Complex dissolved organic matter on the roof of the world – Tibetan DOM molecular characteristics indicate sources, land use effects, and processing along the fluvial-limnic continuum

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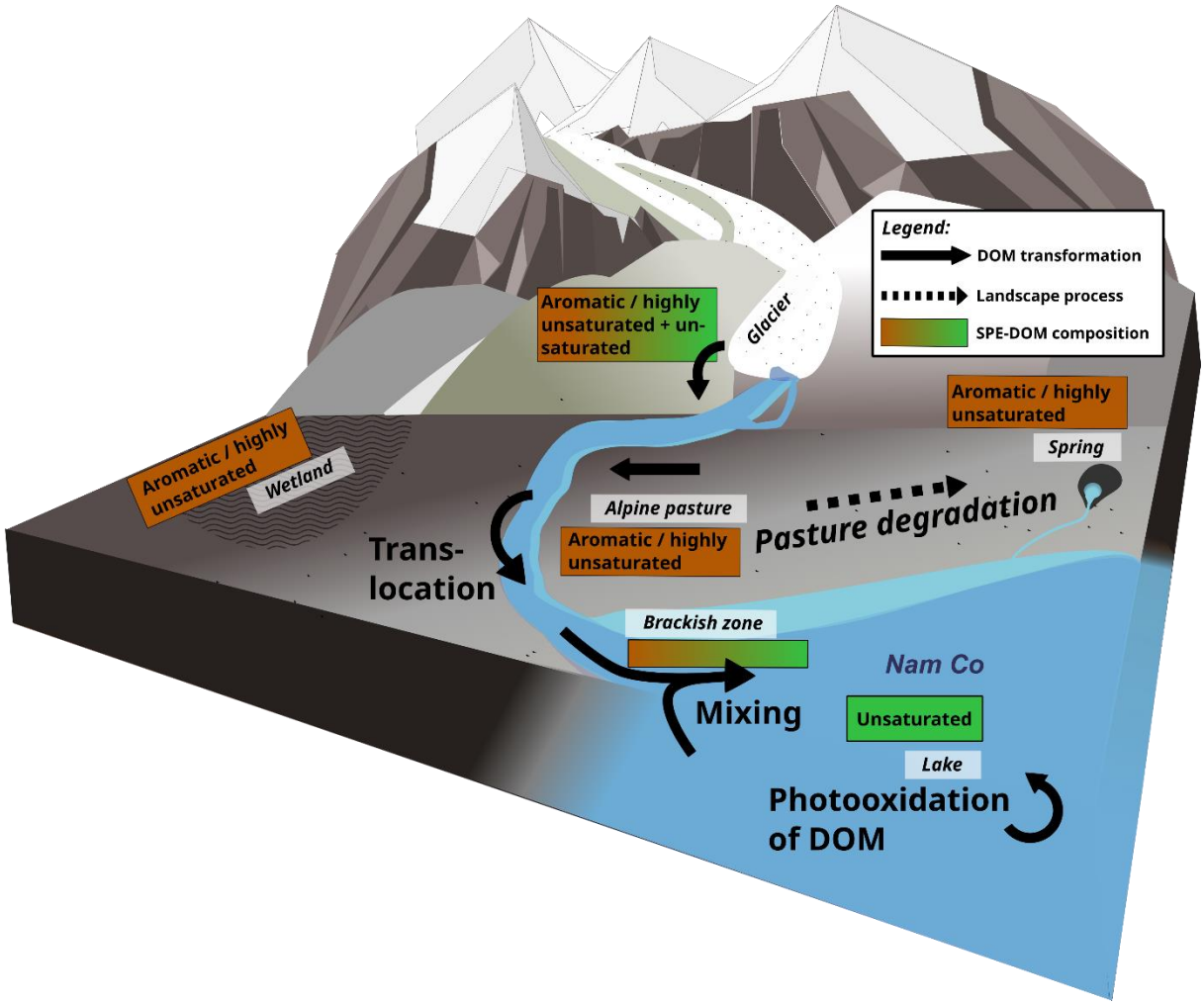
Abstract

The Tibetan Plateau (TP) is the world's largest and highest plateau, comprising the earth's biggest alpine pasture system. It is sensitive to impacts of climate change and anthropogenic pressure. Carbon cycling on the TP is influenced by glaciation and degradation of the pasture ecosystem. Dissolved organic matter (DOM) connects carbon reservoirs, following the hydrological continuum from glaciers and headwaters to lakes. Due to its complexity, DOM cycling along the aquatic continuum and the impact of land use and climate change on DOM characteristics are still not well understood. Here, we study solid phase extracted (SPE) DOM molecular characteristics using ultrahigh-resolution mass spectrometry (FT-ICR-MS) along the TP hydrological continuum from glaciers, groundwater springs, and wetlands including pastures and alpine steppes, to the endorheic Lake Nam Co. Our study revealed that the SPE-DOM composition was largely influenced by local sources of glaciers, wetlands, and groundwater springs as well as pasture degradation. Glacial meltwater SPE-DOM contained more saturated compounds suggesting microbial sources together with aromatic compounds probably derived from aeolian deposition. In comparison, wetland and stream SPE-DOM were characterised by a higher percentage of highly unsaturated and aromatic molecular formulae. These were likely derived from inputs of vascular plants and soils. Groundwater spring SPE-DOM from degraded pastures differed from intact pasture samples. In degraded systems a strongly oxidised signature with lowest counts of P heteroatoms, lower O/C ratio and higher aromaticity of SPE-DOM together with a high degradation index suggested a strong transformation of SPE-DOM. SPE-DOM of the endorheic lake was richer in unsaturated molecular formulae compared to the tributaries. This suggest algae and microbial sources and production in the lake. The SPE-DOM rich in aromatic and highly unsaturated formulae visible in the brackish zone of the lake shore contrasted sharply with that of the lake samples. Aromatic molecular formulae were strongly depleted in the lake deep water suggesting photooxidation of riverine SPE-DOM. This indicates that alpine SPE-DOM signatures are shaped by small-scale catchment properties, land degradation, and the influence of glaciers and wetlands. The close link of alpine SPE-DOM composition to landscape properties is indicative for a strong susceptibility of DOM characteristics to climatic and land use changes in High Asia.



Graphical Abstract: Main processes shaping SPE-DOM molecular characteristics and transformations in the high-alpine Nam Co catchment. DOM processing and sources are indicated by black arrows. Pasture degradation is indicated by a dashed arrow.

1. Introduction

Nutrient and energy cycles of ecosystems are connected by dissolved organic matter (DOM) fluxes (Spencer et al., 2014). DOM can connect fluvial ecosystems over hundreds of kilometres (Seidel et al., 2015) and links terrestrial and aquatic ecosystems (Goodman et al., 2011). Biogeochemical processing and DOM sources are known to shape the molecular composition of DOM (Liu et al., 2020; Roebuck et al., 2020; Seifert et al., 2016; Wilson and Xenopoulos, 2009). But how But how the DOM characteristics in alpine aquatic systems are influenced by different ecosystems and how DOM responds to ecosystem degradation is not well understood yet.

The Tibetan Plateau (TP) comprises the largest alpine pasture system in the world (Miehe et al., 2019) and is known as Asia's water tower (Bandyopadhyay, 2013), forming the source of large river systems. Large amounts of the water are stored in the ice masses of the TP, forming the largest frozen

freshwater reservoir outside the polar regions. This third pole environment is well investigated (Qiu, 2008; Yao et al., 2012), revealing that High Asia's ecosystems are threatened (Hopping et al., 2018) by climate change (Yao et al., 2019) and by intensification of land use (Harris, 2010). Furthermore, emerging freshwater quality issues give reason for concern (Qu et al., 2019).

The Nam Co catchment, located in the southern part of the TP, lies in the transition zone of the *K. pygmaea* dominated alpine pasture ecosystem (Miehe et al., 2008) and the alpine steppe. The unique positioning in this transition zone is expressed between the south, with a more humid glacial-influenced high-mountain ecosystem contrasting the hilly northern margin of the catchment, with more arid climate and vegetation dominated by alpine steppe, where clear signs of pasture degradation are visible (Maurischat et al., 2022). This makes the Nam Co catchment a suitable natural laboratory to test for effects on organic matter (OM) characteristics and the cycling of OM in different ecosystems (Anslan et al., 2020). The effects of pasture degradation of Tibetan soils on OM stocks have been studied previously (Liu et al., 2017), but investigations on degradation induced changes of DOM composition have fallen short. Also DOM characteristics and transformation in this complex natural interplay have only been investigated to limited extent. Spencer et al. (2014) found complex OM sources in glaciers, streams, and Lake Nam Co. A recent study highlighted a coupled decrease of "protein-like" DOM with an increase of "humic-like" DOM along the flowpath (Li et al., 2021), whereas other works found indications for less reactive stream DOM due to the cold and fast flowing water (Maurischat et al., 2022). This leaves important questions of 1) how DOM signatures are influenced by the diverse biotic and abiotic processes in the catchment with differing degrees of glaciation, alpine wetlands, and groundwater sources, as well as land degradation, and the alpine pasture alpine steppe ecotone, and 2) how DOM is processed along the streams and in the lake.

Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is an ultrahigh-resolution method allowing the identification of thousands of molecular formulae, offering an opportunity to better understand molecular, solid phase extracted (SPE) DOM characteristics, sources, and transformations (Hawkes et al., 2020; Leyva et al., 2020; Šantl-Temkiv et al., 2013). Here we used FT-ICR-MS to decipher characteristics and processing of stream SPE-DOM of different subcatchments, glaciers, a groundwater spring, an alpine wetland as well as SPE-DOM of an endorheic lake on the TP.

Our investigations aim at a better understanding of SPE-DOM processing at present conditions which will help to assess the potential consequences of changing environmental conditions in the future. It is important to link the potential vulnerability and responses of sensitive high-alpine ecosystems to ongoing anthropogenic changes. In this respect, we hypothesized:

I) SPE-DOM derived from different ecosystems (glaciers, groundwater springs and wetlands) and streams in degraded land possess unique DOM signatures compared to the integrated DOM of subcatchment streams.

II) The SPE-DOM transformation along the stream path is limited, no major compositional shift is expected in-stream.

III) The SPE-DOM characteristics of lake water are chemically distinct from the terrestrial DOM sources and integrated stream SPE-DOM composition.

2. Materials and methods

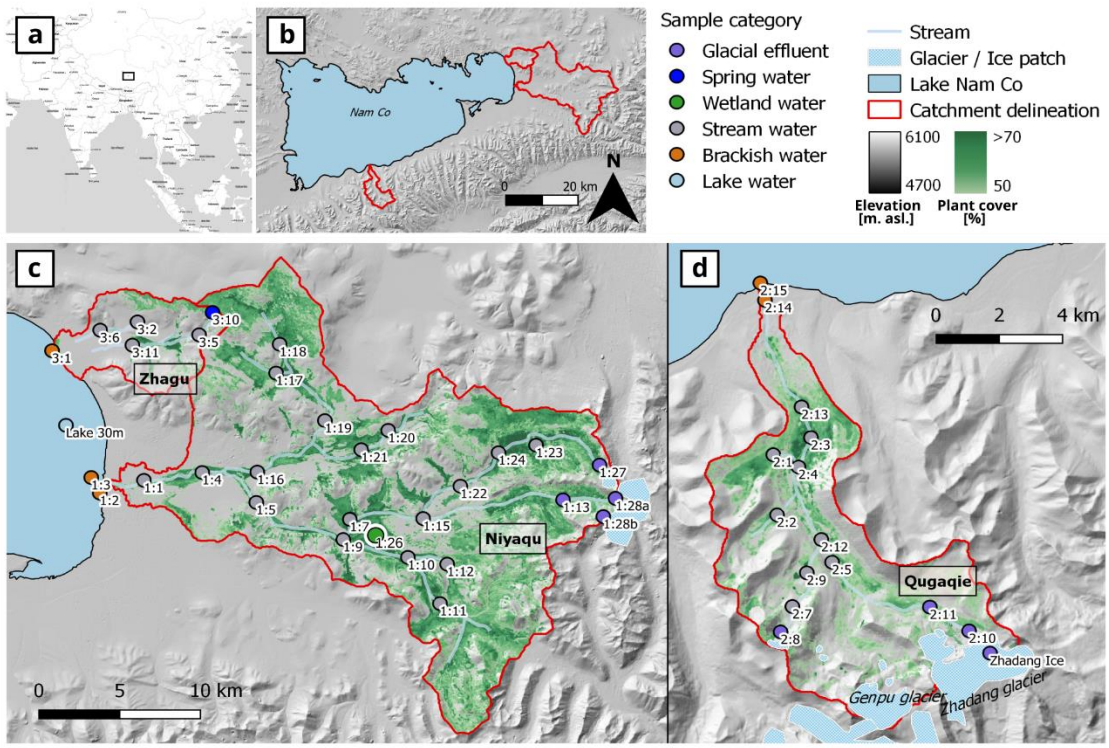
2.1 Site description and sampling

The Nam Co catchment has a total size of ~10800 km^2. Two main landscape units are distinguished, the southern mountainous and the northern upland zone. The south of the catchment is characterised by the Nyainqentanglha mountain ridge (NMR) with glaciation at highest elevations of more than 7000 m asl. (Bolch et al., 2010). Glacial meltwater is the dominant water source of southern streams (Adnan et al., 2019b). *K. pygmaea* pastures developed from 5300 m asl. downward (Anslan et al., 2020) and are associated with a felty organic-rich topsoil (Kaiser et al., 2008). Closer to the lake alpine steppe vegetation is prevailing (Nieberding et al., 2021). The southern part of the catchment is characterised by higher precipitation compared to the north. Up to 530 mm y^{-1} are measured at the NMR (Anslan et al., 2020). In the hilly uplands a less steep relief dominates (Yu et al., 2021), and an annual precipitation of around 300 mm y^{-1} is reported (Anslan et al., 2020). Alpine pasture is developed in north-exposed hill-flanks and valley bottoms, while alpine steppe grows on south exposed flanks, in the upland and at the lake shoreline (Maurischat et al., 2022). Along with this aridity gradient from south to north, land degradation is increasing (Anslan et al., 2020). The endorheic Lake Nam Co with an elevation of 4726 m asl. has a total size of ~2000 km². The lake is dimictic, oligotrophic, and lightly saline (0.9 g salt l^{-1}; Keil et al., 2010) and has a depth of 99 m. It is well supplied with oxygen and has a clear water column (Wang et al., 2020).

Three subcatchments of the Nam Co catchment were selected to represent the natural diversity (Fig. 1 a, b). The Niyaqu catchment (sample IDs 1:n in Fig. 1a, *low glaciation*) in the east has a total area of 406 km². Two streams drain the subcatchment, the southern stream receives meltwater from a glacier of the NMR located 700 m above lake level. This river runs through extensive alpine pastures and feeds a large alpine wetland (Fig. 1b, point 1:26). The northern stream drains a hilly upland area in the transition of the alpine steppe and the alpine pasture. The herding of yaks takes place throughout the year. The Zhagu subcatchment (sample IDs 3:n Fig. 1a, *no glaciation - degraded*) is located in the arid north. It has a size of 46 km² and is mainly characterised by hilly upland relief (Keil et al., 2010). There

is no glacial influence and only a small altitudinal gradient in this catchment, with the highest elevation at 5230 m. asl. Two creeks drain the catchment, both fed by groundwater springs. During sampling, the streams were arheic, and clear signs of degradation of *K. pygmaea* pastures were visible (Maurischat et al., 2022). Besides degraded pastures, alpine steppe is developed and used for animal husbandry. In the south, the Qugaqie subcatchment (sample IDs 2:n Fig. 1b, *high glaciation*) represents the NMR zone. The catchment has a size of 58 km² and is characterised by steep relief and a valley course in south-north direction (Keil et al., 2010). The altitudinal difference between the lake and the summit is 2200 m. This catchment is used as summer pasture.

Water samples were taken in September 2019 following the streams from source to terminus. Glaciers, wetlands, and groundwater springs were sampled directly. Three source groups (glacial effluents, groundwater springs, and alpine wetlands) and three sampling units (stream water, brackish water, and lake water) were distinguished, resulting in six sample categories (Fig. 1). Glacial effluents were drawn directly at or close to the glacial terminus, while groundwater was sampled directly at springs. Alpine wetland samples were taken from the standing water column in the wetlands. Brackish water samples were taken in the mixing zone of stream and lake water offshore in Lake Nam Co.



Data Source: JAXA ALOS World 3D - 30m (AW3030); Coordinate System: WGS 84/ UTM zone 46N EPSG: 3264; Landsat 5, 7, 8 imagery courtesy of the U.S. Geological Survey; Overview map: Data Commons License; openstreetmap.org (OSM)

Figure 1: (a) Overview map (Stamen Design, under CC BY 3.0. Data by Open Street Map contributors 2022, under CC BY SA, distributed under the Open Data Commons Open Database Licence, ODbL, v1.0). The rectangle represents the sampling area. (b) Shows the outline of the Nam Co Lake and the three subcatchments. (c) and (d) Map of the investigated subcatchments and sampling locations

with sample categories. Plant cover estimations from Maurischat et al. (2022) represent *K. pygmaea* pastures, the zones of most prominent yak grazing.

Samples were taken from the middle of the stream profile using a telescopic sampling device. Lake water was sampled from 30 m depth with a submersible sampler. All samples were taken in seven subsamples with a volume of 1 L each, mixed and a 0.5 L aliquot of this was taken for analysis. Samples were filtered on-site using a 0.45 µm mesh size polyethersulfone membrane (Supor, Pall, Port Washington, USA), a filtration device and a portable electric pump. Samples were stored in pre-cleaned high-density polyethylene bottles (Rotilabo, Carl Roth, Karlsruhe, Germany) and kept at -21°C until analysis.

