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DISSERTATION

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“Dissolved organic matter (DOM) biogeochemistry in streams”

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I. Introduction

The role of inland waters for the global carbon cycle

The global carbon cycle links the terrestrial environment – including inland waters, the ocean and the atmosphere. Freshwater ecosystems not only provide essential resources and services to society, e.g. drinking water and natural water purification, but also play a major role in ecological function and have now been recognized as an important and dynamic component of the global carbon cycle (Battin et al. 2009). Climatically and anthropogenically induced environmental changes have resulted in a rise in atmospheric carbon dioxide (CO_2) concentration and increased occurrence of extreme climatic events, such as heavy precipitation and associated floods, and heat waves and associated droughts, with a wide range of possible consequences for freshwater ecosystems. Thus, it is imperative to understand the inter-dependent relationship between the hydrological and the carbon cycle in order to determine and evaluate the possible consequences of changing environmental conditions for ecosystem function and health.

Approximately 70% of the Earth's surface is covered by water, of which inland waters account for only about 1%, a fact which may have resulted in the initial disregarding of the potential contribution of freshwater ecosystems to the global carbon cycle. Traditionally, it was assumed that these systems function as pipes, transporting carbon from the continents to the ocean. However, recent studies highlight the relevance of inland waters for carbon dynamics on a global scale (Cole et al. 2007, Battin et al. 2008, Tranvik et al. 2009, Lapierre et al. 2013), revealing inland waters as active components of the carbon cycle, as they process and release large amounts of organic carbon as CO_2 into the atmosphere (Cole et al. 2007, Battin et al. 2008, Tranvik et al. 2009). Annually, inland waters receive approximately 2.9 Pg C yr^{-1} from the terrestrial environment (Tranvik et al. 2009) and transport 0.9 Pg C yr^{-1} to the oceans (Cole et al. 2007, Tranvik et al. 2009). During this process, approximately 0.6 Pg C yr^{-1} are stored in sediments and 1.4 Pg C yr^{-1} are estimated to evade as CO_2 (Tranvik et al. 2009). Recent research shows that streams and rivers even account for 1.8 Pg C yr^{-1} of the carbon loss in form of CO_2 evasion along their flowpath (Raymond et al. 2013). The relevance of fluvial ecosystems for carbon cycling on a global scale further becomes evident when compared to the estimated 6.4 Pg C yr^{-1} released to the atmosphere via fossil fuel emissions (Burgermeister 2007), and the corresponding terrestrial carbon sink for these emissions of approximately 2.8 Pg C yr^{-1} (Denman et al. 2007).

Dissolved organic matter (DOM) in aquatic ecosystems

The amount and composition of dissolved organic matter (DOM) play a central role for carbon cycling in streams and rivers (Battin et al. 2008, 2009, Lapierre and del Giorgio 2012). DOM is typically comprised of a complex mixture of molecules of highly variable chemical composition and reactivity, originating from a broad range of allochthonous or autochthonous sources. For instance, the autochthonous fraction of DOM often comprises low-molecular-weight compounds and consists of free amino acids, lipids, proteins, urea, sugars, and other carbohydrates, derived from enzymatic degradation of macromolecules, exudation of substances by photoautotrophic organisms, or other processes such as sloppy feeding and viral lysis (Findlay & Sinsabaugh 2003). The most dominant DOM pool in streamwater is of terrigenous origin (Thurman 1985), comprising complex molecules such as humic and fulvic acids (Hedges et al. 1994), largely derived from terrestrial vegetation (Allan and Castillo, 2007). Terrigenous DOM is often characterized by a high content of chromophoric molecules, such as humic and fulvic acids. This chromophoric fraction of DOM (CDOM), which

exhibits a strong absorbance in the visible, UV-A and UV-B regions of the light spectrum (Osburn et al. 2001) is commonly employed as a tracer for the dynamics of the entire DOM pool (Stedmon et al. 2003). Due to its light absorbing properties, DOM can impact the light regime, primary production and related trophic processes (Jaffe et al. 2008, Karlsson et al. 2009, Hansson et al. 2012). For instance, DOM concentration was found to be positively related to UV-B attenuation (Belmont et al. 2009), and thus may shield biota from potentially harmful UV-radiation (Walsh et al. 2003). This effect is of relevance as streams and rivers can account for substantial DOC (Worrall and Burt 2007, Raymond and Saiers 2010) and CDOM export fluxes from the terrigenous environment to downstream ecosystems, even with implications for ocean colour budgets (Spencer at al. 2013). Furthermore, photooxidation of aquatic DOM can lead to the cleavage of large molecules (Kieber et al. 1989), resulting in CO_2 production. This process can be catalyzed by the presence of iron and leads to multiple reactions (e.g. photo-Fenton reaction) which can induce the production of free-radicals, which in turn lead to further DOM degradation (Sulzberger and Kaiser, 2009). In addition to contributing to CO_2 evasion fluxes (Battin et al. 2008, Butman and Raymond 2011, Cole et al. 2007), aquatic DOM can also participate in the complexation of trace metals and facilitate the transport of nutrients and pollutants (McKnight and Bencala 1990, Aiken et al. 2011, Findlay and Sinsabaugh, 2003), with implications for drinking water quality (Kaplan et al. 2006).

Microbial communities mineralize and transform the DOM pool in aquatic systems (del Giorgio and Cole 1998), accounting for a notable proportion of total carbon losses in streams and rivers. Microbial degradation processes and metabolic activity ultimately depend on DOM concentration and composition, as well as the reduction state and stoichiometry of substrates (del Giorgio and Cole 1998). Biodegradable DOM exists along a continuum of reactivity, comprising labile compounds that can be degraded rapidly, semi-labile DOM which is degraded more slowly, and recalcitrant DOM which is degraded over very long time scales (Carlson 2002). For instance, the autochthonous fraction of the DOM pool is believed to be readily available to heterotrophic metabolism (Azam and Cho 1987), yet it occurs only in low concentrations within streamwater. In contrast, the dominant terrigenous DOM fraction is traditionally considered to be recalcitrant (Tranvik 1998, Schmidt et al. 2011) due to its long storage time in soils before its export to adjacent headwaters, with turnover times ranging from years to centuries (Trumbore 1997).

Climatically and anthropogenically induced changes may impact the amount, composition and fluxes of DOM (Striegl et al. 2005, Inamdar et al. 2011, Strohmeier et al. 2013, Wilson et al. 2013), with possible consequences for the delivery of DOM and carbon processing in streams. In order to evaluate the possible consequences for aquatic ecosystem processes and functions and to sustain or restore ecosystem health (Gomi et al. 2002, Bernhardt et al. 2005), it is essential to improve our understanding of DOM composition and transformation, as well as mineralization processes and their resulting fluxes at the terrestrial-aquatic interface.

DOM in Alpine glaciers and pro-glacial streams

In many alpine regions, headwaters - the smallest, but most abundant streams in fluvial networks - are fed by glaciers, which determine downstream ecohydrology and biogeochemistry (Milner et al. 2009). In addition to their prominent role in the hydrological cycle, glaciers are considered as ecosystems where nutrient (Hodson et al. 2005) and carbon cycling take place (Hodson et al. 2007, Hood et al. 2009, Stubbins et al. 2012, Anesio et al. 2012). Increasing evidence indicates that microbial life, which

is adapted to these environments, both metabolically and in the form of lifestyle strategies, mediates nutrient and carbon cycling (Skidmore et al. 2000, Simon et al. 2009). DOM sources in glaciers may include overridden soils and vegetation resulting from glacial advancement during cooler periods, regional emissions from adjacent vegetation and soils, and large-scale emissions such as Saharan dust, which regularly deposits onto the European Alps (Thevenon et al. 2009). Furthermore, cyroconite organic matter, mainly derived from lower order plants (mosses, lichens) and microbial communities, can contribute to the DOM pool in glaciers (Xu et al. 2009). Ancient DOM in Alaskan glaciers was hypothesized to be of terrestrial origin (Hood et al. 2009), a notion that was recently challenged by the discovery of aerosols from fossil fuel burning, imprinting on the age of DOM in supra-glacial meltwater (Stubbins et al. 2012). Yet, in Arctic glaciers, proteinaceous compounds derived from microbes were found to dominate DOM (Pautler et al. 2012). While these findings collectively point to storage and transformation processes of organic carbon in glaciers, which upon release may support microbial life downstream (Hood et al. 2009), the origin, composition and metabolic fate of glacial DOM, and implications for the carbon cycle remain much debated. As climate change strongly affects glacial mass balance (Haeberli et al. in press), this may imply ultimate consequences for the amount and composition of glacial-derived DOM in the glacial streams and carbon cycling.

In order to evaluate the role of glaciers for carbon cycling and determine DOM source and composition in glaciers, the molecular composition, radiocarbon age and bioavailability of DOM in Alpine glaciers was investigated using ultra-high-resolution mass spectrometry, fluorescence spectroscopy and biodegradation experiments. Additionally, carbon dioxide partial pressures in glacier-fed streams were measured to evaluate the role of glacial derived DOM for downstream microbial metabolism.

Terrigenous DOM and carbon cycling in brown-water streams

Increasing evidence suggests an unprecedented export of terrestrial DOC to aquatic systems in Northern America and North and Central Europe (Evans et al 2005, Monteith et al 2007, Hansson et al 2012). This phenomenon is commonly referred to as the "browning" of inland waters, as terrigenous DOM contains large amounts of CDOM, conferring a characteristic brown color to natural waters. For example, humic substances, a typically dominant fraction of CDOM, have increased by more than 50% in Swedish lakes during the past 25 years (Hannson 2012), and DOC concentrations increased by 91% in United Kingdom upland waters within the past 15 years (Evans 2005). The fact that inland waters are often supersaturated with CO_2, largely a result of the mineralization of terrigenous DOC (Sobek et al. 2003, Lapierre and del Giorgio 2012, Teodoru 2009), suggests this putatively recalcitrant DOM pool as an important energy and carbon source for microbial metabolism (Battin et al. 2008). Living as attached biofilms or aggregates, microbial communities have developed lifestyle strategies to enhance their metabolic capacity (Battin et al. 2008), possibly facilitating the degradation of refractory DOM over extended contact times. Recent studies show that chemical recalcitrance and high $\Delta^{14}C$ age, commonly assumed properties of terrigenous DOM, do not necessarily predict its bioavailability (McCallister and delGiorgio 2012, Ward et al. 2013). For example, low-molecular-weight DOM of terrigenous origin was shown to be readily available and also quantitatively important for microbial metabolism in boreal freshwaters (Berggren et al. 2010). Furthermore, microbial communities were shown to degrade more complex fractions of DOM, such as humic substances or polyphenolic compounds (Guillemette and del Giorgio 2011, Berggren et al. 2012), possibly contributing to CO_2 evasion fluxes from aquatic ecosystems. In fact, lignin and related phenolic

compounds were found to be extensively reworked by microorganisms, possibly causing a complete overturn of the DOM pool within weeks in the Amazon River, suggesting metabolism of "recalcitrant DOM" as a possible contributor to carbon dioxide outgassing (Ward et al. 2013).

The notably large increases in DOC concentration have consequences for freshwater ecosystems on multiple levels. Browning does not only impact biomass and abundance of phytoplankton and zooplankton species at various trophic levels (Hannson 2012), but also has consequences for the quality of the drinking water supply (Tuvendal & Elmqvist 2011). Furthermore, given the net heterotrophy of most inland waters (Battin et al. 2008), altered carbon fluxes may impact microbial metabolism and ultimately carbon dioxide emissions from these inland waters. Yet, the implications for carbon cycling and ecosystem functions remain elusive.

I evaluate the effect of browning on carbon dynamics by investigating the metabolic fate of terrestrial DOM in headwater streams along a brown-colouration gradient. In order to illuminate the coupling of DOM quantity and composition, and microbial metabolism and its role for in-stream carbon cycling, biodegradation assays were related to measured CO_2 concentrations in the studied streams.

DOM composition and fluxes in Alpine streams

The mechanisms that control the amount and fluxes of DOM in fluvial ecosystems are complex, involving hydrological, seasonal, and biogeochemical processes operating at various time scales. Furthermore, the geomorphological and hydrological characteristics of catchments dictate the relative contribution of the various DOM sources and their physiochemical properties. Watershed topography exerts a strong control on streamwater DOM composition via the establishment of a range of hydrologic and edaphic conditions (Belmont et al, 2009). Hill slope, soil texture and soil thickness (Berhe et al. 2008), as well as land use (Frost et al, 2005, Wilson and Xenopoulos 2008) can control DOM storage, composition and concentration. For instance, the amount of microbially derived dissolved organic matter was shown to increase with agricultural land use (Wilson and Xenopoulos 2008), possibly impacting microbial degradation processes.

Hydrology has previously been identified as a major control on DOM concentration (Boyer et al. 1996, 1997, Pellerin et al., 2012). Hydrologic events, such as snowmelt, can induce hysteretic responses in the export of terrigenous DOC previously accumulated in adjacent soils (Boyer et al., 1996, Laudon et al., 2004, Pellerin et al., 2012), constituting an important process for the supply of organic carbon and nutrients in seasonally snow covered basins (Murdoch and Stoddard 1992, Hornberger et al. 1994, Boyer et al. 1997). Additionally, floods increase streamwater DOC concentration by temporarily depleting adjective soils (Butturini et al. 2006), thereby accounting for large DOC fluxes to downstream ecosystems (Raymond and Saiers 2010, Yoon and Raymond 2012). Recent studies showed that the flow regime (including hydrologic events such as snowmelt and storms) and season not only impact the timing and amount of DOC released to downstream ecosystems (Boyer et al. 1996, 1997, 2000, Butturini et al. 2006, Raymond and Saiers 2010, Yoon and Raymond 2012), but also DOM composition (Fellman et al. 2009, Nguyen et al. 2010, Singh et al. 2014), with implications for bioavailability (Wilson et al. 2013).

A substantial fraction of the aforementioned controls on the amount, composition and fluxes of DOM are subject to climatically and anthropogenically induced changes. Thus, it is essential to improve our understanding of DOM composition, transformation and mineralization processes and the resulting

fluxes at the terrestrial-aquatic interface in order to evaluate the possible consequences for aquatic ecosystem processes and functions. Such studies are of particular relevance for ecosystems that are susceptible to climatic changes, such as Alpine streams (Barnett et al. 2005) and glaciers, as altered patterns in temperature and discharge are likely to affect DOM composition and export fluxes (Sebestyen 2009, Strohmeier et al. 2013, Laudon et al. 2013) and thus carbon cycling. Despite this, little information currently exists on the temporal variability of DOM composition and the composition of export fluxes from alpine streams.

In my PhD thesis, I aimed to identify the mechanisms that control DOM composition, dynamics and fluxes at different temporal scales in an Alpine headwater stream. Monitoring DOM concentration and composition in the streamwater and hyporheic zone over three years, the effect of hydrological and seasonal controls on the amount and composition of streamwater and hyporheic water DOM, as well as their interactions was investigated. Furthermore, I aimed at illuminating the composition and amount of DOM fluxes to downstream ecosystems and discuss these with respect to carbon cycling and fluxes to downstream ecosystems.

Thesis outline

The overall aim of my PhD thesis was to enhance the understanding of DOM biogeochemistry in fluvial ecosystems, specifically exploring the role of streams for carbon cycling. Encompassing a broad range of streams, including Alpine glaciers and pro-glacial streams, a pristine Alpine stream in Lower Austria and brown-water streams in Waldviertel, Austria, this thesis advances the current knowledge on the coupling of DOM composition and microbial metabolism, and its role for carbon cycling and fluxes. Furthermore, the research highlights the importance of flow regime seasonally induced processes for carbon composition and fluxes to downstream ecosystems.

The main results of the research conducted are presented in chapters II to IV:

In **Chapter II - Biogeochemically diverse organic matter in Alpine glaciers and its downstream fate**, biodegradation assays showed that glacial dissolved organic carbon, although very low in concentration, contributed to carbon cycling in pro-glacial streams. Employing high-resolution mass spectrometry, molecular formulae that were consistent with a predominantly vascular plant source (polyphenols, like lignin- and tannin derivatives), but also carboxylic rich alicyclic molecules (CRAM) and unsaturated aliphatics, of possibly microbial origin, were detected in the glacial DOM.

In **Chapter III - Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams**, a space-for-time substitution approach was used to evaluate the effect of browning on carbon dynamics along a gradient of streams with varying contributions of terrigenous DOM. Biodegradation assays revealed changes in DOM composition associated with browning lead to a decrease in microbial carbon use efficiency, which suggests an impact of browning on carbon cycling in streams. Our findings contradict the common perception that ‘‘brown’’ DOM is resistant to microbial degradation, and show that not only DOC quantity, but also the coupling of CUE with DOM composition contributes to CO_2 evasion to the atmosphere.

In **Chapter IV - Hydrological and seasonal controls on dissolved organic matter (DOM) quality and fluxes in an Alpine headwater stream**, we identified the mechanisms that control DOM

composition, dynamics and fluxes at different temporal scales in an Alpine headwater stream based on a monitoring period of three years. Diurnal, seasonal and event-driven patterns controlled DOM dynamics in the streamwater and hyporheic zone. The results highlight the relevance of hydrology and seasonal-driven processes for DOM dynamics, composition and fluxes to downstream ecosystems.

II. Biogeochemically diverse organic matter in Alpine glaciers and its downstream fate

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*These authors contributed equally to this work

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My contribution to this publication

a. Participated in research design, particularly with respect to biodegradation assays
b. Conducted all biodegradation assays, fluorescence spectrometry, and radiocarbon analysis
a. Aided with the statistical analyses
b. Co-writing, editing and approval of manuscript

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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].

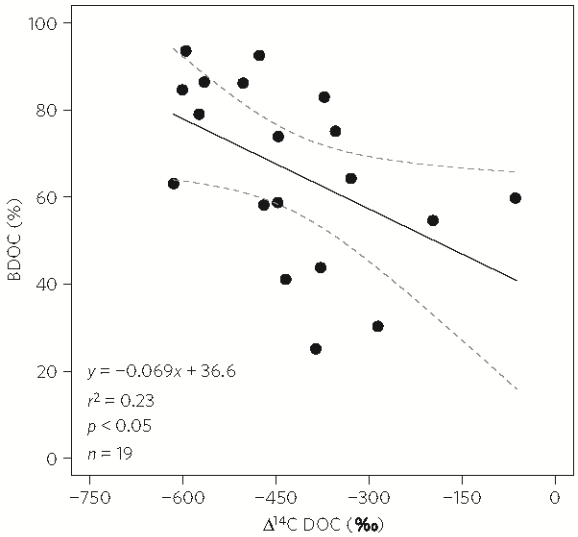

Figure 1 | The percentage of BDOC increases with Δ^{14}C DOC, indicating higher bioavailability of older DOC. Δ^{14}C DOC is inversely related to radiocarbon-derived age. The error bars associated with replicate measurements of Δ^{14}C DOC are smaller than the size of the circles; the dashed lines give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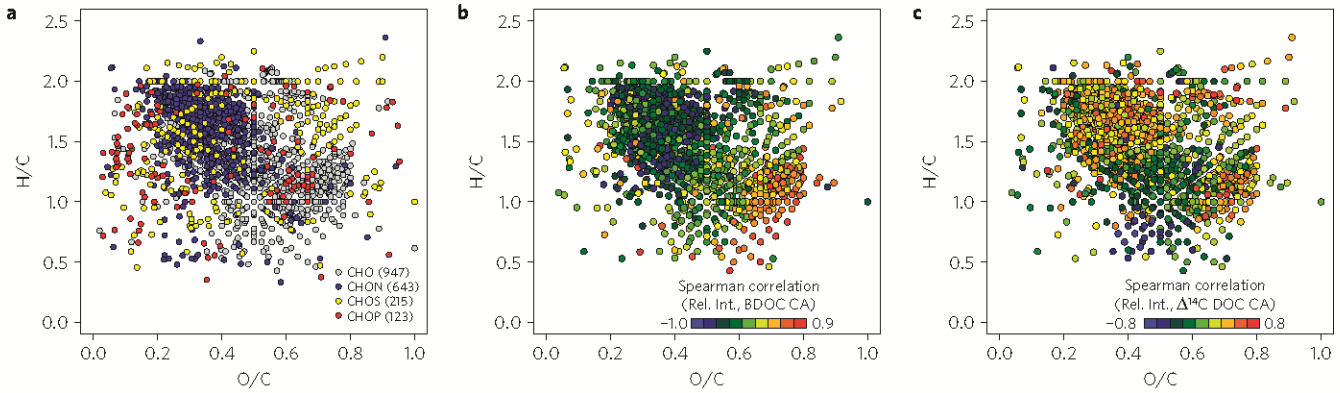

Figure 2 | Van Krevelen diagrams of molecular formulae in ice-locked DOM from the 26 Alpine glaciers. a, DOM molecular composition varied moderately among glaciers with a dominance of carbohydrates (CHO) and nitrogen-containing compounds (CHON). CHOS, sulphur-containing compounds; CHOP, phosphorus-containing 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

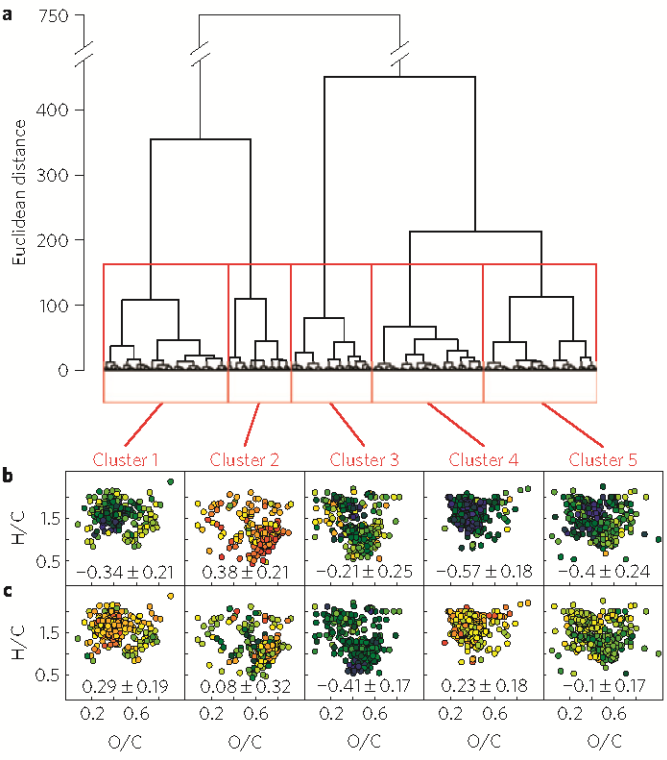

Figure 3 | Biogeochemical groups of glacier DOM. a, Cluster analysis identified five molecular subpopulations on the basis of association patterns of molecules with BDOC, Δ^{14}C DOC, tryptophan-like, tyrosine-like and humic-like fluorescence. The input variables to cluster analysis were five sets of 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 (μg Nl^{-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.

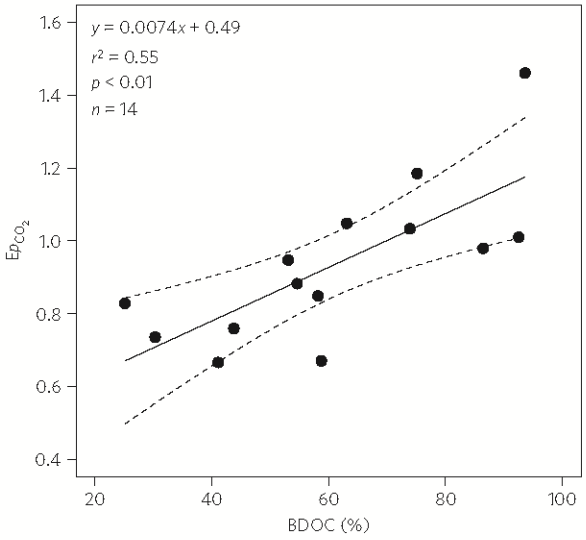

Figure 4 | Ep_{CO_2} in the glacier-fed streams increased with glacier DOC bioavailability. A multiple regression model (adjusted $R^2 = 0.47$, $p < 0.05$, $n = 13$) showed that BDOC (beta = 0.78, $p < 0.01$) rather than stream slope (beta = −0.01, $p = 0.97$) explained the observed variation in Ep_{CO_2}, 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.

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SUPPLEMENTARY INFORMATION

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Biogeochemically diverse organic matter in Alpine glaciers and its downstream fate

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Supplementary Methods

Glaciers, sample collection and preparation

We studied 26 glaciers and their pro-glacial streams located along the main chain of the Austrian Alps (Silvretta-, Ötztaler-, Stubaier-, Zillertaler-, Venediger-, Granatspitz-, Glockner-, and the Ankogel-Hochalmspitze-group) in July and August 2010 (SI-Table 1 and 2). On each glacier, we removed debris and dirty ice down to a depth of at least 0.3 m to collect clean and compact subsurface ice down to at least 0.5 m from 14 randomly selected spots across the ablation zone. Ice was sampled into sterile 1-L Whirl-Paks® with an ice axe with a stainless steel pick which was washed with MilliQ©-water (Millipore, Billerica, U.S.A.), dried and heat-treated by a propane flame to remove possible organic residues prior to sampling.

Ice (ca. 20 L per glacier) was thawed within 1 hour and immediately filtered. One aliquot (8 L) of ice water was filtered through a double layer of precombusted (450° C) glass fibre filters (Whatman GF/F) and used for DOM-extraction[1] on a solid phase (Agilent Bond Elut PPL) after acidification to pH 2 (Suprapur-grade HCl). DOM was eluted from cartridges with LC-MS-grade methanol for ultra-high resolution mass spectrometry on a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR-MS) and black carbon analyses. Another aliquot of ice was sterile-filtered through pre-washed 0.2-µm membrane filters (GSWP, Millipore) for chemical (nutrients and conservative ions) analyses and bioassays. Streamwater was sampled at the glacier terminus and at two further sites downstream (667±315 m and 1593±708 m, respectively) and filtered through prewashed 0.2-µm-membrane filters. All samples for bioassays and $\Delta^{14}C$ analyses were stored in plastic containers (polypropylene copolymer containers; soaked with 0.1 N HCl and rinsed with MilliQ water). Samples for dissolved organic carbon (DOC) and optical analysis were stored in 40 ml glass vials (soaked with 0.1 N HCl, rinsed thoroughly with MilliQ water and combusted for 4h at 450°C), sealed with Teflon-coated septa (soaked with 0.1 N NaOH and rinsed thoroughly with MilliQ water). Samples for nutrient analysis were stored in sterile 15-ml Falcon© tubes. All samples were kept in the dark at 4°C until further processing. Process blanks for all DOM-related analyses were run using 8 L of Milli-Q water frozen in identical sampling bags and otherwise unchanged procedures.

