LETTERS
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Biogeochemically diverse organic matter in Alpine glaciers and its downstream fate

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Besides their role in the hydrological cycle[1], glaciers could play an important role in the carbon cycle[2–6]. They store and transform organic carbon[5,6], which on release could support downstream microbial life[3]. Yet the origin and composition of glacial organic carbon, and its implications for the carbon cycle, remain unclear. Here, we examine the molecular composition, radiocarbon age and bioavailability of dissolved organic matter (DOM) in 26 glaciers in the European Alps, using ultrahigh-resolution mass spectrometry, fluorescence spectroscopy and incubation experiments. We also measure carbon dioxide partial pressures in glacier-fed streams. We show that the glacier organic matter is highly diverse, and that a significant fraction of this material is bioavailable. Phenolic compounds derived from vascular plants or soil dominate, together with peptides and lipids, potentially derived from *in situ* microbial communities. Combustion products, in contrast, seem to contribute only marginally to the DOM sampled. We further show that organic matter bioavailability is positively correlated with in-stream carbon dioxide concentrations. We suggest that glacier-derived DOM contributes to downstream carbon cycling in glacier-fed streams. Our findings highlight the relevance of mountain glaciers for carbon cycling—a role that may change as glaciers recede.

Despite the recent retreat of mountain glaciers in many regions of the world[1,7], they still often feed headwaters—the smallest and most abundant streams in fluvial networks—with noticeable consequences for their ecohydrology and biogeochemistry[8]. To evaluate the biogeochemistry of ice-locked organic matter and its relevance for carbon cycling in glacier-fed streams, we investigated the molecular composition, age and bioavailability of DOM in ice from 26 Alpine glaciers and determined the CO_2 partial pressure (p_{CO_2}) in their streams. Recent work has highlighted the role of the glacier surface, including cryoconite holes and accumulated organic particles[5,6] and DOM in supra-glacial runoff[3,4], for carbon cycling. Complementing these studies, we focused on DOM from subsurface ice that we sampled from approximately 0.5 m beneath the glacier surface in the ablation zone (Supplementary Information). Glaciers ranged from 2,590 to 3,180 m above sea level and covered a broad geographical and environmental range (Supplementary Information). Dissolved organic carbon (DOC) concentrations (mean ± s.d.: 138 ± 96 μg C l^{-1}) in the glacial ice were low and similar to values reported from other glaciers worldwide[4,9]. This highlights glaciers as ecosystems heavily depleted in organic carbon, even more than the deep ocean[10].

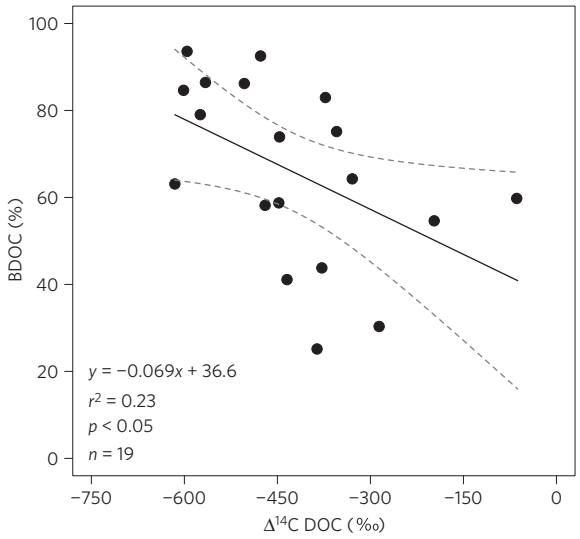

Figure 1 | The percentage of BDOC increases with Δ^{14}C DOC, indicating higher bioavailability of older DOC. Δ^{14}C DOC is inversely related to radiocarbon-derived age. The error bars associated with replicate measurements of Δ^{14}C DOC are smaller than the size of the circles; the dashed lines give the 95% model confidence interval.

