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Original Articles

# Independent response of soil DOM to MAT and MAP: Evidence from a large-scale survey of moss crusts in mainland China

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ABSTRACT

It is known soil dissolved organic matter (DOM) plays an important role in global biogeochemical cycle and is an important factor in soil-forming processes. However, the linkage between the diversity of chemical composition and properties of DOM molecules and the climatic conditions (temperature, precipitation) at regional scales is still not well studied. Therefore, in our study, we selected 33 soil DOM samples which were, divided into temperature-variation and precipitation-variation groups, and used Fourier transform ion cyclotron resonance mass spectrometry to analyze the influence of climate on the chemical characterizations of DOM. Our results showed that although the factors affecting the chemical properties of DOM are complex, mean annual precipitation (MAP) and mean annual temperature (MAT) can explain part of the chemical changes of DOM, and the two data sets in this study can well separate the effects of MAP and MAT on DOM, respectively. The results showed that the content of lignin and tannin were positively related to MAP, while carbohydrate, lipid, unsaturated hydrocarbon and protein decreased with the increase in MAP. The content of nitrogen-containing molecules gradually decreased with the increase in MAT. Meanwhile, the content of sulfur-containing molecules was almost unaffected by the MAP and MAT. In terms of DOM composition, the content of protein decreased with the increase in MAT. The results suggested that the chemical characteristics of DOM is an ecosystem attribute that is closely related to the environment and may be used to predict large-scale soil biochemical processes in the soil carbon cycle.

## 1. Introduction

Dissolved organic matter (DOM) plays an important role in global biogeochemical cycles and is an important factor in the process of soil formation (Delgado-Baquerizo et al., 2013; Sun et al., 2021; Zhao et al., 2021). DOM is an important link between terrestrial and aquatic ecosystem organic matter (Zhao et al., 2019; Zhu et al., 2020). Studying soil DOM will help us better understand the global carbon cycle and other biogeochemical processes associated with it.

The characteristics of DOM in soil can be influenced by factors such as climate, altitude, land use and land management (Gmach et al., 2019; Li et al., 2019; Chen et al., 2021). In general, high temperature accelerates the degradation of DOM and increases the humification degree, leading to an increase in the aromatic content of its components and a decrease in bioavailability, especially carbohydrate-based and peptide-based molecules can be significantly reduced (Wilcke et al., 2020; Du et al., 2021). Reduced precipitation causes drought, and mild drought can increase soil microbial biomass, while moderate and severe drought reduces soil microbial biomass and thus affects soil DOM composition (Wilcke et al., 2020). Plants would grow faster when soil moisture increases, which causes more plant fallout and root secretions to enter the soil environment and thus causes changes in DOM composition (Li et al., 2018b; Guo et al., 2020). In addition, land use can influence soil's physical and chemical properties and the source of DOM, which in turn affects the composition structure of DOM (Li et al., 2018b; Shang et al., 2018). Currently, there have been several studies on the patterns and mechanisms of climate influencing DOM composition, through large-scale sampling to analyze the changes of DOM under climate gradients

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(Zhou et al., 2018; Ding et al., 2020; Jiao and Lu, 2020).

However, there are two main limitations in such studies. Firstly, the effect of land use on DOM components is usually ignored. For example, Gao et al. (2017) analyzed 19 soil samples of farmland types in China, but it is unknown whether the farmland crop types and tillage practices are consistent Secondly, due to the synchronization of rain and heat in a large number of terrestrial areas. More precipitation where the temperature is high and vice versa, makes it difficult to distinguish the effects of the respective factors of temperature or precipitation on soil DOM. Ding et al. (2020) only quantitatively determined how climate factors and soil composition affect DOM molecular diversity.

Currently 3D-fluorescence spectrum-parallel factor analysis method is widely applied in DOM research. However, the molecular structure of DOM mixtures with complex and highly oxidized DOM is still not well studied. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) can be used to obtain more detailed molecular information of DOM, such as molecular formula, element composition, degree of unsaturation (DBE), aromaticity (AI), standard oxidation state of carbon (NOSC), etc. (Leyva et al., 2020). In recent years, FT-ICR-MS has been used to analyze DOM characteristics from different sources, including groundwater, lake water, industrial wastewater, soil, and biochar (Liu et al., 2020; Xu et al., 2020; McKenna et al., 2021; Zhang et al., 2021). Overall, the FT-ICR-MS technique provides great help to study the composition and characteristics of DOM at the molecular level (Miranda et al., 2020).

In this study, 33 soil samples from different regions in mainland China were selected to represent climate gradients. The molecular composition of DOM was analyzed by FT-ICR-MS to quantitatively analyze how climate factors (mean annual temperature and mean annual precipitation) affect the composition and chemical characteristics of DOM. The effects of other important environmental parameters, such as soil nitrogen content on DOM chemical diversity, were also assessed.

## 2. Material and methods

### 2.1. Soil sampling

To exclude the influence of land-use, we chose the same management mode of lawn in the urban residential area, which largely excluded the interference of land-use type in our study. The spatial distribution of sampling points in this study is shown in Fig. 1. In order to distinguish the effects of MAP and MAT on DOM composition and chemical properties, this study selected locations with large differences in MAT (15.9–24.8 °C) in areas with abundant precipitation (MAP greater than 1000 mm) to study the effect of MAT on DOM characteristics. This group of samples was named the MAT-variation group (including sample points 1–18). Similarly, in areas with similar MAT (13–14.6 °C), locations with large differences in MAP (44.1–1072.8 mm) were selected to study the effect of MAP on DOM characteristics. This group of samples was named the MAP-variation group (including points 19–33).

