Temperature sensitivity of permafrost carbon release mediated by mineral and microbial properties

Shuqi Qin1,2, Dan Kou1,3, Chao Mao1,2, Yongliang Chen1, Leiyi Chen1, Yuanhe Yang1,2*

Temperature sensitivity ($Q_{10}$) of permafrost carbon (C) release upon thaw is a vital parameter for projecting permafrost C dynamics under climate warming. However, it remains unclear how mineral protection interacts with microbial properties and intrinsic recalcitrance to affect permafrost C fate. Here, we sampled permafrost soils across a 1000-km transect on the Tibetan Plateau and conducted two laboratory incubations over 400- and 28-day durations to explore patterns and drivers of permafrost C release and its temperature response after thaw. We find that mineral protection and microbial properties are two types of crucial predictors of permafrost C dynamics upon thaw. Both high C release and $Q_{10}$ are associated with weak organo-mineral associations but high microbial abundances and activities, whereas high microbial diversity corresponds to low $Q_{10}$. The attenuating effects of mineral protection and the dual roles of microbial properties would make the permafrost C-climate feedback more complex than previously thought.

INTRODUCTION

Permafrost, defined as ground remaining at or below 0°C for at least two consecutive years, occupies ~24% of the exposed land area in the Northern Hemisphere (1). Current estimate reports that soils in permafrost regions store ~1014 Pg (1 Pg = $10^{15}$ g) carbon (C) to a depth of 3 m (2). Under recent climate warming, a portion of the frozen permafrost C has thawed and been exposed to microbial decomposition, potentially acting as a strong positive feedback to warming due to the high temperature sensitivity of soil organic matter (SOM) decomposition ($Q_{10}$, the factor by which the decomposition rate changes with a 10°C temperature rise) in cold regions (3, 4). This conceptual thinking has been confirmed by mounting evidence, with both in situ experiments and model simulations documenting rapid soil C loss and accelerated warming after permafrost thaw (5–8). However, the inaccurate parameterization of $Q_{10}$ in models constitutes one of the major uncertainties in projecting the C-climate feedback (9), with the predicted soil C loss in permafrost region ranging from 74 to 652 Pg C between 2010 and 2299 under a climate pathway involving little to no mitigation effort [i.e., Representative Concentration Pathway 8.5 (RCP 8.5)] (10). Therefore, accurate evaluation of the temperature sensitivity of permafrost C release and its underlying mechanisms is crucial for more realistic projections of permafrost C dynamics and the associated feedback to climate warming.

Because of the vital role of $Q_{10}$ in evaluating permafrost C fate, studies have increasingly paid attention to the temperature response of C release after permafrost thaw (11–13). Despite all the research conducted so far, our understanding is still limited by the following two aspects. First, previous studies concerning permafrost $Q_{10}$ were mainly confined to site levels (11, 13), with little evidence from large-scale systematic measurements. Although data synthesis based on the site-level research could provide some insights into the spatial variation of $Q_{10}$ (14), the results might be constrained by the inconsistent experimental conditions among individual studies (15).}

A permafrost $Q_{10}$ dataset derived from standardized methodology over a broad geographical scale is thus imperative to benchmark model projections by constraining the temperature response of permafrost C release (10). Second, existing studies predominantly focused on the roles of substrate quality and microbial properties in predicting permafrost $Q_{10}$ (12, 13), whereas the effect of mineral protection, which chemically stabilizes SOM through adsorption, has not been adequately assessed [with an existing study considering only reactive iron (Fe)/aluminium (Al) oxides (16)]. In particular, organo-mineral association through multivalent cation [especially calcium (Ca$^{2+}$)] bridging, functioning as another critical protection mechanism in alkaline and calcareous soils, has received less attention (17). Moreover, how mineral protection, microbial properties, and substrate quality interact to affect permafrost $Q_{10}$ remains largely unidentified. A comprehensive study involving all the potential drivers and quantifying their relative importance is thus greatly needed, which could, in turn, improve the parameterization of $Q_{10}$ in permafrost C models.

The Tibetan Plateau covers the largest permafrost area in the mid- and low latitudes of the world and stores 15.3 Pg C in the top 3-m soils (18). Rapid warming was predicted to result in ~1.9 Pg and ~3.8 Pg permafrost C thawing on the plateau by 2100 under RCP 4.5 (a moderate mitigation scenario) and RCP 8.5, respectively (19), indicating the high vulnerability of permafrost C in this region. However, studies concerning the $Q_{10}$ after permafrost thaw are still limited on the plateau [except for the work of Mu et al. (20), which evaluated $Q_{10}$ values along a permafrost profile on the northern plateau], with existing evidence mainly coming from the Arctic permafrost region. To fill this data gap and the knowledge gap mentioned above, we sampled permafrost soils beneath the active layer (i.e., the seasonally thawed surface ground layer) at 24 sites across a 1000-km transect on the plateau (Fig. 1). On the basis of the collected permafrost samples, we conducted two incubation experiments over 400- and 28-day durations, in combination with first-order kinetic models (21) to quantify the potential C release and $Q_{10}$ after permafrost thaw. We then determined four types of explanatory factors associated with $Q_{10}$, including mineral protection, microbial properties, substrate quality, and edaphic variables (see Materials and Methods). The relative importance of these factors in affecting permafrost C release and $Q_{10}$ was further identified by two types of
statistical analyses, i.e., structural equation modeling (SEM) and variation partitioning analysis, respectively. Overall, this study aimed to explore large-scale patterns and dominant drivers of \( Q_{10} \) after permafrost thaw.