Performing bioassays with a microbial inoculum from the water in the glacier-fed stream (Supplementary Information) we found high fractions of biodegradable DOC (BDOC, 59 ± 20%) in glacial ice. These BDOC values are similar to those reported from glacial runoff in the Gulf of Alaska[3], but noticeably higher than those from headwaters in forested catchments[11]. The radiocarbon age (indicated by Δ^{14}C) of glacial DOC ranged from 600 to 8,500 yr BP, and was positively related to BDOC (Fig. 1), suggesting the utilization of ancient organic carbon by microbial heterotrophs in glacier-fed streams. This relationship confirms previous findings from the Gulf of Alaska[3] and identifies ancient yet labile DOC as a likely common feature of glaciers worldwide. However, the fact that radiocarbon age explained only 23% of the variation in BDOC across our study glaciers suggests complementary biogeochemical factors (for example, chemical composition and source) influencing the bioavailability of ice-locked DOC.

To further elucidate the biogeochemistry of DOM, we applied ultra-high-resolution Fourier transform ion cyclotron resonance

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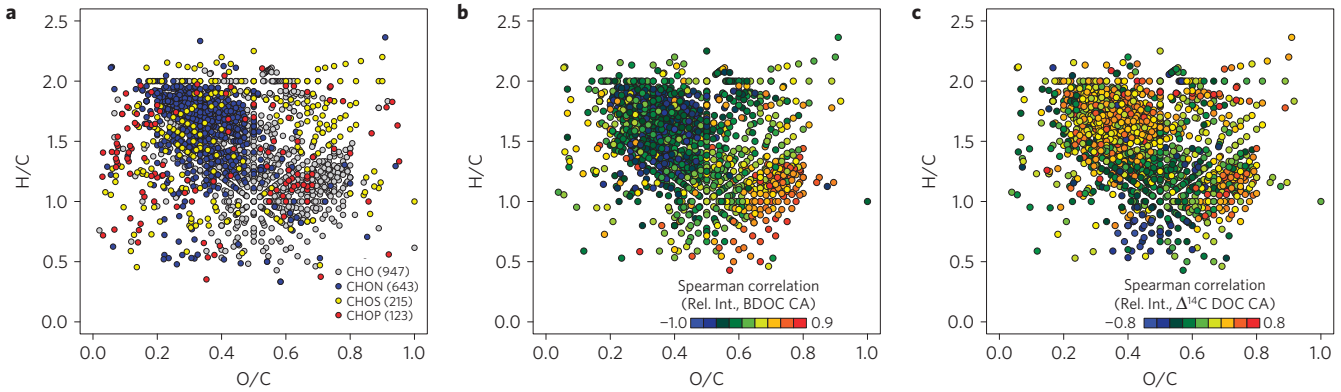

Figure 2 | Van Krevelen diagrams of molecular formulae in ice-locked DOM from the 26 Alpine glaciers. a, DOM molecular composition varied moderately among glaciers with a dominance of carbohydrates (CHO) and nitrogen-containing compounds (CHON). CHOS, sulphur-containing compounds; CHOP, phosphorus-containing compounds. Overall, 20% of formulae have an identical location; the plotting order followed the legend. **b**,**c**, Colour-coding correlations of molecule-specific intensities with the (canonical) axis of DOM molecular space associated with BDOC (**b**) or Δ^{14}C DOC (**c**) allow the identification of distinct molecular subpopulations in Van Krevelen space. For instance, molecules printed in red are associated with a higher bioavailability or lower radiocarbon age of DOM across glaciers. The plotting order was random in **b** and **c**. Rel. Int., relative intensity; CA, canonical analyses of principal coordinates.

