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Whole-soil-profile warming does not change microbial carbon use efficiency in surface and deep soils

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The paucity of investigations of carbon (C) dynamics through the soil profile with warming makes it challenging to evaluate the terrestrial C feedback to climate change. Soil microbes are important engines driving terrestrial biogeochemical cycles; their carbon use efficiency (CUE), defined as the proportion of metabolized organic C allocated to microbial biomass, is a key regulator controlling the fate of soil C. It has been theorized that microbial CUE should decline with warming; however, empirical evidence for this response is scarce, and data from deeper soils are particularly scarce. Here, based on soil samples from a whole-soil-profile warming experiment (0 to 1 m, +4 °C) and ^{18}O tracing approach, we examined the vertical variation of microbial CUE and its response to ~3.3-y experimental warming in an alpine grassland on the Qinghai–Tibetan Plateau. Microbial CUE decreased with soil depth, a trend that was primarily controlled by soil C availability. However, warming had limited effects on microbial CUE regardless of soil depth. Similarly, warming had no significant effect on soil C availability, as characterized by extractable organic C, enzyme-based lignocellulose index, and lignin phenol–based ratios of vanillyls, syringyls, and cinnamyls. Collectively, our work suggests that short-term warming does not alter microbial CUE in either surface or deep soils, and emphasizes the regulatory role of soil C availability on microbial CUE.

microbial carbon use efficiency | whole-soil-profile warming | soil depth | carbon availability | grasslands

Significance

Although surface and deep soils will warm at nearly the same rate throughout the next century, the response of deep-soil organic carbon (C) to climate warming is still unknown. Microbial carbon use efficiency (CUE), the proportion of C ultimately assimilated into biomass, is an important driver of soil C storage. We found that the microbial CUE decreased with soil depth in an alpine grassland, and was mainly controlled by soil C availability. However, short-term (3.3 y) whole-soil-profile warming (0 to 1 m, +4 °C) did not significantly affect either soil available C or microbial CUE across soil depths. Collectively, these results highlight the role of soil C availability in controlling microbial CUE and its response to warming across the soil profile.

Carbon (C) emissions caused by anthropogenic activities have raised the global average temperature by 1.1 °C from 1850 to 2019 (1). Significant climate warming is predicted to have a profound impact on soil organic C (SOC) storage, but the feedback of SOC to warming remains highly uncertain in Earth system models (2–4). Half of the world's SOC is stored below 30 cm (5), while the response of deep SOC (>30 cm) to climate warming is still one of the largest sources of uncertainty in models (6). This is probably related to two common misconceptions: first, that deep SOC is inert and second that deep-soil temperatures lag behind surface SOC when experiencing annual climate warming. Earth system models predict that surface and deep soils will warm at nearly the same rate throughout the 21st century, and soil temperature will increase by 4.5 °C down to 100 cm by 2100 under representative concentration pathways 8.5 (7). Moreover, recalcitrant C in deep soil is more sensitive to warming according to the Arrhenius function (8). Yet available data are scarce to accurately test how SOC dynamics will respond to whole-soil-profile warming (9, 10).

To assess changes in SOC dynamics through the soil profile, a key metric is microbial carbon use efficiency (CUE) (11). CUE describes the allocation of metabolized C between microbial biomass and respiration, and is a key parameter commonly invoked in C cycle models (12, 13). However, there is no consensus on the response of microbial CUE to warming (14–16). Physiologically, microbial respiration is generally considered to be more sensitive to temperature than is growth; thus theoretically, warming would decrease CUE (12, 17). However, both neutral (10, 18, 19) and positive (20, 21) responses of CUE to warming have been reported. Assessing and quantifying the effect of warming on CUE is thus vital to evaluate alterations of microbe-mediated C dynamics and further predict potential responses of soil C storage and terrestrial C feedbacks in a warming world (22).

Soil warming is likely to increase soil enzyme activity (23), accelerate soil organic matter turnover, decrease soil C availability (15, 24, 25), or/and improve soil nutrient availability (26, 27). Warming-induced C limitation is likely to affect the shifts in the metabolized C allocation between microbial growth and respiration. According to enzyme kinetics, introducing available C from low-quality compounds with complex bond structures into microbial biomass requires several enzymatic catabolic reaction processes, which increases microbial investment in resource acquisition and ultimately leads to low CUE (14, 28). Conversely, when available nitrogen (N) increases, it may increase plant growth and aboveground and belowground C inputs into soils (29). Microorganisms may allocate

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more metabolized C for growth due to the lower metabolic cost of C or N acquisition (30), thus showing a higher CUE. Consequently, the changes of soil C and N availability induced by warming may induce inconsistent CUE responses. As the key "engine" driving the Earth's biogeochemical cycles (31), microorganisms are sensitive to climate warming, but with different temperature dependences. For instance, compared with fungi, bacteria are considered to have higher temperature tolerance and thus are more abundant under warming (32). Additionally, opportunistic, fast-growing microbial communities (r-strategists, such as bacteria) show a higher growth rate, but a lower CUE due to their lower biomass C:nutrient ratios and therefore lower biomass C requirements (33). Warming may lead to a decrease in CUE by inducing a reduction in the ratio of fungi to bacteria (F/B). It has also been observed that warming can alter microbial diversity and community composition (34). Yet, limited studies reported the effect of warming-induced microbial diversity and community composition changes specifically on CUE (15, 35, 36).

In addition, multiple pieces of evidence revealed that deep soil (>30 cm) differs from surface soil in resource availability and microbial characteristics (15, 37). The resource availability in deep soil is relatively low due to physical protection or chemical recalcitrance of soil organic matter (38, 39). It has been demonstrated that enzyme activity per unit microbial biomass increased with soil depth (40), which suggests that the low resource availability might increase C-investment into additional enzymes to release limiting resources, so the soil microbial access to resources may become more C-expensive, resulting in a lower CUE in deep soils (41). The difference in microbial community distribution among soil depths could also affect CUE (12, 15). However, there has not been much research on CUE dynamics in deep soils, and our understanding remains insufficient. Due to the interaction between resource availability and microbial community composition on microbial metabolism, a comprehensive study with systematic measurements of CUE together with the potential drivers across soil depths is needed. More importantly, measuring the effects of warming and resultant alteration of resource availability and microbial community on CUE across soil depths will help to resolve these disparate results of CUE response to warming.

Grassland accounts for nearly 41% of the Earth's land area, and can provide a variety of ecosystem services (i.e., nutrient cycling, climate regulation, etc.) (42). Alpine grassland is considered to be one of the most sensitive ecosystems to climate warming (43, 44). Such grassland occupies over 60% of land area of the Qinghai–Tibetan Plateau (45, 46); the high altitude and resultant cold environment limits microbial decomposition, resulting in a large C stock in soils (7.4 Pg C in the top 1 m) (46). Nonetheless, the Qinghai–Tibetan Plateau has experienced a higher rate of temperature increase than the Northern Hemisphere average (47). Exploring the effect of warming on microbial CUE across soil depths and its driving factors is thus crucial to determine the fate of soil C in these alpine grasslands with climate warming. In this study, based on a whole-soil-profile (0 to 100 cm) field warming experiment (48) coupled to a substrate-independent CUE assay (41, 49), we examined CUE across soil depths in an alpine grassland on the Qinghai–Tibetan Plateau. We hypothesized that 1) given the lower soil C availability and microbial substrate at depth, microbes are more likely in a dormant rather than a growth strategy, and would have a lower CUE; and 2) although it was found that warming did not significantly affect the aboveground and underground biomass of plants in the same study area (48), warming may increase enzyme activity, decrease C availability, and affect microbial community properties, which would likely lead to a decrease in microbial CUE across soil depths.

