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1 **Characterization of the vertical size distribution, composition and chemical**
2 **properties of dissolved organic matter in the (ultra)oligotrophic Pacific**
3 **Ocean through a multi-detection approach**

4
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15
16 **Abstract**

17 This work presents a multi-analytical approach for the characterization of marine dissolved
18 organic matter (DOM). The determination of marine dissolved organic carbon (DOC) was
19 performed by size exclusion chromatography (SEC) and validated using a certified reference
20 material (CRM) as well as through an intercomparison exercise. Multi-detection SEC,
21 fluorescence and electrochemical methods were used in order to describe the size distribution
22 spectra, the composition and chemical properties of marine DOM, in the (ultra)oligotrophic
23 West Tropical South Pacific Ocean (WTSP). In this work, we defined the state of degradation
24 of DOC in the different size fractions, operationally defined by SEC. We estimated that on
25 average 62% of DOC was of humic nature (0.5 – 10 kDa), of which ~ 9% was able to
26 complex trace elements, such as iron (Fe). Our results tend to support that non-refractory
27 DOC is of high molecular weight (HMW), nitrogen (N)-rich, aliphatic, and has a weak
28 fluorescence quantum yield and an enhanced binding capacity for Fe. The ageing of marine
29 DOM occurring within mesopelagic waters is mainly driven by microbial respiration and
30 alters these chemical properties. Although our results are in agreement with a paradigm
31 describing oceanic DOM biogeochemistry known as the size-reactivity continuum, 3 μmolC
32 L^{-1} of very HMW (> 10 kDa) were still observed in a water mass mainly composed of Pacific
33 Deep Waters. This persistence could be explained by a significant content (5%) of aromatic
34 carbon that may protect HMW DOM from long term biodegradation.

35
36 **Keywords:** Dissolved Organic Matter (DOM), Pacific Ocean, Humic Substances (HS), Size
37 Exclusion Chromatography (SEC).

39 **1. Introduction**

40 Marine dissolved organic matter (DOM) is a large pool of reduced carbon (Mackenzie et al.,
41 1981), representing an inventory of similar magnitude to atmospheric carbon. DOM is a
42 complex mixture of thousands of compounds (Gu et al., 1995; Stedmond et al., 2003), mainly
43 small molecules, with an extraordinary diversity of composition (Zark et al., 2017), that
44 results from both abiotic and biotic processes occurring throughout the ocean interior (Hansell
45 et al., 2009).

46 The size-reactivity continuum provides a paradigm for describing the oceanic
47 biogeochemistry of DOM (Amon & Benner, 1996; Benner & Amon, 2015). According to this
48 model, the ageing of highly (microbially) bioavailable freshly produced matter of high
49 molecular weight (HMW) induce the production of small, oxidized, nitrogen-depleted
50 compounds that are refractory to heterotrophic respiration. This process is thought to be the
51 main pathway of recalcitrant DOM production, also called the microbial carbon pump (Hach
52 et al., 2020; Jiao et al., 2010). The origin of the recalcitrance of DOM is still debated with on
53 the one hand the dilution hypothesis (Arrieta et al., 2015) and on the other hand the molecular
54 hypothesis (Shen & Benner, 2018) but can also result from a combination of both. The recent
55 work of Shen & Benner (2020) tends to validate the fact that the molecular properties of
56 DOM are the primary control on its microbial utilization in the ocean, highlighting the need to
57 develop quantitative proxies of the chemical properties of DOM.

58 Quantification of DOM can be assessed by the determination of dissolved organic carbon
59 (DOC) concentrations in order to identify the dominant process affecting the entire DOM pool
60 (production vs. degradation) in the global ocean (Hansell et al., 2009). Nevertheless, the DOC
61 concentration does not provide information on the nature of DOM and even less on its
62 composition, therefore oceanic studies often combine DOC distributions to specific (structural
63 characterization, Figure S1) or semi-specific qualitative analyses (inferred classification,
64 Figure S1). Mass spectrometry and nuclear magnetic resonance (NMR) techniques are
65 powerful tools to identify molecular formulae and chemical functions in DOM (Benner, 2003;
66 Hertkorn et al., 2013; Kujawinski, 2011; Kujawinski et al., 2009; Mopper et al., 2007; Ogawa
67 & Tanoue 2003 Osterholz et al., 2021). However, these methods need a solid phase extraction
68 (SPE) step prior to analysis and due to the partial extraction yields of the SPE cartridge. At
69 least a third of bulk DOM is lost during this extraction procedure (Dittmar et al., 2008).
70 Moreover, due to the large seawater volume required, these techniques are not easily

71 applicable for oceanographic expeditions with restricted water budget, involving a large
72 number of parameters. Therefore it is necessary to operationally classify organic compounds
73 according to optical or chemical properties to study DOM biogeochemistry in the ocean.
74 Optical properties of DOM help to identify biogeochemical processes occurring along the
75 water column, and are used as proxies to study the quality (Coble, 2007; Fichot & Benner,
76 2011), to trace the origin (Fellman et al., 2010; Osburn et al., 2016), and also as indicators of
77 the biological and (photo)chemical processing (Del Vecchio & Blough, 2002; Helms et al.,
78 2013; Osburn et al., 2016) of DOM. However, optical measurements by themselves do not
79 provide robust information on the composition of DOM and need to be combined with
80 complementary analyses. This is supported by a recent critical review of fluorescent DOM
81 revealing the ubiquitousness of fluorescence spectra that « are not tied to biogeochemical
82 origin, but exist across a wide range of different environments » (Wünsch et al., 2019). Semi-
83 specific methods and size exclusion chromatography (SEC) are alternative approaches
84 making it possible to easily differentiate the classes of DOM according to their size and
85 polarity (Amy & Her, 2004; Baghoth et al., 2008; Cornelissen et al., 2008; Dittmar & Kattner
86 2003; Dulaquais et al., 2018b; Huber & Frimmel, 1994; Huber et al., 2011). No sample
87 extraction or purification are necessary prior to measurements. Coupled with various detectors
88 (carbon, UV, nitrogen), this type of analysis represents an alternative method to study the
89 biogeochemistry of DOM at large scales (Figure S1).

90 Progress in SEC combined with multiple-detection approaches has made it possible to
91 operationally separate size fractions of DOM, to quantify their DOC content and to describe
92 some of the chemical properties (Amy & Her, 2004, Dulaquais et al., 2018b, Huber et al.,
93 2011). Among others, biopolymers (BP) correspond to a fraction of HMW (> 10 kDa)
94 believed to be mostly constituted of extracellular polymeric substances, such as
95 polysaccharides, proteinaceous material and amino sugars. SEC further permits an operational
96 definition of humic substances (HS) of molecular weight ranging between 0.5 and 10 kDa
97 based on their retention time (e.g. chemical affinity) on a polymethacrylate gel (Huber et al.,
98 2011). Aquatic HS were historically divided into humic acids, which precipitate at $\text{pH} \leq 1$,
99 and fulvic acids soluble at any pH. With SEC, the HS fraction encompasses humic and fulvic
100 acids. According to the literature, HS are believed to derive from phospholipids (Kowalenko
101 & McKercher 1971; Stott & Martin 1990) or produced during the photooxidation of
102 triglycerides and fatty acids (Kieber et al., 1997), but the chemical structure of HS varies
103 depending on their origin (Ertel et al., 1984; Dulaquais et al. 2018a). Recent experiments also

104 proposed the dimerization of the amino acid tyrosine catalyzed by peroxidase as a possible
105 pathway for HS production (Paerl et al., 2020). Carboxylic-rich alicyclic molecules (CRAM)
106 may represent another significant fraction of operationally defined HS (Hedges et al., 1992).
107 CRAM result from the decomposition of biomolecules derived from phytoplankton, exposed
108 to microbial degradation in the ocean interior (Hertkorn et al., 2006) and could be important
109 iron-binding ligands (Bundy et al., 2015). Part of the HS pool could also be formed during
110 condensation reactions by intermolecular collisions of compounds derived from the
111 degradation of BP (Lehmann and Kleber, 2015; Ogawa & Tanoue, 2003). These
112 condensations occur according to Maillard reactions, between carbohydrates and amino acids
113 or proteins, to form melanoidins (Maillard, 1912). SEC also operationally defines fractions of
114 LMW such as building blocks (BB, 0.3 – 0.5 kDa), LMW acids (< 0.3 kDa, Huber et al.,
115 2011), and LMW neutrals. These LMW fractions are thought to be derived from the fractions
116 in the upper size range (Huber et al., 2011) in agreement with the size reactivity continuum.
117 The BB fraction is supposed to correspond to degraded HS of LMW (Huber et al., 2011), the
118 other two fractions contain, among others, mono-protonic organic acids, mono-
119 oligosaccharides, alcohols, aldehydes, ketones and amino sugars (Amon & Benner, 1994;
120 Huber et al., 2011).

121 Among the different chemical properties of DOM, the binding capacity for dissolved iron
122 (dFe) is of particular interest. Indeed, it is a key element for marine life and DOM is well-
123 known to enhance dFe solubility by organic complexation (Powell & Donat, 2001; Rue &
124 Bruland, 1995, 1997; van den Berg, 1995; Wu & Luther III, 1995). Several dFe chelators
125 have already been identified including siderophores, exopolysaccharides and humic-like
126 ligands (Gledhill & Buck, 2012). Due to their relative refractory nature, humic-like ligands
127 can be found in the deep ocean (Dulaquais et al., 2018a; Whitby et al., 2020a) and can thereby
128 play an important role in the biogeochemistry of iron (Fe). Electrochemical methods are used
129 to measure the concentrations of the electroactive humic-like substances in marine
130 environments, which are thought to be the fraction of humic-like matter able to complex trace
131 elements, such as Fe (eHS; Dulaquais et al., 2018a, 2020; Sukekava et al., 2018; Whitby &
132 van den Berg, 2015). The quantification of eHS can be a complementary tool to track changes
133 in the binding properties of DOM during its processing, with broader implications for our
134 understanding of the interactions between DOM and trace elements.

135 In this work we propose a comprehensive view of marine DOM using multiple semi-specific
136 analytical approaches. We present the size fractionation, the aromatic carbon content of DOM
137 and its C:N composition along the entire water column in the oligotrophic gyre of the WTSP.
138 It is commonly assumed that allochthonous DOM is higher in size and more aromatic than that
139 of autochthonous origin. Aromaticity of DOM is one of the main indicators of sources and
140 processes which influence DOM composition (Chen & Hur, 2015; McKnight et al., 2001).
141 C:N elemental ratio of DOM is needed to determine its lability and the state of
142 remineralization of its compounds. N-depleted DOM has been recognized as a typical feature
143 of oligotrophic areas (Kähler & Koeve, 2001), and is further investigated in this manuscript.
144 Samples were collected during the GEOTRACES TONGA (shallow hydroThermal sOurces
145 of trace elemeNts: potential impacts on biological productivity and the bioloGicAl carbon
146 pump, GEOTRACES GPpr14) and US-GP15 expeditions. We also studied the fluorescence
147 properties of DOM and the electroactivity of humic-like substances. The latter analysis
148 provided an estimation of the binding capacity of this humic carbon for dFe.

149 **2. Material and Methods**

150 **2.1 Sampling**

151 Samples were collected during two cruises (Figure 1a): TONGA (20° 24.431'S, 166°
152 35.675'W, GEOTRACES GPpr14 onboard the R/V *L'Atalante* in November 2019) and
153 GEOTRACES US-GP15 (leg 2, 19° 59.99'S, 152° 00.01'W, onboard the R/V *Roger Revelle*
154 in November 2018). During the TONGA cruise, sampling was carried out using a trace metal
155 clean polyurethane powder-coated aluminum frame rosette (TMR) equipped with twenty-four
156 12 L Teflon-lined GO-FLO bottles (General Oceanics) and attached to a Kevlar® wire.
157 Potential temperature (θ), salinity (S), and dissolved oxygen (O_2), were retrieved from the
158 conductivity–temperature–depth (CTD) sensors (SBE9+) deployed on the TMR. The cleaning
159 protocols of all the sampling equipment followed the guidelines of the GEOTRACES
160 Cookbook (<http://www.geotraces.org>). After recovery, the TMR was directly transferred into
161 a clean container equipped with a class 100 laminar flow hood. Samples were then taken from
162 the filtrate of particulate samples (collected on acid cleaned polyethersulfone filters, 0.45 μ m
163 supor) and collected into acid cleaned and sample-rinsed HDPE 125 mL bottles. Immediately
164 after collection, samples were double bagged and stored at -20°C until analysis in a
165 shorebased laboratory. Samples from the GEOTRACES US-GP15 expedition were collected
166 using the Oceanographic Data Facility's (ODF, Scripps Institution of Oceanography) CTD
167 rosette equipped with twelve 30 L Niskin bottles. Samples were filtered through 0.8/0.45 μ m

168 Acropak 500 filter cartridges and were stored in a -15°C freezer on board. Samples were
169 returned frozen to Stanford University and stored at -20°C thereafter. Aliquots were
170 subsampled in acid cleaned and ultrapure water rinsed 30 mL HDPE bottles at Stanford
171 University within a laminar flow hood (class 100) and sent to LEMAR (Plouzané, France) in
172 a cooler with ice packs (travel duration of 2 days) and finally stored at -20°C until analysis. θ ,
173 S, and O₂, were retrieved from the CTD sensors (SBE9+) calibrated with onboard salinity and
174 dissolved oxygen measurements by the ODF group. Details on CTD data access from the
175 TONGA and US-GP15 expeditions are provided in the acknowledgments section.

