



Riverine dissolved organic matter responds to alterations differently in two distinct hydrological regimes from Northern Spain

Selin Kubilay^{1, 2, 3}, Edurne Estévez², José Barquín Ortiz⁴, and Gabriel Singer²

¹Department of Ecohydrology and Biogeochemistry, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, 12587, Germany

²Department of Ecology, University of Innsbruck, Innsbruck, 6020, Austria

³Geography Department, Humboldt University of Berlin, Berlin, 12489, Germany

⁴IHCantabria - Instituto de Hidráulica Ambiental de la Universidad de Cantabria, Santander, 39011, Spain

Correspondence: Selin Kubilay (kubilay.selin@gmail.com) and Gabriel Singer (gabriel.singer@uibk.ac.at)

Abstract. Iberian rivers are characterized by flow regimes with high seasonal flow variation. They also host one-fifth of Europe's reservoirs for hydropower generation, irrigation or water supply needs, and thus many rivers have heavily altered flow regimes. Such flow conditions, also alter the natural dynamics of Dissolved Organic Matter (DOM), with likely implications for carbon cycling due to changed conditions for transformation, transportation and storage of carbon. Here we looked into the effects of flow alteration on the "DOM regime", i.e. the seasonal variation of DOM concentration and composition, in 20 rivers belonging to two different hydrological classes (i.e., Mediterranean and Atlantic) in Northern Spain. To further investigate which flow regime components influence DOM properties, we linked the turnover of DOM composition to a range of hydrological indices.

We found that Atlantic rivers with a natural flow regime have on average lower DOC concentration than their altered equivalents, but this is not mirrored in Mediterranean rivers. Moreover, we did not observe much difference in annual DOM composition due to flow alterations in either hydrological class. However, the turnover of DOM composition is higher in natural Atlantic rivers compared to the altered ones. We linked this turnover in DOM composition to the effects of upstream-located reservoirs, creating flow regimes with homogenized or even reversed seasonality. Our results suggest that Mediterranean rivers may have higher resistance to flow alterations, at least in the sense of not showing unusual DOM behaviour, while Atlantic rivers affected by flow alterations lose their naturally high annual variability of DOM composition.

1 Introduction

The structure and functioning of a river ecosystem are strongly tied to its flow regime (Poff et al., 2006). Climate and weather interacting with catchment properties such as topography, geology and land cover are primary controls of natural flow dynamics (Poff and Zimmerman, 2010), yet these are altered globally by a range of human interventions, including the construction and operation of dams for irrigation, navigation and hydropower generation (Stewardson et al., 2017). Traditionally, flow regimes are described by five components; magnitude, frequency, duration, timing and rate of change; and an altered flow regime is identified by deviation of one or more of these components from its natural tendency (Poff et al., 1997). Given the natural

diversity of flow regimes and the many ways in which they can be modified by dam operations (McManamay et al., 2012; Nadon et al., 2015), the prediction of ecological consequences of flow alterations is not a trivial task.

25 Dissolved organic matter (DOM) is the largest pool of organic carbon and the main energy source for bacteria in aquatic environments (Battin et al., 2008; Riedel et al., 2012). DOM is chemically highly complex because of (i) the numerous sources it originates from, and (ii) various biological and chemical processes it is subjected to during transport along the riverine network (Jaffé et al., 2008). Most of the DOM is of terrestrial origin, characterized by high aromaticity associated with humic acids (Jaffé et al., 2008), while only a small fraction is composed of highly reactive substances such as carbohydrates and
30 proteins which may have terrestrial as well as in-stream origin. This highly reactive fraction is often microbially degraded within minutes to hours after entering the system while the more recalcitrant terrestrial fraction is transported along the river for a longer time. This linkage of differing DOM reactivity with residence times creates a compositional DOM continuum, which varies not only spatially throughout the riverine network, but also temporally (Catalán et al., 2016).

In fact, DOM dynamics are shaped by the flow regime (Hayes et al., 2018). In natural rivers, the variability of DOM
35 concentration and composition is linked to (i) heterogeneity of flow paths (Peter et al., 2020) draining water from the terrestrial surrounding to the river channel, especially during intense hydrologic events induced by snowmelt or rain (Raymond et al., 2016; Raymond and Saiers, 2010), and (ii) residence time of the DOM within the riverine network that is determined by discharge, which cycles through periods of stable base-flow, disturbing and eroding floods and droughts. High discharge spates may be “hot moments” for the mobilization and transport of terrestrial DOM (McClain et al., 2003), yet the decreased residence
40 times in the river corridor may limit microbial access to DOM. In contrast, during base-flow conditions such terrestrial material is more heterogeneous and dependent on soil processes and subsuperficial flow pathways. Moreover, following high-flow events, discharge and concentration (or composition) of terrestrial DOM undergo uncoupling due to the limited terrestrial storage capacity and exhaustion of DOM reservoirs along drainage flow paths (Andrea et al., 2006; Wagner et al., 2019). Such hysteresis in discharge-concentration relationships combined with dynamic residence times prevent simple translations of flow
45 regimes into “DOM” regimes (Andrea et al., 2006).

When flow dynamics are controlled by dams, flood extremes are minimized (Hayes et al., 2018) and water residence time increases due to the storage in the reservoirs, leading to altered composition and timing of the DOM delivery to the downstream river (Xenopoulos et al., 2021). Recent studies indicate that DOM concentration and composition vary according to dam operation and purpose, also determining whether a reservoir acts as a source or sink of carbon (Xenopoulos et al., 2021).
50 A dam with sufficient residence time may dampen event-driven and seasonal terrestrial (allochthonous) signatures of DOM for downstream environments as the increased residence time in the reservoir enables photodegradation and biodegradation and may shift DOM composition towards a more autochthonous and more biolabile composition (Xenopoulos et al., 2021). This rather general model of a DOM regime’s reaction to damming needs fine-tuning, however: The net effect of production, transformation and loss of organic carbon in a reservoir (as in any river reach) depends on several variables including inflowing
55 DOM concentration and composition, algal and bacterial activity, nutrient availability, temperature and solar radiation (Kraus et al., 2011). All these seasonal biotic and abiotic factors are associated also with the river’s natural flow regime; suggesting that the alteration of a specific flow regime may result in quite a specific DOM regime for the downstream river sections.



Notably, compositional turnover of DOM under the influence of flow alteration has yet to be implemented into concepts of riverine carbon cycling.

60 Here, we study the effects of anthropogenic flow regime alterations by dams (which serve mainly hydropower and irrigation purposes) in rivers with two distinct natural (reference) flow regimes. We specifically (i) search for commonalities and differences in flow alteration response with regard to mean and variation of DOM concentration and composition over one year across two naturally defined hydrological classes, and (ii) investigate flow regime characteristics that can be linked to turnover of DOM composition throughout the year. We hypothesize that DOM properties respond to both natural flow regimes and flow
65 alterations. We expect the effect of flow regime alterations on the DOM regime to depend on certain characteristics of the natural flow regime. Despite the high spatial and temporal variance of DOM composition, determining commonalities in the DOM regime response of various natural flow regimes could increase our understanding of the ecological consequences of a dam on the downstream river's structure and functioning.

2 Methods

70 2.1 Study design and sampling strategy

Our study followed a control-impact design comparing rivers with natural and altered flow regimes in two (natural) flow regime types (e.g., hydrological classes) in northern Spain (Fig. 1). Peñas and Barquín (2019) classified Spanish rivers in 20 hydrological classes using data from 282 gauged naturally flowing rivers and relevant environmental and hydrological drivers such as climate, topography, land cover and geology. Using these latter discriminators, they defined natural equivalents of rivers
75 (in the sense of estimated pre-impact reference flow regimes) that are nowadays impacted by various types of flow alteration (Peñas et al., 2016). For our study, we selected 20 rivers within the classes 13 and 10 defined by Peñas and Barquín (2019). In each class, we sampled 4 naturally flowing rivers and 6 rivers with altered flow regimes (where one altered Mediterranean river had to be removed). For simplicity, in this paper, the two classes will be referred to as Atlantic and Mediterranean respectively,
80 1). Overall these rivers form the 4 flow regimes discussed as natural Atlantic(nA), altered Atlantic (aA), natural Mediterranean (nM) and altered Mediterranean (aM) in our study.

A natural Atlantic regime is characterized by highest flows in early spring (February-April) due to snowmelt, followed by a gradual decrease in flow until summer low flows are reached and continue throughout July-September (Fig. 2a). Once minimum flows are reached in October, the flow rate picks up quite quickly with a steep incline due to heavy rains in the fall. Contrary to
85 this, a natural Mediterranean regime (Fig. 2c) has its highest flows a bit later in the spring, in March/April, followed by a sharp decrease in flow in May consequently leading to a long dry summer, reaching the minimum flows between August-September. As opposed to Atlantic rivers, the flow rate increases slowly leading back to the spring peaks, implying comparably less intense fall rains.

The selected altered rivers all have a dam with a reservoir, though the exact alteration of the flow regime may vary depending
90 on the size, purpose and operation of the involved dam (Table 1). Yet, the pre-impact regime of an altered river is expected to be

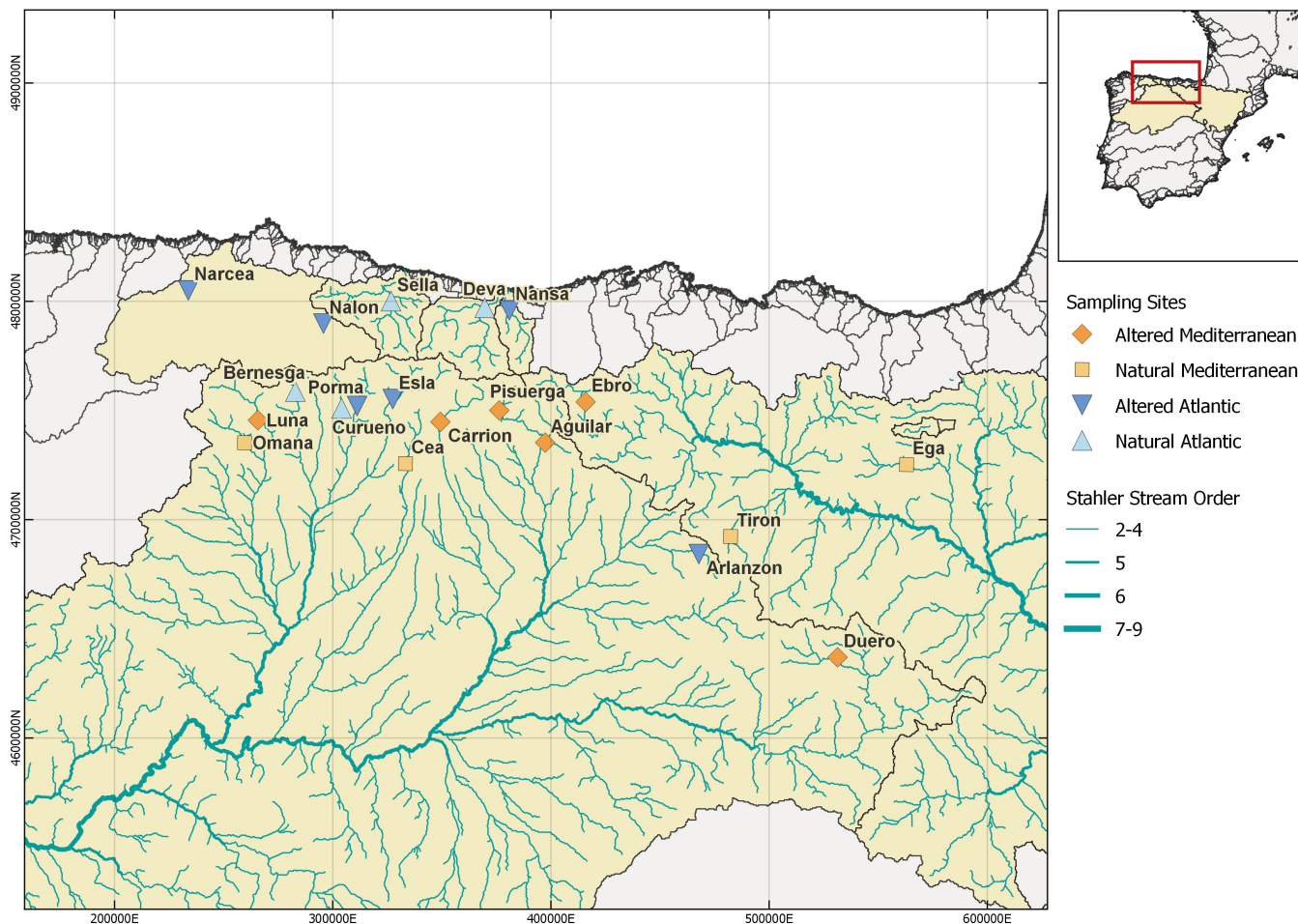


Figure 1. Location of selected Atlantic (blue) and Mediterranean (orange) sites on various rivers in Northern Spain. Light and dark shades of a colour on rivers represent rivers with a natural and altered flow regime, respectively. The rivers and basins are imported from the HydroSHEDS database of World Wildlife Fund's project (Lehner and Grill, 2013)

similar to that of a natural river of the same class. The sampling sites in the altered rivers were located on average 4.1 km (range 1.74 - 33.6 km) downstream of a reservoir and some sites had multiple upstream reservoirs. The catchment areas upstream of the sampling sites ranged from 89 km² to 1258 km² (Table 1) with an average of 429 km². In each river, we characterized DOM properties bi-monthly between October 2017 and August 2018 resulting in a total of 6 sampling occasions.

