Non-conservative patterns of dissolved organic matter degradation when and where lake water mixes

Thibault Lambert¹ · Marie-Elodie Perga¹

Received: 25 March 2019 / Accepted: 25 July 2019 / Published online: 30 July 2019
© The Author(s) 2019

Abstract

In this study, we experimentally investigated the degradation of dissolved organic matter (DOM) during lateral and vertical mixing of different water masses in a peri-alpine lake. River intrusions and vertical winter turnover in Lake Geneva (Switzerland, France) were simulated through short-term laboratory incubations by mixing riverine and lacustrine waters or lacustrine waters collected at different depths in winter. Dissolved organic carbon (DOC) degradation was monitored by dissolved oxygen (DO) measurements and changes in DOM composition were tracked by fluorescence spectroscopy in pure and mixed treatments during 72 h. Initial DOC content and DOM composition were relatively similar between end-members. The amount of DOC respired at the end of the incubation was similar between treatments, but decay constants in mixed treatments derived from a first-order decay model were significantly higher than expected values calculated based on a simple mass balance model. Fluorescent dissolved organic matter (FDOM) in mixed treatments followed non-conservative patterns that could not be predicted based on observations made in pure treatments. Hence, one protein-like and one microbial-like fluorophore that were consumed in lake waters were continuously produced in mixed treatments although lake waters represented 90% of the mix. No relationships were observed between the rate of DOC consumption and the initial DOM composition, suggesting that other factors such as nutrients and/or interactions in microbial communities were involved. Moreover, no relationships were found between DOC and FDOM patterns during incubations, suggesting that these measurements targeted different facets of microbial metabolism of DOM, respectively microbial respiration (catabolism) and microbial production (anabolism). While additional investigations are required in order to identify the drivers of these changes, this study provides evidence of non-conservative behavior of DOM degradation in mixing zones in lakes.

Keywords Dissolved organic matter · Respiration · Mixing events · Metabolism · Conservative behavior · Lakes

Introduction

The pool of dissolved organic matter (DOM) in aquatic ecosystems is a heterogeneous mixture of organic compounds that originate both from terrestrial sources (allochthonous DOM) and from primary production and processes of decomposition within water bodies (autochthonous DOM). Moving downstream along fluvial networks, DOM

is continuously processed by bacterial communities with important ecological and biogeochemical consequences. For example, DOM can be either mineralized as CO₂ and thus contribute to the net heterotrophy observed in many freshwater ecosystems (Battin et al. 2009; Lapierre et al. 2013) or it can be incorporated into microbial biomass and thus become available for higher trophic levels (Jansson et al. 2007; Cole et al. 2011).

The study of DOM degradation has been intensively examined for several decades, and numerous studies have shed light on the role of environmental factors such as temperature, UV radiation, nutrients, and bacterial community composition (Bano et al. 1998; Tranvik et al. 2001; del Giorgio and Davis 2003; Amaral et al. 2016) but also on the importance of intrinsic properties of DOM (Amon and Benner 1996; Volk et al. 1997; Catalán et al. 2013) on microbial DOM degradation. DOM degradation

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00027-019-0662-z>) contains supplementary material, which is available to authorized users.

✉ Thibault Lambert
Thibault.lambert@unil.ch

¹ Institute of Earth Surface Dynamics, University of Lausanne, Lausanne, Switzerland

is typically investigated within a single aquatic environment, i.e. in groundwater (Shen et al. 2015), streams and rivers (Balcarczyk et al. 2009; Lapierre et al. 2013; Catalán et al. 2017), wetlands (Bano et al. 1998) or lakes (Kothawala et al. 2012). Yet, these ecosystems are tightly connected within fluvial networks and DOM degradation at the transition zones of these aquatic ecosystems where DOM and bacterial communities from different origins mix has generated growing interest. The underlying reason is the so-called priming effect (PE), a process through which labile pool of DOM can enhance (“prime”) the degradation of a more recalcitrant DOM pool based on interactions between microbial communities and/or changes in their functions (Kuzakov et al. 2000; Guenet et al. 2010). DOM degradation at mixing interfaces could thus be characterized by a non-conservative behavior or, in other words, a specific pattern that cannot be predicted from the degradation dynamics observed in the adjacent ecosystems.

The occurrence as well as the relevance of the PE concept in aquatic ecosystems is however still debated. While DOM degradation was indeed experimentally enhanced by the addition of simple organic molecules in samples of several aquatic environments such as tropical (Ward et al. 2016) and temperate rivers (Hotchkiss et al. 2014) and lakes (Kankaala et al. 2013; Attermeyer et al. 2014b), neutral or even opposite pattern have also been reported. For instance, the addition of labile DOM did not stimulate the bacterial degradation of riverine DOM in marine coastal environments (Blanchet et al. 2017), neither hyporheic DOM in rivers (Bengtsson et al. 2014) nor lacustrine DOM (Catalán et al. 2015). According to a recent meta-analysis on the occurrence of PE in aquatic ecosystems, the concept relies on assumptions that may limit its incorporation into our understanding of C cycling in aquatic ecosystems (Bengtsson et al. 2018). Indeed, the basic approach for investigating the occurrence of PE consists in adding simple and labile organic molecules into a ambient sample containing the recalcitrant DOM pool. However, although this process remains microbially driven (Farjalla et al. 2009; Guenet et al. 2010), the role of microbial communities and their potential interaction are rarely taken into consideration (Bengtsson et al. 2018). Yet, recent studies have shown that the metabolic fate of DOM could shift between catabolism and anabolism depending on nutrient loadings (Guillemette and del Giorgio 2012) and/or on the relative contribution of terrestrial DOM versus algal DOM (Guillemette et al. 2016). Moreover, it is now well recognized that DOM degradation consists of a continuum of reactivity rather than in distinct pools of specific degradability that can simply be classified as labile or recalcitrant (Koehler et al. 2012). Therefore, the change in of DOM consumption when different water masses mix should be

better described as a conservative or non-conservative behavior rather than positive, null or negative PE (Bengtsson et al. 2018).