Results

Microbial C Metabolic Characteristics. There was no significant interaction between warming and depth on microbial CUE, growth, and respiration (Fig. 1 and *SI Appendix*, Table S1). Along soil depths, microbial CUE, growth, and respiration decreased significantly from 0.79 to 0.30, from 5.57 to 0.14 μg C g^{-1} h^{-1}, and from 1.50 to 0.25 μg C g^{-1} h^{-1}, respectively (Fig. 1). As for microbial biomass–specific growth, there were no significant differences among soil depths in either control or warming plots. However, microbial biomass–specific respiration increased from surface to deep soils in both control and warming plots. After ~3.3-y treatment, warming showed limited effects on microbial CUE, growth, and respiration (Fig. 1 and *SI Appendix*, Table S1).

Soil Resource Changes. Soil C and N pools were strongly affected by soil depth. The SOC, total N, extractable organic C (EOC), and mineral N all decreased with soil depth in both control and warming plots. At all depths, warming did not significantly change soil moisture, SOC, total N, or EOC (*SI Appendix*, Table S2). By contrast, warming resulted in a significant reduction in mineral N by 28.5% in surface soil (0 to 30 cm). The ratio of oxidase to hydrolase—termed lignocellulose index (LCI)—did not differ significantly between control and warming treatments, but increased with soil depth for both treatments (Fig. 2*A*). The ratio of acid to aldehyde forms of vanillyl and syringyl monomers [$(Ac/Al)_V$ and $(Ac/Al)_S$], the ratio of syringyl to vanillyl (S_m/V_m), and the ratio of cinnamyl to vanillyl (C_m/V_m) are usually used to estimate the degree of lignin biotransformation. Similarly, $(Ac/Al)_V$, $(Ac/Al)_S$, S_m/V_m, and C_m/V_m increased with soil depth for both control and warming treatments (Fig. 2). Except for $(Ac/Al)_S$ in 40 to 60 cm, warming had no significant effects on $(Ac/Al)_V$, S_m/V_m or C_m/V_m in different soil layers (Fig. 2). Taken together, these results demonstrated that soil C availability decreased with soil depth, while it showed little response after ~3.3 y of warming.

Microbial Community Properties. We found significant effects of depth on the F/B and the ratio of gram-positive bacteria to gram-negative bacteria (GP/GN) (*SI Appendix*, Table S1). Specifically, the F/B decreased with depth, while the GP/GN increased with depth. Soil depth induced significant changes in bacterial (PERMANOVA test, $P < 0.01$, stress = 0.06) and fungal (PERMANOVA test, $P < 0.01$, stress = 0.15) community compositions (*SI Appendix*, Fig. S1). However, warming had no significant effect on the F/B or the GP/GN (*SI Appendix*, Table S1). Bacterial community α-diversity characterized by the Shannon index only significantly decreased in 10 to 30 cm in response to warming (*SI Appendix*, Table S1). Despite this, ~3.3-y warming did not significantly affect bacterial and fungal community compositions (*SI Appendix*, Tables S3 and S4).

Factors Regulating Microbial CUE. Our results revealed that microbial CUE was negatively correlated with the LCI, $(Ac/Al)_V$, $(Ac/Al)_S$, S_m/V_m, and C_m/V_m (Fig. 3). Correspondingly, these results indicated that microbial CUE was significantly reduced by the decrease in soil C availability. Partial correlation analysis showed that, without controlling the role of soil C availability (zero-order in Fig. 4*A*), microbial CUE was closely correlated with microbial community composition and diversity in addition to fungal community composition (characterized by NMDS1). However, after controlling EOC, LCI, $(Ac/Al)_V$, $(Ac/Al)_S$, S_m/V_m, or C_m/V_m, the correlation coefficients between microbial CUE and microbial community composition and α-diversity decreased

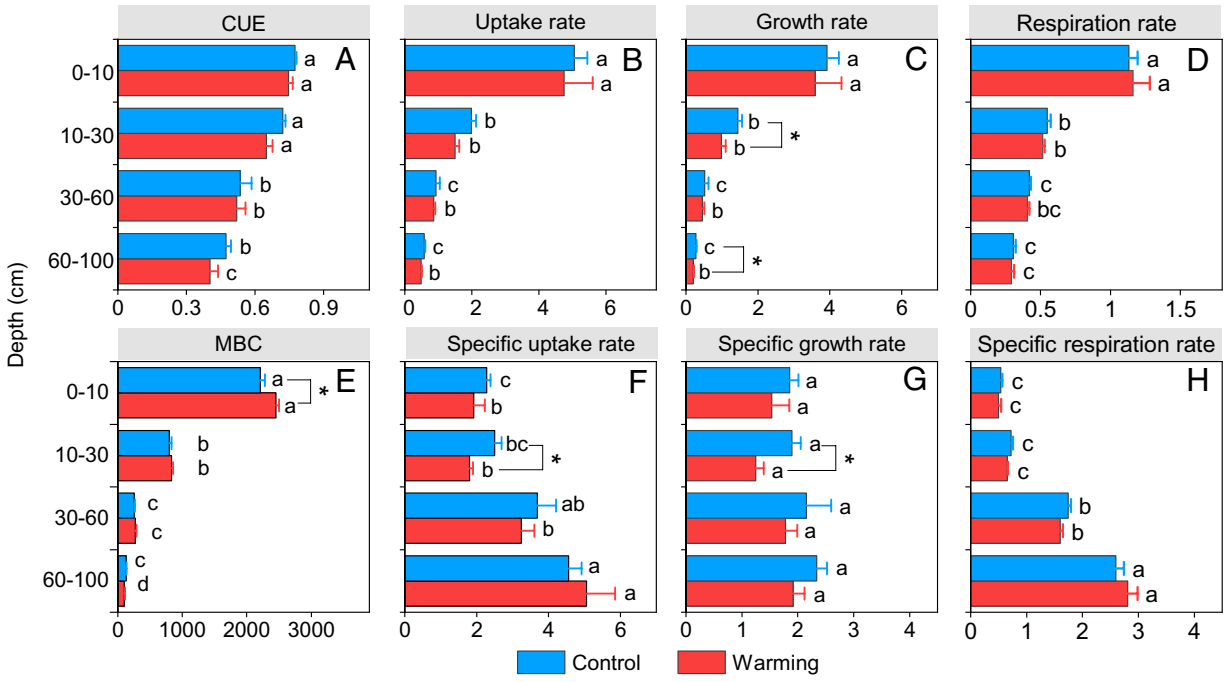

Fig. 1. Effects of whole-soil-profile (0 to 100 cm) warming on (*A*) CUE, (*B*) uptake rate (μg C g^{-1} h^{-1}), (*C*) growth rate (μg C g^{-1} h^{-1}), (*D*) respiration rate (μg C g^{-1} h^{-1}), (*E*) microbial biomass C (mg kg^{-1}), (*F*) microbial biomass–specific uptake rate (ng C kg^{-1} MBC h^{-1}), (*G*) microbial biomass–specific growth rate (ng C kg^{-1} MBC h^{-1}), and (*H*) microbial biomass–specific respiration rate (ng C kg^{-1} MBC h^{-1}) of soil microorganisms in an alpine grassland. Data are presented as mean and SE (n = 4). Different lowercase letters on columns represent significant differences among depths tested separately for each treatment (P < 0.05). The * above the column indicates significant differences between control and warming treatments of the corresponding soil depth (P < 0.05). Note that these microbial processes were measured at a common temperature (10 °C) in the laboratory.