176 **2.2 Reagents**

177 All aqueous solutions and cleaning procedures used ultrapure water (resistivity > 18.2
178 M Ω .cm, MilliQ Element, Millipore®). The mobile phase for SEC multi-detection was a
179 phosphate buffer (pH 6.85), prepared by dissolving disodium phosphate (Na₂HPO₄, 6 g,
180 EMSURE®, 99.5%) and monopotassium phosphate (KH₂PO₄, 10 g, EMSURE®, > 99.5%) in
181 ultrapure water (4 L). The acid phase was prepared by adding orthophosphoric acid (H₃PO₄,
182 16 mL, EMSURE®, 85%) to a solution of potassium persulfate (K₂S₂O₈, 2 g, Alfa Aesar,
183 Ward Hill MA, USA, 97%) in ultrapure water (4 L). Calibrations of the organic carbon
184 detector (OCD) and the ultra-violet detector (UVD) were achieved by potassium hydrogen
185 phthalate (C₈H₅KO₄, Alfa Aesar, 99.95 – 100.05%). The organic nitrogen detector (OND)
186 calibration was done using urea standard solutions (CH₄N₂O, Merck, Germany, >99.5%).
187 Calibrations were made in artificial seawater. The artificial seawater was prepared by
188 dissolving sodium chloride (NaCl, 6.563 g, ChemaLab NV, Belgium, 99.8%), potassium
189 chloride (KCl, 0.185 g, Merck, Germany, 99.999%), calcium chloride (CaCl₂, 0.245 g,
190 Prolabo, France, > 99.5%), magnesium chloride (MgCl₂, 1.520 g, Merck, Germany, 99-
191 101%), magnesium sulfate (MgSO₄, 1.006 g, Sigma-Aldrich, USA, \geq 99%), sodium
192 bicarbonate (NaHCO₃, 0.057 g, ChemaLab NV, Belgium, >99.7 %) in ultrapure water (250
193 mL). Artificial seawater was then UV irradiated for 2 hours in order to remove all traces of
194 organic contaminants. Fulvic (SRFA, 1S101F) and humic acids (SRHA, 1S101H) from the
195 *Suwannee River* used to calibrate the molecular weights (MW) of the HS fraction were
196 provided by the International Humic Substances Society (IHSS). The average molar masses
197 for these fulvic (711 Da) and humic acids (1066 Da) were previously defined by Aiken et al.
198 (1989). Deep seawater reference (DSR) samples were used to validate the DOC
199 measurements and were provided by the *Hansell* research laboratory (batch 19, lot #03-19).
200 Calibration solutions for Total Carbon Analyser (TOC-V) were prepared using C₈H₅KO₄ in
201 ultrapure water. A solution of 4 μ g L⁻¹ quinine sulfate dihydrate (QS, Acros Organics, VWR

202 Chemical, USA, >99%) in 0.1 M sulfuric acid (H_2SO_4 , Fluka®, Sigma-Aldrich, Switzerland,
203 >95%) was used for standardization of fluorescence units. For electrochemical analysis, an
204 acidic solution (hydrochloric acid, HCl , 0.01 M, Suprapur®, >99%) of $1.24 \mu\text{mol L}^{-1}$ iron
205 (III) was prepared from a stock solution (1 g L^{-1} , VWR, Prolabo, France). The borate buffer
206 (H_3BO_3 , 1M, Suprapur®, Merck, Germany, 99.8%) was prepared in 0.4 M ammonium
207 solution (NH_4OH , Ultrapure normatom, VWR Chemical, USA, 20-22%). The potassium
208 bromate solution (BrKO_3 , 0.3 M, VWR Chemical, USA, $\geq 99.8\%$) was prepared in ultrapure
209 water. The SRFA (2S101F) standard stock solution (38.2 mg L^{-1}) was prepared in ultrapure
210 water and was then saturated with Fe and equilibrated overnight before use. Exact
211 concentration was determined by SEC analysis.

212 **2.3 Analysis of marine DOM**

213 **2.3.1 Size exclusion chromatography (SEC)**

214 The distributions of the different organic compound classes were performed by SEC coupled
215 with an OCD, an OND and a UVD (DOC-Labor®, Karlsruhe, Germany) as previously
216 described by Huber et al. (2011) for natural waters, and adapted for marine waters by
217 Dulaquais et al. (2018b). Repeated analysis of DSR samples ($\text{DOC}_{\text{DSR}} = 43.2 \pm 1.7 \mu\text{mol L}^{-1}$;
218 $n = 5$; consensus value of lot #10-18: $43 - 45 \mu\text{mol L}^{-1}$) ensures an accurate determination of
219 DOC. The same method and configuration were applied for our samples, but with injection
220 volumes of 2.5 mL. This sample volume was selected to decrease the limits of detection
221 (LOD, Table S1) and to avoid the condensation of DOM observed for injection volumes
222 greater than 3 mL (Dulaquais et al., 2018b). Two chromatographic columns ($250 \text{ mm} \times 20$
223 mm , TSK HW-50S, 3000 theoretical plates, Toso, Japan) allows the separation of DOM into
224 five fractions of organic compounds with an optimal resolution (Baghoth et al., 2008). These
225 fractions were described in order of retention as BP ($> 10 \text{ kDa}$), HS ($0.5 - 10 \text{ kDa}$), BB ($0.3 -$
226 0.5 kDa), LMW monoprotic acids and neutrals ($< 0.3 \text{ kDa}$, Huber et al., 2011). Their
227 respective hypothetical composition are described in detail in Huber et al. (2011), Dulaquais
228 et al. (2018b) and in Table S1. In this study, C:N ratios were determined in two operationally
229 defined fractions (BP and HS) and the OND was calibrated using urea (instead of nitrate)
230 standards in order to take into account the oxidation yield of organic nitrogen into nitrate. The
231 percentage of aromatic carbon ($\%C_{\text{arom}}$) in an operationally defined given fraction was
232 determined according to Riso et al. (2021). It is defined as the spectral absorption coefficient
233 (SAC in m^{-1}) at 254 nm divided by the organic carbon concentration (OC, in gC m^{-3}) of the
234 fraction, and then multiplied by a coefficient of 4.403 (in $\%C_{\text{arom}} \text{ gC m}^{-2}$). This coefficient was

235 determined by the correlation between the %C_{arom} and SAC to [OC] ratio from NMR data
236 determined for a suite of IHSS standards (Riso et al., 2021).

237 **2.3.1 TOC-V analysis**

238 During the TONGA expedition, samples for DOC analysis by TOC-V were taken from a
239 classical rosette equipped with twenty-four 12 L Niskin bottles. The samples were filtered
240 under low vacuum (< 50 mm Hg) through 25 mm glass fiber filters (porosity ~ 0.7 µm, GF/F
241 Whatmann) and transferred into 10 mL glass ampoules. The filtrates were then acidified with
242 20 µL of H₂SO₄ (95%-98%, Sigma Aldrich), then the ampoules were flame sealed and stored
243 at 4°C until the analysis. DOC concentrations were measured in two replicates on a TOC-V
244 (Shimadzu, Kyoto, Japan) according to Sohrin & Sempéré (2005). The method consist of high
245 temperature (680 °C) platinum (Pt)-catalyzed oxidation coupled to non-dispersive infrared gas
246 detection of carbon dioxide (CO₂). Before injection, the samples were bubbled for 2 min with
247 CO₂-free air to purge inorganic carbon. The accuracy and system blank of the instrument were
248 determined by the analysis of certified water references (batch 19, lot #03-19, *Hansell*
249 Laboratory, Miami, Florida, USA). The nominal precision of the analysis procedure was
250 within 2%.

251 **2.3.3 Fluorescence spectroscopy**

252 Fluorescence analyses were performed on samples from the TONGA expedition. Excitation
253 emission fluorescence matrices (EEM) were generated using a spectrofluorometer Cary
254 Eclipse (Agilent®) equipped with a 150 W xenon excitation lamp. Optical measurements
255 were performed in a 1 cm quartz cell. For each measurement, excitation and emission
256 bandwidths were fixed at 5 nm. EEM were recorded with λ_{Ex} ranging from 200 to 450 nm
257 with an interval of 5 nm between each spectrum, and λ_{Em} ranging from 280 to 510 nm. The
258 scanning speed was set at 1200 nm min⁻¹. The nomenclature defined by Coble (1996)
259 associates peaks B and T to proteinaceous tyrosine-like (λ_{Ex} 225-275 nm, λ_{Em} 300-320 nm)
260 and tryptophan-like (λ_{Ex} 225-275 nm, λ_{Em} 320-380 nm) compounds respectively, and a peak
261 M to marine humic-like substances (λ_{Ex} 310-320 nm, λ_{Em} 380-420 nm) (Coble, 1996; Hudson
262 et al., 2007; Parlanti et al., 2000). To take into account the daily variations of the
263 spectrofluorometer sensitivity, EEM were normalized to the water Raman signal area. A
264 blank correction was carried out by subtracting the mean EEM from ultrapure water samples.
265 Fluorescence signals of EEM were converted into quinine sulfate units (QSU; 1 QSU =
266 fluorescence of 1 µg L⁻¹ of quinine sulfate in 0.1 M H₂SO₄ at λ_{Ex}/λ_{Em} = 350/450 nm). This
267 was performed in order to compare our fluorescent measurements with previous studies since
268 QSU is a widely used fluorescence unit. The LOD were defined as three times the standard

269 deviation of fluorescence intensity for each fluorophore according to the nomenclature
270 defined by Coble (1996), and were 0.9 QSU for peak B (tyrosine-like), 0.7 QSU for peak T
271 (tryptophan-like) and 0.1 QSU for peak M (humic-like). In this work, we mostly focus on
272 three fluorescence intensity indexes. The fluorescence index (FI, λ_{Ex} 370 nm, λ_{Em} 450/500
273 nm; McKnight et al., 2001), the biological index (BIX, λ_{Ex} 310 nm, λ_{Em} 380/430 nm; Huguet
274 et al., 2009) and the humification index (HIX, λ_{Ex} 255 nm, λ_{Em} 435-480/300-345 nm; Zsolnay
275 et al., 1999) were calculated to trace the origin of marine DOM.

276 **2.3.4 Analysis of electroactive humic-like substances (eHS)**

277 The determination of eHS was performed on samples from the TONGA expedition. Analyses
278 were performed by cathodic stripping voltammetry (CSV) using a polarographic Metrohm
279 663VA stand connected to a potentiostat/galvanostat (Autolab, Metrohm®) and to an
280 interface (IME 663, Metrohm®). Data acquisition was done using the NOVA software
281 (version 10.1). The method used in this study was initially developed by Laglera et al. (2007)
282 and adapted by Sukekava et al. (2018). The method is based on the adsorption at pH 8 of an
283 Fe-humic complex at the surface of a mercury drop electrode under oxidative potential (0 V
284 vs Ag/AgCl) and its reduction during linear stripping of potentials (from 0 to -0.8 V vs
285 Ag/AgCl). In the presence of 30 mmol L⁻¹ bromate, the reduction of the Fe-humic complex
286 provides a quantitative peak at -0.5 V (vs Ag/AgCl) with an intensity proportional to the
287 concentration. In this study, the pH of samples was set to 8.00 ± 0.05 by the addition of a
288 borate buffer (final concentration 10 mM) and adjusted by small additions of an ammonia
289 solution. All eHS initially present in a sample were Fe-saturated by the addition of 20 nmol L⁻¹
290 ⁵⁷Fe in order to determine the total eHS concentration. After equilibration, aliquots (15 mL) of
291 the sample were placed into 3 different teflon® vials (Savillex®); among them two were
292 spiked with a SRFA standard (2S101F; spiked of 50 and 100 µg L⁻¹), and left for overnight
293 equilibration. After equilibration, samples were placed into a Teflon® voltammetric cell and
294 analyzed by linear sweep voltammetry after 180 s of nitrogen (N₂) purge (Alphagaz®, Air
295 liquide) and a 90 s deposition step. Since there is no certified reference material (CRM) of
296 marine HS, all the results are provided in equivalent SRFA per liter. The use of SRFA for the
297 determination of oceanic eHS may induce biais due to possible differences in composition
298 between SRFA batches purchased and to the origin of this material. SRFA come from
299 freshwaters with a high DOM load while the marine environment is characterized by low
300 DOM concentrations. Furthermore freshwater and marine HS have different composition,
301 including binding capacity for iron, as previously shown (Dulaquais et al., 2018a, Esteves et
302 al., 2009; Riso et al., 2021). Efforts should be conducted to develop a program for the

303 production of a marine humic reference material. In absence of an oceanic reference material
304 comparable to the DSR (for DOC) or SAFe (for dFe) samples, the use of this material permits
305 the generation of intercomparable data between labs and studies. Actually this consensus
306 material is easy to purchase and widely use by the scientific community (Bundy et al., 2015;
307 Cabanes et al., 2020; Dulaquais et al., 2018a; Gao & Guéguen 2018; Laglera et al., 2019; Riso
308 et al., 2021; Slagter et al., 2019; Sukekava et al., 2018; Whitby et al., 2020a,b). Errors were
309 calculated based on statistical evaluation of a least squares linear fit to the data. The absence
310 of quantitative signals in ultrapure water ensures no contamination all along the analytical
311 process. Because no blank signal can be detected, the LOD was defined as three times the
312 standard deviation of the lowest concentration measured ($23.2 \pm 3.0 \mu\text{g eq-SRFA L}^{-1}$) and was
313 estimated to be $9 \mu\text{g eq-SRFA L}^{-1}$.

