

## Enhancement of saturated fatty acid content in soil microbial membranes across natural and experimental warming gradients

Jinhong He<sup>a</sup>, Xiangping Tan<sup>a</sup>, Yanxia Nie<sup>a</sup>, Lei Ma<sup>b</sup>, Weiping Zhou<sup>c</sup>, Weijun Shen<sup>d,\*</sup>

<sup>a</sup> Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China

<sup>b</sup> Key Laboratory of Geospatial Technology for Middle and Lower Yellow River Regions (Henan University), Ministry of Education, Kaifeng, China

<sup>c</sup> Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, The Key Laboratory for Agro-Environment, Ministry of Agriculture, Beijing, China

<sup>d</sup> Guangxi Key Laboratory of Forest Ecology and Conservation, State Key Laboratory for Conservation and Utilization of Agro-bioresources, College of Forestry, Guangxi University, Nanning, Guangxi, 530004, China

### ARTICLE INFO

#### Keywords:

Phospholipid fatty acids (PLFAs)  
Lipid composition  
Soil microbes  
Global warming

### ABSTRACT

Organisms including microorganisms from aquatic, grassland and glacier ecosystems have been found to regulate the lipid composition of their membranes to acclimate to warming. However, little evidence exists for whether soil microbes in forest ecosystems acclimate to temperature variations through this biochemical mechanism. Here, using phospholipid fatty acid (PLFA) analysis of forest soils subjected to seasonal, latitudinal, and artificial incubation temperature variations, we identified that the lipid composition of soil microbial cell membranes changed significantly with alterations in temperature. Specifically, saturated fatty acids without branches (SAFAs) increased while unsaturated fatty acids (UNFAs) decreased with increasing temperature in a linear fashion. The slope of SAFAs increase (or UNFAs decrease) with temperature was steeper for the long-existing latitudinal temperature gradient than for the short-term incubation temperature gradient, suggesting that longer-term warming may result in larger alterations in lipid constitute of soil microbial membranes. Our results provide evidence that forest soil microorganisms would preserve saturated fatty acids at warmer temperatures and therefore may acclimate to global warming by modifying the lipid composition of their cell membranes to adjust membrane fluidity.

Global surface air temperature is predicted to increase by 0.3–4.8 °C toward the end of this century (IPCC, 2021). This global climatic change has been found to alter soil microbial community structure and functions, for example, to enhance microbial respiration and potentially release more carbon dioxide into the atmosphere (Butler et al., 2012; Grimm et al., 2013). However, soil microorganisms have also been found to acclimate to warming physiologically (e.g., downregulation of respiration) and structurally (e.g., alteration of community composition), which can lower the temperature sensitivity of microbial respiration (Bradford et al., 2008; Crowther and Bradford, 2013; Karhu et al., 2014; Wei et al., 2014; Tian et al., 2022). The microbial community-level thermal response was ascribed to a shift in microbial community composition (Wei et al., 2014; Li et al., 2021), increased network complexity and stability (Yuan et al., 2021), and altered substrate quality and quantity (Moinet et al., 2021). But the physiological or biochemical mechanisms underlying the thermal response of soil

microorganisms remain elusive. Due to the evolutionary trade-offs between the structure and function of both enzymes and membranes (Bradford, 2013), understanding the response pattern of physiological traits to warming at the community-level is critical for improving explanations of microbial thermal acclimation and predictions of soil carbon dynamics.

Organisms can acclimate to temperature changes biochemically by altering their membranes' lipid composition, for example, in plants (Larkindale and Huang, 2004), animals (Hofmann and Todgham, 2010), phytoplankton (Hixson and Arts, 2016), and microbes in lakes or oceans (Hall et al., 2010; Sollich et al., 2017), known as the homeoviscous adaptation theory (Sinensky, 1974; Ernst et al., 2016). Soil microbial membranes contain a broad diversity of fatty acids (Zelles, 1999; Wixon and Balsler, 2013) with distinct thermal properties, for instance, the melting point of membrane lipids is in the order of unsaturated fatty acids (UNFAs) < saturated fatty acids with branches (BRFAs) <

\* Corresponding author. College of Forestry, Guangxi University, 100 Daxue Rd, Xixiangtang District, Nanning, Guangxi, 530004, China.  
E-mail address: [shenweijun@gxu.edu.cn](mailto:shenweijun@gxu.edu.cn) (W. Shen).