The sampling plot was divided into 3 parallel plot (10 m*10 m), with an interval of 5 m between each parallel plot. Six sampling points of 20 cm*20 cm were selected in a serpentine shape in each parallel plot, collected with a 50.46*50 mm ring knife, scraped 2 cm of the soil surface, and mixed thoroughly to form a sample.

The collected samples were placed in marked polyethylene plastic bottles, and transported to the laboratory at low temperature to dry in the shade for subsequent determination of the soil's physical and chemical properties. Note that the effect of air-drying on DOM composition was not significant in this study (see section FT-ICR-MS experiments and data analysis). All soil samples were passed through a 2 mm sieve prior to further analysis and DOM extraction experiments. Standard test methods were used to determine soil pH, dissolved organic carbon (DOC), total nitrogen (TN), total phosphorus (TP), ammonia nitrogen ($NH_4^+$-N), nitrate nitrogen ($NO_3^-$-N), available phosphorus (AV) and available potassium (AK) (Ma et al., 2016). The climate information of the sampling points was gathered from the China Meteorological Network (https://data.cma.cn/). Analyses of soil properties are presented in Table S1.

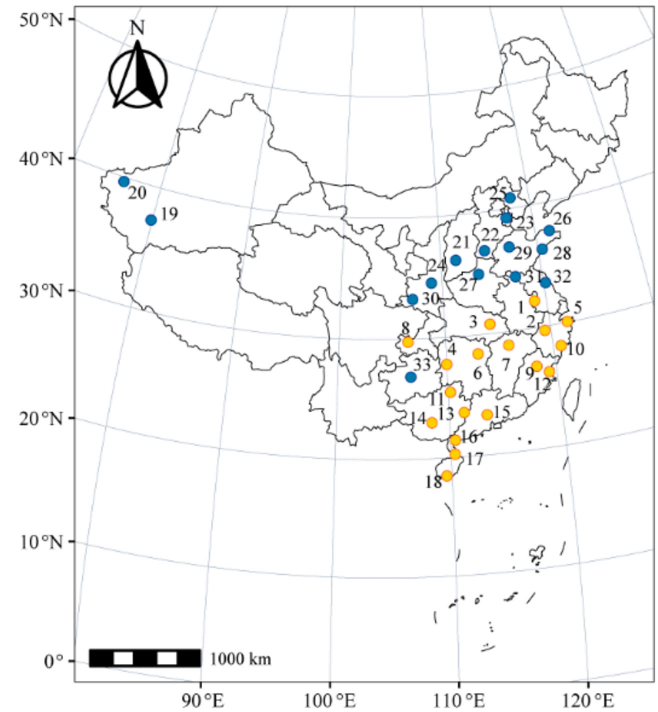


**Fig. 1.** Spatial distribution of sampling sites in China. The blue circle represents the MAP system, the yellow circle represents the MAT system. Samples 1–18 were collected from the areas with MAP of more than 1000 mm. Samples 19–33 were collected from the areas with MAT of 13 to 14.6℃ degrees.

### 2.2. FT-ICR-MS analysis

All extractable organic matter samples were used for FT-ICR-MS analysis, which were prepared by a solid phase extraction (SPE) process (Lv et al., 2016). The extraction efficiency was calculated for nine representative samples (61 ± 4 %, n = 9). We converted the absolute intensities of molecules into relative abundance for subsequent analysis. Based on the aromatic index (AI) of DOM molecules (Koch and Dittmar, 2006), H/C and O/C ratios, the molecules were classified into different compound groups to construct Van Krevelen (VK) diagrams (Kellerman et al., 2018). Further, aliphatic compounds were subdivided into lipid (H/C: 1.5–2.0; O/C: 0–0.3), protein/amino sugar (H/C: 1.5–2.2; O/C: 0.3–0.67) and carbohydrate (H/C: 1.5–2.2; O/C: 0.67–1.2) based on the H/C and O/C ratios of DOM molecules (Ohno et al., 2014). To better characterize the molecular properties of DOM, double bond equivalents (DBE) and nominal oxidation state of carbon (NOSC) were also calculated in this study. DBE reflects the number of double bonds and rings in organic matter molecules, while NOSC reflects the biogeochemical activity and bioavailability of organic matter molecules. The formula for the calculation of the relevant molecular indicators refers to the following references (Stenson et al., 2003; LaRowe and Van Cappellen, 2011).

### 2.3. Statistical analysis

Linear regression and spearman correlation analysis were used to test the relationship between DOM molecular characteristics and environmental variables. If the data were not normally distributed, a natural logarithmic transformation was performed for the data. If the data were still not normally distributed after natural logarithm transformation, bilateral spearman linear correlation analysis was used for correlation analysis (Ma et al., 2018). When the significance level of correlation analysis $p < 0.05$, the correlation was considered significant. The vegan software package of R was used for variation decomposition.

## 3. Results

### 3.1. Molecular characterization of DOM by FT-ICR MS

Molecular characterization of DOM was first performed for all sample sites using FT-ICR-MS. The information including $O/C_w$, $H/C_w$, $N/C_w$, $S/C_w$, $DBE_w$, AI $_{modw}$ and average molecular formula are shown in Table S2.

