

# Ageing and molecular changes of dissolved organic matter between two deep oceanic end members

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## Key points:

- Solid-phase extracted DOM (SPE-DOM) from deep waters in the far North Pacific has a <sup>14</sup>C age of ~6400 years
- Deep Pacific SPE-DOM has an intrinsically stable component.
- Specific molecular formulae are largely preserved in SPE-DOM as it undergoes meridional overturning circulation.

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## Abstract

The global ocean contains a massive reservoir of dissolved organic carbon (DOC), rivaling the atmosphere's pool of CO<sub>2</sub>. The most recalcitrant fractions have mean radiocarbon ages of ~4,000 years in the Atlantic to ~6,000 years in the Pacific. Knowing the radiocarbon signatures of DOC and the molecular composition of dissolved organic matter (DOM) is crucial to develop understanding of the persistence and lifetime of the DOC pool. In this research, we collected samples from the deep North Pacific in August 2013 (aboard the RV *Melville*), to couple the  $\Delta^{14}\text{C}$  content of solid-phase-extracted DOM ( $\Delta^{14}\text{C-SPE-DOM}$ ) with its molecular composition in the ocean's oldest deep waters. We find that deep waters in this region held a mean  $\Delta^{14}\text{C-SPE-DOM}$  value of  $-554 \pm 9 \text{ ‰}$  (~6,400 <sup>14</sup>C years), substantially more depleted than that in the deep Atlantic, which held a mean  $\Delta^{14}\text{C-SPE-DOM}$  value of  $-445 \pm 5 \text{ ‰}$ . While we find a more degraded molecular composition of DOM in the deep Pacific than the deep Atlantic, the molecular formulae within the Island of Stability (Lechtenfeld et al., 2014), are largely retained. These results imply that a fraction of deep DOM is resistant to removal and present in both the deep Atlantic and Pacific Oceans.

## 1. Introduction

A major fraction of dissolved organic matter (DOM) is produced in the surface ocean via primary production where it is either directly released into the water column or solubilized via heterotrophic consumption of particulate organic material (Carlson and Hansell, 2015). Although algal-derived DOM is produced in the euphotic zone, >95% of DOM is contained in the dark ocean, where it survives multiple meridional overturning circulations (Druffel et al., 1992). However, the turnover time and overall biogeochemical fate of this large, deep pool are largely unknown. In this analysis, we investigate the cycling of the deep pool of marine DOM using isotopic and compositional signatures.

The concentration of dissolved organic carbon (DOC; the carbon component of DOM) varies across the deep ocean basins. DOC concentrations are highest at depth ( $48 \mu\text{mol kg}^{-1}$ ) in the North Atlantic near the formation of North Atlantic Deep Water, due to the surface subtropical ocean source of those waters (Hansell and Carlson, 1998; Hansell et al., 2009). Deep waters reach  $\sim 40 \mu\text{mol kg}^{-1}$  in the Southern and Indian Oceans (Ogawa et al., 1999; Bercovici and Hansell, 2016), and  $\sim 34\text{-}37 \mu\text{mol kg}^{-1}$  in the deep Pacific (Hansell et al., 2009; Hansell, 2013).

While early studies suggest that the deep DOC concentration gradient resulted from slow removal over time (Hansell and Carlson, 1998), conserved mixing and localized sinks are now considered to play a role in establishing this gradient as well (Hansell et al., 2009; Hansell, 2013; Bercovici and Hansell, 2016; Lang et al., 2006, Hawkes et al., 2016). The difference in  $^{14}\text{C}$  age of DOM between the deep North Atlantic and the deep North Pacific is  $\sim 2000$  years (Druffel et al., 1992). Apart from transit time and related radiocarbon decay along the deep ocean conveyor belt (Bercovici et al., 2018), an additional aspect that would contribute to this difference in  $^{14}\text{C}$  age is the preferential removal of modern components (Williams and Druffel, 1987; Druffel et al., 1992; Beaupré and Aluwihare, 2010; Hansell et al., 2012, Lechtenfeld et al. 2014). The DOM pool is dynamic, consisting of multiple molecular components cycling on different timescales (Loh et al., 2004; Repeta and Aluwihare, 2006; Walker et al., 2011; Follett et al., 2014; Zigah et al., 2017). Observations suggest that small, dissolved molecules are the most highly aged components of DOM and have much older radiocarbon ages than the larger components of the pool (Loh et al., 2004; Walker et al., 2011; Walker et al., 2016; Broek et al., 2017). A recent study by Zigah et al. (2017) shows that components of DOM separated by size and polarity have varied isotopic signatures, ranging from modern to ancient ( $>10,000$  years).

Recently, the analytical capabilities of Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) allow for advances in our understanding of the relationship between molecular composition of solid-phase extracted DOM (SPE-DOM) and its  $\Delta^{14}\text{C}$  content ( $\Delta^{14}\text{C}$ -SPE-DOM). Two prior studies spanning the Atlantic Ocean provided a comprehensive dataset that coupled radiocarbon and molecular composition (Flerus et al., 2012; Lechtenfeld et al., 2014). These studies isolated the specific formulae present in each FT-ICR-MS spectrum that had a significant positive or negative correlation with  $\Delta^{14}\text{C}$ -SPE-DOM ( $p < 0.05$ ,  $r > 0.65$ ); these formulae represent ~61% of the summed magnitudes of all peaks in the spectrum (Flerus et al., 2012). Lechtenfeld et al. (2014) extended this analysis via a first order degradation between radiocarbon age and individual molecular formulae. They suggested a portion of DOM molecules have a much longer residence time in the water column than the age of the bulk DOC pool would otherwise indicate. Using those molecular formulae that hold longer residence times within the whole SPE-DOM pool, Lechtenfeld et al. identified ~361 distinct molecular formulae that were persistent in virtually all samples ('island of stability'; IOS), representing the most stable fraction in DOM.