2.2 Solid-phase extraction

DOM samples were acidified to pH 2 using 32 % HCl (Rotipuran p.a., Carl Roth, Karlsruhe, Germany). Dissolved organic carbon (DOC) concentrations were measured from 20 mL of sample by high-temperature oxidation on a total organic carbon analyser (varioTOC Cube, Elementar, Langenselbold, Germany). DOM samples were diluted with ultrapure water to a concentration of 1.5 mg C L^{-1}, 250 mL of diluted sample were used for extraction. Cartridges with 100 mg of styrene divinylbenzene polymer (PPL) resin (Bond Elut, Agilent Technologies, Santa Clara, USA) were used for extraction. SPE-DOM was prepared following Dittmar et al. (2008). The SPE elute was transferred to cauterized brown glass flasks (Neochrom, Neolab Migge, Heidelberg, Germany) sealed with polytetrafluorethylen caps (Neochrom, Neolab Migge, Heidelberg, Germany) and stored at -18°C until analysis. The extraction efficiency was evaluated by drying 0.2 mL of SPE-DOM under N_2 atmosphere and resolving the aliquot in ultrapure water. The samples were then analysed for their DOC concentrations by high temperature oxidation and the volumetric proportion of initial DOC to extracted SPE-DOC concentrations was calculated. Reference material (Suwannee River / IHSS) (Green et al., 2015) was compared with routine assays. Blank samples with ultrapure water were used to check for contamination.

2.3 Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry

SPE-DOM samples were diluted in 1:1 methanol/ultrapure water to a final concentration of 5 mg C L^{-1} for analysis. Samples were analysed in duplicates on a SolariX XR 15 Tesla FT-ICR-MS (Bruker Daltonik, Bremen, Germany). Electrospray ionization (ESI) was carried out in negative mode and samples were injected at a flow rate of 122 µL h^{-1}. 200 broadband scans (mass range of 92.14 to 2000 Da) were acquired per sample, accumulation time was 0.2 s per scan. Mass spectra were internally calibrated with a list of known $C_xH_yO_z$ molecular formulae over the mass range in the samples, achieving a mass error of < 0.1 ppm. Instrument variability was assessed with an in–house standard of SPE-DOM from North Equatorial Pacific Intermediate Water (NEqPIW) collected near Hawaii (Natural Energy

Laboratory of Hawaii Authority, NELHA) (Green et al., 2014). Molecular formula attribution was done with ICBM-OCEAN (Merder et al., 2020). The method detection limit (MDL) was applied (Riedel and Dittmar, 2014) with a minimum signal-to-noise ratio (S/MDL) of 2.5. Minimum signal to MDL ratio as backbone for recalibration was 5 using mean recalibration mode. Molecular formulae were assigned with a tolerance of 0.5 ppm as $C_{1-100}H_{1-125}O_{1-40}N_{0-4}S_{0-2}P_{0-1}$ in the mass range 95 to 1000 Da. Molecular formulae assignments were accepted if the molecular formula was present in >5% of the samples. Contaminants were identified and excluded using the contaminant reference mass list and in additional conformity with the SPE-DOM process blanks. Detection limits for peaks were normalised to sample peak intensities. The overall peak intensities were scaled to the local sample maxima using the sum of peaks. Molecular formulae containing isotopes (^{13}C, ^{18}O, ^{15}N, ^{34}S) were removed from the data table for further processing and molecular formulae with molar ratios of oxygen-to-carbon (O:C) = 0, O:C ≥ 1, and hydrogen-to-carbon (H:C) > 2.5 were removed as well. Duplicate samples were normalised, and molecular formulae were retained only when present in both duplicates.

2.4 SPE-DOM molecular descriptive classes, counts, and indices

Molecular formulae were assigned to SPE-DOM molecular compound classes (Leyva et al., 2020). The original compound classification was taken from Šantl-Temkiv et al. (2013), and SPE-DOM compound class labels were modified according to Merder et al. (2020). The modified aromaticity index (AI_{mod}) representing SPE-DOM aromaticity, was calculated for each formula as proposed by Koch and Dittmar (2006; 2016). AI_{mod} > 0.5 was assigned as aromatic, while AI_{mod} ≥ 0.67 was considered as condensed aromatic compounds. The degradation index (I_{Deg}) was calculated as a measure of degradation state of SPE-DOM (Flerus et al., 2012) and the terrestrial index (I_{Terr}) was calculated, as a measure of terrestrial SPE-DOM sources (Medeiros et al., 2016). Molecular formulae that were part of the island of stability (IOS) were evaluated to gain insight into the relative abundance of recalcitrant SPE-DOM (Lechtenfeld et al., 2014). This is based on the assumption that the IOS contains SPE-DOM molecular formulae representing recalcitrant oceanic SPE-DOM that is empirically stable on millennial time scales (Lechtenfeld et al., 2014). The CHO index was calculated as a measure of organic carbon oxidation (Mann et al., 2015). Low CHO values indicate highly reduced molecular formulae and high CHO values indicate highly oxidised molecular formulae. Molecular diversity was interpreted as α-diversity by depicting intracommunity molecular diversity (Thukral, 2017). Here we used the total number of molecular formulae on the group scale of subcatchments and sample categories.

2.5 Statistical analyses

Molecular formula intensities were rescaled between 0 and 1. Grouping was conducted with two independent factors: 1) the three subcatchments: Niyaqu (*low glaciation*), Qugaqie (*high glaciation*), Zhagu (*degraded*) and Lake Nam Co; and 2) the sample categories: glacial effluents, spring, and wetland

as well as stream water, brackish water, and lake water. Samples from spring and wetland, as well as Lake Nam Co, were excluded from statistical group comparisons due to the small sample sizes. Intensity weighted arithmetic means, and standard deviations were calculated for AI_{mod}, number of formulae containing the heteroatoms nitrogen (N), phosphorous (P) and sulphur (S), as well as the total number of assigned formulae and for compound classes.

Due to violations of normal distribution and homoscedasticity in combination with unbalanced sampling design, parametrical tests were considered unreliable (Bortz and Schuster, 2010). Multiple pairwise comparisons were conducted using Kruskal-Wallis tests in combination with Bonferroni post hoc corrected Dunn tests (Birnbaum, 1956). Significance levels of α = 0.05 were applied (Supplementary material, Table S1 and Table S2).

Nonmetric multidimensional scaling (NMDS) was used for dimensionality reduction and ordination (Anderson et al., 2006; Faith et al., 1987) based on a Bray-Curtis dissimilarity index matrix (k = 3). Data for NMDS were scaled and mean-centred (Jolliffe, 2002). NMDS was performed for independent factors (sites and sample categories), while those with low statistical power ($n < 3$) were not incorporated. Loadings, scores, and R^2-coefficients of determination are provided in the supplementary material (Tables S3 – S7). Co-correlation was checked visually and by Pearson's correlation coefficient, defined as $|r| > 0.75$. The following compound classes were combined because of co-correlation: aromatic O-rich and aromatic O-poor = ARO, highly unsaturated O-rich and highly unsaturated O-poor = HUSAT, unsaturated O-rich, unsaturated O-poor and unsaturated with N = USAT (Fig. 4). Collinear variables (section 2.6) were removed.

R software (The R project for statistical computing, v3.6.3, GNU free software) was used for statistics. The R base packages (R Core Team, 2013) and *tidyverse* (Wickham et al., 2019) were used for data organisation, pre-processing, and statistics. The packages *ggplot2* (Wickham et al., 2019) and *vegan* (Oksanen et al., 2020) were used for graphical illustration and for NMDS analysis, respectively.

2.6 Environmental variables for statistical analysis

Several parameters were used as predictor variables in the NMDS. Internal predictor variables were generated from the SPE-DOM dataset obtained by FT-ICR-MS (SPE-DOM indices, compound class percentages and heteroatom counts; described in section 2.4). External predictor variables were taken from a previous study of the same site and the same sampling campaign (Maurischat et al. 2022) and tested for statistical correlations with this SPE-DOM dataset. The selected external variables showed evidence as functional predictors in the prior study and are acknowledged as key parameters for DOM characterisation in numerous applications. The variables included fluorescent DOM (FDOM) components (here used as the product of co-correlated microbial and terrestrial-like FDOM

components) (Fellman et al., 2010), DOC concentrations (Eklöf et al., 2021), $\delta^{13}C$ of DOC (Guo et al., 2006), dissolved inorganic carbon (DIC) concentrations, as a catchment geology parameter (Wang et al., 2016), nitrate concentrations (NO_3^-) (Harms et al., 2016), and the mean plant cover at the sampling point as land use indicator (Sankar et al., 2020).

