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Large-scale importance of microbial carbon use efficiency and necromass to soil organic carbon

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Abstract

Optimal methods for incorporating soil microbial mechanisms of carbon (C) cycling into Earth system models (ESMs) are still under debate. Specifically, whether soil microbial physiology parameters and residual materials are important to soil organic C (SOC) content is still unclear. Here, we explored the effects of biotic and abiotic factors on SOC content based on a survey of soils from 16 locations along a ~4000 km forest transect in eastern China, spanning a wide range of climate, soil conditions, and microbial communities. We found that SOC was highly correlated with soil microbial biomass C (MBC) and amino sugar (AS) concentration, an index of microbial necromass. Microbial C use efficiency (CUE) was significantly related to the variations in SOC along this national-scale transect. Furthermore, the effect of climatic and edaphic factors on SOC was mainly via their regulation on microbial physiological properties (CUE and MBC). We also found that regression models on explanation of SOC variations with microbial physiological parameters and AS performed better than the models without them. Our results provide the empirical linkages among climate, microbial characteristics, and SOC content at large scale and confirm the necessity of incorporating microbial biomass and necromass pools in ESMs under global change scenarios.

KEYWORDS
carbon use efficiency, microbial necromass, microbial physiological parameters, microbial turnover rate, modeling, soil carbon storage

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1 | INTRODUCTION

Soil organic carbon (SOC) is one of most important components of terrestrial carbon (C) cycle, not only because the size of SOC is larger than that in plants and the atmosphere combined (Jobbágy & Jackson, 2000), but also because SOC is very sensitive and vulnerable to climate changes, such as global warming (Crowther et al., 2016). It has been proven that enhancement of C sequestration in soil could help keep the global temperature rise below 1.5°C in 2100 (Huppmann et al., 2018). At present, the processes controlling the formation, transformation, and sequestration of C in soils are still not well understood and the simulation of SOC dynamics in Earth system models (ESMs) needs to be improved (Georgiou et al., 2017; Wieder et al., 2013).

The primary origin of SOC is photosynthetic primary production by vegetation while soil microorganisms are the primary agents of C mineralization, responsible for the loss rate of organic C from soil on the one hand, and the gain of organic C on the other hand via their residues (Crowther et al., 2019; Sokol & Bradford, 2018). The pool size of microbial biomass might affect the size of SOC negatively due to its positive effect on C decomposition (Wieder et al., 2013). However, microbes are also considered as contributors to nonliving organic C, somewhat paradoxically, because of the deposition of microbially derived C through biomass turnover and necromass production (Bradford et al., 2013; Liang et al., 2017; Miltner et al., 2012; Sokol & Bradford, 2018). In addition, SOC could be substrates for microbes and affects the size of microbial biomass positively. Therefore, a positive relationship between microbial biomass C (MBC) and SOC has often been found (Crowther et al., 2019; Xu et al., 2013), different from what Michaelis–Menten kinetics suggest. In order to better simulate SOC accumulation processes, the paradoxical role of microbial biomass in regulating SOC should be better differentiated and more clearly represented in the models.

The role of microorganisms in decomposing SOC has been extensively explored, but how they contribute to the long-term persistence of SOC is less studied. The emerging view suggests that SOC not only is the direct retention of plant debris in soil through selective preservation or humification process, but also includes the sequestration of microbial necromass (Liang et al., 2017; Miltner et al., 2012; Schmidt et al., 2011). Results from models indicated that the contribution of microbially derived necromass C to the formation of SOC could be from 10% to 80% (Fan et al., 2021; Liang et al., 2019; Simpson et al., 2007). Moreover, this C has been found to have different structural components and different decomposition patterns from plant-derived C (Simpson et al., 2007; Wang, Wang, Cotrufo, et al., 2020; Wang, Wang, Pei, et al., 2020). Therefore, the high contribution of microbial necromass to SOC could be due to their relative slow decomposition rate or the higher chance of mineral protection from decomposition by microorganisms or both (Hagerty et al., 2014; Li et al., 2018; Miltner et al., 2012; Wang, Wang, Cotrufo, et al., 2020; Wang, Wang, Pei, et al., 2020). In addition, microbial physiology parameters, such as biomass turnover rate, defined as the rate of microbial growth per unit microbial biomass, and C use efficiency (CUE) could indirectly impact SOC via their impacts on microbial biomass and necromass. Firstly, high microbial biomass turnover rate means fast necromass production and therefore potentially more microbially derived SOC (Hagerty et al., 2014; Spohn et al., 2016). If microbial necromass was readily recycled into microbial biomass and decomposed, it would not contribute significantly to the stable SOM pool. However, recent studies suggest that the decomposition rate of microbial necromass could be quite low once it is protected by soil minerals and oxides (Wang, Wang, Cotrufo, et al., 2020; Wang, Wang, Pei, et al., 2020), making it necessary to model microbial necromass as a separate pool from plant-derived SOC. Secondly, microbial CUE, defined as the proportion of substrate C that microorganisms assimilate for growth out of total uptake C, has been shown to be an important microbial physiology parameter to improve the performance of model prediction of SOC variations under global climate changes (Frey et al., 2013; Georgiou et al., 2017; Malik et al., 2018). Higher CUE generally means more production of microbial biomass and other microbial products when the total uptake of C remains constant, potentially beneficial for the storage of microbially derived C in soils (Cotrufo et al., 2013). Therefore, building the connections among microbial physiology parameters (i.e., CUE and turnover rate), biomass, and necromass will be helpful for understanding the microbially driven soil C cycles.