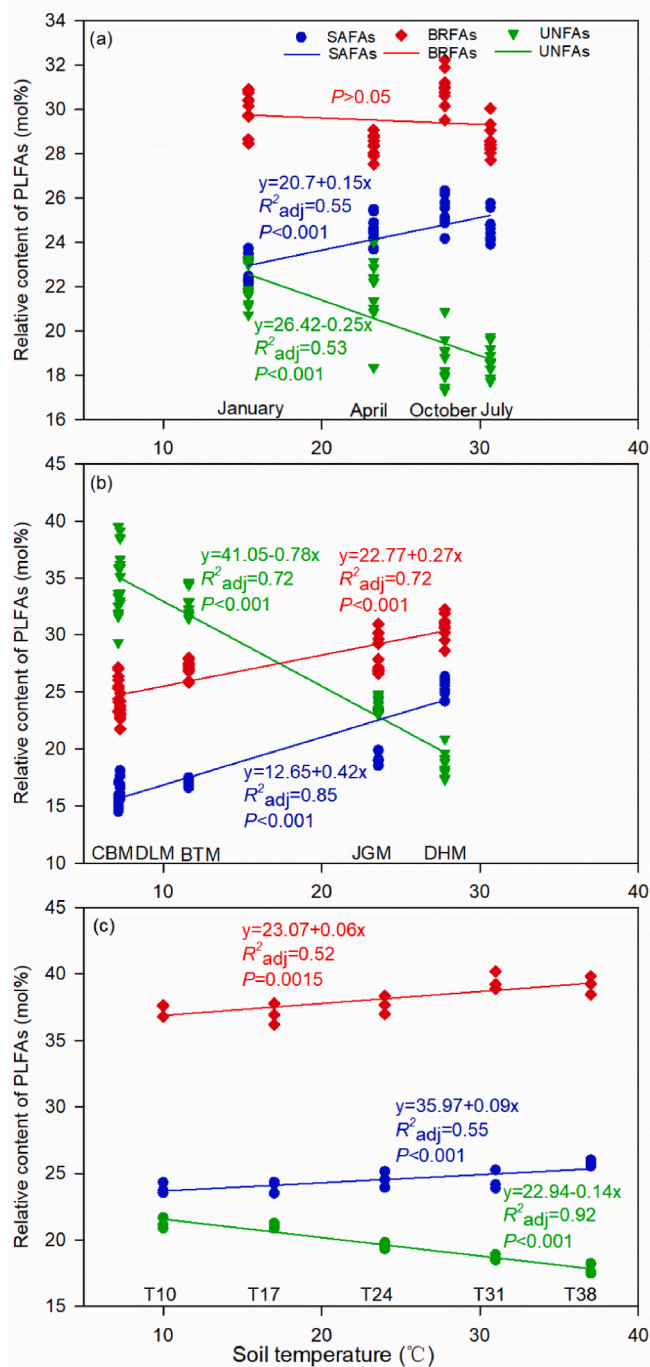
saturated fatty acids without branches (SAFAs) (Hall et al., 2010; Slotsbo et al., 2016). Soil microorganisms are therefore thought to be capable of acclimating to temperature changes by altering the lipid composition and fluidity of their membranes as do the other organisms mentioned above (Wixon and Balsler, 2013). Some previous studies had found that the lipid constitute of microbial membrane was shifted by experimental warming in grassland (Shen et al., 2020) and glacier soils (Bajerski et al., 2017; Hassan et al., 2020); little is known about the membrane lipid composition of microorganisms in forest soils with different temperature histories (e.g., climatic regions and seasonal variation) and their responses to warming.