Lake ecosystems are important features of the carbon cycle at both the regional and global scales (Tranvik et al. 2009). The level of internal transformation increases as their water residence time extends (Catalán et al. 2016), attributing a greater role to large lakes (Weyhenmeyer et al. 2012). Large lakes are also highly dynamic from a hydrological perspective with complex circulation of water masses in both the lateral and vertical dimensions (Bouffard and Wüest 2019), generating water mixing events during which different DOM pools that have been primarily isolated in space and time get in contact and might create potential opportunities for non-conservative behavior of DOM degradation. For instance, littoral areas constitute important zones of primary production and, at the same time, receive substantial inputs of terrestrial DOM from tributaries. Terrestrial DOM is generally less bioavailable than DOM from algal or macrophyte origin (Bertilsson and Jones 2003; Guillemette et al. 2013) and it has been suggested that the release of labile compounds from autochthonous sources may enhance the bacterial degradation of the more recalcitrant terrestrial DOM pool (Farjalla et al. 2009; Guenet et al. 2010; Danger et al. 2013; Attermeyer et al. 2014a). Inputs of terrestrially derived DOM can affect the whole lake metabolism by stimulating bacterial respiration (e.g. Johengen et al. 2008), and recent field observation coupled with laboratory experiments have shown that river-borne turbidity currents—that convey riverine DOM directly into the deep lacustrine waters—could lead to a stimulation of bacterial respiration in Lake Geneva (Bouffard and Perga 2016). The effect of the mix between lake and river waters on DOM degradation has however not been investigated. Besides, the decrease in light penetration along the water column and the strong stratification in deep lakes may contribute to creating and segregating different pools of DOM. Gradual changes in DOM composition may occur along the water column, as a result of primary production being mainly restricted to the epilimnion, while the hypolimnion should host more degraded organic matter and/or organic material originated from sediments (Downing et al. 2008; Gonsior et al. 2013). This vertical gradient from freshly DOM produced in surface lake waters to more degraded material toward the bottom could be analogous to the lateral gradient between riverine terrestrial DOM and algal lacustrine DOM. As lake waters mix vertically by wind action combined with the weakening of the vertical stratification in fall and winter, one might expect that vertical winter turnover, bringing in contact different DOM pools, could be a hot-moment during which DOM from the bottom could be primed by surficial DOM.

We evaluated the potential occurrence of non-conservative behaviors of DOM degradation by simulating both

Table 1 Design of the incubation experiments

Treatment	Sites				
	SHL2-L1	SHL2-L2	SHL2-L3	Dranse (D)	Rhône (R)
SHL2-L1	100	–	–	–	–
SHL2-L2	–	100	–	–	–
SHL2-L3	–	–	100	–	–
Dranse	–	–	–	100	–
Rhône	–	–	–	–	100
L1 + L2	50	50	–	–	–
L1 + L2 + L3	33	33	33	–	–
D + L1 ^a	90	–	–	10	–
D + L2	–	90	–	10	–
D + L3	–	–	90	10	–
R + L1	90	–	–	–	10
R + L2	–	90	–	–	10
R + L3	–	–	90	–	10

Numbers refer to the proportion of each end-member in treatments. SHL2-L refers to Lake Geneva waters with SHL2-L1=0–50 m; SHL2-L2=180 m; SHL2-L3=250 m. Dranse (D) and Rhône (R) rivers were collected at surface (0.5 m)

^aNo dissolved oxygen measurements for this treatment, see text for details

riverine inputs (lateral mixing) and vertical turnover (vertical mixing) in Lake Geneva (Switzerland, France), assuming that the differences in OM quality between the various pools might result in non-conservative patterns. The experiment was conducted in February, i.e. before the time-period of maximal lake mixing that typically occurs in early May. The lateral and vertical structure of the lake was still strong enough so we could sample distinct DOM pools right before they come into contact by winter mixing. Short-term dark bioassays were performed with pure (lacustrine or riverine waters) and mixed treatments with samples collected in February 2017. Dissolved organic carbon (DOC) consumption was monitored by dissolved oxygen (DO) measurements and changes in DOM composition were tracked using excitation-emission fluorescence analyses. With this experimental design, we tested the hypothesis of a non-conservative behavior of DOM degradation at the mixing of river/lake and surface/deep lake waters.

Materials and methods

Experimental design and conceptual approach

The conservative or non-conservative behavior of DOM degradation during mixing was investigated with short-term (72 h) bioassays. The short duration of the incubations was motivated by experimental studies showing that non-conservative patterns of DOM degradation can occur at the daily (Hotchkiss et al. 2014; Bouffard and Perga 2016) or even at the hourly time-scale (Ward et al. 2016). In the

context of Lake Geneva, this duration is representative of the rapid hydrodynamical mixing processes (see study site description).

Pure (e.g. lake or riverine waters) and mixed treatments were incubated in a dark chamber at fixed temperature (6 °C, i.e. at the lake temperature at the time of the experiment). Lateral mixing was simulated by substituting lake waters by riverine waters at a 90:10 ratio while two treatments performed exclusively with lake waters were implemented to simulate an incomplete (L1 + L2) or complete (L1 + L2 + L3) winter turnover (Table 1). Details about sampling and water origin are provided below. Treatments, performed in triplicates, consisted of unfiltered waters incubated in 250 mL hermetically closed glass bottles. Different subsets of vials were prepared and sacrificed at 24 h, 48 h and 72 h in order to investigate changes in DOM composition over the incubation. DO concentrations were measured in vials equipped with SP-PSt7 oxygen planar sensor spots (PreSens). Initial DO was recorded 1 h after the start of the incubation and then DO variations were measured at 24 h, 48 h and 72 h using a PreSens Fibox 4 equipped with a fiber-optic oxygen transmitter. Calibration of the PreSens Fibox 4 (two-point calibration at 0 and 100% oxygen saturation) was performed before incubations. The L1 + D treatment was equipped with other oxygen planar sensor spots (SP-PSt3, PreSens), but it was not possible to record DO concentrations due to instrumental problem with the PreSens Fibox 3 used for data reading of these spots.

Measured oxygen data were used to derive parameters for DOC degradation instead of direct DOC measurements because our analytical sensitivity was not good enough

for capturing carbon losses at the short-time scale of our experiment and for such low DOC concentrations ($\sim 1 \text{ mg L}^{-1}$). Changes in DOC concentrations over time due to heterotrophic respiration are typically modeled by a first-order decay model (Guillemette and del Giorgio 2011; Richardson et al. 2013). In our study DO consumption curves were modeled according to the equation:

$$\text{DO}(t) = \text{DO}_{\text{cons}} \times e^{-k \cdot t} + \text{DO}_{\text{residual}} \quad (1)$$

where $\text{DO}(t)$ is the DO concentration measured during the incubation time (t , in hours), DO_{cons} the amount of DO respired at the end of the incubation, k the decay constant (or consumption rates), and $\text{DO}_{\text{residual}}$ the concentration of the residual pool remaining in solution after 72 h of incubation and for which $k=0$. We used triplicate measurements to derive one model per treatment ($R^2 > 0.98$ for all treatments) and obtained best-fit values as well as upper and lower 95% confidence level values for each parameter of Eq. 1. Cumulative DO respired (DO_{cons}) and oxygen consumption rates (k) were then converted to CO_2 production in order to provide an estimate of DOC consumption (DOC consumed and k in $\mu\text{M C h}^{-1}$) using a respiratory quotient of 1 (Guillemette and del Giorgio 2011).

