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Effects of Litter Input on Temperature Sensitivity of Soil Organic Carbon Mineralization along a Forest Elevation Gradient

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Abstract: The mineralization of soil organic carbon (SOC) is generally stimulated under global warming, known as temperature sensitivity (Q_{10}), which is critical for predicting terrestrial C-climate feedback. However, how Q_{10} varies in different elevations, particularly with litter input, constraining the establishment of accurate models remains poorly understood. Here, the soil samples of three elevations (750, 950, and 1150 m) were collected from the transition zone of subtropical and warm temperate forests in China for incubation. Soils were incubated with and without ^{13}C-labeled *Cunninghamia lanceolata* litter at 15 °C and 17.4 °C for 97 days. Incubation process was divided into two stages (0–37 days and 38–97 days) according to the dynamics of CO_2 emission. The results showed that Q_{10} did not change significantly with elevation in the first stage, but Q_{10} at 950 m was significantly higher than that at 1150 m in the second stage. The variations in Q_{10} with elevation were regulated by pH in the first stage, while soil C/N was the primary factors that regulated Q_{10} in the second stage. Q_{10} showed no response to litter input at 750 m, while it decreased at 950 m in both stages. Following litter input, Q_{10} at 1150 m increased in the first stage but kept stable in the second stage. The change in Q_{10} with litter input was mainly affected by the restriction of soil P availability. Overall, our findings emphasized the importance of vertical spatial heterogeneity of Q_{10} of SOC mineralization in order to improve the prediction accuracy of C dynamics in terrestrial ecosystems.

Keywords: SOC mineralization; temperature sensitivity; vertical heterogeneity; litter input; global warming

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1. Introduction

The mineralization of soil organic carbon (SOC) is the second-largest carbon (C) flux in terrestrial ecosystem [1], releasing 98 ± 12 Pg C every year, thus its tiny changes may greatly affect the concentration of CO_2 in the atmosphere [2,3]. Under the global warming scenario, the accelerated mineralization of SOC and ultimately releasing the largest amount of CO_2 from soils to the atmosphere. This feedback was termed as Q_{10} quantified, a factor by which the rate of SOC mineralization is multiplied when temperature rises by 10 °C [4,5]. Furthermore, there has been promoted plant growth by warming, increasing the amount of litter imported into the soil. The increase of litter input mainly changed Q_{10} through nutrient changes and microbial community composition and activity [3,6]. However, soil nutrients and microbial community composition and activity may differ along the elevation gradient. Thus, there has been considerable uncertainties in the response of Q_{10} to litter input, especially over different spatial gradients. Therefore, it is necessary to determine the vertical heterogeneity of Q_{10} and its response to litter input, in order to improve the prediction of the magnitude and direction of soil C-climate feedback in terrestrial ecosystems.

Q_{10} has been widely studied in terrestrial ecosystems [6,7], yet great uncertainties in the spatial heterogeneity of Q_{10} constrain the establishment of accurate C-climate models [8]. So far, most studies concerning the spatial distribution of Q_{10} focus on the horizontal pattern [7–9]. In contrast, less attention paid to the vertical variations in Q_{10}. However, a growing body of studies demonstrated that the Q_{10} of SOC mineralization varies greatly along vertical spatial gradient, with significant increases [10], decreases [11] or no change in Q_{10} with elevation [12]. In addition, the critical role of soil properties, microbial community composition and activity in regulating Q_{10} have been emphasized in previous studies, however, the mechanism and regulatory factors of the vertical heterogeneity of Q_{10} are not clear [12,13].

In this field, the Q_{10} of SOC mineralization is closely associated with substrate availability, which could be impacted by litter input [14]. Generally, litter input stimulates the mineralization of SOC by stimulating soil microbial activity, which is termed as priming effect [15]. However, whether litter input affects the Q_{10} of SOC mineralization remains unclear [16,17]. Labile organic carbon (LOC) is enriched [18,19] and nutrient availability is altered [20] following litter input, affecting soil extracellular enzyme activity [21], which may lead to changes in Q_{10} [22]. For instance, some recent studies found that litter was recalcitrant to soil microorganisms and thus reduces Q_{10} value [23,24]. Moreover, litter input was affected by differences in microbial community composition at different elevations. Consequently, it is a complicated task to understand the response of Q_{10} to litter input, particularly along elevation gradients. In particular, it is not clear how Q_{10} will react to garbage input at different elevations.

