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Chemically unidentified dissolved organic carbon: A pivotal piece for microbial activity in a productive area of the Northern Patagonian shelf

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ABSTRACT

The biochemical composition and fluorescence properties of DOM were assessed in relation to phytoplankton and major aquatic bacterial clades in a regenerative area of the Argentine Shelf. DOM was mainly of autochthonous biological origin, containing humic- and protein-like substances of medium degree of unsaturation and diagenesis. Biochemical-DOM accounted for 25% of total DOC, being dissolved combined amino acids (DCAA) the dominant fraction followed by free carbohydrates. Phytoplankton was the main source of serine, alanine, and valine, and particulate carbohydrates. *Gammaproteobacteria* abundance correlated negatively with ammonium and positively with DCAA, suggesting a coupling between ammonium consumption and refractory amino acid production. A preferential utilization of alanine, leucine and threonine as nitrogen source was inferred from the distribution of *Cytophaga-Flavobacteria-Bacteroidete* in relation with dissolved free amino acids (DFAA). Notably, *Alpha-* and *Betaproteobacteria* correlated with the large pool (75%) of chemically unidentified DOC and not with DCAA or dissolved combined carbohydrates. Particularly, *Alphaproteobacteria* (~40% of EUB total heterotrophic bacteria) either significantly contribute to the production of the "humic", refractory fraction of marine DOM, or the latter impairs resource control on their abundance. Spatial heterogeneity inherent to coastal-shelf areas drives important regional variability in the biochemical properties of DOM.

1. Introduction

The key role of marine ecosystems in carbon sequestration and climate regulation has put the parameterization of carbon budgets and their ratios in the spotlight of research interest (Bindoff et al., 2019). Assessing the sources and transformations between particulate and dissolved organic matter (POM/DOM) in the pelagic realm is fundamental to understand the trophic pathways and the exportation from the euphotic layers to the deep sea floor. Microbial activity is responsible for production (e.g. phytoplankton), and degradation (e.g. bacterioplankton) of organic matter, where biological and physical interactive processes breakdown large particles into smaller ones through the interplay of the biological carbon pump, the microbial pump and the virus shunt (Legendre et al., 2015; Breitbart et al., 2018; Boyd et al., 2019).

Continental shelves are dynamic systems where multiple environmental drivers modulate the microbial communities, the carbon sources (autochthonous or allochthonous), transformation and fate (Amaral et al., 2016; Garzón-Cardona et al., 2019; Paczkowska et al., 2020). These areas are influenced to different degrees by human activities (e.g. eutrophication, pollution), coastal ecosystems such as saltmarshes and estuaries, bottom sediments and open waters, as well as by the effects of tides and winds. Altogether these drivers determine the supply of nutrients, the water column structure and the circulation regimes, affecting the microbiome and the organic matter composition.

Dissolved organic matter (DOM) is the largest reservoir of organic

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carbon (~70%) in the global ocean (Worden et al., 2015), and has a wide range of molar masses and chemical structures. Therefore, the characterization of DOM sources and diagenetic state implies a complex analytical challenge. However, DOM characterization can be also approached by relatively simple and sensitive optical methods such as spectrofluorimetry (Coble, 1996), which has been shown to strongly correlate with more complex and sensitive analysis (e.g. FTICR-MS, Stubbins et al., 2014). According to Coble (1996) there are two main classes of DOM that can be characterized through fluorescence analysis: 1) labile protein-like material mainly from marine origin (macrophytes, algal exudate and bacterial protein), and 2) refractory humic-like substances from marine and terrestrial origin.

Carbohydrates (CHO) and amino acids (AA) are the main identified components of dissolved organic carbon (DOC) in DOM (Benner, 2002). Dissolved combined AA (DCAA) and CHO (DCCHO) are high-molecular-weight (HMW) components of aquatic DOM, while dissolved free AA (DFAA) and CHO (DFCHO) belong to the low-molecular-weight (LMW) fraction. The chemical heterogeneity of DOM indicates its multifunctional role for heterotrophs in natural environments (Görs et al., 2007), being DOM mineralization rate highly dependent on its composition (Amon and Benner, 1996; Ogawa and Tonue, 2003; Cai et al., 2019).

Dissolved AA and CHO are major growth substrates for most heterotrophic organisms in aquatic ecosystems (Rosenstock and Simon, 2003; Mühlenbruch et al., 2018). Yet, different bacterial groups may show preferences for the utilization of LMW or HMW DOM components (Cottrell and Kirchman, 2000; Sperling et al., 2017). *Alphaproteobacteria* have been identified as main responsible of monosaccharide consumption (Elifantz et al., 2005; Alonso and Pernthaler, 2006), whereas utilization of HMW polysaccharides is widespread among *Actinobacteria*, *Gammaproteobacteria*, *Betaproteobacteria* and *Cytophaga-Flavobacteria* (Elifantz et al., 2005). Furthermore, also *Alphaproteobacteria* frequently dominate DFAA utilization (Cottrell and Kirchman, 2000). Within this context, the abundances of the main clades of aquatic bacteria have been shown to correlate with DOM optical properties (fluorescent Dissolved Organic Matter, FDOM) and concentration in a coastal lagoon in Uruguay (Amaral et al., 2016). This kind of surveys highlights the importance of studies in natural systems to assess the specific association of bacterioplankton with DOM processes. Moreover, these approaches gain especial relevance under the growing need for observational data from marine ecosystems of contrasting environmental settings (e.g. Andersson et al., 2018), in order to contribute to the calibration of global biogeochemical models. So far, there are no reports relating the abundance of these bacteria functional groups with the *in situ* concentrations and chemical and optical characteristics of DOM in other productive shelf regions of the SW Atlantic Ocean.

The present study was conducted in a temperate, inner shelf ecosystem of the Argentine Sea, called El Rincón (Fig. 1). El Rincón area is highly productive (Hoffmeyer et al., 2009; Guinder et al., 2018; Acha et al., 2020) and holds large populations of commercially important fish species (Marrari et al., 2013; Díaz et al., 2018), seabirds, turtles, and marine mammals that use its diverse habitats for refuge, feeding and breeding. Finally, El Rincón area plays a fundamental role in the ecological connectivity between the coastal zone and the outer shelf, and pioneer studies described it as an area of nutrient and organic matter recycling (Carreto et al., 1995; Lara et al., 2010).

The overall goal of the present study was to gain insight into the DOM optical properties and chemical composition in relation with the environmental conditions and the plankton communities in a productive shelf area of the SW Atlantic. Specific aims were to assess (1) the DOM composition in relation with coastal dynamics such as river runoff and nutrient concentrations, (2) the DOM composition in relation with chlorophyll *a* (Chl-a) as a proxy of phytoplankton biomass and major bacterioplankton groups, *i.e. Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Cytophaga-Flavobacteria-Bacteroidete*, and (3) the role of DOM as substrate for heterotrophic microorganisms.

