# Title:

# Climatic seasonality challenges the stability of microbial-driven deep soil carbon accumulation across China

## Running title:

## Climate challenges deep soil C stabilization

Shuhai Wen[1,2], Jiaying Chen[2], Ziming Yang[3], Lei Deng[4], Jiao Feng[2], Wen Zhang[2], Xiao-Min Zeng[2], Qiaoyun Huang[1,2], Manuel Delgado-Baquerizo[5,6], Yu-Rong Liu[1,2*]

[1]National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China

[2]College of Resources and Environment, Huazhong Agricultural University, Wuhan

[3]Department of Chemistry, Oakland University, Rochester, MI 48309, United States

[4]State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi 712100, China

[5]Laboratorio de Biodiversidad y Funcionamiento Ecosistémico, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Sevilla 41012, Spain

[6]Unidad Asociada CSIC-UPO (BioFun), Universidad Pablo de Olavide, 41013 Sevilla, Spain.

Shuhai Wen and Jiaying Chen contributed equally to this work.

*** Corresponding author:**
Dr. Yu-Rong Liu. E-mail: yrliu@mail.hzau.edu.cn; Phone: (+86) 27-87286165

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## Abstract

Microbial residues contribute to the long-term stabilization of carbon in the entire soil profile, helping to regulate the climate of the planet; however, how sensitive these residues are to climatic seasonality remains virtually unknown, especially for deep soils across environmental gradients. Here, we investigated the changes of microbial residues along soil profiles (0-100 cm) from 44 typical ecosystems with a wide range of climates (~3100 km transects across China). Our results showed that microbial residues account for a larger portion of soil carbon in deeper (60-100 cm) vs. shallower (0-30 and 30-60 cm) soils. Moreover, we find that climate especially challenges the accumulation of microbial residues in deep soils, while soil properties and climate share their roles in controlling the residue accumulation in surface soils. Climatic seasonality, including positive correlations with summer precipitation and maximum monthly precipitation, as well as negative correlations with temperature annual range, are important factors explaining microbial residue accumulation in deep soils across the continent. In particular, summer precipitation is the key regulator of microbial-driven carbon stability in deep soils, which has 37.2% of relative independent effects on deep-soil microbial residue accumulation. Our work provides novel insights into the importance of climatic seasonality in driving the stabilization of microbial residues in deep soils, challenging the idea that deep soils as long-term carbon reservoirs can buffer climate change.

## Keywords

Climate-carbon feedback, Soil carbon stabilization, Deep soil, Summer precipitation, Microbial residues, Soil profile

## 1. Introduction

Deep soils represent a huge carbon (C) pool containing over half amount of total C stocks in terrestrial ecosystems, but with a longer resident time compared with topsoils (Balesdent et al., 2018; Jobbágy & Jackson, 2000; Luo et al., 2019). Because of this, deep-soil C storage and dynamics play an important role in the global C cycle in terrestrial ecosystems and climate change regulation (Balesdent et al., 2018; Jobbágy & Jackson, 2000; Rumpel & Kögel-Knabner, 2011). However, unlike for topsoils, C stored in deep soils is far less studied, and the understanding of different environmental factors that drive this C stock is quite limited. Moreover, although deep-soil C is expected to be less exposed to environmental conditions, our knowledge on their vulnerability to climate warming, water scarcity and soil degradation is scarce. Deep soil layer is also directly influenced by the entrance of C from plant inputs and litter decomposition on the surface. For example, deep soils can preserve microbial residues accumulated via lixiviation from shallow soil layers, which supports slow rates of C turnover. These microbial residues are mixtures of dead cell fragments, small biopolymers and enzymes that can be kept for a long time in soils by the protection of minerals, micropores and aggregates (He et al., 2022; Kuzyakov & Mason-Jones, 2018; Six et al., 2006), constituting a large proportion of soil organic C (SOC) (Buckeridge et al., 2022). Numerous studies have demonstrated significant contributions of microbial residues to SOC pools across biomes and climate zones, accounting for about 30-60% of the SOC storage (Deng & Liang, 2022; Ma et al., 2018; Wang et al., 2021b). However, much less is known regarding the influence of climate changes on deep-soil microbial residues, which hampers our ability to predict the role of these components in global climate regulations.