mass spectrometry (FT-ICR-MS; Supplementary Information) and determined thousands of intact dissolved molecules in glacial ice DOM in a mass range of 150–2,000 Da (Fig. 2). In an unprecedented effort, we paired patterns of FT-ICR-MS-derived molecular composition at compound level with bioavailability, radiocarbon age and fluorescence spectrometry data across all glaciers using a multivariate correlative approach[12] (Supplementary Information). DOM molecular composition correlated significantly with DOC radiocarbon age ($r = 0.89$, $p < 0.05$) and BDOC ($r = 0.84$, $p < 0.05$). This is evidence that DOM molecular composition reflects gradients of bioavailability and radiocarbon age across the study glaciers. We further found distinct molecular populations associated with the gradients of bioavailability and radiocarbon age (Fig. 2b,c), as well as specific fluorescence patterns (protein-like and humic-like fluorescence, Supplementary Information). Cluster analysis based on these association patterns revealed five clusters of molecules, which, with the exception of cluster 5, could all be linked to chemical populations as commonly defined in the Van Krevelen space[13–15] (Fig. 3 and Table 1).

Cluster 2, for instance, is prominently associated with higher bioavailability and humic-like fluorescence. These almost nitrogen-free molecules have large but variable mass and a notably heterogeneous association with the radiocarbon age gradient, including apparently younger (H/C > 0.6) and some older more aromatic (H:C < 0.6) compounds. This cluster includes to a large extent polyphenolic diagenetic products from lignins and tannins. The apparent contribution of the tannins to bioavailability suggests that they are largely hydrolysable and lack protective matrices[16]. The characteristics of these polyphenolics are indicative of an either vascular plant or soil origin and imprint a clear terrestrial signature to glacial DOM that is similar to freshwater DOM (refs 13,14). Sources of these compounds may include overridden soils and vegetation in the aftermath of the Holocene climate optimum (6,000–7,000 yr BP; ref. 17), regional emissions from adjacent vegetation and soils but also large-scale emissions, such as Saharan dust, which regularly deposits onto the European Alps[18]. In fact, in some of our study glaciers, estimates of radiocarbon age agree well with the Holocene climate optimum[17] but also with the radiocarbon age (7,000 yr BP) of soil carbon in recently deglaciated terrain in the European Alps[19]. Alpine glaciers may thus incorporate more DOM from regional sources compared with glaciers where DOM relies on aerosol transport over large distances[4]. It is likely

