Temperature sensitivity of SOM decomposition governed by aggregate protection and microbial communities

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Temperature sensitivity ($Q_{10}$) of soil organic matter (SOM) decomposition is a crucial parameter for predicting the fate of soil carbon (C) under global warming. However, our understanding of its regulatory mechanisms remains inadequate, which constrains its accurate parameterization in Earth system models and induces large uncertainties in predicting terrestrial C-climate feedback. Here, we conducted a long-term laboratory incubation combined with a two-pool model and manipulative experiments to examine potential mechanisms underlying the depth-associated $Q_{10}$ variations in active and slow soil C pools. We found that lower microbial abundance and stronger aggregate protection were coexisting mechanisms underlying the lower $Q_{10}$ in the subsoil. Of them, microbial communities were the main determinant of $Q_{10}$ in the active pool, whereas aggregate protection exerted more important control in the slow pool. These results highlight the crucial role of soil C stabilization mechanisms in regulating temperature response of SOM decomposition, potentially attenuating the terrestrial C-climate feedback.

INTRODUCTION

Globally, approximately 2300 Pg (1 Pg = 10^{15} g) carbon (C) is stored in the top 3 m of soils, of which more than 70% is distributed in deep horizons below 20 cm (1). Deep soil C is characterized by high stability over long time scales, because the rate of soil organic matter (SOM) decomposition usually decreases with soil depth (2). Similar to many other biochemical reactions, the decomposition of SOM is temperature dependent (3). Because of this point, global warming is expected to promote carbon dioxide ($CO_2$) release from both the topsoil and the subsoil, thereby triggering a cause of this point, global warming is expected to promote carbon dioxide reactions, the decomposition of SOM is temperature dependent (4). Be- cause of this point, global warming is expected to promote carbon dioxide ($CO_2$) release from both the topsoil and the subsoil, thereby triggering a potential positive feedback (4, 5). However, large uncertainties remain in the magnitude of this C-climate feedback, partly due to the in- accurate parameterization of the temperature sensitivity ($Q_{10}$) of SOM decomposition in different soil depths and C pools in Earth system models (ESMs) (3, 6). It has been estimated that the current use of a globally constant $Q_{10}$ value in most models underestimates the magni- ditude of C-climate feedback by 25% compared to incorporating the spatially heterogeneous $Q_{10}$ values into models (7). Therefore, a deeper understanding of the fundamental mechanisms regulating $Q_{10}$ is pivotal for accurately predicting soil C dynamics and reducing model uncertainties in forecasting terrestrial C-climate feedback.

Historically, SOM quality had been recognized as the major deter- minant of $Q_{10}$. On the basis of enzyme kinetic theory, “carbon-quality temperature” hypothesis predicts that the decomposition of low-quality substrates in the subsoil requires high activation energy and is thus more sensitive to temperature changes (8, 9). However, several emergent conceptual frameworks have challenged the perceived importance of soil C quality in governing the decomposition of SOM and its responses to warming, and argued that processes controlling substrate accessibility (e.g., SOM protection, spatial disconnection, and enzyme diffusion) are also critically important (10–13). Moreover, microbial properties (e.g., microbial abundance, community composition, and enzyme produc- tions) and the existence of energetic barriers to microorganisms regulate SOM decomposition and $Q_{10}$ as well (2, 10, 14, 15). Despite the theoretical predictions of an important role for these factors, direct evidence for the effects of SOM protection and microbial communities on $Q_{10}$ is scarce (16), which, in turn, limits the accurate parameterization of $Q_{10}$ in ESMs. The soil profile provides an ideal natural gradient for exploring the relative contribution of different mechanisms underlying $Q_{10}$ due to vertical variations in substrate quality and environmental and microbial controls over SOM decomposition (9, 17, 18). Moreover, manipulative experiments such as aggregate disruption experiments (19) and microorganism reciprocal transplant experiments (20), combined with laboratory culture, offer the possibility to explore the effects of aggregate protection and microbial communities on $Q_{10}$ respectively. However, these approaches have not been adequately applied in disentangling the complex mechanisms regulating $Q_{10}$ between soil depths.

