Characterization of dissolved organic matter from temperate wetlands: field dynamics and photoreactivity changes driven by natural inputs and diagenesis along the hydroperiod

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Abstract

Wetlands store large amounts of C in biomass, sediments and water. A major C fraction is in the dissolved organic matter (DOM) pool and has multiple regulatory functions in the ecosystem. Patagonian wetlands undergo profound changes in their water cycle due to warming and reduced precipitation, causing shorter hydroperiods and reduced landscape connectivity and overall affecting their C budgets. In this study we characterized the DOM pool of a temporary wetland of North Patagonia during a hydroperiod, using optical DOM proxies obtained by absorption and fluorescence spectroscopy. DOM components were modeled through EEM-PARAFAC. DOC varied between ~4 and ~9 mg L⁻¹, displaying aromatic signals and terrestrial/sediment fingerprints. The humic components C1 (microbial and/or vegetation derived) and C2 (soil/sediment derived) prevailed in the DOM pool, whereas the non-humic component C3 (derived from aquatic production) showed lower contribution. Along the hydroperiod DOM optical proxies allowed identifying allochthonous inputs, degradation and an increasing contribution of the internal production to the DOM pool. Photodegradation experiments showed that exposure to PAR+UVR produced slight changes in the DOC concentration and a reduction in DOM molecular weight/size. The contribution of humic vs. non-humic components influenced DOM photoreactivity. The prevalence of humic components determined high DOM photorecalcitrance.

Additional keywords:

Patagonia, temperate wetlands, hydroperiod, dissolved organic carbon, degradation, photoreactivity

32 Introduction

33 Wetlands occur in different climates and biomes, covering around 9% of the world's
34 land surface, and are considered among the most threatened ecosystems by climate
35 changes, land use, water regulation and pollution (Brinson and Malvárez 2002; Keller
36 2011; van Asselen *et al.* 2013; Were *et al.* 2019; Xu *et al.* 2019).

37 These systems experience profound seasonal variations in water level, which can
38 involve a succession of wet and dry periods, and also undergo high inter-annual
39 fluctuations that imply extended periods of drought or flooding. Natural climate
40 oscillations and human-induced changes impacting on the global water cycle have
41 profound consequences for wetlands. Temperature, evaporation, humidity, and the
42 seasonality and amount of precipitation drive the water cycle, thereby affecting most
43 wetland processes (van Asselen *et al.* 2013; Häder and Barnes 2019).

44 Globally, wetlands are estimated to store ~35% of terrestrial carbon (C) and therefore
45 play an important part in the global C cycle, although the pathways of C flux in these
46 systems is still poorly understood. These ecosystems may affect the atmospheric C
47 cycle through dynamic sequestration-emission processes (Denman *et al.* 2007).

48 Wetlands can act as sinks for C from the terrestrial environment, can sequester CO₂
49 from the atmosphere through photosynthesis of macrophytes and phytoplankton, and
50 also transfer C to connected ecosystems. A large fraction of the total C present in
51 wetlands is in the pool of dissolved organic matter (DOM) which includes a variety of
52 compounds derived from the aquatic production (sediment and plant leachates,
53 metabolites from aquatic organisms, etc.) as well as from soil and vegetation of the
54 catchment. Macrophytes constitute a major source of particulate organic matter (POM)
55 and DOM to the water and sediments of wetlands (Wetzel and Søndergaard 1998;
56 Wetzel 2001; Yuan *et al.* 2020). DOM derived from aquatic production is composed of
57 substances of comparatively lower aromaticity and molecular weight than terrigenous
58 DOM (Cory and McKnight 2005; Stedmon and Markager 2005; Kritzberg *et al.* 2006;
59 Murphy *et al.* 2008; Huguet *et al.* 2009). The concentration, composition and molecular
60 structure of aquatic DOM is variable at spatial and temporal scales, and is ultimately
61 determined by its interaction with environmental factors (climate variables, pH,
62 conductivity, sunlight exposure), other molecules and organisms (Fellman *et al.* 2009;
63 Chen and Jaffé 2014; Hansen *et al.* 2016). In aquatic systems the DOM pool, and
64 particularly its light-absorptive fraction (CDOM), determines the underwater light and

thermal scenarios, and thus, exerts a chief control on their heterotrophic and autotrophic production (Häder *et al.* 2011).

