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Molecular Characterization of Dissolved Organic Matter Unveils their Complexity, Origin, and Fate in Glacier and Glacial-Fed Streams and Lakes on the Tibetan Plateau

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Abstract : Alpine glaciers harbor a large quantity of bio-labile dissolved organic matter (DOM), which plays a pivotal role in global carbon cycling as glacial-fed streams are headwaters of numerous large rivers. To understand the complexity, origin, and fate of DOM in glaciers and downstream-linked streams and lakes, we elucidated the molecular composition of DOM in two different Tibetan Plateau glaciers, eight glacial-fed streams and five lakes, using an ultrahigh-resolution 15 Tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer. The compositional changes of the DOM samples revealed that glacier DOM mostly exhibited sulfur-containing organic compounds (CHOS species). We also found that aliphatic formulae contributed more than 50% of the total abundance of assigned molecules in glacier samples, and those compounds were significantly related to CHOS species. The CHO proportions of glacial-fed streams and lakes samples increased with increasing distance from glacial terminals. The relative contribution of terrestrial-derived organics (i.e., lignins and tannins) declined while microbial-originated organics (aliphatics) increased with increasing elevation. This suggested the gradual input of allochthonous materials from non-glacial environment and the degradation of microbe-derived compounds along lower elevations. Alpine glaciers are retreating as a result of climate change and they nourished numerous streams, rivers, and downstream-linked lakes. Therefore, the interpretations of the detailed molecular changes in glacier ice, glacial-fed streams, and alpine lakes on the Tibetan Plateau could provide broad insights for understanding the biogeochemical cycling of glacial DOM and assessing how the nature of DOM impacts fluvial ecosystems.

Keywords : Tibetan Plateau, FT-ICR MS, dissolved organic matter, fluvial ecosystem, elevation

Introduction

Dissolved organic matter (DOM) is an extremely complicated repertoire of organic molecules that can be degraded photochemically or biologically as well as newly produced from organismal exudates or debris in ecosystems.[1] DOM plays a significant role in ecosystems as a major nutrient and carbon source for organismal metabolic activity.[2] In particular, alpine glaciers harbor a large quantity of bio-labile DOM, which plays a pivotal role in global carbon cycling as glacial-fed streams are headwaters of many large Asian rivers, including Indus, Ganges, Yarlung Tsangpo, and Yangtze. Glaciers are hydrologically connected to downstream environments such as rivers, lakes, and oceans,[3] and therefore, the chemical composition of glacial DOM can affect the downstream fluvial ecosystem.[4] As glacial melting has been accelerated by global warming,[5-7] the impact of glacial runoff on downstream ecosystems will be significantly increased. Thus, a deeper understanding of the biogeochemistry of DOM in glaciers and the downstream-linked environments is important for the study of the response and adaptation of these ecosystems under climate change.[8]

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Glacial DOM on the Tibetan Plateau, which is the highest plateau in the world and includes the largest area of glaciers outside the polar regions, covering a total area of 49,873 km^2,[9] is an important source of bio-labile carbon for downstream-linked fluvial ecosystems around the Tibetan Plateau, and its impact is expected to increase due to the increased glacial melting rate. Glaciers on the Tibetan Plateau are shrinking rapidly, and large amounts of glacier meltwater flows into downstream fluvial ecosystems, including Indus, Ganges, Yangtze, and Huang (Yellow), nourish a population of over a billion.[9] The reactivity and bio-availability of DOM depend largely on its sources and chemical composition. It is therefore of high importance to elucidate the molecular details of DOM in alpine glaciers and downstream-linked stream and lake ecosystems on the Tibetan Plateau.

Recently, a growing number of studies have used ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to clarify the complexity of DOM in various environments, such as aerosols, soil, snow, and water bodies, at molecular level.[10-16] In particular, the molecular composition of DOM in alpine glaciers is associated with its chemical or biological transformations in connected downstream fluvial ecosystems, but only a few reports have addressed molecular information on alpine glacial DOM.[17,18]

Here, we use a 15 Tesla FT-ICR MS to generate ultrahigh-resolution mass spectrum profiles of DOM extracted from Tibetan Plateau glaciers and downstream-linked stream and lake ecosystems to obtain sophisticated chemical information on DOM. The resulting data were interpreted to investigate the molecular composition variability in DOM on the Tibetan Plateau. Environmental variables were also integrated to obtain information on the complexity, origin, and fate of glacial DOM on the Tibetan Plateau. Molecular information provides key constraints for understanding the complexity, origin, and fate of glacial DOM on the Tibetan Plateau.