It is widely recognized that the large heterogeneity of SOM decomposition and its response to warming exists not only between soil depths but also within the composition of SOM itself (16, 21, 22). SOM is considered as a heterogeneous mixture consisting of pools with different turnover times, which is also the premise of classic soil C models such as Century (23) and RothC (24). It has been assumed that the active C pool, consisting for hours to weeks, is composed mainly of chemically labile and unprotected compounds, whereas the slow C pool could persist for decades to centuries due to the physicochemical and biological constraints on decomposition (13). These variations in sub- strate quality and abiotic and biotic regulators between C pools imply that their responses to climate warming and the associated mechanisms may also differ (12). Despite this recognition, current models assume equal $Q_{10}$ value for all C pools (3). The assumption of a single $Q_{10}$ value may obscure the responses of soil C pool to climate warming, especially for the slow C pool, whose temperature response cannot be well captured from short-term incubation experiments (21, 25). With the development of a data-model fusion technology, Bayesian probabilistic inversion, it is possible to estimate $Q_{10}$ values for different soil C pools through the combination of long-term incubations and two-pool models (26). Nonetheless, limited studies have adopted this approach to explore potential mechanisms regulating $Q_{10}$ for different soil C pools.

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This study aimed to address the following three questions: (i) Are $Q_{10}$ values for bulk soil, active C pool, and slow C pool different between the topsoil and the subsoil? (ii) What are the regulatory mechanisms underlying the $Q_{10}$ difference between soil depths? (iii) Are the regulatory mechanisms different between the two C pools? To answer these questions, topsoil samples from 0 to 10 cm depth and subsoil samples from 30 to 50 cm depth were collected from three alpine meadow sites on the Tibetan Plateau (fig. S1). On the basis of these samples, we conducted a 330-day incubation experiment, in combination with three methods (bulk soil $Q_{10}$ by average decomposition rate, $Q_{10}$ values for different respired soil C fractions through the $Q_{10-C}$ method, and active and slow pool $Q_{10}$ by the two-pool model), to estimate the $Q_{10}$ values of various SOM components for the two soil depths. Soil properties including substrate quality, SOM protection, and microbial characteristics were then quantified to explore the determinants of $Q_{10}$. We further performed two manipulative experiments (i.e., aggregate disruption experiment and microorganism reciprocal transplant experiment) to examine the effects of aggregate protection and microbial communities on $Q_{10}$ in different C pools.

**RESULTS**

**Differences in $Q_{10}$ values between soil depths**

Bulk soil $Q_{10}$ in the topsoil was estimated at 2.1 on average, significantly higher than the value (1.8) in the subsoil ($P < 0.05$; Fig. 1A). No significant interactions between site and depth were observed, demonstrating that the depth effect on $Q_{10}$ was independent of the sampling site ($P = 0.28$; table S1). To further explore $Q_{10}$ for various SOM components, we adopted the $Q_{10-C}$ method to evaluate $Q_{10}$ for three soil C fractions (i.e., 1 to 1.5%, 2 to 2.5%, and 3 to 3.5% of cumulative respired soil C), which represent a gradient of decreasing substrate lability. Similarly, the topsoil $Q_{10}$ was significantly higher than the subsoil for 2 to 2.5% and 3 to 3.5% of cumulative respired soil C ($P < 0.05$; Fig. 1B), without interaction effects ($P = 0.20$ and 0.50, respectively; table S1). No significant difference was detected in the $Q_{10}$ value of 1 to 1.5% of cumulative respired soil C between soil depths ($P = 0.05$; table S1). In addition, $Q_{10}$ estimated from the two-pool model also showed significantly lower values in the subsoil for both active pool and slow pool ($P < 0.05$; Fig. 1A), with no site × depth interaction ($P = 0.31$ and 0.26 for active pool and slow pool, respectively; table S1).