2. Materials and methods

2.1. Study area and sampling

The sampling was carried out during austral late-summer (March 2013) in El Rincón area (39.0°–41.0° S and 63.0°–60.0° W) (Fig. 1) on an oceanographic cruise aboard the research vessel Dr. Bernardo Houssay, conducted by the Argentine Institute of Oceanography (IADO-CONICET-UNS). The area of El Rincón is delineated by the coastline on the northwest, between the Bahía Blanca Estuary in the north and the Negro River in the south (Fig. 1), and by the isobath of 50 m, ~220 km from the coastline (Lucas et al., 2005). This large area (*ca.* 120,000 km^2) embraces different coastal environments such as estuaries, tidal flats, saltmarshes, sandy beaches and islands. Offshore the 50 m isobath, a frontal system known as Mid-Shelf Front is established during austral spring and summer (Romero et al., 2006; Díaz et al., 2018). This front separates the vertically homogeneous waters of El Rincón (Elisio et al., 2020) from those stratified in the middle shelf (Piola et al., 2018). A particular feature of El Rincón area is the higher salinity in spring and

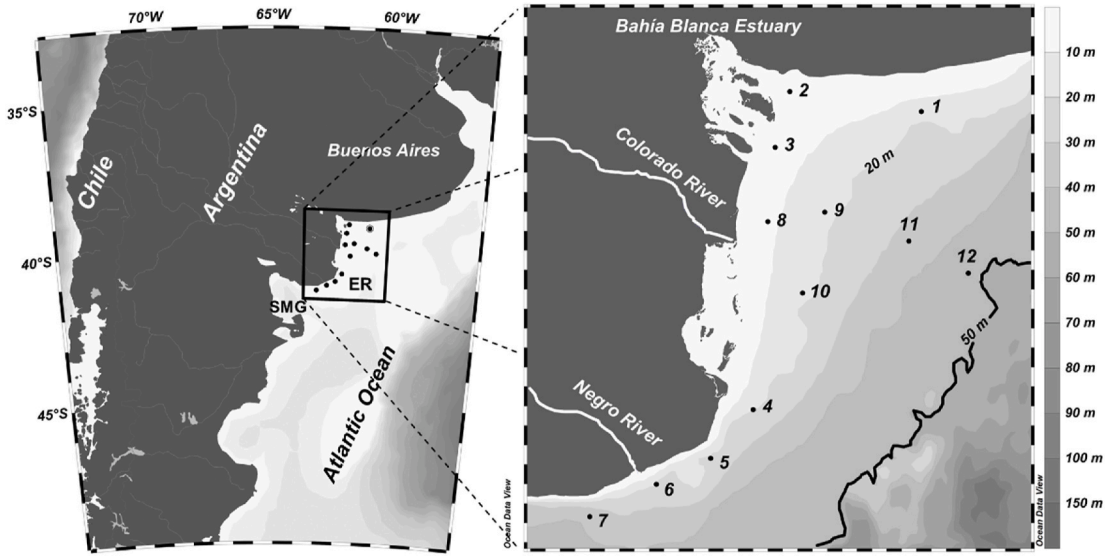

Fig. 1. Location of sampling stations in El Rincón area (ER), in the Argentine Sea. SMG: San Matías Gulf.

summer in the coastal zone compared to the inner shelf, due to the northward advection of saltier and warmer waters alongshore from the San Matías Gulf (Lucas et al., 2005; Auad and Martos, 2012).

Subsurface water samples (5 m) were collected at twelve stations (Fig. 1) using Niskin bottles.

At all stations, continuous profiles of temperature and salinity (CTD, Sea Bird model 911 plus with General Oceanic rosette) were recorded.

Standard methods of water sample collection, conditioning and preservation were followed for the chemical and biological analyses (see sections 2.2. to 2.9). Water samples for inorganic nutrients, DOC, AA, CHO, and FDOM were filtered immediately after collection through Whatman GF/F glass fiber filters pre-combusted at 450 °C for 4 h (Lara et al., 2010). Each filtered sample was split into five aliquots. Samples for inorganic nutrients were stored in alkali-rinsed (NaOH, 0.1M) polyethylene bottles and preserved at −20 °C. Samples for DOC were collected in precombusted 20 mL glass vials and acidifying to pH < 2 with H_3PO_4. Samples for AA, CHO and FDOM analysis were collected in precombusted 10 mL glass vials. Those for the analysis of dissolved CHO, AA, DOC and FDOM were stored in glass vials at −20 °C. Although freezing can alter the structure of some DOM compounds, recent studies have reported minimal changes in DOM due to storage (Hancke et al., 2014; Sánchez-Pérez et al., 2020; Li et al., 2020).

Seston collected on glass fiber filters was used for determination of total particulate carbohydrates (TPCHO) (1.5–3 L) and chlorophyll *a* (Chl-a) (500 mL). Samples for phytoplankton counting were conserved with Lugol solution (1% final concentration) and stored in the dark. Those for evaluation of the bacterial community were fixed with paraformaldehyde at a final concentration of 1%. After 12 h, 10 mL were filtered through polycarbonate filters (type GTTP, 0.2 μm pore size, 47 mm diameter, Millipore). The filters were rinsed twice with sterile phosphate buffered saline and stored at −20 °C until further analysis.

2.2. Phytoplankton biomass and pigment analysis

Samples for phytoplankton and Chl-a determination were collected at 8 and 12 stations, respectively. For the estimation of phytoplankton abundance (in cells L^{-1}), 10–50 mL of preserved seawater samples were settled in Utermöhl chambers during 24 h, and thereafter analyzed under a Wild M20 inverted microscope (Hasle, 1978). Phytoplankton cells >5 μm were counted across the entire chamber under a magnification of 400x. Total phytoplankton biomass (in μg C L^{-1}) was calculated from cell biovolume (in pg C $cell^{-1}$ after Hillebrand et al., 1999), using carbon-to-volume ratios proposed by Menden-Deuer and Lessard (2000). Chl-a was quantified fluorometrically (Shimadzu RF-5301PC) following Holm-Hansen et al. (1965) after overnight cold extraction in 90% (v/v) acetone.

2.3. Bacterial abundance and assemblage composition

The abundance of different microbial taxa in the water samples was determined with horseradish peroxidase-labeled oligonucleotide probes and catalyzed reporter deposition (CARD-FISH; Pernthaler et al., 2002). The following probes were used to characterize the microbial community: EUB338 I-III (most bacteria; Daims et al., 1999), ALF968 (most *Alphaproteobacteria*; Neef, 1997), BET42a (most *Betaproteobacteria*; Manz et al., 1992), GAM42a (most *Gammaproteobacteria*; Manz et al., 1992), CF319a (mainly groups of *Cytophaga-Flavobacteria-Bacteroidete*; Manz et al., 1996).

Signal amplification was performed with Alexa488-labeled tyramides (Molecular Probes). CARD-FISH preparations were counterstained with DAPI at a final concentration of 1 μg mL^{-1}. DAPI- and CARD-FISH-stained cells were counted manually, achieving a minimum of 1000 cells per filter per evaluation.

2.4. Inorganic nutrients

Nitrate, nitrite, ammonium (all in μM N), silicate (μM Si) and phosphate (μM P) were measured using an autoanalyzer (Evolution III, Alliance Instruments) following standard seawater methods (Kattner and Becker, 1991; Grasshoff et al., 1999). The detection limits for nitrate, nitrite, ammonium, silicate and phosphate were 0.10 μM N, 0, 02 μM N, 0.01 μM N, 1.00 μM Si, 0.01 μM P, respectively.

2.5. Dissolved organic carbon

After acidification, duplicate filtrates were analyzed for DOC by high temperature (680 °C) catalytic (Al_2O_3 particles containing 0.5%Pt) oxidation in a TOC analyzer (DohrmannDC-190, CA, USA) followed by quantification of CO_2 by non-dispersive linearized infrared gas analysis (Skoog et al., 1997). A solution of potassium hydrogen phthalate was used as calibration standard.

2.6. Fluorescent dissolved organic matter and indices

Spectral properties of FDOM were determined with a Shimadzu RF-5301 spectrofluorometer with a 150W xenon lamp and a 1 cm quartz cell. Milli-Q water was used as reference and the intensity of the Raman peak was regularly checked. An estimation of dissolved humic-like and protein-like substances was carried out at the wavelengths proposed by Coble (1996). Humic-like fluorophores: $FDOM_C$, containing mostly highly unsaturated components, at Ex/Em: 350/440 nm; $FDOM_A$, with moderate degree of unsaturation, at Ex/Em: 250/425 nm and $FDOM_M$, with low degree of unsaturation, at Ex/Em:310/380 nm. Protein-like fluorophores: $FDOM_T$, with fresh components, at Ex/Em: 270/330 nm and $FDOM_B$, corresponding to DOM transformed by biological or physicochemical factors, at Ex/Em: 260/300 nm. The results were expressed in quinine sulphate equivalent units (QSU), converted with the signal at Ex/Em: 350/451 nm of quinine sulphate dihydrate standard dissolved in 0.05 M H_2SO_4 and the equivalence QSU = 1 μg L^{-1}(Coble, 1996).