In this study, we couple molecular composition of SPE-DOM with its  $\Delta^{14}\text{C}$  values to determine the intrinsic stability of SPE-DOM in the far North Pacific, where deep bulk DOC concentrations and SPE- $\Delta^{14}\text{C}$ -DOM values are at their lowest. In addition, we compare the molecular composition of deep SPE-DOM from two distinctly different ocean basins: in the North Atlantic, deep waters have recently been formed and ventilated; in the North Pacific, deep waters have been out of contact with the atmosphere for centuries. This comparison of the DOM between these two end-members provides a deeper understanding of the intrinsic stability of deep DOM on a global scale.

## 2. Methods:

Samples were collected on a cruise aboard the *R.V. Melville* (MV-1310) in August, 2013 (Fig. 1). In the following subsections, we describe the methods for each variable discussed in this manuscript.

### 2.1. Total organic carbon (TOC) measurements

TOC is comprised of suspended particulate organic carbon (POC) and DOC. DOC is operationally defined as passing through a pore size of 0.2 to 0.7  $\mu\text{m}$ . Here, TOC samples were collected directly from Niskin bottles into acid-cleaned (10% HCl) 60 mL polycarbonate bottles. TOC was measured by high temperature combustion using a Shimadzu TOC-L with auto-injection (precision of  $\pm 1.5 \mu\text{mol kg}^{-1}$ ) (Dickson et al., 2007).

Measurements were quality controlled using Consensus Reference Material (CRM), distributed to the international oceanographic community by the Hansell laboratory. Low C and N reference water was employed to determine system blanks. Hereafter, TOC samples are referred to as DOC, as POC accounts for a minimal portion of TOC (Hansell and Carlson, 2001; further information is described in Section 3.2).

### 2.2. Sampling for Solid Phase Extraction (SPE)

Water samples for SPE were collected with a CTD water rosette sampler at 5 m, 50 m, 1000 m, 2000 m, 3000 m, and a few meters above the seafloor. First, 8 L of each sample were filtered through an 0.2  $\mu\text{m}$  Supor filter and acidified to pH 2 with high purity hydrochloric acid. Mega Bond Elut PPL (1g, Varian) cartridges were used for SPE following the protocol of Dittmar et al (2008). Following the extraction, the cartridges were rinsed with two cartridge-full volumes ( $\sim 8 \text{ mL}$ ) of acidified milliQ (pH 2) and subsequently dried with ultra-pure nitrogen gas. The SPE samples (SPE-DOM) were eluted in 8 mL of methanol and

stored at -20°C until analysis.

To measure the amount of DOC recovered, a 150 µL aliquote of the SPE eluate ( $V_{EL}$ ) was added to a combusted glass vial, dried completely with ultra-pure nitrogen gas, left to dry overnight, and resuspended in 15 mL of milliQ ( $V_{mQ}$ ). The resultant sample was then measured for DOC following the method described in Section 2.1. Extraction efficiencies (EE) for DOC were calculated using following formula:

$$EE = \left( \frac{DOC_{Ex}(\mu M)}{DOC_{orig}(\mu M)} \right) * \frac{V_{mQ}(mL)}{V_{EL}(mL)} * \frac{V_E(mL)}{V_S(mL)} * 100\% \quad (1)$$

where  $DOC_{Ex}$  is the DOC concentration of the extract,  $DOC_{orig}$  is the DOC concentration of the original water sample,  $V_{mQ}$  is the volume of milliQ water added to the dried eluate,  $V_E$  is the volume of the total eluate,  $V_{EL}$  is the volume of the eluate used for analysis, and  $V_S$  is the total sample volume that was enriched using solid-phase extraction.

### 2.3. Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS)

SPE-DOM samples were analyzed in the Analytical Biogeochemistry Laboratory at the Helmholtz Zentrum München (Munich, Germany) using a FT-ICR-MS (SolariX, Bruker Daltonics, Billerica, MA), equipped with a 12 T superconducting magnet (MagneX Scientific Inc., Yarnton, GB). An Apollo II dual electrospray source (ESI, Bruker) was used in negative ion mode (capillary voltage: -4200 V, infusion flow rate: 2 µL min<sup>-1</sup>). Prior to measurement on the FT-ICR MS, extracts were diluted with methanol to obtain the same SPE-DOM concentration for all aliquots. High resolution mass spectra were acquired for the mass range of 147 to 2000  $m/z$  with 512 scans per spectrum. Spectra were externally calibrated with arginine clusters, which are charged solute ions with established  $m/z$  values (Meng & Fenn, 1991).

Ions were singly charged and spectra were evaluated in the range of 200 to 600  $m/z$  according to prior studies (Flerus et al., 2012, Lechtenfeld et al., 2014). Molecular formulae

were calculated from the  $m/z$  values with the same algorithm as described in Lechtenfeld et al. (2014). Relative peak magnitudes were normalized by dividing the mass peak magnitude by the summed magnitude of all mass peaks in that sample. Only mass peaks having a normalized magnitude  $>0.005\%$  were considered in this analysis. An initial quality control was conducted to remove surfactants. The source and quantity of these surfactants are largely unknown, but they are easily ionized and thus have been previously identified in marine FT-ICR-MS samples (Lechtenfeld et al., 2013). While the analytical methods in this study were similar to previous studies (Flerus et al., 2012; Lechtenfeld et al., 2014), a direct comparison between the two datasets might be affected by instrument conditions, as evidenced by a slight shift in the mass distribution of the spectra (Fig. 2). To compare the spectra from the Pacific and the Atlantic, we normalized the FT-ICR-MS data; the sum of the magnitudes of all molecular formulae in a given sample is set to equal 1, and the relative magnitude of each individual formula is described as a percentage of the total magnitude.

