




RESEARCH ARTICLE

Local temperature increases reduce soil microbial residues and carbon stocks

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Abstract

Warming is known to reduce soil carbon (C) stocks by promoting microbial respiration, which is associated with the decomposition of microbial residue carbon (MRC). However, the relative contribution of MRC to soil organic carbon (SOC) across temperature gradients is poorly understood. Here, we investigated the contribution of MRC to SOC along two independent elevation gradients of our model system (i.e., the Tibetan Plateau and Shennongjia Mountain in China). Our results showed that local temperature increases were negatively correlated with MRC and SOC. Further analyses revealed that rising temperature reduced SOC via decreasing MRC, which helps to explain future reductions in SOC under climate warming. Our findings demonstrate that climate warming has the potential to reduce C sequestration by increasing the decomposition of MRC, exacerbating the positive feedback between rising temperature and CO₂ efflux. Our study also considered the influence of multiple environmental factors such as soil pH and moisture, which were more important in controlling SOC than microbial traits such as microbial life-style strategies and metabolic efficiency. Together, our work suggests an important mechanism underlying long-term soil C sequestration, which has important implications for the microbial-mediated C process in the face of global climate change.

KEYWORDS

climate warming, elevation gradient, microbial residue carbon, microbial traits, soil carbon storage, soil properties

1 | INTRODUCTION

Terrestrial ecosystems store an immense amount of soil organic carbon (SOC) in the first centimeters, which plays a vital role in mediating climate-carbon cycle feedback (Hicks Pries et al., 2017; Wang, Qu, et al., 2021). If soil carbon (C) stocks are released back into the atmosphere, it could accelerate climate change with dire consequences for life on Earth (Melillo et al., 2017; Wang et al., 2019). Emerging evidence reveals that soil C storage and release to the atmosphere is ultimately a consequence of microbial catabolism (respiration) and anabolism (biomass synthesis) (Liang & Balser, 2012; Zhu et al., 2021), which may be highly dependent on local temperature (Bradford et al., 2019). Temperature increases are known to stimulate microbial respiration and reduce C stocks (Nottingham et al., 2019). This can be readily seen in global latitude and elevation gradients with the majority of C being sequestered in intermediate-high latitudes and elevations (Dixon et al., 1994; Dong et al., 2020). Consequently, it is essential to understand how soil C is released into the atmosphere and which components of SOC are vulnerable to climate change.

Soil C stocks contain multiple components. One of the most important components is associated with the accumulation of microbial residues, which are derived from the cumulative long-term legacy of anabolic products (e.g., extracellular polymeric substances, *exo*-enzymes, and the dead cell remains) (Liang & Balser, 2011; Luo et al., 2020). Yet, the role of microbial residues in SOC accumulation across temperature gradients remains unclear, hindering our ability to precisely access and predict global C storage under future climate change. Microbial residue C (MRC) is receiving tremendous attention, as it is expected to be an important contributor to the stable SOC pool due to its continuous production and relatively slow decomposition (Liang, 2020; Liang et al., 2020). Because of this fact, MRC could be considered as an important active component in response to climate change. In terrestrial ecosystems, MRC has been reported to contribute up to 60% of the SOC pool (Deng & Liang, 2021). Despite its theoretical resistance to decomposition, MRC is susceptible to increasing local temperature (Ding et al., 2019; Liang & Balser, 2012). Under warming, the decomposition of MRC could increase; in the meanwhile, microbial biomass rises and thus enhances the production of MRC (Liang & Balser, 2012; Wang et al., 2020). However, the net contribution of MRC to SOC remains unclear, because MRC accumulation depends on the balance between microbial production and decomposition (Liu et al., 2021).

The contribution of MRC to SOC and its sensitivity to warming may also be associated with the myriad of microbial traits related to the source, decomposition and accumulation of MRC. Microbial traits (e.g., microbial biomass and metabolic efficiency) may indirectly influence SOC via their impacts on MRC (Ni, Liao, Tan, Wang, et al., 2020; Wang, An, et al., 2021; Wang, Qu, et al., 2021). For instance, soils with larger microbial biomass could result in greater C losses in response to warming due to the increasing SOC decomposition (Wieder et al., 2013). However, microbial biomass could also have indirect positive relationship with SOC through biomass turnover and MRC

production (Liang et al., 2017; Wang, Qu, et al., 2021). Moreover, microbial metabolic efficiency indirectly affects SOC by changing microbial biomass and residues. Higher metabolic efficiency (i.e., lower metabolic quotient and higher C use efficiency) could result in higher microbial biomass and therefore potentially increase SOC sequestration via MRC accumulation (Bhople et al., 2021; Doetterl et al., 2018; Wang, Qu, et al., 2021). Finally, changes in the proportion of microbial communities associated with different life-history strategies (e.g., oligotrophic vs. copiotrophic taxa) can influence the rates of SOC decomposition and persistence (Shao et al., 2021; Wang, Zhang, et al., 2021). Thus, accounting for changes in microbial life-style strategies, linked microbial community composition and metabolic processes to SOC turnover, could improve our understanding of MRC contribution to SOC (Lajoie & Kembel, 2019; Shao et al., 2021). Despite this recognition, the relative contribution of microbial life-style strategies to MRC and SOC remains poorly understood (Shao et al., 2021). Learning more about how the different components of the microbial community influence soil C stocks is crucial for predicting the future of C sequestration under climate change.

In ecology, a model system is a gene, a species, a community, or ecosystem that can be used to test particular research questions in an understandable way (Bowker et al., 2014; Vitousek, 2002). Here, we used elevation gradients as a model system to investigate how local temperature increases influence soil C stocks via changes in MRC. Elevation gradients exhibit dramatic changes in climatic, abiotic, and biotic conditions over short geographic distances (Bhople et al., 2021; Mou et al., 2021). Especially, temperature (i.e., mean annual temperature [MAT]) generally decreases linearly with elevation (Ziello et al., 2009), which makes possible the evaluation of ecosystem responses to local temperature increases. Previous studies have reported that changes in environmental conditions (e.g., temperature and soil properties) influenced microbial traits related to soil C cycling (Bhople et al., 2021; Feng et al., 2021). Therefore, the accumulation of MRC may vary widely along elevation gradients due to the differences in environmental conditions and microbial traits. In this study, we aimed to (1) examine the relationship between MRC and SOC across environmental gradients; (2) identify the role of temperature in driving SOC via changes in MRC. We assumed that (1) MRC accumulation is consistently related to variations in SOC along the elevation gradients; (2) elevated temperature decreases SOC via increasing MRC decomposition. To test our hypotheses, we collected soil samples from 28 and 25 sites along the elevation gradients in the Tibetan Plateau (TP) and Shennongjia Mountain (SNJ), respectively. The TP and SNJ, as model mountain ecosystems, are both considered as hotspots of C sequestration (Praeg et al., 2020). However, alpine ecosystem soils in the TP are characterized by harsh environmental conditions (e.g., low temperature, drought, and poor nutrients) and are more vulnerable to climate change compared with the SNJ (Zhang, Jia, et al., 2021). It remains unclear whether temperature and microbial traits can consistently predict the contribution of MRC to SOC across contrasting ecosystems. We investigated the associations between MRC (represented by amino sugars) and SOC, and quantified the relative contribution of environmental factors to the spatial variations of MRC and

SOC along the elevation gradients. Moreover, in our models, we also considered a variety of important environmental factors, which are known to regulate MRC and SOC across environmental gradients, and that include soil properties such as pH, moisture, and SOC/TN ratio (Mou et al., 2021; Ni, Liao, Tan, Peng, et al., 2020).

