

# The continent-scale variations in soil microbial respiration in forest ecosystems: diverged pattern and mechanism

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## Abstract

Globally rising soil microbial respiration (Rm) is a key process controlling the soil-to-atmosphere CO<sub>2</sub> flux, yet its spatial variation and underlying mechanism at different scales is still poorly understood. A novel experiment based on the annual mean temperature of soil origin sites along a 4,200 km north-south transect of China forests revealed a hump-shaped relationship between Rm and latitude with a latitudinal threshold of 32.5°N. Microbial variables were more important in shaping Rm's spatial variation at the continental scale than at the regional scales, but soil physicochemical property had comparably unique importance at different scales. Labile organic C was the most important factor in regulating the Rm's variation at the continent and in the latitude > 32.5°N region, but fungi biomass was the most important factor in the latitude < 32.5°N region. Overall, our findings suggest different controlling factors of Rm's variations on either side of the latitudinal threshold.

## Title page

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### **Abstract :**

Globally rising soil microbial respiration (Rm) is a key process controlling the soil-to-atmosphere CO<sub>2</sub> flux, yet its spatial variation and underlying mechanism at different scales is still poorly understood. A novel experiment based on the annual mean temperature of soil origin sites along a 4,200 km north-south transect of China forests revealed a hump-shaped relationship between Rm and latitude with a latitudinal threshold of 32.5°N. Microbial variables were more important in shaping Rm's spatial variation at the continental scale than at the regional scales, but soil physicochemical property had comparably unique importance at different scales. Labile organic C was the most important factor in regulating the Rm's variation at the continent and in the latitude > 32.5°N region, but fungi biomass was the most important factor in the latitude < 32.5°N region. Overall, our findings suggest different controlling factors of Rm's variations on either side of the latitudinal threshold.

**Keywords:** heterotrophic respiration, soil organic carbon decomposition, spatial pattern, latitudinal threshold, labile organic carbon, soil microbial community

### **Introduction**

Rising soil microbial respiration (Rm) is an important process that controls CO<sub>2</sub> flux from terrestrial soil to the atmosphere and soil organic carbon (SOC) dynamics (Bond-Lamberty & Thomson, 2010; Chen et al., 2015), and is expected to accelerate under ongoing climate warming (Bond-Lamberty et al., 2018). Given that globally Rm is the second largest C efflux in terrestrial ecosystems (IPCC, 2017), a small change in Rm rate will have the potential to greatly affect atmospheric CO<sub>2</sub> concentration and then modify feedbacks between SOC dynamics and global change (Bond-Lamberty et al., 2018; Rodeghiero and Cescatti, 2005). Therefore, accurately predicting Rm rate is urgently needed for projecting atmospheric CO<sub>2</sub> concentration and reducing the uncertainty in the prediction of SOC dynamics by carbon-climate models (Schmidt, 2011). Despite the importance of Rm in global C cycling, our understanding of the spatial variation in Rm and its shaping mechanism at the continental scale is still quite limited.

Soil Rm is primarily controlled by abiotic and biotic factors, including climate (Karhu et al., 2014), soil physicochemical properties (Delgado-Baquerizo et al., 2016a; Wang et al., 2017) and microbial characteristics (Dacal et al., 2019; Takur et al., 2018). These factors will inevitably affect the spatial variation of Rm at

various scales (e.g., Ali et al., 2018; Meyer et al., 2017; Ngao et al., 2012). However, what the importance of these factors in shaping Rm's spatial variation remains unclear, although these previous studies significantly advanced our understanding of Rm variation and its influencing factors. Furthermore, traditional incubation methods for measuring Rm in previous studies have been questioned because the incubation temperature differs from the ambient soil temperature, with a result that reported Rm is less representative for soil microbial activity (Xu et al., 2017). More importantly, in most of previous studies soils from various sites with different mean annual temperature (MAT) were incubated at the same temperature, resulting in inaccurate estimation of Rm because Rm generally increases with increasing temperature (Hamdi et al., 2013; Liu et al., 2018a). In addition, most of these previous studies focusing on the spatial variation in Rm only at the small (e.g., stand, ecosystem or regional) scales resulted in difficulty scaling up to the continent, even global scale. Therefore, it is urgent to provide a clear picture of spatial variation in Rm at the continental scale. In this study we will use a novel incubation experiment based on MAT of soil origin sites near to the ambient soil temperature to estimate Rm at the continent scale.

Growing studies have emphasized the significance of microbial community composition, activity and diversity (Dacal et al., 2019; Delgado-Baquerizo et al., 2016b; Takur et al., 2018) in driving Rm. A recent study noted that at the regional scale microbial biomass C was a significant variable in explaining basal soil respiration (Ali et al., 2018) because considerable proportion of Rm is controlled by the enzyme activities through mediating the rate-limiting step of SOC depolymerization (e.g., Kandeler et al., 2006). Given that bacteria have lower C use efficiency and prefer more labile organic matter than fungi (Lehmann & Kleber, 2015; Waring et al., 2013) and higher soil-C assimilation of gram-positive bacteria relative to gram-negative bacteria (Creamer et al., 2015), we assume that differences in microbial community composition (e.g., fungi:bacteria and gram-positive and -negative bacteria ratios) may have unique contribution in shaping the spatial variation in Rm. However, this assumption has not been verified, particularly at the continental scale. Furthermore, whether their relative importance in driving Rm's spatial variation varies among different scales remains largely explored.

