzyme activity (βG, CBH, NAG, AP, PHO, PEO) and microbial community (GP, GN, ACT, AMF, Fungi, Bacteria and F:B) on the Q_{10} of warmed and un-warmed SOC mineralization. Each of the composite variables was selected based on the multiple regression for mass loss rate and Akaike's Information Criterion (AIC). Model fit was assessed using Fisher's C statistic, where good-fitting models yield small C statistics and P values > 0.05 indicate that the data is well represented by the model. Piecewise SEM was based on linear mixed-effects models using the R package piecewise SEM. All the statistical analyses were performed in R v. 4.2.2 and with a significance level of 0.05.

3. Results

3.1. Soil Organic Carbon Mineralization and Its Temperature Sensitivity

Throughout the incubation period, the SOC mineralization rates for all treatments consistently declined (Figure 1a). Both soil types and incubation temperatures significantly influenced the mineralization of SOC, and their interaction was also notable (all $p < 0.001$, Table 2). As the incubation temperature rose, a marked increase in the SOC mineralization rates was observed ($p < 0.05$). By the end of the incubation, the cumulative SOC mineralization of un-warmed soil was highest at 40 °C (663.79 ± 6.70 µg CO_2-C g^{-1}), followed by 30 °C (420.72 ± 12.80 µg CO_2-C g^{-1} soil) and 20 °C (322.14 ± 11.75 µg CO_2-C g^{-1} soil). Similarly, the warmed soil exhibited a trend where cumulative SOC mineralization increased with temperature. However, for every temperature level, the un-warmed soil always displayed higher mineralization than the warmed soil (Figure 1b).

Table 2. Results of two-way ANOVA for responses of the cumulative SOC mineralization to soil type and temperature (20, 30, and 40 °C). ***: $p < 0.001$.

Treatment	Cumulative SOC mineralization						
	1 d	7 d	14d	24d	34 d	49d	63d
Temperature	1010.31***	807.56***	1130.97***	1718.71***	1449.05***	1380.53***	1515.73***
Soil type	249.58***	215.57***	228.79***	386.28***	307.45***	308.32***	349.39***
Temperature × Soil type	10.36***	37.93***	45.45***	58.25***	39.60***	31.91***	29.24***

Regarding temperature sensitivity, variations in Q_{10} were observed for QT20 and QT30 between the two soils (Figure 2). The Q_{10} of 20-30 °C (QT20) and 30-40 °C (QT30) of the two soils varied significantly (Figure 2). The QT20 of the warmed soil (1.31 ± 0.03) was higher than that of the un-

warmed soil (1.63 ± 0.07), but the QT30 of the warmed soil (1.58± 0.04) was lower than that of the un-warmed soil (1.41 ± 0.03) (Figure 2).
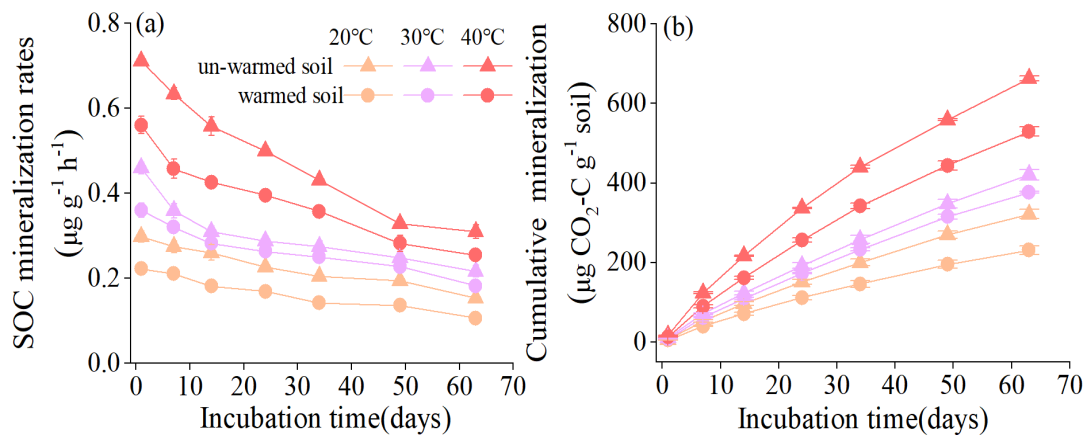