314 The four different analyses applied to the respective cruises and stations are presented in
315 Table S2.

316 **3. Results and discussion**

317 **3.1 Hydrography**

318 The hydrography of the WTSP is described in detail by Fumenia et al. (2018), Wagener et al.
319 (2018), Peters et al. (2018) and Villa-Alfageme et al. (2019). In this section we briefly
320 describe the key features encountered. At the sampling locations, salinities ranged from 35.48
321 to 36.15 and temperatures from 23.6 to 27.1°C in the upper 100 m. The oligotrophy of the
322 domain was evidenced by the extremely low surface nutrient concentrations (Cutter et al.,
323 2018; Guieu & Bonnet, 2019) and surface chlorophyll levels derived from satellite data
324 (Figure 1a; MODIS-Aqua MODISA simulations; data from giovanni.gsfc.nasa.gov). At both
325 stations our shallowest sampling depth (25 m) can be considered in the mixed layer and thus
326 representative of surface waters. The thermocline was deep (100–600 m) and was sampled at
327 several depths (Figure 1b). Along the thermocline, different watermasses were identified. The
328 South Pacific Central Waters (SPCW) can be divided by their salinity into the Western
329 (WSPCW) in the upper thermocline and the Eastern (ESPCW) component in the lower
330 thermocline. Modified Equatorial Subsurface Waters (ESSW) may be identified between
331 these two SPCW components. ESSW are known to be poorly oxygenated (Silva et al., 2009)
332 and to have a similar density to SPCW (Sprintall & Tomczak, 1993; Wyrcki, 1967). Thereby
333 the low oxygen concentrations observed in the middle of the thermocline (Figure 1b) were
334 probably induced by an inclusion of ESSW in the SPCW. Below the thermocline, the
335 intermediate waters (600–900 m) were mostly composed of a mix between Subantarctic Mode
336 Waters (SAMW, $26.80 \leq \sigma\theta \leq 27.06 \text{ kg m}^{-3}$, Hartin et al., 2011) and Antarctic Intermediate

337 Waters (AAIW, $27.06 \leq \sigma\theta \leq 27.40 \text{ kg m}^{-3}$, Hartin et al., 2011). Due to the overlap of their
338 potential density, an exact distinction between these two watermasses remains difficult thus
339 they are often considered as a single watermass in the subtropical area (Fumenia et al., 2018).
340 These two watermasses have distinct origins; SAMW are formed by the mix of Antarctic

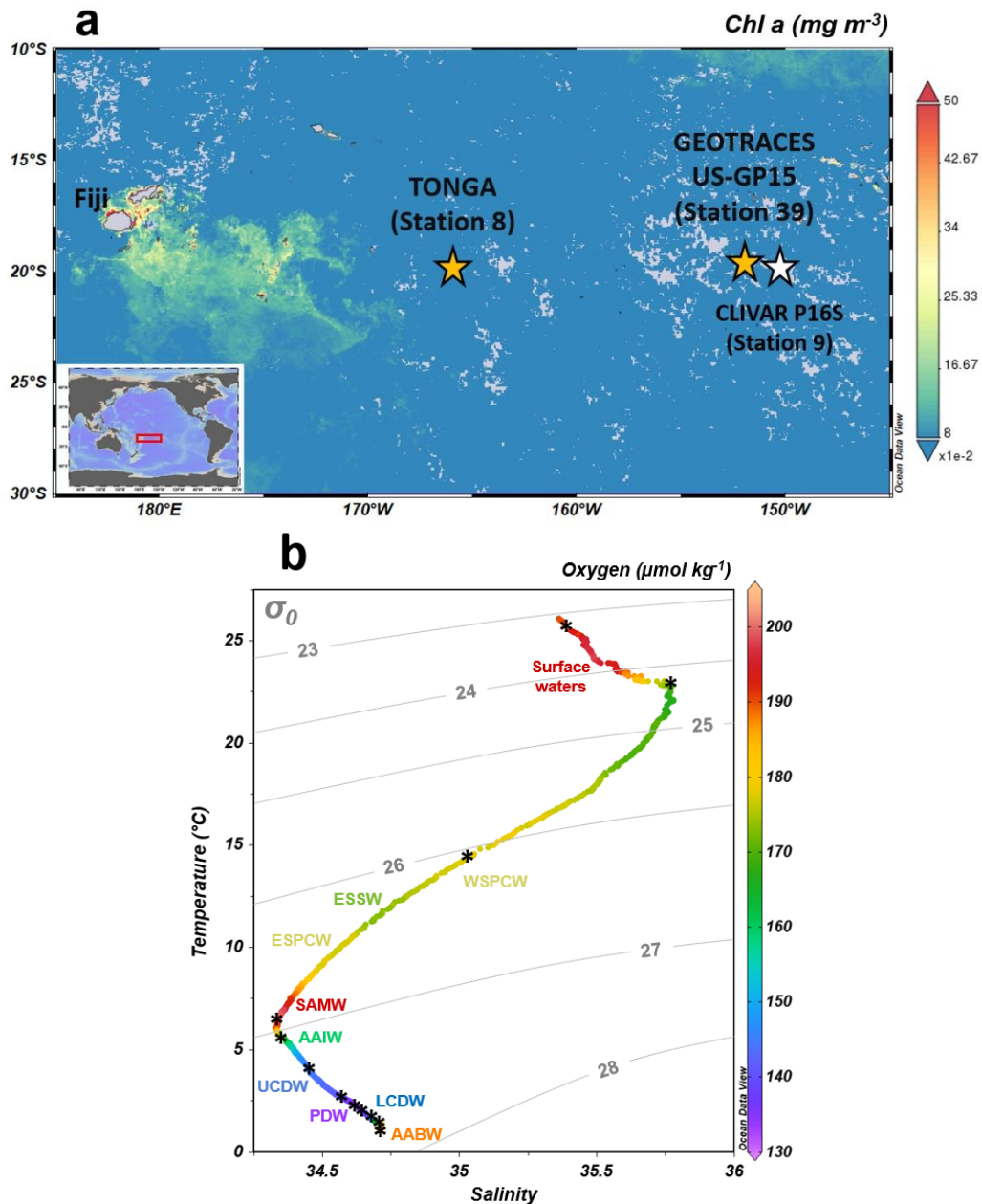


Figure 1. (a) Time averaged map of chlorophyll a (mg m^{-3} ; 8-daily 4-km over 2019-11-09 – 2019-12-09) in the Western Tropical South Pacific Ocean (WTSP). Figure generated using Giovanni (giovanni.gsfc.nasa.gov). The two sampled sites of TONGA and GP-15 cruises are indicated (yellow stars), as well as the selected station from CLIVAR P16 for an intercomparison of dissolved organic carbon (DOC) concentrations (white star). (b) Temperature/salinity diagram of the study area with the color gradation corresponding to dissolved oxygen (O_2) concentrations ($\mu\text{mol kg}^{-1}$) at $20^\circ 24.431'S$, $166^\circ 35.675'W$ during the TONGA expedition. Grey lines indicate the isopycnal horizons (on the basis of potential density referenced to a pressure of 0 dbar). Water masses are indicated (see text for abbreviations). Asterisks (*) indicate the samples collected for Size Exclusion Chromatography (SEC) analyses. Figure generated using ODV software.

341 surface waters with subtropical waters at the subantarctic zone, while AAIW originated from
 342 deep convection of the densest Antarctic surface waters at the polar front zone. These distinct
 343 locations of downwelling imprint the oxygen signature of these two watermasses, allowing us
 344 to distinguish SAMW from AAIW by the higher oxygen content of SAMW ($>200 \mu\text{mol kg}^{-1}$,

345 Figure 1b) according to McCartney (1979). In the deep sea (>1000 m) the two components
346 (upper and lower) of the circumpolar deep waters (CDW) can be differentiated by their
347 potential density ($\sigma_{\theta_{UCDW}} = 27.59$, $\sigma_{\theta_{LCDW}} = 27.79$; Peters et al., 2018; Figure 1b). CDW are
348 the largest water masses of the Southern Ocean and are a mix of AAIW, North Atlantic Deep
349 Waters (NADW) and intermediate water masses from the Pacific Ocean. CDW spread
350 northward in the South Pacific from the Southern Ocean, alongside with the Deep Western
351 Boundary Current (DWBC) of the Pacific Ocean, which flows along the western edge of the
352 Southern basin (Koshlyakov & Tarakanov, 1999). UCDW and LCDW were separated by a
353 layer, centered at 2900 m, mainly composed of PDW ($\sigma_{\theta_{PDW}} = 27.77$; Peters et al., 2018;
354 Figure 1b). This composite of PDW, UCDW and LCDW is transported northward at this
355 location. In this work, this composite will be considered as the PDW. The most abyssal depths
356 (> 4900 m) were occupied by a composite of LCDW and of Antarctic Bottom Waters
357 (AABW), formed in the Ross Sea. These waters spread northward, driven by Ekman transport
358 and forced by seafloor topography (Tomczak & Godfrey, 2003).

359

360 **3.2 Vertical distributions of fractions of dissolved organic marine compounds**

361 **3.2.1 DOC distribution**

362 The distribution profiles of DOC concentrations (Figure 2) of the four datasets showed a
363 decrease from the surface to the deep waters. With the SEC method used for TONGA
364 samples, the concentrations ranged from $73.4 \pm 2.2 \mu\text{molC L}^{-1}$ at 25 m depth to 37.4 ± 1.1
365 $\mu\text{molC L}^{-1}$ in the old PDW composite at 3400 m, reflecting DOM mineralization in the
366 mesopelagic zone. Deeper, DOC increased again to reach $45.1 \pm 1.5 \mu\text{molC L}^{-1}$ at the deepest
367 depth (5461 m). These concentrations were higher than the DOC signature of the AABW
368 ($40.2 \pm 0.7 \mu\text{molC L}^{-1}$ in the deep Southern Ocean, Bercovici & Hansell, 2016) located at
369 these depths and could indicate benthic inputs of DOM by diffusion from sediments as
370 previously proposed by Lahajnar et al. (2005) in the deep Atlantic Ocean. To validate DOC
371 measurements, an intercomparison was performed between the DOC concentrations measured
372 by our SEC-OCD system and data acquired using the « classical » TOC-V method. Results
373 are presented in Figure 2 and the two datasets display similar vertical distribution and ranged
374 of concentration for each depth interval. We further compared our measurements to the
375 historic dataset from the P16 CLIVAR expedition at a station located at the same latitude but
376 different longitude (20°S, 150°W) and it showed a good agreement between the three datasets
377 in surface and deep waters. Scatter plot of the paired datasets shows that all the data were
378 close to the 1:1 line (Figure S2) when uncertainties were considered. Good correlations of the

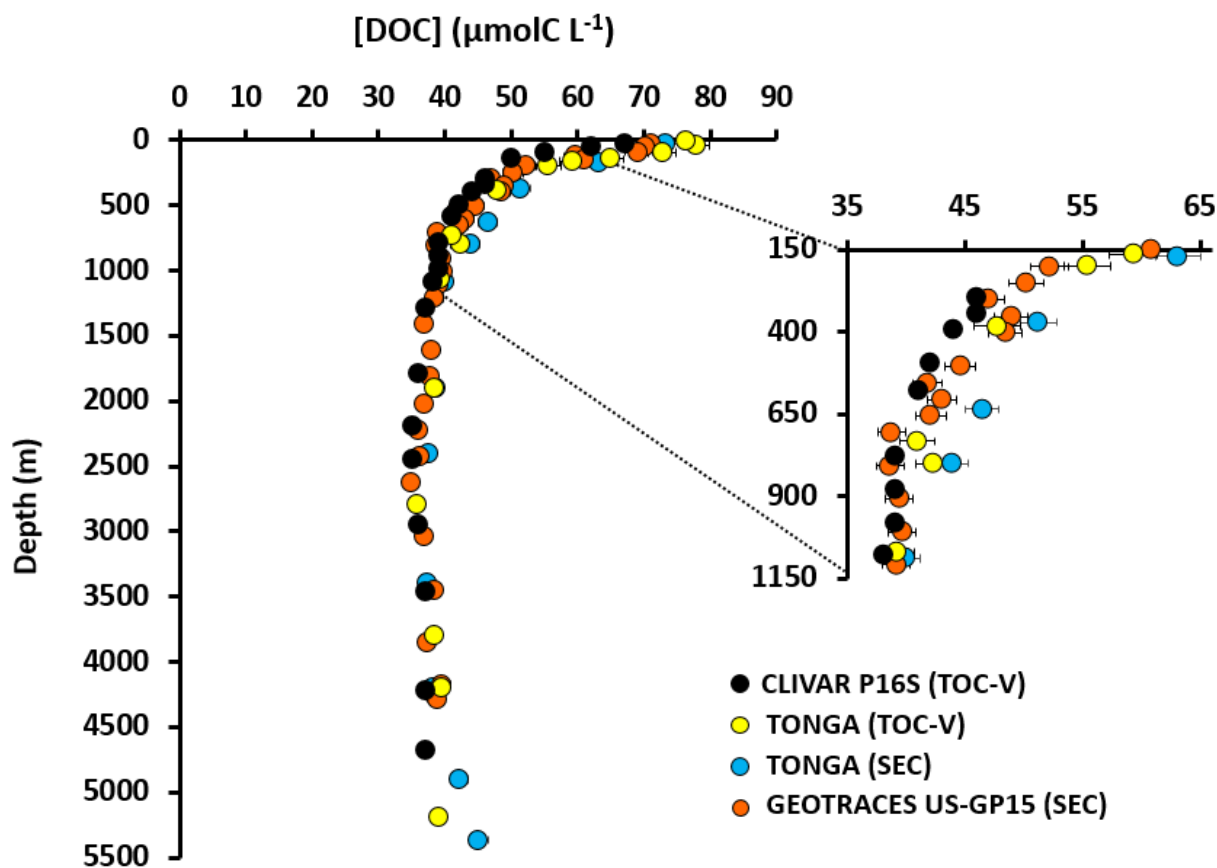


Figure 2. Intercomparison of the DOC concentrations ($\mu\text{molC L}^{-1}$) of the TONGA expedition (GEOTRACES GPpr14) at $20^{\circ} 24.431'S$, $166^{\circ} 35.675'W$ measured by SEC (blue dots) and TOC-V (yellow dots). Data from the CLIVAR P16 cruise ($20^{\circ}S$, $150^{\circ}W$; Swan et al., 2009; dark dots) and from the GEOTRACES US-GP15 cruise ($19^{\circ} 59.99'S$, $152^{\circ} 00.01'W$) were also plotted (orange dots).