The uncertainties about the drivers of microbial residues in deep soils exist for three reasons. First, most studies on microbial residues were based on the top 30 cm of soils; but in fact, microbial-derived C might be much more critical for C storage in deeper soils than topsoils due to the less plant C inputs (Jobbágy & Jackson, 2000;

77 Rumpel & Kögel-Knabner, 2011). Lack of knowledge about microbial residues in
78 deeper soils thus limits our capacity to evaluate the microbial contribution to soil C
79 stocks. Second, most studies on microbial residues were conducted at a local scale or
80 in a specific ecosystem (Chen et al., 2020; He et al., 2022; Zhu et al., 2021), which
81 makes it difficult to provide a reliable assessment on the impact of biotic and abiotic
82 factors on the accumulation of microbial residues across wide gradients of climate and
83 vegetation. We know that microbial residues in topsoils are predominantly regulated
84 by soil properties (Angst et al., 2021; Ni et al., 2020b), climatic variables (Chen et al.,
85 2020; Ma et al., 2018; Zeng et al., 2022), ecosystem type (Liang et al., 2019; Wang et
86 al., 2021a; Yang et al., 2022), and microbiomes (Buckeridge et al., 2020; C. Wang et
87 al., 2021b). However, relative contribution of these environmental factors to explain
88 microbial residues in deeper soils is virtually unknown. Third, unclear association
89 between climate and microbial residues in deeper soils hinders us from clarifying
90 whether stored C in deep soils is stable under climate change. Current knowledge
91 assumes that deeper soils are more independent of climatic conditions than surface
92 soils, but evidences are lacking. Nevertheless, climate might also regulate the
93 accumulation of microbial residues in deeper soils indirectly via regulating the inputs
94 of C from plant and microbial productivity. In particular, climatic seasonality
95 (seasonal variations in meteorological attributes such as range of temperature and
96 frequency and amplitude of rain events), could trigger substantial changes of plant
97 and microbial communities (Engelhardt et al., 2018; Saikkonen et al., 2012),
98 subsequently, influencing their regulations on C storage. Therefore, exploring how
99 microbial residues in deeper soils respond to climate seasonal changes is critical to
100 understanding of the climate sensitivity of soil C pools and its feedback to climate
101 change.

102 Here, we conducted a national-scale standardized survey of soil profiles (topsoil:
103 0-30 cm, subsoil: 30-60 cm, deep soil: 60-100 cm) from 44 ecosystems (from
104 19.95°N to 48.05°N, Fig. S1), covering a broad range of climate, vegetation and soil

types. We investigated the influence of climate on the accumulation of soil microbial residues and compared with other fundamental factors such as soil properties and plant productivity. We used amino sugars as a proxy of microbial residues because they are unique components of fungal and bacterial cell walls but are negligible in the living microbial biomass, making them the most commonly used biomarkers to quantify microbial residues (Buckeridge et al., 2022; Joergensen, 2018; Liang et al., 2019). We first evaluated the variations in content of amino sugars and their proportion in SOC along the soil profile. Then, we explored main driving forces of microbial residues in three soil layers, considering 15 environmental variables relating to geographic, climatic, vegetation and edaphic factors. In particular, we focused the effects of climatic seasonality on the accumulation of microbial residues in deeper soils, because buried microbes in deep soils may be especially sensitive to the changes in nutrients, moisture and temperature pulse driven by the seasonal climate, thus further influence their C assimilation and decomposition processes. Therefore, we assumed that climatic seasonality is the key regulator of microbial residues in deep soils. Furthermore, we identified the most important seasonal climatic variables and clarified the pathways that influence microbial residues in deep soils.

## 2. Materials and methods

### 2.1 Study areas and soil sampling

Soil samples were collected from 44 locations (Fig. S1 and Table S1) across China (from 19.95°N to 48.05°N, stretch across ~3100 km), covering a broad range of climate and environmental gradients. At each sampling site, three soil profiles of 100 cm depth were randomly dug out. We separated topsoils, subsoils and deep soils by specific depths rather than by horizons of soil genesis and development, as done in many previous studies (Fontaine et al., 2007; Balesdent et al., 2018; Luo et al., 2019). This allowed us to better compare the microbial residues across a large-scale using the unified depth of soil layers. Generally, the layers of 0-30 cm soils consisted of humus layer and a transitional horizon and are identified as topsoil; the approximately 30-60

cm soil layers were mineral horizon and are referred as subsoil; the 60-100 cm soil layers consisted of the bottom of subsoil and weathered parent materials which have the characteristics of deep soils, and are therefore identified as the layer of deep soils in this study (Fig. 1b). We mixed the same layer of soil from three soil profiles at each site to obtain composite samples of all the three layers. The composite soil samples were sieved through 2 mm mesh and divided into two sub-samples, one was stored at -20 °C for microbial analysis, and the other was air dried for analyses of soil physical and chemical properties. Soil pH was determined by a pH meter in a 1:2.5 ratio of soil and water suspension. The contents of soil organic carbon (SOC) and total nitrogen (TN) were measured using the Walkley-Black and Kjeldahl methods, respectively (Page et al., 1982).