Abiotic factors, such as temperature and moisture, may influence microbial physiology parameters and then indirectly impact soil C cycling processes (Luo et al., 2017). However, previous studies have not reached a consensus on the relationship between climatic factors and microbial CUE or microbial turnover rate (Manzoni et al., 2012; Zheng et al., 2019) and studies at large spatial scales spanning a wide range of temperature and moisture are particularly scarce. The global spatial pattern in soil MBC is similar to that observed in SOC (Crowther et al., 2019; Xu et al., 2013), showing lower values in areas with higher temperature and moisture, with the reason still unclear. On the one hand, plant C inputs are generally higher in areas with higher temperature and moisture, beneficial for MBC; on the other hand, higher soil moisture and soil respiration due to higher temperature may cause lower level of oxygen, unfavorable to most microbes (Coskun et al., 2019). Understanding the response of microbial physiological parameters to climatic factors could help better explain the global patterns of MBC and SOC. To date, empirical relationships among climate, plant, soil, microbial physiological traits, and SOC formation are still lacking.

The objectives of this study were to establish qualitative relationships between climatic factors and microbial physiological parameters; and to test the correlations between SOC content and microbial physiological traits (i.e., microbial CUE and turnover) and microbial necromass. In addition, examination of these relationships at the large spatial scale should be deployed to generate more general results which could be helpful for building and evaluating the global Earth system modeling. To achieve these aims, we considered soil microbial processes and their controlling parameters simultaneously across a forest transect (~4000 km; Figure S1) and used random forest model, general linear model (GLM), and structural

equation modeling to assess the roles of climate, plant, soil, microbial physiological traits, and necromass in SOC content. We hypothesize that (1) MBC and necromass C had positive relationships with the SOC content across the large spatial scale; (2) soil microbial CUE decreased with mean annual temperature (MAT) and precipitation (MAP) of sampling sites, while microbial biomass turnover rate increased with MAT and MAP; both were important to the spatial variations of MBC and SOC; and (3) the regression model representing microbial physiological parameters could better explain the variations of SOC across the large spatial scale than the one without the microbial physiological parameters.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Soil samples were collected from 16 forest sites along a north–south transect in eastern China (Figure S1). These sites cover three different forest types: temperate forests (NWH, Nanweng River; BKT, Boke Tu; LS, Liangshui; ME, Maoer Mountain; CB, Changbai Mountain; QY, Qingyuan; DL, Dongling Mountain; TS, Tai Mountain), subtropical forests (BZ, Bazhong; JG, Jigong Mountain; XS, Xian Mountain; NP, Nanping; HT, Huitong; DH, Dinghu Mountain), and tropical forests (JFL-N, Jianfengling-natural forest; JFL-S, Jianfengling-secondary forest). MAT at the sampling sites ranges from −2.4°C at NWH to 20.9°C at DH and MAP ranges from 474 at BKT to 2449 mm at JFL. More detailed information for sampling sites can be found in Table S1. At each site, three 20 × 20 m large plots were randomly established with a distance of >10 m between each other within a 100 × 100 m sampling plot and five 2 × 2 m subplots were selected within each large plot (each corner and the center of the plot). The spatial geographical coordinates and elevation of each site were recorded by GPS (eTrex Venture, Garmin). In each 2 × 2 m subplot, five random soil samples (0–10 cm) were collected using a soil corer (2.5-cm diameter) and were homogenized by hand and pooled as one composite sample. The soil samples were collected by 16 groups at the same time from August 18 to 20, 2018 (finished within 2 days). All soil samples were then placed in a box with icebags (to keep the temperature inside between 2°C and 6°C) and shipped to the laboratory in the Institute of Applied Ecology, Chinese Academy of Sciences at Shenyang, China within 4 days. Fresh soil samples were sieved (<2 mm), with all visible roots being removed, and then soils were stored in a refrigerator under 4°C. The subsamples (the composite samples) from five subplots were mixed into a new composite sample and thus the replicate numbers for all analyses were 3 at each site. In the laboratory, one subsample (~50 g) from each replicate was incubated under 60% water holding capacity at the temperature equal to mean growing season temperature of the site where the sample was collected for microbial CUE and turnover analyses. And another subsample was air-dried to determine SOC content, amino sugar (AS) content, soil pH, and soil clay content. Since we were able to finish all the soil sampling and transportation within 1 week, the soil analysis and incubation experiments were done at one time for all the samples from all sites to minimize errors caused by human operation.