2 | MATERIALS AND METHODS

2.1 | Study area and field sampling

Soil samples were collected in two independent elevation gradients: 28 sites (latitude: 29°34'–29°36'N; longitude: 94°19'–94°22'E; elevation: 2974–3559 m) in the TP and 25 sites (latitude: 31°26'–31°32' N; longitude: 110°16'–110°20' E; elevation: 1710–2907 m) in the SNJ (Figure S1). The TP has warmer winters and cooler summers owing to the Indian Ocean monsoon, while SNJ is affected by the southeast sub-tropical monsoon with wet warm summers and cold dry winters. The MAT decreases from 8.6 to 4.7°C and 8.5 to 4.1°C with increasing elevation in the TP and SNJ, respectively. The mean annual precipitation decreases from 673 to 618 mm with elevation in the TP, but increases from 1252 to 1368 mm in the SNJ (data source: WorldClim, <http://www.worldclim.org>). In the TP, the dominant vegetation types shift from temperate coniferous and broadleaved mixed forests (3000–3500 m) to frigid dark coniferous forests (above 3500 m) as the elevation increases (Figure S1) (Xu et al., 2014). In the SNJ, the dominant vegetation types with increasing elevation are described as deciduous broadleaved forests (1710–2000 m), mixed conifer and deciduous broadleaved forests (2000–2400 m), and sub-alpine conifer forests (including sub-alpine shrubs and meadows, above 2400 m) (Wang et al., 2016). The predominant soil types are classified as Luvisols and Cambisols in the TP and Cambisols in the SNJ (IUSS Working Group, 2007).

Soil samples from the TP and SNJ were collected in the growing seasons of 2018 and 2020, respectively. The two samplings used nearly identical random strategies, which were conducted as follows. In each site, five soil replicates (0–10 cm) were collected from the understory or adjacent open grasslands of each sub-plots (1 × 1 m) in a 50 × 50 m plot and then homogenized to form a composite sample. Collected soil samples were transported to the laboratory on ice. The stones and roots were carefully removed, and then the soil samples were divided into two portions. One was stored at –20°C for molecular analysis, while the other portion was stored at 4°C for the subsequent analyses of soil properties and microbial traits.

2.2 | Measurement of soil properties

Soil pH was determined at a 1:2.5 soil/distilled water ratio by a pH electrode. Soil moisture was measured by oven-drying fresh soil for 24 h at 105°C. SOC was quantified by K₂CrO₇ oxidation titration method (Walkley, 1947). Soil total N (TN) was measured with an elemental analyzer (Vario PYRO Cube).

2.3 | Amino sugar analysis

Amino sugars are routinely used as biomarkers of microbial residues, because they are key components of microbial cell walls and stabilized in the soil after cell death (Liang et al., 2019; Luo et al., 2020). We used soil amino sugars to indicate MRC, which was determined according to Liang et al. (2012). Soil samples (containing ≥0.3 mg N) were hydrolyzed with 6 M HCl at 105°C for 8 h. After adding internal standard (myo-inositol), the solutions were filtered, dried at 45°C, re-dissolved in deionized water, adjusted to pH 6.6–6.8 with KOH, and centrifuged. Supernatants were lyophilized and their residues were dissolved in methanol and then dried with N₂ gas at 45°C. Residues were re-dissolved in N-methylglucamine and deionized water, while were then lyophilized. Lyophilized residues were derivatized in 300 µl derivatization containing 32 mg ml^{–1} of hydroxylamine hydrochloride and 40 mg ml^{–1} of 4-(dimethylamino) pyridine in pyridine-methanol (4:1 v/v) at 75–80°C for 35 min. After cooling, the solutions after adding acetic anhydride were reheated at 75–80°C for 25 min. Then, solutions containing 1.5 ml dichloromethane and 1 ml 1 M HCl were washed thrice with deionized water and vortexed to separate the organic phase of amino sugars. Finally, derivatives of amino sugars were dried with N₂ gas at 45°C and then re-dissolved in 300 µl hexane-ethyl acetate solvent (1:1 v/v). Amino sugars were measured using a Shimadzu GC2010 Plus gas chromatograph (Shimadzu Corporation) equipped with an HP-5 column (30 m × 0.25 mm × 0.25 µm).

We used two amino sugars (glucosamine [GluN] and muramic acid [MurA]) to evaluate the MRC accumulation in soil (Zheng et al., 2021). Different microbial groups produce different amino sugars (Liang et al., 2019). GluN primarily originates from fungal cell walls, while MurA is derived exclusively from bacterial cell walls (Murugan et al., 2019). Thus, GluN and MurA can be used to evaluate the accumulation of fungal- and bacterial-derived residues, respectively. The fungal residue C (fungal MRC) and bacterial residue C (bacterial MRC) were calculated using Equations (1) and (2) according to the methods described by Liang et al. (2019).

$$\text{Fungal MRC} = (\text{GluN} / 179.17 - 2 \times \text{MurA} / 251.23) \times 179.17 \times 9 \quad (1)$$

$$\text{Bacterial MRC} = \text{MurA} \times 45 \quad (2)$$

where 179.17 and 251.23 are the molecular weights of GluN and MurA, respectively; 9 and 45 are conversion factors. The total MRC was estimated as the sum of fungal MRC and bacterial MRC.

2.4 | Soil microbial traits analysis

Microbial biomass carbon (MBC) was measured using the fumigation-extraction method (Vance et al., 1987). Microbial metabolic efficiency was assessed by metabolic quotient (qCO₂) and carbon use efficiency (CUE) (Cotrufo et al., 2015; Feng et al., 2021). We used short-term (14 days) incubations at 20°C to estimate microbial

respiration as we have previously described (Feng et al., 2021). Soil qCO_2 was calculated by dividing the hourly microbial respiration by the corresponding MBC and was expressed as $\mu g\ CO_2-C\ \mu g^{-1}\ MBC\ h^{-1}$. Microbial CUE was calculated using the biogeochemical-equilibrium model as described in Cui et al. (2020).

