Bioavailable dissolved organic matter and biological hot spots during austral winter in Antarctic waters

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Abstract
Primary production and heterotrophic bacterial activity in the Antarctic Ocean are generally low during the austral winter. Organic carbon is considered to be a major factor limiting bacterial metabolism, but few studies have investigated the bioavailability of organic matter during winter. Herein, the chemical composition and bioavailability of dissolved organic matter (DOM) were investigated in surface (5–100 m) and mesopelagic (200–750 m) waters off the northwestern Antarctic Peninsula during August 2012. Concentrations of dissolved organic carbon (DOC) were low (42 ± 4 μmol L⁻¹) and showed no apparent spatial patterns. By contrast, the composition of DOM exhibited significant spatial trends that reflected varying ecosystem productivity and water masses. Surface distributions of chlorophyll-a and particulate organic carbon depicted a southward decline in primary productivity from open waters (60.0°S–61.5°S) to ice-covered regions (61.5°S–62.5°S). This trend was evident from concentrations and DOC-normalized yields of dissolved amino acids in the surface waters, indicating decreasing DOM bioavailability with increasing latitude. A different pattern of DOM bioavailability was observed in the mesopelagic water masses, where amino acids indicated highly altered DOM in the Circumpolar Deep Water and bioavailable DOM in the Transitional Weddell Water. Depth distributions of amino acid yields and compositions revealed hot spots of elevated bioavailable DOM at ~75 m relative to surrounding waters at most ice-free stations. Relatively low mole percentages of bacterially derived ε-amino acids in hot spots were consistent with an algal source of bioavailable DOM. Overall, these results reveal the occurrence and spatial heterogeneity of bioavailable substrates in Antarctic waters during winter.

1. Introduction

Ocean waters surrounding the Antarctic continent are dynamic and heterogeneous environments characterized by pronounced spatial and temporal variability in light, ice cover, water masses, and micronutrient conditions [Ducklow et al., 2007]. Primary production in these areas is limited to varying extents by light and iron, with high rates occurring during the austral summer in iron-rich shelf waters (>1 g C m⁻² d⁻¹) and low or negligible rates prevailing during the winter [Moore and Abbott, 2000]. Plankton activity, including direct release from phytoplankton and release during protozoan grazing and viral lysis, produces bioavailable dissolved organic matter (DOM) that supports the microbial loop in which heterotrophic bacteria remineralize DOM [Pomeroy, 1974; Azam et al., 1983] and also produce refractory DOM [Ogawa et al., 2001; Lechтенfeld et al., 2015]. The later process contributes to carbon sequestration and was recently conceptualized as the “microbial carbon pump” [Jiao et al., 2010].

Previous studies of Antarctic marine ecosystems indicate a relatively small fraction (<10%) of primary production is utilized by heterotrophic bacteria compared to that in low-latitude oceans [Bird and Karl, 1999; Kirchman et al., 1995; Duarte et al., 2005]. The low bacterial activity in Antarctic waters has been attributed to low temperature, high grazing pressure, and low bioavailability of DOM [Bird and Karl, 1999; Pomeroy and Wiebe, 2001; Duarte et al., 2005]. However, several studies have suggested a minor effect of temperature on bacterial growth [Carlson et al., 1998; Ducklow and Yager, 2007; Kirchman et al., 2009], and also observed...
minor grazing on bacteria during phytoplankton blooms [Anderson and Rivkin, 2001; Ducklow and Yager, 2007]. Bacterial production is often found to be significantly correlated with chlorophyll-\(a\) concentrations and primary production, suggesting a close coupling between bacteria and phytoplankton [Morán et al., 2001; Ortega- Retuerta et al., 2008; Murray et al., 2011; Ducklow et al., 2012; Teira et al., 2012]. Bioassay experiments and field measurements have shown rapid bacterial responses to the supply of bioavailable DOM [Carlson et al., 1998; Obernosterer et al., 2008; Ducklow et al., 2011]. Kirchman et al. [2009] reported a positive correlation between bacterial growth rates and concentrations of semilabile dissolved organic carbon (DOC) in the Ross Sea. These observations suggest that heterotrophic bacterial activity in Antarctic waters is strongly influenced by the bioavailability of DOM.

Previous research on DOM in the Southern Ocean has mostly focused on measuring concentrations of organic carbon and nitrogen [Kähler et al., 1997; Wiebinga and De Baar, 1998; Ogawa et al., 1999; Carlson et al., 2000; Doval et al., 2002; Clarke et al., 2008], and few studies have addressed the composition and bioavailability of DOM [Kähler et al., 1997; Carlson et al., 2000; Rosenstock et al., 2005; Tremblay et al., 2015]. Logistic challenges have limited most Antarctic expeditions to austral summer months, so little is known about microbial processes in the austral winter. DOM is generally considered to be of limited bioavailability during the winter [Scott et al., 2000; Pearce et al., 2007]. However, active bacterial growth and production have been observed in various dimly lit regions (55°S–65°S) during winter periods [Hanson et al., 1983; Kottmeier and Sullivan, 1987; Mordy et al., 1995; Manganelli et al., 2009]. This suggests the sporadic occurrence of bioavailable DOM, likely derived from production by phytoplankton, ice algae, and chemosynthetic bacteria [Kottmeier and Sullivan, 1987; Cota et al., 1992; Manganelli et al., 2009]. Patches of bioavailable DOM form hot spots that can enhance biogeochemical processes relative to surrounding areas [McCain et al., 2003; Azam and Malfatti, 2007; Shen et al., 2016a, 2016b]. The presence of reactive DOM is not readily reflected in DOM concentrations but is detectable in the chemical composition and bioavailability of DOM [Kirchman et al., 2001; Davis and Benner, 2005; Shen et al., 2012].

