Decomposition of Organic Carbon in Fine Soil Particles Is Likely More Sensitive to Warming than in Coarse Particles: An Incubation Study with Temperate Grassland and Forest Soils in Northern China

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Abstract

It is widely recognized that global warming promotes soil organic carbon (SOC) decomposition, and soils thus emit more CO2 into the atmosphere because of the warming; however, the response of SOC decomposition to this warming in different soil textures is unclear. This lack of knowledge limits our projection of SOC turnover and CO2 emission from soils after future warming. To investigate the CO2 emission from soils with different textures, we conducted a 107-day incubation experiment. The soils were sampled from temperate forest and grassland in northern China. The incubation was conducted over three short-term cycles of changing temperature from 5°C to 30°C, with an interval of 5°C. Our results indicated that CO2 emissions from sand (>50 μm), silt (2–50 μm), and clay (<2 μm) particles increased exponentially with increasing temperature. The sand fractions emitted more CO2 (CO2-C per unit fraction-C) than the silt and clay fractions in both forest and grassland soils. The temperature sensitivity of the CO2 emission from soil particles, which is expressed as Q10, decreased in the order clay>silt>sand. Our study also found that nitrogen availability in the soil facilitated the temperature dependence of SOC decomposition. A further analysis of the incubation data indicated a power-law decrease of Q10 with increasing temperature. Our results suggested that the decomposition of organic carbon in fine-textured soils that are rich in clay or silt could be more sensitive to warming than those in coarse sandy soils and that SOC might be more vulnerable in boreal and temperate regions than in subtropical and tropical regions under future warming.

Introduction

As the largest terrestrial carbon pool, soil plays a vital role in the global carbon cycle [1]. A small change in the magnitude of soil carbon stocks could result in a large impact on the atmospheric enrichment of CO2 [2]. Soil organic carbon (SOC) loss via soil heterotrophic respiration (i.e., SOC decomposition) is one of the largest components of global CO2 flux from the terrestrial ecosystem to the atmosphere [3,4]. It is widely recognized that temperature has a profound influence on SOC decomposition [5] and that soils are expected to release CO2 at faster rates with rising temperature [3]. Therefore, understanding the temperature sensitivity of SOC decomposition is critical for predicting changes in global soil respiration, changes in soil carbon stocks to future warming, and especially, the feedback of soils on climate change [2,6–8].

The temperature sensitivity of SOC decomposition, which is commonly expressed as Q10, refers to the change in decomposition rate with a 10°C increase in temperature. A recent study constructed a database of worldwide soil respiration observations matched with high-resolution historical climate data and estimated that global soil respiration had a Q10 of 1.5 [3]. Nevertheless, soil respiration experiments have unanimously indicated that Q10 is spatially heterogeneous on a global scale [9,10]. Thus, to accurately predict changes in global soil carbon stocks to future warming, knowing which factors determine the temperature dependence of SOC decomposition on large scales is critical [6]. It is known that the Q10 of soil heterotrophic respiration varies with temperature [5,11], soil moisture [12,13], and the quantity and quality of SOC [14,15]. However, it is not clear whether SOC decomposition has different warming responses in different soil textures. It is most likely so because the SOC stock in fine-textured soils is less vulnerable to disturbances, e.g., tillage, than in coarse-textured soils [16,17].

Such different vulnerabilities are assumed to result from fine-textured soils having greater fractions of silt-sized or clay-sized particles, whereas coarse-textured soils have more sand-sized particles; these primary particles contain different mineral compositions and provide various affinities to organic matter [18]. Clay and silt particles (e.g., sesquioxides and layer silicates) provide large specific surface areas and numerous reactive sites at which SOC can be sorbed by strong ligand exchange and...
polysaccharide cation bridges, whereas the sand particles, which is dominated by quartz particles, exhibits only weak bonding affinities to SOC [19, 20]. The bonding of SOC to minerals is recognized to prevent decomposition [21, 22] and may therefore decrease the temperature sensitivity of decomposition [23]. Thus, the decomposition of organic carbon associated with sand, silt, and clay fractions likely respond to warming differently. The temperature sensitivity of SOC decomposition from different particle size fractions is intimately linked to the response of CO₂ emission from different soil textures to future warming and the emission’s feedback on climate change.