Representativeness of the model and potential limitations

We are aware that a two-pools model is a simplification of DOM reactivity and that other models such as the Reactivity Continuum Concept (Koehler et al. 2012) or multiple component first-order decay models (Richardson et al. 2013) describe DOM degradation in more realistic ways. However the number of data points was too limited to apply the Reactivity Continuum Concept and adding one pool in our first-order decay model did not improve significantly the goodness of fits (data not shown). In this scheme, we considered that DOM bioavailability consists of one labile pool that would be consumed within 72 h and a second pool that is recalcitrant, or residual at the time-scale of our experiment. The decay constant k describes the inflexion of the consumption curve over time and provides information on the transition from the more labile pool to the more recalcitrant pool, i.e. the highest the k values, the faster the labile pool is consumed.

Potential limitations of our approach arise from the limited number of DO measurements over the first 24 h as well as from the fact that all treatments were realized with unfiltered waters. Respiration measurements for lake and river waters of the Lake Geneva Basin obtained in June 2017 and November 2018 using the same flasks but with more frequent DO measurement over the first 48 h ($t+5$ h and $t+12$ h, personal data) confirmed that a first-order decay model adequately reproduced the experimental data,

supporting therefore the validity of the model (data not shown). Moreover, the latter were performed with waters filtered at $0.7 \mu\text{m}$, a common pore-size to remove most particles and eukaryotes while leaving the prokaryotic community largely intact. The similar inflexion of consumption curves (e.g. similar range of k values) observed in this study and in June 2017 and November 2018 and the fact that, in each case, the model adequately fits the data strongly suggests that DO decline can be mainly attributed to the microbial respiration. Respiration measured in our study will be nevertheless referred as to community respiration.

Theoretical effects of mixing experiments and statistical analysis

The theoretical dynamic of DOC consumption in the mixed treatments (under the assumption of a conservative behavior) was determined by a linear mass balance approach. The expected k value (k_{exp}) as well as the expected total amount of DOC respired at the end of the incubation ($\text{DOCrespired}_{\text{exp}}$) in each mixed treatment were computed as follows (Eqs. 2 and 3):

$$k_{\text{exp}} = \sum_{i=1}^n k_i \times \% \text{source}_i \quad (2)$$

and

$$\text{DOCrespired}_{\text{exp}} = \sum_{i=1}^n \text{DOCrespired}_i \times \% \text{source}_i \quad (3)$$

where i is the number of source (or pure treatment) used in the mix (from 2 to 3), k the decay constant and DOCrespired_i the total amount of DOC respired at the end of the incubation with the pure source i , and $\% \text{source}_i$ the proportion of the water source i to the mixed sample (see Table 1). Best fit values and values at 95% confidence interval obtained by modeling approaches were used in order to provide a range of theoretical values for each parameter. The observed and expected k and DOCrespired values were then compared using one tailed, paired t test. All analyses were run with GraphPad Prism® version 8.

Study site and sampling

Lake Geneva is the largest lake of Western Europe (volume = 89 km^3 , surface = 580 km^2 , max. depth = 309 m) located at the border between France and Switzerland in the Western Alps. The lake typically overturns partially (down to 100–200 m depth) each winter in late February/March while the complete vertical mixing occurs only every fifth year on average. Vertical turnover typically occurs at the daily scale (CIPEL 2018). About 84% of the water input

originates from the two main alpine rivers, the Rhône (~75%) and the Dranse rivers (~9%), both flowing into the eastern basin. On average, Lake Geneva indeed has long water residence time (11 years), but hydrodynamical processes are quite variable in time and space, from micro- to macro-scales (Bouffard and Wüest 2019). Processes related to lateral mixing occur at short time scales. For instance, the Rhône inflow sinks and mixes quickly when penetrating the lake due to density gradient and turbulences in winter, while most of the riverine waters flows as an interflow in summer that can be traced up to 55 km in the lake after 4–5 days (Halder et al. 2013; Bouffard and Perga 2016). Vertical mixing exerts at both short (daily-weekly convection) and longer (progressive destratification during winter) scales. The water quality of Lake Geneva and its two main tributaries is continuously monitored by the FOEN (Federal Office for the Environment, Switzerland) for the Rhône and by the Observatory of Alpine Lakes for the Dranse and Lake Geneva (SHL2 station, <https://www6.inra.fr/soere-ola>). Public databases were visited in order to obtain nutrient concentrations at closest date to our sampling, i.e. the 15/02/2017 for Lake Geneva at SHL2 (surface), the 20/02/2017 for the Rhône and the 20/02/2017 for the Dranse.

Samples for mixing experiments were collected on 20th February 2017 in Lake Geneva and its main tributaries, the Rhône and Dranse rivers. Water from the lake (30 L per sample) was collected using a 5 L integrated water sampler (HydroBios) at the long-term monitoring station SHL2, located at the central area of the lake and where the influence of river intrusion from the Rhône River is the lowest. The vertical mixing at this day reached down to ~120 m depth based on oxygen concentrations and temperature profiles (Supplementary Fig. 1) measured with a pre-calibrated multi-parameters profiler (Sea&Sun Technology, CTD-90 multi-parameter probe). We chose three different depths in an attempt to collect contrasted water masses that could be a priori associated with different DOM compositions, i.e. epilimnion (L1=0–50 m), upper (L2=180 m) and deeper hypolimnion (L3=250 m). Water from the Rhône and Dranse rivers was collected manually in 10 L HDPE bottles ~1–2 km upstream from the entrance into the lake. Samples were brought to the laboratory and prepared for incubations after one over-night storage at 6 °C in the dark.

DOC concentrations and fluorescence measurements

The DOC concentrations of the lake and river waters (hereafter referred as endmembers) were measured on filtered samples (0.2 µm, nylon syringe filters, Sartorius) using a TOC-L analyzer (Shimadzu). Fluorescent DOM (FDOM) for end-members and during the incubation was measured on filtered waters at a pore size of 0.2 µm using Nylon

syringe filters (Sartorius). Filters were rinsed with ~10 mL of solution before collecting samples in vials. Samples for FDOM analyses were collected in 20 mL amber glass vials and stored in the dark in a refrigerator before analyses that were typically done during the week after sampling. Additional samples collected in the lake were used to build a parallel factor analysis (PARAFAC) model (total number of samples = 216). DOC and FDOM measurements for collected samples were not replicated. Fluorescence intensity was measured with a Fluorolog-3 spectrometer (Horiba) using a 1 cm quartz cuvette across excitation wavelengths of 240–450 nm (5 nm increment) and emission wavelengths of 290–550 nm (2.5 nm increment) in order to build excitation-emission matrices (EEMs). EEMs were acquired in sample emission to lamp reference mode, and a correction matrix provided by the manufacturer in both excitation and emission dimensions was automatically applied during acquisition. EEMs were then decomposed into different components using PARAFAC algorithms (Stedmon et al. 2003). EEMs preprocessing (Raman scattering removal and standardization to Raman units) was performed prior the PARAFAC modeling. Normalization was done using a Milli-Q water sample run the same day as the sample (Zepp et al. 2004). A five components PARAFAC model was obtained using the drEEM Toolbox (Murphy et al. 2013) for MATLAB (MathWorks, Natick, MA, USA). Split-half analysis, random initialization and visualization of residuals EEMs were used to test and validate the model. The spectral loadings of the PARAFAC components (Supplementary Fig. 2) were compared with the open fluorescence database OpenFluor using the OpenFluor add-on for the open-source chromatography software OpenChrom (Murphy et al. 2014).