Most previous studies on Q_{10} mainly focused on subtropical zone and warm temperate zone [25,26]. In addition, transition zones are more sensitive to changes in global change, due to their relatively unstable composition. However, few studies concerned the C dynamics in transition zone with greater vegetation and microbial diversity under global change. Thus, soil C cycling and their responses to climate might be different from those in subtropical zones and warm temperate zones. In this study, soils from three elevations in the transition zone of subtropical and warm temperate forests were collected and incubated with and without ^{13}C-labelled litter at 15 °C and 17.4 °C, respectively. The main objectives of this study were: (1) to reveal the spatial pattern of Q_{10} of SOC mineralization at different elevations; (2) to clarify the response of Q_{10} to litter input at different elevations.

2. Materials and Methods

2.1. Site Description and Soil Sampling

The soil used in this experiment was collected at the Mazongling experimental forestry station in Anhui province (115°31′~115°50′ E, 31°10′~31°20′ N). The climate type is a subtropical humid monsoon climate, with a mean annual temperature (MAT) that range from 13–15 °C and a mean annual precipitation (MAP) of about 1510 mm. The soils of three elevations (750; 950; 1150 m) are sandy loam, loamy sandy and loam, respectively, with distinct soil properties (Table 1). *Cunninghamia lanceolata* is the dominant species.

Table 1. Properties of soils at different elevations. The data are expressed as the means ±SE (n = 4). Different letters followed data in the same row denote significant difference at $p < 0.05$.

Soil Properties	Elevation (m)		
	750	950	1150
SOC (g kg^{-1})	48.56 ± 6.03 b	37.86 ± 2.54 b	70.67 ± 1.9 a
TN (g kg^{-1})	3.60 ± 0.27 b	2.97 ± 0.19 b	5.28 ± 0.20 a
C/N	13.36 ± 0.67 a	12.75 ± 0.14 a	13.41 ± 0.15 a
TP (mg kg^{-1})	0.14 ± 0.01 b	0.29 ± 0.03 a	0.12 ± 0.01 b
Available P (mg kg^{-1})	39.66 ± 4.63 b	56.85 ± 4.60 a	10.22 ± 1.33 c
Mineral N (mg kg^{-1})	13.34 ± 4.22 b	6.94 ± 0.34 b	26.90 ± 4.18 a
LOC (mg kg^{-1})	12.22 ± 2.35 b	9.49 ± 0.52 b	18.43 ± 0.58 a
DOC (mg kg^{-1})	1.22 ± 0.24 b	0.95 ± 0.06 b	1.85 ± 0.06 a
pH	5.07 ± 0.01 a	5.25 ± 0.12 a	5.26 ± 0.11 a
K^+ (mg kg^{-1})	141.90 ± 34.36 a	111.31 ± 23.63 a	201.12 ± 47.44 a
Mg^{2+} (mg kg^{-1})	57.06 ± 24.06 a	37.92 ± 10.03 a	70.41 ± 26.47 a
Ca^{2+} (g kg^{-1})	1.23 ± 0.48 a	0.72 ± 0.13 a	1.18 ± 0.34 a
Na^+ (mg kg^{-1})	5.74 ± 4.08 a	4.26 ± 1.42 a	6.42 ± 2.68 a
Sand content (%)	64.90 ± 4.91 b	81.51 ± 1.38 a	47.53 ± 2.71 c
Silt content (%)	23.12 ± 3.56 b	11.61 ± 1.32 c	41.49 ± 2.57 a
Clay content (%)	11.98 ± 1.82 a	6.88 ± 0.30 b	10.99 ± 0.56 a

The soil samples were collected at a layer of 0–20 cm from four 20 × 20 m plots at three elevations after removing the litter on the soil surface. Twenty soil cores were randomly collected from each plot using a metal sampler and mixed as composite samples. Fresh soil samples were stored in sealed bags and immediately transported to the laboratory and sieved through a 2 mm mesh after removing visible organic debris, roots, and stones. There are four samples with spatially independent samples from each elevation, respectively. In total, 12 soil samples were collected in our study. Part of the soil samples were air-dried naturally for analysis of physical and chemical properties, and the other part was stored in a refrigerator at 4 °C. Samples for PLFA were freeze-dried and stored at −20 °C prior to lipid extraction.