#### 2.4. $^{14}\text{C}$ measurements

Aliquots of the methanol extracts ( $\sim 0.15$  mL) were transferred to pre-combusted Pyrex tubes (6 mm diameter). The contents of each tube were transferred to 1 cm diameter quartz tubes, from which the solvent was removed by vacuum. Each sample was washed with 1 mL of ultrapure water (milliQ), dried and flame sealed under vacuum and combusted to  $\text{CO}_2$  at  $750^\circ\text{C}$  with a  $\text{CuO}/\text{Ag}$  catalyst (Sofer, 1980). The  $\text{CO}_2$  was subsequently reduced with  $\text{H}_2$  to graphite over a  $\text{Co}$  catalyst at the National Ocean Sciences Accelerator Mass Spectrometer (NOSAMS) facility at Woods Hole Oceanographic Institution (Woods Hole, MA). SPE DOC blanks did not yield any detectable amount of carbon ( $<1$  nM C). Radiocarbon ages of SPE-DOM were derived from their respective  $\Delta^{14}\text{C}$ -DOM values following Stuiver and Polach (1977).

### 3. Results and Discussion

#### 3.1. Extraction efficiency of SPE-DOM

The extraction efficiency of the SPE-DOM (equation 1) was  $41\pm 10\%$  and independent of depth and DOC concentration. The SPE extraction method for these Pacific samples was slightly different than that used in the Atlantic data employed here. In this study, we extracted 8 L of seawater through 1 g PPL cartridges, whereas previous studies extracted 5 L of seawater (Flerus et al., 2012; Lechtenfeld et al., 2014). However, the extraction efficiency in the previous studies ( $42\pm 7\%$ ) is effectively the same as here and within the same range ( $\sim 40\%$ ) of other published SPE extraction efficiencies of marine DOM (Dittmar et al., 2008, Coppola et al., 2015).

#### 3.2. Bulk DOC concentrations and $\Delta^{14}\text{C}$ -SPE-DOM values

Bulk DOC concentrations for samples in the far North Pacific ranged from 36 to 71  $\mu\text{mol kg}^{-1}$  (Fig. 3A). Concentrations were highest in the surface layer, varying from 51 to 71  $\mu\text{mol kg}^{-1}$  ( $59\pm 7 \mu\text{mol kg}^{-1}$ ) (depths  $\leq 50$  m,  $n=8$ ; Fig. 3A), as compared to subsurface concentrations (depths  $\geq 1000$  m,  $n=10$ ; Fig. 3A), with a much narrower range of 36 to 39  $\mu\text{mol kg}^{-1}$  ( $37 \pm 1 \mu\text{mol kg}^{-1}$ ; Fig. 3A). Although DOC data here are unfiltered (see Section 2.1), there is typically a minor contribution of POC to the total pool (1 to 4%; Hansell and Carlson, 2001). Given that the concentrations range from 51 to 71  $\mu\text{M C}$  in our surface samples, a 1-4% contribution of POC would imply an addition of  $\sim 0.5$ -3  $\mu\text{M C}$ , similar to the precision of the TOC measurement. Furthermore, at depths  $>1000$  m, where this study focuses, there is no resolvable difference between filtered and unfiltered DOC measurements (Hansell and Carlson, 2001).

In the Pacific dataset, the mean  $\Delta^{14}\text{C}$ -SPE-DOM values at the surface (defined here as depths  $<100$  m) were  $-370\pm 30\%$  ( $\sim 3700$   $^{14}\text{C}$  years). These data are more depleted in  $^{14}\text{C}$  than

those in the Atlantic ( $-293 \pm 34\%$ ;  $\sim 2800$   $^{14}\text{C}$  years; Flerus et al., 2012). Furthermore, the surface values reported here are more depleted in  $^{14}\text{C}$  than bulk  $\Delta^{14}\text{C}$ -DOC surface values, which ranged from  $-180$  to  $-260\%$  in the Atlantic, Pacific, and Indian ocean basins (Druffel et al., 1992, 2015, 2016; Follett et al., 2014; Walker et al., 2011, 2016; Zigah et al., 2017, Bercovici et al., 2018). However, our results are similar to those  $\Delta^{14}\text{C}$ -SPE-DOM values reported by Coppola et al (2016) collected in the East Pacific ( $-318 \pm 11\%$  and  $-335 \pm 7\%$ ).

The offset between the surface values of the  $\Delta^{14}\text{C}$ -SPE-DOM data from this Pacific dataset and others could be due to several factors. Published  $\Delta^{14}\text{C}$ -SPE-DOM data is scarce, and there is variability between experimental and instrumental procedures in between studies. In addition, it is impossible to entirely rule out contamination and methylation in the extraction procedure, however, the impact of methylation was kept to a minimum by cold storage of the extracts (Flerus et al. 2011). In addition, hydrography of the contrasting sampling regions is important to consider. The mean DOC concentration in the surface Atlantic data is  $67 \pm 8$   $\mu\text{mol kg}^{-1}$  (Flerus et al., 2012), whereas that in the surface Pacific was generally lower at  $59 \pm 7$   $\mu\text{mol kg}^{-1}$ . According to NASA Ocean Color, the surface chlorophyll in Nov. 2008 (when the Flerus et al. Atlantic samples were collected) reached up to  $10$   $\text{mg L}^{-1}$  near the African coast, whereas the surface chlorophyll in Aug. 2013 (when the Pacific samples here were collected) reached  $\sim 0.5$   $\text{mg L}^{-1}$ . The lower chlorophyll and the lower DOC concentrations between the two ocean basins indicate that there was likely lower primary productivity in our Pacific sites than in the Atlantic (Flerus et al., 2012).

