Linking dissolved organic matter composition and bacterioplankton communities in an Amazon floodplain system

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Abstract

Dissolved organic matter (DOM) is the main substrate for aquatic prokaryotes, fuelling their metabolism and controlling community composition. Amazonian rivers transport and process large fluxes of terrestrial DOM and yet, the link between DOM composition and its main consumers, heterotrophic bacteria, has not been well explored. The aims of this study were to characterize DOM composition and investigate the coupling between DOM and bacterial community composition (BCC) during a complete hydrological cycle in an Amazon floodplain system (Lake Janauacá). Our study revealed a clear seasonal pattern in DOM composition through the flood pulse, which affected the relative contribution of autochthonous:allochthonous sources and consequently the extent of humification, molecular weight, and aromaticity of the DOM. BCC was tightly coupled to DOM

fluorescence, which was also driven by differences in hydrology with distinct fluorescence components and bacterial taxa being more abundant and correlated with a specific hydrological season. This coupling was particularly well reflected for three of the four identified fluorescence components, two terrestrial-humic like components (C1 and C3) and an autochthonous component (C4). Despite clear changes in DOM composition, dissolved organic carbon concentrations tended to be relatively low and quite stable throughout the year. Overall, our results suggested that in the Amazon, DOM quality is more relevant to bacteria than its quantity, with the rapid turnover labile and freshly produced DOM, being critical to BCC and dynamics.

Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of humic substances, carbohydrates, carboxylic acids, and amino acids, with varying degrees of reactivity (Coble et al., 2014; Roiha et al., 2016). In freshwater lakes and other surface waters, DOM compounds have a strong influence on light attenuation, metal speciation and bioavailability, while also acting as a pH buffer. Importantly, this complex mixture of organic compounds also represents a main substrate and energy source for heterotrophic bacteria (Azam et al., 1983; Coble et al., 2014). The diversity and coupled functional attributes of the inherently complex natural bacterial communities observed in lakes define their potential to process different types of organic matter, and in general, such heterotrophic activity is a major sink for the aquatic DOM pool (Tranvik et al., 2009; Guillemette et al., 2013).

Interactions between heterotrophic bacteria and DOM are complex and may shape the apparent composition of both of these key ecosystem components. There is accumulating evidence that the availability and composition of organic substrates favour specific bacterial groups and in this way shape bacterial community composition (BCC) and community metabolism (Amon & Benner, 1996; Kritzberg et al., 2006; Guillemette et al., 2016). Moreover, bacteria do not only consume and degrade DOM, but also produce and release an array of autochthonous organic compounds during cell growth, division, and death (Kawasaki & Benner, 2006), thereby influencing the availability, composition and biogeochemical cycling of C in the biosphere (Battin et al., 2008; Osterholz et al., 2016).

Interactions between BCC and DOM have largely been explored under controlled conditions in incubation experiments designed to investigate the uptake of

specific substrates driven by differences in bacterial communities while concomitantly monitoring changes in BCC and community function in response to DOM composition (e.g. Kirchman et al., 2004; Krtizberg et al., 2006; Judd et al., 2006; Romera-Castillo et al., 2011; Logue et al., 2016; Ricão Canelhas et al., 2017). However, little is known about the dynamic interactions between BCC and DOM composition in natural aquatic ecosystems (Osterholz et al., 2016; Amaral et al., 2016). Such information is particularly scarce for large tropical floodplain systems, despite the key role these systems play in global carbon budgets processing organic matter inputs from the surrounding landscape (Regnier et al., 2013; Borges et al., 2015).

Amazon floodplains consist of a mosaic of wetland habitats including periodically flooded forests, floating macrophytes and open water environments, periodically inundated by the lateral overflow of rivers and rain coupled to seasonal variations in the annual hydrological pulse (Flood Pulse Concept) (Junk et al., 1989). These freshwater ecosystems stand out as some of the most productive and biologically diverse systems in the world (Bayley, 1995). Large amounts of terrestrial DOM from inundated forests and soil leachates enter floodplains following inundation, together with large accounts of DOM derived from other aquatic primary producers, such as herbaceous plants and phytoplankton. Seasonal variations in hydrodynamics and carbon sources result in an enormous diversity of both terrestrial and aquatic DOM potentially available for bacterial metabolic use, ultimately causing CO_2 super-saturation relative to atmospheric equilibrium, commonly observed in Amazon aquatic systems (Richey et al., 2002; Mayorga et al., 2005; Amaral et al., 2018). Considering the quantitative significance of the Amazon basin in terms of area and water volume as well as the important role of floodplains in DOM processing, it is of central importance to study the composition and reactivity of carbon compounds as well as linkages to heterotrophic bacteria in these environments. Such studies are key to understanding the temporal and spatial DOM dynamics in these systems and their potential role in regional and global biogeochemical cycling.

Previous studies, using isotopic and elemental analyses, revealed that the DOM present in the Amazon rivers system is highly refractory and derived predominantly from terrestrial inputs (allochthonous) (Ertel et al., 1986; Hedges et al., 1986; Hedges et al., 1994; Mayorga et al 2005). However, labile carbon derived from autochthonous sources was also shown to play an important role in the carbon

dynamics of these systems (Mayorga et al. 2005). In Amazon floodplain lakes, there are apparently two distinct DOM pools: a large pool of more refractory DOM from C_3 allochthonous plants sources (terrestrial C from uplands, riverine inputs, leached from flooded forests and soils), and a small pool of labile DOM compounds derived from C_4 macrophytes and phytoplankton, which are consumed and rapidly turned over by heterotrophic bacteria (Waichman, 1996; Melack & Forsberg, 2001; Ellis et al 2012; Mortillaro et al., 2016; Amaral et al 2018;).

The use of optical properties derived from fluorescence spectroscopy is a rapid and cost-efficient method that generates valuable insights about the DOM source and other characteristics, such as the level of processing and overall composition across a wide range of natural waters (Stedmon et al., 2003; Kothawala et al., 2015). These techniques have been used for multiple purposes, including investigating the link between the composition and activity of aquatic bacterial communities and the degradation of DOM (Logue et al., 2016; Amaral et al., 2016). However, no studies have made a systematic characterization of DOM composition and linked natural DOM sources and diagenetic state to BCC in Amazon floodplain systems.

Here, we present results from a systematic characterization of DOM using optical properties (absorbance and fluorescence) and an investigation of the coupling between the apparent DOM properties and BCC over a complete hydrological cycle in an Amazon floodplain system. We also investigated the uptake of distinct natural DOM sources by the indigenous lake bacterial community. We hypothesized that: (i) DOM origin, quantity, and composition would be affected by the strong seasonality in hydrological conditions (variations in water level and connectivity with terrestrial surroundings) typical from these tropical floodplain systems; (ii) bacteria would preferentially consume and mineralize labile DOM from autochthonous sources, e.g. macrophytes and phytoplankton; (iii) seasonal changes in DOM composition would be tracked by parallel changes in BCC as bacteria are the main DOM consumers.

Methods

Study area and sampling

Floodplain lake Janauacá (3°23′ S, 60°18′ W; altitude 32 m) is located in the middle of the Amazon basin, on the south margin of Solimões River, being permanently connect to it year-round by a 12 km long channel (more details can be found in de Melo et al., 2019). We performed seasonal sampling between June 2015

and May 2016, for a total of five campaigns in different phases of the annual hydrological cycle (Fig S1): i) high water (June 2015 - HW), ii) falling water (September 2015 - FW), iii) low water (November 2015 - LW), iv) early rising water (February 2016 - ERW) and v) late rising water (April 2016 - LRW). Water levels in the lake varied 10 m between low and high water seasons, in synchrony with the Solimões River flood pulse (Fig S1).

To track changes to DOM composition we used samples from all of these campaigns, but to specifically assess the BCC-DOM coupling we used samples for only four campaigns (excluding the LW campaign). Samples were collected just below the water surface (50 cm depth) at five sites (see de Melo et al., 2019): (1) drainage basin - a region of the lake draining upland areas, characterized by seasonally variable clear and humic waters; (2) open lake Janauacá - an open water area in the northern region of the floodplain lake, strongly influenced by Solimões waters during high water periods; (3) macrophyte bank - site located near to the lake margin, dominanted by different aquatic grass species throughout the year (*Paspalum repens* P.J.Bergius, *Oryza rufipogon* Griff, *Luziola spruceana* Benth. ex Döll, *Eugenia inundata* D.C.); (4) channel - the permanent connecting channel between the floodplain lake and the Solimões River (12 km long); (5) Solimões River - the main source of river water to the lake. Water samples were stored in acid-washed insulated boxes and transported in the dark to a field laboratory for further analysis and conservation within no more than 3 h. Figure S2 provides a schematic overview of the methodology employed here, which is detailed below.