**2.2 Analysis of microbial residues**

Soil amino sugars are widely used biomarkers to quantify microbial residues, which are only found in cell walls of fungi and bacteria (Buckeridge et al., 2022; Liang et al., 2019). Amino sugars were determined according to the GC-based detection of aldononitrile acetate derivatized glucosamine and muramic acid in the soil (Liang et al., 2012). Briefly, air-dried soil samples (weighing soil that contains ≥ 0.3 mg of N) were hydrolyzed with 6 M HCl at 105 °C for 8 h. After digestion, 100 μL internal standard (1 μg $ml^{-1}$ myo-inositol) was added to each solution. The solutions were filtered, vacuum dried at 45 °C, and then re-dissolved with deionized water and centrifuged after adjusting the pH to 6.6-6.8 using 1 M KOH. The supernatants were freeze-dried, and the dried residues were dissolved with methanol and dried with $N_2$ gas at 45 °C. After that, 100 μL N-methyglucamine was added to each sample, and freeze-dried again. The residues were derivatized with 300 μL of pyridine-methanol (4:1 v/v) at 75-80 °C for 30 min, added acetic anhydride, and reheated at 75-80 °C for 25 min. After cooling, the derivatives were mixed with 1.5 ml dichloromethane and removed the excessive derivatization reagents with 1 M HCl and deionized water. The organic phase containing amino sugar derivatives was dried with $N_2$ gas at 45 °C and

re-dissolved with 300 μL hexane-ethyl acetate solvent (1:1 v/v). Amino sugar derivatives were determined by gas chromatography (Shimadzu GC2010 Plus, Shimadzu Corporation, Kyoto, Japan) equipped with an HP-5 column (25 m × 0.25 mm × 0.25 μm). Two types of amino sugars (glucosamine and muramic acid) were determined as the indicator of the microbial residues in this study. Muramic acid exclusively occurs in bacterial cells, glucosamine is derived both from fungal and bacterial cells, but a higher portion of it is from fungal cells than bacterial cells (Joergensen, 2018). Thus, the contents of bacterial residues are estimated by muramic acid, and the contents of fungal residues are estimated by fungal glucosamine which is figured out by subtracting the bacterial glucosamine from total glucosamine (Liang et al., 2019). The contents of amino sugars are the sum of glucosamine and muramic acid.

### 2.3 Data collection of environmental variables

We collected four categories of environmental factors (geographic, climatic, vegetation and edaphic factors, consisting of 15 individual environmental variables) to identify the main determinants of microbial residues in different soil depths. Geographic variables including longitude, latitude and elevation were recorded by a GPS device at sampling sites. Climatic variables include mean annual temperature (MAT), temperature seasonality (TS), temperature annual range (TAR), mean annual precipitation (MAP), maximum monthly precipitation (PMAX) and summer precipitation (SP), and were extracted from the WorldClim version 2 database (Fick & Hijmans, 2017) (https://www.worldclim.org; ~1 km resolution). Vegetation variable is the normalized difference vegetation index (NDVI) obtained from the MODIS, Global MOD13A1 data (Didan, 2015) (http://modis.gsfc.nasa.gov/; ~500 m resolution). Edaphic variables included SOC, TN, SOC:TN, pH and soil clay content, and were determined using soil samples from the filed survey. Soil clay content was collected from high resolution National Soil Information Grids of China (Liu et al., 2022; Liu et al., 2020) (http://soil.geodata.cn; ~1 km resolution). Detailed information

on the environmental variables and rationale of these variables on the accumulation of microbial residues are shown in Table S2.

### 2.4 Statistical analysis

Differences in the amino sugars between three soil depths were assessed by the Kruskal-Wallis test, and the Wilcox test was conducted for multiple comparisons using "rstatix" package. Hierarchical partitioning analyses were performed to identify the important environmental variables associated with amino sugars accumulation at three soil depths using "rdacca.hp" package (Lai et al., 2021). Hierarchical partitioning allowed us to quantify the independent effect (%) of grouped variables (geographic, climate, vegetation and edaphic) and every single variable (15 environmental variables) on amino sugars. Correlation analysis and ordinary least squares regression were conducted to assess the relationships between climatic variables and amino sugars at three soil depths. These statistical analyses were conducted using R 4.1.1 (http://cran.r-project.org/).

Structural equation modeling (SEM) was conducted to clarify the pathways of four categories of environmental factors influencing amino sugars in topsoil, subsoil and deep soil, respectively. In the prior conceptual model, we assumed that geographic factors generally determined the climate, and climate drives vegetation and edaphic variables, subsequently directly and indirectly affect amino sugars (Fig. S9). Variables involved in SEM analysis include geographic (longitude, latitude and elevation), climatic seasonality (summer precipitation and temperature annual range), vegetation (NDVI) and edaphic (SOC, TN, SOC:TN, clay and pH). Detailed information of these variables was shown in Table S2. The goodness of model fit was examined by chi-square test ($0 \leq \chi^2/df \leq 2$, and $0.05 < p \leq 1$) and RMSEA ($0 \leq$ RMSEA $\leq 0.05$), which further confirmed the fit of model by Bollen-Stine bootstrap test (0.10 < bootstrap $p \leq 1.00$) (Delgado-Baquerizo et al., 2020). SEM analyses were conducted using AMOS 21.0 (SPSS Inc., Chicago, IL, USA).