At depth, the  $\Delta^{14}\text{C}$ -SPE-DOM values reported in the Pacific are  $-554 \pm 9\%$  ( $\sim 6400$   $^{14}\text{C}$  years), whereas those from the Atlantic dataset were  $445 \pm 5\%$  ( $\sim 4700$   $^{14}\text{C}$  years; Table 1; Fig. 3B; Flerus et al., 2012). This offset between the deep Atlantic and Pacific of  $\sim 1700$  years is similar to the  $\sim 2000$  year offset depicted in the first bulk radiocarbon profiles comparing the Atlantic and Pacific Oceans (Druffel et al., 1992). Furthermore, the  $\Delta^{14}\text{C}$ -SPE-DOM data

from the deep Pacific (Table S1) are similar to those  $\Delta^{14}\text{C}$  data of DOC taken from whole water in the deep Pacific (-501 to -564‰, Druffel et al., 1992, Druffel & Griffin, 2015, Walker et al., 2011, 2016, Zigah et al., 2017).

While our  $\Delta^{14}\text{C}$ -SPE-DOM data at depth are similar to the whole water in the Pacific, it is known that there is some variability between bulk  $\Delta^{14}\text{C}$ -DOC and  $\Delta^{14}\text{C}$ -SPE-DOM values. Coppola et al (2015) reported that  $\Delta^{14}\text{C}$ -SPE-DOM data from marine samples were equal to or lower than that of the  $\Delta^{14}\text{C}$  of the total DOC pool. A fraction of the most polar (and presumably labile) portion of the DOM is not recovered with extraction (e.g. molecules such as acetate). Furthermore, in the Bauer et al. (1992) comparison of bulk DOC versus SPE-DOM, DOM extracted on the XAD resin was older than the  $^{14}\text{C}$  age of the bulk DOM.

### 3.3. FT-ICR-MS Analysis

From the FT-ICR-MS spectra (Fig. 2A), a total of 2,726 molecular formulae were identified, with 2,154 of these formulae present in all samples. The magnitude average nominal mass between each sample, was  $421\pm 3$  Da, and the average O/C and H/C ratios were  $0.49\pm 0.13$  and  $1.25\pm 0.21$ , respectively (Table 1). These values are similar to the deep Atlantic, where the magnitude average nominal mass was  $418\pm 1$  Da (Flerus et al., 2012), and the average O/C and H/C ratios were  $0.48\pm 0.14$  and  $1.24\pm 0.22$ , respectively (Table 1). It is important to note that the assigned molecular formulae do not provide information on structural diversity, as different structural isomers can share the same molecular formula.

### 3.4. Extension of the IOS

The IOS is a subset of molecular formulae found in all samples in the deep Atlantic (IOS<sub>A</sub>) and deemed intrinsically stable due to their positive correlation with  $^{14}\text{C}$  age (Lechtenfeld et al., 2014). We found that virtually all of the formulae present in the IOS<sub>A</sub> in

the deep Atlantic were present in the deep Pacific (Fig. 4A and B, blue circles). In the deep Pacific spectra, the mean molecular weight of the IOS<sub>A</sub> was  $439\pm 79$  Da, similar to the average molecular weight of all molecular formulae ( $421\pm 88$  Da; Tables 1 and 2; Fig. 2). In addition, the O/C and H/C ratios for the IOS<sub>A</sub> were  $0.5\pm 0.1$  and  $1.2\pm 0.2$ , respectively, thus occupying a narrower O/C and H/C range than the total distribution of peaks (Table 2; Fig. 4A).

To assess whether the intrinsically stable molecular formulae in the deep Pacific are similar in character to those in the Atlantic, we used the same approach as in Lechtenfeld et al (2014) to calculate a distinct set of formulae for an IOS in the Pacific (termed here as IOS<sub>P</sub>). The IOS<sub>P</sub> formulae were isolated as those molecular formulae with high correlations ( $r > 0.65$ ) with observed gradients in  $\Delta^{14}\text{C-SPE-DOM}$  (termed here as IOS<sub>P</sub>). Examples of molecular formulae with high and low correlations with observed gradients in  $\Delta^{14}\text{C-SPE-DOM}$  are in the Supplementary Materials (Fig. S1). There were 217 molecular formulae in the IOS<sub>P</sub>, with a mean molecular weight of  $438\pm 53$  Da, an O/C ratio of  $0.48\pm 0.06$ , and an H/C ratio of  $1.23\pm 0.12$  (Table 2; Fig. 4A, red circles).