Environmental variables

Water temperature and electrical conductivity were measured using a CTD profiler (Castway, Sontek Inst. Co, San Diego, CA, USA). Other physical parameters such as pH and dissolved oxygen (DO) were determined using specific probes (YSI Inst., Yellow Springs, OH, USA, model Pro-ODO) sampling at 4 Hz with data reported at 0.3 m intervals. Water transparency was determined with a Secchi disc. All of these measurements were obtained directly in the field.

Samples for chlorophyll-*a* (chl-*a*) were filtered through GF/F filters (Whatman®, Maidstone, UK) using a vacuum pump and stored frozen in the dark until analysis. We determined the chl-*a* concentration for each filter using a spectrophotometer, following filter maceration and extraction in 90% acetone (Wetzel

& Likens, 2000). For calculation we used the trichromatic equations of Strickland & Parsons (1992). Dissolved organic carbon (DOC) samples were filtered through pre-combusted (450–500 °C for 1 h) glass fiber GF/F filters (Whatman®, Maidstone, UK) and stored in pre-cleaned insulated amber glass vials and kept at 4 °C until the analysis (maximum of two weeks). DOC was determined using a total organic carbon analyzer (TOC-V Shimadzu, Quioto, Japan).

Coloured dissolved organic matter (CDOM)

Water samples for optical analyses were filtered through 0.2 μm membrane filter (Millipore® Isopore, Burlington, MA, USA) and stored in acid-washed amber glass vials at 4ºC for a few days prior to analysis. Absorbance measurements were conducted at room temperature in the dark. Absorbance spectra of CDOM were performed from 200 to 800 nm in a 0.01 m quartz cuvette (1 nm intervals and dwell time of 0.2 s), and compared against ultrapure water blanks using a FS5 spectrofluorometer (Edinburgh Instruments, Livingston, UK). We further estimated the specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) and the slope ratio (S_R) for each sample. The $SUVA_{254}$ (L mgC^{-1} m^{-1}) was calculated dividing the sample-specific absorbance coefficient at 254 nm by the cuvette path length (1 cm) and then by the DOC concentration (mg L^{-1}). $SUVA_{254}$ is commonly used as a proxy of aromatic content, with higher values associated with greater aromaticity (Weishaar et al., 2003). S_R was defined as the ratio of the spectral slope between 275-295nm by the spectral slope between 350-400 nm. S_R was determined from the linear regression of the log-transformed absorbance spectra. This parameter has been negatively associated to DOM molecular weight (Helms et al., 2008).

Fluorescence dissolved organic matter (FDOM)

Following the same basic filtering and storage procedure as for absorbance measurements, we collected excitation-emission matrices (EEM) of the FDOM in 0.01 m quartz cuvettes using a FS5 spectrofluorometer according to the following method; excitation wavelengths (λ_{ex}) ranged from 240 to 450 nm in 10 nm increments, and emission wavelengths (λ_{em}) from 300 to 560 nm in 2 nm increments with a dwell time of 0.25 s, bandwidth of 5 nm. All EEMs were background corrected against a Milli-Q water. Subsequently the sample signal (S) was corrected for the reference (R)

lamp signal, to get S/R. All fluorescence spectra were corrected for inner filter effects using the absorbance-based approach (Lakowicz, 2006; Kothawala et al., 2013). Fluorescence intensities of the EEM were calibrated to Raman units by dividing the intensity with the Raman area of Milli-Q water integrated at λ_{ex} of 350 nm, and over an λ_{em} range of 380 to 420 nm (Lawaetz & Stedmon, 2009). We used the software Matlab and the DOMcorr toolbox to perform all corrections (Murphy et al., 2010).

Three additional indices were derived from the matrices: Fluorescence Index (FI), Humification Index (HIX) and Freshness Index (Fresh). The FI was determined as the ratio between emission wavelengths (λ_{em}) at 470 and 520 nm with excitation at 370 nm (Cory et al., 2010). Higher FI values (~1.8) are associated with DOM derived from bacterial and algal sources, while lower values (~1.2) indicate DOM derived from terrestrial material (for example, soil, leaves, wood) (McKnight et al., 2001). Intermediate values (~1.4) indicate a mixture of both sources. The HIX was calculated as the area under the emission spectra between λ_{em} 435–480 nm divided by the peak areas between λ_{em} 300–345 nm + 435–480 nm at an λ_{ex} 254 nm. HIX is a proxy of DOM humic content, where higher values indicate higher concentrations of humic substances (Ohno, 2002). The Freshness Index is an indicator of recently produced DOM, with higher values indicating newly produced DOM. This index was estimated as the ratio of the emission intensity at λ_{em} 380 nm by the maximum emission intensity in the interval λ_{em} 420-435, both at an λ_{ex} of 310 nm (Parlanti et al, 2000).

We performed a parallel factor analysis (PARAFAC) to identify components within the EEM that represent independently varying regions of the EEM and represent different types of fluorescencent DOM using Matlab software and the DOMFluor toolbox (Stedmon et al., 2003). The analysis was developed using EEM for altogether 388 samples from Lake Janauacá. After including non-negativity constraints, we removed an outlier and validated the model using split-half analysis and random initialization (Stedmon et al., 2003). We further identified the nature of each PARAFAC component by comparison to previously reported components using the OpenFluor database (Murphy et al., 2014).

Bacterial community composition (BCC)

Samples for determination of BCC were first prefiltered through 3 µm pore size polycarbonate membranes (Whatman® Nucleopore, Maidstone, UK) to remove larger particles. Filtrates were subsequently passed through 0.2 µm polycarbonate membranes (Millipore® Isopore, Burligton, MA, USA) to collect the free-living bacterial size-fraction. Membranes were stored at -80 ºC until bacterial DNA was extracted from filters using the phenol-chloroform method followed by purification with Amicon Ultra-4 centrifugal filters (Millipore® 100kDa) (Ganesh et al., 2014). Using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACT ACHVGGGTATCTAATCC-3') we amplified the V3/V4 region of the 16rRNA gene (Herlemann et al., 2011). We performed all PCR reactions with KAPA High - fidelity Hotstart ready mix (KAPA Biosystems, Boston, MA, USA) with an initial step of 95ºC for 3 min, 25 cycles of 98ºC for 20s, 62ºC for 15s, 72ºC for 15s and finally 72º for 60s. Subsequently, PCR products were purified with magnetic beads in the AMPURE XP kit (Beckman Coulter, Pasadena, CA, USA) and Indexed with Nextera XT kit V2 (Illumina, Inc, San Diego, CA, USA). An additional step of purification with magnetic beads was performed, and then a combined pool was prepared by mixing 5 µl from each library. High-throughput sequencing was performed on an Illumina Miseq2000 instrument using a paired-end approach and 2 x 250 bp chemistry (Laboratório Multiusuário Centralizado para Sequenciamento de DNA em Larga Escala e Análise de Expressão Gênica, Univerdade Estadual Paulista, Jaboticabal, São Paulo, Brazil). All sequences were submitted to the BioSample database hosted by NCBI (https://www.ncbi.nlm.nih.gov/sra/SRP127556).

Quality filtering, denoising and removal of potential chimeras and non-bacterial sequences were performed with UPARSE (Edgar, 2013) following a previously established pipeline (Logares et al., 2014; Logares, 2017). In summary, paired-end reads were merged and all sequences shorter than 100 bp were discarded. Merged sequences were clustered into operational taxonomic units (OTUs) at 97% identity cutoff using UPARSE (Quast et al., 2013), and the taxonomic classification was done with BLASTn against SILVA 119.1 (Zhang & Fang, 2000) with at least 75% similarity threshold. For further analyses, all chloroplasts and Archaea sequences were discarded. To enable comparisons between samples OTU table was randomly subsampled (rarefied) based on the sample with the least number of reads (10,341).