Different soil particle size fractions possess different sizes of carbon pools [20]. Clay and silt have large specific surface areas and can sorb abundant SOC [24]. For example, in temperate arable soils, 50–75% of the total SOC is associated with clay, whereas silt accounts for 20–40% and sand accounts for less than 10% [18]. Additionally, the SOC associated with the clay and silt fractions is assumed to be stable relative to that associated with the sand fraction [21]. In this context, the long-term stabilization of soil carbon pools under climate warming mainly depends on the warming response of the carbon that is associated with the clay and silt fractions.

However, to our knowledge, there were few studies who explored difference in the warming responses of organic carbon associated with these primary particles. In this study, we conducted a 107-day incubation experiment to investigate the temperature sensitivity of SOC decomposition in sand (>50 μm), silt (2–50 μm), and clay (<2 μm) particles from grassland and forest soils. This study aims to answer the following questions: 1) Is there a difference in the Q₁₀ of SOC decomposition among different size soil particles and between the two different ecosystems? 2) Which factors determine the Q₁₀? A further objective is to better understand the features of CO₂ emission and their future trends with global warming for different textured soils.

Materials and Methods

Sites and sampling

Soil samples were collected from two sites at the typical steppe grassland and temperate forest ecosystems in northern China. The grassland site was at the Duolun Restoration Ecology Experimentation and Demonstration Station (42°02′N, 116°17′E, 1324 m a.s.l.) and the forest site was at the Beijing Forest Ecosystem Research Station (39°58′N, 115°26′E, 1252 m a.s.l.) at the Institute of Botany of the Chinese Academy of Sciences. The grassland is permanent grassland situated in the temperate continental semi-arid climate zone. The dominant species are *Stipa baicalensis*, *Thalictrum petaloideum*, and *Artemisia frigida*. The mean annual temperature (MAT) is 1.6 °C, and the mean annual precipitation (MAP) is 386 mm, with 100 frost-free days. The soil is sandy Haplic Calcisols (FAO) composed of 61.2% sand, 23.0% silt, and 15.8% clay, and the pH is 7.0. The forest is an artificial forest (with age >40 years) with a dominant *Pinus tabuliformis* Carriere specie and the climate is warm temperate humid monsoon climate zone. The MAT and MAP are 5 °C and 650 mm, respectively, with 160 frost-free days. The soil is a clay loam that belongs to Eutric Cambisol (FAO) and is composed of 41.0% sand, 42.5% silt, and 16.5% clay, and the pH is 7.0.

At each site, 40 cores of mineral soils at 0–20 cm depths were randomly collected using an auger with 4 cm in diameter and were composited into one soil sample. A small fraction of the soils (approximately 1 kg) was immediately placed in a sterile plastic bag on ice when transported to the laboratory and stored at 4 °C for inoculum preparation. The remaining soils were placed into cloth bags and air-dried at room temperature. The dried soils were finely crumbled and sieved through a 2-mm mesh to remove visibly identifiable plant debris and gravel. Fine roots were removed through static electricity sorption.

Particle-size fractionation

Twenty grams of dried soil were dispersed in 100 ml of deionized water by the ultrasonic probe of a JY92-II Ultrasonic Homogenizer (6 mm diameter probe, 300 W). The ultrasonic time was chosen as 3 min and 8.5 min for grassland and forest soil, respectively, after calibrating against conventional soil texture analyses [25]. The dispersed suspensions were separated into three size factions: sand (>50 μm), silt (2–50 μm), and clay (<2 μm). The sand fraction was separated using distilled water to wash all of the fine particles in the soil suspension through a 50-μm sieve until the elutriate solution that exited the sieve was clear. The elutriate was divided into silt and clay fractions by repeated centrifugation using an Avanti J-25 Beckman Centrifuge [26]. The clay fraction was concentrated from the large volumes of suspensions by flocculation with CaCl₂ and centrifugation [27]. The three size fractions were oven-dried at 40 °C [28], gently crushed, weighed, and then stored for incubation and future analysis.