It is important to address our limitations in this analysis: each molecular formula within SPE-DOM represents a multitude of different compounds that can vary in both  $\Delta^{14}\text{C}$  content and ionization efficiency in the FT-ICR-MS analysis. As a result, correlating  $\Delta^{14}\text{C-SPE-DOM}$  with mass peak magnitudes of molecular formulae within an FT-ICR mass spectrum introduces an analytical uncertainty. However, we observe a strong correlation between the peak magnitude of specific IOS formulas with  $\Delta^{14}\text{C-SPE-DOM}$  values in different ocean basins. Therefore, it is likely that the observed mass peak magnitude of IOS formulae is predominated by the chemical structures that contribute to their persistence. Moreover, normalizing the data is a way of looking at the relative intensity of a molecular

formulae within a given sample, and how that relative intensity would change between samples.

A key to assessing the degradation of DOM in its transit along the deep ocean's conveyer is to compare the relative proportion of  $\text{IOS}_A$  in both datasets. The similar molecular character between  $\text{IOS}_A$  and  $\text{IOS}_P$  is evident in the visual representation in the van Krevelen diagram (Fig. 4A;  $\text{IOS}_A$  is in orange dots,  $\text{IOS}_P$  is in purple dots). For one, both  $\text{IOS}_A$  and  $\text{IOS}_P$  formulae have a clear distribution within the formulae associated with carboxyl-rich alicyclic molecules (CRAM; Hertkorn et al., 2006) (Fig. 4, black outline). These alicyclic groups have been found ubiquitously in marine (Hertkorn et al., 2006; 2013; Arakawa et al., 2017) and freshwater systems (Lam et al., 2007; Arakawa and Aluwihare, 2015).

Additionally, while the molecular formulae in the  $\text{IOS}_A$  and  $\text{IOS}_P$  have the same average molecular weights, O/C ratios, and H/C ratios, the  $\text{IOS}_P$  consists of an even more narrow range of formulae than the  $\text{IOS}_A$  (Fig. 4A, Table 2). We find that the proportion of  $\text{IOS}_A$  in the deep Pacific spectra comprises a larger portion ( $61 \pm 2\%$ ) of the SPE-DOM than in the deep Atlantic spectra ( $\sim 49 \pm 3\%$ ) (Lechtenfeld et al., 2014; Fig. 4). This distinct difference in the relative proportion of  $\text{IOS}_A$  in each deep ocean basin shows that there are more diverse molecular formulae in the Atlantic (and thus the  $\text{IOS}_A$  makes up a smaller proportion of the total DOM). In the Pacific, diversity is reduced due to removal of molecular formulae beyond detectable levels (and thus the  $\text{IOS}_A$  makes up a larger proportion of the total DOM). Contrasting the molecular composition between these two basins indicates that as DOC is transported through the deep ocean conveyer belt, compounds that are not intrinsically stable are removed relative to molecular formulae in the IOS.

DOM in the deep Atlantic carries with it a surface signature reflecting Atlantic Meridional Overturning, in which surface-derived DOC is sequestered into newly formed

deep waters from the North Atlantic (Hansell et al., 2009). This condition is indicated by the higher DOC concentrations (Hansell et al., 1998; 2009), younger radiocarbon ages (Druffel et al., 1992; 2016; 2017), and a greater diversity of molecular formulae (Flerus et al., 2012; Lechtenfeld et al., 2014). In the far North Pacific, deep waters have been out of contact with the atmosphere for centuries (Stuiver et al., 1983; Matsumoto, 2007). As such, less stable compounds in the DOC pool have been removed, and what remains is an intrinsically recalcitrant core of molecular formulae (i.e. those similar in elemental ratios as the IOS), which has residence times greater than the timescales of meridional overturning circulation, explaining why they are in larger proportion to the whole pool.

A recent report suggests that dilution is what primarily controls DOM dynamics in the deep ocean (Arrieta et al., 2015). While dilution may limit microbial response to DOM (i.e. there is not a high enough concentration of a specific substrate), our results demonstrate that the long-term stability of DOM is related to its elemental composition. These results are similar to those concluded by Walker and colleagues (2016a, b), who used  $^{14}\text{C}$  dating of ultra-filtered DOM to suggest that molecular size and composition are important factors in determining the long term storage of DOC in the deep. It is possible that dilution acts to limit the removal of compounds in DOM that are structurally more biologically available. We show that there are molecular formulae in DOM with a certain compositional character that are resistant to removal over long timescales. This concept, where refractory DOC is controlled by both the concentrations of individual molecules (i.e. dilution) and its molecular composition was described as a concept in Jiao et al (2014), but is illustrated here through using molecular composition and  $\Delta^{14}\text{C}$  values of SPE-DOM.

#### 4. Summary

Here we find that the  $\Delta^{14}\text{C}$  content of SPE-DOM in the Pacific is more depleted than in the deep Atlantic by  $-109\pm 10\%$  (or  $\sim 1,700$   $^{14}\text{C}$  years older), and that its molecular composition is more degraded. Consequently, while intrinsically stable molecular formulae (the IOS) are present ubiquitously throughout the ocean, they make up a higher relative proportion of DOM in the deep waters in the Pacific than in the Atlantic. These results show that a portion of deep DOM is intrinsically stable and is present in both Atlantic and Pacific deep waters.