Here, we conducted a novel incubation experiment using mean annual temperature (MAT) of each soil origin site for 238 soils along a 4,200 km north-south transect of forests in China to explore the spatial variation in Rm and its underlying mechanisms at different scales. The MAT of each soil origin site was first used as the incubation temperature of the corresponding soils to assess Rm's spatial variation. In order to identify the key influencing factors and quantify the relative importance of climate, soil physicochemical and microbial properties in shaping Rm's spatial variation, variance partitioning and boosted regression analyses have been performed. The objectives of this study were to explore the Rm's spatial variations in forest ecosystems along a latitudinal gradient at the continental and regional scales, and reveal their potential driving mechanisms. In this study, we hypothesized that Rm would linearly change with increasing latitude and climate (i.e., MAT and mean annual precipitation) would have important roles in shaping the Rm spatial variation. Given the metabolic activity and biomass of soil microorganisms generally increase toward warm, moist tropical regions (Crowther et al., 2019; Tedersoo et al., 2019), as well as that microbial property links closely with soil properties in more productive ecosystems (i.e., tropical forest) (Delgado-Baquerizo et al., 2019). We further hypothesized that there would be different mechanisms in explaining Rm's spatial variations in different regions.

## Materials and methods

### Study area

We collected 238 soil samples from 29 forest sites with a wide array of forest ecosystems (tropical, subtropical, temperate and boreal forests) across 3,800 km (Fig. 1). These sites span a latitudinal range from 18.7°N to 51.5°N and have distinct soil and site characteristics with the mean annual temperature (MAT) ranging from -5.2 °C to 24.4 °C and the mean annual precipitation (MAP) ranging from 468 mm to 1810 mm (Table S1). These forests were divided according to their dominant tree species into broadleaved ( $n = 86$ ), coniferous ( $n = 93$ ), and broadleaved-coniferous mixed forests ( $n = 59$ ). Forest was also categorized into four biomes according to latitudes of the study sites, including tropical, subtropical, temperate and boreal forests (e.g.,

Feng & Zhu, 2019). For each site, information relating to geography (latitude and longitude), climate (MAT and MAP), forest types, and soil chemical properties was obtained (Table S1).

### Field sampling and soil analysis

Soils were collected from each site from late July to early August, which is within the plant growing season. At each site, at least one typical forest was selected. In each forest with 3–5 plots, the upper 10 cm of mineral soil was sampled from 8–10 locations within a plot (100–150 m<sup>2</sup>), coarsely sieved to 5.6 mm to minimize disturbance, and gently combined to produce a composite sample. All soil samples were transported with a low-temperature incubator with ice (< 5 °C) to laboratory, where they were sieved to 2 mm, and then homogenized. One part was stored at 4 °C for incubation and microbial measurement, and the rest was air-dried for chemical determination.

In this experiment, the initial SOC and the total N contents were determined using an elemental analyzer. Labile organic C (LOC) which is oxidized by 333 mM potassium permanganate was measured according to the method described by Blair et al. (1995). Soil pH was measured with a pH meter from a soil slurry with 1:2.5 ratio by volume of soil to distilled water. The soil texture (sand, silt, and clay contents), mineral N, total and available P, and exchangeable cations (K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>) were determined according to the common methods described by Lu (2000).

Microbial biomass and community composition was determined through phospholipid fatty acid (PLFA) analysis described by Bardgett et al. (1996). Phospholipids were extracted from 1.5 g of soil fresh weight and analyzed using an Agilent 6890 Gas Chromatograph. Gram-positive bacteria were identified by the terminal and mid-chain branched fatty acids (i15:0, a15:0, i16:0, i17:0, a17:0), and cyclopropyl saturated and monosaturated fatty acids (16:1 $\omega$ 7c, cy-17:0, 18:1 $\omega$ 7c, 8cy-19:0) were considered indicative of gram-negative bacteria (Rinnan & Bååth, 2009). Fungi were the sum of 18:1 $\omega$ 9c, 18:1 $\omega$ 9t, and 18:2 $\omega$ 9,12c, and actinomycetes were the sum of 10Me16:0, 10Me17:0 and 10Me18:0 (Moore-Kucera & Dick, 2008). The total PLFA concentration was calculated from the identified PLFAs (15:0, 14:0, 16:1, 16:1 $\omega$ 5, 16:0, 17:1 $\omega$ 8, 7Me-17:0, br17:0, br18:0, 18:1 $\omega$ 5, 18:0, 19:1; and those listed above). The ratios of fungal to bacterial (F:B) PLFA and gram-positive to gram-negative (GP:GN) PLFA were taken to represent the relative abundance metrics of these groups.

### Microbial respiration measurement

The fresh soil samples from all forests for incubation were adjusted to 60% of water holding capacity and pre-incubated for 10-day at 20 °C to activate microorganisms and minimize the “pulse effect”. After equilibration, approximately 100 g (dried weight) of soil was placed into 500-ml rectangular plastic containers. These containers had pierced lids that enabled gas exchange, but minimized evaporation and soil drying. Soils were incubated for 72 hours at the corresponding MAT of their origin sites. When MAT of soil origin sites was lower than 10 °C, soils were incubated at 10 °C. To measure R<sub>m</sub>, we connected each 500-ml rectangular soil container (without the lid) to an infrared gas analyzer (Li-Cor 820) in a close-loop configuration. The respired CO<sub>2</sub> was measured by the Li-Cor 820 at every 24 hours. During incubation, all soil samples were maintained with 60% of water holding capacity by regularly weighing the soil containers and adding distilled water to compensate for water loss.

### Statistical analysis

The Kolmogorov–Smirnov test was performed to determine whether all data were normal. Non-normal variables were log-transformed before the analyses. The significant differences of R<sub>m</sub> among different biome zones and forest types were tested by the least significant difference (LSD) multiple-comparison post hoc test after the one-way analysis of variance. Spearman’s correlation analyses were performed to assess the relationships between important variables of soil properties and R<sub>m</sub>. The above statistical analyses were performed using the SPSS software (version 19.0; SPSS Inc., Chicago, Illinois, USA), and significant differences were accepted at the  $p < 0.05$  level of probability. Variation partitioning modeling was performed to assess the relative importance of three groups, namely, climate, soil physicochemical properties and microbial pro-

properties, in driving continental variations in soil Rm. We used the “forward.sel” function to avoid redundancy and multicollinearity in variation partitioning analysis that was conducted using the “Vegan” package.