Microbial life-history strategies (i.e., r/K strategy) were evaluated using oligotroph/copiotroph ratios, the 16S rRNA (*rrn*) operon copy number, and recalcitrant/labile C degradation gene ratios as defined in Li et al. (2021). We used Illumina MiSeq sequencing of 16S rRNA and ITS region to determine bacterial and fungal communities, respectively (Zeng et al., 2022). Based on sequencing data, the main bacterial and fungal taxa have been classified as copiotrophs (r-strategists) and oligotrophs (K-strategists) (Table S1) (Li et al., 2021; Zeng et al., 2022). With respect to the predicted functional guilds, saprotrophic and ectomycorrhizal (ECM) fungi were identified by FUNGuild (Li et al., 2021; Nguyen et al., 2016). We obtained the rRNA operon copy number for each OTU from the rrnDB database (<http://rrnodb.umms.med.umich.edu/>) based on its closest relatives with known rRNA operon copy number (Stoddard et al., 2015). The abundance-weighted average rRNA operon copy number was then calculated by taking the product of the estimated operon copy number and the relative abundance for each OTU, and summing this value across all OTUs in a sample, as done in previous studies (Li et al., 2021; Wu et al., 2017).

The functional genes involved in degrading labile and recalcitrant C could be grouped into r- and K-categories, respectively (Li et al., 2021). Four functional genes related to degrading lignin (*lig* coding for lignin peroxidase), cellulose (*cex* coding for exoglucanase), hemicelluloses (*xylA* coding for xylanase), and starch (*amyA* coding for α -amylase) were measured by a CFX Connect Real-Time PCR Detection System (Bio-Rad). The primer information and the qPCR procedures are provided in Zheng et al. (2018). Additionally, these gene abundances were calculated by normalizing abundances to units per mg MBC and were expressed as the copy number $\mu g^{-1}\ MBC$, in order to avoid the variations induced by biomass changes. We designated the *lig* gene as the recalcitrant C degradation gene, and the sum of *cex*, *xylA*, and *amyA* genes as the labile C degradation gene.

Similar to functional genes, the extracellular enzyme activities involved in degrading labile and recalcitrant C were incorporated into the r/K-classification scheme. Cellulase activities related to labile C (β -1,4-glucosidase [BG]) were measured by a fluorimetric microplate assay using 4-methylumbelliferyl (MUB) substrates (Marx et al., 2001). In brief, 0.50 g of fresh soil was mixed with 50 ml of deionized water and stirred vigorously for 15 min with a magnetic stir plate. Then, 50 μ l of soil homogenate, 100 μ l of substrate solution (200 μ mol L^{-1}) and 50 μ l of acetate buffer (0.2 mol L^{-1} , pH 5.5) were mixed and incubated at 30°C for 3 h. The released fluorescence was measured using a multifunctional microplate reader (Tecan Spark™ 10M, Männedorf, Switzerland) at 360 nm excitation and 450 nm emission wavelengths. Enzyme activities of BG were expressed as nmol $h^{-1}\ g^{-1}$ soil. Ligninase activities related to recalcitrant C (phenoloxidase [PPO] and peroxidase [POD]) were measured

photometrically using L-3, 4-dihydroxyphenylalanine (DOPA) substrate (DeForest, 2009). Then, 200 μ l of soil suspension, 50 μ l of acetate buffer (50 mmol L^{-1} , pH 5.5), and 50 μ l of 25 mmol L^{-1} DOPA solution were mixed and incubated at 20°C for 20 h in the dark. POD required the addition of 10 μ l of 0.3% H_2O_2 . Absorbance at 450 nm was measured using a microplate spectrophotometer. Enzyme activities of PPO and POD were expressed as μ mol $h^{-1}\ g^{-1}$ soil.

2.5 | Statistical analysis

We first identified the patterns of SOC, MRC, and MRC/SOC ratio along two MAT gradients. Statistical differences in MRC and MRC/SOC ratio between the TP and SNJ were tested using the independent-samples t-test by the “stats” package in R 4.0.2 (<http://cran.r-project.org/>). We used correlation analysis to explore the relationships between SOC and MRC, MAT, soil properties (soil pH, moisture, and SOC/TN), and microbial traits (MBC, qCO_2 , CUE and r/K strategy) using the “vegan” package. A Random Forest analysis was conducted to identify the significant predictors of MRC and SOC using the “rfPermute” package. We estimated the importance of different variables by comparing the percentage increase in mean squared error (%IncMSE), with higher %IncMSE indicating more important variables. We then used variation partitioning analysis (VPA) to quantify the relative importance of temperature, soil properties, microbial biomass, metabolic efficiency, r/K strategy, and MRC in regulating SOC (i.e., the unique and shared portion of the variation in SOC explained by the six factors), according to Delgado-Baquerizo, Reich, et al. (2020). This analysis was run using the *varpart* function of the “vegan” package.

Structural equation modeling (SEM), as a multivariate analysis, was conducted to evaluate the direct and indirect associations between elevation, MAT, main soil properties (i.e., soil moisture), microbial traits (i.e., MBC), MRC, and SOC (Delgado-Baquerizo et al., 2016). A conceptual model of hypothetical relationships was constructed based on prior knowledge (Wang, Qu, et al., 2021), assuming that the response of main soil properties and microbial traits to elevation-induced variations in temperature would directly impact SOC, or indirectly influence SOC through altering MRC (see our a priori model in Figure S2). As some of the introduced variables were not normally distributed, the probability that a path coefficient differs from zero was tested using bootstrap test rather than the classical maximum-likelihood estimation (Delgado-Baquerizo, Guerra, et al., 2020). Here we used the chi-squared test (χ^2 ; the model has a good fit when $0 \leq \chi^2/df \leq 2$ and $0.05 < p \leq 1.00$) and the root mean square error of approximation (RMSEA; the model has a good fit when $0 \leq RMSEA \leq 0.05$ and $0.10 < p \leq 1.00$) to test the goodness of fit of our model (Delgado-Baquerizo et al., 2017). Finally, we confirmed the fit of the model using the Bollen–Stine bootstrap test ($0.10 < \text{bootstrap } p \leq 1.00$) (Delgado-Baquerizo, Guerra, et al., 2020). Meanwhile, we used SEM to determine the contribution of different environmental factors in explaining SOC when all these factors are considered simultaneously (Delgado-Baquerizo, Guerra,

et al., 2020). We calculated the standardized total effects of elevation, MAT, moisture, MBC and MRC on SOC. All the SEM analyses were performed using AMOS 17.0 (SPSS Inc.).

3 | RESULTS

3.1 | Variations in MRC and SOC across the temperature gradients

The MAT decreased with increasing elevation in both TP and SNJ (Figure S3). The contents of SOC and MRC, including fungal and bacterial MRC and their sum, decreased with increasing MAT in the TP and SNJ (Figure 1 and Figure S3). The total and bacterial MRC contents were significantly lower in the TP compared with the SNJ, but no significant differences in fungal MRC were found between the TP and SNJ (Figure 2).