The bioavailability of DOM is commonly determined using bioassay incubations that measure biological consumption of DOM over time periods of weeks to months [Sandageraad and Middelboe, 1995; Lenborg et al., 2009]. This approach provides insights about the rates of DOM utilization as well as its relative bioavailability, but it is impractical for large-scale oceanographic surveys of bioavailable DOM. An alternative approach is based on the chemical analysis of specific molecular indicators of bioavailable DOM that have been identified using bioassay incubations [Amon et al., 2001; Benner, 2003; Davis et al., 2009; Goldberg et al., 2010]. Molecular indicators of bioavailable DOM can be directly measured in seawater and can provide insights about the occurrence of bioavailable DOM without the need for lengthy incubations. Amino acids have been used as qualitative and quantitative indicators of bioavailable DOM over broad spatial and temporal scales in various aquatic systems [Davis and Benner, 2007; Shen et al., 2015, 2016b]. Certain amino acids, such as glycine and nonprotein amino acids (\(\beta\)-alanine and \(\gamma\)-aminobutyric acid), increase in relative abundance during DOM degradation, thereby providing additional insights into the extent of DOM alteration [Kaiser and Benner, 2009; Shen et al., 2015]. Furthermore, \(\alpha\)-enantiomers of amino acids are derived from bacteria and are useful biomarkers for tracing the bacterial origin of DOM in the ocean [McCarthy et al., 1998; Kaiser and Benner, 2008].

The objective of this study is to investigate the concentration, chemical composition, and bioavailability of DOM during winter in Antarctic waters and to understand how these features vary with ecosystem productivity and hydrography. Distributions of chlorophyll-\(a\), particulate organic carbon (POC), DOC, and amino acids are examined in different water masses in sea ice-free and ice-covered regions around the South Shetland Islands off the northwestern Antarctic Peninsula during August 2012. Various biochemical indicators are applied to identify specific locations (referred to as biological hot spots) exhibiting elevated concentrations of bioavailable DOM relative to surrounding waters. The western Antarctic Peninsula is among the fastest warming regions on the planet and the most pronounced warming occurs during austral winter, with the mean surface air temperature having risen 5–6°C since 1950 [Vaughan et al., 2003; Ducklow et al., 2007]. Such dramatic winter warming has reduced extent and duration of sea ice, altered phytoplankton communities and production, and affected ecosystem function [Ducklow et al., 2007; Montes-Hugo et al., 2009], highlighting the importance of conducting studies in this region, particularly in winter.
2. Materials and Methods

2.1. Study Area and Sample Collection

Sampling was conducted as part of the Antarctic Marine Living Resources (AMLR) Program aboard the RVIB Nathaniel B. Palmer off the Antarctic Peninsula and South Shetland Islands (SSI) during August 2012 (Figure 1). The sampling areas are characterized by the confluence of different water masses originating from the Antarctic Circumpolar Current, Bellingshausen Sea, and Weddell Sea. A total of 110 seawater samples were collected from discrete depths (5, 10, 15, 50, 75, 100, 200, 750 m) at 25 stations within the historic AMLR research area, extending from the southern Drake Passage to the Bransfield Strait (Figure 1). A rosette sampler system with 24, 12 L bottles and a Seabird Conductivity-Temperature-Depth (CTD) instrument was used for water collections. Water samples for chlorophyll-a (chl-a) were filtered (GF/F; 0.7 μm pore size; Whatman) immediately following collection. Samples for POC measurements were collected mostly at 5 and 200 m, filtered (GF/F; Whatman; precombusted at 450°C for 5 h), and stored frozen until analysis. Water samples for analyses of DOC and amino acids were stored frozen (−80°C) in 60 mL high-density polyethylene screw-cap bottles immediately after collection. Hydrographic data were obtained from the CTD sensors and were used to determine the depth of upper mixed layer and to identify water masses (Table 1).

2.2. Chemical Analyses

The filters for chl-a determinations were extracted in 7 mL of methanol for 24 h, centrifuged, and measured for fluorescence using an acidification module in a Turner Trilogy fluorometer. Readings were calibrated with a 5-point calibration curve using a chl-a standard obtained from Sigma, the concentration of which was determined using a Lambda-18 spectrophotometer. Samples (GF/F filters) for POC analysis were treated with 10% v/v hydrochloric acid (HCl), dried at 60°C, and analyzed using an Exeter Analytical CEC 440HA elemental analyzer.