First, we used “selected.forward” procedure to select most important predictors of Rm and then included these variables in structural equation modelling (SEM). SEM was performed to determine the pathways underlying the observed effects of environmental predictors on microbial respiration. SEM was conducted using “piecewiseSEM”, “nlme” and “lme4” packages. The piecewiseSEM could also account for random effects of sampling sites (to account for having more than one sample per site), by providing “marginal” and “conditional” contribution of environmental predictors in driving microbial diversity. We used the Fisher’s C test (when 0 [?] Fisher’s C/df [?] 2 and  $0.05 < p$  [?] 1.00) to confirm the goodness of the modelling results. We then modified our models according to the significance ( $p < 0.05$ ) and the goodness of the model. We included the same predictors in SEM for Rm at different scales, which could disentangle the best predictors for Rm according to their differences in total standardized effects. We further conducted boosted regression tree analysis to quantify the relative importance of the moderator variables in regulating the spatial variation in soil Rm. A gaussian error structure was assumed during a 10-fold cross-validation to estimate the optimal number of trees. Tree complexity was set to 4, whilst the learning rate was kept at 0.01 and bagging fraction at 0.6. The BRT model was performed using the R package “gbm” (Ridgeway, 2013) combined with the “dismo” package in R 3.6.4.

## Results

### Spatial variation in soil microbial respiration

Across all soil samples, the mean of Rm was  $20.7 \text{ mg C kg}^{-1} \text{ soil}$  for 3-day incubation with a wide range of 3.3 to  $98.2 \text{ mg C kg}^{-1} \text{ soil}$  (Fig. 2a). No simply linear correlation of Rm with latitude along the transect across all forests ( $r = 0.118$ ,  $p > 0.05$ ), instead, a hump-shaped one with the maximum at latitude =  $32.5\text{degN}$  was observed. Interestingly, Rm significantly increased with latitude in the latitude  $< 32.5\text{degN}$  region ( $r = 0.243$ ,  $p < 0.05$ ) and tended to decrease in the latitude  $> 32.5\text{degN}$  region ( $r = -0.166$ ,  $p < 0.05$ ) (Fig. 2a). Thus, latitude =  $32.5\text{degN}$  was the threshold where latitude and Rm interactions diverged. Furthermore, we also found that hump-shaped pattern of Rm with latitude ( $r = 0.488$ ,  $p < 0.01$ ) was observed across broadleaved forest soils (Fig. 2b), but no relationship was observed across coniferous (Fig. 2c) and mixed forests (Fig. 2d). Soils in broadleaved forests and coniferous forests had significantly higher Rm values than those in mixed forests (Fig. 3a), while soils in tropical forest had significant lower Rm than those in other biomes (Fig. 3b).

### 3.2. Influencing factors of the spatial variation in soil microbial respiration

Variables measured in this study had different roles in shaping Rm’s spatial variations at the continental scale and latitude  $< 32.5\text{degN}$  and  $> 32.5\text{degN}$  regional scales, and soil physicochemical properties had greater unique explanation for this variation than others (Fig. 4). Variation partitioning analysis results demonstrated that across all soil samples all variables measured totally explained 56.1% of the Rm’s spatial variation at the continental scale, in which climate, soil physicochemical and microbial properties explained separately 11.5%, 49.5%, and 42.4% of the Rm’s spatial variations (Fig. 4a). Among all factors, soil physicochemical and microbial properties () uniquely explained 13.61% and 6.13% of Rm’s spatial variation, but climate variables less uniquely explained across the continent. In the latitude  $< 32.5\text{degN}$  region, all variables explained 77.4% of the spatial variation in Rm, and soil physicochemical and microbial properties explained uniquely 13.3%, and 4.6% of this variation, separately (Fig. 4b), but in the latitude  $> 32.5\text{degN}$  region, all variables totally explained only 34.6% of the Rm’s spatial variation, and soil physicochemical and microbial properties uniquely explained 13.0%, and 1.3% of this spatial variation, separately (Fig. 4c). These results suggest that the unique role of soil physicochemical properties in regulating the spatial variation in Rm has less change in different regions but the unique role of microbial properties varied.

The piecewise SEM explained 76% (Fig. 5a), 92% (Fig. 5b) and 62% (Fig. 5c) of the Rm’s variations at the continent, latitude  $< 32.5\text{degN}$  and  $> 32.5\text{degN}$  regions, respectively, with all models to be significant even when accounting for the random effects of sampling site. Climate was lest important in directly controlling

Rm's variations than soil physicochemical and microbial properties in all case, but it could indirectly affect Rm's variations by influencing soil physicochemical and microbial properties (Fig. 5), and this indirect effect mainly caused by MAT was stronger in the latitude  $< 32.5^{\circ}\text{N}$  region (Fig. 5b). LOC and fungal biomass were most important among soil physicochemical and microbial property, respectively, in all case (Fig. 5). The SEM results showed that importance of climate, soil physicochemical property and microbial property visa different ways in shaping the Rm's variations varied at different scales.

The boosted regression model analysis was further conducted to quantify the importance of the key influencing factors in shaping the Rm's spatial variation, and the results demonstrated that the key influencing factors varied at different scales (Fig. 6). At the continental scale, LOC and fungi were more important in shaping the Rm's variation, respectively contributing 20.5%, and 18.9% relative importance of the spatial variation regulating Rm, (Fig. 6a), which was also shown by the results of Spearman correlation analysis (Fig. S1). While fungi and LOC were more important in the latitude  $< 32.5^{\circ}\text{N}$  and  $> 32.5^{\circ}\text{N}$  regions, contributing 17.3% (Fig. 6b) and 21.0% (Fig. 6c) relative importance of the spatial variation regulating Rm, respectively.