Streamwater EpCO_2

We determined CO_2 partial pressure (pCO_2) in the streamwater (terminus, stream sites 1 and 2) using the headspace method[2]. Streamwater was collected into a 2-L Schott© bottle by gravity using gas-tight Tygon© tubing; we allowed the sample to overspill for several minutes before the bottle was closed with a gas-tight septum. Subsequently, a 60-ml headspace was created using a syringe and a needle to allow replacement of the removed water volume by an equivalent amount of atmospheric air. After equilibration (by shaking the bottle vigorously for 2 min) duplicate gas samples (15 ml) were obtained from the headspace with a gas-tight syringe and injected into pre-evacuated headspace vials (10 ml) fitted with silicon sealed rubber stoppers and aluminum caps. Duplicate atmospheric air samples (15 ml) were collected at the same sites, again using a gas-tight syringe and pre-evacuated headspace vials. Gas samples were stored at 4°C in the dark and analysed using an Agilent 6890N gas chromatograph (thermal conductivity detector) connected to a headspace autosampler (DANI HSS 86.50). The excess carbon dioxide partial pressure (EpCO_2) was calculated as the quotient of the CO_2 concentration in the stream and the atmosphere.

Nutrients and field parameters

Concentrations of $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$ in the streamwater and in the ice water were determined using Continuous Flow Analysis (Alliance instruments). Detection limits for nutrient analyses were 4 μg L^{-1} for $N\text{-}NH_4$, 100 μg L^{-1} for $N\text{-}NO_3$ and 2 μg L^{-1} for $P\text{-}PO_4$. Dissolved oxygen, pH, electrical conductivity and water temperature were measured in the field using regularly calibrated WTW probes (pH320, OxiCal-SL, Cond340i, Weilheim - Germany).

Bioassays, BDOC and CUE

We investigated the bioreactivity of glacial DOC in bioassays over 50 days at 4°C in the dark. Briefly, we filled sterile-filtered ice water in triplicate 250-ml pre-combusted (450°C, 4h) Schott© bottles and re-inoculated this water with streamwater (less than 10%) from the glacier terminus. Note that ice and streamwater had comparable DOC concentrations. Previous work did not reveal any effect of sterile-filtration on ice water DOC concentration using pre-washed 0.2 μm membrane filters.

Samples for DOC concentration, fluorescence and dissolved inorganic carbon (DIC) were collected at the start of the bioassay and after 6, 12, 24 and 50 days, respectively. They were

filtered through a double layer of Whatman GF/F filters (precombusted, 450°C for 4h) and stored in glass vials (previously soaked with 0.1 N HCL and combusted at 450°C for 4h) sealed with Teflon coated septa (soaked with 0.1 N NaOH and rinsed thoroughly with MilliQ water). All samples were stored at 4 °C in the dark pending analysis.

Samples for cell counts were collected at the same sampling dates, preserved in formaldehyde (2.5% final concentration) and stored at 4°C in the dark. Cells were stained with the nucleic acid stain Sytox Green (Invitrogen), filtered onto black polycarbonate filters (Millipore) and counted using epifluorescence microscopy (Zeiss AxioImager). Per sample, we imaged and analysed 30 fields using AxioVision (4.6.1.0). Cell abundance, length and width on at least 200 cells per sample were determined by image analysis (ImageJ 1.410). The size threshold for cell detection was set by approximating it to manually measured cell sizes. Particles and overlapping cells with an area smaller than 0.1 μm^2 were excluded. Cell volumes were calculated as $V = (w^2 * \pi/4) * (l - w) + (\pi * w^3/6)$ where V (μm^3) is the cell volume, and w and l are cell width and length (in μm), respectively. Cell carbon content (fg) was calculated using the allometric relationship[3] $C = 120 * V^{0.72}$. Microbial biomass was calculated as the product of cell numbers and average cell carbon content for each replicate.

Carbon use efficiency (CUE) was calculated as the change in microbial biomass (until just before the stationary phase, i.e. day 24) relative to the change in microbial biomass plus respiration (as DIC build-up) in the same time period[4]. Bioavailable DOC (BDOC) was calculated as the decrease in DOC concentration over the 24-d incubation period.

DOC analyses

DOC samples from glacier ice, streamwater and from the bioassays were analysed for DOC concentration using a portable Sievers 900 TOC Analyzer operated with an inorganic carbon removal unit. Prior to injection, DOC samples (GF/F-filtered) were automatically acidified in the analyser as recommended by the manufacturer. We determined a method detection limit of the Sievers 900 according to US EPA guidelines[5] and found it typically below 6 μg C L^{-1}. Briefly, the detection limit was calculated as 3 (appr. critical z-value for 99.9% confidence) times the standard deviation of 9 lab replicates of low DOC water (average DOC concentration: 29.71± 1.43 μg C L^{-1}).

Using MilliQ water, we also determined process blanks of the entire workflow for the determination of ice DOC concentration. We filled the same 1-L Whirl-Paks® as used for ice samples with MilliQ water (24 ± 3 μg C L^{-1}, n=3), which was then frozen and thawed, and

subsequently passed through the same filter and vial system as the ice water samples. Performed in triplicates, we found a system blank of 27 μg C L^{-1}.

Excitation emission matrices (EEMs) and PARAFAC

EEMs were generated by measuring fluorescence intensities at excitation wavelengths ranging from 200 to 450 nm (5-nm increments) and emission wavelengths from 250 to 700 nm (2-nm increments) using a Hitachi F-7000. Scan speed was 12,000 nm min^{-1} with a response time of 0.01 s. We used 1-cm quartz cuvettes for measurements and the water Raman peak of Milli-Q water as reference. EEMs were corrected for blanks (MilliQ treated and stored as the samples, n=3) and absorbance, which was measured in 10-cm quartz cuvettes using a UV–VIS spectrophotometer (Shimadzu UV 17000) with Milli-Q water serving as blank. Individual fluorescent components were modeled from EEMs by parallel factor analysis (PARAFAC)[6]. Modelling was performed using the DOMFluor Toolbox (1.7; containing the N-Way toolbox, 3.1)[7] in Matlab (7.11.0). SI-Figure 1 shows the four modeled components and SI-Table 3 provides basic information on the components.

Dating glacial DOC

$\Delta^{14}C$ DOC analyses were conducted on a 3-MV Pelletron tandem AMS facility at the Vienna Environmental Research Accelerator Laboratory (VERA, University of Vienna, Austria). Ice water was acidified with H_3PO_4 to a pH of 2 to remove DIC and the sample volume was reduced by lyophilization to 20 μg C per sample, at least. Prior to analyses, the residual carbon was re-suspended in 500 μl ultrapure water (GIBCO©) and transferred into quartz vials (soaked with 1 N HCL, rinsed with Milli-Q water and combusted at 600°C for 8 h) with a head volume of pure oxygen. UV-light from a low-pressure mercury discharge lamp was applied for 2.5 h. Without additional oxidizing agents, only the 185 nm line is considered effective for oxidizing organic carbon[8]. The samples were repeatedly stirred with the intention to introduce ozone into the liquid and to release produced CO_2. The CO_2 was separated cryogenically and transformed to graphite with established procedures for extremely small samples[9]. This protocol achieved an average carbon yield of 64 ± 17 %. The precision of the AMS measurement for small radiocarbon samples is thoroughly documented[10]. Including graphitization, the accuracy is better than 3% even for samples of about 3 μg carbon. The overall uncertainty for the glacier samples is dominated by the blank correction of the sample

preparation, which was assessed by three full process blanks (20 ± 12 %, n = 3) prepared in parallel to the samples.

$\delta^{18}O$ analysis

Stable isotopes $\delta^{18}O$ from ice, streamwater and from snow samples were analyzed using a Picarro L1115-i Isotopic Liquid Water and Water Vapor Analyzer. The precision of $\delta^{18}O$ measurements was ± 0.1‰.

DOC mass fluxes from glacial wastage

We estimated DOC fluxes from glacial wastage in the European Alps using mass loss data from the Glacier Mass Balance Bulletin[11]. We used an average annual mass balance of -1.236 meter water equivalents (from 2000 to 2009), which, assuming a total glaciated surface area of 2000 km^2 in 2003[12] yields an average annual glacial wastage of 2.47 x 10^6 L. This estimate is close to extrapolations from regional decadal inventories in the Ötztal Alps, Austria[13], which, adjusted for accelerated mass loss from 2000 to 2010, would yield 2.8 x 10^6 L (Andrea Fischer, personal communication). We are aware that these mass balance data provide conservative estimates as other components of runoff beside wastage itself are not included. However, we feel that it better reflects the direct contribution of the glacier – one that is vanishing as glaciers do.

FTICR-MS analyses and statistical analysis

The molecular composition of glacial DOM was studied using ultrahigh-resolution Fourier transform ion cyclotron mass spectrometry (FT-ICR-MS). DOM extracts were analysed in 1:1 MeOH:H_2O on a 15 Tesla Solarix FT-ICR-MS (Bruker Daltonics, Bremen, Germany) in electrospray ionization (ESI) negative mode (400 accumulated scans, 2 s ion accumulation time) searching for masses from 153 to 2000 Da. No peaks were detected for masses >1000 Da. Molecular formulae were assigned to peaks of exported mass lists (153 to 1000 Da, minimum S/N ratio of 3) assuming single-charged deprotonated molecular ions and Cl-adducts for a maximum elemental combination of $C_{100}H_{250}O_{80}N_8P_4S_4$ and a mass tolerance of 0.6 ppm. Potentially valid formulae were further selected based on existence of a ^{13}C- and ^{37}Cl-isotope peak (same tolerance), and a $^{12}C/^{13}C$ peak intensity ratio predicting C within 2 atoms of the suggested formula[14]. Sample-wise confirmed formulae built a database of

chemically feasible compounds for glacial DOM. In a second stage of data processing, peaks from all samples were then reassigned to formulae in the database, neglecting isotope confirmation to recover low-intensity peaks, and resulting in a peak alignment across samples based on assigned formulae rather than observed mass. A sizeable number of formulae could then be ruled out by applying a refined mass tolerance computed as a standard error of the mean 1/sqrt(n), where 1 ppm is the maximum error found for peaks with lowest S/N ratio and n is the frequency of occurrence across all samples. Formulae were finally selected for positive integer double-bond-equivalents, H:C within [0.3,2.5], O:C and N:C within [0,1], $H \leq 2C+2+N$, at least 1 O for each P or S, and no heteroelement (N, S, P) co-occurrence. An aromaticity index was computed in a modified version[15] from sum formulae.

Canonical analyses of principal coordinates (CAP[16], as implemented in the vegan[17] package in R) was used to test for an association of DOM molecular composition (as derived from FT-ICR-MS) with BDOC or $\Delta^{14}C$ DOC, and to identify DOM molecular subpopulations associated with the gradient of bioavailability and age across glaciers. Similarly, molecular composition was linked to fluorescence intensity of humic and protein components derived from EEM and PARAFAC. The two protein components (tryptophan-like and tyrosine-like) differed in their behaviour across glaciers and were analysed separately, while the two humic components were summed due to completely parallel behaviour in CAP. Briefly, this two-stage analysis first reduced the extremely multivariate space of DOM molecular composition (>1900 molecules) to a limited number of principal coordinates (number of glaciers minus 1) derived from a metric scaling of dissimilarities among glaciers. Dissimilarities were Bray-Curtis dissimilarities computed from proportional peak intensities. In a second stage, redundancy analysis identified the single (canonical) linear combination of the principal coordinates most strongly related to one selected constraining variable (BDOC, $\Delta^{14}C$ DOC, tryptophan-like, tyrosine-like or humic fluorescence). This canonical axis can be regarded as one specific dimension of DOM molecular compositional space, along which changes of composition across glaciers parallel the selected constraint (e.g., BDOC). Fluorescence data was log-transformed prior to its use as constraining variable to reduce skewness. Significance of CAP was computed by permutation, and a canonical correlation coefficient (Pearson correlation between constraint and canonical axis) was computed as an indicator for the strength of the relationship. Last, specific molecules associated with the gradient of BDOC and radiocarbon age (as well as tryptophan-like, tyrosine-like or humic-like fluorescence) across the study glaciers were identified by projecting their peak intensities (transformed as above) onto the single canonical axis derived by CAP using a Spearman rank correlation

coefficient. These correlation coefficients were used for color-coding molecules in the Van Krevelen space (e.g., Figure 2b, 2c). The resulting 5 sets of Spearman rank correlation coefficients for the 5 constraining variables were then used in a final analysis to define molecular subpopulations by cluster analysis. Cluster analysis was limited to 1579 molecules occurring in at least 1/3 of all glaciers to reduce noise by unsure Spearman correlation coefficients, and based on Euclidean distance with Ward´s minimum variance as agglomeration method. Lower branches were subjectively cut from a dendrogram visualization (Figure 3) to define 5 clusters, i.e., 5 molecular subpopulations, which were separately graphed in Van Krevelen space and analysed for their chemical properties (e.g., N-prevalence, O:C, molecule mass).

Among the main advantages of the CAP-approach are (i) the expression of DOM molecular composition using all available information (i.e., all identified molecules including rarely occurring ones) without the need for data transformation, (ii) implicit consideration of the inherent dependence among >1900 relatively expressed intensities in individual spectra by condensing DOM compositional information into a simple (Bray-Curtis) dissimilarity measure, and (iii) efficient extraction of a single (canonical) dimension along which changes in composition parallel a chosen constraint across glaciers. However, it has to be noted that our multivariate data analysis approach is still entirely correlative. High variability of a dominant fraction of DOM (e.g., cluster 2) could potentially reduce other signals in FT-ICR-MS spectra with relative peak intensities. Indeed, this could for example explain the counterintuitive association of – quantitatively probably less important – microbially derived material (cluster 1 and 4) with lower bioavailability.

Supplementary Results

Pro-glacial streamwater $\delta^{18}O$ signatures and DOC concentration

To study the fate of the glacial DOC in the pro-glacial stream and its implications for in-stream carbon cycling, we sampled streamwater at the glacier terminus and at two further sites downstream (stream site 1: 667 ± 315 m and stream site 2: 1593 ± 708 m). To assess the relative contribution of glacial wastage to runoff at the terminus, we determined $\delta^{18}O$-values in the ice and in the streamwater. Streamwater $\delta^{18}O$-values (-14.7 ± 0.92 per mille) at the glacial terminus did not significantly (Wilcoxon, $p > 0.05$, n = 22) differ from ice $\delta^{18}O$-values (-14.3 ± 0.95 per mille), and $\delta^{18}O$-values (-15.0 ± 1.21 per mille) from snow samples were variable but slightly lower as in the streamwater. This suggests major contributions from glacial wastage to runoff and little influence from remnant snow patches and other sources in the catchment.

Streamwater DOC concentration did not significantly (Wilcoxon, $p > 0.05$, n = 22) change along the pro-glacial streams from the terminus (266 ± 360 µg C L^{-1}) downstream to stream site 1 (282 ± 322 µg C L^{-1}) and stream site 2 (216 ± 89 µg C L^{-1}).

Pro-glacial E*p*CO_2

To test whether fast downstream increase of ambient pressure in the steep glacial streams can explain the frequently observed low degree of saturation and potentially confound the BDOC effect on E*p*CO_2, we added stream slope adjacent to the glacier terminus besides BDOC in a multiple linear regression model explaining E*p*CO_2. The 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 E*p*CO_2, though the negative regression coefficient for the slope agrees with a downstream pressure effect. A model selection procedure based on the Akaike Information Criterion (AIC,[18]) identifies the simple regression model based on BDOC (Figure 4) as superior.

In a similar fashion, we tested for a potential confounding effect of turbulence-driven gas exchange[19] on the BDOC-E*p*CO_2 relationship. We included stream slope (as a proxy for the reaeration coefficient) interacting with a two-level factor defining CO_2 over- and undersaturation as an additional term besides BDOC in a multiple linear regression model. The interaction term accommodates the two directions of gas exchange between stream water and atmosphere and allows different signs for the effect of stream slope on E*p*CO_2; a similar

approach worked well for us in a large range of streams (data unpublished). The fitted model resulted in insignificant and physically meaningless regression coefficients for the stream slope, and the AIC again selected the simple linear regression model based on BDOC as superior.

EpCO_2 did not significantly (Wilcoxon, $p > 0.05$, n = 20) change along the pro-glacial stream from the terminus to stream sites 2 and 3 (Wilcoxon, $p > 0.05$, n = 19) suggesting that rapid depletion of biodegradable compounds from glacial DOC drives this spatial pattern. These findings show that glacial DOC unexpectedly contributes to carbon cycling in glacial headwaters and ultimately to CO_2 outgassing to the atmosphere.

Supplementary Table 1 Coordinates of each glacier are taken from the World Glacier Inventory[20]. Longitude is given in decimal degrees East and latitude in decimal degrees North. Glacier cover (%) was calculated from total drainage area (Austrian Map 2001) and glacier area (World Glacier Inventory[20]). The elevation is the median glacier altitude in meters above sea level.

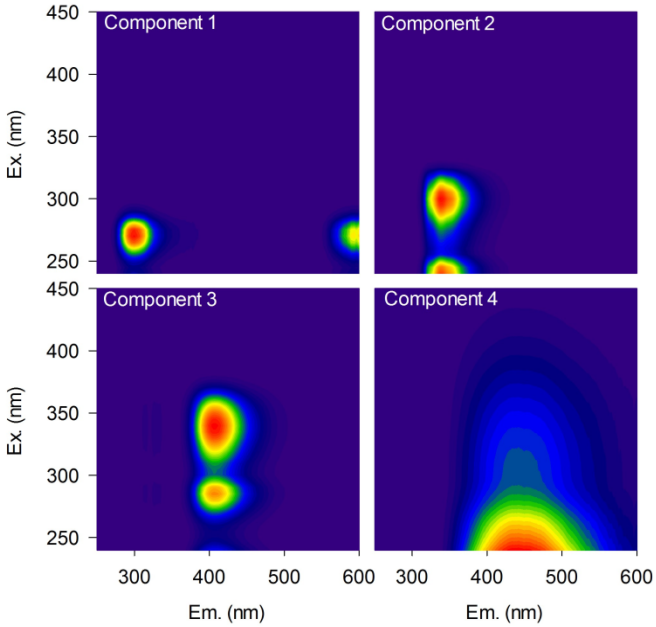
Glacier	Longitude (°E)	Latitude (°N)	Drainage area (km²)	Glacier cover (%)	Elevation (m)
Alpeiner Ferner	11.6417	47.0317	7.31	70	2650
Gaißbergferner	11.065	46.83	2.54	53	2850
Gepatschferner	10.7583	46.855	30.80	57	3090
Hintereisferner	10.77	46.7967	16.88	56	3020
Hornkees	11.8233	47	5.96	51	2790
Jamtalferner	10.1617	46.8617	5.84	71	2810
Kälberstpitzkees	13.2783	47.03	1.27	65	2670
Kesselwandferner	10.7983	46.8383	2.69	100	3180
Kleinelendkees	13.2567	47.0633	2.37	100	2850
Langtaler Ferner	11.0167	46.7933	8.15	43	2910
Niederjochferner	10.86	46.7783	1.04	80	3100
Obersulzbachkees	12.3017	47.1133	13.46	86	2790
Ödenwinkelkees	12.6517	47.1083	6.08	37	2590
Pasterze	12.695	47.1017	30.09	66	2980
Rotmoosferner	11.0483	46.8167	3.85	82	2960
Schalfferner	10.935	46.785	12.35	70	3120
Schlatenkees	12.385	47.1117	11.97	78	3060
Schwarzenbergferner	11.115	47.0433	1.99	80	3090
Schwarzensteinkees	11.855	47.0133	4.33	75	2900
Stubacher Sonnblickkees	12.6	47.1317	1.56	76	2790
Sulztalferner	11.08	47.0017	4.77	87	2960
Taschachferner	10.8583	46.9	11.03	60	3180
Untersulzbachkees	12.35	47.135	8.66	49	2850
Vernagtferner	10.8167	46.8767	3.09	78	3150
Viltragenkees	12.3717	47.1283	3.47	88	2740
Zettalunitzachkees	12.5111	47.1056	3.52	92	n.d.

Supplementary Table 2 DOC concentration, bioavailability, carbon use efficiency (CUE) and Δ^{14}C-DOC.

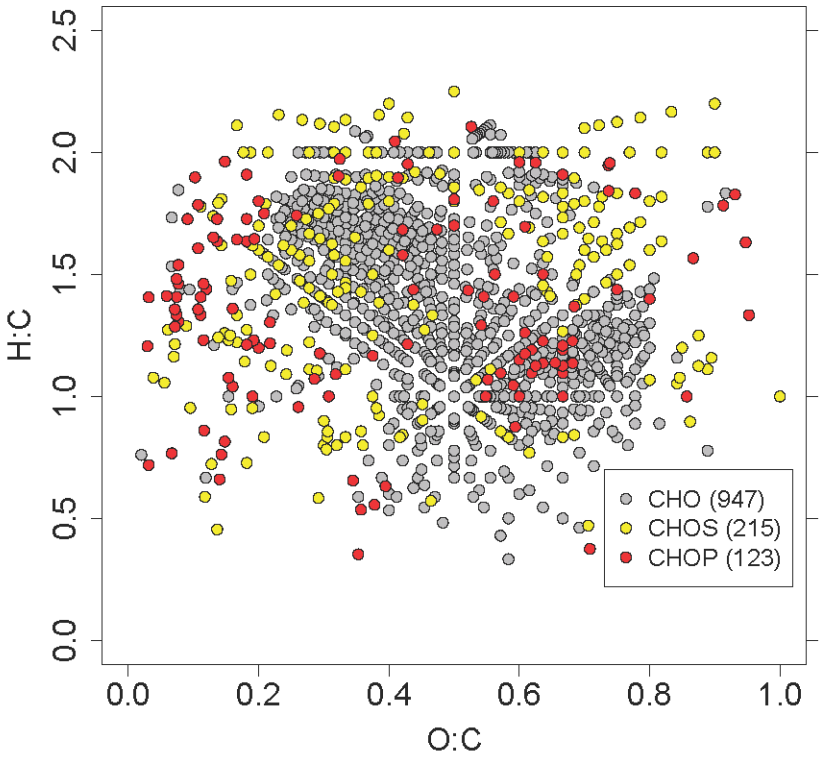
Glacier	DOC ($\mu g\ L^{-1}$)	Bioavailable DOC (% DOC)	CUE (%)	Δ^{14}C-DOC (‰)
Alpeiner Ferner	211.77	30.33	n.d.	-286
Gaißbergferner	101.03	86.19	25.25	-503
Gepatschferner	173.44	28.78	n.d.	n.d.
Hintereisferner	97.43	64.28	18.42	-329
Hornkees	90.10	25.15	n.d.	-386
Jamtalferner	128.43	43.80	n.d.	-378
Kälberstpitzkees	39.60	92.53	21.40	-477
Kesselwandferner	138.43	82.98	29.76	-373
Kleinelendkees	95.33	86.44	19.79	-566
Langtaler Ferner	143.83	79.04	33.16	-574
Niederjochferner	70.50	75.15	n.d.	-355
Obersulzbachkees	120.10	53.09	n.d.	n.d.
Ödenwinkelkees	13.27	73.92	15.80	-447
Pasterze	91.83	63.10	21.53	-615
Rotmoosferner	136.10	84.63	13.18	-601
Schalfferner	143.10	n.d.	n.d.	-62
Schlatenkees	106.20	n.d.	n.d.	-592
Schwarzenbergferner	180.10	n.d.	n.d.	-248
Schwarzensteinkees	185.77	n.d.	n.d.	-290
Stubacher Sonnblickkees	68.30	93.59	13.37	-596
Sulztalferner	187.77	59.77	11.30	-64
Taschachferner	507.43	54.62	n.d.	-197
Untersulzbachkees	241.93	41.11	n.d.	-434
Vernagtferner	90.10	n.d.	n.d.	-225
Viltragenkees	50.10	58.75	n.d.	-448
Zettalunitzachkees	203.77	58.18	n.d.	-470

Supplementary Table 3 Excitation and emission maxima of components modeled by PARAFAC[6] of excitation emission matrices. Secondary peaks are in brackets. Additional fluorophore names[21] and description[21-23] are listed corresponding to the modeled peaks.