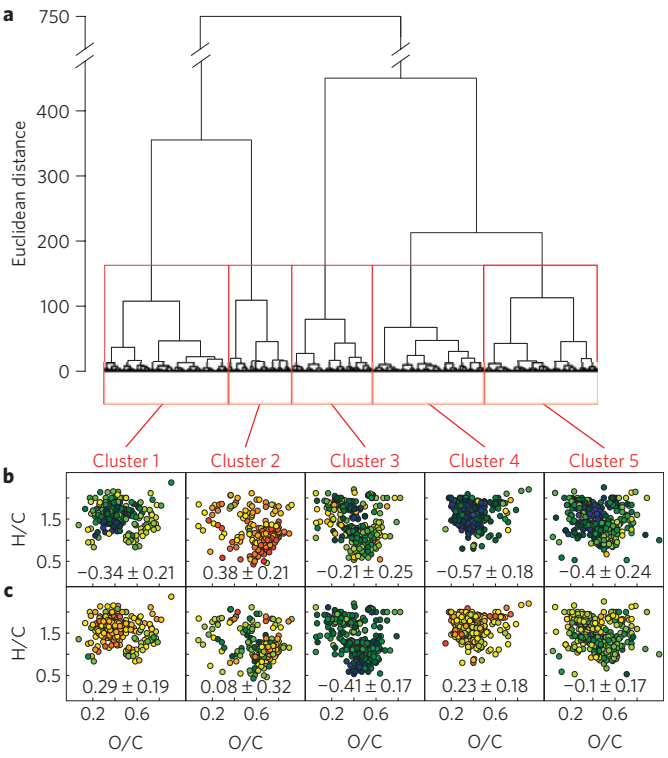

Figure 3 | Biogeochemical groups of glacier DOM. a, Cluster analysis identified five molecular subpopulations on the basis of association patterns of molecules with BDOC, Δ^{14}C DOC, tryptophan-like, tyrosine-like and humic-like fluorescence. The input variables to cluster analysis were five sets of molecule-specific rank-correlation coefficients with canonical axes identified by canonical analyses of principal coordinates[12] of FT-ICR-MS data (Methods and Supplementary Information). **b**,**c**, Location of the five clusters in Van Krevelen space with colour-coded rank correlations of molecule-specific intensities with the (canonical) axis of DOM molecular space associated with BDOC (**b**) or Δ^{14}C DOC (**c**). The colour scale ranges from −1 (blue) to 1 (red) (see colour scales in Fig. 2b,c). The numbers in the panels give the mean (±s.d.) of the correlation coefficients.

Table 1 | Characteristics of DOM molecules belonging to the five clusters shown in Fig. 3.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Percentage of compounds	25.2%	12.8%	16.3%	22.6%	23.1%
Molecule mass (Da)	478.4±79.4	469±120.1	431.9±130.8	412.9±86.3	437.7±99.7
Average percentage of total intensity*	16.2%	20.4%	17.7%	15.6%	27.6%
Average frequency of occurrence of molecules across glaciers	81.5%	71.0%	83.7%	85.0%	85.3%
Variation of total intensity* across glaciers (C.V. in percentage)	67.0%	101.6%	45.0%	43.1%	36.8%
N prevalence (percentage of compounds)	31.4%	5.4%	27.9%	58.3%	36.3%
N count in compounds with N	3.04±1.37	3.09±1.97	2.56±1.67	2.47±1.13	2.24±1.41
O/C ratio	0.4±0.13	0.62±0.17	0.42±0.15	0.38±0.12	0.43±0.16
H/C ratio	1.62±0.23	1.22±0.35	1.39±0.43	1.64±0.24	1.5±0.34
Aromaticity index†	0.08±0.12	0.23±0.2	0.23±0.21	0.1±0.14	0.16±0.18

Clusters have a distinct chemical composition and location in Van Krevelen space, but were identified solely on the basis of across-glacier association patterns between peak intensities and BDOC (Fig. 3b), Δ^{14}C DOC (Fig. 3c), tryptophan-like, tyrosine-like and humic-like fluorescence (Supplementary Information) as achieved by canonical analyses of principal coordinates[12] (Methods and Supplementary Information). Given are means±s.d. *Total intensity of all peaks with a feasible formula assignment; the row does not sum to 100% as the cluster analysis was limited to compounds detected in at least 1/3 of all glaciers. †AI = (1+C−0.5O−S−0.5H)/(C−0.5O−S−N−P). C.V., coefficient of determination.

that DOM that spends considerable time in aerosol transport is subjected to photodegradation selectively removing compounds of terrestrial origin.

Two nitrogen-rich clusters (1 and 4) collectively form a prominent population of unsaturated aliphatics (H/C > 1) whose molecular formulae are consistent with lipids and peptides[14,15,20]. Their association with specific fluorescence patterns that are characteristic for tryptophan- and tyrosine-containing compounds, as well as their comparably younger age as indicated by inverse correlations with Δ^{14}C DOC, supports an *in situ* origin of these partly proteinaceous moieties from microorganisms. The low contribution of these peptides and lipids to bioavailability hints at mechanisms that protect them from degradation, as is known from specific proteins (for example, porins) and peptides from bacteria and prokaryotic algae[21]. Glacial ecosystems are known to harbour microbial communities, including protozoa and algae[2,5], whose membrane constituents following cell death may accumulate in the ice. Atmospheric deposition (for example, dust[18]) can subsidize these microbial communities with nutrients, inducing blooms of snow algae (*Chlamydomonas nivalis*), for instance. The positive relationship ($r^2 = 0.33$, $p < 0.05$, $n = 18$) between total dissolved nitrogen ($\mu g\,N\,l^{-1}$) as an indicator for atmospheric deposition and protein-like fluorescence in glacial ice supports this notion.