In this study, we sampled soils subjected to seasonal, latitudinal, and experimental variations in temperature to examine how the lipid composition of the soil microbial cell membrane varies with temperature. For the seasonal temperature gradient, we collected samples from a subtropical forest at Dinghu Mountain (DHM) in southern China in January (winter), April (spring), July (summer), and October (autumn) in 2014, where the monthly mean soil temperature was 15.4, 23.3, 30.7, and 27.8 °C, respectively. For the latitudinal temperature gradient, soil samples were collected in five zonal forests of eastern China spanning 23° N to 42° N in October 2014: Changbai Mountain (CBM; monthly mean soil temperature 7.2 °C), Dongling Mountain (DLM; 7.3 °C), Baotianman Mountain (BTM; 11.6 °C), Jinggang Mountain (JGM; 23.6 °C) and DHM (27.8 °C) (see more details about the five forests in He et al., 2017). Soils of the laboratory incubation were collected from a subtropical evergreen broadleaved forest at the Heshan National Ecosystem Observation and Research Station (HSM) in May 2011 and incubated under five temperatures (10, 17, 24, 31, and 38 °C) for 30 days (see Zhou et al., 2017).

The lipid composition of microbial cell membranes was assessed by phospholipid fatty acid (PLFA) profiles according to a modified method (Bossio et al., 1998). Briefly, total lipids were extracted from 8 g of freeze-dried soil in a mixture of chloroform/methanol/phosphate (1:2:0.8, v/v/v). After extraction, the resulting fatty acid methyl esters were prepared according to the MIDI protocol and detected by gas chromatography-mass spectrometry (Agilent Technologies, CA, USA). The results were analyzed using the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, USA). The standard nomenclature was used to describe fatty acids (Gifford et al., 2012). Each PLFA concentration was calculated based on the 19:0 (Methyl Nonadecanoate, C<sub>20</sub>H<sub>40</sub>O<sub>2</sub>) internal standard concentrations, and the relative concentration was represented by the mole percentage (mol %) of total PLFAs. We divided the soil microbial PLFAs into three categories: unsaturated fatty acids (UNFAs: 14:1ω5c, 15:1ω6c, 16:1 OH, 16:1 ω5c, 16:1ω7c, 17:1 ω8c, 18:1ω7, 18:1ω9c and 18:2ω6c), saturated fatty acids with branches (BRFAs: 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, 17:0 anteiso, 18:0 iso, 19:0 iso), and saturated fatty acids without branches (SAFAs: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0), to characterize the lipid composition of the microbial cell membrane (Hall et al., 2010; Shen et al., 2019; Zosso et al., 2021).

Before analysis, the normality assumption was assessed using the Kolmogorov-Smirnov test, and the homogeneity of variances was evaluated with the Levene's test. Linear regression was used to illustrate the relationships between soil temperature and the relative content of microbial lipids under the three temperature gradients. One-way ANOVA was used to detect the differences among SAFAs, BRFAs, and UNFAs under each forest with Tukey's HSD test or, when variances were unequal, Tamhae's T2 test was applied. All these statistics were performed in SPSS 22.0 software (SPSS Inc., Chicago, USA).

Under all three types of temperature gradients, the relative contents of SAFAs, BRFAs, and UNFAs showed distinct variation patterns with soil temperature. The relative contents of saturated fatty acids, especially SAFAs, increased significantly with increasing temperature, whereas the relative contents of UNFAs decreased significantly with increasing temperature, both in a linear fashion (Fig. 1). The relative content of BRFAs also increased linearly under the latitudinal and