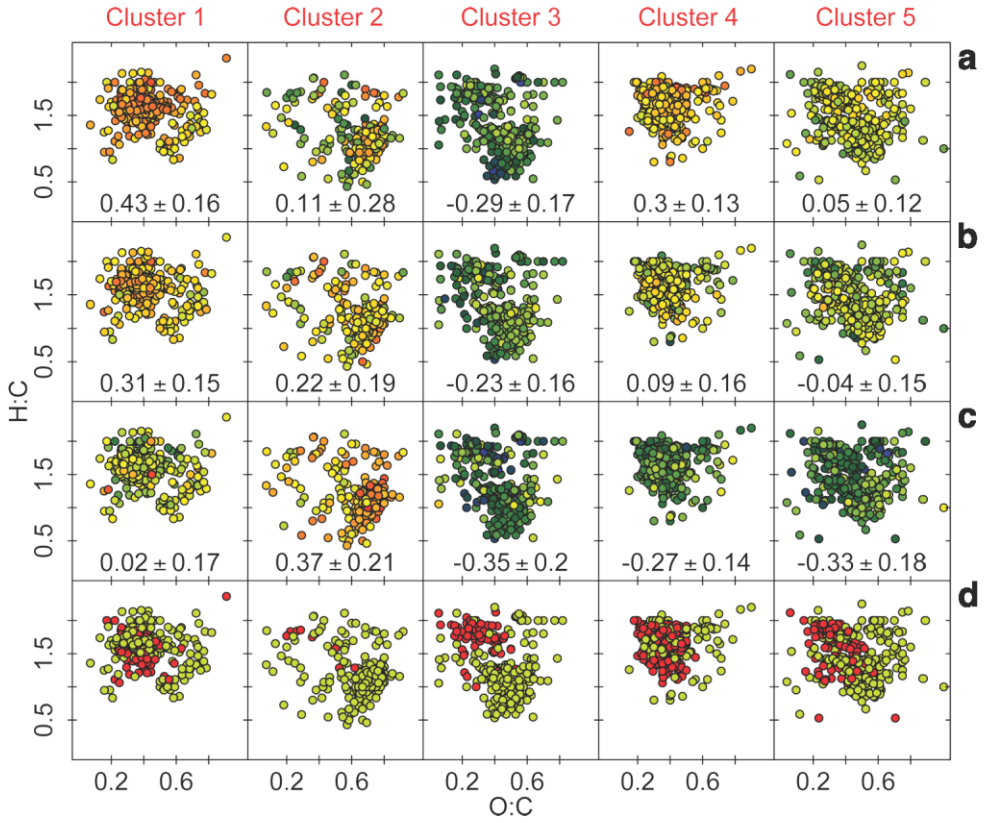
Component number	Excitation maxima (nm)	Emission maxima (nm)	Description	Fluorophore name (Coble,1996)
1	270	298 (<600)	Protein-like (Tyrosine-like)	B
2	>240 (300)	338	Protein-like (Tryptophan-like)	T
3	285 (340)	408	Humic-like	A or C
4	240	442	Humic-like	A



Supplementary Figure 1. Four fluorescent components were detected in excitation emission matrices and modeled by PARAFAC[6]. Component 1 and 2 were assigned as protein-like, component 3 and 4 as humic-like[21-23].



Supplementary Figure 2. Van Krevelen diagram of molecular formulae found in ice DOM from the 26 Alpine glaciers. Nitrogen-containing molecules were excluded (see Figure 2). Appr. 11.5 % of molecules have identical location in Van Krevelen space, plotting order follows legend with CHO compounds plotting beneath CHOS and CHOP.



Supplementary Figure 3. Cluster analysis identified 5 molecular subpopulations based on association patterns of molecules with BDOC, Δ14C DOC, Tryptophan-like, Tyrosine-like and humic-like fluorescence as achieved by CAP[16]. Numbers in brackets give percentage of compounds in each cluster. **(a, b, c)** Three series with 5 panels each show the location of the 5 clusters in Van Krevelen space with cluster-specific colour-coded correlations of molecule-specific intensities with the (canonical) axis of DOM molecular compositional space associated with Tryptophan-like (PARAFAC-component 2) **(a),** Tyrosine-like (PARAFAC-component 1) **(b)** and humic-like fluorescence (sum of PARAFAC-component 3 and 4) **(c)**. Numbers in panels give mean (± SD) of correlation coefficients for each cluster. Color scale in (a, b, c) uniformly ranges from -1 (blue) to 1 (red). Yellow to red colors indicate molecules positively associated with fluorescence of the respective components and identify protein-like and Tannin-like subpopulations in the Van Krevelen space. **(d)** Distribution of nitrogen-containing molecules in each cluster (red indicates at least one N, green indicates no nitrogen). Note that nitrogen-occurrence was not used as a variable in the cluster analysis.

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III. Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams

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a. Research design
b. Aided in conducting bioassays and laboratory analysis
c. Conducted the statistical analyses
d. Co-writing, editing and approval of manuscript

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Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams

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Streams receive substantial terrestrial deliveries of dissolved organic matter (DOM). The chromophoric (CDOM) fraction of terrestrial deliveries confers the brown colour to streamwater, often understood as browning, and plays a central role in aquatic photochemistry and is generally considered resistant to microbial metabolism. To assess the relevance of terrigenous DOM for carbon fluxes mediated by stream microorganisms, we determined the bioavailable fraction of DOM and microbial carbon use efficiency (CUE), and related these measures to partial pressure of CO_2 in headwater streams spanning across a browning gradient. Fluorescence and absorbance analyses revealed high molecular weight and aromaticity, and elevated contributions from humic-like components to characterize terrestrial CDOM. We found that microorganisms metabolized this material at the cost of low CUE and shifted its composition (from fluorescence and absorbance) towards less aromatic and low-molecular weight compounds. Respiration (from CUE) was related to CO_2 supersaturation in streams and this relationship was modulated by DOM composition. Our findings imply that terrigenous DOM is respired by microorganisms rather than incorporated into their biomass, and that this channelizes terrigenous carbon to the pool of CO_2 potentially outgassing from streams into the atmosphere. This finding may gain relevance as major terrigenous carbon stores become mobilized and browning progresses.

A significant amount of the terrestrial primary production is transported laterally along the fluvial continuum from soils to the oceans[1]. Evidence suggests that this lateral flux as DOM into inland waters is increasing in North America, North and Central Europe, and in Southeast Asia[1–5] causing visible browning of these waters[6]. Especially the CDOM fraction of these terrestrial deliveries impacts on freshwater ecosystems as CDOM influences the light regime, primary production and related trophic processes[4,7]. At high concentration, CDOM can even have implications for drinking water supply[8]. The photochemical degradation of CDOM can also contribute to CO_2 outgassing fluxes from streams, rivers and lakes[9].

It is therefore of paramount relevance to improve our understanding of the carbon cycling at the terrestrial-aquatic interface[10–12]. This is especially true for headwater streams, which are most abundant in fluvial networks and tightly connected with the terrestrial milieu. Small streams are also major sources of CO_2 to the atmosphere[10,13–14] but the contributions of DOM metabolism to CO_2 outgassing from these streams remain poorly understood. The common wisdom that terrestrial CDOM (that is, mostly humics) is resistant[15] to microbial metabolism has shaped our thinking over the last decades[12]. This perception is now being re-evaluated based on evidence that chemical recalcitrance and high $\Delta^{14}C$ age as commonly assumed properties of terrigenous DOM do not necessarily predict its bioavailability[12,16–17]. It was shown for instance that terrestrial DOM appears to drive respiration and CO_2 outgassing from the Amazon River[17,18].

The aim of this study was to investigate the metabolic fate of terrestrial DOM in headwater streams and to assess its potential contribution to carbon cycling in these streams. Study streams encompassed a brown-colour gradient and we used a space-for-time substitution approach to evaluate the effect of browning on carbon dynamics. We hypothesize that heterotrophic metabolism in stream ecosystems, as depicted by pCO_2, results from dissolved organic carbon (DOC) concentration, its bioavailable fraction (%BDOC) and the catabolic response of the microorganisms as carbon use efficiency (CUE). We assume that %BDOC and CUE both depend

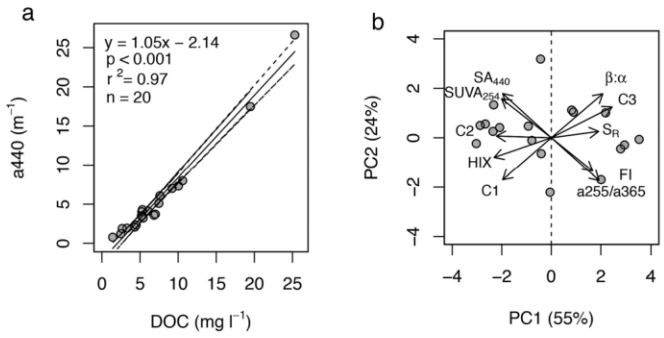

Figure 1 | Quantitative and qualitative aspects of DOM. (a) Colour (as a_{440}) increases with DOC concentration. Dashed lines represent 95% confidence intervals. (b) Principal component analysis (PCA) based on the optical properties of the 20 streams distinguishes terrigenous from autochthonous DOM (PC1). Terrigenous DOM was characterized by high specific absorption at 254 nm ($SUVA_{254}$) and 440 nm (SA_{440}), and a high humification index (HIX). Fluorometry revealed two humic-like compounds of terrigenous origin (C1 and C2), while the third component (C3) was putatively associated with new biological production (*Supplementary Information*). High values of the freshness index (β/α) and the fluorescence index (FI) but also lower apparent molecular weight (indicated by a255/a365 and the slope ratio S_R) describe DOM with a more autochthonous character. Arrows are based on PCA structural coefficients.

on DOM composition[19,20]. To test this hypothesis, we related DOC concentration and DOM optical properties (from fluorescence spectrometry and absorbance) to %BDOC, CUE and to the potential evasion of CO_2 in brown-water streams.

Results

DOM quantity and composition. We investigated attributes of DOM from twenty 1st-order streams in Austria (Table S1 and Table S2) that drain catchments spanning over a gradient in brown colour as a response of varying in peat cover. In fact, the relative contributions of peat and wetlands to land cover are known to imprint on CDOM and its colour as a commonly used indicator of terrestrial DOM deliveries to freshwater ecosystems[21–24]. We found streamwater DOC concentration ranging from 1.41 to 24.31 mg C L^{-1} and CDOM colour (a_{440}) ranging from 0.78 to 26.64 m^{-1} (Table S1) across all streams; concentration and colour correlated significantly (Figure 1a).

We analysed the composition of streamwater DOM as inferred from absorbance and fluorescence measures including fluorescent components modelled from excitation emission matrices (EEMs) by parallel factor analysis (PARAFAC)[25]. A principal component analysis (PCA) based on these measures revealed a gradient of streams where terrigenous DOM mixes in varying proportions with autochthonous DOM. Elevated aromaticity ($SUVA_{254}$), colour (a_{440}), higher degree of humification (HIX) and apparent molecular-weight compounds (S_R), and the humic-like fluorescent components C1 and C2 (Figure 2) are all indicative of terrigenous DOM deliveries to these streams. On the other hand, the freshness index (β/α), low apparent molecular weight and aromaticity, but also the humic-like component C3 (Figure 2) assumedly associated with biological production[26], indicate in-stream production of DOM.

Relating the same set of concentration-independent optical measures that describe DOM composition to a_{440}, we found that the terrigenous imprint on DOM increases with brown colour across all streams (Figure 3a). To rule out the possibility of a spurious correlation driven by DOC concentration, we excluded SA_{440} and $SUVA_{254}$ from the analysis and were able to confirm the observed composition shift towards terrigenous DOM paralleled the gradient of brown colour (Figure S1).

DOM biodegradation and CUE. We used classical batch biodegradation assays (20 days) to monitor changes in DOM composition upon microbial degradation and calculated BDOC (as percent of the initial DOC concentration)[27] and CUE (as biomass production *versus* biomass production plus respiration). Based on canonical correlations, we did not find any obvious relationship between %BDOC and DOM composition as inferred from its optical properties (Figure 3b and Figure S2). To back up our BDOC measurements we also computed microbial carbon demand from respiration and biomass build-up[20], which should essentially equal BDOC. The linear relationship between both measures ($r^2 = 0.80$, $p < 0.001$, $n = 20$) (see *Supplementary Information*) supports our BDOC measurements.

In contrast to %BDOC, CUE varied more broadly (4.57% to 37.10%) over all twenty study streams and averaged 12.73 ± 9.58%; CUE was significantly related to DOM composition (canonical correlation, $r = 0.88$, $p < 0.05$, $n = 20$). CUE increased with putatively fresh DOM of apparently low molecular weight and with a microbial imprint; it declined with increasing contributions from terrigenous DOM (Figure 3c and Figure S3). To test whether coloured DOM affects CUE, we also related the gradient of DOM quality associated with CUE to the gradient of DOM quality associated with

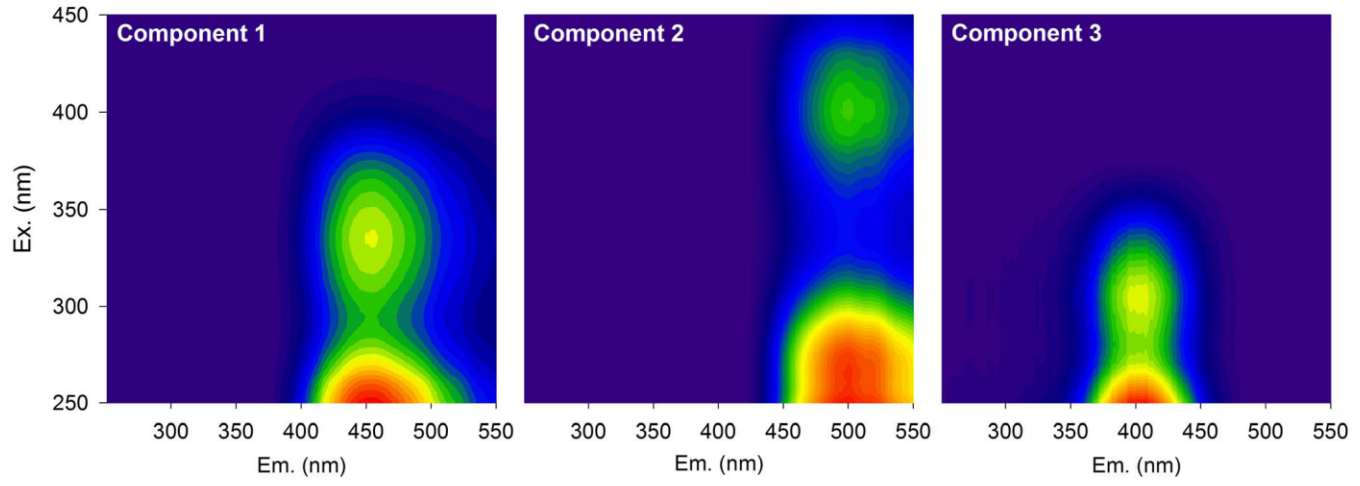

Figure 2 | Three fluorescent components were modeled by parallel factor analysis (PARAFAC) from excitation emission matrices. All components were assigned as humic-like.

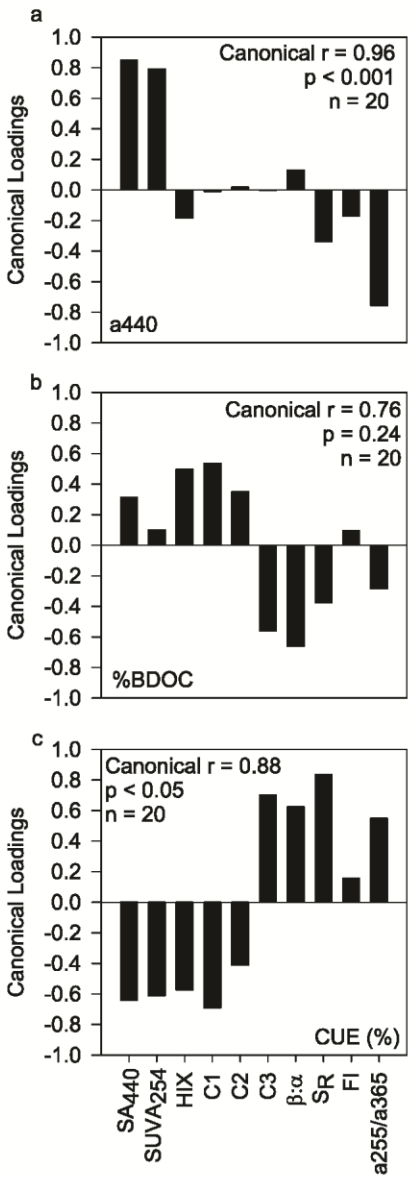

Figure 3 | DOM composition is related to colour (a_{440}) and impacts microbial metabolism. Canonical correlation analyses reveal DOM optical properties most strongly related to (a) colour (a_{440}), (b) the bioavailable fraction of DOC (%BDOC) and (c) carbon use efficiency (CUE). Canonical loadings (correlations between the one canonical variate of our analysis and the respective variables) indicate the strength and direction of the relationship between the respective constraint and individual optical measures (*see Methods*).

brown colour and found a significant correlation (Spearman correlation, $r = -0.50$, $p < 0.05$).

DOM optical properties shifted upon microbial degradation as indicated from a decrease of humification (as HIX) (t-test, $p < 0.001$, $n = 20$; Figure 4a) and a concurrent increase of the apparent freshness (as β/α) of DOM (t-test, $p < 0.001$, $n = 20$; Figure 4b). Especially when the initial terrigenous imprint was strong, apparent freshness increased disproportionally upon degradation. We depicted these shifts in DOM composition occurring during the degradation assays by using the eigenvectors of the PCA describing DOM composition (based on the optical information across the 20 streams before running biodegradation assays) to predict PCA-scores at the end of the experiments (Figure 4c and Figure S4). This revealed that the initial terrigenous imprint on DOM decreased upon microbial degradation, which resulted in an enhanced autochthonous DOM signature. Relating the length of these shifts in carbon composition to CUE, we found that that DOM with a stronger terrestrial imprint induced lower CUE (Figure 5).

Relationship between BDOC, CUE and streamwater CO_2. To evaluate carbon cycling in our study streams we first determined CO_2 partial pressure (pCO_2) in the streamwater and found an average pCO_2 of 1,611 ± 1,055 µatm. We also calculated the degree of CO_2 supersaturation (expressed as ΔCO_2, which is the concentration of streamwater CO_2 minus the CO_2 concentration expected at complete atmospheric equilibrium) as a proxy for stream ecosystem metabolism. Positive ΔCO_2 values (73.00 ± 64.48 µmol l^{-1}) indicate that all streams were supersaturated in CO_2 and hence a potential source of CO_2 to the atmosphere. Next we related respiration rates measured in the biodegradation assays and found them closely related to ΔCO_2 in the streams ($r^2 = 0.66$, $p < 0.001$, $n = 20$). To further illuminate the processes driving this relationship, we propose the following linear model

$$\log \Delta CO_2 = b_0 * \log DOC + b_1 * \log \%BDOC + b_2 * \log(1 - CUE),$$

which is derived from our understanding that stream metabolism is the product of the total amount of DOC *times* its bioavailable fraction (%BDOC) *times* the catabolic response of the microorganisms, as the fraction respired carbon (1-CUE). Regression analysis revealed that DOC concentration and CUE explained 51% ($p < 0.01$) of the variance in ΔCO_2 across all study sites (Figure S5). Although DOC concentration (beta = 0.45, $p < 0.05$, sum of squares = 3.91) was the strongest predictor for ΔCO_2 followed by CUE as suggested by its sums of squares (beta = 0.53, $p < 0.05$, sum of squares = 1.68). BDOC was not a significant predictor (beta = −0.17, $p = 0.43$, sum of squares = 0.2).

Discussion

DOM quantity and composition. Inland waters receive large amounts of DOM from the terrestrial environment[1]. The traditional perception that the chromophoric and humic fraction of this delivery is largely resistant to the microbial metabolism is now changing[12,16–17]. Our findings expand this changing view by showing the link between the microbial degradation of terrestrial CDOM and potential CO_2 outgassing from small headwater streams.

Our results reveal that terrestrial deliveries not only increase the DOC concentration in streams but also shift the composition of DOM. The DOC concentrations we measured are closely bracketed by values from headwaters in the UK[2] and North America[30], for instance, and colour (a_{440}) is in the range of values (0.69–29.94 m^{-1}) from boreal streams and rivers[9]. PARAFAC analyses revealed that humic-like compounds and elevated aromaticity (as $SUVA_{254}$) characterised terrigenous DOM. Values of $SUVA_{254}$ from our streams are in the upper range of values reported from streams and lakes in North America[28] (range: 0.3–8.7 mg L^{-1} m^{-1}) and closely bracketed by values (3.5 to 4.9 mg L^{-1} m^{-1}) reported from channels draining tropical peat swamp forests[5]. The browning gradient that we captured with our study streams may thus be representative for other systems influenced by increased terrestrial DOM deliveries.

The relationship between DOC concentration and colour indicates that the variability in DOC concentration across the streams is driven to a large extent by terrigenous DOM deliveries[9,21,29]. Clearly, this relationship supports the quantitative effect of "brown" and terrigenous DOM deliveries on stream DOC concentration as reported previously[2,31]. The canonical correlation analysis furthermore revealed that an increase in colour was accompanied by high aromaticity, high apparent molecular weight and high humification

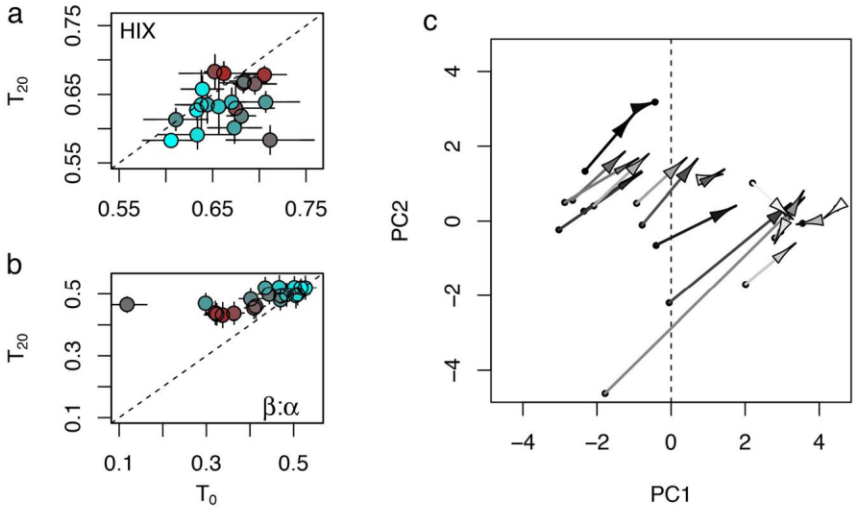

Figure 4 | DOM composition during microbial degradation. (a) The humification index (HIX) and (b) the freshness index (β/α) at start of the degradation assays (T_0) and after the 20-day incubation period (T_{20}). Given is the mean ± SD (n = 3 replicates) and the dashed line indicates a 1 : 1 relationship (i.e., no change during degradation) Original DOM composition as derived from the PCA is indicated by colour. Brown colours denote streams with predominantly terrigenous DOM and blue colours represent streams with relatively more autochthonous DOM. (c) Shifts in DOM composition over the 20-day experiments. Starting coordinates of arrows (indicated by black circles) are identical with scores of the PCA based on optical measures (Figure 1b). End coordinates are predicted scores computed with the PCA eigenvectors and the optical measures at the end of the experiments. Lengths of arrows denote the intensity of the shift in DOM composition. The greyscale indicates CUE; ranging from black (lowest CUE) to white (highest CUE).

collectively pointing at the increasing terrigenous character of DOM. This is also supported by lower FI values indicative of allochthonous DOM sources[32] and by elevated contributions of the humic-like fluorescence components C1 and C2. Both fluorescence components are of terrestrial origin[33] and often encountered in freshwater ecosystems[34], and thus appropriate to track DOM sources[35]. Our findings corroborate previous studies relating DOM colour to chemical composition, including apparent molecular weight[36] or lignin phenols[37].

The PCA based on DOM absorbance and fluorescence also suggests that autochthonous compounds imprint to varying degrees on the optical properties of the DOM pool. Such autochthonous DOM compounds may be of recent microbial origin as indicated by elevated values of the freshness index (β/α)[38,39]. High values of the fluorescence index (FI) commonly used as an indicator of DOM source (i.e., terrigenous *versus* microbially derived)[32] along with lower apparent molecular weight and aromaticity further support our notion of microbial contributions to the DOM pool.

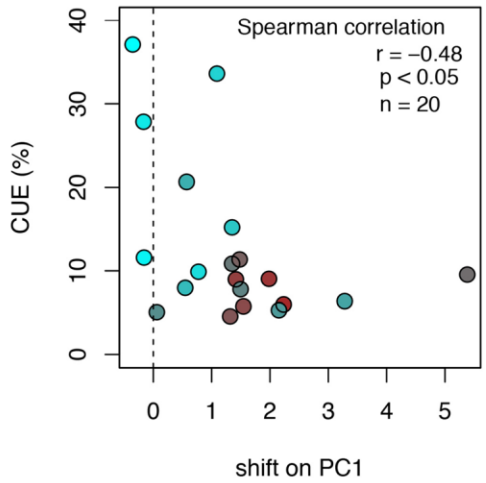

Figure 5 | CUE decreases with the absolute shift in DOM composition along PC1 (i.e., each stream's PC1 score *after* minus the respective score *before* degradation). Original DOM composition as derived from the PCA (PC1) is indicated by colour. Brown colour denotes streams with predominantly terrigenous DOM and blue colour represents streams with relatively more autochthonous DOM.

The relative mix of allochthonous (that is, terrestrial) and autochthonous DOM across the browning gradient studied here possibly affects the susceptibility of the DOM pool to the microbial metabolism in the streams. We could not find any significant effect of DOM composition on its bioavailability (as %BDOC). This seems to run counter to findings from coastal temperate streams, for instance, where protein-like fluorescence predicted %BDOC ranging on average from 12.6% and 30.1%[40]. We attribute this discrepancy to the comparatively low %BDOC values, but close to those reported from other streams draining peat[41], and to their low variability (average ± SD: 4.55 ± 2.14%) across our study streams. CUE, however, varied broadly across the streams and was related to DOM composition. This agrees with previous findings[19], showing, for instance, a relationship between CUE and apparent molecular weight (as *a*254/*a*365)[42]. In line with this, CUE increased with putatively fresh DOM of apparently low molecular weight and with a microbial imprint; it declined with increasing contributions from terrigenous DOM. CUE was lower than values reported from various aquatic ecosystems[19], which may be attributable to the largely terrigenous character of DOM as the consumption of terrigenous carbon may support a low but continuous level of metabolic activity[43]. Our data on nutrient concentration in the biodegradation assays (Table S2) preclude that nutrient limitation affected CUE. This is important to note as nutrient limitation can shift the balance between respiration and biomass production when excess carbon is released by respiration[44]. CUE may also be low in the absence of nutrient limitation. For instance, the relative energy content per unit carbon may decrease with increasing terrestrial imprint due to more oxidized compounds[19,45]. The degradation of such compounds requires the production of exoenzymes (e.g., phenoloxidases), which increases carbon demand but which may also enhance respiration[45].

The fact that the gradient of DOM composition associated with CUE paralleled the gradient of DOM composition (that is, CDOM

associated with the brown colour) indicates that changes in this CDOM lead to a decrease in CUE. This suggests an impact of brown DOM on carbon processing in streams. In streams with elevated brown colour, a smaller fraction of DOC is incorporated into microbial biomass, while a larger fraction is used for the maintenance of microbial metabolism.