2.2. Experimental Design and Soil Incubation

In this study, the elevation represents an increase of MAT, and the temperature increase interval of 2.4 °C was set according to according to temperature decrease by 0.6 m for every 100 m increase. Therefore, four treatments were set up, with four replicates at each elevation: (1) soil without litter at 15 °C; (2) soil with litter added at 15 °C; (3) soil without litter at 17.4 °C; (4) soil with litter added at 17.4 °C. A total of 100 g of soil (dry weight) for each replicate of each treatment was placed in a 1 L Mason jar for incubation. The litter is cut into a 2 mm sieve and mixed evenly with the soil. The added litter is 5% SOC Chinese fir litter, which are labeled ^{13}C and the abundance was 255‰. The temperature is increased by 2.4 °C using a fully automatic incubator. The soil moisture is maintained at 50% of water hold capacity during incubation. Close air compressor pump and the valve on the bottle cap after 1 min ventilation. The Mason jar is sealed for 24 h and then air is collected. Air is collected after 1, 4, 7, 11, 15, 21, 27, 37, 47, 57, 67, 77, 87 and 97 days of the incubation. Soil moisture was supplemented regularly (every 15 days) to ensure that the soil water loss rate is less than 2%.

2.3. Determination of Soil Physical and Chemical Properties

Soil texture (<53 μm, 53–250 μm, >250 μm) was measured by wet screening method [27]. The pH at a soil: water ratio of 1:2.5 (*w*/*v*) was measured using soil pH meter (PB-

10C, Sartorius, Germany). SOC and total nitrogen (N) contents were determined by elemental analyzer (Elemementar Vario ELIII, Germany). The contents of exchangeable cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) in soil, mineral N ($NH4^+$-N and NO_3^--N), total P and available P concentrations were determined using a continuous flow analyzer (AA3, Seal Analytical, Germany), according to Lu (2000) [28]. LOC content was determined through KMnO4 oxidation as described by Blair et al. (1995) [29]. DOC was determined by total organic carbon analyzer (TOC-VCPH, Shimadzu, Kyoto, Japan). Simply, soil solution was shaken by in a shaker (300 r/min) for 30 min to filter and obtain supernatant for measurement.

2.4. ^{13}C labeled Litter Preparation

The two-year old Chinese fir seedlings were planted in a closed incubator with constant temperature and humidity for 5 months. During the incubation period, CO_2 gas labeled with ^{13}C was introduced. After that, the leaves were picked, deactivation of enzymes at 105 °C for 2 h, and dried to constant weight at 80 °C. Finally, the leaves were crushed for subsequent incubation experiments. The basic properties of Chinese fir leaves are as follows: total C content is 465.77 g/kg, total N content is 14.91 g/kg, C/N is 31.4, and $\delta^{13}C$ value is 255‰.

2.5. Partitioning CO_2 Sources and Q_{10} Calculations

In order to calculate the amount of carbon dioxide removed from the soil organic matter and the original organic carbon mineralization during the cultivation period, the following formula was used:

$$C_F = C_T(\delta_T - \delta_S/(\delta_F - \delta_S) \quad (1)$$

$$C_S = C_T - C_F \quad (2)$$

In the Equations (1) and (2), C_T ($C_T = C_L + C_S$) is the total amount of CO_2–C during the considered time interval and δ_T is the corresponding isotopic composition. δ_T corresponds to the isotopic composition; C_F is the carbon amount produced by fresh organic matter (FOM); δ_F is the added FOM isotopic composition; C_S is the carbon amount obtained from SOC; δ_S is the control soil ^{13}C abundance of exhaled CO_2. To determine the temperature sensitivity of mineralization rate of SOC, the following formula was used:

$$Q_{10} = (F_1/F_2)^{10/(T1-T2)} \quad (3)$$

F_1 is the mineralization rate of SOC at a higher temperature [mg(C–CO_2)·kg^{-1}·h^{-1}], F_2 is the mineralization rate of SOC at a lower temperature [mg(C–CO_2)·kg^{-1}·h^{-1}]; T_1 and T_2 are the incubation temperature (°C).