Figure 1. Temporal patterns of SOC mineralization rates (a) and cumulative SOC mineralization during the incubation period (b) at different incubation temperatures (20 °C, 30 °C, 40 °C) in un-warmed soil, and warmed soil. Bars are standard deviation (n = 3). Different capital letters denote significant difference among incubation temperatures and different lower-case letters denote significant difference between un-warmed soil and warmed soil at the same incubation temperature ($p < 0.05$).
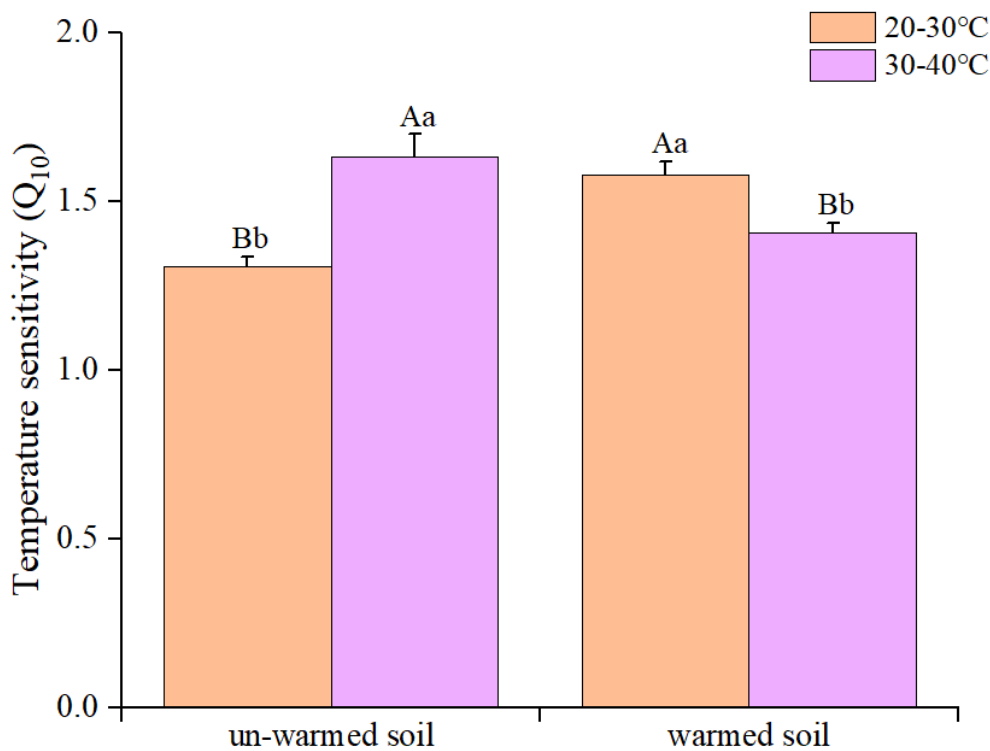
Figure 2. The Q_{10} values of soil organic carbon mineralization of un-warmed soil and warmed soil over two temperature ranges (20-30 °C (QT20) and 30-40 °C (QT30)), respectively. Bars are standard deviation (n = 3). Different capital letters denote significant difference among incubation temperatures and different lower-case letters denote significant difference between un-warmed soil and warmed soil at the same incubation temperature ($p < 0.05$).

3.2. Soil Nutrients, MBC, Microbial Metabolic Quotients

With an increase in incubation temperature, Soil NO_3^--N levels rose significantly in both soil types. For the 40 °C-incubation temperature, significant differences between the two soil types were observed for NH_4^+-N and DON levels (Table 3). Meanwhile, as temperatures rose, there was a decline in DOC and MBC, with un-warmed soils consistently recording higher levels than the warmed soils. For MBN, levels were notably higher at 40 °C for both soil types, with the un-warmed soil showing a more pronounced increase. Variations in soil qMBC and qCO_2 were also influenced by temperature changes, with distinct patterns observed between the two soil types at certain temperatures (Table 3).

Table 3. Mean post-incubation soil ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), dissolved organic N (DON), dissolved organic C (DOC), microbial biomass carbon (MBC), nitrogen (MBN), microbial quotient, and metabolic quotient at different incubation temperatures in un-warmed and warmed soil. Values are expressed as (mean ± standard deviation; n = 3). Different capital letters denote significant difference among incubation temperatures and different lower-case letters denote significant difference between un-warmed soil and warmed soil at the same incubation temperature ($p < 0.05$).

Treatment		NH_4^+-N (mg·kg^{-1})	NO_3^--N (mg·kg^{-1})	DON (mg·kg^{-1})	DOC (mg·kg^{-1})	MBC (mg·kg^{-1})	MBN (mg·kg^{-1})	Microbial quotient (%)	Metabolic quotient (mg CO_2-C g^{-1} MBC h^{-1})
un-warmed soil	20 °C	15.50±0.76Ba	7.73±0.67Ca	1.42±0.27Ba	26.03±1.99Aa	233.18±14.38Aa	23.31±3.11Aa	1.80±0.13Aa	0.66±0.06Ca
	30 °C	14.14±0.55Ba	11.08±0.76Bb	2.57±0.8Ba	18.67±1.68Ba	167.65±9.02Ba	25.82±4.54Aa	1.34±0.22Ba	1.30±0.09Ba
	40 °C	27.88±0.72Aa	15.96±0.51Ab	5.68±0.18Aa	15.88±0.43Ca	94.26±6.12Ca	17.54±2.28Ba	0.78±0.07Ca	3.30±0.38Aa
warmed soil	20 °C	15.95±1.47Ba	6.88±0.28Ca	1.48±0.37Ba	15.71±1.05Ab	178.58±12.37Ab	18.03±3.31Aa	1.44±0.15Ab	0.60±0.08Ca
	30 °C	13.46±2.09Ba	13.32±0.66Ba	1.71±0.15Ba	13.45±0.74Bb	136.89±9.21Bb	20.69±2.27Aa	1.15±0.08Ba	1.34±0.10Ba
	40 °C	19.22±0.63Ab	17.20±0.30Aa	4.54±0.41Ab	10.96±1.08Cb	79.23±7.18Cb	13.32±1.72Bb	0.67±0.06Ca	3.23±0.25Aa

3.3. Soil Enzyme Activities and Microbial PLFAs

Increasing the incubation temperature from 20 °C to 30 °C boosted the activity of several soil enzymes, except AP (Figure 3). When the temperature was further increased, certain enzyme activities, like CBH and NAG, showed a decline. However, oxidase activity in warmed soil was consistently higher than in un-warmed soil across all temperatures (Figure 3).