Biodegradation assays revealed that microbial activity apparently imparted an autochthonous signature on an otherwise allochthonous DOM pool characterised by chromophoric and humic-like terrigenous compounds. Our findings suggest that microorganisms removed humic-like compounds while simultaneously producing novel compounds. This is consistent with the perception of the dual role of microorganisms as consumers and producers of DOM[46]. Uptake of humic substances or polyphenolic compounds by microorganisms in streams has been reported previously[43,45,47]. DOM with a strong initial terrigenous imprint was extensively reworked as indicated by the pronounced shifts towards autochthonous DOM compared to DOM that initially has a more autochthonous signature. The apparent freshness for instance increased disproportionally when the initial terrigenous imprint on DOM was strong, which is reflected in the overall shifts in DOM composition upon degradation. This agrees with the observation of lignin and related phenolic compounds being extensively reworked by microorganisms and possibly causing a complete overturn of the DOM pool within weeks in the Amazon River[17]. Moreover, low-molecular-weight DOM of terrigenous origin was readily available and also quantitatively important for microbial metabolism in boreal freshwaters[20]. However, optical properties of relatively more autochthonous DOM changed comparatively less upon microbial degradation, which may be due to the simultaneous production of novel compounds which may resemble the optical properties of the initial material.

DOM with a strong terrigenous imprint induced lower CUE upon degradation, supporting the results from the canonical correlation analysis. This confirms that this carbon pool fuelled microbial respiration rather than sustaining microbial growth. This unbalance between microbial respiration and growth may impart the microbial imprint on DOM observed during biodegradation. As evoked by the shifts in DOM composition this may occur especially when the initial terrestrial imprint was strong. Our results suggest that elevated catabolism was likely required to maintain microbial metabolism of terrigenous DOM[19].

It is now well established that headwater streams are supersaturated in CO_2[9,14,13,48–51], yet it remains largely unclear to what extent this CO_2 originates from catchment or from *in situ* respiration[12]. While there is evidence that groundwater delivers CO_2 from terrestrial respiration into small headwater streams[49,50,52], it is also recognized that these streams are net heterotrophic (that is, they metabolize terrigenous organic carbon)[10]. That inland waters are to a large extent net heterotrophic is also supported by surveys showing that lake and stream pCO_2 is linked to *in situ* metabolism of DOC[9,48,51]. In line with these observations, the respiration measured in our biodegradation assays was closely related to streamwater ΔCO_2, which indicates indeed that metabolism contributes to the build-up of CO_2 in headwater streams. This result is remarkable because it links DOC metabolism to CO_2 supersaturation in the streams despite the fact that these are independent measurements.

To further explore the relationship between respiration and CO_2 supersaturation in the streams we proposed a simple model based on the fact that the fraction of respired DOC can be inferred from BDOC and CUE. Our model revealed DOC concentration and CUE as significant predictors for streamwater ΔCO_2. DOC concentration may affect streamwater ΔCO_2 on a mass basis, which complies with large-scale surveys from lakes and streams[48,51]. We recognise that a common terrestrial origin of both DOC and CO_2 may drive to some extent at least the observed relationship between DOC and streamwater CO_2 as reported from Amazonian headwater streams[52]. It is clear that quantitative contributions of DOC and CUE to CO_2 supersaturation cannot be inferred from our model; however, it points to the microbial metabolism of terrigenous DOM as an important driver of CO_2 dynamics in brown-water streams. While DOC represents the potential energy basis for the heterotrophic metabolism, BDOC better reflects the amount of organic carbon that is actually available for microbial metabolism. That %BDOC was not a significant term points to bioavailable DOM as a relatively constrained fraction of the carbon pool, which in fact agrees with the missing relationship between %BDOC and DOM composition. In contrast, model results stress that the coupling of CUE with DOM composition constitutes a control on ΔCO_2 besides DOC quantity. Our findings thus provide evidence that DOM quantity and composition affect CO_2 build-up in headwater streams. The quantity of terrigenous DOM deliveries to streams may increase the pool of carbon that is potentially available for microorganisms, while its composition affects its metabolic fate in the microbial compartment. Coloured DOM was preferentially respired with little trophic transfer to the microbial food web, which may contribute to CO_2 supersaturation and evasion to the atmosphere.

Our findings highlight terrestrial deliveries of chromophoric and humic DOM as a relevant component of the carbon cycle in headwater streams and thus complement recent findings from boreal[16,20] and tropical[17] systems. Upon entrance into streams, this DOM pool becomes subject to microbial degradation at the cost of low carbon use efficiency and potentially contributes to CO_2 evasion to the atmosphere even without photochemical facilitation. Collectively, these findings contradict the common wisdom that "brown" DOM is resistant to microbial degradation and therefore largely exempt from metabolism en route from terrestrial sources to marine sinks[9]. Our findings may have important implications as terrigenous DOM deliveries into inland waters are predicted to increase and to cause browning[3], especially where carbon loss from major stores such as peat and permafrost can be massive as climate change progresses.

Methods

Site description and sampling. We sampled twenty streams (1st-order, Austria) draining catchments with differing coverage of coniferous forest and peat (Table S1 and S2). Streamwater samples for DOC and optical analyses were filtered (Whatman GF/F filters) and stored in clean borosilicate vials. Triplicate streamwater samples were collected for the determination of pCO_2 into 50-ml glass vials pre-conditioned with NaN_3 (0.02% final concentration); at each site we also collected samples for atmospheric CO_2. Streamwater for degradation assays was filtered as DOC samples and collected in clean polypropylene copolymer containers.

Biodegradation assays. We conducted biodegradation assays to determine BDOC and CUE, and to relate them to DOM composition and to the observed shifts in DOM composition during microbial degradation. For each stream we conducted triplicate assays over 20 days (in the dark, 18°C). Sterile-filtered streamwater was inoculated with the native microbial community from the respective stream in 250 ml Schott-bottles with a headspace (38%). Samples for DOC concentration, DOM fluorescence and absorbance and CO_2 were obtained at the start of the incubation and at day 3, 6, 10, 15 and 20, respectively. DOM samples were filtered and stored as described above. CO_2 samples (10 ml) were collected from the headspace and injected into pre-evacuated exetainers. Abundance of microbial cells at each time point was determined using epifluorescence microscopy (Zeiss AxioImager) and microbial biomass was estimated from cell size and conversion factors. BDOC was calculated as the loss in DOC concentration over the 20-d incubation period. CUE was calculated as the increase in microbial biomass versus the increase in microbial biomass plus respiration (as dissolved inorganic carbon increase calculated from CO_2 measurements over the same time period). Further details are provided in the *Supplementary Information*.

DOM analyses. DOC concentration was measured using a total organic carbon analyzer (Sievers 5310C, GE, USA). DOM fluorescence and absorbance were determined using an Aqualog (Horiba, USA). Parallel factor analysis (PARAFAC) on excitation emission matrices[25] resulted in three humic-like components but no protein-like component; this is plausible given the humic character of the streamwater (Figure 2). Components (C1, C2, C3) are expressed in percent relative to their total fluorescence. From fluorescence we further derived the humification index (HIX) indicative of the extent of humification[53], the β/α index indicative of fresh microbially produced DOM[41], and the fluorescence index (FI) as a proxy for DOM source (i.e., terrigenous *versus* microbially derived DOM)[42]. From absorption

coefficients we derived the brown colour of DOM (a_{440})[54], the specific UV absorption ($SUVA_{254}$) as a proxy for aromaticity[55], and the slope ratio (S_R)[36] and the ratio of absorption coefficients a_{254}:a_{365}[42], which are both related to apparent DOM molecular weight. Further details are provided in the *Supplementary Information*.

Streamwater ΔCO_2 and respiration. CO_2 concentration was measured using gas chromatography (Agilent) directly from the exetainers containing headspace samples from the degradation assays or after equilibration from the headspace (N_2) in the streamwater samples. Anticipating shifts in carbonate fractions during sample equilibration we recalculated dissolved inorganic carbon (DIC) concentration using Henry's law and considering alkalinity and the respective equilibrium constants for the carbonate fractions (adjusted for temperature and ionic strength)[45,56].We derived streamwater CO_2 concentration (mol l^{-1}) from DIC, pH and the equilibrium constants for the carbonate fractions[56]. The degree of CO_2 supersaturation (expressed as ΔCO_2 in µmol l^{-1}) in the streams is the concentration of streamwater CO_2 minus the CO_2 concentration expected at complete atmospheric equilibrium; it indicates the CO_2 evasion potential from the streams and serves as a proxy for heterotrophic metabolism. Further details are provided in the *Supplementary Information*.

Statistical analyses. Principal component analysis (PCA) using optical measures normalized for DOC concentration served to explore DOM composition and to differentiate bulk DOM characterised by terrigenous or by autochthonous DOM contributions. Three separate canonical correlation analyses identified three linear sets of optical measures (as the respective first canonical axis) most strongly related to a_{440} (brown water colour), %BDOC and CUE as the constraints. Canonical loadings (i.e., correlations between the underlying variables and the one canonical axis) indicate the strength and the direction of the relationship of the individual DOM optical measure to the constraint. The strength of the relationship between the canonical axis and the constraint was computed as the canonical correlation coefficient, significance of each canonical correlation was computed by permutation.

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Author contributions

C.F., G.A.S. and T.J.B. planned the research. C.F. and B.B. accomplished the fieldwork and the biodegradation experiments. C.F. conducted the CO_2 measurements and all microbial work, B.B conducted the fluorescence spectrometry. C.F. performed the statistical analyses aided by G.A.S. T.J.B. wrote the manuscript with assistance from G.A.S., B.B. and C.F.

Additional information

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Supplementary Information for

Microbial degradation of terrigenous dissolved organic matter and possible consequences for carbon cycling in brown-water streams

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Methods

1. Degradation experiments

We conducted degradation experiments to determine bioavailable DOC (BDOC) and carbon use efficiency (CUE), and to relate these measures to DOM composition and to shifts in DOM composition during degradation as inferred from fluorescence and absorbance. For each stream we conducted triplicate degradation experiments over 20 days. We filled 180 ml sterile filtered streamwater into triplicate 250-ml pre-combusted (450°C, 4h) Schott© bottles and re-inoculated this water with unfiltered streamwater (10%) of the same stream, thereby leaving appr. 1/3 headspace. Bottles were incubated in the dark in a water bath at constant 18°C. After an equilibration period of 6 hours, during which bottles were loosely capped with combusted aluminum foil to avoid contamination but allow gas exchange, they were closed air-tight using screw caps and Teflon-coated septa (soaked with 10% $Na_2S_2O_8$ solution at 60°C for 1 h and rinsed thoroughly with MilliQ water). Samples for cell counts, DOC, optical analysis of DOM, and respiration (CO_2) were obtained at the beginning of the incubation period and after 1, 3, 6, 10, 15 and 20 days, respectively. Gas samples (i.e., 10 ml headspace of each bottle) were obtained with a gas tight syringe and transferred into 7-ml pre-evacuated headspace vials (exetainers), fitted with silicon sealed rubber stoppers and aluminum caps. Samples for DOC and optical analysis of DOM were filtered through a double layer of Whatman GF/F filters (precombusted, 450°C for 4h) into 40 ml glass vials (soaked with 0.1 N HCl, rinsed thoroughly with MilliQ water and combusted for 4h at 450°C); these were sealed with Teflon-coated septa (soaked with a 10% $Na_2S_2O_8$ solution at 60°C for 1 h and rinsed thoroughly with MilliQ water)

and stored at 4°C in the dark. Samples for cell counts (1 ml) were preserved in formaldehyde (2.5% final concentration) and stored at 4°C in the dark pending further processing.

Samples for cell counts were analysed using epifluorescence microscopy (Zeiss AxioImager) and the nucleic acid stain Sytox Green (Invitrogen). Stained cells were filtered onto black polycarbonate filters (Millipore) and 30 fields were photographed using AxioVision (4.6.1.0). We determined cell abundance, length and width for at least 200 cells per sample using image analysis (ImageJ 1.410). Image analysis was automated based on a size threshold (0.1 μm^2) identified by manually measured sizes. Cells with an area smaller than this threshold were excluded, as well as overlapping particles. We calculated cell volumes as $V = (w^2 * \pi/4) * (l - w) + (\pi * w^3/6)$ where V (μm^3) is the cell volume, and w and l are cell width and length (in µm). Employing the allometric relationship[1] $C = 120 * V^{0.72}$ we computed individual cell carbon content (fg). Microbial biomass was then calculated as the product of cell numbers and average cell carbon content for each sample. Bioavailable DOC (BDOC) was calculated as the change in DOC concentration over the 20-d incubation period. BDOC values were significantly related ($r^2 = 0.80$, $p < 0.001$, $n = 20$) to BDOC values derived from the sum of respiration and biomass (Figure S6). Carbon use efficiency (CUE) was computed as the increase in microbial biomass versus the increase in microbial biomass plus respiration (as DIC production, see below) over the identical time period (i.e., until stationary phase; day 15).

2. Analysis of DOC and optical properties of DOM

DOC samples from streamwater and the degradation experiments were analysed within 10 days of sampling using a Sievers 5310C TOC Analyzer operated with an inorganic carbon removal unit. Prior to injection, DOC samples were automatically acidified in the analyser as recommended by the manufacturer. The method detection limit of the Sievers 5310C according to US EPA guidelines[2] was typically below 6 µg C l^{-1}.

Samples for optical analysis of DOM were measured within 14 days of sampling. Absorbance scans and excitation emission matrices (EEMs) were generated simultaneously using an Aqualog® (Horriba Scientific). Fluorescence intensities were measured at excitation wavelengths ranging from 250 to 450 nm (5-nm increments) and emission wavelengths from 250 to 550nm (2-nm increments). The water Raman peak of Milli-Q water served as reference. EEMs were corrected for blanks (MilliQ) and absorbance (inner filter effect). Using parallel factor analysis (PARAFAC)[3], we modeled individual fluorescent components from the obtained EEMs. Modeling was performed in Matlab (7.11.0) using the DOMFluor Toolbox (1.7; containing the N-Way toolbox, 3.1)[4]. PARAFAC identified three humic-like components but no protein-like component, which is plausible given the humic character of the water (Figure 2). Component 1 (Em 455 nm / Ex < 250 (335) nm) and 2 (Em 500 nm / Ex < 250 (400 nm) resembled the classical peaks C and A, respectively, as described by Coble[5]. Both peaks are of terrestrial origin and commonly found in freshwater environments[6]. In contrast, component 3 (Em 408

nm/ Ex < 250 (305) nm) is putatively associated with biological production *in situ* and therefore diagenetically younger[7].

To characterize DOM properties and composition we computed the following indices from DOM absorbance and fluorescence: The humification index (HIX) was calculated as the peak area of the emission wavelengths from 435 to 480 nm divided by the peak area of the emission wavelengths from 300 to 445 nm, at an excitation wavelength of 254 nm[8]. Higher values indicate higher humic substance content or extent of humification[8].The ß/α index was computed as the ratio of emission intensity at 380 nm (ß) to the maximum emission intensity between 420 and 435 nm (α) at an excitation wavelength of 310 nm[9]. High ß/α values indicate autochthonous inputs, i.e., recently microbially produced DOM[10]. The fluorescence index (FI) was calculated as the ratio of emission intensity at 450 nm to that at 500 nm for an excitation wavelength of 370 nm[11]; it is commonly used as an indicator of DOM source (i.e. terrigenous vs. microbially derived DOM)[11]. The slope ratio (S_R) was calculated as the ratio of $S_{275-295}$ to $S_{350-400}$; it is reported to correlate inversely with DOM molecular weight[12]. The specific UV absorption ($SUVA_{254}$) was calculated as the absorption coefficient at 254 nm (m^{-1}) relative to the DOC concentration (mg l^{-1})[13]. The $SUVA_{254}$ was reported to correlate positively with increasing DOM aromaticity[13]. Additionally we calculated the ratio of absorbance at 254 and 365nm (a254/a365), which was shown to be negatively correlated to molecular weight of DOM[14, 15].

3. Nutrients, ions and field parameters

Concentrations of $N\text{-}NH_4$, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$ in the streamwater were determined using Continuous Flow Analysis (Alliance instruments). Detection limits for nutrient analyses were 4 µg l^{-1} for $N\text{-}NH_4$, 100 µg l^{-1} for N-NO3 and 2 µg l^{-1} for $P\text{-}PO_4$. Ion concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3, and SO_4^{2-}) were measured using ion chromatography (761 Compact IC; Methrom). Water temperature and pH were measured using regularly calibrated WTW probes (Cond340i, pH320, Weilheim -Germany) and a calibrated high-precision glass electrode for pH (Metrohm), respectively. Alkalinity was determined by Titration with 0.1 mM HCl.

4. Streamwater ΔCO_2 and respiration

To determine the streamwater CO_2 concentration we carefully submersed triplicate 50-ml glass vials (pre-conditioned with precipitated NaN_3, 0.02 % final concentration) into the streamwater, and allowed them to overflow before closing them under water with a 1 cm thick gas-tight rubber stopper. While closing the vials a needle was inserted into the rubber stopper to avoid disturbance and allow excess water to emerge from the vial. Triplicate atmospheric samples (10 ml each) were obtained using a gas-tight syringe and injected into pre-evacuated 7-ml headspace vials, fitted with silicon sealed rubber stoppers and aluminum caps. Gas samples were stored at 4°C in the dark for a maximum

of 2 days. Prior to measurement a headspace of known volume was generated with N_2 in the liquid samples. Equilibration of the gaseous and the liquid phase was then achieved by means of a water bath at controlled temperature aided by shaking for 45 min. Samples were analysed using gas chromatography (Agilent Technologies 7890A) and a headspace autosampler. Temperature was kept constant during measurement.

During sample equilibration at lab conditions, CO_2 evades from the liquid phase into the N_2-headspace, causing the equilibria of the carbonate fractions (CO_2, HCO_3^- and CO_3^{2-}) to shift. Thus, the CO_2 concentration measured in the headspace cannot be directly related to the CO_2 concentration in the liquid phase in the stream and at the respective field conditions. We accounted for this by calculating the total DIC concentration of the sample assuming constant alkalinity and employing the equilibrium constants for the respective carbonate fractions[16, 17] and respective temperature and pressure conditions. Briefly, we computed the amount of moles of CO_2 in the liquid phase from measured CO_2 partial pressure in the headspace and Henry´s law assuming full equilibration. This computed sample CO_2 concentration (mol l^{-1}), alkalinity and the respective equilibrium constants for the carbonate fractions (adjusted for temperature and ionic strength) were used to infer pH. Then the concentration of HCO_3^- and CO_3^{2-} could be calculated using pH and the adequate equilibration constants (adjusted for temperature and ionic strength). The sum of all carbonate fractions (in moles) in the liquid phase and the total amount of CO_2 (in moles) in the headspace gave the total amount of DIC (in moles).

We then calculated the CO_2 concentration (mol l^{-1}) in the streamwater from computed DIC values and measured pH values using the equilibrium constants (adjusted for temperature and ionic strength). A potential streamwater CO_2 concentration in mol l^{-1} at equilibrated conditions was calculated from the measured atmospheric CO_2 concentrations using Henry´s law. The difference of the CO_2 concentration in the stream in mol l^{-1} to the concentration expected from the atmospheric equilibrium gives the degree of CO_2 oversaturation (ΔCO_2).

During the degradation experiments CO_2 production causes the equilibrium of the carbonate fractions (CO_2, HCO_3^- and CO_3^{2-}) to shift. Thus, we used the same calculation scheme as for the liquid samples to recalculate total DIC concentrations. From measured pCO_2 concentrations we derived the amount of moles of CO_2 in the liquid phase using Henry´s law. Employing the equilibrium constants for the respective carbonate fractions and alkalinity (constant and measured at the start of the degradation experiments) we recalculated a pH value for each time point [Dickson et al., 2007; Berggren *et al.*, 2012]. Taking into account the pH values and the equilibrium constants for the respective carbonate fractions we computed all carbonate fractions. The sum of headspace CO_2 concentration (in moles) and the carbonate fractions (in moles) gave the total DIC concentration for each incubation time point.

Table S1 Geographical location, dissolved organic carbon (DOC) concentration, bioavailability (BDOC), color (a_{440}) and carbon use efficiency (CUE) of all 20 streams. Longitude is given in decimal degrees East and latitude in decimal degrees North.

Stream	Longitude (°E)	Latitude (°N)	DOC (mgl^{-1})	a440 (m^{-1})	BDOC (%)	CUE (%)
A	15.0075	48.3736	9.21 ± 0.04	7.04 ± 0.4	3.62	5.77
B	15.0028	48.3586	5.25 ± 0.04	4.02 ± 0.02	4.11	6.01
C	14.9853	48.3111	1.41 ± 0.02	0.78 ± 0.06	8.18	9.55
D	14.9675	48.3547	7.63 ± 0.02	6.08 ± 0.1	5.04	8.97
E	14.9633	48.3694	2.66 ± 0.01	1.90 ± 0.02	9.30	5.31
F	14.9656	48.3786	5.31 ± 0.06	4.32 ± 0.1	8.18	9.04
G	15.1053	48.3842	5.16 ± 0.05	3.49 ± 0.17	4.67	7.81
H	15.0808	48.3792	19.49 ± 0.23	17.52 ± 0.03	4.25	4.57
I	15.0450	48.4086	6.85 ± 0.04	3.59 ± 0.06	1.63	15.24
J	15.0314	48.4028	10.04 ± 0.05	7.36 ± 0.1	4.04	10.85
K	15.0439	48.3975	3.28 ± 0.03	1.96 ± 0.08	4.07	6.36
L	15.0436	48.3806	10.60 ± 0.02	8.00 ± 0.05	3.72	11.39
M	14.8722	48.5047	6.07 ± 0.04	4.15 ± 0.07	3.33	20.63
N	14.8619	48.5047	25.31 ± 0.07	26.64 ± 0.19	5.09	5.07
O	14.8533	48.5222	7.48 ± 0.12	5.09 ± 0.17	4.26	8.00
P	14.8367	48.5594	4.33 ± 0.12	2.09 ± 0.02	7.06	9.91
Q	14.7619	48.5961	2.48 ± 0.01	1.26 ± 0.06	4.38	37.10
R	14.7306	48.5969	4.50 ± 0.01	2.33 ± 0.01	2.33	11.58
S	14.7069	48.5564	7.02 ± 0.02	3.73 ± 0.14	1.87	27.86
T	14.6861	48.5511	5.42 ± 0.05	3.25 ± 0.16	1.92	33.65

Table S2 PH, electrical conductivity and nutrients (NH_4, NO_2, NO_3, PO_4) of all 20 streams.

			Nutrients at the start of the biodegradation assays				Nutrients at the end of the biodegradation assays			
Stream	pH	Conductivity ($\mu S\ cm^{-1}$)	NH_4 ($\mu g\ l^{-1}$)	NO_2 ($\mu g\ l^{-1}$)	NO_3 ($\mu g\ l^{-1}$)	PO_4 ($\mu g\ l^{-1}$)	NH_4 ($\mu g\ l^{-1}$)	NO_2 ($\mu g\ l^{-1}$)	NO_3 ($\mu g\ l^{-1}$)	PO_4 ($\mu g\ l^{-1}$)
A	6.69	37.60	5.33 ± 2.03	3.37 ± 0.35	301.77 ± 3.96	21.37 ± 0.71	6.33 ± 3.73	3.20 ± 0.10	302.53 ± 8.37	20.43 ± 1.15
B	6.92	49.20	< 4	2.60 ± 0.44	787.70 ± 3.04	16.30 ± 0.35	5.60 ± 4.55	2.33 ± 0.40	801.37 ± 20.05	15.63 ± 0.06
C	7.30	81.50	< 4	1.00 ± 0.36	1414.93 ± 9.00	13.17 ± 0.68	7.40 ± 2.19	1.13 ± 0.15	1427.27 ± 4.91	14.53 ± 0.64
D	6.81	44.70	4.43 ± 1.82	4.03 ± 0.23	451.40 ± 2.74	17.90 ± 0.35	5.63 ± 1.58	2.83 ± 0.06	457.40 ± 6.68	17.37 ± 0.32
E	7.00	71.40	4.35 ± 1.34	1.37 ± 1.12	662.23 ± 10.84	4.10 ± 0.00	13.03 ± 3.42	1.30 ± 0.61	665.33 ± 8.20	5.30 ± 0.36
F	7.02	56.10	6.30 ± 4.24	6.23 ± 0.75	534.17 ± 17.47	5.75 ± 0.78	15.70 ± 3.45	2.60 ± 0.26	534.13 ± 7.97	6.13 ± 0.12
G	6.83	59.60	< 4	2.37 ± 0.25	515.77 ± 5.80	21.60 ± 3.00	< 4	2.20 ± 0.53	503.80 ± 6.90	18.07 ± 0.78
H	6.36	43.00	< 4	1.47 ± 0.67	519.10 ± 0.85	10.80 ± 0.95	14.73 ± 1.46	9.27 ± 0.15	< 100	54.87 ± 0.85
I	6.84	51.30	< 4	< 1	714.40 ± 4.83	9.47 ± 0.40	< 4	< 1	155.23 ± 4.75	9.90 ± 0.95
J	7.14	72.40	12.27 ± 0.25	8.93 ± 0.23	< 100	59.37 ± 0.59	4.57 ± 1.27	3.57 ± 0.21	690.60 ± 5.80	14.20 ± 0.53
K	6.77	67.50	7.30 ± 1.65	5.27 ± 0.61	700.10 ± 3.58	14.73 ± 0.40	4.27 ± 3.57	1.23 ± 0.12	722.93 ± 8.46	11.23 ± 1.55
L	6.90	59.40	6.07 ± 0.78	5.60 ± 0.66	596.13 ± 3.44	21.70 ± 0.26	9.70 ± 3.08	3.87 ± 0.31	596.33 ± 4.01	19.20 ± 0.10
M	6.88	53.00	< 4	3.57 ± 0.31	571.93 ± 3.18	28.63 ± 2.17	7.77 ± 1.19	3.07 ± 0.12	567.40 ± 3.99	39.80 ± 1.30
N	6.53	55.40	251.40 ± 1.14	16.80 ± 0.44	267.43 ± 0.91	102.23 ± 2.72	19.53 ± 1.80	n.d.	n.d.	77.17 ± 5.15
O	7.14	76.30	7.80 ± 0.96	2.20 ± 0.50	373.00 ± 1.21	48.05 ± 0.35	7.47 ± 3.93	3.20 ± 0.50	368.70 ± 4.88	32.77 ± 1.88
P	7.16	122.50	4.73 ± 0.25	1.13 ± 0.21	649.70 ± 1.71	13.60 ± 0.71	< 4	1.80 ± 0.30	626.77 ± 3.09	14.17 ± 1.06
Q	7.40	110.70	< 4	1.33 ± 0.51	592.50 ± 5.41	11.67 ± 0.23	4.97 ± 1.70	< 1	591.70 ± 8.41	12.67 ± 0.93
R	6.75	62.20	4.57 ± 1.15	1.07 ± 0.45	146.17 ± 2.51	6.65 ± 0.64	8.40 ± 1.14	1.10 ± 0.53	155.83 ± 4.72	8.33 ± 0.59
S	6.93	64.40	4.23 ± 1.39	1.80 ± 0.20	232.60 ± 2.95	9.50 ± 0.46	5.73 ± 2.34	1.97 ± 0.46	253.30 ± 2.27	11.70 ± 0.98
T	6.81	131.60	< 4	2.37 ± 0.15	283.07 ± 2.37	9.50 ± 1.08	4.87 ± 2.27	1.97 ± 0.12	295.87 ± 3.36	10.73 ± 1.30

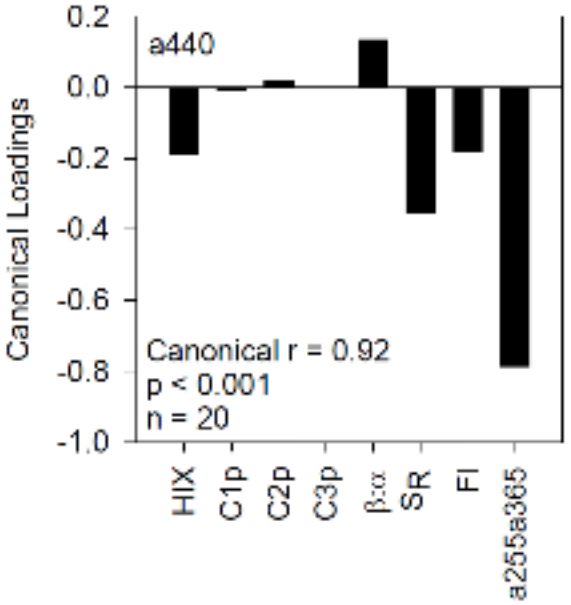

Figure S1 Canonical correlation analysis based on the optical information of the 20 streams reveals the DOM optical properties which are most strongly related to absorbance at 440 nm (a_{440}). This analysis is identical to the one presented in Figure 3 but excluding specific absorbance at 440 nm (SA_{440}) and specific UV absorbance at 254 nm ($SUVA_{254}$) in order to test for any spurious effect of DOM quantity. Canonical loadings (correlations between the one canonical variate of our analysis and the respective variables) indicate the strength and direction of the relationship between the constraint (a_{440}) and individual optical measures: component 1 (C1), component 2 (C2), component 3 (C3), humification index (HIX), fluorescence index (FI), freshness index (β/α), slope ratio (S_R), the ratio of absorbance at 254 to 365nm ($a254/a365$). Despite the exclusion of the concentration-dependent parameters $SUVA_{254}$ and SA_{440} canonical loadings indicate an increase of the terrigenous signal with a_{440}.