## Discussion

Relative to previous studies determining Rm with traditional method that incubation temperature differs from the ambient soil temperature (Jenkinson & Ladd, 1981), we used a novel method to incubate soils at the MAT of their corresponding sites when MAT was higher than  $10^{\circ}\text{C}$ . As Xu et al. (2017) reported, our measured Rm based on MAT may be more representative for soil microbial activity and also more useful in process modeling because MAT is near to ambient soil temperature. Furthermore, in the previous studies estimating Rm at the same incubation temperature for all soils collected from different sites may have a deviation in Rm values because Rm is generally positively related to incubation temperature (Hamdi et al., 2013; Wang et al., 2019). That is to say, Rm in high latitude biomes with low MAT (e.g., boreal forests) would be overestimated when incubation temperature was higher than their MAT, while in low latitude biomes with high MAT (e.g., tropical forests) Rm would be underestimated. Therefore, we believe that the findings from our novel incubation experiment may somewhat advance our understanding of Rm's spatial variations and enhance the accuracy of predicting Rm and its feedback to global change under the environment change scenario.

### Spatial variations in soil microbial respiration along latitude at different scales

In this study, we investigated Rm along a 4,200 km and found that Rm ranged from 3.3 to  $98.2 \text{ mg C kg}^{-1}$  soil with a mean of  $20.7 \text{ mg C kg}^{-1}$  soil within 3-day incubation (Fig. 2a), which was within the general range of the Rm measured in laboratory (Li et al., 2018; Priess & Folster, 2001). Our results suggested that Rm values had large spatial variations across China forest ecosystems. To the best of our knowledge, we for the first time revealed a hump-shaped relationship between Rm and latitude with a threshold at latitude  $= 32.5^{\circ}\text{N}$  at the continental scale (Fig. 2a), which was contrary to the general pattern previously reported (e.g., Colman & Schimel, 2013; Wang et al., 2016) and our first hypothesis that Rm would linearly change with increasing latitude. The hump-shaped relationship between Rm and latitude in broadleaved forests (Fig. 2b) may have large contribution to the opposite relationship between Rm and latitude for below and above latitude  $= 32.5^{\circ}\text{N}$  at the continental scale across China forest ecosystems. The opposite relationship between Rm and latitude for below and above latitude  $= 32.5^{\circ}\text{N}$  suggests that climate has less importance in shaping the Rm's spatial variation, and controlling factors and potential mechanisms of Rm are different in areas below and above this threshold.

The threshold at latitude  $= 32.5^{\circ}\text{N}$  occurring at the continental scale can be potentially explained by the following reasons. The biome near to the latitude  $= 32.5^{\circ}\text{N}$  was a sensitive and transitional zone that climate is from subtropics to temperate and dominated vegetations from evergreen species to deciduous species (Jing et al., 2019). This ecotone will lead to a peak of soil bacterial diversity (i.e., richness) (Liu et al., 2020). Alternatively, soils in this zone were characterized with higher C availability and SOC quality, which was supported or reflected by the latitudinal pattern of LOC contents (Fig. S2) and lower C:N ratios

of leaves and roots in mid-latitudinal regions (Zhang et al., 2020), resulting in the higher Rm than other regions.

### Primary factors controlling variations in soil microbial respiration at different scales

As our second hypothesis, in this study we found that factors controlling Rm's spatial variation and underlying mechanisms were different at the different scales, and revealed that the relative importance of climate, soil physicochemical and microbial properties in driving Rm's spatial variation varied with scales according to variance partitioning results (Fig. 4), although they together affected the Rm's spatial patterns through their various impacts on microbial activities (Colman & Schimel, 2013). We found that climate affected indirectly Rm's variation by changing different soil physicochemical and microbial properties at different scales (Fig. 5), although some previous studies demonstrated Rm was related to MAT and MAP at different scales (e.g., Li et al., 2020). At the continental scale, climate affected indirectly Rm by altering mainly LOC, fungal and gram-positive bacterial biomass (Fig. 5a), which was supported by the observation of Wang et al. (2018), while in the latitude  $< 32.5^{\circ}\text{N}$  region, the alteration of fungal and gram-positive bacterial biomass by climate played a critical role in Rm's variation (Fig. 5b), and the changes in LOC and SOC caused by climate controlled Rm's variation in the latitude  $> 32.5^{\circ}\text{N}$  region (Fig. 5c). In comparison with climate and soil microbial variables, the largest total (49.5%) and unique (13.6%) explanation of soil physicochemical property for the spatial variation in Rm (Fig. 5a) suggested that soil physicochemical property had the most importance in regulating the Rm's spatial variation across China forest ecosystems. As some previous studies reported (Dai et al. 2017; Herbst et al. 2011; Meyer et al. 2017), some physicochemical variables such as the quality and quantity of the substrate as the direct factors of soil microbial metabolisms exert a crucial role in determining the spatial patterns of microbial activity. We further revealed that LOC was the most significant explanatory variable among physicochemical factors in regulating the Rm's spatial variation based on boosted regression analysis (Fig. 6a), which was supported by the positive relationship between LOC content and Rm (Fig. S1b). This is because high LOC content is usually associated with high microbial availability and C energy, and preferentially utilized by soil microorganisms.