Temperate wetlands of the southern hemisphere have been comparatively less studied than tropical and subtropical wetlands with regard to their role in the global C cycle. In Andean Patagonia (Southern South America) temporary wetlands usually develop during autumn and last until early summer, experiencing freezing conditions at the beginning of the hydroperiod and warming up from late spring. Due to their geographical location and shallowness, their water columns are highly exposed to elevated ultraviolet (UV) radiation levels (Villafañe *et al.* 2001; Perotti *et al.* 2005; Díaz *et al.* 2006; Pérez *et al.* 2010). Challenging radiation levels enhance photochemical weathering affecting the C pool of aquatic systems, with profound effects on abiotic and biotic compartments (Zagarese *et al.* 2001; 2017; Pérez *et al.* 2003; Bastidas *et al.* 2009, 2010; Gerea *et al.* 2016).

In this investigation we focus on DOM dynamics in a cold temperate ephemeral wetland of Northwestern Patagonia over the course of a hydroperiod, aiming to characterize the natural DOM pool in terms of concentration and quality. We applied optical analysis of the light-absorbing properties of the chromophoric and fluorescent DOM fractions (CDOM and FDOM, respectively) in order to characterize the DOM quality in terms of potential sources, molecular features and diagenetic state. Additionally, we evaluated through laboratory assays the photoreactivity of natural DOM pools collected at different moments of the hydroperiod by exposing natural DOM to photosynthetically active radiation and ultraviolet radiation (PAR+UVR). We hypothesize that changes in DOM optical properties and photoreactivity along the hydroperiod reflect DOM inputs from different sources and degradation processes.

Methods

Study area and field sampling

The study was carried out in Fantasma Pond (FP; 41°07' S, 71°27' W; 798 m a.s.l.), a shallow (~2 m), temporary piedmont wetland of Andean Patagonia (Nahuel Huapi National Park, Argentina). The climate of the area is cold temperate and markedly seasonal with ~60% of the precipitation concentrated from May to September

(hereinafter the wet season). The wet season is characterized by cold temperatures and a short photoperiod, while the dry season (spring-summer) has much lower rainfall levels, warmer temperatures and a long photoperiod with enhanced ultraviolet radiation (UVR) levels, particularly in spring (Paruelo *et al.* 1998; Villafañe *et al.* 2001). In order to evaluate changes in the concentration and quality of the DOM pool of FP, water samples were collected monthly throughout the entire hydroperiod, from June to December. At each sampling occasion water level was recorded at the sampling point. Water temperature, pH and conductivity were measured using multiparameter probes. In November, the vertical profile of downward irradiance (380-750 nm, every 1 nm) was measured at noon with a radiometer (USB2000, Ocean Optics). Sampling was conducted at a central point of the wetland using a Kemmerer bottle (4.7 L). Water samples were poured into acid-cleaned polycarbonate carboys which were immediately transported to the laboratory thermally insulated and in darkness. In the laboratory the samples were filtered through 0.22 μm polyvinylidene difluoride membranes (PVDF, Millipore) and stored in sealed dark flasks at 5°C for DOM characterization and photodegradation assays. Precipitation and air temperature data for the studied period were retrieved from the meteorological station EMMA (INIBIOMA-CONICET) (Fig. S1).