3. Results

3.1 Sample treatment and quality assessment

SPE-DOM extraction efficiencies were 61.4 % ± 18.6 % (Supplementary material, Table S8). DOM extraction efficiencies and the number of SPE-DOM molecular formulae (detected by FT-ICR-MS) were only weakly correlated (R^2=0.08, F (1, 43) =5.144, β=-0.007, p=0.02) indicating that extraction efficiency was not an important driver of molecular variability in our samples. Duplicate SPE-DOM process blanks had below 1000 molecular formulae assigned and the Suwannee River / IHSS reference material had ~3500. Minimum molecular formulae count to accept NOM samples was set to 2000, roughly in the range of the number of molecular formulae expected from terrestrial aquatic samples (Seidel et al., 2017; Spencer et al., 2014). The in house-control standard (NEqPIW SPE-DOM) was run repeatedly during analysis (*n* = 38) to account for instrument variability. On average, 3558 (± 218) molecular formulae were assigned: no significant instrument drift was detected (Supplementary material Figure S1).

3.2 Molecular group counts and statistics

The number of assigned molecular formulae, here termed α-diversity, decreased in the order Qugaqie (*high glaciation*) > Zhagu (*degraded*) > Niyaqu (*low glaciation*) > Lake Nam Co. Lake Nam CO SPE-DOM had 50 % less assigned molecular formulae compared to the subcatchments (Table 1). SPE-DOM samples from wetland and brackish environments had the highest numbers of assigned molecular formulae (Table 2), compositional shifts in the H/C and O/C ratio of brackish and lake SPE-DOM are displayed in Figure 6a with a zoom on one example mass range (371-371.3 *m/z*, Fig. 6b).

Lake Nam Co SPE-DOM had relatively more N-containing molecular formulae compared to SPE-DOM samples from the subcatchments (Table 2). The relative abundance of S-containing molecular formulae was significantly lower in *low glaciation* Niyaqu compared to *high glaciation* Qugaqie SPE-DOM (p=0.005). P-containing molecular formulae were enriched in the lake compared to SPE-DOM of the subcatchments (Table 1, Table S1). N-containing molecular formulae were more abundant in wetland water and brackish SPE-DOM samples, while groundwater spring SPE-DOM samples had the lowest count. Glacial SPE-DOM had the highest relative abundances of S-containing molecular formulae.

SPE-DOM of Lake Nam Co had lower AI_{mod} and I_{Terr} values, compared to the subcatchments (Table 1). For the sample categories, brackish SPE-DOM had higher I_{Terr} values but no significant differences were

observed (Table 2). The H/C ratios were highest in Lake SPE-DOM (Table 1) with significantly higher relative numbers of hydrogen compared to Niyaqu (p=0.005). Additionally, the CHO index showed that Lake SPE-DOM and SPE-DOM of the *high glaciated* Qugaqie catchment were less oxidized compared to SPE-DOM of the *degraded* Zhagu catchment (p=0.042| statistically tested only for subcatchments). Correspondingly, I_{Deg} values, were significantly higher for *degraded* Zhagu compared to the *glaciated* Niyaqu and Qugaqie subcatchments SPE-DOM (p=0.0002). IOS values, indicative of recalcitrant SPE-DOM with relatively long residence times, showed a significantly higher contribution in *degraded* Zhagu and Lake Nam Co compared to *low glaciated* Niyaqu and *high glaciated* Qugaqie (p= 0.04, 0.05, respectively| statistically tested only for subcatchments). For sample categories (Table 2), H/C ratios were higher in glacial and lake SPE-DOM and the CHO index suggested less oxidized SPE-DOM in glacial effluents and the lake compared to terrestrial sources (Fig. 5). Correspondingly highest I_{Deg} values were observed in SPE-DOM of the brackish zone and in groundwater springs. The percentage of IOS values increased in lake and groundwater springs compared to glacial effluents, indicating differences in the contribution of recalcitrant SPE-DOM.

Compound classes (Fig. 2) give an overview of the composition of SPE-DOM. Largest differences among the sites were found for aromatic classes. Lake Nam Co had 80 % less aromatic (O)-rich molecular classes compared to the subcatchments. The lake had only 5 % of the abundance of aromatic O-poor compounds compared to subcatchment SPE-DOM. For highly unsaturated O-rich molecular formulae, Lake Nam Co had higher values compared to subcatchment SPE-DOM, while highly unsaturated O-poor molecular formulae decreased in the lake. Furthermore, the unsaturated O-poor and unsaturated N-containing SPE-DOM classes were higher in lake SPE-DOM compared to the terrestrial systems. Highly unsaturated O-rich formulae were more abundant in SPE-DOM of *high glaciation* Qugaqie compared to *low glaciation* Niyaqu (Fig. 2).

Table 1: Overview on mean and standard deviation of indices and elemental composition ratios and mean and standard deviation of molecular formulae counts for sites.

Variable	Niyaqu (*low glaciation*) n=26		Qugaqie (*high glaciation*) n=15		Zhagu (*degraded*) n=4		Lake Nam Co ǂ n=1
	Mean	***SD (±)***	***Mean***	***SD (±)***	***Mean***	***SD (±)***	***Value***
AI_{mod}	0.33 [a]	0.05	0.32 [a]	0.02	0.36 [a]	0.02	0.25
O/C	0.46 [a]	0.02	0.45 [a]	0.02	0.45 [a]	0.02	0.44
H/C	1.12 [a]	0.08	1.17 [b]	0.05	1.13 [a,b]	0.08	1.20
CHO	-0.21 [a,b]	0.11	-0.26 [b]	0.08	-0.17 [a]	0.04	-0.29
I_{Deg}	0.55 [a]	0.15	0.59 [a]	0.10	0.77 [b]	0.02	0.33
I_{Terr}	0.32 [a]	0.13	0.35 [a]	0.03	0.34 [a]	0.04	0.08
IOS [%]	14.4 [a]	1.7	14.7 [a]	1.6	18.0 [b]	2.2	17.2
Number of formulae containing N	1412 [a] (49.2)	941	1928 [a] (54.9)	1083	1933 [a] (56.5)	951	1284 (58.8)
Number of formulae containing P	143 [a] (4.9)	112	129 [a] (3.6)	96	130 [a] (3.8)	54	196 (8.9)
Number of formulae containing S	69 [a] (2.4)	96	146 [b] (4.1)	101	46 [a,b] (1.3)	43	37 (1.6)
Total number of molecular formulae (α-diversity)	2867 [a]	1060	3509 [a]	1340	3416 [a]	848	2183

[a, b] Significant differences (α=0.05) are indicated by superscript letters. ǂ Single sample, standard deviations were not calculated and statistical tests were not performed. For heteroatoms (N, P, S), percentages of the total number of molecular formulae are given in parentheses. Boxplots of data are presented in the supplementary material (Fig. S2).

Table 2: Overview on mean and standard deviation of indices and elemental composition ratios and mean and standard deviation of formulae counts for sample categories.

Variable	Glacial effluent n=8		Spring ǂ n=1	Wetland ǂ n=1	Stream water n=38		Brackish water n=4		Lake water ǂ n=1
	Mean	***SD (±)***	***Value***	***Value***	***Mean***	***SD (±)***	***Mean***	***SD (±)***	***Value***
AI_{mod}	0.31 [a]	0.04	0.35	0.33	0.33 [a]	0.05	0.35 [a]	0.02	0.25
O/C	0.45 [a]	0.03	0.44	0.46	0.46 [a]	0.02	0.46 [a]	0.01	0.44
H/C	1.17 [a]	0.08	1.15	1.09	1.13 [a]	0.07	1.13 [a]	0.07	1.20
CHO	-0.28 [a]	0.14	-0.18	-0.19	-0.22 [a]	0.09	-0.18 [a]	0.07	-0.29
I_{Deg}	0.53 [a]	0.13	0.77	0.53	0.58 [a]	0.15	0.66 [a]	0.02	0.33
I_{Terr}	0.34 [a]	0.06	0.32	0.34	0.32 [a]	0.11	0.39 [a]	0.04	0.08
IOS [%]	13.7 [a]	2.24	17.4	16.0	15.1 [a]	1.9	14.5 [a]	1.33	17.2
Number of formulae containing N	1548 [a] (52.2)	1135	1261 (44.7)	2549 (62.3)	1511 [a] (44.2)	922	2586 [a] (57.5)	1231	1284 (58.8)
Number of formulae containing P	103 [a] (3.4)	90	117 (4.1)	291 (7.1)	130 [a] (3.8)	91	231 [a] (5.1)	160	196 (8.9)
Number of formulae containing S	134 [a] (4.5)	125	9 (0.3)	68 (1.6)	77 [a] (2.2)	90	163 [a] (3.6)	126	37 (1.6)
Total number of formulae (α-diversity)	2965 [a]	1132	2819	4091	3416 [a]	1053	4492 [a]	1639	2183

[a, b] Significant differences (α=0.05) are indicated by superscript letters. [‡] Single sample, standard deviations were not calculated and statistical tests were not performed. For heteroatoms (N, P, S), percentages of the total number of molecular formulae are given in parentheses. Boxplots of data are presented in the supplementary material (Fig. S3).