DOM uptake experiments

To study microbial uptake of DOM we performed a batch culture experiment with two distinct and abundant natural sources of DOM found in lake Janauacá: soil and macrophytes (*Panicum repens*). We chose *P. repens* because this was the most abundant macrophyte species observed in the study area during the experiment (May 2016). *P. repens* is a floating grass, that converts atmospheric carbon dioxide into biomass through a C_4 metabolic pathway and can cover large areas of Amazon floodplains (Hess et al., 2003; Silva et al., 2013). Macrophyte-derived DOM represents an autochthonous source with high amounts of labile DOM components (Bertilsson & Jones, 2003) and has been suggested as the main source of labile DOM to bacterial communities in Amazon floodplain lakes (Waichman, 1996; Melack & Forsberg, 2001). During the initial stages of flooding (rising waters), large amounts of terrestrially derived DOM are leached from surrounding soils (Junk et al., 1989), which is an allochthonous source with high content of humic substances, high degree of aromaticity, and low reactivity (Thurman, 1985).

We first prepared a slurry with 200g of margin soil collected from the lakeshore and 800 ml of Milli-Q water that was subsequently stirred for 3 hours. For macrophytes, we collected and washed leaves, roots and stems of *P. repens* from the lake and mixed this with 500 ml of Milli-Q water. This extract was kept in an ultrasonic water bath for 3 hours, to ensure that sufficient amounts of DOM were extracted. We then filtered each mixture (macrophytes and soil) sequentially through 20, 3, 0.7 and 0.2 μm pore size filters to remove particulate organic matter and microbial cells. We then diluted DOM solutions with Milli-Q water to a concentration analogous to in situ conditions (<5 mg C L^{-1}) and stored these extracts at 4^{o}C until the beginning of the incubations (3 days).

We used a dilution culture approach to cultivate bacterial cells from lake Janauacá by filtering lake water twice through 0.7 µm filters and adding the filtered inoculum to DOM solutions 1:10 (v:v). The experiment consisted of three different treatments incubated in triplicate with the addition of lake bacteria: (i) soil DOM (S), (ii) macrophyte DOM (M) and (iii) a mixture of soil and macrophyte DOM (50% each) (S+M). Incubations were carried out in amber glass bottles (500 ml) for 124 hours in the dark at 30^{o}C (in situ temperature) with constant shaking (x RPM), to avoid photochemical alteration of the DOM and sedimentation, respectively. We took samples for bacterial production (BP) initially (T0) and after 24, 48, 72, and 124 hours of incubation. BP was estimated by the standard H^3-leucine incorporation

method (Kirchman et al., 2004), using a carbon:protein conversion factor of 0.86 (Simon & Azam, 1989).

Statistical Analyses

To estimate the link between DOM and BCC composition we performed a Canonical Analysis of Principal Coordinates (CAP) (Anderson & Willis, 2003). For this purpose we used data of relative abundance of bacterial operational taxonomic units (OTUs) and PARAFAC component intensities (in Raman units) to compute dissimilarity matrices based on Bray-Curtis and Euclidean distance, respectively. We then performed a Principal Coordinate Analysis (PCoA) to reduce the number of orthonormal axes to be included in CAP analysis. To avoid negative eigenvalues, we took the square root of all dissimilarity matrices (Legendre & Legendre, 1998). The choice of the number of PCoA axes is an important step in CAP analysis in order to ensure that patterns seen on our plots would not be over-parameterized and misleading (Anderson & Willis, 2003). Here, this was done with the aim of choosing the number of axes representing at least 75% of the total variation of each data set to ideally lose minimal information and also avoid too many dimensions contributing noise and hampering the analysis (Osterholz et al., 2016).

The next step was to check the significance of canonical correlations (Canonical Correlation Analysis – CCorA) computed by permutation (n = 9999). Finally, we considered correlations of individual PARAFAC components (intensity in Raman units) and OTUs (relative abundance) with the first two canonical axes using Spearman rank correlation followed by a false discovery rate (FDR) test for multiple comparisons. For this analysis, we included all OTUs with an average relative abundance ≥ 0.1 and significant relationships (FDR adjusted p-value ≤ 0.05).

DOM optical properties (coefficients, indexes, and components) were tested for differences among group means (seasons and sites) using one-way ANOVA and a post hoc Tukey test ($p \leq 0.05$). Results were plotted in violin plots to visualise the distribution of the data and its probability density (kernel density estimation). All statistics (sections 2.7 and 2.8) were performed in R (version 3.3.3, R Development Core Team, http://cran.r-project.org/) using the packages “vegan”, “ape” and “psych” (Oksanen et al., 2015; Paradis et al., 2004, Revelle, 2018). To create figures, we used the package ggplot2 (Wickham, 2009).

Results

DOM characterization

DOC concentrations were generally low, ranging between 2.7 and 5.9 mg C L^{-1} (with the exception of one sample where the concentration was 9.0 mg C L^{-1}), with a mean annual average of 4.8 mg L^{-1} (table 1). The specific absorbance ($SUVA_{254}$) index varied between 1.8 and 5.2 L mg C^{-1} m^{-1} and showed a negative and strong correlation to DOC concentrations (R^2=0.59 $p<0.001$). The S_R ranged between 1.1 and 1.7, and had a strong positive relationship with the Freshness Index, suggesting that recently produced DOM were of overall low molecular weight. The Freshness Index also showed a strong positive relationship with chl-*a* concentrations and water temperature (both were correlated, Fig. 1a and 1b), indicating that the fresh DOM likely originated from recent primary production (Figs. 1b and 1c).

The parallel factor analysis (PARAFAC) identified four components (Fig. 2) that were matched against the OpenFluor database to identify similar components identified in other studies (Murphy et al., 2014). Three components are associated with terrestrial humic-like material (C1, C2 and C3) and one was a protein-like component (C4) (Fig. 2). C1 exhibits both UVC and UVA excitation maxima (260 and 350 nm, respectively) and an emission peak at 480 nm, similar to peak C, which is described as humic-like material exported from terrestrial sources (Coble, 1996). This component is characterized by DOM rich in aromatic compounds and of high molecular weight and is widespread in aquatic environments (Stedmon et al., 2003; Williams et al., 2010). C2 has been described as a proxy for terrestrial DOM, rich in fulvic acids and is often referred to as A + M peaks (Coble, 1996). C3 represent terrestrially derived DOM of intermediate molecular weight (Lambert et al., 2016). Finally, C4 has properties similar to tryptophan-like fluorescence, an amino acid signal indicative of autochthonous sources (Stedmon et al., 2003).

Seasonal patterns in DOM composition

Our results revealed that the contribution of each optical index and PARAFAC component varied strongly across different seasons, but this temporal dynamics was still less evident than the differentiation between sites (Figs. 3 and 4). Nevertheless, DOC concentration did not vary significantly across either time or space (ANOVA p-value$\geq$ 0.05). DOM spectral slope (S_r; indicator of molecular weight) and Freshness

Index tended to be lower during the low water (LW) period concomitant to the lowering of the water level (Figs. 3a and 3b). HIX was significant higher in late rising water season (LRW) (Fig. 3c), indicating that the inundation of terrestrial surroundings provided humic substances to the lake. Fluorescence Index (FI) did not present a clear seasonal pattern (Fig. 3d, ANOVA p-value≥0.05), but a significant difference was seen between open lake and basin sites, being higher at the lake site the farthest away from land (ANOVA p-value≤0.05). C1, C2 and C3 were the most abundant PARAFAC components identified for the studied waters and varied over the year (Figs. 4 and S3). We found that C1 and C2 responded to water level and increased along the hydrological cycle with significantly higher values during LRW (Figs. 4a and 4b, ANOVA p-value≤0.05). C3 decreased over the sampling period, reaching maximum values during high water (HW) and falling water (FW) seasons while being at its lowest during the LRW (Fig. 4c). C4 was the least abundant component and was significant lower in HW and LRW (Fig. 4d, ANOVA p-value≤0.05).

Linking bacterioplankton and DOM composition

To link bacterioplankton and DOM composition, we first investigated the production rates of heterotrophic bacteria from lake Janauacá exposed to distinct sources of DOM using a batch culture experiment. We calculated BP rates in three treatments over 124 hours and found that this proxy for bacterial growth typically increased during the course of the incubations for all treatments and varied depending on DOM substrate (Fig. 5). BP in the macrophyte treatment (an autochthonous source) increased rapidly during the first 48 hours, and was always higher than in the soil treatment (allochthonous source). The highest BP rates were seen in the mixed treatment (soil+macrophyte), which reached high values already in the first 24 hours and then stabilized.