All five components matched the excitation and emission spectra of previously identified components from 39 independent studies (similarity score > 0.95, Table 2). Components C1 and C4 were classified as humic-like and are often associated with a terrestrial origin (Stedmon and Markager 2005; Graeber et al. 2012; Lambert et al. 2017). Components C2 and C3 are respectively identified as tyrosine- and tryptophan-like fluorophores and are associated with proteins derived from autochthonous production (Romera-Castillo et al. 2011; Amaral et al. 2016). The last component C5 is similar to protein-like fluorophores derived from microbial activity (Williams et al. 2010).

Results

Oxygen consumption

No statistical differences were observed in the cumulative amount of DOC respired after 72 h across treatments (Fig. 2a, b) despite differences in the inflexion in the DO/

Fig. 1 Time-course of DO/DO_i curves during the 72 h of incubation for all treatments except L1 + D (see text for details). Measured data (black circles) and best fit models (black lines) are shown for both pure (grey backgrounds) and mixed (white backgrounds) treatments. The best fit for models calculated from mass balances are plotted for

comparison for mixed treatments (red lines). Using DO concentration curves or DO/DO_i curves provided the same estimations for k and DOC consumed estimations but DO/DO_i curves were preferred for visual comparison (color figure online)

DO_i vs. time curves (Fig. 1). The total amount of DOC respired ranged from 12.3 to 16.8 $\mu\text{M C}$ in pure treatments and from 12.8 to 14.9 $\mu\text{M C}$ in mixed treatments for which observed values matched those expected from the linear mixing model. Decay constants were however significantly higher in the mixed treatments ($0.117 \pm 0.02 \mu\text{M C h}^{-1}$, $n=6$) compared to pure treatments ($0.057 \pm 0.017 \mu\text{M C h}^{-1}$, $n=5$; Fig. 2c, d) as illustrated by the strongest inflexion in the DO/DO_i vs. time curves in the mixed treatments (Fig. 1). The observed DOC consumption rates significantly exceeded those predicted by the mass balance approach by a factor from 1.5 to 2.9. No relationships were found between the total amount of DOC respired or the decay constants and the fluorescence intensities of PARAFAC components (Supplementary Fig. 3).

Initial DOM content and composition and fluorescence patterns during incubations

DOC concentrations ranged from 1.0 to 1.6 mg L^{-1} , with highest values in the upper layer of the lake and in the

Dranse River (Fig. 3a). Total fluorescent intensities (F_{Tot}) were however not related to DOC concentrations: F_{Tot} was higher in the Rhône River compared to the Dranse River despite a lower DOC content and the highest fluorescence signal in the lake was recorded at 180 m (L2), where DOC was the lowest. The fluorescence signal in Lake Geneva was dominated by protein-like fluorophores C2 and C3 at all depths, the value of F_{Max} for C2 being especially high at the 180 m depth. Overall, the F_{Max} values for C1 and C4 components were the highest in the Rhône and the Dranse but both rivers exhibited similar (for the Dranse) or even higher (for the Rhône) fluorescence intensities for C2 and C3 components compared to lake waters. The microbial-like component C5 showed the lowest F_{Max} values both in lake and in river waters.

Overall, the humic-like components C1 and C4 exhibited stable F_{Max} values along the incubations in all treatments (Fig. 4). The tyrosine-like component C2 remained relatively stable in the lake and river waters, with only a significant increase in the Dranse River. Yet, the F_{Max} values for this C2 component were highly variable in the mixed treatments

Fig. 2 **a** DOC consumed at the end of the incubation in pure lake and river waters, **b** expected and observed DOC consumption in mixed treatments, **c** decay constants k in pure lake and rivers waters and **d** expected and observed k values in mix. Error bars represent standard deviation based on best fit values and 95% confidence level values (see text for details). Differences between observed and expected values in mixed treatments were tested using one tailed paired t test, where ns corresponds to not significant, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

Table 2 Fluorescence properties and general description of the five PARAFAC components identified in this study

Component	Ex _{max} (nm)	Em _{max} (nm)	Description
C1	< 250 (320)	420	Humic-like fluorophore, associated with terrestrial sources ^{a-c}
C2	270	310	Protein (tyrosine)-like fluorophore, derived from autochthonous production ^{a,f}
C3	275	330	Protein (tryptophan)-like fluorophore, derived from autochthonous production ^{a,d}
C4	< 250 (370)	475	Humic-like fluorophore, associated with terrestrial sources ^{a-c}
C5	295	365	Protein-like fluorophore, microbial-produced ^e

Comparisons were made using the OpenFLuor database with a similarity score > 0.95

^aStedmon and Markager (2005)

^bLambert et al. (2017)

^cGraeber et al. (2012)

^dAmaral et al. (2016)

^eWilliams et al. (2010)

^fFellman et al. (2010)

so no clear trend was apparent. More diverse patterns were however observed for the tryptophan-like C3 and the microbial-like C5 components between the sources and between the pure and mixed treatments. Thus, both the C3 and C5 components exhibited decreasing F_{Max} values in the lake waters (especially C3 that was depleted after 24 h) but they remained stable in the Rhône and Dranse rivers (C3 even increased in the Dranse). More unexpectedly, despite the fact that lake waters were substituted by only 10% of river waters in mixed treatments, C3 and C5 behave in the mixed

treatments as in the pure riverine waters (stable to increasing signal).