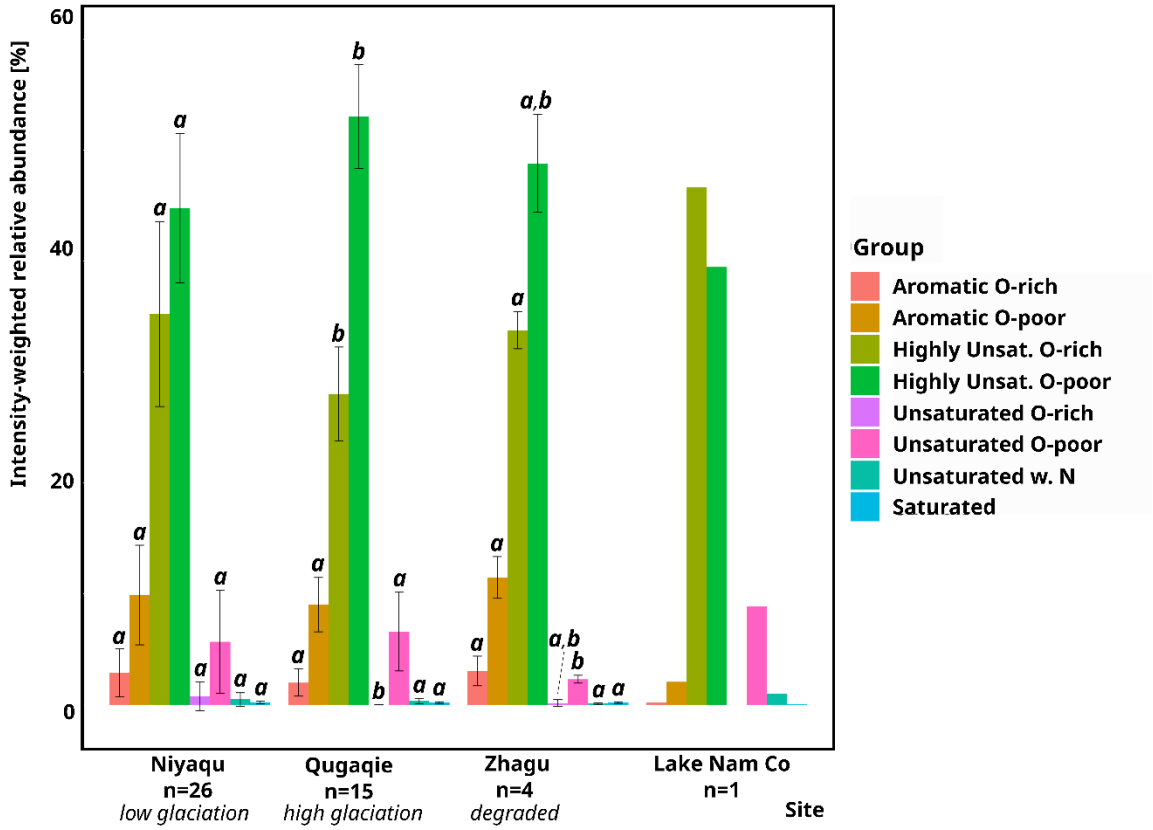


Figure 2: Relative intensity-weighted averages of SPE-DOM compound classes for stream water of the three subcatchments and Lake Nam Co (sites). For sample sizes n<3, no standard deviations are given. Error bars indicate standard deviations, significant differences (α=0.05) are indicated by superscript letters (a, b).

The relative abundances of aromatic compounds were different between SPE-Dom of the lake and SPE-DOM assigned to other sample categories, with samples from Nam Co having the lowest relative abundances (Fig. 3). Brackish SPE-DOM had significantly more aromatic O-poor compounds compared to glacial effluents (p=0.05). SPE-DOM from the Nam Co Lake had the highest relative abundances of highly unsaturated O-rich compounds compared to all other groups, especially glacial SPE-DOM had on average 40% less O-rich compounds.

Unsaturated O-poor compounds were relatively more abundant in lake SPE-DOM and glacial effluents compared to the other environmental sample categories; this class was especially depleted in spring SPE-DOM. Alongside Lake and glacial SPE-DOM were relatively rich in unsaturated N-containing formulae. Spring water had the fewest of this compound class (Figure 3).

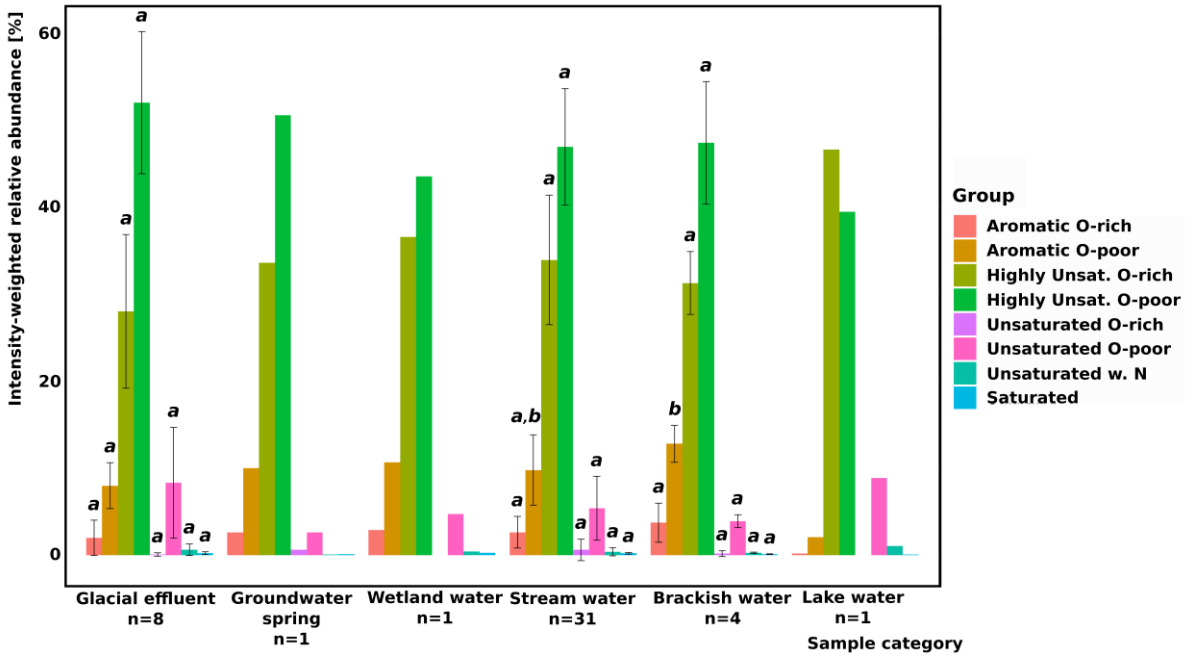


Figure 3: Relative intensity-weighted averages of DOM compound classes according to environmental sample categories. Error bars indicate standard deviations. For sample sizes n<3, no standard deviations are given. Error bars indicate standard deviations, significant differences (α=0.05) are indicated by superscript letters (a, b).

3.3 Multivariate statistical analysis

NMDS was conducted with a graphical overlay for sites and sample categories. The stress value of 0.14 is within tolerance (< 0.2; Dexter et al., 2018). In NMDS ordination (Fig. 4a) molecular formulae expanded in the ordination plane above the coordinate origin. Samples were distributed in two groups depending on aromaticity and related indicators (AI_{mod}, I_{Deg}, and I_{Terr}). AI_{mod} values increased from the positive to the negative direction of the first dimension. In the positive direction of dimension 1, samples containing relatively more unsaturated and saturated SPE-DOM compound classes as well as S-containing compounds were distributed (Fig. 4b) these samples also had lower AI_{mod}, I_{Deg}, and I_{Terr}. In the negative direction of dimension 1, samples with a higher abundance of aromatic compound classes were distributed. The external predictors DIC and plant cover were loading in this direction along with DOC concentrations and values of FDOM and $\delta^{13}C$ of DOM (black vectors in Fig. 4b) suggesting that these samples were influenced by DOM inputs of plants and soils. The second dimension of the NMDS ordination separated samples according to the abundance of highly unsaturated and saturated SPE-

DOM compounds, together with the N, P and S heteroatoms. These samples were also characterized by elevated NO_3 concentrations. Samples located closer to the origin of predictor variable vectors were related to higher IOS percentages and suggested a higher abundance of recalcitrant SPE-DOM.

Samples of the *degraded* Zhagu subcatchment were located close to the centre of predictor variable vectors (Fig. 4b), while samples of *high glaciation* Qugaqie were scattered on the dimension plane. SPE-DOM from the *low glaciation* Niyaqu subcatchment as more uniformly placed in the lower part. Stream water SPE-DOM was scattered, indicating large chemical diversity. Most brackish SPE-DOM samples were placed in the upper left of the plane, associated with higher terrestrial indicators (AI_{mod}, I_{Deg}, and I_{Terr}) and more abundance of N and P heteroatoms. Glacial SPE-DOM was associated with heteroatoms, saturated and unsaturated molecular compounds, and less depleted $\delta\ ^{13}C$ of DOM, visible by its positioning in the bottom left to top right of the ordination plane.