379 linear regressions ($R^2 \geq 0.96$) and Spearman coefficients close to 1 (Figure S2), both indicate
 380 the strong correlation between DOC concentrations determined by SEC and TOC-V. Together
 381 with the accurate determination of DOC in DSR samples, this statistical demonstrate that the
 382 SEC-OCD system has an excellent recovery of marine DOC and can be used for accurate
 383 quantitative DOC assessment in an open ocean environment. Small discrepancies (see insert
 384 Figure 2), higher than uncertainties of the methods, were observed in the mode waters and
 385 could be explained by the hydrography. The θ -S diagram shows only small differences in the
 386 water composition between P16 and the two stations sampled (Figure S3a) with mode waters
 387 being less haline at our locations than during the CLIVAR expedition. This difference of
 388 intermediate water composition between ~ 400 and ~ 700 meters depth was however visible
 389 by the higher apparent oxygen utilization (AOU) during both the CLIVAR and GP15
 390 expeditions compared to TONGA (Figure S3b). The AOU represents the integrated oxygen
 391 consumption by heterotrophic bacteria in the breakdown of organic matter and it is computed
 392 as the difference between the oxygen saturation concentration, which depends on
 393 thermohaline properties (Weiss, 1970) and the observed oxygen concentration. Thereby the

394 slightly higher DOC concentration we observed during TONGA (Figure 2) can be linked to a
 395 lower DOC mineralization state at these depths than during CLIVAR P16. These observations
 396 highlight the imprint of hydrography on DOC distribution.

397 3.2.2 Size class distribution and composition of DOM

398 The distribution of the different fractions composing DOM are presented in Figures 3a and in
 399 Figure S4 (for TONGA and US-GP15 cruises, respectively). Along the water column, HS had
 400 a quasi constant molecular weight (705 ± 20 Da) and was systematically the major fraction of
 401 DOC (61.6 ± 2.6 %), with concentrations between 22.2 and $43.2 \mu\text{molC L}^{-1}$. LMW neutral
 402 compounds represented the second most abundant fraction, contributing on average to 17.3%
 403 of DOC, with an interval of concentration between 6.2 and $11.2 \mu\text{molC L}^{-1}$. The BB fraction
 404 accounts for an average of 9.7 ± 1.3 % of DOC throughout the water column, with
 405 concentration varying between 3.3 and $6.5 \mu\text{molC L}^{-1}$. BP contributed to $6.7 \pm 1.6\%$ ($2.0 - 8.0$
 406 $\mu\text{molC L}^{-1}$) and the fraction containing LMW acids ($1.1 - 4.5 \mu\text{molC L}^{-1}$) was the minor
 407 component of DOC contributing to only $\sim 4.7\%$.

408 Within all the different fractions, DOC concentrations decreased from surface to deep waters,
 409 but small variations in the partitioning were however observed. For instance, the contribution
 410 of the BP and of the LMW acids to DOC decreased from 25 m depth to the 2500-3000 m
 411 depth and at the same time the contribution of LMW neutrals increased. These variations can
 412 be interpreted as differences in the reactivity of the fractions. HMW compounds seem more
 413 labile and consumed more rapidly than small neutral compounds that accumulate with ageing

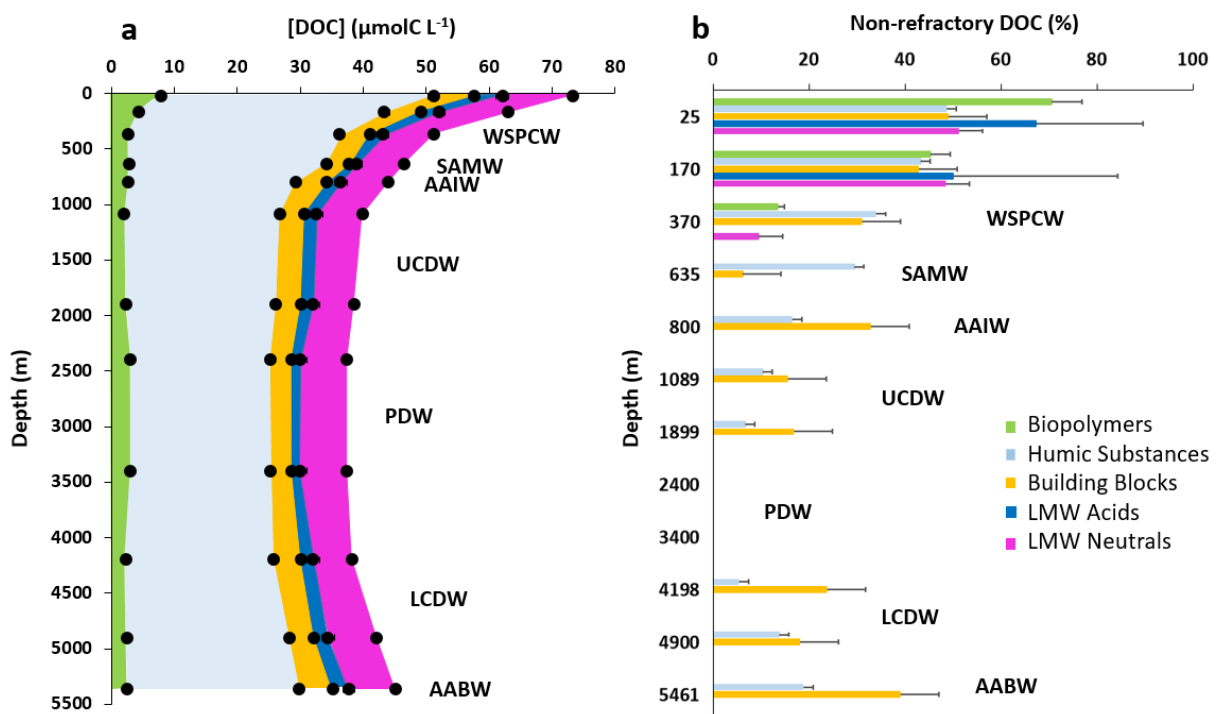


Figure 3. (a) Vertical profiles of the DOC concentrations ($\mu\text{molC L}^{-1}$) in the five operational fractions defined by SEC. Non-visible error bars are covered by the symbols. (b) Percentage of non-refractory DOC calculated for each fraction (see text for explanation) as a function of depth (m). Depth of water masses are indicated (see text for abbreviations) at $20^\circ 24.431'S$, $166^\circ 35.675'W$ during the TONGA expedition (station 8).

414 of water masses. These observations are in agreement with the size reactivity continuum
415 described by Benner & Amon (2015) and with the recent study of Zigah et al. (2017) that
416 reports the size partitioning of DOC along the water column from surface to 3500 m depth in
417 the oligotrophic North Pacific Ocean (Station ALOHA). Zigah et al. (2017) estimated a
418 contribution of HMW DOC (defined in their study as > 1kDa) to be between 23 and 35%
419 while LMW hydrophobic compounds (defined in their study as < 1kDa) retained by SPE
420 cartridge accounted for 45-52% of DOC and LMW hydrophilic compounds (non-retained by
421 SPE cartridge) were estimated to account for 22-33% of DOC. Similar to our observations,
422 Zigah et al. (2017) measured HMW DOM throughout the water column and found humic-like
423 substances (called LMW hydrophobic compounds in their study) to be the main fraction
424 composing DOC. The authors further described a distribution of LMW hydrophilic
425 compounds as quasi-homogeneous with a slight enrichment in the surface. The distribution
426 they described is in excellent agreement with those we observed for both LMW acids and
427 neutrals that are LMW hydrophilic compounds. In our study, seawater samples were analyzed
428 without any pre-processing of samples. The excellent agreement of the size-distribution
429 between our study and Zigah et al. (2017) shows that our method allows describing the size
430 distribution of marine DOC with 2.5 mL of sample and without any preliminary filtration and
431 extraction.

432 *DOC refractivity within fractions*

433 At the latitude of sampling, the PDW has an age of over 1000 years (estimated using
434 CLIVAR P16 inorganic $\Delta^{14}\text{C}$ at 21°S and ageing equation proposed by Hawco et al., 2018). It
435 has been shown that the organic $\Delta^{14}\text{C}$ is even more depleted in the deep Pacific Ocean than
436 the inorganic $\Delta^{14}\text{C}$ (Zigah et al., 2017). Thereby DOC can be considered as almost only
437 composed of refractory DOC (RDOC) in this watermass. Due to its refractory nature, the
438 distribution of RDOC is assumed to be conservative (Ogawa & Tanoue, 2003) and can
439 therefore be considered homogeneous throughout the water column. As a result, the DOC
440 concentrations measured for each fraction in the PDW composite can be used to define the
441 contribution of non-RDOC in the different fractions at each depth ($[\text{non-RDOC}] = [\text{DOC}] -$
442 $[\text{DOC}]_{\text{PDW}}$). Despite caveats implied by this assumption (e.g. possible photodegradation of
443 RDOC in the upper euphotic zone, Polimene et al., 2018) it provides a working hypothesis to
444 investigate the chemical properties and reactivity of semi-labile and refractory compounds.
445 The contribution of non-RDOC for each fraction during the TONGA expedition was
446 calculated for both stations throughout the water column (Figure 3b) and showed a decrease

447 from surface to the PDW composite depths (2400-3400 m). In surface waters more than 50%
448 of DOC was non-refractory reaching up to 70% for the BP fractions. The decrease of non-
449 RDOC with depth, down to the PDW composite, is observed for all fractions (Figure 3b) and
450 is in agreement with the lability paradigm. However some differences among the fractions
451 were observed with non-RDOC being undetectable in the LMW acids, LMW neutrals and BP
452 fractions below 400 meters depth (Figure 3b) while non-refractory HS, and BB were still
453 depicted. These differences in the non-RDOC distributions are representative of variabilities
454 in the reactivity of the fractions and can be interpreted as the existence of semi-refractory
455 compounds in the BB and HS fractions that were not measured in the other fractions.