2.6. PLFA Analysis

PLFA extraction was used to determine the abundance of microbial communities, and analyses were carried out, according to Wang et al. (2013b) [30]. In short, 5 g of freeze-dried soil was extracted for 2 h with a chloroform:methanol:phosphate buffer (1:2:0.8), and the phospholipids were separated from the other lipids on a silicic acid column. Samples were analyzed on an Agilent 6890 gas chromatograph with a flame ionization detector and an Ultra-2 column after the addition of fatty acid 19:0 as an internal standard. To identify the peaks, we employed the fatty acid methyl ester and BAME controls. Biomarkers PLFAs were used to access the major taxa of microorganisms and classify them according to Joergensen (2021) [31]. The i14:0, i15:0, a15:0, i16:0, i17:0, i18, a15:0, a16:0, a17:0, a18:0 and a 19:0 PLFAs served as markers for gram-positive (G+) bacteria, whereas the

cy17:0, cy19:0, 16:1ω7, 16:1ω9, 17:1ω8 and 18:1ω7 functioned as markers for gram-negative (G-) bacteria. Besides, fungi were designated by the letters 16:1ω5c, 18:1ω9c, 18:2ω6c and 18:3ω6,9,12 and the 10Me 16:0, 10Me, 17:0 and 10Me 18:0 PLFAs were markers for actinomycetes. The total number of PLFA biomarkers was considered to be representative of the microbial biomass.

2.7. Enzyme Kinetics

β-glucosidase (BG), N-acetyl aminopeptidase (NAG), Cellulase (Cello), Xylanase (Xyl), Leucine aminopeptidase (Leu) and Tyrosinase (Tyr) were determined by 96-well enzyme plate. According to the method provided by German et al. (2011) [32] and Razavi et al. (2015) [33]. The specific steps are as follows: 1 g fresh soil was weighed in a hydrolytic flask, 50 mL ultra-pure water was added, and the soil was shaken on a shaker for 40 min, followed by low energy ultrasound (40 J S^{-1} output energy) 2 min. Then add 50 μL soil solution, 100 μL substrate solution and 50 μL buffer solution [MES ($C_6H_{13}NO_4SNA_{0.5}$), (pH:6.5) for MUF substrate, TRIZMA ($C_4H_{11}NO_3$ HCl, $C_4H_{11}NO_3$), (pH: 7.2) for AMC substrates]. The plate was put into the microplate reader and measured at 0 min, 1 h and 2 h under the excitation wavelength of 360 nm and emission wavelength of 460 nm. Enzyme activity was expressed as MUF or AMC release in nmol per g dry soil per hour (nmol g^{-1} soil h^{-1}). The enzyme activity was calculated by Michaelis–Menten equation, designated as formula:

$$V = Vmax[S]/(Km + [S])$$

Vmax is the maximum rate of enzyme activity. *K*m is the half-full sum constant, the concentration of the substrate at 1/2 Vmax, and [S] is the concentration of the substrate.

2.8. Statistical Analysis

One-way ANOVA was used to analyze differences in soil's physical and chemical properties and microbial community composition among different elevations. Repeated measurement ANOVA was used to analyze the influence of elevation, litter input and their interaction to Q_{10}, and Tukey's HSD test was used to test the significance of differences at $p = 0.05$ level. Pearson correlation was used to detect the relationship between Q_{10} and soil's physical and chemical properties and microbial community composition. Besides, Origin (2021) was used to perform linear fitting of Q_{10} with soil's physical and chemical properties and *K*m of N enzyme, respectively, the linear fitting of SOC cumulative mineralization and available nutrients were also carried out. SPSS Version 23.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the data.

3. Results

3.1. Soil Environmental Conditions at Different Elevations

The content of SOC, TN, mineral N, LOC and DOC were generally lower at medium elevation (950 m) than 750 m and 1150 m (Table 1). The soil samples at medium elevation have the highest sand content ($p < 0.05$). Furthermore, fungal PLFA were significantly higher at 750 m than those at medium elevation (Table 2, $p < 0.05$). The gram-negative bacteria at medium elevation were significantly lower than those at 750 m and 1150 m ($p < 0.05$).

Table 2. PLFAs of soils at different elevations. The data are expressed as the means ± SE (n = 4). Different letters followed data in the same row denote significant difference at $p < 0.05$.