115

116 **DOM photodegradation experiments**

117

Laboratory incubations were conducted to analyze the natural DOM photoreactivity of water samples collected in FP in six consecutive months of the hydroperiod. The experimental design targeted the effect of exposure to photosynthetically active radiation and UV radiation (PAR+UVR) on the concentration and quality of natural DOM. The experiments consisted in natural DOM incubation under two treatments (each with 3 replicates): exposed to PAR+UVR or in darkness (control). Filter-sterilized DOM samples were placed in capped quartz test tubes (28 mL) and the control tubes were wrapped with aluminum foil to prevent exposure to light. The replicates were set to rotate in a custom-made wheel at 1 RPM in an environmental test chamber (Sanyo, MLR5) for 5 days at 20°C, with a photoperiod of 12 h PAR+UVR: 12 h darkness. Irradiation was provided through an array of 2 PAR lamps (Sanyo 40 W PAR = 69.1 $\mu\text{Em}^{-2} \text{ s}^{-1}$), 2 UV-B lamps (Q-panel 320, UVB = 4.1 kJ m^{-2}) and 3 UV-A lamps (Q-panel 340 UVA = 44 kJ m^{-2}) (Fig. S2a).

131 **Laboratory analysis**

132

133 DOM characterization in terms of concentration and quality was performed on the
134 filtered water samples collected throughout the hydroperiod and before and after
135 exposure to the different treatments (PAR+UVR and darkness) of the photodegradation
136 experiment.

137 The concentration of dissolved organic carbon (DOC) was measured in filtered water
138 samples as non-purgeable organic carbon using a Shimadzu TOC-L high temperature
139 analyzer with a high sensitivity catalyst (detection limit of 4 $\mu\text{g L}^{-1}$).

140 Optical analyses were conducted to characterize the chromophoric and fluorescent
141 DOM fractions (CDOM and FDOM, respectively). The absorbance spectra of filter-
142 sterilized water samples were obtained at 1 nm intervals from 200 to 800 nm in a UV-
143 Visible spectrophotometer (Hewlett Packard 8450), using a quartz cuvette (1 cm path
144 length). ASTM1 grade water (Milli-Q) was used as a blank. For analysis of the FDOM
145 fraction, the filtered, sterilized water samples were scanned in a Perkin-Elmer 55B
146 spectrofluorometer equipped with a 150-W Xenon arc lamp and a Peltier temperature
147 controller, using a 1 cm quartz fluorescence cell. Excitation-emission matrices (EEMs)
148 were collected at specific excitation (240-450 nm at 5 nm intervals) and emission (300-
149 600 nm at 0.5 nm intervals) wavelengths. The spectrofluorometer was set up with 10
150 nm excitation and emission slits and a scan speed of 1500 nm min⁻¹. As detailed above,
151 ASTM1 grade water was used as a blank.

152

153 **Data analysis**

154 The averaged UV-visible absorbance between wavelengths 700 to 800 nm was
155 subtracted from the absorbance spectra to correct for offsets due to several instrument
156 baseline effects, following Helms et al. (2008). The absorbance data were converted to
157 absorption coefficients as follows:

$$158 \quad a_{\lambda} = \text{Napierian coefficient (m}^{-1}\text{)} = 2.303 A_{\lambda}/l$$

159 A = absorbance value

160 l = quartz cuvette path length (m) = 0.1 m

161 The absorption coefficient at 350 nm (a_{350}) was associated with terrigenous DOM
162 and/or with the concentration of lignin phenols (Hernes and Benner 2003; Spencer et al.

2008). The specific absorption coefficient at 350 nm ($a_{350}:\text{DOC}$) was calculated and used as a surrogate for DOM aromatic content (Weishaar *et al.* 2003; Hansen *et al.* 2016). The spectral slopes for the intervals 275-295 nm ($S_{275-295}$) and 350-400 nm ($S_{350-400}$) were calculated by fitting the log-transformed spectral data to a linear regression (Helms *et al.* 2008). These slopes and the slope ratio S_R ($S_R = S_{275-295} : S_{350-400}$) are inversely related to DOM molecular size and aromaticity and are customarily used as proxies for DOM degradation (Helms *et al.* 2008; Spencer *et al.* 2010; Fichot and Benner 2012). The $S_{275-295}$ and $S_{350-400}$ and the S_R generally increase on irradiation (Helms *et al.* 2008; Hansen *et al.* 2016). The Climate Forcing Optical Index (CFOI) was calculated as $a_{320}:\text{DOC}$ divided by the spectral slope $S_{275-295}$, and was applied as a proxy of sunlight/photochemical weathering and terrestrial DOM inputs (Williamson *et al.* 2014).