Soil microbial property had a unique explanation (6.13%) for the spatial variation in Rm at the continental scale (Fig. 4a). Our results provide a first direct experimental evidence that soil microbial characteristics play a unique role in regulating Rm's spatial variation at the continental scale and suggest the relationships between microbial community composition and Rm potentially linked to microbial life strategies and functional capabilities (Takur et al., 2018; Trivedi et al., 2016). Various microbial groups with different C use efficiency (Austin et al., 2004; Waring et al., 2013) and preferences to soil organic matter (Lehmann and Kleber, 2015; Ramirez et al., 2012) maybe lead to this unique explanation of microbial variables to the Rm's spatial variation, which was in accord with recent studies suggesting the significant role of soil microbial biomass and community in controlling ecosystem multiple functions (Bradford et al. 2017; Delgado-Baquerizo et al., 2016b; Liu et al., 2018b). We quantified the importance of major microbial groups measured by PLFAs in predicting Rm based on the boosted regression analysis and revealed fungi were the most important drivers of the spatial variation in Rm (Fig. 6a), supporting the growing literature that demonstrates the significance of fungi in driving soil functions (de Boer et al., 2005; Dacal et al., 2019; Delgado-Baquerizo et al., 2016b; Wagg et al., 2014). Together with other results, we highlight the importance of including microbial community composition in Earth system model to improve our ability to predict C feedbacks in terrestrial ecosystems.

More importantly, we found an interesting result that microbial variables had larger unique explanations for Rm's spatial variation in the latitude  $< 32.5^{\circ}\text{N}$  region than in the latitude  $> 32.5^{\circ}\text{N}$  region (Fig. 4b,c) and for the first time revealed the differences in the primary factors regulating the Rm's variations in the latitude  $< 32.5^{\circ}\text{N}$  and  $> 32.5^{\circ}\text{N}$  regions. Microbial property, especially fungal biomass as the primary predictors in regulating Rm's variations was probably related to higher microbial activity because of high MAT (Fig. 5b; Fig. 6b). Although some previous studies demonstrated that in cropland or grassland ecosystems soil microbial biomass was a strong regulator of soil respiration or litter decomposition at regional or continental scales (Colman & Schimel, 2013; Bradford et al., 2017), they did not identify its

relative importance and also not observe that fungal biomass had different importance in regulating Rm's spatial variation in different regions. Therefore, our findings advanced our understanding of Rm's spatial variation and its controlling mechanisms at different scales.

Although we have some important findings that to some extent enhance our understanding of Rm's variation and its mechanisms, here we noted that the unique explanation of soil microbial property may be underestimated because some key enzyme activities associating with SOC process were not measured. Some experiments have emphasized the important role of enzyme activities in controlling Rm through mediating the rate-limiting step of SOC depolymerization (Ali et al., 2018; Dungait et al., 2012; Kandeler et al., 2006). Therefore, we considered that the unique explanation of soil microbial property to Rm's spatial variation would be higher than our current estimation if some key enzyme activities are measured. Another important aspect to mention here is that we controlled soil samples under 60% water holding capacity, which made Rm independent of soil moisture (Ali et al., 2018; Wang et al., 2018). That is to say, the influence of soil moisture on Rm was not considered in our study, although soil moisture has strong effect on Rm (Chang et al., 2014; Stoyan et al., 2000). Therefore, we should be careful when directly using these results in predicting Rm in field and its dynamics under global change environment.

In conclusion, we highlighted the importance of incorporating Rm's spatial variation in C-climate models for better predicting responses of forest soil C dynamics to future environmental change using a novel incubation method and for the first time revealed the hump-shaped relationship between latitude and Rm with a latitudinal threshold of 32.5degN at the continental scale. The factors controlling Rm's spatial variation and underlying mechanisms varied in different regions, although labile organic C was the most important variable in regulating the Rm variation at the continental scale. Importantly, soil microbial variables, particularly fungal biomass, played an important and unique role in regulating Rm's spatial variation, but their significances were higher in shaping Rm's variation across the continental and latitude < 32.5degN region than at latitude > 32.5degN region. Overall, our findings suggest labile organic C and fungal have critical roles in controlling Rm in China's forest ecosystems, and suggest that including Rm spatial variation in Earth system models can potentially improve our capacity to predict changes in soil organic C balance under changing environment.

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## Competing interests

The authors declare no competing financial and other interests.

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## Figure captions

**Fig. 1** Site locals (black dots) of studied forests along a latitude gradient in China.

**Fig. 2** Spatial patterns of soil microbial respiration along latitude across all forests (a), broadleaved (b), coniferous (c) and mixed (d) forests in China.

**Fig. 3** Differences in soil microbial respiration among forest types (a) and biomes (b) across China forests. Different letters denoted significant differences at  $p < 0.05$ .

**Fig. 4** Variance partitioning for three different categories: climate, soil physicochemical property (PC) and microbial property (MP) in explaining the soil microbial respiration across the continent (a), in the latitude  $< 32.5\text{degN}$  (b) and  $> 32.5\text{degN}$  (c) regions.

**Fig.5** PiecewiseSEM accounting for the direct and indirect effects of climate predictors, soil properties and microbial properties on the microbial respiration (MR) across the continent (a), in the latitude  $< 32.5\text{degN}$  (b) and latitude  $> 32.5\text{degN}$  (c) regions. Climate, soil properties and microbial properties are both composite variables. Numbers adjacent to measured variables are their coefficients with composite variables. Numbers adjacent to arrows are path coefficients are the directly standardized effect size of the relationship. The thickness of the arrow represents the strength of the relationship. Total standardized effects of composite variables on microbial respiration are showed in conditional and marginal  $R^2$  represent the proportion of variance explained by all predictors without and with accounting for random effects of “sampling site”. Significance levels of each predictor are \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Fig. 6** Predictor relative importance of key variables among climate, soil physicochemical and microbial properties in driving soil microbial respiration based on boosted regression model analysis for soils across the continent (a), latitude  $< 32.5\text{degN}$  (b) and  $> 32.5\text{degN}$  (c) regions.