by 35% on average (Fig. 4*A*). Structural equation model analysis further showed that soil C availability was the dominant driver of microbial CUE across soil depths (Fig. 4*B*). The soil C availability and microbial properties were responsible for 74% and 8% of the explained variance in microbial CUE, respectively, as indicated by the multiple regression models (Fig. 4*C*). Taken together, these

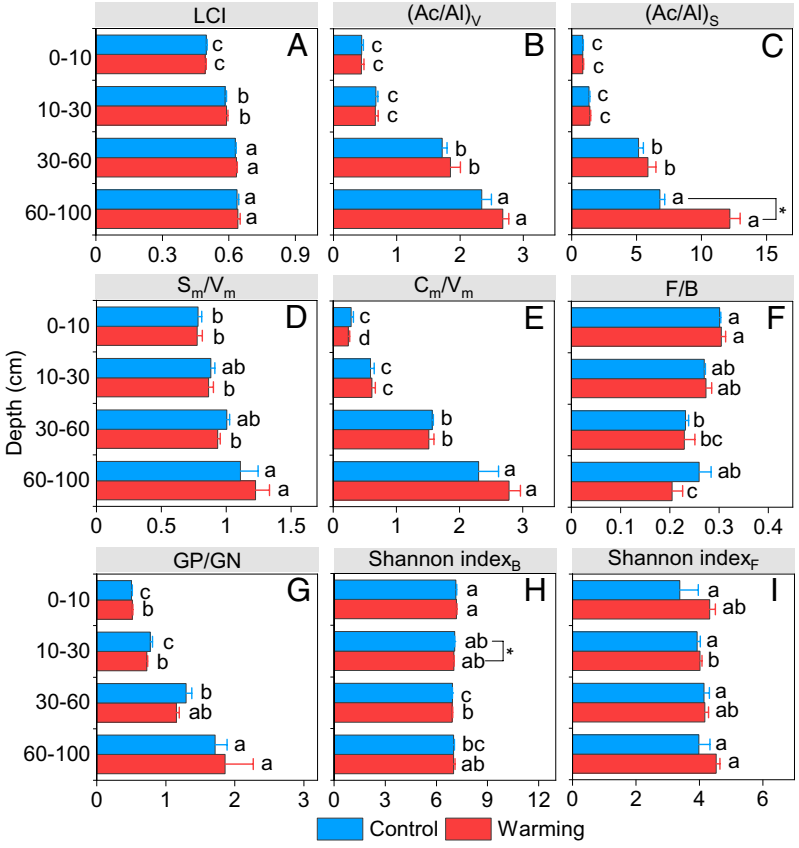

Fig. 2. Effects of whole-soil-profile (0 to 100 cm) warming on (*A*) the LCI, (*B* and *C*) ratios of acid to aldehyde forms of vanillyls and syringyls [$(Ac/Al)_V$ and $(Ac/Al)_S$], (*D*) ratio of syringyls to vanillyls (S_m/V_m), (*E*) ratio of cinnamyls to vanillyls (C_m/V_m), (*F*) F/B, (*G*) ratio of gram-positive bacteria to gram-negative bacteria (GP/GN), (*H*) bacterial community α-diversity (Shannon $index_B$), and (*I*) fungal community α-diversity (Shannon $index_F$) of soils in an alpine grassland. Data are presented as mean and SE (n = 4). Different lowercase letters on columns represent significant differences among depths tested separately for each treatment (P < 0.05). The * above the column indicates significant differences between control and warming treatments of the corresponding soil depth (P < 0.05).

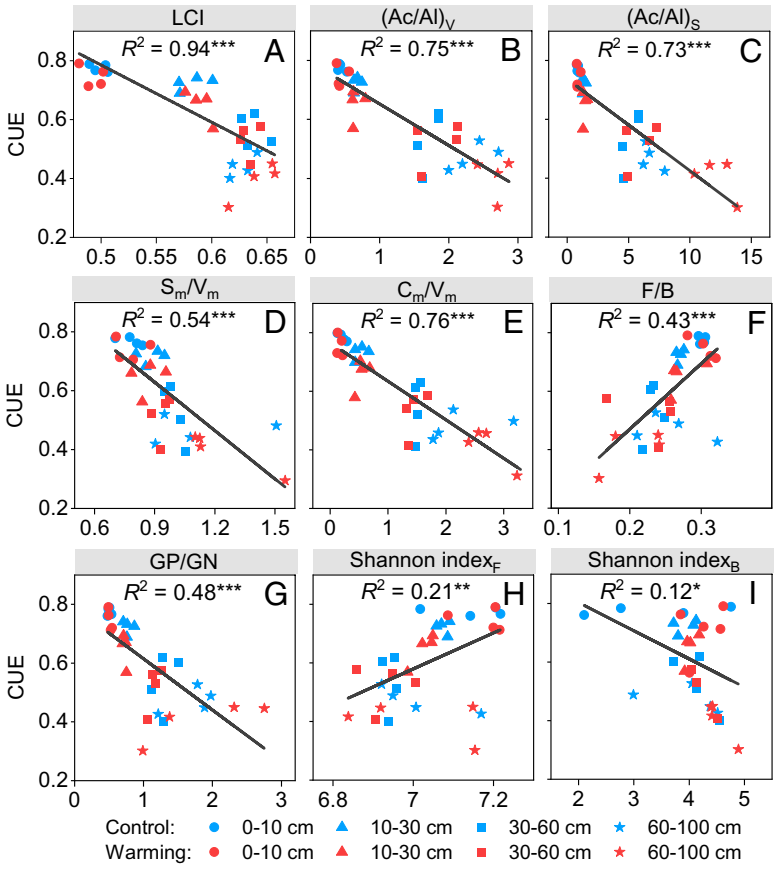

Fig. 3. Relationships of microbial CUE with (*A*) the lignocelluloses index (LCI), (*B* and *C*) ratios of acid to aldehyde forms of vanillyls and syringyls [$(Ac/Al)_V$ and $(Ac/Al)_S$], (*D*) ratio of syringyls to vanillyls (S_m/V_m), (*E*) ratio of cinnamyls to vanillyls (C_m/V_m), (*F*) F/B, (*G*) GP/GN, (*H*) bacterial community α-diversity ($Shannon\ index_B$), and (*I*) fungal community α-diversity ($Shannon\ index_F$) of soils in an alpine grassland. *P < 0.05; **P < 0.01; ***P < 0.001.

three analyses jointly demonstrated that soil available C was a key factor regulating microbial CUE across soil depths.