Materials and Methods

Sampling sites and sample preparation

In this study, we sampled DOM from glaciers ($n = 2$), glacial meltwater ($n = 8$) and lakes with different elevations ($n = 5$) on the Tibetan Plateau (Figure 1 and Suppl. Table S1). Specifically, ice and meltwater samples were collected from two glaciers and eight glacial fed-streams in September 2017 as previously reported.[19] Five lake water samples were collected from lakes in the southern Tibetan Plateau in June (HL1 and HL2) and November (HL3, LL1, and LL2) 2018 (see Fig. 1 for the location of sampling sites). HL1 and HL2 locate in temperate continental climate and have low annual rainfall. Although the stream and lake samples were collected during different campaigns, they were all collected in low water seasons to avoid mountain torrents and minimize the impacts of lateral DOM inputs other than glacial-fed streams.[20] The detailed information on the measurements of dissolved organic carbon (DOC), dissolved total phosphorus (DTP), absorption coefficient of chromophoric DOM ($CDOM_{254}$), ratio of $CDOM_{250}$ to $CDOM_{365}$, i.e. X250:365, and biological freshness index (BIX) and humification index (HIX) obtained from DOM fluorescence spectroscopy have been previously reported.[19] 5–667 mL filtrates (volumes depending on the DOC concentrations to ensure a similar DOC load (60 μgC) with each cartridge) through 0.22 μm Millipore filters were acidified to pH = 2 using 10 M HCl, and solid-phase extracted with Bond Elut PPL cartridges (Agilent, Santa Clara, CA). Finally, the dried extracts were redissolved in 1 mL of 50% (v/v) methanol for 15 T Fourier transform ion cyclotron resonance mass spectrometry (Bruker Daltonics, Billerica, MA).

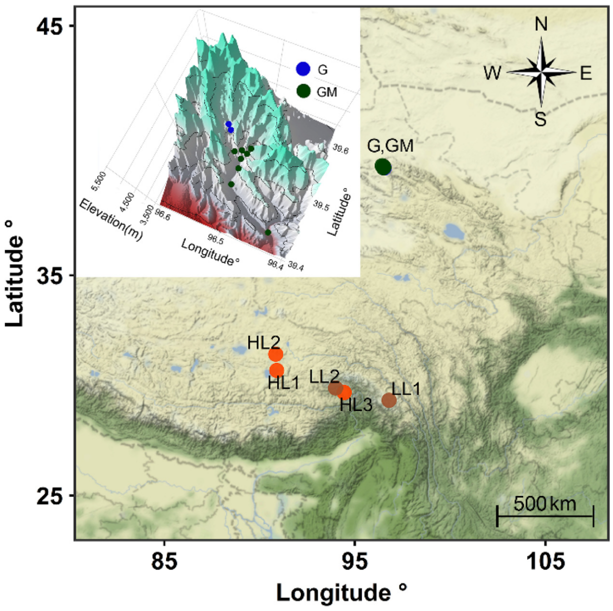

Figure 1. Geographical information of the sampling sites in this study. G, GM, HL and LL denote glaciers, glacial meltwater, lakes with higher elevations and lakes with lower elevations, respectively. The inset shows the G and GM sampling points and their connections.

FT-ICR MS measurements and data processing

Highly accurate mass measurements of DOM samples were carried out with ultrahigh-resolution FT-ICR MS. The DOM extracts (each 5 μL) were nanoelectrosprayed using a chip-based nanoelectrospray system (TriVersa NanoMate, Advion BioSciences, Ithaca, NY) with a spray voltage of 1.4 kV and a gas pressure of 0.3 bar (resulting in a flow rate of approximately 300 nL/min), directly infused into 15 T FT-ICR MS, and were analyzed in negative ion mode within the mass range of *m/z* 150-1200, as reported previously.[21,22] Ultrahigh-resolution mass spectra were acquired by collecting 200 scans per sample with an 8 M transient, resulting in a mass resolving power greater than 900,000 (FWHM at *m/z* 400).