2.2 | Microbial traits analysis

After preincubation (24 h), soil microbial CUE and turnover time were determined using the ^{18}O-H_2O tracer method (Spohn et al., 2016). Briefly, duplicate aliquots of 600 mg of each preincubated soil were placed into 2 ml brown chromatographic vials. For one replicate, the ^{18}O-H_2O (97.0 at% ^{18}O) was added in order to adjust the ^{18}O content of soil water to 20.0 at% ^{18}O and another replicate was added the same volume of non-labeled water. Subsequently, the vials containing samples were transferred to 20 ml headspace bottles, capped, and flushed with CO_2-free air to obtain headspace concentrations of CO_2 to ~0 ppm. Three blank bottles without soils were prepared and processed through all steps to serve as negative controls for CO_2 concentration analysis. The samples were incubated for 24 h at the temperature equal to mean growing season temperature of the site where the sample was collected. After 24 h of incubation, 10 ml gas sample was collected from each bottle with a syringe and the CO_2 concentration was determined immediately using a gas chromatograph system (GC-7890B; Agilent Technologies). Then the brown vials containing soil were retrieved and capped, immediately frozen in a lyophilizer, and were subsequently stored at −80°C until DNA extraction. Total soil DNA was extracted using a DNA extraction kit (MoBio, Powersoil) following the manufacturer's procedures. The DNA concentration was determined by the Picogreen fluorescence assay (Quant-iT^{TM} PicoGreen dsDNA Reagent, Thermo Fisher) using a Microplate spectrophotometer (Infinite® M200, Tecan). The remaining DNA extract was dried in a silver capsule at 45°C for 5 h to remove any water. Subsequently, the ^{18}O abundance and the total O content were measured using an IRMS-TC/EA (Thermo Scientific). MBC was determined by the CH_3Cl fumigation extraction method and the extraction efficiency factor 0.45 was used for calculation.

Microbial growth rate, turnover rate, and CUE were calculated as per the method described by Spohn et al. (2016). Total dsDNA produced ($DNA_{produced}$, μg) during the 24-h incubation period was calculated according to the following equation:

$$DNA_{produced} = O_{total} * \frac{at\%_{excess}}{100} * \frac{100}{at\%_{final}} * \frac{100}{31.21}, \quad (1)$$

where O_{total} is the total O content (μg) of the dried DNA extract, $at\%_{excess}$ is the difference between at% ^{18}O of the labeled sample and at% ^{18}O of the non-labeled sample, and 31.21 is the average percentage of O in DNA ($C_{39}H_{44}O_{24}N_{15}P_4$). The $at\%_{final}$ is the ^{18}O at% of soil water at the beginning of incubation (20.0% in this study). In addition, an assumption that O in new DNA only derived from water was made (Qu et al., 2020). Because of the short incubation time, the mortality of newly produced ^{18}O-labeled microbial cells is negligible in the experiments.

A conversion factor (f_{DNA}) was calculated at each specific sample to represent the ratio of soil MBC to soil DNA content (μg g^{-1} soil). Microbial growth rate (Growth, ng C g^{-1} soil h^{-1}) was calculated by multiplying the DNA production rate and f_{DNA}:

$$\text{Growth} = \frac{f_{DNA} * \text{DNA}_{produced} * 1000}{\text{DW} * t}, \quad (2)$$

where DW (g) is the dry weight of soil and t is the incubation time (h). Additionally, microbial basal respiration rate (Respiration, ng C g^{-1} soil h^{-1}) was calculated by the following equation:

$$\text{Respiration} = \frac{R_s}{\text{DW} * t} * \frac{p * n}{R * T} * V * 1000, \quad (3)$$

where p is the atmosphere pressure (kPa), n is the molecular mass of the element C (12.01 g mol^{-1}), R is the ideal gas constant (8.314 J mol^{-1} K^{-1}), and T is the absolute temperature of the gas (295.15 K). V is the headspace volume (L) of the vials. R_s (ppm) is the amount of CO_2 concentration produced during the 24-h incubation period.

Microbial CUE and microbial biomass turnover rate (τ, $year^{-1}$) were calculated by the following equations (Spohn et al., 2016):

$$\text{CUE} = \frac{\text{C}_{growth}}{\text{C}_{growth} + \text{Respiration}}, \quad (4)$$

$$\tau = \frac{\text{DNA}_{produced} * 24}{\text{DNA}_{content} * t} * 365, \quad (5)$$

where $\text{DNA}_{content}$ (μg) is the DNA content in each soil, and t is incubation time in hours.