Discussion

Decreases in Microbial CUE across Soil Depths. Consistent with our first hypothesis, microbial CUE decreased with soil depth in both control and warming treatments (Fig. 1). Although soil depth had strong effects on soil C and N availability, and on microbial properties (*SI Appendix*, Tables S2–S4 and Fig. S1), soil C availability had the highest predictive power in explaining soil depth patterns of microbial CUE; this was based on a variety of analytical and statistical methods including partial correlation analysis (Fig. 4*A*), structural equation model (Fig. 4*B*), and multiple regression models (Fig. 4*C*). Why does soil C availability have a significant effect on CUE? We propose two possible mechanisms. First, because of its proximity to aboveground vegetation, surface soil usually receives a greater load and range of fresh, labile, C inputs from surface litter and plant roots (39) that can be used with high efficiency. Typically, the microbial substrate (i.e., available C) declines with soil depth due to a combination of microbial processing, selective transport to deep soils in the form of dissolution, and preferential stabilization of microbial C in association with soil minerals (39, 40). In the present study, soil EOC concentration decreased significantly with soil depth (*SI Appendix*, Table S2). The decrease in microbial C uptake with depth was synchronized with that of EOC ($slope_{uptake}$ = –0.033, R^2_{uptake} = 0.82; $slope_{EOC}$ = –0.016, R^2_{EOC} = 0.93), suggesting that EOC has a direct impact on the energy supply of microorganisms and the energy constraint of microbial C utilization increases with soil depth. At the same time, the microbial biomass–specific hydrolase activity increased with soil depth (*SI Appendix*, Table S5), suggesting that microbes need to invest more in enzymes for the same community size to grow. This result contributes to the idea that the investment needed for growth is higher in the deep soils compared to the upper soils which have more labile/easily degradable C (23). Overall, microorganisms responded to lower C availability in deep soil by reducing the C uptake rate (41, 50), which could result in a decrease in CUE in deeper soil.

Second, in addition to reducing uptake, soil microbial communities may adapt to use previously inaccessible C pools to promote their metabolic activities (23). Soil microorganisms require metabolized C to meet their energy requirements for maintenance (i.e., respiration) and synthesizing structural molecules to build biomass (30). The regulation of soil available C on CUE can therefore be attributed to its potential impact on microbial energy allocation. Microbial utilization of recalcitrant substrates (i.e., polyphenolics) requires depolymerization, which is a process catalyzed by enzymes (51). At the level of compound category, the lignin phenol, hydrophilic part, and aromatic C—namely plant-derived organic molecules, decrease along soil depth (39). Consistently, our results showed that lignin phenol concentration [adding together the vanillyls (V_m), syringyls (S_m), and cinnamyls (C_m) monomers] decreased with soil depth. However, the LCI, $(Ac/Al)_V$, $(Ac/Al)_S$, S_m/V_m, and C_m/V_m increased with soil depth (Fig. 2), indicating that the degree of lignin degradation increased with soil depth. There were significant negative relationships between microbial CUE and LCI, $(Ac/Al)_V$, $(Ac/Al)_S$, S_m/V_m, and C_m/V_m (Fig. 3),

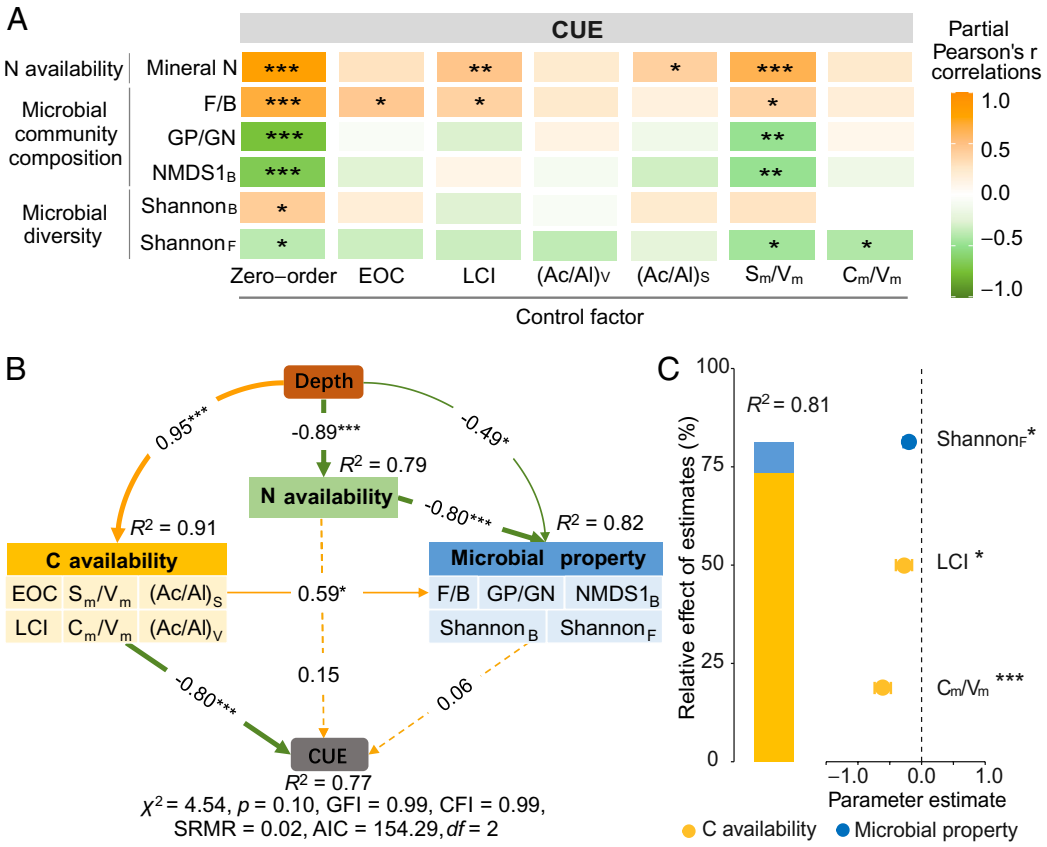

Fig. 4. (*A*) Partial correlations between microbial CUE and soil N availability and microbial community properties after controlling soil C availability. The *x* axis represents the zero-order (without controlling any factors) and the factors being controlled. The *y* axis represents the factors (N availability, microbial community composition, and microbial diversity). The color in the figure shows the strength of the correlation. Differences in color between the zero-order and controlled factors represent the level of dependency of the correlation between microbial CUE and the examined factor on the controlled variable (no change in color between the controlling factor and zero-order = no dependency; a decrease/increase in color intensity = loss/gain of correlation). (*B*) Structural equation modeling showing the multivariate effects on microbial CUE. Orange and green lines indicate positive and negative relationships, respectively. Solid and dashed lines suggest significant and insignificant paths, respectively. The width of solid line is directly proportional to the correlation strength. Double-layer rectangles represent the first component from the principal component analysis conducted for soil C availability and microbial community properties. Soil C availability variables include EOC, the lignocelluloses index (LCI), ratios of acid to aldehyde forms of vanillyls and syringyls [$(Ac/Al)_V$ and $(Ac/Al)_S$], ratio of syringyls to vanillyls (S_m/V_m), and ratio of cinnamyls to vanillyls (C_m/V_m). Soil N availability is represented by mineral N; Microbial community properties include F/B, GP/GN, bacterial α-diversity ($Shannon_B$), fungal α-diversity ($Shannon_F$), and bacterial community composition ($NMDS1_B$). The goodness-of-fit statistics of the model are displayed below the model. (*C*) Relative effects of multiple predictors of microbial CUE. It shows the relative importance of each predictor, expressed as the percentage of explained variance, as well as the average parameter estimates (standardized regression coefficients) of the model's predictors and their 95% CIs. *P < 0.05; **P < 0.01; ***P < 0.001.

suggesting that there is a trade-off between microbial growth and decomposition. Microorganisms invested more metabolized C in the production of enzymes to oxidize recalcitrant substrates, so as to obtain a return on energy or C gain, which ultimately leads to lower CUE (12, 23). Moreover, the oxidase activity and microbial biomass–specific respiration rate increased with soil depth, while the microbial biomass–specific growth rate did not change significantly with soil depth (*SI Appendix*, Table S5), suggesting that relatively more metabolized C is allocated to maintain respiration and recalcitrant substrates degradation with increasing soil depth, while the cost of growth is relatively fixed compared to respiration (25). Overall, with lower soil C availability at depth, microbes are more likely in a dormant, rather than a growth strategy. Thus, at depth, they would have a lower CUE. Our results together demonstrate that soil available C is a vital predictor for the observed variation in CUE across soil depths.