Figure 1. Sampling sites off the South Shetland Islands during the Antarctic Marine Living Resources (AMLR) cruise in August 2012. The sampling regions include various water masses from the Antarctic Circumpolar Current (ACC), Bellingshausen Sea, and Weddell Sea. The sea ice-free (n = 11) and sea ice-covered (n = 14) stations are denoted by red and blue solid circles, respectively. Stations with 5–7 sampling depths (5–750 m) are labeled with station names (n = 13) and represent the vertical profiles in Figures 5 and 6. The dashed rectangles include the stations plotted in Figure 2b. Map was generated using Ocean Data View 4.7.4 (Schlitzer, R., Ocean Data View, http://odv.awi.de, 2015).
Table 1. Summary of Oceanographic Parameters at the Sampling Stations

| Station | Latitude (°S) | Longitude (°W) | Sea Ice Coverage | SSS (‰) | SST (°C) | Z_{TOC} (m) | Depth (m) | Major Water Mass
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>W07-01</td>
<td>60.001</td>
<td>-56.501</td>
<td>Ice-free</td>
<td>33.96</td>
<td>1.26</td>
<td>75</td>
<td>5-750</td>
<td>WW, CDW</td>
</tr>
<tr>
<td>W08-02</td>
<td>-60.252</td>
<td>-57.000</td>
<td>Ice-free</td>
<td>33.96</td>
<td>1.38</td>
<td>97</td>
<td>5-750</td>
<td>WW, CDW</td>
</tr>
<tr>
<td>W02-02</td>
<td>-60.244</td>
<td>-54.008</td>
<td>Ice-free</td>
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<td>1.54</td>
<td>100</td>
<td>5-750</td>
<td>WW, CDW</td>
</tr>
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<td>-56.998</td>
<td>Ice-free</td>
<td>33.93</td>
<td>0.78</td>
<td>120</td>
<td>5-750</td>
<td>WW, CDW</td>
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<tr>
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<td>-56.994</td>
<td>Ice-free</td>
<td>33.91</td>
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<td>5, 200</td>
<td>WW, CDW</td>
</tr>
<tr>
<td>W07-05</td>
<td>-60.993</td>
<td>-56.496</td>
<td>Ice-free</td>
<td>33.97</td>
<td>1.47</td>
<td>115</td>
<td>5, 200</td>
<td>WW, CDW</td>
</tr>
<tr>
<td>W055-05</td>
<td>-61.000</td>
<td>-55.753</td>
<td>Ice-free</td>
<td>34.14</td>
<td>1.57</td>
<td>nd (n=1)</td>
<td>5–100</td>
<td>W</td>
</tr>
<tr>
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<td>-61.248</td>
<td>-56.995</td>
<td>Ice-free</td>
<td>34.00</td>
<td>1.35</td>
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<td>5, 200</td>
<td>WW, CDW</td>
</tr>
<tr>
<td>W08-07</td>
<td>-61.507</td>
<td>-57.001</td>
<td>Ice-free</td>
<td>34.06</td>
<td>1.83</td>
<td>177</td>
<td>5-450</td>
<td>WW, TW</td>
</tr>
<tr>
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<td>-55.010</td>
<td>Ice-free</td>
<td>34.06</td>
<td>1.53</td>
<td>77</td>
<td>15–750</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W07-07</td>
<td>-61.500</td>
<td>-56.506</td>
<td>Ice-covered</td>
<td>34.06</td>
<td>1.82</td>
<td>172</td>
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<td>WW</td>
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<tr>
<td>W02-07</td>
<td>-61.496</td>
<td>-54.026</td>
<td>Ice-covered</td>
<td>34.10</td>
<td>1.80</td>
<td>98</td>
<td>5–750</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W08-08</td>
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<td>-57.122</td>
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<td>34.07</td>
<td>1.84</td>
<td>154</td>
<td>5, 200</td>
<td>WW</td>
</tr>
<tr>
<td>W07-08</td>
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<td>-56.498</td>
<td>Ice-covered</td>
<td>34.12</td>
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<td>5, 200</td>
<td>WW, TW</td>
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<tr>
<td>W055-08</td>
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<td>-55.768</td>
<td>Ice-covered</td>
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<td>1.84</td>
<td>145</td>
<td>5, 200</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W08-09</td>
<td>-62.000</td>
<td>-56.998</td>
<td>Ice-covered</td>
<td>34.09</td>
<td>1.86</td>
<td>126</td>
<td>5, 200</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W07-09</td>
<td>-62.003</td>
<td>-56.540</td>
<td>Ice-covered</td>
<td>34.11</td>
<td>1.83</td>
<td>111</td>
<td>5–750</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W055-09</td>
<td>-61.998</td>
<td>-55.768</td>
<td>Ice-covered</td>
<td>34.12</td>
<td>1.83</td>
<td>127</td>
<td>5–750</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W04-09</td>
<td>-61.982</td>
<td>-55.024</td>
<td>Ice-covered</td>
<td>34.07</td>
<td>1.83</td>
<td>155</td>
<td>15–750</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W03-09</td>
<td>-62.003</td>
<td>-54.515</td>
<td>Ice-covered</td>
<td>34.10</td>
<td>1.79</td>
<td>72</td>
<td>5, 200</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W10-10</td>
<td>-62.256</td>
<td>-58.001</td>
<td>Ice-covered</td>
<td>34.02</td>
<td>1.83</td>
<td>54</td>
<td>15–750</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W08-10</td>
<td>-62.240</td>
<td>-57.008</td>
<td>Ice-covered</td>
<td>34.10</td>
<td>1.85</td>
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<td>WW, TW</td>
</tr>
<tr>
<td>W07-10</td>
<td>-62.250</td>
<td>-56.516</td>
<td>Ice-covered</td>
<td>34.12</td>
<td>1.86</td>
<td>91</td>
<td>5, 200</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W09-11</td>
<td>-62.503</td>
<td>-57.501</td>
<td>Ice-covered</td>
<td>34.16</td>
<td>1.86</td>
<td>101</td>
<td>5, 200</td>
<td>WW, TW</td>
</tr>
<tr>
<td>W07-11</td>
<td>-62.489</td>
<td>-56.536</td>
<td>Ice-covered</td>
<td>34.21</td>
<td>1.87</td>
<td>44</td>
<td>5, 200</td>
<td>WW, TW</td>
</tr>
</tbody>
</table>