We showed that MRC was consistently and positively correlated with SOC in two independent MAT gradients (Figure S4), and fungal MRC contributed more to SOC than bacterial MRC in the TP and SNJ (Figure 2 and Figure S5; fungal MRC/SOC > bacterial MRC/SOC). The contribution of MRC to SOC (MRC/SOC ratio) increased with increasing MAT in the TP and SNJ (Figure S5). Moreover, the

contribution of fungal MRC to SOC (fungal MRC/SOC) was significantly higher in the TP than those in the SNJ, although there were no significant differences in the contribution of total MRC to SOC (total MRC/SOC) between the TP and SNJ (Figure 2).

3.2 | Variations in microbial traits across the temperature gradients

Our results showed that MBC and qCO_2 decreased, while CUE increased with increasing MAT in the TP and SNJ (Figure S6). The microbial r/K strategy ratio shifted significantly along two MAT gradients. Specifically, the ratios of bacterial oligotroph/copiotroph, and recalcitrant/labile C degradation genes and enzyme activities consistently decreased with increasing MAT in the TP and SNJ (Figure 3). The ratios of ECM/saprotrophic fungi also decreased significantly with increasing MAT in the TP, while there were no significant changes in the SNJ. Moreover, the weighted average rRNA operon copy number of bacterial community increased significantly with increasing MAT in the TP, but the SNJ exhibited an opposite pattern (Figure S7).

There were significant shifts in the specific microbial taxa along the MAT gradients. The relative abundances of potential oligotrophic

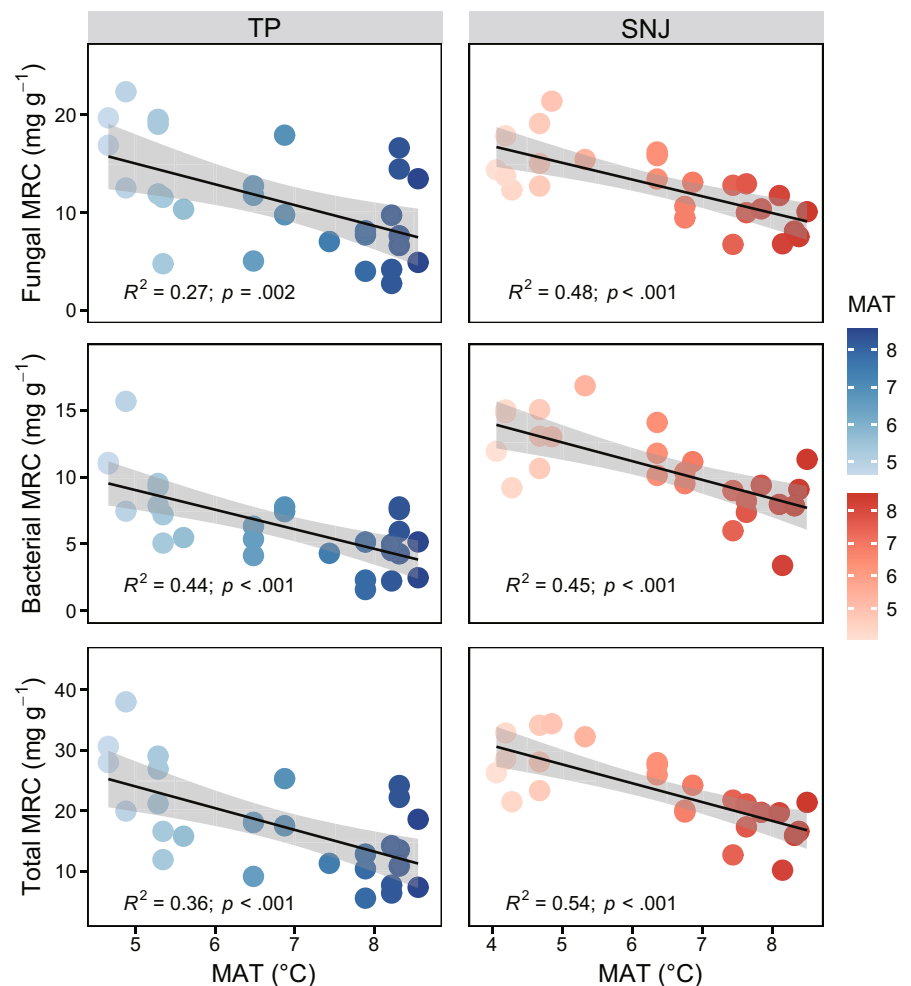


FIGURE 1 Patterns of microbial residue carbon (MRC) along the mean annual temperature (MAT) gradients of the Tibetan Plateau (TP) and Shennongjia Mountains (SNJ). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16347)]