Limited Effect of Warming on Microbial CUE. Early efforts have been devoted to investigate the responses of microbial community composition as well as SOC dynamics to warming (34, 52), but the effects of warming on microbial metabolism remain unclear. Here, we observed that warming had no significant effect on microbial CUE at different soil depths from 0 to 100 cm (Fig. 1). This is in line with the observation that warming had no significant effect on CUE of surface soil microorganisms in a grassland (18). Due to the technical difficulty and high cost of warming deep soils in situ, potential warming-induced alteration of CUE in deep soils has been uncertain. Dove et al. did not detect differences in CUE measured by the metabolic flux analysis method between deep soils for control and warming treatments in a 4.5-y whole-soil-profile warming experiment in a temperate forest (15). This result was contrary to their expectations, and they suggested that it may be the result of the method used (16, 49), because the metabolic flux analysis method does not include the C cost change related to the depolymerization of polymeric C compounds into simple compounds (14). In this study, we assessed CUE by a substrate-independent ^{18}O-H_2O labeling approach, and also found that warming had no significant effects on CUE in deep soils (Fig. 1 and *SI Appendix*, Table S1). Taken together, our experiment provides strong evidence that warming has a limited effect on microbial CUE in both surface and deep soils. This falsifies our second hypothesis that warming would decrease CUE across soil depths. The neutral response of microbial CUE across soil depths to warming may be attributed to the following reasons.

First, previous results from laboratory incubations in the absence of plants showed that increasing temperature decreased CUE (14, 53–55). This difference could be explained by the continuous supply of C by plants in the field. In previous studies, we found that whole-soil-profile warming had no significant effect on plant biomass and productivity in the alpine grassland (48). Combined with the neutral responses of soil EOC, $(Ac/Al)_V$, $(Ac/Al)_S$, S_m/V_m, and C_m/V_m to warming (Fig. 2 and *SI Appendix*, Table S1), we deduce that short-term (i.e., 3.3 y in this study) warming had no significant effect on soil C availability, which probably translates into minor changes in microbial CUE. However, a long-term (26 y) warming experiment in the Harvard Forest showed that the stages of substantial soil C loss alternate with the stages of undetected loss (52). This long-term warming was reported to reduce the soil organic matter quality, that is, the depletion of microbially accessible C pools (25), which may alter the CUE. Moreover, Frey et al. investigated the effects of warming on microbial CUE in two warming experiments (2 and 18 y) at the same site, and found that only older warming had significant effects on CUE (14). Recently, Domeignoz-Horta et al. revealed that long-term warming (13 and 28 y) affects microbial physiology by reducing C availability (25). Thus, as the duration of warming progresses, warming is likely to induce a decrease in microbial CUE in response to warming-induced substrate depletion, which will be detrimental to SOC storage. Overall, these results reinforce the importance of focusing on the effects of long-term warming on CUE.

Second, stoichiometric perspectives as well as recent advances provide evidence that soil heterotrophic microorganisms are primarily limited by energy or C (30, 56). Changes in microbial community dynamics have been reported to alleviate C limitation, leading to a shift in CUE (57). The insignificant change of soil C availability may not have shifted the microbial community composition and α-diversity in this study (*SI Appendix*, Fig. S1 and Tables S1, S3, and S4), leading to a limited response of microbial CUE to warming.

Third, warming had no significant effect on microbial biomass–specific growth or respiration in this study (Fig. 1), in part because the lack of significant changes in soil C availability under warming. Furthermore, according to the thermodynamic theory (58, 59), organisms may not have been exposed to a temperature near or beyond their physiological limits from a 4 °C increase in temperature. Walker et al. found similar results that microbes did not adjust their growth and respiration in a subarctic grassland where the temperature increased by 6 °C (18). Therefore, microbial CUE showed a remarkable resistance to experimental warming.

Finally, warming led to a lower soil available N concentration in the present study (*SI Appendix*, Table S2), which may be a result of increased N uptake by forbs and/or decreased N fixation by legumes (48). Microorganisms with low N supply may allocate less C to growth due to the high metabolic cost of N acquisition, or dispose C as CO_2 through overflow respiration to meet their N demands, which decreases their CUE (13). However, we did not find a significant warming effect on CUE (Fig. 1). Additionally, warming had no significant effect on N-acquiring enzymes (e.g., N-acetyl-glucosaminidase and leucine aminopeptidase; *SI Appendix*, Table S5), indicating that microorganisms may not be limited by N under warming. This concept of microorganisms is not limited by N is supported by the fact that warming has no significant effect on microbial properties (i.e., microbial community composition and α-diversity). Alternatively, CUE remained unchanged under warming probably because the changes in soil N availability have not yet reached the threshold of microbial metabolic change. This is consistent with Dove et al., who demonstrated that CUE did not change significantly even though soil N availability was changed with whole-soil-profile warming in a temperate forest (15). Overall, it is reasonable to think that soil N availability plays a small role in regulating the response of microbial CUE to warming.

To sum up, microbial CUE showed a dramatic decline with soil depth. We found that this depth-induced reduction in microbial CUE was mainly owing to the reduction of soil C availability (Fig. 5). That is, under lower C availability, microorganisms regulated the change of CUE by reducing C uptake and changing metabolized C allocation. Moreover, by conducting a field experiment of whole-soil-profile warming in an alpine grassland, our study provides strong evidence that short-term warming has a limited effect on microbial CUE at all soil depths (Fig. 5). This is probably related to the maintenance of soil available C and microbial community composition, as well as the thermal adaptation of microbial metabolism to warming. Taken together, these results show that short-term warming does not alter microbial CUE in either surface or deep soils, and highlight the key role of soil C availability in controlling microbial CUE across the soil profile. Further long-term studies of the warming effects on soil C availability, microbial community composition and CUE, particularly across the whole-soil profile, can help predict the fate of SOC in a warmer world.

Materials and Methods

Study Site. The whole-soil-profile warming experiment was conducted at the Haibei National Field Research Station of Alpine Grassland Ecosystems on the northeast of the Qinghai–Tibetan Plateau, Qinghai Province, China (37°37′ N, 101°12 E, 3,200 m a.s.l.). The soil is classified as Mat-Cryic Cambisol. The mean annual air temperature of this study site is 1.1 °C, with the hottest month in summer averaging 22.0 °C and the coldest month in winter averaging −17.6 °C. Mean annual precipitation is 485 mm, 80% of which occurs in the growing season (May to September) (60). Within the study area, the dominant plant species include *Kobresia humilis*, *Kobresia pygmaea*, *Elymus nutans*, *Stipa aliena*, and *Gentian astraminea*.