**RESULTS**

**Spatial variations of permafrost C release and \( Q_{10} \)**

Both cumulative C release and \( Q_{10} \) exhibited large variations across the 24 sampling sites. During the 400-day incubation at 5°C, the cumulative C release normalized to per unit organic C (OC) ranged from 12.3 to 268.6 mg CO\(_2\)-C g\(^{-1}\) OC and averaged 90.7 ± 14.7 mg CO\(_2\)-C g\(^{-1}\) OC (hereafter, values were reported as means ± SE unless noted; Fig. 1A and fig. S1A). Higher cumulative C release was observed at 15°C for the soils from all the sites, with an average value of 176.8 ± 29.6 mg CO\(_2\)-C g\(^{-1}\) OC over 400 days (fig. S1A). To further explore the temperature response of C release after permafrost thaw, we used a one-compartment model and a two-pool model, combined with the 400-day incubation (at 5° and 15°C) to calculate the \( Q_{10} \) values of bulk soil and different C pools (i.e., active C pool persisting for days to weeks and slow C pool stabilizing for years to decades or even longer), respectively. Bulk soil \( Q_{10} \) varied between 1.3 and 2.6, averaging 2.1 ± 0.1 (Fig. 1B). Active and slow C pool \( Q_{10} \) ranged from 1.3 to 3.3 and 1.8 to 2.7, respectively (fig. S1, B and C). We also performed the 28-day incubation (at −3°, 1°, 5°, 10°, and 15°C) to acquire the \( Q_{10} \) from below to above freezing temperatures. \( Q_{10} \) obtained from this short-term incubation was significantly correlated with the bulk soil \( Q_{10} \) derived from the 400-day incubation (\( P < 0.01 \)) but was much higher (\( P < 0.001 \)), ranging between 5.7 and 8.0 and averaging 6.6 ± 0.1 across the 24 sampling sites (fig. S2, A and B).

**Direct and indirect drivers of permafrost C release**

We established relationships of cumulative C release and \( Q_{10} \) with the potential predictors, including mineral protection, microbial properties, substrate quality, and edaphic variables (Figs. 2 and 3 and fig. S3). The cumulative amount of C released over the 400-day incubation at 5°C was significantly associated with the examined factors (Fig. 2 and fig. S3A). Specifically, permafrost C release after thaw was negatively correlated with the degree of mineral protection characterized by the content of reactive minerals (except for total pedogenic Fe and exchangeable Ca\(^{2+}\)) and organo-mineral associations (all \( P < 0.05 \); Fig. 2, A to F). Higher microbial abundances (especially fungal abundance quantified by phospholipid fatty acid (PLFA) analysis) and hydrolase activity were indicative of higher C release (all \( P < 0.05 \); Fig. 2, G to J). Moreover, C release decreased with recalcitrant pool proportion and humification index, which represent the SOM recalcitrance (all \( P < 0.05 \); fig. S3A). Edaphic variables also significantly affected C release, with C release decreasing with soil moisture but increasing with active layer thickness and soil pH (all \( P < 0.01 \); fig. S3A). The combination of the four types of predictors explained 92% of the variance in C release as revealed by the SEM (Fig. 4A). Among them, mineral protection and microbial properties had direct negative and positive effects on
Fig. 2. Relationships of cumulative CO₂-C release with mineral protection and microbial properties. (A to F) Factors characterizing mineral protection, including the content of SOC stored in MAOM fraction (OC-MAOM), the content of SOC associated with Fe oxides (OC-Fe) and Ca bridges (OC-Ca), the content of poorly crystalline and organically complexed Fe/Al oxides (Fe₂ + Al₂ and Fe₃ + Al₃), and the ratio of amorphous Si to poorly crystalline Al (Si/Al₆). (G to L) Factors characterizing microbial properties, including microbial abundances, community structure, enzyme activities, and microbial diversity. F/B, fungal/bacterial ratio; hydrolase activity, the sum of activities of BG, NAG, LAP, and AP; oxidase activity, phenol oxidase activity; microbial diversity, the average of the standardized bacterial and fungal diversity indexes. The linear regression lines with 95% confidence intervals reflect the predicted effects of fixed factors. *P < 0.05, **P < 0.01, and ***P < 0.001.

