Threshold conditions for the accumulation of microbial residues in terrestrial ecosystems

Zhiguo Hao
Lanzhou University

Yunfei Zhao
College of Earth and Environmental Sciences & MOE Key Laboratory of Western China's Environmental Systems, Lanzhou University.

Xia Wang (submitmanuscript@126.com)
College of Earth and Environmental Sciences & MOE Key Laboratory of Western China's Environmental Systems, Lanzhou University. https://orcid.org/0000-0002-5256-0611

Silong Jiang
College of Earth and Environmental Sciences & MOE Key Laboratory of Western China's Environmental Systems, Lanzhou University.

Jinjin Xiao
College of Earth and Environmental Sciences & MOE Key Laboratory of Western China's Environmental Systems, Lanzhou University.

Kaichang Wang
College of Earth and Environmental Sciences & MOE Key Laboratory of Western China's Environmental Systems, Lanzhou University.

Xiaohe Zhou
College of Earth and Environmental Sciences & MOE Key Laboratory of Western China's Environmental Systems, Lanzhou University.

Huiying Liu
College of Earth and Environmental Sciences & MOE Key Laboratory of Western China's Environmental Systems, Lanzhou University.

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Threshold conditions for the accumulation of microbial residues in terrestrial ecosystems

Highlight

1. We obtained 268 soil amino sugar levels from field studies.
2. Globally, soil organic carbon, soil C:N ratio, and aridity index are important factors for predicting microbial residue accumulation.
3. In different ecosystem types and climate zones, the dominant factors for predicting microbial residues are different.
4. In the subtropical forest, the effect of soil pH on microbial residue accumulation is close to that of soil organic carbon.
5. In the process of increasing soil nutrients in arid climate, the content of microbial residues has a sharp decrease threshold.

Abstract

Microbial residues play important roles in the formation and stability of soil carbon pools; however, the factors affecting large-scale accumulation of microbial residues remain unclear. Here, we collected data of 268 amino sugar levels (biomarkers of microbial residues) from previous field studies and found that soil organic carbon (SOC), soil C:N ratio, and aridity index mainly determine the accumulation of microbial residual carbon. Moreover, we found that the threshold of the aridity index where microbial residue starts decreasing is in the range of humid climate type, while the threshold of soil C:N ratio represents a point of sharp decrease

in fungal abundance. Although SOC and aridity index were important in all cases, the dominant factors for predicting microbial residues varied across different ecosystems and climate zones, with pH being particularly important. Hence, climate and soil environment play important roles in the process of microbial residue accumulation.

Introduction

Soil is the largest carbon reservoir in terrestrial ecosystems. Small changes in the carbon budget can cause large fluctuations in atmospheric carbon dioxide concentration, which in turn can have a profound impact on the structure and function of terrestrial ecosystems[1-3]. As the "native inhabitants" of soil, microorganisms can regulate the carbon dynamics of soil through catabolism and anabolism[4-7]. Although the living microbial biomass only accounts for 2%–4% of soil organic carbon (SOC)[8,9], *in vivo* turnover of microbial products can contribute to soil carbon through the microbial carbon pump (MCP)[6]. These products are stable in the soil due to the mineral protection mechanism[6]. This process results in a thick layer of underground microbial residue, and their relatively stable storage contributes to the persistence of organic carbon in the soil[6,9]. Consequently, dynamic changes of these residues can significantly affect the balance of the terrestrial carbon budget[6,9-11]. Dead remains of microorganisms (residual substance) can be traced by amino sugars; this can help in characterizing the source of SOC and estimating its stability potential[11-13]. Studies using advanced research methods have revealed that microbial carbon is important for the formation of stable soil SOC[10,14-17]. However, the environmental factors controlling the preservation of microbial-derived carbon are not well understood[17].

Microbial communities regulate ecosystem functions through interactions between individuals or between individuals and the environment[18]. Moreover, microorganisms modify environmental factors in ecosystems. For example, the deterioration of environmental conditions (by aridity aggravation or nutrient reduction)

reduces the activity of microorganisms, thereby reducing the utilization efficiency of substrates[19,20]. Similarly, a higher quality plant litter increases the metabolic activity of microorganisms and enhances their ability to decompose organic carbon[20,21]. Thus, changes in soil properties and climate can lead to changes in microbial characteristics, and thereby affect the storage of SOC[20,22-24]. However, little is known about the optimal environmental conditions for microbial carbon accumulation.

Amino sugar biomarkers are increasingly being used to explore the mechanism of soil carbon storage[17,25,26]. This enables extensive quantification of the global heterogeneity of soil amino sugar content and its predictors. Forest and grassland ecosystems account for approximately 30%[27] and 26%[28] of the earth's land surface area, respectively, while accounting for approximately 47% and 20% of the earth's land SOC content, respectively[28,29]. Therefore, this study examined microbial residues in 0–20 cm depth of soil in forest and grassland ecosystems. We aimed to determine the environmental variables important for predicting microbial residues and to explore the variation in levels of microbial residues with changes in environmental variables. We hypothesized that climate, geographical location, and soil chemical factors have significant effects on the accumulation of microbial residues, due to the coupling relationships between various environmental variables in the ecosystem. Moreover, we speculated that the response pattern of microbial residues to different environmental variables may not always be gradual. Hence, we predicted the occurrence of an optimal range or thresholds of environmental conditions for the accumulation of microbial residues.

Results

Geographical pattern of microbial residues

We analyzed data from 268 datasets that we extracted from 64 scientific articles and 1 unpublished study on microbial residues in tropical grasslands, subtropical grasslands, temperate grasslands, tropical forests, subtropical forests, and temperate forests worldwide. The values of microbial residues in topsoil ranged from 0.04 to 11.21 (mg/g soil), with an average of 2.25 ± 0.13 (mg/g soil) and a median of 1.68 (mg/g soil) (Fig. 1c). Scheirer–Ray–Hare test with ecosystem type and climate zone as fixed factors revealed no significant difference in microbial residues between forests (2.21 ± 0.22 mg/g soil) and grasslands (2.29 ± 0.17 mg/g soil) ($P = 0.111$, Table 1). However, significant differences were detected between climatic zones ($P = 0.019$, table 1) with significantly higher microbial residues in forests and grasslands in the temperate zone than those in forests and grasslands in the subtropical zone ($P = 0.004$, Table 1). No significant interaction was found between vegetation types and climatic zones on microbial residues ($P = 0.094$, Table 1). Kruskal–Wallis test across the six categories (tropical grasslands, subtropical grasslands, temperate grasslands, tropical forests, subtropical forests, and temperate forests) revealed significant differences in microbial residues between subtropical forests and temperate forests ($P = 0.009$) and between subtropical forests and temperate grasslands ($P = 0.047$, Fig. 1b). Globally, microbial residues were significantly correlated with absolute latitude (Spearman's correlation $R^2 = 0.044$, $P < 0.05$), SOC ($R^2 = 0.490$, $P < 0.001$), aridity index ($R^2 = 0.003$, $P < 0.05$), and soil clay content ($R^2 = 0.017$, $P < 0.005$, Fig. 2b).

Predictors of microbial residues on a global scale

We used Random Forest modeling to determine the most important environmental factors (among absolute latitude, aridity index, soil clay content, SOC, soil C:N ratio, and soil pH) that influence microbial residues. The Random Forest model showed that all the environmental variables we considered were important for predicting the levels of microbial residues (Overall model: $R^2 = 0.768$, $P < 0.001$; environment variable $P < 0.05$, Fig. 2d). We also used structural equation modeling (SEM) to test whether the relationship between microbial residues and environmental factors remains unchanged when considering the causal relationship of multiple environmental factors at the same time. Our SEM explained 65.4% of the variance in microbial residues (Fig. 2a). SOC, soil C:N ratio, and aridity index still had more standard total effects (STEs) (0.929, -0.289, -0.226, respectively, Fig. 2c) after considering the causal relationship between variables.

Nonlinear responses of microbial residues to drought, soil C:N ratio, and soil organic carbon

Three important environmental variables (SOC, soil C:N ratio, and aridity index) suggested by SEM were selected for further analysis (Fig. 2c). Linear models, quadratic models, and general additive models (GAM) were fitted to determine the relationship between microbial residues and environmental variables. Comparisons of Akaike information criteria (AIC) values among these models showed that non-linear

117 relationships used in the GAMs were best fits for the relationship between microbial
118 residues and aridity index, soil C:N ratio, and SOC (Table 2, Supplementary Fig. 2).
119 We identified threshold levels for the increase in aridity index (0.768) and soil C:N ratio
120 (9.583 [0.57 after ln (x + 1) conversion, and 2. 26 after ln (x) conversion, respectively],
121 Fig. 3a1, b1) for the accumulation of microbial residues. After this threshold, the levels
122 of microbial residues decreased significantly (Fig. 3a2, b2). According to the
123 generalized climate classification scheme of aridity index values formulated by the
124 United Nations Environment Programme (UNEP) in 1997, this threshold level would
125 lie within the humid climate class (aridity index > 0.65: humid; Supplementary Table
126 1). The threshold level of soil C:N ratio was between the lower quartile and the lower
127 decile, and was less than the average value of the C:N ratios (14.16 ± 0.35) observed in
128 our study (Supplementary Fig. 1). For SOC, the curvature at the threshold did not affect
129 the original trend, and we observed a linear increase in accumulation of microbial
130 residues with SOC (Fig. 4).
131
132 **Predictors of microbial residues in different ecosystem types and climatic zones**
133 Since most forests and grasslands are located in subtropical and temperate zones
134 (87.50%, 99.32%, respectively, Fig. 1), we conducted independent analysis for
135 subtropical forests, temperate forests, subtropical grasslands, and temperate grasslands.
136 Our Random Forest models suggest that SOC and aridity index were the most important
137 predictors of microbial residues in subtropical grasslands, temperate grasslands,
138 subtropical forests, and temperate forests (environment variable: $P < 0.05$, Fig. 7). Soil

pH and C:N ratio also significantly predicted microbial residues in grasslands ($P < 0.05$, Fig. 7a, b), while the absolute latitude could predict microbial residues in forests ($P < 0.05$, Fig. 7c, d). In contrast to other categories, soil clay content was the most influential factor determining microbial residues in temperate forests ($P < 0.05$), while the effect of soil pH was not significant (Fig. 7).

Our SEMs explained 88.2%, 74.4%, 74.6%, and 62.4% of the variance in microbial residues in subtropical grasslands, temperate grasslands, subtropical forests, and temperate forests, respectively (Fig. 5). According to the STEs of SEMs of all categories, we identified SOC as the main positive regulatory factor of microbial residues (Fig. 6). In grasslands, aridity index and soil pH were the next most important predictors (Fig. 6a, b), while the absolute latitude strongly predicted microbial residues in temperate grasslands (STE: 0.280, Fig. 6b). In subtropical forests, the effect of soil pH on microbial residues was second only to SOC, followed by soil C:N ratio and soil clay content (Fig. 6c). The soil C:N ratio in the temperate forests showed a strong negative effect on microbial residues (STE: -0.272, Fig. 6d).