The experiment consists of four blocks and each block consists of one control and one warming plot. Each plot measures 3.5 m in diameter, including a sampling experimental area with a diameter of 3 m and a circular buffer zone extending 25 cm outward. Twenty vertical heating cables (BriskHeat, Ohio, USA) of 1.2-m-long (1 m belowground) were installed around the warming plots, and two concentric rings (1 and 2 m in diameter, respectively) of heating cables were installed in the soil at a depth of 5 cm from the surface layer to compensate for surface heat loss. To reduce the interference caused by the installation of heating cables, unheated steel pipes were installed similarly in each control plot. At different soil depths of each plot, the temperature (thermistors, custom-made) and moisture sensors (Delta-T, UK) were installed to measure soil temperature and moisture, respectively. The temperature and moisture sensors were 75 cm away from the center of the plot, and the distance between them was 20 cm. Each paired control and warming plots were connected to a thermostatic system (SCRs, Watlow, Missouri, USA) to maintain the soil temperature of the warming plot at 4 °C above the ambient temperature at 0 to 100-cm depth (48, 61).

Soil sampling occurred in August 2021 after ~3.3 y of experiment warming (starting from June 2018), and samples were collected at 0 to 10-cm, 10 to 30-cm, 30 to 60-cm, and 60 to 100-cm soil depths from all eight plots. Three soil cores (3.5 cm in diameter) were collected from each plot and pooled together by soil depth for homogenization. The soils were transported to the laboratory immediately in the incubator with ice bags. Then, samples were sieved through a 2-mm sieve with all visible roots and rocks were removed. Each sample was divided into three subsamples for different analysis. The first subsample was air-dried at room temperature before analyzing the SOC and total N. The second subsample was stored at 4 °C for the determination of EOC, mineral N, soil microbial biomass and enzyme activities. The third subsample was stored at −20 °C before measuring microbial CUE, lignin phenol, microbial community composition and diversity.

We determined microbial CUE using ^{18}O incorporation from ^{18}O-H_2O into microbial DNA following the method described by Spohn et al. (41). The method is direct (growth is measured by DNA replication, not C incorporation into biomass) and substrate independent, thus avoiding the addition of energy or nutrients that could alter the relationship between temperature and microbial growth (49).

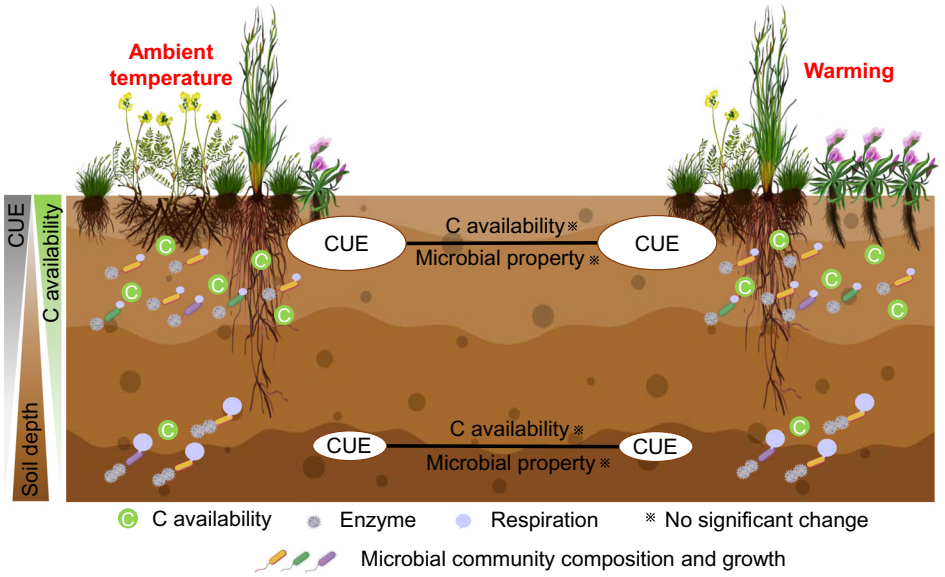

Fig. 5. Conceptual diagram of the effect of whole-soil-profile warming on microbial CUE in an alpine grassland. The CUE shows a dramatic decline along the soil profile, which is mainly owing to the reduction of soil C availability. In the case of microbial C starvation, microorganisms regulate the change of CUE by reducing uptake and changing metabolized C allocation. For example, microbial biomass–specific enzyme activity and respiration rate increase with soil depth, while microbial biomass–specific growth rate does not change significantly with soil depth. Microorganisms invest more metabolized C in the production of enzymes to oxidize recalcitrant substrates and maintenance (i.e., respiration), so as to obtain a return on energy or C gain, which ultimately leads to lower CUE. Conversely, warming has limited effects on microbial CUE regardless of surface or deep soils. Similarly, warming has no significant effects on soil C availability, which probably translates into minor changes in microbial CUE. The insignificant change of soil C availability may not cause microbial community composition and diversity changes, which further reveals the reason for the neutral response of microbial CUE to warming.

Briefly, after a 7-d preincubation in darkness at 10 °C, two replicates of 500-mg soil samples from each plot were weighed into 2-mL centrifuge tubes. We incubated 500-mg soil for 24 h at 10 °C with ^{18}O-H_2O to 20 atom% enrichment and 60% of water holding capacity, alongside a duplicate containing the same volume of molecular-grade nonlabeled H_2O as a natural abundance control. Microbial respiration (R, μg C g^{-1} h^{-1}) during this time was analyzed using gas chromatography (GC, Agilent 7890A, USA). After the incubation, the resultant DNA was extracted (FastDNA® SPIN Kit for Soil, MP Biomedicals), quantified (Quant-iT™ PicoGreen® dsDNA Reagent, Life Technologies), and analyzed for ^{18}O abundance and total O content using IRMS-TC/EA (Delta V Advantage, Thermo Fisher, Germany). DNA production was then calculated and used to derive microbial community growth. We calculated DNA production (DNAp) as:

$$DNA_p = O_t \times \frac{O_e}{100} \times \frac{100}{O_l} \times \frac{100}{31.21}, \quad [1]$$

where O_e is the ^{18}O atom% excess of the labeled sample, O_l is the ^{18}O enrichment (atom%) of the labeled sample, and the constant 100/31.21 is a factor to convert oxygen in DNA to total DNA.

Then, we converted DNA production to equivalent microbial biomass C production, that is, microbial growth (G, μg C g^{-1} h^{-1}), for each sample separately using:

$$G = \frac{C_{mic}}{DNA_{mic}} \times DNA_p, \quad [2]$$

where C_{mic} and DNA_{mic} are a sample's microbial biomass C concentration and DNA content. We calculated microbial C uptake (U, μg C g^{-1} h^{-1}) as the sum of microbial growth (G) and microbial respiration (R):

$$U = G + R. \quad [3]$$

The microbial CUE was calculated as:

$$CUE = \frac{G}{U} = \frac{G}{G + R}. \quad [4]$$

Additional detailed experimental methods for field collection and analyses are provided in *SI Appendix*.