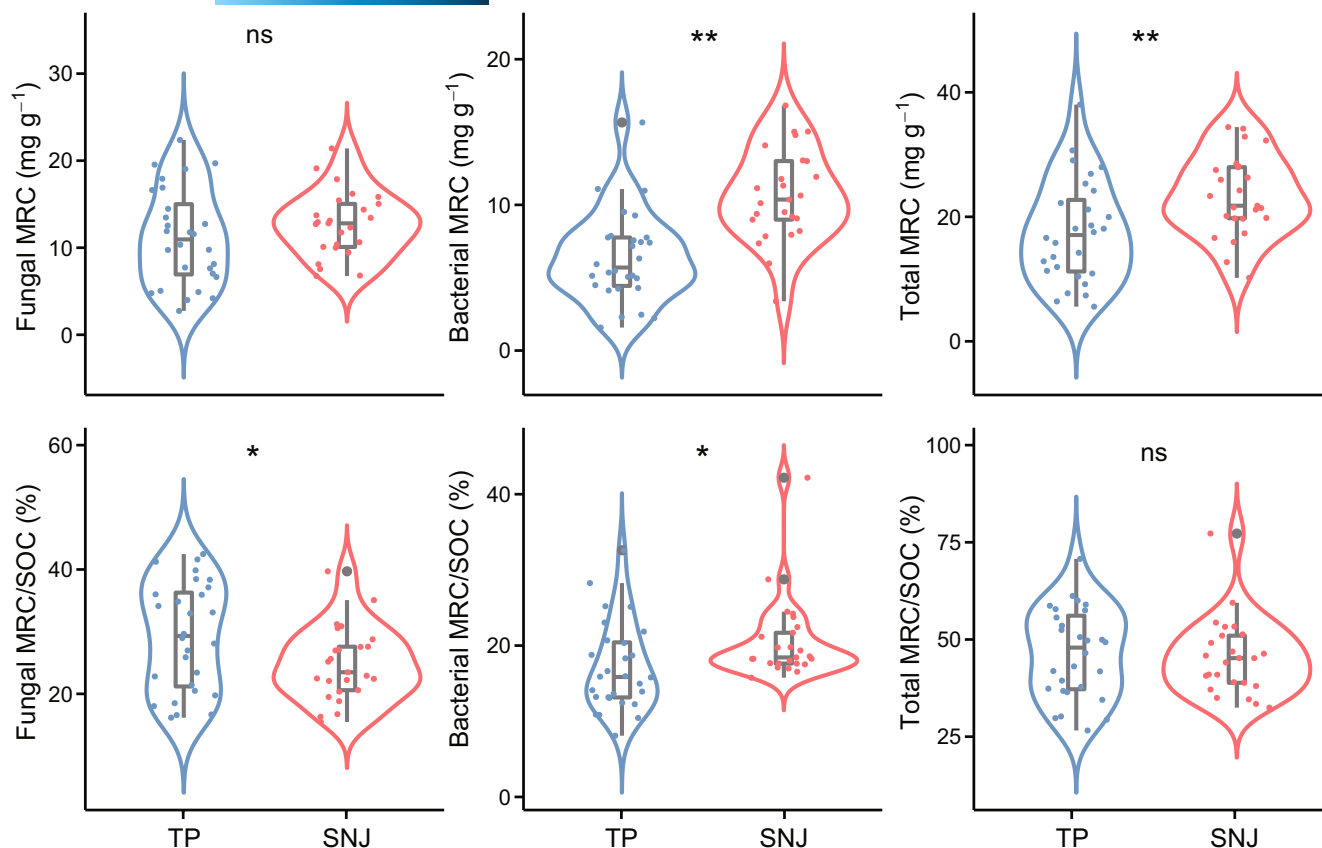


FIGURE 2 Comparisons of microbial residue carbon (MRC) and its contribution to soil organic carbon (SOC) between the Tibetan Plateau (TP) and Shennongjia Mountains (SNJ). Asterisks indicate significant differences in the independent-samples *t*-test. **p* < .05; ***p* < .01; ns, no significance. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16347)]

taxa (Acidobacteria, Deltaproteobacteria and Chloroflexi) decreased with increasing MAT. In contrast, the proportions of likely copiotrophic taxa—Actinobacteria and Firmicutes increased with increasing MAT in the TP and SNJ, respectively (Figure S8). Moreover, the abundances of the functional genes related to C also varied along the MAT gradients (Figure S9). Specifically, *lig*/MBC, *amyA*/MBC, and *xylA*/MBC decreased with increasing MAT in the TP, whereas all gene abundances increased with increasing MAT in the SNJ. For enzyme activities involved in C degradation, PPO and POD showed overall decreasing trends with increasing MAT in the TP, but only POD decreased in the SNJ (Figure S10).

3.3 | Associations of soil and microbial factors with MRC and SOC

SOC was consistently correlated with MAT, soil pH, moisture, SOC/TN, MBC, Enzyme_R/L, and MRC in the TP and SNJ (Figure 4a). Specifically, SOC had positive relationships with soil moisture, SOC/TN, MBC, Enzyme_R/L, and MRC, but was negatively associated with MAT and soil pH. More importantly, Random Forest analysis showed that shifts in MRC explained the highest proportion of variations in SOC after considering temperature, essential soil, and microbial factors. The results from VPA also supported that MRC

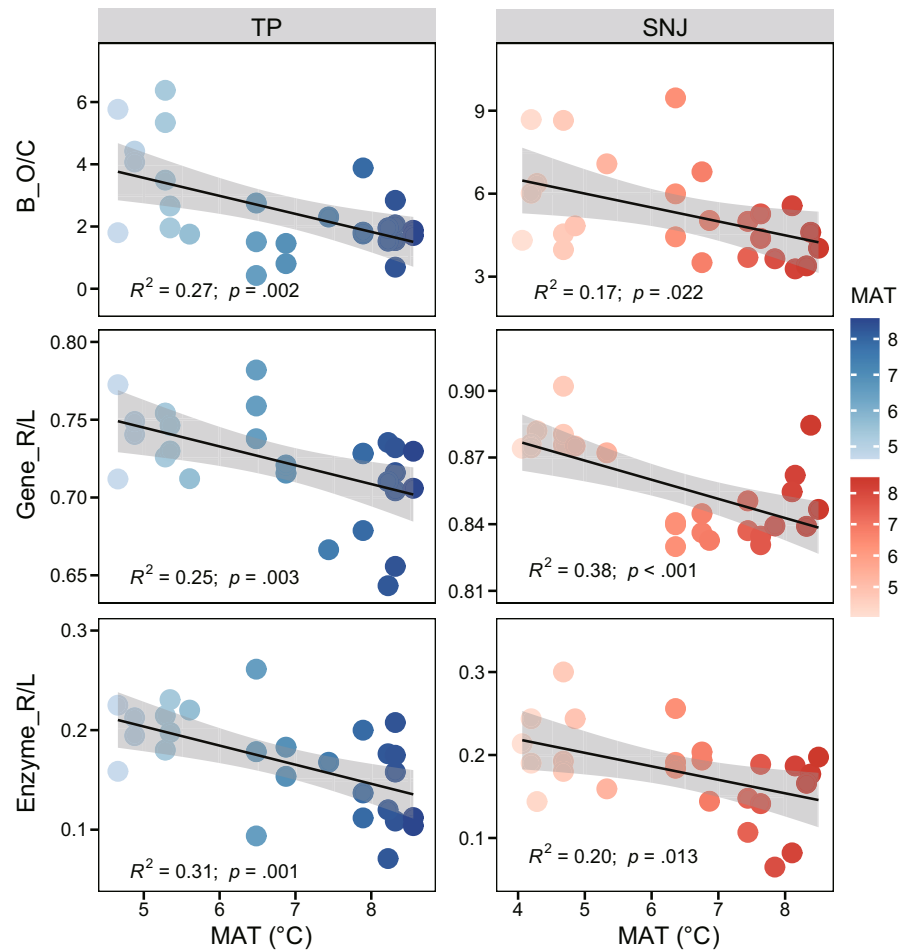
explained a relatively high portion of shared variation (with environment; 67%), while a relatively small portion of unique variation (Figure 4b). Moreover, the SEM provided further statistical evidence that MRC had a direct and positive relationship with SOC. In particular, temperature (i.e., MAT) had a strong indirect link with SOC via the negative association with MRC in both TP and SNJ (Figure 5).

Regarding MRC, correlation analysis consistently showed significant associations of MAT, soil pH, moisture, MBC, and r/K strategy with MRC in the TP and SNJ (Figures S11 and S12). Random Forest analysis indicated that shifts in MAT, pH, and moisture were essential predictors of MRC, when we concurrently considered essential microbial traits including microbial metabolic efficiency (i.e., $q\text{CO}_2$ and CUE) and r/K strategy (Figure S13). Regression analysis further indicated that MRC was negatively correlated with MAT and soil pH but positively correlated with soil moisture (Figure S14).