2.3 | Measurement of AS

We used ASC in soil to indicate the soil microbial necromass C (MNC; Joergensen, 2018). ASs, including glucosamine (GluN), galactosamine (GalN), mannosamine (ManN), and muramic acid (MurA), were determined according to the protocol of Zhang and Amelung (1996). Briefly, soil samples (containing about 0.3 mg N) were hydrolyzed with 6 M HCl (10 ml) for 8 h, and the solution was subsequently filtered, centrifuged, and freeze-dried. Methanol was then added to the freeze-dried supernatants and centrifuged to extract ASs from the residues. The ASs were reacted with hydroxylamine hydrochloride and 4-(dimethylamino) pyridine and acetic anhydride, and the AS derivatives were separated on a DB-5MS column (30 m × 0.25 mm × 0.25 mm) with temperature program set by Zhang and Amelung (1996). Separation of AS derivatives was carried out on an Agilent 6890 GC (Agilent Technologies) equipped with a Scientific Ultra-2 column (25 m × 0.2 mm × 0.33 μm) and flame ionization detector. The individual AS derivatives were separated by referencing the retention times of authentic standards containing GluN, GalN, ManN, and MurA. We normalized AS-C contents by their molecular mass and calculated total soil AS-C as the sum of GluN-C, GalN-C, ManN, and MurA-C (Joergensen, 2018).

2.4 | Soil properties

Soil pH was measured at a ratio of fresh soil to water ratio of 1:2.5 (soil:water) by a pH electrode (Leici). SOC and total nitrogen content of air-dried soils were measured with an elemental analyzer after being ground by a ball mill (MAT-253; Thermo Fisher Scientific). Contents of clay and silt of air-dried bulk soil were determined by the hydrometer method.

2.5 | Climate and plant biomass

The MAP and MAT of each sampling site were extracted from maps of global temperature and precipitation provided by WorldClim database (www.worldclim.org). The plant aboveground standing biomass C (AGBC, Mg C ha^{-1}) and belowground standing biomass C (BGBC, Mg C ha^{-1}) for each site were extracted from maps that were developed by Spawn et al. (2020). These data are accessible through the Oak Ridge National Laboratory (ORNL) DAAC data repository (https://doi.org/10.3334/ORNLDAAC/1763). Finally, all maps were imported into ArcGIS (version 10.2, ESRI) and then the values of climate variables and plant biomass for each site were extracted using coordinates (latitude and longitude) of our sampling sites.

2.6 | Statistical analyses

First, we explored the relationships between SOC and C pools (MBC, MNC, AGBC, and BGBC), climate variables (MAP and MAT), soil variables (soil pH and clay), and microbial physiological variables (CUE and τ). Second, we conducted a random forest analysis to identify the relative importance of a given variable compared with other variables. This analysis was run using the *randomForest* (https://www.stat.berkeley.edu/~breiman/RandomForests) and *rfPermute* (https://github.com/EricArcher/rfPermute) packages in R 3.5.3 (R Core Team, 2019). Third, we used GLM to examine if including microbial physiological variables (CUE, τ, and MBC) and MNC could better explain the variations of SOC than the model without them. To do this, we built three models with different numbers of SOC response variables: model A included all predictors; model B included all except MNC; and model C included all except for microbial physiological variables (CUE, τ, and MBC) and MNC. The Akaike information criterion (AIC) was used to select the best model and the lower AIC means a better-fit model. This analysis was run using the *MuMIn* package (version 1.43.15, https://cran.r-project.org/web/packages/MuMIn/index.html). Finally, we used structural equation modeling (SEM) to evaluate the direct and indirect effects of MAT, BGBC, soil clay, and microbial physiological variables (MBC, CUE, and τ) and necromass on SOC. We started the SEM procedure with

the specification of a conceptual model of hypothetical relationships, based on a priori and theoretical knowledge (Figure S2). In the SEM analysis, we compared the model-implied variance–covariance matrix against the observed variance–covariance matrix. Data were fitted to the models using both the maximum-likelihood estimation method and the Bayesian analysis. For simplicity, the least significant path was deleted and the model was re-estimated; then the next least significant path was removed, and so on, until the paths that remained in the final SEM were all significant. Model fit statistics include the degree of freedom (*df*), ratio of chi-square and *df* (χ^2/df), probability level (*p*), R^2 (proportion of variance explained), and comparative fit index. The SEM analysis was conducted in the environment of Amos 20.0 (Amos Development Company).

3 | RESULTS

3.1 | SOC across large spatial scales

For soils sampled from 16 spatially dispersed forest sites across eastern China (Figure S1; Table S1), the SOC content generally decreased from northern sites to southern sites, with a range of 18.3–108.7 mg C g^{-1} soil and an average of 43.6 mg C g^{-1} soil (Table S2). Similarly, soil MBC and ASC showed a declining pattern from north to south with a range from 0.11 to 3.78 mg C g^{-1} soil and from 0.38 to 2.31 mg C g^{-1} soil, respectively (Table S2).

Across all sites, MBC and AS were significantly positively correlated with SOC (Figure 1a,b). After removing the two points with the highest SOC values, the correlation between AS and SOC got less strong (R^2 = 0.64 vs. R^2 = 0.90 with the two samples), but still indicating the robust correlation between the two parameters. A marginal positive correlation between belowground biomass C (BGBC) and SOC was observed (Figure 1d; R^2 = 0.19, p = 0.09), but aboveground biomass C (AGBC) was not correlated with SOC (Figure 1c). There were significant negative relationships between SOC and MAP (Figure 2a) and MAT (Figure 2b), while positive relationships between SOC and soil clay content (Figure 2d) and microbial CUE (Figure 2e) were observed.

