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# 42 Abstract

Hundreds of studies have surveyed plastic debris in surface ocean gyre and convergence zones, 43 however, comprehensive microplastics (MP,  $\leq 5$  mm) assessments beneath these surface 44 accumulation areas are lacking. Using *in-situ* high-volume filtration, Manta net and MultiNet 45 46 sampling, combined with micro-Fourier-transform-infrared imaging, we discovered a high abundance (up to 244.3 pieces per cubic meter [n m<sup>-3</sup>]) of small microplastics (SMP, 47 characteristically <100 um) from the surface to near-sea floor waters of the remote South Atlantic 48 Subtropical Gyre. Large horizontal and vertical variations in the abundances of SMP were 49 50 observed, displaying inverse vertical trends in some cases. SMP abundances in pump samples were over 2 orders of magnitude higher than large microplastics (LMP,  $>300 \mu m$ ) concurrently 51 collected in MultiNet samples. Higher density polymers (e.g., alkyd resins and polyamide) 52 comprised >65% of the total pump sample count, highlighting a discrepancy between polymer 53 compositions from previous ocean surface-based surveys, typically dominated by buoyant 54 polymers such as polyethylene and polypropylene. Contrary to previous reports stating LMP 55 preferentially accumulated at density gradients, SMP with presumably slower sinking rates are 56 much less influenced by density gradients, thus resulting in a more even vertical distribution in 57 the water column, and potentially longer residence times. Overall, our findings suggest that SMP 58 is a critical and largely underexplored constituent of the oceanic plastic inventory. Additionally, 59 our data support that weak current systems contribute to the formation of SMP hotspots at depth, 60

61 implying a higher encounter rate for subsurface particle feeders. Our study unveils the prevalence 62 of plastics in the entire water column, highlighting the urgency for more quantification of the 63 deep-ocean MP, particularly the smaller size fraction, to better understand ecosystem exposure 64 and to predict MP fate and impacts.

# Key words: FTIR imaging, *in-situ* pump, microplastics, plastic marine debris, water column, South Atlantic Gyre

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### 68 Introduction

It has been estimated that between 19 and 23 million metric tons of plastic waste entered the 69 world's aquatic ecosystems in 2016 alone (1). Much of this plastic waste ultimately enters the 70 marine environment, where it is fragmented via physical, chemical and biological processes (2), 71 and subsequently a portion of these plastics are transported across the World Ocean. Currently, up 72 to 51 trillion microplastic (MP) particles are estimated to be floating in the surface waters of the 73 global ocean (3). However, a size-selective loss of MP has been observed at the sea surface (4, 5), 74 suggesting the surface ocean is not the ultimate sink for plastic debris, bringing the ultimate fate 75 76 of MP into question. Empirical evidence and modelling studies suggest that biological and physical processes of the upper ocean could redistribute MP drifting at sea throughout the pelagic 77 water column (6). A vast majority of plastic marine debris surveys, however, have focused on 78 surface waters (4, 5, 7), and to a lesser extent, deep-sea sediments (8-10). 79

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Although the deep sea is Earth's largest ecosystem by volume and area (11), waters below 200 m 81 82 (12) are just beginning to be explored for MP distributions and include the following: a report by La Daana et al. (13) showed that MP depth profiles ranged from 0-375 n m<sup>-3</sup> in the Arctic Central 83 Basin, which were analyzed and reported from Niskin bottles collected water samples (7-12 L); a 84 study by Choy et al. (14) employed a large volume (1007-2378 m<sup>3</sup> per sample) in-situ filtration 85 technique to harvest across epi- and mesopelagic depths to quantify MP abundances (~1.0-15.0 n 86 m<sup>-3</sup>) in Monterey Bay: and a study by Ross et al. found average MP was 37.3 n m<sup>-3</sup> in Niskin-87 bottle collected seawater samples (29-67 L) and determined these plastics to be pervasive 88 throughout the water column in the Beaufort Sea (15). Nevertheless, all of these studies were 89 limited to spectroscopic measurements that relied on visual presorting of plastic-like particles and 90 inherently could not reliably include MP <100 µm, a fraction previously demonstrated by other 91 workers to be numerically abundant in the ocean (16, 17). Using micro-Fourier transform infrared 92 (µFTIR) spectroscopic imaging with automated data analysis, Tekman et al. (18) measured MP 93 concentrations from surface waters to deep-sea sediments by filtering >200 L of seawater in the 94

Arctic. The authors unveiled typical concentrations spanning 0-1287 n m<sup>-3</sup>, with MP  $\leq$ 25 µm accounting for more than 50% of the particle counts in each sample.

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As plastic particles disintegrate into smaller size fractions, they can become harmful in different 98 and unpredictable ways that are only beginning to be understood. For example, MP smaller than 99 150 µm can translocate across the gut epithelium (19), become trapped in biomass, and have the 100 potential to transfer through marine food webs, posing an unknown ecological risk and 101 biogeochemical impacts. The occurrence of these smaller MP in food webs and surrounding 102 seawaters, however, is underreported due to the difficulty of sampling high volumes of water and 103 the need for specialized analytical methods required for characterizing small particles from 104 samples (20). 105

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The presence of accumulation zones of plastic marine debris located within the five subtropical 107 gyres has been verified (4, 5, 7). These studies all focused on LMP collected via surface-trawl 108 plankton nets and were sorted visually. To date, only two studies have reported the presence of 109 smaller MP (<300 µm) in the surface waters of the North Atlantic Gyre (16, 21). Most recently, 110 11.6-21.1 million tonnes of polyethylene (PE), polypropylene (PP), and polystyrene (PS) MP (32-111 651 µm) were estimated to be in the top 200 m of the Atlantic Ocean after collection from three 112 discrete depths along a north-south transect with in-situ pumps and µFTIR polymer identification 113 (22); however, this study was limited to only PE, PP and PS, presenting a possible knowledge gap 114 of other plastic polymers (22). Recently, other polymers (e.g., polyamide, polyurethane) rather 115 than PE, PP and PS have been reported to be the dominant plastics of sub-surface marine samples 116 (18, 23). Therefore, a more complete dataset containing all identified polymers is necessary to 117 provide a comprehensive understanding of ocean plastic pollution. It is plausible that small MP 118 are ubiquitous in the deep-ocean waters underneath offshore plastic accumulation zones, based on 119 their high abundances in the upper layer (<200 m) of mid-latitude oceanic gyres mentioned above 120 (16, 21, 22). Furthermore, the observations of open-ocean SMP in the shallow and deep-pelagic 121 waters have only been reported in two studies (18, 22), both of which disclosed the inconsistency 122 of the vertical trends of plastic abundance and distribution across sampling stations. This unclear 123 pattern in vertical profiles of water-column plastics has been attributed to the unknown 124 redistribution and removal processes in the ocean interior (6). A better mechanistic understanding 125 of how plastics sink from the ocean surface beyond the mixed layer and ultimately to abyssal 126 depths is critical to predicting its fate and impacts on the marine ecosystems. 127