The FT-ICR MS datasets were subjected to further processing, including peak detection and recalibration, using DataAnalysis (ver. 4.4, Bruker Daltonics) and elemental formula assignment using Composer (Sierra Analytics, Modesto, CA). Briefly, the empirical molecular formulas were determined for the masses of singly charged ions in the range of *m/z* 150–1,000 by combinations of $C_{1–100}H_{1–200}O_{1–60}N_{0–4}S_{0–1}$, and then the molecular formulas with assignment errors > 0.3 ppm were excluded from further interpretation. The assigned chemical compositions were displayed based on the atomic hydrogen-to-carbon (H/C) and oxygen-to-carbon (O/C) ratios on a van Krevelen diagram.[23] The modified aromaticity index (AI_{mod}) was used to interpret aromaticity of DOM molecules.[24,25] The double bond equivalent (DBE) value, representing the sum of rings and double bonds in each molecule, can be calculated from the number of atoms in the chemical formulas by the following equation: $DBE = 1 + C - 0.5\,H + 0.5\,N$. The nominal oxidation state of carbon (NOSC) was calculated by the following equation: $NOSC = 4 - [4 + H/C - 3\,N/C - 2O/C - 2S/C]$. Organic constituents can be categorized into four chemical classes based on their elemental composition [i.e.,compounds containing only C, H and O (CHO), compounds containing C, H, O and N (CHON), compounds containing C, H, O and S (CHOS), and compounds containing C, H, O, N and S (CHONS)].[16]

The assigned peaks were classified into seven categories of molecules: (i) lipids ($0 \le O/C < 0.3$, $1.5 < H/C < 2.0$), (ii) proteins associated with amino acids ($0.3 \le O/C \le 0.67$, $1.5 < H/C < 2.2$, $0.05 \le N/C$), (iii) lignins ($0.1 \le O/C \le 0.67$, $0.7 \le H/C \le 1.5$, $AI < 0.67$), (iv) carbohydrates ($0.67 < O/C < 2.0$, $1.5 < H/C < 2.3$), (v) unsaturated hydrocarbons ($0 < O/C < 0.1$, $0.7 \le H/C \le 1.5$), (vi) condensed aromatics ($0 < O/C \le 0.67$, $0.2 \le H/C < 0.67$, $AI \ge 0.67$), and (vii) tannins ($0.67 < O/C < 2.0$, $0.5 \le H/C \le 1.5$, $AI < 0.67$).[26,27]

Multivariate statistical analysis

Nonmetric multidimensional scaling (NMDS; stress: 0.05, $k = 2$) analysis was performed to identify the patterns of molecular variations across samples using the mean intensity of each formula with the Bray-Curtis dissimilarity index. The normality of variables was tested by the Shapiro-Wilks test through all variables. Chemical properties, molecular classes, chemical compositions and environmental variables (nutrients and optical indices) were fitted as vectors to evaluate a variable's contribution to a variety of ordinations. Major variables were selected by assessing significance using a permutation test ($p < 0.05$) and displayed as a vector on the NMDS plots. All steps were computed in R applying the 'vegan' package,[28] and the correlation test was conducted using the 'stats' package.[29] Pearson's correlation between the proportion of molecular classes and chemical compositions was calculated. Spearman's rank correlation test was used to determine the relationship between elevation and molecular classes.

Results and Discussion

Comparison of the environmental factors of glaciers, glacial-fed streams and lakes on the Tibetan Plateau

The sampling sites of glaciers and glacial-fed streams were located at high elevations (3,752~4,391 meters above mean sea level) with no residents, so it was assumed that these samples were not contaminated by anthropogenic activity, whereas lakes can be influenced by human activities such as farming and household heating. Three of the lakes with higher elevations (i.e., HL1~3, altitudes above 4,000 meters) were not surrounded by villages and had few effects of human activity, i.e., only intake by rainwater or snow-melt water. In contrast, two other lakes (LL1 and 2, altitudes below 4,000 meters) were surrounded by many houses, so those locations might be readily affected by anthropogenic activities.