Laboratory incubation

For each soil particle, a 10-g dried sample was placed into a 150-ml brown flask. Then, 2 ml of inoculum were added to each sample, and the inoculum solution was prepared by shaking 100 g of fresh soil with 1000 ml of deionized water and leaving to stand overnight [28]. To improve soil aeration and make the inoculum disperse uniformly, the sample plus inoculum was loosened, stirred, and leveled slightly. The soil moisture was adjusted to 60% water-filled pore space (WFPS) [29]. The incubation lasted 107 days (Figure 1). The soil samples were incubated at room temperature (ca. 20 °C) when there was no measurement. The samples were regularly corrected for water loss by adding deionized water. Four replicates were conducted in the experiment. Empty flasks were set as blank. The volume of head space (flask volume minus soil solid) was accurately measured after complementing the experiment by the method of water replacement [30].

Following Fang et al. [31] and Xu et al. [32], soil respiration rates under changing temperature were measured in sequence. Three temperature-change cycles were started at the 9th, 47th, and 100th days after the beginning of the incubation, and each cycle lasted 8 days (Figure 1). The first temperature-change cycle began after 8 days of incubation to stabilize the microbial activity to avoid an undesired microbial peak [33]. Within each cycle, the temperature was continuously increased from 5 °C to 30 °C in intervals of 5 °C and then gradually decreased from 30 °C to 5 °C with the same interval. After each temperature was changed to the next, the incubation flasks were kept open for 3 hours to allow the soil samples to adapt to the changed temperature [30]; the flasks were then sealed using butyl rubber septa and flushed with ambient air for one minute using a pump with an “inlet-outlet” needle inserted through the septa to produce the same initial gas conditions among all flasks. After specific times of enclosure (20, 15, 9, 6, 4, and 3 hours for 5, 10, 15, 20, 25, and 30 °C, respectively), 5 ml of gas was taken through a gas-tight injection syringe from the headspace of the flask. The CO₂ concentrations were analyzed with a gas chromatograph (Agilent 7890A, USA).

Determination of soil physicochemical properties

The soil texture was measured using the hydrometer method [34]. The SOC and soil total nitrogen (STN) concentrations were
with temperature, the Q10 values were calculated separately (DOC) and total dissolved nitrogen (TDN) [38,39]. Soil samples were considered as the dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) [38,39].

Calculations and statistical analysis

Similar to Lang et al. [40], the CO2 emission rate was calculated using the following equation:

$$ R = V \times \frac{273}{T} \times (C_s - C_b) \times \rho \times \frac{12}{44} \frac{(W \times c)}{t} $$

where \( R \) is the CO2 emission rate (\( \mu g \) CO2-C g \(^{-1}\) OC h \(^{-1}\)); \( V \) is the flask headspace volume (L); \( T \) is the incubation temperature (°C); \( C_s \) and \( C_b \) are the CO2 concentrations in the sample and blank flasks (ppm), respectively; \( \rho \) is the standard state CO2 density (g L \(^{-1}\)); \( W \) is the dry weight of the soil particles (kg); \( c \) is the SOC concentration of the soil particles (g kg \(^{-1}\)); and \( t \) is the time encosed (h). The final CO2 emission rate at a given temperature was calculated as the average of the values between the increasing temperature and decreasing temperature periods [31,32]. To illustrate the temperature sensitivity of CO2 emission from soil particles, we used two methods to calculate Q10. One method involves the commonly used first-order exponential model:

$$ R = Ae^{BT} $$

where \( R \) is the CO2 emission rate (\( \mu g \) CO2-C g \(^{-1}\) SOC h \(^{-1}\)), \( T \) is the temperature (°C), and A and B are model parameters that are to be determined. The parameter A is a carbon quality index, which refers to the availability and lability of the carbon substrates [15,41]. The Q10 value was then computed as following:

$$ Q_{10} = e^{10 \times B} $$

The Q10 calculated by Eqn. 3 was a constant value in temperature range of 5–30°C. To examine the Q10 variation with temperature, the Q10 values were also calculated separately for each temperature interval of 5 degrees (e.g., 5–10°C) and 10 degrees (e.g., 5–15°C) based on the following equation [11]:

$$ Q_{10} = (R_2 - R_1)^{10/(T_2 - T_1)} $$

where \( R_2 \) and \( R_1 \) are the CO2 emission rates (\( \mu g \) CO2-C g \(^{-1}\) SOC h \(^{-1}\)) measured at temperatures \( T_2 \) (high temperature) and \( T_1 \) (low temperature), respectively.