Fig. 3. Relationships of bulk soil and slow C pool Q₁₀ with mineral protection and microbial properties. (A) Standardized regression coefficients of the factors of mineral protection and microbial properties with bulk soil and slow C pool Q₁₀, with the color indicating the strength and sign of the relationship. (B to E) Relationships between Q₁₀ and the predictors retained in the optimal model by the stepwise selection procedure. The linear regression lines with 95% confidence intervals reflect the predicted effects of fixed factors. *P < 0.05. OC-MAOM, the content of SOC stored in MAOM fraction; OC-Fe and OC-Ca, the contents of SOC associated with Fe oxides and Ca bridges; Fe₂, pedogenic Fe oxides; Fe₂ + Al₂, poorly crystalline Fe/Al oxides; Fe₃ + Al₃, organically complexed Fe/Al oxides; CaExch, exchangeable Ca²⁺; Si/Al₆, the ratio of amorphous Si to poorly crystalline Al; F/B, fungal/bacterial ratio; hydrolase activity, the sum of activities of BG, NAG, LAP, and AP; oxidase activity, phenol oxidase activity; microbial diversity, the average of the standardized bacterial and fungal diversity indexes.
C release, respectively. Mineral protection also showed an indirect effect by negatively affecting microbial properties (Fig. 4A). In addition, edaphic variables exhibited indirect effects on C release after permafrost thaw (Fig. 4A and fig. S4).

**Key predictors of \(Q_{10}\) after permafrost thaw**

Mineral protection and microbial properties were key predictors of \(Q_{10}\) variation (Fig. 3). Specifically, both bulk soil and slow C pool \(Q_{10}\) derived from the 400-day incubation were negatively correlated with OC-Ca (the content of SOC associated with Ca bridges), \(\text{Fe}_p + \text{Al}_p\) (the content of organically complexed Fe/Al oxides), and microbial diversity (the average of the standardized bacterial and fungal diversity indexes) but were positively associated with hydrolase activity (all \(P < 0.05\); Fig. 3A). In addition, bacterial PLFAs and oxidase activity had positive effects on slow C pool \(Q_{10}\), while bacterial diversity showed negative effects (all \(P < 0.05\); Fig. 3A). No relationship was detected between the active C pool \(Q_{10}\) and any of the potential predictors examined in this study (\(P > 0.05\)). We then adopted a stepwise selection procedure to select the optimal models for \(Q_{10}\) and found that OC-Ca and microbial diversity were dominant predictors of bulk soil and slow C pool \(Q_{10}\) (Fig. 3, B to E).

We conducted variation partitioning analysis for \(Q_{10}\) to further estimate the relative contributions of the predictors that remained in the optimal model (i.e., OC-Ca representing mineral protection and microbial diversity characterizing microbial properties). Mineral protection and microbial properties explained 57.6% of the bulk soil \(Q_{10}\) variation in total, with the unique effects being 34.8 and 21.5% for the two respective groups of factors (Fig. 4B). For the slow C pool \(Q_{10}\), the unique effects of mineral protection and microbial properties were 24.5 and 29.2%, respectively, which jointly accounted for 58.5% of the total variation (Fig. 4C). Consistently, the analyses of \(Q_{10}\) derived from the 28-day incubation from below to above freezing temperature also demonstrated the crucial roles of mineral protection and microbial properties in predicting \(Q_{10}\) over large scales (fig. S2, C to E).

**DISCUSSION**

On the basis of large-scale permafrost sampling and laboratory incubations, combined with systematic measurements of potential predictors, this study explored the spatial patterns and drivers of permafrost C release and its temperature response after thaw across...
the Tibetan Plateau. Our results revealed spatial variations in both C release and Q10. The bulk soil Q10 from 5°C to 15°C ranged between 1.3 and 2.6 and averaged 2.1 ± 0.1 across the study area, comparable with the corresponding results from the Arctic permafrost, where a 10°C increase from 5°C to 15°C accelerated C release by a factor of 2.0 (95% confidence interval, 1.6 to 2.6) (14). Further analyses demonstrated that mineral protection and microbial properties were important predictors of permafrost C dynamics upon thaw (Fig. 4). Despite its associations with C release (fig. S3A), substrate quality did not show any significant relationship with Q10 (fig. S3, B and C), conflicting with the kinetic theory, which predicts higher intrinsic temperature sensitivity for lower-quality SOM (22, 23). This discrepancy indicates that mechanisms other than intrinsic recalcitrance could predominate in affecting the observed temperature sensitivity (23–25), highlighting the dependence of permafrost C dynamics on the abiotic and biotic factors (such as mineral protection and microbial properties detected here), which currently stabilize soil C in this unique geographic area.

Mineral protection attenuates C release and its temperature response after permafrost thaw

Mineral protection had negative effects on C release and Q10 (Figs. 2 and 3), possibly by restricting substrate accessibility or inhibiting microbial activities (17). On the one hand, organo-mineral associations might transfer SOM from soil solution to a solid phase, reducing the diffusion of SOM and its contact with degrading enzymes (17). On the other hand, soil extracellular enzymes responsible for SOM decomposition could show a strong affinity for mineral surfaces and thus be rendered inactive upon adsorption (26). These mineral protection processes can occur in different forms: Apart from the recognized importance of adsorption by reactive minerals such as Fe/Al oxides, Ca2+ would predominate in alkaline and calcareous soils by forming cation bridges between the negatively charged phyllosilicates and SOM (17). Soils on the Tibetan Plateau have been reported to be rich in Ca under neutral to alkaline conditions (27). Accordingly, the proportion of SOC associated with minerals via Ca bridges was even higher than that with Fe/Al oxides (14 ± 2% versus 8 ± 1%; fig. S5A), and it increased with soil pH across our study area (fig. S5B), reflecting the importance of Ca bridges in affecting permafrost C dynamics on the plateau.