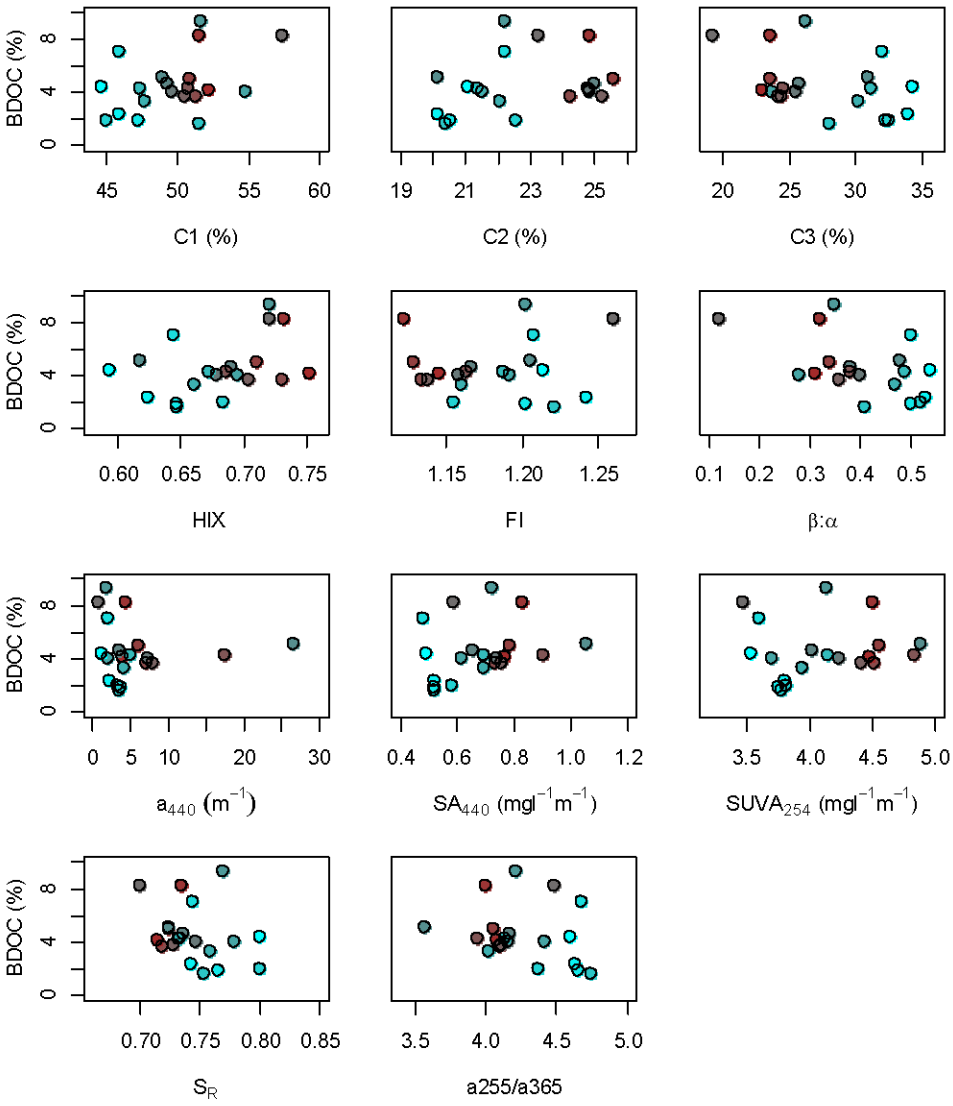

Figure S2 Relationships of bioavailable DOC (%BDOC) with various optical measures: component 1 (C1), component 2 (C2), component 3 (C3), humification index (HIX), fluorescence index (FI), freshness index (ß/α), absorbance at 440 nm (*a*440), specific (i.e., normalized for DOC) absorbance at 440 nm (SA_{440}), specific UV absorbance at 254 nm ($SUVA_{254}$), slope ratio (S_R), and the ratio of absorbance at 254 to 365 nm (*a*254/*a*365). Original DOM quality as derived from the PCA is indicated by colour. Brown colors denote streams with predominantly terrigenous DOM and blue colors represent streams with relatively more autochthonous DOM.

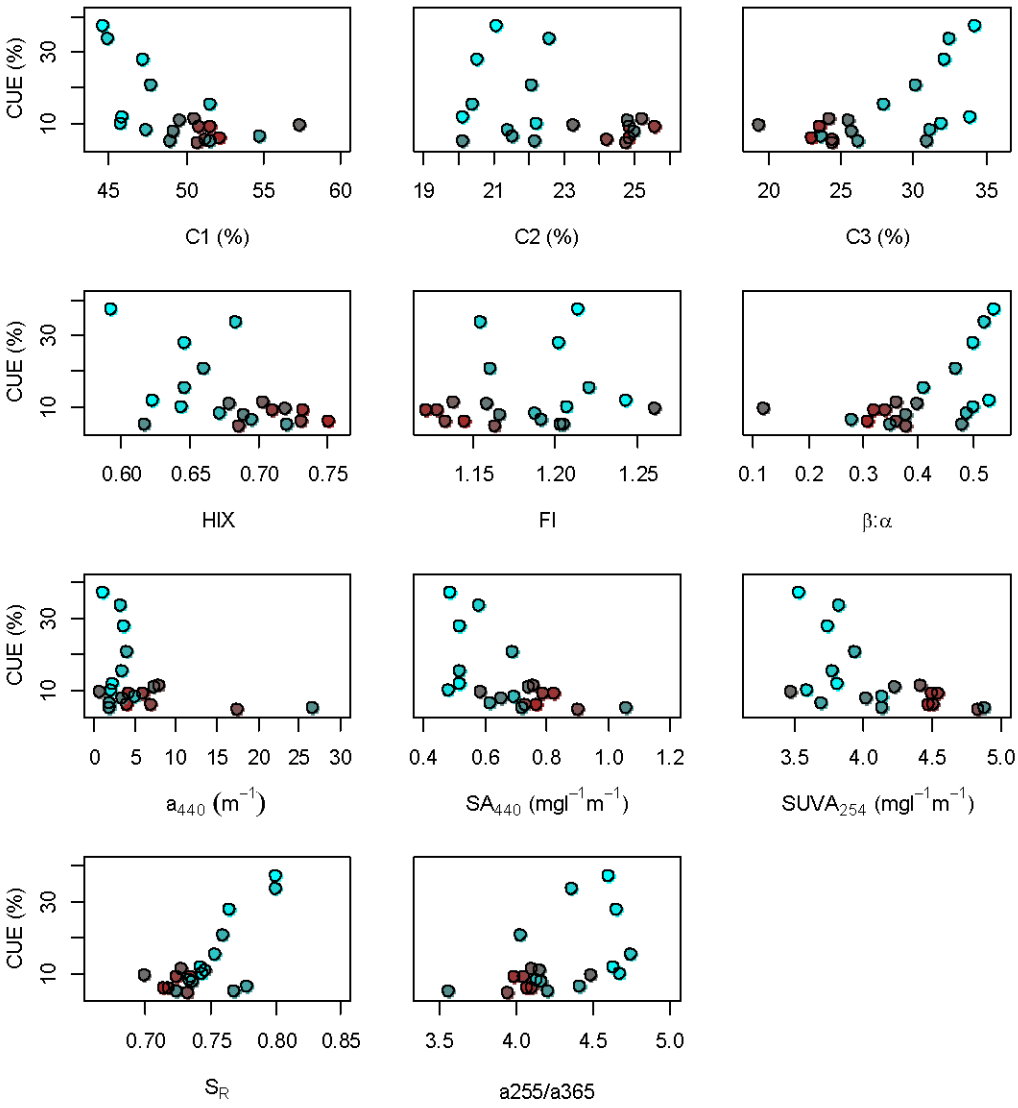

Figure S3 Relationships of carbon use efficiency (CUE) and various optical measures: component 1 (C1), component 2 (C2), component 3 (C3), humification index (HIX), fluorescence index (FI), freshness index (ß/α), absorbance at 440 nm (*a*440), specific (i.e., normalized for DOC) absorbance at 440 nm (SA_{440}), specific UV absorbance at 254 nm ($SUVA_{254}$), slope ratio (S_R), and the ratio of absorbance at 254 to 365 nm (*a*254/*a*365). Original DOM quality as derived from the PCA is indicated by colour Brown colors denote streams with predominantly terrigenous DOM and blue colors represent streams with relatively more autochthonous DOM.

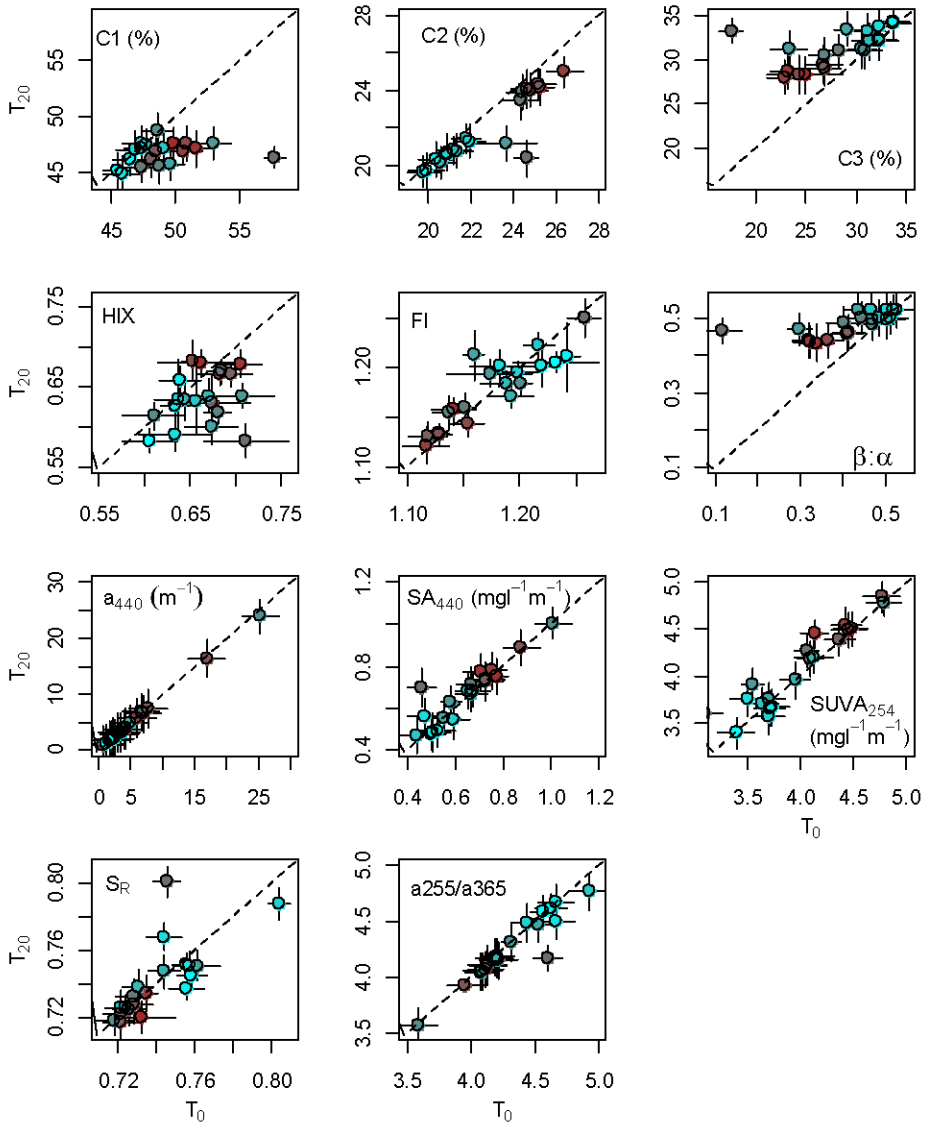

Figure S4 Optical measures at the start of the degradation experiments (T0) and after the 20-day incubation period (T20): component 1 (C1), component 2 (C2), component 3 (C3), humification index (HIX), fluorescence index (FI), freshness index (ß/α), absorbance at 440 nm (*a*440), specific (i.e., normalized for DOC) absorbance at 440 nm (SA_{440}), specific UV absorbance at 254 nm ($SUVA_{254}$), slope ratio (S_R), and the ratio of absorbance at 254 to 365 nm (*a*254/*a*365). Given is the mean ± SD (n=3 replicates) and the dashed line indicates a 1:1 relationship (i.e., no change during degradation) Original DOM quality as derived from the PCA is indicated by colour. Brown colors denote streams with predominantly terrigenous DOM and blue colors represent streams with relatively more autochthonous DOM.

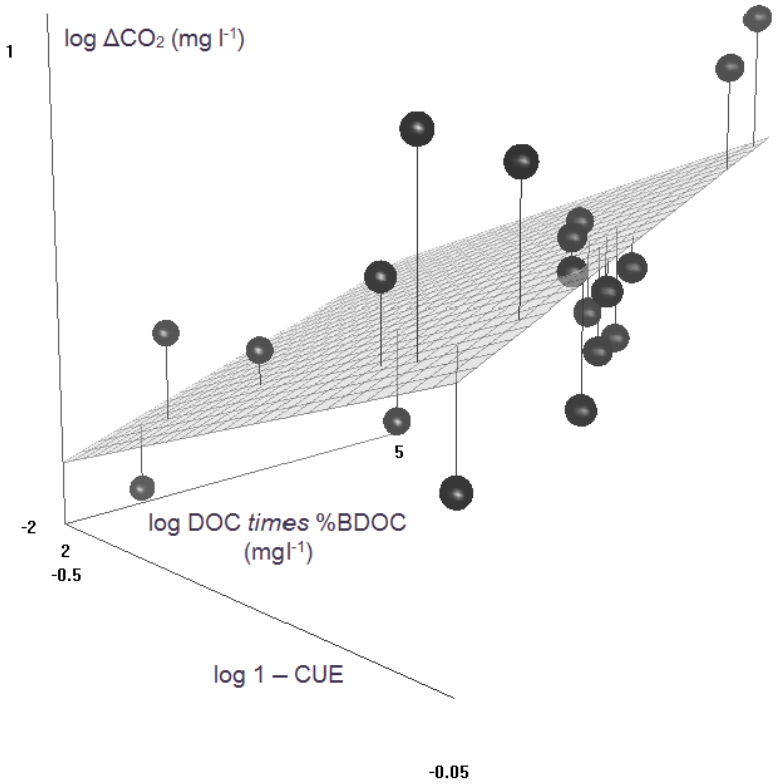

Figure S5 Bioavailable dissolved organic carbon and carbon use efficiency predict streamwater CO_2 partial pressure. Symbols indicate measured values with lines (residuals) connected to the modeled surface.

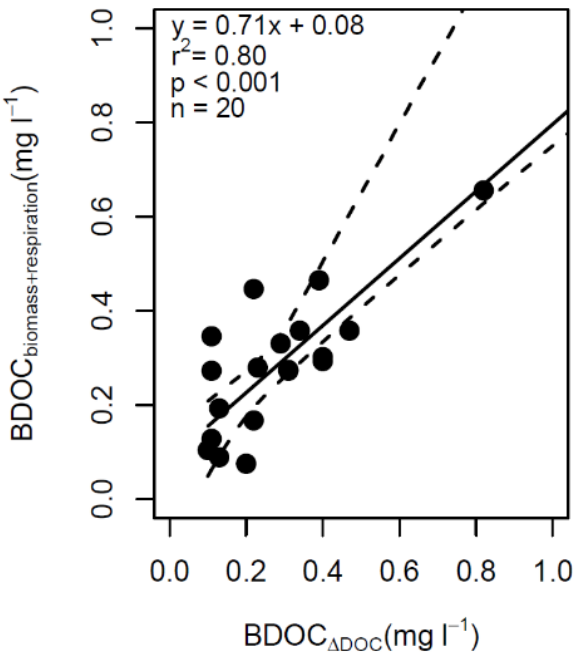

Figure S6 Ranged major axis regression of the BDOC values measured as the decrease in DOC concentration over the incubation period ($BDOC_{\Delta DOC}$) and BDOC values derived from the sum of respiration and biomass. Significance was computed by permutation and dashed lines give the 95% model confidence level.

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IV. Hydrological and seasonal controls on dissolved organic matter (DOM) quality and fluxes in an Alpine headwater stream

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My contribution to this publication

a. Mathematically analysed DOM absorbance and fluorescence using parallel factor analysis (PARAFAC)
b. Constructed the database for DOM optical properties, hydrological and environmental data
c. Conducted the statistical analyses
d. Writing, editing and approval of manuscript

Hydrology controls dissolved organic matter export and composition in an Alpine stream and its hyporheic zone

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Running head: Hydrology controls stream and hyporheic DOM

Abstract

Streams and rivers transport dissolved organic matter from the terrestrial environment to downstream ecosystems. In light of climatically and anthropogenically induced changes it is crucial to understand the quantity, composition and dynamics of dissolved organic matter (DOM) and its drivers for elucidating the nature and composition of DOM exports to downstream ecosystems. We monitored DOM concentration and composition based on a diurnal sampling design for over three years in an Alpine stream. We found hydrologic variability to control DOM composition and the coupling of DOM dynamics in the streamwater and the hyporheic zone, which were modulated by seasonally regulated processes. High-flow events increased DOM inputs from terrestrial sources (as indicated by the contributions of humic- and fulvic-like fluorescence), while summer baseflow enhanced the autochthonous imprint of DOM. Diurnal and seasonal patterns of DOM composition were likely induced by biological processes linked to temperature and photosynthetic active radiation (PAR). Floods frequently interrupted diurnal and seasonal patterns of DOM, which led to a decoupling of streamwater and hyporheic water DOM composition and delivery of aromatic and humic-like DOM to the streamwater. Accordingly, DOM export fluxes (4.03±0.02 g C m^{-2} yr^{-1}) were largely of terrigenous origin as indicated by fluorescent components modeled by parallel factor analysis and aromaticity. Our study highlights the relevance of hydrologic and seasonal dynamics for the origin, composition and fluxes of DOM in an Alpine headwater stream.

Introduction

Streams and rivers are major contributors to global carbon fluxes (Cole et al. 2007; Battin et al. 2009). Headwaters, the smallest and most abundant streams in fluvial networks, are tightly connected with the terrestrial milieu from which they receive dissolved organic matter (DOM) that they transform or export to downstream ecosystems and ultimately to the oceans (Battin et al. 2008). Headwater streams therefore link the terrestrial and aquatic carbon cycle as conveyed by the "boundless carbon cycle" (Battin et al. 2009).

Building evidence shows that hydrology drives this biogeochemical link to a large extent. Export budgets of DOM from catchments, DOM composition and even its bioavailability to the heterotrophic metabolism in streams all are at least to some extent controlled by precipitation and discharge. For instance, near-stream soils are often rich in organic carbon, which can act as a source of DOM to streams during elevated precipitation and snowmelt (Boyer et al. 1997, Sawyer et al. 2014). During storms, the delivery of DOM from these soils to the streams can be substantial and even lead to the transient depletion of soil organic matter reservoirs (Boyer et al. 1997; Butturini et al. 2006). Storm-induced terrestrial deliveries of DOM to the streams may be transiently facilitated by groundwater flowpaths (Sawyer et al. 2014). The combined effects of changed hydrological connectivity and hydrological flow paths, and the depletion of organic matter reservoirs at the catchment scale can lead to significant DOM export fluxes from catchments during storm events (Raymond and Saiers 2010, Yoon and Raymond 2012). Using high-resolution monitoring, Yoon and Raymond (2012) showed that almost 40% of the annual export flux of dissolved organic carbon (DOC) from Esopus Creek occurred during Hurricane Irene within only five days. As sources of DOM change during storms, its chemical composition and bioavailability to the microbial heterotrophs in streams can change as well (e.g., Strohmeier et al. 2013, McLaughlin and Kaplan 2013, Singh et al. 2014). Reduced residence times and elevated water depth along with low bioavailability of the terrestrial DOM constituents during storms may provide downstream subsidies rather than stimulating *in situ* metabolism (Battin et al. 2008). This

pattern may reverse during extended baseflow when hydrological conditions favor in-stream DOM sources, such as leaf litter fall (Meyer et al. 1998) and algal blooms, and their metabolism. However, relatively little is known about the DOM dynamics between storm events (Vázquez et al. 2012, Wyatt et al. 2014).

The hyporheic zone at the interface between groundwater and streamwater is critical for stream ecosystem processes, which seem particularly important in streams with steep slope and alluvial geology that facilitate hydrodynamic exchange and hyporheic flow (Boulton et al. 1998, Boano et al. 2014). While the involvement of the hyporheic zone for stream metabolism and nutrient cycling is well recognized (e.g., Schindler and Krabbenhoft 1998, Sobczak and Findlay 2002), the contributions of hyporheic processes to DOM sources and dynamics remain poorly understood at present (Wong and Williams 2010). To fully appreciate the contributions of streams to carbon cycling we need a better understanding of the various ecosystem components (e.g., hyporheic zone) to DOM sources and fluxes, and how these may respond to climate change and related shifts in hydrology and temperature, for instance.

The aim of the present study was to unravel controls on DOM dynamics and composition in the streamwater and hyporheic zone of an Alpine headwater stream. Alpine ecosystems are particularly susceptible to climate change with predicted changes in precipitation and hydrological regime (Barnett et al. 2005, Berghuijs et al. 2014). We monitored diurnal patterns of DOM concentration, absorbance and fluorescence in the streamwater and the hyporheic zone, and computed export fluxes of bulk DOM and its major fluorescent components for over three years. We hypothesized varying controls on DOM dynamics across three temporal scales, namely seasonality, day-night changes and both storms and extended baseflow as discrete hydrological events. We expect storms to increase export fluxes of terrestrial DOM and periods of extended baseflow to contribute autochthonous constituents to the DOM fluxes. Moreover we expect sources and composition of DOM to differ in streamwater and in the hyporheic zone, and that discharge modulates this divergence.

Methods

Study site – Oberer Seebach (OSB) is a second-order stream draining a largely pristine catchment in the eastern Alps (Lunz am See, Austria) of approximately 25 km^2. *Fraxinus excelsior, Acer pseudoplatanus, Fagus sylvatica, Salix caprea and Picea abies* dominate the catchment vegetation. Long-term annual air temperature averaged 6.7°C and annual precipitation averaged 1,608 mm. The catchment is characterized by glacial alluvial deposits, underlain by a layer of ancient lake sediment and calcareous rock (Battin, 1999). OSB streamwater pH and electrical conductivity average 8.3 ± 0.2 and 231.6 ± 17.0 μS cm^{-1}, respectively. Nutrient concentrations ($P\text{-}PO_4$: <3 μg L^{-1}; $N\text{-}NH_4$: 4.4 μg L^{-1}; $N\text{-}NO_3$: 0.57 mg L^{-1}) and turbidity (5.4 ± 36.2 NTU) are generally low and the streamwater is typically well oxygenated. Additional key properties of the OSB are summarized in Table 1. The study reach is characterized by gravel (median grain size 23.1 mm), high porosity (29%) and a slope of 0.41 %, facilitating high hydrological exchange between streamwater and the hyporheic zone (Battin et al. 1999). Snow cover can extend from December until April and the OSB typically has a snowmelt dominated runoff regime with a pronounced discharge peak in the spring.