The microbial community composition was also significantly impacted by temperature changes (Figure 4). All the PLFA biomarkers showed a decreasing trend (except for GP in the un-warmed soil). The PLFA of total microbes, total bacteria, GN, AMF, ACT, and fungus of the warmed soil significantly ($p < 0.05$) decreased compared to those of the un-warmed soil (Figure 4). 40 °C incubation significantly increased the ratio of gram-positive bacteria to gram-negative bacteria (GP:GN) for both soils and the ratio was significantly higher in the warmed soil than in the un-warmed soil (Figure 4). Increasing the incubation temperature significantly decreased F:B for both soils and the ration was also significantly higher in the warmed soil than the un-warmed soil for each incubation temperature (Figure 4). The first principal component of the PLFA pattern explained 82.35 % of the variation in the data, while PC2 explained another 11.21 % (Figure 5). According to the results of anosim analysis,

there were significant differences in microbial community results between soil treatment and temperature treatment ($p < 0.05$) (Figure 5).
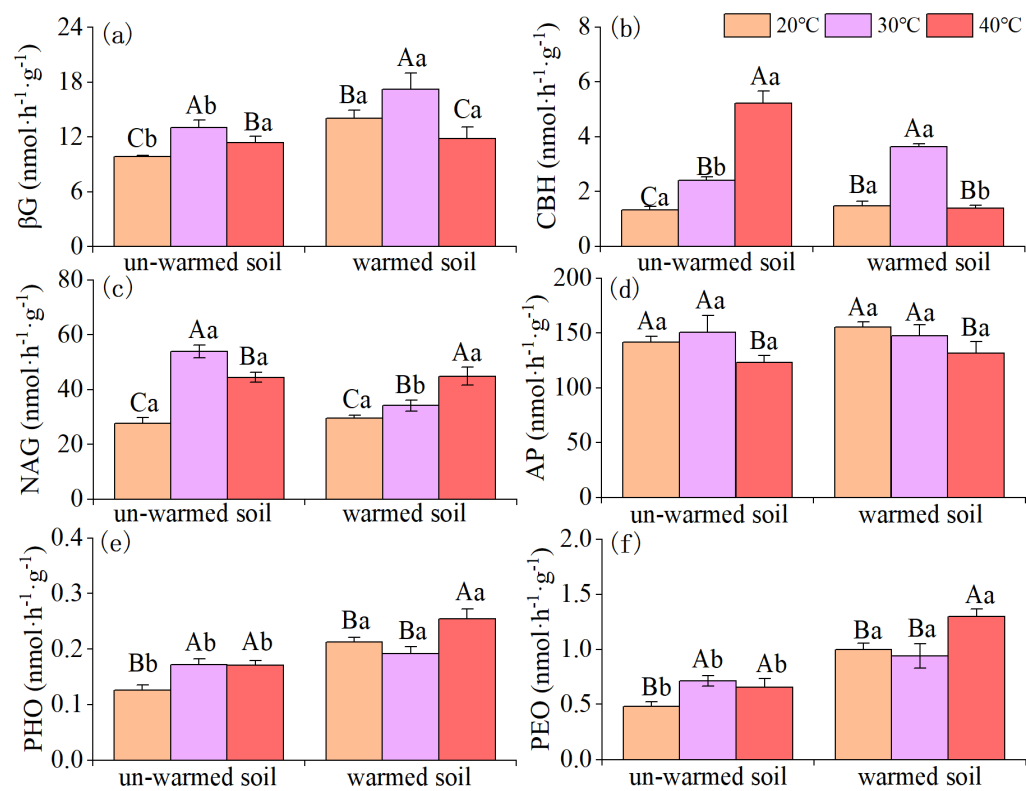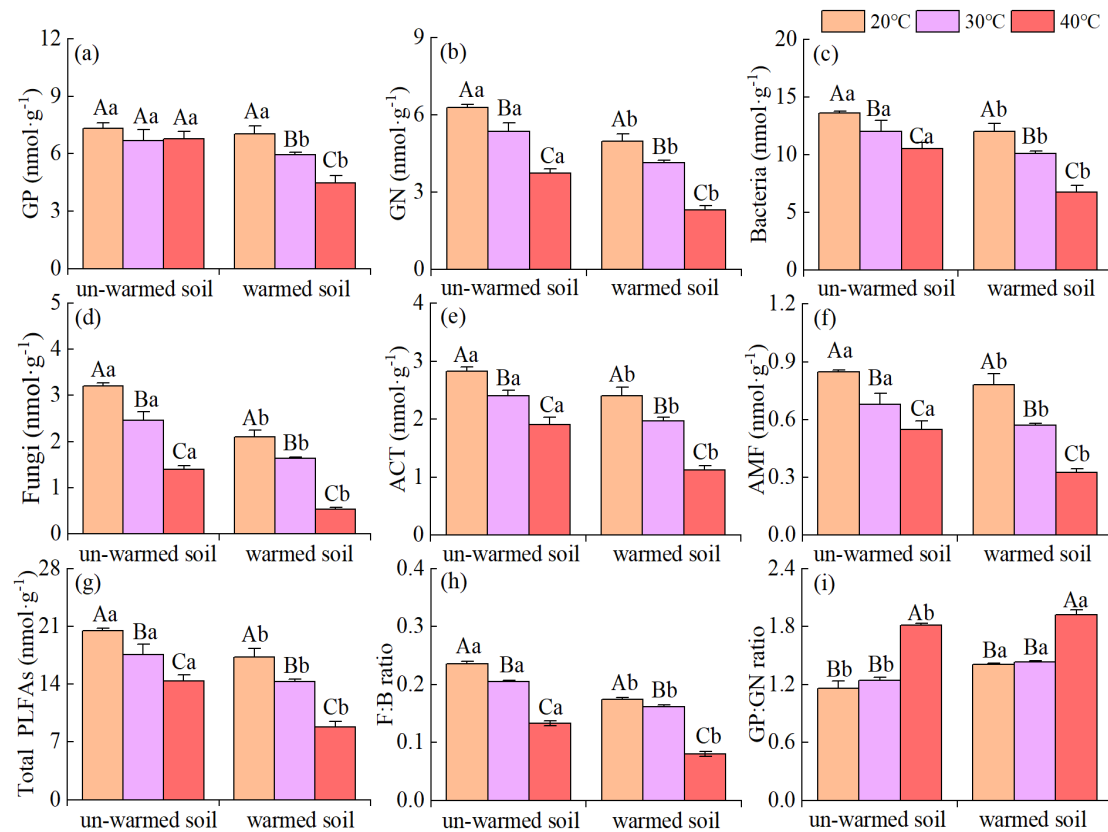