Discussion

On a global scale, Random Forest analysis showed that all environmental variables (absolute latitude, aridity index, soil clay content, SOC, soil C:N ratio, and soil pH) affected the levels of microbial residues in terrestrial ecosystems (increase in Mean Square Error (MSE) (%) $P < 0.05$, Fig. 2d). In terrestrial ecosystems, latitude significantly affects light intensity and thus photosynthesis in surface vegetation, resulting in the inhibition of carbon input and changes in carbon fluxes[24,30,31]. Although the microbial residues closely related to SOC had a significant correlation with latitude ($P < 0.05$, Fig. 2b), in our study, the SEM revealed low STE of latitude (STE: 0.030, Fig. 2c). This suggests a weak effect of latitude on the accumulation of microbial residues. However, we found that SOC, soil C:N ratio, and aridity index were the main predictors of microbial residues (Fig. 2c). Aridity is an important driving force of biological and geochemical processes[32,33]. Increasingly arid climatic conditions have changed the balance of chemical elements (carbon, nitrogen, etc.) in the ecosystem[34]. This could lead to a decline in microbial functions, thereby affecting the sustainability of the ecosystem[35]. Additionally, drought conditions weaken the evaporative cooling effect of plant leaves, and increase the effects of high temperatures[36]. This feedback between soil water and temperature may lead to a heat wave[37], which would further threaten the survival of microorganisms. Although SOC increased significantly with the decrease in aridity (increase in aridity index; Fig. S3a), the levels of microbial residues did not increase continuously (STE: -0.226, Fig. 2c). Thus, no significant linear relationship was detected between aridity index and microbial residue (amino sugars as

177 biomarkers, Supplementary Fig. 3b). Several studies have shown strong positive
178 correlations between amino sugars (markers of microbial residues) and SOC[38,39], with
179 higher levels of microbial residues associated with a higher soil organic matter content.
180 Our results are consistent with this rule; although microbial necromass carbon accounts
181 for a large proportion of SOC[40], it does not completely determine the amount of SOC.
182 However, SOC can be used as a predictor of microbial residue levels. The aggravation
183 of aridity leads to a decrease in available soil carbon (C) and nitrogen (N), damage of
184 soil nutrient supply, and increased nitrogen limitation on the growth of plants and
185 microorganisms in the soil[34,41]. Additionally, aridity indirectly affects the accumulation
186 of microbial residues through the soil C:N ratio [42].

187 Furthermore, our analysis showed no simple linear relationship between aridity
188 index, soil C:N ratio, SOC, and microbial residues (Fig. 3, 4). Instead, we found support
189 for our second conjecture since changes in microbial residues with climate and
190 ecosystem attributes occurred after an irreversible critical point, which may be
191 considered a threshold.

192 An increase in aridity results in limitation of water[17,43], reduction of soil hydraulic
193 conductivity[44], formation of disconnected resource islands[45], and reduction of
194 metabolic activity and substrate utilization efficiency of the soil microbial
195 community[46,47]. Moreover, aridity can reduce the ability of microorganisms to utilize
196 SOC and plant litter for growth and reproduction (microorganisms as decomposers),
197 and thus reduce the efficiency of accumulation of microbial residues[19,48]. Drought
198 conditions can also reduce the production of plant litter and root biomass, resulting in

199 the reduction of plant carbon input[42]. The consequent lack of available substrates in turn
200 can delay the production and accumulation of microbial residues. If the global climate
201 situation improves and aridity stress decreases, soil water content could increase, and
202 ecosystem processes could gradually recover. However, extremely humid climates and
203 active microorganisms are not conducive to the accumulation of SOC. The movement
204 of oxygen in the soil is limited when the soil moisture content is extremely high[45].
205 Under such conditions, growth efficiency of microorganisms is reduced, resulting in
206 lower microbial carbon use efficiency, which in turn affects the production,
207 accumulation, and stabilization of microbial residues in the soil. Moreover, lignin
208 decomposition increases with an increase in water content, and promotes the activity of
209 microorganisms through the "priming effect"[17,21]. The active microbial growth process
210 accelerates the decomposition and utilization of SOC, resulting in the loss of SOC.
211 Hence, these two seemingly contradictory mechanisms coexist in the ecosystem.
212 Additionally, the rapid leaching channel of soil is reused in humid climates[49], and the
213 microbial residues in the soil move to the deep layer, resulting in the sharp reduction of
214 microbial residues in the surface soil.

215 In our study, we also found that aridity significantly affected the accumulation of
216 microbial residues through the soil C:N ratio. Carbon (C) is the primary energy source
217 of microorganisms and nitrogen (N) is an important nutrient, closely related to the
218 growth and development of microorganisms in the biogeochemical cycle[50]. In an
219 ecosystem with limited nutrients and resources, microorganisms tend to synthesize
220 extracellular enzymes, and decompose and release complex organic matter[51]. This

221 weakens the efficiency of MCP and ultimately reduces the relative contribution of
222 microbial residues to SOC[20]. The contribution of microbial-derived carbon to the SOC
223 pool could be promoted by application of fertilizers with a high N content to alleviate
224 environmental pressure[52,53]. This indicates that environments with sufficient nutrient
225 supply accelerate the accumulation of microbial residues in the soil[20,54]. Hence, the
226 proportion of microbial residues in SOC is higher in soil with low soil C:N ratio[13,55]
227 due to a higher soil nutrient content enabling greater anabolism in microorganisms.
228 Moreover, unstable substrates can improve carbon use efficiency of microorganisms,
229 which is conducive to the formation and accumulation of microbial necromass in
230 mineral soils[14,41]. However, we found that soil nutrient supply does not continue to
231 promote the accumulation of microbial residues after reaching a certain level (Fig. 3b1).
232 This may be explained by the effect of the soil C:N ratio on the relative abundance ratio
233 of bacteria/fungi[56]. The distribution of fungi is strongly limited by the availability of
234 resources, and low nutrition and acidic environments are more conducive to bacteria
235 than fungi[56]. However, fungal necromass constitute the majority of the microbial
236 residues. Hence, microbial residues decrease when the conditions do not match the
237 optimal accumulation conditions of fungal residues. Alternatively, we speculate that
238 this could be the result of interactions between drought, plants, and microorganisms.
239 Drought reduces the absorption of nitrogen by plants, resulting in higher contents of
240 ammonium nitrogen and nitrate nitrogen in the soil[57]. Furthermore, the weakening of
241 microbial activity reduces the mineralization rate of nitrogen[58], which leads to a higher
242 C:N ratio in the soil; this shows that the levels of microbial residues are not high at the

243 macro level in the high nutrient soil. In order to clearly identify the changes in microbial
244 residues caused by drought, and the coupling between plants and microorganisms, we
245 fit the values of amino sugars using a GAM. The results showed a clear threshold for
246 the reduction in microbial residue levels (Supplementary Fig. 4).

247 We also analyzed the controlling factors of microbial residue accumulation in
248 different areas. The dominant factors for predicting microbial residues were different in
249 different ecosystems and climatic zones, while SOC and aridity index were important
250 factors in all cases (Fig. 6, 7). Although Random Forest analysis showed that soil C:N
251 ratio was not important in the forest (Fig. 7c, d), it still had large STEs (Fig. 6c, d).
252 Among other environmental variables, soil pH had a large effect on microbial residues
253 in all datasets except temperate forests. Previous studies have shown that soil pH can
254 control the structure of soil microbial community, affect the adsorption of soil minerals
255 on soil organic matter, and even affect the recycling efficiency of microbial residues by
256 the microbial community[56,59]. Therefore, dynamic changes in soil pH can affect the
257 microbially mediated carbon sequestration process[7]. Moreover, we noticed that the
258 effect of pH in subtropical forests was almost similar to that of SOC, which was
259 significantly stronger than the effect of these factors in other regions. This may be
260 because pH in subtropical forests is relatively low, and the accumulation of microbial
261 residues is more sensitive to changes in pH. Similarly, clay mineral content also plays
262 an important role in determining microbial residues[17,60]. The stable storage of SOC is
263 related to both its anti-decomposition property as well as the protection mechanism of
264 soil minerals on SOC[61]. Microbial residues are stabilized in fine-grained soil minerals

by adsorption and binding, and physical protection prevents microbial residues from being reused by microbial communities[52,61,62]. Therefore, soil with a higher clay mineral content generally has a higher organic carbon content[63]. Moreover, clay can maintain the moisture and nutrient elements of the surface soil, resulting in greater surface microbial activity[64,65]. Hence, the effect of clay on the accumulation of microbial residues is not the same in different data sets due to these two factors.

Our research has some limitations. First, most of our data are from the northern hemisphere, especially from the temperate and subtropical regions, with little to no data from the boreal and tropical regions (Figure 1). Although our selection criteria for reducing publication bias of different articles exclude some data, we were able to obtain heterogeneous data. The lack of data presents significant limitations for estimating the global accumulation of microbial residues. Improvements in data collection and accurate estimation of such residues will be the focus of our future study. Second, we used data from the global high-resolution (250 m) gridded soil properties database (http://data.isric.org) because some data were not shown in the original studies. This type of grid prediction data may not match the data from the actual research studies.

In conclusion, our study identified the main factors affecting microbial residues globally and regionally, and we provide evidence for optimal accumulation conditions for microbial residues on a global scale. Our findings provide a useful reference for terrestrial carbon storage management.

Materials and methods

Microbial residues data sources

We extensively searched the Web of Science (http://apps.webofknowledge.com) and the China Knowledge Resource Integrated Database (www.cnki.net) for scientific articles on microbial residues. Data were collected from published field trials before December 2020. The search terms were "amino sugars" or "microbial necromass" or "microbial residues," and were combined with "grassland" or "forest" or "woodland." To reduce the publication bias of different articles, only studies meeting the following six criteria were selected:

1. The sampling depth was clearly defined. We only selected studies on microbial residues in the mineral soil layer at depths of 0–20 cm.
2. Studies in which total contents of glucosamine, galactosamine, muramic acid, and mannosamine were provided in the absence of total amino sugar concentration, since these four types of amino sugars are easy to quantify[12].
3. Manipulation experiments (e.g., warming up, CO_2 rising, and nitrogen addition) were used with the concentration value of amino sugar in the "control" treatment.
4. Data from soil fractions with different aggregate sizes were not used, only those from bulk soil were adopted.
5. Data obtained from the average of a large range of sample points, where accurate location information could not be obtained, were not used.
6. Only data of articles using the method of hydrolyzation with 6 mol/L

hydrochloric acid at 105 °C for 6 to 8 h and determination of the contents by gas or liquid chromatography were used.

To study the factors affecting microbial residues in different communities, grassland and forest ecosystems were divided into tropical, subtropical, and temperate zones. If data from the same study were published in different journals, only the results of one study were used, to avoid pseudoreplicates. The amino sugar content of different altitudes, grassland, or forest types was regarded as an independent duplication in the global analysis for a single study.

We obtained 265 datasets from 64 articles that met the criteria for global analysis, and 3 unpublished grassland data (near Longnan City, Gansu Province, China [Supplementary data, Ref. ID: 65]). This included 1, 52, 95, 15, 40, and 65 datasets of tropical grasslands, subtropical grasslands, temperate grasslands, tropical forests, subtropical forests, and temperate forests, respectively (Supplementary data). At each study site, we also recorded other information from the original publications, such as geographic variables (latitude, longitude, and altitude) and climate variables (mean annual temperature [MAT], mean annual precipitation [MAP], and aridity index). Each data point is indicated in the graph (Fig. 1a).