In this study we sampled plastic particles in the South Atlantic Subtropical Gyre (SASG, Fig. 1) 129 by deploying a combination of surface Manta nets (SI Appendix, Table S1), multiple 130 opening/closing nets at discrete depths in the top 100 m (SI Appendix, Table S1), and McLane in-131 situ Large Volume Water Transfer System (WTS-LV) pumps at four different stations from 132 surface to near-seafloor depths (SI Appendix, Table S2) in the South Atlantic plastic accumulation 133 zone (4). Our combined analysis procedure, including the automated interpretation of spectral 134 datasets created by µFTIR imaging, provide a more integrative view of the distribution, 135 abundance, dimensions and chemical nature of plastic particles in the interior of an ocean gyre. 136

#### 137 Materials and Methods

Study Area and Sample Collection. Samples were collected while onboard the RV Pelagia cruise 138 64PE-448 during the 21-day long "Sinergia" SASG Cruise (January 4th-24th, 2019). Two stations 139 (A, D) located in the outer accumulation zone of the SASG, and two stations (B, C) located in the 140 inner accumulation zone, were chosen to collect oceanic particle samples in the water column 141 (Fig.1). According to previous model results (4, 5), the outer accumulation zone of the SASG has 142 a modeled average concentration of surface plastics of  $\sim 100$  g per km<sup>2</sup>, while the inner 143 accumulation zone shows a mean concentration of plastics of  $\sim 600$  g per km<sup>2</sup>. Manta net 144 (Oceaninstruments) and MultiNet (Hydro-bios) samples (Fig.1 and S1, Table S1) were collected 145 before deploying the WTS-LV pumps (LV08, McLane Research Laboratories). Four WTS-LV 146 pumps were used in total: three pumps were fitted with three standard radial filter holders and one 147 pump was fitted with a 3-tier filter holder. All filter holders were made of black acetal which we 148 analyzed for our polymer workflow library. The site of deploying the WTS-LV pumps at each 149 station were purposely distant (on average about 5 nautical miles) from the location where the 150 Manta net and MultiNet were retrieved. These intervals assured the prevention of potential 151 contamination of pump samples from the possible shedding of nylon fiber from the mesh material 152 of the Manta net and MultiNet collection devices. For the details of net and pump samplings, 153 please refer to Table S1 and S2. Manta trawls were carried out using a Manta net (0.86 m wide  $\times$ 154 0.15 m vertical opening) with a 500 µm mesh net and a closed cod end. The MultiNet had an 155 opening of  $0.5 \times 0.5$  m, with 200 µm mesh nets and plastic cod ends with 100 µm mesh windows. 156 The Manta nets were towed in a straight line for 30 minutes at 2 knots at each Manta net tow 157 158 station (n = 5, MT-05, -14, -16, -18, -22, Fig.1). The MultiNet samples were collected at five different depths (Table S1). The net was lowered to the maximum depth with all nets closed, then 159 nets were opened/closed individually and towed for 25-48 min at each depth. The net depth was 160 monitored continuously and maintained within 3 m of the target depth, then closed before raising 161

the frame to the next depth. Three MultiNet deployments (MN-01 at depths of 20, 40, 60, 80 100 162 m; both MN-02 and MN-03 at depths of 5, 10, 20, 40, 60 m) were conducted around the WTS-LV 163 pump stations A, B and C (Fig.1, Table S1 and S2). The volume of seawater filtered was 164 determined from the readings of a mechanic flowmeter (2030RC, General Oceanics) within the 165 mouth of the net. Once the net (Manta net or MultiNet) was retrieved from the sea, it was 166 thoroughly rinsed on board with a seawater hose from the outside inwards, from the mouth 167 toward the end to accumulate all the material in the cod-end. Then, the cod-end was removed and 168 rinsed down into a clean bucket. In the onboard laboratory, any plastic-like particles floating in 169 each bucket were picked out visually. Then remaining samples in the bucket were poured onto a 170 300-um mesh size sieve. Once sieved, the content retained was carefully examined, and plastic-171 like particles were transferred to the white plastic tray. When in doubt, the particle was checked 172 173 with the aid of a stereomicroscope and retained for FTIR analysis. Counts were documented for each Manta net and MultiNet sample. Finally, particles were dried and stored in the dark in glass 174 scintillation vials with foil lined caps (20 ml) for polymer analysis (see below). 175

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177 At each station, a conductivity-temperature depth (CTD, Sea-Bird Electronics) profile was collected to measure standard parameters (e.g., conductivity, temperature, dissolved oxygen) of 178 the deployment area. Using the profiles of these parameters (Fig. S2), distinct water layers of 179 interest were identified such as the mixed layer, pycnocline, Antarctic Intermediate Water (AAIW) 180 181 and the Antarctic Bottom Water (AABW). The WTS-LV pumps were tethered to a single wire and deployed at predetermined depths (Fig.S2, Table S2) using a modified pumping method (24). 182 For each pump unit, seawater was directed through a series of filters: The samples passed through 183 stacked pre-combusted 200 and 40 µm stainless steel (SS) mesh screens followed by the pre-184 combusted 2.0 µm quartz fiber filter (Whatman QM-A). Thus, >200, 40-200, and 2-40 µm size-185 fraction particles were retained by the SS and QM-A filters. The effective filtration area of these 186 filters was ~125 cm<sup>2</sup>. The reasons for selecting QM-A filters were as follows: 1) quartz fiber 187 filters can be pre-combusted to avoid potential contamination, which is particularly important for 188 studying small microplastic; and, 2) in comparison to membrane filters, QM-A filters have better 189 particle loading and uniformity (24). The disadvantage of using QM-A filters, however, is that its 190 pore tortuosity (a fluid dynamic term for the complex path a particle takes with twists and turns) 191 is better suited for trapping particles at the surface and inside the filter media. However, retaining 192 particles within the filter layers is not conducive to particle extraction and thus probably results in 193 an underestimation of plastic abundances. During particle extraction in this study disintegration of 194

QM-A filters was observed which would release the entrapped particles and improve recoveryrate.

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All pumps were programmed to run for five hours and stop if flow rates fell below 3-4 L/minute, resulting in a range of 440 to 1765 L seawater filtered, depending on *in-situ* particle concentrations (Table S2). Of all filters, only QM-A filters, which collected 2-40 µm sized particles, were analyzed in this study. The QM-A filters and SS meshes were folded and wrapped with pre-combusted foil, and immediately stored at -80 °C until analysis. At the end of the cruise, all filters were transported on dry ice to the laboratory and stored at -80 °C prior to further processing. Calm seas prevailed during the WTS-LV pump sampling (Table S1).