PLFAs	Elevation (m)		
	750	950	1150
Fungi (nmol g^{-1})	7.65 ± 0.63 a	3.27 ± 1.83 b	6.13 ± 0.33 ab
Bacteria (nmol g^{-1})	24.67 ± 3.26 a	19.36 ± 3.97 a	21.76 ± 1.27 a
Fungi/Bacteria	0.32 ± 0.04 a	0.15 ± 0.07 b	0.28 ± 0.03 ab
Gram-positive bacteria (nmol g^{-1})	7.51 ± 0.86 a	7.01 ± 0.96 a	6.30 ± 0.40 a
Gram-negative bacteria (nmol g^{-1})	12.82 ± 0.96 a	7.48 ± 1.58 b	11.15 ± 0.32 a
Gram-positive/Gram-negative bacteria	0.59 ± 0.05 b	1.07 ± 01.7 a	0.57 ± 0.04 b
Actinomycetes (nmol g^{-1})	2.77 ± 0.47 a	2.15 ± 0.51 a	2.58 ± 0.46 a
AM Fungi (nmol g^{-1})	1.00 ± 0.01 a	0.76 ± 0.10 a	0.81 ± 0.06 a
Total microbial PLFA (nmol g^{-1})	27.35 ± 5.20 a	22.23 ± 6.27 a	23.81 ± 1.97 a

3.2. The Influence of Elevations on Q_{10}

The CO_2 emission rate roughly showed a similar trend in the whole incubation period at different elevations. It declined gradually during the first 37 days, and then remained basically unchanged with some fluctuations (Figure 1). Hence, incubation process was divided into two stages (0–37 days and 38–97 days), according to the dynamics of CO_2 emission. The Q_{10} of SOC mineralization at different elevations varied from 1.92 to 4.15 in two stages (Figure 2). Q_{10} kept stable with elevation in the first stage (Figure 2A), while Q_{10} at medium elevation was significantly higher than at high elevation during the second stage (Figure 2B, $p < 0.05$). Q_{10} was mainly influenced by soil's physical and chemical properties (Figure 3). Specifically, Q_{10} was positively related to pH in the first stage (Figures 3 and 4A, $p < 0.05$), while negatively correlated with C/N and soil fertility (SOC, TN, mineral N) in the second stage (Figures 3 and 4B, $p < 0.05$).

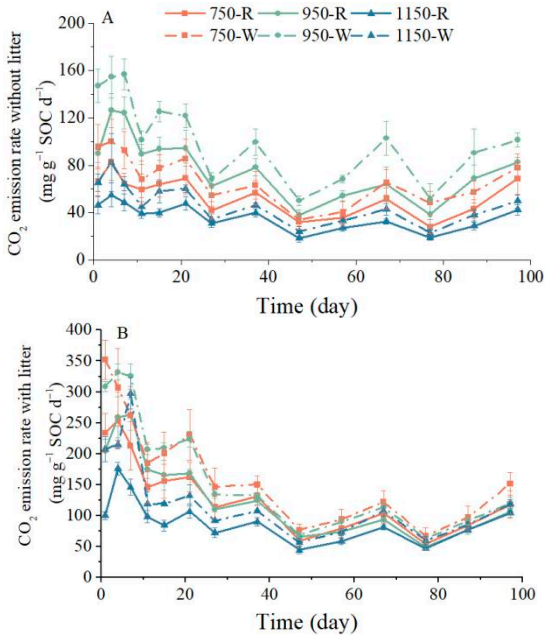

Figure 1. Dynamics of CO_2 efflux rate from control (**A**) and litter-mended soils (**B**) along the different elevations (750, 950, 1150 m) during the 97 day incubation period. R and W represent room temperature and warming temperature, respectively. The vertical bars are standard errors.