Other climate and soil attribute data sources

Since most published articles do not include such data, a global climate database (Worldclim, version 2.0) was used to obtain climate information (MAT, MAP). Similarly, the global high-resolution (250 m) gridded soil properties database

(http://data.isric.org) was used to obtain some soil physical and chemical properties (i.e., soil clay content, soil pH, SOC content, and soil total nitrogen (TN) content). Aridity index data were obtained from the Global Aridity and PET Database (http: www//www.cgiar-csi.org/data/global-aridity-and-pet-database). The Global Land Cover Characteristics Database v2.0 was used to obtain altitude data (https://lta.cr.usgs.gov/GLCC). In order to evaluate the consistency of the predictors of microbial residues, we ensured that the depth of data used in the database is consistent with the depth of sample points. These data were obtained by ESRI ArcMap (Environmental Systems Research Institute, Redlands, CA, USA).

Calculation of amino sugar concentrations

We calculated the absolute concentration of amino sugars (mg/g soil) in the surface layer (0–20 cm) of soil. The total amino sugar content is equal to the sum of glucosamine, galactosamine, muramic acid, and mannosamine contents.

Some studies only reported the content of fungal necromass carbon and bacterial necromass carbon in SOC, which is based on the concentration of glucosamine and muramic acid combined with a conversion factor. The absolute concentrations of muramic acid (mg/g soil) and glucosamine (mg/g soil) were calculated as follows[25]: equation (1), equation (2).

In these equations, 45 is the conversion factor from muramic acid to bacterial necromass C, and 9 is the conversion factor from glucosamine to fungal necromass C. It is assumed that the ratio of muramic acid and glucosamine in bacterial cells is 1:2[25,66].

In some studies, only glucosamine carbon, galactosamine carbon, muramic acid carbon, and mannosamine carbon were reported. Since muramic acid is formed of 9 carbon atoms, while the other amino sugars are formed of 6 carbon atoms, the absolute concentration of each amino sugar (mg / g soil) was calculated as follows[40]:

equation (3), equation (4), equation (5), equation (6).

Statistical analysis

Significance test of microbial residues

Unless otherwise specified, all statistical analyses were conducted using R 4.0.3. Before conducting statistical analysis, we tested the normality of all data. Scheirer–Ray–Hare test was used to test the differences in microbial residues across vegetation types, climate zones, and to determine their interactions. Additionally, differences in microbial residues among tropical grasslands, subtropical grasslands, temperate grasslands, tropical forests, subtropical forests, and temperate forests were directly analyzed by the Kruskal–Wallis test. Two-tailed Spearman's linear correlation was used to explore global correlations between microbial residues and environmental variables. Statistical significance was assessed at $P < 0.05$.

Evaluating the importance of environmental variables

We used all the microbial residue data for model analysis. We used Random Forest models to determine the importance of environmental variables (absolute latitude, aridity index[17], soil clay content, SOC, soil C:N ratio, and soil pH)[67,68]. The Random

Forest model is a machine learning algorithm for regression and classification. The importance of variables is evaluated by classifying multiple decision trees[67]. Since our purpose is only to determine the importance of predictors, and not to predict the data, we used the whole dataset for analysis, without dividing the data into training set and prediction set. These analyses were performed using the randomForest[69] package in R 4.0.3 (http://cran.R-project.org/). The significance of the model and the cross-validation R^2 were evaluated by using the A3 package. Similarly, the rfPermute package was used to assess the significance of each predictor's importance to microbial residues.

We used SEM to evaluate the direct and indirect relationships between environmental factors (absolute latitude, aridity index, soil clay content, SOC, soil C:N ratio, and soil pH) and microbial residues. Because the correlation between SOC and TN was significant (Spearman's R = 0.91), only SOC was selected as the organic component of SEM. Before performing the SEM, we performed logarithmic transformation for non-normal variables and standardized each variable using the Z-score transformation to improve the comparability of the data[70]. We built a prior model (Supplementary Fig. 5) based on existing knowledge, and determined the final SEM through maximum likelihood estimation and based on an overall goodness-of-fit, including chi-square (χ^2) statistics, whole-model P value, goodness-of-fit index, and the root-mean-square error of approximation[71]. Since some variables were non-normal, the Bollen–Stine bootstrap test was used to recalculate the overall fit of the model[71]. When the bootstrap P value was greater than 0.1, the model was considered to have a good fit[71]. In order to integrate the function of SEM, we calculated the STE of each

environmental variable. Since most studies on forests and grasslands were located in subtropical and temperate zones (87.50%, 99.32%, respectively, Fig. 1), we only conducted independent Random Forest models and SEM analysis on temperate and subtropical forests and grasslands. SEM analysis was performed using the Amos 26.0 (Amos IBM, USA).

Evaluation of linear and nonlinear responses of environmental variables

For global data, we fitted linear and non-linear (e.g., GAM[72]) regressions to the relationships between variables with the large effect values and microbial residues shown by SEM. The linear model assumes that the response of microbial residues to environmental variables is gradual[73]. The GAM models show that the gradient of environmental variables is nonlinear but continuous[73]. We chose the GAM model to describe the complexity of nonlinear trends (through smoothing parameters[72]). We then used the AIC to determine the best fit model for each environmental variable[73]. In general, a difference in AIC values greater than 2 indicates that the models are significantly different, with the most likely model being the one with the lowest AIC value[73].

Threshold detection

The existence of thresholds can be explored and nonlinear trends can be determined only when the nonlinear model is suitable[73]. Following Goffman et al[74] and Miguel et al[73], we fitted segmented regressions by actively searching for continuous thresholds

418 (abrupt change of slope on both sides of the threshold[75]) and searching for
419 discontinuous thresholds or breakpoint to fit step + segmented (stegmented) regressions
420 (change of intercept and slope on both sides of the threshold[75]). In addition, when
421 segmented or stegmented regression is fitted to the GAM regression model, segmented
422 or stegmented regression can prove the maximum curvature point of fitting[73]. This can
423 be considered as a threshold because it shows the extreme value of microbial residue
424 response to environmental variables, even if the fitting of segmented or stegmented
425 regression is worse than that of the GAM model[73].

426 Therefore, for those environmental variables that the GAM models fit better than
427 linear models, we fit segmented and stegmented regressions. These models all provide
428 a threshold point for prediction, which proves the change of functional relationship
429 (slope or slope + intercept of segmented and stegmented regressions, respectively[75]).
430 We consider this as the threshold of the GAM regression model. We used the AIC to
431 select the most suitable threshold model for data. Segmented/stegmented and GAM
432 regressions were fitted with chngpt[75] and gam packages in R 4.0.3, respectively.

433

434 ***Verifying the importance of the determined threshold***

435 To test whether the determined threshold significantly affects the intercepts of
436 stegmented regressions, we conducted linear regressions on both sides of the
437 threshold[73]. Then, we extracted the intercepts, we used the boots package in R to
438 perform 1000 bootstrap samplings before and after the threshold of environmental
439 variables for prediction, and tested the difference using the Mann–Whitney U test.

The global map, fitting curve, and histogram of sample distribution in this study were all plotted using R 4.0.3.

Data Availability

The data that support the findings of this study are available in the Supplementary Data.

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Competing interests

The authors declare no competing interests.

Figure legends

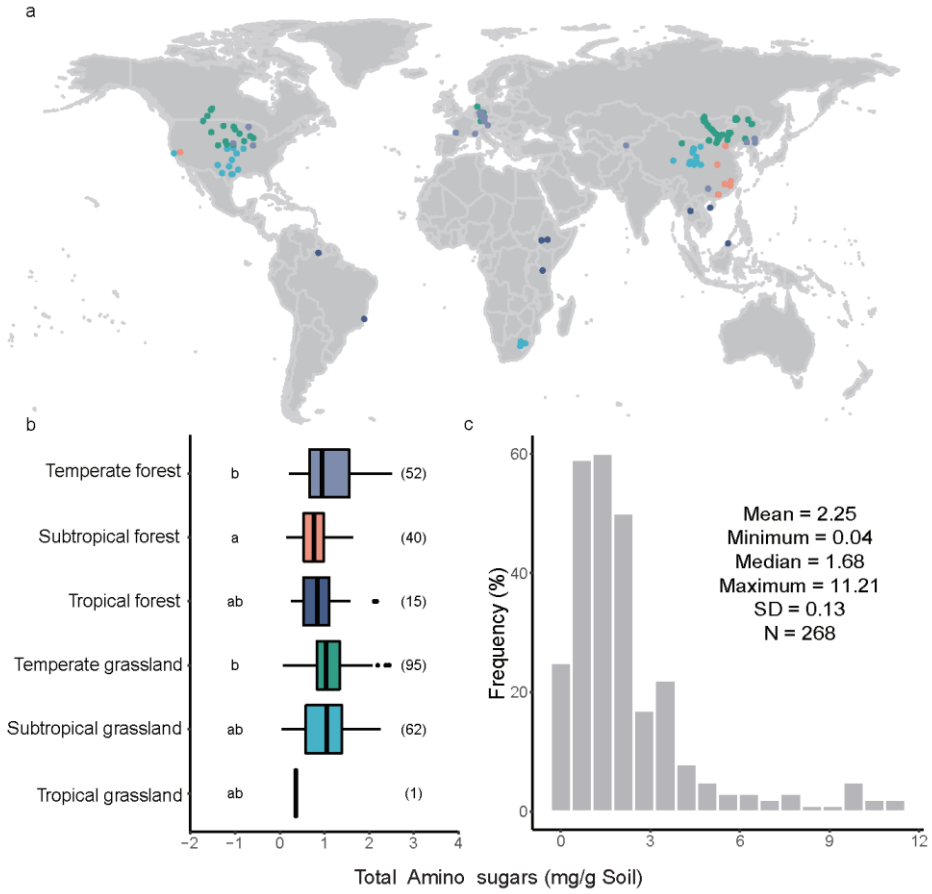
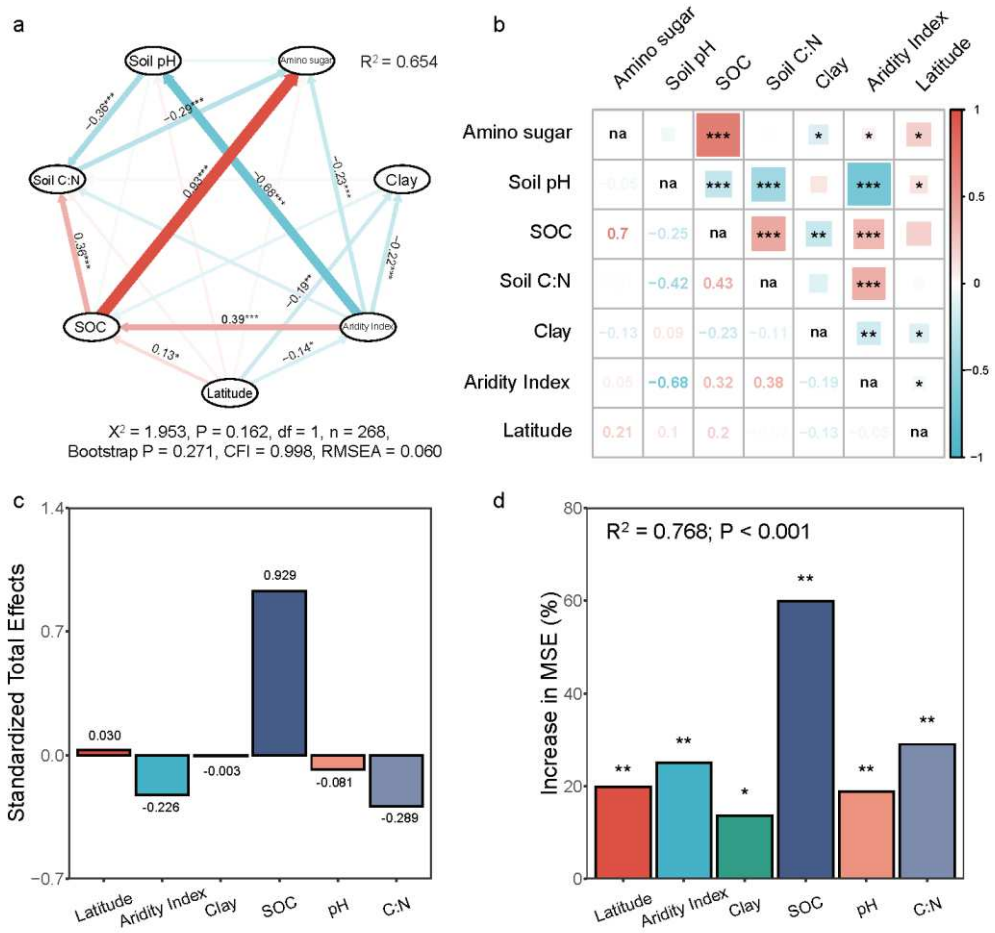