**Determinants of the $Q_{10}$ difference between soil depths**

To explore determinants of the $Q_{10}$ difference between soil depths, we analyzed four types of variables associated with $Q_{10}$, including substrate quality, SOM association with minerals, SOM protection by aggregates, and microbial communities, and compared them between the two soil depths. Of these variables, substrate quality revealed by SOM composition differed between the topsoil and the subsoil. Specifically, the intensity of O-alkyl C acquired from solid-state $^{13}$C CP/MAS (cross polarization with magic-angle spinning) NMR (nuclear magnetic resonance) was higher in the topsoil (41%) than in the subsoil (29%) ($P < 0.01$; Fig. 2, A and B), whereas the contribution of alkyl C was significantly higher in the subsoil (53%) than in the topsoil (28%) ($P < 0.01$; Fig. 2, A and B). The calculated alkyl/O-alkyl ratio was thus significantly higher in the subsoil ($P < 0.01$; Fig. 2B), indicating more recalcitrant SOM in deep soils. Consequently, on the basis of the “quality-temperature” hypothesis, the poorer substrate quality could not be responsible for the lower $Q_{10}$ in the subsoil.

We then measured the proportion of soil organic carbon (SOC) associated with Fe to total SOC (i.e., Fe-bound SOC) to quantify SOM protection by minerals. The results revealed no significant difference in the Fe-bound SOC between the topsoil and the subsoil ($P = 0.17$; Fig. 2C). In contrast, the proportion of SOC associated with silt + clay (<53 μm) acquired by the wet-sieving technique did not show significant difference ($P = 0.83$; Fig. 2C), jointly indicating that SOM protection by minerals might be less responsible for the $Q_{10}$ difference between soil depths.

**Microbial communities,** as determined through phospholipid fatty acid (PLFA) analysis, also differed between the topsoil and the subsoil. The abundances of the microbial groups, including total PLFAs, bacterial PLFAs, and fungal PLFAs, were all higher in the topsoil than in the subsoil ($P < 0.01$; table S2). The community structure was different in
certain aspects, with significantly higher fungal/bacterial (F/B) ratio ($P < 0.05$) and relative abundance of fungal PLFAs (fungal PLFAs/total PLFAs, $P < 0.01$) in the topsoil (table S2), while there was no difference in the relative abundance of bacterial PLFAs (bacterial PLFAs/total PLFAs) between the two depths ($P = 0.10$; table S2). These variations in microbial communities also contributed to the $Q_{10}$ differences: Bulk soil $Q_{10}$, active pool $Q_{10}$, and slow pool $Q_{10}$ were all positively associated with the relative abundance of fungal PLFAs (Fig. 4, A to C).

**Mechanisms of depth-associated variations in $Q_{10}$ in various C pools**

To test whether the underlying mechanisms of depth-associated variations in $Q_{10}$ were C pool dependent, we conducted variation partitioning analyses for the active and slow C pools. During these analyses, we incorporated aggregate protection and microbial communities, which exerted significant controls over $Q_{10}$ (Figs. 3 and 4). The results demonstrated that $Q_{10}$ variations in the bulk soil and slow pool were primarily explained by aggregate protection, with the pure effects being 24.5% (Fig. 5A) and 36.9% (Fig. 5C), respectively. The $Q_{10}$ variations in the active pool, however, were mainly explained by microbial communities, whose pure effect was 16.2% (Fig. 5B). These results demonstrated that microbial communities governed $Q_{10}$ in the active pool, while aggregate protection governed $Q_{10}$ in the slow pool.

The different underlying mechanisms of $Q_{10}$ between the two C pools were confirmed by the microorganism reciprocal transplant experiment and aggregate disruption experiment. The former experiment showed that, in the active pool, inoculating the topsoil with the subsoil microorganism (away inoculum) significantly decreased $Q_{10}$ compared with its own inoculum ($P < 0.01$; fig. S2A), whereas inoculating the subsoil with the topsoil microorganism (away inoculum) resulted in a significantly higher $Q_{10}$ ($P < 0.01$; fig. S2A). Consequently, $\Delta Q_{10}$ (i.e., $Q_{10}$topsoil $- Q_{10}$subsoil) was significantly lower for the treatment of away inoculum in the active pool ($P < 0.01$; Fig. 6A). However, in the slow pool, no significant difference was detected between the two inoculums in both the topsoil and the subsoil ($P = 0.88$ and 0.21, respectively; fig. S2B), and thus, no change was observed for $\Delta Q_{10}$ in this pool ($P = 0.20$; Fig. 6A). In contrast, the later experiment demonstrated that the SOM protection by aggregates mainly affected $Q_{10}$ in the slow pool. The removal of aggregate protection by crushing resulted in a significantly declined $\Delta Q_{10}$ in the slow pool ($P < 0.01$) but had no effect in the active pool ($P = 0.21$; Fig. 6B).
DISCUSSION