Discussion

Non-conservative effects on DOM degradation

This study tested the hypothesis of a non-conservative behavior in DOM degradation during mixing events in a large peri-alpine lake. DO measurements were used to

Fig. 3 **a** Initial DOC concentration (grey bars) and F_{Tot} values (black circles) and **b** initial FDOM composition in water samples collected in Lake Geneva, the Rhône and Dranse rivers at the time of sample collection. Error bars for DOC concentrations represent the analytical precision

derive parameters for organic carbon dynamics instead of direct DOC measurements because of the short duration of our incubation and the lack of analytical sensitivity that prevented us from measuring carbon losses in the flasks at the low ambient concentrations. Samples were collected in winter, i.e. at a moment of low river discharge and low primary production in the lake, and similar DOC concentrations were measured amongst end-members. Moreover, the composition of the initial lacustrine and riverine pools both presented high contribution of the protein-like fraction (C2 + C3) known as being more bioavailable than the humic-like component of the DOM pool (Fellman et al. 2010; Cory and Kaplan 2012). The elevated relative contribution of these compounds in the FDOM pool in the Dranse (37%) and Rhône (66%) rivers likely reflects the influence of agricultural and urban areas around Lake Geneva, as human land use favors the production of proteinaceous organic material in aquatic (Wilson and Xenopoulos 2009) and terrestrial (Lambert et al. 2017) environments. The lack of large differences in initial DOM pools at this specific sampling period was probably the reason why no significant differences were observed in the total DOC respired in the pure treatments.

However, a non-conservative behavior was clearly observed in mixed treatments for which measured decay constants k were higher by a factor of 1.6–2.9 than the expected values while total DOC consumed matched expectations. It is noteworthy that a stimulation of respiration was also observed in treatments simulating a vertical mixing in the water column of the lake. The apparent discrepancy between higher k but similar DOC consumed in mixed treatments compared to expectations could simply indicate that a very small pool of DOC is fueling respiration. This pool would be exhausted faster in mixed treatments, denoting that respiration at the timescale of our experiment was C limited. Indeed, DOM exported during baseflow period has a typically low bioavailability compared to DOM exported during high flow events (Ågren et al. 2008; Fellman et al. 2009b), and similarly low level of easily degradable DOM can be expected in Lake Geneva because of the low winter primary production. In a recent study, Bouffard and Perga (2016) found that the substitution of 1–10% of Lake Geneva waters collected at 100 m depth at the SHL2 station by water from the Dranse River stimulated the bacterial respiration by increasing the amount of DO consumed by 60% after 86 h of incubation. In their study, waters were collected during another period, in autumn, when primary production is still significant in Lake Geneva. The strong effect of the mixing on bacterial respiration and DOC consumption could therefore be linked to more distinct initial pools. In contrast, they detected no significant effect when lake waters collected at 200 m depth were mixed with the same riverine waters. Hence, this study and our results point that mixing of different water masses in the Lake Geneva Basin does not necessarily result in changes in respiration rates and/or DOC consumption.

Contrary to our initial hypothesis, we found no relationships between the patterns of DOC consumption and initial DOM composition, neither in terms of total DOC respired nor in the decay constant k (Supplementary Fig. 3). Yet, bioassays revealed that bacteria imparted different FDOM signatures depending on sources (lake waters *versus* river waters) and treatments (non-linear behavior in mix treatments). It is now well documented that bacterial communities have the ability to degrade and/or generate various types of fluorescent compounds. Although considered recalcitrant and mainly from terrestrial origin, consumption (Moran et al. 2000; Romera-Castillo et al. 2011; Fasching et al. 2014) as well as production (Guillemette and del Giorgio 2012; Amaral et al. 2016; Kinsey et al. 2018) of humic-like components by heterotrophic bacteria have been previously reported. Similarly, proteinaceous compounds are considered as a highly labile pool of FDOM (Fellman et al. 2009a; Cory and Kaplan 2012) but net production of protein-like fractions during bioassays have also been reported elsewhere

Fig. 4 Time-course of the different fluorescent components identified in this study. The two left panels show the evolution of PARAFAC components in pure treatments, those in the middle in treatments sim-

ulating a vertical mixing and the two right panels in treatments simulating lateral mixing. Standard deviation for triplicate measurements are shown, but error bars may eventually be smaller than the symbols

(Cammack et al. 2004; Lønborg et al. 2009; Guillemette and del Giorgio 2012). Consequently, both humic-like and protein-like fractions of FDOM can be considered as a substrate or a by-product of heterotrophic activity and thus their net production or consumption may provide information on microbial metabolism (Guillemette and del Giorgio 2012).

At the time-scale of our incubations, humic-like components C1 and C4 appeared to be biologically inert and no clear difference in the behavior of the protein-like component C2 could be observed between the sources and the mix treatments (Fig. 4). Divergent bacterial-FDOM interactions across treatments were illustrated by the evolution of the

protein-like component C3 and the microbial-like component C5. If we acknowledge that variations in the F_{Max} values resulted from the balance between production *versus* consumption processes, decreasing intensities for C3 and C5 in lake waters implied that these compounds were consumed during incubations. Increasing or stable patterns found in the Dranse and Rhône rivers implied however a net production (e.g. C3 in the Dranse River) or an equilibrium between production and consumption in rivers. Relatively stable F_{Max} values for these components in mixed treatments denoted therefore a clear and persistent non-consistent behavior considering that river waters represented only 10% of the mix or were even absent (vertical mixing). Applying a similar mass balance approach as for DOC consumption (e.g. Eq. 2 where k is replaced by F_{Max}) confirmed that F_{Max} values reached at the end of the incubation in mixed treatments were significantly higher ($p < 0.01$ in all cases) than expected values. We found no relationships with respiration patterns, suggesting that these measurements targeted different facets of microbial metabolism of DOM. Therefore, we hypothesize that FDOM patterns were indicative of changes in bacterial production where C3 and C5 could represent microbial metabolites continuously produced in mixed treatments while consumed in lake waters.

Potential drivers of non-conservative behaviors

Our DO consumption measurements and the evolution of FDOM components C3 and C5 support the idea that DOM degradation during mixing events can operate distinctively from what can be observed and predicted from original water masses, even in low-DOM environments such as Lake Geneva. This result is noteworthy since it was observed in mixing endmembers having similar DOC concentrations and similar DOM composition while contrasting DOM pools are often the main cause considered when investigating PE (Bengtsson et al. 2018). Although FDOM is only a portion of the DOM pool that does not necessarily correlate with the biodegradable pool of DOM (Lu et al. 2013; Coble et al. 2019), our study suggests that non-consistent patterns cannot solely be attributed to DOM composition and that other factors are involved.