4 | DISCUSSION

In agreement with our hypotheses, we found that MRC is an important contributor to SOC across local temperature gradients. The positive relationship between MRC and SOC suggests that MRC could play an essential role in SOC accumulation, as described in earlier studies (Liang et al., 2019; Zheng et al., 2021). Importantly,

FIGURE 3 Variations in microbial r/K strategy ratio along the mean annual temperature (MAT) gradients of the Tibetan Plateau (TP) and Shennongjia Mountains (SNJ). B_O/C, bacterial oligotroph/copiotroph ratio; gene_R/L, the ratio of recalcitrant/labile organic C degradation gene abundance; enzyme_R/L, the ratio of recalcitrant/labile organic C degradation enzyme activities. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16347)]



our Random Forest analysis, VPA and SEM consistently demonstrate a strong association between MRC and SOC, even when considering key climate, soil, and microbial variables. Besides, our model suggests that elevation-induced variations in temperature, soil properties, and microbial traits could be indirectly related to SOC mainly through their regulations on MRC. Interestingly, the contribution of MRC to SOC increased with rising temperature. This indicates that an important portion of SOC is formed by MRC, which is vulnerable to local temperature increases. Furthermore, recalcitrant forms of C are known to require higher temperature to be degraded, which may happen in the warm areas with high MAT. On the contrary, cold environments contribute to more accumulation of MRC. We showed that increases in temperature indirectly reduced SOC via decreases in MRC. Thus, our work contextualizes the importance of MRC relative to other factors in explaining SOC. As such, the reduction in SOC with increasing MAT could be partially explained by the decreased accumulation of MRC under warmer soil conditions. To confirm whether temperature is driving SOC storage in terrestrial ecosystems mainly by variations in MRC, new investigations considering other components of SOC such as plant-derived C need to be conducted in the future.

Our analyses showed that the relative contribution of fungal and bacterial MRC to SOC differed under contrasting elevation gradients. Fungal MRC had a closer relationship with SOC in the TP

(elevation: 2974–3559 m), while bacterial MRC was more strongly related to SOC in the SNJ (elevation: 1710–2907 m). This suggests a greater contributor of fungal MRC to SOC in high-alpine ecosystems such as those of the TP, as directly reflected by a larger fungal MRC/SOC ratio in the TP than in the SNJ. Fungi are known to be more tolerant to the harsh conditions (i.e., low substrate quality and temperature) compared with bacteria (Alotaibi et al., 2020; Murugan et al., 2019). Furthermore, fungal residues are more resistant to decomposition (Wang, An, et al., 2021) and the remaining fungal MRC might reflect the actual C input to SOC in the high-alpine ecosystem. Thus, the inconsistent contribution patterns of fungal and bacterial MRC in the TP and SNJ are closely related to the different composition of main microbial groups and their residues as a consequence of their intrinsic elevations. Overall, these findings jointly highlight the importance of fungal and bacterial MRC on SOC, and a further understanding of the factors influencing MRC across temperature gradients can improve predictions of soil C sequestration under climate warming.

Our results further revealed a relatively weak influence of microbial traits on MRC and indirectly on SOC. Here, we made the first attempt to link MRC with microbial r/K strategy based on specific taxonomic groups, functional genes and enzyme activities. Our results are consistent with previous studies showing that microbial life-style strategies can explain a unique, but relatively