Fig. 1 Location of data points used in the study (a). For the Kruskal–Wallis test between different ecosystems and climatic zones, the letters in the figure indicate significant differences such that conditions with the same letters are not significantly different (b). The frequency distribution histogram of the original data of microbial residues and the numbers in the graph show basic statistical information (c).



665

666 **Fig. 2. Models of relationships between environmental variables and microbial**
667 **residue.** Panels show structural equation model (SEM) (a), correlations between
668 environmental variables (b), the standard total effect of SEM (c), and Random Forest
669 model (d) on a global scale. The Random Forest model shows the average predictive
670 importance (mean square error (MSE) increase percentage) of each environmental
671 factor in the absolute content of soil amino sugar (characterization of microbial
672 residues). SEM shows the causality and correlation between the absolute content of
673 amino sugar (characterization of microbial residues) in soil and the environmental
674 variables. Red lines indicate positive effects, while blue lines indicate negative effects.

675 The thickness and color of the line were directly proportional to the standardized path
676 coefficient on the single arrow. SOC: soil organic carbon. R^2 represents the variance of
677 biomarkers explained by the model. The asterisk (*) indicates the significance of the
678 path, and "*," "*," and "***" indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.
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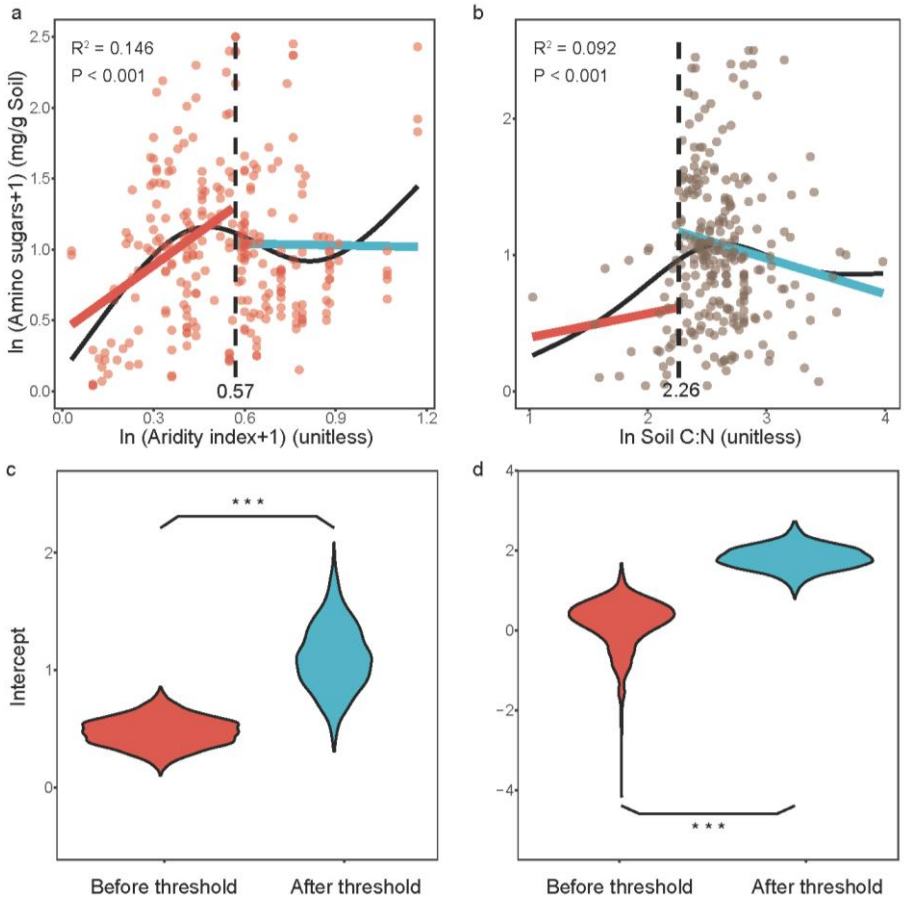

680

681 **Fig. 3 Modeling of microbial residues in response to aridity index and soil C:N**

682 **ratio.** Nonlinear responses of microbial residues to aridity index and soil C:N ratio (a,

683 b), and differences of predicted values of variables under aridity and soil C:N ratio

684 threshold (c, d). The data of each variable (a, b) are log-transformed. In (a, b), the black

685 solid line, the red solid line, and the blue solid line represent the smoothed trendline

686 fitted by the general additive model (GAM), and the linear fitting on the left and right

687 sides of each threshold, respectively. Black illustrated numbers and vertical dashed lines

688 describe the identified threshold. Violin graphs (c, d) show the bootstrap predictions of

689 each variable on each side of each threshold (red plots: regression before the threshold;

690 blue plots: regression after the threshold). The asterisks "***" indicate a significant

691 difference according to the Mann–Whitney U test before and after the threshold at $P <$

692 0.001.

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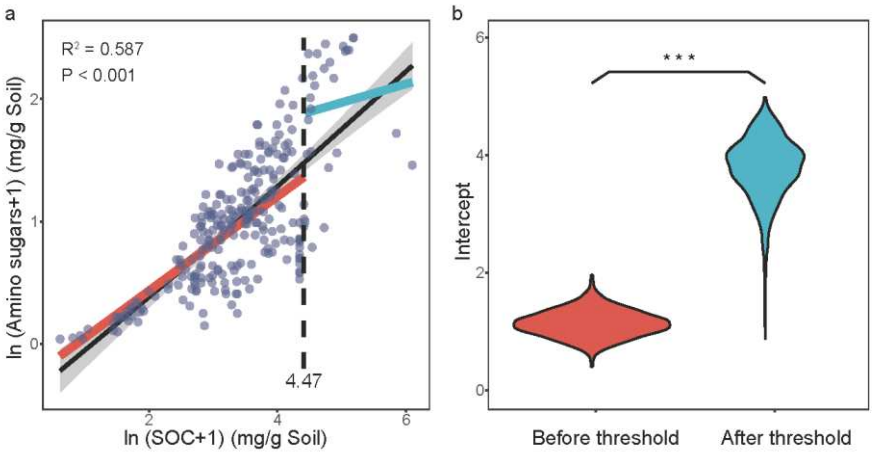


694

695 **Fig. 4 Modeling of microbial residues in response to soil organic carbon (SOC).**

696 Nonlinear responses of microbial residues to SOC (a), and differences of predicted

697 values of variables under SOC threshold (b). The data are logarithmically transformed

698 (a). Rest of the details similar to figure 3.

699

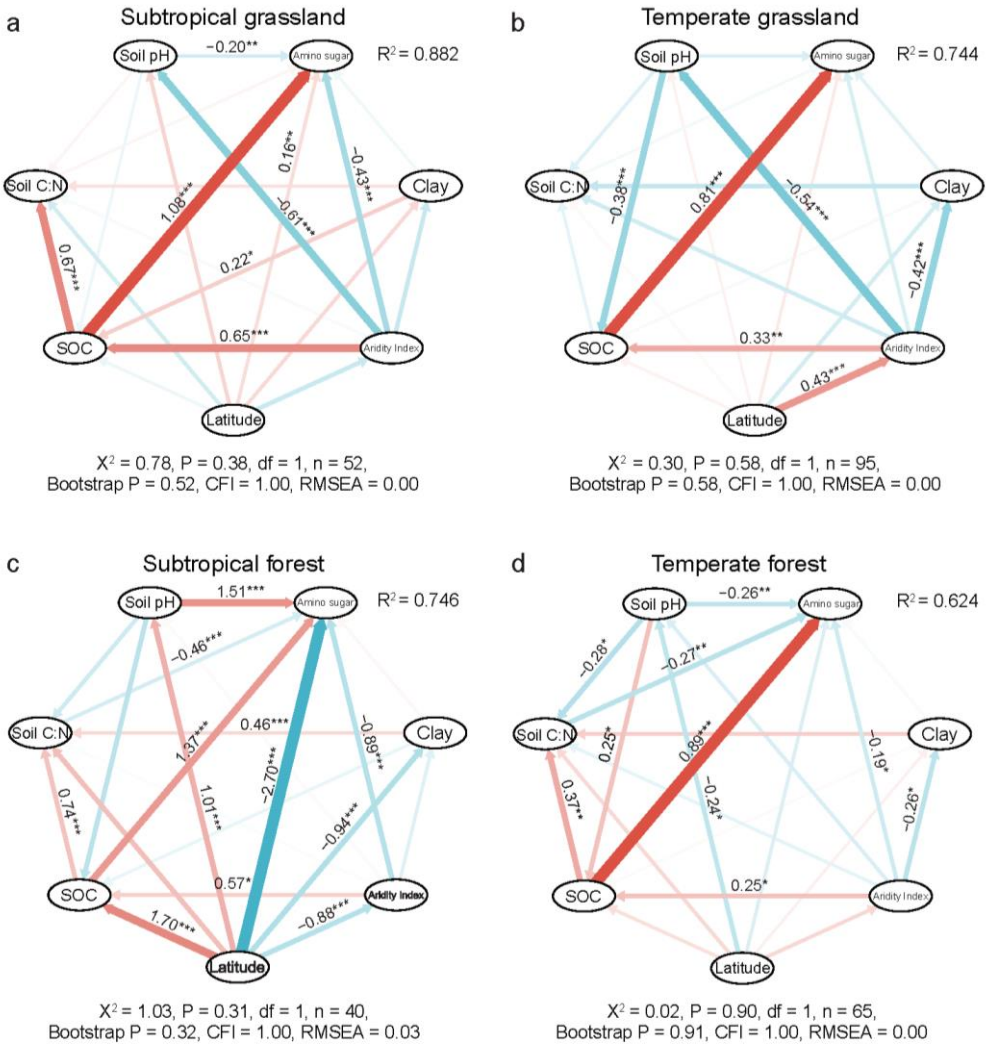
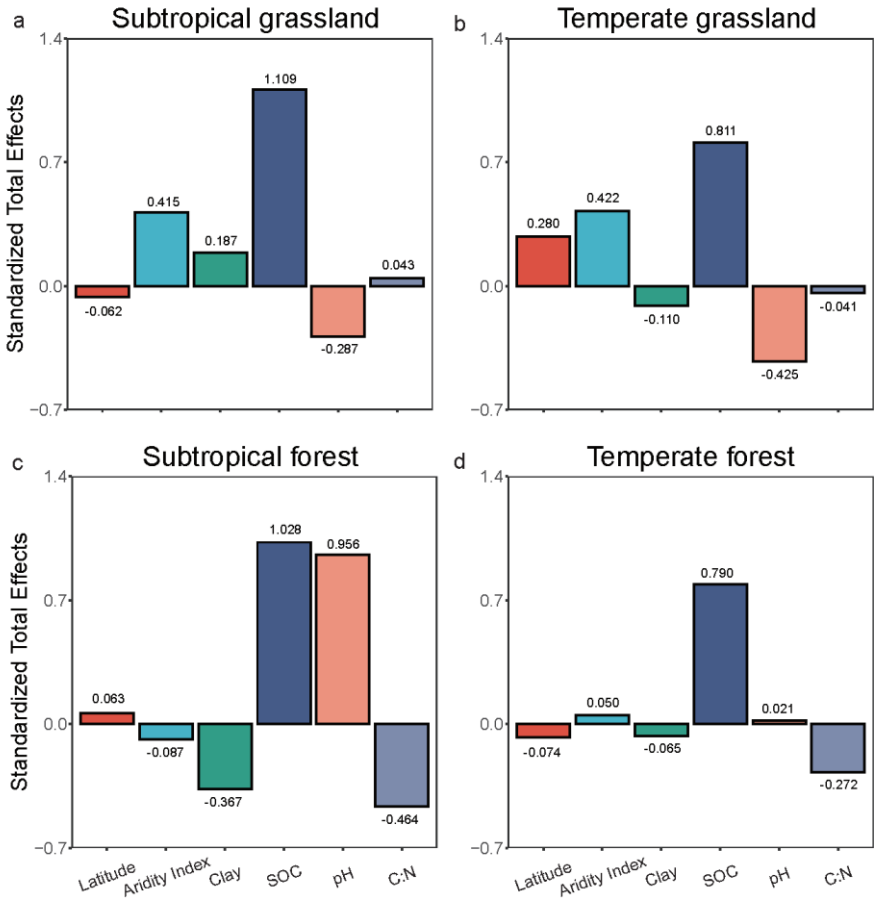


