Supporting information for

Biogeochemical controls on ammonium accumulation in the surface layer of the Southern Ocean

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Introduction

Supporting information is provided here in the form of text, one table, and six figures. **Text S1** provides the definitions of the Southern Ocean fronts and zones used in the main text. **Text S2** gives a detailed description of the methods used to enumerate cells via flow cytometry. **Text S3** discusses the degree of coupling between net primary production (NPP) and nitrogen (N) uptake. **Table S1** shows a correlation matrix, indicating the Pearson correlation coefficient (and significance) of the relationships between each of the nutrient uptake rates, and concentrations of nitrate (NO_3^-), ammonium (NH_4^+), urea, bulk particulate organic nitrogen (PON), bulk particulate organic carbon (POC), and bulk chlorophyll-a (chl-a). **Figure S1** shows the cruise tracks for summer 2018/19, winter 2019, and spring 2019 overlaid on the *in situ* sea surface

temperature. **Figure S2** shows the cell sizes of the heterotroph, nanoeukaryote, picoeukaryote, and *Synechococcus* populations, as well as their autofluorescence. **Figure S3** shows surface concentrations of dissolved phosphate, silicate, and nitrite. **Figure S4** shows the bulk (>0.3 µm) particulate organic carbon and nitrogen (POC and PON) concentrations and the contribution of the nano+ (>2.7 µm) plankton size class to the bulk concentrations. **Figure S5** summarizes expands on Fig. 7 in the main text, highlighting spatial variations in the relative abundance of detritus (DNA-negative particles), photosynthetic cells, and heterotrophic cells, and their relationships to the ambient NH_4^+ concentration. **Figure S6** shows the relationship between the specific rates of total N uptake (i.e., the sum of NO_3^-, NH_4^+, and urea) and inorganic carbon fixation for the bulk and nano+ size classes in each of the Southern Ocean zones.

Text S1: Hydrographic fronts and zones of the Southern Ocean

The Agulhas Front (AF) forms at the southern edge of the Agulhas Return Current in the Subtropical Zone (STZ) and is present between 15°E and 70°E (Belkin & Gordon, 1996; Lutjeharms, 1985; Lutjeharms & van Ballegooyen, 1988; Lutjeharms & Ansorge, 2001). High mesoscale activity in the STZ between the AF and Subtropical Front (STF) (Lutjeharms & Valentine, 1984) has been observed to drive elevated levels of biological activity that reaches a minimum in winter (Machu & Garçon, 2001). South of the STF, the Subantarctic Zone (SAZ) is a region of subduction where Subantarctic Mode Water (SAMW) forms (McCartney, 1977). The SAZ is bordered at its southern edge by the Subantarctic Front (SAF) that also forms the northern boundary of the Polar Frontal Zone (PFZ) (Orsi et al., 1995; Tréguer & Jacques, 1992). Antarctic Intermediate Water (AAIW) is formed in the PFZ and subducts at the SAF (Sloyan & Rintoul, 2001). The subduction of SAMW and AAIW is critical for the solubility pump (Daly et al., 2001) and global ocean fertility, with SAMW supplying nutrients to the low-latitude thermocline that support 33-75% of net community production north of 30°S (Marinov et al,. 2006; Palter et al., 2010; Sarmiento et al., 2004; Fripiat et al. 2021). The PFZ is a transition zone between Antarctic and Subantarctic waters and is characterised by high physical variability that drives eddy formation (Emery, 1977; Gordon et al., 1977). The highly turbulent Polar Front (PF), which constitutes the southern boundary of the PFZ, can meander considerably, merging at times with the SAF (e.g., Langlais et al., 2011; Sokolov & Rintoul, 2009) and splitting in some regions into two separate fronts, the PF and the South Polar Front (SPF) (Moore et al., 1999; Pollard et al., 2002). The PF (or SPF in the case of branching) is the northern boundary of the Open Antarctic Zone (OAZ), the northern domain of the Antarctic Zone (AZ) that remains ice-free year round (Orsi et al. 1995). The OAZ is bounded to the south by the Southern Antarctic Circumpolar Current Front (SACCF), which constitutes the southern edge of the ACC core and is the only