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Extraction of SMP in Pump Samples. To isolate SMP, the QMA filters wrapped within the 206 aluminum foil were thawed to room temperature for  $\sim 3$  h under a laminar flow hood. Three 207 subsamples of the QM-A filters from each pump were taken with a flamed metal punch (23 mm 208 diam.) sampling along the surface. Based on the filtration efficiency and filtration area 209 210 calculations of the QM-A filters, each subsample corresponded to ~100 L of filtered seawater and were therefore recognized as one technical replicate. A total of three technical replicates were 211 analyzed for each pump, and the filtered seawater volume of each replicate ranged from 102.3 L 212 to 139.9 L (Table S1). For each replicate, the sub-sampled QM-A filters were placed into separate 213 pre-combusted borosilicate glass scintillation vials (20 ml, DMK, Life Sciences Kimble) 214 containing sodium iodide (NaI: 1.65-1.70 g cm<sup>-3</sup>) and sonicated for 5 min. These subsamples were 215 then pooled into a 50 ml conical centrifugation tube (DMK, Life Sciences Kimble) and 216 centrifuged at 1600 rpm for 15 minutes. The supernatant was filtered onto a pre-combusted 217 stainless-steel filter (5 µm), and this procedure was repeated at least three times per replicate. The 218 sample on the stainless-steel filter was stored in a scintillation vial with 5 ml 5% potassium 219 hydroxide at 60 °C for 24 hours for removal of organic matter. Potassium hydroxide was 220 neutralized with hydrochloric acid, and particles on the stainless-steel filter were detached by 221 sonication for 5 minutes. Particles in the liquid were concentrated onto an Anodisc filter (0.2 µm 222 pore size, GE Whatman) held in a glass filter holder (Advantec, 13 mm in diameter), followed by 223 a thorough vial rinsing step with 50% ethanol to remove SMP that could possibly adhere to the 224 glass vessel. The rinsing steps was done three times to increase SMP yields. The Anodisc filter 225 was then dried at 37 °C overnight prior to µFTIR analyses. 226

To test the influences of the sample storage and experimental steps, aged plastics (n=10) from our Manta net samples were placed into a pre-combusted glass scintillation vial and stored at -80 °C for 3 days. Plastics were subsequently thawed at room temperature for 3 hours and processed following the identical procedure as the samples. The mechanical transformation was evaluated by comparing the geometries (i.e., Feret diameter) and weights of these particles at pre-test to post-test. No apparent changes were found.

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Polymer Analysis. SMP particles were identified by µFTIR spectroscopy (Thermo Scientific™ 235 Nicolet<sup>™</sup> iN10, USA) in a HEPA filtered laminar flow hood. The entire sample-filter area (13 236 mm in diameter) was mapped using the FTIR microscope in transmission mode using an MCT/A 237 detector (aperture size: 25  $\mu$ m × 25  $\mu$ m, 1 scan at 16 cm<sup>-1</sup> resolution, step size: 21 and 22  $\mu$ m). 238 The background position was selected as an area clear of particles on the Anodisc filter. Upon 239 acquiring the FTIR chemical images of samples (more than 17 million individual spectra were 240 generated in total), the particle polymer types were confirmed using two steps (Fig. S3): (Step I) 241 All spectra of the resulting chemical maps were subject to preprocessing with the auto-baseline 242 243 correction and normalization in OMNIC Picta (Thermo Fisher Scientific, USA). All spectra within the whole chemical map were exported to CSV files and SPA files. Then all the spectra 244 (CSV files) were compared automatically against a transformed reference library (25) with the 245 search algorithm (Pearson's correlation) using a Python script. When Pearson's correlation coeffi-246 cient between the experimental and the library spectrum was larger than 0.8, the position and 247 polymer types of each identified spectrum were recorded; (Step II) Based on the coordinates, 248 spectra were tracked in chemical maps, and the polymer identity was confirmed by comparing 249 with the Hummel Polymer Sample Library in OMNIC Picta. The software interpretation was 250 systematically validated (>70% match with reference spectra) or rejected (<60% match with 251 reference spectra). Spectra with a match between 60% and 70% were individually reinterpreted 252 by confirming the clear presence of specific polymer peaks. The particle was only verified to be 253 plastic if its spectrum was confirmed as the same polymer type by both steps. Procedure blanks 254 were scanned and analyzed with the same methods. 255

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To verify the polymer composition in the Manta net samples, the FTIR Spectrometer (Thermo Scientific<sup>TM</sup> Nicolet<sup>TM</sup> iN10, USA) in the attenuated total reflection mode in the spectral range from 3600 to 1250 cm<sup>-1</sup> was applied to selected particles. A total of 33% (n = 457) of the total particles collected in nets (n = 1406) was analyzed. The polymer type of each particle was

confirmed by comparing its FTIR spectra against the Hummel Polymer Sample Library in
 OMNIC Picta. The polymer composition was identified according to the criteria mentioned above.

**Oxidation Degree.** Plastics are manufactured with different additives (e.g., plasticizers, stabilizers) 264 based on polymer application (e.g., plasticizers, stabilizers) and therefore may differ in their 265 reaction to various levels of environmental exposure to temperature and ultraviolet light. 266 Consequently, plastic debris may exhibit a large variety of degradation signatures. Characterizing 267 weathering degree is critical to better understand the origin and fate of environmental plastics (15). 268 The carbonyl indices have been often used to estimate the weathering degree of plastics in 269 literature (15, 26, 27). Therefore, PE polymers, the most common resin types found in the open 270 ocean, were selected to study oxidation degree by comparing carbonyl indices. The carbonyl 271 272 indices of PE large microplastics (PE-LMP) in the Manta net samples and the PE small microplastics (PE-SMP) collected by the WTS-LV pumps were calculated by measuring the ratio 273 of the absorbance band area of the carbonyl group at 1630-1850 cm<sup>-1</sup> and the olefinic band area at 274 1420-1490 cm<sup>-1</sup> (28, 29). Although little degradation of plastics due to the 10% KOH processing 275 276 step has been documented (30-32) we took the extra precaution in our study to employ 5% KOH to purify samples and calculated carbonyl index based on two band areas, which differs from 277 previously published methods (two spectral peaks), thus further minimizing the potential artifacts 278 of KOH treatment on FTIR spectra and carbonyl index determination. 279

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SMP Characteristics. The optical images of particles were often partially blurred due to the 281 combination of irregularly shaped particles and the motion of particles during the high-speed 282 imaging (10 steps per second). Therefore, the major (the maximum Ferret's diameter) and the 283 minor axes (the longest axis perpendicular to the major axis) of each SMP were measured on the 284 pseudo-color infrared image according to a method by Simon et al. (33). Briefly, the infrared 285 image of each identified particle was located with the OMNIC Picta depending on its x, y 286 coordinates. Based on the polymer assignment, the infrared image was highlighted in the specific 287 band region of the assigned polymer. Band regions for distinguishing polymer types were 288 provided by Löder et al. (34). Then, all neighbor pixels showing the same polymer assignment 289 were confirmed and recognized as the same particle. By observing the pseudo-color image, the 290 boundary of the particle, which shows significant contrast with the ambient pixels, can be clearly 291 determined (Fig. S3). The particle dimensions were measured with the ruler tool in the OMNIC 292 Picta software. In this study, the uncertainty of plastic size measured on infrared images lies 293

within 5  $\mu$ m when comparing to particle images obtained in the visible wavelengths. However, such an uncertainty would not cause a significant error in the particle size distribution.