Two fluorescent DOM compositional indicators were calculated from the fluorescence scans of the samples. The humification index HIX, an indicator of humic content or extent of humification, was calculated from the ratio of two integrated regions of an emission scan ($\text{Em}_{435-480} : \text{Em}_{300-345}$) at Ex_{254} (Zsolnay *et al.* 1999; Ohno 2002). High HIX values indicate a high degree of humification (Hansen *et al.* 2016). The biological index (BIX), a proxy for autotrophic production, was calculated as the ratio of the emission intensities 380 and 430 nm (Em_{380} and Em_{430}) at Ex_{310} , following Huguet *et al.* (2009). BIX values >1 correspond to recently-produced DOM of autochthonous origin (Hansen *et al.* 2016).

A total of 60 EEMs were processed using the software FL–WinLab[®] (Perkin-Elmer). The absorbance spectra (200-800 nm) were used to develop a matrix of correction factors for each EEM using the Matlab FDOMcorr toolbox, accounting for inner filter effects, blank subtraction and normalization to the area under the water Raman peak of the blank at 350 nm. The resulting data were expressed in Raman units according to Murphy *et al.* (2010).

Parallel Factor Analysis (PARAFAC) was applied to deconvolve DOM fluorescence signals into specific components using the drEEM toolbox for MATLAB (MATLAB R2015a) (Murphy *et al.* 2013).

A principal component analysis (PCA) was used to analyze the variation in the DOM pool during the hydroperiod taking into account the water depth, the DOC concentration, CDOM parameters ($a_{350}:\text{DOC}$, $S_{275-295}$, $S_{350-400}$, S_R) and FDOM variables

196 (HIX, BIX and the PARAFAC components). The analysis was performed using the
197 FactoMiner package in the R environment.

198 A linear model was fitted to the relationship between the absorption coefficient a_{350} and
199 DOC concentration. The results of the photodegradation assays were analyzed through
200 two-way ANOVA, using as factors the radiation treatments (PAR+UVR and Dark) and
201 the hydroperiod month (6 levels), using the free software R (package rstatix).

202

203 **Results and discussion**

204 **Dynamics of the dissolved organic matter pool during the hydroperiod**

205 The hydroperiod studied in FP lasted from June to December and reflected the
206 precipitation and temperature patterns, reaching its maximum volume in early spring
207 with the snowmelt water input, and then contracting until it completely dried up in early
208 summer (Fig. S1; Table 1). Similar hydroperiod patterns have been observed in
209 temporary wetlands adjacent to FP, which are typical of piedmont systems of the
210 Andean Patagonian region, showing slight differences due to local fluctuations in
211 precipitation volume (Cuassolo *et al.* 2012, 2015, 2020; Jara 2019).

212 The vegetation present in FP was characterized by a profuse ring of *Carex*
213 *aemathorrhyncha* covering ~60% of the pond and a mixed cover of *Eleocharis*
214 *pachycarpa* and *Potentilla anserina* (~10% and ~30%, respectively) in the central
215 sector.

216 The water level at the sampling point varied from ~ 0.6 m to ~1.3 m, showing higher
217 depths in August and September (Fig. S1; Table S1). Conductivity ranged from 75 μ S
218 and 106.3 μ S, with higher values in September and October coinciding with higher
219 depths and water inputs due to increased runoff. The pH varied slightly around
220 neutrality showing a mean value of 7.4 ($\pm$ 0.4) and similarly lower values at the
221 beginning and at the end of the hydroperiod (~6.5).