456 *Aromatic carbon content of DOM*

457 The aromaticity of DOC was determined for all fractions except for the fraction of LMW
458 neutrals wherein no quantifiable aromatic signal was detected (Figure 4a). In the oligotrophic
459 WTSP, DOM was poorly aromatic with the percentage of aromatic carbon always below 5%
460 in all fractions. In agreement with Medeiros et al. (2015) and Osterholz et al. (2021), our
461 measurements showed surface samples of the Pacific Ocean with extremely low aromatic
462 carbon content and the percentage of aromatic carbon increasing with depth in the upper 1000
463 m. The higher percentages were observed for the BP and BB fractions in the PDW (2500 –
464 3500 m). The maximum of aromaticity of BP and BB (~ 5%) is close to those observed for
465 SPE-DOM in the Atlantic Ocean (grey area Figure 4a, 4.57 – 5.13 %C_{arom}; Hertkorn et al.,
466 2013) suggesting that the BP and BB are partly representative of the compounds retained on a
467 SPE cartridge. According to the size-reactivity continuum, occurrence of BP should be
468 limited in old water-masses. However, recent findings suggest that, among others, dissolved
469 black carbon is likely an important fraction of refractory DOM (RDOM) in the ocean
470 (Benner, 2003; Coppola & Druffel, 2016; Dittmar & Koch, 2006; Dittmar & Paeng, 2009;
471 Nakane et al., 2017; Ziolkowski & Druffel, 2010). Black carbon are compounds with

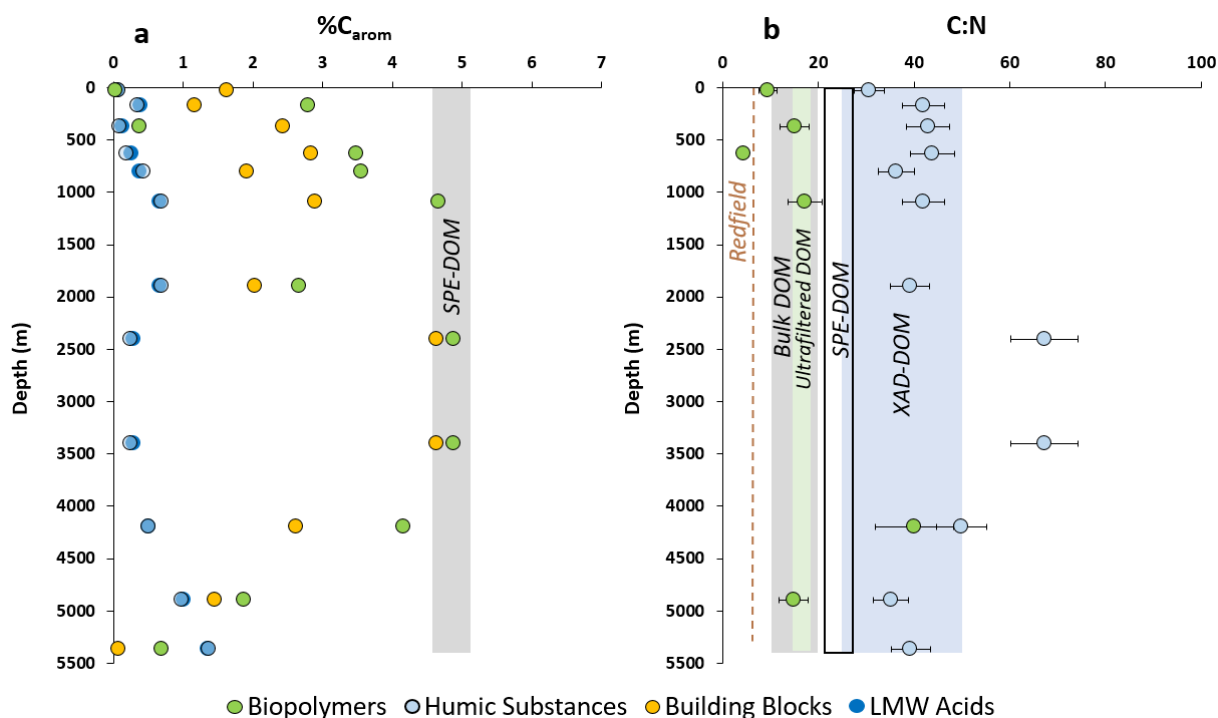


Figure 4. (a) Percentage of aromatic carbon against depth (m) measured in four of the five fractions defined by SEC. Aromatic signal was undetectable in the low molecular weight (LMW) neutral fraction. Range of percentage of aromatic carbon in marine SPE-DOM from the Atlantic Ocean (Hertkorn et al., 2013) is also provided for comparison. (b) Elemental C:N ratios measured in the biopolymers (BP) and humic substances (HS) fractions along the water column at 20° 24.431'S, 166° 35.675'W during the TONGA expedition (station 8). Redfield ratio as well as the different ranges of marine C:N ratio in bulk, ultrafiltered, XAD-resin isolated DOM (Hessen & Tranvik, 2013) and SPE-DOM of the Central Pacific Ocean (Green et al., 2014) are provided. Non-visible error bars are covered by the symbols.

472 condensed rings, ultra-refractory to biodegradation (Dittmar, 2008; Marques et al., 2017;
 473 Nakane et al., 2017; Wagner et al., 2018) and can be found under HMW compounds in the
 474 deep Pacific Ocean (Ziolkowski & Druffel, 2010). The high percentage of aromatic carbon
 475 measured in the PDW for BP and BB possibly indicates the presence of black carbon of both
 476 HMW and LMW at these depths, explaining the persistence of these fractions in the PDW.
 477 Deeper, $\%C_{\text{arom}}$ decreases in the water masses containing non-RDOC. This feature suggests
 478 that the $\%C_{\text{arom}}$ increases with the refractory nature of BP and BB. The aromaticities of HS
 479 and LMW acids were significantly lower (0.1 to 1.4% of C_{arom}) than those of BP and BB. The
 480 extremely low aromatic carbon content of HS contrast with those measured in humic
 481 standards (e.g. SRFA_{2S101F} = 22% C_{arom} , IHSS) and in estuarine humic-like substances (Riso et
 482 al., 2021) confirming the difference in the chemical composition of terrestrial and marine HS,
 483 the latter being known to have extremely low aromaticity (Harvey et al., 1983; Hertkorn et al.,
 484 2006; Malcolm, 1990; Repeta et al., 2002). The near-zero values in aromatic content
 485 measured at the surface may result from a production of HS mainly aliphatics together with
 486 the UV-oxidation of their aromaticity (Corin et al., 1996; Miranda et al., 2020; Zhao et al.,

487 2020). Then, the increase of %C_{arom} in the mesopelagic layer down to 2000 m can be
488 interpreted as a condensation of HS during their biodegradation/transformation by bacteria
489 into semi-refractory DOM. In the PDW, the %C_{arom} of refractory HS was extremely low
490 (0.25%) and contrasted with the BP and BB fractions. This result possibly indicates that long
491 term biogeochemical processes occurring in the abyssal waters alter the aromaticity of HS
492 (aromatic rings and conjugated double bounds) formed in the mesopelagic layer. The
493 percentage of C_{arom} increased again in the younger water masses (LCDW, AABW) found
494 deeper and close to sediments in line with the occurrence of HS with an advanced state of
495 biodegradation that are a mix of semi-refractory and refractory HS (Figure 3b).

496 BB are considered as by-products of HS since ultrasonification experiments were conducted
497 on this DOC fraction (Huber et al., 2011). However, significant correlations were observed
498 between the %C_{arom} of BP and BB ($R^2 = 0.5408$; $r_s = 0.85$) and between HS and LMW acids
499 ($R^2 = 0.9895$; $r_s = 0.92$). For the two datasets tested, *Spearman* coefficients r_s close to 1,
500 further suggesting links existing between these fractions. According to the size-reactivity
501 continuum, small compounds are produced from the degradation of larger compounds. Based
502 on the aromatic signature we measured in the fraction, we propose to reconsider the BB as by-
503 products of the degradation of BP and LMW acids to be generated during the degradation of
504 HS. This simple definition could then be used to monitor the state of DOM degradation by
505 following a simple ratio (BP/BB or HS/LMW acids) at large scales. Distribution of the
506 BP/BB ratio (data not shown) evidenced a global decrease from surface to deep in agreement
507 with a gradual increase in the state of degradation of DOM with ageing of watermasses.
508 Based on our measurements, we suggest to no longer consider BB as byproducts of HS, as
509 initially postulated in Gaid (2011), in open ocean studies. Overall the results of this study lead
510 to similar conclusions to those obtained from the molecular approach of Osterholz et al.
511 (2021) that shows a significant increase of the DOM degradation index from surface waters to
512 the deep layers of the south east oligotrophic Pacific Ocean.

513 *C:N elemental ratios in BP and HS*

514 N-content of DOM is of interest to investigate the lability of DOM (Vallino et al., 1996). Due
515 to the analytical challenge to measure these extremely low organic nitrogen concentrations
516 studies reporting the C:N ratios of specific DOM compounds in the open ocean are still
517 scarce. In this work we succeed to measure sub-micromolar organic nitrogen concentrations
518 in the HS and BP fractions and present the vertical distribution of their respective C:N ratios
519 (Figure 4b).

520 During the TONGA expedition, the mean C:N ratio in the HS fraction was relatively
521 homogenous (44.7 ± 4.7 ; $n = 12$) with the exception of PDW samples wherein the C:N ratio
522 was significantly higher (67.4 ± 7.1) and can be defined as the C:N ratio of refractory HS. In
523 the shallowest sample (25 m depth) the C:N ratio of HS was 30.7 ± 3.2 . These values are
524 higher than those measured in SPE DOM of the Pacific Ocean (21.3 – 27.6; Green et al.,
525 2014) and lower than those of the *Suwannee River* IHSS standards (e.g. C:N SRFA_{2S101F} =
526 91.1; C:N SRHA_{1S101H} = 51.5; IHSS) except for the PDW samples. These values are however
527 in agreement to the C:N ratios determined in marine HS isolated using XAD resins (Druffel et
528 al., 1992; Hedges et al., 1992; Lara et al., 1993) including those measured in the open Pacific
529 Ocean (e.g. 32 – 43, Meyers-Schulte & Hedges, 1986; Hedges et al., 1992). Vertical
530 variations of the C:N values we measured indicate differences in the bioavailability of
531 nitrogen and carbon in humic matter. Occurrence of a low C:N ratio (30.7 ± 3.2) at 25 m
532 depth suggests two pools of humic-like substances, the refractory HS (high C:N) depleted in
533 nitrogen and the non-refractory HS (low C:N), a N-rich organic matter. Non-refractory HS are
534 possibly generated during the surface degradation of marine phytoplankton (N-rich) and its
535 processing by marine bacteria (cellular lysis, microbial transformation). Culture experiments
536 showing the formation of humic-like compounds (identified by absorbance and fluorescence)
537 during phytoplankton degradation by bacteria (Osburn et al., 2019) support this pathway of
538 production. The increase of the C:N elemental ratio with depth indicate a non-redfieldian
539 mineralization of HS and suggests a preferential remineralization of organic nitrogen
540 compared to organic carbon.

541 In the BP fraction, C:N ratios (Figure 4b) varied between 4.5 (at 635 m) to 40.1 (at 4198 m).
542 In the upper 1000 meters depth, the C:N ratios of this fraction fall into the range reported for
543 ultrafiltered DOM (HMW DOM; 15-18; Hessen & Tranvik, 2013) which is the definition of
544 BP. In this fraction no organic nitrogen signal can be detected between 1899 m and 3400 m,
545 indicating that refractory HMW compounds are totally nitrogen depleted in the deep Pacific
546 Ocean. BP are mainly composed of proteinaceous material in surface water and with only
547 30% of refractory matter. They can be considered freshly produced and very labile. Absence
548 of a nitrogen signal in the deep ocean evidences a total utilization of the nitrogen content in
549 the BP fraction during remineralization. Here again a preferential mineralization of nitrogen
550 compared to carbon is observed, suggesting that BP is an important nitrogen reservoir for
551 heterotrophic bacteria. Similar non-redfieldian stoichiometry during DOM mineralization has
552 already been proposed and modeled (Aminot & K erouel, 2004; Letscher & Moore, 2015;
553 Osterholz et al., 2021). Our results confirm the preferential mineralization of nitrogen

554 compared to carbon and suggest that the lability of organic compounds may be tightly linked
555 to their nitrogen content.

556 **3.3 Fluorescent properties of DOM**

557 Fluorescence intensities of three different fluorophores (peaks T, B and M) were measured
558 along the water column. Measured values largely exceed LOD (see Figure S5), the intensities
559 and the vertical distributions we report are in agreement with existing data for marine waters
560 (Tedetti et al., 2011; Yamashita & Tanoue, 2004). Despite the flaws of fluorescence methods,
561 such as the quenching effect (Chen et al., 2013; Poulin et al., 2014; Yamashita & Jaffé, 2008)
562 and the ubiquity of some fluorophores (Wünsch et al., 2019), it can provide useful
563 information on the transformation of DOM in the environment. In this section, we focus on
564 the vertical distributions of fluorescent indices during the TONGA expedition. Three
565 fluorescent indices (FI, BIX and HIX) were measured along the water column to further
566 characterize DOM (Figure 5). These indices were determined based on the works of
567 McKnight et al. (2001), Huguet et al. (2009) and Zsolnay et al. (1999) respectively. FI is
568 commonly used to track the DOM sources, in particular to distinguish microbially-derived (FI
569 > 1.9) and terrestrially-derived DOM (FI < 1.4) in marine waters. Along the water column FI
570 ranged between 0.2 (5461 m) and 7.0 (370 m). At 25 m and close to the bottom, values were
571 below 1.4, indicating the existence of a DOM with a FI comparable to terrestrial material. A
572 low FI close to the sediments may be conceivable however it is unusual in surface waters far
573 away from terrestrial influence and without terrestrial eolian inputs (monitored using air mass
574 backward trajectories from the ensemble Hysplit model, ready.noaa.gov/HYSPLIT.php). In
575 absence of a clear terrestrial influence we relate the low surface FI value to photobleaching.
576 Photobleaching of FI is not systematic for freshwater DOM (Helms et al., 2014) and, to our
577 knowledge, has not been reported in marine environment. If proven to be true, the
578 photobleaching of FI in marine waters have implications for the use of the low FI as a proxy
579 of terrestrial DOM. A low FI in marine environment should not be systematically assimilated
580 to a terrestrial influence, especially in environment receiving high solar irradiation (e.g.
581 subtropical domains). Below the euphotic layer, the FI sharply increased in the mesopelagic
582 waters to reach 7.0 ± 0.4 and remained high down to 1000 meters depth. These high FI values
583 suggest the production of microbially-derived DOM (Burdige et al., 2004; Coble 1996) in this
584 layer (100-1000 m). This increase of FI could be related to the mineralization of a sinking
585 biomass, produced in the surface, as well as to a microbial transformation of the labile DOM
586 derived from biomass degradation. The lower values of the FI in the deep sea (Figure 5a)

587 suggest little to no production of microbial DOM below 1089 m (with the exception of FI =
 588 2.9 ± 0.1 at 3400 m).