The optical indices of CDOM were based on our previous study.[19] BIX which shows the biological properties of DOM were compared between samples (Suppl. Figure S1 and Suppl. Table S1). DOM with high BIX values (greater than 1) has been considered to be a more likely biological- or microbial-derived DOM,[30] while HIX represents the proportion of terrestrial- or plant-derived DOM. The HIX value generally increases with a decrease in the BIX value and vice versa. As shown in Supplementary Figure S1, the average BIX values of glaciers and glacial meltwater samples were significantly higher than those of lake samples, likely implying more intensive biological activities in these samples.

Chemical composition and molecular distribution of DOM samples from glaciers, glacial-fed streams and lakes on the Tibetan Plateau

The DOM extracts were analyzed using an ultrahigh-resolution 15 T FT-ICR MS equipped with an ESI source in negative ion mode. After calculation of the elemental formulas from the raw spectral datasets, 8941 chemical formulas of total organic substances were assigned to the ESI (-) FT-ICR MS data. Organic constituents were categorized into four chemical classes based on their elemental composition, i.e., CHO, CHON, CHOS, and CHONS. The overall chemical attributions of the organic compounds belonging to the DOM samples are summarized in Supplementary Table S2. We investigated the molecular differences in the relative abundance of all the assigned formulas identified by FT-ICR MS for the glacier, glacial-fed streams and lake DOM samples.

The detailed chemical compositions of DOM samples

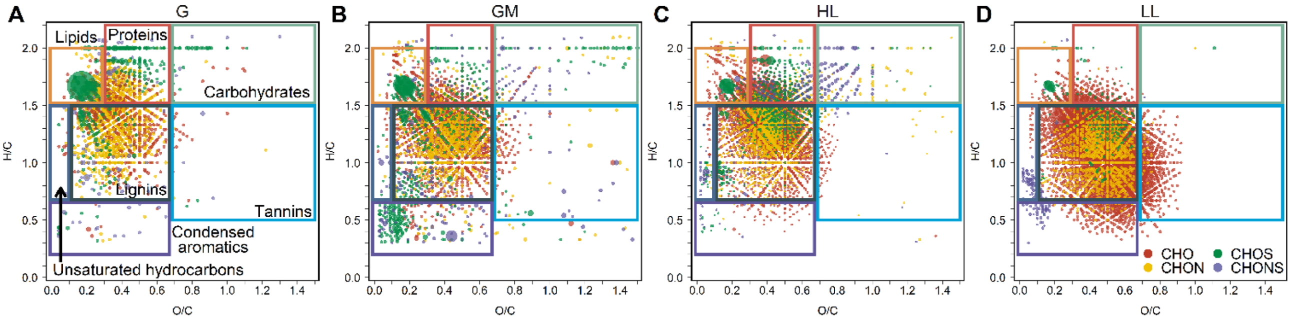

Figure 2. Representative van Krevelen plots showing the intensity-weighted distributions of chemical classes based on the molar H/C and O/C ratios of the assigned elemental compositions of DOM in (A) glacier, (B) glacial meltwater, (C) high lake (HL), and (C) low lake (LL) samples from the Tibetan Plateau, analyzed by ESI (-) FT-ICR MS (color code: CHO, red; CHON, yellow; CHOS, green; CHONS, purple). The size of each circle is proportional to the intensity of the molecular formula.