front that is not a boundary between distinct surface water masses (Orsi et al., 1995). Instead, the SACCF denotes the southernmost extent of Upper Circumpolar Deep Water (Talley et al. 2011). South of the SACCF is the southern domain of the AZ, the Polar Antarctic Zone (PAZ), which experiences seasonal sea-ice cover (DiFiore et al., 2009). In the western Indian sector of the Southern Ocean, the PAZ encompasses the easternmost reaches of the Weddell Gyre and the Marginal Ice Zone (MIZ) (Orsi et al., 1995), where the latter lies south of the Southern Boundary (SB) of the ACC and is a biologically-active zone of partial sea-ice cover (e.g., Squire, 1998).

Text S2: Flow cytometry methods

Autofluorescence was detected in the following bandpass filter sets, named for commonly-used fluorochromes: allophycocyanin (APC, 660/20), R-phycoerythrin (PE) (575/25), fluorescein isothiocyanate (FITC) (525/20), PE-cyanine 7 (PE-Cy7) (780/40), PE-Texas Red (610/20), and Pacific Blue (450/50). Background "noise" was gated out based on the forward- and side light scatter values (FSC = 800 and SSC = 200). DNA-containing cells were isolated in each sample based on their detected autofluorescence on the FITC bandpass filter (above a minimal fluorescence threshold of 10^3 RFU).

Based on their detected autofluorescence on the APC bandpass filter relative to the PE bandpass filter, the isolated DNA-containing cells were subsequently grouped into the following populations: Nano- and picoeukaryotes, and *Synechococcus*. Additionally, small heterotrophic cells were identified, as containing DNA but with the lowest detected autofluorescence across all bandpass filters, except the FITC (Marie et al., 1997; Gasol & del Giorgio, 2000). All particles lacking DNA were considered detritus. For each sample, data acquisition was terminated when a minimum of 5000 and maximum of 10000 events was recorded. The populations of interest were gated using FlowJo 10.3 software (TreeStar, Inc.; www.flowjo.com).

Relative cell sizes were determined using 60 µL of SPHERO™ Blank Calibration Particles, 1.8 – 2.2 µm in diameter, added to 1 mL of selected samples to yield a final concentration of ~$6x10^5$ particles mL^{-1}. Relative to the 1.8 – 2.2 µm calibration beads, the fixed nanoeukaryotes were larger than 2.2 µm, picoeukaryotes and heterotrophic cells were smaller than 1.8 µm, and *Synechococcus* exhibited a range of sizes around 2 µm, with two distinct subgroups; one of ~2 µm in size and another slightly larger than 2.2 µm (see Fig. S2). *Synechococcus* was isolated from the nanoeukaryotes by its pigment characteristics – both subgroups of *Synechococcus* had high PE relative to APC content (Barlow et al., 1985; Marie et al., 1997), whereas nanoeukaryotes had high APC and PE. The "true" sizes of the phytoplankton cells may have been greater than

those reported here as post-collection fixation of samples with glutaraldehyde can lead to cell shrinkage, as well as cell lysis (Eckford-Soper & Daugbjerg, 2015).

Text S3: Coupling of NPP and N uptake

The specific carbon fixation rate (V_C) was calculated as ρC/[POC] and the specific uptake rate of total N (V_{Ntot}) was calculated as ρN_{tot}/[PON] (where $\rho N_{tot} = \rho NH_4^+ + \rho NO_3^- + \rho Urea$). No urea uptake experiments were conducted at the underway stations at 50.7ºS and 55.5ºS (both AZ); here, V_{Ntot} was calculated omitting V_{urea}.