3.2 | Responses of microbial physiological variables to climatic and edaphic factors

Regression analyses showed that microbial CUE was negatively correlated with MAP (Figure 3a; R^2 = 0.38, p < 0.01) and MAT (Figure 3b; R^2 = 0.52, p < 0.01), but was marginally positively correlated with soil pH (Figure 3c; R^2 = 0.21, p = 0.07). In addition, microbial turnover rate (τ) increased with soil pH (Figure 3g; R^2 = 0.31, p < 0.05) and decreased with clay content (Figure 3h; R^2 = 0.33, p < 0.01).

3.3 | SOC models representing MBC and MNC pools

The results from random forest analysis suggested that AS and MBC were the two most important predictors of SOC variations (Figure S3). In addition, climate (MAT and MAP) and microbial CUE were selected as the significant drivers of SOC (Figure S3). Further, GLMs were adapted to assess the importance of microbial physiological variable (MBC, CUE, and τ) and AS as predictors of SOC (Table S3). Results indicated that the full model with all variables had the highest explanations for SOC (the lowest AIC value)

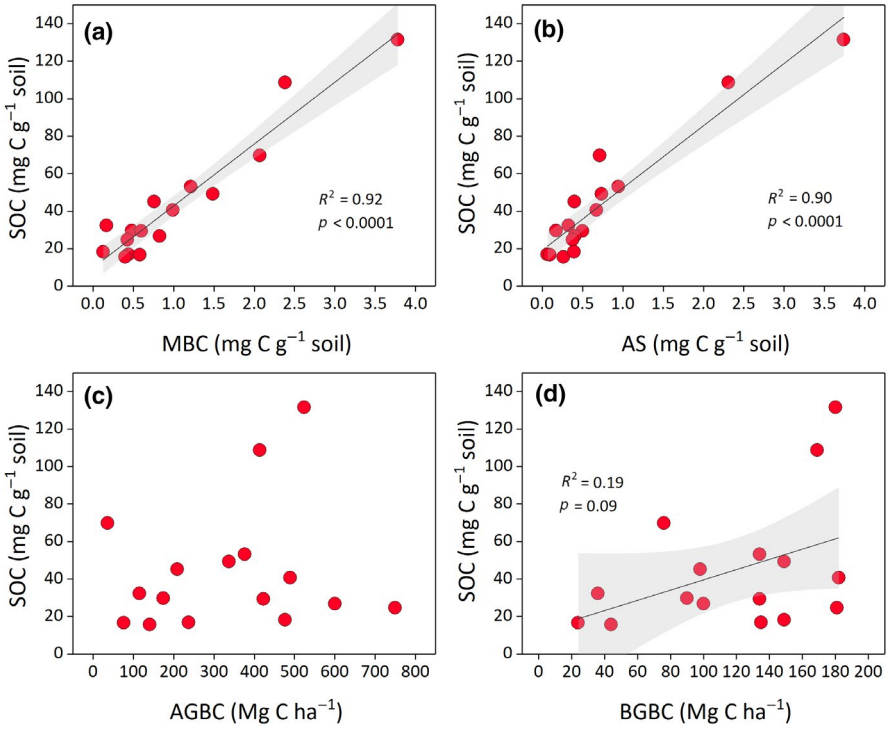

FIGURE 1 Relationships between soil organic carbon (SOC) and those of microbial and plant pools along a forest transect. (a) SOC and microbial biomass C (MBC), (b) SOC and soil amino sugar (AS), (c) SOC and standing plant aboveground standing biomass C (AGBC), and (d) SOC and standing plant belowground standing biomass C (BGBC) [Colour figure can be viewed at wileyonlinelibrary.com]