Figure 3. Effects of incubation temperature on soil enzyme activity between un-warmed soil and warmed soil. βG: β-1, 4-glucosidase, CBH: Cellobiohydrolase; NAG: β-1, 4-N-acetylglucosaminidase; AP: Acid phosphatase. PHO: Phenol Oxidase; PEO: Peroxidase. Bars are standard deviation (n = 3). Different capital letters denote significant difference among incubation temperatures and different lower-case letters denote significant difference between un-warmed soil and warmed soil at the same incubation temperature ($p < 0.05$).

Figure 4. Effects of incubation temperature on the phospholipid fatty acid biomarker contents (in nmol g^{-1} soil) between un-warmed soil and warmed soil. Total, total microbial PLFAs; fungi, total fungi PLFAs; total bacteria PLFAs, the sum of GP, GN and unspecific bacteria; AMF, Arbuscular mycorrhiza fungi; ACT: actinomycetes; F:B ratio, the ratio of total fungi to total bacteria PLFAs; GP, total gram-positive bacteria PLFAs; GN, total gram-negative bacteria PLFAs; GP:GN, the ratio of total gram-positive bacteria to gram-negative bacteria PLFAs. Bars are standard deviation (n = 3). Different capital letters denote significant difference among incubation temperatures and different lower-case letters denote significant difference between un-warmed soil and warmed soil at the same incubation temperature ($p < 0.05$).
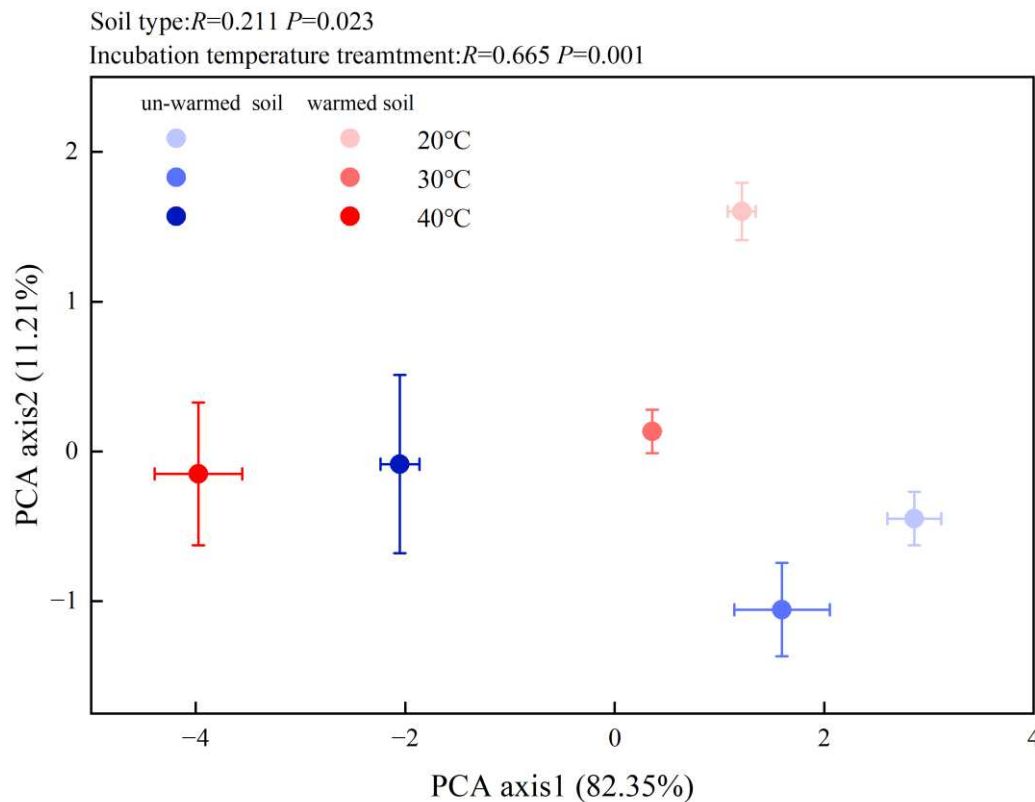
Figure 5. Principal components analysis (PCA) of microbial communities in the soils with three incubation temperatures: 20, 30, and 40 °C. Blue circle: un-warmed soil, red circle: un-warmed soil. The higher the incubation temperature, the darker the color.