## 3. Result

### 3.1 Distribution patterns of amino sugars along the soil profile across the continent

The concentration of amino sugars decreased with increasing soil depth across China (Fig. 1a and 1c). The average level of the amino sugars in deep soils (0.50 ± 0.04 mg $g^{-1}$) was lower compared to topsoils (0.69 ± 0.08 mg $g^{-1}$) ($p < 0.05$, Fig. 1c). However, when normalizing amino sugars by the total amount of SOC, we show that the proportion of amino sugars in SOC increased with soil depth, being 1.8 times higher in deep soils than that in topsoils ($p < 0.05$, Fig. S3). Consistently, the proportion of muramic acid (proxy of bacterial residues) and fungal glucosamine (proxy of fungal residues) in SOC were higher in deep soils than in topsoils, even though the concentrations of muramic acid and fungal glucosamine decreased or were independent of soil depth ($p < 0.05$, Fig. S4). We observed general decreases of amino sugars with increasing latitude at all three soil depths (Fig. 1d), which exhibited a large geospatial variability across different climate zones. For instance, temperate climates are associated with a higher concentration of soil amino sugars than arid and cold climates (Fig. 1e).

### 3.2 Climatic seasonality explains amino sugar concentration in deep soils

Hierarchical partitioning analyses reveal that overall climate factor was closely associated with amino sugars in soil profiles, especially in deeper soil layers (Fig. 2). The independent effect of climate explained a much higher proportion in the variation of amino sugars in deep soils (27.1%) compared to that in topsoils (10.6%) (Fig. 2a). Among the climatic variables, precipitation explains a larger variation of amino sugars than temperature, and the discrepancy of explanations increased with the soil depth (Fig. 2b). Furthermore, we find that variables of climatic seasonality such as summer precipitation, maximum monthly precipitation, temperate seasonality and temperature annual range have strong associations with amino sugars in deep soils. Particularly, summer precipitation has the highest relative independent effect (37.2%) on amino sugars in deep soils. We further reveal that the content of amino sugars was

positively correlated with summer precipitation, maximum monthly precipitation and mean annual precipitation, while negative correlations with temperature seasonality and temperature annual range were observed in all three soil depths (Fig. 3). In addition, climatic variables had stronger associations with bacterial residues in deeper soils, but with fungal residues in topsoils (Fig. S5).

### 3.3 Pathways of seasonal climatic effects on microbial residues in different soil depths

Structural equation modeling (SEM) analyses further illustrated the pathways by which climatic seasonality influenced amino sugars in soil profiles combined with other environmental variables (Fig. 4). In topsoils, climatic seasonality was indirectly associated with amino sugars through affecting edaphic variables (Fig. 4a). In deep soils, summer precipitation had a direct association with the concentration of amino sugars, while vegetation was indirectly associated with amino sugars through influencing the SOC (Fig. 4c). Overall, summer precipitation had the highest positive total effect on amino sugars in deep soils, followed by temperature annual range (Fig. 4f). Moreover, amino sugars in deep soil had stronger associations with seasonal variations in precipitation than in temperature (Fig. 4c and 4f).

## 4. Discussion

Soil C pools in deep soil layers have been of great concern due to the enormous quantity of C stored which strongly affect global climate (Balesdent et al., 2018). Deep soils contain a huge amount of C associated with microbial residues, playing a key role in long-term C storage in the terrestrial ecosystems (Liang et al., 2017). However, the distribution of microbial residues in soil profiles and their associations with climate have been rarely assessed, and the major forces driving this C stock in deep soils remains virtually unknown. Moreover, we know little about the sensitivity of deep-soil microbial residues to changing climate, which limits our ability to predict deep-soil C budget under the ongoing climate change. Our study provides empirical

evidence that microbial residues contribute a larger proportion of SOC in deep soils than in topsoils across China, and climatic seasonality is a determining factor for the accumulation of microbial residues in deeper soil layer. Our work highlights a greater importance of climatic regions and seasonality on regulating the deep-soil C sequestration than we previously thought.