Fig. 5 Structural equation models (SEMs) of different ecosystems and climatic zones. The SEMs show the causality and correlation between the absolute content of soil amino sugar (characterization of microbial residues) and environmental variables. Red lines indicate positive effects, while blue lines indicate negative effects. The thickness and color of the line are directly proportional to the standardized path coefficient on the single arrow. SOC: soil organic carbon. R2 represents the variance of biomarkers explained by the model. The asterisk (*) indicates the significance of the path, and "*," "**," and "***" indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.



709

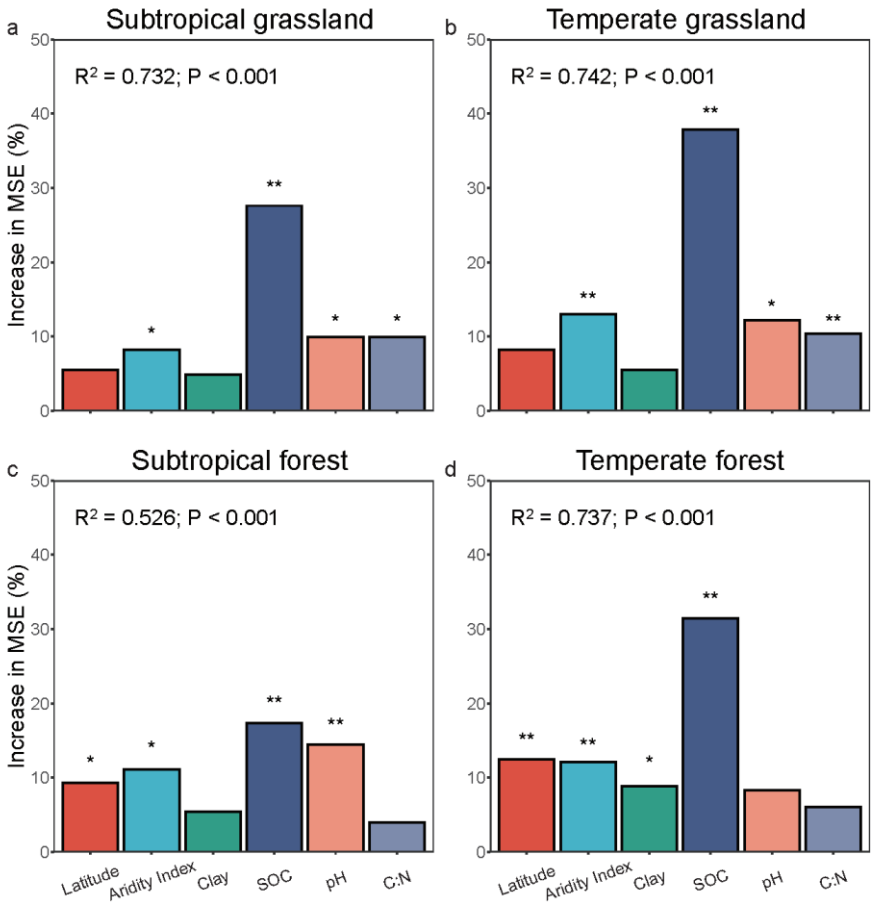
710 **Fig. 6 Standard total effects of environmental variables on the absolute content of**

711 **soil amino sugars**. Standard total effects (direct and indirect effects) in structural

712 equation models of subtropical grasslands (a), temperate grasslands (b), subtropical

713 forests (c), and temperate forests (d).

714



715

716 **Fig. 7 Random Forest models of different ecosystems and climatic zones.** Random

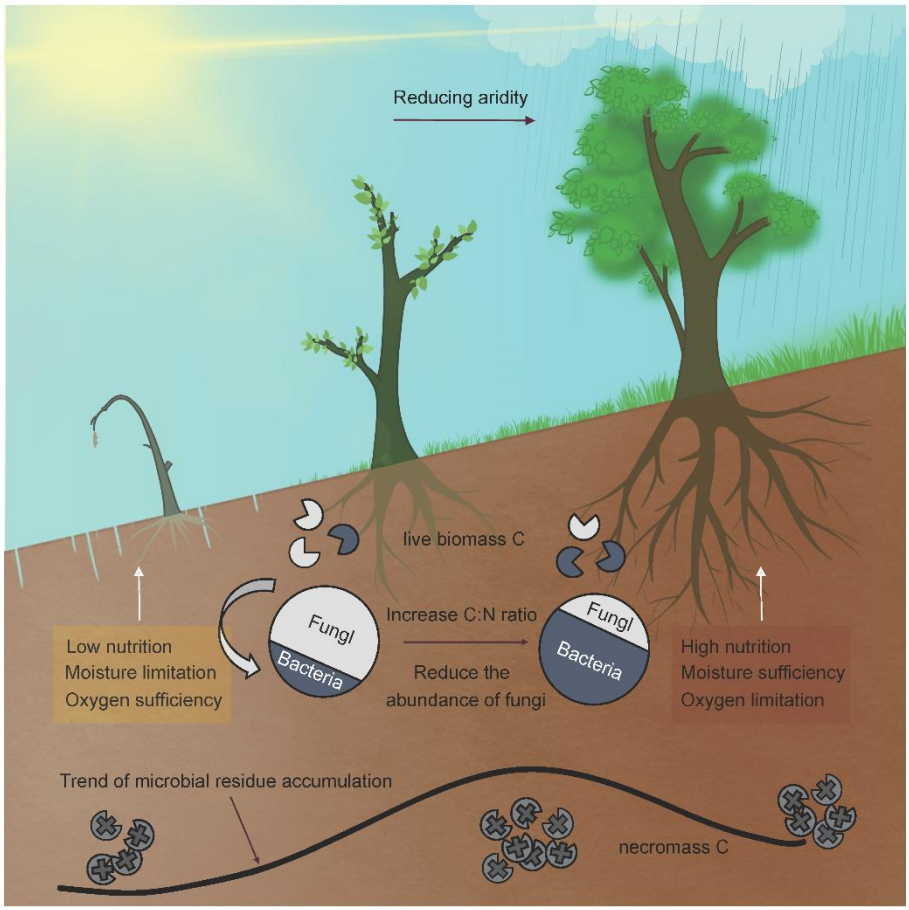
717 Forest models show the average predictive importance (mean square error (MSE)

718 increase percentage) of each environmental factor in the absolute content of soil amino

719 sugar (characterization of microbial residues). An asterisk (*) indicates the significance

720 of each predictor, and "*" and "**" indicate $P < 0.05$ and $P < 0.01$, respectively.

721



722

723 Fig. 8 **Schematic diagram of microbial residue accumulation.** With a decrease in
724 aridity, the climate became humid, and the soil C:N ratio increased. In this process, the
725 content of microbial residues increased at first and then decreased, and there was a sharp
726 decrease in threshold. This is due to the impairment of biological processes that
727 contribute to the accumulation of microbial-derived carbon, and the shift in relative
728 abundance of fungi and bacteria.

729

730 **Tables**

731 **Table 1** Scheirer–Ray–Hare test of the effects of ecosystem type (forest and grassland),
732 climate zone (tropical, subtropical, and temperate), and their interaction on soil
733 respiration amino sugar content (characterization of microbial residues).

	SS	df	H	P
Climatic zone	47057	2	7.8332	0.019
Ecosystem type	15248	1	2.5381	0.111
Ecosystem type * Climatic zone	28441	2	4.7344	0.094

SS, sum of squares.

734

735

736 **Table 2 The best model of microbial residue response to each variable.** Akaike
737 information criteria (AIC) are used to compare the fits of linear, nonlinear, and optimal
738 threshold models. Models with lower AIC values show the best fits. The determination
739 coefficients (R^2) of linear and best-fit threshold models are also shown. GAM: general
740 additive model.