The molecular formulas were classified into four subcategories: CHO, CHON, CHOS, and CHONS (Fig. 2). It could be seen that CHO molecules at each sample point are the most abundant (43.9–73.9 %), followed by CHON (18.6–46.3 %), and CHOS molecules were the least abundant (6.8–15.3 %) (Fig. 2a). According to the proportion of elements, the molecular formulas were classified into protein, lipid, tannin, lignin, carbohydrate, unsaturated hydrocarbon and unsaturated hydrocarbon. It could be seen that the content of lignin was the largest (35.7–61.4 %), followed by protein (10.3–27.3 %), the lowest content was carbohydrate (0.3–1.7 %) (Fig. 2c). The Shannon diversity and Pielou evenness indices of the DOM molecules distribution of the samples are shown in Fig. 2b. The Shannon index reflects the number of DOM molecular types at the sampling point, and the Pielou index reflects the uniformity of the distribution of the individual DOM molecules in the overall DOM molecules.

The specific categories were detected as $N_3O_{0\text{-}10}$, $N_2O_{0\text{-}10}$ and $NO_{1\text{-}11}$(Fig. 3). These categories could explain the biochemical changes of DOM at the molecular level. Sample 30 contained the least variety of $N_xO_x$, $N_3O_x$, $N_2O_x$ and $NO_x$, and sample 18 contained the most variety of $N_xO_x$.

### 3.2. Influences of MAP on the chemodiversity of DOM

Fig. 4a shows the correlation between DOM molecular characteristics and MAP and MAT, respectively. It could be seen that the influence of MAP on DOM molecular characteristics was greater than that of MAT. In the MAP-variation group, with the increase of precipitation gradient, the index $H_w$ decreased, $N_w$ and $O_w$ increased; other molecular characteristics also increased with the increase of precipitation, although not

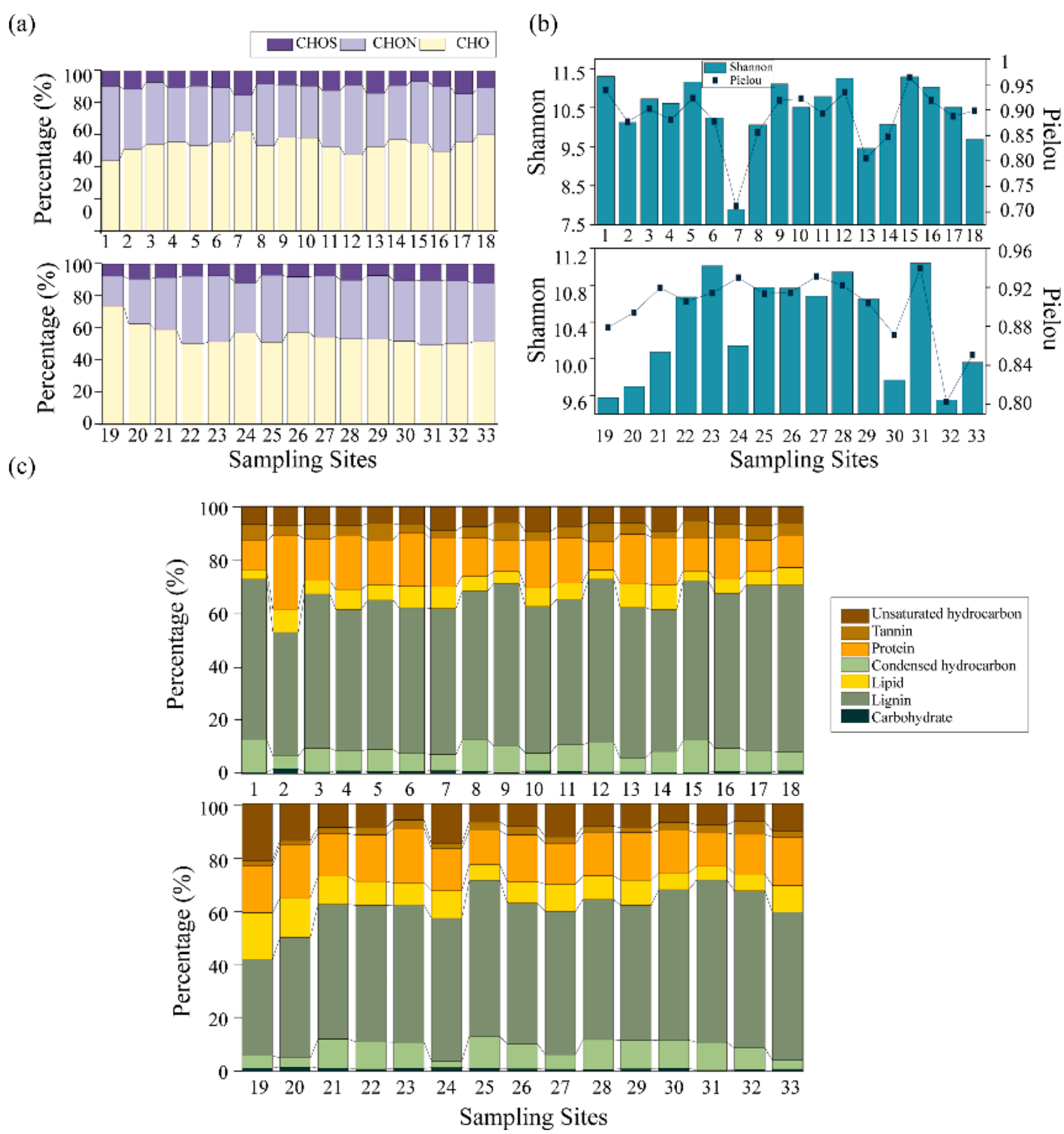


**Fig. 2.** Comparison of DOM compositions in the sampling sites. Bar diagram (a) shows the contribution of the major subcategories (CHO, CHON, CHOS). (b) shows the α-diversity index of DOM. Bar diagram (c) shows the contribution of the major biochemical classes recognized in the Van Krevelen diagrams.