All statistical analysis was conducted using SPSS 13.0 (SPSS Inc., 2004). The means were compared using the Duncan's multiple range test (DMRT).

Results

Physicochemical characteristics of soils

The SOC and STN concentrations of the bulk soil were very similar between the grassland and forest (Table 1). The organic carbon concentrations in grassland and forest soil particles both decreased with increasing particle size, from 55 g kg \(^{-1}\) and 73 g kg \(^{-1}\) in the clay particle (<2 μm) to 9 and 15 g kg \(^{-1}\) in the sand particles (>50 μm), respectively. Similar trends were observed for the total nitrogen concentrations. The C:N ratios were similar between the clay and silt particles, which were lower than those of the bulk soil and the sand particles. The total percentages of the carbon masses associated with clay and silt particles in the total SOC were more than 60% for both grassland and forest soils, and the percentages of their nitrogen masses in the total STN were both approximately 75%.

Relationship between soil organic carbon decomposition rate and temperature

The SOC decomposition rate from soil particle size fractions, which is expressed as the CO2-C emission rate per unit of fraction-C, had an exponential relationship with the incubation temperature in all measurements (Figure 2). The sand fractions, which had a lower organic carbon content (Table 1), exhibited greater SOC decomposition rates than the silt and clay fractions, which latter two were similar to one another. The decomposition rates in all the soil fractions decreased continuously as incubation time progressed (from an earlier temperature-change cycle to a later one), except for the sand fraction from the second to the third cycle. As a whole, the decomposition rates from grassland soil particles were greater than those from the corresponding forest soil particles under same condition (Figure 2).

The parameter A in the fitted equation (Eqn. 2) for the relationship between the decomposition rate and temperature is an index for carbon availability and lability. The value of parameter A was greater for the sand fraction than for the silt and clay fractions in both the grassland and forest soils (Figure 2). Furthermore, the values of parameter A were approximately equal between the two land uses for the clay and silt fractions during each temperature change cycle but were greater in the grassland than in the forest for the sand fraction (Figure 2).

Temperature sensitivity of soil organic carbon decomposition for different soil particle-size fractions

The Q10 values calculated using the exponential model (Eqn. 3) were significantly different among the soil particle size fractions during each temperature-change cycle; the clay fraction exhibited the greatest value, followed by the silt and sand fractions (Table 2). The calculated mean Q10 values of the three cycles were 2.68±0.16 and 2.43±0.16 for the clay fraction, 2.45±0.06 and.
2.22±0.10 for the silt, and 2.33±0.08 and 2.09±0.09 for the sand, in the grassland and forest soils, respectively. The mean Q10 values of the grassland soil particles were significantly greater than those of the corresponding forest soil particles (Table 2). As the incubation time progressed (from an earlier temperature-change cycle to a later one), Q10 increased for the grassland clay and silt fractions (Table 2).

The Q10 values decreased in power-law with increasing temperature for all soil particles when calculating using Eqn. 4 during each individual temperature interval (Figure 3). The average Q10 values of all soil particle fractions were 4.22±0.14 during the minimum temperature interval and 1.81±0.17 during the maximum temperature interval (Figure 3). The grassland soil particles had greater Q10 than the corresponding forest soil particles during the temperature interval of 5–15°C, whereas the Q10 values for the two land uses tended to approach one another during 15–30°C (Figure 3). Nevertheless, the grassland soil particles exhibited a more rapid decline in Q10 with increasing temperature (i.e., greater absolute values of the power-law exponents in Figure 3) than did the forest soil particles. Furthermore, the Q10 values calculated using Eqn. 4 followed as clay>silt>sand (Figure 3), which was consistent with the pattern in Q10 calculated using the exponential model (Eqn. 3) (Table 2).