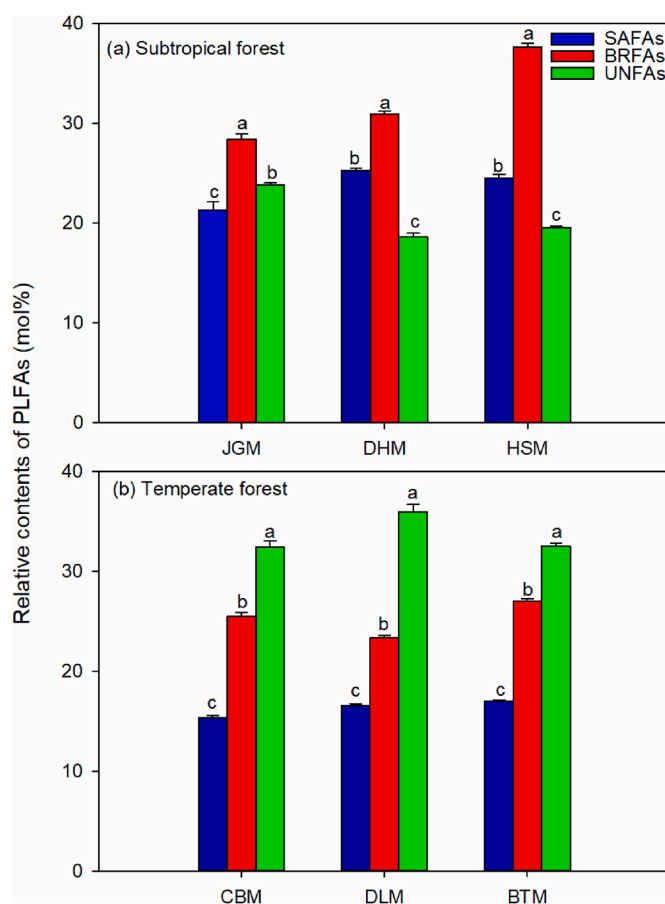


**Fig. 1.** Variation pattern of soil microbial membrane lipid contents with seasonal (a), latitudinal (b) and experimental temperature changes (c). The number of samples analyzed was 10 for the seasonal gradient, 10 for the latitudinal gradient, and 3 for the incubation experiment. A set of fatty acids were chosen to represent the saturated fatty acids without branches (SAFAs), including 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0. The sum of 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 anteiso, 17:0 iso, 18:0 iso, and 19:0 iso was calculated as an indicator of saturated fatty acids with branches (BRFAs). The unsaturated fatty acids (UNFAs) were indicated by the PLFAs: 14:1ω5c, 15:1ω6c, 16:1 OH, 16:1 ω5c, 16:1ω7c, 17:1 ω8c, 18:1ω7, 18:1ω9c and 18:2ω6c. The CBM, DLM, BTM, JGM, and DHM represent the Changbai, Dongling, Baotianman, Jinggang, and Dinghu, Mountains, respectively. T10, T17, T24, T31 and T38 are the incubation temperatures (10, 17, 24, 31, and 38 °C).

incubation temperature gradients, although it changed little with the seasonal temperature gradient (Fig. 1a). This result is in line with the homeoviscous hypothesis that cell membranes of soil microbes tend to have higher contents of saturated than unsaturated fatty acids under warmer conditions. Our results also indicated that soil microorganisms could alter the fatty acid composition of their cell membranes in response to both the short-term temperature variation (as in the 30-day laboratory incubation experiment) and the long-existing seasonal and latitudinal temperature gradients. The cell membranes of soil microorganisms play an essential role in substrate transportation, energy production and information interchange (Budin et al., 2018), and their fluidity would be raised when temperature rises (Hall et al., 2010). Our results showed that soil microbes preserved more saturated fatty acids under warmer environments, most likely for the purpose of lowering membrane fluidity and stabilizing cell structure. This strategy of soil microbes to maintain membrane fluidity within a defined range of viscosity would contribute to optimize the functions of transmembrane proteins for resource acquisition and substrate transportation, and maintain the electrochemical gradient for ATP synthesis under warmer conditions, and further effect on the microbial regulated processes (e.g., microbial respiration; Hall et al., 2010; Wixon and Balsler, 2013; Budin et al., 2018).