Hydrology– Streamwater gage and groundwater levels adjacent to OSB were recorded in 30-min intervals using pressure transducers (GPRS data logger type 255, HT-Technik, Germany). We derived stream discharge (L s^{-1}) from a rating curve based on salt additions. Specific groundwater discharge

into and out of OSB (L m^{-2} s^{-1}) was estimated from hydraulic gradients (m m^{-1}) and sediment permeability (m s^{-1}) (Battin 1999). Overall discharge of the OSB during our study period averaged 1,152 L s^{-1} (5.7 mm d^{-1}) with peak values of 5,575 ± 3,352 L s^{-1}. Based on a flow-duration curve of average daily discharge, we defined discharge lower than 75% of the time (< 306 L s^{-1}) as baseflow. Similarly, high flows were defined as the discharge of the highest 10% of the days (discharge > 2939 L s^{-1}). Discharge between these 10% and 75% duration limits (306 to 2,939 L s^{-1}) were considered as intermediate. These thresholds agree approximately with previous estimates of median baseflow (289 L s^{-1}) and threshold values of bed scouring discharge (3,400 L s^{-1}) for OSB (Peter et al. 2014).

Sampling and sample preparation– During the study period (May 2010 to August 2013) samples from streamwater (n =3695) and hyporheic water at 1.5 m below sediment surface (n =1761) were collected (ISCO Autosampler) into clean bottles (soaked in 0.1 N HCl, rinsed with MQ water) at 01:30 h, 07:30 h, 13:30 h and 19:30 h each day for DOC and DOM absorbance and fluorescence measurements. Samples were filtered (Whatman GF/F filters) and stored in borosilicate vials (soaked in 0.1 N HCl, rinsed with MQ water and combusted at 450°C for 4 h) at 4°C in the dark until analysis. Streamwater temperature (°C), electrical conductivity (µS cm^{-1}), pH and dissolved oxygen (mg L^{-1}) were measured using a multiparameter sonde (YSI 600R, Ohio, USA). We also recorded photosynthetic active radiation (PAR) (W m^{-2}), air temperature (°C) and precipitation (mm) on site.

DOC concentration and DOM composition – DOC samples were analyzed using a TOC analyzer (GE-Sievers 900) operated with an inorganic carbon removal unit. DOC samples were automatically acidified in the analyzer, prior to injection. DOM absorbance scans were performed in a 5-cm quartz cuvette using a UV–VIS spectrophotometer (ShimadzuUV 17000); Milli-Q water served as the blank. Excitation emission matrices (EEMs) were generated using a fluorescence spectrophotometer (Hitachi F-7000) and a 1-cm quartz cuvette. Fluorescence intensities were measured at excitation (Ex) wavelengths ranging from 240 to 450 nm (5-nm increments) and emission (Em) wavelengths from 250 to 550nm (2-nm increments). EEMs were corrected for blanks (MilliQ) and the inner filter effect using corresponding absorbance measurements. To express fluorescence intensities in Raman units, the Raman peak of Milli-Q water was used as the reference value. We modeled individual fluorescent components from the EEMs employing parallel factor analysis (PARAFAC, Stedmon and Bro, 2008). Modeling was performed in Matlab (7.11.0) using the DOMFluor Toolbox (1.7; containing the N-Way toolbox, 3.1) (Andersson and Bro, 2000). PARAFAC analysis revealed 5 fluorescent components: Components 1 to 3 are commonly found in freshwater environments and resemble humic and fulvic-like fluorescence (Stedmon and Markager 2005, Cory 2012). While the humic-like component 1 (Ex 240 (305) nm/ Em 412 nm) was previously related to forest streams and wetlands, and sources of terrigenous origin (Stedmon and Markager 2005), components 2 (Ex 240 (405) nm/ Em 486 nm) and 3 (Ex 240 (365) nm/ Em 458 nm) resembled those of the fulvic acid fluorophore group and may be of terrestrial and/or autochthonous origin (Stedmon and Markager 2005). Components 4 and 5 resemble protein-like fluorescence, with C4 (Ex 240 (280) nm/ Em 338 nm) being similar to Tryptophan-like fluorescence and C5 (Ex 270 nm/ Em 300 nm) to Tyrosine-like fluorescence (Coble 1996). The relative fluorescence of each component was then calculated as the percentage of the total fluorescence (sum of streamwater and hyporheic water DOM fluorescence).

DOM was further characterized by the naperian absorption coefficient, $a254$ (m^{-1}) calculated as $a_\lambda = 2.303A_\lambda/l$, where A_λ is the absorbance and l is the cell path length (m) (Blough and Del Vecchio, 2002). The specific UV absorption $SUVA_{254}$, indicative of aromaticity, was calculated as the absorbance (A) at 254 nm (m^{-1}) divided by DOC concentration (mg L^{-1}) (Weishaar et al. 2003). The

slope ratio (S_R) was computed as the ratio of $S_{275-295}$ to $S_{350-400}$ and is reported to correlate inversely with DOM molecular weight (Helms et al. 2008). The humification index (HIX) was calculated by dividing the peak area of the Em wavelengths from 435 to 480 nm by the peak area of the Em wavelengths from 300 to 445 nm, at an Ex wavelength of 254 nm (Zsolnay et al. 1999). The freshness index (ß/α) is the ratio of Em intensity at 380 nm (ß) to the maximum Em intensity between 420 and 435 nm (α) at 310 nm Ex wavelength; high ß/α values are commonly associated with autochthonous DOM from recent microbial production (Wilson and Xenopoulos 2008). The fluorescence index (FI) was determined according to McKnight *et al.* (2001) and serves as indicator of DOM sources (i.e., allochthonous *versus* authochthonous).

Flux calculations – We estimated average annual discharge and DOC exports from OSB similar to the approach by Raymond and Saiers (2010). First, we binned DOC concentrations by averaged daily discharge. The highest discharge bin contained DOC concentrations equated to discharge higher than 58 mm d^{-1} (= the lower bound of the highest bin). The bounds of the remaining bins were calculated employing an exponential function. We estimated the annual discharge associated with each bin by multiplying the binned discharge by the fraction of days at which the corresponding discharge was measured. Bin averaged DOC concentrations (mg l^{-1}) were then described as a power law function of discharge (Q, mm d^{-1}), which allows calculating DOC concentrations for all discharges, including discharges for which DOC measurements were unavailable (Raymond and Saiers 2010). The same procedure was repeated for the rising and falling hydrograph:

rising hydrograph: $DOC = 1.19 + 0.29\ Q^{0.403}$ (r^2=0.98, p < 0.001), (1)

and the falling hydrograph: $DOC = 1.26 + 0.24\ Q^{0.396}$ (r^2=0.94, p < 0.001). (2)

Employing these power law functions, we calculated the DOC export for the rising (Equation 1) and falling (Equation 2) hydrograph. We used bin-averaged values for discharge and DOC concentration to calculate DOC export at baseflow.

We also calculated export fluxes of DOC and fluorescent components (using absolute values; Raman Units (R.U.)) for the whole study period and for each month individually using LOADest (Runkel et al. 2004). LOADest requires a broad range of flow conditions and estimates loads by applying the method of adjusted maximum likelihood estimation (AMLE). To eliminate co-linearity LOADest centers discharge and constituent quantity. A predefined regression model based on available measurements of constituent quantity and corresponding measurements of discharge is then selected to fit the data based on *Akaike Information Criteria* (AIC). Loads were computed for days in which DOC measurements were unavailable by applying the regression model. Individual models were validated using the correlation coefficients (r^2) of the AMLE, residuals data, and the serial correlation of residuals according to Dornblaser and Striegl (2009).

Data analysis – A principal component analysis (PCA) based on DOC concentration, absorbance and fluorescence measurements was used to explore the overall variability of DOM composition. The PCA allowed differentiating between allochthonous and authochthonous DOM in the streamwater and the hyporheic zone, respectively. To analyze the coupling of DOM composition in the streamwater and the hyporheic zone we computed a canonical correlation analysis based on a set of environmental variables (e.g., discharge, streamwater temperature) and DOM optical measures (e.g., HIX, $SUVA_{254}$, fluorescent components) in the streamwater and the hyporheic zone. To explore the coupling between

streamwater and hyporheic DOM biogeochemistry, we use the difference between values of streamwater DOM optics and hyporheic water DOM optics. Positive values indicate higher DOM concentration, absorbance and fluorescence in the streamwater as compared to the hyporheic zone. The strength of the relationship between the canonical axis and the constraints was computed as the canonical correlation coefficients (canonical r); the significance of each canonical correlation was computed as Wilks' Lambda, using F-approximation (Rao's F). We further explored the effect of discharge and streamwater temperature on streamwater DOM concentration and composition using general additive models (GAM) with a residual temporal correlation structure (auto-regressive model of order 1). GAMs were fitted with R version 2.15.1 (R Development Core Team, 2012) using the 'mgcv' package (Wood et al. 2015).

Results

Hydrological variability and DOC concentrations – The flow regime of OSB was characterized by distinct snowmelt events with a mean peak discharge of 28 mm day^{-1} in spring. In contrast, summer extended baseflow (0.45 to 1.47 mm day^{-1}) was regularly interrupted by storm events (up to 60 mm day^{-1}) (Figure 1A). The study reach received shallow groundwater from the left hill slope which markedly contributed to streamwater electrical conductivity dynamics (Figure 1 B). Streamwater electrical conductivity therefore reflected discharge dynamics with elevated values (231 ± 18 µS cm^{-1}) during summer and lower values during snowmelt (214 ± 14 µS cm^{-1}), for instance.

Hydrological variability caused significant fluctuations in stream DOC concentrations. Overall, DOC concentration in OSB streamwater was low with an annual average of 1.71 ± 0.35 mg L^{-1} and with maximum concentrations of 2.18 ± 0.52 mg L^{-1} during storms (Table 1). DOC concentration in the hyporheic zone ranged from 0.82 to 3.12 mg L^{-1} and its average (1.5 ± 0.3 mg L^{-1}) was significantly lower (t-test, $p < 0.001$, $n = 1746$) than in the streamwater; hyporheic DOC concentration was related to streamwater DOC concentration and discharge (multiple linear regression, adjusted $r^2 = 0.68$, $n = 5238$, $p < 0.001$).

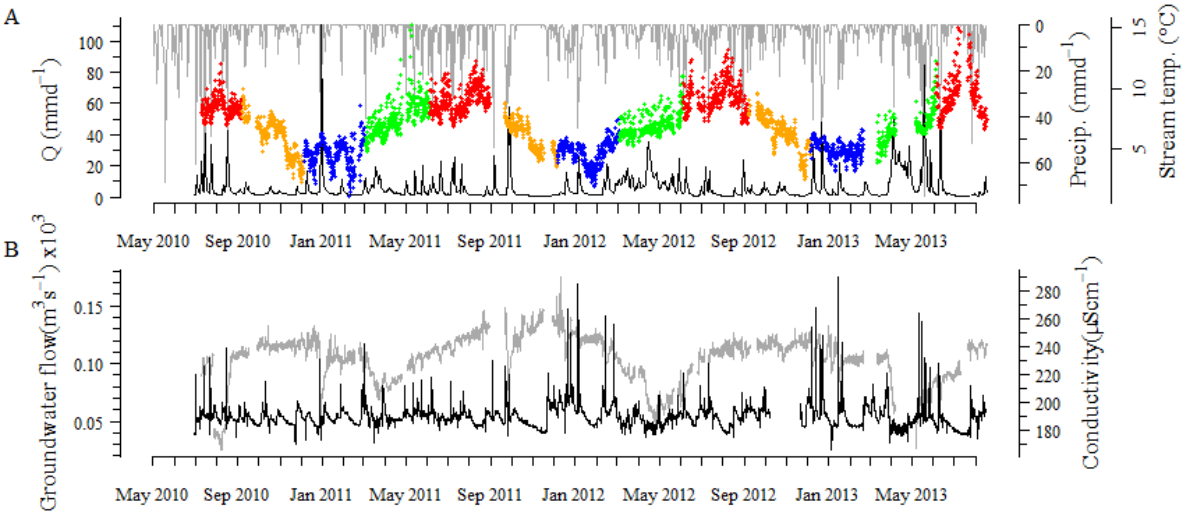

Figure 1 *(A) Daily discharge, daily precipitation and stream water temperature (color indicates seasons: spring (green), summer (red), autumn (orange), winter (blue)), (B) groundwater flow and electric conductivity during the study period in the Oberer Seebach, Austria.*

Streamwater and hyporheic DOM composition – Streamwater and hyporheic DOM was predominantly of terrestrial origin throughout the year as indicated by strong contributions from the humic-like (C1, 49 ± 2%) and the fulvic-like components C2 and C3, (C2: 18 ± 2%; C3: 18 ± 1%). Temporal variability of these components was similar in the streamwater and hyporheic zone (Figure 2). However, humic and fulvic-like fluorescence was generally higher in the streamwater than in the hyporheic zone, while protein-like (C4 and C5) fluorescence was similar in both, streamwater and hyporheic water.

Streamwater $SUVA_{254}$ and FI averaged 1.70 ± 0.25 mg^{-1} m^{-1} and 1.37 ± 0.05, respectively. $SUVA_{254}$ displayed large variability (0.47 - 1.20 mg^{-1} m^{-1}) with highest values during elevated discharge (Figure 2). Hyporheic $SUVA_{254}$ and FI were similar to streamwater values with averages of 1.87 ± 0.19 mg^{-1} m^{-1} and 1.36 ± 0.04, respectively. S_R values in the streamwater (0.47 to 1.20 mg^{-1} m^{-1}) and hyporheic water (0.65 to 3.37 mg^{-1} m^{-1}) varied broadly during the study period.

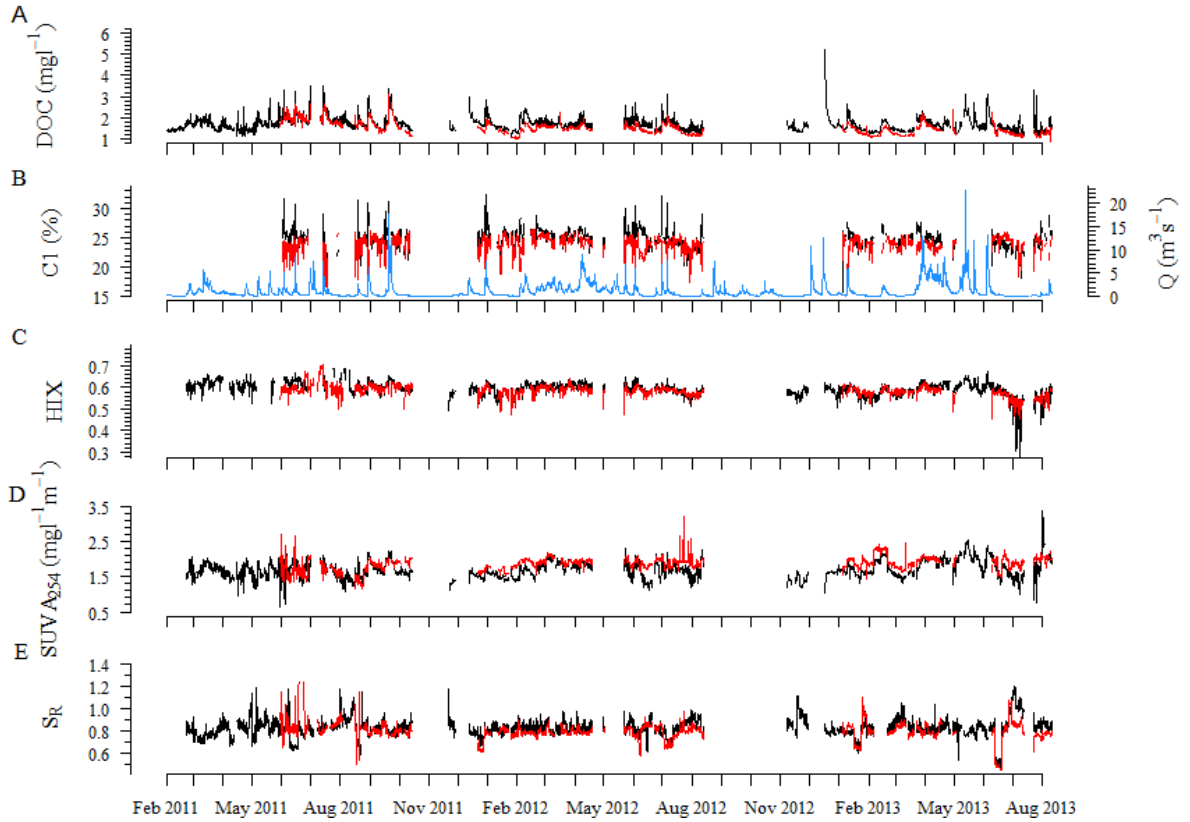

Figure 2 *(A) DOC concentration (mg l-1), (B) stream discharge (Q, in m3 s-1)) and the fluorescent component C1 (%) modeled by parallel factor analysis (PARAFAC), (C) HIX, (D) SUVA254 (mg l-1 m-1), and (E) the slope ratio (SR) during the study period.*

At low to intermediate discharge the relative contributions of autochthonous DOM to streamwater DOM, as indicated by ß/α, S_R and FI, tended to increase and displayed high variability (Figure 3 A - B). In contrast, elevated discharge increased the humic (C1) and fulvic-like components (C2, C2) and decreasing the absolute values and variability of the proteinaceous components (C4, C5), and of FI, ß/α and S_R. Hyporheic DOM optical properties followed a similar pattern; however, variability was

most pronounced at intermediate discharge (Figure 3 C - D). For instance, the coefficient of variation (CV) of S_R was highest (CV = 11.72) at intermediate flow but decreased at both low (CV = 7.74) and high flow (CV = 5.68). For DOC concentrations larger than 2 mg C L^{-1}, humic and fulvic fluorescence and elevated HIX values characterized DOM in the streamwater and the hyporheic zone.

The PCA suggests discharge as a strong driver of streamwater DOM composition with various contributions from allochthonous and autochthonous sources (Figure 3 E). DOM composition in the hyporheic zone had a similar gradient of sources as streamwater DOM, yet with a less pronounced control of streamwater discharge on its composition (Figure 3 F). However increasing discharge induced a terrigenous imprint on streamwater and hyporheic DOM as indicated by elevated $SUVA_{254}$ and contributions of C1, C2 and C3. This DOM pool was further characterized by elevated HIX and lower S_R values. At baseflow values of ß/α, FI and S_R increased while $SUVA_{254}$ decreased, which suggests autochthonous deliveries to the DOM pool. This pattern was further supported by elevated contributions of tryptophan-like (C4) and tyrosine-like fluorescence (C5).

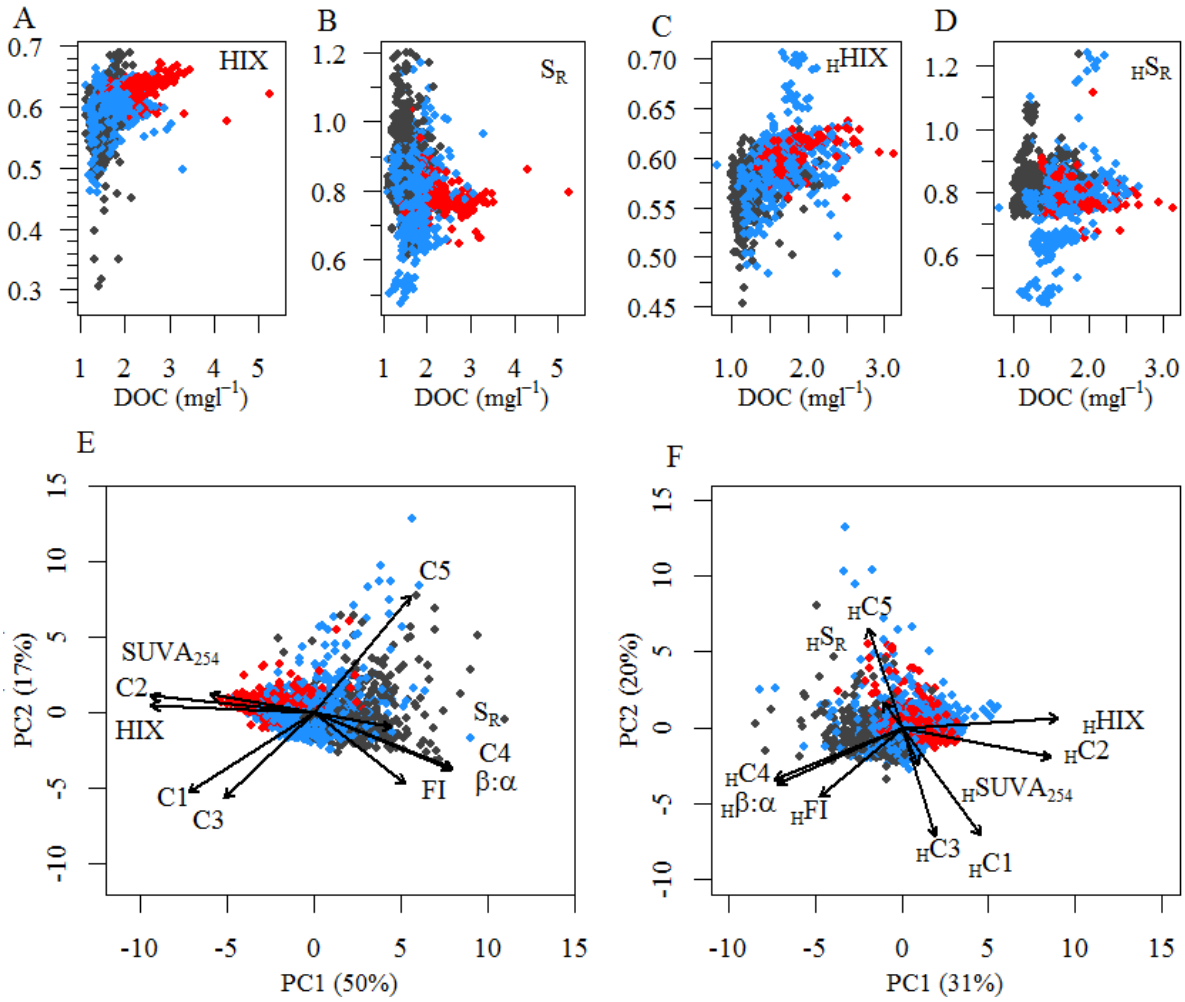

Figure 3 *Dissolved organic matter composition in the Oberer Seebach: Shown are representative relationships of the dissolved organic carbon (DOC) concentration and representative measures derived from absorbance and fluorescence measurements in the (A-B) streamwater and the (C-D) hyporheic zone: humification index (HIX) and the slope ratio (SR). Principal component analysis (PCA) based on absorbance and fluorescence measurements in the (E) streamwater and the (F)*

hyporheic zone: Humic- like components (C1-C3), protein-like components (C4 and C5), specific absorption at 254 nm (SUVA254), humification index (HIX), fluorescence index (FI), freshness index (ß/α), slope ratio (SR) (see Methods). Arrows are based on PCA structural coefficients. Color coded by discharge (Q) bins: Black symbols indicate baseflow (< 306 L s-1), blue and red symbols indicate intermediate flow (306 – 2,939 L s-1) and high flow (>2,939 L s-1), respectively.

Overall, we found that the DOM optical properties were more variable at low discharge. For instance, CV for streamwater S_R increased from 5.39 at high discharge to 10.39 at baseflow. Similarly, the CV of HIX changed from 3.08 to 6.63. Streamwater DOM composition generally exhibited a greater variability than hyporheic water DOM. The CV of streamwater $SUVA_{254}$ was 14.94 and 10.42 for the hyporheic zone, and 5.48 and 4.58 for streamwater and hyporheic HIX, respectively.

Controls on streamwater and hyporheic DOM composition – DOM temporal dynamics differed in the streamwater and hyporheic zone. The coupling between these dynamics was partially driven by discharge, streamwater temperature and PAR (canonical r = 0.67, p < 0.001, n = 1040) (Figure 4, Table 2 and 3). Generally the contributions from autochthonous sources (that is, elevated ß/α and S_R values) were higher in the streamwater than in the hyporheic zone at baseflow. For instance, during summer baseflow, streamwater ß/α and S_R increased with increasing streamwater temperature and PAR while hyporheic $SUVA_{254}$ was highest during baseflow. During snowmelt and summer storms this pattern reversed with increased contributions of the humic-like components (C1, C2 and C3), HIX and $SUVA_{254}$ to streamwater DOM compared to hyporheic water.