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The aspect ratio of the SMP, a measure of particle shape, can be estimated by calculating the ratio of the minor and major axes. To estimate the mass of the identified plastic particle, each SMP was assumed to be an ellipsoid. Based on the ellipsoid volume model (*35*), the volume of SMP was calculated using the major (a), minor (b), and intermediate (c) axes:

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$$V = \frac{\pi a b c}{6}, (1)$$

The intermediate axis (c) of each SMP was estimated by multiplying the minor axis and the median aspect ratio (0.67 ± 0.12) of all SMP (*33*). The mass of SMP was calculated from the volume and the density ( $\rho$ , Table S5) of the assigned plastic type:  $Mass_{MP} = \rho V$ . (2).

Theoretical Sinking Rates of SMP. Because of their small sizes, SMP particles (n=148) in our study, which are heavier than the averaged seawater density of the top 200 m, are characterized by low Reynolds numbers. Therefore, the theoretical sinking velocities (v) of SMP particles composed of polymers denser than the seawater was calculated using the Stokes' formula:

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$$v = 2(\rho_p - \rho_f)gR^2/9\mu$$
, (3)

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where  $\rho_p$  is the density of the particles (kg m<sup>-3</sup>, Table S5).  $\rho_f$  is the mean density of the seawater of the top 200 m in our study area (1.026 kg m<sup>-3</sup>). *g* is the gravitational field strength (m s<sup>-2</sup>). R, the radius of the equivalent spherical diameter, was calculated on the basis of a best-fit ellipse used in the formula (1).  $\mu$  is the dynamic viscosity of seawater (~10<sup>-3</sup> kg m<sup>-1</sup> s<sup>-1</sup>) taken from Ardekani and Stocker (*36*).

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*LMP Data Processing.* All identified polymer particles from the Manta net samples were weighed on an analytical balance. The averaged MP mass per piece was the total weight of plastics divided by the total number and subsequently employed to estimate the plastic mass in both Manta net and MultiNet samples. Because of the wind-induced vertical mixing, MP counts collected by Manta net may underestimate the total number of buoyant plastics in the area sampled (*37*). Thus, the integrated MP abundance/mass for the top 5 m in our study area was approximated with the equation and parameters used in literature (*38,39*)

*Hydrodynamic data.* The climatology of sea surface height and geostrophic current was computed using the daily data obtained from the Copernicus Marine and Environment Monitoring Service (CMEMS, http://www.marine.copernicus.eu). It has a spatial resolution of 0.25° spanning from 1993 to 2018. The Simple Ocean Data Assimilation (SODA) reanalysis version 3.4.2 (40) was used for constructing the depth-dependent velocity profiles. The product is available from 1980 to 2018 with a spatial resolution of 0.5° and 50 vertical levels.

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333 The stratification of the water column was cast by the buoyancy frequency,

334 
$$N = \sqrt{\frac{g \,\partial \rho}{\rho_f \partial z}} \,, (4)$$

where *g* is the gravitational acceleration,  $\frac{\partial \rho}{\partial z}$  is the vertical gradient of the potential density, and  $\rho_f$ is the reference density defined above.

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Contamination Prevention. To avoid potential contamination, we employed the following 338 protective measures (41): (1) Prior to the cruise, all filters were combusted at 450 °C for >5 h to 339 remove organic matter; (2) Aboard ship, all sample handling was conducted in a laminar flow 340 hood (AirClean 600 Workstation; AirClean Systems), located in a dedicated, clean room with 341 restricted entry where only natural fiber clothing was allowed; vents in this room had AirClean 342 pre-filters taped over them to minimize dust; (3) WTS-LV filter holders were disassembled and 343 soaked in fresh water for 20 minutes, and then thoroughly rinsed with pressurized fresh water, and 344 finally followed by three rinses using 0.2 µm filtered MilliQ water; (4) The clean filter holders 345 were immediately wrapped with pre-combusted foil and stored in the laminar flow hood in the 346 clean room; (5) The laminar flow hood was cleaned and run for at least for 15 min prior to usage 347 and all loading and removal of filters were performed in the laminar flow hood; (6) Tweezers for 348 handling filters were rinsed with 6 nm filtered seawater (from a tangential flow filtration unit) 349 between processing different pore sized filters; and (7) Pre-combusted 47 mm GF/A filters were 350 exposed in the hood during filter loading and sample processing to monitor possible MP 351 contamination. Nitrile gloves and cotton lab coats were worn during field and laboratory work. 352

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In the laboratory at Harbor Branch Oceanographic Institute: (1) All liquids used for sample processing and analysis (e.g., Milli-Q water, potassium hydroxide solution, ethanol) were filtered through glass-fiber filters prior to use (Whatman GF/F, 0.7  $\mu$ m pore size, 47 mm diam.); (2) All glass-fiber filters and glassware (e.g., beakers, bowls, scintillation vials, Pasteur pipettes) were covered with aluminum foil, and combusted at 450 °C for >5 h; (3) Steel tweezers were washed with filtered Milli-Q water and flame combusted; (4) All sample preparation was performed in the clean laminar flow under HEPA (high-efficiency particulate air) filtration; (5) Procedural blanks in the lab (n=4) were performed by exposing GF/F filters under the laminar flow when extracting particles from QM-A filters. After the analysis of samples, these four laboratory controls together with four onboard blanks were processed employing the identical procedure as the pump samples. The resultant particles were concentrated on Anodisc filters and scanned by FTIR imaging.

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Statistical evaluation. Given that the datasets were not normally distributed (Kolmogorov-Smirnov test), and their variances were not homogeneous (Levene's test), the nonparametric Kruskal-Wallis statistical test was used for multiple comparisons. If the test was significant, pairwise comparisons were performed using the nonparametric Mann-Whitney-Wilcoxon test. Values of P < 0.05 were considered significant. A generalized additive model (GAM) was used to estimate the relationship between the length and the aspect ratio of the identified SMP. All statistical tests and graphs were performed using R software (v.3.4.3, R Development Core Team).

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#### 374 **Results**

**Contamination Controls.** The analysis results of µFTIR images of procedural blanks showed no 375 positive signals of SMP at Stations B, C and D. One polyurethane particle was confirmed at 376 Station A. In addition, 38 polyacetal particles, determined to originate from WTS-LV pump heads, 377 were identified on 17 out of 48 (35.4%) QM-A filters. The maximum quantity of polyacetal 378 particles detected on a sub-filter was nine, and that was detected at 10 m depth of Station A (SI 379 Appendix, Table S3). The size of polyacetal MP ranged from 25.6 to 3778.3 µm. The spectra of 380 polyacetal particles are shown in the Fig. S4 (SI Appendix). In subsequent data analysis, the 381 number of observed plastics in blanks at each station was subtracted from all datasets of pump 382 samples corresponding to the same station. 383