This mechanism of microbial acclimation to temperature may come from the long-term adaptation of soil microbes to their habitats. By examining the slope of the linear regression lines for SAFAs (blue lines in Fig. 1), we found that it increased from 0.09 under the incubation temperatures (Fig. 1c) to 0.15 under the seasonal temperature gradient (Fig. 1a), and reached 0.42 under the latitudinal temperature gradient (Fig. 1b). Correspondingly, the slope of the linear regression lines for UNFAs (green lines in Fig. 1) decreased from  $-0.14$  under the incubation temperatures (Fig. 1c) to  $-0.25$  under the seasonal temperature gradient (Fig. 1b), and reached  $-0.78$  under the latitudinal gradient (Fig. 1b). That is also to say, the rate of SAFAs increase (or UNFAs decrease) with temperature was faster under the long-existing latitudinal temperature gradient than under the short-term incubation temperature gradient, suggesting long-term warming might result in larger changes in soil microbial membrane lipid constitute. Furthermore, by comparing the two groups of the six studied forests (Fig. 2), we found that soil microbes in the subtropical forests originating from a warmer climate had significantly higher saturated fatty acid contents (23.74% for SAFAs and 32.28% for BRFAAs) than those of the temperate forests (16.33% for SAFAs and 25.26% for BRFAAs) originating from a cooler climate (Fig. 2a). In contrast, the temperate forests had significantly higher UNFAs (33.95%) than those of subtropical forests (19.34%; Fig. 2b). These results further suggest that soil microorganisms coming from warmer regions are more acclimated to high temperatures than those from colder areas (also see Wu et al., 2010; Wang et al., 2016). A previous study of ours (He et al., 2017) using high throughput sequencing technology revealed that soil microbial community composition of the same forests exhibited obvious seasonal and spatial variation patterns. These, together with the results of the present study indicate that shifts in microbial community composition co-occur with lipid resynthesis under temperature variations (Wixon and Balsler, 2013; Mooshammer et al., 2017). Therefore, we argue that soil microorganisms do not endure warming in a conservative manner but take an aggressive strategy to adapt to it via changing the lipid composition of cell membranes and community composition.

In summary, we have demonstrated that the lipid composition of soil microbial cell membranes varies with temperature, regardless of whether the changes are natural (seasonal and latitudinal) or artificial (laboratory incubation). The patterns showed that the relative proportion of saturated fatty acids without branches (SAFAs) was positively correlated with increasing temperature, whereas unsaturated fatty acids (UNFAs) were negatively correlated. This study provides evidence that soil microbes, similar to plants and animals, can modify the lipid composition of cell membranes to acclimate to altered temperature



**Fig. 2.** Comparison of soil microbial membrane lipid contents between the subtropical (a) and temperate forests (b). The JGM, DHM, HSM, CBM, DLM, BTM and indicate the Jinggang, Dinghu, Heshan, Changbai, Dongling and Baotianman Mountains in eastern China, respectively. Different lowercase letters indicate that soil microbial membrane lipid contents were significantly different among the three types of lipids (one-way ANOVA  $P < 0.05$ ) for a given forest.

conditions. Whether and how this mechanism affects community-level microbial functions (e.g., soil organic matter decomposition and greenhouse gas emission) deserves further study, especially connecting the lipid composition of cell membranes to the soil microbial community through high-resolution techniques (e.g., high-throughput sequencing).

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

#### Acknowledgments

We thank Dr. Naoise Nunan for his suggestions on the writing and Mrs. Chunqing Long for her assistance in field sampling and laboratory incubation. Two anonymous reviewers provided constructive comments that were helpful for revising the manuscript. This study was financially supported by the National Natural Science Foundation of China (32271847 and 31425005), the National Key Research and Development Program of China (No. 2021YFC3100405) and the Open Fund of

Key Laboratory of Geospatial Technology for the Middle and Lower Yellow River Regions (Henan University), Ministry of Education (No. GTYR202101).

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