Mineral protection has typically been regarded as less important in the Arctic permafrost zones than in other regions (28, 29) [except for a recent study proposing the importance of Fe/Al oxides in Siberia (16)]. However, we observed a large proportion of SOC associated with minerals across the Tibetan permafrost (~82 ± 3% of the total SOC; fig. S5A), which attenuated both permafrost C release and Q10 (Fig. 4). This discrepancy in organo-mineral interactions between the two permafrost regions might be attributed to the distinct sources of SOM in permafrost deposits. The active layer is typically shallow in the Arctic [~0.7 m (30)], and the entire active layer can be composed of just organic horizon in some regions (29). Consequently, large amounts of organic-rich plant residues could be transported into near-surface permafrost by cryoturbation (i.e., the vertical soil mixing by frequent freezing-thawing processes), leading to a large proportion of SOC in the unprotected particulate organic matter (POM) fraction in the Arctic permafrost soils (usually >30% or even >70% of total SOC) (31, 32). By contrast, the active layer is much thicker over the Tibetan permafrost region [1.9 m on average (33)], and ~90% of the root biomass is distributed in the surface 30 cm of soil (34), making it difficult for the undecomposed plant materials to enter into permafrost soils. The SOM derived from the deep active layer leaching or cryoturbation processes would thus be mainly composed of microbial products (35), which could then be adsorbed to minerals more easily (36). In addition to SOM sources, the temperature difference between the two regions might lead to the distinct degree of mineral protection despite the similar formation history and clay mineralogy for the Arctic and Tibetan permafrost. Most of the permafrost was formed during the late Pleistocene in both permafrost regions, with the predominant clay minerals being illite, chlorite, vermiculite, and kaolinite (37, 38). Nevertheless, the low temperature [mean annual temperature (MAT) of ~7.8°C from 92 sites (39)] in the Arctic has constrained the weathering process (40), while the warmer climate on the plateau (MAT of ~2.0°C across the study area) might be more favorable for mineral weathering and thus promote mineral protection, which, however, needs to be further tested in future studies.

Dual roles of microbial properties in driving C release and Q10 after permafrost thaw

Microbial abundances indicated by PLFAs and hydrolase activity were positively associated with C release and Q10, while high microbial diversity attenuated Q10 (Figs. 2 and 3). The positive effects of microbial abundances and activities reflected the constraints on substrate conversion and consumption by the small pool sizes of enzyme and microbial population in permafrost soils (13). Nevertheless, microbial functional redundancy among various species existed after permafrost thaw, so a reduction in certain species might have minor impacts on the overall SOM decomposition because other microbes could carry out the same function (41). As a result, microbial diversity had no effect on C release after permafrost thaw (Fig. 2L). In contrast to C release, Q10 declined with microbial diversity (Fig. 3), which was in line with a recent finding in the subsoil of an alpine ecosystem (42). This negative effect of microbial diversity on Q10 might be attributed to the increase in functional resistance (43). Increased microbial diversity would enhance functional resistance by providing a pool of species that could moderate ecosystem functioning (e.g., SOM decomposition and nutrient cycling) under changing environments (43). Thus, the microbial communities with higher diversity exhibited a weaker response of C release to experimental warming, supporting the positive biodiversity-ecosystem stability hypothesis (42).

Microbial communities have been observed to experience significant shifts in response to permafrost thaw in both short-term laboratory incubations (44, 45) and long-term in situ experiments (46, 47). Hence, it is possible that the associations between C release and microbial properties would change over time. To test this possibility, we determined the microbial properties after the 400-day incubation and found that variables such as bacterial abundance and diversity increased, while fungal diversity and fungal/bacterial ratio (F/B) decreased (fig. S6, A to I). These changes might be due to the greater competitive advantage of bacteria over fungi with increased nutrient availability [as indicated by positive net rates of nitrogen mineralization reported by Mao et al. (48)] during incubation (49). Despite these changes, C release was still positively correlated with microbial properties after incubation (including microbial abundances, F/B, and hydrolase activity; fig. S6, J to M), demonstrating the important role of microorganisms in affecting permafrost C dynamics even at the later stage after permafrost thaw.
Similarly, on the basis of an in situ permafrost thaw experiment, Monteux et al. (46) revealed the effects of bacterial community structure on soil respiration in the thawed permafrost, although bacterial communities tended to converge with those in the active layer, further highlighting the close linkages between permafrost C release and microbial communities after thaw. Nevertheless, some uncertainties might exist concerning the importance of microbial communities revealed by the DNA-based analysis in this and previous studies (13, 45, 46), since this technique may not depict the active members. RNA-derived sequences are thus encouraged to more directly reflect the microbial processes after permafrost thaw. In addition, this study conducted laboratory incubations under optimal conditions, precluding the effects of in situ factors such as geology, hydrology, and vegetation (50). Natural physical and biogeochemical processes such as permafrost collapse and C input, which could alter microbial communities and SOM composition, were also excluded. More knowledge about in situ processes is needed to enable a better understanding of permafrost C dynamics under a changing environment.