Variable name	AIC linear	AIC nonlinear	Best AIC	R^2 linear	R^2 threshold
Aridity index	434.88	431.50	GAM=406.68	0.016	0.130
Soil C/N ratio	439.06	433.19	GAM=423.83	0.000	0.108
Soil organic carbon	223.35	223.92	GAM=211.03	0.553	0.596

741

742

Equations

$$\text{Muramic acid}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)=\frac{\text{Bacterial necromass C}\left(\frac{\text{mg}}{\text{g}}\text{SOC}\right)\times\text{C}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)}{1000\times45} \quad (1)$$

$$\text{Glucosamine}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)=\left(\frac{\text{Fungal necromass C}\left(\frac{\text{mg}}{\text{g}}\text{SOC}\right)\times\text{C}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)}{1000\times9\times179.17}+\frac{2\times\text{Muramic acid}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)}{251.23}\right)\times179.17 \quad (2)$$

$$\text{Muramic acid}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)=\frac{\text{Muramic acid C}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)\times251.23}{108} \quad (3)$$

$$\text{Glucosamine}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)=\frac{\text{Glucosamine C}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)\times179.12}{72} \quad (4)$$

$$\text{Galactosamine}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)=\frac{\text{Galactosamine C}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)\times179.12}{72} \quad (5)$$

$$\text{Mannosamine}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)=\frac{\text{Mannosamine C}\left(\frac{\text{mg}}{\text{g}}\text{soil}\right)\times179.12}{72} \quad (6)$$

Figures

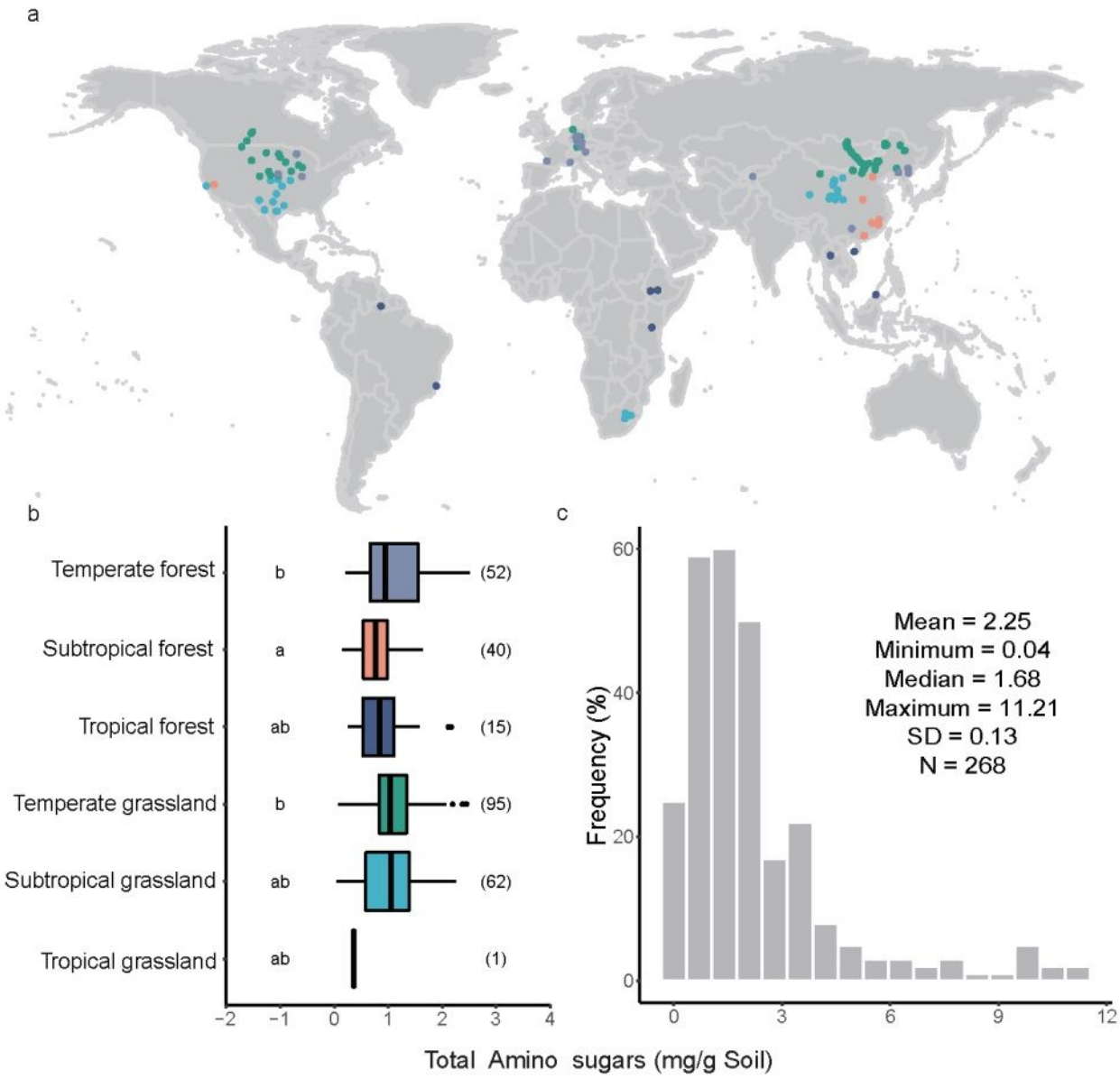


Figure 1

Location of data points used in the study (a). For the Kruskal–Wallis test between different ecosystems and climatic zones, the letters in the figure indicate significant differences such that conditions with the same letters are not significantly different (b). The frequency distribution histogram of the original data of microbial residues and the numbers in the graph show basic statistical information (c).Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country,

territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

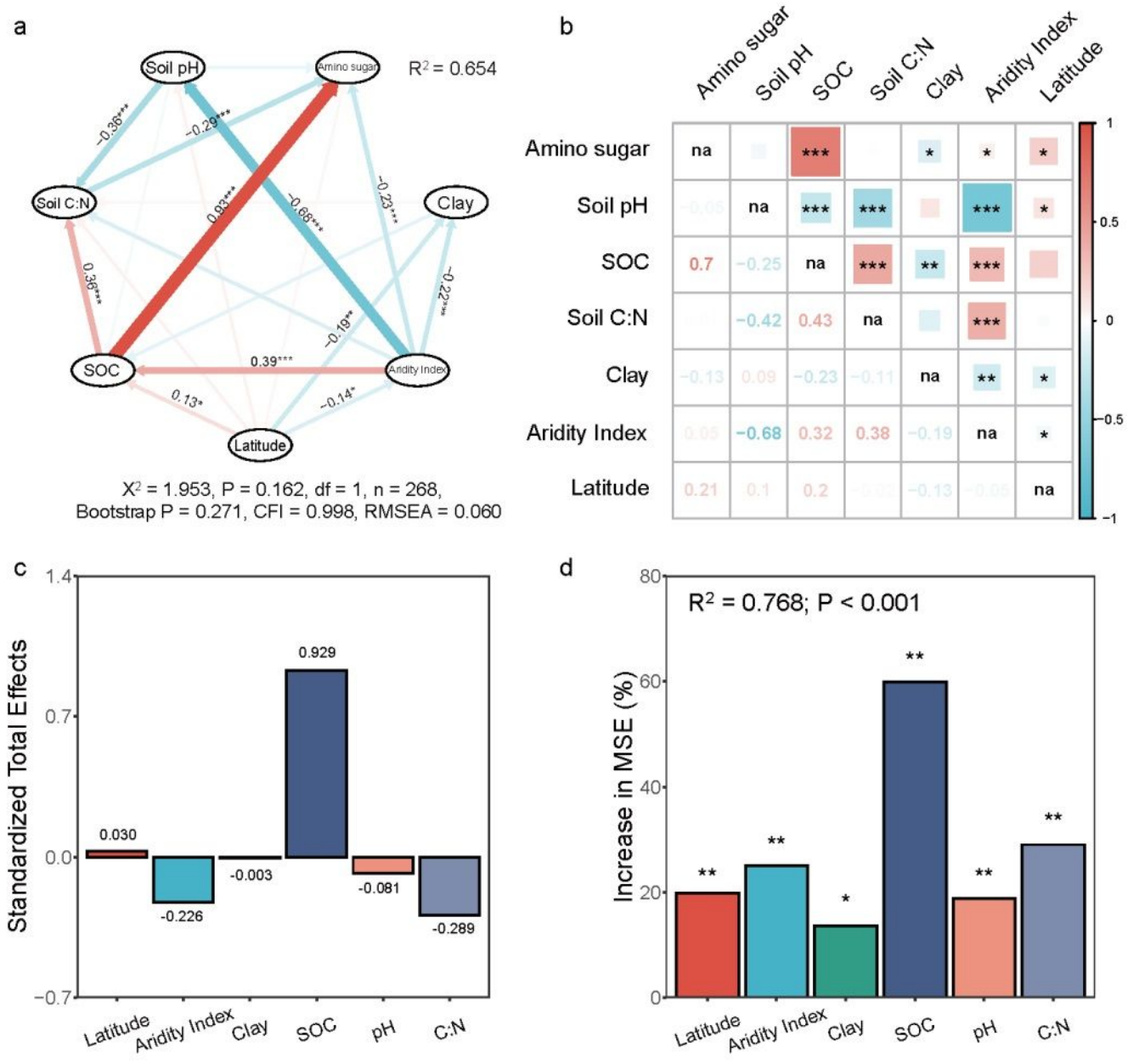


Figure 2

Models of relationships between environmental variables and microbial residue. Panels show structural equation model (SEM) (a), correlations between environmental variables (b), the standard total effect of SEM (c), and Random Forest model (d) on a global scale. The Random Forest model shows the average predictive importance (mean square error (MSE) increase percentage) of each environmental factor in the absolute content of soil amino sugar (characterization of microbial residues). SEM shows the causality and correlation between the absolute content of amino sugar (characterization of microbial residues) in

soil and the environmental variables. Red lines indicate positive effects, while blue lines indicate negative effects. The thickness and color of the line were directly proportional to the standardized path coefficient on the single arrow. SOC: soil organic carbon. R2 represents the variance of biomarkers explained by the model. The asterisk (*) indicates the significance of the path, and "*," "*," and "***" indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