3.4. Factors Affecting the Temperature Sensitivity of SOC Mineralization

For the un-warmed soil, the Q_{10} of SOC mineralization showed several distinct relationships. It showed a significant positive correlation with the response ratios (RRs) of NH_4^+-N (Figure 6a) and DOC (Figure S1c). On the other hand, it had a significant negative correlation with the response ratios of NO_3^--N (Figure 6b), MBN (Figure S1b), and enzyme activities such as βG (Figure 6c), NAG, APC (Figure S1f,h), PHO, PEO (Figure 6d, e), and microbial communities such as GN, Fungi, F:B and GP:GN (Figure 6f,g,h,i).

For the warmed soil, it showed a significant negative correlation with the response ratios of NH_4^+-N (Figure 6a) and DON (Figure S1e), as well as with PHO and PEO (Figure 6d,e) and GP:GN (Figure 6i). Conversely, a significant positive correlation was observed with the RRs of NO_3^--N (Figure 6b), βG (Figure 6c) and CBH (Figure S1d), GN, Fungi, F:B (Figure 6f,g,h), AMF, ACT, Total PLFAs and Bacteria (Figure S1i,j,k,l).

The structural equation model explained 97 % and 98 % of the variance in the Q_{10} of un-warmed and warmed soil, respectively (Figure 7). This model depicted that incubation temperature, enzyme activity, microbial community, and soil properties directly and indirectly affected the Q_{10} of both soil types. Notably, while the effects of soil properties on Q_{10} were similar for both soil types, other factors, such as incubation temperature, enzyme activity, and microbial community, showed contrasting impacts on the two soils. For instance, in un-warmed soil, while the incubation temperature exhibited a positive effect on Q_{10}, the soil properties, enzyme activity, and microbial community seemed to diminish Q_{10} (Figure 7a). On the other hand, in warmed soil, both incubation temperature and soil properties had a detrimental effect on Q_{10}, while the enzyme activity and microbial community enhanced its value (Figure 7b).
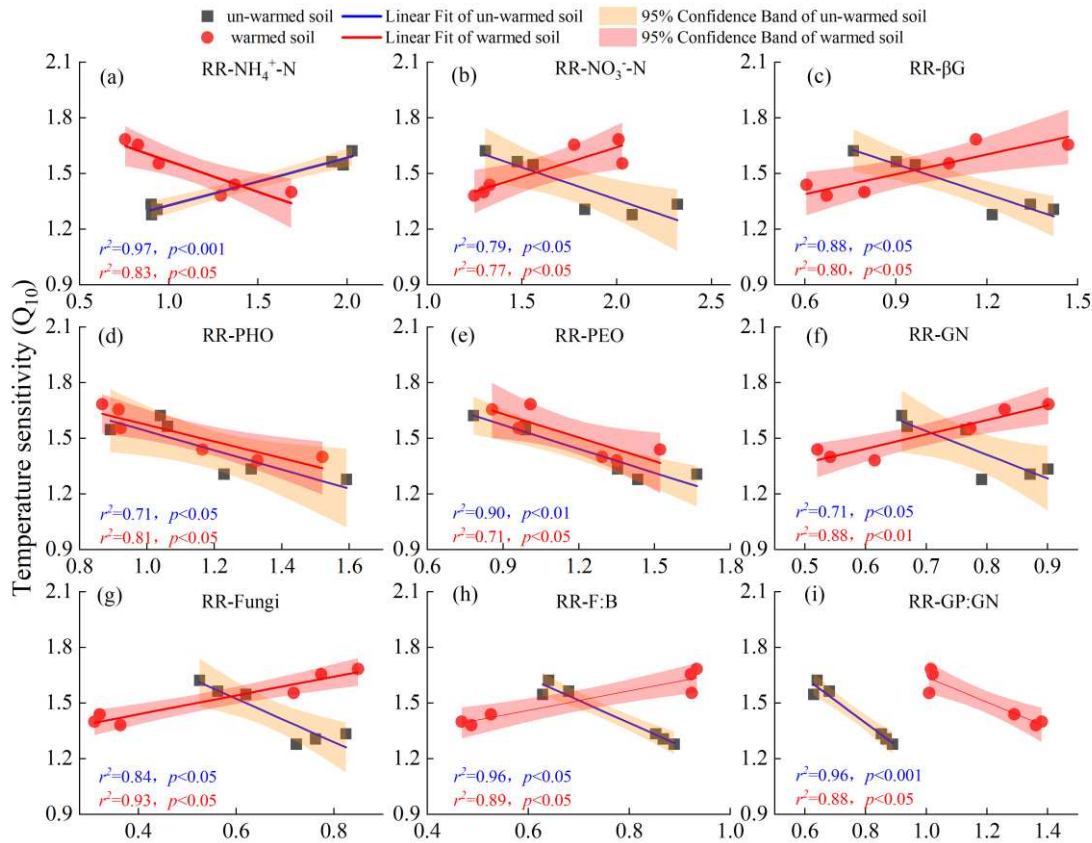