95 2.2 DOM composition

We measured DOC concentration, DOM absorbance and fluorescence, and DOM molecular size distribution. In the field, we filtered water samples through pre-combusted (450 °C, 4 h) glass fiber filters of 0.3 μm nominal pore size (Advantec GF-75,



Table 1. Study design and environmental characteristics of the altered (a) and natural (n) flow regimes within the Atlantic (A) and Mediterranean (M) hydrological classes. The altered rivers encompass dams of various purposes/operations with varying reservoir volume(s). Sampling sites were located at similar distances to the next upstream reservoir, yet some sites had multiple upstream reservoirs. Storage indexes were taken from (Pompeu et al., 2022) and show storage volume/average annual runoff.

River	Hydro Class	Alteration	Reservoir Usage	Upstream Catchment Area (km ²)	Elevation (m)	Reservoir (hm ³)	Storage Index	Distance to reservoir(s) (km)
Aguilar	M	a	Irrigation	579.87	882	247 - 65	0.82	5.5 - 33.6
Pisuerga	M	a	Irrigation	255.53	1014	65	0.46	2.2
Carrion	M	a	Irrigation	318.34	744.96	95 - 70	0.72	2.8 - 12
Duero	M	a	Irrigation	590.31	1032	249	0.74	8.3
Ebro	M	a	Irrigation	473.66	802	540	3.71	5.8
Luna	M	a	Irrigation	507.69	1008	308	0.84	5.2
Arlanzon	A	a	Irrigation	173.24	1023	75 - 23.2	0.63	2.3 - 9.7
Esla	A	a	Irrigation	612.04	1001	664	0.86	3.1
Nalon	A	a	Hydropower	356.26	317.33	4-34	0.13	4.2 - 9.5
Nansa	A	a	Hydropower	363.37	66	2-12	0.06	1.74 - 29.1
Narcea	A	a	Hydropower	1258.45	122.33	33	0.03	4.4
Porma	A	a	Irrigation	296.46	986	318	1.64	4.7
Cea	M	n	-	370.23	893.67	-	-	-
Ega	M	n	-	455.78	489	-	-	-
Omana	M	n	-	403.74	974	-	-	-
Tiron	M	n	-	186.59	815	-	-	-
Bernesga	A	n	-	88.95	1132	-	-	-
Curueno	A	n	-	168.29	1035.33	-	-	-
Deva	A	n	-	647.17	23.68	-	-	-
Sella	A	n	-	476.28	60	-	-	-

Japan) into acid-washed, MilliQ water-rinsed, pre-combusted (450 °C, 4 h) glass vials. We stored all the samples in the dark at 4 °C until analysis. We measured DOC concentration (mg L⁻¹) by high-temperature catalytic combustion of an acidified and sparged water sample with a Shimadzu TOC-V Analyzer. We took DOM absorbance and fluorescence measurements simultaneously using an Aqualog (Horiba Ltd, Kyoto, Japan). We measured absorbance in 5 nm increments from 250 nm to 600 nm and fluorescence as excitation-emission-matrices (EEMs); we excited the samples from 250 nm to 600 nm in 5 nm increments with emission from 212 nm to 620 nm in 2 nm increments. We subtracted MilliQ water EEMs from all sample EEMs to remove optical scatter phenomena but did not apply any inner filter correction since the decadal absorption coefficient at 254 nm (A_{254}) was lower than the threshold of 0.3 for all samples (Ohno, 2002).

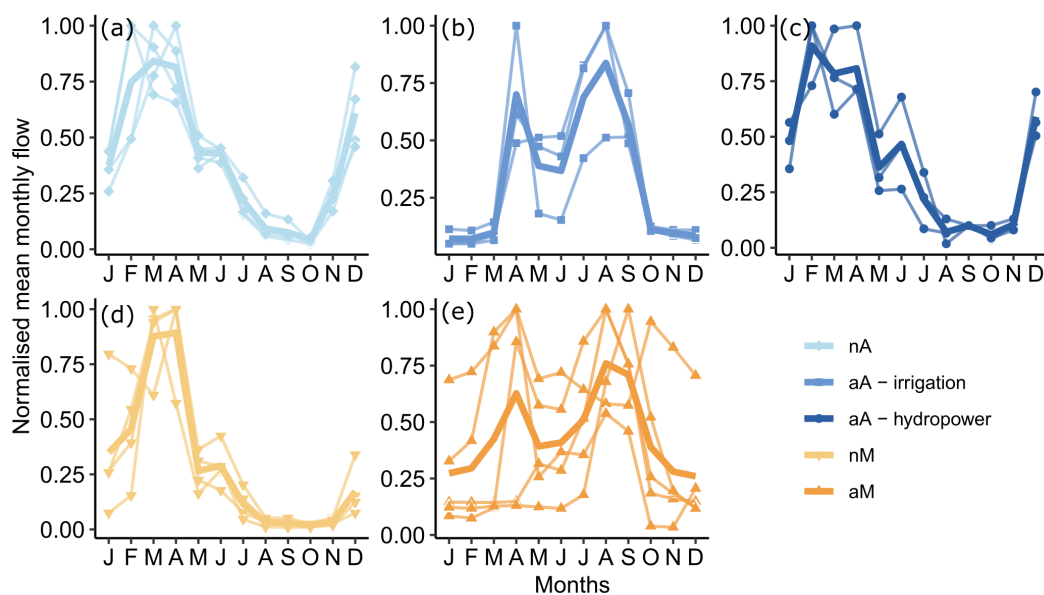


Figure 2. Normalized (to maximum) mean monthly flow of each river grouped by flow regime (a) natural Atlantic (nA) in light blue, (b, c) altered Atlantic (aA) in dark blue shades according to their reservoir purpose, (d) natural Mediterranean (nM) in light orange, and (e) altered Mediterranean (aM) in dark orange (only reservoirs with irrigation purpose by default). The thick line represents the average of the flow regimes.

We used the Aqualog measurements to calculate 6 optical indices; fluorescence index (FI), humification index (HI), freshness index (β/α), E2:E3 ratio, slope ratio (SR) and specific UV absorbance ($SUVA_{254}$), as summarised in Table 2. Further, we performed a parallel factor analysis (PARAFAC) after Raman-normalizing, smoothing and normalizing each EEM by setting the maximum emission to 1 (Coble et al., 2019). We used the StaRdom package (Pucher et al., 2019) in R version 4.0.3 (R Core Team, 2020) to partition the EEMs into individual components in accordance with the PARAFAC modelling strategy. We chose the 8-component PARAFAC model as the one achieving the best representation of the 699 EEMs dataset. We verified the model with split-half analysis with random sample selection following residual analysis. Sample-specific fluorescence of components was expressed in Raman units by reverting the normalization step through the multiplication of F_{max} values with the original EEMs' maximum emission values. We scanned the literature via the OpenFluor database (Murphy et al., 2013) for similar components. The components C1, C2, C3, C4 and C5 were reported as old humic-like DOM with strong terrestrial signals composed of mostly high MW substances. C6, C7 and C8 were all protein-like compounds of recently produced fresh material (Table 3). We grouped the relative contribution of the first 5 components (C1-C5) into an indicator of humic and terrestrial signatures ($C_{Terrestrial}$) by dividing their sum of relative contributions by the sum of all components. In the same manner, we turned the last 3 components into an indicator of freshly produced microbial input ($C_{Microbial}$).

We used liquid size-exclusion chromatography (SEC) with organic carbon and nitrogen detection (LC-OCD-OND; Huber et al. (2011)) for the molecular size distribution measurements. This allowed us to estimate the abundance of non-humic high



Table 2. Calculated optical indices, their meaning and used method

Indice	Description	Method
FI	Fluorescence index; the relative contribution of terrestrial to microbial fluorophores	Ratio of emissions of 450 and 500 nm at 370 nm excitation (McKnight et al., 2001)
HI	Humification index	Dividing the sum of emission intensities from 435 to 480 nm by the sum of intensities from 300 to 345 nm and from 435 to 480 nm (Ohno, 2002)
β/α	Freshness index, a proxy for relative microbial (fresh) contribution	Ratio of emission intensity at 380 nm to the maximum emission observed between 420 and 435 nm at 310 nm excitation (Harjung et al., 2019; Wilson and Xenopoulos, 2009)
E2:E3	Inverse indicator of molecular size	Absorbance ratio of 250 to 365 nm
SR	Slope ratio; inverse indicator of molecular weight	Slope ratio of short slope between 275 and 295 nm and long slope between 350 nm and 400 nm (Loiselle et al., 2009)
SUVA ₂₅₄	Aromaticity indicator	Decadal UV absorbance at 254 nm divided by DOC concentration

125 molecular weight substances (C_{HMWS} , like polysaccharides and proteins, mg C L^{-1}); humic substances (C_{Humic} , mg C L^{-1}) and neutral, hydrophilic to amphiphilic low molecular weight substances (C_{LMWS} , e.g., aldehydes, sugars and amino acids, mg C L^{-1}). For samples below the detection limit, we used half the value of the detection limit in the final dataset to allow multivariate data analysis. To have an estimate of the relative contributions of each of the defined size fractions, we divided each by total dissolved organic carbon concentration measured by SEC (Heinz and Zak, 2018).

2.3 Statistical analysis

130 To assess differences among the flow regimes, we did a PCA using the 81 flow indices reported in Peñas et al. (2016) and grouped the rivers according to their hydrological regime (Supp Fig. S1) to see if flow alterations homogenize or diversify the flow regimes in terms of their flow characteristics. To this aim, we also used the average distance to the centroid of a particular flow regime as a measure of dispersion in the same way as for DOM data (see below for assessment of among-river variation of annual mean DOM composition). The magnitude of 1-30-90 day High Flow Events (1HF, 30HF, 90HF) and the magnitude of 1-30-90 day Low Flow Events (1LF, 30LF, 90LF) were selected to compare the four flow regimes following the findings of natural and altered rivers' flow differences of Goldenberg-Vilar et al. (2022) on the same rivers.

135 As measures of mean DOM concentration and mean composition for each river, we computed annual averages of the DOC concentration, optical indices and SEC fractions. To express the seasonal variability of these variables we computed river-specific temporal coefficients of variation (CVs). Annual means and temporal CVs were then used as univariate responses describing DOM regimes to test for differences between the four flow regimes. For this, we followed a 1-way approach in 2 steps for each response variable: First, we tested mean and CV differences of all four hydrological regimes with a one-



Table 3. PARAFAC components with the modelled maximum emission and excitation values (Ex_{max} , Em_{max}) were compared to the literature using OpenFluor (TCC combined > 0.90). For each component, the main sources, the peak names and selected keywords from the literature are given.