In summary, on the basis of large-scale permafrost sampling, together with long-term and short-term laboratory incubations, our study depicted spatial patterns and drivers of permafrost C dynamics after thaw. We observed substantial variations in both C release and $Q_{10}$ across the Tibetan permafrost, suggesting the necessity of incorporating spatially heterogeneous $Q_{10}$ values into permafrost C models. We also found that mineral protection and microbial properties were important predictors of permafrost C release and $Q_{10}$. Mineral protection attenuated C release and $Q_{10}$, especially in the slow C pool. By contrast, microbial properties played dual roles in affecting permafrost C fate, with higher microbial abundances and activities increasing while greater diversity decreasing $Q_{10}$. These abiotic and biotic modifications would lead to a more complex permafrost C-climate feedback than previously considered. Further model development should thus incorporate both mineral protection and microbial properties and distinguish their functions among C pools to accurately project permafrost C dynamics and the associated climate feedback.

**MATERIALS AND METHODS**

**Study area and permafrost sampling**

Permafrost is widely distributed across the Tibetan Plateau, covering an area of $1.1 \times 10^6$ km$^2$ and occupying 40% of the total area of the plateau (51). The active layer thickness exhibits large spatial variations and averages 1.9 m over the region (33). Similarly, mineral protection, microbial properties, and edaphic variables all cover broad gradients on the plateau (52), providing an ideal platform to explore large-scale patterns and dominant drivers of potential C release and $Q_{10}$ after permafrost thaw. In this study, we collected the uppermost permafrost soils at 24 sites across a 1000-km transect on the plateau, which covered three representative permafrost regions, i.e., Elashankou-Qingshuixue section on the eastern plateau (10 sites), Qilian Mountains on the northeastern plateau (3 sites), and Golmud- Amdo section in the middle part of the plateau (11 sites) (48). The MAT of these study sites ranges between $-4.5^\circ$ and $1.8^\circ$C, and the mean annual precipitation varies from 245 to 504 mm. The dominant vegetation of the sampling sites includes alpine steppe, alpine meadow, and swamp meadow, with *Stipa purpurea* and *Carex moorcroftii*, *Kobresia pygmaea* and *Kobresia humilis*, and *Kobresia tibetica* as dominants, respectively (53). Soil type is mainly Cambisols and also includes Calci-sols and Cryosols according to the World Reference Base for Soil Resources (54).

We conducted permafrost sampling in 2016, obtaining 120 soil cores in total from the 24 sites (48, 53). Specifically, we set up five 1 m $\times$ 1 m quadrats at the center and four corners of a 10 m $\times$ 10 m square plot. Within each quadrat, we used a borehole drilling machine to drill a soil core to a depth of 1.5 to 3.5 m according to the active layer thickness. To collect the uppermost 50-cm-thick permafrost soil, we removed the unfrozen active layer soil and took care to avoid the transition zone between the active layer and permafrost deposits during drilling (48, 53). All the collected samples were classified as mineral soils with initial C < 20% (55). The cores were transported frozen back to the laboratory, and the outer surface of each core was scraped in a cold room to avoid possible contamination (13). Then, one part of the samples was stored at $-20^\circ$C until incubation. The remaining part was air-dried to determine the physicochemical properties after passing through a 2-mm sieve and removing roots and gravel. Before incubation, we quickly cut the frozen samples into small pieces under low room temperature. Considering the high experimental costs, we mixed soils from the five replicates at each site to form a composite sample for the subsequent analyses.

**Incubation experiments**

We conducted two incubation experiments (referred to as “long-term incubation” and “short-term incubation”) to quantify permafrost C release and its temperature response. Of them, the long-term incubation was performed at 5$^\circ$ and 15$^\circ$C for 400 days, and the data at 5$^\circ$C were used to analyze the patterns and drivers of potential C release after permafrost thaw. We chose these two temperatures to facilitate the comparison of $Q_{10}$ with the corresponding results derived from the Arctic permafrost incubations (14, 16). Given that the temperature response of C release would be higher under frozen conditions (56), we also conducted a short-term incubation with two additional lower temperatures ($-3^\circ$ and $1^\circ$C) to further explore the $Q_{10}$ from below to above freezing temperature. In total, we selected five temperatures (i.e., $-3^\circ$, $1^\circ$, $5^\circ$, $10^\circ$, and $15^\circ$) to perform an additional 28-day incubation. The combination of the short-term and long-term incubations enabled us to not only capture the temperature response of permafrost C release in the case of thawing but also monitor the long-term permafrost C dynamics after thaw.