Three fluorescence indices were used to assess possible sources and diagenetic state of the DOM. The fluorescence index (FIX) can be used to differentiate DOM origins. FIX values ≤ 1.4 correspond to terrestrially-derived DOM, while values ≥ 1.9 refer to aquatic and microbial sources. If FIX is between 1.4 and 1.9, DOM components are affected by both terrestrial and marine inputs (McKnight et al., 2001). The following formula was used to calculate the fluorescence index: $FIX = \frac{I_{370/450}}{I_{370/500}}$, where $I_{370/450}$ represents the fluorescence intensity at Ex/Em: 370/450 nm and $I_{370/500}$ is the intensity at Ex/Em: 370/500 nm.

The humification index (HIX) can be used to assess DOM diagenetic condition. A high HIX value ≥ 0.9 indicates a high degree of DOM humification (Hansen et al., 2016). HIX was calculated after Zsolnay et al. (1999) and Ohno (2002) as the ratio H/L of two spectral region areas from the emission spectrum scanned for excitation at 254 nm. These two areas are quantified between emission wavelengths of 435 and 480 nm for H, and between 300 and 345 nm for L. When the degree of aromaticity increases, the H/L ratio and hence HIX, increases. This corresponds to a shift in maximum fluorescence intensity from shorter to longer wavelengths associated with an increasing number of highly substituted aromatic nuclei and/or with conjugated unsaturated systems of high molecular weight (Senesi et al., 1991).

The biological index (BIX) can be used to estimate the relative contribution of biogenic DOM (Huguet et al., 2009). Values > 0.8 indicates that DOM is mainly derived from microbial and other biological sources, where as those <0.6 indicate a low amount of biogenic DOM. BIX is calculated as the ratio $BIX = \frac{I_{310/380}}{I_{310/430}}$, where $I_{310/380}$ is the fluorescence intensity at Ex/Em: 310/380 nm and $I_{310/430}$ is the fluorescence intensity at Ex/Em: 310/430 nm.

2.7. Identification and quantification of dissolved amino acids

Amino acids were transformed into fluorescent derivatives by o-phthaldialdehyde (OPA) and 2-mercaptoethanol (Lindroth and Mopper, 1979; Pantoja and Lee, 1999), separated by HPLC (Thermo Scientific) using a gradient of K_2HPO_4/methanol, and detected with an on-line fluorometer (Pantoja et al., 1997). The peaks of the fluorescent products were converted to concentrations with the external AA standard Pierce N° 20088, containing aspartic acid (asp), glutamic acid (glu), serine (ser), histidine (his), glycine (gly), threonine (thr), arginine (arg), alanine (ala), tyrosine (tyr), valine (val), methionine (met), phenylalanine (phe), isoleucine (ile) and leucine (leu). Total dissolved amino acids (TDAA) were quantified after chemical hydrolysis with 6 N HCl at 150 °C for 2 h under N_2 and neutralized with 12 N KOH. DFAA were measured without chemical hydrolysis. DCAA resulted from the difference between TDAA and DFAA.

2.8. Dissolved and total particulate carbohydrates

Total dissolved carbohydrate (TDCHO) concentrations, including DFCHO and DCCHO, were determined with the TPTZ (2,4,6-tripyridyl-s-triazine) method of Myklestad et al. (1997). Accordingly, 4 mL of filtered seawater samples were placed in 5 mL glass ampoules, acidified with 0.40 mL of 1 M HCl, sealed and hydrolyzed for 1 h at 150 °C. The hydrolyzed samples were neutralized with 1 M NaOH after cooling and TDCHO concentrations were measured by oxidizing the free reduced sugar with Fe^{3+} under alkaline conditions, followed by spectrophotometric quantification at 595 nm of a colored complex of Fe^{2+} and TPTZ. DFCHO were quantified directly without hydrolysis, and DCCHO concentrations were obtained by the difference between TDCHO and DFCHO. D (+)-glucose (analytical standard, 47829, Supelco) was used as a standard. To optimize analytical precision, the procedure was conducted in the dark after addition of the potassium ferricyanide solution.

Concentrations of total TPCHO were measured with the phenol-sulphuric acid method (Dubois et al., 1956). Absorbance was measured at 490 nm using a Jenway 67 spectrophotometer.

2.9. Composition of DOC

Carbon normalized yields of TDAA and TDCHO were calculated as the percentages of DOC accounted by TDAA, $\%TDAA-C = \frac{TDAA-C}{DOC} * 100$ and TDCHO, $\%TDCHO-C = \frac{TDCHO-C}{DOC} * 100$, where DOC, TDAA-C, and TDCHO-C are concentrations of bulk DOC, and carbon contained in TDAA and TDCHO, respectively. %TDAA-C and %TDCHO have been demonstrated to be molecular indicators of DOM bioavailability in freshwater and marine environments (Benner and Kaiser, 2011; Shen et al., 2015, 2016). The fraction of DOC not accounted by TDAA + TDCHO was named chemically-unidentified DOC (cuDOC).

Table 1
Physical, biogeochemical and biological parameters measured in the El Rincón area during austral summer 2013.

Parameter (n)	Coastal shelf (station 1 to 7)				Inner shelf (station 8 to 12)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Salinity (12)	33.4	0.9	31.9	34.5	32.2	0.2	32.0	32.5
Temperature (°C) (12)	18.2	0.5	17.7	18.9	17.1	0.7	16.3	17.9
Inorganic nutrients (μM)								
NO_3^- (12)	0.7	0.5	0.2	1.6	0.4	0.5	0.1	1.2
NO_2^- (12)	0.2	0.2	<DL	0.5	0.2	0.2	0.1	0.4
NH_4^+ (12	0.5	0.4	0.1	1.2	0.6	9.0	0.2	1.4
$Si(OH)_4$ (12)	17.6	9.1	5.0	31.3	10.3	0.3	2.8	21.5
PO_4^{-3} (12)	1.2	0.2	0.9	1.4	1.1	0.5	0.7	1.5
Fluorescent DOM (QSU)								
$FDOM_A$ (12)	2.3	0.9	1.5	4.1	1.5	0.5	1.0	2.3
$FDOM_C$ (12)	1.2	0.5	0.7	2.0	0.8	0.4	0.4	1.4
$FDOM_M$ (12)	1.9	0.9	1.0	3.2	1.2	0.6	0.5	2.1
$FDOM_B$ (12)	1.9	0.5	1.2	2.9	1.5	0.8	0.8	2.9
$FDOM_T$ (12)	2.1	1.3	0.6	3.8	1.2	1.1	0.2	2.8
FIX (12)	2.0	0.2	1.8	2.3	2.1	0.2	1.9	2.3
BIX (12)	1.5	0.2	1.2	1.9	1.5	0.2	1.3	1.7
HIX (12)	1.1	0.4	0.7	1.6	0.9	0.1	0.7	1.0
Biochemical-DOC (μM-C)								
DOC (10)	118.5	74.6	73	266	77.5	10	70.0	92.0
TDAA (11)	13.1	5.6	3.7	18.2	8.8	3.9	5.3	14.9
DCAA (11)	12.6	5.5	3.4	17.9	8.4	3.9	5.1	14.6
DFAA (11)	0.5	0.3	0.3	1.0	0.3	0.1	0.2	0.5
TDCHO (12)	9.5	1.9	6.5	12.1	14.9	7.0	7.1	22.3
DCCHO (12)	3.1	1.6	1.4	5.6	7.2	5.1	1.9	14.4
DFCHO (12)	6.4	3.2	2.7	10.6	7.7	2.7	5.1	12.1
TPCHO (μM) (12)	191.1	116.3	38.5	376.9	85.0	24.4	53.4	121.6
Total phytoplankton (log cells L^{-1})								
Phytoplankton (8)	4.2	3.9	3.9	4.4	3.7	3.5	3.1	4.0
Diatoms (8)	4.2	4.0	3.9	4.4	3.5	3.9	3.0	3.7
Dinoflagellates (5)	2.4	2.3	2.0	2.7	3.4	3.4	1.7	3.7
Other flagellates (6)	2.8	2.7	2.3	3.0	3.1	3.3	1.7	3.7
Chl-a (μg L^{-1}) (12)	4.4	3.6	1.0	11.4	1.8	0.5	1.1	2.6
Heterotrophic Bacteria (log cells mL^{-1})								
DAPI (8)	6.0	0.2	5.8	6.2	5.9	0.2	5.7	6.1
EUB (8)	5.8	0.2	5.5	6.0	5.8	0.2	5.6	6.0
CFB (8)	4.8	0.3	4.6	5.2	5.1	0.3	4.7	5.3
Gama (8)	4.8	0.4	3.8	5.4	4.6	0.4	4.3	5.1
Alfa (8)	5.3	0.3	5.0	5.7	5.2	0.3	4.9	5.6
Beta (8)	4.7	0.1	4.5	4.9	4.7	0.1	4.6	4.7

n: number of samples, SD: standard deviation, Min: minimum, Max: maximum, DL: Detection Limit.