Our study shows that climate effects on the accumulation of microbial residues increased along the soil profile, and the main driving forces shifted from edaphic attributes in topsoils to climate variables in deep soils. Generally, climate and vegetation construct topsoils with abundant C and nutrient resources, microbial biomass and activities, thus microbial processes and residues accumulation are greatly influenced by soil properties such as SOC and N availability (Ni et al., 2020a; Ni et al., 2020b). The role of climate in microbial residues could be accompanied by soil properties in surface soils. However, deep soils have relatively deprived substrates (Fig. S2) which could suppress the turnover of microbial communities, wherein climate rather than soil properties or vegetation becomes crucial in regulating microbial processes. For example, frequent precipitation events and relatively large precipitation in summer can cause water logging and thus form anoxic conditions in deep soils due to poor drainage (Agboma & Itenfisu, 2020; Zhang et al., 2022), which slows down aerobic soil C respiration and leads to higher accumulation of microbial residues (García-Palacios & Chen, 2022). Nevertheless, the climate effects on deep-soil microbial community and their accumulated residues are not evident in small-scale experiments or regional studies (He et al., 2022; Moritz et al., 2009), owing to the little variations of climate variables. Consequently, our results suggest that seasonal climate is a more important driver of soil microbial residue accumulation in deep soils than other environmental attributes.

In general, soil C cycle in terrestrial ecosystems was considered predominantly controlled by the geo-climate (Doetterl et al., 2015; Patoine et al., 2022); however, the effects of climatic seasonality, climate annual changes on the storage of SOC and

microbial-derived C remained largely unexplored. In the present study, we show that summer precipitation, which represents the precipitation in the growing season, was a key climatic variable related to the accumulation of microbial residues in deep soils. High precipitation during the growing season is conducive to plant growth and decomposition of organic matter (Campos et al., 2017), which increases microbial assimilation and residue formation due to the high C inputs (Bell et al., 2014). Moreover, dissolved organic matter can be permeated into the deep soil through mass flow and diffusion through seasonal precipitation events (Belnap et al., 2005). Increased available substrates and moisture resulted from rainfall facilitate microbial turnover (Serna-Chavez et al., 2013), and thereby promote the formation of microbial residues in deep soils. In top soils, however, we did not observe a similar contribution of summer precipitation to variations of microbial residues, even though C input and nutrient availability were greater than those in deep soils. This might be attributed to that increased plant C input in topsoils was offset by the enhanced microbial respiration and SOC decomposition under favorable moist and warm conditions (Campos et al., 2017). Differently, in the deeper soils, summer precipitation-regulated water and nutrient supplies were essential for microbial assimilation under the harsh condition (Fierer et al. 2003; Fontaine et al., 2007; Suseela et al., 2012), and thus had stronger effects on the accumulation of microbial residues.

Our results also revealed that temperature seasonality and temperature annual range are important climatic variables associated with microbial residues in deep soils. Previous studies showed that microbial C use efficiency declined relative to respiration in subsoils under warming due to temperature seasonality (Maxwell et al., 2022; Sistla et al., 2014), and thus increased the loss of old C in deep soils (Pegoraro et al., 2021). For example, temperature switching from cold winter to hot summer can stimulate microbial activity more than that from warm winter to hot summer (Koven et al., 2017). Moreover, wide ranges of temperature between seasons can also indicate a soil environment under relatively long-term temperature changes, providing more

time for microbial decomposition. Consequently, large variations in temperature without timely substrates supply in deep soils may lead to more investment by microbes in catabolism rather than anabolism (Feng et al., 2021), impeding the formation of microbial residues in the soil (Buckeridge et al., 2020). This may explain why we observed that temperature seasonality and temperature annual range had negative correlations with microbial residues in deep soils, and low residue accumulation in high latitudes could be associated with the relatively wide ranges of temperature in these areas (Fig. S6). Thus, we suggest that seasonal variation of temperature is a key regulator of C sequestration in deep soils.

We did additional analyses to explore the contribution of various environmental factors to explain soil microbial residues excluding variables of climatic seasonality. The total explanations of these factors for amino sugars sharply reduced from 0.5 to 0.2 for deep soil layer (Fig. S7a). Mean annual temperature and precipitation as climatic factors can well explain the accumulation of microbial residues in topsoils but not for deep soils. The dominant role of climate in explaining deep-soil amino sugars (Fig. 2a) was replaced by edaphic factors when only considering the mean annual climate (Fig. S7b). These analyses suggest that lacking consideration of climatic seasonality factors may underestimate climate impacts on deep-soil microbial residues, or overestimate the stability of such a C pool under climate change. We compared the relative independent effects of various climatic variables on SOC (Fig. S8), and found that climatic seasonality is a nonnegligible factor for C storage in deep soils. Moreover, plant C input associated with climatic seasonality is an important factor influencing microbial turnover and residue accumulation at different soil layers (He et al., 2022). In this study, plant C input was not included due to the great challenges of measuring plant biomass during such a large-scale field sampling. Instead, we did additional analyses using net primary production (NPP) as plant C input (ANPP+$BNPP_{0\text{-}30}$ for the shallow soil layer, $BNPP_{30\text{-}100}$ for the deeper soil layer, as commonly used in many previous studies (Balesdent et al., 2018; Luo et al., 2019).