**Long-term incubation**

The permafrost samples used for incubation were first allowed to thaw at 5$^\circ$C for 48 hours. Given that the Tibetan alpine permafrost is mainly distributed in uplands (1), the samples were drained freely using a sand bath to mimic natural drainage following thaw (57). Then, we weighed approximately 15 to 50 g of thawed samples into 250-ml amber jars, with increasing weight for low-SOC samples to ensure reliable accumulation in the headspace CO$_2$ concentration. Soil moisture was adjusted to 60% of water holding capacity and was kept constant by adding deionized water (58). We first conducted a 7-day preincubation to exclude pulses in microbial activities caused by the disturbance of permafrost thaw and moisture adjusting. During the following 400-day incubation, we measured CO$_2$ release rate every 2 to 4 days for the first month, then every 1 to 2 weeks for the following 2 months, and in the later stage every 20 to 50 days. Before each measurement, the jars were sealed and flushed with CO$_2$-free air. Then, 15 ml of headspace gas was immediately
removed by a gas-tight syringe for initial CO₂ analysis, and the jars were equalized to atmospheric pressure by injecting CO₂-free air (58). After incubation at 5°C and 15°C for 24 to 28 hours, gas samples were collected again. On the basis of the CO₂ concentration determined by an infrared gas analyzer (EGM-5; PP Systems, Amesbury, MA, USA), we calculated CO₂ release rate according to the accumulation of headspace CO₂ concentration during the incubation interval and normalized it to per unit OC to control the effect of SOC content among sites. Using the data derived from the long-term incubation experiment, we calculated Q_{10} (termed “bulk soil Q_{10}” throughout) based on a first-order kinetic one-compartment model (16, 21). Specifically, the cumulative amount of C released on any measurement day was acquired by linear interpolation between sequential measurement points and fitted as follows:

\[ C_{\text{cum}}(t) = C_0 \times (1 - e^{-kt}) \]  

(1)

where \( C_{\text{cum}}(t) \) represents the cumulative amount of C released until time \( t \) (mg CO₂·C g⁻¹ OC), \( C_0 \) is the preincubation SOC content (mg C g⁻¹ OC), and \( k \) is the decay rate constant (day⁻¹). The model fitted the incubation data well (table S1), and bulk soil \( Q_{10} \) was then calculated as the ratio of decay rate constant at 15°C (\( T_w \)) to that at 5°C (\( T_c \)):

\[ Q_{10} = \left( \frac{k(T_w)}{k(T_c)} \right)^{0.10 \frac{T_w}{T_c}} \]  

(2)

We then calculated the \( Q_{10} \) values for active and slow C pools using the two-pool model (termed “active C pool \( Q_{10} \)” and “slow C pool \( Q_{10} \)”, respectively) (59):

\[ R(t) = \sum_{i=1}^{2} k_i f_i C_0 e^{-kt} \]  

(3)

\[ Q_{10}^{i} = \left( \frac{k(T_w)}{k(T_c)} \right)^{0.10 \frac{T_w}{T_c}} \]  

(4)

\[ f_1 + f_2 = 1 \]  

(5)

where \( R(t) \) denotes the CO₂ release rate measured at time \( t \) (mg CO₂·C g⁻¹ OC day⁻¹). \( C_0 \) is the initial SOC content (i.e., 1000 mg C g⁻¹ OC). \( k_1 \), \( f_1 \), and \( Q_{10} \) are the parameters for the active C pool, representing the decay rate constant (day⁻¹), fraction relative to the total C pool, and \( Q_{10} \), respectively. \( k_2 \), \( f_2 \), and \( Q_{10}^{2} \) are the corresponding parameters for the slow C pool. \( k(T_w) \) and \( k(T_c) \) are the decay rate constants at 15°C (\( T_w \)) and 5°C (\( T_c \)). We set the prior range of the model parameters (i.e., \( k_1 \) and \( k_2 \) at 5°C, \( Q_{10}^{1} \), \( Q_{10}^{2} \) and \( f_1 \); table S2) according to previous studies (55, 58, 60) and estimated the posterior probability density function of the parameters using the Bayesian probability inversion and a Markov chain Monte Carlo technique as described by Qin et al. (60). On the basis of the posterior parameter distributions, maximum likelihood estimates and means were then estimated for well-constrained and poorly constrained parameters, respectively (55). Notably, the two-pool model performed well in estimating the decomposition rate for the 24 sites (fig. S7).

**Short-term incubation**

Permafrost samples for each site were simultaneously incubated at −3°C, 1°C, 5°C, 10°C, and 15°C for 28 days. During the incubation, we determined CO₂ release rate at each temperature as performed in the long-term incubation. Given that CO₂ release rate declined rapidly under low temperatures, the incubation interval was extended to reach a reliable accumulation of headspace CO₂ concentration (56). We then adopted a first-order exponential equation to acquire \( Q_{10} \). Briefly, we fitted changes in CO₂ release rate with incubation temperatures as follows (56, 61):

\[ R = A e^{(BT)} \]  

(6)

where \( R \) is the CO₂ release rate at a given temperature in μg CO₂·C g⁻¹ OC day⁻¹, and \( T \) is the incubation temperature (°C). \( A \) and \( B \) are fitted parameters. The equation described the relationships well for all soils (table S3). Then, we used the fitted \( B \) values to calculate \( Q_{10} \) for each permafrost sample (56, 61):

\[ Q_{10} = e^{(10B)} \]  

(7)

**Mineral protection**

To explore the role of mineral protection in affecting permafrost C dynamics, we first measured the content of Fe/Al oxides and exchangeable Ca²⁺ in soil samples. Specifically, the total amount of pedogenic Fe (Feₚ) was extracted using citrate-bicarbonate-dithionite (CBD) method (16). Acid-ammonium oxalate was used to extract poorly crystalline oxyhydroxides (Feₜ₀ and Alₜ₀) and amorphous silicon (Si), and Si/Alₜ₀ was then calculated to reflect the weathering degree of parent materials, of which the higher value represented the lower degree of soil weathering (62). Organically complexed Fe and Al (Feₚ and Alₚ) were extracted by sodium pyrophosphate (37). We also used NH₄Cl-ethanol to extract the exchangeable Ca²⁺ (52). The element contents in the solution were measured on an inductively coupled plasma optical emission spectrometer (ICAP 6300, Thermo Fisher Scientific, Waltham, MA, USA).