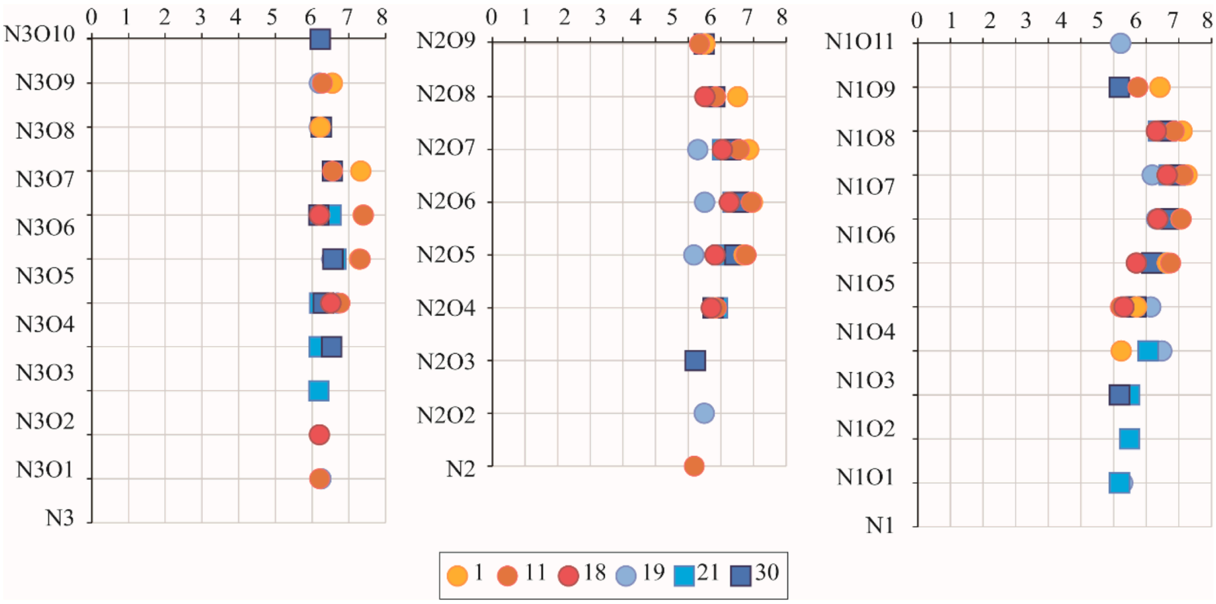


**Fig. 3.** Classification of CHOS compounds into different subgroups according to the number of N and O atoms in their molecules. The units of measure on the x-coordinate have been logged.

significantly. However, in the MAT-variation group, the change in the DOM molecular characteristics was irregularly.

With the increase of precipitation, the proportion of CHO type molecules decreased significantly ($P < 0.05$) in the MAP-variation group, while the CHON type molecules increased. The proportion of CHOS type molecules was relatively stable. In the MAT-variation group, the proportion of CHON decreased with the increase of temperature, while the proportion of CHO and CHOS did not change significantly.

The correlations of the seven DOM components with the MAP and MAT are shown in Fig. 5. In the MAP-variation group, the proportions of carbohydrate, lipid, protein and unsaturated hydrocarbon decreased with the increase of precipitation, and the decrease trend of lipid was the largest ($P < 0.05$, $R^2 = 0.585$), while the proportions of lignin and tannin showed an opposite trend. In the MAT-variation group, when the temperature increased, the proportion of protein decreased, and the proportion of lignin increased.

### 3.3. Molecular associations with climatic factors and edaphic factors

The correlation analysis results of DOM molecular characteristics, molecular types and molecular composition with edaphic factors and climate factors are shown in Fig. 6. In the MAP-variation group, excluding the influence of precipitation on DOM components, the content of DOC in soil was directly proportional to the properties of DOM. With the increase of DOC content, the index $H_w$ decreased while $O_w$ and $N_w$ increased. Besides, the relative proportion of CHOS type molecules was related to the change of soil pH. In the MAT-variation group, TN content had a great effect on the change of DOM. When TN content increased, $H_w$ decreased, $O_w$ and $N_w$ increased, and the increase of TP also led to the decrease of CHO type molecules and the increase of CHON type molecules.