Our results demonstrate that SOM decomposition in the topsoil was more sensitive to warming than in the subsoil. This significant difference could be due to the following two reasons. First, the stronger SOM protection by aggregates in the subsoil could attenuate $Q_{10}$. Aggregate protection exerts important control over the stabilization of SOM (27), and it has been reported that small sizes of aggregates need more energy to be disrupted (11). That is to say, the degree of protection increases with the decline in aggregate sizes and would be stronger in microaggregates (27). In our study, a higher proportion of C stored in microaggregates in the subsoil compared with the topsoil indicated that aggregate protection was stronger in the subsoil, which further resulted in a lower $Q_{10}$ value through the following two aspects. On the one hand, SOM is occluded in the interior of microaggregates, inducing spatial disconnection between substrates and enzymes, and thus protecting SOM from microbial decomposition (27). On the other hand, the small sizes of microaggregates could restrict the diffusion of oxygen (11), which limits the activity of microorganisms and results in low $Q_{10}$ (28). Overall, the determining role of SOM protection by aggregates observed here, together with previous proposals (3, 10), jointly demonstrates the importance of aggregate protection in governing the response of SOM decomposition to global warming.

Second, variations in microbial communities between soil depths could lead to the $Q_{10}$ difference. Microorganisms are addressed to play key roles in regulating the terrestrial C cycle, mediating not only the rate of SOM decomposition but also its responses to warming (14). In our study, the lower microbial abundance in the subsoil (table S2) that resulted from the alterations in soil resource availability (e.g., the decline in soil C and nitrogen; table S3) might constrain SOM decomposition even under high temperature and thus attenuate $Q_{10}$ (15). Moreover, microbial community composition would also affect $Q_{10}$. It has been reported that fungi are the dominant decomposers of recalcitrant substrates (29). Because the decomposition of these low-quality substrates requires greater activation energy (8), the shift in microbial community composition with decreasing proportion of fungi with soil depth may contribute to the lower $Q_{10}$ in the subsoil (30). These deductions were further confirmed by the microorganism reciprocal transplant experiment: By applying inoculums with high microbial abundance and fungi proportion to the subsoil, $Q_{10}$ significantly increased, while applying inoculums with low microbial abundance and fungi proportion to the topsoil significantly decreased $Q_{10}$ (fig. S2A). In addition to the variations in microbial communities, the activities of subsoil microorganisms might not be sustained by enough energy due to the lack of fresh substrates (2, 31, 32), which would further attenuate $Q_{10}$ in the subsoil (33).

Our results also demonstrated that the $Q_{10}$ variation in the active pool was primarily mediated by microbial communities, whereas the
its temperature response. Extending studies incorporating these on soil physical properties, and despite a widely used sterilization technique with limited effects on enzymes and oxygen (40, 41); enzyme activities and dynamics (34, 42); organic matter–mineral interactions (6); and hydrologically driven diffusion of substrates or enzymes (43). In view of the simple assumption about soil C turnover in current models, uncertainties might exist in model estimations. Hence, future studies should improve model structure to better reflect the mechanisms of soil C stabilization (6) and also measure Q_{10} of different soil C pools directly to provide benchmark for model development.

In summary, through a multiple approach–based analysis, we observed that, across soil depths, lower microbial abundance and stronger SOM protection by aggregates are two coexisting mechanisms responsible for the lower Q_{10} in the subsoil. We also found that the underlying mechanisms of Q_{10} were different between the active and slow pools, as microbial communities dominated in the active pool, whereas aggregate protection was more important in the slow pool. Given that the slow C pool is the largest component of SOM with a longer turnover time, SOM protection via microaggregates could be the key mechanism that regulates the long-term response of SOM decomposition, especially for the large deep and slow C pools, thereby attenuating the predicted terrestrial C-climate feedback.