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LMP and SMP Abundance. In total, 1406 presumptive plastic particles were recovered in the 385 Manta net samples. In a subsample of these particles (n = 457), 452 particles were confirmed as 386 plastics and five could not be classified. The total weight of the Manta net collected particles 387 identified as LMP (n = 452) was 0.619 g, with the averaged LMP mass per particle calculated as 388 0.0014 g. Wind-corrected LMP abundance in the upper 5 m of the SASG varied across the Manta 389 net tow stations, with a range of 0.4-3.0 n m<sup>-3</sup> ( $0.6 \times 10^3$ -4.3  $\times 10^3$  µg m<sup>-3</sup>) and an average of 1.5 n 390 m<sup>-3</sup> (2.1  $\times$  10<sup>3</sup> µg m<sup>-3</sup>). The highest abundance was recorded at Station MT-14 (3.0 n m<sup>-3</sup>; 4.3  $\times$ 391 10<sup>3</sup> µg m<sup>-3</sup>), followed by Stations MT-16 (2.5 n m<sup>-3</sup>;  $3.5 \times 10^3$  µg m<sup>-3</sup>), MT-18 (0.8 n m<sup>-3</sup>;  $1.1 \times$ 392

<sup>393</sup>  $10^3 \ \mu g \ m^{-3}$ ), MT-22 (0.7 n m<sup>-3</sup>;  $0.9 \times 10^3 \ \mu g \ m^{-3}$ ), with the lowest abundance recorded at Station <sup>394</sup> MT-05 (0.4 n m<sup>-3</sup>;  $0.6 \times 10^3 \ \mu g \ m^{-3}$ ). These findings display a general increase in the LMP <sup>395</sup> abundance with proximity to the inner accumulation zone (Fig. 1).

396

LMP (n = 21) was found in eight out of 15 MultiNet bags at different depths (range 5-60 m, SI 397 Appendix, Table S1). LMP abundances were from 0 to  $1.1 \times 10^{-2}$  n m<sup>-3</sup> (0-15.3 µg m<sup>-3</sup>) (Fig. 2 A, 398 B, C). Disparities, spanning from 2.4 to over 5 orders of magnitude, were found in the numerical 399 abundances of plastics when comparing SMP from WTS-LV pump to LMP from MultiNet 400 samples in the upper 60 m. After correction of control values and subtraction of all polyacetal 401 polymer signal from our WTS-LV pump samples, SMP particles were detected in 34 of 48 402 analyzed subsamples. A total of 229 particles were confirmed as plastic polymers by µFTIR 403 imaging. The SMP abundance profiles ranged from 0-244.3 n m<sup>-3</sup> and 0-20.83 µg m<sup>-3</sup> (Fig. 2). No 404 plastics were found at the depth of 3490 m at Station B. SMP abundances were highly variable in 405 our four vertical profiles (Kruskal-Wallis test, P < 0.05, SI Appendix, Table S4), with the highest 406 mean abundance detected at 10 m at Station A ( $218.3 \pm 45.1 \text{ n m}^{-3}$ ), followed by 60 m at Station 407 D (85.4  $\pm$  9.5 n m<sup>-3</sup>), and 4835 m at Station C (71.1  $\pm$  21.5 n m<sup>-3</sup>) (Fig. 2 A, C, D). Generally, 408 SMP abundances in the vertical profiles decreased with depth, except at Station C (Fig. 2C). In 409 the upper 60 m, the mean abundance of SMP in the outer accumulation zone (Stations A and D, 410 mean:  $105.3 \pm 93.9$  n m<sup>-3</sup>, median: 85.4 n m<sup>-3</sup>) was significantly higher than samples taken from 411 the inner accumulation zone (Stations B and C, mean:  $24.4 \pm 24.8$  n m<sup>-3</sup>, median: 17.1 n m<sup>-3</sup> 412 (Mann-Whitney-Wilcoxon test, W = 85, P = 0.026, SI Appendix, Fig. S5). 413

414

**Polymer Composition.** A total of 12 polymer types, including theoretically buoyant polymers, 415 were identified in the SMP samples (SI Appendix, Fig. S6). Five polymer types accounted for 416 more than 80% of the particle count, including polyamide 6/6.6 (PA 6/6.6, 29.3%, n=67), alkyd 417 resin (16.2%, n=37), PP (15.3%, n=35), PE (11.0%, n=25) and polyethylene/polypropylene 418 copolymer (PE/PP, 8.7%, n=20). The polymer species of each pump sample differed, ranging 419 from 0 to 10 polymer types. PE, PP, PE/PP and Ethylene-ethyl acrylate copolymer (EEA) 420 particles that are lighter than the *in-situ* seawater (~1.025 g cm<sup>-3</sup>) were also detected in the 421 ocean's interior (Fig. 3 and SI Appendix, Table S5). In the Manta net LMP subsamples, PE was 422 the dominant polymer, corresponding to 79.2% (n = 358) of the particles, followed by PP (12.2%, 423 n = 55) and PE/PP copolymer (7.3%, n = 33). The 1.3% remaining particles (n = 6) were 424 identified as PA 6/6.6 (n = 3), polyester (n = 2) and polystyrene (PS, n = 1). 425

Size and Theoretical Sinking Rates of SMP. The length of SMP particles ranged from 20.1 to 427 321.2  $\mu$ m (mean = 58.6  $\pm$  32.4  $\mu$ m, median = 49.6  $\mu$ m), while the width spanned from 15.9 to 428 126.3  $\mu$ m (mean = 35.3 ± 11.6  $\mu$ m, median = 35.5  $\mu$ m, Fig. S7). A total of 25% (n = 57) in the 429 length and 88% (n = 202) in the width of all identified plastic particles were less than 40  $\mu$ m long 430 (SS mesh pore size), while 62% (length) and 97% (width) of particles were <56.6 µm (the 431 theoretical diagonal of square SS mesh sieve aperture). The aspect ratio of particles steadily 432 increased with decreases in length (GAM,  $R^2 = 0.63$ ,  $P \ll 0.01$ , SI Appendix, Fig. S8) indicating 433 smaller particles became spherical. The theoretical sinking velocity of SMP particles (n = 148)434 with densities higher than seawater ranged from  $2.4 \times 10^{-9}$  to  $1.0 \times 10^{-6}$  m s<sup>-1</sup>(Table S6). 435

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Oxidation Degree. In the Manta net samples, PE-LMP from the outer accumulation zone (n = 99) of the SASG exhibited modestly higher carbonyl indices in contrast to PE-LMP (n = 259) from the inner accumulation zone (Mann-Whitney-Wilcoxon test, W = 8738,  $P = 1.6 \times 10^{-6}$ , Fig. 4A). Overall, PE-SMP from WTS-LV pump samples presented significantly higher carbonyl indices than those captured in Manta nets from the identical sampling region within the SASG (Mann-Whitney-Wilcoxon test, the outer accumulation zone, W = 479,  $P = 9.3 \times 10^{-4}$ ; the inner accumulation zone, W = 27,  $P = 1.4 \times 10^{-6}$ , Fig. 4A).