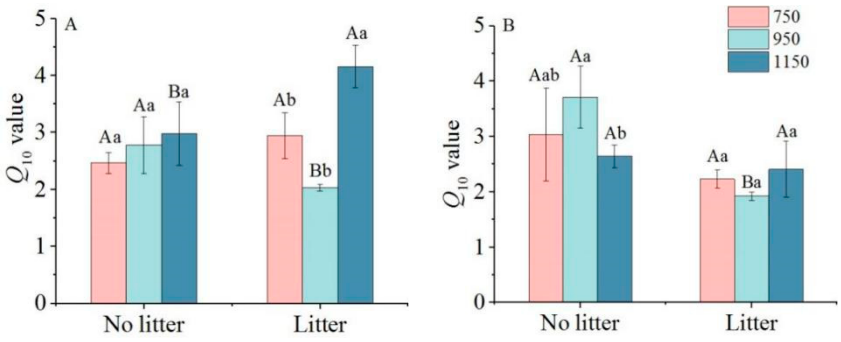

Figure 2. Temperature sensitivity (Q_{10}) of SOC in the control (no litter) and treated (litter input) soils from different elevations (750 m, 950 m, 1150 m) during the 0–37 days (**A**) and 37–97 days (**B**) incubation period, respectively. Values are expressed as the means ± SE (n = 4). Lower case letters and the capital letters indicate significant differences between elevations and litter, respectively (p < 0.05).

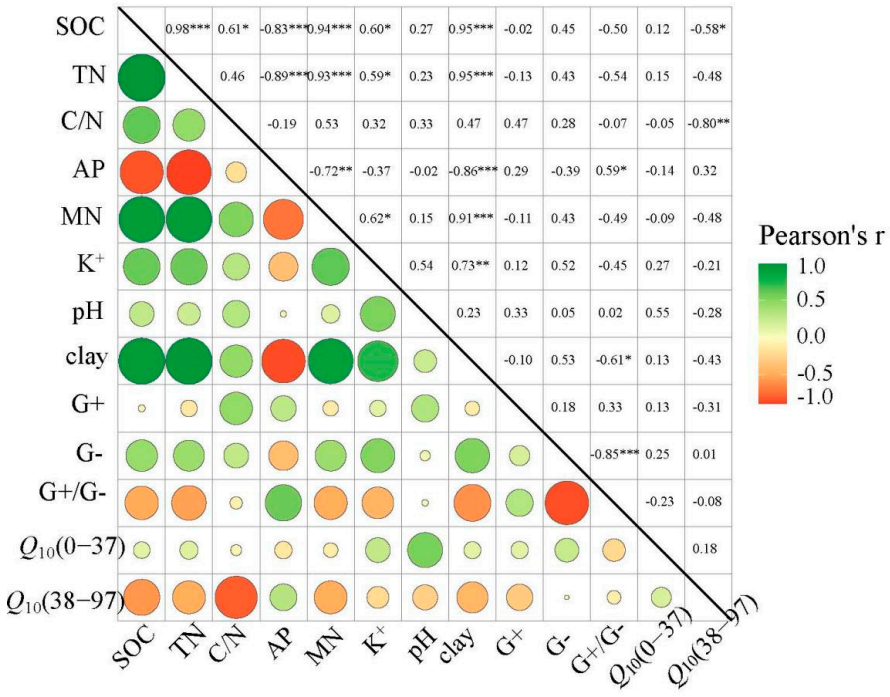

Figure 3. Pearson's correlation coefficient matrix of Q_{10} with key important influencing variables. Soil properties include soil organic carbon (SOC), total N (TN), C/N, Available P (AP), Mineral N (MN), pH, clay content, Soil microorganisms include Gram-positive (G+), Gram-negative (G-), Gram-positive / Gram-negative (G+/G−). *, ** and *** Denote significant difference at p < 0.05, 0.01 and 0.001, respectively.

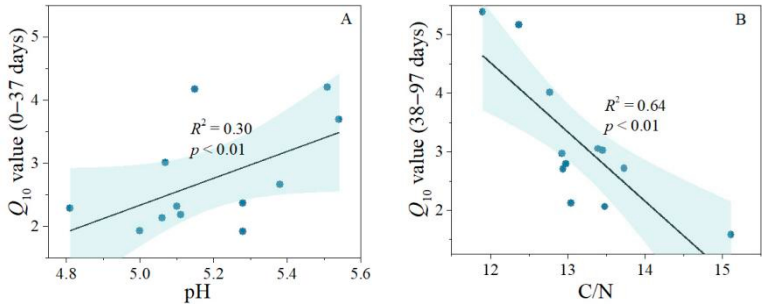

Figure 4. Relationships between the Q_{10} without litter and pH (**A**) during the 0–37 days incubation period. Relationships between the Q_{10} and C/N (**B**) during the 38–97 days incubation period.