**Fig. 1**

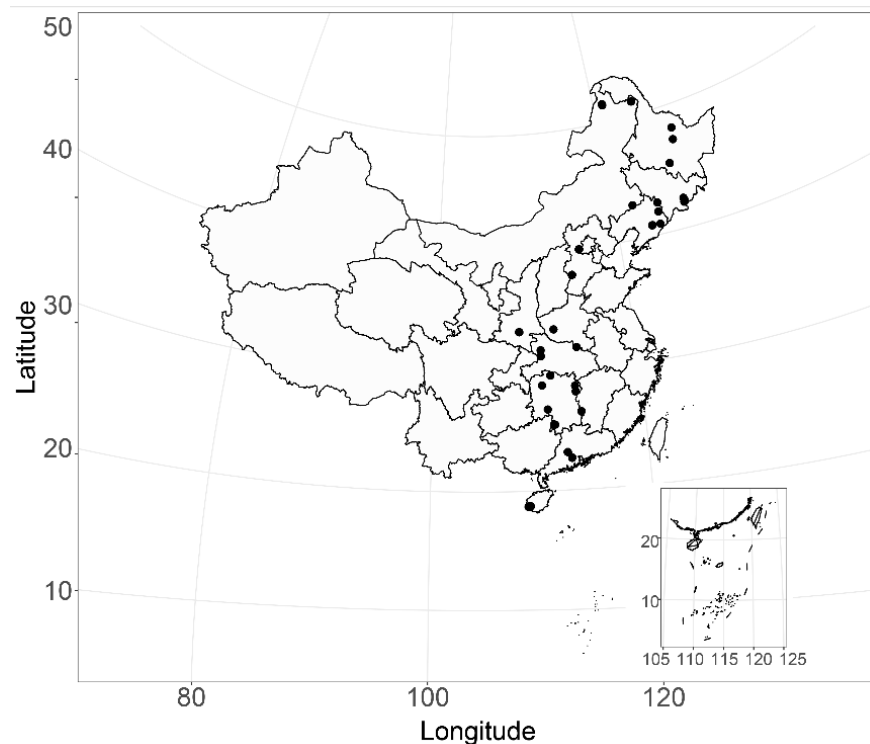


Fig. 2

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Fig. 3

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Fig. 4

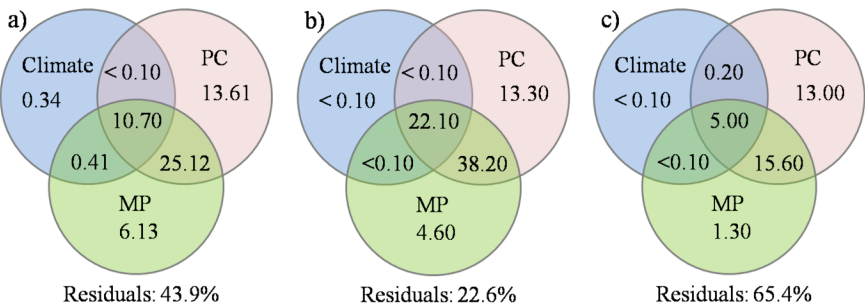
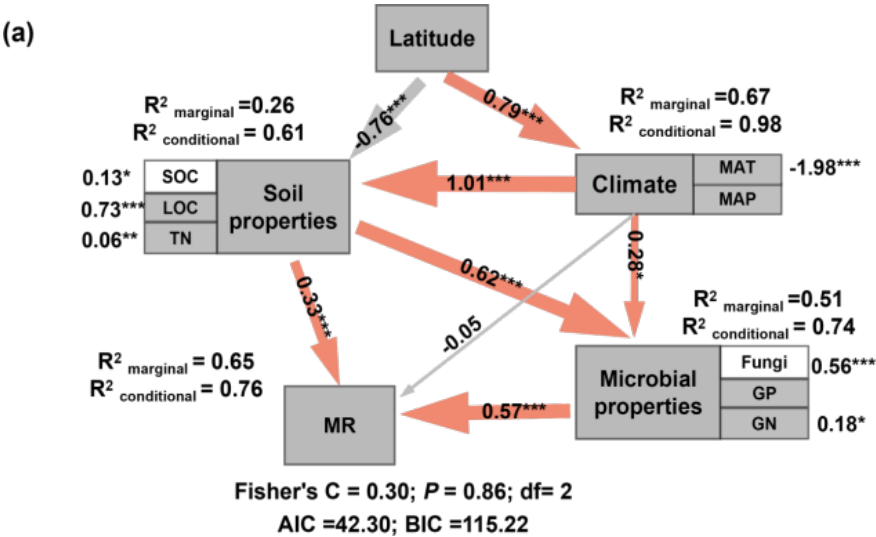