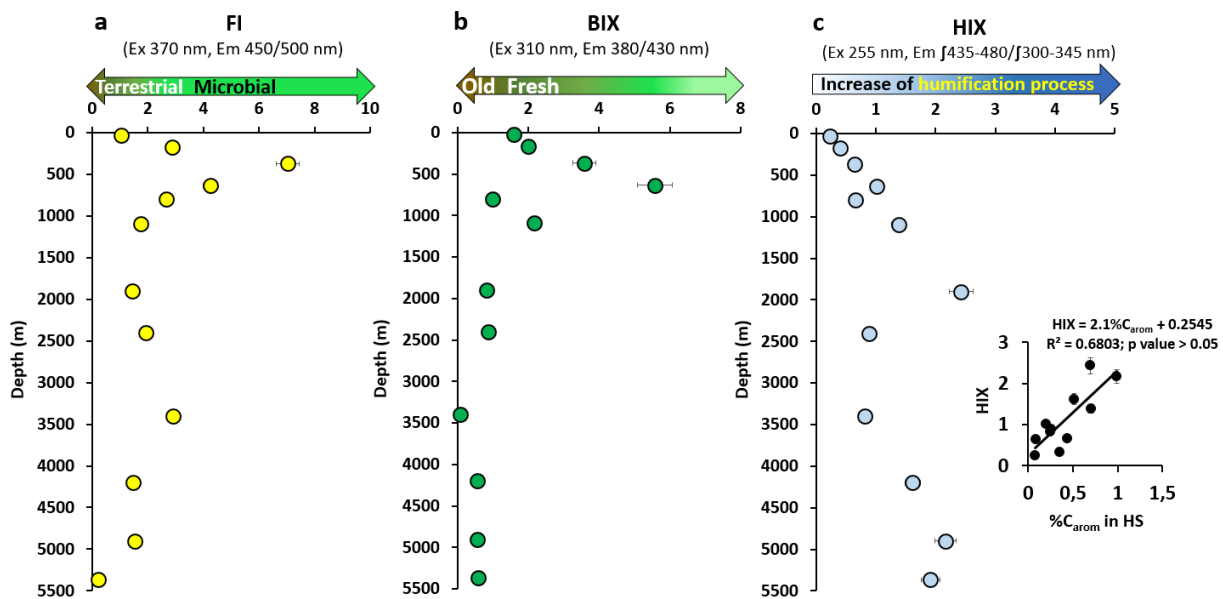


Figure 5. Vertical profiles of (a) fluorescence index (FI); (b) biological index (BIX); (c) humification index (HIX) at 20° 24.431'S, 166° 35.675'W during the TONGA expedition (station 8). Nomenclature of index follows those defined by McKnight et al., (2001), Huguet et al., (2009) and Zsolnay et al., (1999) respectively. Insert in (c) shows the significant correlation (p value = 0.002) recorded between the HIX and the percentage of aromatic carbon in the SEC HS fraction (Figure 4a).

589 The distribution of BIX (Figure 5b) corroborates FI results and provides an estimate of the
 590 freshness of DOM compounds. A BIX higher than 1 is representative of relatively young
 591 DOM, produced *in situ*, while a BIX below 0.6 indicates the occurrence of older DOM that
 592 may suffer from long term processing (e.g. biodegradation, photodegradation, Huguet et al.,
 593 2009). The BIX values measured ($0.02 \leq \text{BIX} \leq 5.57$) show that DOM can be considered
 594 relatively young from the surface down to 1089 m, confirming the production of DOM in this
 595 zone. Abyssal waters had $\text{BIX} < 1$ implying the presence of old DOM in the deep sea, in
 596 agreement with the high proportion of RDOM (Figure 3b). Combining FI and BIX results as
 597 well as the distribution of non-RDOM allowed us to better characterize the dynamics of DOM
 598 processing in the mesopelagic waters. In the upper 600 meters, the degradation of sinking
 599 biomass by heterotrophic bacteria produced young HS and BP that were non-refractory and
 600 N-rich (Figure 3b). These new compounds represented a substantial percentage of DOM.
 601 Deeper, BB and LMW acids were newly produced (increase of non-refractory percentage) as
 602 a result of the degradation of the non-refractory BP and HS (Figure 3b). This production of
 603 BB and LMW acids was observed in the zone (~ 300-700 m) where FI and BIX were
 604 maximum. These observations suggest that BB and LMW acids could be proxies of microbial
 605 mineralization products.

606

607 HIX is another indirect proxy of the chemical properties of DOM, highlighting the degree of
608 humification of organic matter in aquatic environments (Miranda et al., 2018; Zsolnay, 2003).
609 HIX ranged from 0.24 at 25 m to 2.4 at 1899 m depth (Figure 3c). HIX increased from the
610 shallow waters (0.24 ± 0.02) to the deep sea ($\text{HIX} > 1$) with the exception of the PDW
611 wherein relatively low HIX were measured ($\text{HIX} = 0.88 \pm 0.02$). Throughout the water
612 column, HIX were on average much lower than values measured for vascular plants (1.15 –
613 4.33; Ohno et al., 2007), soils (> 8 ; Kalbitz et al., 2003), terrestrial aquatic humics from the
614 IHSS (20 – 50; Birdwell & Engel, 2010) and estuarine and coastal DOM samples ($\sim 4 - 16$;
615 Huguet et al., 2009). HIX measured in this work were in agreement with those of Chen et al.
616 (2017) in the Arctic sector of the Pacific Ocean and we propose to define that a HIX below \sim
617 2.5 indicates a DOM of primarily marine origin. The very low HIX at 25 m depth (Figure 5c)
618 is in agreement with previous observations in the oligotrophic waters of the Indian Ocean (0.9
619 ± 0.4 ; Tedetti et al., 2011) and in the Mediterranean Sea (0.90 ± 0.35 at 5 m depth; Para et al.,
620 2010). Low HIX in the surface can be related to the phenomenon of photobleaching. Solar
621 irradiance is known to strongly decrease HIX of DOM in freshwater samples (Helms et al.,
622 2014; Para et al., 2010) and a similar process was suggested for Mediterranean sea surface
623 samples (Para et al., 2010). The longterm exposition of marine DOM to solar irradiance and
624 subsequent fluorescence extinction is the most likely process explaining the low HIX we
625 measured in the oligotrophic Pacific surface ocean. The increasing values of HIX with depth
626 across the mesopelagic layer (Figure 5c) suggest a humification of DOM. We relate it to the
627 transformation of fresh organic matter into an oxidized fluorescent DOM by the microbial
628 loop. A similar process was previously invoked to explain the increase of the humic-like
629 fluorescence of DOM in this oceanic basin (Yamashita & Tanoue, 2008; 2009; Yamashita et
630 al., 2010). According to Paerl et al. (2020), the production of humic-like fluorescent
631 compounds can also be related to a dimerization of tyrosine catalyzed by peroxidase enzymes
632 on a short timescale (\sim hours). During microbial degradation of sinking biomass, this amino-
633 acid may be released and dimerized by ambient microbial peroxidase therefore increasing
634 humic-like fluorescence in the mesopelagic waters. HIX often correlates with the aromaticity
635 of organic matter, as observed in estuarine (Huguet et al., 2009) and soil (Segnini et al., 2010)
636 samples as well as for humic reference materials (Ateia et al., 2017). The low HIX values we
637 determined indicate that DOM was poorly aromatic (Birdwell & Engel, 2010) in agreement
638 with the low aromatic carbon content we measured in the different fractions (Figure 4a).
639 Similar to previous works, we observed a significant correlation ($R^2 = 0.6803$; p value < 0.05)

640 between HIX and %C_{arom} in the HS fraction (insert in Figure 5c). This result demonstrates
641 that the HS fraction operationally defined by SEC is well representative of the humic material.
642 Such simultaneous increase of HIX and aromaticity has been observed during microbial
643 degradation experiments of soil organic matter (Kalbitz et al., 2003). Moreover, there is recent
644 evidence that HIX increases during the microbial degradation of phytoplankton biomass
645 (Kinsey et al., 2018), indicating that microbial processing also controls the humification of
646 DOM in marine systems. Low HIX in the older PDW are consistent with the low aromatic
647 carbon content of HS (Figure 5a) further suggesting an alteration of aromaticity with ageing
648 of refractory humic matter. The increase of HIX in the deepest samples (Figure 5c) is likely
649 representative of the mixing between watermasses formed in different areas with DOM
650 content of different ages and thus of different degrees of decomposition. Finally, the
651 concomitant increase of humic-like fluorescence (e.g. peak M, Figure S5 and HIX Figure 5c)
652 and decrease of humic-like carbon evidence a gain of fluorescence quantum yield by the
653 humic material during its microbial processing in the mesopelagic waters. Fluorescence
654 spectroscopy is thereby useful to identify the humification process but not to quantify the
655 fraction of DOC related to humic matter in marine environments.

656
657 Our results show that fluorescence data are usable proxies for the study of DOM
658 bioavailability when associated to quantitative DOM data ([DOC] and %C_{arom} in the isolated
659 fractions). In this work, the use of this combination on field samples confirms experimental
660 observations during heterotrophic bacteria incubations (Zheng et al., 2019). Microbial ageing
661 of a relatively labile DOM, originating from oligotrophic phytoplankton, induces the
662 consumption of a low aromatic DOM with a low humic-like fluorescent signature and its
663 transformation to RDOM displaying a high humic-like fluorescence signature and enriched in
664 aromatic carbon.

665 **3.4 Iron binding properties of oceanic humic substances**

666 We further characterized the chemical properties of HS in the WTSP by determining their
667 electroactivity and thereby their complexing capacity for dFe. The concentrations of eHS
668 ranged from 23.2 to 160.2 µg-eqSRFA L⁻¹ (Figure 6a).

669 These concentrations are in agreement with those reported in other marine environments
670 including the Pacific Ocean (Cabanes et al., 2020; Dulaquais et al., 2018a; Laglera et al.,
671 2019; Whitby et al., 2020a). The vertical distribution of eHS displays the typical profile
672 reported in these previous studies, with a surface enrichment and low concentrations in the
673 deep sea. The surface enrichment can be related to a production of eHS during bacterial

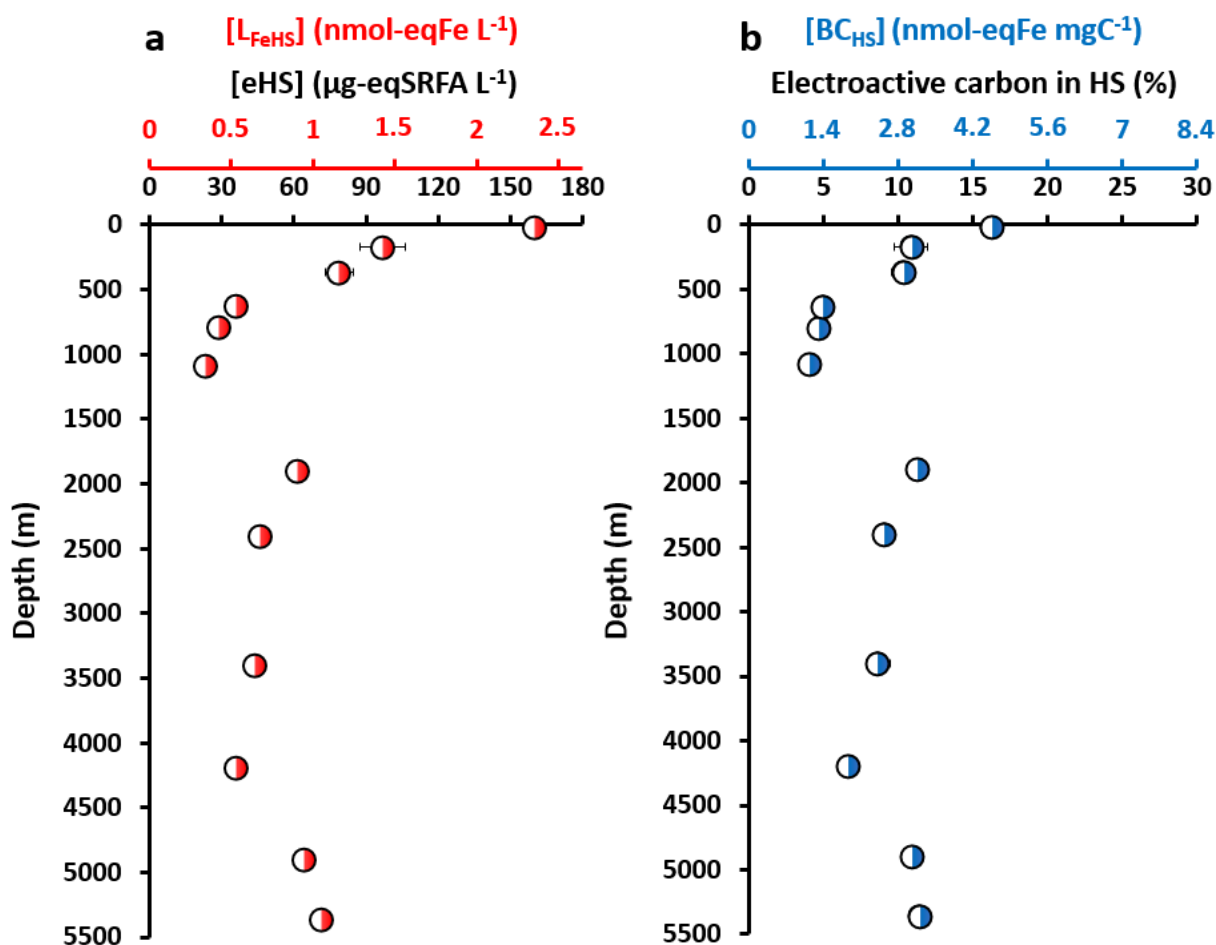


Figure 6. Vertical distributions of the concentrations of (a) electroactive humic substances (eHS, expressed in $\mu\text{g-eqSRFA L}^{-1}$) and humic-type ligands (L_{FeHS}) expressed in nmol-eqFe L^{-1} (calculated following Dulaquais et al., 2018b and Sukekava et al., 2018); (b) the percentage of electroactive carbon in the HS fraction along with the binding capacity of humic carbon for iron (BC_{HS} expressed in $\text{nmol-eqFe mgC}^{-1}$) at $20^{\circ} 24.431'S$, $166^{\circ} 35.675'W$ at the TONGA station.