222 The DOC concentration ranged between ~4 and ~9 mg L⁻¹, with higher values at the
223 beginning and at the end of the hydroperiod, and lower values coinciding with the
224 higher depths recorded (Fig. 1a; Table S1). These DOC levels are within the range of
225 previously-reported concentrations for this wetland (Pérez *et al.* 2010; Cuassolo *et al.*
226 2012, 2020). The CDOM analysis resulted in high values of the coefficient a_{350} ,
227 indicative of terrigenous DOM and/or dissolved lignin content, particularly in the

228 samples from the beginning and the end of the hydroperiod (Fig. 1b; Table S1). These
 229 high values of a_{350} likely reflected the contribution of DOM from surrounding soils [soil
 230 organic matter (SOM)], hygrophilous plants and bottom sediments, as indicated also by
 231 the direct relationship found between DOC concentration and a_{350} ($R^2=0.98$; $p=0.001$).
 232 The a_{350} :DOC values indicated high aromaticity of the DOM pool and was sensitive to
 233 eventual external inputs, such as recorded in September following a heavy rainfall that
 234 increased the runoff (Table S1). In ephemeral wetlands such as FP it is difficult to
 235 establish what is allochthonous and autochthonous, since the system alternates between
 236 dry (terrestrial phase) and flooded periods. The major sources of POM and DOM are the
 237 soils, bottom sediments and hygrophilous plants that can tolerate drought or flooding.
 238 Within FP, *C. aemathorrhyncha* and *E. pachycarpa* tolerate the drought period and
 239 develop large stands during the flooded period. Whereas, *P. anserina* dominates during
 240 the dry phase and decays during the hydroperiod, contributing substantially to the pools
 241 of POM, DOM and nutrients. This species decomposes faster than native species
 242 leaching coloured, high molecular weight DOM (Cuassolo *et al.* 2012, 2020). Thus,
 243 inputs from the decomposition of *Potentilla* and bottom sediments are likely important
 244 DOM sources during the initial flooding, along with contributions from the terrestrial
 245 vegetation and soils surrounding the pond that are mobilized by the runoff. The bottom
 246 sediments are known to contain large amounts of organic matter (Chimner *et al.* 2011),
 247 and the leaching that occurs on inundation sends an initial pulse of DOC into the water
 248 column of wetlands. Accordingly, the initial DOM pool of FP showed optical signatures
 249 indicative of high humic content and prevalence of compounds of high molecular
 250 weight/size and aromaticity (Table S1), resembling terrigenous inputs (García *et al.*
 251 2018).
 252 The different optical analyses performed on the natural water samples revealed changes
 253 in DOM quality over the course of the hydroperiod. Analysis of the CDOM fraction,
 254 and particularly the increase found in the spectral slopes $S_{275-295}$, $S_{350-400}$ and the S_R ,
 255 indicated a decrease in the molecular weight/size of the DOM pool during the
 256 hydroperiod (Fig. 1c,d; Table S1). These changes point to degradation, probably the
 257 combined effect of biological and photochemical processing in the water column
 258 (Osburn *et al.* 2011; Lee *et al.* 2018). Moreover, the CFOI index, tracked the
 259 allochthonous inputs (reflected in high CFOI values) from June to August and indicated
 260 the impact of photodegradation on the DOM pool from September towards the end of
 261 the hydroperiod (lower CFOI Values). Biodegradation may be considered slow during

the initial part of the hydroperiod (June to early September) due to the prevailing low temperatures ($< 8^{\circ}\text{C}$) and even freezing conditions; however, during spring the system starts warming up, which favors biological activity, contributing to explain the stronger degradation signals observed towards the end of the hydroperiod.