In order to estimate the relative importance of potential drivers of DOM transformation, the effects of edaphic factors, MAP and MAT on DOM diversity were analyzed by variation decomposition. The results of variation decomposition clearly showed the individual effects of MAP and MAT on soil DOM (Fig. 7). In the MAP-variation group (Fig. 7a), the selected parameters could explain 81.5 % of the molecular variation. Edaphic factors were the most important predictor (37.4 %), followed by MAP (29.9 %) and the combined MAP and MAT (5.6 %). In the MAT-variation group, the parameters explained 47 % of the molecular variation. MAT was the dominant predictor (explaining 18.3 % of the molecular variation), followed by edaphic factors (16.7 %). MAP and MAT jointly explained 5.5 %, while more than 50 % of the variation remained unexplained.

## 4. Discussion

In this study, a series of sampling points with relatively fixed temperature and significant variation in precipitation (MAP-variation group) and a series of sampling points with obvious temperature variation but not limited precipitation (MAP over 1000 mm) (MAT-variation group) were selected for distinguishing the effects of precipitation and temperature on DOM components, respectively. According to the results of variation decomposition, although the contributions of MAP and MAT to the simultaneous influence of the two groups were 5.5 % and 5.6 %, respectively, in the MAP-variation group, the independent contribution value of MAP to DOM was close to 30 %, while the independent impact of MAT on DOM is almost negligible. The same trend was true for the MAT-variation group. This demonstrated that the two sets of data in this study could well separate the influences of MAP and MAT on DOM.

The variation decomposition results showed that in the MAP-variation group, the contribution of edaphic factors to DOM was 37.4 %, which was higher than the impact of MAP on DOM. In the MAT-variation group, the contribution of edaphic factors to DOM was 16.7 %, only half of that in the MAP-variation group. However, more than 50 % of DOM variation was still unexplained, indicating that other environmental variables could contribute to the change of DOM components. This unexplained DOM variability may be related to soil texture and heavy metal pollutants, or the composition of microbial communities (Huang et al., 2022). Previous studies had shown that the chemical diversity of DOM was significantly positively correlated with bacterial diversity ($r = 0.46$, $P = 0.048$), but not with fungal diversity ($R = -0.36$, $P = 0.127$), confirming the influence of bacterial community on DOM (Roth et al., 2019).

Edaphic factors also had a great influence on the composition of DOM. In the MAP-variation group, the composition of DOM had a significant positive correlation with the content of soil DOC, as DOM in soil was mainly derived from soil organic matter (Sokol et al., 2019). However, in the MAT-variation group, the composition of DOM was significantly correlated with TN content. The increase of TN input could