674 degradation of the biomass as previously demonstrated by Whitby et al. (2020a). High surface
 675 eHS concentrations and their decrease with depth in the mesopelagic layer suggest that the
 676 electroactivity of humic matter is a property affected by all the biogeochemical processes
 677 governing the distribution of HS (e.g. surface production, bacterial degradation). The deep sea
 678 concentrations of eHS were quite homogeneous ($50 \pm 12 \mu\text{g-eqSRFA L}^{-1}$) indicating a partial
 679 recalcitrance of the electroactivity to long term degradation. Conversion of eHS (in $\mu\text{g-}$
 680 eqSRFA L^{-1}) into Fe-binding ligands of humic type (L_{FeHS}) can be estimated using the binding
 681 capacity of the model humic-type ligand SRFA (1S101F) for dFe in seawater (14.6 ± 0.7
 682 $\text{nmolFe mgSRFA}^{-1}$; Sukekava et al., 2018). A similar conversion is commonly used in the
 683 recent literature (Dulaquais et al., 2018a, 2020; Laglera et al., 2019; Whitby et al., 2020a). At
 684 this station we estimated L_{FeHS} values of between 0.3 and $2.4 \text{ nmol-eqFe L}^{-1}$. This interval fell
 685 into the range of Fe-binding ligand (L_{Fe}) concentrations previously measured in the south

686 oligotrophic Pacific Ocean ($0 - 4 \text{ nmol-eqFe L}^{-1}$; Buck et al., 2018; Cabanes et al., 2020).
687 This result demonstrates that part of L_{Fe} were of humic nature.
688 In the oligotrophic Pacific Ocean, dFe is extremely depleted in surface waters ($\leq 0.1 \text{ nM}$,
689 Buck et al., 2018; Cabanes et al., 2020; Guieu et al., 2018). The implication of L_{FeHS} in the
690 stabilization of surface dFe can be considered limited due to the possible occurrence of very
691 strong iron binding ligands in this layer (e.g. siderophores, Boiteau et al., 2016). In contrast,
692 in the deep ocean ($> 1000 \text{ m}$) dFe exists at higher concentrations ($> 0.4 \text{ nM}$). At these depths,
693 intermediate iron chelators dominate (called L_2 in Buck et al., 2018) and humic-type ligands
694 are thought to be a major component of this class of ligand (Bundy et al., 2015), therefore the
695 role of humics in the stabilization of dFe is probably enhanced in the deep Pacific Ocean. To
696 monitor the modifications of the binding properties of HS along with biogeochemical
697 processes we determined the contribution of eHS to the HS fraction defined by SEC and
698 estimated the binding capacity for Fe of humic DOC (BC_{HS} , Figure 6b). This calculation
699 considers the carbon content of the SRFA standard used (52.44%) to normalize the
700 concentrations expressed in $\mu\text{g eq-SRFA L}^{-1}$ into $\mu\text{gC L}^{-1}$ and assumes that all eHS elute in
701 the HS fraction operationally defined by SEC (Riso et al., 2021). At this station the average
702 contribution of eHS to the total HS carbon was $\sim 9.1 \pm 0.6 \%$ (Figure 6b). This contribution
703 varied along the water column with a maximum at 25 m (16.2%) and a minimum at 1089 m
704 (4.1%). The non-constant contribution of eHS to HS DOC implies a non-conservativity of the
705 binding properties of HS during their biogeochemical processing.
706 The BC_{HS} were systematically below those of the SRFA standard ($BC_{\text{SRFA}} = 27.8 \pm 1.3 \text{ nmol-}$
707 eqFe mgC^{-1}) indicating a lower BC of marine humic carbon compared to the terrigenous
708 humic matter. BC_{HS} in the shallowest sample ($4.5 \pm 0.1 \text{ nmol-eqFe mgC}^{-1}$) was significantly
709 higher than in the deep sea ($2.7 \pm 0.5 \text{ nmol-eqFe mgC}^{-1}$; $n = 6$). A large part of HS in the
710 upper 100 m depths was non-refractory (50%; Figure 3b), thereby we deduce that the binding
711 capacity for dFe of non-refractory HS is higher than that of refractory HS. The decrease of the
712 BC_{HS} from surface to 1089 m and the strong decrease of the contribution of eHS to humic
713 DOC in the mesopelagic waters (Figure 6b) indicate an alteration of the Fe-binding properties
714 during the microbial mineralization of HS DOC. Statistical analysis of the data did not reveal
715 significant correlations between BC_{HS} , $\%C_{\text{arom}}$ nor the C:N elemental ratio of HS. This result
716 suggests that Fe-binding moieties of marine HS are aliphatic and that the N-containing
717 functional groups may not be involved in the formation of the Fe-humic complex in the
718 marine environment. Based on these results, we suspect carboxylates to be the main chemical
719 functional group involved in the Fe-binding properties of oceanic HS. Carboxylates are of

720 intermediate strength (Bundy et al., 2015) compared to N-containing (strong, Gledhill &
721 Buck, 2012; Rue & Bruland, 1995) and aromatic/phenolic (weak, González et al., 2019)
722 moieties for Fe complexation. The correlation between Fe-binding HS and CRAM in
723 estuarine samples (Bundy et al., 2015) and the observations of humic type ligands falling into
724 the intermediate class of Fe chelators (Buck et al., 2018; Bundy et al., 2015) both support this
725 hypothesis. The decrease of BC_{HS} in the mesopelagic waters can be interpreted as a decrease
726 of the abundance of carboxylates with HS during microbial transformation. This latter
727 hypothesis is supported by the long-term experimental study (1 year) on the degradation of a
728 large size spectra of DOM (108-7410 Da) by marine bacteria from Daoud & Trembley
729 (2019). Their study showed a substantial decrease of carboxylate abundance in seawater
730 DOM that suffered from microbial degradation compared to the control sample. There is
731 currently no NMR data on oceanic HS samples to confirm this and the acquisition of such
732 data along a full water column in the future would allow for the testing of this hypothesis.

733 Our results and interpretations of the complexation of dFe by humic matter contrast with
734 terrestrial HS. For terrestrial HS, the binding capacity for dFe is thought to be linked to their
735 aromaticity (Kikuchi et al., 2017) as it is observed in the two most used humic type standards
736 SRFA ($\%C_{arom} = 24\%$; $BC_{SRFA\ 1S101F} = 14.6\ \text{nmolFe mgSRFA}^{-1}$; Sukekava et al., 2018) and
737 SRHA ($\%C_{arom} = 37\%$; $BC_{SRHA\ 1S101H} = 31\text{-}32\ \text{nmolFe mgSRHA}^{-1}$; Abualhaija & van den
738 Berg, 2014; Laglera & van den Berg, 2009). This difference further denotes the uniqueness of
739 marine HS compared to terrestrial humic matter, as previously discussed by Malcolm (1990)
740 in terms of chemical properties. We note that our results also contrast with Whitby et al.
741 (2020b) where it was hypothesised that the Fe-binding capacities increased with depth due to
742 higher HS aromaticity. In their study the higher Fe-binding capacities of HS in deep waters of
743 the North Atlantic could be a result of a high fraction of terrestrial HS, in part derived from
744 the subduction of humic-rich Arctic waters, which have been shown to have particularly high
745 Fe-HS stability constants (Laglera et al., 2019). Lignin, an unambiguous molecular tracer for
746 terrestrial DOC, is also higher in the Atlantic Ocean than in the Pacific Ocean (Hernes &
747 Benner, 2002, 2006; Opsahl & Benner 1997). This suggests that the contribution of HS from
748 terrestrial vs. marine sources could result in differences in Fe-complexing behaviour between
749 oceanic regions.

750 **4. Conclusions**

751 This work presents the opportunity provided by multi-detection SEC to study the size-
752 reactivity continuum and the chemical properties of oceanic DOC without any prior

753 extraction. This method has an excellent recovery of DOC and requires only a small sample
754 volume (2.5 mL).

755 We used a combination of three semi-specific methods (SEC, fluorescence, CSV) to study
756 DOM chemical modifications induced by biogeochemical processes along the water column
757 of the oligotrophic Pacific Ocean. We identified N-contents of the BP and HS fractions as
758 pools of bioavailable nitrogen. Our results also suggest the condensation, the decrease in N-
759 content, the increase of quantum yield of fluorescence and a loss of complexing capacity for
760 dFe of HS during microbial degradation of DOM. Marine HS seem to be produced in surface
761 waters during biomass degradation. HS are initially non-refractory, nevertheless their ageing
762 induces their transformation into refractory DOM. This process was identified to occur in two
763 steps. First the microbial transformation in the mesopelagic waters increases their aromaticity
764 and their fluorescence, secondly, long term (> centuries) processing alters their aromatic
765 content making marine HS more aliphatic. All these changes in the chemical properties could
766 alter and gradually degrade the newly produced labile DOM into RDOM trapped in the deep
767 ocean. This work provides insights to reconcile the quantitative (loss of matter) and
768 qualitative (increase of fluorescence) views in humic oceanic biogeochemistry.

769 Results of this work and the conclusions drawn are consistent with studies that used molecular
770 approaches to characterize fate of DOM in field samples (Osterholz et al., 2021) and in
771 bacterial incubation experiments (Lian et al., 2021; Zheng et al., 2019). A larger scale study
772 and the use of a molecular approach to each DOM fraction isolated in this work would
773 confirm the main hypotheses.

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796

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1221 **Supplementary material**

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1223 **Table S1.** Limits of detection (LOD) for the different detectors coupled to SEC in a seawater matrix
 1224 (Bay of Brest, S = 35 psu). Injection volumes were 2 mL. LOD were calculated according to the
 1225 IUPAC recommendation (1978): $LOD = 3 \times SE$ where « SE » is the mean standard error (or standard
 1226 error) of the blanks over 10 consecutive measurements of a coastal water of the Bay of Brest,
 1227 previously irradiated (3h) with UV light (Dulaquais et al., 2018b). The expected contents in each
 1228 fraction operationally defined by SEC are those proposed by Huber et al. (2011) and in this study.

Organic carbon				(μMC)		
Fraction	DOC	BP	HS	BB	LMW acids	LMW neutrals
Expected content	Huber et al. (2011)	Proteinic matter Polysaccharides	Humic matter including	HS-like material of LMW	Anions at the pH of the buffer (~ 7)	Hydrophilic/amphiphilic compounds of low ion density
	This study		Fulvic and humic acids	Degradation by-products of BP		
LOD	2.3	0.4	0.2	0.1	1.0	3.0
Organic nitrogen (μMN)				Aromatic carbon (%)		
Fraction		BP	HS	BP		HS
LOD		0.2	0.2	0.6		0.1

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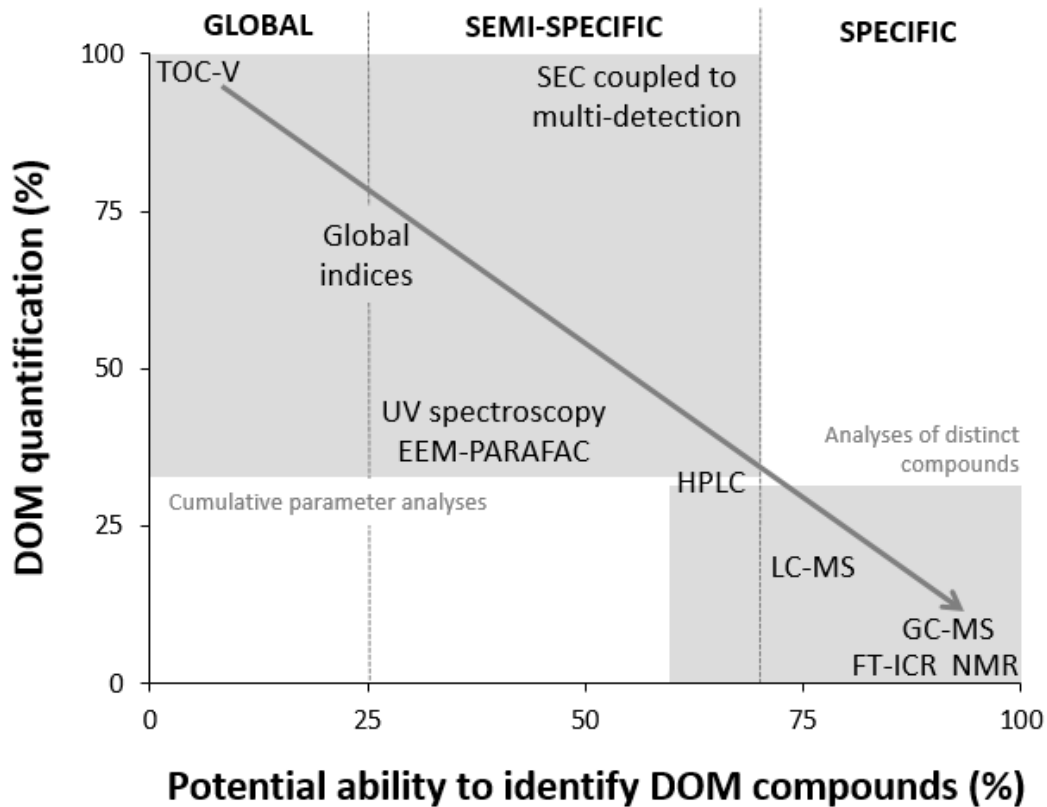
1234 **Table S2.** Analyses performed on DOM for each different cruise presented in this study. TOC-V
 1235 analyses during CLIVAR (*) were carried out by Swan et al. (2009).