The first hypothesis is related to the effect of nutrients. We did not measure directly the nutrient concentrations, but waters of Lake Geneva are typically nutrient-depleted compared to inflowing tributaries (CIPEL 2018), as illustrated by water quality data obtained from local agencies near the period of sampling showing lower level of phosphorus and nitrogen in the surface of the lake at SHL2 ($10 \mu\text{g-P L}^{-1}$ and $680 \mu\text{g-N L}^{-1}$) compared to the Rhône ($24 \mu\text{g-P L}^{-1}$ and $900 \mu\text{g-N L}^{-1}$) and the Dranse ($14 \mu\text{g-P L}^{-1}$ and $1600 \mu\text{g-N L}^{-1}$). Increasing the level of nutrients in mixed treatments could have therefore alleviated nutrient limitations in Lake

Geneva waters and increased respiration rates in mixed treatments. A support for this hypothesis is the fact that decay constants tended to be higher in the Dranse and Rhône treatments compared to treatments performed with Lake Geneva waters (Fig. 2a, c). Moreover, both nitrogen and phosphorus can modulate the production of particular FDOM fractions by controlling the bacterial growth efficiency (Biers et al. 2007; Guillemette and del Giorgio 2012), an effect that could explain the specific patterns observed for C3 and C5 components in mixed treatments.

Another and complementary hypothesis would be that the higher respiration rates and the changes in bacterial production in mix treatments would result from a greater microbial diversity (Farjalla 2014). Different aquatic ecosystems or water bodies with contrasted limnological properties may not only transport DOM of various quality, but also different bacterial communities with specific community trait structure (Ruiz-González et al. 2015; Logue et al. 2016). As ecosystem functioning increases with increasing species diversity (Bell et al. 2005; Hooper et al. 2005), interactions between microbial communities such as co-metabolism in confluence zones of high microbial diversity could be of prime importance for the metabolic fate of DOM.

Conclusion

In this study we investigated the potential occurrence of non-conservative patterns in DOM degradation during mixing events by simulating both lateral river inputs into the lake and vertical turnover occurring during the winter period. Our results showed that while the amount of DOC consumed after 72 h was similar across treatments, the dynamic of this consumption was faster in the mixed rather than in the pure waters. The higher decay constants k in mixed treatments clearly pointed towards a non-conservative behavior, which was not related to the initial conditions of the end-members as they had similar DOC concentration and composition inferred from optical properties at the period of sampling. Keeping in mind that DOM concentration and composition vary seasonally in inland waters, our observations nevertheless imply that contrasting levels of DOM content and/or composition are not necessarily a prerequisite to non-conservative patterns during mixing events. Additionally, a specific evolution of the protein-like and microbial-like fluorescent components were observed in the mixed treatments, suggesting a change in microbial production. While our results clearly show that DOM degradation patterns during mixing events deviate from what can be expected based on observations made in pure water masses, the nutrient-bacterial communities interactions and their net effect on

the DOM metabolic fate should deserve more attention in future work.

Acknowledgements This work was funded by the Fonds National Suisse (FNS, CARBOGEN). Water quality data were kindly provided by the FOEN (Federal Office for the Environment, Switzerland) for the Rhône) and by the Observatory of Alpine Lakes for the Dranse and Lake Geneva (SHL2 station, <https://www6.inra.fr/soere-ola>). We thank Laetitia Monbarron and Micaela Faria for assistance in the laboratory, Marttiina Rantala and Nicolas Escoffier for their comments on the manuscript as well as anonymous reviewers and the editor whose comments helped to improve the first version of the manuscript.

Data availability Full data-set will be made publicly available at <http://www.zenodo.org> should the manuscript be accepted in Aquatic Sciences. The PARAFAC model (including details on measurements, correction and PARAFAC loadings) will be uploaded on the OpenFluor database.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Ågren A, Berggren M, Laudon H, Jansson M (2008) Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshw Biol* 53:964–972. <https://doi.org/10.1111/j.1365-2427.2008.01955.x>
- Amaral V, Graeber D, Calliari D, Alonso C (2016) Strong linkages between DOM optical properties and main clades of aquatic bacteria. *Limnol Oceanogr* 61:906–918. <https://doi.org/10.1002/lno.10258>
- Amon RMW, Benner R (1996) Bacterial utilization of different size classes of dissolved organic matter. *Limnol Ocean* 41:41–51
- Attermeyer K, Hornick T, Kayler ZE et al (2014a) Enhanced bacterial decomposition with increasing addition of autochthonous to allochthonous carbon without any effect on bacterial community composition. *Biogeosciences* 11:1479–1489. <https://doi.org/10.5194/bg-11-1479-2014>
- Attermeyer K, Tittel J, Allgaier M et al (2014b) Effects of light and autochthonous carbon additions on microbial turnover of allochthonous organic carbon and community composition. *Microb Ecol* 69:361–371. <https://doi.org/10.1007/s00248-014-0549-4>
- Balcarczyk KL, Jones JB, Jaffé R, Maie N (2009) Stream dissolved organic matter bioavailability and composition in watersheds underlain with discontinuous permafrost. *Biogeochemistry* 94:255–270. <https://doi.org/10.1007/s10533-009-9324-x>
- Bano N, Moran MA, Hodson RE (1998) Photochemical formation of labile organic matter from two components of dissolved organic carbon in a freshwater wetland. *Aquat Microb Ecol* 16:95–102. <https://doi.org/10.3354/ame016095>
- Battin TJ, Kaplan LA, Findlay S et al (2009) Biophysical controls on organic carbon fluxes in fluvial networks. *Nat Geosci*. <https://doi.org/10.1038/ngeo101>
- Bell T, Newman JA, Silverman BW et al (2005) The contribution of species richness and composition to bacterial services. *Nature* 436:1157–1160. <https://doi.org/10.1038/nature03891>
- Bengtsson MM, Wagner K, Burns NR et al (2014) No evidence of aquatic priming effects in hyporheic zone microcosms. *Sci Rep* 4:1–6. <https://doi.org/10.1038/srep05187>
- Bengtsson MM, Attermeyer K, Catalán N (2018) Interactive effects on organic matter processing from soils to the ocean: are priming effects relevant in aquatic ecosystems? *Hydrobiologia*. <https://doi.org/10.1007/s10750-018-3672-2>
- Bertilsson S, Jones JB (2003) Supply of dissolved organic matter to aquatic ecosystems: autochthonous sources. In: *Aquatic Ecosystems*. Academic Press, pp 3–24
- Biers EJ, Zepp RG, Moran MA (2007) The role of nitrogen in chromophoric and fluorescent dissolved organic matter formation. *Mar Chem* 103:46–60. <https://doi.org/10.1016/j.marchem.2006.06.003>
- Blanchet M, Pringault O, Panagiotopoulos C et al (2017) When riverine dissolved organic matter (DOM) meets labile DOM in coastal waters: changes in bacterial community activity and composition. *Aquat Sci* 79:27–43. <https://doi.org/10.1007/s00027-016-0477-0>
- Bouffard D, Perga ME (2016) Are flood-driven turbidity currents hot spots for priming effect in lakes? *Biogeosciences* 13:3573–3584. <https://doi.org/10.5194/bg-13-3573-2016>
- Bouffard D, Wüest A (2019) Convection in lakes. *Annu Rev Fluid Mech* 51:189–215. <https://doi.org/10.1146/annurev-fluid-010518-040506>
- Cammack WKL, Kalf J, Prairie YT, Smith EM (2004) Fluorescent dissolved organic matter in lakes: relationship with heterotrophic metabolism. *Limnol Ocean* 49:2034–2045
- Catalán N, Obrador B, Felipe M, Pretus JL (2013) Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake. *Aquat Sci* 75:581–593. <https://doi.org/10.1007/s00027-013-0302-y>
- Catalán N, Kellerman AM, Peter H et al (2015) Absence of a priming effect on dissolved organic carbon degradation in lake water. *Limnol Oceanogr* 60:159–168. <https://doi.org/10.1002/lno.10016>
- Catalán N, Marcé R, Kothawala DN, Tranvik LJ (2016) Organic carbon decomposition rates controlled by water retention time across inland waters. *Nat Geosci* 9:501–504. <https://doi.org/10.1038/ngeo2720>
- Catalán N, Casas-Ruiz JP, von Schiller D, et al (2017) Biodegradation kinetics of dissolved organic matter chromatographic fractions, a case study in an intermittent river. *J Geophys Res Biogeosciences* 122:131–144. <https://doi.org/10.1002/2016JG003512>
- CIPEL (2018) Evolution physico-chimique des eaux du Léman. Rapport de la Commission Internationale de la Protection des Eaux du Lac Léman, Campagne 2017:18–66
- Coble AA, Koenig LE, Potter JD et al (2019) Homogenization of dissolved organic matter within a river network occurs in the smallest headwaters. *Biogeochemistry* 143:85–104. <https://doi.org/10.1007/s10533-019-00551-y>
- Cole JJ, Carpenter SR, Kitchell J et al (2011) Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proc Natl Acad Sci* 108:1975–1980. <https://doi.org/10.1073/pnas.1012807108>
- Cory RM, Kaplan LA (2012) Biological lability of streamwater fluorescent dissolved organic matter. *Limnol Oceanogr* 57:1347–1360. <https://doi.org/10.4319/lo.2012.57.5.1347>
- Danger M, Cornut J, Chauvet E et al (2013) Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams : a case of aquatic priming effect ? Antoine Lecerf Published by : Wiley Stable. <http://www.jstor.org/stable/23596949> REFERENCES Linked references are available on. *Ecology* 94:1604–1613. <https://doi.org/10.1890/12-0606.1>
- del Giorgio PA, Davis J (2003) Patterns in DOM lability and consumption across aquatic ecosystems. In: *Aquatic Ecosystems*. Academic Press, pp 399–424
- Downing BD, Bergamaschi BA, Evans DG, Boss E (2008) Assessing contribution of DOC from sediments to a drinking-water reservoir