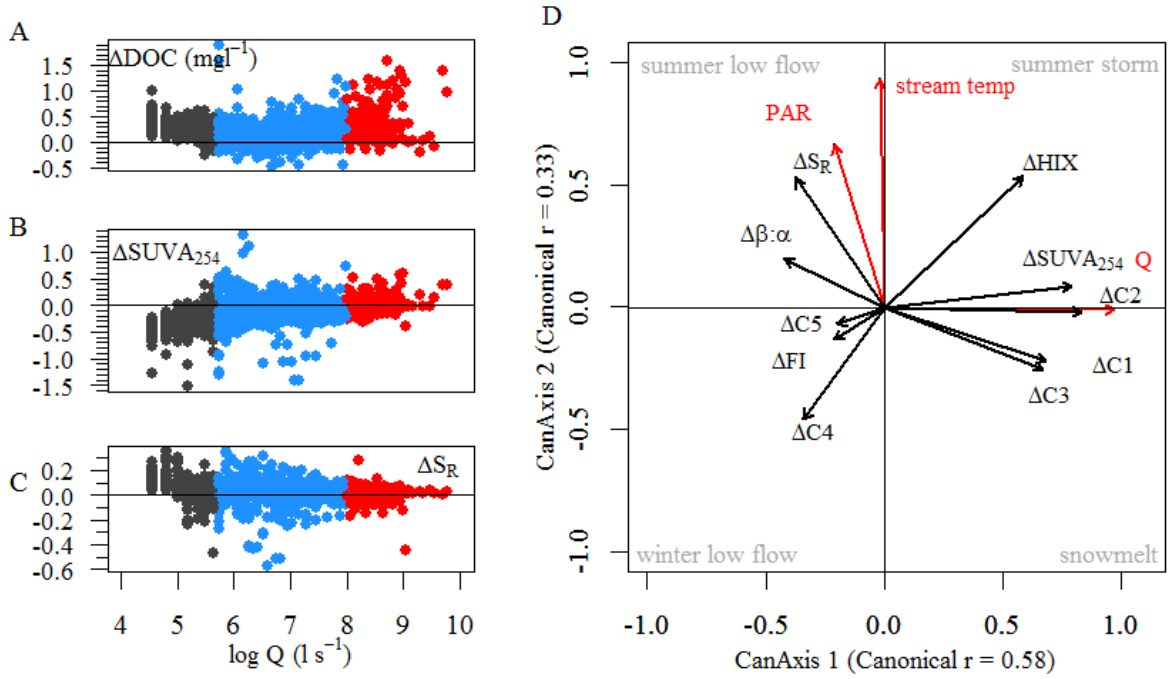

Figure 4 *Representative patterns of DOM optical measures in the streamwater and the hyporheic zone (hyporheic water optical measures were subtracted from streamwater optical measures at the same time points): (A) dissolved organic carbon (ΔDOC), (B) specific UV absorbance at 254 nm (ΔSUVA254) and (C) slope ratio (SR). Color coded by discharge (Q) bins. (E) Canonical correlation analysis based on environmental parameters and DOM composition in the streamwater and the hyporheic zone in Oberer Seebach (n = 1,040) reveals the main drivers of the coupling between*

streamwater and hyporheic water DOM: Shown are (A) streamwater temperature (stream temp), discharge (Q), photosynthetic active radiation (PAR) and (B) DOM optical measures derived from absorbance and fluorescence measurements (for more information see text). Arrows represent standardized canonical coefficients.

Diurnal patterns of DOM composition – At baseflow, streamwater and hyporheic DOC concentration followed diurnal patterns, which were most pronounced during summer but less in winter (Figure 5). Diurnal variations in streamwater DOC concentration ranged from 0.01 to 1.25 mg C L^{-1}, averaging 0.22 ± 0.16 mg C L^{-1} in summer and 0.08 ± 0.04 mg C L^{-1} in winter, respectively. Maximum daily DOC concentrations occurred around 19:30 h as PAR decreased. To specifically explore the variation and potential drivers of the diurnal amplitude of streamwater DOC concentration (ΔDOC) at base flow, we propose the following linear model:

$$\Delta DOC = b_0 * PAR + b_1 * T_{Streamwater} + b_2 * time\ after\ storm. \quad (3)$$

Based on AIC criteria, we found PAR (beta = 0.25, $p < 0.001$, sum of squares = 0.16), streamwater temperature (beta = 0.28, $p < 0.001$, sum of squares = 0.37) and the time since the last storm event (beta = 0.20, $p < 0.01$, sum of squares = 0.08) to explain 45% of the daily amplitude of ΔDOC (n = 149).

In the hyporheic zone, diurnal amplitudes of DOC concentration (ΔDOC_H; 0.01- 0.26 mg C L^{-1}) were less pronounced than in the streamwater. The maximum amplitude of ΔDOC_H occurred between 19:30 h and 01:30 h. Hydrodynamic exchange between streamwater and the hyporheic zone in OSB is controlled by discharge (Battin 1999), and we therefore propose the following linear model to explore variations in ΔDOC_H

$$\log \Delta DOC_H = b_0 * \log \Delta DOC + b_1 * \log Q_{baseflow} \quad (4)$$

Based on AIC criteria, we found that ΔDOC (beta = 0.69, $p < 0.001$, sum of squares = 18.45) and discharge at baseflow ($Q_{baseflow}$; beta = 0.66, $p < 0.05$, sum of squares = 2.58) explained 31% of the variance in ΔDOC_H at baseflow (n = 119).

Besides the diurnal patterns of DOC concentration, we also found that S_R changed on a diurnal basis with peak values at 13:30 h and minimal values at night (Figure 3). Diurnal variations of S_R occurred predominantly during summer in streamwater (0.07 ± 0.05), but were less pronounced in the hyporheic zone (0.05 ± 0.04). Also, variation of S_R was small during winter in both the streamwater and the hyporheic zone as compared to the summer.

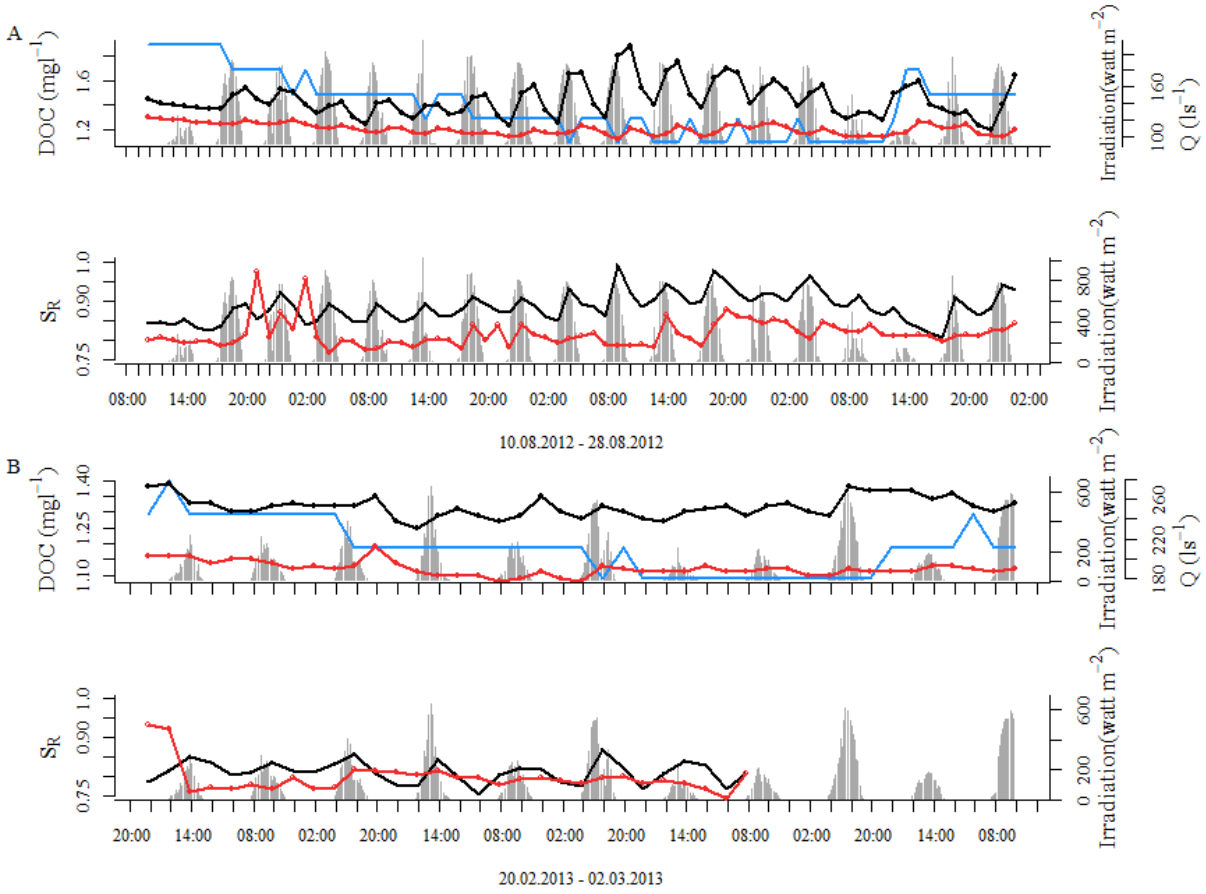

Figure 5 *Representative patterns of diurnal variation of streamwater (black) and hyporheic water (red) DOC concentration (mg L-1) and slope ratio (SR) in (A) summer and (B) winter. Diurnal amplitudes were more pronounced in summer than in winter. Discharge (Q) is indicated in blue and photosynthetic active radiation (PAR) in grey.*

Seasonal patterns of DOM composition – Streamwater DOM composition varied among seasons. For instance, baseflow peak S_R was 0.9 ± 0.09 in summer but only 0.8 ± 0.04 in winter. HIX varied broadly in summer (0.28 to 0.74, CV = 7.48) but less in winter (0.50 to 0.64, CV = 4.27). The protein-like component C4 displayed maximum values (up to 30 %) in summer and decreased in autumn (8 %). Streamwater DOC concentration (GAM, edf = 8.64, deviance explained = 54%, $p < 0.001$, n = 2972), β/α (GAM, edf = 8.98, deviance explained = 46.3%, $p < 0.001$, n = 4813) and tryptophan-like fluorescence (GAM, edf = 8.89, deviance explained = 49.8%, $p < 0.001$, n = 1152) were positively related to streamwater temperature (Table 4). However, β/α (GAM, edf = 8.98, deviance explained = 46.3%, $p < 0.001$, n = 4813) and tryptophan-like fluorescence were inversely related to discharge (GAM, edf = 8.89, deviance explained = 49.8%, $p < 0.001$, n = 1152). Values of ß/α and S_R were elevated in summer at low to intermediate discharge. In contrast, the humic-like component C1 (GAM, edf = 8.88, deviance explained = 28.4, $p < 0.001$, n = 1152), fulvic-like components C2 (GAM, edf = 8.92, deviance explained = 61.3%, $p < 0.001$, n = 1152) and C3 (GAM, edf = 8.47, deviance explained = 0.45.6, $p < 0.001$, n = 1151), and HIX (GAM, edf = 8.99, deviance explained = 54%, $p < 0.001$, n = 2262) were positively related to discharge.

DOM export fluxes – We estimated an annual water flux of 2002 mm y^{-1} and 2028 mm y^{-1} as based on binning discharge (Raymond and Saiers 2010) and LOADest (Runkel et al. 2004), respectively. From

these water fluxes, we calculated DOC export fluxes from OSB at 4.00 g m^{-2} y^{-1} and 4.03 ± 0.02 g m^{-2} yr^{-1} using either two approaches. Adding up DOC export fluxes calculated separately for baseflow, high and intermediate discharge yielded a total DOC export of 4.01 g m^{-2} y^{-1}. The flux of aromatic DOM (*a*254) was estimated at 24.71 ± 0.14 yr^{-1}, while fluxes of the C1, C2 and C3 components (all humic-like) averaged 0.91 ± 0.005 R.U m yr^{-1}, 0.38 ± 0.003 R.U m yr^{-1} and 0.35 ± 0.002 R.U m yr^{-1}, respectively (Table 5). The export fluxes of the protein-like components, C4 and C5, were consistently lower with 0.17 ± 0.003 R.U m yr^{-1} and 0.05 ± 0.002 R.U m yr^{-1}, respectively, than the fluxes of the humic-like components.We found 66% of DOC and 59- 65% of chromophoric DOM (as depicted by the fluorescent components) to be exported in spring and summer.

Discussion

The present study highlights the role of hydrology, seasonal and diurnal variability for DOM composition and export from an Alpine stream. Our findings on DOM source partitioning with varying discharge and in-stream biological processes expand current knowledge on carbon dynamics in stream ecosystems (e.g., Raymond and Saiers 2010, Inamdar et al. 2012, Singh et al. 2014). Our findings also shed new light on the relevance of the hyporheic zone as a contributor to stream DOM dynamics.

Drivers of DOM source and composition — Our optical measurements suggest that streamwater and hyporheic DOM was to a large extent of terrestrial origin throughout the year. This notion is supported by elevated values of humic-like (C1) and fulvic-like components (C2, C3), elevated aromaticity (as $SUVA_{254}$) and by FI values. Snowmelt and summer storms mobilized terrestrial DOM likely originating from humic-rich riparian soils in the floodplains adjacent to OSB. These findings are consistent with reports from other streams and support the notion of large lateral carbon fluxes entering headwater streams (e.g., Hornberger et al. 1994; Lambert et al. 2013). On top of this dynamic baseline of allochthonous DOM, autochthonous sources contributed to the DOM composition depending on discharge and season. This observation is essentially supported by the diurnal and seasonal variation of apparent molecular weight (S_R), aromaticity ($SUVA_{254}$), freshness (α/β) and by results from the PARAFAC.

Hydrology shaped DOM composition and dynamics in the streamwater and hyporheic zone of the OSB, permitting seasonal and diurnal patterns at baseflow. The marked seasonal and diurnal variations of apparently autochthonous DOM evoke biological processes linked to PAR and temperature, which are likely coupled yet may act at different temporal scales. While streamwater temperature closely reflects seasonal conditions in OSB (Figure 1), diurnal patterns are potentially driven by PAR.

Elevated PAR and low discharge augmented the contributions of freshly produced compounds with lower molecular weight to the DOM pool. It is reasonable to assume that benthic biofilms are major sources of this DOM as, for instance algae exude low-molecular weight compounds (Kaplan and Bott 1989) and bacteria produce fluorescent DOM (Guillemette and del Giorgio 2012). Less pronounced temporal variations of this autochthonous DOM in the hyporheic zone than in the streamwater corroborate the notion of benthic algae as a DOM source to streamwater. This observation is in agreement with earlier findings in OSB showing that hydrodynamic exchange between streamwater and the hyporheic zone is moderate at baseflow (Battin 1999). In OSB, shallow groundwater entering the hyporheic zone may entrain aromatic DOM (enriched in humics and fulvics) from riparian soils.

These terrestrial DOM deliveries are less subject to temporal variation than autochthonous sources of DOM and may therefore blur autochthonous signatures upon mixing in the hyporheic zone.

We observed more pronounced patterns of diurnal variations in DOC concentration and DOM apparent molecular weight in the streamwater during summer baseflow than in the hyporheic zone. These diurnal patterns also coincided with PAR dynamics. Interestingly, apparent molecular weight decreased with PAR, while DOC concentrations peaked as daily PAR decreased. This suggests that biological activity of benthic biofilms, including algae, act concurrently with photo-oxidation generating these conspicuous patterns. It is in fact well known that photo-oxidation of high-molecular-weight DOM from terrestrial sources can produce low-molecular-weight DOM (Kragh et al 2008), also in clear-water streams such as OSB (Fasching and Battin 2011). However, the fact that streamwater DOC concentrations peaked on a diurnal basis, namely when PAR decreased, points to in-stream sources (that is, from primary producers) rather than to photo-oxidation as the predominant source of autochthonous DOM. Additional evidence for the importance of DOM from in-stream sources in OSB may be derived from the recognition that the day-night oscillations of streamwater DOC concentration increased with the time elapsed since the last storm event. Benthic biomass accumulates as baseflow extends, thereby producing stronger signatures of autochthonous DOM when photosynthesis peaks. Storms may then disrupt this oscillating DOM patterns by scouring the streambed and collapsing primary production (Uehlinger, 2006) – a pattern consistent with our observations from OSB.

The fact that seasonally and diurnally controlled processes at baseflow conditions lead to a dynamic, yet notable autochthonous DOM signature in the streamwater and hyporheic zone may be of relevance for stream metabolism. In fact, Peter et al. (2014) showed a similar coupling of diurnal variations of streamwater and hyporheic CO_2 concentrations at baseflow for the OSB which could largely be attributed to in-stream metabolism modulated by streamwater temperature and primary production as inferred from PAR.

Our study further supports findings on diurnal variations of DOM in a larger river (San Joaquin River, USA), which were attributed to both biological and photochemical processes (Spencer et al. 2007). At the same time our data on hyporheic DOM expand the current picture of diurnal DOM patterns in streams. We suggest that mixing of allochthonous and autochthonous DOM sources, as mediated by discharge, shapes the observed differences in diurnal DOM patterns in the streamwater and the hyporheic zone. In fact, the downward mixing of streamwater into the hyporheic zone would influence DOM composition therein given that DOM from two sources mix in the hyporheic zone. Previous work showed that mixing between streamwater and hyporheic water is a function of discharge (Battin 1999). At baseflow, downward transport of streamwater is weak and DOM from benthic sources is therefore poorly entrained into the hyporheic zone where aromatic and humic compounds from adjacent riparian soils dominate the DOM pool. As discharge increases more streamwater enters the hyporheic zone but because of concomitant physical disturbance of the benthic zone and dilution effects, signatures of benthic-derived DOM in the hyporheic zone become reduced.

Seasonal hydrological dynamics, including summer droughts and snowmelt, may further shape DOM composition and concentration (Laudon et al. 2004, Singh et al. 2014). In OSB, seasonal changes in streamwater DOM composition may result from shifts in sources potentially ranging from soils, leaf litter, groundwater and from in-stream primary production. Our results suggest, for instance, that

during the spring freshet terrigenous DOM is flushed into OSB, while baseflow in winter favored autochthonous DOM production.

Our results on hyporheic DOM composition do not support clear seasonal patterns as observed by others (Wong and Williams 2010). Instead, our results indicate that hyporheic DOM is a composite of aromatic DOM from riparian soils that enter the hyporheic zone along subsurface flow paths. In the hyporheic zone, these terrestrial deliveries mix with DOM downwelling from the streamwater, which depends on discharge. We suggest that the increased variability of hyporheic DOM composition results from the mixing of these two end members, which is most pronounced at intermediate discharge. This mixing would translate into reduced DOM variability at elevated discharges when allochthonous rather than autochtonous DOM enters the hyporheic zone from the streamwater. Although DOM composition in the streamwater exhibited higher variability than hyporheic DOM composition it followed clear seasonal trends. Similarly Singh et al. (2014) found pronounced seasonality in streamwater DOM composition, yet this was muted or absent in groundwater and during baseflow conditions. However, in OSB, seasonal differences were pronounced at baseflow conditions. For instance, diurnal variations of apparent molecular weight (S_R), indicating photo-oxidation, and elevated freshness (β/α) were dominant during summer baseflow, while microbially derived DOM, as indicated by tryptophan-like fluorescence (C4) and higher FI values (McKnight et al. 2001, Osburn et al. 2012) imprinted the DOM during baseflow in winter. These results indicate different processes in summer and winter may contribute to the production of autochthonous DOM. Primary production and photo-oxidation may be important in summer, while microbial degradation may dominate in winter. These processes likely provide labile DOM to the streamwater during baseflow.

Storm events interrupted seasonal and diurnal patterns of DOM composition and diverged its dynamics in the streamwater and the hyporheic zone. Storms likely changed the DOM deliveries from shallow groundwater into OSB at baseflow (Battin et al. 1999) to deliveries from top soils and A-horizons via surface runoff and shallow subsurface flow. This conjecture is supported by the observation that summer storms increased the contributions of DOM with high humification, aromaticity and lower FI. Similar shifts in DOM composition were also observed in other streams interpreted as a shift in DOM source from mineral soils to top soils enriched in aromatic substances and lignin (Vidon et al. 2008, Inamdar et al. 2011, 2012). In OSB, storms further depressed the temporal variability of autochthonous DOM constituents, such as protein-like fluorescence and S_R. We propose that in OSB storms may entrain aromatics from the surface water and groundwater into the hyporheic zone, thereby turning the hyporheic DOM over, which may be relevant for hyporheic biogeochemistry (Sawyer et al. 2014). Collectively, our findings suggest that hydrology and both seasonal and diurnal controls shape DOM composition in the streamwater, and that hydrodynamics determine DOM composition in the hyporheic zone on top of the allochthonous baseline.

DOM export fluxes –The DOC flux from OSB was in the range of fluxes from small, forested watersheds in the north-eastern USA (0.5 – 5.7 g m^{-2} yr^{-1}; Raymond and Saiers 2010). The *a*254 yield from the OSB was comparatively low compared to large rivers (Spencer et al. 2013), which can be expected given the difference in catchment size. However, the areal flux of *a*254 from the OSB (24.71 ± 0.14 yr^{-1}) was bracketed by the fluxes from large rivers (0.038 – 67.53 yr^{-1}) (Spencer et al. 2013), indicating approximate agreement per unit catchment area.

Clearly, discharge rather than concentration was the more important control on DOM export fluxes from OSB, as fluxes largely mirrored variations in discharge. This resulted in relatively high humic

and fulvic export fluxes throughout the year. These fluxes peaked in spring and summer, which is attributable to snowmelt and summer storms. Hydrologic events have previously been shown to impact DOC fluxes and induce a hysteretic DOC response to snowmelt (Boyer et al. 1997, Laudon et al. 2004, Pellerin et al. 2012) and storm events (Hornberger et al. 1994, Butturini et al. 2006). We found that streamwater DOC concentrations increased more rapidly with increasing discharge during the rising limb of the hydrograph than during the falling limb. Accordingly, DOC export during the rising (equation 1) and falling (equation 2) hydrograph accounted for the majority (52.9% and 47.0%) of all DOC export, while only 0.13% of DOC was exported during periods of baseflow. This is consistent with the observation that the majority of DOC export (86%) takes place during rising and falling hydrograph in forested watersheds (Raymond et al. 2010) and may point to an initial flushing of DOC from soils, resulting in temporary DOC depletion, as indicated by the lower DOC concentrations during the falling hydrograph (Raymond et al. 2010). Likewise, a clockwise DOC–discharge hysteresis has previously been attributed to a temporary depletion of the terrestrial DOC supply flushed prior to peak flow (Ågren et al. 2008; Boyer et al. 2000; Hornberger et al. 1994). Our results confirm that frequent hydrological events like storms and snowmelt determine the timing and amount of DOM fluxes (Boyer et al. 2000, Raymond et al. 2010) particularly in headwater streams which are well embedded in the terrestrial environment. This may be of relevance for downstream ecosystems, were the timing and composition of DOM fluxes determine stream metabolism (Battin et al. 2008).

Ecosystem implications – Our results suggest seasonal, diurnal and event-driven processes linked to hydrology, temperature and PAR to drive DOM concentration, composition and export fluxes from an Alpine headwater stream. It seems that the biological processes occurring in the benthic zone impart a dynamic autochthonous fingerprint onto an otherwise largely allochthonous DOM pool. Our findings emphasize the hyporheic zone as an interface for DOM between catchment and in-stream processes driven to some extent by hydrology and hydrodynamic exchange between the streamwater and the hyporheic zone. The temporal dynamics of DOM in the hyporheic zone is less subject to fluctuations, which is consistent with the concept that the hyporheic zone is buffered against environmental fluctuations (Boulton et al. 1998). Furthermore, the hyporheic zone receives terrestrial DOM which may be relatively resistant to microbial metabolism but which is continuously delivered. This large and steady reservoir of recalcitrant DOM in the hyporheic zone may confer stability to the stream ecosystem (sensu Wetzel 2001). In contrast, benthic processes sporadically deliver DOM that is more readily available for heterotrophic metabolism. Interactions between these two DOM pools as encapsulated by the notion of priming still remaining to be unveiled in stream ecosystems (Bengtsson et al. 2014).

The involvement of the various components of stream ecosystems, even including the catchment itself, in DOM fluxes is susceptible to changes in hydrology. At the seasonal and diurnal scales, biological processes (e.g., primary production) and ensuing concentration seem to be relevant drivers of DOM composition, whereas at the event-driven scale (e.g., storms) discharge is the major driver of DOM composition — a processes that also shapes DOM export fluxes to downstream ecosystems. Untangling these processes and patterns is crucial to better understand how seasonal hydrology but also the hydrodynamic exchange between streamwater and the hyporheic zone likely affect DOM export fluxes as climate changes. In fact, shifts in precipitation patterns and snow pack are predicted to have major impacts on the hydrological regime of streams (Berghuijs et al. 2014) and their connectivity with the catchment and groundwater. Our study paves the way towards a better

understanding of the possible consequences of such environmental changes on the DOM biogeochemistry in Alpine streams and on the downstream implications.

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Tables

Table 1 Environmental data during May 2010 to August 2013. Shown are mean values for spring (20.03-21.06), summer (20.06 – 22.09); autumn (23.09 – 21.10), winter (22.10- 10.03).

	Spring	Summer	Autumn	Winter
Discharge (l s^{-1})	1972.74 ± 2141.28	957.83 ± 1589.81	609.28 ± 1216.7	1079.75 ± 2019.64
	(150.4 - 22461.35)	(94.44 - 16714.77)	(94.44 - 17674.32)	(180.29 - 28065.27)
Streamwater temperature (°C)	7.13 ± 1.31	8.99 ± 1.28	6.12 ± 1.4	4.77 ± 0.95
	(3.82 - 15.29)	(6.6 - 14.98)	(2.3 - 10)	(1.1 - 8.54)
Air temperature (°C)	10.53 ± 6.81	15.76 ± 5.34	3.63 ± 6.68	-1.14 ± 5.77
	(-6.9 - 33.2)	(1.2 - 34.3)	(-20 - 22.9)	(-23 - 17.9)
Conductivity (µS cm^{-1})	214.52 ± 13.71	230.95 ± 18.17	243.79 ± 8.23	237.1 ± 10.81
	(167 - 238)	(166 - 266)	(209 - 276)	(189 - 290)
DOC (mg l^{-1})	1.74 ± 0.33	1.79 ± 0.4	1.56 ± 0.3	1.67 ± 0.37
	(1.11 - 3.87)	(1.11 - 5.43)	(1.16 - 3.38)	(1.13 - 5.25)
DOC_H (mg l^{-1})	1.48 ± 0.21	1.55 ± 0.35	1.58 ± 0.35	1.37 ± 0.21
	(1.11 - 2.37)	(0.82 - 2.67)	(1.12 - 3.12)	(1 - 1.9)
PAR (w m^{-2})	154.84 ± 234.47	168.32 ± 246.46	50.67 ± 118.37	50.81 ± 122.14
	(0 - 983)	(0 - 979)	(0 - 603)	(0 - 734)

Table 2 Canonical correlation analysis based on the environmental parameters discharge (Q), streamwater temperature and photosynthetic active radiation (PAR) and dissolved organic matter properties derived from absorbance and fluorescence measurements from the Oberer Seebach, where hyporheic water optical measures were subtracted from streamwater optical measures at the same time points (n = 1040): Δ humification index (ΔHIX), Δ fluorescence index (ΔFI), Δ freshness index (Δß/α), Δ slope ratio (ΔSR), Δ specific UV absorbance at 254 nm (ΔSUVA254), humic- like components (ΔC1-ΔC3), protein-like components (ΔC4 and ΔC5). Shown are tests of canonical dimensions. The strength of the relationship between the canonical axis and the constraints were computed as the canonical correlation coefficients (canonical r), significance of each canonical correlation was computed as Wilks' Lambda, using F-approximation (Rao's F).