Cruise – Station – Coordinates	Analytical method			
	SEC	TOC-V	Fluorescence	CSV (eHs)
TONGA – St. 8 – 20° 24.431'S, 166° 35.675'W	✓	✓	✓	✓
US-GP15 – St. 39 – 19° 59.99'S, 152° 00.01'W	✓			
CLIVAR P16 – St.9 – 20°S; 150°W		✓*		

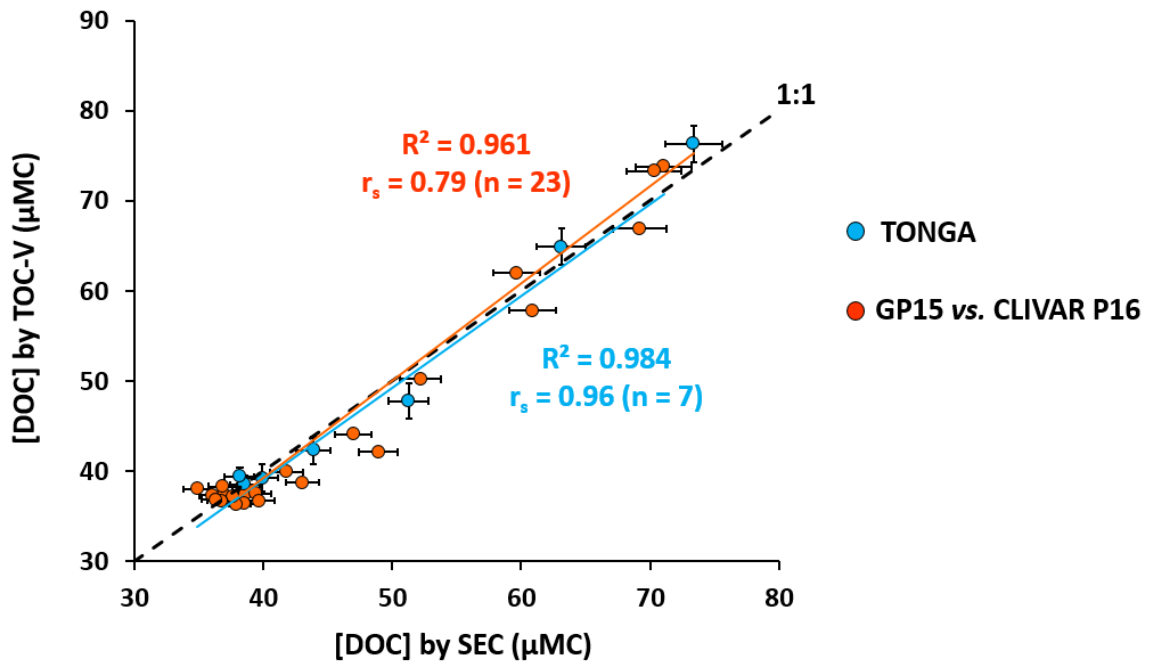
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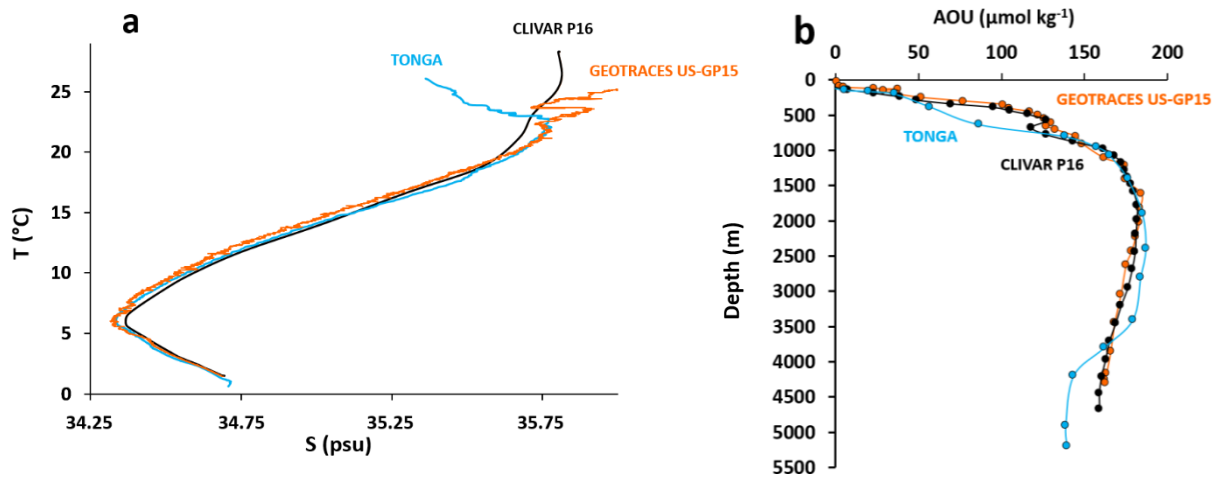


1239 **Figure S1.** Analytical window of the different approaches applied to DOM analysis. TOC = Total
 1240 Organic Carbon; SEC: Size Exclusion Chromatography; EEM-PARAFAC: Excitation Emission
 1241 fluorescence Matrice-Parallel Factor Analysis; HPLC: High Performance Liquid Chromatography;
 1242 LC-MS: Liquid Chromatography-Mass Spectrometry; GC-MS: Gas Chromatography-Mass
 1243 Spectrometry; FT-ICR: Fourier Transform Ion Cyclotron Resonance; NMR: Nuclear Magnetic
 1244 Resonance. Figure based on http://doc-labor.de/?page_id=13.

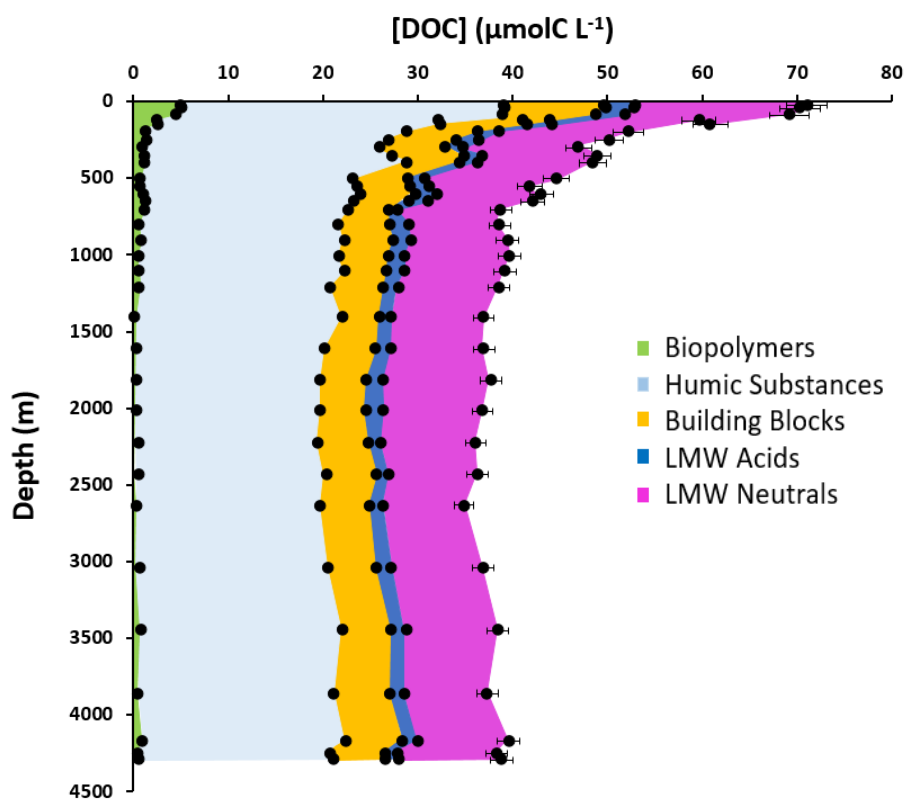


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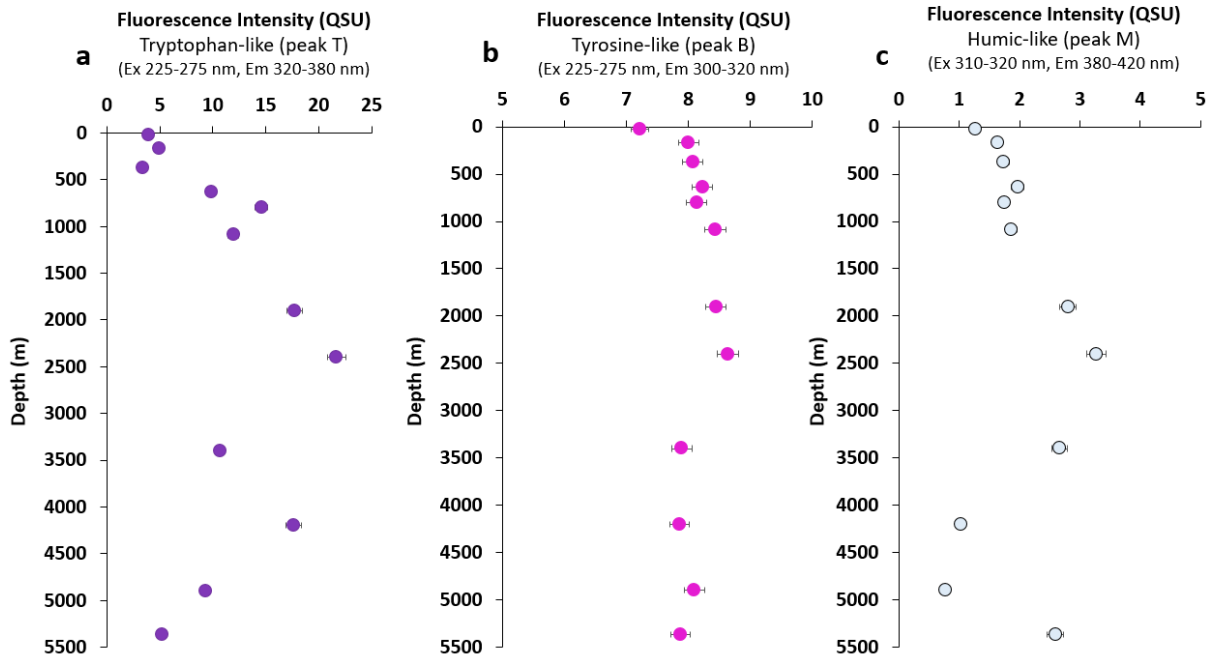
Figure S2. Comparisons between the datasets from GP15 St. 39 ($19^\circ 59.99'S$, $152^\circ 00.01'W$) by SEC and historical data from CLIVAR P16 St. 9 ($20^\circ S$; $150^\circ W$) by TOC-V, as well as the two TONGA datasets (SEC and TOC-V) at St. 8 ($20^\circ 24.431'S$, $166^\circ 35.675'W$). Spearman coefficient r_s close to 1 in both cases indicates the strong correlation between DOC concentrations determined by TOC-V and SEC.



1251 **Figure S3. (a)** Intercomparison of the temperature/salinity AOU diagram between TONGA St. 8 (20°
 1252 24.431'S, 166° 35.675'W), GEOTRACES US-GP15 St. 39 (19° 59.99'S, 152° 00.01'W) and
 1253 CLIVAR P16 St. 9 (20°S; 150°W). **(b)** Intercomparison between vertical profiles of apparent oxygen
 1254 utilization (AOU) for TONGA, GEOTRACES and CLIVAR P16 (same stations).



1255 **Figure S4.** Vertical profiles of the DOC concentrations (μMC) in the five operational fractions
 1256 defined by SEC, along the water column at $19^\circ 59.99'S$, $152^\circ 00.01'W$ during the GEOTRACES US-
 1257 GP15 expedition. Non-visible error bars are covered by the symbols.



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Figure S5. Vertical profiles of (a) tryptophan-like fluorescence intensity (T); (b) tyrosine-like fluorescence intensity (B); (c) marine humic-like fluorescence intensity (M); according to the nomenclature defined by Coble (1996).