A further cluster (cluster 3) contained molecules associated with high radiocarbon age but with no clear relationship to bioavailability and no association with any fluorescence. This cluster comprised non-aromatic nitrogen-containing molecules at high H/C (1.5–2.0), polyphenolic aromatics and to a lesser extent sulphur-containing aliphatics (H/C > 0.5, O/C > 0.5, Fig. 2a). The aromatics are evidently strongest associated with older radiocarbon age. These signatures are suggestive of moderate contributions from combustion products to glacial DOM (refs 4,20). The very low contributions (0.07±0.05%) of molecularly determined polycyclic aromatic carbon—a measure for black carbon[22]—to DOC and their lacking relationship with radiocarbon age ($r^2 = 0.01$, $p = 0.91$, $n = 24$) confirmed these FT-ICR-MS results. This finding seems surprising given that the European Alps are surrounded by highly industrialized regions and contrast those from the Gulf of Alaska where combustion products in aerosols characterized glacial surface runoff[4]. This difference is most likely attributable to the fact that we sampled the englacial environment, which may even include pre-industrial ice, rather than the glacial surface directly exposed to the atmosphere. Regionally constrained black carbon deposition from biomass burning in pre-industrial times[18] may explain the scattered signatures of combustion products in the Alpine glaciers.

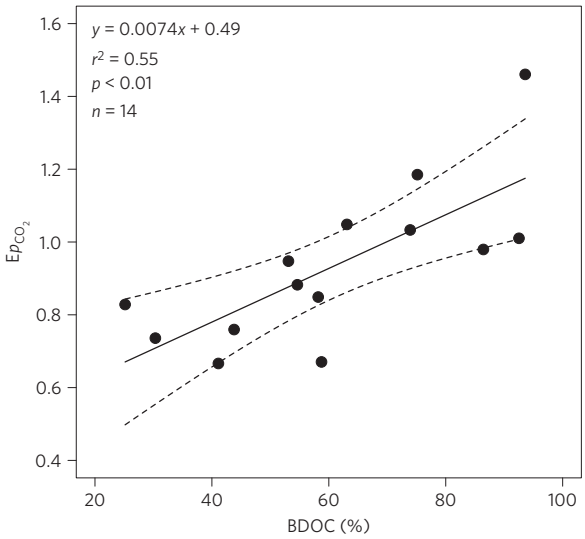

Figure 4 | Ep_{CO_2} in the glacier-fed streams increased with glacier DOC bioavailability. A multiple regression model (adjusted $R^2 = 0.47$, $p < 0.05$, $n = 13$) showed that BDOC (beta = 0.78, $p < 0.01$) rather than stream slope (beta = −0.01, $p = 0.97$) explained the observed variation in Ep_{CO_2}, although the negative regression coefficient for the slope agrees with a downstream pressure effect. Ep_{CO_2} values greater (respectively, less) than 1 denote supersaturation (respectively, undersaturation) of the streamwater p_{CO_2} relative to the atmospheric p_{CO_2}.

In our study, DOM from geographically dispersed small Alpine glaciers proves to be of high biogeochemical diversity, which may contribute to the scattered relationship between DOC radiocarbon age and bioavailability. Young microbial-derived material and low amounts of anthropogenic combustion products constitute the endmembers of the DOM age gradient, with vascular plant and soil material as an intermediate component. Across all glaciers, compounds of vascular plant and soil material had the lowest frequency of occurrence but the highest variation of peak intensity (Table 1). This suggests that this material contributed to overall DOM occasionally but then markedly, thereby driving the observed gradient of bioavailability.