Source	Comp.	Ex_{max}	Em_{max}	Keywords
$C_{Terrestrial}$	C1	330	444	Terrestrial humic-like, Peak C (Kothawala et al., 2012), high aromaticity, high MW, photosensitive (Lambert et al., 2016a)
	C2	<260 (340)	520	Terrestrial, humic-like DOM, high MW, aromatic (Kothawala et al., 2012; Lambert et al., 2016a, b), Peak A and Peak C (Lin and Guo, 2020)
	C3	<325	390	Humic-like, low MW, UV-A, fresher DOM, Peak M (Kothawala et al., 2012)
	C4	260 (300)	420	High MW, aromatic (Kothawala et al., 2012), photoproduct of a terrestrial, Peak A, Terrestrial humic-like (Lambert et al., 2016b)
	C5	<260 (380)	460	High MW (Kothawala et al., 2012), photochemically degradable, terrestrial humic-like (Harjung et al., 2019), aromatic, fulvic acid (Yang et al., 2017)
$C_{Microbial}$	C6	<260 (295)	380	Protein-like, microbial-humic like fluorescence (Lambert et al., 2016a), tryptophan-like (Derrien et al., 2018), microbially produced, Peak T/Peak M mixture (Kothawala et al., 2012)
	C7	260	340	Tryptophan-like recent biological production (Lambert et al., 2016a), Protein-like (Lambert et al., 2017)
	C8	270	310	Protein-like recent biological activity fluorophores (Lambert et al., 2016a), tyrosine-like fluorophores (Painter et al., 2018)

140 way test (Welch, 1951), which - unlike classical ANOVA - does not need variance homogeneity as a prerequisite. In case of
 significance, this was then followed by planned paired t-tests between (i) natural Atlantic and natural Mediterranean (nA vs
 nM), (ii) altered Mediterranean and natural Mediterranean (aM vs nM), (iii) altered Atlantic and natural Atlantic (aA vs nA).
 We corrected p-values with the Bonferroni method assuming 3 a-priori planned tests. We also compared the spatial variation
 of annual means and temporal CVs across the rivers of the four flow regimes, nA, aA, nM, and aM. For this, we followed a
 145 similar 1-way approach in 2 steps, where we first tested for variance homogeneity of annual means and of temporal CVs across
 all four regimes with a Bartlett test, which - when significant - was followed by planned F-tests between (i) nA vs nM, (ii) aM
 vs nM and (iii) aA vs nA. Taken together, our response variables included measures of mean location and temporal variation
 for each river's DOM indicators, which serve as DOM regime descriptors, and measures of (spatial) variation of these DOM
 regime descriptors across rivers belonging to the same flow regime. The latter was specifically computed to assess whether,
 150 in any given hydrological class, flow alteration by various dams diversifies DOM regimes compared to DOM regimes under
 natural flow.



Additionally, to assess the similarities and differences in DOM composition and its annual turnover between natural and altered rivers for each hydrological class in a more integrative, multivariate way, we did a principal component analysis (PCA) using the relative fluorescence of PARAFAC components, optical indices and SEC fractions. We looked for recognizable differences in DOM composition among flow regimes (i.e., between natural and altered regimes of both hydrological classes) with regard to location (mean DOM composition) and specifically with regard to temporal turnover of DOM composition in the various rivers. To statistically test for differences in DOM composition among flow regimes we use a 2-way permutational multivariate ANOVA (Anderson, 2001) on annual average locations (river-specific centroids) in the PCA-space with the same paired strategy as above. Temporal turnover of DOM composition is graphically represented in 2D PCA-space by convex hulls for each river (Fig. 5c-f), where each corner represents the DOM composition of a sampling date in the PCA space and the centroid represents the mean DOM composition of that river over the entire sampling period. The average distance of individual sampling dates to the centroid of a river serves as a measure of temporal turnover of DOM composition; it is computed as a dispersion (i.e., multivariate variation) on all dimensions of the PCA. To statistically test the temporal turnover of DOM composition we followed the same analysis approach as described above for a univariate response variable, i.e. a one-way ANOVA comparing dispersion as a response among all 4 flow regimes followed by 3 planned t-tests as post hoc tests.

In analogy to the analysis of univariate responses, we also compared the among-river variation of annual mean DOM composition and of temporal turnover of DOM composition across the four flow regimes given the multivariate dataset. This step was again motivated in order to assess if, in any given hydrological class, flow alteration by various dams diversifies DOM regimes (in either mean location or temporal turnover) compared to DOM regimes under natural flow. Again, we followed a similar 1-way approach in 2 steps. For the among-river variation of annual mean DOM composition, we tested for dispersion homogeneity of river centroids across all four regimes with PERMDISP, a permutational equivalent of the Bartlett test (Anderson, 2006), which - when significant - was followed by planned permutational F-tests between (i) nA vs nM, (ii) aM vs nM and (iii) aA vs nA. For the among-river variation of temporal turnover of DOM composition, we used the river-specific measure of temporal dispersion as a univariate response and followed the same approach as described above for variation of univariate responses (Bartlett-test followed by planned t-tests). As the individual axes of the PCA can be understood as distinct meaningful components of a river's DOM regime, we also tested the differences in average location and variance along PC1 and PC2 axes following the same univariate strategy of a one-way test followed by planned t-tests described above. Similarly, we compared across-river variance of average location and variance along the PC1 and PC2 axis, with a Bartlett test followed by planned F-tests as post hoc analysis.

To look for links between characteristics of the flow regime and annual turnover of DOM composition, we performed three partial least squares (PLS) analyses (one for the overall turnover of DOM composition, one each for PC1 and PC2 variance). We chose PLS analysis due to its reputed good performance in situations with high collinearity of predictors and small sample size (Carrascal et al., 2009; Coble et al., 2019). As predictors, we used 81 hydrological indices computed by Peñas and Barquín (2019) that were grouped into 5 flow regime components: magnitude, duration, frequency, timing, and rate of change (Supplementary Table S1). As dependent variables we used the multivariate dispersion (for the overall turnover of DOM composition) and the two variances on the first 2 PCA axes for each river, which can be understood as the two most



important components of DOM turnover, each having a specific qualitative character captured by the respective PCA-axis. In PLS analysis, we selected the hydrological indices with VIP-scores (Variable Importance on the Predictor) higher than 1 as the most important explanatory variables and reported only the first component of the PLS analysis for simplicity.

190 3 Results

3.1 Flow Regimes

Both Atlantic and Mediterranean natural flow regimes are characterized by high flows in early to late spring and low flows throughout the summer with a minimum in August-September before fall rains begin (Fig. 2a and c). Additionally, natural Atlantic flow regimes have a second high flow peak in early winter (November-December) compared to the natural Mediterranean regime.

Contrarily, altered Mediterranean rivers, with their irrigation reservoirs, do not experience the natural succession of spring high flows followed by summer droughts as observed in the nM rivers. From previous studies, we know that our altered Mediterranean rivers collect water during winter and spring and release it from May to September (Pompeu et al., 2022) which is the reason behind the summer high flows. We also know that this behaviour is limited by the storage capacity of these reservoirs where a lower storage capacity requires an additional ‘pre-release’ in winter-spring due to the capacity being reached, resulting in a double-humped shape (Fig. 2e).

When the mean of the altered Atlantic rivers is compared to its natural equivalent (Fig. 2b), we see that the above-mentioned introduction of summer high flows is not as consistent, i.e. 3 rivers have distinct summer high flows while 3 others have summer droughts in late summer. When grouped according to their reservoir purposes, rivers with hydropower reservoirs (Fig. 2c, Narcea, Nalon, Nansa) still possess summer droughts even though they have altered flow regimes; they somewhat mimic the natural flow regime expected of that river. Contrarily, rivers with irrigation reservoirs (Fig. 2b, Porma, Esla, Arlanzon) are relatively small and hence have summer high flows due to storage capacity limitations. Thus, in altered Atlantic rivers flow regime heterogeneity among the rivers comes from the different purposes of the reservoirs (irrigation vs hydropower) whereas in altered Mediterranean rivers, flow regime heterogeneity is derived mostly from the reservoir storage capacity differences since they are all irrigation reservoirs.

From previous studies on our rivers, we know that altered rivers show a higher diversity of flow regime compared to natural rivers (Goldenberg-Vilar et al., 2022). To distinguish this diversity for Atlantic and Mediterranean classes, we repeated a PCA analysis with these respective groups and found that altered Mediterranean rivers have 13-fold and altered Atlantic rivers 44-fold higher dispersion in the PCA space compared to their natural equivalents, while both natural flow regime classes showed similarly small dispersions (Supp Fig. S1). This furthermore confirms that altered Atlantic regimes encompassing both irrigation and hydropower reservoirs are somewhat more diversified than altered Mediterranean regimes which only have irrigation reservoirs.

As a final step, we looked at some flow regime characteristics (Fig. 3), specifically to the magnitude of 1-30-90 Day High and Low Flow Events (selected from Goldenberg-Vilar et al. (2022)) According to these resulting boxplots, both natural

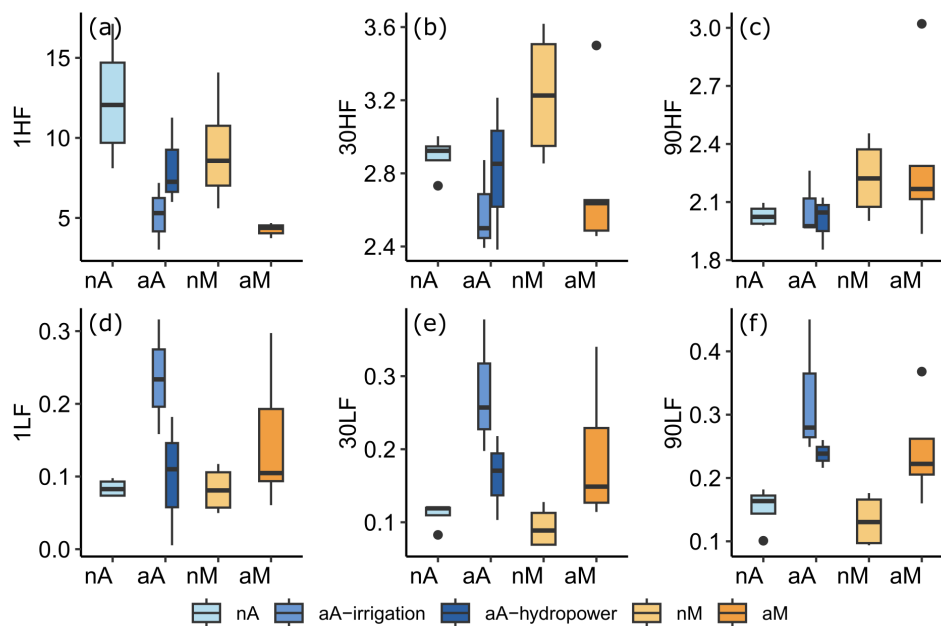


Figure 3. Boxplot of magnitude of extreme flow events representing flow variability; Magnitude of (a) 1-Day High Flow Event (PCA abbreviation, 1HF), (b) 30-Day High Flow Event (30HF), (c) 90-Day High Flow Event (90HF) and (d) 1-Day Low Flow Event (1LF), (e) 30-Day Low Flow Event (30LF), (f) 90-Day Low Flow Event (90LF). Natural Atlantic (nA) rivers are light blue, altered Atlantic (aA) in dark blues, separated by different shades according to their dam purpose, natural Mediterranean (nM) in light orange and altered Mediterranean (aM) in dark orange where all rivers are altered with irrigation dams by default.

220 flow regimes have higher 1HF than their altered counterparts. Natural Mediterranean rivers have higher 30HF than altered Mediterranean rivers whereas this difference was not as clear between Atlantic rivers. There was not much visible difference between regimes in 90HF. Similarly, natural Atlantic rivers have lower 1LF than altered Atlantic-irrigation but not so much from altered Atlantic-hydropwer. Natural rivers have lower 30LF and 90LF than altered counterparts for both Atlantic and Mediterranean rivers.

225 3.2 Average and temporal variation of DOC concentration

The DOC concentration varied 10-fold from 0.59 mg L^{-1} to 5.3 mg L^{-1} across rivers throughout the year. Planned t-tests could not show any significant difference in annual average DOC concentration (Fig. 4a, Table 4) between the two natural flow regimes (nA-nM), nor between the altered and natural rivers within each climate (aA-nA and aM-nM, respectively). The consistently higher median in all altered Atlantic rivers compared to the natural Atlantic rivers and partly significant results prior to Bonferroni correction point to a lack of statistical power due to low sample size causing a lack of significance.