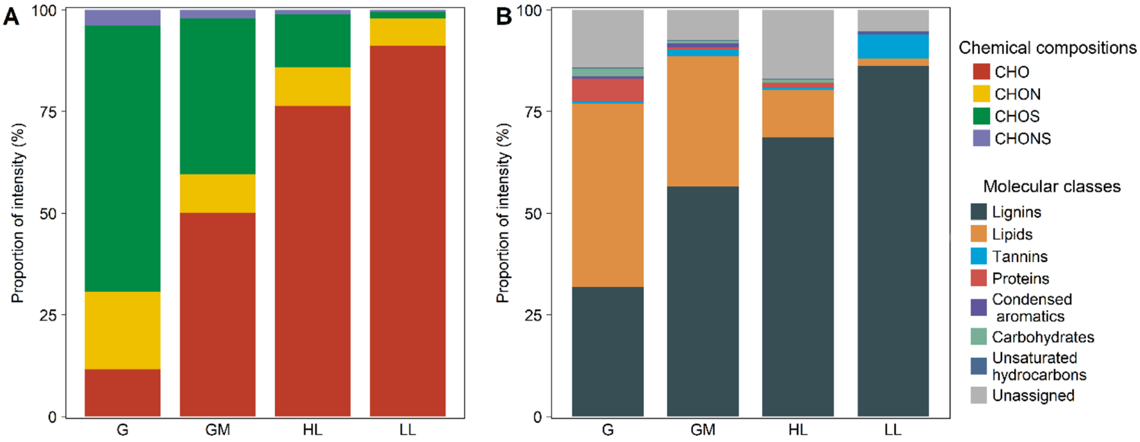

Figure 3. DOM properties of sampling site distribution. (A) Chemical compositions and (B) molecular classes.

elucidated by 15T FT-ICR MS illustrate the sophisticated molecular features of DOM. The representative molecular distributions of the DOM samples were displayed on van Krevelen plots, as shown in Figure 2. The assigned formulas from the DOM samples in the van Krevelen plots were categorized by different molecular classes (i.e., proteins, lignins, carbohydrates, lipids, tannins, unsaturated hydrocarbon and condensed aromatics) using various chemical metrics (Figure 2).[5,11] The lipid, protein and carbohydrate class compounds are mostly considered to be derived from microbial sources, whereas lignin and tannin class compounds are considered to be derived from leachate of terrestrial soil organic-rich substances [also called plant-derived products]. Furthermore, the chemical properties (i.e., AI_{mod} and DBE values, atomic O/C and H/C ratios) of the DOM samples can be used to interpret the molecular characteristics of the organic substances in the DOM.

As shown in Figures 2 and 3, the chemical compositions and molecular class distributions of glacially derived DOM differed markedly from those of lake samples. Glacial DOM dominantly consisted of sulfur-containing organic compounds (CHOS, 65.5%), and the proportions of CHOS species decreased to 38.8, 13.0 and 1.7% along the glacier fed-streams and downstream lakes (see Supplementary Table S3). In addition, the glacier and glacial-fed compounds (51.8% and 34.8%, respectively, Figure 3b), which agreed well with the fact that the excretion of microbes and degradation of microorganisms are considered the main sources of stream DOM samples exhibited large proportions of lipid-like supraglacial DOM.[31-35] The microbial-derived organic constituents in glacial DOM can fuel the biogeochemical cycling of downstream riverine and lake ecosystems through meltwater export.[36,37] Comparatively, CHO compounds increased from 11.6% to 91.2% from glaciers to downstream lakes, and lignin class species also followed similar trends, being a more predominant fraction from glacier samples to glacier fed-water and lake water (Fig. 3b). It was assumed that the downstream water received a more significant terrestrial inputs that can be typical allochthonous soil- and plant-derived materials.