Table 1. Physicochemical composition of the soils.

| Land uses and particle size fractions | Fraction * (%) | Organic carbon | Total nitrogen | C:N ratio |
|--------------------------------------|----------------|----------------|----------------|-----------|
|                                      |                | Concentration (g kg⁻¹) | Share of the total (%) | Concentration (g kg⁻¹) | Share of the total (%) |
| Grassland                            |                |                      |                        |                     |
| Clay (<2 μm)                         | 11.2 (1.6)     | 55.3               | 28.5               | 5.8       | 31.9       | 9.5       |
| Silt (2–50 μm)                       | 28.0 (1.3)     | 30.9               | 40.0               | 3.1       | 43.2       | 9.9       |
| Sand (>50 μm)                        | 57.4 (1.0)     | 8.9                | 23.6               | 0.7       | 18.7       | 13.4      |
| Bulk soil                            |                | 21.7               | 2.0                |           | 10.7       |           |
| Recovery (%)                         | 96.6 (1.1)     |                    | 92.1               |           | 93.9       |           |
| Forest                               |                |                      |                        |                     |
| Clay (<2 μm)                         | 18.1 (2.8)     | 72.6               | 44.6               | 6.1       | 51.2       | 12.0      |
| Silt (2–50 μm)                       | 35.1 (2.7)     | 17.4               | 20.8               | 1.5       | 24.2       | 11.8      |
| Sand (>50 μm)                        | 43.9 (1.2)     | 13.0               | 19.4               | 0.7       | 14.1       | 18.9      |
| Bulk soil                            |                | 29.5               | 2.1                |           | 13.8       |           |
| Recovery (%)                         | 97.1 (1.0)     |                    | 84.8               |           | 89.5       |           |

*Values in the parentheses represent SD.
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Figure 2. Relationship between CO2 emission rates from particle-size fractions and incubation temperature for grassland (A, B, C) and forest soils (D, E, F). The panels from left to right represent temperature-change cycles in the order 1, 2, 3. The vertical bars denote the standard deviations (n = 4).
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Dependence of Q10 on soil biochemical properties

Generally, there were large differences among various particle-size fractions in most soil biological and chemical properties at the end of incubation (Table 3). Specifically, MBC, MBN, DOC, and TDN were greatest in the clay fraction and were generally of the same order of magnitude as Q10 (Table 2). However, the ratios of MBC/OC and MBN/TN shifted to be greatest in the sand fraction. The microbial C/N ratios were not significantly different among the soil particles, but they were generally greater than the soil particles’ C/N ratios (except for the forest sand particles; see Table 1). Additionally, the grassland soil particles had greater MBC/OC and less qCO2 values than the corresponding forest soil particles.

The soil biological and chemical properties at the end of incubation represented the soil conditions during the 3rd temperature-change cycle; thus we analyzed the relationship between the Q10 in the 3rd cycle and these soil biological and chemical properties. Table 4 presents the Pearson correlation coefficients of Q10 during an intermediate temperature range of 15–25°C with various soil biochemical properties. Clearly, the Q10 values were highly correlated with MBC and TDN. The Q10 had significant linear relationships with MBC (Figure 4A) and TDN (Figure 4B) for the 5–15°C and 20–30°C temperature intervals and also for other temperature intervals (not shown). It should be noted that the slopes of regression line were different between the temperature ranges of 5–15°C and 20–30°C. Over an increasing temperature interval of 10°C, the slopes with low baseline levels of 5°C were steeper than those with high baseline levels of 20°C. Note that the datasets were well-mixed for the grassland and forest, thereby suggesting that these two land use soils had similar features in terms of the dependence of Q10 on MBC and TDN.

Discussion

Carbon stability and microbial activity among different size fractions

The carbon associated with different particle-size fractions has different availability and lability [42]. The sand fraction had greater organic carbon availability and lability (greater parameter A), and thus lower carbon stability, than did the silt and clay fractions (Fig. 2). This pattern of SOC stability among the different size fractions was consistent with previous studies [43,44]. The difference in SOC stability among soil primary particles is the reason why coarse sandy soils that are rich in sand particles are less

Table 2. The Q10* values (±SD) of SOC decomposition for different particle-size fractions in grassland and forest soils (n = 4).