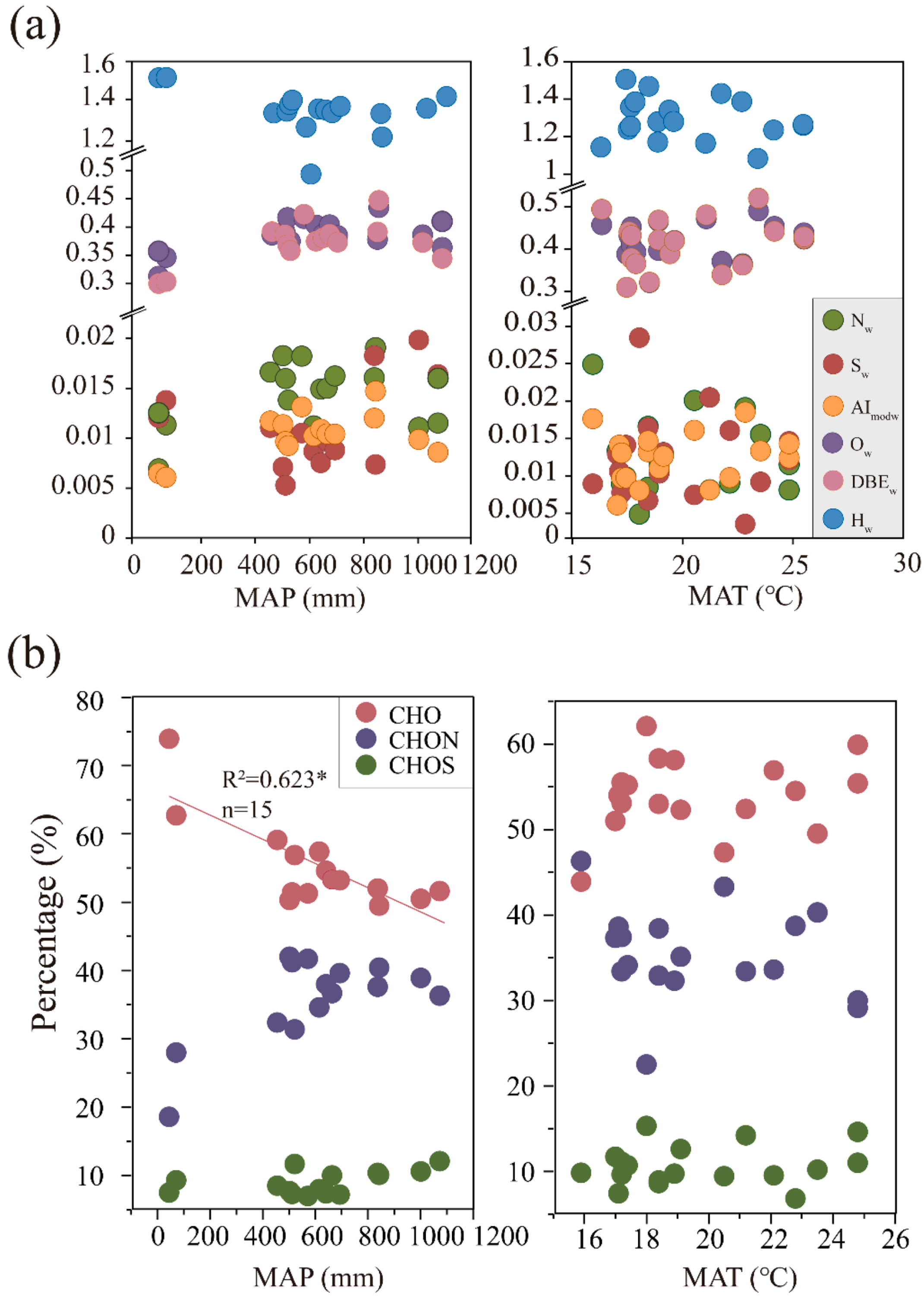


**Fig. 4.** Relationship between DOM molecular level and MAP and MAT. (a) Relationship between intensity weighted averaged (w) values for molecular composition and MAP and MAT. (b) Relationship between molecular type (CHO, CHON, CHOS) and MAP and MAT. Significance levels $0.01 < p \leq 0.05$ is denoted with″*″.

significantly reduce the content of hydrogen element in soil DOM, increase the aromaticity of soil and hence the relative proportion of refractory organics (Ling et al., 2022; Hu et al., 2022). In addition, TP content in soil could increase the content of nitrogen element in soil DOM, which may be related to the stability of the N/P ratio in plant growth (Zhang et al., 2018).

### 4.1. *Influence of MAP on DOM molecules of crust*

In the MAP-variation group, with the increase of precipitation, CHO type molecules in soil DOM significantly decreased, while CHON type molecules increased. Meanwhile, the number of $N_xO_x$ in the area with high precipitation was less than that in the area with low precipitation. In terms of composition, with the increase in precipitation, the proportion of lignin and tannin increased, while the proportion of protein,

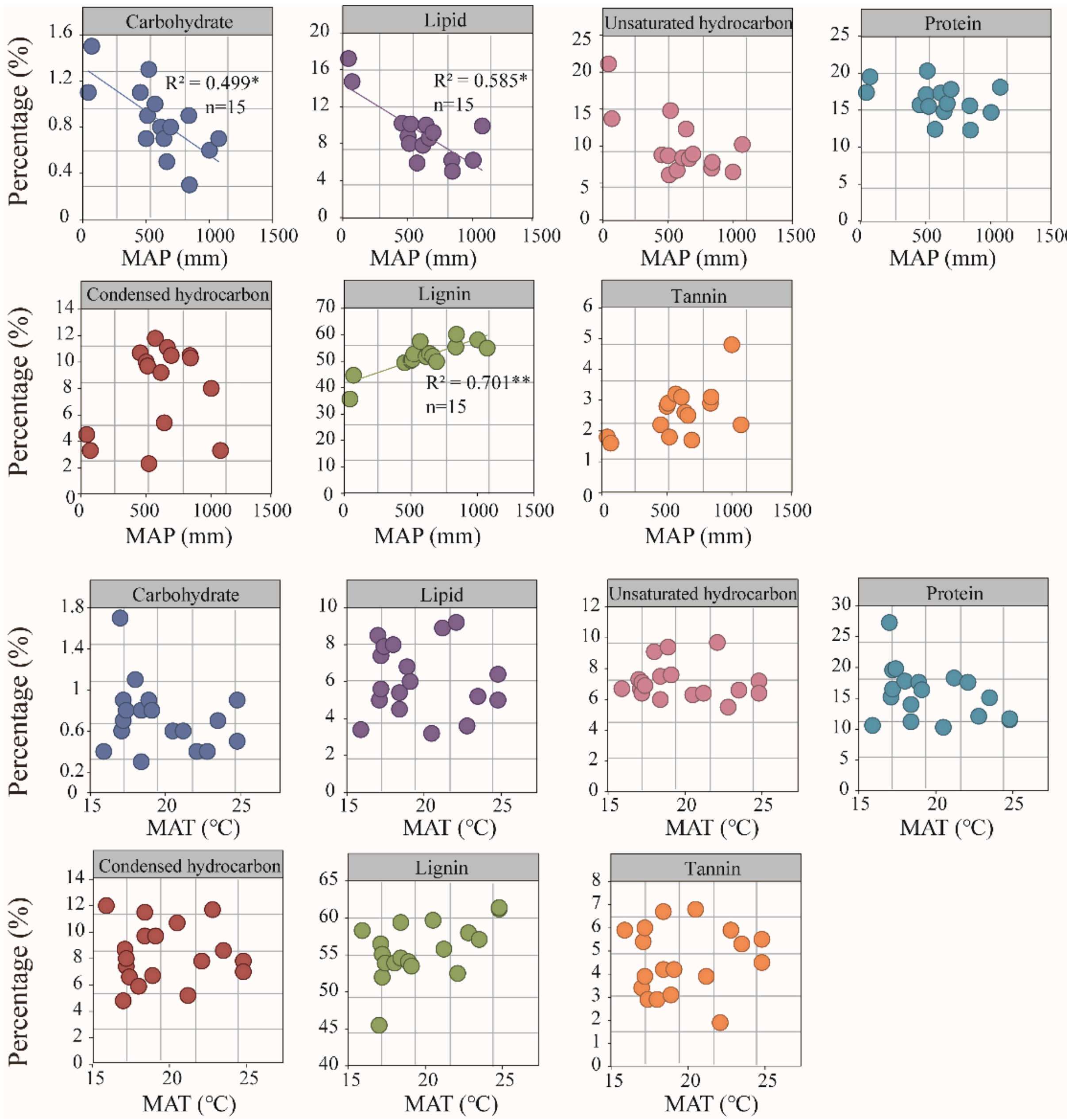


**Fig. 5.** Relationship between the major biochemical classes and MAP and MAT. Significance levels 0.001 < p ≤ 0.01 and 0.01 < p ≤ 0.05 are denoted with "* *" and "*", respectively.