Figure 6. Relationships between temperature sensitivity (Q_{10}) over two temperature ranges (20-30 ℃ (QT20) and 30-40 ℃ (QT30)) and the response ratios (RRs) of NH_4^+-N (a), NO_3^--N (b), βG (c), PHO (d), PEO (e), GN (f), Fungi (g), F:B (h) and GP:GN (i). Black square and red point represent correlations in un-warmed and warmed soil, respectively. Blue and red lines represent relationships in un-warmed soil and warmed soil, respectively. Orange and red ranges represent 95% confidence interval in un-warmed soil and warmed soil, respectively.
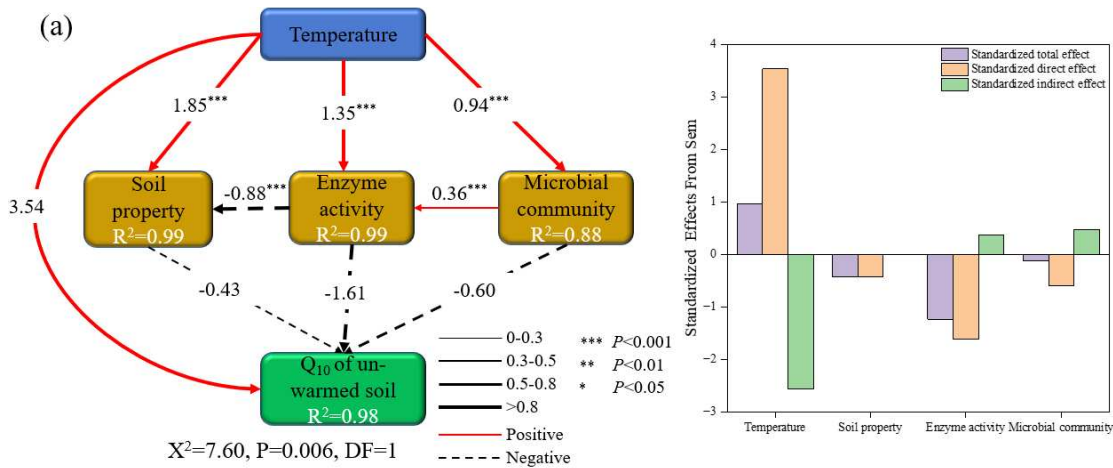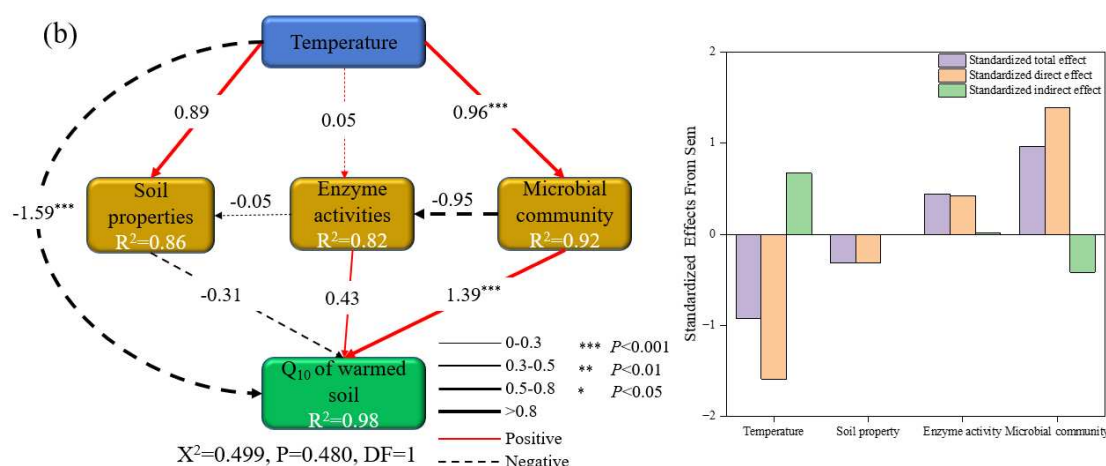
Figure 7. Structural equation model exploring the direct and indirect effects of incubation temperature, soil propertiy, enzyme activity and microbial communitiy on the Q_{10} of soil organic carbon mineralization (a: un-warmed soil: The final results of model fitting were: $C = 7.60$, $df = 1$, $P = 0.006$, AIC = 35.60, BIC=32.68, b: warmed soil: The final results of model fitting were: $C = 0.50$, $df = 1$, $P = 0.48$, AIC =28.50, BIC=25.58). The soil properties are contents of NH_4^+-N, NO_3^--N, MBC, MBN, DON and DOC. The enzyme activities are contents of βG, CBH, APC, NAG, PHO and PEO. The microbial communities are contents of GP, GN, ACT, AMF, Fungi, Bacteria and F:B. The blue boxes indicate the incubation temperature; the green boxes indicate the target factor (e.g. the Q_{10} of soil organic carbon mineralization of un-warmed soil and warmed soil, respectively); the orange boxes indicate the Biological and abiotic factors (e.g. soil properties, microbial communities and enzyme activities). The numbers adjacent to the arrows are standardised path coefficients. The solid lines indicate a positive effect and the dashed lines indicate a negative effect. The thickness of the lines indicates the size of the coefficients. The proportion of variance explained (R^2) appears alongside each response variable in the model.