4. Discussion

4. 1 Catchment properties shape SPE-DOM composition at Lake Nam Co

The subcatchments (*high glaciation, low glaciation and degraded*) of Lake Nam Co differed significantly in their molecular SPE-DOM composition. The *high glaciation* subcatchment had the largest molecular α-diversity and a larger abundance of S heteroatoms (Table 1). This can be influenced by the productivity of the glacial ecosystem (Hodson et al., 2008). S heteroatoms in DOM are likely related to high sulphide contents in the runoff of Zhadang glacier (Yu et al., 2021). Sulphate reduction takes place in glacial sediments and ice (Wu et al., 2012). Under sulfidic conditions, sulphide can be incorporated into DOM ("sulfurization") (Pohlabeln et al., 2017). The higher abundance of O-poor compounds, namely with depleted unsaturated O-rich and highly unsaturated O-rich molecular formulae and increased highly unsaturated O-poor and unsaturated O-poor formulae alongside with negative CHO values, indicates less intensively microbial transformed SPE-DOM compounds (Anesio et al., 2009; Hood et al., 2009; Spencer et al., 2014) compared to the *degraded* and *low glaciation* subcatchments. Accordingly, D'Andrilli et al. (2019) observed the relative increase of O-enriched molecular formulae after bio-incubations of DOM. Likely, low water temperatures of the glacial meltwater in Qugaqie hamper the microbial decomposition of DOM (Adams et al., 2010).

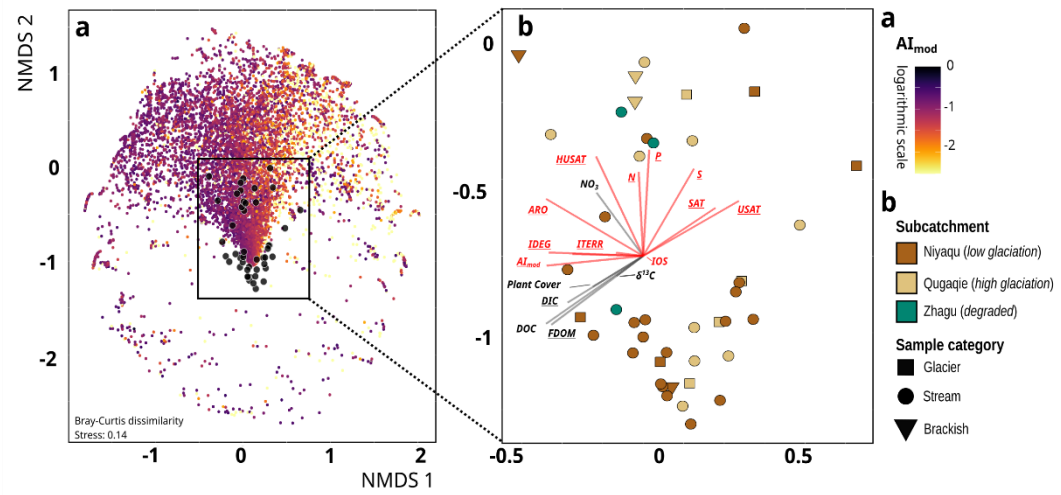


Figure 4: Non-metric multidimensional (NMDS) scaling analysis based on the relative abundances of SPE-DOM molecular formulae (calculated on Bray–Curtis dissimilarity matrix) (a) with the colour-coded modified aromaticity index (AI_{mod}, logarithmic scale) (Koch and Dittmar 2006; 2016). b) Zoom of NMDS analysis based on the relative abundances of SPE-DOM molecular formulae with post-hoc fitted SPE-DOM parameters (internal predictor variables, red) and environmental parameters (external predictor variables, black) shown as vectors. Underlined parameters were significantly ($p \leq 0.05$) related to NMDS ordination (stress = 0.14, k = 3). ARO= aromatic O-rich and aromatic O-poor, HUSAT= highly unsaturated O-rich and highly unsaturated O-poor, USAT= unsaturated O-rich, unsaturated O-poor and unsaturated with N, and SAT= saturated. Subcatchments are represented by the colour of sample points, while sample categories are represented by shapes.

Compared to the Qugaqie catchment, the contribution of glacial meltwater was smaller in the Niyaqu subcatchment and absent in Zhagu. The main water sources for Zhagu are precipitation and groundwater (Adnan et al., 2019a; Anslan et al., 2020; Tran et al., 2021). In the *degraded* Zhagu subcatchment, a stronger indication of aromaticity with higher AI_{mod} and higher relative abundance of aromatic compounds was found. Likewise, the higher oxidation state indicated by the CHO index (Fig. 5b, Table 2) and higher degradation index (I_{Deg}, Table 2) suggest a larger share of soil-borne, aromatic DOM compounds This was also corroborated by the higher percentage of the IOS (+ ~3 %), indicating a larger input of degradation products. The *low glaciation* Niyaqu catchment in comparison had a higher contribution of aromatic and unsaturated compounds together with a lower H/C ratio, suggesting less oxidised DOM. In combination with the AI_{mod} and I_{Terr} indices this suggests inputs of soils and plants, with e.g. lignin and hemicellulose and their degradation products (Roebuck et al., 2018; Seifert et al., 2016).

The *high glaciation* Qugaqie catchment comprises of a signature rich in highly unsaturated O-poor and unsaturated O-poor formulae likely derived from a glacial-borne microbial source. Furthermore, the high aromatic indices (AI_{mod}, I_{Terr}) and high percentages of aromatic compound classes are indicative of SPE-DOM derived from soil and plant material. Likely, there is a steady input of soil-derived material into the streams from pastoral land as demonstrated for other grassland systems (Seifert et al., 2016;

Lu et al., 2015). Notably, this influence became smaller, when glacial-borne more unsaturated DOM was more dominant on subcatchment level (Fig. 3b).

4.2 The effect of pasture degradation on SPE-DOM composition

Molecular α-diversity defined by the number of molecular formulae and SPE-DOM characteristics can be pinpointed to landscape units / environmental sample categories (summarized in Fig. 7). Glacial SPE-DOM from Qugaqie and Niyaqu subcatchments contained two different signatures from different sources. First, with high abundances of unsaturated compounds with and without nitrogen as well as a low oxidation state of carbon (Fig. 5a), high ratios of H/C and low percentages of recalcitrant SPE-DOM visible by the IOS. These parameters indicate a relatively fresh, reduced (oxygen-poor) SPE-DOM of low-molecular mass probably derived from microbial activity at the partly anoxic ice shield. This is in-line with findings from other glacial environments worldwide (Hood et al., 2009; Telling et al., 2011; Anesio et al., 2009). Second, aromatic and highly unsaturated compound classes and aromatic and terrestrial indices (I_{Terr}, AI_{mod}) were suggesting plant- and soil-borne SPE-DOM sources, despite the absence of plant cover in the glacial zones. Glaciers receive compounds with higher molecular mass from aeolian deposition, either condensed (poly)aromatics, e.g. from the burning of fossil fuels (Takeuchi, 2002) or compounds uncondensed but rich in phenolics, usually associated with dust from degraded vascular plant material and soils (Singer et al., 2012). Local dust formation on the TP is projected to increase with ongoing pasture degradation (Wang et al., 2008), likely affecting the DOM composition of glaciers. The coexistence of microbial produced, autochthonous DOM and airborne aromatic, allochthonous DOM renders the understanding of the downstream fate of glacial DOM difficult.

Groundwater spring SPE-DOM from the *degraded* Zhagu subcatchment mainly contained aromatic and highly unsaturated compounds. Molecular α-diversity and the number of N, P and S heteroatoms were low. Together with this, high I_{Deg} and CHO indices suggested a strong degradation of spring SPE-DOM (Fig. 5b) further 17% of DOM being IOS DOM indicates a large contribution of recalcitrant DOM. Spring water is generally expected to inherit aquifer and catchment characteristics in its DOM signature, also partly preserving its terrigenous source (Osterholz et al., 2022; Yoo et al., 2020). The shallow groundwater table of Zhagu (Tran et al., 2021), is in contact with soil OM and yak faeces (Maurischat et al., 2022), which can leach soil-borne OM to the groundwater (Connolly et al., 2020) which re-emerge at groundwater springs. Hence, the highly degraded SPE-DOM compounds likely originated from the degraded pedosphere and have been transported with the groundwater. The connection of degraded pastures of Zhagu (Fig. 1 & 4b) with the molecular composition of groundwater spring SPE-DOM indicates that highly modified SPE-DOM signatures are transported with the spring water and retrieved in streams.