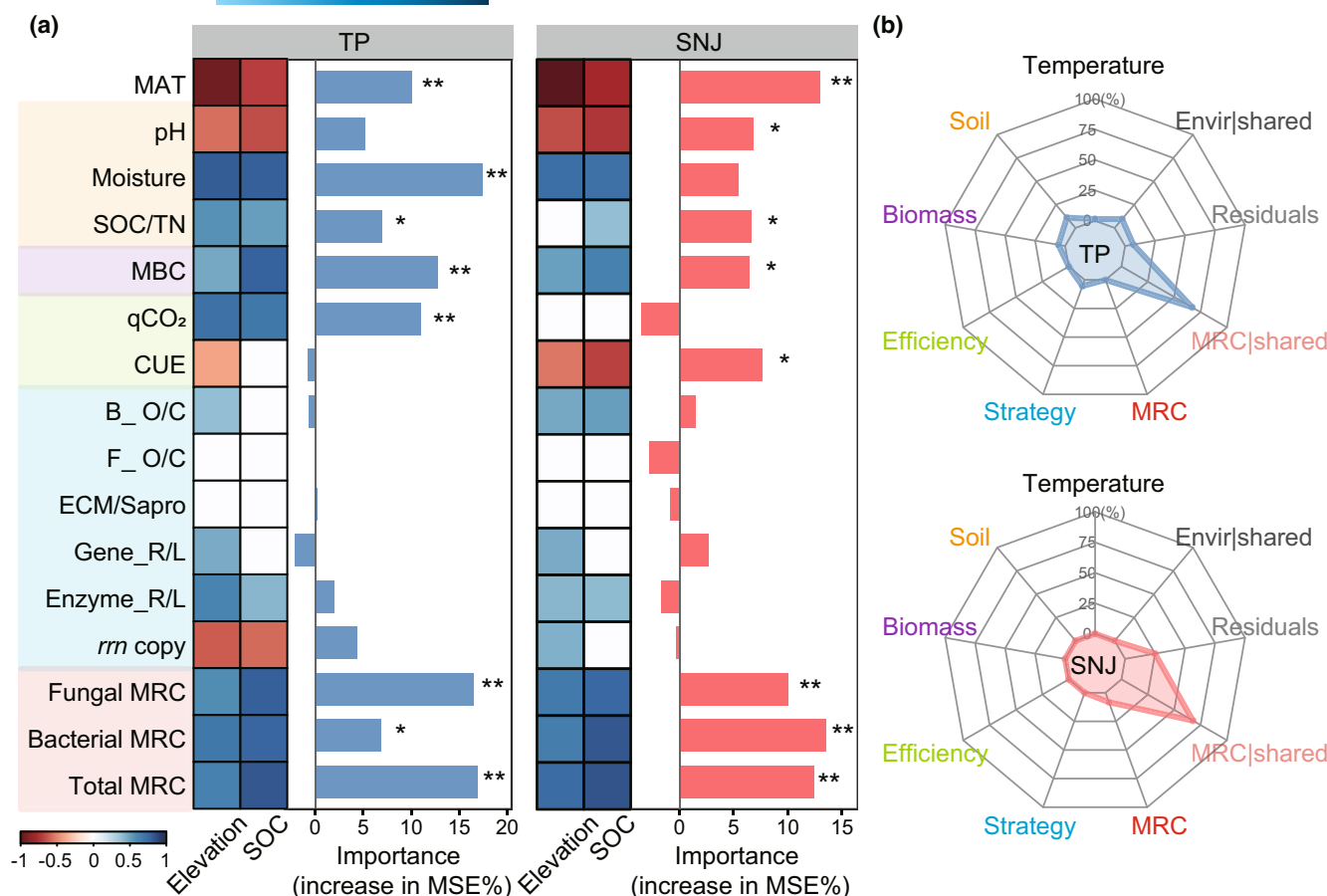


FIGURE 4 Major predictors of soil organic carbon (SOC) along the mean annual temperature (MAT) gradients of the Tibetan Plateau (TP) and Shennongjia Mountains (SNJ). (a) Spearman correlations between elevation, SOC, and environmental variables and the percentage increases in mean squared error (%IncMSE) of SOC evaluated by Random Forest analysis; (b) the unique and shared portions of variation in SOC explained by temperature, soil properties (soil), microbial biomass (biomass), metabolic efficiency (efficiency), microbial life-style strategies (strategy), and MRC based on variation partitioning analysis. The percentage variance of SOC explained by these variable groups are indicated by the points falling on the radial lines. Environment | shared (Envir | shared) refers to the percent of shared variation in SOC explained by temperature, soil, biomass, efficiency, and strategy. MRC | shared refers to the percent of the shared variation in SOC explained by temperature, soil, biomass, efficiency, strategy, and MRC. CUE, carbon use efficiency; ECM/Sapro, ectomycorrhizal/saprotrophic fungi ratio; F₋O/C, fungal oligotroph/copiotroph ratio; MAT, mean annual temperature; MBC, microbial biomass carbon; qCO₂, metabolic quotient; *rrn* copy, the weighted average rRNA operon copy number of bacterial community. **p* < .05; ***p* < .01. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16347)]

minor, portion of variation in MRC (Shao et al., 2021). We observed that the MRC was negatively associated with the prevalence of microbial communities with r-strategy, characterized by low bacterial oligotroph/copiotroph ratios and ECM/saprotrophic fungi ratios, and also low ratios of recalcitrant/labile C degradation genes and enzyme activities, with a high *rrn* operon copy number (Li et al., 2021). These associations were also supported by previous views that r-strategists (i.e., copiotrophs) with low substrate use efficiency could stimulate SOC turnover and subsequent C loss by mineralizing soil C to CO₂ (Luo et al., 2020; Saifuddin et al., 2019). In contrast, recent studies have documented that copiotrophs typically have faster growth and turnover rates, which may result in the greater residue yields. The opposite is expected for oligotrophs, which may have lower residues production and accumulation due to the slower growth rates (Cotrufo et al., 2015; Shao et al., 2021; Wang, An, et al., 2021). However, MRC accumulation not only depends on the production of MRC but also on its decomposition

(Zhang, Deng, et al., 2021). Even with more residues production, the residues of r-strategists (e.g., bacterial MRC) could be more susceptible to degradation than those of K-strategists (e.g., fungal MRC) (Fernandez et al., 2016; Li et al., 2021; Ye et al., 2019), thus likely leading to the less MRC retention. Therefore, the decomposition of MRC could be more important in shaping patterns of MRC compared with its production across temperature gradients (Mou et al., 2021; Yuan et al., 2021). This point was supported by the negative relationship between metabolic efficiency (qCO₂ and CUE) and MRC across our study areas. Our study further exhibited the potential role of microbial life-history strategies and metabolic efficiency in regulating MRC accumulation. Despite the significant relationships with MRC, microbial traits are not major determinants of MRC across environmental gradients, after considering other key environmental factors.

Our results showed that soil properties are important factors associated with MRC and SOC, which is in line with a great body of

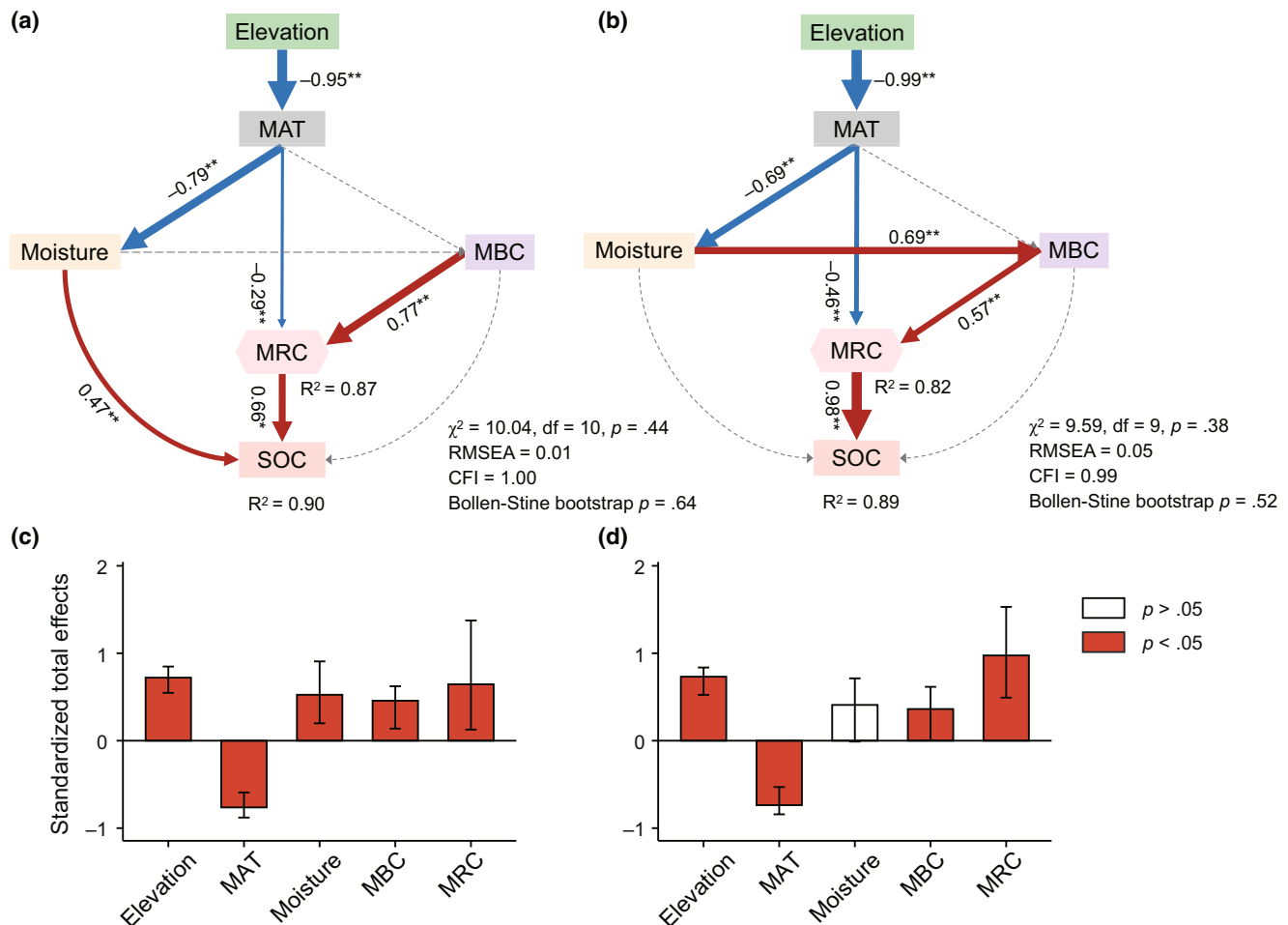


FIGURE 5 Direct and indirect associations between elevation, environmental variables, and soil organic carbon (SOC), as evaluated by the structural equation model (SEM). (a, b) The final SEM of the Tibetan Plateau (TP) and Shennongjia Mountains (SNJ), respectively; (c, d) the total standardized effects (sum of the direct and indirect effects, $STE \pm$ bootstrap confidence interval 95%) on SOC of the TP and SNJ, respectively. Microbial residue carbon (MRC) was used as a composite variable, including fungal and bacterial MRC. Red arrows indicate a positive relationship, whereas blue arrows indicate a negative relationship. Solid and dashed arrows indicate significant and insignificant relationships, respectively. The arrow width is proportional to the strength of the relationship. $*p < .05$; $**p < .01$. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16347)]