In aquatic systems of Andean Patagonia, UV radiation is a limiting factor for aquatic biota and a key driver of biochemical processes in the water column, constituting a governing factor of DOM transformation (Pérez *et al.* 2003; Marinone *et al.* 2006; Zagarese *et al.* 1998, 2017). The region experiences high exposure to solar radiation in spring and summer and high UV levels year-round due to its lati/altitudinal location (Villafañe *et al.* 2001; Díaz *et al.* 2006). Moreover, from spring to autumn the longer photoperiod with high UV levels enhances photochemical processes in the water column (Queimaliños *et al.* 2019). In shallow wetlands a major fraction of the UV radiation is absorbed in the first 10-20 cm (Fig. S2a), however, the water column is mixed by the action of strong winds, which enhances photochemical weathering (Zagarese *et al.* 1998, 2017). In spring, the pond displays a lower DOC concentration due to the increase in water input as a consequence of snowmelt, promoting diluted conditions and enhancing light penetration in the water column; such a pattern has been observed in shallow lakes of the area (Soto Cárdenas *et al.* 2017).

The analysis of the FDOM fraction also supported the observed changes in DOM quality reported above. First, the outcome of the PARAFAC modelling showed that the FDOM pool of FP is composed of two humic-like components (C1 and C2) and one non-humic component (C3) (Table S2). The fluorescent components C1 and C2 showed two excitation maxima at a single emission spectrum, as a combination of two fluorescent peaks [C1 (A + M peaks) and C2 (A + C peaks)]. Whereas, C3 was characterized by a single peak, corresponding to peak T. The fluorescent peaks A and M included in C1 likely correspond to humic substances originating from microbial activity, while peaks A + C included in C2 have been attributed to terrestrially-derived humic compounds. Component C3, characterized by the T peak, has been associated with non-humic compounds of autochthonous origin (aquatic production) (Table S2). Throughout the hydroperiod the humic components C1 and C2 prevailed in the DOM pool, whereas C3 showed a smaller contribution (Fig. 2b).

The intensities of the three fluorescent components followed the DOC concentration pattern (Fig. 1a; Fig. 2a). However, the DOC-normalized components displayed a relatively stable and prevalent contribution of the humic components C1 and C2 and an

296 increase in C3 during the hydroperiod (Fig. 2b). This pattern was supported by the high
297 HIX values highlighting the contribution of vegetation and soil/sediment DOM. In
298 addition, the increasing contribution of C3 and BIX mirrored the growing input of the
299 aquatic production over the course of the hydroperiod (Fig. 2b, c).

300 The PCA analysis performed to analyze variation in the DOM pool during the
301 hydroperiod, taking into account DOC concentration, CDOM parameters and FDOM
302 variables, resulted in two valid principal components (PC). PC1 and PC2 contributed to
303 explain 57.2% and 29.9%, respectively, accounting to explain 87.11% of the total
304 variance (Table S3). PC1 correlated positively with the DOC concentration, $a_{350}:\text{DOC}$,
305 C1, C2, and HIX, and showed a negative correlation with water depth, $S_{275-295}$ and S_R
306 (Fig. 3; Table S3). PC2 correlated positively with the degradation proxies $S_{275-295}$ and
307 $S_{350-400}$ and with the proxies of autochthonous production, C3 and BIX. Along the PC1
308 axis two groups of samples were distinguished. On the right of the plot, samples from
309 June, July and December were grouped, based on their higher DOC, $a_{350}:\text{DOC}$, high C1
310 and C2 intensities and HIX and corresponding with lower depths of the wetland. Based
311 on contrasting values of the contributing variables, samples from August, September
312 and October were clustered on the left of the plot (Fig. 3). PC2 separated the samples
313 from September, October and December with higher values of $S_{350-400}$, C3 and BIX,
314 indicative of higher degradation and the contribution of autochthonous biological
315 activity (Fig. 3). Overall, the PCA allowed discriminating samples with high DOC
316 concentrations supported by differences in DOM quality. Samples from the beginning
317 of the hydroperiod, derived from vegetation and soil/sediment inputs, showed a strong
318 humic fingerprint. In contrast, those from the end of the hydroperiod reflected
319 autochthonous production. Regarding the vegetation inputs, it is difficult to discriminate
320 between the contributions of terrestrial and hygrophilous vegetation to the DOM pool of
321 the pond, since they are both sources of high molecular weight and aromatic DOM due
322 to their lignin and cellulose contents (Talbot and Treseder 2012; Hansen *et al.* 2016).