444

#### 445 **Discussion**

This study provides the first dataset on the distribution of MP throughout the water column 446 beneath an offshore plastic accumulation zone. Although the dataset is modest and comes from a 447 single expedition, the abundance and composition of MP at different depths from our study 448 provide unique insight into how MP are distributed from the epi- to the abyssopelagic layer within 449 the SASG. Overall, we observed that LMP abundances in net samples (Manta net and MultiNet) 450 were at least two orders of magnitude lower than the SMP abundances concurrently sampled with 451 in-situ pumps (Fig. 2, refer to Table S1 for the exact abundance values). These disparities 452 between abundances in the pump and net samples confirm that smaller-sized MP has been largely 453 undercounted and is an important contributor to plastic mass balance in the SASG. Our surface 454 SMP abundances are comparable to existing data (13-501 n m<sup>-3</sup>) from surface waters of the North 455 Atlantic Ocean by Enders et al. (16), who used Raman spectroscopy to identify MP down to 10 456 μm and found over 64% of particles were less than 40 μm. In comparison to a recent study that 457 employed similar large-volume sampling and automated interpretation µFTIR imaging methods 458 to study the plastic pollution in the deep-water column, SMP abundance (0-244.3 n m<sup>-3</sup>, median: 459 25.6 n m<sup>-3</sup>) in our study was higher than reported for the Arctic Ocean (size fraction: 25-50 µm, 460

abundance: 0-96 n m<sup>-3</sup>, median: 13.0 n m<sup>-3</sup>) (*18*). It has been shown that smaller-sized MP (typically <150  $\mu$ m) can translocate into the tissues and organs of organisms upon ingestion, exerting potential negative impacts on marine organisms (*19*). Investigation of smaller-sized plastics, largely missed in net-based and visual detection-based collections, is pivotal to understanding the ecological impacts of plastic debris as a whole in the ocean ecosystem.

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Horizontal Distribution. Floating plastic debris is generally understood to increase in abundance 467 towards the center of oceanic gyres (3-5). In the SASG, Cózar et al. (4) reported differences of a 468 hundred-fold in plastic concentration (>200 µm) between debris within inner and outer 469 accumulation zones, which agree with our Manta net data (Fig. 1). Our observations of lower 470 SMP abundances at water depths <60 m in the inner SASG accumulation zone (Stations B and C) 471 472 was observed, compared to those in the outer accumulation zone (Stations A and D) (Mann-Whitney-Wilcoxon test, P < 0.05, Fig. 2, SI Appendix, Fig. S2) are seemingly conflicting with 473 these previous observations. In particular, Station A has the highest abundance of SMP, followed 474 by Stations D, B and C. At present, we are not able to interpret this mismatch with certainty. 475 476 However, known processes provide a possible explanation for this anomaly. A previous modelling study predicted that 60%-80% of floating debris in the South Atlantic accumulation 477 zone was from South America (42). As the basin-scale circulation largely flows along the isolines 478 of sea surface height, this floating debris from the western border of the South Atlantic would 479 likely arrive first at Station B, followed by Stations A, D and finally Station C (Fig. 5). The 480 floating plastics probably disperses along the proposed route, which may partially contribute to 481 the lower SMP numbers in the near-surface layer of Station C. Additionally, the velocity fields 482 and sea level anomalies on the sampling day of each station (SI Appendix, Fig. S9) showed that 483 Stations A and D were closer to the core of the anticyclonic eddies than Stations B and C. 484 Previous model results documented that particles were prone to aggregate at the edges of the 485 anticyclonic eddies (43). Based on a MP field survey in the North Atlantic Gyre, data from 486 satellite observations and models, Brach et al. (44) provided evidence that the anticyclonic eddies 487 could retain, concentrate, and transport MP. High accumulations of near-surface SMP at Stations 488 A and D might also be closely related to these meso- and submesoscale features (Fig. S9). More 489 extensive *in-situ* observations of plastics in the water column will be necessary to resolve the 490 temporal-spatial patterns of water-column MP and verify the possible mechanisms at play. 491

492

493 Vertical profiles. Contrasting profile distribution patterns of MP were observed in the Arctic 494 Ocean among four stations by Tekman et al. (18), who regarded the inconsistent vertical

distributions among stations as indications for different mechanisms in the MP flux, highlighting 495 the role of local ocean circulation in the distribution of MP. Similar vertical differences were also 496 observed in our study. SMP abundances at Stations (A, B, D) generally decreased with depth, but 497 contrary to this trend, Station C showed a striking increase of over 20-fold (Fig. 2) at depth. To 498 compare this with the current velocity structure at each station, we computed the climatological 499 depth-dependent velocity profile at the closest grid point of the Simple Ocean Data Assimilation 500 database in 1993-2018 (Fig. 4B). It clearly indicates that the near bottom velocity of bottom 501 currents at Station C was 2-4 times weaker than the other three Stations A, B, and D. We 502 hypothesize that the slower bottom current velocity at Station C was less dilutive and appears to 503 be entraining SMP particles transported to this depth. Although the concurrent presence of both 504 high-abundance SMP and slow currents was only observed at one station, it agrees well with 505 506 Kane et al. (45) who reported that low intensity currents may accumulate plastic debris in the Tyrrhenian Sea based on a combination of *in-situ* MP measurements and numerical modelling. 507 The accumulation of SMP at depth may also be attributed to the interactions with biological 508 processes (e.g., biofouling, marine aggregate formation and incorporation into fecal pellets), 509 510 which have been shown to accelerate the downward transport of plastics (46, 47). But the knowledge of water-column MP interactions with biological processes is limited and warrants 511 further research. Additionally, the three-dimensional transportation of plastic particles might 512 contribute to the observed distribution patterns of SMP along the depth continuum (6). Non-513 metric multidimensional scaling ordination analysis of the polymer composition at each depth 514 supports the clustering of samples into three distinct groups (Fig. 4C, analysis of similarities 515 R=0.32, p=0.005). In general, high similarity of polymer types between the deep samples and 516 surface samples at different stations (Fig. 4C) was observed, which suggests oceanic SMP 517 particles are dispersed in lateral advection, vertical convection, or a combination of these 518 movements. Further studies of vertical measurements will aid in the understanding of MP 519 transport pathways in the water column. 520

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Steep density gradients, termed pycnoclines, are known to considerably decelerate settling rates of particles (such as suspended solids, algae or detritus, marine snow, etc.) and prolong their residence times above or within the pycnocline, resulting in a preferential accumulation (*48, 49*). Recently, several studies have reported higher abundances of MP particles in the pycnocline layers (*14, 50, 51*). In this study, we deliberately placed high volume pumps within the pycnocline at each station to investigate this phenomenon. At our sampling stations, the maximum buoyancy frequency values (0.021-0.025 s<sup>-1</sup>, Fig. S10) were larger than 0.016 s<sup>-1</sup>, indicating that the local