Second, we used the canonical analysis of principal coordinates (CAP) to investigate the link between BCC and DOM composition *in situ*. One important step of CAP analysis was to select the number (m) of principal coordinate axes (PCoA) to be included in the analysis. For all PARAFAC components, the first four PCoA axes (m=4) explained more than 75% of total variation in DOM quality, and for bacterial OTUs, the first ten axes (m=10) (cumulative eigenvalues, table S1). Then we performed canonical correlation analysis (CCorA) that revealed significant

correlations between BCC and DOM composition (p-value≤0.05), for three of the four identified components (C1, C3, and C4). In contrast, CCorA did not reveal significant correlation between the component C2 and BCC.

A further use of the CAP analysis is its potential for identifying the original variables (OTUs and components) responsible for the association between BCC and DOM composition in the form of a pair of canonical axes. We found this multivariate pattern was driven by differences in hydrology, with distinct components and OTUs being simultaneously more abundant and correlating with a specific hydrological season (Fig. 6 and table S2). Also, we found that different sampling sites shared more similarities for certain hydrological season, e. g. the channel and Solimões River were very similar at LRW and ERW periods, and the channel was close to the drainage basin and macrophytes bank during FW and HW, respectively (Figure 6A). We identified three PARAFAC components, C1, C3 and C4, and 23 OTUs from 6 different phyla (*Proteobacteria* (alpha, beta, gamma), *Actinobacteria, Planctomycetes, Verrucomicrobia, Bacteriodetes* and *Cyanobacteria*) that were significant correlated with this pattern (Fig. 6b, Pvalue-FDR≤0.05).

C1 was most strongly correlated with HW while C4 and C3 were associated with the FW period, with C3 being in between (Figs. 6a and 6b). Regarding OTUs, we identified a similar pattern, indicating that some taxa were correlated with a specific DOM component. For example, the OTU_127 (genus *Synechococcus*) followed the same pattern as component C4, suggesting that they were linked (Fig. 6).

All representatives of *Betaproteobacteria* (9 OTUs) seemed to be linked with C1, being more abundant in HW and ERW. In contrast, all representatives of *Alphaproteobacteria* (3 OTUs) were related with FW and HW and negatively correlated with ERW and LRW (Fig. 6). The same pattern was seen for C3.

Discussion

Aquatic DOM provides organic carbon and nutrients required for bacterial metabolism and growth, and to tracing the compositional variability within DOM over time and space may help elucidate potential interactions between bacteria and specific DOM components (Kujawinski, et al. 2011). Here, we identified a clear seasonal pattern in DOM composition coupled to the flood pulse, which had a prominent role in controlling the influence of terrestrial organic matter and the relative contribution of autochthonous:allochthonous C to the lake. Bacterial

community composition (BCC) also changed, with major differences in the relative abundance of the main bacterial groups over the annual cycle (Figure S4).

The strong relationship between primary production (chl-*a*) and DOM age (Freshness Index) appeared to be due to strong seasonal changes. For instance, an increase in water temperature was related to decreased water level (during the falling water season - FW) and a subsequent increase in phytoplankton biomass (chl-*a*). In turn, the freshly produced DOM from primary producers enhanced the Freshness Index and low-weight molecular content (S_R) of bulk DOM. While Amazonian floodplains are among the most productive ecosystems in our biosphere (Melack & Forsberg, 2001), phytoplankton production is generally considered a minor C source, at least at the regional scale (only 8%, compared to 52% of macrophytes and 32% of flooded forest trees) (Junk, 1985; Melack & Forsberg, 2001; Melack et al., 2009). However, in some lakes phytoplankton biomass can still reach high levels (Forsberg et al., 2017), especially during low waters periods as found here (falling, low, and early rising water). Additionally, high phytoplankton excretion rates have been reported in tropical systems, associated with high incident light and rapid nutrient exhaustion, promoting the release of labile DOM that fuels bacterial growth and respiration (Morana et al., 2014; Freitas et al., 2017; Amaral et al 2018).

Our results suggested that this fresh DOM is mainly produced and released by phytoplankton (an autochthonous source). Nonetheless, we highlight the direct role of macrophytes as an alternative source of labile DOM and also propose an indirect effect as a natural fertilizer for Amazon floodplains, where nitrogen and phosphorus are limiting factors (Devol et al., 1984; Forsberg, 1984). The dominant species in Lake Janauacá during this study was *P. repens* (C_4 metabolism), a perennial aquatic grass suggested to host N_2 fixing bacterial symbionts (Martinelli et al., 1992; Junk & Piedade, 1997). When the water level retreats (falling water season), macrophytes begin to decompose, persisting only in the rhizomes, which in turn release large amounts of nutrients and stimulate phytoplankton growth (Rai & Hill, 1984; Junk & Piedade, 1997; Melack & Forsberg, 2001). Concomitantly, we observed increased chl-*a* concentrations in Lake Janauacá.

During rising waters (ERW and LRW), we observed an increase in the degree of humification (HIX) and in aromatic content ($SUVA_{254}$) of the DOM. These are common features of allochthonous materials (leaves, soil, wood, etc). These materials could have entered into the water upon temporary flooding of terrestrial habitats (Junk

et al., 1989). They could have also entered the lake with inflowing river water which has been shown to contain high concentrations of degraded terrestrial humic substances (Ertel et al 1986). In addition, large amounts of senescent biomass decay and both inorganic and organic materials (deposited during the terrestrial phase) are hydrologically mobilized at the aquatic-terrestrial transition zones during rising water seasons. The increase in water level and terrestrial organic matter inputs will then affect environmental conditions and influence on aquatic biota (Junk et al., 1989; Thomaz et al., 2007).

Concerning the identity of fluorescence components (PARAFAC), we observed a marked change in the dominant DOM components throughout the flood pulse. C1 and C2 dominated during ERW and LRW, and there was a considerable input from allochthonous materials to the lake. Both components are indicators of terrestrially-derived humic matter, reported to have great aromatic content and high molecular weight. Another terrestrial-humic like component found in these waters was the abundant C3. In spite of the terrestrial nature of C3, it had a seasonal pattern very different from C1 and C2, being more abundant at high water (HW) and falling water (FW). This different hydrological response implies that this component (C3) may have a different origin from C1 and C2. These findings are in agreement with previous studies that demonstrated the predominance of terrestrial and largely refractory DOM in the Amazon River and its tributaries (Ertel et al., 1986; Hedges et al., 1986). Another study conducted in Brazilian wetlands (Pantanal) also report a dominance of terrestrial-humic materials compared to protein-like compounds in these dynamic waters (Dalmagro et al., 2018). The increased presence of the C3 componente at high water may reflect inputs from inundared floodplain forest which surrounds the lake at this time.

In contrast, C4 provided a signature of autochthonous protein-like DOM and was less abundant compared to the other components, which is typical as this protein-like peak is only a small fraction of the total DOM fluorescence. We believe that compounds represented by C4 are continuously produced by primary producers but, due to their reactive nature, they are rapidly consumed by bacteria and, thus, do not accumulate appreciably in the water column. Primary producers, phytoplankton and macrophytes could be responsible for producing this DOM component. It is well known that a wide range of bacterial taxa have the capacity to assimilate and metabolize low-weight labile molecules (such as those represented by C4), but the

capacity to cleave the larger molecules in the supposedly "recalcitrant" DOM requires a set of specialized hydrolytic enzymes that are absent in many taxa (Kritzberg et al., 2006). An investigation of the autotrophic carbon sources of bacterioplankon in a similar floodplain lake, based on the isotopic analysis of plants, DOC and respired CO_2, demonstrated that the DOC consumed by bacteria was derived predominantly from herbaceous macrophytes, while the DOC that accumulated on the lake was predominantly refractory carbon derived from terrestrial sources, largely consistent with our interpretation Waichman (1996).