2.10. Statistical analysis

Cluster analysis using Ward's method was applied to different environmental variables e.g. sea surface temperature, salinity, chlorophyll and dissolved inorganic nutrients at the sampling stations, to assess the physical and chemical spatial structure of the study area. After the identification of two zones (coastal and inner shelf), the means and standard deviations were estimated for all the biogeochemical and biological variables in each zone using the individual stations as replicates ($n = 12$ for most variables, e.g. 8 for EUB and DAPI, Table 1). We tested if multivariate models could improve the explanation of some biochemical parameters, e.g. fluorescence indices (FIX; BIX and HIX) in relation to physical, chemical and biological variables, using the corrected Akaike information criterion AICc. From all combinations of linear models, lowest AICc's have always been obtained with univariate models. Hence, Pearson's rank correlation coefficient was applied to assess the relationship between biogeochemical and biological parameters of interest. Due to the low number of replicates, test for normality were not sufficiently confident; we applied known transformations when required (e.g. log transformation for cell abundance of phytoplankton and bacteria). The softwares JMP 11.0.0 (SAS Institute Inc) and PAST 2.17c were used for the analyses.

3. Results and discussion

3.1. Temperature, salinity, nutrients and phytoplankton

Based on the distribution of surface salinity, temperature, and *in situ* Chl-a (Fig. 2a), the stations 1 to 7 were grouped into the coastal zone, and the stations 8 to 12 were grouped into the inner shelf zone (Table 1). In the vertically homogeneous waters of El Rincón, the coastal zone was warmer and saltier than the inner shelf (Fig. 2a), consistent with previous characterization of the hydrology in the area during spring and summer (Lucas et al., 2005; Palma et al., 2008).

Distribution of inorganic nutrients in El Rincón displayed higher concentrations in the coastal zone (Table 1 and Fig. 2b), with maxima nearby the eutrophic Bahía Blanca Estuary influenced by anthropogenic activities (Guinder et al., 2010; López Abbate et al., 2019) and northward the mouth of Colorado and Negro Rivers (Barrera, 2015; Kopprio et al., 2017). In particular, the high concentrations of silicate in the Bahía Blanca estuarine area are likely due to the contribution of interstitial water from the wide mud banks, tidal flats and islands (Negrin et al., 2016). Ammonium showed notable maxima at coastal stations 1 and 2 and at station 11 in the inner shelf, denoting large spatial variability related to intrinsic *in situ* biogeochemical processes and anthropogenic inputs (Kopprio et al., 2017; López Abbate et al., 2019).

Chlorophyll values varied between 1.0 and 11.4 $\mu g\, L^{-1}$ (Table 1), with maxima in the coastal zone, especially in the highly productive Bahía Blanca Estuary (stations 2 and 3) (Fig. 2a), where diatoms are the dominant phytoplankton group all-year round with recurrent winter-early spring and summer blooms (Guinder et al., 2010; López Abbate et al., 2017). Surface Chl-a followed a similar spatial pattern as silicate ($r = 0.64$, $p = 0.03$, $n = 12$) and temperature ($r = 0.69$, $p = 0.01$, $n = 12$), yet not as ammonium, which is frequently a preferential nitrogen source for phytoplankton, although nitrogen utilization depends on eutrophic conditions and microbial assemblages (Glibert et al., 2016). In the innermost zone of the Bahía Blanca Estuary, empirical studies (López Abbate et al., 2019) and sustained field observations (Berasategui et al., 2021) have shown that severe local eutrophication frequently exceeds the tolerance threshold of plankton to ammonium, and diatoms switch to nitrate uptake. Chlorophyll raised towards the mouth of the San Matías Gulf, reaching up to 3.4 $\mu g\, L^{-1}$ at station 7. Phytoplankton abundance co-varied with Chl-a ($r = 0.92$, $p < 0.001$, $n = 8$), being diatoms from the microplankton the dominant group at coastal stations, accounting for a biomass of $25.1 \pm 18.6\ \mu g\, C\ L^{-1}$ (4.2 ± 4.0 log cells L^{-1}, Table 1) in the coastal area and of $10.0 \pm 11.0\ \mu g\, C\, L^{-1}$ (3.5 ± 3.9 log cells L^{-1}, Table 1) in the inner shelf, in agreement with sustained studies in the area (Guinder et al., 2010; Garibotti et al., 2011; López Abbate et al., 2017). The most frequent diatom species were commonly related to mixed, nutrient-rich and turbid conditions (e.g. Delgado et al., 2019). Conversely, nanoflagellates and dinoflagellates dominated in the inner shelf, as documented in other surveys in El Rincón in spring and summer (Guinder et al., 2018), likely related to more oligotrophic waters dominated by ultra- and picophytoplankton (Silva et al., 2009).

3.2. Heterotrophic bacteria

The abundance of major bacterial groups at stations 1, 2, 11 and 12 was not assessed for technical issues, which constrains the assessment of their distribution between coastal and inner shelf. EUB and DAPI counts (in log cell mL^{-1}) were highly correlated (Fig. 3a). EUB counts were on average $97 \pm 1\%$ of DAPI's, and EUB were used for subsequent analyzes.

The amount of heterotrophic bacteria did not show a significant correlation with autotrophic biomass (EUB vs Chl-a: $r = 0.27$, $p = 0.52$), and hence bacteria might be more dependent on the availability of dissolved substrates than of particulate organic matter (EUB vs DOC: $r = 0.69$, $p = 0.05$, $n = 8$). The most abundant groups were

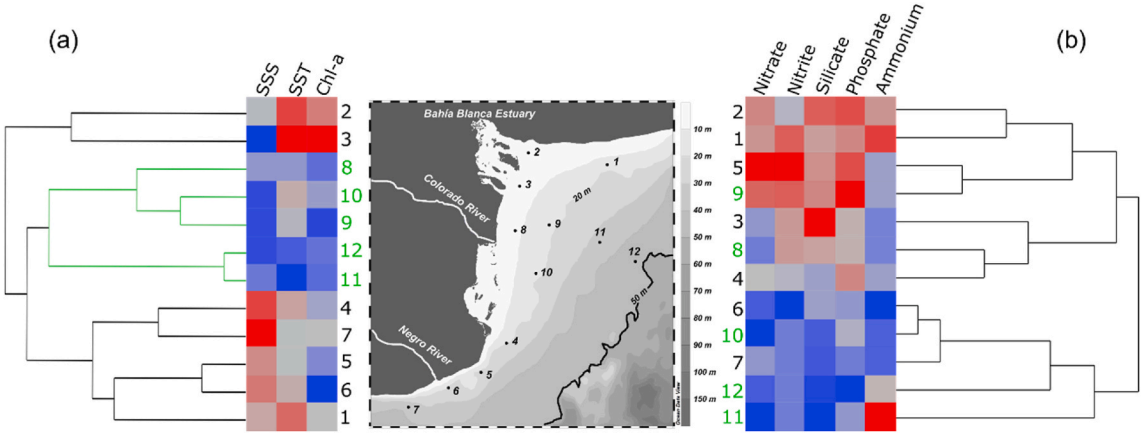

Fig. 2. (a) Cluster analysis showing the distribution of sea surface salinity (SSS) temperature (SST), and chlorophyll *a* (Chl-a) in El Rincón area. The cluster shows the warmer and saltier coastal zone (stations 1 to 7) in relation to the inner shelf zone (stations 8 to 12 indicated in green). (b) Cluster analysis of dissolved inorganic nutrients. In the color map, red is maximum and blue is minimum, the values are shown in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