*SSS, sea surface salinity; SST, sea surface temperature; nd, not determined; and n, number of samples.

$Z_{TOC}$, Depth of the upper mixed layer, determined as the depth in which potential density is 0.05 kg m$^{-3}$ greater than the value at 5 m (Hewes et al., 2008). Similar results were obtained by using a temperature-based criterion (i.e., SST < 0°C).

Three major water masses were identified in this study: Winter Water (WW), Circumpolar Deep Water (CDW), and Transitional Weddell Water (TW).
Concentrations of TDAA and TPAA were determined as the total concentrations of the eighteen dissolved and particulate amino acids, respectively. DOC-normalized yields of TDAA were calculated as the percentage contributions of amino acid carbon to the total DOC, using equation (1) and being reported in units of \%DOC:

\[
TDAA(\%DOC) = \left( \frac{[TDAA-C]}{[DOC]} \right) \times 100
\]

where \([DOC]\) and \([TDAA-C]\) are the concentrations of bulk DOC and carbon measured in the total dissolved amino acids, respectively. This calculation excluded the two nonprotein amino acids (β-Ala and γ-Aba) that are thought to be byproducts of decomposition [Cowie and Hedges, 1994].

2.3. Statistical Analyses

The significance of least squares linear regression analyses between variables was determined using the enter approach in SPSS 20.0 (IBM Statistical Package for the Social Sciences Inc.). Normality of residuals was tested using a Kolmogorov-Smirnov test (two-tailed, \(a = 0.05\)). Statistical differences between variables were analyzed using the nonparametric Mann-Whitney \(U\) test (two-tailed, \(a = 0.05\)), which makes no assumptions of equal group size and normality of data distribution.

3. Results

3.1. Hydrography

Areas around the South Shetland Islands (SSI) are influenced by various water masses during austral winter, including Winter Water (WW), Transitional Bellingshausen Water (TBW), Circumpolar Deep Water (CDW), and Transitional Weddell Water (TWW). These water masses were identified at the sampling stations (5–750 m; Table 1; Figure 2a) according to their temperature and salinity characteristics as described in previous studies [Sangrà et al., 2011; Teira et al., 2012]. Cold (\(-1.9\) to \(-0.5^\circ\)C) and less saline (33.5–34.3) WW was located in the upper 100 m within the surface mixed layer (Figure 2b). Below the WW intrusions of relatively warm and dense waters from adjacent seas resulted in elevated temperatures and salinities that were characteristic of different water masses in the mesopelagic zone (200–750 m) in the region of the SSI (Figure 2b). In the Drake Passage, the Antarctic Circumpolar Current brought warm (0–2\(^\circ\)C) and salty (34.4–34.7) CDW that dominated the mesopelagic waters north of the SSI (60.0–60.25\(^\circ\)S). By contrast, areas south of the SSI (SSI shelf and the Bransfield Strait; 60.5–62.5\(^\circ\)S) were influenced by inflows from the TBW and Weddell Sea (Figure 1). The TBW was not well sampled in this study (\(n = 3\)) and is therefore not further discussed.