230 The annual DOC variation of the rivers (CV_{DOC}) ranged from 14% to 53%. The two natural regimes (nA-nM) showed no significant difference in CV_{DOC} (Table 4). The natural Mediterranean rivers had significantly higher CV_{DOC} than their altered

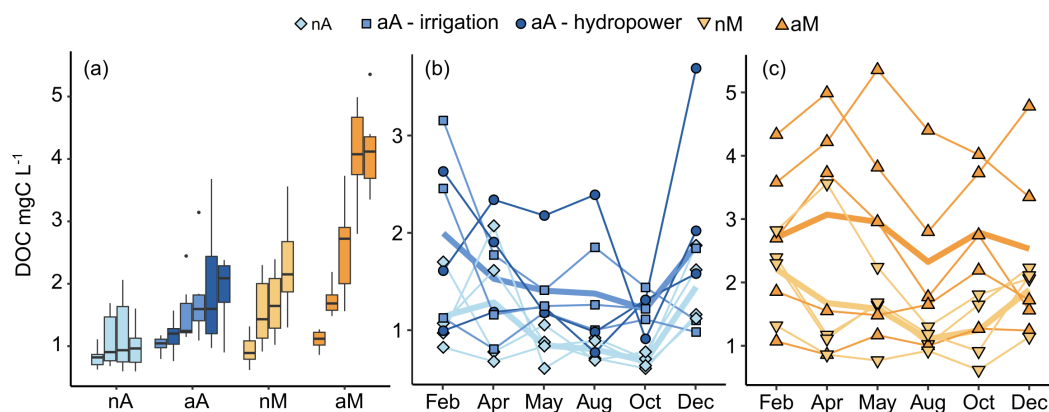


Figure 4. (a) DOC of each river as individual boxplots grouped by flow regime; natural Atlantic (nA) in light blue, altered Atlantic (aA) in dark blue, natural Mediterranean (nM) in light orange and altered Mediterranean (aM) in dark oranges. (b) Atlantic and (c) Mediterranean rivers' temporal DOC behaviours are represented as lines following the same colour scheme. The aA rivers are separated into two reservoir purpose groups, irrigation and hydropower, for the sake of visualisation. All aM rivers have reservoirs with irrigation purposes by default. The individual points represent single DOC measurements in various rivers of each flow regime in each sampling month, thus thin lines represent the trajectory of a river throughout the year. The thick lines are the average of that month for all the rivers of the represented flow regime.

equivalents (nM-aM), while this difference was not significant between natural and altered Atlantic rivers (nA-aA). Looking at the change in DOC through the sampled months in Atlantic rivers (Fig. 4b), we observe that both altered and natural rivers' average DOC time-series follow similar paths through the year with lowest values in August followed by an increase until spring high flows. In Mediterranean rivers, however (Fig. 4c), altered rivers' DOC time-series increase in April and May, while most natural rivers have low values in these months.

In both flow regimes, the among-river variation of DOC and CV_{DOC} seems lower in natural rivers than in their altered equivalents, but we could not find any significant difference between either of the pairs (data not shown), likely again because of low statistical power.

3.3 Average DOM composition and temporal turnover

In all regimes, DOM showed strong terrestrial characteristics throughout the year with relatively low FI (<1.4) and β/α (<1) and high HI (0.86 ± 0.04) and SUVA₂₅₄ (>2.4). Also, with values $>60\%$, the indicator $C_{Terrestrial}$ showed dominance of soil-derived materials over microbially produced materials (McKnight et al., 2001; Fellman et al., 2010; Hansen et al., 2016). The FI values ranged from 1.09 to 1.38 among the rivers throughout the year, typical for natural waters (Hansen et al., 2016). The SR values were relatively low for all flow regimes ($SR < 1$) indicating dominance of higher MW compounds and aromatic, plant-derived materials (Oliver et al., 2016). The relative contribution of SEC fractions also indicates that, in all of the regimes,



humic substances were most abundant (C_{HS}), followed by simple monomers like sugars and amino acids (C_{LMWS}) and polysaccharides and proteins (C_{HMWS}).

250 When we compare the two natural regimes of Atlantic and Mediterranean rivers, there was no significant difference in any annual average optical indicator or SEC fraction (Table 4). However, CV_{SR} and $CV_{E2:E3}$ were significantly higher in natural Atlantic rivers compared to natural Mediterranean rivers. The F-test results for all annual averages and annual turnover indicators show similar among-river variances between the two natural regimes (nA-nM, data not shown).

When we compare the natural regimes to their altered equivalents in terms of average DOM composition, we could not
255 observe any significant difference in the annual average optical indices (Table 4) in either of the two hydrological classes. Among the temporal turnover indicators, we observed that in Atlantic rivers, $CV_{E2:E3}$, $CV_{CProtein}$ and $CV_{CTerrestrial}$ were significantly higher in natural rivers compared to their altered equivalents (Table 4). In Mediterranean rivers, CV_{SR} was significantly higher in altered rivers compared to their natural equivalents. From the F-test results, we observe that all of the average DOM composition and temporal turnover indicators showed similar among-river variances between the natural
260 and altered regimes (data not shown).

We further analyzed the similarities and differences in the annual average and temporal turnover of DOM composition in a multivariate approach with a PCA. The first and second axes of the PCA (Fig. 5, PC1) explained 47 % and 16 % of the total variation of DOM composition. PC1 was negatively correlated with terrestrial and humic components ($C_{Terrestrial}$), humic substances (C_{HS}) and HI, and positively correlated with microbially sourced components ($C_{Microbial}$), FI, β/α , and C_{HMWS} ,
265 representing non-humic biopolymers (Huber et al., 2011). PC2 was negatively correlated with SR, E2:E3 and SUVA₂₅₄ and positively correlated with C_{LMWS} . Together the PCA space from the bottom left to the top right corner shows a gradient of terrestrially sourced, aromatic, high MW and presumably diagenetically older material to microbially sourced, fresher, low MW material. Notably, PC1 and PC2 capture indicators of DOM composition described with different methods: PC1 groups mostly fluorescence-based indices ($C_{Microbial}$, $C_{Terrestrial}$, FI, HI, β/α) and PC2 groups absorbance indices (E2:E3, SUVA₂₅₄, SR)
270 and SEC fractions.

We used the centroid location and average distance to the centroid of each river (represented as polygons in 2D space in Fig. 5c-f) as proxies for the annual average and temporal dispersion of DOM composition, respectively. We did not find a significant difference in centroid location between any of the compared pairs of flow regimes (Supp Table 1). In terms of temporal dispersions, we could not identify a significant difference between the natural rivers nor between the Mediterranean
275 rivers, yet we observe that natural Atlantic rivers have a higher average distance to the centroid compared to their altered Atlantic equivalents (Fig. 5c-d, Fig. 6a, Supp Table 1). Thus, in Atlantic rivers DOM composition was more variable over time when the flow regime was natural.

The global tests comparing the 4 flow regimes could not show any significant difference in among-river variance regarding the mean centroid location and the mean dispersions from the river centroids (Supp Table 1). Still, DOM regimes did not appear
280 similarly variable among the various flow regimes: Specifically, the seasonal turnover of DOM in natural Mediterranean flow regimes was quite variable. Here, by optical assessment, alteration caused a homogenization across rivers with seasonally



Table 4. Means of river-specific annual averages and temporal coefficients of variation for each of 4 flow regimes; natural Atlantic (nA), altered Atlantic (aA), natural Mediterranean (nM), altered Mediterranean (aM). The table provides means \pm standard deviations. Superscripted letters above the values of mean and standard deviation indicate significant differences according to t-tests and F-tests done pairwise between two flow regimes. These letters allow pairwise comparisons between any two flow regimes, but Bonferroni-correction was applied to p-values assuming only 3 a-priori planned tests of relevance (nA vs. nM, nA vs. aA, nM vs. aM). The letters were dropped when the pairwise comparisons did not indicate a significant difference.

	Atlantic		Mediterranean	
	natural	altered	natural	altered
<i>Water Chemistry</i>				
DOC (mg C L ⁻¹)	1.02 ^A \pm 0.15	1.55 ^{AB} \pm 0.39	1.62 ^{AB} \pm 0.56	2.73 ^B \pm 1.37
CV _{DOC}	0.38 \pm 0.13	0.33 \pm 0.14	0.33 \pm 0.04	0.19 \pm 0.07
<i>Optical Indices</i>				
FI	1.20 \pm 0.02	1.18 \pm 0.04	1.23 \pm 0.04	1.22 \pm 0.03
CV _{FI}	0.04 \pm 0.01	0.03 \pm 0.005	0.03 \pm 0.02	0.03 \pm 0.01
HI	0.86 \pm 0.02	0.86 \pm 0.02	0.88 \pm 0.02	0.84 \pm 0.03
CV _{HI}	0.06 \pm 0.03	0.03 \pm 0.009	0.03 \pm 0.01	0.05 \pm 0.02
β/α	0.66 \pm 0.03	0.66 \pm 0.05	0.66 \pm 0.02	0.70 \pm 0.02
CV _{β/α}	0.07 \pm 0.01	0.07 \pm 0.03	0.05 \pm 0.02	0.09 \pm 0.03
E2:E3	5.80 ^A \pm 0.05	6.44 ^{AB} \pm 0.06	6.30 ^{AB} \pm 0.04	7.10 ^B \pm 0.06
CV _{E2:E3}	0.31 ^A \pm 0.10	0.13 ^B \pm 0.04	0.13 ^B \pm 0.03	0.14 ^B \pm 0.04
SR	0.89 \pm 0.07	0.89 \pm 0.10	0.82 \pm 0.05	0.97 \pm 0.09
CV _{SR}	0.34 ^A \pm 0.05	0.20 ^B \pm 0.10	0.11 ^B \pm 0.02	0.20 ^{AB} \pm 0.05
SUVA ₂₅₄	2.93 \pm 0.32	2.74 \pm 0.37	2.63 \pm 0.26	2.40 \pm 0.33
CV _{SUVA254}	0.18 \pm 0.04	0.20 \pm 0.05	0.23 \pm 0.06	0.27 \pm 0.05
<i>PARAFAC Components</i>				
C _{Terrestrial}	0.69 \pm 0.02	0.69 \pm 0.04	0.71 \pm 0.05	0.64 \pm 0.07
CV _{C_{Terrestrial}}	0.19 ^A \pm 0.07	0.09 ^B \pm 0.02	0.12 ^{AB} \pm 0.04	0.12 ^{AB} \pm 0.04
C _{protein}	0.31 \pm 0.02	0.31 \pm 0.04	0.29 \pm 0.05	0.36 \pm 0.07
CV _{C_{protein}}	0.40 ^A \pm 0.10	0.20 ^B \pm 0.05	0.28 ^{AB} \pm 0.04	0.21 ^B \pm 0.05
<i>Relative Contribution of SEC fractions</i>				
C _{HMWS}	0.09 \pm 0.02	0.06 \pm 0.01	0.07 \pm 0.02	0.09 \pm 0.03
CV _{C_{HMWS}}	0.60 \pm 0.25	0.45 \pm 0.27	0.59 \pm 0.22	0.65 \pm 0.18
C _{LMWS}	0.13 \pm 0.01	0.13 \pm 0.02	0.14 \pm 0.02	0.14 \pm 0.01
CV _{C_{LMWS}}	0.27 \pm 0.08	0.25 \pm 0.10	0.27 \pm 0.07	0.11 \pm 0.10
C _{HS}	0.77 \pm 0.03	0.81 \pm 0.07	0.79 \pm 0.03	0.76 \pm 0.04
CV _{C_{HS}}	0.12 \pm 0.03	0.09 \pm 0.09	0.11 \pm 0.06	0.07 \pm 0.02



similar invariable DOM (Fig. 5e-f). Also, seasonal turnover of DOM in natural Mediterranean regimes seemed to be more variable than in Atlantic counterparts.

Since individual PCA axes represent distinct gradients of compositional DOM turnover, we looked at the timelines, average
285 locations and variances of rivers along the PC1 and PC2 axes separately as well. Timelines of DOM composition throughout
the year show similar patterns on PC1 for all flow regimes (Supp Fig. 2a-b). Contrarily, on PC2, natural rivers differ from their
altered equivalents both in August and October (Supp Fig. 2c-d). Plotting the β/α index as a particular example, it is higher
in October in altered Mediterranean rivers than in its natural equivalents; such a difference was not observed in other months
nor in Atlantic rivers (Supp Fig. 2 e-f). We could not find any significant difference in average location along the PC1 or PC2,
290 neither between the two natural regimes nor between altered and natural Mediterranean rivers (Fig. 6b, Supp Table 1). But, we
can visually see that aA - irrigation rivers have the highest 3 and aA - hydropower has the lowest 3 PC1 median.