| Land uses | Temperature-change cycles | Particle size fractions |
|-----------|---------------------------|------------------------|
|           |                           | Clay (<2 μm)           | Silt (2–50 μm) | Sand (>50 μm) |
| Grassland | 1                         | 2.56 ±0.07a*           | 2.41 ±0.05b    | 2.39 ±0.04b    |
|           | 2                         | 2.60 ±0.04a            | 2.45 ±0.06b    | 2.34 ±0.09c    |
|           | 3                         | 2.88 ±0.04a            | 2.51 ±0.02b    | 2.27 ±0.07c    |
|           | Mean*                     | 2.68 ±0.16aB           | 2.45 ±0.06bA   | 2.33 ±0.08cA   |
| Forest    | 1                         | 2.45 ±0.07a            | 2.32 ±0.08b    | 2.19 ±0.04c    |
|           | 2                         | 2.24 ±0.01a            | 2.13 ±0.08b    | 2.08 ±0.03b    |
|           | 3                         | 2.59 ±0.04a            | 2.23 ±0.05b    | 2.00 ±0.05c    |
|           | Mean*                     | 2.43 ±0.16aB           | 2.22 ±0.10bB   | 2.09 ±0.09cB   |

*Q10 value was calculated using Eqn. 3 with the parameters for the fitted curves in Figure 2.

1The mean was calculated including each replicate among the three temperature-change cycles (n = 12).

2Different lowercase letters in the same row indicate significance differences (p<0.05) among soil fractions.

3Different capital letters in the same column indicate significance differences (p<0.05) between two land uses.

Figure 3. Dependence of Q10 on incubation temperature for (A) clay, (B) silt, and (C) sand. The Q10 for each temperature interval of 5 degrees (e.g., 5–10°C) and 10 degrees (e.g., 5–15°C) was calculated using Eqn. 4. The average temperature for each interval was taken as representative temperature. Data from all the three temperature-change cycles were included. Vertical bars denote the standard errors (n = 12).

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Table 3. Soil biological and chemical properties in different particle-size fractions at the end of incubation (mean ± SE, n = 4).

| Land-use type | DOC (mg/kg) | MBC (mg/kg) | MBN (mg/kg) | MBN/TN (%) | MBC/OC * (mg CO₂-C kg⁻¹ MBC h⁻¹) | qCO₂ * (mg CO₂-C kg⁻¹ MBC h⁻¹) |
|---------------|-------------|-------------|-------------|-------------|-----------------------------------|-------------------------------|
| Grassland     | 1096 ± 26a  | 35 ± 2a     | 28 ± 1a    | 21 ± 1c    | 36 ± 1a                           | 25 ± 1b                      |
| Clay (≥ 2 µm) | 2356 ± 25a  | 26 ± 6a     | 23 ± 3a    | 23 ± 1c    | 35 ± 4a                           | 25 ± 1b                      |
| Silt (2–50 µm)| 22 ± 1a     | 21 ± 1a     | 39 ± 4b    | 33 ± 2a    | 21 ± 2a                           | 28 ± 1a                      |
| Sand (≤ 2 µm) | 27 ± 1a     | 22 ± 1a     | 39 ± 4b    | 33 ± 2a    | 21 ± 2a                           | 28 ± 1a                      |
| Clay (≤ 2 µm) | 29 ± 1b     | 23 ± 2b     | 39 ± 4b    | 33 ± 2a    | 21 ± 2a                           | 28 ± 1a                      |
| Silt (≤ 2 µm) | 29 ± 1b     | 23 ± 2b     | 39 ± 4b    | 33 ± 2a    | 21 ± 2a                           | 28 ± 1a                      |

qCO₂ is the metabolic quotient calculated by the CO₂ emission rate at 25°C in the third incubation cycle divided by MBC. Different lowercase letters in the same column for the same land use indicate significance differences (p < 0.05) among soil fractions.

Effect of climatic and soil parameters on Q₁₀

First, temperature can determine the Q₁₀ of SOC decomposition. The lower Q₁₀ of soil respiration at higher temperatures was not only assumed by the Arrhenius equation [6] but also confirmed by two comprehensive analyses of incubation studies of soils from all types of terrestrial ecosystems [11,54]. Similarly, we found that the Q₁₀ values were negatively correlated with the incubation temperature (Figure 3). The relationship was fitted by a power function: \( y = ax^b \). Clearly, higher values of \( x \) produce higher values of Q₁₀. Otherwise, with larger absolute values of \( b \), Q₁₀ decreases more rapidly with increasing temperature. The parameters \( a \) and \( b \) in the Q₁₀-T curves were found to be significantly correlated with the MBC and soil C:N ratio (Figure 5).