In spite of the clear seasonal pattern in DOM composition, DOC concentration tended to remain low and quite stable (ranging between 2.7 and 5.9 mg L^{-1}) throughout the year of monitoring. This highlights the importance of DOM quality and DOM availability for biological utilization. Our result aligned with the hypothesis of co-occurrence of a small fraction of labile and freshly produced DOM, which is rapidly consumed, with a larger pool of recalcitrant DOM (mostly humic) at the Amazon Basin (Mayorga et al 2005) and in other aquatic ecosystems (Bertilsson & Jones, 2003). Small seasonal variations in DOC concentrations were previously reported at Lake Janauacá (Albéric et al., 2018). Similar results were reported by Waichman (1996), in a similar Amazon floodplain lake. However, in this study there was no significant variation in DOM quality as measured by isotopic composition of DOC, suggesting relatively constant and homogeneous sources of DOM from upland soils and flooded forests (Albéric et al., 2018). Here, we demonstrated that optical properties of DOM is a useful and practical approach for revealing seasonal and spatial patterns in DOM quality and could prove to be very useful for future work in Amazonian aquatic systems and elsewhere.

A widespread view in aquatic biogeochemical studies is that the bacterial communities who preferentially consume autochthonously produced DOM, also obtain energy more efficiently than they would from allochthonous DOM, essentially due to greater accessibility and higher nutritional value (Kitzberg et al., 2006; Guillemette et al., 2013). Here, we also found higher rates of BP in macrophyte leachates (more labile DOM) than soil treatments (humic source). However, the mixed treatment had the highest BP rates, indicating that a synergistic effect of allochthonous and autochthonous sources could contribute to even more effective uptake of DOM. Possible explanations for this synergistic effect could be microbial niche diversification and substrate specialization in the microbial communities or

resource/nutrient complementarity. Niche diversification concerns the differential response in growth rates of different groups of bacteria on distinct types of DOM. Such differences in BCC explain differential uptake and utilization of autochthonous and allochthonous C (Judd et al., 2006). Another explanation could be resource complementarity, where soil leachates may provide some limiting nutrients and trace elements while macrophyte leachates supply readily available energy sources in form of organic substances. Another speculation is that there could be a priming effect, where labile DOM substrates enhance the overall utilization of less reactive compounds by promoting the activity of bacterial communities (Guenet et al., 2010). Despite being previously reported in incubations experiments in the Amazon (Ward et al., 2016) as well as in other aquatic ecosystems (Bianchi, 2011; Kuehn et al., 2014), further studies would be necessary to evaluate if there is any such priming effect in bacterial DOM degradation in these ecosystems.

Identifying the links between DOM and BCC has been a subject of great interest, triggering research efforts at multiple scales and in different experimental settings. Still, these interactions have remained less explored under natural conditions in aquatic systems (Ruiz-González et al., 2015; Osterholz et al., 2016; Amaral et al., 2016), and to our knowledge this is the first attempt in a tropical floodplain system. Although the link between BCC and DOM does not always come out clearly (Langenheder et al., 2005), most studies have reinforced the central role of bacteria for DOM transformation and the importance of DOM availability and quality as drivers of bacterial metabolism and composition (Kritzberg et al., 2006; Judd et al., 2006). Experimental approaches are fundamental because they allow the manipulation of community composition and tight control of environmental conditions, enabling researchers to isolate the effects of specific environment factors on composition and functioning of microbial communities (Reed et al., 2007). However, these approaches also have limitations since the so-called “bottle effect” promotes growth of specific fast-growing taxa that are typically rare in natural ecosystems, and this may accordingly have a great effect on the outcome of the experiments (Krammer et al., 2008; Hammes et al., 2010). Additionally, studies under laboratory conditions often focus on a narrow range of organic substrates of known compositions, and do not fully consider the interplay between the complex DOM mixtures seen in natural habitats and the equally complex indigenous microbial communities.

In this study we used Canonical Analysis of Principal Coordinates (CAP) to investigate the link between PARAFAC components and BCC in an Amazon floodplain under natural conditions. We found that BCC was tightly coupled to the wide natural variation we observed in DOM composition, which broadly followed a seasonal pattern. This coupling was particularly well reflected for three of the four identified fluorescence components (C1, C3 and C4). Additionally, we found that specific bacterial taxa were correlated with a specific component within the DOM pool. For example, we found an interesting link between C4 and the OTU_137 (*Synechococcus*) during FW. This may indicate a role of these pico-cyanobacteria in producing and releasing labile DOM during this period, and this was paralleled by an increase in phytoplankton biomass (chl-*a*) and Freshness Index of the DOM. Another interesting finding was that all representatives of *Betaproteobacteria* were associated with C1 (ERW and HW), while all representatives of *Alphaproteobacteria* were associated with C3 (HW and FW). This implies specialization of these groups in using contrasting sets of substrates and relates to the availability and concentration of such resources during specific seasons. In agreement with this, a preference for fulvic acid-rich compounds by *Alphaproteobacteria* has already been suggested in a previous study (Amaral et al., 2016). In general, a major factor that distinguish these two classes is that members of freshwater *Alphaproteobacteria* are commonly described as oligotrophs, i.e. good competitors at lower-nutrient availability and able to degrade a variety of organic compounds (Salcher et al., 2013). In contrast, *Betaproteobacteria* require higher amounts of organic nutrients to be competitive.

A previous study conducted by our group demonstrated that bacterial communities in Lake Janauacá were strongly shaped by dispersal processes, driven by water exchange and connectivity with the main river and that these varied throughout the flood pulse cycle (de Melo et al., 2019). Here we observed that the DOM quantity, source and composition are also affected by changes in hydrology indicating that hydrodynamics shape the interchanges of the floodplain with the feeding river in wetlands, controlling both BCC and DOM composition, but also regulating the coupling between them. We believe that the flood pulse is a strong force that has a great impact on dispersal processes, but also affects environmental filters, especially the availability of organic substrates to bacteria, controlling their activity and composition.

While the optical measurements used may not have captured the full complexity of DOM composition and dynamics (i.e. access all compounds contained in each PARAFAC component, or detailed patterns in chemodiversity), the approach providing considerable insight into the seasonal patterns in DOM composition, reactivity and aromaticity, and revealed clear correlations between BCC and DOM quality. Moreover, the optical characteristics of DOM have previously been demonstrated to accurately reflect high-resolution molecular-level data at wide geographic and temporal scales (Kellerman et al., 2015).

In conclusion, we found that the flood pulse modulated the source and aromaticity of DOM, and that there were strong links between these characteristics and bacterial community composition. We reiterate that the CAP approach did not resolve causal interactions (i.e. when and at what rate DOM was being consumed or produced by bacteria) but it was very effective in revealing bacterial-DOM relationships under natural conditions. Overall, our results support the hypothesis that a small portion of rapidly recycled autochthonous DOM is an important resource for bacterioplankton. We believe that the concomitant use of between experimental and *in situ* approaches could help researchers better understand when bacterial degradation is a source or a sink for DOM and uncover interaction across contrasting scales, as needed for a better understanding of the global carbon cycle.

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Conflict of interest

The authors declare no conflict of interest.

Authors Contribution

MM, JHM, BF and HS designed the field approach. MM and JHM collected environmental samples and did laboratory measurements. MM performed molecular analyses and statistical processing. MM and DK performed DOM and PARAFAC analyses. MM wrote the first draft of the manuscript and all authors contributed to data interpretation and analysis and subsequent revisions of the manuscript.