![Figure 2](image_url)

Figure 2. (a) Temperature versus salinity of all water samples (\(n = 110\)) collected in the present study, WW, Winter Water; CDW, Circumpolar Deep Water; TBW, Transitional Bellingshausen Water; TWW, Transitional Weddell Water. TBW was not well sampled in this study and were excluded from the discussion. (b) Latitudinal distributions of temperature and salinity in the upper 750 m along the two transects extending from the Drake Passage to the Bransfield Strait (see Figure 1).
The relatively cold (−1.8 to −0.5°C) and saline (34.3–34.6) TWW entered Bransfield Strait from the Weddell Sea and was a major component of mesopelagic waters in the Bransfield Strait. The sampling areas encompassed both sea ice-free and sea ice-covered waters (Figure 1 and Table 1). Surface waters in the north of the SSI were largely ice-free, whereas waters south of the SSI were mostly ice-covered. Sea ice affects light reflection and penetration in the water column, and the varying sea ice extent could strongly affect the spatial variability in primary production during this low-light season.

3.2. Latitudinal Distributions of chl-α, Organic Carbon, and Amino Acids

Concentrations of chl-α and POC in the surface WW were quite variable and decreased significantly toward the south from 0.22 to 0.01 µg L⁻¹ ($r^2 = 0.53$, $p < 0.01$) and from 3.2 to 0.8 µmol L⁻¹ ($r^2 = 0.29$, $p < 0.01$), respectively (Figures 3a and 3c; Table 2). In comparison, concentrations of chl-α and POC in the CDW and TWW were significantly lower (Mann-Whitney U test, $p < 0.01$), with most values below 0.04 µg L⁻¹ and 2 µmol L⁻¹ (Figures 3b and 3d; Table 2). No significant difference was observed in concentrations of chl-α (Mann-Whitney U test, $p > 0.05$) and POC (Mann-Whitney U test, $p > 0.05$) between the CDW and TWW.

Concentrations of DOC around the SSI ranged from 35 to 58 µmol L⁻¹ (average: 42 ± 4 µmol L⁻¹) and showed no latitudinal gradients in the surface waters ($r^2 = 0.00$, $p > 0.5$) or vertical gradients between surface and mesopelagic layers (Mann-Whitney U test, $p > 0.1$) (Figures 4a and 4b; Table 2). In comparison, concentrations and DOC-normalized yields of TDAA were more variable, ranging ~3-fold from 84 to 257 nmol L⁻¹ and from 0.6% to 1.7% in the surface WW and ~2-fold from 83 to 144 nmol L⁻¹ and from 0.5% to 1.1% in the mesopelagic waters, respectively (Figures 4c–4f; Table 2). Concentrations and yields of TDAA in the surface WW displayed weak but significant latitudinal trends ($r^2 = 0.11–0.13$, $p < 0.05$), while in the mesopelagic waters these values exhibited significant differences between the CDW and TWW ($p < 0.05$).

Figure 3. Latitudinal distributions of (a, b) chlorophyll-α (chl-α) concentrations and (c, d) particulate organic carbon (POC) concentrations in Winter Water (WW, 5–100 m), Circumpolar Deep Water (CDW, 200–750 m), and Transitional Weddell Water (TWW, 200–750 m).
3.3. Vertical Distributions of chl-\(a\), DOC, and Amino Acids

Depth profiles of chl-\(a\), DOC, and amino acids at seven sea ice-free and six sea ice-covered stations are presented in Figure 5. At the ice-free stations, concentrations of chl-\(a\) were elevated near the surface and decreased with increasing depth (Figure 5a). Concentrations of DOC were generally low (37–45 \(\mu\)mol \(L^{-1}\)) and showed no apparent depth trends (Figure 5b). Maximal concentrations of DOC (40–57 \(\mu\)mol \(L^{-1}\)) were observed at a depth of 75 m at four stations (W0701, W0202, W0804, and W0407). Surface concentrations of chl-\(a\) and POC at these four stations were among the highest values measured in all profiles.

### Table 2. Physical and Chemical Properties of Major Water Masses and the Biological Hot Spots

<table>
<thead>
<tr>
<th>Latitude (({^\circ}\text{S}))</th>
<th>Salinity</th>
<th>Temp. (^\circ\text{C})</th>
<th>Depth (m)</th>
<th>Chl-(a) (\mu)g (L^{-1})</th>
<th>POC (\mu)mol (L^{-1})</th>
<th>DOC (\mu)mol (L^{-1})</th>
<th>TDAA (\mu)mol (L^{-1})</th>
<th>TDAA (%DOC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW ((n=76))</td>
<td>60.00–62.50</td>
<td>34.05 ± 0.15</td>
<td>1.52 ± 0.45</td>
<td>5–100</td>
<td>0.09 ± 0.05</td>
<td>1.9 ± 0.5</td>
<td>42 ± 4</td>
<td>122 ± 32</td>
</tr>
<tr>
<td>CDW ((n=10))</td>
<td>60.00–61.50</td>
<td>34.61 ± 0.09</td>
<td>1.48 ± 0.78</td>
<td>200–750</td>
<td>0.01 ± 0.01</td>
<td>1.3 ± 0.3</td>
<td>41 ± 3</td>
<td>88 ± 16</td>
</tr>
<tr>
<td>TWW ((n=21))</td>
<td>60.25–62.50</td>
<td>34.42 ± 0.10</td>
<td>−0.95 ± 0.38</td>
<td>200–750</td>
<td>0.02 ± 0.01</td>
<td>1.3 ± 0.3</td>
<td>41 ± 3</td>
<td>104 ± 16</td>
</tr>
<tr>
<td>Hot spots ((n=4))</td>
<td>60.00–61.50</td>
<td>34.04 ± 0.08</td>
<td>−1.24 ± 0.42</td>
<td>75</td>
<td>0.12 ± 0.02</td>
<td>nd</td>
<td>47 ± 7</td>
<td>205 ± 43</td>
</tr>
<tr>
<td>All</td>
<td>60.00–62.50</td>
<td>34.17 ± 0.24</td>
<td>−1.14 ± 0.98</td>
<td>5–750</td>
<td>0.07 ± 0.05</td>
<td>1.7 ± 0.5</td>
<td>42 ± 4</td>
<td>116 ± 30</td>
</tr>
</tbody>
</table>