To further evaluate the degree of mineral protection, we adopted a fractionation technique based on the combination of density and size (63) and calculated the content of SOC stored in mineral-associated organic matter (MAOM) fraction (OC-MAOM) as well as the content of SOC associated with Fe oxides (OC-Fe) and Ca bridges (OC-Ca). First, we separated soils into free POM and heavy fraction (HF) using 1.6 g cm⁻³ NaCl, after which residual NaCl was rinsed by deionized water. Then, 1.6 g cm⁻³ NaCl NaCl was added to the HF again, and the samples were shaken with 5-mm-diameter glass beads at 60 rpm for 16 hours to disrupt soil aggregates (64). The floating material was the occluded POM. The remaining soil residue was rinsed thoroughly of NaI and then sieved through a 53-μm sieve to separate the sand-sized fraction (>53 μm). The remaining silt + clay (<53 μm) fraction was the MAOM. The mass recovery errors of all the samples were >95%. After fractionation, we measured the SOC content of the MAOM fraction to calculate the OC-MAOM. OC-Ca and OC-Fe were determined following Ye et al. (65). Briefly, the HF was extracted by 0.5 M Na₂SO₄ to release OC-Ca. The remaining soil residue was sequentially extracted by CBD as the treatment group and by NaCl as the control group, with the difference in SOC content between the two groups as OC-Fe (66). All the SOC contents were calculated as milligrams per gram of bulk soil.
Microbial properties

Given the importance of microbial properties in predicting SOM decomposition and $Q_{10}$, we determined a suite of microbial parameters before and after the long-term incubation, including abundances, community composition, activity, and diversity. The initial measurements were conducted after preincubation to reflect the microbial properties at the beginning of incubation and then used to examine the microbial effects on C release and $Q_{10}$. After the 400-day incubation, we performed the same measurements for soils at the two temperatures to explore microbial changes and to test whether the microbial effects on permafrost C release retained with incubation time. We estimated microbial abundances and community composition by PLFA analysis. PLFAs were first extracted following previously described procedures (67). Then, the extracted PLFAs were analyzed by an Agilent 6850 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE, USA), with methyl nonadecanoate fatty acid (19:0) as the internal standard. We quantified PLFA biomarkers specific to bacteria (i14:0, a15:0, i15:0, i16:0, 16:1ω7c, a17:0, cy17:0, i17:0, 18:1ω7c, and cy19:0) and fungi (18:2ω6,9c) (48, 58) and assessed the microbial community composition by F/B.

We assayed the potential extracellular enzyme activities of four hydrolases [responsible for labile SOM decomposition, including β-1,4-glucosidase (BG), β-1,4-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and phosphatase (AP)] and one oxidase (phenol oxidase involved in recalcitrant SOM degradation). In brief, the activity of hydrolases was measured by adding 4-methylumbellif erone (MUB)-β-D-glucoside for BG, 4-MUB-N-acetyl-β-D-glucosaminidase for NAG, 1-leucine-7-amino-4-methylcoumarin for LAP, and MUB phosphate for AP to soil slurries of each sample. After incubation at 15°C for 5 hours, the fluorescence of the mixture was measured by a multifunction detector (DTX 880 Multimode Detector, Beckman Coulter Inc., Fullerton, CA, USA) at 365 nm in excitation and 450 nm in emission (68). Considering the quenching effects, we established standard curves for each sample with soil slurries and standard solutions (4-MUB for BG, NAG, and AP and 7-amino-4-methylcoumarin for LAP). We expressed hydrolase activities as nmol g$^{-1}$ OC hour$^{-1}$ and summed the activities of the four hydrolases to represent the total hydrolase activity. Oxidase was measured by spectrophotometry with L-3,4-dihydroxy-phenylalanine as a substrate (68). The microplates were incubated at 15°C for 20 hours, and then the absorbance was determined by the multifunction detector at 450 nm. The oxidase activity was calculated as μmol g$^{-1}$ OC hour$^{-1}$.

We adopted high-throughput sequencing to further explore the role of microbial diversity. Briefly, we extracted DNA by using the PowerMax ® Soil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). The primers 515F/806R were chosen to amplify the V4 region of the 16S ribosomal RNA genes for bacteria (69), and the fungal ITS2 region was amplified with the primers ITS3/ITS4 (70). For a detailed description of the polymerase chain reaction conditions and data processing, see the work of Chen et al. (44). Then, on the basis of the operational taxonomic unit abundance tables, we acquired the Shannon-Wiener index for bacteria and fungi. To calculate the microbial diversity, we standardized the diversity indexes of bacteria and fungi respectively as follows (71)

$$Z_i = \frac{H_i - H_{\text{min}}}{H_{\text{max}} - H_{\text{min}}}$$ (8)

where $H_i$ represents the Shannon-Wiener index of bacteria/fungi for sample $i$, $H_{\text{max}}$ denotes the maximum Shannon-Wiener index for bacteria/fungi across all the samples, and $H_{\text{min}}$ is the minimum. Microbial diversity was calculated as the average of the standardized diversity indexes of bacteria and fungi (71), which ranged between 0 and 1 in value.