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Figure and Table Captions

Table 1: Dissolved organic matter properties and environmental variables in Lake Janauacá sampling stations during the studied period. Abbreviations: dissolved organic carbon (DOC), Slope ratio (S_r), Specific absorbance at 254 nm ($SUVA_{254}$), Florescence Index (FI), Freshness Index (Fresh), Humification Index (HIX), water temperature (Temp), dissolved oxygen (O_2), electrical conductivity (Condut)

Figure 1: Relationships between (A) log water temperature and log chlorophyll-*a*, (B) log chlorophyll-*a* and log Freshness Index and (C) log Freshness Index and log Slope ratio. A and B (*n*=20), C (*n*=23). Abbreviations: high water (HW), falling water (FW), low water (LW), early rising water (ERW), late rising water (LRW)

Figure 2: Excitation-emission spectra highlighting the four fluorescing components (C1, C2, C3 and C4) describing the major fluorescing regions as identified in our study using parallel factor analysis (PARAFAC)

Figure 3: Violin plots showing: (A) Slope ratio (SR), (B) Freshness Index (Fresh), (C) Humification Index (HIX) during high water (HW), falling water (FW), low water (LW), early rising water (ERW) and late rising water (LRW) seasons; and (D) Fluorescence Index (FI) in different sampling sites. Note that the scales of the y-axis and labels of x-axis are variable between graphs

Figure 4: Violin plots showing the contribution of each PARAFAC component (C1, C2, C3 and C4 in Raman units) in sampled seasons: high water (HW), falling water (FW), low water (LW), early rising water (ERW) and late rising water (LRW)

Figure 5: Mean and standard deviation of bacterial production in treatments during incubation. Abbreviations: Soil+bacterial DOM (S), macrophytes DOM (M), soil+macrophytes DOM (S+M)

Figure 6: (A) Ordination with Canonical Analysis of Principal coordinates axes between bacterial community and all DOM components; (B) the correlated fluorescence components (intensities) and OTUs (relative abundance) along the two first canonical axes

Figure 1

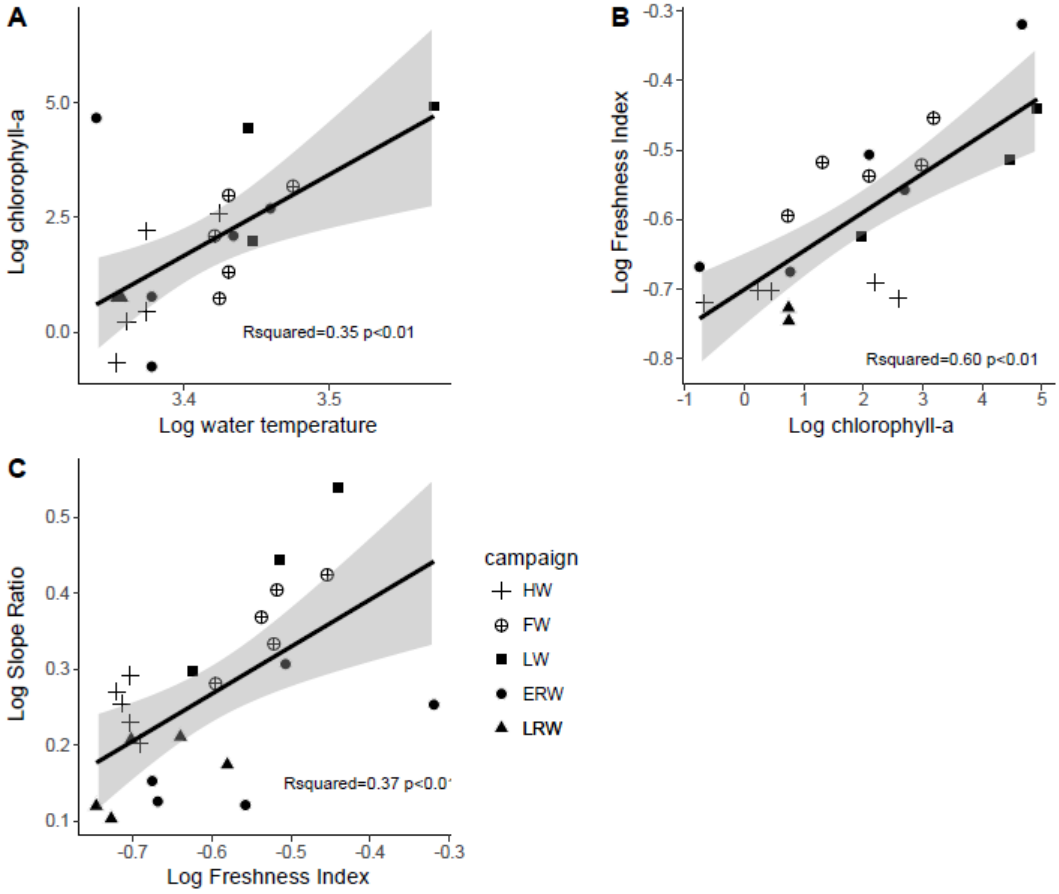
A
Log chlorophyll-a
5.0
2.5
0.0
3.4
3.5
Log water temperature
Rsquared=0.35 p<0.01
B
Log Freshness Index
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
-1
0
1
2
3
4
5
Log chlorophyll-a
Rsquared=0.60 p<0.01
C
Log Slope Ratio
0.5
0.4
0.3
0.2
0.1
-0.7
-0.6
-0.5
-0.4
-0.3
Log Freshness Index
Rsquared=0.37 p<0.0
campaign
HW
FW
LW
ERW
LRW

Figure 2

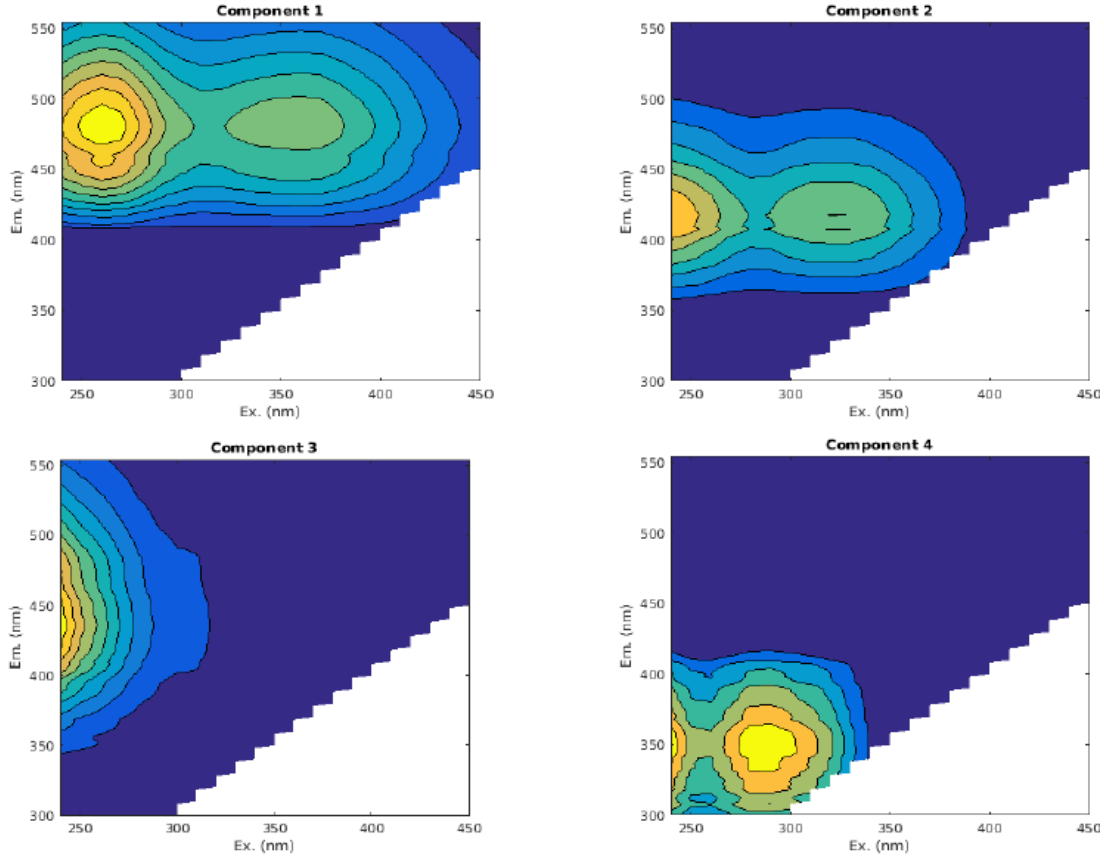
Component 1
Component 2
Component 3
Component 4
Em. (nm)
Ex. (nm)
550
500
450
400
350
300
250
300
350
400
450