carbohydrate, lipid and unsaturated hydrocarbon decreased. The above results indicated that when the MAT is stable and MAP increases, the non-degradable substances in soil DOM increase while the easily degradable organics decrease.

The variation of precipitation resulted in the change in plant growth and microbial community structure in soil systems (Hutchins et al., 2019; Jansson and Hofmockel, 2020). In general, increased precipitation can make plants grow faster and lead to greater microbial activities (Li et al., 2018a). More plant litter increased organic content in the soil, which was also the reason for the increased content of oxygen and nitrogen elements (Fig. 4a). At the same time, when the temperature was appropriate, more energy input and more water would make microbes more active (Na et al., 2019). With the suitable temperatures, the number of microorganisms increased with the increase of soil moisture, and more microorganisms consumed soil DOM (Bapiri et al., 2010; Dong et al., 2021). In previous studies, the multiple dry-wet alternate experiments showed that dissolved organic carbon was reduced after rehydration, mainly because rehydration stimulates the mass reproduction of microorganisms, which caused soil DOM to be decomposed and mineralized by microorganisms in a short period for their growth and reproduction (Hicks et al., 2019). In this study, the content of element hydrogen decreased with the increase of precipitation, which confirmed the degradation effect of microorganisms on DOM. On the one hand, plant litter increased the content of TN in the soil; microorganisms used nitrogen for growth and metabolism. While the substances that were difficult to utilize, such as tannin and lignin, remained in soil for a long time (Wu et al., 2021; Zhang et al., 2022). Thus, in the MAP-variation group, soil DOM composition was more dominated by microbial response to precipitation.

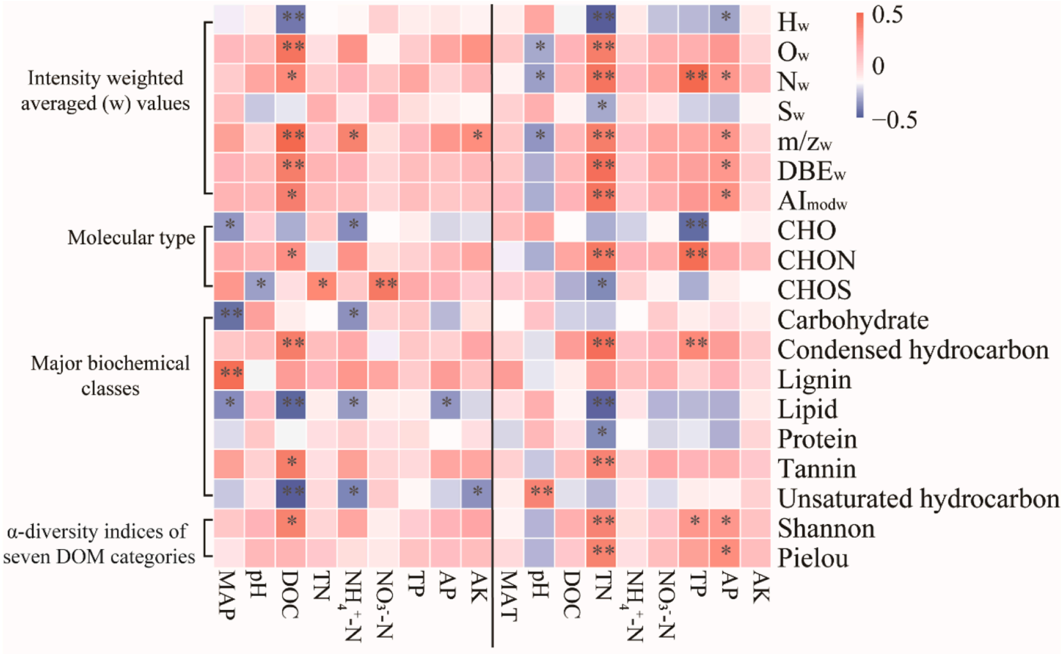


**Fig. 6.** Spearman correlation matrix of the molecular characteristics and compositions of DOM with MAP and MAT and edaphic factors. Significance levels $0.001 < p \leq 0.01$ and $0.01 < p \leq 0.05$ are denoted with "* *" and "*", respectively. Colors from blue to red represent changes in the Pearson correlation coefficient from − 1 to 1. TN: total nitrogen, TP: total phosphorus, $NH_4^+$-N: ammonia nitrogen, $NO_3^-$-N: nitrate nitrogen, AP: available phosphorus, AK: available potassium.