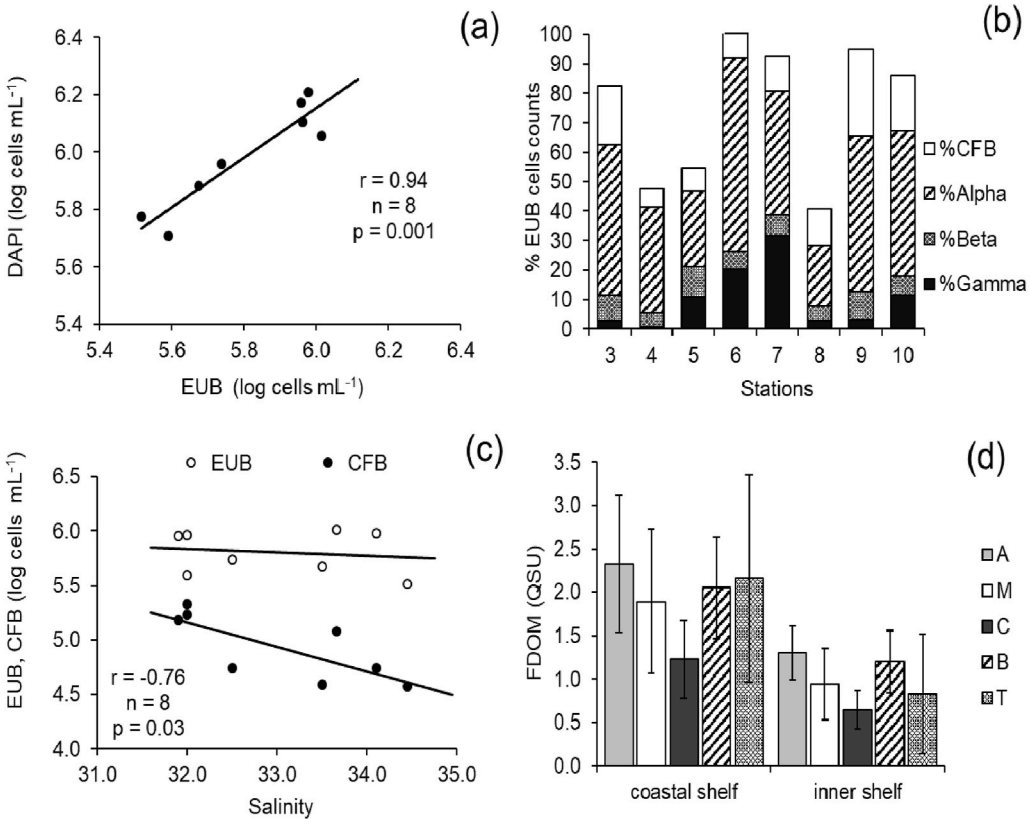

Fig. 3. (a) Relationship between total bacteria cell counts (log cells mL^{-1}) determined by EUB338 and DAPI staining. (b) Proportions of Cytophaga-Flavobacteria-Bacteroidete (CFB), Alpha-, Beta- and Gammaproteobacteria (Gamma) (percentage of EUB cell counts). (c) Relationship of total bacteria counts (EUB) and CFB with salinity. (d) Fluorescence intensity (in quinine sulphate units, QSU) of DOM characterized by the main bands: humic-like ($FDOM_C$, $FDOM_A$ and $FDOM_M$) and protein-like peaks ($FDOM_B$, $FDOM_T$).

Alphaproteobacteria and *Cytophaga-Flavobacteria-Bacteroidete*, which on average accounted for 42 ± 15% and 14 ± 7% of EUB338 total bacterial abundance, respectively. The less abundant groups, *Gammaproteobacteria* and *Betaproteobacteria,* accounted for 12 ± 11% and 7 ± 2% of EUB338 counts, respectively (Fig. 3b).

Notably, *Cytophaga-Flavobacteria-Bacteroidete* displayed a significant negative correlation with salinity (Fig. 3c) despite the relatively narrow salinity range (31.9–34.5). Moreover, the salinity gradient in summer in the study area is opposite to the common gradients in coastal-open sea transition zones, where higher abundances of *Cytophaga-Flavobacteria-Bacteroidete* are expected in the freshwater end (i.e. coastal zone) of the salinity gradient (Zhang et al., 2006). This highlights the large regional variability displayed by microbial assemblages driven by local coastal processes (e.g. Aldunate et al., 2018), which also depends on their substrate preferences (Amaral et al., 2016; Cai et al., 2019). The case of El Rincón area emphasizes the need of further regional studies for the parameterization of microbial communities in continental shelves with contrasting hydrological settings.

3.3. Characterization of DOM: fluorescence properties and DOM composition

3.3.1. Fluorescent dissolved organic matter

All five main bands $FDOM_C$, $FDOM_A$, $FDOM_M$ and $FDOM_B$, $FDOM_T$ showed higher intensities in the coastal area (Table 1, Fig. 3d), where the highest proportion of humic-like components ($FDOM_A$) with moderate degree of unsaturation was observed. The three humic fluorophores ($FDOM_{A,\ C,\ M}$) correlated strongly with each other ($r > 0.90$, $p < 0.001$, $n = 12$), suggesting similar sources. The range of humic fluorophores intensities observed in the temperate El Rincón area is within the range observed in subpolar regions of the Southwestern Atlantic Shelf (Garzón-Cardona et al., 2016, 2019). The protein-like bands ($FDOM_{T,\ B}$) showed similar average proportions of fresh and modified material in the coastal area, likely from biological activity, salt marshes and intertidal mudflats, e.g. Gao et al. (2010), with a predominance of components ($FDOM_B$) that had undergone transformation processes by biological or physicochemical factors (Coble, 1996) in the inner shelf.

The distribution of the three fluorescence indices in El Rincón area is shown in Fig. 4a. The fluorescence index (FIX) ranged between 1.8 and 2.3 (Table 1), pointing to a predominantly biogenic character of DOM, mainly derived from bacterial activity (Huguet et al., 2009). Yet, FIX did not increase with indicators of heterotrophic (EUB, counts of bacterial groups) or autotrophic biomass (Chl-a, phytoplankton abundance), but rather it showed a slight trend to decrease with increasing values of these parameters, notably with *Betaproteobacteria* ($r = -0.67$, $p = 0.08$, $n = 8$) and Chl-a ($r = -0.49$, $p = 0.1$, $n = 12$). The fact that highest FIX values are found at low microbial plankton biomasses suggests that a fraction of DOM in El Rincón could be predominantly older biogenic material.

The biological index (BIX) ranged between 1.2 and 1.9 (Table 1), also indicating a predominantly autochthonous biogenic character of DOM. Finally, the humification index (HIX) ranged between 0.7 and 1.6 (Table 1), and was correlated with phytoplankton, both in terms of Chl-a ($r = 0.87$, $p = 0.001$, $n = 12$) and abundance ($r = 0.87$, $p = 0.01$, $n = 7$), as well as with bacteria abundance,. HIX also showed a significant correlation with TPCHO ($r = 0.76$, $p = 0.005$, $n = 12$), which in turn highly correlated with Chl-a (see section 3.3.3). In the four linear regressions between HIX and Chl-a, phytoplankton abundance, bacteria abundance (EUB) and TPCHO, the mean intersection value was 0.69, which corresponds to DOM of an intermediate humification degree. This is in agreement with Chen et al. (2011), who reported a mean HIX value of ~0.61 for seawater samples, and with Ohno (2002) who associated a HIX value of 0.57 to CDOM at early stages of the humification processes.