**MATERIALS AND METHODS**

**Site description and soil sampling**

We collected soil samples from three alpine meadow sites on the Tibetan Plateau, China, where substantial quantities of soil organic C (15.3 Pg) were stored in the top 3 m (44). In the past decades, mean annual temperature on the plateau increased at a rate about twice that of global warming (45). The high rate of climate warming, together with the high Q_{10} on the plateau (46), could induce potential positive C-climate feedback in this unique geographic region. Moreover, because of the detected differences in substrate quality, environmental constraints, and microbial properties among soil depths (17), there might be considerable differences in Q_{10} through soil profile. To test this possibility, we selected three sampling sites (Halejing, Xihetai, and Zhagamu) on the northeastern Tibetan Plateau (fig. S1A) and examined Q_{10} in different soil depths and various soil C components, as well as the associated mechanisms.

The sites are situated at latitudes of 37.02°N to 37.61°N and longitudes of 100.11°E to 101.24°E, with an elevation of 3200 to 3400 m. The mean annual temperature varies from 0.12° to 2.20°C, and the mean annual precipitation ranges from 340 to 378 mm. The vegetative type belongs to alpine meadow, characterized by the dominant species of Kobresia pygmaea in Halejing and Zhagamu, and Kobresia humilis in Xihetai. The soil type at the three study sites is Cambisol according to the World Reference Base for Soil Resources (46). Cambisols are widespread not only on the Tibetan Plateau but also worldwide (47), accounting for ~38% of the Tibetan Plateau and ~11% of the

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**Fig. 6.** Δ Q_{10} in active and slow pools derived from two manipulative experiments. (A) Microorganism reciprocal transplant. (B) Aggregate disruption. Δ Q_{10} is the Q_{10} difference between the topsoil and the subsoil, with a positive value representing larger Q_{10} in the topsoil and a negative value representing larger Q_{10} in the subsoil. Error bars denote SE (n = 9). *P < 0.01, significant difference between control and treatment. ns, no significant difference.
global land surface. Soil physicochemical properties at these sites are given in table S3.

We conducted soil sampling in July and August 2014 (44). At each sampling site, we set up a 10 m × 10 m square plot and excavated three replicate soil pits at two corners and the center along a diagonal line (fig. S1B). We then collected topsoil at depths of 0 to 10 cm and subsoil at depths of 30 to 50 cm (fig. S1C). Soils from each depth were divided into two subsamples: one of which was passed through a 2-mm sieve and stored at −20°C to determine SOM composition and microbial communities; the other set was air-dried and processed for measurements of other physicochemical properties.

**Experimental design**

To examine the \( Q_{10} \) difference between soil depths and the associated mechanisms, we established two manipulative experiments based on 330-day incubations at 10° and 20°C: one was conducted to compare \( Q_{10} \) in the topsoil and subsoil, and also to test the effects of aggregate protection through the uncrushed (control) and crushed (remove aggregate protection) soils (termed “aggregate disruption experiment”). The other was performed to examine the depth difference in microbial controls by inoculating soils with active microorganism from away (collected in the other depth) and own (the same depth) (termed “microorganism reciprocal transplant experiment”).

In the aggregate disruption experiment, we compared \( Q_{10} \) between the topsoil and the subsoil and then examined the effects of aggregate protection on \( Q_{10} \). The treatments included uncrushed (control) and crushed soil samples. For the crushed treatment, air-dried soil samples were crushed for 1 min by a ball mill to disrupt aggregates and remove aggregate protection (19). Together, a \( 3 \times 2 \times 2 \times 3 \) factorial experiment was conducted corresponding to three study sites, two soil depths, two levels of aggregate disruption, two incubation temperatures, and three replicates. For each of these treatments, 10 g of air-dried topsoil and 20 g of air-dried subsoil from each replicate were evenly distributed into 250 ml of airtight amber jars and incubated at both 10° and 20°C for 330 days (fig. S1D). Soil moisture was adjusted to 60% of water holding capacity (46). The rate of CO\(_2\) release was determined on the basis of the changes in headspace CO\(_2\) concentration during the incubation interval (17) using an infrared gas analyzer (EGM-5; PP Systems, Haverhill, MA, USA). The measurements were taken every 1 to 4 days for the first 3 weeks and then 1 to 2 weeks for 3 months, followed by 1 to 2 months for the rest of the incubation. It should be noted that the CO\(_2\) production from the uncrushed (control) soils was also used to explore the difference in \( Q_{10} \) between soil depths.