3.3. The Influence of Litter Input on Q_{10} at Different Elevations

The effects of litter input on Q_{10} differed in soils of different elevations (Figure 2). Specifically, Q_{10} at medium elevation (950 m) decreased significantly in both stages, whereas Q_{10} at low elevation kept stable after litter input (Figure 2). The responses of Q_{10} to litter input at high elevation changed with incubation: litter input increased Q_{10} in the first stage, whereas it derived no impact on Q_{10} in the second stage. The response to litter input was closely related to nutrient availability, with cumulative CO_2 positively correlated with available P, mineral N (Figure 5A,B). Besides, *K*m of the N-acquisition enzyme was negatively correlated with Q_{10} (Figure 5C).

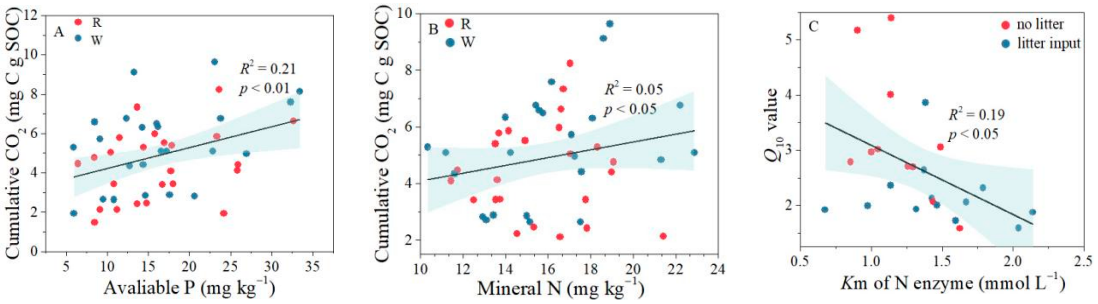

Figure 5. Relationships between the cumulative CO_2 (38–97 days) and available P (**A**) and mineral N (**B**) after 97 days incubation. The relationship between *K*m of N enzyme and Q_{10} (**C**) in the second stage. R and W represent room temperature and warming temperature, respectively.

4. Discussion

In this study, a 2.4 °C interval of incubation temperature, which was different from the previous experiment with 5 or 10 °C [33,34]. It has been evidenced that the intervals of incubation temperatures significantly affect the Q_{10} values, mainly from substrate consumption and microbial thermal adaptability [35] A growing number of studies have shown that warming temperature increases microbial activity and accelerates substrate consumption, which may reduce the Q_{10} of SOC mineralization [36]. On the other hand, the thermal adaptability of microorganisms can also have a certain impact [37]. Therefore, a 2.4 °C range we chose, which is based on the fact that every 100 m of elevation will decrease by 0.6°C, was used for the accurate assessment of Q_{10} values. Meanwhile, a 2.4 °C range is closer to the predicted increase in the global mean temperature at the end of the 21st century [38]. In addition, in many studies of SOC dynamics, the incubation time is less than 100 days, such as 10 days in Li et al. (2017) [18], 14 days in Liu et al. (2021) [39] and 90 days in Zhou et al. (2021) [40]. Therefore, time is not an absolute factor, and we pay more attention to the dynamic change of CO_2. We can see the CO_2 release rate has stabilized (Figure 1). So, 100 days is not a short period of time and can be a solid result. Additionally, longer incubation means more measurements, which is likely to introduce larger measuring error in cumulative SOC mineralization given the large number of jars measured a time in combination with the decrease in SOC mineralization rate. The priming effect after litter input also influenced the selection of incubation days. Ultimately, a short-term incubation (i.e., 97 days) was adopted in our experiment accordingly.