We found that altered Atlantic rivers (Supp Table 1) have lower average PC2 scores than natural Atlantic rivers. Similarly,
we could not find any significant difference in temporal variation along the PC1 and PC2, neither between the natural regimes
nor between the altered and natural Mediterranean rivers (nM-aM, Fig. 6b, Supp Table 1). However, we found that natural
295 Atlantic flow regimes have significantly higher temporal DOM turnover than their altered equivalents along both PC1 and PC2
axes (nA-aA, Supp Table 1).

Moreover, along PC1, the among-river variation of mean DOM was similar among the rivers. Along PC2 we found a
difference between the natural rivers (nA-nM; Supp Table 1). Similarly, along PC1, among-river variation of temporal DOM
turnover was similar for all the rivers. Contrarily, along PC2 it was different both between the natural rivers and between natural
300 and altered in the Atlantic Rivers (nA-nM, nA-aA; Supp Table 1). These results confirm our visual assessments (Fig. 5c-f, Fig.
6).

3.4 Linking DOM regimes to flow regimes

The first component of the partial least square regression (PLSR) models explained 30.86 % of the variance of the dispersion
in the entire multidimensional PCA space, 18.72% of the variance on the PC1 axis, and 23.99 % of the variance on the PC2
305 axis. For ease of interpretation, only the 1st component was selected from each of the three PLS models. Based on $VIP > 1$ as
a criterion, 34, 34, and 29 indices were selected to explain the multidimensional dispersion and the variance of PC1 and PC2
axes, respectively. Mostly, indices identified as important for "PCA dispersion" - as the global model of DOM turnover - were
then also found as relevant to explain variances of PC1 or PC2 or both axes. A few indices, however, were identified as relevant
in only one of the three models, suggesting multivariate dispersion to be a maybe blurry but more encompassing measure of
310 DOM turnover, i.e. capturing DOM turnover that cannot be attributed solely to either PC1 or PC2.

The global model for dispersion was driven by high flow in the wet winter and spring months (November, December, January
Flow, and March, April, May Flow, Fig. 7a) and low flow in the dry summer months (June, July, August, September Flows,
Fig. 7a). Standard deviations of these flow magnitude indices show a similar trend of positive correlation in wet months and
negative correlation in dry months. Moreover, the global dispersion increases with increasing magnitude and frequency of
315 short duration high flow events (1-3-7 Day Maximum and 1-3-7 Day Frequency, Fig.7a). Regarding the rate and frequency of

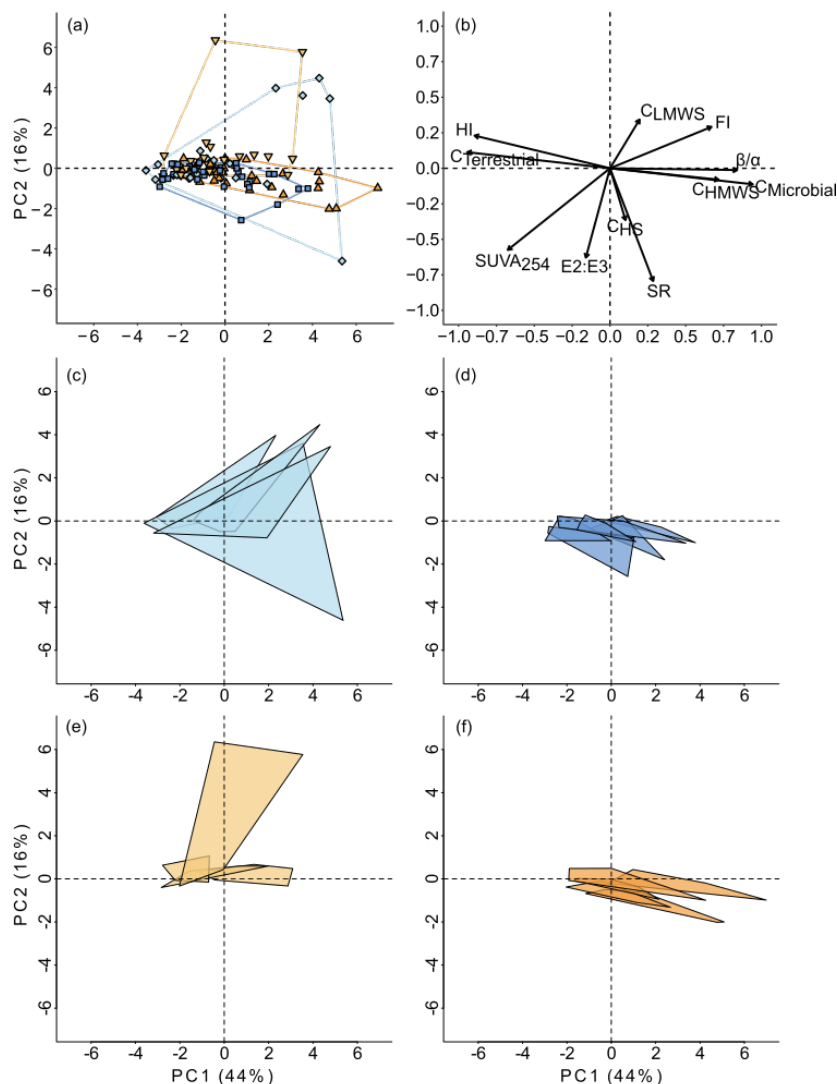


Figure 5. Principal component analysis of DOM composition. Graphical representation of (a) scores and (b) loadings of the PCA results where scores of each sampling occasion show a large overlap of flow regimes regarding DOM composition. The bottom panels show the temporal turnover of DOM composition in each river as a polygon in the same PCA space, where (c) the natural Atlantic rivers (nA) are represented in light blue (diamond), (d) the altered Atlantic (aA) rivers are represented in dark blue (square), (e) the natural Mediterranean (nM) rivers are represented in light orange (inverse triangle) and (f) the altered Mediterranean (aM) rivers are represented in dark orange (triangle). The PCA is based on absorbance and fluorescence measurements; humic-like components ($C_{Terrestrial}$), protein-like components ($C_{Microbial}$), specific absorption at 254 nm ($SUVA_{254}$), humification index (HIX), fluorescence index (FI), freshness index (β/α), slope ratio (SR), inverse molecular weight indicator (E2:E3) and size exclusion chromatography fractions: high MW biopolymers (C_{HMWS}), humic substances (C_{HS}) and low MW substances (C_{LMWS}).

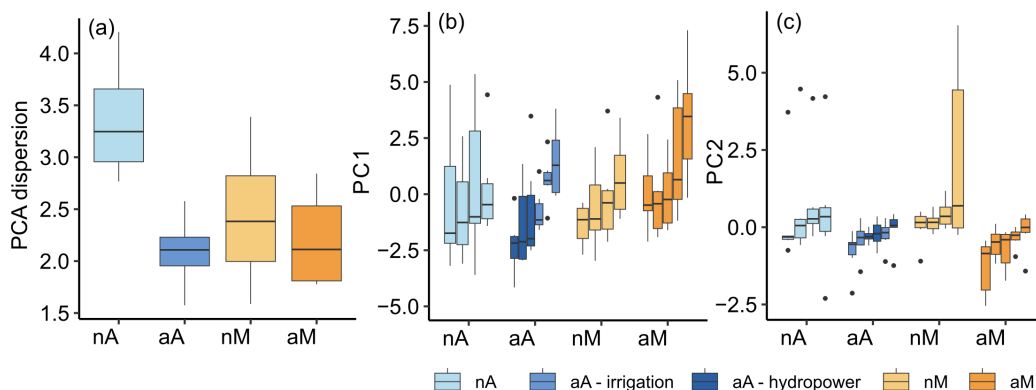


Figure 6. Temporal turnover of DOM composition in the various rivers belonging to four flow regimes, i.e., natural and altered flow regimes in two hydrological classes: Annual DOM turnover as average dispersion (distance to river centroid) along all the PCA axes (a); temporal variation of DOM in each river along PC1 (b) and PC2 (c). The rivers are grouped by flow regime; natural Atlantic (nA, light blue), altered Atlantic (aA, dark blues), natural Mediterranean (nM, light orange) and altered Mediterranean (aM, dark orange) rivers. The aA rivers are separated into two reservoir purpose groups, irrigation and hydropower, for the sake of visualisation. All aM rivers have reservoirs with irrigation purposes by default.

flow change, the count of increasing flow days decreases and the count of decreasing flow days increases this dispersion (No Increasing Flow Days, No Decreasing Flow Days, Fig. 7a). Regarding the timing indices, the later date of the annual minimum flow is related to higher dispersion whereas variation in this date (i.e. its standard deviation) is related to lower dispersion.

In addition to the global dispersion drivers, we observe that PC2 variance decreases with increasing long-duration minimum flows specifically, while PC1 variance increases with increasing high flood frequency and counts. Similarly, PC1 variance increases with increasing rise rate and decreases with increasing fall rate. Additionally, we found that PC1 variance decreases with increasing SD of minimum flood-related indices especially while most SD indices are negatively correlated.

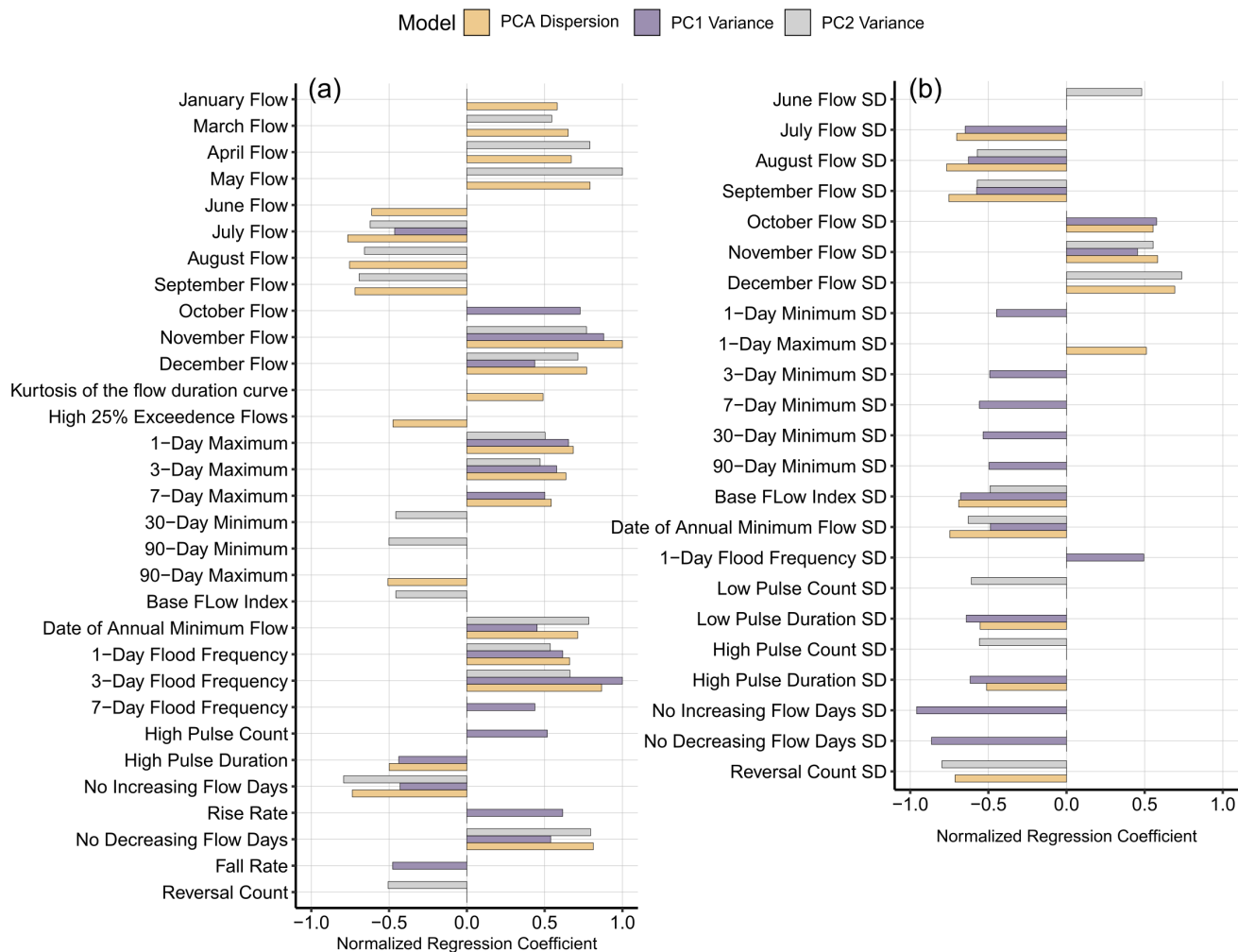


Figure 7. Following the PLSR model the indices with VIP scores higher than 1 were assessed for each model. (a) The mean indices and (b) the intra-annual standard deviation indices are separated for ease of interpretation. The yellow bars represent normalized regression coefficients for the indices in the model explaining multidimensional dispersion, the purple and grey bars represent the regression coefficients for the indices in the models explaining variance along PC1 (ranging from -0.07 to 0.07) and along PC2 (ranging from -0.06 to 0.06), respectively. For better comparison regression coefficients were normalized to ranges [-0.02, 0.02], [-0.07, 0.07] and [-0.06, 0.06] for dispersion, PC1 and PC2, respectively. The complete list of indices used and their abbreviations are given in Supp Table 2.