Consistently, the result still indicated that climatic seasonality was the dominant driver of deep soil microbial residues even after involving belowground C input (Fig. S10). Therefore, we suggest that considering seasonal climatic variations in future work can improve our ability to predict SOC sequestration.

Microbial composition, turnover and recycling of dead residues vary along the soil profiles, influencing the accumulation of microbial-driven C (Throckmorton et al., 2012; Liang et al., 2016; Liu et al., 2018). Our results indicated that climate had a strong association with bacterial residues in deep soils, while with fungal residues in topsoils (Fig. S5), suggesting that climatic seasonality probably regulates soil microbial residues by influencing soil microbial communities. In topsoils, the input of a large amount of plant matter is conducive to the metabolism and turnover of fungi that typically utilize fresh litter, and thus fungal residues could sensitively respond to the climatic seasonality. However, the harsh conditions in deep soils may shift the microbial communities to bacteria-dominated communities because most bacteria are facultative anaerobes while fungi prefer aerobic environments (Moritz et al., 2009; De Vries & Shade, 2013). Moreover, fungal residues are considered to be more stable than bacterial residues owing to their large body size, thick cell walls, hyphae, and some recalcitrant components, such as melanins and chitin (Martin & Haider, 1979; Schweigert et al., 2015; Luo et al., 2021). Hence, bacteria communities and residues could be more susceptible to climatic seasonality in deeper soils due to their faster turnover and shorter resident time than fungi. The turnover of microbial residues is important for interpreting the stability of microbial residues and SOC pools in deep soils under climate change. Therefore, further evidence on microbial physiology, biomass and composition will deepen our understanding of soil microbial-driven C cycles.

Taken together, our study clarifies distribution patterns and drivers of microbial residues along the soil profile across China, and provides new insights into the associations between climate seasonal changes and C stability in deep soils. Microbial

residues are the major contributor to the organic C pools in deeper soils, which represent an enormous reservoir of terrestrial C. We identified that climatic seasonality plays a more important role in the deep-soil accumulation of microbial residues compared to other environmental attributes, and summer precipitation is a key climatic variable predicting microbial residues in deeper layer of soils. This knowledge is critical to our understanding of C stability in terrestrial ecosystems and the role in global climate regulation. Our work also highlights the necessity of reassessing deep-soil C stability and potential in response to ongoing climate changes at a global scale.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

**Author contributions**

Y.-R.L. designed the study. J.C. and S.W. carried out the field survey and lab analyses. S.W. analyzed the data and wrote the first draft of the paper. Y.-R.L., M.D.-B., J.C., Z.Y., L.D., J.F., W.Z., X.-M.Z. and Q.H. revised the paper. All authors reviewed the paper and approved the final version of the manuscript.

**Data availability statement**

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.21524778.v1.

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## Figures

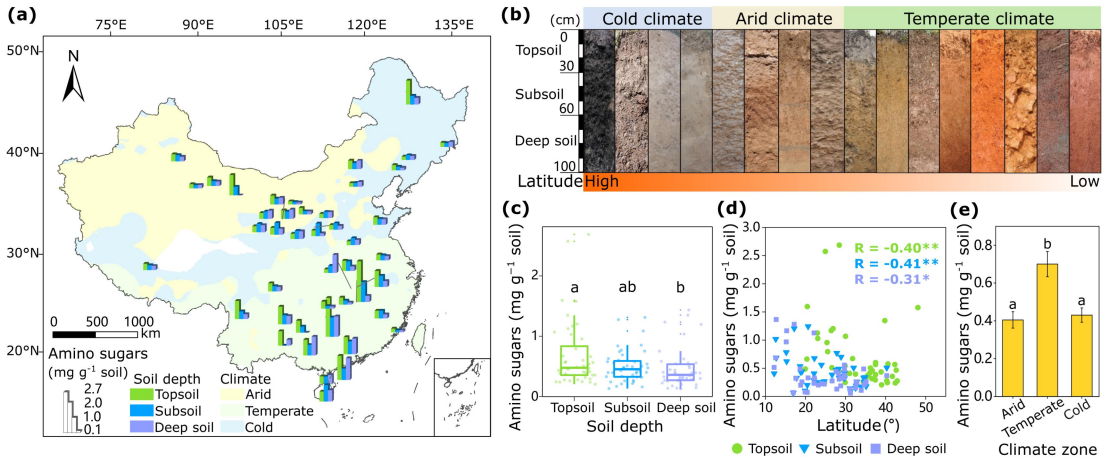


**Fig. 1 Spatial distribution pattern of microbial residues across China.** (a) The vertical distribution pattern of amino sugars (proxy of microbial residues) in the soil profiles across China (total n = 129, Topsoil: n = 44, Subsoil: n = 43, Deep soil: n = 42). (b) The typical soil profiles of 100 cm depth from distinct climate zones. (c) The concentration of amino sugars in three soil depths. (d) Correlations between latitude and amino sugars in three soil depths. (e) Concentrations of soil amino sugars in different climate zones (Arid: n = 29, Temperate: n = 61, Cold: n = 39). Lowercase letters in (c) and (e) represent significant differences at $p < 0.05$ level between soil depths and climate zones, respectively. Pearson correlation coefficients are shown in (d), "*" and "**" represent significant levels at $p < 0.05$ and $p < 0.01$, respectively. Soil depths include topsoil: 0-30 cm, subsoil: 30-60 cm, deep soil: 60-100 cm.