- using optical profiling. *Lake Reserv Manag* 24:381–391. <https://doi.org/10.1080/07438140809354848>
- Farjalla VF (2014) Are the mixing zones between aquatic ecosystems hot spots of bacterial production in the Amazon River system? *Hydrobiologia* 728:153–165. <https://doi.org/10.1007/s10750-014-1814-8>
- Farjalla VF, Marinho CC, Faria BM et al (2009) Synergy of fresh and accumulated organic matter to bacterial growth. *Microb Ecol* 57:657–666. <https://doi.org/10.1007/s00248-008-9466-8>
- Fasching C, Behounek B, Singer GA, Battin TJ (2014) Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams. *Sci Rep* 4:1–7. <https://doi.org/10.1038/srep04981>
- Fellman JB, Hood E, D'Amore DV et al (2009a) Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rain-forest watersheds. *Biogeochemistry* 95:277–293. <https://doi.org/10.1007/s10533-009-9336-6>
- Fellman JB, Hood E, Edwards RT, D'Amore DV (2009b) Changes in the concentration, biodegradability, and fluorescent properties of dissolved organic matter during stormflows in coastal temperate watersheds. *J Geophys Res Biogeosci* 114:1–14. <https://doi.org/10.1029/2008JG000790>
- Fellman JB, Hood E, Spencer RGM (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: a review. *Limnol Oceanogr* 55:2452–2462. <https://doi.org/10.4319/lo.2010.55.6.2452>
- Gonsior M, Schmitt-Kopplin P, Bastviken D (2013) Depth-dependent molecular composition and photo-reactivity of dissolved organic matter in a boreal lake under winter and summer conditions. *Biogeosciences* 10:6945–6956. <https://doi.org/10.5194/bg-10-6945-2013>
- Graeber D, Gelbrecht J, Pusch MT et al (2012) Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams. *Sci Total Environ* 438:435–446. <https://doi.org/10.1016/j.scitotenv.2012.08.087>
- Guenet B, Danger M, Abbadie L, Lacroix G (2010) Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology* 91:2850–2861. <https://doi.org/10.1890/09-1968.1>
- Guillemette F, del Giorgio PA (2011) Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. *Limnol Oceanogr* 56:734–748. <https://doi.org/10.4319/lo.2011.56.2.0734>
- Guillemette F, del Giorgio PA (2012) Simultaneous consumption and production of fluorescent dissolved organic matter by lake bacterioplankton. *Environ Microbiol* 14:1432–1443. <https://doi.org/10.1111/j.1462-2920.2012.02728.x>
- Guillemette F, McCallister SL, Del Giorgio PA (2013) Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes. *J Geophys Res Biogeosci* 118:963–973. <https://doi.org/10.1002/jgrg.20077>
- Guillemette F, Leigh McCallister S, Del Giorgio PA (2016) Selective consumption and metabolic allocation of terrestrial and algal carbon determine allochthony in lake bacteria. *ISME J* 10:1373–1382. <https://doi.org/10.1038/ismej.2015.215>
- Halder J, Decrouy L, Vennemann TW (2013) Mixing of Rhône River water in Lake Geneva (Switzerland–France) inferred from stable hydrogen and oxygen isotope profiles. *J Hydrol* 477:152–164. <https://doi.org/10.1016/j.jhydrol.2012.11.026>
- Hooper DU, Chapin FS, Ewel JJ et al (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr* 75:3–35. <https://doi.org/10.1890/04-0922>
- Hotchkiss ER, Hall RO, Baker MA et al (2014) Modeling priming effects on microbial consumption of dissolved organic carbon in rivers. *J Geophys Res Biogeosci* 119:982–995. <https://doi.org/10.1002/2013JG002599>
- Jansson M, Persson L, De Roos AM et al (2007) Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends Ecol Evol* 22:316–322. <https://doi.org/10.1016/j.tree.2007.02.015>
- Johengen TH, Biddanda BA, Cotner JB (2008) Stimulation of lake michigan plankton metabolism by sediment resuspension and river runoff. *J Great Lakes Res* 34:213–227
- Kankaala P, Lopez Bellido J, Ojala A et al (2013) Variable production by different pelagic energy mobilizers in boreal lakes. *Ecosystems* 16:1152–1164. <https://doi.org/10.1007/s10021-013-9674-z>
- Kinsey JD, Corradino G, Ziervogel K et al (2018) Formation of chromophoric dissolved organic matter by bacterial degradation of phytoplankton-derived aggregates. *Front Mar Sci* 4:1–16. <https://doi.org/10.3389/fmars.2017.00430>
- Koehler B, Von Wachenfeldt E, Kothawala D, Tranvik LJ (2012) Reactivity continuum of dissolved organic carbon decomposition in lake water. *J Geophys Res Biogeosci* 117:1–14. <https://doi.org/10.1029/2011JG001793>
- Kothawala DN, von Wachenfeldt E, Koehler B, Tranvik LJ (2012) Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations. *Sci Total Environ* 433:238–246. <https://doi.org/10.1016/j.scitotenv.2012.06.029>
- Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biol Biochem* 32:1485–1498. [https://doi.org/10.1016/S0038-0717\(00\)00084-5](https://doi.org/10.1016/S0038-0717(00)00084-5)
- Lambert T, Bouillon S, Darchambeau F et al (2017) Effects of human land use on the terrestrial and aquatic sources of fluvial organic matter in a temperate river basin (The Meuse River, Belgium). *Biogeochemistry* 136:191–211. <https://doi.org/10.1007/s10533-017-0387-9>
- Lapierre JF, Guillemette F, Berggren M, Del Giorgio PA (2013) Increases in terrestrially derived carbon stimulate organic carbon processing and CO₂ emissions in boreal aquatic ecosystems. *Nat Commun*. <https://doi.org/10.1038/ncomms3972>
- Logue JB, Stedmon CA, Kellerman AM et al (2016) Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *ISME J* 10:533–545. <https://doi.org/10.1038/ismej.2015.131>
- Lønborg C, Álvarez-Salgado XA, Davidson K, Miller AEJ (2009) Production of bioavailable and refractory dissolved organic matter by coastal heterotrophic microbial populations. *Estuar Coast Shelf Sci* 82:682–688. <https://doi.org/10.1016/j.ecss.2009.02.026>
- Lu Y, Bauer JE, Canuel EA et al (2013) Photochemical and microbial alteration of dissolved organic matter in temperate headwater streams associated with different land use. *J Geophys Res Biogeosci* 118:566–580. <https://doi.org/10.1002/jgrg.20048>
- Moran MA, Sheldon WM, Zepp RG (2000) Carbon loss and optical property changes during long-term photochemical and biological degradation of estuarine dissolved organic matter. *Limnol Oceanogr* 45:1254–1264
- Murphy KR, Stedmon CA, Graeber D, Bro R (2013) Fluorescence spectroscopy and multi-way techniques. *PARAFAC*. *Anal Methods* 5:6557. <https://doi.org/10.1039/c3ay41160e>
- Murphy KR, Stedmon CA, Wenig P, Bro R (2014) OpenFluor— an online spectral library of auto-fluorescence by organic compounds in the environment. *Anal Methods* 6:658–661. <https://doi.org/10.1039/C3AY41935E>
- Richardson DC, Newbold JD, Aufdenkampe AK et al (2013) Measuring heterotrophic respiration rates of suspended particulate organic carbon from stream ecosystems. *Limnol Oceanogr Methods* 11:247–261. <https://doi.org/10.4319/lom.2013.11.247>
- Romera-Castillo C, Sarmiento H, Alvarez-Salgado XA et al (2011) Net production and consumption of fluorescent colored dissolved organic matter by natural bacterial assemblages growing