4 Discussion

In this study, we compared the effects of flow alteration on the DOM regime in two distinct natural flow regimes. Our results
325 contribute to the increasing evidence that dams do not influence all rivers alike (McManamay et al., 2012). Indeed, the likely
high diversity of DOM regimes associated with natural flow regimes may give the impression that similar reactions to flow
alterations are improbable. On the other hand, a suite of common ecological effects associated with reservoirs, e.g. the simple
fact of increased residence time, may indeed drive commonalities of DOM regime response to a dam-induced alteration of
natural flow dynamics. In reality, the natural flow regime forms a baseline background, on top of which a dam with a reservoir
330 of a certain size and a certain type of operation, thus residence time, may cause flow alterations with consequences for the
DOM regime. When designing this study, we acknowledged this by restricting study rivers to 2 distinct hydrological classes,
namely Atlantic and Mediterranean. In our study of 20 rivers, we indeed could not show similar responses in Atlantic and
Mediterranean regimes to flow alterations neither in DOC concentration nor in DOM composition when comparing altered
rivers to their natural equivalents. Across the considered natural flow regime classes, DOM regimes responded differently
335 yet consistently to flow alteration. We suggest that (1) reservoir effects (i.e. Hydraulic retention times and operation schemes
Maavara et al., 2020), (2) flow seasonality shifts and (3) flow regime homogenization may work towards altered DOM regimes
in downstream systems along a range of pathways, with ultimately unique outcomes for each hydrological class.

4.1 Investigated flow regimes and observed flow alterations

This study benefits from Peñas and Barquín (2019) who classified natural flow regimes on the Iberian Peninsula into 20 distinct
340 regime types (or hydrological classes), of which we selected two. Indeed, natural rivers in both classes have pretty distinct flow
regimes, each with common features. Hence, both classes have a low variance of flow descriptors across their rivers. Contrarily,
the high among-river variation of flow variability boxplots in most altered regimes (Fig. 3), combined with the hydrographs
(Fig. 2), indicate little commonality in flow alteration across the altered rivers: while some rivers have smoother hydrographs,
others show a tendency towards increased temporal variation in their flow regime (eg. introducing summer high flows and/or
345 winter low flows). This is in accordance with the literature, which suggests that flow alterations may not affect all flow regime
classes alike, and while it may push some rivers outside of their normal river function (McManamay et al., 2012), others may
still behave similarly to their expected natural flow regime. Moreover, as visible by their higher across-river variation, flow
alterations diversified altered Atlantic rivers more than altered Mediterranean rivers. Since flow alterations within each class
diversify flow regimes in different directions, mostly due to by design with reservoirs of different purposes and sizes together
350 as one group, altered flow regimes are not as easily classified and not as distinct as their natural equivalents. This is supported
by the higher dispersion of these rivers in the PCA space of the flow regime analysis (Supp Fig. 3). Despite this obvious
diversification of flow regimes by dams and reservoirs built for diverse purposes in both hydrological classes, we also observe
2 common features of altered flow regimes: (1) the general loss of dynamics of the flow regime throughout the year by less
intense 1-Day high flows (Fig. 3a) and more intense seasonal low flows (Fig. 3e-f) and (2) the newly introduced summer high
355 flows resulting in a shift in flow seasonality (Fig. 2 b and e). The latter was especially pronounced in Mediterranean rivers,



likely caused by them all being irrigation reservoirs with differing storage capacities that result in a need for water release during summer. Additionally, the reservoir capacity is surpassed in smaller reservoirs in winter-spring flows, resulting in a second “release”, hence the double-humped shape of their flow regime. A similar seasonal water release does not exist in the altered Atlantic rivers, especially those flowing to the North (Nalon, Nanca, Narcea) since they are all hydropower reservoirs without much storage capacity, hence they mimic the intense summer low flows of their natural equivalent.

This grouping had its challenges of its own; for instance, while hydropower production often drives hydropeaking fed by seasonal water storage, our hydropower reservoirs in our study tend not to hydropeak and while some irrigation reservoirs in this study tend to be small for their spring high flows and have to release twice within a year, some are big enough to produce one high flow during summer low flows. Moreover, we also had to group reservoirs of 2 hm³ to 664 hm³ in the same “flow alteration” group even though reservoirs of different sizes imply different residence times, stratification patterns, warming potential, etc., which have different implications for the carbon dynamics of the downstream rivers. Thus, in our study, flow regimes diversify through common, yet varied types of alteration. One thing our study cannot achieve by design is to look for commonalities in DOM regimes for reasonably categorized, distinct types of alteration. But, as a result, although grouping reservoirs for irrigation and hydropower production as “flow alteration” despite their very distinct flow features resulted in mostly little difference between the studied flow regime responses, we believe any commonality or difference we find has high importance due to this grouping design.

4.2 Responses of annually averaged DOM quantity and composition towards flow regimes

In our comparison of annual averages of natural and altered rivers, we saw clearly higher median values in altered Atlantic rivers compared to their natural equivalents and a weaker replication of the same trend in Mediterranean rivers. These patterns were not significant, likely due to low statistical power.

A clearcut response of DOM quantity to flow regimes or their alteration is not easy to predict. DOC of natural rivers is affected by various environmental factors such as topography, climate and land cover, while in altered rivers it is additionally influenced by factors such as dam operation, reservoir stratification and reservoir water temperature (Maavara et al., 2020). Various studies have found conflicting results on reservoirs as increasing, decreasing or not altering DOC concentrations for the downstream systems (Ulseth and Hall, 2015). The existing diversity of influences makes a common response to flow alteration across Atlantic and Mediterranean rivers unlikely, especially when considering the highly integrative nature of a variable like annually averaged DOC. Yet, in our study, we observed an increase in DOC from natural to altered rivers when Atlantic and Mediterranean rivers were separated. One explanation for this effect of flow alteration by dams on DOC concentration is the efficient capture, transient storage and averaging of extreme DOM amounts delivered to the river network from the catchment during flood events (Raymond et al., 2016). In this explanation, dams interfere with the transmission of a DOM pulse during a flood, but more importantly, some DOM is transiently stored in the reservoir and passed on later over a longer time. Thanks to this hydrological averaging, sampling at discrete points in time in downstream rivers thus results in relatively high and stable values, while it is highly unlikely that we were able to capture a pulse on any of our natural rivers. In addition or alternatively to this effect of temporal smoothing of terrigenous DOM pulses, productivity in reservoirs may contribute additional DOM



390 of autochthonous origin. Both mechanisms are related to the increased residence time of water in the reservoirs, yet they
expectedly result in very different DOM compositions passed on to downstream river reaches.

Strictly looking at annual average measures for DOM composition between natural and altered flow regimes we found only
limited evidence for differences; we observed a significant difference only in mean DOM composition along PC2 between
natural and altered Atlantic rivers. The lack of significant effects between naturally flowing and flow-altered rivers with re-
395 gard to (average) DOM composition to some extent supports the idea of reservoirs as smoothers of a lowly variable DOM
regime, or it may imply that other factors such as hydrology or catchment characteristics have an overriding influence on DOM
composition, thereby masking any potential reservoir effect by a lot of noise.

In fact, the literature on linking DOM composition to dams has mixed results. Some studies show that a high export of
autochthonous and biolabile DOM caused by increased primary production in reservoirs and tailwaters may result in a shift
400 from terrestrially sourced DOM to protein-like DOM downstream of dams (Xenopoulos et al., 2021). Contrarily, other studies
claim that dams of certain size and function may have no significant effect on downstream DOM composition. In boreal rivers,
for example, no shift in DOM composition from terrestrial to protein-like was observed downstream of dams (Xenopoulos
et al., 2021). This diversity of responses points to a particular source of noise in our study that may cause difficulties in finding
clearcut effects of flow alteration on DOM composition: the fact that we grouped various dams of different purposes and sizes
405 under the term “flow alteration”. In this respect, dam operations such as the timing of the reservoir filling or water release
are also of concern, specifically regarding their influence on the composition of DOM transported to downstream reaches by
interfering with residence times and transmission of upstream source signals (Xenopoulos et al., 2021). Indeed, dams can be
a carbon source or a carbon sink depending on many such factors related to dam nature and operation; in the worst case, they
lead to DOM composition to respond to “flow alteration” in opposing directions even if rivers are expected to belong to the
410 same hydrological class.

4.3 DOM regime reactions: Atlantic and Mediterranean rivers show divergent responses to flow regime alterations in annual DOM turnover

A DOM regime may be better described than just by annually averaged DOC and DOM composition, especially when these
variables are subject to dynamic processes and show pronounced temporal variability. Indeed, when looking at the temporal
415 change of DOM composition indicators (Supp Fig. 2e-f), we observed specific differences in summer and fall months between
the natural and altered regimes in both Atlantic and Mediterranean rivers. The divergence between natural and altered rivers
is even more obvious in the multivariate analysis, specifically along PC2, where particularly during summer, DOM in altered
rivers behaved differently than in natural rivers (Supp Fig. 2c-d). To make use of the temporally spaced information without
having to resort to time series analyses, we computed measures of variation of DOC and of turnover of DOM composition
420 at the annual timescale as descriptors of the DOM regime. We reasoned that such integrative measures based on variance can
also be quite flexibly computed in cases where the temporal spacing of measurements is uneven, not aligned among rivers, or
sampling is sparse or spread randomly over multiple years.



Hydrological seasonality and event-induced flow variation are the main drivers of annual variation in carbon concentration in natural rivers (Fasching et al., 2016). However, we could not observe an effect of flow regime on the annual DOC variation
425 nor observe any clear difference among annual timelines (Fig. 4b-c). Contrarily, we found several DOM indices showing differences in their annual variance (CV values) and PCA dispersion among Atlantic flow regimes. Such common patterns in Atlantic rivers but lack thereof in altered Mediterranean rivers imply that climatically different flow regimes respond differently to flow alterations in terms of DOM (or that flow alterations have climate-specific effects on DOM).

Our PLSR results linking flow regime characteristics to PCA dispersion and variance along PC1 and PC2 axes yield more
430 insights into the mechanisms driving temporal DOM turnover in the various flow regimes. Short-duration high-flow events are key in mobilizing terrestrially stored carbon into the riverine system via increased riparian connectivity. The PLSR results show an increase in DOM turnover with an increase of indices describing magnitude (1-3-7 Day Maximum) and frequency (1-3-7 Day Flood Frequency) of such short-duration high flow events (Fig. 7a). These relationships may be partly driven by natural differences in terrestrial DOM mobilization, but more likely they are associated with the ability of reservoirs to interfere with
435 the downstream transmission of a terrigenous DOM pulse.