Figure 3

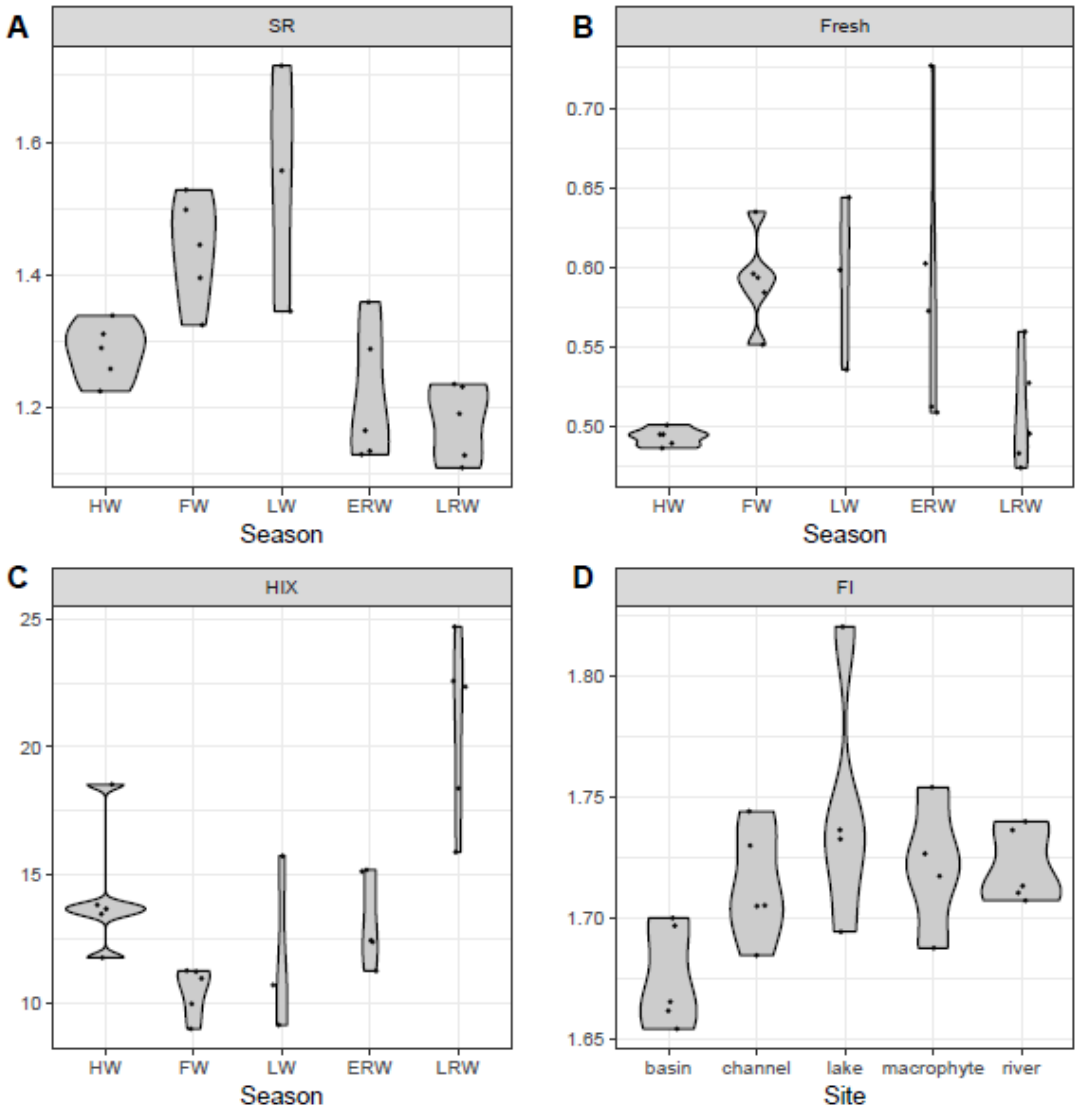
A
SR
1.6
1.4
1.2
HW
FW
LW
ERW
LRW
Season
B
Fresh
0.70
0.65
0.60
0.55
0.50
HW
FW
LW
ERW
LRW
Season
C
HIX
25
20
15
10
HW
FW
LW
ERW
LRW
Season
D
FI
1.80
1.75
1.70
1.65
basin
channel
lake
macrophyte
river
Site

Figure 4

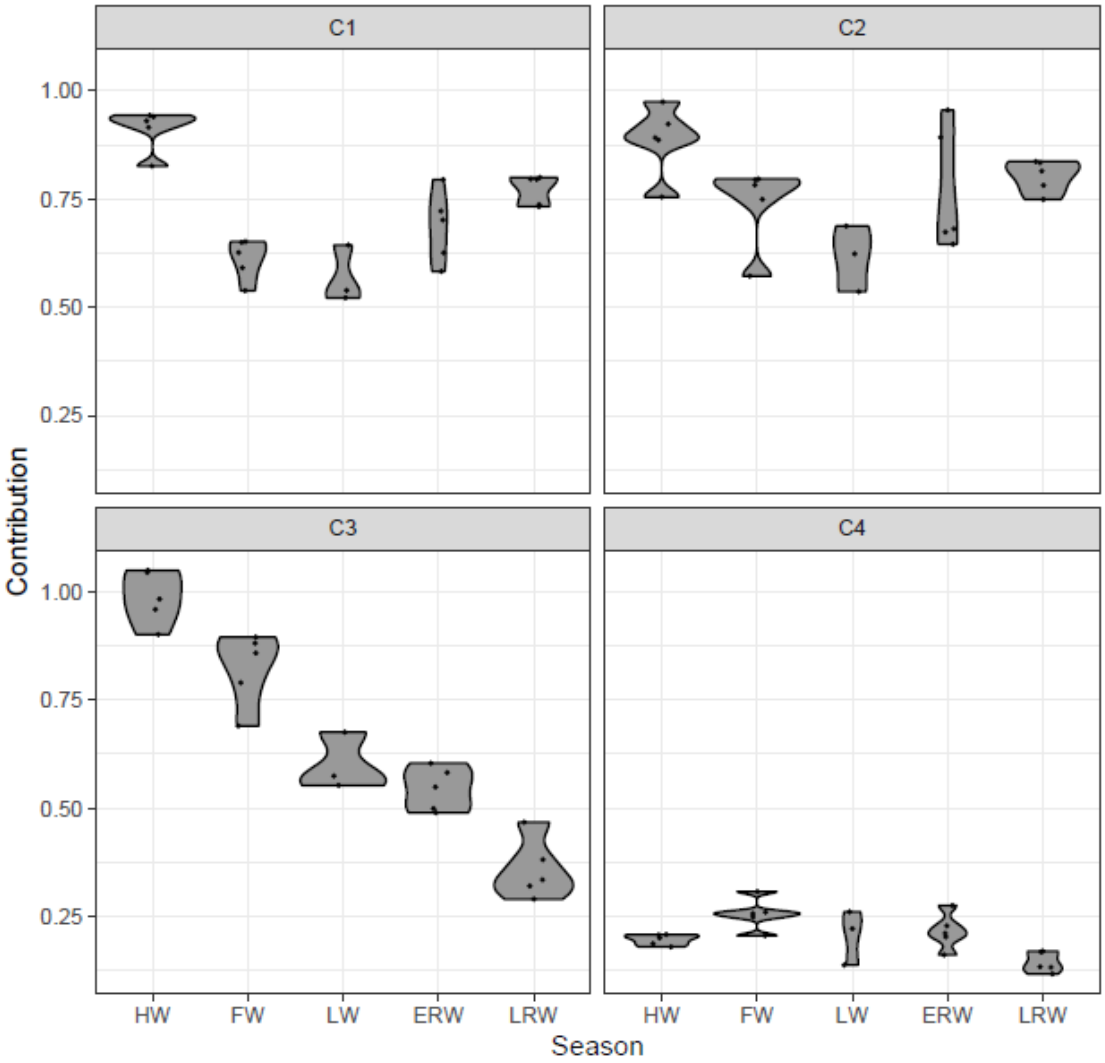
C1
C2
C3
C4
Contribution
1.00
0.75
0.50
0.25
HW
FW
LW
ERW
LRW
Season