Statistical Analysis. Before analysis, the Shapiro–Wilk (function *shapiro.test*) and Levene's tests (function *leveneTest*) were used to check the normality and homogeneity of variances for all variables, respectively. All statistical analyses were performed with *R* (version 4.1.3) using the *lme4* (62), *vegan* (63), *lavaan* (64), *psych* (65), *MuMIn* (66), and *rdacca.hp* (67) packages. The significance of all statistical tests was determined at the $\alpha = 0.05$ level. We tested the fixed effects of warming, depth, and their interaction on soil microbial CUE, physicochemical property, enzyme, lignin phenol, as well as microbial community composition and diversity using linear mixed-effects models with plot nested within block as random effects (function *lme*). At each depth, differences in the above variables between control and warming treatments were measured using paired *t* test (function *t.test*). To assess significant differences in the above variables among soil depths, a linear mixed-effects model with "depth" as fixed factor and "block" as random factor was used (function *lme*).

The relationships between CUE and soil resource and microbial community properties were analyzed by univariate linear regression (function *lm*). As there was no significant correlation between CUE and fungal community composition (characterized by $NMDS1_F$), the microbial community property in the following analysis did not include $NMDS1_F$. We applied three types of statistical analyses (partial correlation, structural equation model, and multiple regression) to quantify the relative importance of soil C availability [EOC, LCI, $(Ac/Al)_V$, $(Ac/Al)_S$, S_m/V_m, and C_m/V_m), N availability (mineral N), and microbial community properties (F/B, GP/GN, bacterial α-diversity ($Shannon_B$), fungal α-diversity ($Shannon_F$), and bacterial community composition (characterized by $NMDS1_B$)] in regulating CUE.

For partial correlation analysis, after controlling every single variable of soil C availability, we assessed the relationships between CUE and N availability and microbial community properties (function *pcor.test*). The greater the difference in partial correlation coefficient between zero-order and controlling correlation, the stronger the effect of control factor. Prior to constructing structural equation model, we performed principal component analysis separately for variables related to soil C availability and microbial community properties to simplify our analysis and facilitate interpretation (function *principal*). The first components (PC1), which explained 86% and 63% of the soil C availability and microbial community properties, respectively, were then introduced as two new variables into the structural equation model analysis. The fit of the final model was indicated by a nonsignificant χ^2 test ($P \geq 0.05$), high goodness-of-fit index (GFI > 0.90),