4.1. Q_{10} Response to Elevations

The Q_{10} values ranging from 2.04 to 4.15 in this study meet the global Q_{10} range from 1.9 to 5.7 [41]. Q_{10} did not respond to elevation in the first stage, however, varied significantly along the elevation gradient in the second stage (Figure 2). Elevation mainly affects Q_{10} through soil's physical and chemical properties, based on empirical results [9]. Our results are in line with this idea because soil pH is more correlated with Q_{10} than other factors in the first stage (Figure 3). Previous studies reported that pH is considered to be an important determinant of microbial activity and composition [42]. Given the forest soils

in our study are acidic (Table 1), the increase of microbial activity with soil pH could promote SOC mineralization, so Q_{10} was significantly positively correlated with pH (Figure 4A). This means that a range of soil acidification reduce the risk of C loss under the global warming scenario. In addition, no variation was detected in Q_{10} among soils of different elevation with similar pH values. In contrast, the soil C/N and clay content are key explanatory variables to Q_{10} in the second stage (Figure 3). Soil C/N has long been recognized as an indicator of soil C quality [39]. We found that Q_{10} was significantly and negatively correlated with soil C quality (Figure 4B), in line with the C-quality temperature hypothesis, which suggested that decomposition of higher quality organics has lower Q_{10} than that of lower quality organics [43]. Besides, given no significant variations in the C/N ratio at different elevations, the weaker physical protection by clay particles at medium elevation could be the primary cause of the higher Q_{10} [44,45]. Furthermore, SOC, TN and Mineral N, representing soil fertility, were negatively correlated with Q_{10} in the second stage. This finding indicated that the abundance of microorganisms groups (gram-positive bacteria) in soil with distinct fertilities could regulate the risk of soil C loss in response to global warming. Thus, soil carbon sequestration can be enhanced by improving soil fertility.

4.2. Q_{10} Response to Litter Input with Different Elevations

Q_{10} response to litter input varied along the elevation gradient (Figure 2). Specifically, Q_{10} response to litter input was not observed at 750 m, while a decrease in Q_{10} was detected at 950 m. These two distinct reactions may be owing to the decreased availabilities of nutrients in soil, differentiating the responses of SOC mineralization to global warming [46]. Changes in soil nutrients and subsequent microbial community composition and activity following litter input, result in different rates of SOC mineralization. Well supporting this suggestion, the mineralization of SOC closely associated with soil mineral N and available P at the second stage of incubation, with closer association between SOC mineralization and available P (Figure 5A,B). Furthermore, decrease in the affinity of N enzyme to substrate (increased *K*m of N-acquisition enzyme) makes Q_{10} decrease (Figure 5C), indicating that microorganisms will increase their relative investment in enzymes that require P rather than N following litter input in order to maintain a stable stoichiometry of microbial nutrients [47]. Together, these findings indicated that soil P availability regulated the response of Q_{10} to litter input, which was in agreement with previous studies [48,49]. Therefore, in medium elevation soil with abundant P, activated microbial community by litter input would mine less SOC to acquire P, resulting in lower Q_{10} with litter input at 950 m. Compared with decreased Q_{10} at 950 m, Q_{10} at 750 m has no response to litter input because of the lower availability of soil P (Table 1). In addition to the change of available nutrients by litter input, soil texture may also limit microbial access to available P. Studies have shown that sand particles have higher available P accumulation [50], and litter input does not increase sand particles content in the short term. Furthermore, there was a significant negative correlation between clay particles and available P (Figure 3), which further verified that the higher the content of clay particles, the less available P content (Table 1). As a result, microbes could not obtain more available P, and thus Q_{10} at 1150 m did not significantly increase following litter input.

5. Conclusions

In this study, the vertical spatial heterogeneity between subtropical and warm temperate transitional zones at different elevations was studied in laboratory. The pH and C/N are the main factors regulating the vertical heterogeneity of Q_{10} in forest ecosystems. The negative C/N-Q_{10} correlations in the second stage imply that improving soil fertility contributed to the enhancement of soil carbon sequestration, which partly supports the soil C-quality temperature hypothesis. In addition, the Q_{10} of second stage response to litter input was regulated by available P along the elevation gradient. Overall, our results emphasize the importance of vertical spatial heterogeneity in Q_{10} of SOC mineralization.

Therefore, in order to improve the prediction of the magnitude and direction of soil C-climate feedback in terrestrial ecosystems, it should be considered in the earth system model.

Author Contributions: This specific study was designed by P.T.; P.T. collected soil samples; J.W. conducted the laboratory analysis and analyzed the data with the assistance of Q.Z.; J.W. wrote the manuscript with assistance from P.T. and Q.W. All authors commented preliminary versions of the manuscript and contributed to improve the final version. All authors have read and agreed to the published version of the manuscript.

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