Plotting the specific rate of total dissolved N uptake (V_{Ntot}) against that of inorganic carbon fixation (V_C) at each station provides a means of assessing the coupling between autotrophic N consumption and primary production (Fig. S7). If the data fall on a 1:1 line in this space, the implications are that 1) primary production is well represented by total N uptake (i.e., we have not overlooked a potentially significant N source to phytoplankton in designing our experiments), and 2) all measured N uptake can be attributed to phytoplankton. Deviations from the 1:1 relationship can thus provide information about the biogeochemical functioning of the plankton system (Flynn et al., 2018; Mdutyana et al., 2020; Peng et al., 2018).

The bulk specific rates at the AZ stations fall slightly above the 1:1 line, with $V_{Ntot} > V_C$ (circles in Fig. S7). This can be interpreted to evince assimilation of dissolved N (likely NH_4^+ and/or urea) by heterotrophic bacteria, which would occur in the absence of carbon fixation. Indeed, significant rates of NH_4^+ uptake by heterotrophic bacteria have been inferred previously for the winter Southern Ocean from a decoupling of NPP and total N uptake (Mdutyana et al., 2020). Values of V_{Ntot} in excess of V_C could alternately be due to the stimulation of phytoplankton NH_4^+ uptake (to values in excess of the "true" *in situ* rates) following $^{15}NH_4^+$ tracer addition (Lipschultz, 2008). However, the ambient NH_4^+ concentrations in the AZ were the highest of the transect, and $^{15}NH_4^+$ was added at 10-20% of these ambient concentrations, which is unlikely to have stimulated V_{NH4}. See also section 3.2.6 of the main text

For the STZ stations, the bulk values of V_C considerably exceed V_{Ntot} (triangles in Fig. S7). One interpretation of such a deviation is that some fraction of the measured NPP was supported by an N source that we did not account for (Mdutyana et al., 2020; Peng et al., 2018). However, V_{Ntot} in the STZ includes NO_3^-, NH_4^+, and urea uptake, the three species that typically fuel nearly all phytoplankton growth. A further potential N source is N_2 fixation, but the available data suggest that this process is very limited in the open subtropical South Atlantic (Mather et al., 2008; Moore

et al., 2009), as is atmospheric N deposition (Baker et al., 2003; Jickells et al., 2016; Yan et al., 2013). An alternate possibility, as has been suggested for oligotrophic subtropical waters (Fawcett et al., 2018), is that phytoplankton growing under nutrient-limited conditions fix carbon in excess of their stoichiometric requirements (and thus in excess of $V_{N_{tot}}$) and then exude it into the environment where it will ultimately contribute to the formation of transparent exopolymer particles (TEP; Alldredge et al., 1993; Chin et al., 1998; Corzo et al., 2000; Engel, 2004; Mari et al., 2017). While we cannot rule out this possibility, particularly given that phytoplankton were probably iron-limited at the time of sampling, the macronutrient concentrations at the STZ stations (NO_3^- of 2.4-3.4 µM and PO_4^{3-} of 0.39-0.49 µM; Fig. 2c) were unlikely to be limiting to subtropical phytoplankton. A perhaps more plausible explanation, therefore, is that the apparent surface decoupling of V_C and $V_{N_{tot}}$ is an artefact of our sampling design insofar as it may not hold over the entire mixed layer given the far stronger light dependence of photosynthesis (i.e., V_C) compared to inorganic N uptake (particularly NH_4^+ and urea; Dortch, 1990). In other words, if we had depth-resolved data, mixed-layer integrated V_C and $V_{N_{tot}}$ may not be decoupled.

Tables

Table S1: Correlation matrix of leg S and N surface ocean concentrations of bulk PON, bulk POC, bulk chl-a, NH_4^+, NO_3^-, and urea, and rates of net primary production (NPP), N uptake (as NH_4^+, NO_3^-, and urea), f-ratio (excluding ρUrea), and NH_4^+ oxidation. *, **, ***, and **** indicate levels of significance of the Pearson correlation coefficients of 0.05, 0.01, 0.001, and 0.0001, respectively.