The response of HIX to phytoplankton and bacteria abundance changes suggests a mixture of DOM components of different "freshness" overlaying on a background of older DOM. Moreover, both microbial groups could be independently contributing to HIX with their respective exudates or degradation/lysis products, which could constitute

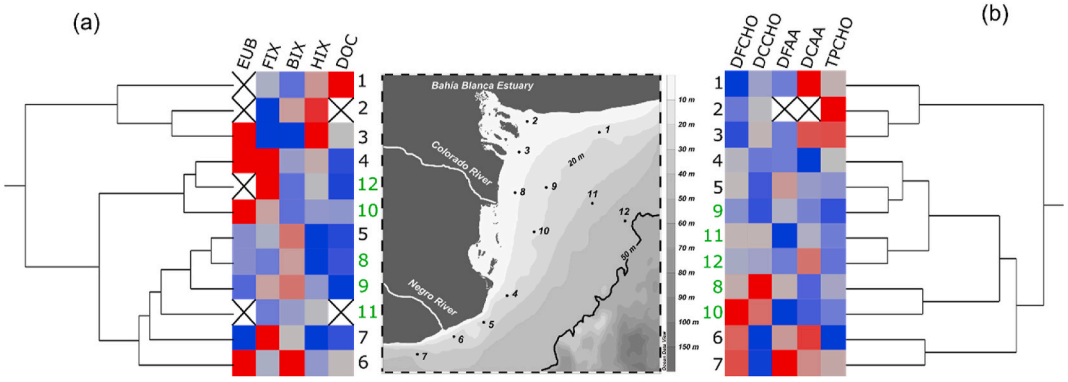

Fig. 4. Cluster analysis showing the distribution of (a) total bacterial abundance (EUB), fluorescence indices FIX (fluorescence), BIX (biological) and HIX (humification), and dissolved organic carbon (DOC). (b) Cluster analysis of the dissolved free (DF-) and combined (DC-) carbohydrates (CHO) and amino acids (AA), and the total particulate carbohydrates (TPCHO) in El Rincón area. The stations 1 to 7 correspond to the coastal zone, and he stations 8 to 12 correspond to the inner shelf zone and are indicated in green. In the color map, red is maximum and blue is minimum, the values are shown in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

precursors of humic material. In this context, it is also remarkable that HIX positively correlated with the ratios DCAA/DFAA ($r = 0.68$, $p = 0.02$, $n = 11$) and DCCHO/DFCHO ($r = 0.51$, $p = 0.09$, $n = 11$), increasing its value with the higher proportion of polymeric material (Hubberten et al., 1994).

In summary, these results indicate that in El Rincón, DOM is on average in a medium degree of maturation or humification (HIX < 4) and that it is mostly derived from autochthonous biogenic sources (BIX ˃1). Both characteristics are consistent with the DOM dynamics of a regenerative area (Carreto et al., 1995).

3.3.2. Composition of DOC

Carbon-normalized yields were defined as the percentage of organic carbon contributed by carbohydrates and amino acids. Surface DOC concentrations ranged between 70 and 226 μM-C (Table 1), with the highest value at station 1 (Fig. 4a), probably related to heterotrophic activity. Although bacterial abundance was not estimated for that station (Fig. 4a), the highest DCAA (Fig. 4b) and ammonium concentrations support this assumption.

In most samples, the largest contribution to DOC was from DCAA (12 ± 6%), followed by DFCHO (8 ± 4%), DCCHO (4 ± 3%) and DFAA (1%) (Fig. 5). Thus, these chemically characterized components accounted for almost one fourth of DOC in El Rincón. A large proportion of the remaining DOC, 75 ± 8%, was chemically unidentified (cuDOC), likely humic substances which have been reported to represent about 60% of DOC in marine systems (Görs et al., 2007; Zhang et al., 2016).

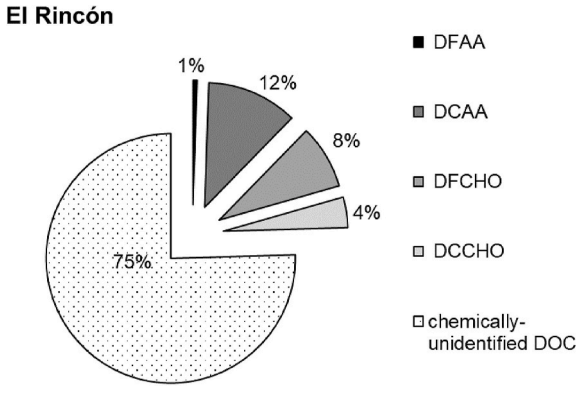

Fig. 5. Average chemical composition of dissolved organic carbon (DOC) in surface waters of El Rincón area. Dissolved free (DF-) and combined (DC-) amino acids (AA) and carbohydrates (CHO).

3.3.3. Concentrations and composition of carbohydrates

The average concentrations of particulate and dissolved carbohydrates in surface waters of El Rincón (Fig. 4b) were within the concentration ranges reported in other shelf regions (North Pacific Ocean, Norwegian Sea, northern Gulf of Mexico and Southwest Atlantic) (Benner et al., 1992; Myklestad et al., 2007; Shen et al., 2016, Garzón-Cardona et al., 2019). TPCHO in the coastal zone was on average higher and more variable (coefficient of variation CV = 61%) than in the inner shelf (CV = 21%) (Table 1, Fig. 4b). It showed a significant relationship with phytoplankton abundance ($r = 0.93$, $p = 0.002$, $n = 8$) as well as with Chl-a ($r = 0.87$, $p < 0.001$, $n = 12$), pointing to phytoplankton as main source of particulate carbohydrates in the coastal and inner shelf. Further, the correlation between phytoplankton and TPCHO can also reflect the formation of aggregates from exudates of plankton and bacteria, which coalesce to form transparent exopolymer particles (TEPs) (Passow and Alldredge, 1994; Nichols et al., 2004).

DCCHO and DFCHO comprised 39 ± 19% and 61 ± 19% of TDCHO, respectively; indicating a dominance of monomeric compounds in the dissolved carbohydrate pool. Riverine input and *in situ* production can be associated with high TDCHO concentrations (Hung et al., 2001; Khodse et al., 2010; Hu et al., 2020). Moreover, estuarine mud banks and tidal flats also influence their distribution probably contributing with significant amounts of HMW lignocellulosic material from terrestrial or salt marsh vegetation (Bortolus et al., 2009; Negrin et al., 2016), aside from microalgal-derived carbohydrates (Yamashita et al., 2015; Garzón-Cardona et al., 2019).

There was a slight trend to higher DFCHO concentrations with decreasing Chl-a and TPCHO ($r = -0.49$, -0.50, $p = 0.09$, $n = 12$). If TPCHO is considered as the primary source of dissolved CHO, the inverse relationship could be attributed to the effect of recent grazing or bacterial activity on phytoplankton, producing a DOM rich in DFCHO (see section 3.4). In addition, the lack of relationship between DCCHO and TPCHO or Chl-a could indicate that DCCHO are part of older and more refractory material compared to DFCHO which showed a reaction to changes in autotrophic biomass.

3.3.4. Concentrations and composition of amino acids

The concentration of dissolved combined and free amino acids was within the range reported for other coastal and shelf waters (Pettine et al., 2001; Görs et al., 2007; Zhang et al., 2015; Shen et al., 2016; Garzón-Cardona et al., 2019). The major constituents of DFAA and DCAA in El Rincón were his, asp, ala, glu, thr and val (Fig. 6) with an average contribution of 70% to DFAA and 60% to DCAA.