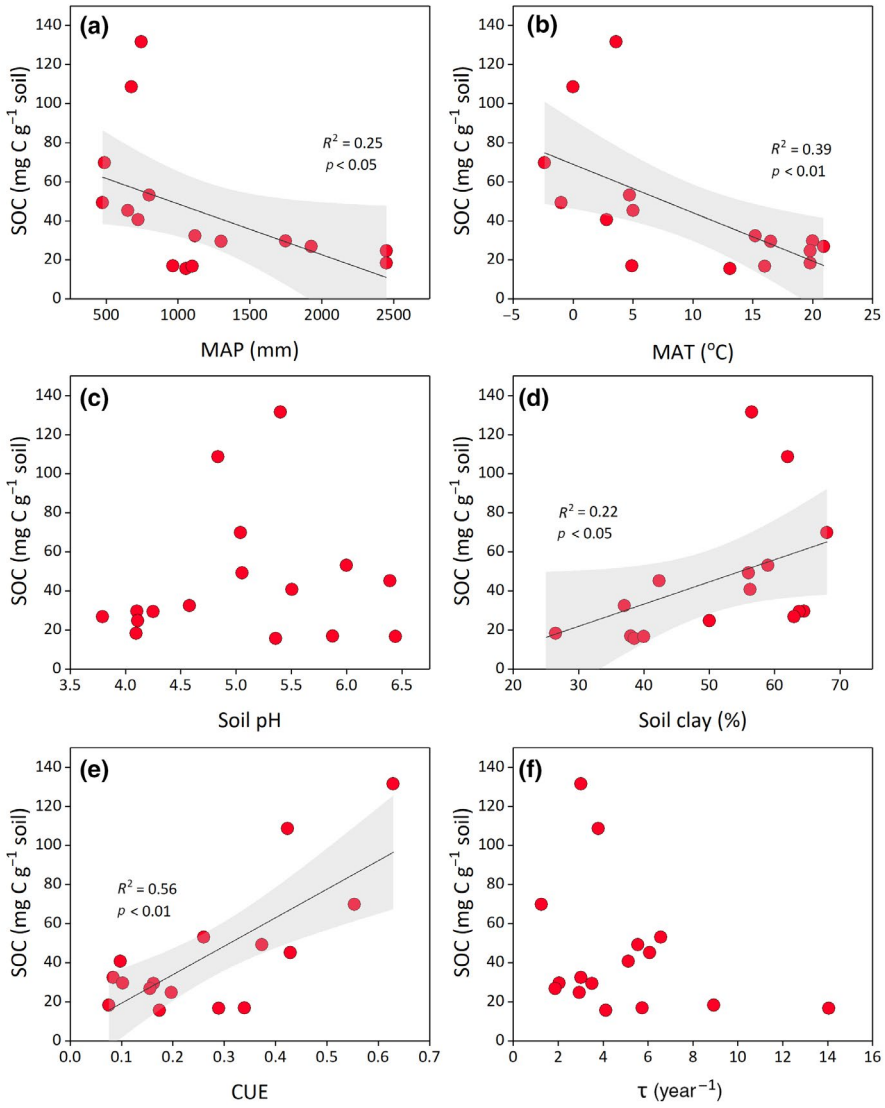

FIGURE 2 Relationships between soil organic carbon (SOC) and climatic factors, soil variables, and microbial variables. Linear regression is tested between (a) mean annual precipitation (MAP), (b) mean annual temperature (MAT), (c) soil pH, (d) soil clay, (e) microbial carbon use efficiency (CUE), and (f) microbial turnover rate (τ) along the large-scale gradient in Chinese forests and only significant relationships ($p < 0.05$) are shown with solid lines [Colour figure can be viewed at wileyonlinelibrary.com]

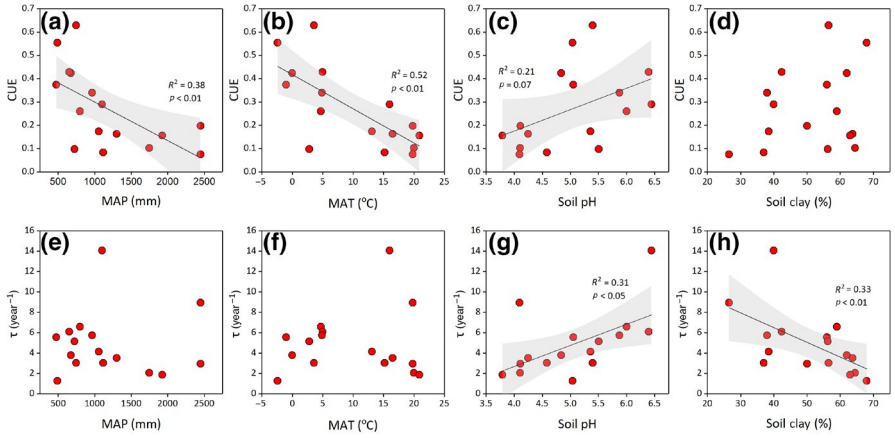

FIGURE 3 Relationships between climatic and soil variables and microbial physiological variables. Linear regression is tested between (a) mean annual precipitation (MAP) and microbial carbon use efficiency (CUE), (b) mean annual temperature (MAT) and CUE, (c) soil pH and CUE, (d) soil clay and CUE, (e) MAP and biomass turnover rate (τ), (f) MAT and τ, (g) soil pH and τ, (h) soil clay and τ along the large-scale gradient in Chinese forests [Colour figure can be viewed at wileyonlinelibrary.com]

and the exclusion of AS (model B), and the exclusion of both AS and microbial physiological variable (model C) would decline the prediction power of models.