We also investigated the chemical properties of the

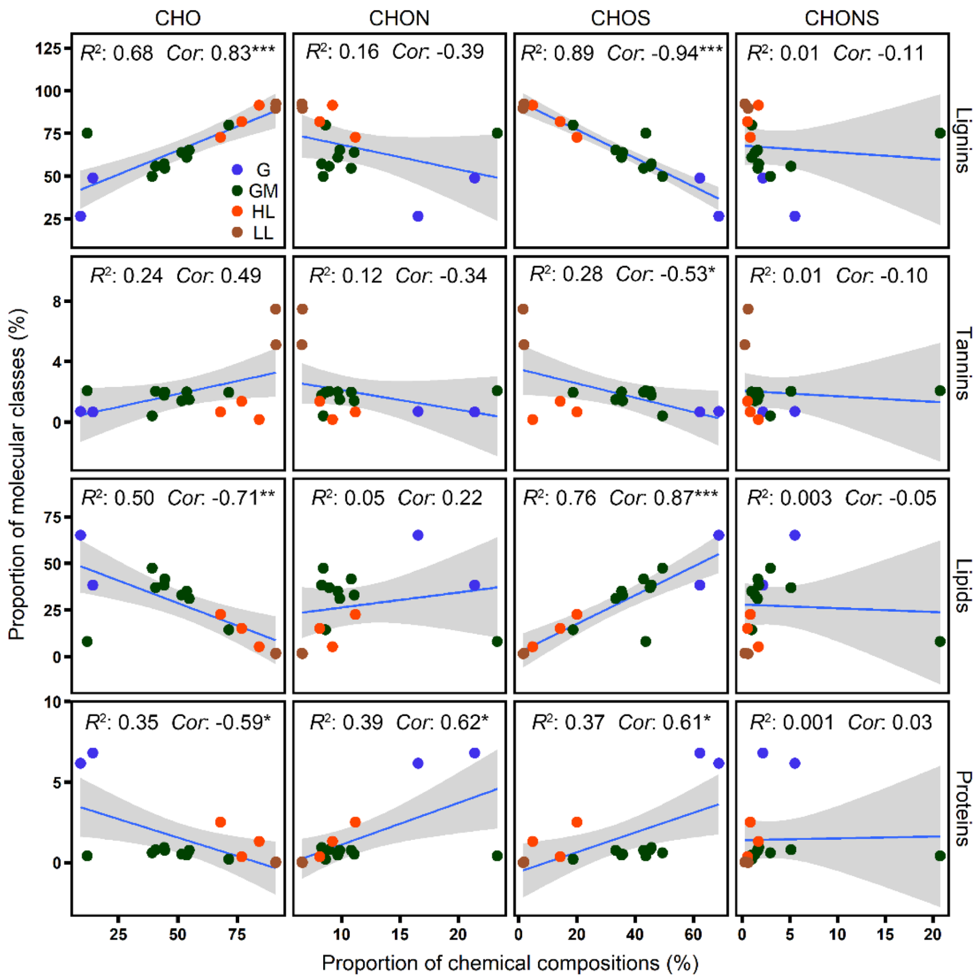

Figure 4. Relationship between molecular classes and chemical compositions. Cor denotes the Pearson correlation coefficient between variables. (***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$)

individual chemical species groups to determine the significant features of those chemical species (see Suppl. Figure S2). The CHOS species in glaciers and glacial fed-streams had lower DBE, atomic O/C, and nominal oxidation state of carbon (NOSC) values, as well as higher AI_{mod} and atomic H/C values than that in lakes, suggesting that the glacial CHOS species exhibited a less oxidized and more aliphatic nature. The sulfur-containing organic compounds are thought to likely either originate from organic aerosols by complete or incomplete combustion of fossil fuels or microbial accumulation in cryoconite holes.[17] In contrast, the CHO compounds exhibited higher DBE, AI_{mod}, NOSC, and O/C values in lakes with low elevations and lower H/C ratios than those in glaciers and glacial meltwater, showing their more oxidized and aromatic characteristics.

Pearson correlation coefficients between chemical compositions and molecular classes of DOM were calculated to investigate their relationships. In particular, CHO species were highly positively related to lignin-like compounds (Cor = 0.83, $p < 0.01$) and showed negative relationships with lipid and protein compounds. This relationship indicates that CHO class species in the DOM samples mostly originated from lateral input of terrestrial soil organic matter. In contrast, CHOS species were significantly and positively correlated to lipid constituents (Cor = 0.87, $p < 0.01$), while negatively correlated to the contribution of lignin formulae (Figure 4). Note that the linkages between molecular classes and CHON-, CHONS-containing compounds can be closer when a glacial-fed stream sample was excluded from the correlations (Figure 4).

Investigation of regional characteristics using NMDS analysis

To investigate the factors affecting the clusters of each sample group, we performed NMDS analysis using all elemental formulas assigned from those samples. All points were clustered by each region (Figure 5). To determine any environmental variable or chemical/molecular property that affected the clustering, those variables were selected as significant loading vectors ($p < 0.05$) contributing to the ordination in the NMDS plots (Figure 5).