Figure 5

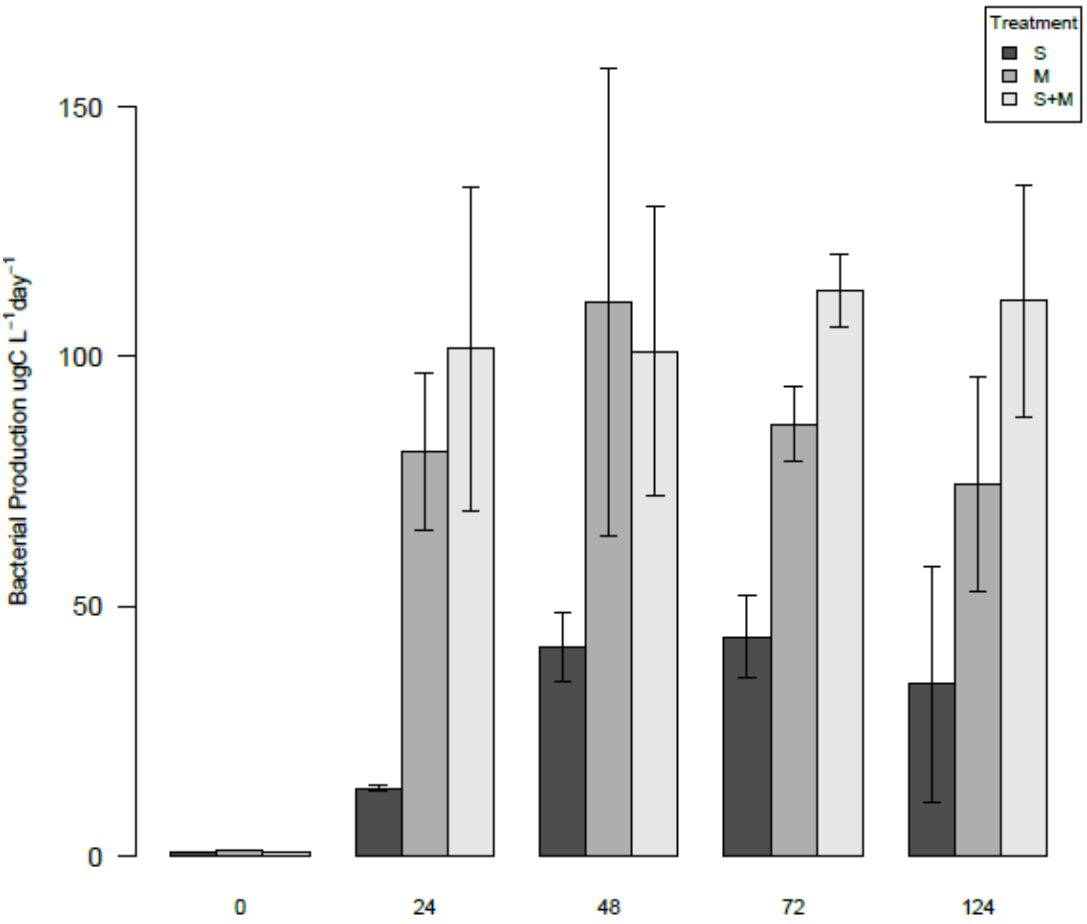

Figure 6

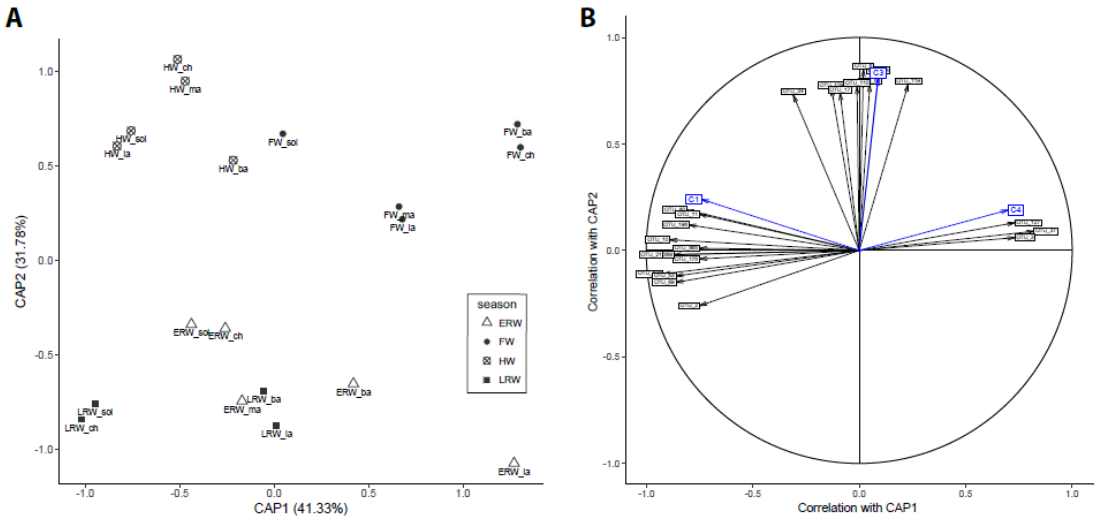

Table 1

Campaign	Site	Sample	DOC (mg L-1)	SR	SUVA254 (L mgC-1 m-1)	FI	Fresh	HIX	Temp (°C)	secchi (m)	pH	O2 (mg L-1)	Condut (µS cm-1)	Chlorophyll-*a* (ug L-1)	POC (mg L-1)
High water (HW)	basin	HW_ba	4.93	1.22	4.03	1.67	0.50	13.84	29.2	1.6	6.6	3.2	42.3	6.0	0.4
	lake	HW_la	4.37	1.26	4.12	1.69	0.50	13.68	28.8	1.1	6.2	1.7	40.8	1.3	0.4
	macrophyte	HW_ma	5.24	1.29	3.47	1.69	0.49	18.54	30.7	1.6	6.6	0.8	58.0	13.3	0.3
	channel	HW_ch	5.13	1.34	3.20	1.68	0.50	13.48	29.2	1.2	6.6	1.6	53.5	1.6	0.6
	river	HW_sol	5.13	1.31	3.02	1.71	0.49	11.77	28.6	0.7	6.8	1.8	56.4	0.5	0.7
Falling water (FW)	basin	FW_ba	9.02	1.50	1.83	1.70	0.60	11.27	30.9	2.0	6.4	4.3	23.9	8.3	0.8
	lake	FW_la	3.59	1.44	4.01	1.73	0.58	10.96	30.6	1.0	6.8	5.1	48.1	8.2	1.3
	macrophyte	FW_ma	3.92	1.53	4.14	1.72	0.63	9.00	32.3	1.5	6.8	1.2	39.0	24.1	1.9
	channel	FW_ch	3.91	1.40	4.09	1.73	0.59	11.23	30.9	0.9	6.4	2.6	33.8	15.6	1.9
	river	FW_sol	2.70	1.32	4.44	1.74	0.55	9.97	30.7	0.4	6.4	3.1	35.8	2.1	3.2
Low water (LW)	basin	LW_ba	5.90	1.56	2.91	1.65	0.60	9.15	31.3	0.2	6.3	5.6	20.5	86.0	4.3
	channel	LW_ch	5.88	1.72	2.33	1.74	0.64	10.70	32.4	0.0	5.9	4.1	34.3	137.8	23.2
	river	LW_sol	3.69	1.34	3.36	1.74	0.54	15.74	31.4	0.2	6.9	5.7	69.9	7.2	1.9
Early rising water (ERW)	basin	ERW_ba	4.30	1.36	4.05	1.70	0.60	11.26	31.0	0.5	6.8	7.6	46.9	19.8	2.0
	lake	ERW_la	5.38	1.29	3.35	1.82	0.73	12.39	28.2	0.5	7.3	7.3	86.9	106.7	4.5
	macrophyte	ERW_ma	5.54	1.13	4.02	1.75	0.57	15.20	31.8	0.2	6.3	2.4	62.5	14.8	2.8
	channel	ERW_ch	3.62	1.16	5.17	1.71	0.51	12.45	29.3	0.1	7.3	5.6	72.1	2.2	2.3
	river	ERW_sol	3.55	1.13	5.25	1.71	0.51	15.14	29.3	0.1	7.2	5.5	79.7	1.4	2.5
Late rising water (LRW)	basin	LRW_ba	5.35	1.23	4.60	1.66	0.50	22.35	29.4	1.2	6.6	4.2	46.0	-	-
	lake	LRW_la	4.52	1.19	4.38	1.74	0.56	18.38	29.7	0.7	6.4	4.4	72.0	-	-
	macrophyte	LRW_ma	5.17	1.23	4.18	1.73	0.53	15.90	30.5	1.0	6.4	4.4	49.6	4.0	-
	channel	LRW_ch	4.90	1.11	4.82	1.71	0.48	22.58	28.6	0.2	6.4	3.0	48.7	2.1	0.9
	river	LRW_sol	4.95	1.13	4.71	1.71	0.47	24.69	28.7	0.2	6.5	3.0	49.6	2.1	1.0