323 Collectively, these results indicate that over the course of the hydroperiod, the DOM
324 pool of FP showed pronounced concentration and quality changes. Although the
325 prevailing DOM sources appear to be terrestrial and hygrophilous vegetation and the
326 soils and bottom sediments, the contribution of aquatic production increases from early
327 spring towards the end of the hydroperiod. The aquatic contribution could be tracked
328 through the increase in the C3 and BIX, likely enhanced by the activity of aquatic

vegetation, microbiota (plankton, sediment microbiota, epiphytic microbial communities, etc.) and their metabolites. The changes observed in the concentration and quality of the DOM pool along the hydroperiod suggested that inputs and degradation might trigger DOM reactivity changes. This hypothesis was tested through photodegradation experiments performed with DOM samples collected monthly during the hydroperiod.

Photodegradation experiments

Photodegradation experiments were performed by exposing water samples to PAR+UVR and dark treatments. Significant differences were detected in CDOM and FDOM proxies between treatments and across samples from different months of the hydroperiod (Table S4), suggesting that natural changes in DOM concentration and quality influenced the magnitude of photodegradation.

The effect of irradiation on DOC concentration was rather idiosyncratic among the water samples studied. Notably, samples from September and December showed a significant reduction in DOC concentration on exposure to PAR+UV, whereas in the other samples the trend was opposite or not significant (Fig. 4a).

In terms of changes in DOM quality, the PAR+UV treatment produced a significant loss of absorbance in the water samples, reflected in lower values of the a_{350} :DOC, and a significant increase in $S_{275-295}$ and S_R , indicative of a reduction in DOM molecular weight/size (Table S4; Fig. 4 b, c). The intensity of the three fluorescent components decreased significantly on irradiation; however, differences in the loss of fluorescence were detected among the three components (Fig. 4 d, f; Table S4). The humic component C1 and the non-humic C3 showed higher loss of fluorescence intensity than the humic component C2, which was comparatively more stable or photoresistant (Fig. 4d-f).

The negative slopes of the relationship between a_{350} and $S_{275-295}$ computed for all the incubations (Fig. 5a) indicated that exposure to radiation consistently reduced DOM molecular weight/size, although the range of the slope values suggested differences in the degree of transformation among samples, even in those with similar a_{350} values (Fig. 5a). The relationship a_{350} vs. $S_{275-295}$ allowed the detection of differences in photodegradation of the DOM pools from different moments of the hydroperiod, regardless of DOC concentration. The magnitude of photodegradation reflects changes in DOM, likely related to its composition and/or diagenetic state. This idea is also supported by

the relationship found between the intensity of the three fluorescent components and $S_{275-295}$, which showed differences in photoreactivity. Steeper slopes were found in the case of C1 and C3, while a smoother pattern was observed in the case of C2, indicating its photo-resistant condition (Fig. 5 b-d). The photoreactivity of the components can therefore be characterized as $C1 > C3 > C2$. The humic component C1 includes compounds probably derived from microbial sources, and was more photolabile/photoreactive than the humic component C2, which has a stronger soil/sediment fingerprint. Furthermore, the stability displayed by C2 in the DOM pool throughout the hydroperiod may be taken as an indication of its recalcitrance also to biological degradation (Fig. 2b). Other investigations focusing on the photoreactivity of DOM in running waters and shallow permanent lakes in the same catchment have also reported high recalcitrance of the humic component C2 (or A+C peaks) (Soto Cárdenas *et al.* 2017; García *et al.* 2018).