As shown in Figure 5, the glacier (G) samples were separately clustered from others on the left side of the axis of NMDS-1, and the loading vectors determining the clustering were the atomic N/C and H/C ratios, CHON species, and protein-like compounds. The glacial meltwater (GM) samples were clustered in the middle of the axis of NMDS-1, and the low lake (LL) samples were placed along the slightly right direction of the GM samples. The atomic O/C ratio, DBE, lignins and tannin-like compounds were significant factors for LL samples, supporting the input of terrestrial organic constituents in downstream lakes. Shrubs and trees occupy a large area in valleys in the studied lake watersheds on the southeastern Tibetan Plateau. In particular, the NMDS plots in Figure 5 with the molecular/chemical properties showed that high lakes and low lakes clustered differently. The high lake (HL) samples were clustered on the negative side of the axis of NMDS-2, while all other samples were clustered on the zero line of the NMDS-2 axis.

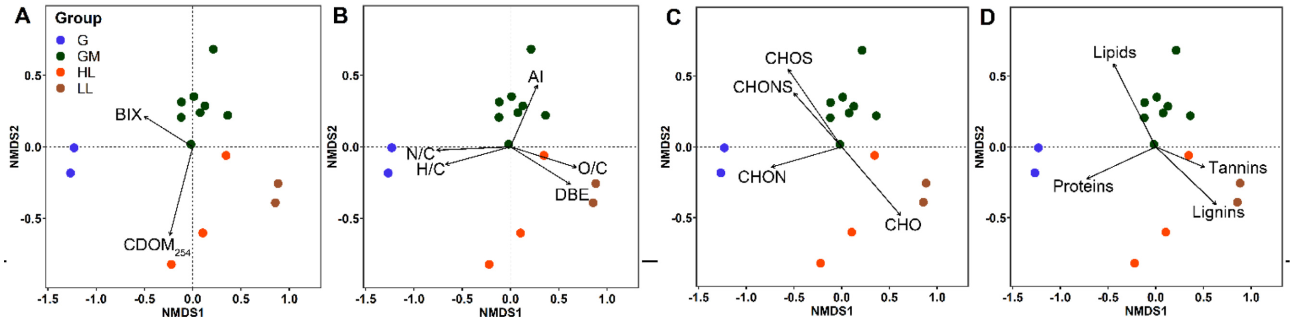

Figure 5. Nonmetric multidimensional scaling (NMDS) plot showing the physicochemical properties of the DOM samples in this study. Four regions (G, GM, HL and LL) of sampling sites. (A) Environmental variables, (B) chemical properties, (C) chemical compositions, and (D) molecular classes. All arrows are the significant properties ($p < 0.05$) contributing to each ordination.