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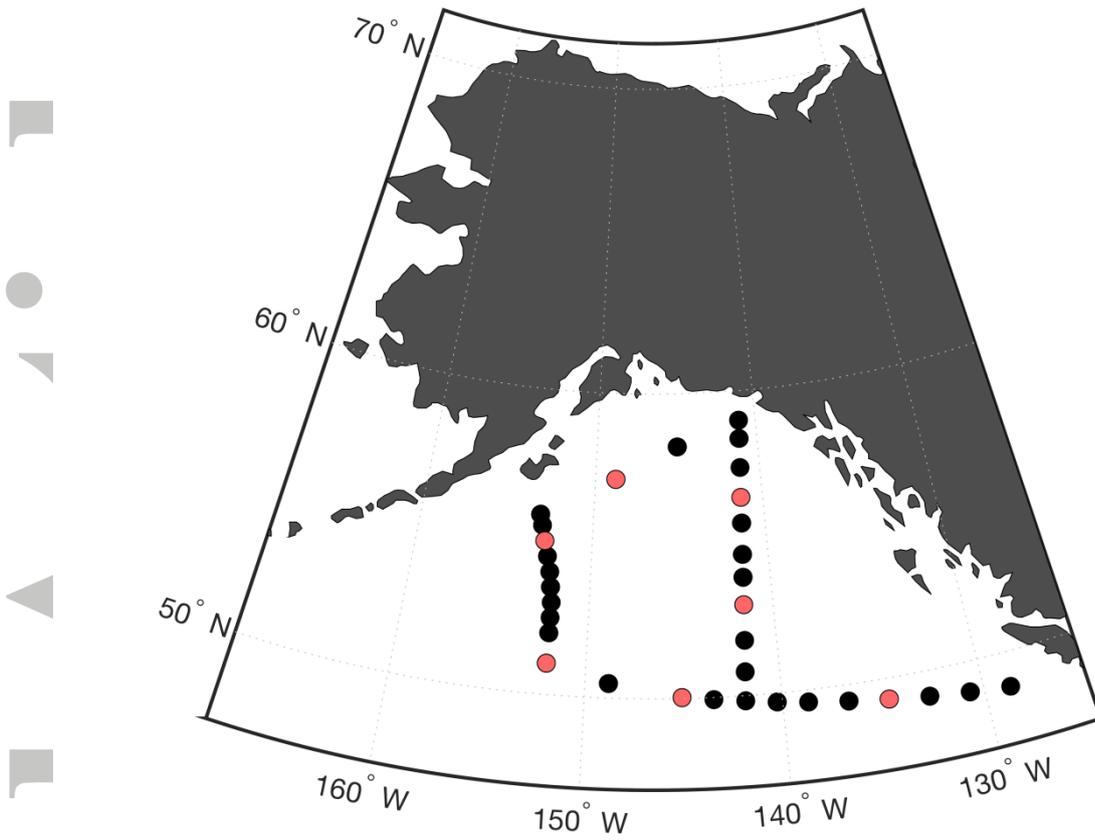
**Table 1.** Mean ( $\pm$ standard deviation) nominal mass, O/C ratio, H/C ratio,  $\Delta^{14}\text{C}$ -SPE-DOM value (with corresponding  $^{14}\text{C}$  age in parentheses), and relative proportion of the  $\text{IOS}_A$  in the deep Atlantic and Pacific SPE-DOM samples.

	Nominal Mass (Da)	O/C	H/C	$\Delta^{14}\text{C}$ -SPE-DOM (‰)	Relative proportion of $\text{IOS}_A$
Deep Atlantic	418 $\pm$ 99	0.48 $\pm$ 0.14	1.24 $\pm$ 0.22	-445 $\pm$ 5‰ (~4700 y)	49 $\pm$ 2%
Deep Pacific	421 $\pm$ 88	0.49 $\pm$ 0.13	1.25 $\pm$ 0.21	-554 $\pm$ 9‰ (~6400 y)	61 $\pm$ 2%

**Table 2.** Mean nominal mass, O/C ratio and H/C ratio, for the IOS constructed in the Atlantic (IOS<sub>A</sub>) and Pacific (IOS<sub>P</sub>).