Data are reported as average ± standard deviation.

WW, Winter Water; CDW, Circumpolar Deep Water; TWW, Transitional Weddell Water; and nd, not determined.

3.3. Vertical Distributions of chl-\(a\), DOC, and Amino Acids

Depth profiles of chl-\(a\), DOC, and amino acids at seven sea ice-free and six sea ice-covered stations are presented in Figure 5. At the ice-free stations, concentrations of chl-\(a\) were elevated near the surface and decreased with increasing depth (Figure 5a). Concentrations of DOC were generally low (37–45 \(\mu\)mol \(L^{-1}\)) and showed no apparent depth trends (Figure 5b). Maximal concentrations of DOC (40–57 \(\mu\)mol \(L^{-1}\)) were observed at a depth of 75 m at four stations (W0701, W0202, W0804, and W0407). Surface concentrations of chl-\(a\) and POC at these four stations were among the highest values measured in all profiles. TDAA

![Figure 5](image-url)
Concentrations (60–257 nmol L$^{-1}$) were more variable than DOC concentrations and the maximal values (>160 nmol L$^{-1}$) also occurred at 75 m at the four stations (Figure 5c). TPAA were measured in a subset of unfiltered samples and showed markedly high concentrations at 75 m (40–110 nmol L$^{-1}$; Figure 5d), coinciding with the depth of maximal DOC and TDAA concentrations.

Concentrations of chl-a, DOC, TDAA, and TPAA at the ice-covered stations (Figures 5e–5h) were much lower and less variable than those in the open waters. Chl-a concentrations were below 0.1 μg L$^{-1}$ near the surface and gradually decreased with increasing depth (Figure 5e). Concentrations of DOC and TDAA were distributed relatively uniformly throughout the water column and ranged mostly from 40 to 45 μmol L$^{-1}$ and from 85 to 140 nmol L$^{-1}$, respectively (Figures 5f and 5g). Concentrations of TPAA displayed considerable variability and ranged from 6 to 95 nmol L$^{-1}$ (Figure 5h). None of these parameters showed a notable subsurface maximum at the sea ice-covered stations.

### 3.4. DOM Bioavailability

DOM bioavailability at the ice-free and ice-covered stations was investigated using four independent amino acid-based indicators (DOC-normalized yields of TDAA and mole percentages of glycine, nonprotein amino

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**Figure 5.** Depth profiles of (a, e) chlorophyll-a (chl-a) concentrations, (b, f) dissolved organic carbon (DOC) concentrations, (c, g) total dissolved amino acid (TDAA) concentrations, and (d, h) total particulate amino acid (TPAA) concentrations at (upper plots) seven sea ice-free stations and (lower plots) six ice-covered stations. Note that the ice-free and ice-covered stations are labeled separately as in plots a and e, respectively.
acids and D-amino acids) (supporting information Figure S1). DOM of high bioavailability is typically enriched in total amino acids with a low relative abundance of glycine, β-Ala and γ-Aba, and D-amino acids. As microbial alteration proceeds, TDAA yields decrease due to preferential removal of most amino acids relative to bulk DOC, whereas the mole fractions of glycine, β-Ala and γ-Aba, and D-amino acids increase as a result of selective preservation, decarboxylation of glutamine and asparagine, and release of bacterial D-amino acids, respectively [Cowie and Hedges, 1994; Nguyen and Harvey, 1997; Kaiser and Benner, 2008, 2009]. An outstanding feature was found at the four ice-free stations with subsurface maxima of DOC and amino acid concentrations (Figure 6). Yields of TDAA at these four stations showed maximal values (1.3–1.7% DOC) at 75 m (Figure 6a). Remarkable declines in mole percentages of glycine (20–30%), nonprotein amino acids (β-Ala1 γ-Aba: 6–9%), and D-enantiomers of amino acids (20–30%) was observed also at 75 m (Figures 6b–6d). These molecular indicators were consistent in showing elevated bioavailability of DOM in subsurface waters. This feature was not evident at other sea ice-free or ice-covered stations (supporting information Figure S1).