Substrate quality

Considering the importance of chemical recalcitrance in affecting C release and intrinsic $Q_{10}$, we adopted acid hydrolysis approach (72) and Fourier transform infrared (FTIR) spectroscopy analysis (73) to quantify substrate quality. For the acid hydrolysis approach, soil samples were hydrolyzed with 2.5 M H$_2$SO$_4$ for 30 min at 105°C. The samples were centrifuged and decanted to recover the hydrolysate. The residue was washed with deionized water, after which the supernatant was combined with the above hydrolysate as labile pool I [mainly consisting of noncellulosic polysaccharides (72)]. The remaining residue was then hydrolyzed using 13 M H$_2$SO$_4$, under shaking at room temperature overnight. Thereafter, the acid was diluted by deionized water to 1 M, and the samples were hydrolyzed at 105°C for 3 hours. The hydrolysate was recovered, and the residue was washed to combine the supernatant. This hydrolysate was regarded as labile pool II, which was mainly composed of cellulose (72). Last, the remaining soil residue was washed and dried to acquire the recalcitrant pool fraction [consisting of fats, waxes, resins, suberins, and lignin (72)]. The OC content in each pool was quantified, and then the proportion of C in each fraction relative to the total SOC was calculated.

We also conducted FTIR spectroscopy analysis using a spectrometer (Tensor-27, Bruker, Germany). Briefly, we mixed ground soil samples with KBr and prepared a pellet using a hydraulic press. Spectra were recorded by averaging 32 scans at 4-cm$^{-1}$ resolution (wave number) over the range of 4000 to 400 cm$^{-1}$. The spectra were baseline corrected, and the absorption bands at ~1030 cm$^{-1}$ (polysaccharides), ~1630 cm$^{-1}$ (aromatics and aromatic or aliphatic carboxylates), ~1720 cm$^{-1}$ (carboxylic acids and aromatic esters), and ~2850 and ~2920 cm$^{-1}$ (aliphatics) were selected as indicative of the four chemical classes of SOM (73). Then, we calculated the humification indexes as the ratios between absorbances at the three other chemical classes with respect to polysaccharides (73).

Soil physicochemical characterization

We measured soil physicochemical properties to illustrate the effects of edaphic variables on permafrost C release and $Q_{10}$. Specifically, we determined soil moisture of the permafrost samples by drying fresh soils at 105°C and weighing before and after drying. We analyzed soil texture by a particle size analyzer (Malvern Masterizer 2000, Malvern, Worcestershire, UK) after removal of organic matter and carbonates to acquire the contents of clay, silt, and sand (48). We also determined soil pH in a 1:2.5 soil-to-water mixture and SOC content by an element analyzer (Multi EA 4000, Analytik Jena, Germany) after removing inorganic C.

Statistical analyses

We used linear mixed-effect models [R package: nlme (74)] to investigate the relationships of cumulative C release and $Q_{10}$ with mineral protection, microbial properties, substrate quality, and edaphic variables. In the model (also in all the following analyses), we included the examined predictors as fixed effects and the sampling
region (i.e., Elashankou-Qingshuihe section, Qilian Mountains, and Golmud-Amdo section) as a random effect. The residues were checked for normality, and log transformation was conducted when necessary. Goodness of fit was determined by the marginal $R^2$, which described the variance explained by fixed factors as proposed by Nakagawa and Schielzeth (75). The $P$ values were adjusted with the Benjamini-Hochberg method for multiple testing (76). Then, two statistical approaches, SEM and variation partitioning analysis, were used to further explore the roles of mineral protection and microbial properties in affecting C release and $Q_{10}$, respectively.

First, we constructed SEM to evaluate the direct and indirect effects of various factors on C release after permafrost thaw. In the priori model (fig. S8), active layer thickness induced the variation in other factors among study sites. Mineral protection, microbial properties, and substrate quality had direct effects on C release, while edaphic variables functioned through the indirect effects. Considering the correlations among factors, we performed principal components analysis (PCA) using the predictors significantly associated with C release to create a new index for mineral protection, microbial properties, and substrate quality. The first component, which accounted for >70% of the variance of each group (table S4), was then introduced in the SEM. The goodness of fit of the SEM was evaluated using Fisher’s C statistic, Akaike information criterion, and the whole-model $P$ value (77). The SEM was conducted using R packages piecewiseSEM (77) and nlme (74).

Second, we conducted variation partitioning analysis to quantify the relative importance of mineral protection and microbial properties in affecting $Q_{10}$ (78). Before the analysis, we performed a stepwise selection procedure (79) to select the best predictors of $Q_{10}$ based on a multiple linear regression model, which included all the variables significantly affecting $Q_{10}$ in the pairwise analyses. Then, the factors retained in the optimal model (table S5) were used to perform the variation partitioning analysis. All the analyses were completed using R 4.0.3 (80).

SUPPLEMENTARY MATERIALS
Supplemental material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/eabe3596/DC1

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