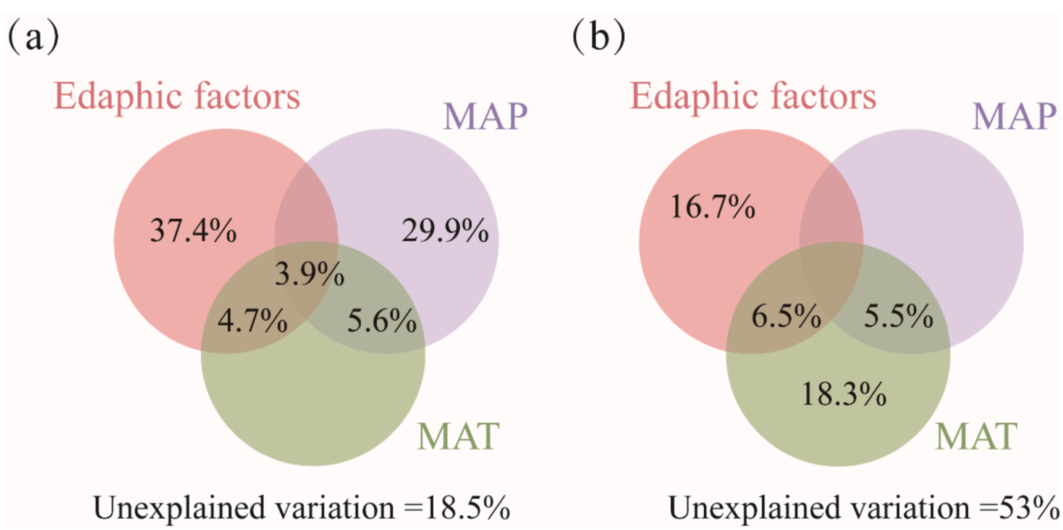


**Fig. 7.** Variation partitioning for potential drivers of DOM molecular compounds. (a) Variation explained by edaphic factors, MAP and MAT in sampling 1–18. (b) Variation explained by edaphic factors, MAP and MAT in sampling 19–33.

### *4.2. Influence of MAT on DOM molecules of crust*

The results showed that in the MAT-variation group, the proportion of CHON type organic matter in DOM decreased with the increase in temperature. In addition, in CHON type organic matter the number of molecules with more nitrogen element ($N_3O_x$) was significantly reduced compared with those with less nitrogen element ($N_1O_x$ and $N_2O_x$). At the molecular level, with the increase in temperature, the protein organic components decreased significantly, while the lignin organic components increased significantly. The above results indicate that under the condition that precipitation is not limited, the increase of temperature not only reduces the proportion of nitrogen-containing (*N*-containing) organic matter in DOM, but also reduces the content of N in *N*-containing organic matter.

The increase in temperature promoted the growth of land plants and also significantly promoted the activity of soil microorganisms (Liu et al., 2019; Payandi-Rolland et al., 2020). Studies had shown that with the increase in temperature, nitrogen content in plant leaves gradually decreased (Chen et al., 2018; Hussain et al., 2018; Zhang et al., 2019a). This is mainly due to the different nitrogen content required by plants at different growth stages, and temperature rise accelerates this process (Hu et al., 2018). Therefore, although the increase in temperature can promote the growth of land plants and make soil obtain more organic matter supplement such as litters, the nitrogen content of these supplemented organic matter would decrease with the increase in temperature (Zhang et al., 2019b). On the other hand, microorganisms were also selective when degrading organic matter (Huang et al., 2021). Generally, amino acids and some proteinaceous substances of small molecules are easy to be decomposed by microorganisms, while lignin and humic acids are more difficult to be decomposed by microorganisms (Morán et al., 2020). Recent mineralization experiments in our laboratory showed that the half-life of amino acids and protein substances decomposed by microorganisms was less than 30 days. The half-life of humic acids is greater than 45 days (data not presented in this paper). Amino acids and proteins were likely to be decomposed more quickly as the temperature rises promoted microbial activity. The above two processes and the input of soil nitrogen by plants were the main reasons for the changes in soil DOM components in the MAT-variation group.

## 5. Conclusions

The two sets of data in this study can well separate the influences of precipitation and temperature on DOM. The results showed that when the MAT was within a certain range (13–14.6℃), the proportion of CHO in molecular composition decreased gradually with the increase of MAP, while the proportion of *N*-containing molecules increased gradually. From the DOM composition, the content of lignin and tannin had a positive relationship with MAP and carbohydrate, lipid, unsaturated hydrocarbon and protein decreased with the increase of precipitation. When MAP was high enough and the sampling point was not limited by MAP (>1000 mm), the content of nitrogen molecules decreased gradually with the increase MAP. Meanwhile, the content of molecules containing sulfur element was almost unaffected by MAP and MAT. In terms of DOM composition, protein content decreased with the increase of temperature, while other substances had no obvious response to the temperature change.

## CRediT authorship contribution statement

**Siwan Liu:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Linhua Fan:** Data curation, Resources. **Chao Chang:** Visualization, Formal analysis. **Zhengkui Ge:** . **Ning Ma:** Software, Methodology. **Wenbin Chen:** Software, Methodology. **Fang Yang:** Writing – original draft, Methodology, Supervision, Validation, Writing – review & editing. **Baozhu Pan:** Writing – original draft, Methodology, Supervision, Validation, Writing – review & editing. **Ming Li:** Project administration, Funding acquisition. **Li Gao:** Writing – original draft, Methodology, Supervision, Validation, Writing – review & editing.

## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.109240.

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