To infer the possible downstream fate of this bioavailable and biogeochemically diverse DOM, we measured the excess CO_2 partial pressure (Ep_{CO_2}) as a proxy for in-stream metabolism in the glacier-fed streams. We found that the bioavailability of glacial

organic carbon explained 55% of the variation in Ep_{CO_2} in these streams below (approximately 10 m) the glacier terminus (Fig. 4), where bulk discharge originated from glacial ice melt as shown by oxygen stable isotopes ($\delta^{18}O$; Supplementary Information). The relationship between BDOC and Ep_{CO_2} suggests that respiration of organic carbon released from glaciers contributes to p_{CO_2} build-up in glacier-fed streams. Overall, 65% of the streams were undersaturated in CO_2 (that is, $Ep_{CO_2} < 1$), which contrasts with the common view of headwaters as a source of CO_2 to the atmosphere[23,24]. Besides low in-stream metabolism, CO_2 undersaturation in these streams may be attributable to the low pre-industrial atmospheric p_{CO_2} during ice formation, to reduced gas exchange between water and atmosphere at low temperatures, and to the fast downstream increase in atmospheric pressure experienced by these high-slope streams. We found no evidence for confounding of the relationship between BDOC and Ep_{CO_2} by turbulence-driven gas exchange[24] or downstream pressure increase (Supplementary Information).

Glaciers are DOC-poor ecosystems and yet recent work has highlighted their role for carbon fluxes[3,5,6]. Notably, respiration and primary production in cryoconite holes at the glacier surface were shown to contribute to large-scale carbon fluxes[5,6]. Our findings on Alpine glaciers expand this view of glaciers, suggesting them as a stimulus for downstream heterotrophic activity in the glacier-fed stream. On the basis of an annual mean wastage of 2.5 km^3 in the European Alps[7] and on our DOC concentrations, we estimated an average annual DOC flux from Alpine glaciers at 0.34 Gg C yr^{-1}. This flux is small when compared with the DOC export into the Gulf of Alaska[3], for instance, but the area-weighted DOC flux (0.17 g C m^{-2} yr^{-1}) corresponds to the lower end of DOC export from small non-glaciated catchments[25]. Given the high bioavailability of glacial DOC, this underscores the regional relevance of Alpine glaciers for downstream biogeochemistry. Approximately 0.20 Gg C yr^{-1} of the glacial DOC flux may experience fast processing within the glacier-fed streams with the remaining 0.14 Gg C yr^{-1} transported further downstream. On the basis of estimates of carbon use efficiency ($19.4 \pm 7.24\%$, $n = 11$; Supplementary Information)—the amount of microbial biomass produced per unit of organic carbon assimilated[26]—we further estimated that in-stream metabolism of glacial DOC potentially contributes 0.16 Gg C yr^{-1} to respiratory CO_2 that eventually leaves the glacier-fed stream to the atmosphere. Even if these numbers seem small when compared with other systems[24], they illustrate that melting mountain glaciers link ice-locked stores of organic carbon, some of it ancient, with downstream and atmospheric carbon fluxes.