4. Discussion

4.1. Response of SOC Mineralization of Un-Warmed and Warmed Soil to Warming

The enhancement of SOC mineralization rate by the increased incubation temperatures was evident for both un-warmed and warmed soil (Figure 1a). Additionally, the cumulative SOC mineralization from un-warmed soil was consistently higher than that from warmed soil at the same incubation temperature (Figure 1b). This aligns with many studies suggesting that higher temperature can intensify C mineralization and subsequently increase soil CO_2 emission [22,48,50]. For instance, Gudasz et al. [51] also found that cumulative SOC mineralization escalated with increasing temperatures. Furthermore, our study indicated that greater SOC mineralization in un-warmed soil could be linked to the higher DOC and MBC levels in un-warmed soil than in warmed soil. Specifically, labile SOC contents, including DOC and MBC, were found to be 39-66 % and 20-31 % greater in the un-warmed soil (Table 3). For example, in the Great Hing'an Mountains in temperate northeast China, between 1.3-2.1 % of SOC was mineralized over a 42-day period at 15 °C without ^{13}C-glucose [19]. Similarly, research from Northeastern China's permafrost peatlands demonstrated 0.6-11.1 % decreases in SOC after 90-day incubation at 15 °C [18]. Contrastingly, in tropical and subtropical forests, SOC mineralization recorded between 2-7 % [52,53]. The positive relationship we observed between incubation temperature and soil NO_3^--N, NH_4^+-N, and DON (Table 3) mirrors previous findings. Warming has been shown to enhanced net N mineralization and nitrification rate, leading to N loss from ecosystems [54]. Additionally, Dawes et al. [55] and Melillo et al. [56] reported that experimental soil warming in temperate forests stimulated soil organic N mineralization. Lastly, temperature also impacts SOC mineralization by modulating soil microbial activity and community composition [57]. At the same temperature, the cumulative SOC mineralization of warmed soil was lower than un-warmed soil, because the PLFA of total microbes, total bacteria, fungi, and F:B

significantly ($p < 0.05$) decreased (Figure 4). The greater SOC in the temperate region than the tropical and subtropical regions but the cumulative mineralization is not much higher, which imply that there was likely more sensitive to global warming than temperate and boreal forests in the tropical and subtropical region [58].

4.2. Response of the Q_{10} of SOC Mineralization of Un-Warmed and Warmed Soil to Rising Incubation Temperature

Q_{10} is strongly affected by substrate availability and soil microbial characteristics [23,59]. In our observations, the QT20 treatment displayed a peculiar trend where the Q_{10} of warmed soil exceeded that of un-warmed soil. This suggests that at this range, the sensitivity of the un-warmed soil changes might be somewhat reduced. This behavior aligns with Bradford et al. [60], who pointed out potential reductions in labile C and thermal adaptation of microbial decomposers as factors reducing temperature sensitivity of SOC mineralization. Furthering this line of thought, Domeignoz-Horta et al. [12] proposed that metrics closely associated with microbial biomass-such as total organic carbon and size of the extracellular enzymatic pool- alongside the temperature sensitivity of extracellular enzyme activity, played pivotal roles in dictating respiration temperature sensitivity. Delving deeper into enzyme activity, we noticed a significant uptick in PEO and PHO enzyme activities for un-warmed soil as incubation temperature rose from 20 ℃ to 30 ℃, whereas warmed soil showcased no such pronounced change. Both the un-warmed soil and warmed soil displayed a negatively correlation with the response ratio of oxidases enzymes activity. Interestingly, in the QT20 treatment, the Q_{10} of the warmed soil surpassed that of the un-warmed soil, hinting at the former's heightened sensitivity within this temperature range. This observation contrasts with findings from numerous studies that have reported a higher Q_{10} at reduced temperatures [61]. Another intriguing point is that warmed soil SOC mineralization Q_{10} was not correlated with the RRs of MBC and DOC (Figure S1a,c). This is different from many studies, which reported substrate availability had a significant effect on temperature sensitivity [22,62,63]. The decrease in microbial quotient combined with the increase in metabolic quotient indicated that warming increased microbial respiration rather than microbial growth, and the higher qCO_2 indicated a lower assimilation rate and higher maintenance C demand [64]. According to the metabolic theory [65], if microbes are active for a long period, cumulative maintenance respiration would be large but the population size of microbes would be small. This might explain the higher qCO_2 yet reduced MBC at the highest incubation temperature.