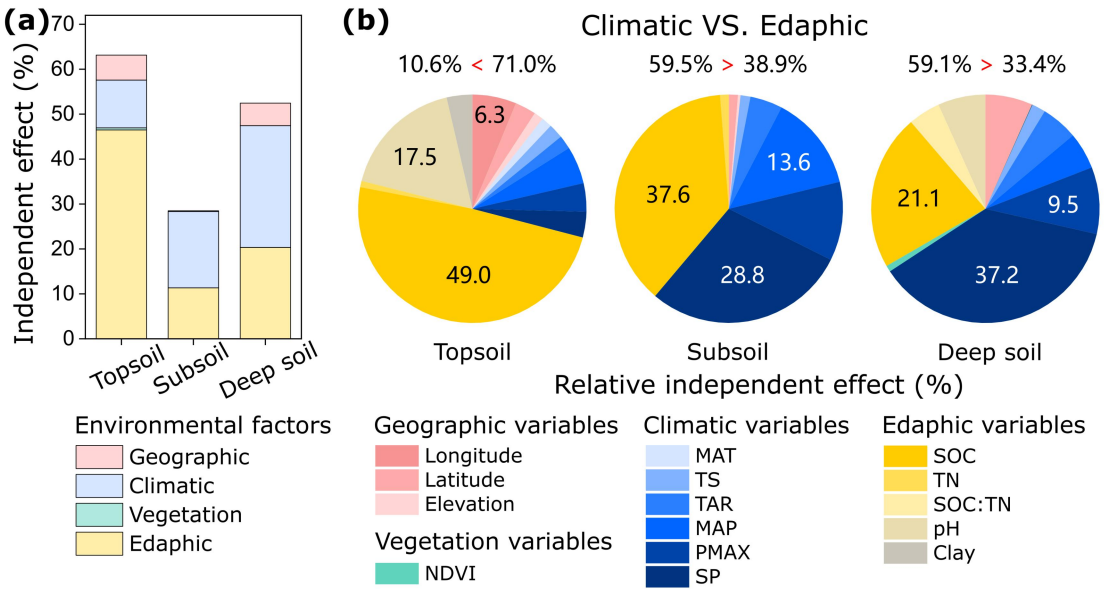


**Fig. 2 Hierarchical partitioning analyses illustrate the importance of environmental factors for the microbial residues in three soil depths.** (a) Comparison of environmental factors in explaining amino sugars in three soil depths (Topsoil: n = 44, Subsoil: n = 43, Deep soil: n = 42). (b) Relative independent effects of individual variable on amino sugars in three soil depths. Geographic variables include latitude, longitude and elevation. Climatic variables include MAT (mean annual temperature), TS (temperature seasonality), TAR (temperature annual range), MAP (mean annual precipitation), PMAX (maximum monthly precipitation), SP (summer precipitation). Vegetation variable includes NDVI (normalized difference vegetation index). Edaphic variables include SOC (soil organic carbon), TN (total nitrogen), SOC:TN, pH and Clay. Detailed information on environmental variables is shown in Table S2.