375

376 **Conclusions**

377

The DOC concentration and quality of the wetland varied markedly over the hydroperiod in response to inputs from terrestrial, hygrophilous vegetation and soil/sediment DOM.

Higher DOC concentrations were found at the beginning and at the end of the hydroperiod, coinciding with the lower water levels, while lower DOC concentrations were recorded at the end of winter and in early spring, following the rise in water level due to snowmelt.

The DOM pool of the wetland was characterized by the prevalence of high molecular weight, aromatic DOM with strong soil/sediment and vegetation (terrestrial and hygrophilous) fingerprints.

The progression of degradation and a low but increasing contribution of internal production to the DOM pool was detected over the hydroperiod.

In laboratory experiments, DOM photochemical transformation involved only minor changes in DOC concentration but significant changes in quality, as indicated by several optical proxies of degradation and biological production.

Changes in the relative contribution of the humic vs. non-humic components, specifically by the components C1 and C3, determined the reactivity and photo-degradability ($C1 > C3 > C2$) of the DOM pools at different moments of the hydroperiod.

Our results suggest that the cold temperate wetlands of Andean Patagonia can process a moderate amount of the C pool they receive/produce due to the combined effect of their DOM quality (highly recalcitrant) and the low temperature that prevails during most of the hydroperiod, which dampens biodegradation. Despite the importance of photodegradation processes, the high resistant DOM pool prevents photo-mineralization during the short hydroperiod, leading to accumulation of recalcitrant material in the sediments that may be subjected to weathering and microbial processing during the dry phase.

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Conflicts of interest

The authors declare no conflicts of interest.

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608 **Figure captions**

609 **Figure 1.** Monthly variation in different parameters of the dissolved organic matter
610 (DOM) pool during a hydroperiod of Fantasma pond (North Patagonia, Argentina): **a)**
611 Dissolved organic carbon (DOC) concentration; **b)** Absorption coefficients a_{254} and a_{350}
612 (applied as proxies for aromaticity); **c)** Spectral Slope $S_{275-295}$, and **d)** Slope ratio S_R
613 ($S_{275-295} : S_{350-400}$) (applied as proxies for molecular weight and degradation).

614 **Figure 2.** Variation in FDOM parameters over a hydroperiod of Fantasma pond: **a)**
615 Intensity of the PARAFAC components C1, C2 and C3; **b)** DOC-normalized intensity
616 of the FDOM components C1, C2 and C3, and **c)** Humification index (HIX: black
617 dotted line) and Biological Index (BIX: red solid line).

618 **Figure 3.** Plot of the Principal Component Analysis (PCA) PC1 vs. PC2 performed to
619 study variation in the DOM pool of Fantasma Pond over a hydroperiod, including DOC
620 concentration and CDOM (a_{254} ; a_{350} ; SUVA; $a_{350}:\text{DOC}$; $S_{275-295}$; $S_{350-400}$ and S_R) and
621 FDOM proxies (fluorescent components C1, C2 and C3; and the humification and
622 biological indexes: HIX and BIX).

623 **Figure 4.** Changes in DOM properties in laboratory incubations under PAR+UVR and
624 DARK treatments performed to study the DOM photoreactivity of DOM samples
625 collected monthly during a hydroperiod in Fantasma Pond: **a)** DOC concentration; **b)**
626 Spectral slope $S_{275-295}$; **c)** Slope ratio S_R ($S_{275-295} : S_{350-400}$); **d)** fluorescent component C1;
627 **e)** fluorescent component C2, **f)** fluorescent component C3.

628 **Figure 5.** Relationship between the absorption coefficient a_{350} (**a)**, the fluorescent
629 components C1 (**b**), C2 (**c**) and C3 (**d**) and the spectral slope $S_{275-295}$ in DOM samples
630 collected monthly from Fantasma Pond and incubated under PAR+UVR in the
631 laboratory.

Dark PAR+UVR