Figures

Figure S1: Cruise tracks for a) summer 2018-2019, b) winter 2019, and c) spring 2019 overlaid on sea surface temperature (SST) measured by the ship's thermosalinograph. Solid lines indicate the positions of the fronts. Abbreviations for zones: STZ – Subtropical Zone; SAZ – Subantarctic Zone; PFZ – Polar Frontal Zone; AZ – Antarctic Zone. Figure produced using the package ggplot2 (Wickham, 2016).

Figure S2: Size distributions (inferred from forward scatter area, FSC-A) of a) heterotrophic bacteria, c) nanoeukaryotes, e) picoeukaryotes, and g) *Synechococcus* relative to SPHERO™ Blank Calibration Particles (1.8 – 2.2 µm in diameter; indicated by the red band), and cytograms showing allophycocyanin content (APC-A) relative to phycoerythrin content (PE-A) for the

populations of b) heterotrophic bacteria, d) nanoeukaryotes, f) picoeukaryotes, and h) *Synechococcus*.

Figure S3: Surface concentrations of dissolved a) phosphate (PO_4^{3-}), b) silicate ($Si(OH)_4$), and c) nitrite (NO_2^-) during Legs S and N. Abbreviations for zones are as in Figure S1, with the addition of OAZ – Open Antarctic Zone; PAZ – Polar Antarctic Zone (collectively, the AZ). Abbreviations for fronts: AF – Agulhas Front; STF – Subtropical Front; SAF – Subantarctic Front; PF – Polar Front; SACCF – Southern Antarctic Circumpolar Current Front; SBDY – Southern Boundary. Figures produced using the package ggplot2 (Wickham, 2016).

Figure S4: Leg S and N surface ocean concentrations of bulk (>0.3 µm) a) POC and b) PON, and nano+ (>2.7 µm) plankton contributions (%) to bulk c) POC and d) PON. Note that the colour scales in panels c and d have been skewed to show 50 to 100% since the nano+ size class is dominant across the transect. Only stations where PON and POC concentrations for the nano+ size class were measured are shown in panels c and d (black circles). Station 58.5°S, where the nano+ contribution was >100% of the corresponding bulk PON concentration, likely due to a measurements artefact, was excluded from panel d. Abbreviations are as in Figure S1 and S3. Figure produced using the package ggplot2 (Wickham, 2016).

Figure S5: Relative abundances of a) total photosynthetic versus heterotrophic bacteria and b) detritus versus heterotrophic bacteria at the surface for Leg S. The surface NH_4^+ concentration at each station is indicated by the yellow dots. The values in maroon text on the right side of panel a are the photosynthetic-to-heterotrophic cell ratios. The upper x-axis in panel b begins at 75% in order to highlight the (much smaller) heterotrophic bacterial contribution to the summed detrital + heterotrophic particles. Frontal abbreviations are as in Figure S1 and S3.

Figure S6: Specific rates of total nitrogen uptake (V_{Ntot}, = ρN_{tot} / [PON]) relative to carbon fixation ($V_{C,}$ = NPP / [POC]). Error bars represent ± 1 standard error for duplicate experiments, with error propagated according to standard statistical practices. Light grey error bars are associated with values calculated by subtraction (0.3-2.7 µm (i.e., pico) size class) and black error bars indicate the error for experimental duplicates. The specific rates are shown in relation to a 1:1 line, on which the data points are expected to fall if NPP is supported by the sum of NO_3^-, NH_4^+, and urea uptake. Circles indicate AZ stations (OAZ and PAZ), squares indicate PFZ stations, diamonds indicate SAZ stations, and open triangles indicate STZ stations. Open symbols are used for the STZ to differentiate them from "true" Southern Ocean samples. Dotted circles indicate samples where no V_{urea} was measured. Figure produced using the package ggplot2 (Wickham, 2016).

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