Indeed, many reservoirs in Northern Spain are associated with a decreased frequency of high flow events due to the storage of water in the reservoir and downstream flow homogenization (Aristi et al., 2014). In our study, in both natural flow regimes, summer low flows follow high winter and spring flows (with some climate-specific variation, for instance in the exact timing and intensity of drought). Thus, flow alteration largely leads to a more or less strong reversal of seasonal dynamics by retaining
440 water in reservoirs in winter and spring for later release during the summer. This effect is greater in Mediterranean rivers. These seasonalities of the flow regimes in our study are reflected in patterns of DOM turnover: a positive correlation of mean monthly flows of winter and spring high discharges but a negative correlation of mean monthly flows in summer droughts with the DOM turnover. This is corroborated by relatively higher terrestrial signals in the rainy spring months in natural rivers (Supp Fig. 2a-b) and also shown by the positive correlation of aromaticity and molecular weight variance with seasonal high
445 flows (March, April, May Flows). Natural flow regimes allow the transmission of flood-induced terrigenous DOM pulses in spring while such DOM is rare in these rivers during summer low flows. On the other hand, in altered flow regimes, reservoirs pass on seasonally smoothed and thus rather invariable terrigenous DOM to downstream systems long into summer (Supp Fig. 2e-f). This is also supported by the negative correlation of summer high flows as part of the altered regimes with global PCA dispersion, indicating the lack of summer droughts lowers the natural DOM variance of a river.

In addition to the importance of seasonal high flows for driving seasonal variance of DOM composition, naturally dry
450 summer months contribute to DOM turnover as well as higher microbial signals from autochthonous production of DOM in the merely flowing rivers. This is shown by higher PC1 and PC2 values during summer low flow in the natural Atlantic rivers while DOM remained comparably invariable in the altered Atlantic rivers (Supp Fig. 2a). A similar microbially sourced carbon pulse was observable in altered rivers in October, coinciding with the lowest flow rate for these rivers.

A counter-effect to the lowered autochthonous contribution to DOM variance in the altered rivers may be given by the
455 potential production of DOM in the reservoirs, especially when these have longer residence times, reasonable nutrient supply and support warming. This seems to be the case for altered Mediterranean rivers that show a stronger microbial and weaker



terrigenous signal in summer compared to their natural equivalents (see time series of PC1, PC2, and beta/alpha, Supp Fig. 2b, d and f).

460 Notably, the buffering effect of reservoirs may also imply a change of DOM composition due to higher degradation as a result of longer residence time. We observed that in August - October measurements, PC2, which is strongly correlated to SR and E2:E3, diverges between natural and altered rivers in both Atlantic and Mediterranean regimes. Here, natural rivers have positive values and altered rivers have negative values. We think this may be related to the higher irradiation particularly since SR indicates higher photodegradation. This might be due to two factors; longer residence times in the reservoir leading up
465 to these months and water release from the reservoirs from May/June till October where water depth decreases hence light penetration increases.

4.4 Dams drive homogenization of DOM regimes across rivers

Statistical tests involving the pairwise flow regime comparisons could not identify among-river differences based on annually integrated DOC variables, yet visual data inspection of among-river variation of DOC and CV_{DOC} suggests a trend towards
470 increasing among-river variation from natural to altered rivers. This indicates a spatial diversification of DOM regimes in terms of quantitative behaviour, which parallels the flow regime diversification due to dams. This conclusion, however, could not be corroborated by a similar behaviour when analyzing DOM composition. We observed that flow regime alterations homogenized the DOM regime across rivers despite our dams of differing sizes and purposes which alter the flow regime in different directions. This loss of natural DOM diversity indicates that dams have a very strong homogenizing effect on DOM
475 regimes.

5 Conclusions

In our study, we investigated how dams, serving various purposes, alter the flow regime in rivers with distinct natural flow patterns to uncover commonalities and variations in DOM dynamics, we looked into two distinct natural flow regimes and compared natural and altered DOM regimes within each. We concluded that a dam may move a river outside of its normal river
480 function both in terms of its annual average and annual variance of DOC concentration and composition through its influences on the natural flow regime expected of a river. Among such influences, introducing high flows during natural seasonal drought and interfering with natural high and low flow events are likely the driving factors for the decrease in annual turnover of carbon quantity. Moreover, these alterations created long periods of low flow in downstream rivers, especially in the winter and spring months, preventing the transmission of terrigenous DOM sourced upstream towards downstream located systems.
485 In conclusion, these drastic seasonality reversals of the flow regime may impact the seasonality of the DOM composition negatively. It appears that in our study area, reservoirs act as a buffer, where longer residence times average out the naturally high turnover of DOM composition, and send relatively invariable DOM further downstream.

Flow alterations also resulted in the homogenization of DOM downstream of dams across time and space. This may have important consequences for riverine carbon cycling. In natural flow regimes, considerably variable DOM meets benthic bac-



490 terial consumers that undergo frequent succession due to disturbance by flow. The likely resulting mismatch between the
chemical traits of incoming DOM and the microbial functions needed to process this DOM as a resource limits its metabolism
(Talluto et al., 2024). In contrast, in altered flow regimes, there is an increased chance for a match between carbon resources
and their heterotrophic consumers in now less disturbed microbial communities. Ultimately, this would translate to increased
metabolism of terrigenous DOM and increasingly higher CO₂ emissions in rivers downstream of dams. Notably, this effect
495 on riverine carbon cycling comes on top of already-known effects on intensified DOM processing caused by higher residence
times in reservoirs. Consequently, more research is needed to determine which flow components are influencing the concen-
tration and composition of carbon more than others in their differing response. This could also help to design flow alteration
strategies that prevent impacts on ecosystem structure and functioning and move one step forward in restoring natural riverine
habitats.

500 *Code availability.* The R code used for data analysis and visualisations in this paper is openly available on Zenodo at zenodo.13354231
(Kubilay, 2024b)

Data availability. The optical data used in the analysis is openly available on Zenodo at zenodo.13354316 (Kubilay, 2024a). Hydrological
data is obtained from Pompeu et al. (2022) and Goldenberg-Vilar et al. (2022) and can be accessed through the authors.

505 *Author contributions.* SK: Conceptualization, Methodology, Software, Formal Analyses, Visualization, Writing – Original Draft and Review
and Editing. EE: Conceptualization, Data Curation, Writing – review & editing. JBO: Funding acquisition, Conceptualization, Writing –
review & editing. GS: Funding acquisition, Conceptualization, Methodology, Supervision, Writing – review & editing. All authors have read
and agreed to the published version of the paper

Competing interests. Some authors are members of the editorial board of the journal Biogeosciences

510 *Acknowledgements.* We thank our colleagues from IHCantabria for their help in sample collection. We also thank our colleagues from
Leibniz Institute of Freshwater Ecology and Inland Fisheries for sample processing. This project has received funding from the European
Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765553.



References

- Anderson, M. J.: A new method for non-parametric multivariate analysis of variance, *Austral Ecology*, 26, 32–46, <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>, _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1442-9993.2001.01070.pp.x>, 2001.
- 515 Anderson, M. J.: Distance-based tests for homogeneity of multivariate dispersions, *Biometrics*, 62, 245–253, <https://doi.org/10.1111/j.1541-0420.2005.00440.x>, 2006.
- Andrea, B., Francesc, G., Jérôme, L., Eusebi, V., and Francesc, S.: Cross-site Comparison of Variability of DOC and Nitrate c–q Hysteresis during the Autumn–winter Period in Three Mediterranean Headwater Streams: A Synthetic Approach, *Biogeochemistry*, 77, 327–349, <https://doi.org/10.1007/s10533-005-0711-7>, 2006.
- 520 Aristi, I., Arroita, M., Larrañaga, A., Ponsatí, L., Sabater, S., von Schiller, D., Elosegi, A., and Acuña, V.: Flow regulation by dams affects ecosystem metabolism in Mediterranean rivers, *Freshwater Biology*, 59, 1816–1829, <https://doi.org/10.1111/fwb.12385>, _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/fwb.12385>, 2014.
- Battin, T. J., Kaplan, L. A., Findlay, S., Hopkinson, C. S., Marti, E., Packman, A. I., Newbold, J. D., and Sabater, F.: Biophysical controls on organic carbon fluxes in fluvial networks, *Nature Geoscience*, 1, 95–100, <https://doi.org/10.1038/ngeo101>, number: 2 Publisher: Nature Publishing Group, 2008.
- 525 Carrascal, L. M., Galván, I., and Gordo, O.: Partial least squares regression as an alternative to current regression methods used in ecology, *Oikos*, 118, 681–690, <https://doi.org/10.1111/j.1600-0706.2008.16881.x>, _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1600-0706.2008.16881.x>, 2009.
- 530 Catalán, N., Marcé, R., Kothawala, D. N., and Tranvik, L. J.: Organic carbon decomposition rates controlled by water retention time across inland waters, *Nature Geoscience*, 9, 501–504, <https://doi.org/10.1038/ngeo2720>, number: 7 Publisher: Nature Publishing Group, 2016.
- Coble, A. A., Koenig, L. E., Potter, J. D., Parham, L. M., and McDowell, W. H.: 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>, publisher: Springer International Publishing, 2019.
- 535 Derrien, M., Kim, M.-S., Ock, G., Hong, S., Cho, J., Shin, K.-H., and Hur, J.: Estimation of different source contributions to sediment organic matter in an agricultural-forested watershed using end member mixing analyses based on stable isotope ratios and fluorescence spectroscopy, *Science of The Total Environment*, 618, 569–578, <https://doi.org/10.1016/j.scitotenv.2017.11.067>, 2018.
- Fasching, C., Ulseth, A. J., Schelker, J., Steniczka, G., and Battin, T. J.: Hydrology controls dissolved organic matter export and composition in an Alpine stream and its hyporheic zone, *Limnology and Oceanography*, 61, 558–571, <https://doi.org/10.1002/lno.10232>, _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/lno.10232>, 2016.
- 540 Fellman, J. B., Hood, E., and Spencer, R. G. M.: Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review, *Limnology and Oceanography*, 55, 2452–2462, <https://doi.org/10.4319/lo.2010.55.6.2452>, _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.4319/lo.2010.55.6.2452>, 2010.
- Goldenberg-Vilar, A., Delgado, C., Peñas, F. J., and Barquín, J.: The effect of altered flow regimes on aquatic primary producer communities: Diatoms and macrophytes, *Ecohydrology*, 15, e2353, <https://doi.org/https://doi.org/10.1002/eco.2353>, 2022.
- 545 Hansen, A. M., Kraus, T. E. C., Pellerin, B. A., Fleck, J. A., Downing, B. D., and Bergamaschi, B. A.: Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation, *Limnology and Oceanography*, 61, 1015–1032, <https://doi.org/10.1002/lno.10270>, _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/lno.10270>, 2016.