In the microorganism reciprocal transplant experiment, we explored whether microorganisms were functionally different between soil depths (20). This experiment was conducted by applying two microbial inoculums (derived from topsoil and subsoil) to both sterilized topsoil and subsoil. Specifically, soil samples were sterilized using \( \gamma \)-irradiation (\(^{60}\)Co) at a dose of 30 kGy (37). The sterilized soil was then divided into two subsamples, receiving the inoculum derived from itself (own) and from the other corresponding depth (away), respectively; the former treatment (own) was treated as control. In total, 72 microcosms (3 sites × 2 soil depths × 2 inoculums × 2 temperatures × 3 replicates) were constructed. Inoculums from each soil depth were made by adding 1 g of dry weight fresh soil to 100 ml of sterilized deionized water. Then, the inoculum was shaken at 150 rpm for 0.5 hour and filtered through a Whatman GF/C filter. Inoculum (0.4 ml) was applied to per gram soil. The procedure of subsequent CO\(_2\) measurement was the same as the first experiment.

**Calculation of \( Q_{10} \) values**

We adopted three methods to calculate \( Q_{10} \) values for bulk soil and various SOM components. First, \( Q_{10} \) for bulk soil was calculated according to the decomposition rate at two incubation temperatures

\[
Q_{10} = \frac{(R_w/R_c)^{10/(T_w - T_c)}}{} (1)
\]

where \( R_w \) and \( R_c \) denote the average rate of SOM decomposition at the warmer and colder temperature (mg CO\(_2\)-C g\(^{-1}\) SOC day\(^{-1}\)), respectively. \( T_w \) and \( T_c \) represent the warmer and colder temperature (°C). \( Q_{10} \) obtained by this method was referred to as bulk soil \( Q_{10} \).

Second, the \( Q_{10-q} \) method, which is based on the measured cumulative percentage of soil C respired during incubation, was applied to calculate the \( Q_{10} \) values of different SOM fractions (22)

\[
Q_{10-q} = \frac{(t_c/t_w)^{10/(T_w - T_c)}}{} (2)
\]

where \( t_c \) and \( t_w \) are the time needed to decompose a given soil C fraction at the colder and warmer temperature (day), respectively. \( T_w \) and \( T_c \) denote the warmer and colder temperature (°C). In this study, we chose the upper limit of soil C fraction according to the lowest cumulative percentage of respired C at 10°C (3.9%; fig. S3), which was within the range of previous incubations (0.4 to 17.0%) based on the same temperature and the approximate duration (48, 49). According to a previous study (16), we then set the size of the fractions as 0.5% of total soil C and estimated \( Q_{10} \) values for three fractions of soil C (i.e., 1 to 1.5%, 2 to 2.5%, and 3 to 3.5% of cumulative respired soil C), which represent a gradient of decreasing substrate lability.

Last, we used a two-pool (that is, active and slow pools with different turnover times) model to estimate \( Q_{10} \) for each C pool using data from our 330-day incubation experiment. The two-pool model performed well in simulating the soil C flux (fig. S4) and was applied to each sample at the two temperatures as follows (26)

\[
R(t) = \frac{3}{t} \sum_{i=1}^{3} k_{fi} C_{tot} e^{-kt} \quad (3)
\]

\[
Q_{10} = \left( \frac{k_{fi}(T_w)}{k_{fi}(T_c)} \right)^{w_{fi}} \quad (4)
\]

\[
f_1 + f_2 = 1 \quad (5)
\]

where \( R(t) \) is the measured decomposition rate at time \( t \) (mg CO\(_2\)-C g\(^{-1}\) SOC day\(^{-1}\)), \( C_{tot} \) denotes the initial SOC content (i.e., 1000 mg C g\(^{-1}\) SOC), \( k_1 \) and \( k_2 \) are the decay rates of active and slow pool (day\(^{-1}\)), and \( f_1 \) and \( f_2 \) denote the fractions of the active pool and slow pool. \( Q_{10} \) and \( Q_{10}^2 \) represent \( Q_{10} \) in the active pool and slow pool. \( k(T_w) \) and \( k(T_c) \) are the decay rates at the warmer (\( T_w \)) and colder (\( T_c \)) temperature, respectively. Before modeling, the prior range of the five parameters \( k_1 \) and \( k_2 \) at 10°C, \( Q_{10} \), \( Q_{10}^2 \), and \( f_1 \); table S4) was set on the basis of previous studies (17, 50) and then determined by a Markov chain Monte Carlo (MCMC) approach as follows (26): Probabilistic inversion approach based on Bayes’ theorem (Eq. 6) was applied to optimize parameters (θ) in the model. In this approach, the posterior probability density
function (PDF) \( P(\theta|Z) \) was acquired from the prior knowledge of parameters and the information of incubation data, represented by a prior PDF \( P(\theta) \) and a likelihood function \( P(Z|\theta) \), respectively