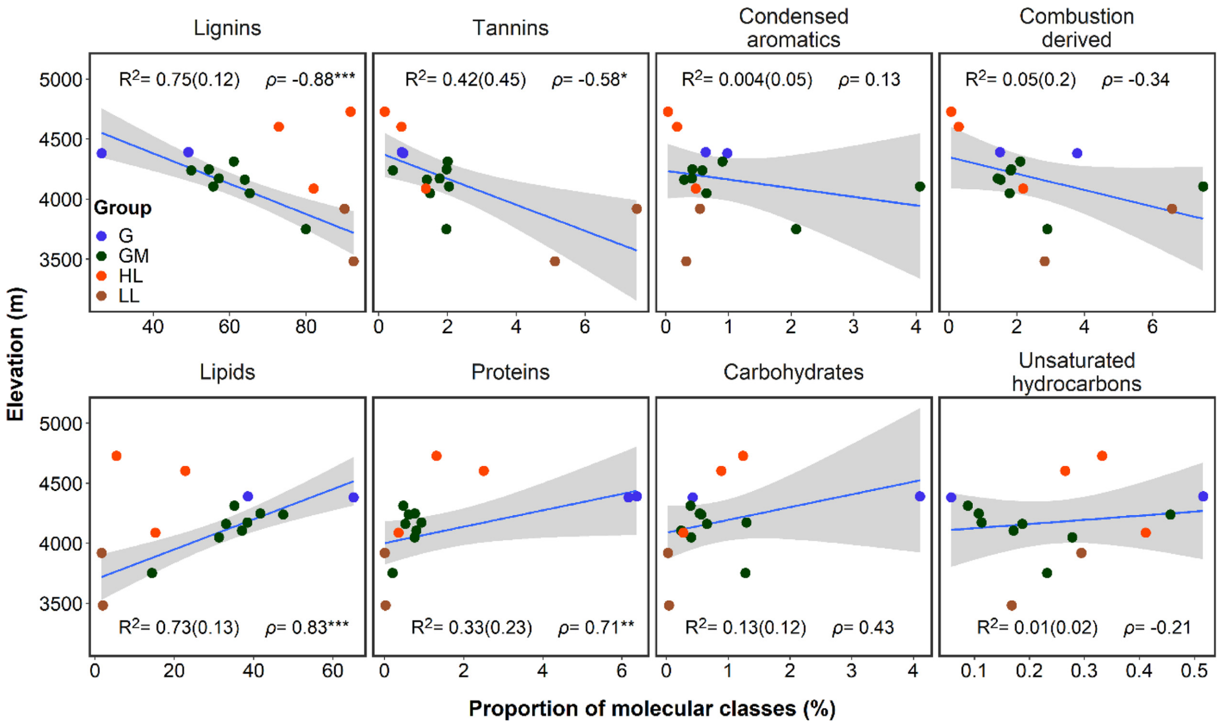

Figure 6. The correlation between elevation and the proportion of molecular classes. The correlation coefficients (R^2) were determined without the HL samples, and the values in parentheses indicate the R^2 determined including the HL samples. Significant differences among different chemical properties are indicated by letters above the boxes. (***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$)

Relationship of the molecular classes of DOM in the fluvial ecosystem of the Tibetan Plateau with elevation and distance from glacial terminals

The high relative contribution of lignins and tannins in the low elevation lakes in the Yarlung Tsangpo watershed (Figure 6) can be explained by the high precipitation and forest coverage in these low elevation lake watersheds.[20] Similarly, in well-forested Alaskan glacial-fed stream watersheds, soil organic-rich substance from lateral environments contributed importantly to these stream DOM pool.[38] As the molecular distributions of DOM on the Tibetan Plateau regionally differed along its fluvial ecosystems, we thought that the differences in the DOM compositions would be associated with the elevation-mediated precipitation and forest coverage. We also observed elevational trends in the characteristics of DOM (Figure 6). As the elevation of sampling sites decreased, corresponding to an increased distance from glacier terminals, the proportions of lignins and tannins increased, whereas those of proteins and lipids decreased. In particular, the elevational gradient of the molecular classes of lake DOM samples was distinct from that of the glacier meltwater samples, indicating that compared with non-glacial input or in-lake biogeochemical processing, the meltwater from the upstream alpine glaciers likely contributed limited organic carbon to downstream-linked lakes.

Conclusions

In this study, we elucidated the molecular compositions of DOM on the Tibetan Plateau and downstream ecosystem using ultrahigh-resolution FT-ICR MS, which is a powerful tool for accurately identifying the molecular characteristics of DOM. Highly bioavailable DOM associated with CHOS-containing compounds in glaciers and glacial-fed streams and lakes can be a significant source fueling downstream heterotrophic activity. We also found that the molecular features of DOM in alpine ecosystems changed depending on their elevation and distance from glacial terminals, demonstrating the inflow of glacial meltwater and allochthonous soil organic-rich DOM to fluvial ecosystems. The interpretations of the detailed molecular changes in glaciers, meltwater streams, and alpine lakes on the Tibetan Plateau could provide broad insights for understanding the biogeochemical cycling of glacial DOM and assessing how the nature of glacial DOM impacts fluvial ecosystems.

Supplementary Information

Supplementary information is available at https://drive.google.com/file/d/1Z7TaHwmUV8uBlCxXg-RObCwP8n54maFk/view?usp=sharing.

Acknowledgments

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