An increase in the MBC concentration would lead to a Q₁₀ rising (Figure 5A), which is consistent with Figure 4A. Significant inverse relationships were found between the parameter \( a \) and the soil C:N ratio (Figure 5B), thereby suggesting that higher soil C:N ratios was linked with higher tolerance to increasing temperature. A more rapid rate of Q₁₀ decrease with increasing temperature (greater absolute value of \( b \)) in grassland versus forest soil particles could be related to lower C:N ratios (Table 1) or greater MBC in grassland soil particles (Table 3). It should be mentioned that no significant relationship was found between the values of \( a \) and \( b \) and the soil properties of MBN, DOC, and DTN.
The negative relationship between Q₁₀ of SOC decomposition and temperature has two implications. First, in a given region, SOC decomposition should be more sensitive to warming during winter than warming during summer. This trend was observed in the Alaskan tundra, with an increase of greater than 50% in CO₂ fluxes during the snow-covered period by winter warming and a 20% increase during the growing season by summer warming [55]. Nevertheless, considering that the initial soil respiration rate is much less in winter than in summer, the absolute magnitude of the CO₂ emission increase from warming could be still less in winter than in summer. Second, the SOC stock in boreal and temperate regions (low MAT), especially peat lands and permafrost, should be more vulnerable to warming than that in

Table 4. Pearson correlation coefficient for Q₁₀ (15–25°C) in the third temperature-change cycle and soil biological and chemical properties.

|               | Q₁₀ (15–25°C) | MBC | MBN | DOC | TDN | Micro C/N | DOC/TDN | MBC/OC | MBN/TN | qCO₂ |
|---------------|---------------|-----|-----|-----|-----|-----------|---------|--------|--------|------|
| Q₁₀ (15–25°C) | 1             |     |     |     |     |           |         |        |        |      |
| MBC           | 0.87***       | 1   |     |     |     |           |         |        |        |      |
| MBN           | 0.52***       | 0.44* | 1   |     |     |           |         |        |        |      |
| DOC           | 0.38**        | 0.32 | 0.38| 1   |     |           |         |        |        |      |
| TDN           | 0.90***       | 0.80*** | 0.45* | 0.81*** | 1   |           |         |        |        |      |
| Micro C/N     | 0.51**        | 0.59** | 0.29| 0.25| 0.54** | 1       |         |        |        |      |
| DOC/TDN       | -0.85***      | -0.73*** | -0.42| -0.20| -0.59** | -0.45* | 1       |        |        |      |
| MBC/OC        | -0.02         | 0.19 | -0.09| 0.65*** | 0.29| 0.13      | 0.19    | 1       |        |      |
| MBN/TN        | -0.43*        | -0.29| 0.04 | -0.59** | -0.54** | -0.44* | 0.19    | 0.71*** | 1    |      |
| qCO₂          | -0.66***      | -0.70*** | -0.24| 0.18 | -0.35 | -0.37 | 0.85*** | -0.54* | -0.05| 1    |

*Q₁₀ (15–25°C) was calculated using Eqn. 4 for each replicate.
**Significant at probability levels of 0.05, 0.01, and 0.001, respectively.
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The Q₁₀ (5–15°C) and Q₁₀ (20–30°C) were calculated using Eqn. 4 for each replicate.