Dimension	Canonical r	F-value	df1	df2	p-value
1	0.67	27.70	33	3023.49	< 0.001
2	0.33	10.68	20	2054	< 0.001
3	0.28	9.50	9	1028	< 0.001

Table 3 Canonical correlation analysis based on the environmental parameters discharge (Q), streamwater temperature and photosynthetic active radiation (PAR) and dissolved organic matter properties derived from absorbance and fluorescence measurements from the Oberer Seebach (see Table 2). Shown are standardized canonical coefficients for the first two canonical dimensions.

Dimension	**1**	**2**
Q	0.97	-0.01
Streamwater temperature	-0.02	0.66
PAR	-0.02	0.93
ΔHIX	0.59	0.54
ΔFI	-0.20	-0.07
Δβ/α	-0.42	0.20
ΔS_R	-0.37	0.53
$\Delta SUVA_{254}$	0.79	0.08
ΔC1	0.68	-0.22
ΔC2	0.83	0.01
ΔC3	0.67	-0.26
ΔC4	-0.34	-0.46
ΔC5	-0.21	-0.13

Table 4 General additive models (GAM) including a residual temporal correlation structure (auto-regressive model of order 1) of discharge and streamwater temperature and the individual fluorescent components (in %) and DOM optical measures derived from fluorescence and absorbance measurements: dissolved organic carbon (DOC), humification index (HIX), fluorescence index (FI), freshness index (ß/α), slope ratio (SR), specific UV absorbance at 254 nm (SUVA254), humic- like components (C1-C3), protein-like components (C4 and C5). Shown are standardized regression coefficients (ß), t-values, estimated degree of freedom (edf) and the deviance explained (%).

		Discharge			**Streamwater temperature**				
	Intercept	ß	t- value	p-value	ß	t- value	p-value	edf	Deviance Explained (%)
DOC	1.45	1.15×10^{-4}	50.85	p< 0.001	1.59×10^{-2}	4.13	p< 0.001	8.64	54
HIX	0.62	5.29×10^{-6}	13.46	p< 0.001	-3.07×10^{-3}	-4.84	p< 0.001	8.99	49
FI	1.38	-5.65×10^{-6}	-12.66	p< 0.001	-9.25×10^{-4}	-1.3	p = 0.020	8.83	45.8
β/α	0.63	-6.07×10^{-6}	-16.57	p< 0.001	4.90×10^{-3}	8.3	p< 0.001	8.98	46.3
S_R	0.64	-4.21×10^{-6}	-4.95	p< 0.001	2.51×10^{-2}	16.45	p< 0.001	8.80	17.3
$SUVA_{254}$	2.08	4.97×10^{-5}	24.44	p< 0.001	-6.28×10^{-2}	-17.07	p< 0.001	8.93	45.9
C1	25.50	3.98×10^{-4}	12.68	p< 0.001	-1.52×10^{-1}	-3.39	p< 0.001	8.88	28.4
C2	9.89	3.45×10^{-4}	23.64	p< 0.001	-1.27×10^{-1}	-5.19	p< 0.001	8.92	61.3
C3	9.93	2.06×10^{-4}	17.48	p< 0.001	-1.43×10^{-1}	-7.35	p< 0.001	8.47	45.6
C4	3.78	-2.03×10^{-4}	-10.38	p< 0.001	2.98×10^{-1}	9.04	p< 0.001	8.89	49.8
C5	1.91	-1.71×10^{-4}	-4.03	p< 0.001	5.21×10^{-1}	0.74	p = 0.46	8.58	9.82

Table 5 Loads and export fluxes of DOC (including standard error of prediction calculated for the modeled flow period), the fluorescent components (C1-C5 in R.U; from PARAFAC analysis) and the absorbance at 440 nm (a254) estimated using LOADest (Runkel et al. 2004).

	LOAD	FLUX
DOC	$7.27 \times 10^{7} \pm 4.33 \times 10^{5}$ g yr^{-1}	4.03 ± 0.02 g m^{-2} yr^{-1}
*a*254	$4.46 \times 10^{8} \pm 2.59 \times 10^{6}$ m^2 yr^{-1}	24.71 ± 0.14 yr^{-1}
C1	$1.64 \times 10^{7} \pm 8.43 \times 10^{4}$ R.U m^3 yr^{-1}	0.91 ± 0.005 R.U m yr^{-1}
C2	$6.84 \times 10^{6} \pm 5.11 \times 10^{4}$ R.U m^3 yr^{-1}	0.38 ± 0.003 R.U m yr^{-1}
C3	$6.25 \times 10^{6} \pm 3.61 \times 10^{4}$ R.U m^3 yr^{-1}	0.35 ± 0.002 R.U m yr^{-1}
C4	$3.09 \times 10^{6} \pm 5.15 \times 10^{4}$ R.U m^3 yr^{-1}	0.17 ± 0.003 R.U m yr^{-1}
C5	$9.58 \times 10^{5} \pm 3.58 \times 10^{4}$ R.U m^3 yr^{-1}	0.05 ± 0.002 R.U m yr^{-1}

Beside three papers that constitute the core of my PhD thesis, I also contributed to the following papers:

1. UV oxidation - a new extraction method for radiocarbon dating

Authors: Peter Steier, Christina Fasching, Klaus Mair, Jakob Liebl, Tom J. Battin, Alfred Priller, Robin Golser

Publication status: published 2013 in *Radiocarbon* Volume 2-3, pages 373-382

My contribution to this publication
a. Aided with statistical analyses
b. Editing of manuscript

2. Microbial biodiversity in glacier-fed streams

Authors: Linda Wilhelm, Gabriel A. Singer, Christina Fasching, Tom J. Battin and & Katharina Besemer

Publication status: published 2013 in The ISME Journal Volume 7, pages 1651-1660

My contribution to this publication
a. Participated in research design
b. Editing of manuscript

3. Rare but active taxa contribute to community dynamics of benthic biofilms in glacier-fed streams

Authors: Linda Wilhelm, Katharina Besemer, Christina Fasching, Tim Ulrich, Gabriel A. Singer, Christopher Quince and Tom J. Battin

Publication status: published 2014 in Environmental Microbiology Volume 16, pages 2514-2524

My contribution to this publication
a. Participated in research design
b. Aided with statistical analyses
c. Editing of manuscript

VIII. Conclusions and Outlook

This thesis contributes to our current understanding of the mechanisms which govern carbon cycling and fluxes in streams along DOC and colour gradients, ranging from Alpine glaciers and clear water streams to brown-water streams. This study informs how hydrology and seasonally regulated processes influence the temporal dynamics of DOM composition and fluxes at different scales. Furthermore, it enhances our current knowledge on how microbial degradation and metabolism contribute to the mineralization and flux of DOM within these freshwater environments and impact the global carbon cycle.

C**hapter II - Biogeochemically diverse organic matter in Alpine glaciers and its downstream fate**, highlights the diversity of glacial DOM and the role of these low-DOC ecosystems for carbon cycling. Our results indicate DOC bioavailability and biogeochemical factors (for example, chemical composition and source) influence the bioavailability of ice-locked DOC and impact the metabolism in the glacier-fed stream. The findings of this study are corroborated by several previous studies indicating the notable occurrence of nutrient (Hodson et al. 2005) and carbon cycling within alpine glaciers (Hodson et al. 2007, Hood et al. 2009, Stubbins et al. 2012, Anesio et al. 2012). Previous findings from the Gulf of Alaska suggested ancient, but labile DOC to support downstream metabolism (Hood et al. 2009), and primary production and respiration in cryoconite holes at the glacier surface to contribute to large-scale carbon fluxes (Anesio et al. 2009, 2012). Our findings on Alpine glaciers expand this view, suggesting glaciers to be sources of bioavailable and highly diverse DOM, which act as a control for downstream heterotrophic activity in lacier-fed streams.

The results from C**hapter II** highlight glaciers as notable contributors to carbon cycling, governed by complex interactions of DOM released via glacial melt and specific microbial communities adapted to these ecosystems (Wilhelm et al 2013, 2014). Climate change alters the glacial mass balance and may have consequences for the amount and composition of DOM. Furthermore, it may affect the activity and composition of microbial communities in pro-glacial streams, thereby impacting carbon cycling and CO_2 evasion fluxes from these ecosystems. The findings of this and other studies highlight the need for a deeper understanding of how climatic changes affect carbon cycling and microbial communities in glacial ecosystems.

C**hapter III - Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams**, expands upon the findings of **Chapter II,** highlighting that in-stream metabolism of terrigenous DOM contributes to CO_2 evasion fluxes from streams to the atmosphere. However, not only the amount of DOM, but the coupling of microbial metabolism and DOM composition was found to control streamwater CO_2 partial pressures. Terrigenous DOM constituted the dominant fraction of the carbon pool in brown-water streams and was a major contributor of the biodegradable fraction of DOM in glacial streams. Although traditionally considered refractory, this carbon pool was readily metabolized by stream microorganisms, but at the cost of low carbon use efficiency, with potential contributions to CO_2 evasion to the atmosphere. Thus, our findings contradict the common perception that ‘‘brown’’ DOM is resistant to microbial degradation (Schmidt et al. 2011) and highlight the role of terrigenous DOM for stream metabolism. This supports recent research, reporting large amounts of CO_2 evasion from streams and rivers to the atmosphere (Sobek et al. 2003, Lapierre and del Giorgio 2012, Teodoru 2009) and highlights streams as active components of the global carbon cycle. This seems relevant as terrigenous DOM deliveries into inland waters are predicted to increase in the future (Monteith et al.

2007); particularly in areas where carbon loss from major stores such as peat and permafrost can be substantial as climate change progresses.

C**hapters II** and **III** highlight that not only the quantity, but also DOM composition, impacts streamwater microbial metabolism, underscoring the importance of DOM dynamics and composition for in-stream carbon cycling. However, in fluvial ecosystems, the amount and composition of DOM varies considerably over time due to complex mechanisms, involving hydrological, seasonal, and biogeochemical processes (Kalbitz et al. 2000, Aitkenhead-Peterson et al. 2003, Singh et al. 2013).

Chapter IV - Hydrological and seasonal controls on dissolved organic matter (DOM) quality and fluxes in an Alpine headwater stream identifies hydrology and seasonally induced processes as major drivers for DOM composition and dynamics, which display distinct diurnal, seasonal and event-driven patterns. While discharge directly controlled the amount of DOM exported, at seasonal and diurnal scales, biological processes (e.g., primary production) seem to be relevant drivers of DOM composition in the streamwater and the hyporheic zone. These findings are corroborated by recent research highlighting that the flow regime (including hydrologic events such as snowmelt and storms) and season not only impact the timing and amount of DOC released to downstream ecosystems (Boyer et al. 1996, 1997, Butturini et al. 2006, Raymond and Saiers 2010, Yoon and Raymond 2012), but also DOM composition (Fellman et al. 2009, Nguyen et al. 2010, Singh et al. 2014). Our results expand upon these findings by providing information about how hydrology, seasonal and diurnal variability impact the coupling between streamwater and hyporheic water DOM dynamics and of DOM export. Complementing studies on DOM export from small forested watersheds in the Eastern USA (Raymond and Saiers 2010) and large US rivers (Spencer et al. 2013), I calculated a DOC export of 4.03 ± 0.02 g m^{-2} yr^{-1} and a CDOM ($a254$) export of 24.71 ± 0.14 yr^{-1} from the Oberer Seebach, Austria. Export fluxes of humic and fulvic-like fluorescence were comparatively higher than protein-like fluorescence export, with values in the range of that of large USA rivers (Spencer et al. 2013), further highlighting the importance of terrigenous DOM in fluvial ecosystems. In fact, terrigenous DOM was a major contributor to the carbon pool in all investigated fluvial ecosystems (**Chapter II**, **Chapter III** and **Chapter IV**), contributing to carbon cycling and fluxes to downstream ecosystems as well as CO_2 evasion to the atmosphere. This is in line with studies showing that inland waters are often supersaturated in CO_2, largely resulting from the mineralization of terrigenous DOC (Sobek *et al.* 2003, Lapierre and del Giorgio 2012, Teodoru 2009).

Climate change may lead to changes in the frequency and intensity of heavy precipitation events and associated floods, droughts and other characteristic flows, potentially altering the typicalsurface and groundwater flow regimes. Such alterations in the natural flow regime may ,induce shifts in hyporheic flow paths and temperature patterns, with possible consequences for the delivery of DOM, carbon processing and other functions within streams. In fact, increases in temperature, in addition to changes in precipitation and discharge patterns, have been suggested to alter DOM composition and export fluxes from rivers (Striegl et al. 2005, Sebestyen 2009, Inamdar et al. 2011, Strohmeier et al. 2013, Wilson et al. 2013). For instance, increasing intensities of storms are expected to increase the concentration and humic content of DOM (Inamdar et al. 2011). Altered coupling and seasonal timing of water and DOM fluxes (Laudon et al. 2013) in response to changes in hydrological exchange of streamwater and and groundwater within the hyporheic zone may be ultimate consequences of climatic changes, with implications for in-stream carbon cycling and fluxes to downstream ecosystems.

This thesis illuminates how hydrology and seasonally regulated processes – both of which are subject to climate change – influence the temporal dynamics of DOM composition and fluxes at different

scales. It enhances our understanding of how fluvial ecosystems contribute to the mineralization of terrigenous DOM and informs on the role of the coupling of DOM composition and microbial metabolism for in-stream carbon cycling. Furthermore, the findings highlight the need for long-term studies of how environmental controls impact the coupling of DOM composition and microbial metabolism, as well as the amount and composition of DOM export fluxes to downstream ecosystems in order to gain a deeper understanding on how climatic changes may affect carbon cycling in fluvial ecosystems.

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Appendix

Summary

Streams and rivers are major contributors to carbon cycling and account for large dissolved organic matter (DOM) export fluxes from the terrigenous environment to downstream ecosystems. Yet climatically and anthropogenically induced changes may impact the amount, composition and fluxes of DOM, with possible consequences for the delivery of DOM and carbon processing in streams. This thesis investigates the role of streams for carbon cycling, spanning ecosystems predicted to be particularly susceptible to climate change such as Alpine Glaciers and Alpine streams. It informs on the amount and composition of carbon fluxes from these environments and reveals the underlying controlling mechanisms. Furthermore, it reveals the relevance of DOM composition and dynamics for microbial metabolism.

In many alpine regions, headwaters - the smallest, but most numerous elements of fluvial networks- are fed by glaciers, which impact downstream ecohydrology and biogeochemistry. In addition to their prominent role in the hydrological cycle, glaciers are increasingly considered as ecosystems, where storage and transformation processes of organic carbon take place. Glacial melting may thus imply ultimate consequences for the amount and composition of glacial-derived DOM in the pro-glacial streams and carbon cycling. In order to evaluate the role of glaciers for carbon cycling and determine DOM source and composition, the molecular composition, radiocarbon age and bioavailability of DOM of 26 Alpine glaciers was investigated, employing a combination of ultra-high-resolution mass spectrometry, fluorescence spectroscopy and biodegradation experiments. Glacial DOM was dominated by a high-intensity polyphenolic population – typical for vascular plants and soils – alongside a large population of peptide-like unsaturated aliphatics – presumably of microbial origin and produced in-situ. These molecular populations were closely mapped by fluorescence signals derived from excitation-emission spectroscopy and parallel factor analysis (PARAFAC). Biodegradation assays were employed to study microbial degradation of glacial organic matter and calculate export fluxes of labile and recalcitrant moieties. Results suggest a metabolic link between ancient terrestrial organic carbon stored in Alpine glaciers and carbon cycling in pro-glacial streams. I calculated that the European Alps deliver 340 tons C yr^{-1}, of which 162 tons carbon are potentially respired as CO_2 to the atmosphere, highlighting the relevance of mountain glaciers for carbon cycling.

In order to further illuminate the coupling of DOM composition and microbial metabolism and its role for carbon cycling, DOM composition and dynamics in brown-water streams was investigated over a gradient of varying terrigenous inputs. Evidence suggests an increasing export of terrestrial DOC to aquatic systems in Northern America and North and Central Europe. This phenomenon commonly referred to as “browning” of inland waters is the result of the presence of terrigenous DOM containing large amounts of CDOM, which confers a brown color to natural waters. In order to evaluate the effect of browning on carbon cycling, streamwater CO_2 concentrations were related to microbial metabolism in biodegradation assays. The relationship of microbial metabolism and degradation of terrigenous DOM showed that microbial carbon use efficiency (CUE) was ultimately related to DOM composition. Thus, the amount of DOM as well as the coupling of CUE with DOM composition constituted an important control on streamwater CO_2. Changes in DOM composition associated with browning led to a decrease of CUE, highlighting the effect of browning on carbon cycling in streams.

To identify the mechanisms that control DOM composition, dynamics and fluxes we monitored DOM concentration and composition in the streamwater and hyporheic zone of an Alpine headwater stream over three years. Hydrology and seasonal parameters, such as photosynthetic active radiation (PAR) and streamwater temperature controlled streamwater and hyporheic DOM concentration and composition, and induced diurnal, seasonal and event-driven patterns. During periods of low flow, diurnal fluctuations in DOM concentration were pronounced; DOM composition was characterized by an autochthonous imprint, possibly from originating benthic algae. Increasing discharges frequently interrupted diurnal patterns and led to a relatively higher allochthonous DOM imprint, likely derived from the flushing of adjacent riparian soils. Accordingly, DOM export fluxes were largely of terrigenous origin as indicated by the aromaticity (a254: 24.71 ± 0.14 yr^{-1}) and the fluorescent components modeled by parallel factor analysis. I calculated a DOC export flux of 4.03 ± 0.02 g m^{-2} yr^{-1} and a chromophoric DOM (a254) flux of 24.71 ± 0.14 yr^{-1} from the stream. Export fluxes of humic and fulvic-like fluorescence were comparatively higher than protein-like fluorescence export, further highlighting the relevance of terrigenous DOM in fluvial ecosystems. Our results underscore the relevance of the flow regime and seasonal-driven processes for DOM dynamics, composition and fluxes in Alpine streams and highlight the need for further research on how these climatically influenced factors may affect carbon cycling as a result of climate change.

The findings presented in this thesis underscore the strong link between the flow regime, DOM composition and dynamics, and microbial metabolism in natural streams. They enhance our understanding of DOM biogeochemistry in fluvial ecosystems and illuminate the role of streams and terrigenous DOM for carbon cycling.

Zusammenfassung

Flüsse und Bäche tragen substantiell zum Kohlenstoffzyklus bei und sind verantwortlich für große Exportflüsse von der terrestrischen Umwelt in abwärts gelegene Ökosysteme. Klimatische und anthropogen hervorgerufene Veränderungen der Umwelt könnten die Menge, Zusammensetzung und Flüsse von gelösten organischen Kohlenstoff beeinflussen, mit möglichen Konsequenzen für den Eintrag und den Umsatz von gelösten organischen Kohlenstoff in Bächen. Diese Dissertation befasst sich mit der Rolle von Bächen für den zum Kohlenstoff Zyklus in Alpinen Gletschern und Bächen, welche besonders sensitiv gegen über klimatischen Veränderungen reagieren. Diese Arbeit informiert über die Menge und Zusammensetzung von Kohenstoffflüssen von diesen Ökosystemen und identifiziert die treibenden Mechanismen. Desweitern unterstreicht sie die Relevanz der Kohlenstoff-Zusammensetzung und Dynamik für den mikrobiellen Metabolismus.

In vielen alpinen Regionen speisen Gletscher Bäche - die kleinsten, aber zahlreichsten Elemente fluvialer Netzwerke, und beeinflussen so deren Ökohydrologie und Biogeochemie. Zusätzlich zu ihrer prominenten Rolle im Wasserkreislauf, werden Gletscher immer mehr als Ökosysteme angesehen wo Kohlenstoffspeicherung und Transformationsprozesse stattfinden. Das rapide Abschmelzen der Gletscher könnte daher unmittelbare Konsequenzen für die Quantität und Zusammensetzung von Kohlenstoff in Gletschergespeisten Bächen und den Kohenstoffzyklus haben. Um die Rolle von Gletschern für den Kohlenstoffzyklus zu evaluieren, sowie dessen Quelle und Zusammensetzung zu erforschen untersuchten wir die die molekulare Zusammensetzung, das Alter und die Bioverfügbarkeit des Kohlenstoffs von 26 Alpinen Gletschern. Dazu setzten wir hochauflösende Massenspektrometrie, Fluoreszenz-Spektrometrie und Inkubationsexperimente ein. Kohlenstoff glazialen Ursprungs wurde

neben einer großen Gruppe von Peptid-ähnlichen Substanzen – möglicherweise mikrobiellen Ursprungs und in-situ produziert, von polyphenolischen Substanzen - typische für Gefäßpflanzen und Böden- dominiert. Diese Molekülgruppen waren stark mit Fluoreszenz Signalen, stammend von der Exzitation-Emission Spektroskopie und anschließender Parallelen Faktoren Analyse (PARAFAC), assoziiert. Mit Hilfe von Inkubationsexperimenten konnten wir mikrobielle Abbauprozesse von organischem Kohlenstoff glazialen Ursprungs untersuchen und Export Flüsse von labilen und rekalzitranten Kohlenstoff berechnen. Resultate zeigten, dass Europäische Alpen 340 Tonnen Kohlenstoff pro Jahr exportieren, wovon 162 Tonnen potenziell als respiriert werden und als CO2 in die Atmosphäre verloren gehen. Diese Ergebnisse unterstreichen die Relevanz von Gletschern für den Kohlenstoffzyklus.

Um das Zusammenwirken der Zusammensetzung von Kohlenstoff und des mikrobiellen Metabolismus sowie dessen Rolle für den Kohlenstoffzyklus weiter zu erforschen wurde die Kohlenstoff-Zusammensetzung und Dynamik in Braunwasserbächen über einen Gradienten von variierenden terrestrischen Inputs untersucht. In Nord Amerika und Nord und Zentral Europa stiegen die Kohlenstoff Exporte von terrestrischen zu aquatischen Ökosystemen über die letzten Jahre an. Dieses Phänomen wird als "browning" bezeichnet und resultiert aus der Präsenz terrestrischen Kohlenstoffs. Dieser enthält große Mengen und chromophoren Kohlenstoff und ist daher verantwortlich für die braune Färbung des Gewässers. Um den Effekt von "browning" auf den Kohlenstoffzyklus zu evaluieren, erfolgte die Messung der CO_2 Konzentration im Gewässer und Inkubationsexperimente wurden eingesetzt um den mikrobiellen Metabolismus und Abbau von terrestrischen organischen Kohlenstoff zu untersuchen. Die Ergebnisse zeigen, dass die mikrobielle Effizienz Kohlenstoff in Biomasse umzuwandeln (carbon use efficiency; CUE) unmittelbar mit der Kohlenstoffzusammensetzung zusammenhängt. Somit stellt die Kohlenstoffmenge, sowie die Koppelung zwischen der CUE und der Zusammensetzung des Kohlenstoffs eine wichtige Kontrolle für die CO_2 Konzentration im Bach dar. Änderungen in der Zusammensetzung des Kohlenstoffs, welche mit "browning" assoziiert werden können, führten zu einer Herabsetzung der CUE und unterstrichen somit den Einfluss von "browning" auf den Kohlenstoffzyklus in Bächen.

Um jene Mechanismen zu erforschen welche die Zusammensetzung, Dynamik und Exportflüsse von Kohlenstoff kontrollieren erfolgte ein 3-Jahres Monitoring der Kohlenstoffkonzentration und Zusammensetzung im Bachwasser und der hyporheischen Zone eines alpinen Baches. Die Ergebnisse zeigten, dass die charakteristisch saisonalen Parameter, wie Abflussregime und Temperatur, die Menge und Zusammensetzung des Kohlenstoffs im Bachwasser und der hyporheischen Zone kontrollierten. Des Weiteren wurden diurnale, saisonale und Ereignis- getriebene Muster der Menge und Zusammensetzung des Kohlenstoffs identifiziert. Während Perioden geringen Abflusses könnten klare diurnale Fluktuationen in der Kohlenstoffkonzentration identifiziert werden. Dabei war der Kohlenstoff hauptsächlich autochthonen Ursprungs, möglicherweise von benthischen Algen. Diurnale Muster wurden regelmäßig von höheren Abflüssen unterbrochen, welche zu einem relativ stärkeren autochthonen Signal führten, welches von der Auswaschung von angrenzenden Böden herrühren könnte. Entsprechend waren Kohlenstoff Exporte hauptsächlich terrestrischen Ursprungs wie durch die Aromatizität (a254: 24.71 ± 0.14 yr^{-1}) und die fluoreszenten Komponenten, welche mit der PARAFAC modelliert wurden, bestätigt. Es ergab sich ein Kohlenstoffexport von 4.03 ± 0.02 g m^{-2} yr^{-1} und ein chromophorer Kohlenstoffexport (a254) von 24.71 ± 0.14 yr^{-1} von dem Bach. Die Erkenntnis, dass Exportflüsse von Humin- und Fulvosäuren- ähnlicher Fluoreszenz höher als jener Protein-ähnlicher Fluoreszenz sind, unterstreicht die Relevanz von terrestrischem Kohlenstoff in fluvialen Ökosystemen. Die Resultate betonen die Relevanz des Abflussregimes und von saisonalen Prozessen für die Dynamik, Zusammensetzung und Exportflüsse von Kohlenstoff und die Wichtigkeit

der weiteren Erforschung der möglichen Auswirkungen dieser Faktoren auf den Kohlenstoffzyklus infolge des Klimawandels.

Die Ergebnisse dieser Arbeit unterstreichen den starken Zusammenhang zwischen dem Abflussregime, der Zusammensetzung und Dynamik von aquatischen Kohlenstoff und dem mikrobiellen Metabolismus in natürlichen Bächen. Diese Arbeit trägt dazu bei unser Verständnis von der Biogeochemie von Kohlenstoff in fluvialen Systemen und der Rolle von Bächen und terrestrischen Kohlenstoff für den Kohlenstoffzyklus, zu vertiefen.

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