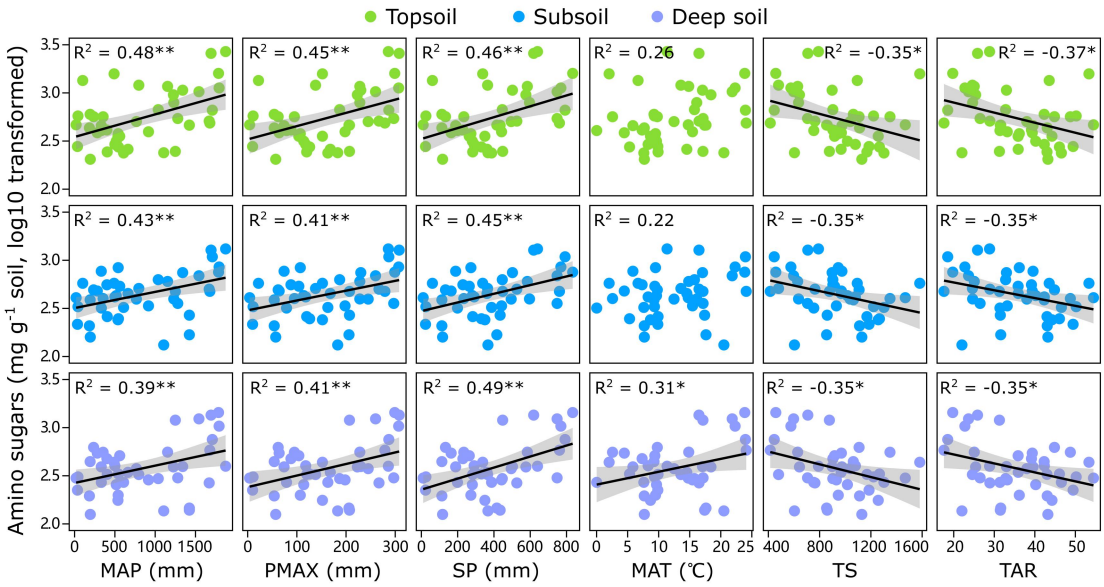


**Fig. 3 Relationships between various climatic variables and microbial residues in three soil depths.** Pearson correlation coefficient is shown in each panel (Topsoil: n = 44, Subsoil: n = 43, Deep soil: n = 42), "*" and "**" represent significant levels at $p < 0.05$ and $p < 0.01$, respectively. The solid lines were fitted by linear regressions, and the shadow areas corresponded to 95% confidence intervals. MAP, mean annual precipitation; PMAX, maximum monthly precipitation; SP, summer precipitation; MAT: mean annual temperature; TS, temperature seasonality; TAR, temperature annual range.

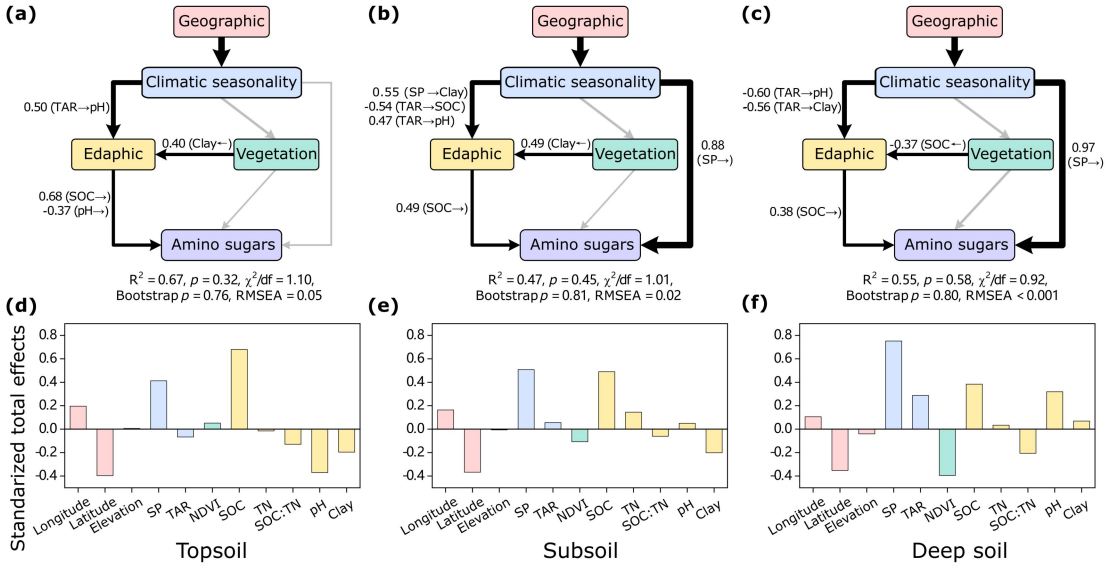


**Fig. 4 Structural equation models (SEMs) show the pathways of climatic seasonality affecting microbial residues in three soil depths.** (a), (b), (c) Direct and indirect associations of geographic, climate, edaphic and vegetation factors with amino sugars in topsoils (n = 44), subsoils (n = 43) and deep soils (n = 42), respectively. (d), (e), (f) The standardized total effects of different factors on amino sugars in three soil depths, respectively. Different variables of each environmental factor (geographic, climate, edaphic properties and vegetation) were grouped in the same box for the simple illustration of SEM. The width of arrows is proportional to the potential causal effects between variables, and standardized path coefficients are shown by numbers adjacent to arrows. Paths with significant effects ($p < 0.05$) are plotted in black lines, and insignificant paths are plotted in gray lines. Considering the potential collinearity of climatic variables, we selected summer precipitation (SP) and temperature annual range (TAR) as the most important variables of climatic seasonality in our SEM. The prior SEM model and more detailed information on the environmental factors included in SEM are shown in Fig. S9 and Table S2.