stratified layers were well-defined (32). Stratified conditions such as these have been observed to 529 entrap marine snow ( $\geq 200 \ \mu m$ ) particles (48), yet we only saw a modest peak in pycnocline SMP 530 numbers at one (Station B) of the four sampling stations. It should be noted that Station B 531 displayed the most intense pycnocline conditions from our sample set. However, LMP 532 abundances were observed to increase within pycnocline proximity in the MultiNet samples, 533 particularly MN-01 and -02 (Fig. 2). This difference may reside in the smaller particle size of 534 SMP investigated in this study compared to previous studies (14, 50, 51), whose detectable sizes 535 were limited to MP larger than 100 µm. The retention time of particles at ocean pycnoclines, 536 increases quadratically with particle size (52), with stratification effects being assessed by 537 comparing the size of settling objects with the fundamental length scale O (100 µm to 1 mm). 538 Thus, objects smaller than 100 µm are much less influenced by water stratification (36, 53). 539 Moreover, the theoretically predicted sinking velocities  $(2.4 \times 10^{-9} \text{ to } 1.0 \times 10^{-6} \text{ m s}^{-1})$  of SMP 540 particles in our study were at least three orders of magnitude smaller than previously measured 541 settling speeds ( $1.0 \times 10^{-3}$  to  $250 \times 10^{-3}$  m s<sup>-1</sup>) of large MPs (>300 µm) and fecal pellets (SI 542 Appendix, Table S6). Notably, SMP size are much smaller than microplastics and fecal pellets of 543 544 which the settling speeds were previously measured. These extremely low settling velocities of SMP imply higher dispersion, even distribution, and longer lifetime in the water column. 545 Altogether, these factors could explain the lack of retention of SMP particles at isopycnal 546 interfaces. However, the sinking behaviors of SMP may be altered by microbial colonization or 547 incorporation into marine snow and fecal pellets of zooplankton (46, 47). Incorporation of SMP 548 into egested organic materials and marine aggregates, could significantly increase the settling 549 velocity of plastic particles and thus enhance their export from oceanic surface layers and sinking 550 to the seafloor (46), which could result in the reduction of plastic abundances in the upper water 551 column. This undercounting seems especially true with respect to plastics that are denser than the 552 ambient seawater. In contrast, buoyant plastics may be less influenced because these particles can 553 resurface upon aggregate disintegration (54). 554

555

556 **Chemical Characteristics of Polymer Particles.** The polymer compositions of plastics in WTS-557 LV pump samples and Manta net samples varied significantly. Overall, PE and PP (91.4%) 558 dominated in the trawl samples, in agreement with findings extensively documented in the 559 literature (4, 5, 7). In contrast, the polymers in the WTS-LV pump samples included PA 6/6.6 560 (29.1%), alkyd resin (16.1%) and polyolefins (PP: 15.2% and PE:10.9%, PE/PP: 8.7%). This 561 incongruity between polymer types collected from pump and Manta net samples verify plastic 562 compositional changes with sampling depth due to size variation, buoyancies and chemical

properties of specific polymers. The prevalence of PA in the pelagic water column has also been 563 reported in the Arctic and Pacific Oceans (14, 18). PA is a typical polymer used for fishing nets 564 and ropes. The high number of PA particles indicates that active fisheries in the South Atlantic 565 Ocean (55), may be a sea-based source of PA plastics. Plastic debris sourced from the fishing and 566 other marine activities accounted for over 70% of marine plastic litter by mass on a remote island 567 in the central South Atlantic Ocean (56). Furthermore, polymers with functional groups, like the 568 amide bonds in PA, are susceptible to degradation such as biotransformation and disintegration, 569 relative to more recalcitrant polymers such as PE (57, 58). Enhanced susceptibility to general 570 degradation would accelerate generation of SMP from larger pieces, which could contribute to the 571 higher abundance of smaller PA in the marine environment. The second most abundant polymer 572 type found in our study was alkyd resin. The dominant alkyd resin in marine environments is 573 574 likely linked to the degradation of the painted surfaces of metal ships (23, 59). As an increasingly busy shipping route from South America to Asia, and a fishing hotspot (55, 60), the South 575 Atlantic Ocean is reasonably susceptible to these paints shedding from commercial and fishery 576 vessels. Ship paints have been shown to disintegrate more rapidly when compared with other 577 578 plastic polymers and this observation could explain their smaller size distribution in our samples (23, 59). 579

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The overall inventory from our WTS-LV pumps shows abundant plastic particles smaller than 581 100 µm below the surface of the South Atlantic (SI Appendix, Fig. S4). Our pump sampling 582 method was selective for particles that passed through the 40 µm mesh and the size distribution of 583 SMP shows a peak in abundance of fragments around 40 µm (Fig. S4), suggesting the meshes 584 were efficiently selecting for SMP. Meanwhile, SMP abundance peaking around 40 µm also 585 implied that SMP size was not apparently altered by our sample processing steps such as frozen 586 storage at -80°C, water bath sonication treatments and centrifugations. However, several large 587 plastics (>100 µm) were observed in the 2-40 µm size fraction. A similar phenomenon was 588 reported in a previous study where a 333 µm neuston net sampling yielded over 60% of non-589 string-like MP (0.4-1.0 mm) was able to pass through the mesh (61). A possible explanation for 590 the observed size anomaly is the pre-combusted stainless-steel mesh (40 µm) may be deformed 591 under increasing pressure during sampling, allowing larger particles through the mesh. Larger and 592 softer plastic particles could also have worked their way through the 56.6 µm minimal theoretical 593 diagonal of the square SS mesh sieve aperture. Finally, but less likely, the presence of larger 594 plastic particles may also be the result of the overlay or aggregation of small particles during the 595 processing of the samples. For instance, samples were concentrated on the 25 mm Anodisc 596

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membrane which was assembled in an open-face 13 mm filter holder, and finally generated an effective filtration area of 133 mm<sup>2</sup> (~13 mm in diameter) for  $\mu$ FTIR imaging, which may lead to particles overlaying. It should be mentioned that the boundary geometry of SMP particles measured by this approach suffers from uncertainties, to some extent. The uncertainty in the SMP dimension measurement can be attributed to the low peak-signal-to-noise ratios, which are caused by the irregularities of particle morphology (*34*), inhomogeneity in chemical composition of plastic materials, or insufficiently removed biofilm (*62*).

604

Manta net collected PE-LMP from the outer accumulation zone had higher carbonyl indices than 605 from the inner accumulation zone (Fig. 4A) suggesting a longer residence time for these LMP. 606 However, this finding is in disagreement with previous research (63, 64), that reported plastic 607 608 particles displaying a higher degree of oxidation offshore along a transect. A possible explanation for this discrepancy is that the literature datasets were from the inshore to offshore samples, in 609 contrast to all samples from the SASG in our study, where plastic particles with different 610 oxidative history were entrapped and move with the flow in the gyre. Therefore, it is plausible 611 612 that PE-LMP retained in the gyre for a longer time was coincidentally captured in the outer accumulation zone. Higher carbonyl indices were observed for SMP collected by WTS-LV 613 pumps compared to LMP collected with Manta nets, suggesting SMP were potentially more 614 degraded than LMP. A similar result was obtained by Ter Halle et al. (65), who found a 615 significant decrease in the molar mass from mesoplastic to MP sampled from the North Atlantic 616 Gyre, which suggests that small plastics are more oxidized than larger ones. These data also agree 617 with previous findings for the model of plastic degradation, which denotes that plastic degrades 618 into smaller fragments and these daughter fragments are likely to be more oxidized than parent 619 fragments due to their different chemical and physical properties (2). It should be noted that 620 specific polymer types exhibit variation in carbonyl indices, which underlines the need for a 621 larger database and development of other reliable methods to evaluate the weathering degree of 622 plastics in the environment. 623