- Harjung, A., Perujo, N., Butturini, A., Romaní, A. M., and Sabater, F.: Responses of microbial activity in hyporheic pore water to biogeochemical changes in a drying headwater stream, *Freshwater Biology*, 64, 735–749, <https://doi.org/10.1111/fwb.13258>, <https://onlinelibrary.wiley.com/doi/pdf/10.1111/fwb.13258>, 2019.
- Hayes, D. S., Brändle, J. M., Seliger, C., Zeiringer, B., Ferreira, T., and Schmutz, S.: Advancing towards functional environmental flows for temperate floodplain rivers, *Science of The Total Environment*, 633, 1089–1104, <https://doi.org/10.1016/j.scitotenv.2018.03.221>, 2018.
- Heinz, M. and Zak, D.: Storage effects on quantity and composition of dissolved organic carbon and nitrogen of lake water, leaf leachate and peat soil water, *Water Research*, 130, 98–104, <https://doi.org/https://doi.org/10.1016/j.watres.2017.11.053>, 2018.
- Huber, S. A., Balz, A., Abert, M., and Pronk, W.: Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography - organic carbon detection - organic nitrogen detection (LC-OCD-OND), *Water Research*, <https://doi.org/10.1016/j.watres.2010.09.023>, 2011.
- Jaffé, R., McKnight, D., Maie, N., Cory, R., McDowell, W. H., and Campbell, J. L.: Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties, *Journal of Geophysical Research: Biogeosciences*, 113, <https://doi.org/10.1029/2008JG000683>, [eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1029/2008JG000683](https://onlinelibrary.wiley.com/doi/pdf/10.1029/2008JG000683), 2008.
- Kothawala, D. N., von Wachenfeldt, E., Koehler, B., and Tranvik, L. J.: Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations, *Science of The Total Environment*, 433, 238–246, <https://doi.org/10.1016/j.scitotenv.2012.06.029>, 2012.
- Kraus, T., Bergamaschi, B., Hernes, P., Doctor, D., Kendall, C., Downing, B., and Losee, R.: How reservoirs alter drinking water quality: Organic matter sources, sinks, and transformations, *Lake and Reservoir Management*, 27, 205–219, <https://doi.org/10.1080/07438141.2011.597283>, publisher: Taylor & Francis [eprint: https://doi.org/10.1080/07438141.2011.597283](https://doi.org/10.1080/07438141.2011.597283), 2011.
- Kubilya, S.: Chemical Data used in the "Riverine dissolved organic matter responds differently to alterations in two distinct hydrological regimes from Northern Spain", <https://doi.org/10.5281/zenodo.13354316>, 2024a.
- Kubilya, S.: Selkubi/Cantabria-Paper-Data-Analysis-and- Visualisations: Latest version used in the paper draft, <https://doi.org/10.5281/zenodo.13354231>, 2024b.
- Lambert, T., Bouillon, S., Darchambeau, F., Massicotte, P., and Borges, A.: Shift in the chemical composition of dissolved organic matter in the Congo River network, *Biogeosciences*, 13, 5405–5420, <https://doi.org/10.5194/bg-13-5405-2016>, 2016a.
- Lambert, T., Teodoru, C. R., Nyoni, F. C., Bouillon, S., Darchambeau, F., Massicotte, P., and Borges, A. V.: Along-stream transport and transformation of dissolved organic matter in a large tropical river, *Biogeosciences*, 13, 2727–2741, <https://doi.org/10.5194/bg-13-2727-2016>, publisher: Copernicus GmbH, 2016b.
- Lambert, T., Bouillon, S., Darchambeau, F., Morana, C., Roland, F. A. E., Descy, J.-P., and Borges, A. V.: 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>, 2017.
- Lehner, B. and Grill, G.: Global river hydrography and network routing: baseline data and new approaches to study the world's large river systems, *Hydrological Processes*, 27, 2171–2186, <https://doi.org/https://doi.org/10.1002/hyp.9740>, 2013.
- Lin, H. and Guo, L.: Variations in Colloidal DOM Composition with Molecular Weight within Individual Water Samples as Characterized by Flow Field-Flow Fractionation and EEM-PARAFAC Analysis, *Environmental Science & Technology*, 54, 1657–1667, <https://doi.org/10.1021/acs.est.9b07123>, publisher: American Chemical Society, 2020.



- Loiselle, S. A., Bracchini, L., Dattilo, A. M., Ricci, M., Tognazzi, A., Cózar, A., and Rossi, C.: The optical characterization of chromophoric dissolved organic matter using wavelength distribution of absorption spectral slopes, *Limnology and Oceanography*, 54, 590–597, <https://doi.org/10.4319/lo.2009.54.2.0590>, eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.4319/lo.2009.54.2.0590>, 2009.
- Maavara, T., Chen, Q., Van Meter, K., Brown, L. E., Zhang, J., Ni, J., and Zarfl, C.: River dam impacts on biogeochemical cycling, *Nature Reviews Earth & Environment*, 1, 103–116, <https://doi.org/10.1038/s43017-019-0019-0>, publisher: Nature Publishing Group, 2020.
- McClain, M. E., Boyer, E. W., Dent, C. L., Gergel, S. E., Grimm, N. B., Groffman, P. M., Hart, S. C., Harvey, J. W., Johnston, C. A., Mayorga, E., McDowell, W. H., and Pinay, G.: Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems, *Ecosystems*, 6, 301–312, <https://doi.org/10.1007/s10021-003-0161-9>, 2003.
- McKnight, D. M., Boyer, E. W., Westerhoff, P. K., Doran, P. T., Kulbe, T., and Andersen, D. T.: Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity, *Limnology and Oceanography*, 46, 38–48, <https://doi.org/10.4319/lo.2001.46.1.0038>, eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.4319/lo.2001.46.1.0038>, 2001.
- McManamay, R. A., Orth, D. J., and Dolloff, C. A.: Revisiting the homogenization of dammed rivers in the southeastern US, *Journal of Hydrology*, 424–425, 217–237, <https://doi.org/10.1016/j.jhydrol.2012.01.003>, 2012.
- Murphy, K. R., Stedmon, C. A., Graeber, D., and Bro, R.: Fluorescence spectroscopy and multi-way techniques. PARAFAC, *Analytical Methods*, 5, 6557–6566, <https://doi.org/10.1039/C3AY41160E>, publisher: The Royal Society of Chemistry, 2013.
- Nadon, M. J., Metcalfe, R. A., Williams, C. J., Somers, K. M., and Xenopoulos, M. A.: Assessing the effects of dams and waterpower facilities on riverine dissolved organic matter composition, *Hydrobiologia*, 744, 145–164, <https://doi.org/10.1007/s10750-014-2069-0>, 2015.
- Ohno, T.: Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter, *Environmental Science and Technology*, <https://doi.org/10.1021/es0155276>, 2002.
- Oliver, A. A., Spencer, R. G. M., Deas, M. L., and Dahlgren, R. A.: Impact of seasonality and anthropogenic impoundments on dissolved organic matter dynamics in the Klamath River (Oregon/California, USA), *Journal of Geophysical Research: Biogeosciences*, 121, 1946–1958, <https://onlinelibrary.wiley.com/doi/abs/10.1002/2016JG003497>, publisher: John Wiley & Sons, Ltd, 2016.
- Painter, S. C., Lapworth, D. J., Woodward, E. M. S., Kroeger, S., Evans, C. D., Mayor, D. J., and Sanders, R. J.: Terrestrial dissolved organic matter distribution in the North Sea, *Science of The Total Environment*, 630, 630–647, <https://doi.org/10.1016/j.scitotenv.2018.02.237>, 2018.
- Peter, H., Singer, G., Ulseth, A. J., Dittmar, T., Prairie, Y. T., and Battin, T. J.: Travel Time and Source Variation Explain the Molecular Transformation of Dissolved Organic Matter in an Alpine Stream Network, *Journal of Geophysical Research: Biogeosciences*, 125, e2019JG005616, <https://doi.org/10.1029/2019JG005616>, eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1029/2019JG005616>, 2020.
- Peñas, F. J. and Barquín, J.: Assessment of large-scale patterns of hydrological alteration caused by dams, *Journal of Hydrology*, 572, 706–718, <https://doi.org/10.1016/j.jhydrol.2019.03.056>, 2019.
- Peñas, F. J., Barquín, J., and Álvarez, C.: Assessing hydrologic alteration: Evaluation of different alternatives according to data availability, *Ecological Indicators*, 60, 470–482, <https://doi.org/10.1016/j.ecolind.2015.07.021>, 2016.
- Poff, N. L. and Zimmerman, J. K. H.: Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows, *Freshwater Biology*, 55, 194–205, <https://doi.org/10.1111/j.1365-2427.2009.02272.x>, eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-2427.2009.02272.x>, 2010.
- Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegard, K. L., Richter, B. D., Sparks, R. E., and Stromberg, J. C.: The Natural Flow Regime, *BioScience*, 47, 769–784, <https://doi.org/10.2307/1313099>, 1997.



- Poff, N. L., Bledsoe, B. P., and Cuhacyan, C. O.: Hydrologic variation with land use across the contiguous United States: Geomorphic and
625 ecological consequences for stream ecosystems, *Geomorphology*, 79, 264–285, <https://doi.org/10.1016/j.geomorph.2006.06.032>, 2006.
- Pompeu, C. R., Peñas, F. J., Goldenberg-Vilar, A., Álvarez Cabria, M., and Barquín, J.: Assessing the effects of irrigation and hy-
dropower dams on river communities using taxonomic and multiple trait-based approaches, *Ecological Indicators*, 145, 109–662,
<https://doi.org/10.1016/j.ecolind.2022.109662>, 2022.
- Pucher, M., Wunsch, U., Weigelhofer, G., Murphy, K., Hein, T., and Graeber, D.: staRdom: Versatile Software for Analyzing Spectroscopic
630 Data of Dissolved Organic Matter in R, *Water*, 11, 2366, <https://doi.org/10.3390/w11112366>, number: 11 Publisher: Multidisciplinary
Digital Publishing Institute, 2019.
- Raymond, P. A. and Saiers, J. E.: Event controlled DOC export from forested watersheds, *Biogeochemistry*, 100, 197–209,
<https://doi.org/10.1007/s10533-010-9416-7>, 2010.
- Raymond, P. A., Saiers, J. E., and Sobczak, W. V.: Hydrological and biogeochemical controls on watershed dissolved organic matter transport:
635 pulse-shunt concept, *Ecology*, 97, 5–16, <https://doi.org/10.1890/14-1684.1>, [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1890/14-1684.1](https://onlinelibrary.wiley.com/doi/pdf/10.1890/14-1684.1), 2016.
- Riedel, T., Biester, H., and Dittmar, T.: Molecular Fractionation of Dissolved Organic Matter with Metal Salts, *Environmental Science &
Technology*, 46, 4419–4426, <https://doi.org/10.1021/es203901u>, publisher: American Chemical Society, 2012.
- Stewardson, M. J., Acreman, M., Costelloe, J. F., Fletcher, T. D., Fowler, K. J. A., Horne, A. C., Liu, G., McClain, M. E., and Peel, M. C.:
640 Chapter 3 - Understanding Hydrological Alteration, in: *Water for the Environment*, edited by Horne, A. C., Webb, J. A., Stewardson,
M. J., Richter, B., and Acreman, M., pp. 37–64, Academic Press, ISBN 978-0-12-803907-6, <https://doi.org/10.1016/B978-0-12-803907-6.00003-6>, 2017.
- Talluto, L., del Campo, R., Estévez, E., Altermatt, F., Datry, T., and Singer, G.: Towards (better) fluvial meta-ecosystem ecology: a research
perspective, *npj Biodiversity*, 3, 1–10, <https://doi.org/10.1038/s44185-023-00036-0>, publisher: Nature Publishing Group, 2024.
- 645 Ulseth, A. and Hall, R.: Dam tailwaters compound the effects of reservoirs on the longitudinal transport of organic carbon in an arid river,
Biogeosciences, 12, 4345–4359, <https://doi.org/10.5194/bg-12-4345-2015>, 2015.
- Wagner, S., Fair, J. H., Matt, S., Hosen, J. D., Raymond, P., Saiers, J., Shanley, J. B., Dittmar, T., and Stubbins,
A.: Molecular Hysteresis: Hydrologically Driven Changes in Riverine Dissolved Organic Matter Chemistry During a
Storm Event, *Journal of Geophysical Research: Biogeosciences*, 124, 759–774, <https://doi.org/10.1029/2018JG004817>, [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1029/2018JG004817](https://onlinelibrary.wiley.com/doi/pdf/10.1029/2018JG004817), 2019.
- 650 Welch, B. L.: On the Comparison of Several Mean Values: An Alternative Approach, *Biometrika*, 38, 330–336,
<https://doi.org/10.2307/2332579>, publisher: [Oxford University Press, Biometrika Trust], 1951.
- Wilson, H. F. and Xenopoulos, M. A.: Effects of agricultural land use on the composition of fluvial dissolved organic matter, *Nature Geo-
science*, <https://doi.org/10.1038/ngeo391>, 2009.
- 655 Xenopoulos, M. A., Barnes, R. T., Boodoo, K. S., Butman, D., Catalán, N., D’Amario, S. C., Fasching, C., Kothawala, D. N., Pisani,
O., Solomon, C. T., Spencer, R. G. M., Williams, C. J., and Wilson, H. F.: How humans alter dissolved organic matter composition
in freshwater: relevance for the Earth’s biogeochemistry, *Biogeochemistry*, 154, 323–348, <https://doi.org/10.1007/s10533-021-00753-3>,
2021.
- Yang, L., Zhuang, W.-E., Chen, C.-T. A., Wang, B.-J., and Kuo, F.-W.: Unveiling the transformation and bioavailability of dis-
660 solved organic matter in contrasting hydrothermal vents using fluorescence EEM-PARAFAC, *Water Research*, 111, 195–203,
<https://doi.org/10.1016/j.watres.2017.01.001>, 2017.