\[
P(\theta|Z) \propto P(Z|\theta)P(\theta)
\]

To calculate the likelihood function \( P(Z|\theta) \), we assumed that errors between modeled and observed values followed a multivariate Gaussian distribution with a zero mean

\[
P(Z|\theta) \propto \exp\left\{-\frac{1}{2\sigma^2} \sum_{i=1}^{2} \sum_{j=1}^{5} \left| \frac{Z_i(t) - X_i(t)}{\sigma(t)} \right|^2\right\}
\]

where \( Z_i(t) \) and \( X_i(t) \) denote the measured and modeled data, and \( \sigma(t) \) represents the SD of the measurements. Metropolis-Hastings (M-H) algorithm, an MCMC technique, was used to complete the construction of \( P(\theta|Z) \) of parameters (51, 52).

**Measurements of abiotic and biotic variables associated with \( Q_{10} \)**

We determined four types of variables, including substrate quality, SOM association with minerals, SOM protection by aggregates, and microbial communities, to explore potential mechanisms responsible for the \( Q_{10} \) difference between the two soil depths. Specifically, to determine substrate quality, SOM composition was investigated by solid-state \(^{13}\)C CPMAS NMR. Having been treated with 10% hydrofluoric acid (HF) repeatedly (33), soil samples were rinsed with deionized water and then freeze-dried. Approximately 100 mg of HF-treated samples was measured on an AVANCE III 400 WB spectrometer (Bruker BioSpin, Rheinstetten, Baden-Württemberg, Germany) at 100.62 MHz. The spectrometer was equipped with a 4-mm CPMAS probe, and the parameters were set with a spinning rate of 8 kHz, a contact time of 2 ms, and a recycle delay of 6 s. We then used MestRe Nova 9.0 (Mestrelab Research S.L., Santiago de Compostela, Galicia, Spain) to integrate the spectra into the following chemical shift regions and acquire the relative intensity of each region: alkyl C (0 to 50 parts per million (ppm)), O-alkyl C (50 to 110 ppm), aromatic C (110 to 165 ppm), and carboxylic C (165 to 220 ppm) (54).

To quantify SOM protection by minerals, Fe-bound SOC, which directly reflects the proportion of SOC associated with reactive Fe, was determined on the basis of the citrate-bicarbonate-dithionite (CBD) method (55). Briefly, in the reduction treatment, a solution containing trisodium citrate and sodium bicarbonate was added to 0.25 g of soil and heated to 80°C by water bath. A reducing agent, sodium dithionite, was then added, and the mixture was held at 80°C for 15 min. Instead of CBD extraction, soil samples in the control treatment were extracted with sodium chloride (NaCl) at an equivalent ionic strength. After rinsing the soil residues three times with 1 M NaCl, SOC content of the residues in each treatment was measured. Fe-bound SOC was then calculated as follows

\[
\text{Fe-bound SOC (\%)} = \frac{\text{SOC}_{\text{NaCl}} - \text{SOC}_{\text{CBD}}}{\text{SOC}} \times 100
\]

where \( \text{SOC}_{\text{NaCl}} \) and \( \text{SOC}_{\text{CBD}} \) refer to the SOC content (g kg\(^{-1}\)) of the control treatment and reduction treatment, respectively.