studies on C (Mou et al., 2021; Wang, An, et al., 2021). Importantly, we showed that soil pH and moisture were better predictors of MRC and SOC than microbial traits. Specifically, soil pH was closely related to the production and decomposition of MRC with a negative link to MRC. Previous studies found that high soil pH favored bacteria compared with fungi (Liu et al., 2021). Higher pH normally depresses the fungal growth and its metabolic activity, thus reducing the production of fungal MRC (Wang, An, et al., 2021). Additionally, higher pH could increase extant fungal MRC decomposition, because fungal chitin and soil protein decomposition were correlated with high soil pH (Hu et al., 2020). Given that fungal MRC contains higher C content and decomposes more slowly compared with bacterial MRC (Nakas & Klein, 1979; Strickland & Rousk, 2010), the decreased production and increased decomposition of fungal MRC would decline MRC accumulation with increasing MAT. Our study also suggests that soil moisture has a positive association with MRC mainly by altering MRC production. Previous studies showed that soil moisture can affect MRC by regulating the

size of living microbial biomass (Ni, Liao, Tan, Peng, et al., 2020; Wang, An, et al., 2021). In our study, we also observed the indirect association between soil moisture and MRC mainly through MBC in the SNJ, although the link was much weaker in the TP. This may be because warm conditions reduced soil moisture by strong evaporation in the SNJ, which possibly led to a decrease in production and accumulation of MRC due to the limited nutrient diffusion and aeration in soils (Liang & Balser, 2012; Liu et al., 2021). Moreover, we also found the direct effect of soil moisture on SOC in TP, indicating that the decomposition of SOC could be dependent on soil moisture other than temperature in high-alpine ecosystems (Curiel Yuste et al., 2007). As a whole, the increased soil pH and decreased moisture with increasing MAT (i.e., with decreasing elevation) reduced the accumulation of MRC, consequently a lower amount of SOC in regions with higher MAT.

We synthesize the present results into a simplified conceptual framework that portrays the effect of local temperature on SOC across environmental gradients (Figure 6). The MRC and its

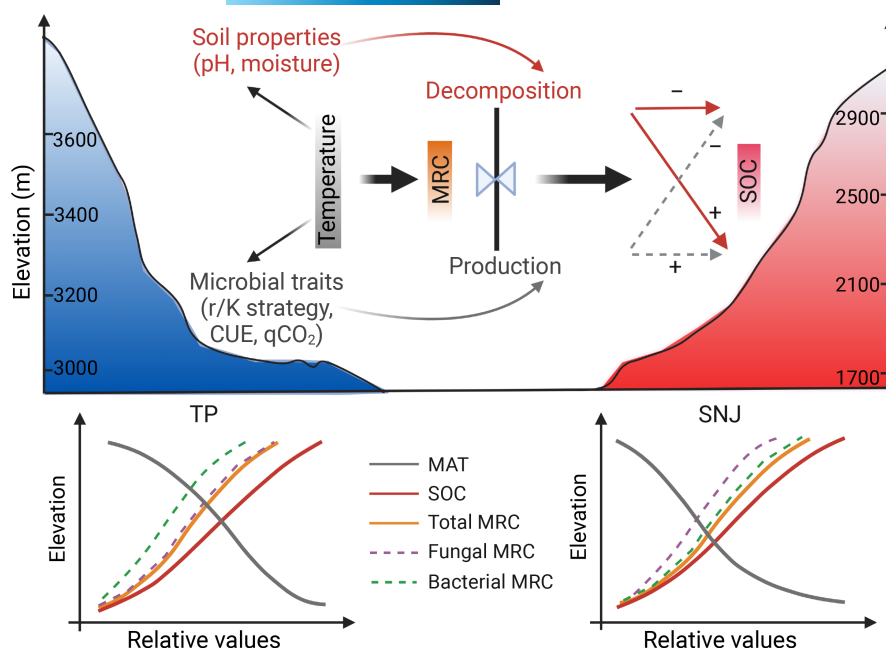


FIGURE 6 A conceptual diagram illustrating the potential mechanism of local temperature regulation on microbial residue carbon (MRC) and soil organic carbon (SOC) along the mean annual temperature (MAT) gradients of the Tibetan Plateau (TP) and Shennongjia Mountains (SNJ). The color gradient in the above figure reflects the changes in temperature, MRC and SOC content, and the darker colors indicate higher values. The symbols “+” and “−” represent positive and negative effects, respectively. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16347)]

contribution to SOC in two elevation gradients are dependent on temperature (Chen, Ma, et al., 2020; Ding et al., 2019). Local temperature increases may reduce the accumulation of MRC mainly by soil properties (e.g., soil pH and moisture) rather than microbial traits, resulting in decreased SOC with rising temperature. Furthermore, losses in SOC with local temperature increases might be associated with the increased decomposition of MRC, which is consistent with our second hypothesis. The MRC accumulation depends on the balance between microbial production and decomposition (Wang, An, et al., 2021). Elevated temperature could increase microbial growth and proliferation, thereby leading to increased input of MRC (Ding et al., 2019; Wang et al., 2020), as observed for the increased metabolic efficiency and the prevalence of r-strategist with increasing MAT. However, the temperature along our elevation gradient was strongly associated with the decomposition rather than the production of MRC with negative effects on MRC accumulation (García-Palacios et al., 2021; Liang & Balser, 2012; Mou et al., 2021). It has been reported that elevated temperature enriched microbial genes involved in degrading and modifying microbial cell walls and consequently promoted a highly active microbial community capable of rapid mineralization of MRC (Donhauser et al., 2021; Wang, An, et al., 2021). Accordingly, global warming may decrease SOC accumulation by increasing the decomposition rate of MRC associated with variation in soil properties, which has important implications for potential C loss under climate change scenarios. In comparison with the SNJ, the SOC in the TP is prone to loss via increasing MRC decomposition, as its higher sensitivity and vulnerability of both C cycling and SOC stability to climate change (Chen, Li, et al., 2020; Zhang, Jia, et al., 2021). The degree of responses of MRC and SOC to changing temperature may be different under contrasting elevation gradients and further work on the decomposition of MRC induced by temperature is worth pursuing.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad Digital Repository at <https://doi.org/10.5061/dryad.dncjsxm31>.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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