- on marine phytoplankton exudates. *Appl Environ Microbiol* 77:7490–7498. <https://doi.org/10.1128/AEM.00200-11>
- Ruiz-González C, Niño-García JP, Lapierre J-F, del Giorgio PA (2015) The quality of organic matter shapes the functional biogeography of bacterioplankton across boreal freshwater ecosystems. *Glob Ecol Biogeogr* 24:1487–1498. <https://doi.org/10.1111/geb.12356>
- Shen Y, Chapelle FH, Strom EW, Benner R (2015) Origins and bio-availability of dissolved organic matter in groundwater. *Biogeochemistry* 122:61–78. <https://doi.org/10.1007/s10533-014-0029-4>
- Stedmon CA, Markager S (2005) Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnol Oceanogr* 50:686–697. <https://doi.org/10.4319/lo.2005.50.2.0686>
- Stedmon CA, Markager S, Bro R (2003) Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Mar Chem* 82:239–254. [https://doi.org/10.1016/S0304-4203\(03\)00072-0](https://doi.org/10.1016/S0304-4203(03)00072-0)
- Tranvik L, Bertilsson S, Letters E (2001) Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecol Lett* 4:458–463. <https://doi.org/10.1046/j.1461-0248.2001.00245.x>
- Tranvik LJ, Downing JA, Cotner JB et al (2009) Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol Oceanogr* 54:2298–2314. https://doi.org/10.4319/lo.2009.54.6_part_2.2298
- Volk CJ, Volk CB, Kaplan LA (1997) Chemical composition of biodegradable dissolved organic matter in streamwater. *Limnol Oceanogr* 42:39–44. <https://doi.org/10.4319/lo.1997.42.1.0039>
- Ward ND, Bianchi TS, Sawakuchi HO et al (2016) The reactivity of plant-derived organic matter and the potential importance of priming effects along the lower Amazon River. *J Geophys Res Biogeosciences* 121:1522–1539. <https://doi.org/10.1002/2016JG003342>
- Weyhenmeyer GA, Fröberg M, Karlun E et al (2012) Selective decay of terrestrial organic carbon during transport from land to sea. *Glob Chang Biol* 18:349–355. <https://doi.org/10.1111/j.1365-2486.2011.02544.x>
- Williams CJ, Yamashita Y, Wilson HF et al (2010) Unraveling the role of land use and microbial activity in shaping dissolved organic matter characteristics in stream ecosystems. *Limnol Oceanogr* 55:1159–1171. <https://doi.org/10.4319/lo.2010.55.3.1159>
- Wilson HF, Xenopoulos MA (2009) Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nat Geosci* 2:37–41. <https://doi.org/10.1038/ngeo391>
- Zepp RG, Sheldon WM, Moran MA (2004) Dissolved organic fluorophores in southeastern US coastal waters: correction method for eliminating Rayleigh and Raman scattering peaks in excitation-emission matrices. *Mar Chem* 89:15–36. <https://doi.org/10.1016/j.marchem.2004.02.006>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.