high comparative fit index (CFI > 0.95), low standardized rms residual (SRMR < 0.08), and low Akaike information criterion (AIC) value. Finally, we fitted a multiple regression model to evaluate the relative importance of soil C availability, N availability, and microbial community properties on microbial CUE. We started with the full model. Then the optimal model was obtained based on the minimum value of AIC (function *dredge*). To assess the relative importance of the predictors as drivers of CUE, we calculated the relative effect of the parameter estimates for each of the predictors compared with the effect of all parameter estimates in the model (function *rdacca.hp*). In the final optimal model, three variables with high explanatory degrees are retained, including C_m/V_m, the LCI and $Shannon_F$.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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1. IPCC, *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2021), p. 2391.
2. K. E. O. Todd-Brown, B. Zheng, T. W. Crowther, Field-warmed soil carbon changes imply high 21st century modeling uncertainty. *Biogeosciences* **15**, 3659–3671 (2018).
3. N. van Gestel *et al.*, Predicting soil carbon loss with warming. *Nature* **554**, E4–E5 (2018).
4. M. M. Wang *et al.*, Global soil profiles indicate depth-dependent soil carbon losses under a warmer climate. *Nat. Commun.* **13**, 5514 (2022).
5. E. G. Jobbágy, R. B. Jackson, The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol. Appl.* **10**, 423–436 (2000).
6. M. A. Bradford *et al.*, Managing uncertainty in soil carbon feedbacks to climate change. *Nat. Clim. Change* **6**, 751–758 (2016).
7. J. L. Soong, C. L. Phillips, C. Ledna, C. D. Koven, M. S. Torn, CMIP5 models predict rapid and deep soil warming over the 21st century. *J. Geophys. Res. Biogeo.* **125**, e2019JG005266 (2020).
8. E. A. Davidson, I. A. Janssens, Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* **440**, 165–173 (2006).
9. C. E. Hicks Pries, C. Castanha, R. C. Porras, M. S. Torn, The whole-soil carbon flux in response to warming. *Science* **355**, 1420–1423 (2017).
10. A. T. Nottingham *et al.*, Microbial diversity declines in warmed tropical soil and respiration rise exceed predictions as communities adapt. *Nat. Microbiol.* **7**, 1650–1660 (2022).
11. F. Tao *et al.*, Microbial carbon use efficiency promotes global soil carbon storage. *Nature* **618**, 981–985 (2023), 10.1038/s41586-023-06042-3.
12. S. Manzoni, P. Taylor, A. Richter, A. Porporato, G. I. Ågren, Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* **196**, 79–91 (2012).
13. R. L. Sinsabaugh, S. Manzoni, D. L. Moorhead, A. Richter, Carbon use efficiency of microbial communities: Stoichiometry, methodology and modelling. *Ecol. Lett.* **16**, 930–939 (2013).
14. S. D. Frey, J. Lee, J. M. Melillo, J. Six, The temperature response of soil microbial efficiency and its feedback to climate. *Nat. Clim. Change* **3**, 395–398 (2013).
15. N. Dove, M. Torn, S. Hart, N. Taş, Metabolic capabilities mute positive response to direct and indirect impacts of warming throughout the soil profile. *Nat. Commun.* **12**, 2089 (2021).
16. Q. F. Zhang, W. K. Qin, J. G. Feng, B. Zhu, Responses of soil microbial carbon use efficiency to warming: Review and prospects. *Soil Ecol. Lett.* **4**, 307–318 (2022).
17. J. K. Apple, P. A. del Giorgio, W. M. Kemp, Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. *Aquat. Microb. Ecol.* **43**, 243–254 (2006).
18. T. W. N. Walker *et al.*, Microbial temperature sensitivity and biomass change explain soil carbon loss with warming. *Nat. Clim. Change* **8**, 885–889 (2018).
19. L. A. Domeignoz-Horta *et al.*, Microbial diversity drives carbon use efficiency in a model soil. *Nat. Commun.* **11**, 3684 (2020).
20. P. Dijkstra *et al.*, Effect of temperature on metabolic activity of intact microbial communities: Evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency. *Soil Biol. Biochem.* **43**, 2023–2031 (2011).
21. Q. Zheng *et al.*, Growth explains microbial carbon use efficiency across soils differing in land use and geology. *Soil Biol. Biochem.* **128**, 45–55 (2019).
22. S. D. Allison, M. D. Wallenstein, M. A. Bradford, Soil-carbon response to warming dependent on microbial physiology. *Nat. Geosci.* **3**, 336–340 (2010).
23. J. Chen *et al.*, Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. *Global Change Biol.* **24**, 4816–4826 (2018).
24. J. M. Melillo *et al.*, Soil warming, carbon–nitrogen interactions, and forest carbon budgets. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 9508–9512 (2011).
25. L. A. Domeignoz-Horta *et al.*, Substrate availability and not thermal acclimation controls microbial temperature sensitivity response to long-term warming. *Global Change Biol.* **29**, 1574–1590 (2023).
26. E. Bai *et al.*, A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytol.* **199**, 441–451 (2013).
27. S. W. Liu *et al.*, Increased soil release of greenhouse gases shrinks terrestrial carbon uptake enhancement under warming. *Global Change Biol.* **26**, 4601–4613 (2020).
28. M. R. Ullah, Y. Carrillo, F. A. Dijkstra, Drought-induced and seasonal variation in carbon use efficiency is associated with fungi:bacteria ratio and enzyme production in a grassland ecosystem. *Soil Biol. Biochem.* **155**, 108159 (2021).
29. N. Wang *et al.*, Effects of climate warming on carbon fluxes in grasslands-A global meta-analysis. *Global Change Biol.* **25**, 1839–1851 (2019).
30. J. L. Soong *et al.*, Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global Change Biol.* **26**, 1953–1961 (2020).
31. P. G. Falkowski, T. Fenchel, E. F. Delong, The microbial engines that drive Earth's biogeochemical cycles. *Science* **320**, 1034–1039 (2008).
32. J. K. Jansson, K. S. Hofmockel, Soil microbiomes and climate change. *Nat. Rev. Microbiol.* **18**, 35–46 (2020).
33. K. M. Keiblinger *et al.*, The effect of resource quantity and resource stoichiometry on microbial carbon-use-efficiency. *FEMS Microbiol. Ecol.* **73**, 430–440 (2010).
34. A. T. Nottingham, P. Meir, E. Velasquez, B. L. Turner, Soil carbon loss by experimental warming in a tropical forest. *Nature* **584**, 234–237 (2020).
35. G. Pold *et al.*, Carbon use efficiency and its temperature sensitivity covary in soil bacteria. *mBio* **11**, e02293-19 (2020).
36. T. P. Smith, T. Clegg, T. Bell, S. Pawar, Systematic variation in the temperature dependence of bacterial carbon use efficiency. *Ecol. Lett.* **24**, 2123–2133 (2021).
37. Y. H. Hou, Y. Chen, X. Chen, K. Y. He, B. Zhu, Changes in soil organic matter stability with depth in two alpine ecosystems on the Tibetan Plateau. *Geoderma* **351**, 153–162 (2019).
38. S. Q. Qin *et al.*, Temperature sensitivity of SOM decomposition governed by aggregate protection and microbial communities. *Sci. Adv.* **5**, eaau1218 (2019).
39. V. Roth *et al.*, Persistence of dissolved organic matter explained by molecular changes during its passage through soil. *Nat. Geosci.* **12**, 755–761 (2019).
40. M. M. Stone, J. L. DeForest, A. F. Plante, Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory. *Soil Biol. Biochem.* **75**, 237–247 (2014).
41. M. Spohn, K. Klaus, W. Wanek, A. Richter, Microbial carbon use efficiency and biomass turnover times depending on soil depth–Implications for carbon cycling. *Soil Biol. Biochem.* **96**, 74–81 (2016).
42. R. P. White, S. Murray, M. Rohweder, *Grassland Ecosystems* (DC World Resources Institute, Washington, 2000).
43. S. L. Piao *et al.*, Impacts of climate and CO_2 changes on the vegetation growth and carbon balance of Qinghai-Tibetan grasslands over the past five decades. *Global Planet. Change* **98–99**, 73–80 (2012).
44. H. Y. Liu *et al.*, Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 4051–4056 (2018).
45. W. H. Li, X. M. Zhou, *Ecosystems of Qinghai-Xizang (Tibetan) Plateau and Approach for Their Sustainable Management., Guangzhou* (Guangdong Science & Technology Press, 1998).
46. Y. H. Yang *et al.*, Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. *Global Change Biol.* **14**, 1592–1599 (2008).
47. M. Dong, Y. Jiang, C. Zheng, D. Zhang, Trends in the thermal growing season throughout the Tibetan Plateau during 1960–2009. *Agr. Forest Meteorol.* **166–167**, 201–206 (2012).
48. W. K. Qin *et al.*, Whole-soil warming shifts species composition without affecting diversity, biomass and productivity of the plant community in an alpine meadow. *Fundam. Res.* **3**, 160–169 (2023).
49. K. M. Geyer, P. Dijkstra, R. Sinsabaugh, S. D. Frey, Clarifying the interpretation of carbon use efficiency in soil through methods comparison. *Soil Biol. Biochem.* **128**, 79–88 (2019).
50. J. Q. Li, J. M. Pei, F. A. Dijkstra, M. Nie, E. Pendall, Microbial carbon use efficiency, biomass residence time and temperature sensitivity across ecosystems and soil depths. *Soil Biol. Biochem.* **154**, 108117 (2021).
51. R. L. Sinsabaugh, Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol. Biochem.* **42**, 391–404 (2010).
52. J. M. Melillo *et al.*, Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science* **358**, 101–105 (2017).
53. X. J. A. Liu *et al.*, Soil aggregate-mediated microbial responses to long-term warming. *Soil Biol. Biochem.* **152**, 108055 (2021).
54. C. Cruz-Paredes, D. Tájmel, J. Rousk, Can moisture affect temperature dependences of microbial growth and respiration? *Soil Biol. Biochem.* **156**, 108223 (2021).
55. J. T. Li *et al.*, Low soil moisture suppresses the thermal compensatory response of microbial respiration. *Global Change Biol.* **29**, 874–889 (2023).
56. R. W. Sterner, J. J. Elser, *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere* (Princeton University Press, Princeton, NJ, 2002).
57. C. Kaiser, O. Franklin, U. Dieckmann, A. Richter, Microbial community dynamics alleviate stoichiometric constraints during litter decay. *Ecol. Lett.* **17**, 680–690 (2014).

58. L. A. Schipper, J. K. Hobbs, S. Rutledge, V. L. Arcus, Thermodynamic theory explains the temperature optima of soil microbial processes and high Q10 values at low temperatures. *Glob. Change Biol.* **20**, 3578–3586 (2014).
59. C. J. Alster, J. C. von Fischer, S. D. Allison, K. K. Treseder, Embracing a new paradigm for temperature sensitivity of soil microbes. *Glob. Change Biol.* **26**, 3221–3229 (2020).
60. H. Wang *et al.*, Alpine grassland plants grow earlier and faster but biomass remains unchanged over 35 years of climate change. *Ecol. Lett.* **23**, 701–710 (2020).
61. P. J. Hanson *et al.*, Attaining whole-ecosystem warming using air and deep-soil heating methods with an elevated CO_2 atmosphere. *Biogeosciences* **14**, 861–883 (2017).
62. D. Bates, M. Maechler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
63. J. Oksanen *et al.*, vegan: Community ecology package. (2022). https://cran.r-project.org/web/packages/vegan/vegan.pdf.
64. Y. Rosseel, lavaan: An R package for structural equation modeling. *J. Stat. Softw.* **48**, 1–36 (2012).
65. W. Revelle, D. M. Condon, Reliability from alpha to omega: A tutorial. *Psychol. Assess.* **31**, 1395–1411 (2019).
66. K. P. Burnham, D. R. Anderson, *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* (Springer-Verlag, New York, ed. 2, 2002).
67. J. S. Lai, Y. Zou, J. L. Zhang, P. R. Peres-Neto, Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. *Methods Ecol. Evol.* **13**, 782–788 (2022).