In the QT30 treatment, the Q_{10} of warmed soil was significantly lower than that of un-warmed soil. Our results indicated that both DOC and MBC decreased with increasing incubation temperature; moreover, the levels of DOC and MBC in un-warmed soil were significantly higher than those in warmed soil at the same incubation temperature (Table 3). With the increase of incubation temperature (30 ℃increased to 40 ℃), the PEO and PHO of warmed soil did not increase significantly, but un-warmed soil was no significantly. Our study showed that increased incubation temperature stimulated the activity of C-degrading enzymes (βG and CBH) and the acid-resistant C-degrading enzymes (PHO and PEO) (Figure 3). The Q_{10} also was significantly related to the response ratios of βG and CBH enzymes activity, as well as PHO and PEO activities. The N-degrading enzyme activity increased with increased incubation temperature, but the P-degrading enzyme activity showed a downward trend. The Q_{10} of un-warmed soil was significantly correlated with the RRs of NAG and AP. The result on enzyme activity in the present study indicates that incubation temperature can differentially affect the extractable C- and acid-resistant C-degrading enzymes and consequently change the SOC decomposition, and the decrease of labile SOC of warmed soil will also accelerate the decomposition rate of non-labile SOC, because oxidases enzymes are responsible for decomposing recalcitrant C fractions, such as lignin and humus [66]. Compared with warmed soil, the Q_{10} of organic carbon decomposition of un-warmed soil is higher at 40 ℃, which may be because its microorganisms still have higher nutrient acquisition as the temperature increases, since nutrient availability also has a significant effect on the Q_{10} values [67].

The profound of global warming on the microbial mineralization of soil organic matter are well-documented [68]. The important role microbes play in modulating the temperature sensitivity of

SOM decomposition has seen increased interest [23,69]. Our research corroborates this, highlighting that a surge in incubation temperature drastically reduces the total PLFAs, bacteria, fungi, GN, AMF, and ACT. Interestingly, warmed soil consistently higher than un-warmed soil under identical temperature treatment (Figure 4), likely because warming amplifies environmental stressors affecting soil microorganisms. SOC Mineralization is predominantly by bacteria and fungi [2]. In our study, soil microbial community composition shifted after incubation, there were significant differences in microbial community results between soil treatment and temperature treatment ($p < 0.05$) (Figure 5). Fungi are associated with a slow energy channel (slow turnover of more acid resistant and N-poor substrates, leading to high soil-C accumulation). In contrast, bacteria are associated with a fast energy channel (fast turnover of extractable and N-rich substrates), leading to low soil-C accumulation [70]. Our result showing that the higher GP:GN at 40 °C (Figure 4) and F:B decreased with increasing incubation temperature, which implies possible bacterial-dominated microbial communities. The shift in microbial communities could be the underlying reason for disparities in SOC mineralization across different incubation temperatures. Furthermore, Li et al. [8] show that the Q_{10} increased with the decrease of F:B. In our study, the Q_{10} of warmed soil SOC mineralization was negatively correlated with GP:GN (Figure 6i), while demonstrating a positively correlation with GN, Fungi, F:B (Figure 6f,g,h), AMF, ACT, Total PLFAs and Bacteria (Figure S1i,j,k,l). The structural equation model showed that incubation temperature exerts both direct and indirect effects on the Q_{10} of un-warmed soil. In contrast, for warmed soil, these effects manifest predominantly through microbial community shift (Figure 7). Therefore, it is plausible that the divergent temperature sensitivity between un-warmed soil and warmed soil across varied incubation temperatures are primarily driven by microbial community structure.

5. Conclusions

Our comprehensive study underscores the multifaceted impacts of experimental warming on SOC mineralization dynamics. The research distinctly illuminates the intensified SOC mineralization under elevated temperatures, concomitant with a marked reduction in soil DOC concentrations. Intriguingly, the escalated incubation temperatures induced discernible shifts in soil microbial communities, characterized by a decline in total microbial biomass, PLFAs, and the abundance of key microbial taxa, including bacteria, fungi, and GN. The qMBC and qCO_2 patterns further attest to the dominance of microbial respiration processes in the warmed conditions, as opposed to microbial growth. Central to our findings is the revelation that the Q_{10} of SOC mineralization is intricately tethered to alterations in soil nutrients, enzymatic activities, and microbial community structures, especially the fungi to bacteria ratio. The nuanced disparities in Q_{10} values between un-warmed and warmed soils across varying incubation temperatures accentuate the evolving nature of the warming effects on SOC mineralization. As global temperatures continue their upward trajectory, these insights become paramount, urging the scientific community and policymakers to factor in the dynamic responses of SOC mineralization when forecasting the soil C feedbacks to ongoing climatic changes.

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