4. Discussion

4.1. Concentrations of Organic Carbon
There is a paucity of data on concentrations of organic carbon in Antarctic waters during austral winter due to difficult sampling conditions. Concentrations of POC (1–3 μmol L⁻¹) and DOC (35–58 μmol L⁻¹) measured in this study were comparable to the few existing winter values (POC: 0–5 μmol L⁻¹; DOC: 35–45 μmol L⁻¹) in the region of Antarctic Peninsula [Cota et al., 1992; Manganelli et al., 2009]. Much higher and more variable winter concentrations of DOC were reported in coastal waters near the Davis Station (90–170 μmol L⁻¹) [Pearce et al., 2007] and in the northern Marguerite Bay and western Antarctic Peninsula (40–100 μmol L⁻¹) [Clarke et al., 2008]. Other field surveys of organic carbon in the Southern Ocean were mostly conducted during spring-summer (October to January), and they covered regions of Antarctic Peninsula [Doval et al., 2002; Ducklow et al., 2007; Clarke et al., 2008; Ortega-Retuerta et al., 2010], Weddell Sea [Wedborg et al., 1998], Ross Sea [Carlson et al., 2000; Gardner et al., 2000], and northern areas (40–60°S) of the Southern Ocean [Kähler et al., 1997; Wiebinga and De Baar, 1998; Ogawa et al., 1999; Tremblay et al., 2015]. With a few exceptions, surface concentrations of DOC measured in these studies (mostly 45–60 μmol L⁻¹) are comparable to our winter results but are lower than most values found in other major ocean basins (60–100 μmol L⁻¹) [Kaiser and Benner, 2009; Hansell, 2013; Shen et al., 2016a].
4.2. Composition and Bioavailability of DOM
Significant latitudinal changes in DOM composition were observed in surface waters around the SSI and appeared to reflect variability in ecosystem productivity. Winter primary production in this dimly lit environment is light-limited and the surface distributions of chl-a and POC revealed a significant decrease in phytoplankton biomass and potential production from open waters (north of the SSI) to sea-ice-covered regions (south of the SSI). This pattern appeared to parallel the trends in the surface concentrations and DOC-normalized yields of dissolved amino acids, which decreased significantly from the Drake Passage to Bransfield Strait and indicated a southward decline in DOM bioavailability. The latitudinal change in amino acid yields (from 1.7% to 0.7%) in these surface waters approximated vertical gradients occurring in other ocean basins [Kaiser and Benner, 2009], indicating substantial spatial variability in DOM bioavailability in this region. The coincident pattern of ecosystem productivity and DOM bioavailability also appears to occur during the austral summer, but in a reverse latitudinal trend. An increasing north-south trend in protein-like fluorescence of DOM has been measured around the SSI in summer and it corresponds to a southward increase in primary production [Teira et al., 2012]. Unlike the wintertime scenario, summer primary production is generally lower in the Drake Passage, where phytoplankton growth is limited by iron, and is elevated toward the south in the Bransfield Strait, where the mixing of iron-replete waters from the adjacent Weddell Sea supports high phytoplankton biomass and production [Holm-Hansen and Hewes, 2004; Ardelan et al., 2010; Teira et al., 2012; Garcia-Munoz et al., 2014].

DOM composition in the mesopelagic layer displayed a different pattern that was related to hydrographical conditions. Concentrations and yields of amino acids between 200 and 750 m were lower in the Drake Passage than those in the Bransfield Strait, and this appeared to be associated with varying sources and utilization of bioavailable DOM in the different water masses. CDW and TWW dominate the mesopelagic layers of the Drake Passage and the Bransfield Strait, respectively. CDW is a thick layer (~200–3000 m) formed by mixing deep waters from other ocean basins [Rintoul et al., 2001] and it receives a very limited supply of bioavailable DOM [Kaiser and Benner, 2009]. In addition, heterotrophic activity may decompose bioavailable substrates during transport in the CDW. The low oxygen level typically found in the upper CDW (oxygen <180–200 μmol kg^{-1}) [Rintoul et al., 2001] is consistent with the extremely low amino acid yields (0.5–0.8% DOC), indicating DOM in the CDW has undergone extensive microbial alteration and is of limited bioavailability. By contrast, higher amino acid yields (mostly 0.8–1.2% DOC) were observed in the TWW, indicating elevated concentrations of bioavailable DOM. Intermediate and deep waters in the eastern and southern regions of the Bransfield Strait originate primarily from the Weddell Sea and have a chlorofluorocarbon (CFC)-derived age younger than ~8 years (CFC-11: 3–5 pmol kg^{-1}) [Gordon et al., 2000; Sangrà et al., 2011]. The relatively rapid ventilation allows some bioavailable DOM (e.g., semilabile DOM with a turnover time of months–decades) produced on the Weddell shelf to be transported to the Bransfield Strait.