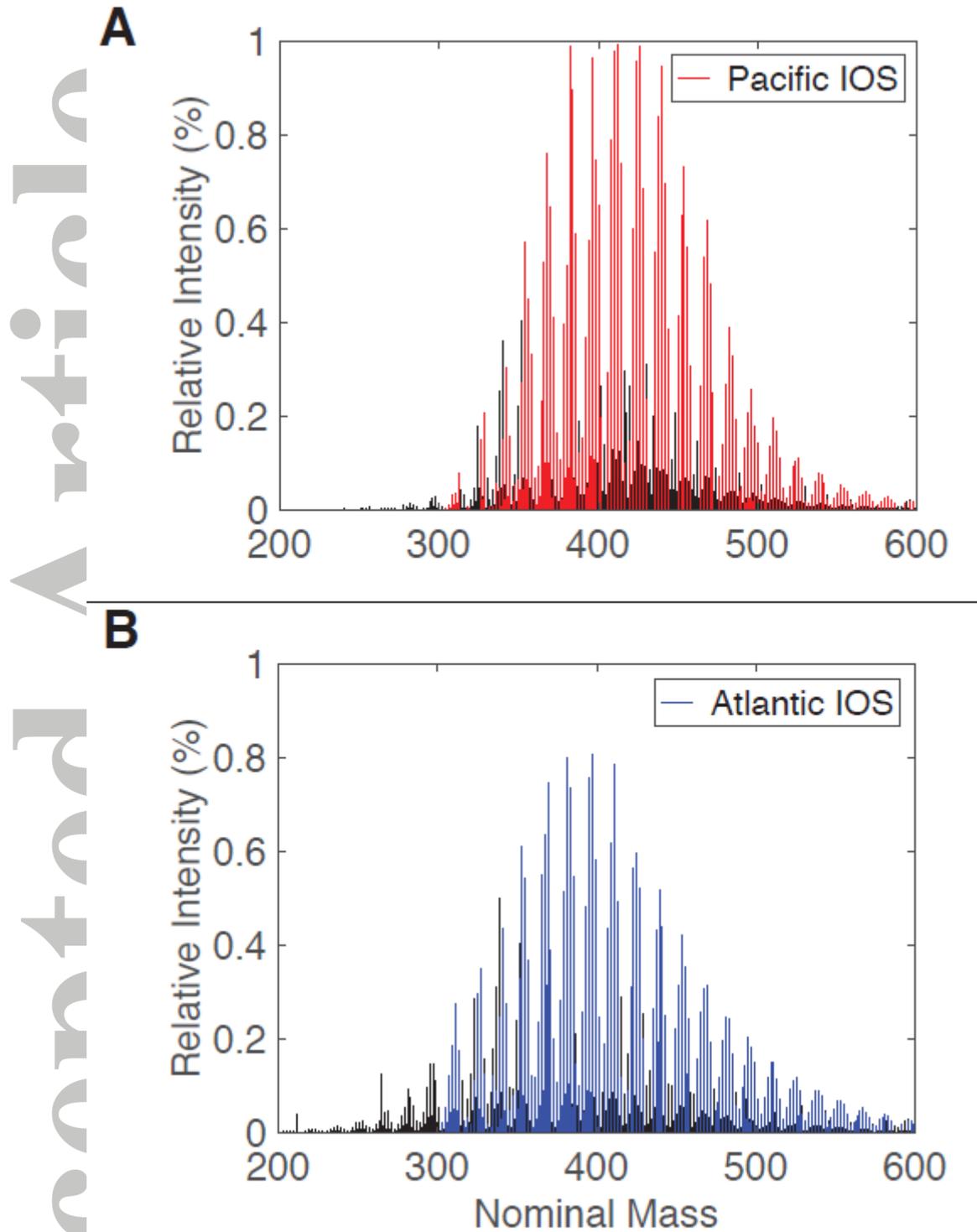
	Nominal Mass (Da)	O/C	H/C
IOS <sub>A</sub>	439±79	0.46±0.10	1.24±0.17
IOS <sub>P</sub>	438±53	0.48±0.06	1.23±0.12

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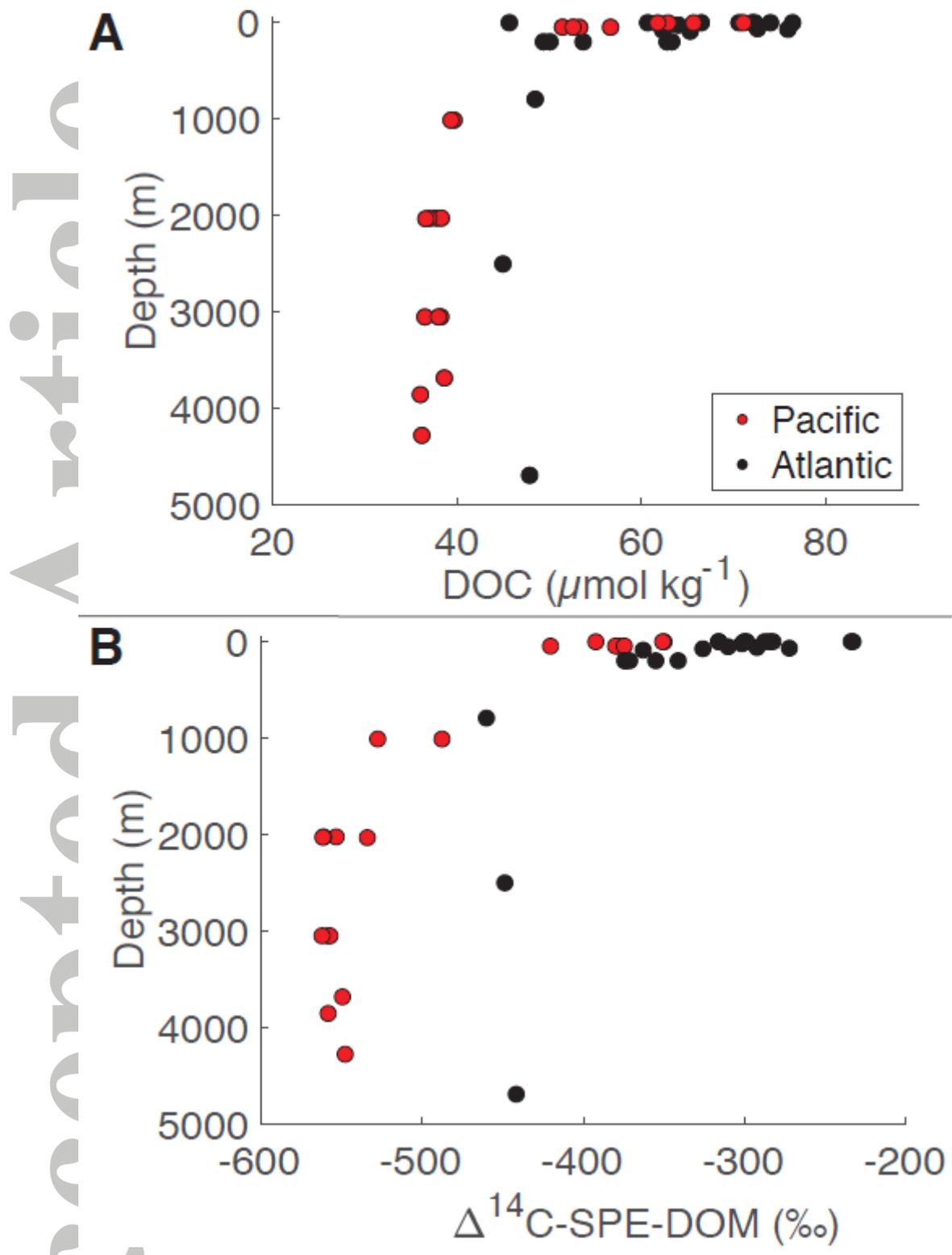


**Figure 1.** Map of station occupations for the *MV-1310* cruise. Pink dots represent where samples were collected for this analysis.