Figure 4. Dependence of Q₁₀ in the third temperature-change cycle on microbial biomass carbon (A) and total dissolved nitrogen (B). The Q₁₀ (5–15°C) and Q₁₀ (20–30°C) were calculated using Eqn. 4 for each replicate.
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Figure 5. Dependence of parameters in the Q₁₀–T curve on the soil microbial biomass carbon (A) and soil C: N ratio (B). Vertical bars denote the 95% CI of the estimated parameters in Fig. 3. For each parameter α and β, six points represent the three particles of clay, silt, and sand from the grassland and forest soils. Horizontal bars represent the standard errors (n = 4).
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The question of whether the decomposition of recalcitrant and labile carbon has different temperature dependencies is important because most experimental work has been done on the temperature sensitivity of the decomposition of more labile fractions, yet the long-term effect of warming on soil carbon storage should be primarily determined by the temperature dependence of the more recalcitrant carbon pool [7]. Kinetic theory and the Arrhenius equation both suppose that recalcitrant carbon pools are more warming-sensitive than labile carbon pools [6,63]. Many laboratory incubation studies have supported this supposition [32,64,65]. Our study further affirmed this result by showing that the decomposition of the carbon associated with the clay and silt fractions (recalcitrant carbon pool), with slower turnover rates (Figure 2), yet had higher Q_{10} than that with the sand fraction (labile carbon pool) (Table 2). Furthermore, the organic carbon associated with the clay and silt fractions usually accounts for a large portion of the total SOC, i.e., more than 60% in this study (Table 1). Therefore, long-term response of the SOC stock to climate warming would be more severe than the current predictions that assume the same Q_{10} for labile and recalcitrant carbon pools.

However, the supposition that recalcitrant carbon pools have greater warming dependence has not obtained wide acceptance [31,66]. Based on this supposition, Q_{10} in the incubation studies is expected to rise with prolonged incubation time because SOC is generally composed of a large proportion of labile carbon during the early incubation period, yet of a small proportion of labile carbon during the late incubation period due to depletion of labile carbon [67]. And yet, Fang et al. [31] and Reichstein et al. [66] found that Q_{10} was stable and independent of the incubation time. Similarly, our study only observed increasing trends in Q_{10} for the grassland clay and silt fractions from the 1st temperature-change cycle to the 2nd and the 3rd (Figure 2), and did not find such trends in Q_{10} for the forest soil fractions or the grassland sand fractions. The inconsistency between these observations and the expectation derived from the above supposition could be explained by that Q_{10} having a significant positive relation with MBC and lower MBC values resulting in a smaller Q_{10} (Figure 4A); MBC generally declines with prolonged incubation because of the depletion of labile carbon [31,68]. Consequently, as incubation is prolonged, increasing trend of Q_{10} due to increased recalcitrant carbon proportion could be counteracted by decreasing trend of Q_{10} as a result of declined MBC [67]. If the MBC did not decrease sharply from early to late incubation stage, the Q_{10} would have increased as incubation progressed in Fang et al. [31] and Reichstein et al. [66], as well as in our study. However, the mechanism for how Q_{10} is affected by MBC is not clear. Assuming that the microbial community composition does not change and that every single microbe maintains the same warming response with prolonged incubation time, a decline in MBC alone should not induce lower Q_{10}. Possible mechanisms could be either microbial community shift to species with low thermal response [69] or microbe acclimatization to temperature increase [70]. On the other hand, soil carbon recalcitrance did not certainly increase continuously with prolonged incubation. In other words, carbon availability and lability did not certainly decrease continuously. Such as, we can see that the parameter A for the sand fraction increased from the second to the third cycle both for grassland and forest soils (Figure 2). Tian et al. [71] also observed the phenomenon and affirmed that a pulse-dynamic pattern of soil respiration and increased carbon availability during a long incubation could exist. Overall, these results suggest that it is unreliable to judge whether the decomposition of recalcitrant and labile carbon have different temperature dependencies by comparing Q_{10} values after different incubation time.

Conclusions

This study presented three implications for understanding the soil-carbon response to climate warming. First, the Q_{10} of SOC decomposition in soil particles decreased with increasing particle size (clay>silt>sand) for grassland and forest soils. This result suggested that SOC decomposition from fine-textured soils rich in clay or silt content could be more sensitive to warming than coarse sandy soils. Second, Q_{10} decreased with increasing temperature, thereby implicating that the SOC might be more vulnerable in low MAT regions than in high MAT regions under future warming. Third, the soil nitrogen availability facilitated a temperature dependence of SOC decomposition, suggesting that atmospheric nitrogen deposition could stimulate positive feedback of soil respiration on climate warming.

Author Contributions

Conceived and designed the experiments: YH WS. Performed the experiments: FD GJ YC. Analyzed the data: YH FD WS. Wrote the paper: FD WS.
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