Fig. 5



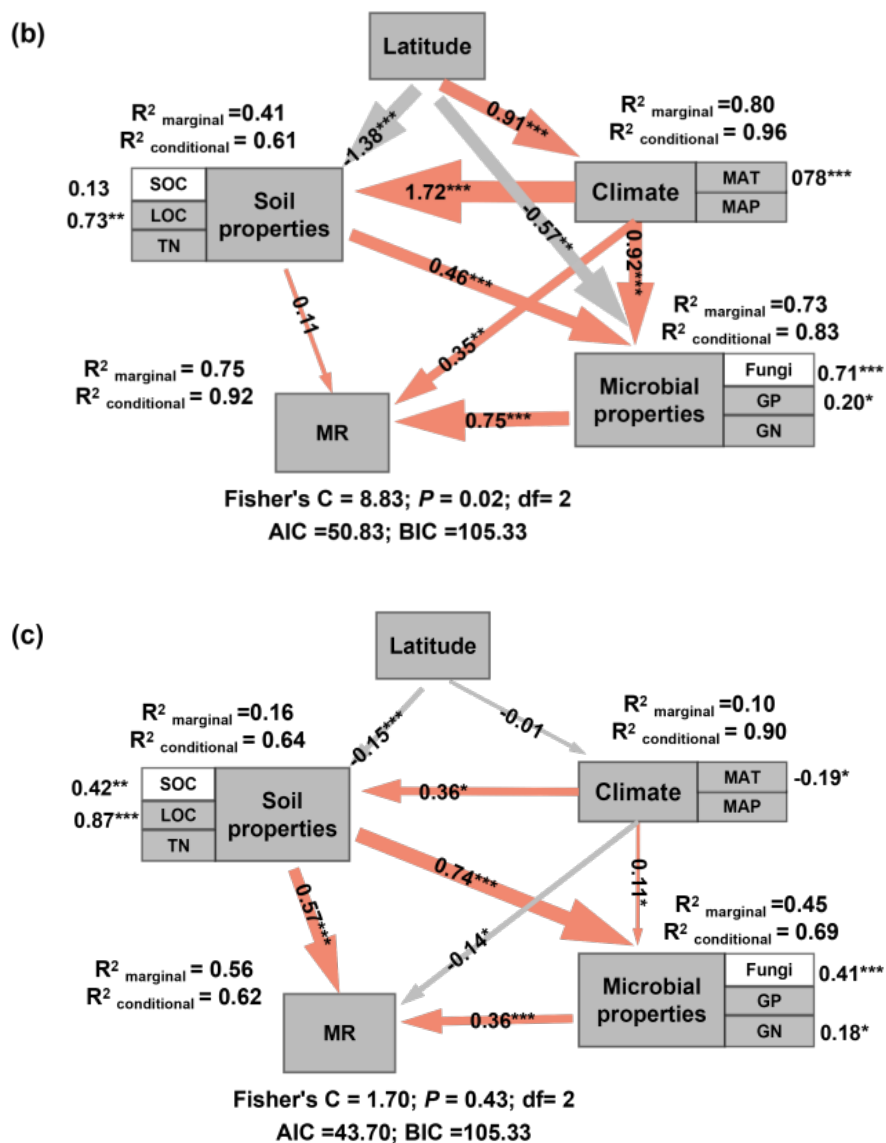
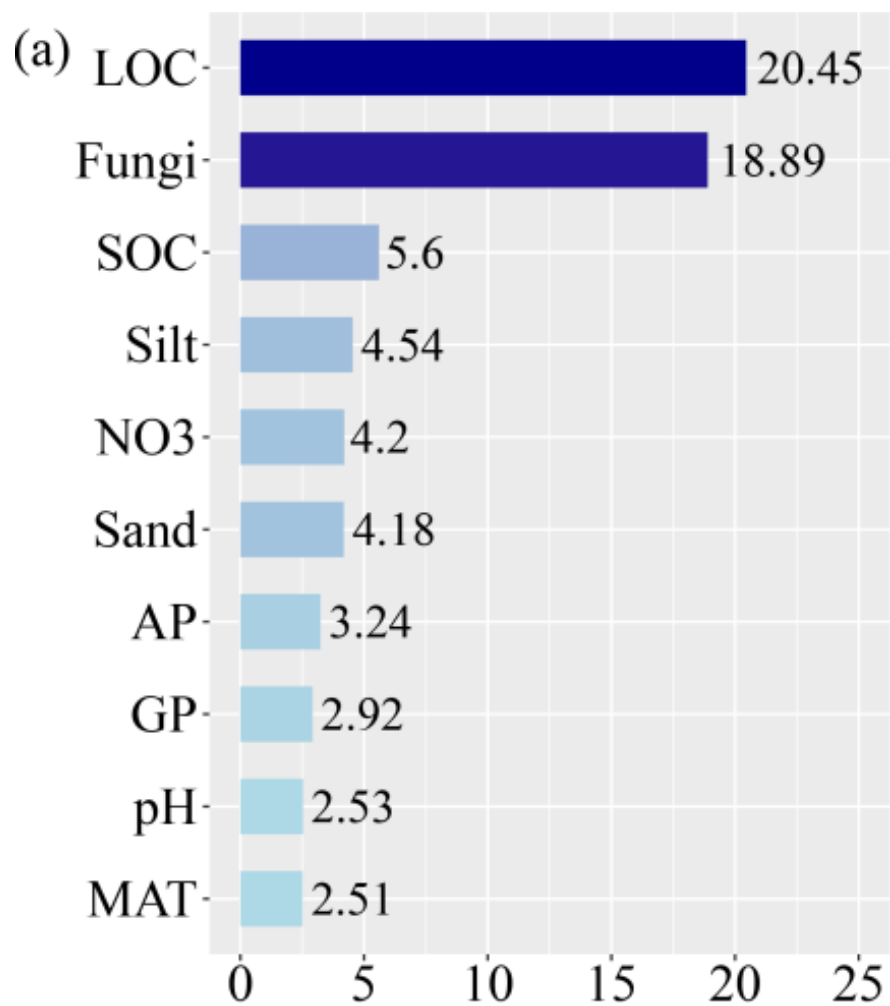
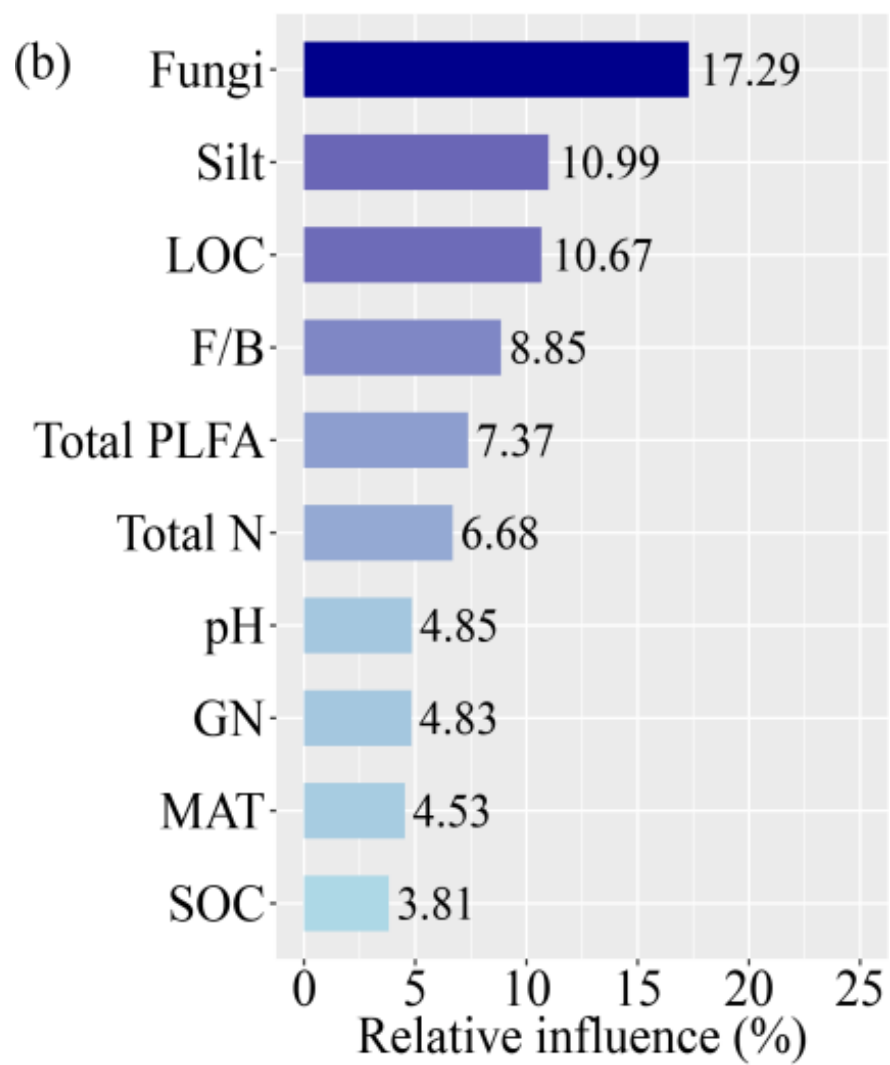
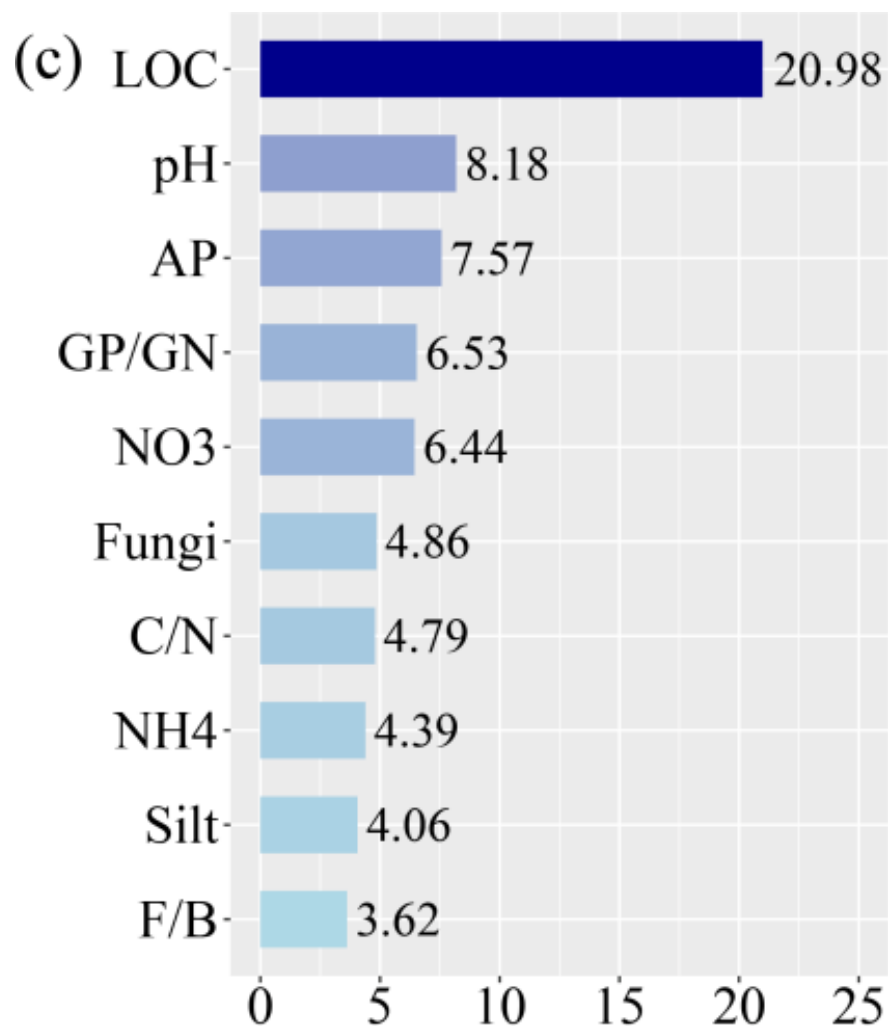


Fig. 6









### Supporting Information

**Table S1** Site information and soil key physicochemical properties used in this study.

**Fig. S1** Spearman correlations of soil microbial respiration with key important influencing variables.

**Fig. S2** The latitudinal pattern of labile organic carbon (LOC) contents across all soils in China forest.