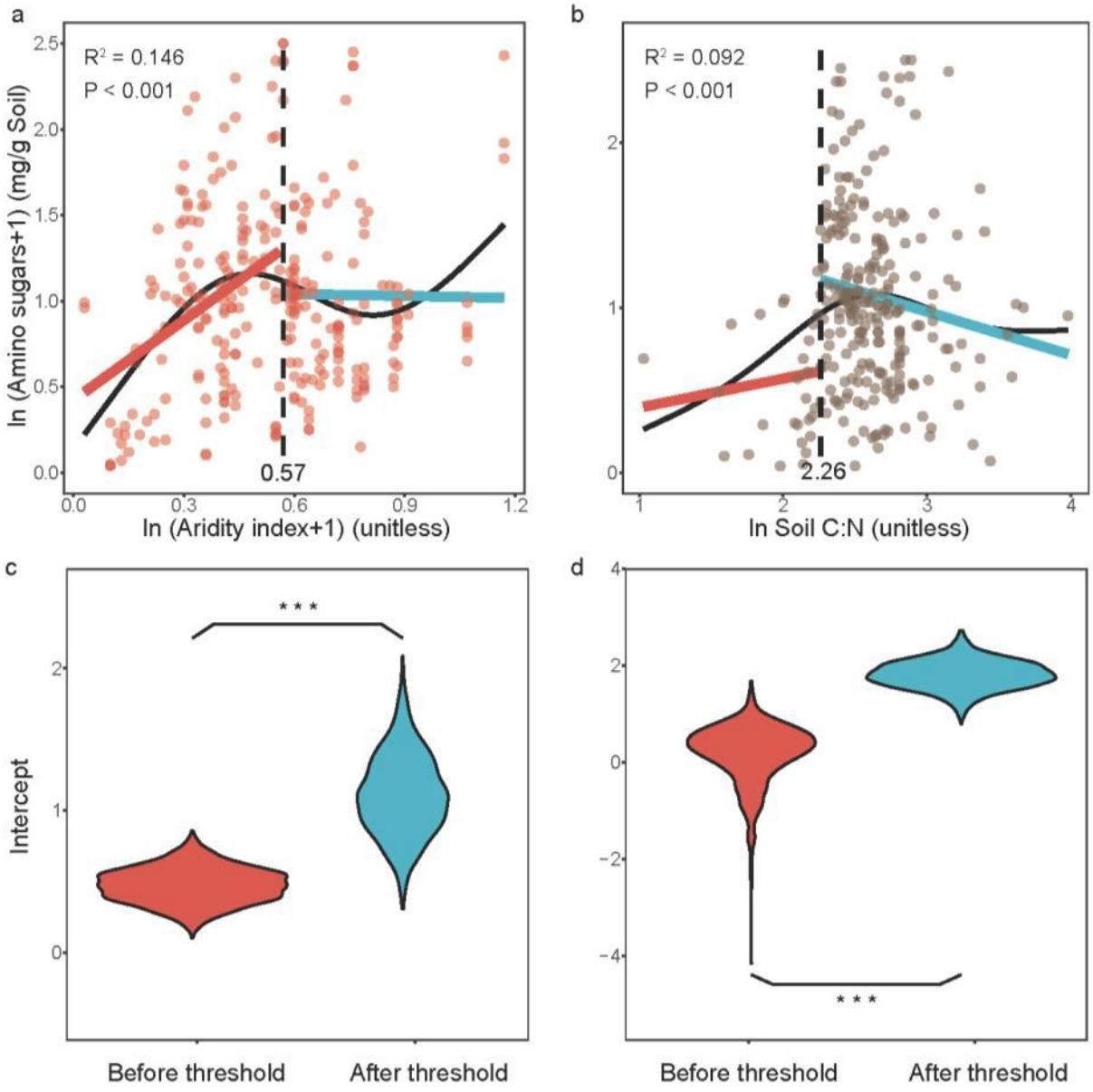


Figure 3

Modeling of microbial residues in response to aridity index and soil C:N ratio. Nonlinear responses of microbial residues to aridity index and soil C:N ratio (a, b), and differences of predicted values of variables under aridity and soil C:N ratio threshold (c, d). The data of each variable (a, b) are log-transformed. In (a, b), the black solid line, the red solid line, and the blue solid line represent the smoothed trendline fitted by the general additive model (GAM), and the linear fitting on the left and right sides of

each threshold, respectively. Black illustrated numbers and vertical dashed lines describe the identified threshold. Violin graphs (c, d) show the bootstrap predictions of each variable on each side of each threshold (red plots: regression before the threshold; blue plots: regression after the threshold). The asterisks "***" indicate a significant difference according to the Mann–Whitney U test before and after the threshold at P < 691 0.001.

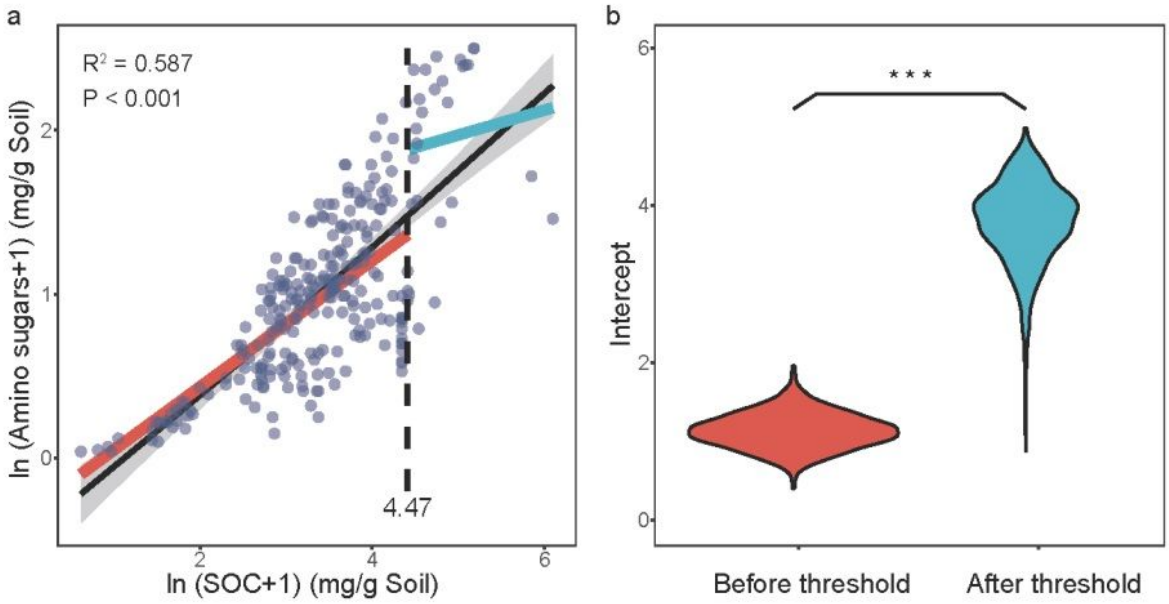


Figure 4

Modeling of microbial residues in response to soil organic carbon (SOC). Nonlinear responses of microbial residues to SOC (a), and differences of predicted values of variables under SOC threshold (b). The data are logarithmically transformed (a). Rest of the details similar to figure 3.

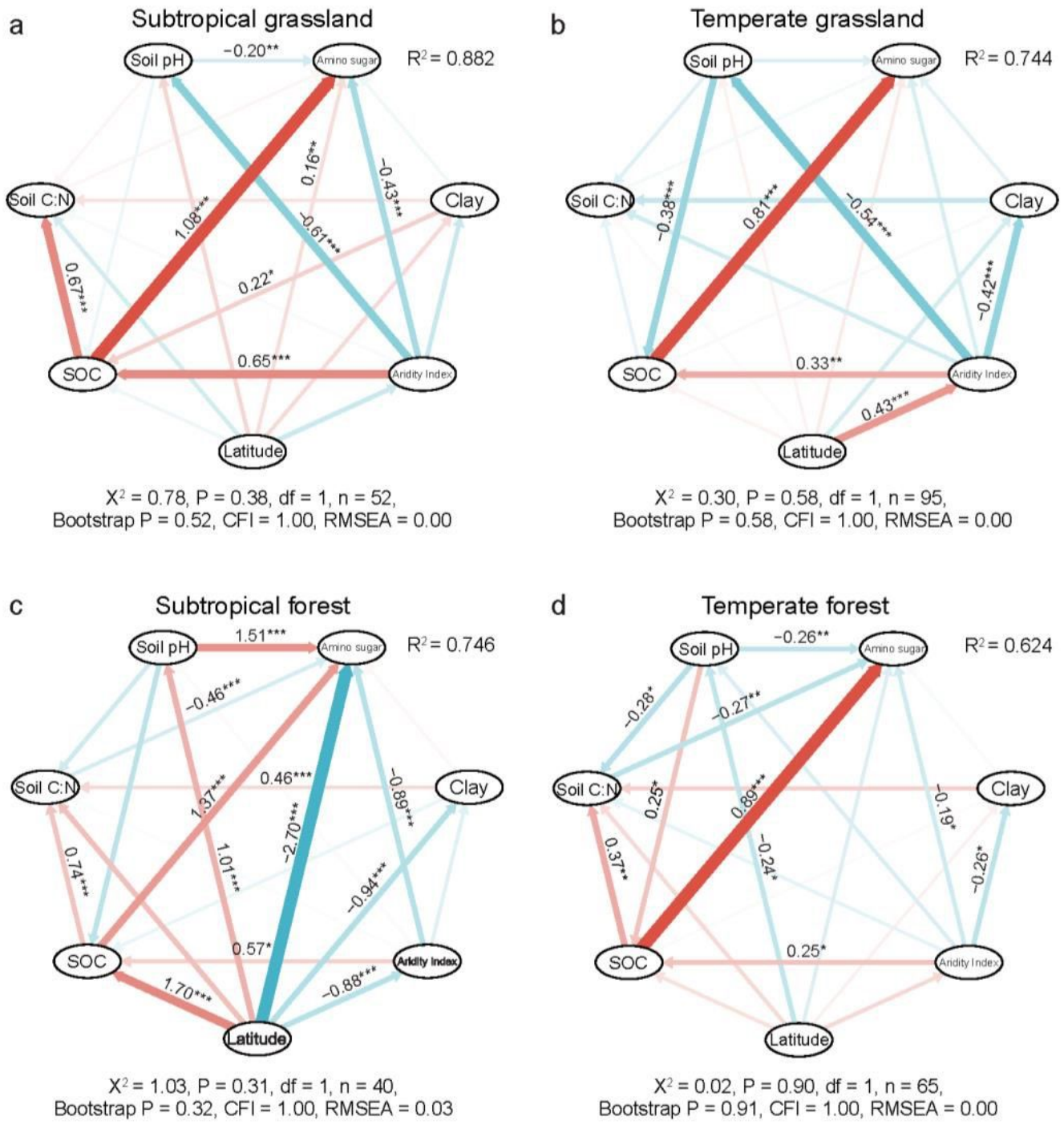


Figure 5

Structural equation models (SEMs) of different ecosystems and climatic zones. The SEMs show the causality and correlation between the absolute content of soil amino sugar (characterization of microbial residues) and environmental variables. Red lines indicate positive effects, while blue lines indicate negative effects. The thickness and color of the line are directly proportional to the standardized path coefficient on the single arrow. SOC: soil organic carbon. R2 represents the variance of biomarkers

explained by the model. The asterisk (*) indicates the significance of the path, and “*,” “**,” and “***” indicate P < 0.05, P < 0.01, and P < 0.001, respectively.