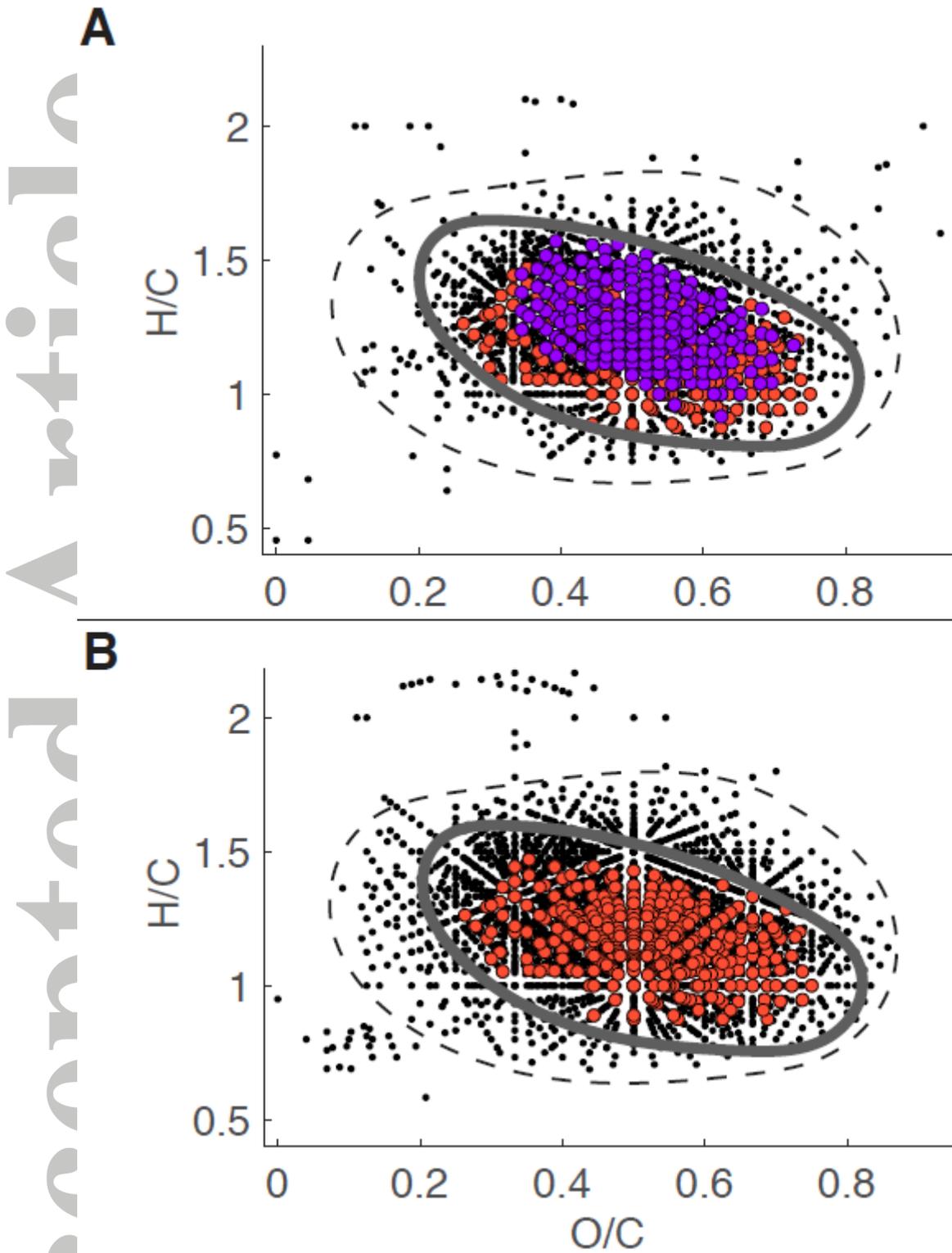
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**Figure 2.** FT-ICR-MS mass spectra of A.) a deep Pacific and B.) a deep Atlantic sample. The red and blue highlighted peaks represent those in the IOS.



**Figure 3.** A.) DOC concentration and B.)  $\Delta^{14}\text{C-SPE-DOM}$  vs. depth in the Pacific (red, data from this cruise) and the Atlantic (black, data from Flerus et al., 2012).



**Figure 4** Van Krevelen plots of A.) the deep Pacific and B.) the deep Atlantic, where the x-axis is the oxygen to carbon ratio (O/C) and the y-axis is the hydrogen to carbon ratio (H/C). The orange and purple colored dots represent the IOS<sub>A</sub> and IOS<sub>P</sub>, respectively. The grey circle represents a general outline of the CRAM regions in van Krevelen space, and the

dashed black line is a reference around the majority of the molecular formulae present in the deep Atlantic van Krevelen plot to show the decrease in molecular formulae from the deep Atlantic to the deep Pacific. The relative magnitude of IOS compounds increases 10% from the deep Atlantic ( $IOS_A \sim 49\%$  of total; Lechtenfeld et al., 2014) to the deep Pacific ( $IOS_A = 60\%$  of the total).

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