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The appreciable amounts of SMP in the ocean's interior unveiled here suggest a potential ecosystem-wide impact. Compared to LMP, SMP are more readily ingested by marine particle feeders because of their small size and the smaller-sized plastic has the ability to translocate to internal tissues in organisms (*66*), resulting in bioaccumulation of MP and its associated chemicals at multiple trophic levels (*67*). Methods for the quantification of water-column SMP in the open ocean is new and results are therefore relatively uncommon, however, the current study

together with two previous studies all revealed unexpectedly high numbers of SMP in the ocean's 631 interior and varied with depth (18, 22). Previous reports have estimated mean MP abundances in 632 the upper 200 m of an Atlantic North-South transect as high as 2272 n m<sup>-3</sup> (22). Based on all these 633 depth-resolved observations, it is reasonable to consider the following scenario: large amounts of 634 SMP accumulate at certain depths, forming a high plastic-to-marine biota ratio, thus increasing 635 the probability of encountering and ingesting plastic particles by marine organisms (14, 68). For 636 instance, comparing our measured SMP abundances (0-244.3 n m<sup>-3</sup>) to previously measured total 637 copepod abundance (0-12 n m<sup>-3</sup>) at depth of 0-3000 m (69) in the South Atlantic, it is plausible 638 that copepods would encounter these abundant plastics at certain depths. Furthermore, the impacts 639 of SMP on marine fauna could be exacerbated by the combination of more aged surface 640 properties and large-aspect ratios (SI Appendix, Fig. S5), both of which have been experimentally 641 documented to enhance MP ingestion (47, 70). Filed evidence showed that MP (40-200 µm) 642 constituted roughly 26% of all plastic particles ingested by mesopelagic fishes in the northwest 643 Atlantic Ocean (71), and 45% of deep-sea benthic amphipods in the North East Atlantic contained 644 translocated MP in soft tissues, of which PA was the dominant polymer type consistently found 645 646 (72). As commercial fishing efforts scale up to harvest marine species for human consumption, studies focusing on smaller MP ingestion are urgently needed to assess the extent of plastic 647 contamination in biomass, which may represent a large fraction of the 'missing' plastics (73). 648 Marine organisms grazing on MP also have potential impacts on global biogeochemical cycling. 649 Employing an Earth system model, Kvale et al (74) predicted that zooplankton ingesting 650 microplastics could accelerate global deoxygenation by an extra 0.2-0.5% relative to 1960 values 651 by the year 2020 and reduce the oxygen inventory in the North Pacific by up to 10%. 652

653

Our results show that highly abundant SMP dominate surface to near-seafloor waters in the 654 plastic accumulation zone of the South Atlantic and form microplastic hotspots at certain depths, 655 implicating the ocean interior as a crucial pool of 'missing' plastics, particularly in low-flow 656 regimes. The abundances and distributions patterns of SMP varied geographically and vertically 657 due to the diverse and complex redistribution processes interacting with different plastic particles. 658 Compared with net-collected LMP, SMP particles are more highly oxidized and appear to have a 659 longer lifetime in the water column, suggesting increased marine ecosystem health risks through 660 possible bio-uptake of plastic particles and associated chemicals (75) and potential impacts to 661 global biogeochemical cycles (74). SMP are distinguished from LMP with respect to their high 662 abundance, chemical nature, transport behavior, weathering stages, interactions with ambient 663 environments, bioavailability and the release efficiency of plastic additives (57). These distinct 664

characteristics impact their environmental fate and potential impacts on marine ecosystems. Given the fundamental elements of our findings, this study of the SASG strongly suggests that SMP, largely unaccounted in previous studies, is a critical and unique component in ocean plastic inventories. Additionally, our study provides a stimulus for additional work to interpret and understand the fate and potential impacts of microplastics in aquatic systems in general.

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S.Z. wrote the first draft of the paper, all coauthors were involved in editing subsequent versions.
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- **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.
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## 915 Figure legends

and and

Fig. 1. Map of the Manta net, MultiNet, and WTS-LV pumps sampling locations. Blue 916 triangles represent four WTS-LV pump sampling stations (A, B, C, D). Green shaded bubbles 917 indicate the five Manta net tow stations (MT-05, -14, -16, -18, -22) and size of circles reflects the 918 wind-corrected LMP (0.3-5.0 mm) abundances. Red diamonds represent the three Multinet 919 sampling stations (MN-01, -02, and 03). Surface LMP abundances (n m<sup>-3</sup>) in the South Atlantic 920 Ocean by Eriksen et al. (5) are contoured using the Kriging interpolation method based on the 921 surface-trawl datasets (gray dots). The blue arrows represent the schematic circulation in the 922 South Atlantic. 923

Fig. 2. Numerical, mass abundances of MP collected with the Manta net, MultiNet, and WTS-LV pump. (A) Station A, (B) Station B, (C) Station C, (D) Station D. The filled colors of points correspond to the respective x-axis. The values of the Manta net sample have been corrected for wind-induced mixing in the 5-m-thick ocean layer. The numerical abundances of plastics in the Manta net and MultiNet samples were log transformed. Yellow triangles represent the MultiNet samples in which no plastic fragments were found. Gray and light blue shades 930 indicate the top 5 m of water column and approximate pycnocline layer, respectively. Error bars

931 represent standard deviation.

Fig. 3. Polymer composition profiles of SMP collected with WTS-LV pumps at four stations.
(A) Station A, (B) Station B, (C) Station C, (D) Station D.

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Fig. 4. (A) Carbonyl indices of polyethylene particles from the Manta net (orange boxplots) and 940 WTS-LV pump (yellow boxplots) in the SASG. The "Outer" and "Inner" indicate the sampling 941 regions located in the outer and inner accumulation zones of the SASG as shown in Fig. 1. Bold 942 black horizontal lines represent boxplot medians; top and bottom of colored boxes represent 25th 943 and 75th percentiles; and whiskers indicate the largest and the smallest measured values within 944 945 1.5 interquartile ranges from the box. Asterisks denote statistically significant differences between LMP and SMP (Mann-Whitney-Wilcoxon test, p < 0.05). (B) The depth-dependent velocity 946 profiles (m s<sup>-1</sup>  $\times$  10<sup>-2</sup>) at the four sampling stations averaged over 1993-2018. The dataset is from 947 the Simple Ocean Data Assimilation reanalysis. (C) Non-metric multidimensional scaling (NMDS) 948 949 ordination of polymer type composition identified in WTS-LV samples at each depth from all stations based on Bray-Curtis dissimilarities (Stress = 0.11). Ellipses in NMDS denote 95% 950 confidence levels for the distinct clustering. Colored shapes represent four WTS-LV pump 951 stations. The text labels indicate the sampling depth. 952

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Fig. 5. Surface circulation in the South Atlantic. The mean sea surface height (m, color) with
surface geostrophic velocities overlayed (m s<sup>-1</sup>, vectors) over the South Atlantic during 1993-2018.
The sampling locations are marked as red triangles. The dataset was obtained from the CMEMS.