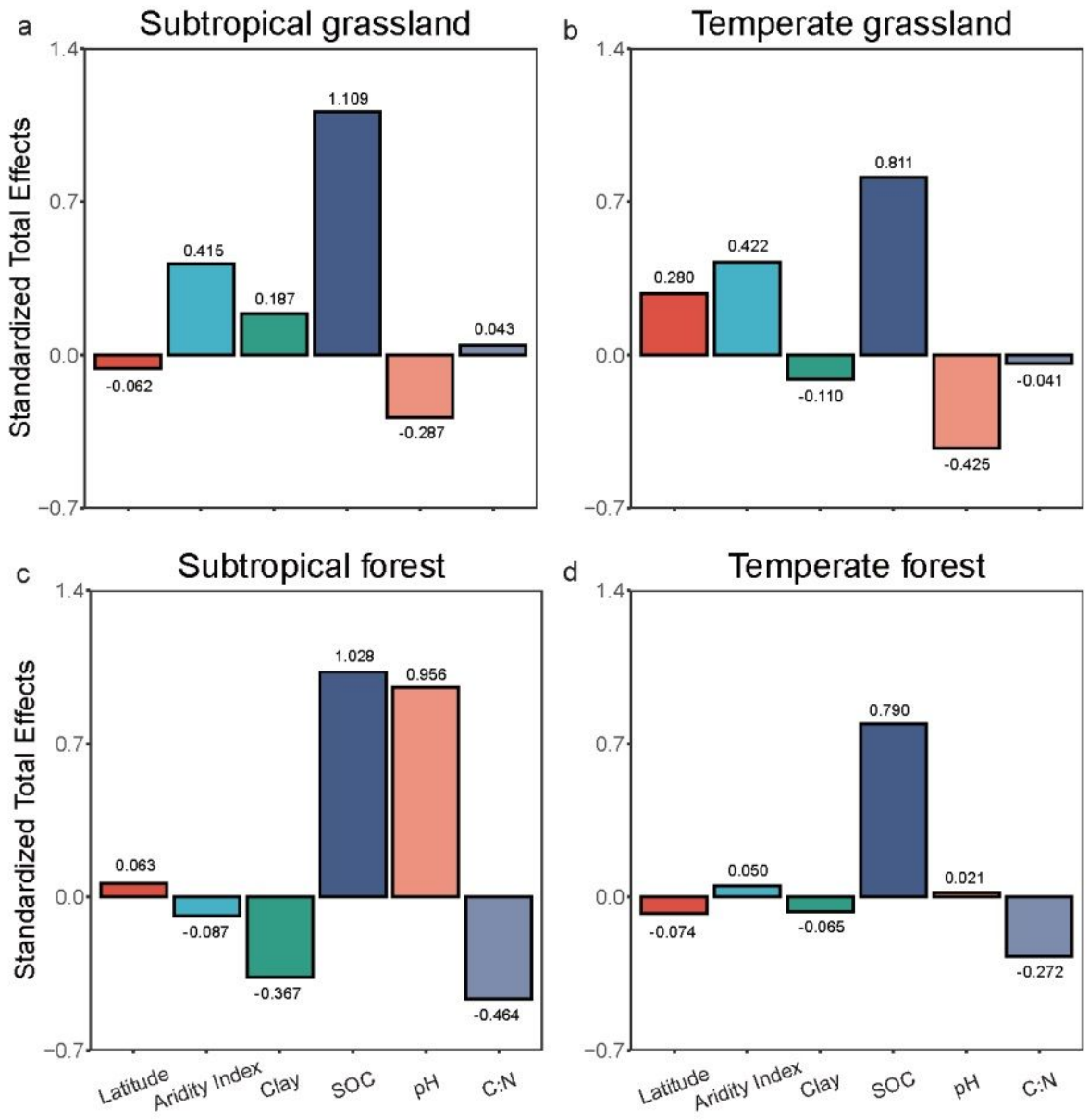


Figure 6

Standard total effects of environmental variables on the absolute content of soil amino sugars. Standard total effects (direct and indirect effects) in structural equation models of subtropical grasslands (a), temperate grasslands (b), subtropical forests (c), and temperate forests (d).

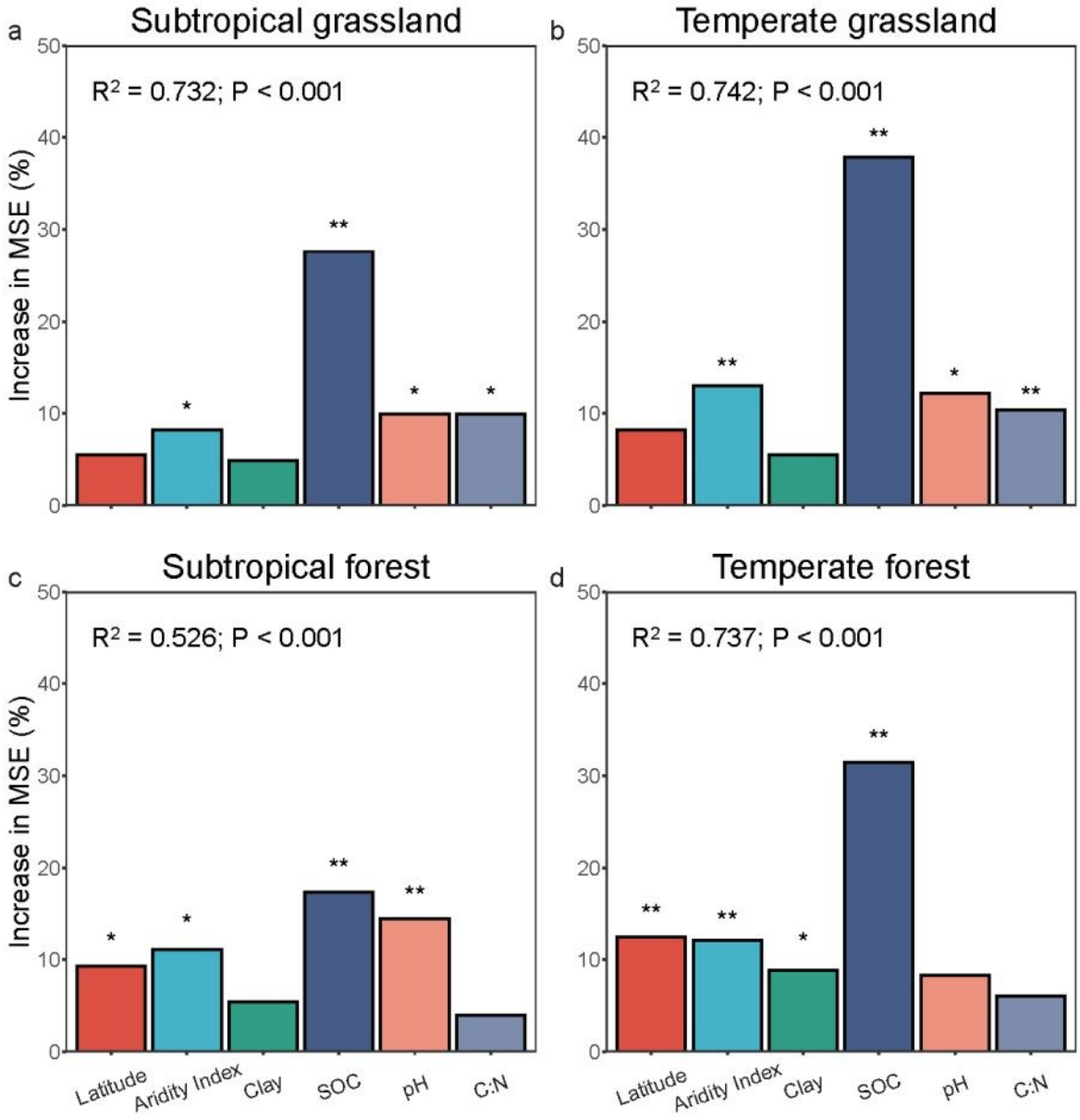


Figure 7

Random Forest models of different ecosystems and climatic zones. Random Forest models show the average predictive importance (mean square error (MSE) increase percentage) of each environmental factor in the absolute content of soil amino sugar (characterization of microbial residues). An asterisk (*) indicates the significance of each predictor, and “*” and “**” indicate P < 0.05 and P < 0.01, respectively.

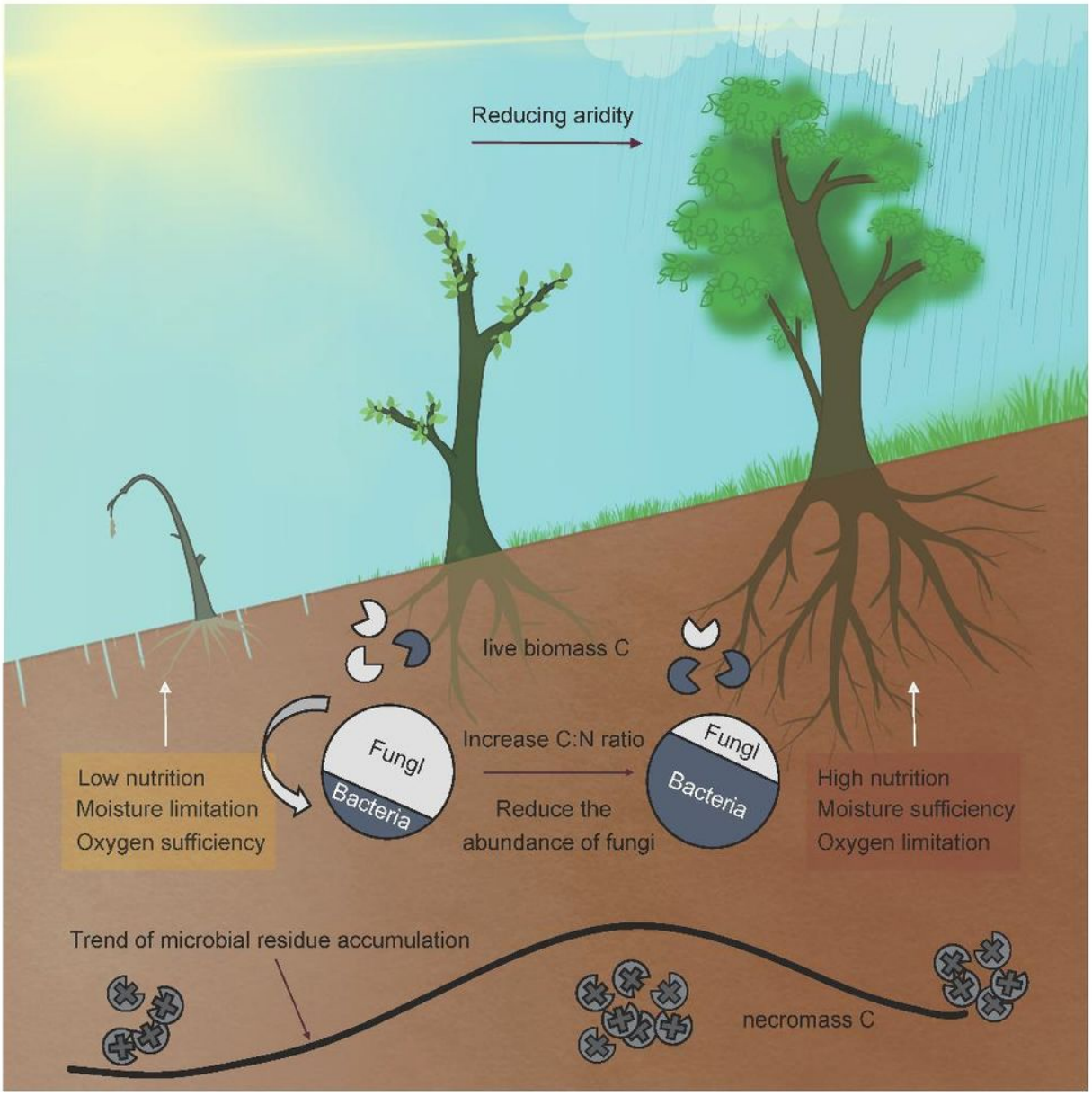


Figure 8

Schematic diagram of microbial residue accumulation. With a decrease in aridity, the climate became humid, and the soil C:N ratio increased. In this process, the content of microbial residues increased at first and then decreased, and there was a sharp decrease in threshold. This is due to the impairment of biological processes that contribute to the accumulation of microbial-derived carbon, and the shift in relative abundance of fungi and bacteria.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata.xlsx
- Supplementaryinformation.pdf