The present study corroborated previous findings that common biochemicals, such as amino acids and carbohydrates, can reveal insights about DOM bioavailability that are not apparent from DOC concentrations. The dynamics of ecosystem productivity and ocean circulation around the SSI were reflected in the composition and bioavailability of DOM but not in DOC concentrations. Similar features have been noted in other polar waters. Kirchman et al. [2001] observed a ~10-fold increase in concentrations of dissolved neutral sugars during a summertime phytoplankton bloom in the Ross Sea, while concentrations of DOC increased by only ~20%. Likewise, Shen et al. [2012] measured similar concentrations of DOC in two Arctic systems with contrasting productivity (Chukchi and Beaufort Seas) but observed substantial accumulations of amino acid-rich DOM over the more productive Chukchi shelf. Recent observations in a highly productive ocean margin also indicated pronounced temporal and spatial variability in plankton-derived DOC in surface waters, in sharp contrast to the low variability of DOC concentrations [Shen et al., 2016b]. Plankton production and heterotrophic microbial processes rapidly cycle amino acids and carbohydrates. These biomolecules generally comprise a relatively small fraction of seawater DOM (<15% DOC) [Benner, 2002; Kaiser and Benner, 2009], and their production and consumption are often not apparent from changes in DOC concentrations.

4.3. Biological Hot Spots and Implication for Carbon Cycling
Biochemical indicators revealed the occurrence of biological hot spots around the SSI during winter. Maximal concentrations and yields of amino acids were observed near a depth of 75 m at most sea ice-free stations and the values were twice those in surrounding waters. The maximal amino acid yields (1.3–1.7%
The observation of hot spots during austral winter is surprising and intriguing given the low water temperatures and primary productivity. The effects of temperature on bacterial metabolism are complex [Pomeroy and Wiebe, 2001; Kirchman et al., 2005; Ducklow et al., 2012], but there is evidence that bacteria in the polar oceans are adapted to low temperature [Carlson et al., 1998; Kirchman et al., 2009]. Field observations in the Ross Sea (~−1.7°C) and off the Kerguelen Island (2–4°C) showed remarkable increases in bacterial biomass and production with the supply of bioavailable substrates produced during phytoplankton blooms [Carlson et al., 1998; Obernosterer et al., 2008].

The sources of bioavailable DOM to the hot spots are difficult to determine but are interesting to consider. The deep chl-a maximum, a common feature at 50–100 m in the AMLR sampling region during austral summer [Holm-Hansen and Hewes, 2004], was not observed during the winter [Manganelli et al., 2009; this study]. All hot spots were characterized by the absence of sea ice cover during sampling, and the concentrations of chl-a and POC at these stations were among the highest in the present study. This implies surface phytoplankton production could be a potential source of bioavailable DOM in the subsurface waters. The relatively low mole percentages of ω-amino acids in the hot spots were consistent with an algal source of the bioavailable DOM. Depth distributions of particulate amino acids indicated the occurrence of amino acid-rich particles in the hot spots. It is likely that biogenic particles produced locally or elsewhere release bioavailable DOM into the subsurface waters via dissolution, grazing, and viral lysis [Strom et al., 1997; Carlson, 2002; Azam and Malfatti, 2007]. Another possible source is chemoautotrophic production, which has been found to be substantial (0.1–40 ng C L−1 d−1, ~10% of the total prokaryotic carbon production) during austral winter around the Antarctic Peninsula [Manganelli et al., 2009; Gryzinski et al., 2012].

The observations of biological hot spots reveal spatial heterogeneity in Antarctic waters and highlight a potential role of heterotrophic bacteria in carbon cycling during the winter. There is growing evidence that bacterial metabolism and processes can transform labile DOM into refractory DOM and thereby contribute to the long-term storage of carbon in the ocean [Brophy and Carlson, 1989; Ogawa et al., 2001; Lechentfeld et al., 2015], a process recently conceptualized as the “microbial carbon pump” [Jiao et al., 2010; Benner, 2011]. The biological hot spots likely represent important sites of bacterial activity and the potential for sequestration of carbon through the microbial carbon pump in a low primary production regime. It has already been noticed in various Antarctic oceanic regions that bacteria are abundant and active during the austral winter [Kottmeier and Sullivan, 1987; Mordy et al., 1995; Manganelli et al., 2009], suggesting bacterial transformation of bioavailable carbon to more bioresistant forms occurs during periods of diminished primary productivity in high-latitude environments.

References

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Figure S1. Depth profiles of (a, e) DOC-normalized yields of total dissolved amino acids (TDAA), (b, f) mole percentages of glycine, (c, g) mole percentages of two non-protein amino acids: β-alanine (β-Ala) and γ-aminobutyric acid (γ-Aba), and (d, h) percentages of D-enantiomers of amino acids at seven sea ice-free stations (upper panels) and six ice-covered stations (lower panels). Note that the ice-free and ice-covered stations are labeled separately as in panels a and e, respectively. Percentages of D-amino acids were calculated as:

\[ D - \text{amino acids} \% = \frac{[D - \text{AA4}]}{[D - \text{AA4}]+[L - \text{AA4}]} \times 100 \]

where [D-AA4] and [L-AA4] were concentrations of the four D- and L-amino acids (Asx, Glx, Ser, and Ala).