We further used SEM to explore the direct and indirect roles of different variables in SOC storage (Figure 4). The SEM estimated by using both maximum-likelihood estimation method and Bayesian analysis showed that ~93% of variations in SOC could be explained by the selected variables (Figure 4a; Table S4). Specifically, MBC and AS were directly associated with SOC, while the influences of climate, plant biomass, and soil variables on SOC were mediated through microbial CUE and MBC (Figure 4). Consistent with the results from random forest analysis and GLMs, MBC, microbial

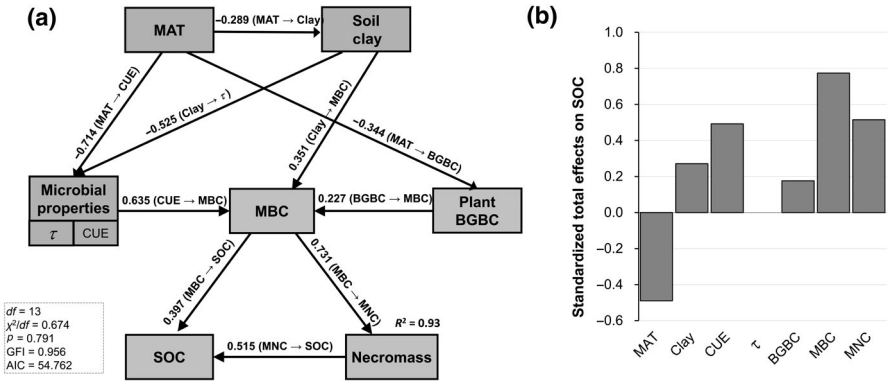

FIGURE 4 Structural equation modeling (SEM) for the effects of the response variables on the variation of soil organic carbon. (a) Parameters between the designed path in SEM were estimated by the maximum-likelihood estimation method. Microbial properties include microbial carbon use efficiency (CUE) and microbial turnover rate (τ, year^{-1}). Carbon pools in this SEM include microbial biomass carbon (MBC, mg C g^{-1} soil), microbial necromass C (MNC, it was indexed by amino sugar content in soil, mg C g^{-1} soil), and soil organic carbon (SOC, mg C g^{-1} soil). The plant carbon pool only includes belowground biomass carbon (BGBC, Mg C ha^{-1}). Numbers adjacent to arrows are standardized path coefficients, analogous to relative regression weights, and indicative of effect size of the relationship. Information about a priori model is provided in Figure S2. (b) Total standardized effects of each variable on SOC from SEM

necromass, microbial CUE, and MAT could be the major predictors of SOC along this national-scale forest transect (Figure 4b).

4 | DISCUSSION

4.1 | Importance of microbial parameters to SOC

This study is the first attempt, to the best of our knowledge, to demonstrate the national-scale links among key microbial physiological traits (MBC, CUE, and τ), microbial necromass, environmental variables, and SOC content. In agreement with our first hypothesis that microbial necromass is one of the important precursors of soil organic matter (Bradford et al., 2013; Cotrufo et al., 2013; Liang et al., 2017; Schmidt et al., 2011), our study provides empirical evidence of the positive relationship between microbial necromass index (content of AS) and the content of SOC across sites spanning a large gradient of climatic and edaphic factors, although this correlation could be a little weaker when removing two samples with the highest SOC values (Figure 1). Besides, the effects of climatic and edaphic factors on SOC were mainly via their regulation on the size of MBC and MNC pools, instead of direct effects (Figure 4), confirming the necessity of considering these pools in SOC modeling (Fan et al., 2021). Microbes should be considered not only as a controlling factor of the consumption of SOC in ESMs (Todd-Brown et al., 2013), but also as an influencing factor of the production of SOC. We believe the best way to represent this paradoxical role of microbes is to establish the MNC pool and link it to MBC and SOC via microbial turnover rate (τ) and CUE (Frey et al., 2013; Georgiou et al., 2017; Hagerty et al., 2014). GLM analyses suggested that the regression modeling including microbial physiological variables (MBC, CUE, and τ) had higher prediction power than other ones without these variables (Table S3). As researchers pay more attention to microbial necromass, data on soil AS content have increased dramatically in the past 5 years (Joergensen, 2018). We believe as we understand microbial necromass more and more, incorporation of the microbial necromass pool into the ESMs will become possible. Overall, our results indicate that incorporation of both the "microbial biomass" and the "microbial necromass" pools into ESMs and consideration of their relationships with SOC as the ones demonstrated in our SEM (Figure 4) should greatly improve the prediction of global variations of SOC under the context of changing climate.

4.2 | Variation of microbial CUE and turnover rate across the large spatial scale

In order to represent MBC and MNC pools in ESMs, we should know the relationships between environmental factors and microbial turnover rate (τ) and CUE. Our study provides empirical relationships among environmental factors and these microbial physiological parameters, which can facilitate future model development. Empirical observations of microbial CUE across large environmental gradients in soils are scarce (Manzoni et al., 2012). We used the ^{18}O incubation approach and found the averaged CUE along this forest transect was 0.27, which is at the lower end range of CUE values of a global synthesis (Qiao et al., 2019; Zheng et al., 2019). The ^{18}O incubation approach has been found to have lower estimation of CUE than other approaches due to its consideration of the whole microbial community and all substrates (Geyer et al., 2019).