The concentration of ser, ala and val accounted for a molar contribution of 27% to DCAA and each AA showed a significant correlation

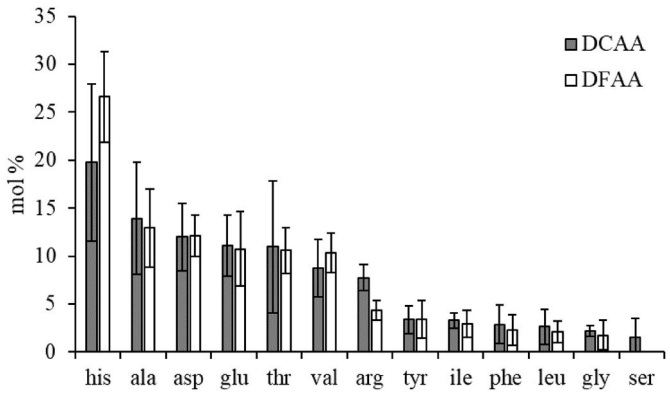

Fig. 6. Average mol fraction ± standard deviations of individual amino acids in the dissolved free (DFAA) and dissolved combined amino acids (DCAA) pools in El Rincón surface waters. asp: aspartic acid, glu: glutamic acid, ser: serine, his: histidine, gly: glycine, thr: threonine, arg: arginine, ala: alanine, tyr: tyrosine, met: methionine, val: valine, phe: phenylalanine, ile: isoleucine, leu: leucine.

($p < 0.001$, $n = 8–12$), with phytoplankton abundance ($r = 0.82$ to 0.90) and Chl-a ($r = 0.85$ to 0.91), indicating a significant influence of algal exudate production on TDAA distribution (Ogawa and Tanoue, 2003).

In all stations, DCAA values were much higher than DFAA with an average contribution of 95% to TDAA. This predominance of polymeric AA contrasts with CHO, where monomeric compounds represented a larger proportion of the total. The average concentration of DFAA decreased from coastal to inner shelf while average DCAA displayed a slight decrease offshore (Fig. 7).

The high DCAA/DFAA ratios suggest that DFAA are likely consumed by heterotrophic organisms (see section 3.4) or alternatively, that a lower exudate production takes place by phytoplankton. The uptake of freshly produced monomeric amino acids could leave behind polymeric material of lower availability for other bacterial groups. Since DCAA/DFAA and DOC positively correlated ($r = 0.70$, $p = 0.03$, $n = 9$), increases in autochthonous DOM could be linked to a larger proportion of polymeric to monomeric amino acids (Fig. 7). A large fraction of DCAA has been reported to form part of dissolved humic matter fractions (Hubberten et al., 1994; Rosenstock and Simon, 2003).

DCAA was not correlated with Chl-a or EUB, probably for being part of older HMW material, alike DCCHO. Surprisingly, DCAA neither correlate with the protein-like peaks $FDOM_B$ or $FDOM_T$. However, DFAA correlated significantly with both $FDOM_B$ (Fig. 8a) and $FDOM_T$ (Fig. 8b). This sets a question mark on both the chemical environment in which DCAA are embedded or are part of, and about the suitability of the denomination "protein-like" for these peaks, taking into account that precisely the compounds more likely to be part of proteins (combined amino acids) did not show any correlation with $FDOM_B$ or $FDOM_T$. Keil and Kirchman (1993) proposed that rapid abiotic complexation of labile organic compounds such as proteins with existing DOM may be a critical first step in the formation of refractory organic materials in seawater. Our data suggest that DCAA eventually produced by bacterial activity (see section 3.4) could suffer such a transformation, which besides providing protection from bacterial attack, it may affect its fluorescence properties.

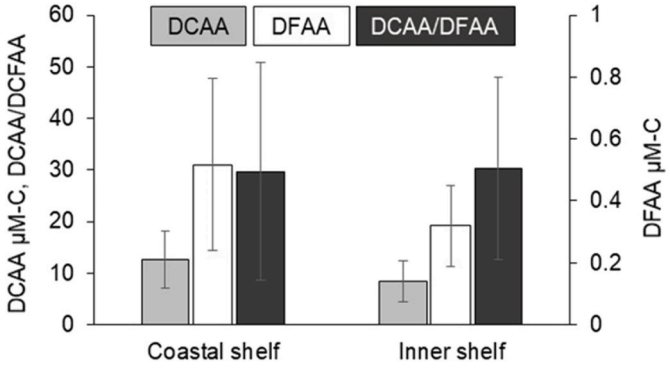

Fig. 7. Average ± standard deviations of dissolved combined amino acids (DCAA), dissolved free amino acids (DFAA) and DCAA/DFAA ratios in El Rincón surface water.

3.4. Relationship among major bacterial groups, ammonium and chemical and fluorescence properties of DOM

The affinity of a particular clade for a specific substrate must be differentiated from the overall uptake of organic components by heterotrophs. Therefore, here we performed the correlation of each individual clade with EUB counts. Only *Alpha-* ($r = 0.68$, $p = 0.06$, $n = 8$) and *Betaproteobateria* ($r = 0.58$, $p = 0.06$, $n = 8$) correlated with EUB. *Gammaproteobateria* and *Cytophaga-Flavobacteria-Bacteroidete* counts did not show a clear trend to increase with total bacteria and thus their dependence on a specific substrate may be biogeochemically more meaningful.

There was an indication of preferential DFAA uptake by EUB and CFB, based on their inverse correlations (DFAA vs EUB: $r = -0.65$, $p = 0.08$, $n = 8$; DFAA vs CFB: $r = -0.62$, $p = 0.09$, $n = 8$). EUB significantly correlated ($p < 0.01$, $n = 8$) with the individual amino acids arg ($r = -0.86$), ser ($r = -0.85$), ala ($r = -0.82$), his and asp ($r = -0.70$), while CFB correlated with thr, leu and ala ($r = -0.70$ to -0.60, $n = 8$, $p < 0.05$). In the present study, his (22%), asp (12%), and ala (12%) were the most abundant amino acids of DFAA and TDAA. Preferential uptake of DFAA by bacteria can significantly differ in aquatic ecosystems, and no further data are available so far for the Southwest Atlantic. Mudryk and Skórczewski (1998) found that the highest percentage of the planktonic bacteria in Baltic waters utilized glu, asp, his and cys for their optimal growth, whereas only a small percentage of bacterial strains utilized ser, phe, orn and gly. These authors also remarked that the utilization of individual amino acids by bacteria differed among distinct water layers. Grover and Chrzanowski (2000) determined that bacteria preferred asp than other amino acids in temperate lakes. This is supported by the fact that decarboxylation of asp can also be a source of alanine, a precursor of pantothenic acid, one of the components of the mureine complex of the bacterial cell wall (Cole and Lee, 1986). Frette et al. (2004) reported that most marine and estuarine bacterial isolates were able to utilize DFAA as their sole N source. In contrast to our results, Cottrell and Kirchman (2000) reported that *Cytophaga--Flavobacteria-Bacteroidete* were generally underrepresented in the assemblage consuming DFAA in incubation experiments.

The abundance of *Gammaproteobacteria* was negatively correlated with NH_4^+ and positively with DCAA (Fig. 8c and d) and DFCHO ($r = 0.70$, $p = 0.05$, $n = 8$). The positive correlation with DFCHO and DCAA (Fig. 8d) suggests a contribution of this clade to these DOM fractions, or alternatively, a resource control of *Gammaproteobacteria*. Middelboe et al. (1995) found that bacterioplankton can release DCAA especially during the stationary phase. The inverse relationship between *Gammaproteobacteria* and NH_4^+ (Fig. 8c) may indicate an active bacterial uptake of this nutrient over autotrophs (Kirchman, 1994; López Abbate et al., 2019) in the regenerative El Rincón region.