SPE-DOM of an alpine wetland had a high α-diversity (>4000 molecular formulae), was rich in N, and P heteroatoms, as well as in highly unsaturated O-rich, and unsaturated O-poor compounds. The wetland was also enriched in ammonium and DOC compared to the streams (Maurischat et al., 2022). Alpine wetlands are highly productive and contain large amounts of nutrients in the biomass and soil OM (Bai et al., 2010; Zhang et al., 2020). Wetlands on the TP have been massively degrading (Zhang et al., 2011), enhancing microbial decomposition in wetland mire soils (Ma et al., 2018) and driving a subsequent release of nutrients into adjacent streams (Gao, 2016). For Nam Co Lake this would pose a severe biodiversity threat to oligotrophic streams and the sensitive lake shore (Hu et al., 2016).

Stream samples are concentrated in the lower centre of the NMDS (Fig. 4b). these samples had a dominance of aromatic compounds either associated with highly unsaturated O-poor or highly unsaturated O-rich formulae, suggesting mostly inputs of terrestrial compounds, such as lignin and tannin and their degradation products (Mann et al., 2015), further corroborated by depleted $\delta^{13}C$ DOC signatures (Fig. 4b; Maurischat et al., 2022). The *K. pygmaea* ecosystem spreads as an azonal pasture along the streams (Fig. 1). Lu et al. (2015) pointed out that grassland sites provide terrestrial inputs of aromatic and highly unsaturated compounds to surface waters. The *K. pygmaea* pasture browsed by yak has potential influence of their faeces (Du et al., 2021), suggesting lower molecular mass and negative CHO inputs due to the reductive conditions in the yak gastrointestinal tract and decomposition of plant-borne material therein (Fahey et al., 1983). Stream samples can therefore also be under influence of changing inputs from the pasture ecosystem. Faeces inputs and products of their microbial utilisation are likely associated with increases of N-containing unsaturated formulae and saturated formulae (Vega et al., 2020). Pastoral land-use and pasture degradation both had a considerable impact on stream SPE-DOM composition. While intact pastures released a highly unsaturated and aromatic signature related to the pasture soils and plants, streams close to degraded pastures were characterised by highly oxidized aromatic signatures and low molecular α-diversity.

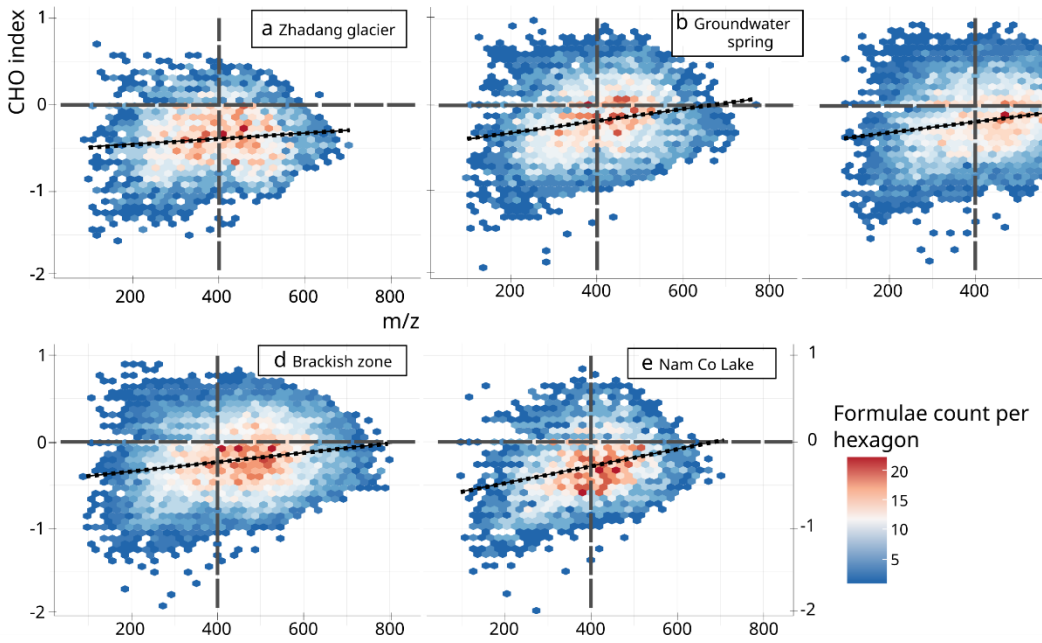


Figure 5: Hexagon scatters plot the count of the chemical formula in the space of the CHO index (Mann et al., 2015) and *m/z*. The black dotted lines represent the linear model of the CHO index and *m/z* of the respective sample, and the grey dashed lines split the plot into quadrants for orientation. The regression and ledger lines are printed to guide the eyes only. a) Ice from a glacier in *high glaciation* Qugaqie, b) groundwater spring from the upland of *degraded* Zhagu (3:10), c) water from the standing water column of a wetland (1:26), d) water from the brackish zone of Lake Nam Co and a tributary stream (2:15) and e) water sample from 30 m depth of Lake Nam Co. Axis scales are fixed.

4.3 The Lake reactor: photooxidation changes the SPE-DOM molecular composition

Brackish SPE-DOM samples had the highest molecular α-diversity, highest number of N and S heteroatoms and highest number of aromatic O-poor compounds together with high AI_{mod} and I_{Terr} (Tab. 2). Thus, brackish SPE-DOM retained and accumulated the terrestrial signal of streams. Brackish regions are not only zones of gradual mixing of different water masses (Van Dongen et al. 2008), where terrestrial-derived DOM of streams is exported to lakes and the ocean (Benner et al., 2004; Dittmar and Kattner, 2003; Ruediger, 2003) but are also zones of chemical transformation and uptake of riverine DOM, e.g. by flocculation and osmotrophy (Hoikkala et al. 2015). The relative increase of aromatic compounds in brackish SPE-DOM compared to stream SPE-DOM (Fig. 3) suggests a relative enrichment, for example, by selective degradation and oxidation of lower-molecular mass compounds in the wash of the waves as indicated by the increased CHO index and, I_{Deg} values (Fig. 5d) as shown for estuaries (Asmala et al., 2014). We conclude that the high molecular α-diversity in brackish samples represents both, the terrestrial input from streams mixing with the SPE-DOM signature of lake water and selective degradation of SPE-DOM in the high-energy wave zone.

Lake water differed in its SPE-DOM composition most strongly as compared to all other sample categories (Fig 7). Lake SPE-DOM was relatively enriched in unsaturated and saturated compounds,

484 which can include lipids and carbohydrates, but it was relatively depleted in aromatic and highly
485 unsaturated O-rich molecular formulae. Correspondingly, AI_{mod} and I_{Terr} decreased. Photooxidation and
486 microbial degradation can both remove aromatic DOM, such as lignin-derived phenolics (Spencer et
487 al. 2009; Helms et al. 2014; Vähätalo and Wetzel, 2004). Given the clear water column and high
488 irradiation at the TP (Wang et al., 2020), photooxidation can take place down to greater depths in the
489 lake, making it a probable important mechanism. Photooxidation in combination with microbial
490 degradation can explain the depletion of aromatic constituents when comparing brackish and lake SPE-
491 DOM (blue points in Fig. 6a and mass spectra in Fig. 6b).

492 In the estuary-lake gradient, riverine terrigenous SPE-DOM likely underwent a transformation, leaving
493 more recalcitrant SPE-DOM behind (Goldberg et al., 2015) as corroborated by the 2.1 % and 2.7 %
494 increase of IOS compared to stream and brackish SPE-DOM, respectively (Table 2). Along with
495 transformation of imported riverine SPE-DOM, primary production in the lake plays a key role for the
496 SPE-DOM characteristics of Lake Nam Co. The increase in unsaturated and unsaturated N-containing
497 compound classes and the more negative CHO with lower molecular mass (Fig. 5e) in lake SPE-DOM
498 (Fig. 3, yellow points in Fig. 6a) act as indicators for bacterial and algal primary production in the lake.
499 Microbial DOM sources have been suggested for Lake Nam Co (Spencer et al., 2014; Maurischat et al.,
500 2022) and other large lakes of the TP (Liu et al., 2020). Performing a food web study in Lake Nam Co,
501 Hu et al. (2016) reported that mainly lake-borne organic carbon sources are utilised by zooplankton,
502 showing the importance of this type of DOM as substrate. The comparably low CHO index (Fig. 5e)
503 underlines the existence of low molecular mass reduced DOM produced in the lake, while higher
504 molecular masses of aromatic and highly unsaturated riverine origin are more oxidised in the lake
505 environment compared to other systems (indicated by the greatest steepness of the regression line in
506 Fig. 5e), indicative of the strong processing described above. Lake SPE-DOM exhibited sources derived
507 from algal and microbial production. Also it is low in aromatic compounds and terrigenous indices and
508 had a much larger percentage of IOS as compared to subcatchment streams SPE-DOM, suggesting
509 more recalcitrant compounds. The shift in SPE-DOM molecular composition, hence, shows that the

terrigenous riverine aromatic DOM is processed in the lake, while microbiota derived DOM is produced.