We found microbial CUE decreased with increasing MAP or MAT from northern sites to southern sites (Figure 2), consistent with our second hypothesis. This finding indicates that microbes allocate more C to the respiration process and less C to biomass formation, consequently a lower amount of microbial residual and SOC formation in regions with higher MAP or MAT. This was probably because the maintenance energy cost of microbes increased with increasing temperature and precipitation (Dijkstra et al., 2011).

The differences in substrate composition (Tian et al., 2018) and microbial community composition among sites (Xia et al., 2016) may also contribute to the decline of CUE with increasing MAP or MAT (Frey et al., 2013). Our finding of lower CUE in subtropical areas also explains why subtropical areas were found to have lower MBC despite the higher C inputs compared to temperate areas (Xu et al., 2013). Although soil pH was previously suggested as an important factor controlling microbial CUE (Malik et al., 2018; Manzoni et al., 2012), its relationship with CUE was weak in our studied soils (p = 0.07; Figure 3), while clay content did not really influence the variation of microbial CUE (Figure 3). Soil pH and clay content instead seemed to be better predictors of microbial turnover rate than MAT and MAP (Figure 3). With the increase in soil pH, microbial turnover rate significantly increased, probably because of the improvement in the living environment as previous studies found soil pH was the most important controlling factor of microbial community composition and activity (Fierer & Jackson, 2006; Malik et al., 2018). Similarly, as clay content increased, microbial turnover rate significantly decreased, probably due to the limitation of movement and accessibility to resources (Dungait et al., 2012). It seemed that microbial turnover rate had optimum MAP and MAT above or below which this rate would decrease (Figure 3). Besides, it should be noted that the soil samples in this study were collected only in the growing season. The microbial CUE and turnover rate may be different in the nongrowing season and further work on temporal variations of these variables is worth pursuing.

4.3 | Implication for future model development

Plants provide the primary C source of SOM via aboveground litter and belowground roots (Liang et al., 2017; Sokol & Bradford, 2018). Our results indicated that plant belowground C biomass was more strongly correlated with SOC than aboveground C biomass (Figures 1 and 4), which is consistent with previous findings (Chen et al., 2018; Miao et al., 2019). In areas with high MAT and MAP, aboveground litter is quickly decomposed and the remaining biomass might not reflect the actual C input to SOC. However, we found net primary productivity was not correlated with SOC either (Figure S4) due to the impacts of decomposition. Previous studies also suggest that belowground rhizosphere dissolved organic C entry may be more efficient than aboveground entry, due to the direct entry of C into the soil (Kallenbach et al., 2016; Sokol & Bradford, 2018). However, it should be noted that the plant biomass data used in this study were derived from modeling with a ~300 m spatial resolution, which may be incompatible with the SOC data from field sampling. More studies on the linkage between plant biomass and SOC content are needed. Nevertheless, our study re-emphasizes the necessity to separately model aboveground C inputs and belowground C inputs (Kallenbach et al., 2016; Sokol & Bradford, 2018).

In order to represent MBC and MNC pools in SEM as proposed by us (Figure 4), another important parameter besides CUE and τ needs to be studied more, that is, the decomposition rate of microbial necromass. Because microbial necromass may be more tightly bound in the soil matrix than plant debris due to the nature of microbes living on particle surfaces (Cotrufo et al., 2013; Schmidt et al., 2011), necromass C may be highly protected by soil minerals. This might explain the positive relationship between clay content and soil AS content (Table S5). However, very few studies have quantified the decomposition rate of microbial necromass and its temperature sensitivity (Wang, Wang, Cotrufo, et al., 2020; Wang, Wang, Pei, et al., 2020). Future research is needed to study this parameter and its response to environmental factors in order to better incorporate the microbial necromass pool in ESMs. In addition, whether the use efficiency of MNC by microorganisms is equal to that of plant debris or SOM is unclear and should be studied further in the future.

5 | CONCLUSION

Our results supported a more nuanced perspective on SOC production and storage underpinning the importance of microbial necromass pool to SOC storage across a broad spatial and climatic scale (Figure 4). We provided empirical relationships among major climatic factors, edaphic factors, and microbial CUE and turnover rate, which can facilitate future model development. The better performance of GLM with microbial physiological parameters than the models without microbial physiological parameters (Table S3) calls for our attention to the properties of microbial necromass under the changing environment. Our study provides a platform for developing a new model representing microbial necromass and for enhancing our understanding on microbial controls on soil C cycling.

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AUTHOR CONTRIBUTIONS

Chao Wang and Edith Bai designed the study. Chao Wang designed the field study and coordinated all field and laboratory operations. Laboratory analyses were done by Lingrui Qu, Chao Wang, Liuming Yang, Dongwei Liu, Renhui Miao, and Ziping Liu. Data analysis was conducted by Chao Wang, Lingrui Qu, and Edith Bai. The paper was written by Chao Wang, Lingrui Qu, and Edith Bai, and the rest of the co-authors contributed to its improvement.

DATA AVAILABILITY STATEMENT

All data are available in the main text or the Supporting Information and raw data are available upon request from the corresponding author. Further reference and link to public data sources are given in Section 2.

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SUPPORTING INFORMATION

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