To determine SOM protection by aggregates, we isolated three SOM fractions to measure C distributions in each fraction. Specifically, using the wet-sieving technique (56), 30 g of air-dried soil (5 mm) was submersed in water for 5 min and then wet-sieved over 250 and 53 mm of sieves, consecutively. The fraction collected on the 250-μm sieve was macroaggregates (250 to 2000 μm), and that collected on the 53-μm sieve was microaggregates (53 to 250 μm). The fraction in the remaining suspension was silt + clay (<53 μm). Each fraction was then dried at 60°C. After isolating sand in macroaggregates and microaggregates with sodium hexametaphosphate, SOC concentrations of the three fractions were measured. Of the three fractions, the proportions of SOC distributed in macroaggregates and microaggregates were used to quantify SOM protection by aggregates (27).

We adopted the PLFA approach to assess soil microbial abundance and community composition. PLFAs were extracted according to the protocol described by Bossio and Scow (57). With 19:0 (methyl nonadecanoate, \( \text{C}_{20}\text{H}_{40}\text{O}_{2} \)) as the internal standard, samples were then analyzed with a gas chromatograph (Agilent 6850, Agilent Technologies, Santa Clara, CA, USA). The identification of the extracted fatty acid was based on a MIDI peak identification system (Microbial ID Inc., Newark, DE, USA). PLFAs specific to fungi (18:2\( \Delta 9,9 \)) and bacteria (11:0, 15:0, 16:0, 17:0, 16:1\( \omega 7c \), cy-17:0, 18:1\( \omega 7c \), cy19:0) were quantified (17). The composition of the microbial community was represented by the relative abundance of fungal PLFAs and F/B ratio.

**Statistical analyses**

We conducted mixed effects models (R package: nlme) to examine the difference in \( Q_{10} \) values between the topsoil and the subsoil. In the model, site and soil depth were set as fixed factors, and depth nested in replicate was treated as a random factor. Having detected no interaction effect between site and soil depth (table S1), we used paired \( t \) tests to compare the means of physicochemical properties between the topsoil and the subsoil. Linear regression models were then performed to identify the relationships of \( Q_{10} \) with aggregate protection and microbial communities. Before the analyses, the variance inflation factor (VIF) was calculated to assess the collinearity of the variables (acceptable collinearity VIF \( \leq 2 \) (58). On the basis of this criterion, the collinearity between SOC distribution in macroaggregates and microaggregates could be partly omitted (VIF = 1.6), whereas the relative abundance of fungal PLFAs was closely correlated with F/B (VIF = 4.4), and thus, only the former was included in the model.

To further analyze the relative importance of different variables, we conducted variation partitioning analysis (R package: vegan) to partition the effects of aggregate protection and microbial communities on \( Q_{10} \) in various C pools. \( \Delta Q_{10} \) (\( Q_{10 \text{topsoil}} - Q_{10 \text{subsoil}} \))—the difference in \( Q_{10} \) between the topsoil and the subsoil)—for the paired manipulative experiments was then calculated to test the role of aggregate protection and microbial communities. A positive value of \( \Delta Q_{10} \) represents larger \( Q_{10} \) in the topsoil, while a negative value represents larger \( Q_{10} \) in the subsoil. The means of \( \Delta Q_{10} \) between control and crush/inoculation treatments were compared using paired \( t \) tests. The decrease in \( \Delta Q_{10} \) compared with the control indicates that crush/inoculation treatment reduces the difference in \( Q_{10} \) between the topsoil and the subsoil and vice versa. All of the statistical analyses were conducted with R version 3.2.1 (39), with a significance level of 0.05.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://advances.sciencemag.org/cgi/content/full/5/7/eaau1218/DC1

Fig. S1. Field soil sampling and laboratory incubation.
Fig. S2. Effects of microorganism reciprocal transplant on QO2 for the two soil depths.
Fig. S3. Changes in the cumulative respired soil C with incubation time.
Fig. S4. Comparison between modeled and measured soil C flux.
Table S4. Prior parameter ranges for C pool partitioning coefficients (\(Q_i\)).

Fig. S3. Changes in the cumulative respired soil C with incubation time.

Table S3. Physicochemical characteristics in the two soil depths at three sites.

Table S4. Prior parameter ranges for C pool partitioning coefficients (\(Q_i\)).

Fig. S2. Effects of microorganism reciprocal transplant on QO2 for the two soil depths.

**REFERENCES AND NOTES**


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Temperature sensitivity of SOM decomposition governed by aggregate protection and microbial communities
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