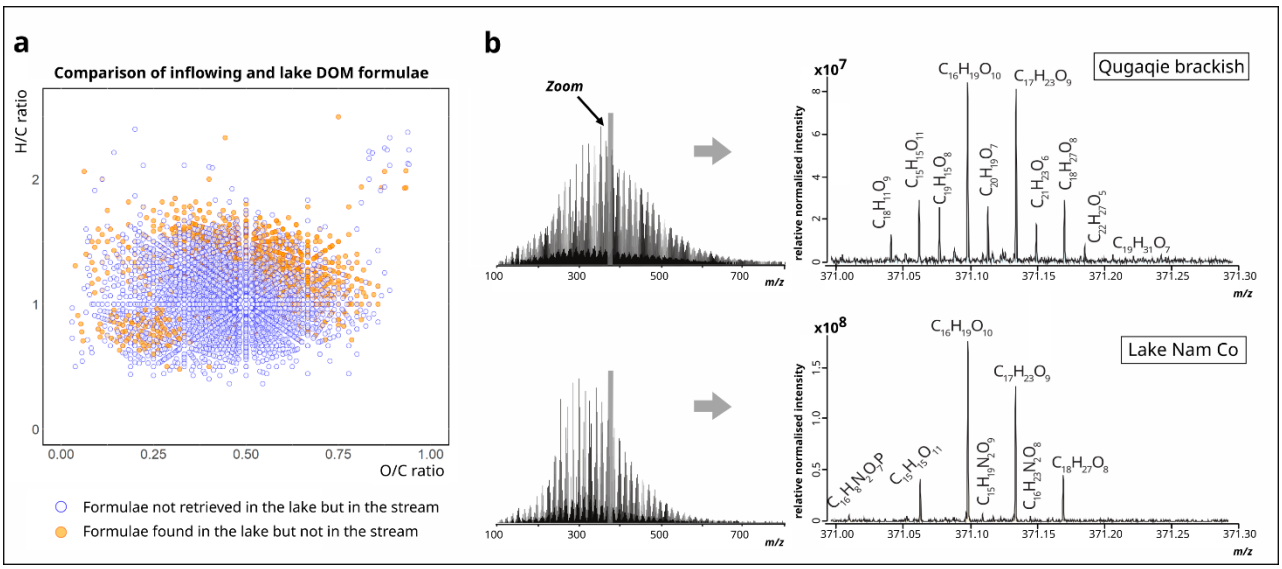


Figure 6: A) van Krevelen diagram of molecular formulae retrieved in lake DOM but not in brackish DOM and vice versa. B) Left: Intensity normalised overview mass spectra (m/z: 100-800) of Qugaqie brackish DOM (Id 2:15) and a water sample from 30 m depth of the Lake Nam Co. Right: Exemplary zoom into one mass range (371.0-371.3 *m/z*) of the respective samples. Note the axis scales shift.

5. Conclusions

We elucidated the molecular composition and the processing of SPE-DOM in the High Asian endorheic Nam Co catchment. We investigated three subcatchments: *high glaciation, low glaciation, no glaciation / degraded*, including SPE-DOM samples of glaciers, groundwater springs, alpine wetlands, streams, the brackish mixing zone, and the weakly saline lake. The subcatchments differed in the molecular composition of SPE-DOM.

In the *high glaciation* catchment, we identified a unique dual source of a microbial, low-molecular mass SPE-DOM relatively enriched in S heteroatoms and unsaturated compounds with and without nitrogen, suggesting autotrophic sources in the glacial ice shield. Meanwhile, aromatic SPE-DOM with high abundance of highly unsaturated compounds, such as plant-derived lignin degradation products or polycondensed aromatics derived from the combustion of fossil fuels or household burning of yak faeces, hint at a depositional source by aeolian transport. The influence of glacial meltwater greatly modified SPE-DOM signatures along the *high glaciation* Qugaqie stream, probably delivering more bio-available compounds to the southern lake shoreline. The *low glaciation* Niyaqu catchment comprised a lower molecular α-diversity and had a mainly terrestrial-borne SPE-DOM source of highly unsaturated and aromatic compounds, attributed to the input of the surrounding *K. pygmaea* pastures to the streams. In comparison, spring SPE-DOM of the *degraded* Zhagu subcatchment was influenced by degradation of *K. pygmaea* pastures visible by highly degraded and oxidized signatures with a higher percentage of recalcitrant compounds. DOM signatures can thus be used to trace pasture degradation. Wetland SPE-DOM exhibited high molecular α-diversity and was relatively enriched with N-heteroatoms. Wetland SPE-DOM reflected the comparably high primary productivity of these systems. The reported degradation of alpine wetlands in High Asia can drive a large release of DOM to the streams; at Nam Co this can be considered a threat to the oligotrophic lake. The stream samples were mostly associated with the input of terrestrial-borne materials, originating from vascular plants and soils. These are attributed to the *K. pygmaea* ecosystem stretching along the paths of streams. From the pastures, aromatic and highly unsaturated SPE-DOM was constantly refuelled into the streams. Brackish SPE-DOM represented the mixing zone of stream and lake water. Here riverine terrestrial DOM entered the lake, indicating that DOM transformation along the stream was limited. However, lake SPE-DOM was different compared to the tributaries. Its molecular composition suggested photooxidation and microbial degradation as transformation of imported stream DOM, together with a source of microbial and algal production in the oligotrophic lake.

Our study shows that DOM cycling in the Nam Co catchment needs a thorough assessment, as it can be diverse on subcatchment level and between landscape units. In order to safeguard water resources and related ecosystem services, knowledge about the different sources and the fate of DOM is

indispensable. SPE-DOM molecular properties have proven to be selective for the source systems and hence allow to decipher landscape processes. In the case of the sensitive TP, they might be a way forward to better understand the local effects of global change.

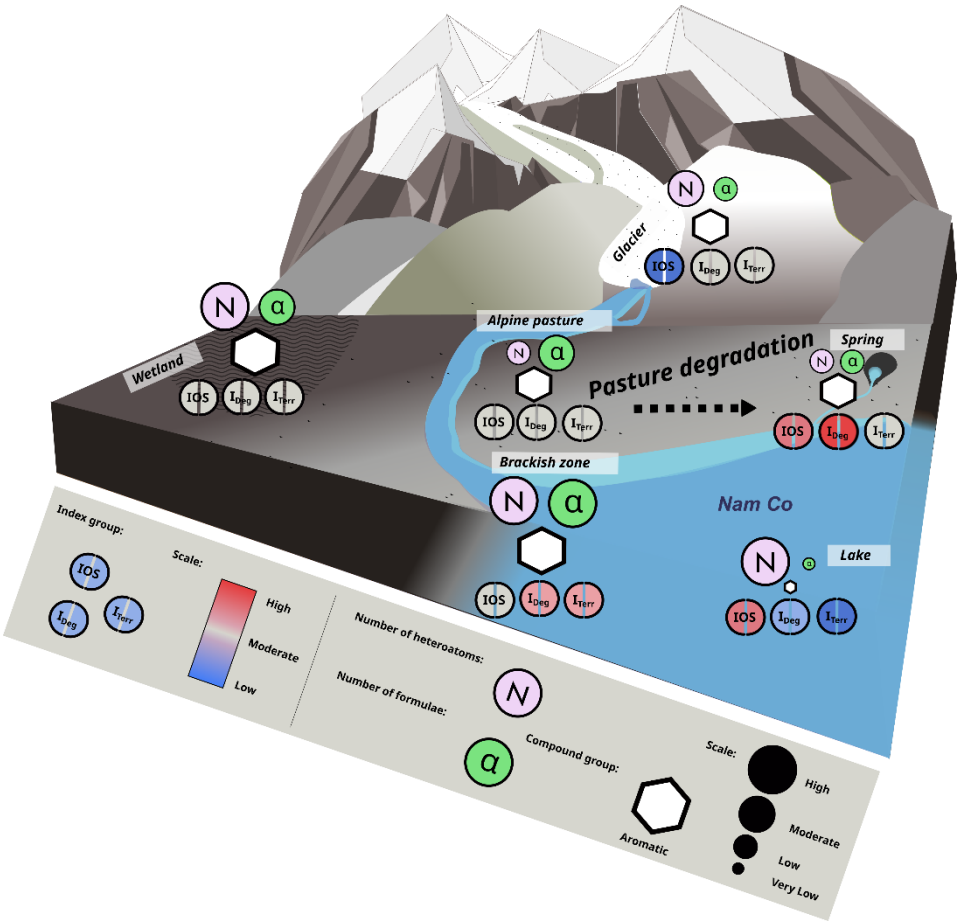


Figure 7: Overview of the molecular information from SPE-DOM along the water continuum of the TP. The number of N-heteroatoms is indicated relative to the total number of molecular formulae. The island of stability (IOS), degradation index (I_{Deg}), and terrestrial index (I_{Terr}) are ranging in a red-blue colour. Alpine steppe and groundwater spring samples are spatially correlated, and thus subject of a common evaluation. The local pasture degradation gradient is indicated by a dashed arrow.

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