EUB counts showed a significant positive correlation with DOC (Fig. 8e), but did not correlate with DCCHO or DCAA. Instead, the correlation with DOC is generated by the chemically-unidentified fraction, cuDOC ($r = 0.77$, $p = 0.02$, $n = 8$). *Alpha-* and *Betaproteobacteria* exhibited the same behavior as EUB towards DOC, DCCHO and DCAA. Cottrell and Kirchman (2000) also reported a low affinity of *Alphaproteobacteria* for DCAA. Additionally, both groups also highly significantly correlated with cuDOC (Fig. 8f). Even though the abundance of both clades increased with EUB, they showed a low correlation with each other ($r = 0.37$, $p = 0.36$, $n = 8$). This, together with the high correlation

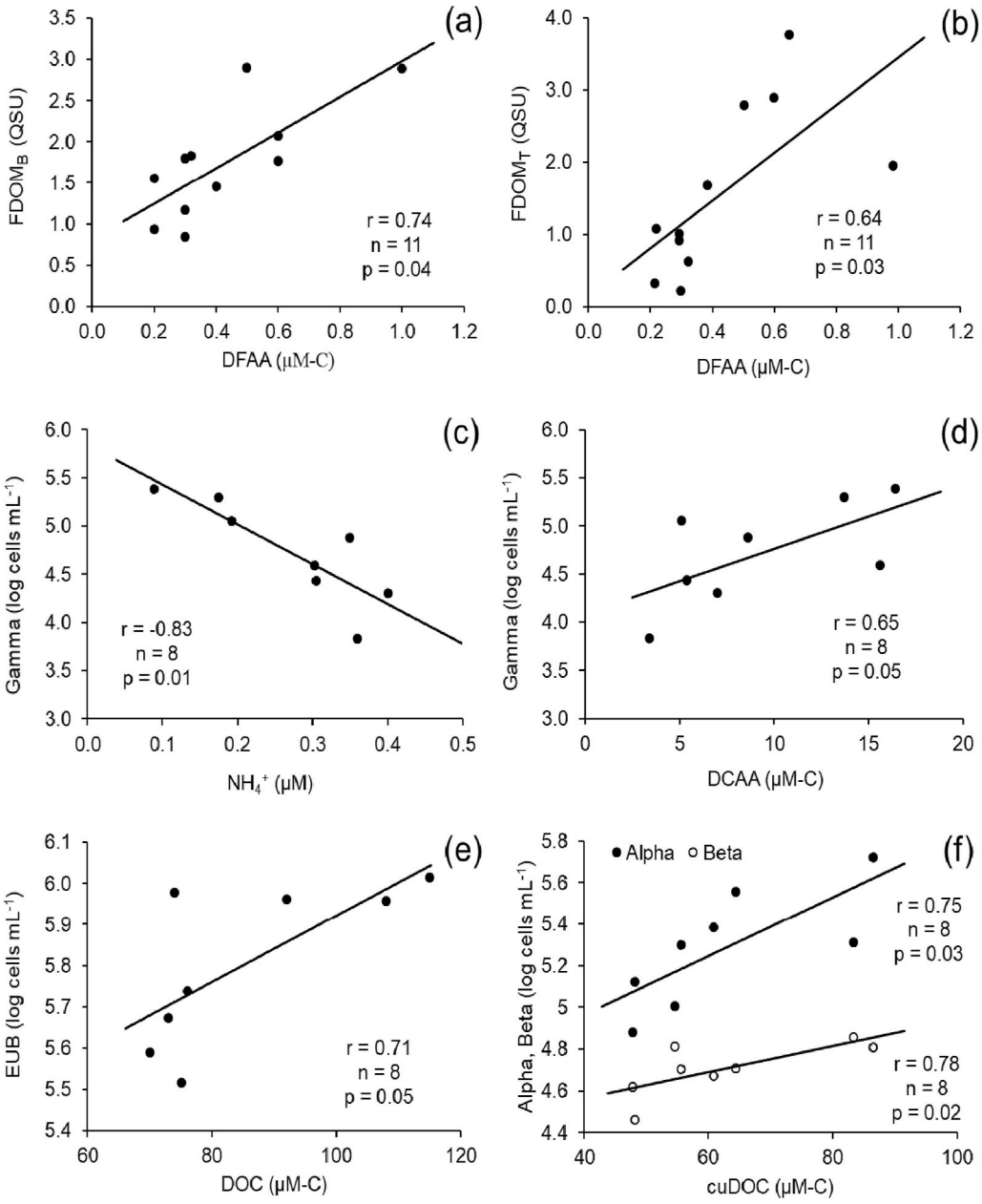

Fig. 8. Relationship between chemical and fluorescence characteristics of DOM: (a) "Protein-like" fluorescent dissolved organic matter ($FDOM_B$) vs dissolved free amino acids (DFAA), and (b) "Protein-like" fluorescent dissolved organic matter ($FDOM_T$) vs DFAA. Relationship among major bacterial groups, ammonium (NH_4^+) and dissolved organic matter: (c) *Gammaproteobacteria* (Gamma) vs NH_4^+, (d) Gamma vs dissolved combinate amino acids (DCAA), (e) total bacteria counts (EUB) vs dissolved organic carbon (DOC), and (f) *Alphaproteobacteria* (Alpha) and *Betaproteobateria* (Beta) vs chemically-unidentified DOC (cuDOC).

with cuDOC seems to exclude a cross correlation as the reason for their similar relationship with cuDOC. However, these positive correlations with cuDOC are intriguing, and particularly relevant in the case of *Alphaproteobacteria,* which accounts for 42 ± 15% of EUB total bacterial abundance. This group has been reported to show affinity for terrestrial humic DOM enriched in fulvic acids (Amaral et al., 2016). However, although cuDOC in the present study might be considered the "humic", uncharacterized fraction of DOM, it is most likely not of terrestrial origin, since it does not inversely correlate with salinity as might be expected in the coastal zone. Furthermore, cuDOC did not correlate with FDOM peaks or indices that could point to terrestrial humic components. Thus, comparisons with results from other authors on *Alphaproteobacteria* preferences remain elusive, until the chemical structure of the marine "humic" fraction is better known. Additionally, the positive correlations of *Alphaproteobacteria* and *Betaproteobacteria* with cuDOM could imply either a significant carbon contribution of these groups to the DOM pool, or a resource control of their abundance by cuDOC, same as suggested for *Gammaproteobacteria* and DCAA. To our knowledge, this is the first report of a significant relationship between the abundance of these bacterial groups and cuDOC in marine environments.

4. Conclusions

The dissolved fraction of suspended organic matter comprises the largest reservoir of reduced carbon in marine ecosystems, where microbes play a critical role in the interconversions between dissolved and particulate pools. Interactive physical and chemical processes in coastal shelves add complexity to the characterization of the organic matter composition and stocks. Strikingly, a large proportion of the DOM pool still remains chemically unidentified. In fact, in our study, cuDOC represented around the 75% of the total DOM, likely made up of humic substances where *Alphaproteobacteria* seem to be a pivotal player. In a global frame, our study adheres to the growing evidence of the large regional environmental effect on DOM biogeochemistry and microbial plankton configuration, where the multiple paths in the organic carbon cycle largely depend on the ecosystem settings and the specific

ecological traits of the microorganisms. Our exploratory survey emphasizes the need for further multidisciplinary sustained observations to embrace the spatial and temporal variability of DOM composition at a regional scale.

An open question remains in relation to the nature of the compounds detected by the so-called "protein-like" peaks. Neither $FDOM_B$ nor $FDOM_T$ correlated with DCAA, the fraction where dissolved proteins are contained. However, both peaks correlated highly significantly with DFAA, which was more than one order of magnitude lower than DCAA. In the same context, it remains unclear to what extent DCAA were available for heterotrophs or rather were part of recalcitrant components. This is particularly relevant for regenerative, productive shelf areas where nitrogen recycling is essential. This topic is currently under research in El Rincón and adjacent frontal zones. Overall, this study highlights the global need for chemical characterization of DOM in continental shelves in relation to microbial communities to parameterize regional carbon budgets and identify source and sink areas.

CRediT authorship contribution statement

John E. Garzón-Cardona: Conceptualization, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Valeria A. Guinder:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Investigation. **Cecilia Alonso:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Ana M. Martínez:** Formal analysis, Funding acquisition, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Silvio Pantoja-Gutiérrez:** Funding acquisition, Writing – original draft, Writing – review & editing. **Germán A. Kopprio:** Writing – original draft, Writing – review & editing. **Bernd Krock:** Writing – original draft, Writing – review & editing. **Rubén J. Lara:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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