Methods

We studied 26 glaciers and their streams located along the central chain of the Austrian Alps in July and August 2010 (Supplementary Information). On each glacier, ice (0.3–0.5 m depth) was collected and pooled from 14 single sites across the ablation zone (about 100–200 m above terminus) using a cleaned and heat-treated stainless-steel ice axe and sterile and acid-washed 1-l Whirl-Paks. Once thawed, one aliquot (8 l) of ice water was filtered (pre-combusted Whatman GF/F) and DOC was extracted after acidification to pH 2 on a solid phase (Agilent Bond Elut PPL; ref. 22) and eluted with methanol for FT-ICR-MS and black carbon analyses. Another aliquot was sterile-filtered (pre-washed 0.2-μm membrane filters) for chemical analyses and bioassays. Samples were either stored in glassware (soaked with 0.1 N HCl and combusted) or in plastic containers (polypropylene copolymer containers; soaked with 0.1 N HCl and rinsed with Milli-*Q* water) for bioassays and $\Delta^{14}C$ DOC analysis. All samples were kept in the dark (4 °C) until further processing. Process blanks of the entire workflow for all DOC-related analyses were run using Milli-*Q* water frozen in 1-l Whirl-Paks and used to correct DOC and BDOC concentrations. Streamwater p_{CO_2} was measured using the head-space method[27] and gas chromatography. DOC concentration was determined using a total organic carbon analyser (Sievers 900) and individual fluorescent components were modelled from excitation emission matrices employing parallel factor analysis[28]. Analyses for $\Delta^{14}C$ DOC were carried out at the Vienna Environmental Research Accelerator Laboratory on a 3-MV Pelletron tandem accelerator. Black carbon was determined on an unambiguous molecular level as benzenepolycarboxylic acids after nitric acid oxidation[22] using a UPLC-PDA (Waters). The molecular composition of glacial DOM was analysed using a 15 T Solarix FT-ICR-MS (Bruker Daltonics) with electrospray ionization (ESI negative); 400 broadband scans were accumulated for each sample. All detected compounds had a molecular mass <1,000 Da. Molecular formulae were assigned to peaks with a minimum signal-to-noise ratio of 3 and for a maximum elemental combination of $C_{100}H_{250}O_{80}N_8P_4S_4$ on the basis of stringent tolerance criteria[29] and conditional on $^{12}C/^{13}C$ isotope confirmation (adequate mass shift and peak ratio). Canonical analyses of principal coordinates[12] (CAP) was used to test for a relationship of DOM molecular composition with one of the following five linearly related constraints: BDOC, $\Delta^{14}C$ DOC, tryptophan-like, tyrosine-like and humic fluorescence. CAP views DOM molecular composition as a multi-dimensional space where it identifies the single dimension (also known as the canonical axis) most strongly related to the constraint. The significance of CAP was computed by permutation, and a canonical correlation coefficient was computed as an indicator for the strength of the relationship. We further rank-correlated (Spearman) the canonical axis with relative peak intensities to identify DOM molecular subpopulations related to the across-glacier gradient of the selected constraint (for example, BDOC). Thus, we obtain a compound-specific assignment to age and bioavailability indirectly from the variation across all glaciers. In a follow-up analysis, the five sets of compound-specific correlation coefficients (one for each constraint) were simultaneously used in a cluster analysis to differentiate DOM molecular subpopulations.

We inoculated the sterile-filtered water from glacial ice with an aliquot of stream water (from the terminus) of the respective glacier and incubated these systems at 4 °C in the dark. Samples for DOC and fluorescence, dissolved inorganic carbon, and microbial cell counts were collected at the start of the bioassay and after 6, 12 and 24 days, respectively. Carbon use efficiency was calculated as the change in microbial biomass relative to the change in microbial biomass plus respiration (as dissolved inorganic carbon) in the same time period[26]. BDOC was calculated as the decrease in DOC concentration over the incubation period.

DOC fluxes were extrapolated to the European Alps on the basis of mass loss data from the Glacier Mass Balance Bulletin[30] and, comparatively, from regional glacier inventories of the Ötztal Alps, adjusted for mass loss data from 5 glaciers from 2000 to 2010.

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Author contributions

G.A.S., C.F. and T.J.B. conceived and designed the research. G.A.S. and L.W. organized and coordinated fieldwork. C.F. conducted all bioassays, fluorescence spectrometry, and radiocarbon dating assisted by P.S. G.A.S. ran all FT-ICR-MS analyses guided by T.D. and J.N. J.N. performed BPCA analyses. G.A.S. performed all statistical analyses assisted by C.F. T.J.B. wrote the manuscript with significant assistance from G.A.S. and C.F. All authors commented on the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to T.J.B.

Competing financial interests

The authors declare no competing financial interests.